California created an urgent need for a great increase in mosquito surveillance and control programs. Helicopters were used extensively for larval surveillance. When used in conjunction with the concentrator, approximately 20 to 30 square miles were inspected per hour depending upon the number of sources.

SUMMARY AND CONCLUSION. The mosquito larval-concentrator devised by Husbands (1969) was evaluated against the conventional medicine dropper technique; it has been shown that the new technique is nearly three times faster in operation and that samples collected by the two methods agree in species composition as well as in numbers of each developmental stage.

This new method is an especially useful device for mosquito control agencies. By using the larval-concentrator, which reduced the time spent on each collection, the Fresno Westside Mosquito Abatement District was able to expand its program and nearly quintuple the total number of samples.

When used in conjunction with larval surveillance by helicopter, the new technique increased the efficiency of inspections and greatly reduced the cost per sample.

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CROSS-OVER SUPPRESSORS AND STERILITY IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

SATISH C. BHALLA

Division of Medical Entomology and Ecology, Institute of International Medicine, University of Maryland School of Medicine, Baltimore, Md. 21201

INTRODUCTION. Translocations have often been proposed as possible means for genetic control of insect populations because of the semisterility associated with them (Serebrovskii, 1940; Burnham, 1962; Rai and Asman, 1968; Laven, 1969). On the other hand, inversions, based on the massive data accumulated on *Drosophila,* have not been considered as possible candidates for genetic control because they are not usually associated with a high degree of sterility. Evidence reported herein associates two sex-linked cross-over suppressors, which are perhaps inversions, and a "cross-over suppressor-enhancer system" with semisterility in the yellow fever mosquito, *Aedes aegypti*. Their possible use as tools for genetic control is discussed.

Two sex-linked mutant strains, bronze body, designated as *bz* (Bhalla and Craig, 1967) and white eye, designated as *w* (Bhalla, 1968), and a wild-type strain, ROCK were used in these investigations. Homozygous bronze females are sterile and stocks are maintained by crossing *bz*/+ females to *bz*/*bz* males. The strains were provided through the courtesy of Professor G. B. Craig, Jr., University of Notre Dame, Notre Dame, Indiana. Three batches each of 1–2 day old adults and 1 day old pupae were x-irradiated at 1000, 2000, and 4000 r. The irradiated males were screened for the presence of cross-over suppressors on the sex chromosomes using suitable crossing schemes. The genetic schemes utilized permitted the maintenance of the suppressors in heterozygous condition.

Sex in culicine mosquitoes is determined by a single gene located on chromosome I, or by a small segment of this chromosome. The males are heterogametic (*M/m*) while the females are homogametic (*m/m*). The sex locus can be used as a marker for linkage studies. The normal arrangement of the genes on the chromosome is of the order *w,bz,m*. Linkage distances are 13 between *w* and *bz*, 3 between *bz* and *m*, and 16 between *w* and *m* (Bhalla and Craig, 1970).

RESULTS AND DISCUSSION. One hundred eighty-nine sperms were tested for cross-over suppressors on the w^{+} *bz m* chromosome and 137 on the *w M* chromosome. Two suppressors were recovered, one from each group, and designated as C_1 and C_2. In addition a "cross-over suppressor-enhancer system" was isolated from the first group and designated as COSES. The two suppressors and COSES were recovered from adults irradiated at 2000 r. None of these is associated with any morphological characters, either in homozygous or heterozygous condition.

C_1 is maintained in heterozygous condition and is passed from one sex to the other in alternating generations. The homozygous bronze females cannot be used for perpetuation of this suppressor, since they are sterile. Table 1 reveals low hatch rates for the eggs laid both by the females carrying C_1 in heterozygous condition and those inseminated by C_1 heterozygote males. Hatch rates of 40 percent for the first category, and 35 percent for the second category do not compare favorably with 95 percent hatch rate of control ROCK females. Earlier Bhalla and Craig (1967) reported egg hatches of 97 percent from ROCK and 96 percent from *bz*/+ females. High zygotic lethalities in both cases, i.e., C_1 females and normal females inseminated by C_1 males, suggest that aneuploid cross-over products are not eliminated either in spermatogenesis or oogenesis. In order to avoid the possibility of the suppressor-free mating partner contributing sterility factors, which might have been induced by irradiations, suppressor heterozygotes were always crossed to males or females of the desired genotypes synthesized from non-irradiated stocks. Maintenance of this suppressor for six generations without increase in fertility beyond approximately 50 percent indicated the association of C_1 with partial sterility.

