Theor. Appl. Genet. 51, 153-157 (1978)

Laboratory Evalution of a Translocation Double Heterozygote for Genetic Control of *Aedes aegypti*

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Summary. Two pure translocation homozygote stocks, T_1/T_1 and T_3/T_3, were used to produce a double translocation heterozygote system designated T_1/T_3, employing T_1/T_1 as the male and T_3/T_3 as the female parent. The double heterozygote showed 73% sterility when mated to wild females. Tests on mating competitiveness, recombination frequency in the differential segment, insemination rate and inheritance of sterility after release, for four generations in laboratory cages, have been carried out to evaluate the efficiency of this strain as an agent for a population control programme.

Key words: Translocation Double Heterozygote - *Aedes aegypti*

Introduction

The possible use of chromosome translocations as a mechanism for genetic control of various insect pests has received much attention in the last few years. This type of structural re-arrangement produces varying degrees of sterility due to the production of chromosomally unbalanced gametes, and a proportion of the balanced gametes also transmit the property of partial sterility to the progeny. Translocation heterozygotes are semi-sterile whereas homozygotes are generally fully fertile when mated with the wild population (Serebrovskii 1940; Curtis 1968) but all the progeny would be translocation heterozygotes. The idea of using a multiple translocation or double translocation heterozygote, involving two or more pairs of chromosomes, to achieve high levels of sterility was discussed by Serebrovskii (1971) and Curtis and Robinson (1971). The other advantages, apart from higher sterility in using a double translocation are

(a) such a strain, which is the product of two homozygotes, may be more fit than the parents due to heterosis (Seawright et al. 1975), and

(b) each male reared and released would introduce two translocations into the population.

Rai et al. (1974) have reported several multiple translocations in *Aedes aegypti* involving two or three pairs of chromosomes with different levels of sterility. Normally, double heterozygotes involving two chromosome pairs produce about 50% sterility while complexes involving three chromosome pairs show approximately 75% sterility, so that the use of the latter for a population control programme would seem advantageous. However, the only viable translocation homozygotes available to us both involved chromosomes 1 and 3. They were crossed to produce a double translocation heterozygote and this yielded unexpectedly high sterility (Uppal et al. 1974). This combination was evaluated in the laboratory in various ways to assess its suitability for a population control programme and this paper reports the results.

Materials and Methods

The following stocks were used:

1. A sex-linked homozygous reciprocal translocation involving chromosomes 1 and 3 (Lorimer et al. 1972) designated T_1. It was induced in 'ROCK' genome, Indian genetic background was introduced by backcrossing it with the Delhi wild type stock and it was then re-established as a homozygote. The egg hatchability on inbreeding ranged from 35-60%. The translocation breakpoint on chromosome 1 is 3-4 cross-over units from the sex locus (Lorimer, personal communication and Uppal, unpublished data).

2. Another sex-linked reciprocal translocation homozygote involving the same two chromosomes and designated T_3 was induced and isolated in Delhi wild stock at the University of Notre Dame (Rai et al. 1974). The egg hatchability on inbreeding of this stock ranged from 70-90%. The translocation break point on chromosome 1 is about 15 units from sex and

0040-5742/78/0051/0153/$ 1.00

close to the white eye (*w*) locus (Lorimer, personal communication and Uppal, unpublished data).

3. A wild type strain collected from Sonepat (Haryana State, India).

Double translocation heterozygotes were generally produced by crossing T_1/T_1 homozygote male with T_3/T_3 homozygote females. These double heterozygotes are designated T_1/T_3. For comparison in one test the reciprocal cross was made, yielding double heterozygotes designated T_3/T_1.

Survival of immatures of T_1/T_3 was compared with that of the Sonepat strain by rearing to pupation 250 1st instar larvae of each type. The samples of each stock were divided into three batches and reared side by side under identical conditions of food, water, temperature and space. The insemination capacity of T_1/T_3 males was compared with that of Sonepat males by providing a batch of four young virgin females to each male on two successive days. All the males were of the same age (approximately 2 days old) at the beginning of the test. The females were dissected and their spermathecae were examined for the presence of sperms.

Mating competition tests were carried out in 2 m cube outdoor cages and in 30 cm cube laboratory cages. T_1/T_3 males, Sonepat males and Sonepat virgin females aged 2-3 days were mixed in a 1:1:1 ratio. After a 24 hour mating period the females were fed on chicken blood, collected and allowed to deposit eggs in individual vials. After conditioning for 72 hrs, the eggs were floated for 48 hrs and the hatchability determined. The total number of eggs and number of eggs hatched from all the matings were pooled and the competitiveness was calculated using the following formula (Haisch 1970):

$$e = \frac{(q-f)}{n(f-p)}$$ where e = competiveness of T_1/T_3 males
f = egg hatchability from mixed populations
q = hatchability from wild type × wild type matings
p = hatchability from wild type × T_1/T_3 matings
n = no. T_1/T_3 males/no. wild type males (= 1 in these experiments).

