# MICROEVOLUTIONARY CHANGES AT THE ESTERASE-C LOCUS IN *ANOPHELES PUNCTIPENNIS*

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ABSTRACT. The EST-C locus alleles of *Anopheles punctipennis* were monitored for 2 breeding seasons from May to November in 1970 and 1971. Allelic frequencies were determined and were seen to change each month. One of the 4 alleles remained stable but the other 3 changed significantly in frequency. Hardy-Weinburg calculations based on chi-square analysis showed the genotypes not to be in equilibrium. Heterosis apparently is not a mechanism for the maintenance of the polymorphism since homozygotes greatly outnumbered heterozygotes, however, the lack of heterozygotes contribute significantly to the lack of Hardy-Weinburg equilibrium. The changes in allelic frequencies were statistically analyzed to determine if there was any correlation with environmental factors. Daylength and temperature showed some significant correlation. These were not always consistently correlated with allele frequency changes, suggesting that either some other component of the environment can significantly influence allelic frequencies or that other unknown mechanisms are involved. The tentative conclusion is reached that behavioral and ecological preference may be factors which affect the observed allele and phenotype distribution patterns.

The study of mosquito allozymes may be significant from both genetic and ecological points of view. Genetically, most mosquito genomes have not been studied very well, yet such information may be of singular importance to those interested in genetic methods of control. Because genetic variability, in the Neo-Darwinian sense, is supposed to contribute to the ability of organisms to adapt to and evolve with environmental and ecological change, the importance of allozymic variability is underscored.

In most mosquitoes studied to date, the esterases have proven to be such a variable allozyme system. Their genetics have been established for *Aedes aegypti* (Trebatoski and Craig 1969), *Culex pipens quinquefasciatus* (Garnett and French 1971) and *Cx. pipiens pipiens* (Stordeur 1976). In addition, the possible adaptive significance of the variability at the esterase locus has been under investigation. Scott and McClelland (1975) found differences in allelic frequencies of various enzymes between sympatric ecotypes which represented populations breeding in human dwellings and those breeding outdoors in treeholes. The variation in allelic frequency of the 2 gene pools was postulated to be the result of strong behavioral habitat selection. Saul et al. (1976) studying *Ae. aegypti* showed that the *EST-6* locus has 14 identifiable alleles with significant differences in gene frequency between an indoor and an outdoor strain. Likewise strains from villages only ¼ km apart frequently had different alleles.

The present report concerns an investigation into the possible adaptive significance of the polymorphic *EST-C* locus of *Anopheles punctipennis*. The esterase polymorphism of this species has been demonstrated by Narang and Kitzmiller (1971a) and the mode of inheritance of the *EST-C* locus established by Narang and Kitzmiller (1971b). In this study the *EST-C* locus alleles were monitored throughout 2 breeding seasons at the same locality with the purpose of investigating whether or not the 4 alleles changed in frequency and, if a change did occur, whether it could be correlated with

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changes in specific environmental variables.

## MATERIALS AND METHODS

SOURCE OF SPECIMENS. *Anopheles punctipennis* was chosen for the study of population dynamics because the genetics of the Esterase—*C* locus is known (op. cit.) and the organism was readily available in Allerton Park near Monticello, Illinois. Larvae were collected during the breeding season from the time of their first appearance in spring (late May) until the weather became too severe (early November) in both 1970 and 1971. Collection samples were made approximately every 2 weeks during this period. Fourth instar larvae were usually used in the assay but adults were also used at times. When time did not allow for the fresh material to be assayed it was stored at −20°C. Storage appeared to have no significant detrimental effect on isozyme activity.

Single pair crosses were done by induced copulation to establish lines homozygous for a given allele. These selected lines were used as marker stocks to serve as a standard reference point to help assign each band in a zymogram to its correct allelic designation. Dr. J. L. Hubby, University of Chicago, kindly provided a strain of *Drosophila virilis* (stock number 1018) homozygous for its esterase loci which also served as a standard reference.

SPECIMAN PREPARATION. The individual mosquitoes were homogenized in 0.015 ml of grinding solution (0.1 M Tris-EDTA-borate buffer plus 5% sucrose, pH 7.0) in 0.1 ml centrifuge tubes and centrifuged for 10 minutes in a refrigerated centrifuge. Then 0.01 ml of supernatant was placed into preformed gel slots with a microsyringe. The individual samples were then subjected to electrophoresis.

