

A METHOD FOR DETECTING AND MEASURING CONCEALED VARIABILITY IN THE MOSQUITO, *CULEX TRITAENIORHYNCHUS*¹

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ABSTRACT

A natural population of *Culex tritaeniorhynchus* was sampled to test the efficacy of a proposed method to detect and measure concealed variability. Thirty-nine lethals and ten recessive visible mutations were uncovered out of a total of 125 chromosomes tested.

RECENTLY, there has been considerable interest in controlling mosquitoes by introducing genetic factors such as dominant lethals, chromosomal aberrations, segregation distorters and cytoplasmic incompatibility into natural populations (LAVEN 1967; CRAIG and HICKEY 1967; PATTERSON, LOFGREN and BOSTON 1968; LAVEN 1969; WHITTEN 1971). However, very little is known about the genetic variability present in wild populations. KITZMILLER, FRIZZI and BAKER (1967) have found evidence suggestive of considerable variability in the salivary chromosomes of some species while CRAIG and HICKEY (1966) have demonstrated the presence of morphological variability. Aside from the preliminary investigations of these workers, little has been done to assess the genetic variability of natural populations. Thus there remains a dearth of data concerning natural populations, particularly in the areas of recessive lethals and visible mutations, both important factors which should be accorded considerable attention in control programs utilizing genetic techniques. This lack of information is probably due to the absence of methods such as the *CIB* in *Drosophila* by which this variability could be readily assessed. BHALLA (1970) has proposed a method to detect sex-linked lethals in *Aedes aegypti*. However, this method is not applicable for wild-type chromosomes as lethals can only be detected from marked stocks. This paper describes a method for a mosquito, *Culex tritaeniorhynchus*, whereby recessive visible and lethal mutations can be detected in natural populations. Moreover, the technique is applicable for the quantification of the effects of mutagenic agents upon this mosquito.

MATERIALS AND METHODS

Many techniques have been described for *Drosophila* in which an entire chromosome or a

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major portion of one is rendered homozygous to detect recessive mutations. Usually the tester stock is characterized by two readily identifiable dominant morphological markers and an inversion which prevents crossing over between the experimental chromosome and the genetically marked tester chromosome. Recently these requirements have been fulfilled in *Culex tritaeniorhynchus* by the experimental introduction of inversions on the sex chromosome and the fortuitous discovery of a dominant gene, *Delta*. *Delta* (*D*) is a dominant gene on chromosome 1 which affects the shape of the eyes and the orientation of the wings. Both sexes show complete penetrance of this gene although there is some variability in expressivity. The second dominant marker is sex itself which in this species appears to be determined by a single pair of alleles, *M* and *m*, for which the males are heterozygous *m/M* and the females homozygous *m/m* (BAKER 1968). *Delta* and sex are less than one map unit apart.

No recombination of sex-linked markers has been found in females of *Culex tritaeniorhynchus* (BAKER and RABBANI 1970) but recombination is a regularly observed phenomenon in males. A number of experimentally induced pericentric inversions on the sex chromosomes have been previously described (BARKER, SAKAI and MIAN 1971). The particular inversion used in this study *I(1)101*, completely suppresses crossing over between *go* and *M* (25–27 map units). With the fulfillment of these three criteria, it was possible to synthesize a tester stock for the detection of recessive lethals and visible mutations on chromosome 1.

The tester stock was synthesized as follows (Figure 1): White-eyed (*w*) females heterozygous