A laboratory test of the degree of inherited partial sterility induced by T_1/T_3 releases was conducted as follows:

Into a 30 cm cube laboratory cage, Sonepat virgin females and males and T_1/T_3 males (all 2-3 days old) were mixed in a 1:1:10 ratio. Egg papers were collected, conditioned and egg hatchability was determined. The larvae were reared and equal numbers of the males and females were mixed with T_1/T_3 males in a 1:1:10 ratio. This procedure was repeated for four discrete generations with a check on sterility level at each generation. After the fourth generation no further additions of T_1/T_3 males were made and the cage population was allowed to breed with discrete generations using the techniques already described.

Results

The average fertility of T_1/T_3 males when outcrossed to normal virgin females was found to be 27.7 % (Table 1) whereas the males from the reciprocal cross gave higher fertility (32 %). This difference was significant at the 1 % level (Table 1).

Table 1. Fertility of T_1/T_3 and T_3/T_1 double heterozygote males mated to wild type females

Type of mating	Hatch range in %	Number of individuals	Average fertility
Sonepat ♀♀ × T_1/T_3 ♂♂ (49 single-pair matings)	11 - 20	5	27.7 %
	21 - 30	29	
	31 - 40	13	
	41 - 50	2	
Sonepat ♀♀ × T_3/T_1 ♂♂ (44 single-pair matings)	11 - 20	0	32.0 %
	21 - 30	20	
	31 - 40	23	
	41 - 50	1	

Comparison of means : $t_{81} = 3.31$, $P < 0.01$

Table 2. Data from rearing larvae of T_1/T_3 and the Sonepat stock based on results from 250 1st instars of each stock

Type of male	% survival to pupal stage	Female: male ratio	Mean duration of larval life in days	
			Female	Male
T_1/T_3	96.1	1.26:1	5.18	4.88
Sonepat	96.8	1.14:1	4.92	4.63

From these matings 155 male and female progeny were further backcrossed to wild type and all were found to carry at least one translocation; there were no wild type recombinants as all these matings were semi-sterile. It is concluded that there is little or no recombination between these two translocations. T_3 and T_1 are distinguishable by their linkage distance from white eye (*w*), and it was shown in tests on 25 individuals that male progeny had inherited T_1 and female progeny had inherited T_3, i.e. the translocations had retained their original linkage to sex in that T_3 remained linked to the *m* locus and T_1 to *M*.

The comparative study on the survival of immature stages revealed no difference between T_1/T_3 and the Sonepat stocks. Table 2 shows that in all respects both types of immatures behaved similarly, except that the development time for T_1/T_3 was slightly longer than for the Sonepat stock. The results of tests of the insemination potential of five

Table 3. Results of mating competition tests of T_1/T_3 males versus Sonepat for Sonepat females in a 1:1:1 ratio in field and laboratory cages

Cage used	Age of males & females at the time of mating (days)	Number of matings: Hatch range in percent 1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total no. matings	Hatched eggs/ Total eggs	Estimated competitiveness of T_1/T_3
Field	2-3	0	2	29	19	6	4	4**	1**	8	20	93	3005/5110 = 59%	121%
Field	2-3	3	18	14	11	2	1	-	1*	4	28	82	2205/4125 = 53.4%	176%
Field	2-6	-	-	8	9	5	-	-	-	3	12	37	1331/2184 = 60.8%	116%
Laboratory	2-3	-	3	5	3	1	-	-	1**	1	11	25	1121/1739 = 64.3%	96%

* Double insemination was proved to have occurred by finding both semi-sterile and normal F1 males
** Probably due to double insemination

Table 4. Egg hatchability from mass matings between various translocation genotypes

Serial No.	Type of mating: Female	Male	Number of eggs counted	% egg hatch
1	Sonepat	× Sonepat	1110	97
2	Sonepat	× T_1/T_3	2573	27
3	T_1/T_3	× Sonepat	1132	32
4	$T_1/+$	× Sonepat	570	45
5	$T_3/+$	× Sonepat	560	50
6	T_1/T_1	× Sonepat	281	80
7	T_3/T_3	× Sonepat	615	95
8	T_3/T_3	× T_1/T_3	1463	18
9	$T_3/+$	× T_1/T_3	1172	18
10	T_1/T_3	× T_1/T_3	565	11
11	$T_1/+$	× T_1/T_3	508	17
12	$T_3/+$	× $T_1/+$	1146	25
13	$T_1/+$	× $T_3/+$	65	28
14	$T_1/+$	× $T_1/+$	146	37
15	$T_3/+$	× $T_3/+$	647	34
16	$T_3/+$	× T_1/T_1	399	19
17	T_3/T_3	× T_1/T_1	1482	43
18	T_3/T_3	× T_3/T_3	1135	86
19	T_1/T_1	× T_1/T_1	316	52
20	T_3/T_3	× $T_1/+$	548	41
21	$T_3/+$	× T_3/T_3	446	24

T_1/T_3 males showed that they inseminated a total of 26 females, whereas five Sonepat males inseminated 20 females.

