FIELD COMPETITIVENESS OF MALES OF *AEDES AEGYPTI* (L.) HETEROZYGOUS FOR A TRANSLOCATION

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ABSTRACT. Genetically altered males of *Aedes aegypti* (L.) heterozygous for a translocation were competitive with normal males in mating with normal females in a field test on a small island. Expected and observed sterility of eggs was 66.5 and 66.7%, respectively. The normal mosquitoes were from a wild-type strain (VOYLE), and the translocation heterozygotes were the F_1 progeny of a cross between VOYLE and T1:2–26, a male-linked (*M*) translocation strain. Egg hatch from females captured 2 days after the releases also demonstrated that the translocation males were competitive.

Serebrovsky (1940) first proposed the control of insect pests by using reciprocal chromosomal translocations that cause semi-sterility of eggs of all heterozygotes bearing the translocation. More recently, researchers have used computer simulations to demonstrate that insect populations are theoretically vulnerable when 1 or more translocation types are introduced into them (Curtis 1968, McDonald and Rai 1971). Reciprocal translocations have been isolated and studied in a number of insect species, and interest is presently increasing in the use of translocations for control of some mosquito species (Rai et al. 1970, Laven 1969, Sakai et al. 1971, Rabbani and Kitzmiller 1972).

Using gamma irradiation from a ^{60}Co source, we have produced several reciprocal translocations in *Aedes aegypti* (L.) in our laboratory. The procedures for detecting the translocations are simple and effective. However, the techniques required to determine the potential of translocations for genetic control of mosquitoes are time consuming. Also, since there has been only limited research into the utilization of genetic aberrations, few criteria exist for evaluating the effects of the translocations on field populations. In the laboratory one can determine the linkage groups involved, the degree of semi-sterility, the chromosome breakpoints, the sex ratio, and other attributes of a reciprocal translocation; however, this information still leaves unanswered the question of the fitness of the translocation type in the natural environment as compared to the normal type. It is possible that a gain in overall fitness could be accomplished by a breeding program designed to increase vigor, and this could be done in the simplest cases by outcrossing to a wild type with the subsequent release of the F_1 heterozygotes.

In our research we found that translocation heterozygote males were able to compete well with males of a mutant strain in mating competitiveness studies in the laboratory and in outdoor cages, but to our knowledge, there has been no effort to determine the competitiveness of translocation heterozygotes under field conditions.

In this paper, we present the results of a field test conducted to compare the mating competitiveness of normal males and males heterozygous for T1:2–26, a male-linked (*M*) translocation.

METHODS AND MATERIALS. Three strains of *A. aegypti* were used in the study:

VOYLE—A wild type stock started from several hundred mosquitoes field collected in Gainesville, Florida, during July 1972.

RED—A strain with markers for *red eye (re)*, *spot (s)*, and *black tarsi (blt)* on chromosomes 1, 2, and 3, respectively, that was originally obtained from G. B. Craig, Notre Dame, Indiana, but has since been outcrossed 3 times with subsequent selection for the markers.

T1:2–26—A male-linked (*M*) translocation stock maintained in the laboratory for 12 generations by backcrossing to RED. This stock is homozygous for *black tarsi (blt)* on chromosome 3. The fertility of the heterozygous male is ca. 35%.

The VOYLE strain was considered representative of wild-type *A. aegypti* in northern Florida and was selected as the "normal" stock. Males heterozygous for T1:2–26 were crossed with VOYLE females to produce F_1 males for release. The cross to VOYLE was intended to increase the vigor of the F_1 males and also to incorporate the translocation onto the genetic background of VOYLE, which should have minimized behavioral differences between the translocation heterozygotes and the VOYLE males. This type of manipulation will probably be necessary in releases of strains with genetic aberrations; however, for many genetic mechanisms, increasing the vigor will require much more than a simple outcross.

All the mosquitoes used in the release were reared in a large outdoor cage. Groups of 1400 first stage larvae were set in trays containing 6 liters of water infused initially with liver powder (0.93 g) and brewer's yeast (0.47 g); 4 days later, 2.8 g of ground hog supplement was added. Most of the larvae pupated after 6–8 days at the prevailing weather conditions. Sexual separation was accomplished with a pupal separator similar to the one described by Fay and Morlan (1959) with >99% efficiency.