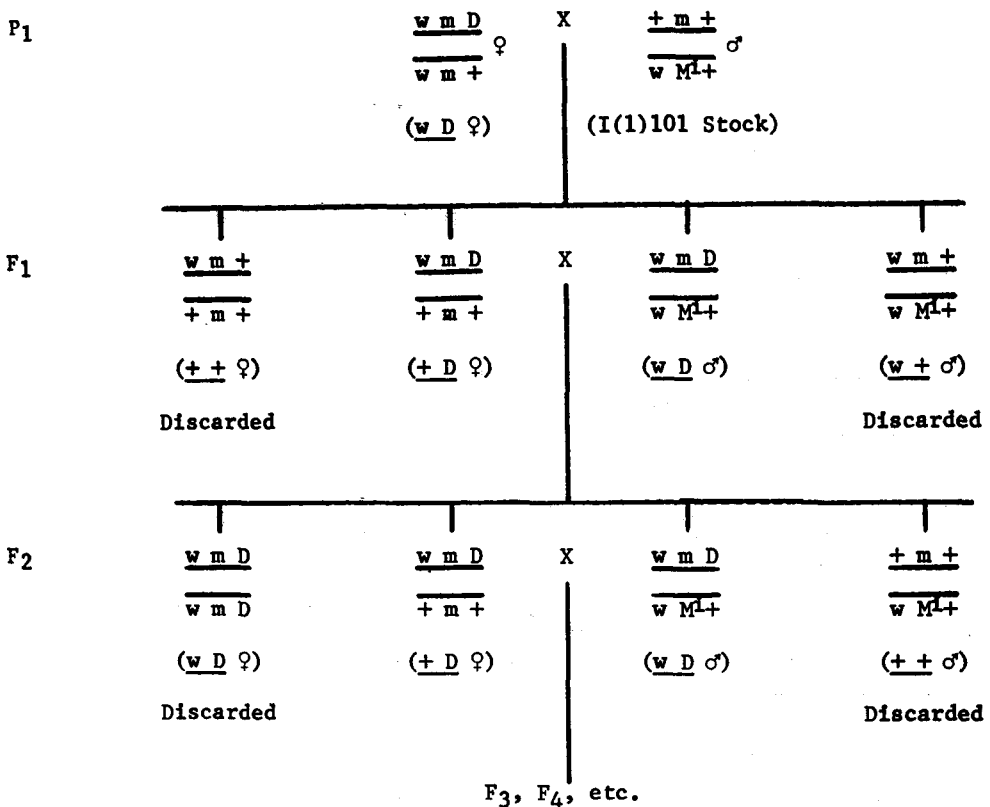


FIGURE 1.—Diagrammatic representation of the mating scheme used to synthesize the tester stock. *w* = white eye. *D* = delta. *m*, *M* = sex-determining factors. *M*ⁱ = inversion on the *M*-bearing chromosome.

for the *Delta* (*D*) gene were crossed with *I(1)101* males which carry the pericentric inversion on the *M*-bearing chromosome (*M*ⁱ). White-eye, a recessive sex-linked marker (BAKER 1969), is also present in the inverted segment. Among the F₁ offspring, the normal females and the white-eyed males were discarded and the +*D* females were crossed with *wD* males. This procedure is repeated every generation to maintain the tester stock.

Figure 2 is a diagrammatic representation of the crosses employed to achieve homozygosis of the *m* chromosome collected from a natural population which is carrying a presumed mutant

gene, a. Males from the natural population were mated to the tester females ($\frac{+ w m D}{+ + m +}$).

The +*D* females were selected from the F₁ progeny and crossed with tester *wD* males. From the progeny of this mating, the +*D* ♀♀ and the ++ ♂♂ were selected and crossed. Four phenotypes are expected among the progeny of this cross: ++ ♀, +*D* ♀, ++ ♂ and *wD* ♂. If a recessive lethal were present on the tested wild chromosome, no ++ ♀ would be found among the progeny of the above cross. Moreover, if a recessive visible mutation were present on the tested chromosome, all the ++ ♀♀ would show the character. Both lethals and visible mutations can be maintained in subsequent generations by repeatedly selecting and crossing +*D* ♀♀ and ++ ♂♂.

The controls for the experiment consisted of crossing +*D* ♀♀ from one chromosome line with ++ ♂♂ from a different chromosome line. The ++ ♀♀ among the progeny are thus heterozygous for two different *m* chromosomes from the natural population. Similarly, allelism tests for lethals could be made by crossing the +*D* ♀♀ from one lethal strain to the ++ ♂♂ from another lethal strain.