ELECTROPHORESIS AND STAINING. Vertical acrylamide gel electrophoresis (E.C. apparatus) was utilized using a 5% cyanogum solution made in 0.1 m Tris-EDTA-borate buffer, pH 8.9. A volume of 2000 ml of 0.1 M buffer was used to circulate through the system. The gel box was cooled with ethylene glycol and maintained at a temperature of −7°C. The current was 400 volts with 80–110 ma for 1.5 hr. At the end of a run, the gel was preincubated in 0.5 M boric acid for 1.5 hr in a cold room at 40°F. The gel was rinsed in distilled water and was then ready for staining. Fifty ml of 0.1 M phosphate buffer (pH 6.5) containing 12.5 mg each of alpha-naphthylacetate and beta-naphthylacetate (dissolved in 2 ml acetone) and 50 mg of Fast Blue BB salt was poured onto the gel slab. (See Johnson et al. 1966, for an explanation of the staining procedure.) Gels were shaken 1 hr on a shaker at room temperature to develop distinct bands and stored in a refrigerator until the isozyme bands could be identified as a specific allele.

ENVIRONMENTAL MEASUREMENTS. Four environmental factors were measured for the time periods covering the 2 breeding seasons. The data for the environmental variables of total rainfall, average temperature, average amount of moisture in the air, and average length of daylight were taken from the University of Illinois Weather Resources records. Table 1 lists the tabulated environmental variables for 12 consecutive 2-week periods.

STATISTICAL METHODS. The statistical methods used standard chi-square values to determine if the population phenotype distribution was in Hardy-Weinberg equilibrium.

A contingency chi-square test was used to determine if the phenotype change was significant between collections. A correlation analysis was then undertaken to determine if allele frequencies were associated with specific environmental variables. A similar test for phenotype frequencies was not conducted because heterozygotes constituted less than 7% of the total for each year samples were collected; thus phenotype frequencies should parallel the allele frequency changes in each year. Description of the analytical techniques are described in Snedecor and Cochran (1969).

Table 1. Environmental variables and allele frequencies used for correlation analysis based on twelve 2-week periods.

| Collection periods | Average duration of daylength, moisture in air, air temperature, and total rainfall | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Duration Daylength | | Moisture | | Temperature | | Rainfall | | $C^{1.89}$ | | $C^{1.81}$ | | $C^{1.72}$ | | $C^{1.63}$ | |
| | | | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 |
| May 28–June 10 | 1 | 891 min. | 52.6 | 61.9 | 69 | 72 | 1.18″ | .04″ | — | .092 | — | .618 | — | .276 | — | .013 |
| June 11–June 24 | 2 | 900 min. | 65.3 | 67.0 | 73 | 77 | 2.08″ | .76″ | .000 | .111 | .667 | .511 | .333 | .378 | .000 | .000 |
| June 25–July 8 | 3 | 897 min. | 65.1 | 69.2 | 74 | 79 | 2.02″ | 3.98″ | — | — | — | — | — | — | — | — |
| July 9–July 22 | 4 | 884 min. | 65.3 | 47.9 | 73 | 72 | 1.37″ | 5.44″ | .436 | .079 | .445 | .667 | .118 | .254 | .000 | .000 |
| July 23–Aug. 5 | 5 | 861 min. | 45.4 | 61.4 | 75 | 68 | 1.39″ | 1.96″ | — | .375 | — | .625 | — | .000 | — | .000 |
| Aug. 6–Aug. 19 | 6 | 832 min. | 66.5 | 64.6 | 74 | 73 | 2.85″ | .70″ | .294 | .083 | .471 | .752 | .235 | .166 | .000 | .000 |
| Aug. 20–Sept. 2 | 7 | 799 min. | 65.3 | 65.3 | 73 | 73 | .18″ | .69″ | .000 | .262 | .000 | .608 | 1.000 | .096 | .000 | .035 |
| Sept. 3–Sept. 16 | 8 | 764 min. | 63.3 | 64.7 | 70 | 74 | 3.05″ | 1.94″ | .105 | .141 | .579 | .668 | .316 | .174 | .000 | .016 |
| Sept. 17–Sept. 30 | 9 | 729 min. | 59.2 | 57.3 | 67 | 66 | 3.83″ | 2.83″ | — | .105 | — | .737 | — | .053 | — | .105 |
| Oct. 1–Oct. 14 | 10 | 691 min. | 52.1 | 50.6 | 59 | 60 | 1.95″ | .38″ | — | .220 | — | .659 | — | .073 | — | .049 |
| Oct. 15–Oct. 28 | 11 | 655 min. | 47.1 | 58.2 | 53 | 65 | 1.12″ | .34″ | .212 | — | .636 | — | .152 | — | .000 | — |
| Oct. 29–Nov. 11 | 12 | 622 min. | 40.6 | 39.0 | 45 | 52 | .43″ | .32″ | .000 | .371 | 1.000 | .571 | .000 | .029 | .000 | .029 |

