PILOT RELEASE OF A SEX-LINKED MULTIPLE TRANSLOCATION INTO A *CULEX TARSALIS* FIELD POPULATION IN KERN COUNTY, CALIFORNIA[1]

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ABSTRACT. A pilot-release study was carried out with genetically-altered *Culex tarsalis* at an isolated area in Kern Co., California. The results indicated that males carrying sex-linked double heterozygous translocations could be mass-produced, could be transferred as pupae to the field and emerge from artificial ponds, and could survive in the field as immatures and adults. Mark-release-recapture studies provided estimates of the size of the native population and survival rates of native and released adults. The release did not affect the density of the native population nor was the translocation recovered from field-collected egg rafts. One known contributing factor to this outcome was that the ratio of the translocated to the native population was never adequate.

INTRODUCTION

Culex tarsalis males heterozygous for a sex-linked multiple translocation, T(1;2;3)1*A*, have competed successfully against males of both laboratory colonies and field-derived populations in mating tests with field-derived females (Terwedow et al. 1977). The tests were done in both small laboratory and large outdoor cages. When the genetically altered males competed with wild-type males in a 1:1 ratio, the egg hatch rate was suppressed in a significant portion of egg rafts because of inherited sterility. Progeny of the translocation-carrying males survived through the immature stages in semi-outdoor artificial rearing ponds, and the complex translocation was genetically identified in the subsequent generation.

A search began in 1975 for sites that might be suitable for field trials to determine the effectiveness of releases of the heterozygote translocation. One site, Poso West, was found satisfactory, and intensive monitoring of adult and larval populations was begun. This site in Kern County was within reasonable distance of our Bakersfield Field Station, and supported almost a pure population of *Cx. tarsalis*. We developed estimates of population densities throughout the mosquito season in 1975 and 1976. The population data, along with reports on the biology of this species from other studies, were used to develop a simulation model that estimated the size of the *Cx. tarsalis* population at different times in the season and predicted the population suppression that could be expected upon introduction of genetically modified mosquitoes in different ratios into the field population. These baseline preparations led to the current program for mass-production of the heterozygote stock, and its release into the field area beginning in the spring of 1977. Our primary objectives were to ascertain if: 1) the translocation line could be mass-produced; 2) the introduction of pupae into the field would lead to effective insertion of an adult population; 3) the released population could effectively interact and compete with the wild population; 4) the translocation could be recovered from the field population in subsequent generations; and 5) areas of research could be identified that were

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needed if genetically modified populations are to be useful in the control of *Cx. tarsalis*.

MATERIALS AND METHODS

The translocation complex that was tested is a multiple heterozygous interchange involving all 3 linkage groups, T(1;2;3;)1*A*. The 3 chromosomes involved are transmitted as a unit from males to male progeny since the interchange is closely linked to the male (*M*) locus. A normal set of chromosomes is conveyed from the translocation-carrying male to the female progeny; thus a mating of normal females with the T(1;2;3)1*A* males results in normal females and translocated males. There is less than 1% recombination between the interchange and the sex-determining factor. The line is maintained by outcrossing to a multiple-marker stock and checks for purity of culture are based on eye-color. The mean hatch of egg rafts fathered by these males is 29% (Terwedow et al. 1977).

The Poso West field site is an isolated arid area about 15 miles north of Bakersfield (Figure 1). The breeding area is 0.7 mile long, in a ravine, and surrounded by barren foothills for 3–5 miles. The water supply is stable, as it is supplied by waste waters pumped from an oil field, and the native population is almost exclusively *Cx. tarsalis*. Vital baseline information that had been obtained and continued to be collected on *Cx. tarsalis* females included: population density, survival, host preference, vector competence for arboviruses, resistance to organophosphorous insecticides, and autogeny and parity rates. Similar baseline measurements were maintained at an ecologically similar comparison field area (McVan) located approximately 4 miles away. Weekly monitoring of the population at Poso West was begun in 1975 by using 5–8 CO_2 bait traps and a battery-operated New Jersey light trap. In 1976, 3 CO_2 baited CDC light traps were operated at least 1 night each week, and provided an index of females per trap night. That same year a series of mark-release-recapture studies provided estimates of the absolute size of the adult population (Nelson et al. 1978). A sufficient number of marked females was recaptured to permit estimations to be made of mean survival rates. Based on the 1976 population estimates, the computer simulation of the introduction of males heterozygous for a sex-linked multiple interchange indicated that we would need to release approximately 100,000 translocated males in April and May to achieve an effective insert that would reduce the mid-summer population to about one-half its normal size.

