

# DOUBLE TRANSLOCATION HETEROZYGOTES AND THEIR FERTILITY IN *Aedes aegypti* (L.)<sup>1</sup>

1264 9

J. A. SEAWRIGHT AND P. E. KAISER

Insects Affecting Man Research Laboratory, Agr. Res. Serv., USDA, Gainesville, Florida 32604

**ABSTRACT.** Double translocation heterozygotes (DTH) involving all 3 chromosomes were obtained in *Aedes aegypti* (L.) by 2 methods of crossing translocation heterozygotes. The average fertility of 7 different DTH males was 23.6 per-

cent. DTH breeding schemes, utilizing translocation heterozygotes rather than translocation homozygotes, are discussed in relation to utilization of conditional lethals for separating genetic types and inversions for stabilizing desirable genotypes.

The use of reciprocal translocations for the genetic control of insect pests was originally proposed by Serebrovsky (1940), who considered the potential use of translocation homozygotes as a control mechanism. Curtis (1968) simulated theoretical populations with a computer to show the applicability of translocations for genetic control of species with a low reproductive potential. However, there is some doubt as to the applicability of single heterozygote translocations for control of insect populations with high growth rates because their lethal genetic load is limited. Most single translocation heterozygotes are usually 40 to 50% fertile, although this will vary and can be higher or lower depending on the particular chromosomal arrangement. The suitability of the single heterozygote is thus suspect when considered as a mechanism for control. It would be much better to use a release strain with a higher genetic load, which would cause greater population suppression. In this regard, McDonald and Rai (1971) suggested the production of double translocation heterozygotes (DTH) involving all 3 linkage groups, for control of the mosquito *Aedes aegypti* (L.). They used computer simulations of populations based on data acquired in laboratory tests to confirm the theoretical validity of this system. DTH combinations for release could be obtained by crossing appropriate homozygous translocations, if these strains

were readily available. Unfortunately, homozygous translocation strains have been difficult to isolate (Lorimer et al. 1972) in mosquito species. In our laboratory, for example, only one translocation homozygote was found out of 40 heterozygous strains of *A. aegypti* tested.

In the present paper we describe (1) the fertility of some DTH combinations (made up of male-linked and autosomal translocations), (2) possible methods for obtaining DTH, and (3) the limitations of these methods for obtaining DTH by means other than by crossing homozygotes.

**METHODS AND MATERIALS.** Larval rearing was conducted in an incubator at 30° C. Groups of 150 larvae were placed in enamel pans containing 800 ml of distilled water infused with liver powder (0.67 mg/larva) and brewer's yeast (0.33 mg/larva); 4 days later, pulverized ground hog supplement (2 mg/larva) was added. The following translocation stocks were used:

Stock	Type	% Fertility of heterozygote
T2:3-11	Autosomal	50
T2:3-22	Autosomal	50
T2:3-11/22	Autosomal	25
T1:3-19	M-autosomal*	46
T1:3-31	M-autosomal	42
T1:2-43	M-autosomal	45
T1:2-46	M-autosomal	47
T1:3-42	M-autosomal	47

\* Sex determination in *A. aegypti* is due to a single locus on chromosome 1, where M/m is male and m/m is female (McClelland, 1962).

<sup>1</sup> Mention of a pesticide in this paper does not constitute a recommendation of this product by the USDA.

Most of these heterozygous translocation

strains were described by Seawright et al. (1975). The T<sub>2</sub>:3-11/22 strain is a combination of T<sub>2</sub>:3-11 and T<sub>2</sub>:3-22 and produces only T<sub>2</sub>:3-11/22 hybrids each generation, since the translocations are inviable as homozygotes. It is not a homozygote, but behaves the same in outcrosses, where the observed fertility was as high as 70% in a cross to a wild-type strain. We have designated strains of this type as pseudohomozygotes. (Dobzhansky and Sturtevant, 1931, described the viability of heterozygotes of this type in *Drosophila*.) We also used RED, a mutant marker strain (referred to as GVR by Lorimer et al., 1972) homozygous for *red eye* (*re*) on chromosome 1, *spot* (*s*) on chromosome 2, and *black tarsi* (*blt*) on chromosome 3 and usually about 85% fertile. The translocation strains, with the exception of T<sub>2</sub>:3-11/22, were maintained by backcrossing to RED so the genotype of the translocation stocks was essentially RED.