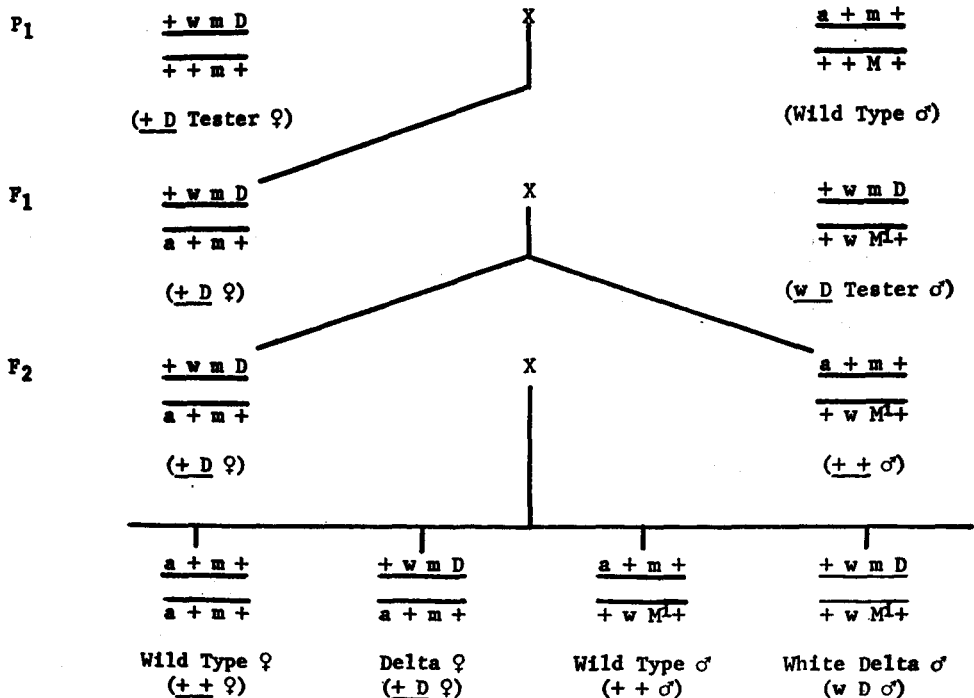


FIGURE 2.—Diagrammatic representation of the crosses employed to achieve homozygosis of *m*-bearing chromosomes (a/a) collected from a natural population. a = *m*-bearing chromosome from a natural population. w = white eye. D = delta. m, M = sex-determining factors. Mⁱ = inversion on *M*-bearing chromosome.

One of the difficulties in using this mosquito species is that single pair matings, although possible, do not occur sufficiently often for regular use in genetic experiments. Evidence from other species of mosquitoes suggests a high frequency of monogamy (CRAIG 1967). Preliminary studies in our laboratory using mass matings of marked stocks suggest that the frequency of multiple fertilizations occurs less than 3% of the time. Moreover, each female of *Culex tritaeniorhynchus* lays a discrete egg raft which can easily be isolated. Therefore, each raft represents a single family, the progeny of a single female and one male (97% of the time). Determinations of total number of eggs per raft and percent hatch can easily be done with a high degree of accuracy.

To test the effectiveness of the lethal and visible detecting system, a small survey was made of a wild population collected at Balloki Head Works about 40 miles south of Lahore, Pakistan. Collections were made by capturing fully blood-fed females from buffaloes. The females were then taken to the laboratory and individual egg rafts were isolated and reared separately.

Four collections were made at Balloki the spring of 1971: Females collected on 29 March 1971 produced 21 rafts; 15 April: 17 rafts; 22 April: 29 rafts; and 5 May: 77 rafts. The density of mosquitoes at this time was high although the highest frequency usually occurs later in July, August and September.