A production process was begun in January 1977, in an effort to produce 10,000 males for release every 3 days in April. Twelve separate production lines were established, and the lines were staggered so a new line began pupation every 3 days from March 28 through April 30. The last generation of each line represented an outcross of translocation males to virgin females from a vigorous laboratory colony (Knights Landing). Prior to the last generation outcross the purity of the translocation stock was maintained by crossing translocation males with carmine black (*car/blc*) marker females (Asman 1975), and by culling pupae and adults to eliminate possible recombinants. This system required that 3 separate strains of *Cx. tarsalis* be reared simultaneously for the various crosses.

The 12 production lines were developed as 3 successive sequences of 4 lines each. The first 2 lines of each sequence were carried through the culling and crossing process described, and were progressively expanded. When the Knights Landing females (crossed to translocation males) first oviposited, they were promptly re-fed and allowed to oviposit a second time. In this way, 4 lines of final pupal production were achieved although only 2 went through the culling process.

Larvae were reared in 5 x 23 x 33 cm white enamel pans, each with 1.5 liters of

Fig. 1. Aerial view, facing south, of Poso West study site, Kern County, California.

water. With normal colony mosquitoes, 4 egg rafts, a total of about 700 eggs, were placed in each pan. With females mated with translocation males, hatch averaged only about 29%, so 14 rafts were placed in each pan for these populations. Larvae were fed a powdered mixture of Tetramin®, rabbit pellets, and brewer's yeast (Nelson and Bruen 1976), and the pans were aerated. Larvae were subjected to a photoperiod of 14 hr of light with an additional 30 min semi-light period at dusk and dawn.

Pupae were separated from larvae with the device described by Fay and Morlan (1959). The same device was used, with fine adjustment, to separate the relatively small male from the large female pupae when the former were harvested for field release. Since the lines were staggered, translocation pupae could be harvested and released daily once production began. For each line, pupae were harvested the day after they first appeared and again on the 3rd and 5th days. The remaining pupae, mainly females, were discarded.

Estimates were made of the number of male pupae harvested. The procedure was to count and isolate a known number of pupae (usually 500); they were then weighed to the nearest 50 mg on a torsion balance. Then all pupae were weighed and their total number estimated. The pupae were transferred to the field ponds each day in water-holding plastic bags.

To insure that the released males were carrying the translocation, quality-control tests were conducted on 3 occasions during the course of releases. In these tests 25–50 males, reared from a pupal sample that was scheduled for release, were mated with *car/ble* females in the laboratory. The egg rafts obtained from these crosses were categorized for hatchability as low (30% or less), medium (31–74%), or high (more than 75%).

At the study site, translocated pupae were released into artificial ponds designed for rearing *Cx tarsalis* from egg through adult stages in the field (Nelson and Bruen 1976). Each pond had a pyramid-shaped cover that tapered to a round 9 cm hole at the apex to permit escape of adult males. Four of these ponds were sub-divided to provide a total of 8 sections each measuring 85 x 115 cm. Water was added to a depth of 10 cm. Each day's harvest of pupae was placed in 1 of the 8 sections. To estimate adult emergence, a sub-sample of 100 pupae was placed in a plastic emergence "tube" 8 cm in diameter and topped with a screened paperboard carton which captured emerged adults. The tube was placed on a pond bottom and was screened on the bottom section, to allow passage of water but retain pupae. Emerged adults were removed and counted by sex daily until emergence was completed.

Two groups of adult translocation males were marked with fluorescent dusts and released in the field on April 4 and May 5. Shelter collections were made for 10 days following each release to allow us to estimate survival of the marked translocated males. The number of released males in the population on day i (N_i) could then be estimated as: $N_i = s\ N_{i-1} + R_i$, where s is the mean daily survival rate, and R_i is the number of translocated males emerging or released on day i.