Both C_1 and C_2 markedly reduce crossing over in the regions w^{+} *m* and *w M* respectively (Table 1). C_1 suppresses crossing over from 16 to 1.7 in the females and 2.0 in the males. C_2, which is carried only in the males (*M* chromosome), suppresses crossing over from 16 to 3.0. C_2 also showed low egg hatch (33 percent) over a period of five generations, again indicating that the aneuploid cross-over products of spermatogenesis are being passed on to the eggs.

Cytological examination of the dividing male germ cells of C_1 and C_2 heterozygotes showed high frequencies of anaphase bridges and fragments (Fig. 1–4). Twenty-seven percent of 216 C_1 anaphases and 18 percent of 72 C_2 anaphases showed bridges and fragments. Significant suppression of crossing over and the presence of anaphase bridges strongly suggest that these suppressors are paracentric inversions.

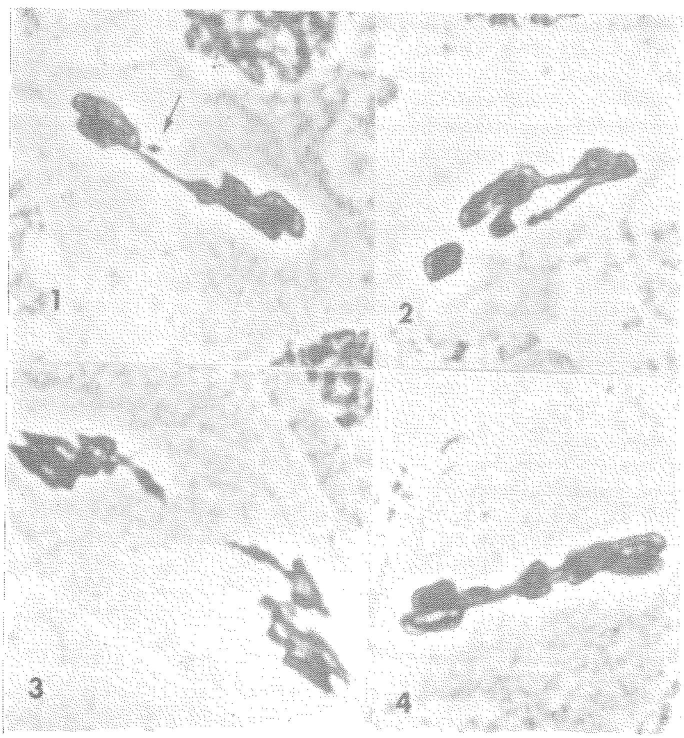
COSES is of special interest because it suppresses crossing over in the females but enhances crossing over in the males, i.e., the cross-over rate is reduced from 13 to 0.9 in the region *w bz*, while it is increased from 16 to 32.6 in the region *w M* (Table 1). This might suggest that a break has occurred between *bz* and *m*, and the two loci have moved farther apart. Typical heterozygote translocation

Table 1.—Egg hatch rates and cross-over suppression or enhancement associated with the two suppressors and COSES.

Suppressor	Chromosome carrying suppressor	Sex carrying suppressor	Cross-over region	No. of Eggs	% egg hatch	Total progeny	C.O*±S.E.†
C_1	w^+ *bz m*	♀	*w bz*	5506	40	1663	1.7±.31
		♂	*w m*	11001	35	3619	2.0±.23
C_2	*w M*	♂	*w M*	5871	33	1844	3.0±.39
COSES	w^+ *bz m*	♀	*w bz*	6279	36	1892	0.9±.22
		♂	*w m*	9674	43	3412	32.6±.80
Control (ROCK)	..	..	..	2013	95	..	..

* The normal cross-over rates are 13 in the region *w bz*, 3 in the region *bz m* and 16 in the region *w m*.

† S.E. $= \sqrt{\frac{p(1-p)}{n}}$, where p represents the cross-over value, as fraction of 1, and n the number of individuals, (Serra, 1965).



Figs. 1–4—Anaphase bridges from meiosis in the C_1 males, X790. Fig. 1, Note a bridge and a fragment (arrow). Fig. 2, A complete and a broken bridge. Fig. 3, A bridge in the process of breaking up. Fig. 4, A bridge overlapping a large piece of chromatin, perhaps a fragment.

configurations and a high frequency of anaphase bridges (14 percent in 74 cells examined) were observed in the dividing meiotic cells. This suggests the presence of both, a paracentric inversion and a translocation. The exact nature of this system, however, is not known and further studies are planned.