From each of the individual families approximately 25 males were put into a cage with five virgin $+D$ tester females (Figure 2). Only one raft was then saved from this cross (P_1). In the F_1 cross, approximately 20 virgin $+D$ females were crossed to five wD tester males. Here again only one raft was saved for the next generation. In the F_2 cross nearly all of the resulting $+D \text{♀}$ and $++ \text{♂}$ were put in a cage. Three rafts were then collected and each was reared individually for scoring in the adult stage.

The handling of the adults and the rearing of the larvae were similar to those reported earlier (RABBANI and BAKER 1970). The individual egg rafts were collected before hatching and isolated into vials. After hatching, the total number of larvae and of hatched and unhatched (embryonated and non-embryonated) eggs were recorded for each raft (family) in every cross and generation.

RESULTS AND DISCUSSION

From the initial collection of 144 rafts (144 m chromosomes to be tested), 125 reached the final test in the F_3 generation. The remaining 19 chromosomes failed to complete the test for various reasons: Some failed to produce egg rafts in either the P_1 , F_1 or F_2 generations, and a few were accidentally lost or contaminated. In the final generation (F_3) a minimum of 3 rafts was collected from each cross. This was usually adequate to obtain a sample of over 100 adults (the average sample size for each chromosome was 212). However, if fewer than 100 adults emerged or if a particular chromosome appeared interesting (no $++ \text{♀}$ were found or a visible mutation was detected), F_4 and successive generations were set up. If $++ \text{♀}$ did appear, they were tested for sterility by mating them to their $++$ brothers. In this preliminary survey, control data were not obtained for all chromosomes tested but only for those F_3 cultures in which no or nearly no $++ \text{♀}$ were found. This was done by serially mating $+D \text{♀}$ from one lethal chromosome line to the $++ \text{♂}$ of another lethal chromosome line. For example, the lethal-1 chromosome $+D \text{♀}$ were crossed to lethal-2 $++ \text{♂}$, the lethal-2 $+D \text{♀}$ were crossed to lethal-3 $++ \text{♂}$, etc., for all lethal chromosomes so that each lethal chromosome was tested twice.

The distribution of the viabilities of the $++$ females from the 125 homozygous tested chromosomes and the heterozygous controls are given in Table 1 and Fig-

TABLE 1
*Viability distribution of homozygotes and heterozygotes for m chromosomes expressed
 as availability ratios, ++♀ : 1/3 (+D♀ plus ++♂ plus uD♂)*

m chromosome	Viability ratio													Total			
	0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	1.1	1.2		1.3	1.4	1.5
Homozygotes	#	39	2	2	2	0	4	5	11	18	18	8	8	5	2	1	125
	%	31.2	1.6	1.6	1.6	0	3.2	4.0	8.8	14.4	14.4	6.4	6.4	4.0	1.6	.8	
Heterozygotes	#	0	0	0	0	0	0	0	2	8	9	7	8	4	1	0	39
	%	0	0	0	0	0	0	0	5.1	20.5	23.1	17.9	20.5	10.3	2.6	0	

= Number of chromosomes falling in the different viability classes.
 % = Percent of total number of chromosomes tested.