Weekly CO_2 light trap and shelter collections were made from March through October. Mark-release-recapture studies, similar to those done in 1976 (Nelson et al. 1978) were done in June, July, August, and September 1977, and allowed us to estimate the absolute population size.

The field population was monitored weekly to ascertain the effectiveness of the released genetically-altered males into the native population. This was started 2 weeks prior to the first release to establish an expected hatch rate for egg rafts from the native population. Monitoring consisted of 3 different sampling methods: 1) up to 100 females collected in light traps were returned to the laboratory and blood fed, oviposited egg rafts were graded for percent hatch as low, medium, and high; some collections were also made at McVan for comparisons; 2) egg rafts collected from the field also were graded for percent hatch; 3) up to 100 pupae were collected from the field and returned to the laboratory where they were allowed to emerge. Single males from this sample group were mated with 5 genetically-labeled virgin *car/ble* females in gallon cages. Difficulty in obtaining rafts from these females prompted a change during the program. Large colony cages, 60 × 60 × 45 cm, were used for mass mating instead of gallon cages. Larvae from low hatch rafts were reared. From these a random sample of males was crossed to *car/ble* females and the progeny were scored for phenotype to confirm the presence or absence of the translocation.

Additional field experiments were carried out in the summer of 1977 to compare the larval population dynamics of translocated and wild strains of *Cx. tarsalis* in a field environment. Experimental populations were reared in 5-liter buckets placed in the natural aquatic habitat. The enclosures had mesh sides which prevented the passage of larvae but allowed water to flow. Populations of the native *Cx. tarsalis* and the translocated stock were initiated from either 1st-instar larvae or eggs. The proportion of hatched eggs or viable larvae were counted when introduced. Wild strain eggs or larvae were derived from captured wild females which had fed on blood and oviposited in the laboratory. The translocated strain, eggs or larvae, was derived from virgin wild females that were hatched from field-collected pupae, mated with translocation-bearing males, blood-fed, and allowed to oviposit. Survival and development times were determined by noting when larvae first developed to a certain instar, counting the sample, and replacing them into the buckets. Some populations received food supplementation, consisting of the same mixture used to rear laboratory colonies. Screen cones placed over the enclosures prevented access by wild mosquitoes and also funneled emerging mosquitoes into containers. Emerging adults were removed, sexed, and counted to complete the documentation.

RESULTS AND DISCUSSION

The quality control tests on random samples of released males indicated that the mass-produced males were carrying the translocation. Out of 151 rafts fathered by these males 21 gave no hatch, 129 gave low hatch (30% or less), and 1 gave medium hatch (Table 1).

An estimated 64,730 translocated males emerged from the field rearing ponds between April 2 and May 6. An additional 11,583 marked translocation-bearing male adults that had emerged in the laboratory were also released. Thus, a total of 76,313 translocated males were released. This was approximately 24,000 less than was planned. An estimated 10,880 females, which did not carry the interchanges, were inadvertently released. One of the difficulties in the mass-production phase of this pilot study, other than the low percent hatch of egg rafts and the need to cull expanding lines

Table 1. Quality control tests based on egg hatch rates when released males were mated with *car ble* females.

Sources of specimens	Dates of collections	No. of rafts	Rafts with no hatch	% rafts hatching	No. hatching rafts[1] low	medium	high	% (low+ medium)
Release ♂ ♂	March 23	42	6	86	36	0	0	100
Release ♂ ♂	April 21	30	3	90	27	0	0	100
		30	6	80	24	0	0	100
Release ♂ ♂	May 4	49	6	88	42	1	0	100
	Total	151	21	86	129	1	0	100

[1] Hatchability grades were: low <30%, medium 31–74%, and high >75%.

for eye color, was that a manual sexing process had to be used and it did not always remove small-sized female pupae.

Of the 11,583 translocated males released as marked adults (5,703 on April 4 and 5,880 on May 5th), 245 were recaptured in subsequent shelter and CO_2/light trap collections. The mean daily survival rate (estimated by the regression technique described in Nelson et al. 1978) of these males was 72%. Application of this survival rate to the estimated numbers released each day indicated that the translocated male population in the field area probably never exceeded 11,000 on any day during the entire release period (Figure 2).