The adult males and females were allowed to emerge in the laboratory and were maintained on 2.5% sugar water until they were 3 and 2 days old, respectively. Then they were taken to the release site in insulated containers designed by B. J. Smittle and R. S. Patterson (1974). The male mosquitoes were held until they were 3 days old to insure that all would be sexually mature before the release. This precautionary measure obviated any bias in the results due to possible differences in the rates of maturation of the VOYLE and translocation males.

The release site was on the island of Seahorse Key, 2 miles west of the town of Cedar Key, Florida, in the Gulf of Mexico. This island is 1 mile long and at points as much as 0.25 mile wide with an elevated central ridge running lengthwise. Dense vegetation covers the island except for a centrally located T-shaped clearing where a lighthouse is located at the top of the sandy ridge, with a marine laboratory situated next to the dock. Repeated surveys on the island failed to detect *A. aegypti,* but positive collections were made in nearby Cedar Key. The absence of a population of *A. aegypti* made Seahorse Key ideal for testing the competitiveness of T1:2–26 males against VOYLE males.

On June 23, 1973, 2900 F_1 males (VOYLE x T1:2–26), 2900 VOYLE males and 3300 VOYLE females were released in the middle of the clearing on the island. Both sexes were liberated at the same site, but the females were released about 2 hours before the males.

Estimation of the degree of competitiveness of T1:2–26 males was based on the fertility of eggs collected from twenty 1-gallon buckets lined with filter paper and eleven 55 gallon barrels lined with white cloth that were located throughout the clearing and were about half-filled with water. There was an abundance of wildlife on the island, including a large number of nesting white ibis, which assured ample blood meal opportunities for the females. In addition to the egg collections, adult females were caught in biting collections and returned to the laboratory where the fertility of each female was determined from the hatch of individual egg batches held for 7 days after oviposition. Originally, another method for assaying competitiveness was contemplated. Because T1:2–26 had been backcrossed to RED for 12 generations, it was homozygous for *blt* on chromosome 3, and this marker should have been useful for tracking competitiveness by crossing a sample of the progeny from the released insects to RED, the frequency of mosquitoes homozygous for *blt* would correspond to the degree of competitiveness. However, this procedure was abandoned for reasons to be discussed later.

RESULTS AND DISCUSSION. After the mosquitoes were released at mid-morning, dispersal from the release point was complete within 20 minutes. The mosquitoes withstood the 90-minute trip from Gainesville to the island quite well, and mortality (16 ♀ and 40 ♂) was negligible.

Four hours after the release, mosquitoes were observed over most of the T-shaped clearing, the females were actively seeking blood meals, and mating behavior was exhibited by the males. The males were attracted to man and flew in a loose figure eight pattern around the host. However, this behavior was not typical of a swarm, since each male appeared to act individually. Mating was observed to occur when a female was attracted to man; the male would break from the hovering pattern and dart out and seize the female. At times several mating pairs were observed close to the host. These observations on mating behavior are almost identical to those reported by Hartberg (1971) in East Africa.

Two days after the release, 63 adult females were caught with a battery-powered aspirator. Egg hatch data from these females showed that 30 had mated with normal males and 26 had mated with translocation heterozygotes; 7 laid no eggs. These results indicated the T1:2–26 males were competitive.

Eggs were first observed on the filter paper in buckets and cloth in barrels 4 days after the release; thus, the females were 6 days old before they oviposited. A total of 3708 eggs was collected on the island.

The egg hatch data showed the observed value for the released females was in close agreement with the expected value (66.7% vs. 66.5%), which was derived from egg hatchability in control crosses. In laboratory tests VOYLE females mated to VOYLE males were 96.8% fertile while VOYLE females mated to translocation males were only 36.2% fertile. Our field data confirmed the competitiveness of the translocation heterozygotes and satisfied our principal objective, which was to assess the ability of a genetically altered stock to compete with a wild type stock.

Laven et al. (1971) reported on a field experiment near Montpellier, France in which males of *Culex pipiens* (L.) heterozygous for a male-linked translocation were released into a well with a naturally-breeding population of this mosquito. The translocation males were rated as highly competitive, although no data were presented to support this conclusion.

