RECIPROCAL TRANSLOCATIONS IN *AEDES AEGYPTI* (L.)

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ABSTRACT. Reciprocal translocations were induced in males of *Aedes aegypti* (L.) by treatment with 4 kR of either X rays or gamma rays (from a ^{60}Co source). Thirty-three heterozygous translocation stocks were maintained in the laboratory and characterized in terms of linkage groups, fertility, and percentage recombination between markers. Of 11 of the stocks tested for competitive mating, 9 either equalled or exceeded the standard stock, RED (a mutant marker strain). Twenty-five of the translocations were evaluated for viability of the homozygote, but only one stock was viable when homozygous.

The value of chromosomal interchanges as genetic tools was recognized many years ago, but only during the past decade has considerable attention been directed at the use of interchanges, especially reciprocal translocations, for the control of insect pests. The basic concept was discussed by Serebrovsky (1940), whose paper was largely neglected for over 20 years, and later by Curtis (1968) and Whitten (1971). The production of reciprocal translocations in insect pests was reported for *Musca domestica* L. (Wagoner et al. 1969), *Glossina morsitans* (Westwood) (Curtis 1970), *Phaenicia* (= *Lucilia*) *cuprina* (Wiedemann) (Whitten 1970), and four species of mosquitoes, *Aedes aegypti* (L). (Rai et al. 1970), *Culex pipiens* (L.) (Laven 1969), *Culex tritaeniorhynchus* Giles (Sakai et al. 1971) and *Anopheles albimanus* Wiedemann (Rabbani and Kitzmiller 1972). We are currently involved in producing reciprocal translocations in *A. aegypti* as a part of a program to develop genetic aberrations that can be included in a practical scheme for the control of this species. This present paper summarizes information dealing with the isolation and characterization of reciprocal translocations.

MATERIALS AND METHODS. The following stocks of *A. aegypti* were used: RED —A mutant marker stock with *sex* (*m*) and *red eye* (*re*) on chromosome 1, *spot* (*s*) on chromosome 2, and *black tarsi* (*blt*) on chromosome 3. This stock was originally received from G. B. Craig, Notre Dame, Indiana. *Siws blt*—A mutant marker stock with *Silver* (*Si*), and *spot* (*s*) on chromosome 2 and *black tarsi* (*blt*) on chromosome 3.

OCALA—A wild type stock collected in Ocala, Florida. INDIA—A wild type stock obtained from New Delhi, India, through the courtesy of R. S. Patterson. VOYLE—A wild type stock collected in Gainesville, Florida.

Males from the wild stock were irradiated with 4 kR of gamma (^{60}Co, 1076 r/min) or X-rays (X-ray machine operated at 50 KUP, 222 r/min) when they

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were less than 24 hours old. These treated males were crossed to virgin RED females, and the F_1 male progeny were backcrossed to RED. All crosses were individual pair matings to ensure the detection of translocations. Tentative identification of reciprocal translocations was based on the fertility of the backcross eggs and the pseudolinkage of the mutant markers in the backcross progeny. The stocks were maintained in subsequent generations by backcrossing to RED, and the recombinant types were discarded each generation.

To determine whether the translocation stocks were debilitated due to the radiation treatment, males from 11 stocks were tested against RED males in competitive mating trials. These tests were conducted in .5 m^3 plastic cages with 100 T males: 100 RED males:100 RED females; the T males were heterozygous for the translocation and the markers *s* and *blt*. Competitiveness was assessed by measuring both the percentage egg hatch and the phenotype of the progeny. The expected hatch was calculated by summing the products for hatch and frequency of the two types of males. Hatch values were obtained from previous observations and from controls observed concurrently with the tests. The expected values were compared with observed values by χ^2 analysis. Competitiveness (c) was calculated by the following formula:

$$c=\frac{T}{N}=\frac{W_{1\alpha}}{W_2(1-)}$$

where: T=frequency of translocation males
N=frequency of normal males
W_1=fertility of a normal male
W_2=fertility of a translocation male
=frequency of wild type male progeny

This formula is valid as stands for actual ratios of 1T:1N, but with greater ratios, division of the calculated ratio by the actual ratio would be required. For the autosomal translocations, competitiveness was calculated by $c=\frac{T}{N}=\frac{W_{1\alpha}}{W_2(.5-)}$ because only one-half of the male progeny carry the translocation. These formulas used to calculate competitiveness are similar to the procedure outlined by Fried (1971) for calculation of competitiveness of sterile males.