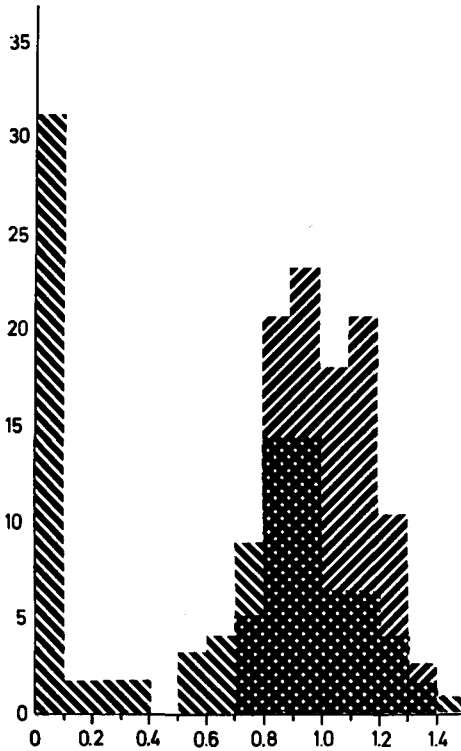


FIGURE 3.—Distribution of frequencies of viability ratios, $++\text{♀} : 1/3 (+D\text{♀♀ plus } ++\text{♂♂ plus } wD\text{♂♂})$. Ordinate = frequency of chromosomes in percent. Abscissa = viability ratios. /// = heterozygous controls. Reverse hatch = homozygotes. Cross-hatched area = area of overlap.

ure 3. The viables are expressed as the ratio of the $++\text{♀}$ to the average of the three other classes, $++\text{♀} : 1/3 (+D\text{♀♀ plus } ++\text{♂♂ plus } wD\text{♂♂})$. The bimodal distribution for the homozygotes is similar to that found in *Drosophila* (GREENBERG and CROW 1960; WALLACE 1968). One peak in this distribution lies at the level of complete or nearly complete lethality. The second peak occurs in the 0.80–1.0 interval, suggesting that most of the homozygotes are not fit as the mean optimal phenotype which was arbitrarily assigned a fitness of 1.0. Although no attempt was made in this study to categorize the viabilities into semilethals or subvitals, as in *Drosophila*, an entire spectrum of viability effects were observed. The criteria for selection of what is classified as a lethal are, therefore, somewhat arbitrary. If one considers as lethal those chromosomes which give fewer than ten percent as many wild type as the control or expected theoretical mean, then the frequency of lethals in this experiment was 31.2% (39 out of 125 tested chromosomes). Twenty-seven of these stocks produced no $++$ females, while twelve produced only a few $++$ females.

The viabilities of the $++\text{♀}$ from the serial control (heterozygous) matings of the 39 lethal chromosomes ranged from 0.7 to 1.4. The average viability for all these serial heterozygous crosses was 1.02, which does not differ significantly from the expected theoretical viability ratio of 1.0 ($P > 0.30$).

TABLE 2

Pooled data of adult phenotypes for all homozygote and heterozygote crosses

	$++\text{♀}$	$+D\text{♀}$	Phenotypes $++\text{♂}$	$wD\text{♂}$	Total
Homozygous					
Alive	3538	5730	6268	4828	20364
Dead*	442	1006	695	1837	3980
Total	3980	6736	6963	6665	24344
Percent dead	11.1	14.9	10.0	27.6	16.3
Heterozygous					
Alive	2622	2342	2571	1898	9433
Dead*	30	245	58	668	1001
Total	2652	2587	2629	2566	10434
Percent dead	1.1	9.5	2.2	26.0	9.6

* Dead on water or not completely emerged at time of scoring.

The results reported above were based on scoring all the adults. An individual mosquito was counted if the head had emerged from the pupal skin as all marker genes (sex, eye color and eye shape) can be easily recognized. In a few cases the $++\text{♀}$ from some homozygous crosses were not able to successfully complete emergence from the pupal skin and died in the water. When analysis was made on viabilities based solely on adults that successfully completed emergence and were flying in the culture bottle, the frequency of lethals are slightly higher, 32% (41 chromosomes of 125 tested).

The total numbers of mosquitoes examined is given in Table 2. In the homozygous chromosomal crosses, the $++\text{♀}$ class is the least frequent, whereas, in the heterozygous chromosomal crosses, the $++\text{♀}$ occurred in the highest frequency. Of the remaining three classes in both homozygous and heterozygous chromosome tests, the double mutant, $wD\text{♂}$, is the smallest class. This class also has the highest percentage of failure to emerge successfully from the pupal skin or dying in the water after emergence. Two other phenotypic classes were found ($wD\text{♀♀}$ and $+D\text{♂♂}$) which may represent crossovers. Thus the pericentric inversion used in this study appeared effective in eliminating recombination within the tested segment of the *m* chromosome as only fourteen (0.04%) of these apparent crossover types were found out of a total of 34,792 adults examined.