Two methods were employed to obtain DTH males. The first method, as diagrammed in Figure 1, was a cross of females heterozygous for an autosomal translocation and males heterozygous for an *M*-linked translocation. This method was employed in obtaining DTH combinations between T<sub>2</sub>:3-11 and T<sub>2</sub>:3-22 and the *M*-linked stocks, T<sub>1</sub>:3-19, and T<sub>1</sub>:3-31. The mutant markers *s* and *blt* were used to trace the DTH males and separate them phenotypically from the males bearing only an *M*-linked translocation. Those male progeny that were wild type (++) carried both translocations (DTH), and those that were homozygous for a single marker, *s*+, were heterozygous for only the *M*-linked translocation. Half of the female progeny were expected to be ++ and heterozygous for a T<sub>2</sub>:3, and the remainder were expected to be *s blt* with no translocation. The second method was simpler and consisted of a

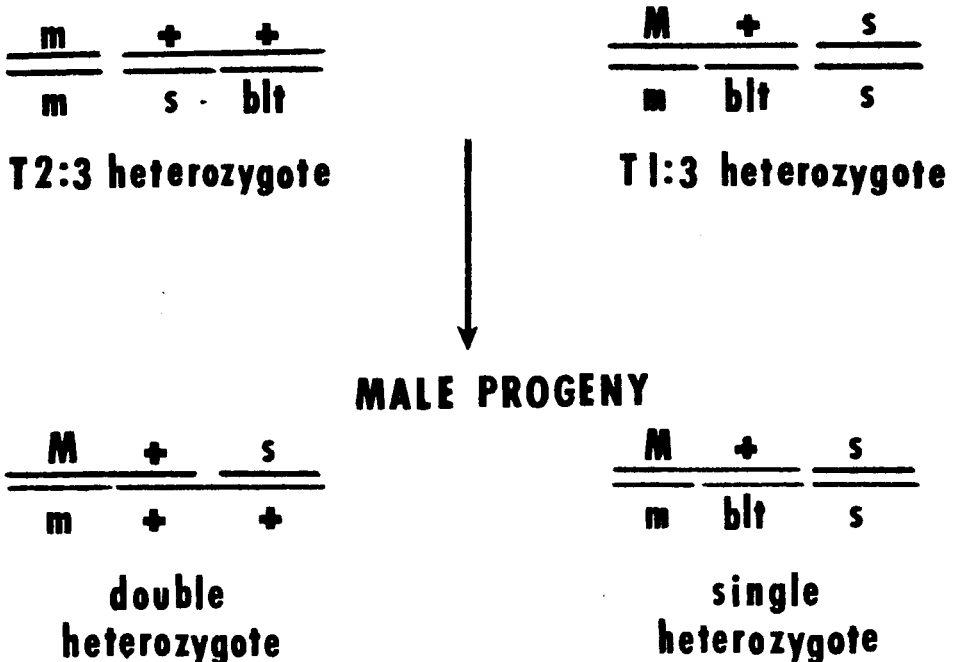


Fig. 1. Crossing scheme used to produce males of *A. aegypti* heterozygous for T<sub>1</sub>:3 and T<sub>2</sub>:3.

cross of T<sub>2</sub>:3-11/22 ♀ to the ♂ of the M-linked stocks, T<sub>1</sub>:2-43, T<sub>1</sub>:2-46, and T<sub>1</sub>:3-42. All of the male progeny were expected to carry both translocations, and the female progeny were to be heterozygous for one of the autosomal translocations, T<sub>2</sub>:3-11 or T<sub>2</sub>:3-22. The fertility of the DTH males was assayed by mating them to VOYLE (a wild-type strain) females with a subsequent count on percentage egg hatch.