Each apparent translocation was confirmed cytologically by examination of chromosome squashes of larval brain or adult gonadal tissue. The cytogenetic data are not presented here, but will appear in a separate paper.

The translocations that appeared to be simple, i.e., those involving only an interchange between two chromosomes and not complicated by unidentified aberrations, were outcrossed for five generations and then tested for viability as homozygotes. The crossing schemes used to isolate homozygotes were similar to those reported by Lorimer et al. (1972), except that the *Silver* (*Si*) mutant on chromosome 2 was used in the schemes that evaluated the T1:2 and T2:3 stocks. Since it is possible to distinguish Si^+/Si^+, Si/Si^+, and Si/Si phenotypically, this gene was very useful in screening for homozygotes.

RESULTS AND DISCUSSION. From the 71 males treated with X rays, 129 F_1 males produced backcross progeny, of which 6 (4.7%) were heterozygous for a reciprocal translocation. These results were similar to those of Sakai et al. (1971) with *C. tritaeniorhynchus* (3.6% calculated from their data) and of Wagoner et al. (1969) in *M. domestica* (4.2%). In contrast, 61 males irradiated with ^{60}Co produced a total of 154 F_1 males, of which 26 (16.9%) were heterozygous for a translocation. With the detection system (pseudolinkage and fertility) we used, the efficiency of translocation induction was greater with the ^{60}Co treatment.

The translocation stocks are characterized in Table 1 in terms of fertility. As expected, the fertility of each stock was

Table 1. Summary of the egg viability of reciprocal translocation heterozygotes in *Aedes aegypti*.

Linkage groups involved	No. of stocks	Fertility (% hatch) Range	Mean
T1:2	11	15–49	32.6
T1:3	9	39–53	44.2
T2:3	9	20–51	43.4
T1:2:3	2	7–27	17.0

consistent each generation; however, the fertility between stocks varied over a broad range (7–53%), even though the linkage data in some cases indicated the presence of a single reciprocal translocation. Low fertility (<20%) was observed in two of the T1:2 stocks and one of the T2:3 stocks; none of the T1:3 stocks were in that category. The lowest fertility was observed in T1:2:3–54, which involves all 3 chromosomes, but the fertility of most of the stocks was >35 percent. According to Burnham (1962) the fertility of a translocation heterozygote is usually close to 50 percent, but this can vary somewhat and depends on the relative frequencies of adjacent and alternate segregation during meiosis. Presumably, the location of the breakpoints will exert influence on the chromosomal pairing, which in turn could affect the rates of balanced and unbalanced gametes. Inversions present in the interchanged chromosomes could also influence the degree of fertility, either directly by causing unbalanced gametes as a result of crossing-over, or by enhancing the frequency of adjacent segregation imposed by the unusual chromosomal pairing. Whatever the reason, the fertility in the translocation stocks varied considerably. The cytogenetic data for these stocks are not complete enough at this time to determine accurately the mechanism(s) responsible for the low fertilities, but inversions have been identified in some cases.

Recombination between mutant markers was consistent each generation for each stock, but variable, as expected, between stocks. The percentage crossing-over tended to be lower in the T2:3 stocks; indeed, in two of these stocks, no recombinants were recovered over a period of six generations.

The results of the competitive mating tests (Table 2) were encouraging, since

Table 2. Mating competitiveness of translocation stocks of *A. aegypti* in caged populations of equal numbers of translocation heterozygote males, RED males, and RED females (mean of 3 replications).

Stock	Competitiveness (c) Based on hatch[a]	Based on marker analysis
T2:3–11	1.06	1.29
T1:3–19	2.51	1.22
T2:3–22	2:00	1.66[b]
T1:2–26	1.59	1.55[b]
T1:3–27	1.08	0.85
T1:3–28	1.03	1.57[b]
T1:3–29	0.59	1.31
T1:3–34	1.09	0.96
T1:2–35	0.95	1.15
T1:3–36	6.66	1.34
T1:2–37	0.71	0.53[b]

[a] Calculated according to Fried (1971); χ^2 not calculated.