Rai (1967) has indicated the possibility of using inversions for genetic control of mosquitoes under certain circumstances, but a World Health Organization Scientific Group (1968) subsequently concluded that the use of inversions to control vectors is remote. The argument against using inversions stems mainly from the evidence accumulated in *Drosophila,* where the inversions are not usually associated with a high degree of sterility. However, the situation in *Drosophila* is unique for two reasons. First, there is no crossing over in *Drosophila* males; the aberrant chromosomes, i.e., dicentrics, acentrics, duplications, and deficiencies, expected as a result of crossing over in the inversion heterozygote males, giving rise to aneuploid gametes, are not produced. Second, the females have a built-in mechanism which prevents the inclusion of aberrant chromosomes in the functional ovum (Sturtevant and Beadle, 1936). In contrast, crossing over regularly occurs in male culicine mosquitoes. Therefore, their inversion heterozygote males regularly produce cross-over aneuploid sperms, which after fertilization give rise to non-viable zygotes thus leading to partial sterility. The female culicine mosquitoes are perhaps unable to prevent the inclusion of at least a part of the aberrant cross-over products in the eggs, thus manifesting partial sterility.

Theoretically sterility induced by inversions may or may not be of the same order as that induced by translocations. However, if it can be augmented by sterility from other sources, i.e., deficiencies, deleterious genes, translocations etc., it may provide a potential mechanism for genetic control. A massive field release of males carrying inversions either in one or both of their sex determining chromosomes plus other deleterious genes, might curtail the fertility of culicine populations. The population reduction obviously will depend on the numbers released and the degree of semisterility induced.

The above mentioned suppressors, which evidence suggests are inversions, can be utilized in a number of ways. 1. They provide a tool for the detection of sex-linked recessive lethals (Rai, 1967). Work is already in progress along these lines. 2. C_1 can be employed as a "balancer" to develop a balanced lethal system for maintaining bronze stocks without recourse to selection and crossing of $bz/+$ females and bz/bz males in every generation. 3. They may be used for reducing fertility in mosquito populations. Those inversions which are associated either with translocations or some other deleterious genes such as the sex-ratio distorter of Hickey and Craig (1966) would be especially useful. Further confirmation of these suppressors as inversions, and if so the length of these inversions, the break points on the chromosomes and their interchromosomal effects, are being investigated.

SUMMARY. Two sex-linked cross-over suppressors, one on *m* chromosome and the other on *M* chromosome, have been isolated. Crossing-over in the region *w bz m* is suppressed to the extent of about 80–90 percent. Significant suppression of crossing-over and the presence of high frequencies of anaphase bridges and fragments in the male germ cells strongly suggests that the suppressors are paracentric inversions. Of special interest is a suppressor-enhancer system which suppresses crossing-over in females but enhances crossing-over in males. Both cross-over suppressors and the suppressor-enhancer system, induce partial sterility. Their possible use in genetic control is discussed.

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MOSQUITO CONTROL IN SEWAGE OXIDATION PONDS WITH DRIP AND POUR-IN LARVICIDES[1]

M. S. MULLA,[2] H. A. DARWAZEH[2] AND D. R. PETERS[3]

The Cucamonga sewage water ponds operated by the Cucamonga Water District, Cucamonga, California are located 5 miles east of Ontario and 6 miles south of Cucamonga. Raw sewage flows southward from the City of Cucamonga in a pipeline underground into the ponds at an average rate of one million gallons per day. The effluent from the initial two stabilization ponds or lagoons which are connected in series, subsequently passes into four percolation ponds connected in parallel. The four percolation ponds were periodically rotated. All ponds were found to be an ideal breeding source for several species of mosquitoes. During a 2-year period (December 1967–November 1969) larvae of four species were recovered and identified. They were: *Culex peus* Speiser, *C. quinquefasciatus* Say, *C. tarsalis* Coquillett, and *Culiseta inornata* (Williston), all biting man or pestiferous to man and domestic animals at times.

High, dense vegetation growth around the edges of the ponds, where mosquito larvae are found, hinders mosquito control operations from the levees. Mosquito larvicidal sprays failed to penetrate the heavy vegetation to yield adequate control. Weed control programs could reduce the intensity of the mosquito problem in the ponds, but the cost of material, equipment and labor is somewhat high. In order to achieve satisfactory control of mosquito breeding in this type of habitat at a low

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[2] Department of Entomology, University of California, Riverside, California.

[3] Cucamonga County Water District, Cucamonga, California.