The stage of mortality for the various lethals has not been determined. The inversion (*I(1)101*) present in the $++\text{♂♂}$ itself produces lethality in the egg stage from the duplications and deficiencies produced by crossovers within the inversion loop. The sterility (ratio of unhatched eggs/total number of eggs) for this inversion (BAKER *et al.* 1971) was 28%. The amount of sterility present among the lethals and controls is shown in Figure 4. It is difficult on the basis of sterility alone to determine if mortality in the eggs is due to the inversion or to both the inversion and homozygosity for a lethal gene(s). Possibly a few of the chromosomes showing significantly higher sterilities than the one characteristic of the inversion may represent lethals with mortality in the egg stage. Many stocks contained deformed and grossly abnormal larvae, larvae which lived for

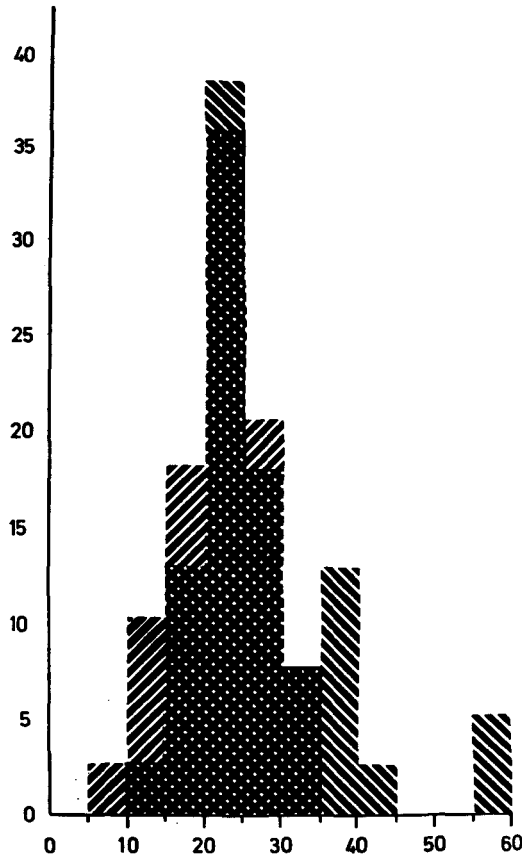


FIGURE 4.—Distribution of sterilities among the 39 lethals and heterozygous controls. Ordinate = frequency of chromosomes in percent. Abscissa = sterility in percent. /// = heterozygous controls. reverse hatch = lethals. cross-hatched area = area of overlap.

many weeks but remained in the second instar stage until death occurred or those which appeared normal but were not able to pupate. Other stocks were characterized by $++\text{♀}$ which could not successfully eclose.

Currently allelism tests among the thirty-nine lethals are being done. Detailed analysis of this experiment will be reported elsewhere. In addition to the thirty-nine lethal-bearing chromosomes, we found ten chromosomes with apparently recessive visible mutations: (1) pepper—the dark bands on the dorsal surface of the abdomen are reduced to a few scattered black scales. (2) bandless—the dark bands on the ventral surface of the abdomen are missing. (3) jointed palpi—the palpi of the females bear one or two extra joints. This was found in three different chromosomal lines. Two of the lines apparently carry the same mutation as females heterozygous for the *m* chromosomes from two of the lines show the characteristic phenotype. (4) long wings—the wings are extremely long. (5) short antennae—the antennae are drastically shortened, often with fusion of

some segments. Fertility of the homozygous females is very low. (6) tent wings—the wings are folded tent-like over the body. Homozygous females are sterile. (7) rose eyes—eyes are a bright rose color. Both homozygous females and males are fertile. This mutant is apparently an allele of the white-eye locus. (8) rough eyes—eye facets are irregularly spaced, giving the eye a rough appearance.