To establish population estimates during the post-release period, 31,853 adult *Cx. tarsalis* were marked and released, and 154,692 mosquitoes were collected in recovery efforts. A total of 90 marked males and 1,121 marked females were recaptured. The light trap indices and estimates of female population in 1977 are shown in Figure 2. Population estimates for 1977 surpassed those of 1976 for most of the April-July period but were much lower in August-September (Table 2). Survival estimates (Table 3) also differed between 1976 and 1977 in that survival rates were lower in 1977 indicating a faster population turnover that year, thought to be due to higher temperatures. The number of adult females at any time approximated 500 times the female light trap index. An insufficient number of marked males was recaptured in these studies to allow direct estimations of the size or survival rates of the adult male population. However, based on the sex ratio of adults collected in artificial shelters, it is assumed that the adult male population did not exceed the adult female population.

Table 2. Estimated size of adult female *Culex tarsalis* population at Poso West in 1976 and 1977, based on extrapolations from CO_2/light trap indices for April and May, and on mark-release-recapture studies from June through September.

Month	Population estimates	
	1976	1977
April	<1,000	40,000
May	7,000	15,000
June	154,892	150,368
July	68,475	156,182
August	56,448	12,682
September	52,836	15,153

Table 3. Estimated mean daily survival rate of female *Culex tarsalis*, Poso West, in 1976 and 1977.

Month	Mean daily temperature		% daily survival
	Max.	Min.	
	1976		
June	31	18	73
July	37	23	64
August	32	19	67
September	29	18	77
	1977		
June	35	20	66
July	38	23	54
August	36	20	65
September	37	22	67

As stated earlier, the collection of adult females from the field to monitor the rate of mating with released males was begun in the pre-release period and continued in the post-release period. Thus, this time span covered 3 weeks (March 13–April 2) in pre-release, 5 weeks (April 3–May 7) during release, and 16 weeks (May 8–August 27) post-release. In the pre-release period only 6 rafts were recovered from captured females and all were high hatch. During the release period, 653 captured females oviposited, and of the 600 egg rafts that hatched, 170 had low hatch and 4 had medium hatch. In the post-release period among the 1,279 hatched rafts there were 43 low hatch and 4 medium hatch rafts. From the control site, McVan, 438 females oviposited in the post-release weeks, and of the 381 rafts that hatched 13 were low hatch and 2 were medium hatch (Table 4).

Egg rafts were collected from the water in the field at Poso West during the release and post-release periods. There were 12 low-hatch rafts in 151 hatched rafts during the release, and 24 low hatch