**RESULTS AND DISCUSSION.** Average fertility values for the DTH ♂ resulting from the various translocation combinations are presented in Table 1. The fertility values are close to that expected from a double heterozygote, composed of independent translocations, but are higher than expected from a combination of all 6 chromosomes. If 6 chromosomes in a ring are segregated at random, there are 20 possible combinations of 3 chromosomes, of which 2 gametes would be viable. This would then correspond to 10% fertility. Obviously, segregation for balanced gametes is favored and results in the higher than expected hatch.

In the crosses of the heterozygous strains (Figure 1) the expected numbers of the 2 classes of males were recovered, and the ++ and s+ ♂ proved to be double heterozygotes and single heterozygotes, respectively. The marker strain RED was

used to monitor the translocations by crossing the ++ and s+ ♂ to RED ♀ with subsequent scoring of the progeny for mutant markers and fertility level. The results of these crosses were as expected, except a minor (<2%) number of recombinant types.

The utility of translocations for the control of mosquitoes will depend primarily on the ability to produce strains with genetic lethal loads sufficient to cause natural populations to decline. Most mosquito species are probably capable of population increases to offset the possible 50% load in a single heterozygote, thus it is advisable to attempt to release strains with higher loads. Double heterozygotes would provide the mechanism for accomplishing this objective. They can be obtained by crossing translocation homozygotes or heterozygotes. Unfortunately, homozygous strains are difficult to obtain, and the use of heterozygotes requires the laborious and expensive necessity of culling the resulting single heterozygote males. Even the use of a visible genetic marker, *blt*, would be unsuitable for a large release; however, for laboratory studies, mutants can be used to good advantage.

One possible approach to the use of heterozygous strains for the production of DTH males involves the use of conditional lethals, which are sensitive to a

Table 1. Summary of production and fertility of males of *Aedes aegypti* heterozygous for autosomal and M-linked translocations.

Cross	% Egg hatch*	Sex	Phenotype of samples of progeny				% Fertility of <sup>b</sup> DTH produced (++)
			++	s+	+ <i>blt</i>	<i>sblt</i>	
T <sub>2</sub> :3-11 x T <sub>1</sub> :3-19	28(23)	♂	312	280	0	0	19
		♀	296	8	8	288	
T <sub>2</sub> :3-11 x T <sub>1</sub> :3-31	23(21)	♂	240	210	0	0	24
		♀	180	15	0	150	
T <sub>2</sub> :3-22 x T <sub>1</sub> :3-19	26(23)	♂	170	160	0	0	22
		♀	136	16	0	160	
Δ <sup>c</sup> x T <sub>1</sub> :2-46	27(28)					25	
Δ x T <sub>1</sub> :2-43	13(27)					24	
Δ x T <sub>1</sub> :3-42	28(28)					20	

\* Expected % egg hatch in parentheses.

<sup>b</sup> DTH ♂ were crossed to wild type ♀.

<sup>c</sup> Designation for the autosomal double heterozygote (T<sub>2</sub>:3-11/T<sub>2</sub>:3-22).