## RESULTS

GENETIC DESCRIPTION. Narang and Kitzmiller (1971a) identified 3 alleles at the Esterase C-locus. These 3 alleles produced bands with RF values of $C^{1.89}$, $C^{1.81}$, $C^{1.72}$ expressed as differential migration with respect to a known marker-band (RF 1.0). The same naming system is used here. The present study has revealed a 4th allele, $C^{1.63}$. All 4 alleles at the *C* locus are codominant; the observed zymogram pattern therefore represents both phenotype and genotype. The presence of 1 band indicates an individual homozygous for a given allele while 2 bands represent a heterozygous individual. The individual allelic frequencies can thus be directly calculated. In this case, the locus is considered polymorphic because the frequency of the most common allele is no greater than 0.95.

Table 2 gives the allelic frequencies based on the number of individuals collected during 1970 and 1971. Note the absence of the rare $C^{1.63}$ allele from the 1970 samples. Table 2 shows that $C^{1.81}$ gradually increases in frequency while $C^{1.72}$ fluctuates in 1970. In 1971, $C^{1.89}$ increases more than $C^{1.81}$ while $C^{1.72}$ shows a consistent decline.

Table 3 shows the observed and expected genotype observations at the *EST-C* locus. The $X^2$ values indicate the significance of the deviation between the 2 under Hardy-Weinberg (H-W) conditions. The P values are significant at the .01 level. The H-W formulation and calculations for Table 3 were based on the 4 *EST-C* locus alleles and their respective monthly frequencies as shown in Table 2. Note in Table 2 for 1970 that the $C^{1.63}$ allele was not recorded nor was $C^{1.89}$ for June and November. Similarly the $C^{1.63}$, $C^{1.89}$, and $C^{1.72}$ alleles were not recorded for each month in 1971. Thus one has to be aware of the number of classes generated by 4 alleles or 3 alleles in determining the degrees of freedom for the $X^2$ value. The Yates correction term for small samples was used each time in calculating the $X^2$ values for each month. Based on the 1% level of significance, it

Table 2. Allele frequencies for the Esterase-C locus monthly collection periods.

| | | | 1970 | | | | |
|---|---|---|---|---|---|---|---|
| Allele | May | June | July | Aug. | Sept. | Oct. | Nov. |
| $C^{1.89}$ | — | .000 | .436 | .294 | .100 | .212 | .000 |
| $C^{1.81}$ | — | .667 | .445 | .471 | .550 | .636 | 1.000 |
| $C^{1.72}$ | — | .333 | .118 | .235 | .350 | .152 | .000 |
| $C^{1.63}$ | — | .000 | .000 | .000 | .000 | .000 | .000 |
| # of ind. | 0 | 3 | 55 | 17 | 20 | 33 | 4 |
| | | | 1971 | | | | |
| Allele | May | June | July | Aug. | Sept. | Oct. | Nov. |
| $C^{1.89}$ | .000 | .104 | .113 | .117 | .135 | .264 | .750 |
| $C^{1.81}$ | .417 | .587 | .662 | .676 | .680 | .639 | .250 |
| $C^{1.72}$ | .583 | .300 | .226 | .128 | .153 | .056 | .000 |
| $C^{1.63}$ | .000 | .009 | .000 | .019 | .032 | .042 | .000 |
| # of ind. | 6 | 115 | 71 | 324 | 111 | 72 | 4 |

appears that individual collection months rarely find the mosquito population to be in Hardy-Weinberg equilibrium for the *C* locus, and that the months most affected are those of July, August, September and October. The lack of equilibrium in the 1971 sample is especially impressive because not only is the same pattern followed as observed in 1970, but it occurs even though the sample sizes are much higher in most of the months sampled.