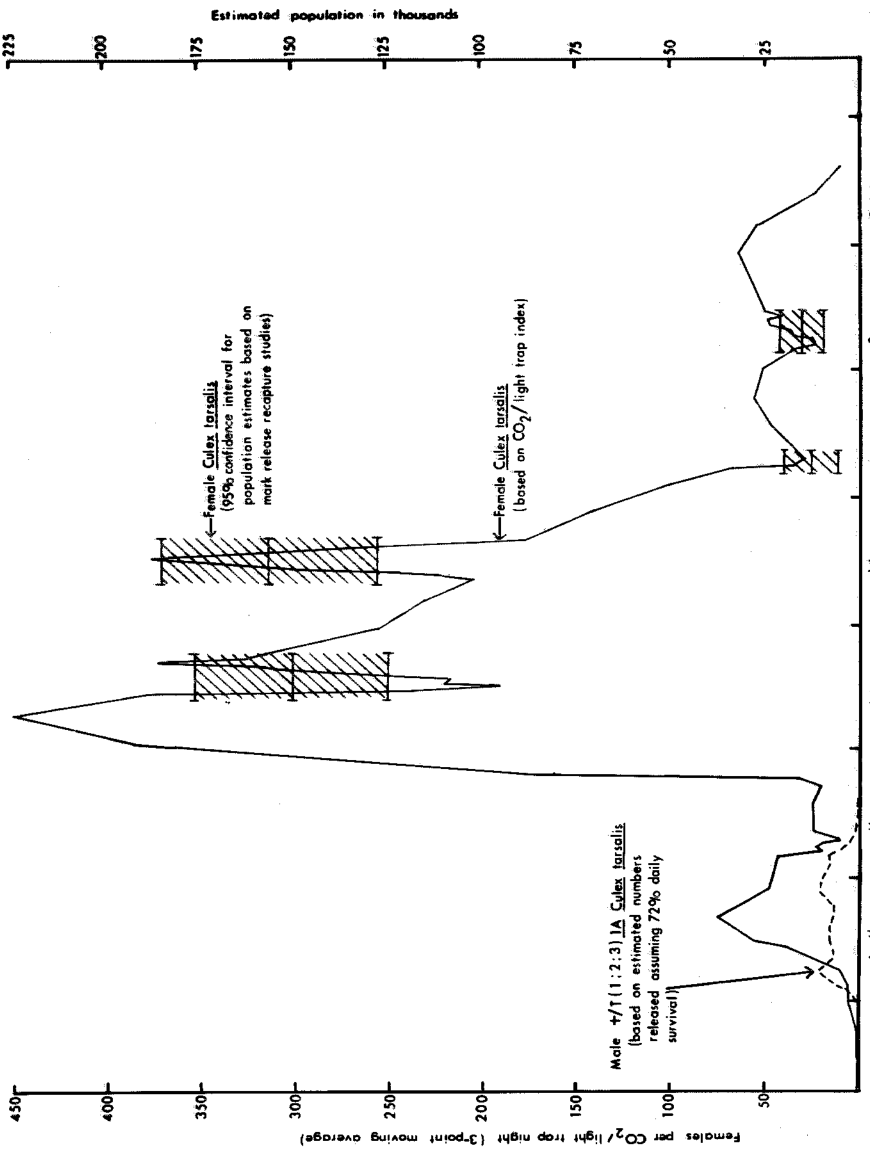

Fig. 2. CO_2/light trap indices and population estimates for *Culex tarsalis* at Poso West, Kern County, 1977.

Table 4. Monitoring of percent of hatch of egg rafts, Poso West and McVan, 1977.

Source of females or rafts	Time period	No. ♀ ♀	No. rafts	Rafts with no hatch	% rafts htch'g	Hatching rafts[1]			
						Low	Med.	High	% low and medium
Poso West field collected adult ♀ ♀	Prerelease	7	6	0	100.0	0	0	6	0
	Release	723	653	53	91.9	170	4	426	29.0
	Postrelease	1721	1486	207	86.1	43	4	1232	3.7
McVan field-collected adult ♀ ♀ (control site)	Postrelease	541	438	57	86.9	13	2	366	3.9
Rafts from Poso West water surface	Release		158	7	95.6	12	0	139	7.9
	Postrelease		1458	27	98.1	24	1	1406	1.7
Rafts from laboratory females mated to males emerged from Poso West pupae	Initial—SC[2]		131	103	21.4	7	2	19	32.1
	Intermediate—SC		195	159	18.5	10	0	26	27.8
	LC[2]		369	151	59.1	125	0	93	57.3
	Final—SC		31	31	0.0	0	0	0	—
	LC		1226	257	79.0	366	8	595	38.6

[1] Hatchability grades were low <30%, medium 31–74% and high >75%.
[2] SC: small cages; LC: large cages.

and 1 medium hatch rafts among 1,431 hatching rafts collected in the post-release period (Table 4).

Egg rafts were obtained from laboratory females mated to males reared from field-collected pupae during the 3 time periods. Pupal collections were made 4 times in the initial period when no translocation-carrying progeny were possible (March 23–April 13), 5 times in the intermediate period when the direct descendents of released males could have been represented in pupal collections (April 20–May 13), and 14 times in the final period (May 26–August 25) (Table 4). Low-hatch rafts were oviposited by females mated to males derived from field-collected pupae even during the pre-release time. Each of 53 groups of male sibs from such low hatch rafts were mass-mated to marker females and the resultant progeny were observed for continued low hatch and genetic evidence of the translocation. Four groups of larvae that had derived from these matings were reared to adults, and none carried the translocation.

