SEX-LINKED CROSSOVER SUPPRESSORS IN THE MOSQUITO, *AEDES AEGYPTI*

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INTRODUCTION. Crossover suppressors have been utilized to detect lethals in the X chromosome of Drosophila. Techniques like CLB and 'Basc' or Muller-5, can be developed for species other than Drosophila, if the necessary mutations and crossover suppressors are available. However, little progress has been made along these lines with mosquitoes. Bhalla (1970) utilized two paracentric inversions as crossover suppressors for detecting sex-linked recessive lethals in *Aedes aegypti*, but these involved only a part of the known genetic length of chromosome I. This work has now been extended to detect and isolate more crossover suppressors, which either individually or in combination will effectively cover the total known genetic length of chromosome I. This paper presents 11 new suppressors, thus bringing the total number of sex-linked crossover suppressors available in *Aedes aegypti* to 13.

MATERIALS AND METHODS. A detailed description of mutant strains of *Aedes aegypti*, methods of rearing and basic genetic information can be found in earlier publications (Craig and Hickey, 1967; Bhalla, 1970; Bhalla and Craig, 1970). Sex-linked recessive mutants used in the experiments reported here, include rust eye (*ru*), red eye (*re*), bronze body (*bz*) and white eye (*w*). Sex (*m*) is also used as a marker; homogametic *mm* are females and heterogametic *Mm* are males. Homozygous bronze females are sterile and hence can not be used for test crosses.

Two breeding schemes were employed to screen cross-over suppressors on *m* and *M* chromosomes respectively. In scheme

1 (Fig. 1) bronze male pupae were x-irradiated at 3000 r and crossed to rust-red females. Wild-type female progeny, heterozygous for the irradiated *m* chromosome, were test-crossed to rust-red bronze males. Non-recombinant progenies of females showing suppression of crossing over in *ru-re-bz* region were isolated. From among these, bronze males carrying irradiated *m* chromosome were selected and test-crossed to rust-red females heterozygous for bronze (step 3). In the following generation wild-type females heterozygous for irradiated *m* chromosome were again selected and test-crossed to rust-red-bronze males (same as step 2). Thus the irradiated *m* chromosome passes alternately from females→males→females ——— and can be maintained indefinitely by repeating steps 2 and 3 *ad infinitum*.

In scheme 2 (Fig. 2), wild-type male pupae were x-irradiated at 3000 r and crossed to rust-red females. Wild-type male progeny heterozygous for the irradiated *M* chromosome were test crossed to rust-red females. Non-recombinant progenies of the males showing cross-over suppression were isolated and crossed. Thus the irradiated *M* chromosome, which passes only through the males can be maintained indefinitely.

RESULTS AND DISCUSSION. Following x-irradiation, 46 *m* chromosomes and 36 *M* chromosomes were tested for the presence of crossover suppressors. Six suppressors were detected and isolated on *m* chromosome and 5 on *M* chromosome (Table 1). The suppressor lines have passed through 4–6 generations.

Varying levels of fertility were observed among various suppressor lines (Table 1). Egg hatch rates of the females, heterozygous for suppressors, were taken as indicators of their fertility. Fertility of males heterozygous for suppressors was measured by the egg hatch rates of normal females inseminated by them. In two lines –*In. (1)C9* and *In. (1)C10,* the males showed higher fertility than the females, while in one line *In. (1)C31* females showed higher fertility than the males. In the remaining three *m* chromosome suppressor lines, the fertility among the two sexes was comparable. Among the *M* chromosome suppressor lines, the egg hatch rates varied from 56 to 89 percent.

Linkage analyses were carried out along the total known genetic length of chromo-

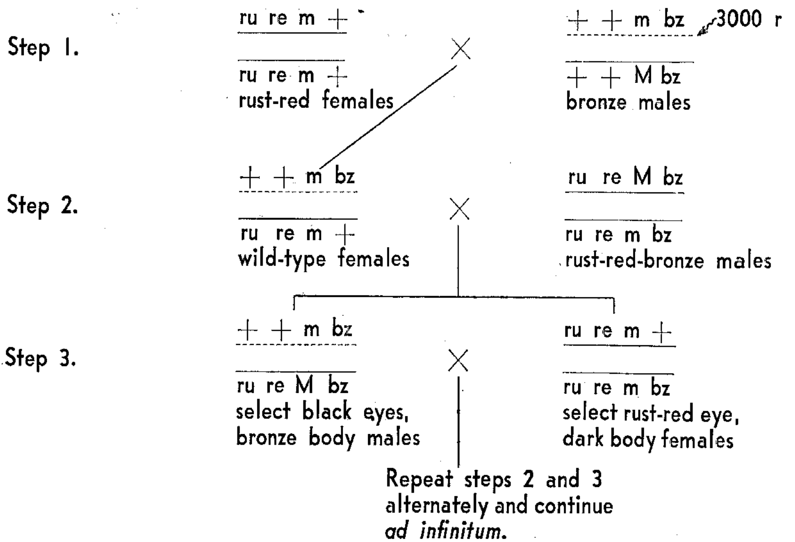

FIG. 1.—A breeding scheme to isolate and maintain crossover suppressors on *m* chromosome. Dashed line indicates the irradiated chromosome-carrying suppressor.

some I for each suppressor line (Table 1). The normal sequence of genes is *ru re m bz w* (Bhalla and Craig, 1970). Normal crossover values for various segments are shown in the table. The chromosomal length affected by suppressors varies with each line. *In. (1)C10* is especially interesting, since it suppresses crossing over along the total known genetic length of this chromosome, suppression being complete in *ru-re* segment and partial in *re-m-bz-w* segment. *In. (1)C9* affects *ru-re-m region* and *In. (1)D50* affects *re-m-w* region. The remaining suppressor lines manifest crossover suppression along smaller regions of the chromosome, the smallest region affected is *re-m* in *In. (1) D9* and *In. (1)D39*. *In. (1)C56* suppresses crossing over in two distant segments of the chromosome *ru-re* and *m-w;* the intermediate segment *re-m-bz* remains unaffected. All of the remaining suppressor lines affect only *ru-re* region.