The chi-square method was also used to determine if heterozygotes occur in significantly greater numbers than expected. Table 4 is a comparison of the numbers of observed and expected homozygous and heterozygous genotypes. The critical value of chi-square is 3.84 at the .05 level of significance. The data show that homozygotes are significantly more prevalent than heterozygotes in the July-October samples, explaining the lack of Hardy-Weinberg equilibria in both years.

Calculation of contingency chi-squares for the phenotypes occuring within breeding seasons gives values for 1970 ($X^2$ = 27.8, df = 12) and 1971 ($X^2$ = 47.7, df = 12) which are highly significant for each year. These values were calculated using the June-October samples and dropping all individuals for the $C^4$ allele in 1971 in order to obtain a picture which could parallel the 1970 sample. Because of the low occurrence of heterozygotes, these were summarized into one class. Comparisons were then made using the above adjustment to the data on a month-by-month basis between years to determine if the phenotype classes differed significantly in numbers between years. These chi-square values are summarized in Table 5 and show that the June, August and September samples for 1970 and 1971 do not differ significantly in their relative phenotype numbers. The July and October samples as well as a value calculated for all samples do differ significantly, however, most likely because of the excess of $C^1C^1$ homozygotes in the July 1970 collection and the excess of $C^2C^2$ and greater number of heterozygotes for the October 1970 collection. The insignificant chi-square associated with the June analysis may be an artifact because of only 3 individuals in the June 1970 sample compared to 115 in the June 1971 sample.

CORRELATIONS WITH ENVIRONMENTAL DATA. In this study the environmental factors used for correlation were limited to the specific and measurable data of total rainfall, average temperature, average amount of moisture in the air and the average amount of daylight. If these factors are associated with allelic frequency

Table 3. Observed and expected genotype observations at the *EST-C* locus for 1970 and 1971. The $X^2$ values indicate the significance of the deviation between the two under Hardy-Weinberg conditions. The P values are significant at the .01 level.

| Collection Period | | Genotype | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C^1C^1$ | $C^2C^2$ | $C^3C^3$ | $C^4C^4$ | $C^1C^2$ | $C^1C^3$ | $C^1C^4$ | $C^2C^3$ | $C^2C^4$ | $C^3C^4$ | $X^2$ | p |
| May 1970 | obs | — | — | — | — | — | — | — | — | — | — | | |
| | ex | — | — | — | — | — | — | — | — | — | — | — | — |
| June | obs | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | ex | 0.00 | 1.33 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | 1.33 | 0.00 | 0.00 | .63 | .73 |
| July | obs | 24 | 24 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | | |
| | ex | 10.45 | 10.89 | 0.76 | 0.00 | 21.34 | 5.65 | 0.00 | 5.78 | 0.00 | 0.00 | 88.68 | .001 |
| Aug. | obs | 5 | 8 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | ex | 1.47 | 3.77 | 0.94 | 0.00 | 4.71 | 2.35 | 0.00 | 3.76 | 0.00 | 0.00 | 24.95 | .001 |
| Sept. | obs | 2 | 11 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | ex | 0.20 | 6.05 | 2.45 | 0.00 | 2.20 | 1.40 | 0.00 | 7.70 | 0.00 | 0.00 | 27.04 | .001 |
| Oct. | obs | 6 | 19 | 3 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | | |
| | ex | 1.48 | 13.35 | 0.76 | 0.00 | 8.90 | 2.13 | 0.00 | 6.38 | 0.00 | 0.00 | 24.88 | .001 |
| Nov. | obs | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | ex | 0.00 | 4.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | .99 |
| May 1971 | obs | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | | |
| | ex | 0.00 | 1.04 | 2.04 | 0.00 | 0.00 | 0.00 | 0.00 | 2.92 | 0.00 | 0.00 | .99 | .65 |
| June | obs | 10 | 63 | 30 | 1 | 2 | 2 | 0 | 7 | 0 | 0 | | |
| | ex | 1.24 | 39.62 | 10.35 | 0.01 | 14.03 | 7.18 | 0.21 | 40.50 | 1.20 | 0.62 | 166.35 | <.001 |
| July | obs | 8 | 47 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | ex | 0.90 | 31.11 | 3.62 | 0.00 | 10.62 | 3.62 | 0.00 | 21.24 | 0.00 | 0.00 | 127.58 | .001 |
| Aug. | obs | 54 | 207 | 32 | 4 | 3 | 3 | 1 | 16 | 3 | 0 | | |
| | ex | 10.14 | 148.04 | 5.28 | 0.10 | 77.50 | 14.64 | 2.14 | 56.05 | 8.29 | 1.56 | 565.02 | .001 |
| Sept. | obs | 13 | 72 | 15 | 2 | 3 | 1 | 0 | 2 | 2 | 1 | | |
| | ex | 2.02 | 51.33 | 2.60 | 0.11 | 20.38 | 4.57 | 0.95 | 23.09 | 4.82 | 1.07 | 170.22 | .001 |
| Oct. | obs | 19 | 45 | 4 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | | |
| | ex | 5.01 | 29.40 | 0.22 | 0.12 | 24.28 | 2.12 | 1.58 | 5.14 | 3.86 | 0.33 | 139.87 | .001 |
| Nov. | obs | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | ex | 2.25 | 0.25 | 0.00 | 0.00 | 1.50 | 0.00 | 0.000 | 0.00 | 0.00 | 0.00 | .94 | .65 |
| | | $C^1=C^{1.89}$ | | $C^2=C^{1.81}$ | | | $C^3=^{1.72}$ | | | $C^4=^{1.65}$ | | | |