We also recovered one stock which produced many $++\♀$, all of which were sterile. Dissection of the spermathecae from these females revealed that they had been fertilized. Examination of the ovaries after a blood meal showed grossly abnormal egg development with only a very few apparently normal eggs and many immature eggs. Another stock was isolated in which the $++\♀$ were characterized by low fertility (32%).

This experiment demonstrates the efficacy of the proposed method in elucidating the hidden variability in a natural population. This technique can be utilized equally well to assess the effects of various mutagenic agents on this mosquito. Most important this method has the advantage that intact wild-type chromosomes from the natural populations or from laboratory stocks can be directly tested without the prior placement of mutant markers upon the chromosomes to be tested. Information about the hidden variability in a natural population would be important for a program utilizing biological control techniques. Thus a method whereby this variability could be readily assessed would be a valuable addition to the armamentarium of an integrated control program.

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LITERATURE CITED

- BAKER, R. H., 1968 The genetics of "golden," a new sex-linked colour mutant of the mosquito *Culex tritaeniorhynchus* Giles. *Ann. Trop. Med. Parasitol.* **62**: 193-199. —, 1969 White eye, a female sterile and sex-linked mutant of *Culex tritaeniorhynchus*. *Mosq. News.* **29**: 571-573.
- BAKER, R. H. and M. G. RABBANI, 1970 Complete linkage in females of *Culex tritaeniorhynchus* mosquitoes. *J. Heredity* **61**: 59-61.
- BAKER, R. H., R. K. SAKAI and A. MIAN, 1971 Linkage group-chromosome correlation in a mosquito. Inversions in *Culex tritaeniorhynchus*. *J. Heredity* **62**: 31-36.
- BHALLA, S. C., 1970 Paracentric inversions and the detection of sex-linked recessive lethals in *Aedes aegypti*. *Can. J. Genet. Cytol.* **12**: 635-650.
- CRAIG, G. B., 1967 Mosquitoes: female monogamy produced by male accessory gland substance. *Science.* **156**: 1499-1501.
- CRAIG, G. B. and W. A. HICKEY, 1966 Genetic variability in *Aedes aegypti* (Diptera: Culicidae). IV. Mutation load in some African populations. *Ann. Entomol. Soc. Am.* **59**: 1228-1234.
- , 1967 Genetics of *Aedes aegypti*. In: *Genetics of Insect Vectors of Disease*. Edited by J. WRIGHT and R. PAL. Elsevier, Amsterdam.
- GREENBERG, R. and J. F. CROW, 1960. A comparison of the effect of lethal and detrimental chromosomes from *Drosophila* populations. *Genetics* **45**: 1153-1168.

- KITZMILLER, J. B., G. FRIZZI and R. H. BAKER, 1967 Evolution and speciation within the maculipennis complex of the genus *Anopheles*. In: *Genetics of Insect Vectors of Disease*. Edited by J. WRIGHT and R. PAL. Elsevier, Amsterdam.
- LAVEN, H., 1967 Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature, Lond.* **216**: 383-384. —, 1969 Eradicating mosquitoes using translocations. *Nature, Lond.* **221**: 958-959.
- PATTERSON, R. S., C. S. LOGGREN and M. D. BOSTON, 1968 Sterile males for mosquito control: a field cage study with *Culex pipiens quinquefasciatus*. *Mosq. News.* **28**: 540-544.
- RABBANI, M. G. and R. H. BAKER, 1970 Red-spotted eye in the mosquito, an autosomal conditionally codominant mutant. *J. Heredity* **61**: 135-138.
- WALLACE, B., 1968 *Topics in Population Genetics*. W. W. Norton and Company. New York.
- WHITTEN, M. J., 1971 Use of chromosome rearrangements for mosquito control. In: *Sterility Principle for Insect Control or Eradication*. Joint FAO/IAEA Symposium, Athens, September, 1970.