Chromosome preparations from testes, ovaries and brain cells were examined. None of the mitotic metaphases showed shifts in the position of centromere indicating that none of the suppressors are pericentric inversions. On the other hand all lines showed dicentric anaphase bridges and acentric fragments in the male meiotic cells. Lewis and John (1966) caution against regarding all cases of bridges and fragments as diagnostic of paracentric inversion hybridity. However, in the present experiments, bridges and fragments occur frequently and are accompanied by crossover suppression. Moreover, dicentric bridges accompanied by acentric fragments are rarely observed in the normal meiosis of *Aedes aegypti*. The evidence is strongly in favor of assuming that all crossover suppressors described above are paracentric inversions. Also, two previously reported inversions, which suppressed crossing-over effectively in *m-w* region, were found to be paracentric (Bhalla, 1970). Thus, the evidence available indicates that the centromere is not included in the presently known genetic length of chromosome I; in other words, all known mutant loci are located on one arm of the chromosome. Suitable genetic markers on the other arm of this chromosome are needed to compile the genetic map.

SUMMARY. Eleven sex-linked crossover

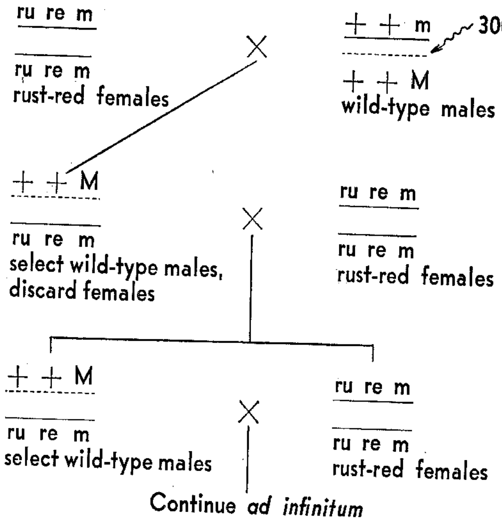

FIG. 2.—A breeding scheme to isolate and maintain crossover suppressor on *M* chromosome. Dashed line indicates the chromosome-carrying suppressor.

TABLE 1.—Fertility and crossing over in eleven strains of crossover suppressors

Strain designation	Suppressor carried by: chromosome	Suppressor carried by: sex	Egg sample examined	% egg hatch	No. of* adults scored: a	No. of* adults scored: b	% crossing over**: ru-re	% crossing over**: re-m	% crossing over**: re-bz	% crossing over**: m-w
In.(1)C9	m	♀	720	37	2145	-	2.7	-	15.2	-
		♂	1898	90	1535	2640	2.7	3.9	-	21.1
In.(1)C10	m	♀	927	45	1249	-	0	-	2.3	-
		♂	2169	70	1675	1223	0	2.1	-	5.6
In.(1)C19	m	♀	784	54	777	-	1.2	-	21.1	-
		♂	1498	57	1780	2703	2.4	9.4	-	23.7
In.(1)C26	m	♀	841	69	1765	-	2.3	-	7.6	-
		♂	1659	71	2954	2712	1.6	8.6	-	11.9
In.(1)C31	m	♀	722	93	836	-	3.6	-	15.3	-
		♂	1184	77	1862	4308	4.4	5.2	-	14.4
In.(1)C56	m	♀	502	73	477	-	2.5	-	12.6	-
		♂	920	65	1885	3325	2.5	6.7	-	8.7
In.(1)D4	M	♂	1953	64	3421	2111	2.4	7.7	-	13.0
In.(1)D6	M	♂	2566	56	1448	1783	1.1	6.4	-	16.3
In.(1)D9	M	♂	1927	89	1266	1467	12.8	1.5	-	16.5
In.(1)D39	M	♂	2421	79	1924	1828	16.4	3.0	-	13.9
In.(1)D50	M	♂	2377	89	2787	1117	16.6	3.3	-	8.2
Normal***	None	♀	-	-	-	-	9.9	-	11-18	-
		♂	-	-	-	-	16.6	7.5	-	15.0

* a—recombinations in columns *ru-re, re-m* and *re-bz* are based on these scores.
b—recombinations in column *m-w* are based on these scores.
** *ru* = rust; *re* = red; *bz* = bronze; *m* = sex; *w* = white.
*** Linkage data based on previous analysis (Bhalla and Craig, 1970).

suppressors were isolated and established in heterozygous lines. These strains have passed through 4–6 generations and manifest varying levels of sterility. All lines manifest crossover suppression along various segments of chromosome I. One line suppresses crossing over along the total known genetic length of chromosome I, while the rest affect smaller regions of the chromosome. Cytological data strongly suggest that all suppressors isolated are paracentric inversions.

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