Table 4. Comparison of observed and expected homozygous and heterozygous genotypes at the C locus. Critical value of $X^2$ is 3.84 at .05 level of significance.

| | 1970 | | | | | 1971 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Homozygotes | | Heterozygotes | | | Homozygotes | | Heterozygotes | | |
| | obs. | Ex. | obs. | Ex . | $X^2$ | obs. | Ex. | obs. | Ex. | $X^2$ |
| May | — | — | — | — | — | 5 | 3.08 | 1 | 2.92 | 2.46 |
| June | 3 | 2.66 | 0 | 1.33 | 1.34 | 104 | 51.22 | 11 | 63.74 | 98.03 |
| July | 54 | 22.1 | 1 | 32.77 | 76.84 | 71 | 35.63 | 0 | 35.48 | 70.59 |
| Aug. | 17 | 6.18 | 0 | 10.82 | 29.76 | 297 | 163.56 | 26 | 160.18 | 220.00 |
| Sept. | 20 | 8.7 | 0 | 11.3 | 25.97 | 102 | 56.06 | 9 | 54.88 | 76.00 |
| Oct. | 28 | 15.59 | 5 | 17.41 | 17.72 | 70 | 34.75 | 2 | 37.31 | 69.16 |
| Nov. | 4 | 4.00 | 0 | 0 | 0.00 | 4 | 2.5 | 0 | 1.5 | 2.6 |

Table 5. Contingency chi-square values obtained by comparing the numbers in the phenotype classes for 1970 to those for 1971. Individuals carrying allele $C^4$ have been dropped since 1970 samples did not have this allele present. Three degrees of freedom were used in all cases to determine significance.

| Month of Collection | $X^2_c$ | p |
|---|---|---|
| June | .68 | >.90 |
| July | 19.28 | <.001 |
| August | 6.08 | >.10 |
| September | 5.82 | >.10 |
| October | 11.75 | <.01 |
| Totals | 15.22 | <.01 |

changes, then there should be a significant correlation between one of these and the genetic parameter.

Table 1 lists the data for the environmental variables and the frequency of each allele grouped into twelve, 2-week intervals. Meaningful correlations are best detectable when a larger sample size is used, i.e. more collection dates. Therefore, the collection periods have been divided into 2-week intervals instead of months. Two-week intervals are also desirable because a 2-week period approximates the mosquito life cycle.