A study was initiated to identify factors that would explain the low hatch rate of rafts fathered by males collected in the field, and the low yield of hatching rafts derived from matings of field collected "normals". Experiments were conducted in large cages in which females derived from 3 laboratory colonies (*car/ble,* KL and PWC) and field collected females were mated to males of 2 types collected at Poso West. The males were either reared from pupae or collected as adults from shelters. There was a consistent low hatch among rafts fathered by these Poso West males regardless of the origin of the females (Table 5). The low-hatch phenomenon of rafts fathered by these males evidently was not due to the presence of the translocation as the same pattern occurred whenever males from this field area were outcrossed to long-term colonized females (Table 4). When the field-collected males were mated with field-collected females or with females recently colonized from Poso West, the incidence of low hatch was effectively reduced by approximately one-half. In al-

Table 5. Hatching characteristics of rafts from laboratory or field-collected females mated with males collected as pupae (reared) or adults at Poso West.

Virgin females		Males	No. rafts	Rafts with no hatch	Htch'g. rafts No.	Htch'g. rafts Percent htch'g.	Low hatch rafts No.	Low hatch rafts Percent low hatch
car/ble	X	*car ble*	339	44	295	87	125	42
(lab.	X	Poso West reared	1595	408	1187	74	491	41
	X	Poso West shelter collection	176	38	138	78	57	41
Knights Landing	X	Knights Landing	100	2	98	98	5	5
(lab.)	X	Poso West reared	14	9	5	36	5	100
	X	Poso West shelter collection	120	60	60	50	27	45
PWC[1]	X	PWC[1]	50	3	47	94	3	6
(lab.)	X	Poso West reared	44	34	10	23	1	10
	X	Poso West shelter collection	37	4	33	89	2	6
Poso West	X	Poso West reared	55	38	17	31	2	12
(field-collected)	X	Poso West shelter collection	49	27	22	45	3	14

[1] PWC: recently colonized Poso West Strain.

most all instances a low proportion of rafts hatched when Poso West males were used for mating. This could indicate in part a lack of adaptability to laboratory conditions.

Field studies to compare survival of the released translocation strain with the wild population at Poso West did not reveal major qualitative differences once the hatched larvae survived the first day (Table 6). First-day larval mortality was much greater in translocated populations than in wild populations under laboratory conditions; however, mortality soon after hatch has been considered as part of the sterility effects of this translocation (Terwedow et al. 1977). The major results were: survival and development time, from first instar to emergence, were about the same for wild and translocated populations; the sex ratios at emergence had wide variation but averaged 1:1 for both populations (this high variance also was observed in field populations in 1976); addition of food greatly shortened the development time for both strains.

A second pilot release is in progress in the spring of 1978 with the same translocated stock but with modified techniques based on the 1977 experiences.

CONCLUSIONS

With the first release, a T(1;2;3)1*A* translocation stock of *Cx. tarsalis* did not establish itself sufficiently to affect the density of the natural population. A satisfactory ratio of translocated to natural male population was not achieved. It is also possible that the translocated males were unsuccessful in mating competition with the natural population.

Some objectives of the study were met. These were demonstration that: the T(1;2;3)1*A* translocation could be mass-produced, and an adult population could be derived by transfer of pupae to artificial ponds in the field for subsequent adult emergence. The advantages to this method are that there is minimum handling of release materials and a simple method can be used to estimate the numbers released. To be successful this approach requires an improved sexing system.

There was equal development time and survival of immature stages of T(1;2;3)1*A* and field populations in the field.

More research and data are necessary to improve techniques for mass-rearing, release strategies, estimates of male populations, and field monitoring of the translocation.

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Table 6. Comparison of larvae population dynamics of wild, Poso West, and translocated populations of *Culex tarsalis*.

Population	Laboratory		Field enclosures		
			% survival from day 1 to emergence	Median days from hatch to emergence for females	
	% hatch	% survival to day 1		With food supplement	No food supplement
Translocated	50	55	50	12.3	20.0
Wild	78	92	63	12.5	16.8

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