given stimulus when present in a homozygous state. This method requires the development of a strain homozygous for the lethal which would then be irradiated for production of an *M*-linked translocation bearing the lethal gene. These males would then be crossed to females that were heterozygous for both an autosomal translocation and the lethal (on a normal chromosome). In the progeny from such a cross, the DTH males would be heterozygous for the lethal and die. Half of the females would be heterozygotes for the lethal and the autosomal translocation, and the remainder would be homozygous for normal chromosomes and the lethal. After application of the lethal factor, only DTH males (for release) and heterozygous females (for production of the next generation) would survive. Sexing of *A. aegypti* can be done mechanically in the pupal stage. In the context of conditional lethals, it seems that the dieldrin resistance gene on linkage group 2 in *A. aegypti* (Klassen and Brown, 1964) would seem to be suitable. In order to make a system of this type function as intended, an inversion covering the region of dieldrin resistance on chromosome 2 would be necessary to limit recombination, which occurs in both sexes of *A. aegypti*. The potential for using conditional lethals is certainly unbounded in developing techniques to eliminate certain genotypes in a rearing and release program. Also, conditional lethals could be included on the normal chromosomes of the insects to be released in the field and hence would become a detriment to those that inherit the normal chromosomes.

While the previous scheme should work, we believe the easiest approach for obtaining DTH males involves the second methods we used. The  $T_{2:3-11}/T_{2:3-22}$  double heterozygote (or pseudohomozygote) was easy to obtain, and the use of this strain for the production of DTH males eliminates the single heterozygotes, and thus obviates any need for separation of 2 types of males. Stability of the *M*-linked translocation could be accomplished

either by induction of an inversion in the area covering sex determination or by synthesis of a sex-linked stock similar to the autosomal pseudohomozygote.

It is interesting to note that a pseudohomozygote stock could be useful for a variety of purposes in breeding schemes as a substitute for homozygotes. Since these strains are composed of translocations that are viable only as heterozygotes, the fertility upon inbreeding is 25%, or that expected from 4 recessive lethals. We have isolated 4 such strains, composed of combinations of different, autosomal translocations. One of our combinations has a fertility approaching 50% which would be observed if one of the chromosomes in an interchange was viable; in this case the viable chromosome would replace the lethal one in the stock and the fertility would then be that expected from a balanced system of two lethals. Manipulation of heterozygous translocation strains that have a single lethal chromosome could produce a manufactured translocation homozygote. We have used the pseudohomozygote as a means of maintaining autosomal heterozygotes. The use of this balanced lethal system eliminates culling the normal individuals and in this respect represents a savings of valuable time.

It must be pointed out that the effect of a release in the field of DTH males obtained by the two methods described here would be similar to a sterile-male release, except that the lethal load in the released males would always be less than 100%. There is little potential for population replacement of the native strain by the released material since the fertility of the single heterozygote greatly exceeds that of the pseudohomozygote. The fixation point for these translocation arrangements would theoretically equal a frequency of 1.0. We should emphasize that translocations should not be considered as a possible control mechanism except in the case of species whose potential rate of population growth can be contained by the actual lethal load that can be obtained in the released insects.

*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.
- Dobzhansky, T. and A. H. Sturtevant. 1931. Translocations between the second and third chromosomes of *Drosophila* and their bearing on *Oenothera* problems. Carnegie Inst. Washington Publ. 421:29-59.
- Klassen, W. and A. W. A. Brown. 1964. Genetics of insecticide-resistance and several visible mutants in *Aedes aegypti*. Can. J. Genet. Cytol. 6:61-73.
- Lorimer, N., E. Hallinan, and K. S. Rai. 1972. Translocation homozygotes in the yellow fever mosquito, *Aedes aegypti*. J. Hered. 63:159-66.
- McClelland, G. A. H. 1962. Sex linkage in *Aedes aegypti*. Trans. R. Soc. Trop. Med. Hyg. 56:4.
- McDonald, P. T. and K. S. Rai. 1971. Population control potential of heterozygous translocations as determined by computer simulations. Bull. WHO 44:829-45.
- Seawright, J. A., P. E. Kaiser, J. L. Yeh and H. R. Stevenson. 1975. Reciprocal translocations in *Aedes aegypti* (L.). Mosquito News 35(3): 384-388.
- Serebrovsky, A. S. 1940. On the possibility of a new method for the control of insect pests. Zool. Zh. 19:618-30.