Table 6 lists the correlation coefficients which were determined (using the arc sine transformation) by using the allelic frequencies and the measurements for the environmental variables. In 1971 the $C^{1.89}$, $C^{1.72}$, and $C^{1.63}$ alleles show significant correlation with daylength. Temperature also gives some significant correlations. It would appear that the amount of daylight and average temperature are correlated with changes in allele frequencies more so than moisture and rainfall. On a random basis, 1.4 (5%) correlations are expected by chance but 21.4% are observed.

## DISCUSSION

The genetic analysis of the *EST-C* locus in the Allerton Park population of *An. punctipennis* reveals a dynamic picture both with respect to allele and the

Table 6. Correlation coefficients between *EST-C* allele frequencies and specific environmental variables. Significance of the critical values are at the .05 level.

| | Daylength | | Moisture | | Temperature | | Rainfall | |
|---|---|---|---|---|---|---|---|---|
| | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 | 1970 | 1971 |
| $C^{1.89}$ | .6529 | −.7663* | .2926 | −.4966 | .3263 | −.7182* | −.5043 | −.4449 |
| $C^{1.81}$ | −.9736* | −.1039 | −.8381 | .0562 | −.8569 | .0370 | −1509 | .3471 |
| $C^{1.72}$ | −.1158 | .9205* | .3114 | .5332 | .2788 | .7951* | .9238 | .1508 |
| $C^{1.63}$ | — | −.6899* | — | −.2240 | — | −.5328 | — | −.1418 |

* Critical value—1970 .9500.
1971 .6664.

phenotype changes. Hardy-Weinberg calculations based on the chi-square method to determine if observed numbers of genotypes fit the expected distribution show that the population is not in equilibrium. There are 2 factors which affect the chi-square distribution in this study and consequently whether or not the sample is a H-W equilibrium. The deficiency of heterozygotes suggests that they are experiencing some form of selection or that assortative mating is going on. It is not due to the presence of a null allele since Narang and Kitzmiller (1971b) did not detect such an allele in 17 laboratory crosses which they made. It is interesting that these workers also found a consistent deficiency of heterozygotes, and examination of their data in their Table 1 indicates that crosses generating all heterozygotes had the poorest fitness, and crosses made between only heterozygous parents had the next lowest fitness. If assortative mating is going on, then there may be niche subdivision occurring with within-niche mating taking place (i.e. Wahlund's principle is in evidence). To conclude that assortative mating is indeed taking place would require further investigation; however, it should be pointed out that Miles (1976, 1977) has observed assortative mating in natural and laboratory populations of *Culex*.

Secondly, the $X_2$ test itself becomes biased as the number of alleles increases and many genotype classes are generated with no or small numbers (less than 5 individuals in each class) as can be seen from Table 3 for the genotypes $C^1C^2$, $C^1C^3$, $C^2C^3$ of July 70.

Although the maintenance of the allelic variability at this locus may be due to assortative mating, other factors may also play a role. The data presented in Table 4 suggest we can rule out heterosis as a factor since homozygotes predominate. The environmental analysis reveals that the 2 breeding seasons are not identical environmentally with unseasonably cool and warm situations developing in July and Oct. of 1971 as shown in Table 1. Similarly, rainfall and moisture patterns varied for each season with considerable rainfall in Aug. 1970 and much less in Aug. of 1971. Examination of the allele frequency data show that the allelic frequency patterns are not identical for both years. A time period of 2 weeks approximates the time for the mosquito life cycle. Therefore it is probable that each generation or every 2nd generation was presented with a significantly different set of environmental interactions. It may be plausible, then, that the *C* locus polymorphism is maintained because it is advantageous to this species in adapting to the constantly changing environment. Johnson et al. (1969) found significant correlation with respect to what was called "weather" and the pattern of variability in 2 esterases in the harvester ant in different geographic areas. They proposed that natural selection was important in determining allelic frequency patterns and thus the value of the polymorphism. Johnson (1971) working with an esterase and 2 other enzymes in *Drosophila ananassae* found that the frequency differences among localities were consistent with adaptiveness of the polymorphisms to variations in the environment.

Table 6 shows significant correlations of various alleles with daylight and temperature. The patterns do not appear to be totally consistent. For example, $C^{1.81}$ shows significant correlation (−.9736) to daylight for 1970 but a nonsignificant correlation in 1971. One would think that if daylength is a significant environmental factor in allelic selection one year it would show its effect the following season also. Likewise temperature is seen to be a significant factor to allelic selection but appears to be more of a significant factor one year and less another. This suggests daylight and temperature are not the only factors influencing selection. We feel it would be best to view daylight and temperature as not having sole responsibility for selection of allelic frequency patterns but instead work in combination with the totality of the environment which could perhaps include such elements as the

source and population density of animals used by adult females for a blood meal.