Table 3 gives the data of four mating competition tests, of which three were carried out in field cages and one in a laboratory cage. Each time T_1/T_3 males were found to compete approximately equally with Sonepat males, for mating with Sonepat females.

Table 4 lists the fertility of matings between various possible genotypes involving the two translocations. Mating nos. 8, 9 and 12 are of interest because of unexpectedly high sterility.

In the laboratory cage release experiment the fertility level declined to 17% after three generations of release and the fourth release caused no further decrease (Table 5). Subsequently the progeny were bred through successive discrete generations without further releases of T_1/T_3 and the level of fertility slowly increased from 20% to 72% over eight generations of breeding. The frequency of translocations in a sample of the population was checked from time to time by outcrossing the males with wild type females and, at the last generation, 44% of males were single or double translocation heterozygotes.

The outcross progeny of 31 fully fertile males at generations 5 and 6 were tested and all proved to be fully fertile; thus there is no evidence for the production of T_3/T_3 homozygous males, which would be fully fertile and give semi-sterile progeny on outcrossing.

Table 5. Effects of releases of T_1/T_3 males on fertility of a population during and after the period of release

Number of generation	Ratios			Sample of eggs counted	% egg hatch
	Cage ♀♀	Cage ♂♂	T_1/T_3 ♂♂		
1	1*	1*	10	1245	46
2	1	1	10	1640	33
3	1	1	10	1575	17
4	1	1	10	1810	20
5		Inbreeding	-1	1716	28
6			-2	1700	29
7			-3	1700	31
8			-4	1800	41
9			-5	1700	49
10			-6	1704	62
11			-7	1235	69
12			-8	1770	72

* Sonepat strain

Discussion

The T_1/T_3 double translocation heterozygote shows much more than 50 % sterility but there was no evidence for recombination between the two translocations. This strongly suggests that crossing over occurs freely in one of the differential segments between the translocation breakpoints, thus contributing to the numbers of unbalanced gametes produced, and raising the sterility level well above 50 %. However, in the other differential segment there appears to be little or no crossing over, so that the translocations cannot complete the process of recombination. Thus the T_1/T_3 combination appears to be analogous to a double translocation involving chromosomes 2 and 3 in *Drosophila* studied by Robinson and Curtis (1972). The T_1/T_3 combination differs markedly from a translocation complex in *A.aegypti* involving all three chromosome pairs, in which crossing over in the single differential segment, generating "new" chromosome combinations, was enhanced in frequency (McDonald and Rai 1970).

The data in Table 3 indicate good mating competitiveness of T_1/T_3. However, unexpectedly, another set of cage tests and a field test indicated poor competitiveness of T_1/T_3 males (Grover et al. 1976). This discrepancy was unexplained and there is now no opportunity to investigate it further.

The effect on fertility of releases of T_1T_3 males into a population maintained with discrete generations resembled the results achieved in a field cage experiment with overlapping generations (Curtis et al. 1976). The results illustrate several general principles of translocation releases:

1. A point is reached at which further releases do not reduce the fertility level.

2. The minimum population fertility level is appreciably less than the fertility of the released males in cases where females inherit partial sterility and contribute to the overall sterility (in the present case this remains true even if most of the females are T_3/T_3 homozygotes because the $T_3/T_3 \times T_1/T_3$ mating has unexpectedly low fertility as shown in Table 4).

3. After the termination of releases partial sterility is inherited. However, it is almost inevitable that natural selection will eventually restore full fertility. It might have been expected that this would involve the production of a population of T_3/T_3 homozygous males and females, the males being produced as a result of cross-overs in $T_3/+$ single heterozygotes. However, in the present experiment and that described by Curtis et al. (1976) T_3/T_3 males were not detectable, suggesting that they may have reduced fitness (Lorimer, personal communication).

The efficiency of T_1/T_3 as a population suppression system can be enhanced by the incorporation of sex ratio distortion (Suguna and Curtis 1974; Curtis et al. 1976). It may be possible to further enhance the efficiency of the system by incorporation of the closely sex linked recessive gene short wing (Uppal, Curtis and Soni 1976) into the system.

Acknowledgement

We are grateful to M.K. Gupta, Miss V. Tandon and K.N. Joshi for their excellent technical assistance. We thank Prof. K.S. Rai and Dr. N. Lorimer for providing the T_1 and T_3 translocation stocks to this Unit and to Dr. N. Lorimer for her valuable contribution to the earlier stages of this work. We acknowledge the support and encouragement of Dr. G.D. Brooks, Project Leader.

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Received June 1, 1977
Communicated by B.R. Murty

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