[b] Significant at 0.05 level (χ^2).

males of 9 of the 11 stocks were competitive with the RED males. The χ^2 analysis to determine competitiveness was based on the number of mutant progeny. There was disagreement between the observed values for hatch and mutant progeny in some cases. For instance, according to the mutant analysis, T1:3–28 appeared to be more competitive than RED, but the hatch data indicated that the stocks were equally competitive. If the hatch data were completely accurate, then an increase in the survival of wild type progeny would account for any discrepancy. However, in 6 of 7 comparisons of translocation heterozygotes and RED, no difference in larval survival was observed; only the T2:3–22 heterozygotes were more viable than RED (Table 3). Hatch data, especially with mutants such as RED, can be quite variable and this was the most probable source of the disagreement.

Table 3. Survival in laboratory of larvae from translocation and RED strains in the same containers (mean of 4 replications).

Stock	Survival in control	χ^2 Deviation from expected survival in mixed groups[a]
T1:3–19	0.86	NS[b]
T1:2–26	0.93	NS
T1:3–28	0.76	NS
T1:3–29	0.81	NS
T2:3–11	0.88	NS
T1:2–32	0.73	NS
T2:3–22	0.82	S
RED	0.78	..

[a] Seventy-five translocation and 75 RED larvae per rearing container.
[b] NS=Not significant; S=significant.

Data on sex ratios were not included in this paper, because the RED strain is sensitive to *distorter* (*d*) and this would bias the sex ratio data from the translocation strains. There was a slight distortion in favor of males in all of the sex-linked stocks, but a distortion of the same magnitude is usually observed in the RED stock. There was no indication that sex distorter mechanisms caused any problems in the mutant-marker analysis of the competitive trials.

DISCUSSION. We did not pursue competitive testing with all of the translocation stocks because the results of the other tests indicated that most of the translocations caused little or no loss in competitiveness. Since all the competition trials were conducted using the mutant stock, RED, there could be reservations about the performance of translocation heterozygotes against a wild population. However, the genetic background of a translocation stock can be modified by crossing to a wild type stock, and the translocation should not cause loss of viability although this may not be true in all cases. Data have been presented that substantiate the premise that translocations (or mutant genes) do not cause a loss in competitiveness provided they are part of the natural genome of a strain. Seawright et al. (1975a and 1975b) have shown that translocation heterozygotes were equally competitive with a wild type strain in limited field tests. Also, in an outdoor cage study, Seawright et al. (1975c) found the hybrid males from a cross of RED and a wild type strain were competitive with males of the wild type strain. Therefore, we accept the results of the competitive testing with RED in the laboratory as a valid estimate of the capability of the translocation heterozygotes.

Out of 25 translocations tested for viability when homozygous, only one strain was found to be viable. This strain, T1:3–34 was one of the X-ray induced translocations. Another strain was suspected of being viable as a homozygote, but outcrossing of this stock and cytological examination revealed that a recessive lethal was present on one of the mutant chromosomes that caused the death of the mutant (*s blt*) homozygotes, so that only translocation heterozygotes were produced when this strain was inbred. Considerable difficulty was encountered in our evaluation of homozygote viability due to the uncontrolled genetic recombination occurring in the crosses. We pursued many false leads that could have been avoided if inversion strains had been available to suppress recombinant types. In retrospect, the best way to efficiently study translocations in *A. aegypti* is to first isolate suitable inversions.

The results reported in this paper are a small part of a larger scheme that is aimed at developing translocation strains which can be incorporated into a practical system for use in the suppression of natural populations of *A. aegypti*. Our program has advanced to the point that we have: (1) an efficient system for isolating and studying the translocations, which is the subject matter in this paper, (2) procedures for testing competitiveness in laboratory and field tests (Seawright et al. 1975a and 1975b), (3) methods for manipulating heterozygous translocations in balanced lethal stocks that allow the ability to produce double translocation heterozygotes (unpublished data). We are currently refining methods for mass production of

double heterozygotes, and hopefully a major field trial with translocations against a natural population of *A. aegypti* will be completed in the near future.

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