We should point out, however, that the possibility of a single environmental factor acting to determine allele frequencies can not be ruled out since we have chosen only the environmental variables which were easily monitored. We know little of what microenvironmental features might be important in affecting the genetic structure at this locus either directly or indirectly by affecting, for example, mating behavior, phenotype movement and distribution patterns, or blood meal choice. That correlations between environmental factors and allozyme frequencies exist is witnessed by the studies of Merritt (1972) for lactate dehydrogenase, Schopf and Gooch (1971) for leucine aminopeptidase and Steiner (1979) for phosphoglucomutase, and isocitrate dehydrogenase as well as an esterase.

Although high mutation pressure (Wright 1966, Kimura 1968) might also act to maintain the variability we see here, we believe it unlikely for 2 reasons. First the great fluctuations in allele frequencies demonstrated in Figures 1 and 2 suggest that we would also have to see fluctuations in the mutation rate between generations, a feature that does not seem likely with respect to current evidence concerning the stability of mutation rates. Furthermore, Tobari and Kojima (1972) have shown that allozymes in *Drosophila* do not have mutation rates any higher than any other locus on the average. We have no reason to believe the case may differ for mosquitoes.

Finally, frequency-dependent selection, as observed by Kojima and Huang (1972) , can be suggested to maintain allele polymorphism. Allele $C^{1.81}$ which usually has the highest frequency, does not follow the criteria necessary here; that is, it is above 50% in frequency in 9 of 12 samples across both years and appears never to reach a threshold which might spark a sudden reversal in selection pressures (i.e., frequencies below 25%). Although we can not rule out frequency-dependent selection as a maintenance factor without time-dependent relative fitness tests for each phenotype, this type of selection appears unlikely to be a major balancing force because the allele fluctuations do not follow consistent and related trends.

*An. punctipennis* is polymorphic for 9 different chromosome inversions, and we would wonder what the role of these might be here. Obviously, if the *EST-C* locus is closely linked or tied up with an inversion, then a larger segment of the chromosome would be indicated as being involved in any maintenance scheme. EST-C would be acting as a marker for that segment. We have no information on the frequency of chromosome inversions or their seasonal abundance. Currently, plans are under way to determine these and to establish the linkage relationships for this and other polymorphic loci in this species in order to determine the relationship to chromosome variability. If *EST-C* is linked to an inversion, presumably the inversion would exist primarily in the homozygous state as well. Prakash (1976) has observed specific alleles at an alkaline phosphates locus associated with inversions in *Drosophila pseudoobscura*. Like esterases, phosphateses are known to hydrolyze ester bonds.

We must conclude, then, that no clear-cut relationships can be seen between the parameters we used to estimate gene-environment relationships, although we see significant differences between months, seasons and years with respect to allele and phenotype frequencies. The most consistent and significant effect seems to be a lack of heterozygotes across 2 breeding seasons. Because significant phenotype changes are seen and lack of heterozygotes exist, we do not see random genetic drift as playing any significant role here. Rather, we tentatively suggest, pending further investigation, that behavioral features of this population possibly associated with ecological subdivision may be at work to genetically differentiate the population, similar to that observed by Scott and McClelland

(1975) and Saul et al. (1976) for *Aedes aegypti*. If true, then this phenomenon may be more widespread than previously thought for mosquitoes and the evolutionary implications of this type of assortative mating would deserve serious consideration.

ACKNOWLEDGMENTS: We wish to thank an anonymous reviewer whose comments forced us to critically evaluate and clarify some of the statements we make.

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## ERRATUM

Dr. P. G. Jupp has called attention to an error in his paper, "*Culex quinquefasciatus, Culex pipiens* and other Culicines ovipositing in the Karoo Region of South Africa," which appeared in Vol. 38(4) pp. 594–595. In the last paragraph but one in the third line "There were collections . . ." should read "These were collections . . ." Also, on the inside front cover the word "America" was substituted for "Africa."