Laven et al. (1971) made an error in assuming that a frequency of .66 of the male-linked translocation heterozygotes would represent a saturation or equilibrium point at which the translocation would displace the normal chromosomes. There is no calculable equilibrium point for a male-linked translocation, except the fixation frequency of 1.0. Their statement contained a premise that displacement would occur if the translocation heterozygotes and the normal were equal in reproductive capacity. If a hypothetical situation were established under that premise, then a mating by a translocation heterozygote and a normal male would produce the same number of offspring, thus preserving intact the frequency of each type of male. The frequency of the translocation certainly would not increase, but instead, it would decrease because the assumption of equal reproductive capacity is artificial and unrealistic. Larval mortality will exact the same toll on the offspring from both types of matings, so that the frequency of the translocation will decline in each generation after releases are discontinued, and the decline in frequency of the translocation will be related to the genetic load in the heterozygotes. With semi-sterility, the frequency of .66 translocation males would decline to .50 in the F_1, .34 in the F_2, etc., and the decline would continue until the male-linked translocation would ultimately be eliminated from the population. At any frequency other than the fixation point, which equals 1, the number of translocation males will decrease each generation unless there are continual releases or the survival potential of offspring of translocation males is greater (the margin exceeding the working level of the genetic load) than that from normal matings.

In spite of the comments about population equilibria Laven et al. (1971) should be commended for demonstrating that a genetically altered stock could mate and reproduce in a field situation. For even though the direct use of male-linked translocations for population control is limited somewhat, there does exist a potential to use them to force desirable characteristics into a natural population.

In our experiment we considered, as mentioned previously, the possibility of using the marker *blt* to track competitiveness and lend some support to the sterility data collected. We did not use this method because of the loss of *blt* in one of the control crosses. When the F_1 male (VOYLE x T1:2–26) was crossed to RED, the progeny were expected to consist of equal numbers of blt^+ and *blt*. Instead of a 1:1 ratio, the blt^+ dominated by a ratio of 6:1. This unexpected result precluded the use of the marker analysis since the basis of this distortion is unknown. However, some sort of autosomal distorter phenomenon could be responsible. Further work is planned with this type of distortion in autosome recovery. If there is a fairly simple basis for this type of aberration, then it might be possible to incorporate such phenomena into genetic control schemes.

Literature Cited

Curtis, C. F. 1968. A possible genetic method for the control of insect pests, with special reference to tsetse flies (*Glossina* spp.). Bull. Entomol. Res. 57:509–523.

Fay, R. W. and H. B. Morlan. 1959. A mechanical device for separating the development stages, sexes, and species of mosquitoes. Mosq. News 19:144–147.

Hartberg, W. K. 1971. Observations on mating behavior of *Aedes aegypti* in nature. Bull. Wld. Hlth. Org. 45:847–850.

Laven, H. 1969. Eradicating mosquitoes using translocations. Nature 221:958–959.

Laven, H., J. Cousserans and G. Guille. 1971. Inherited semisterility for control of harmful insects. III. A first field experiment. Experientia 27:1355–1357.

McDonald, P. T. and K. S. Rai. 1971. Population control potential of heterozygous translocations as determined by computer simulations. Bull. Wld. Hlth. Org. 44:829–845.

Rabbani, M. G. and J. B. Kitzmiller. 1972. Chromosomal translocations in *Anopheles albimanus* Wiedemann. Mosq. News 32:421–432.

Rai, K. S., P. T. McDonald and Sr. M. Asman. 1970. Cytogenetics of two radiation-induced, sex-linked translocations in the yellow-fever mosquito, *Aedes aegypti*. Genetics 66:635–651.

Sakai, R. K., R. H. Baker and A. Mian. 1971. Linkage group-chromosome correlation in a mosquito. J. Hered. 62:90–100.

Serebrovsky, A. S. 1940. On the possibility of a new method for the control of insect pests. Zool. Zh. 19:618–630.

Smittle, B. J. and R. S. Patterson. 1974. Container for irradiation and mass transport of adult mosquitoes. Mosq. News 34:406–408.