male offspring were produced in K; but all alleles in other crosses showed the 1 : 1 segregation ratio. Meanwhile, all linkage chi[2] values estimated were not significant at the 5% level, which reveals that *M, Wb* and *b* were inherited independently of each other. The 3 alleles may be separately located in all 3 linkage groups in this species. Before this can be proved linkage studies on more mutants are required.

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RELEASE OF HETEROZYGOUS TRANSLOCATED ADULT MALES FOR GENETIC CONTROL OF *CULEX TARSALIS* AT AN ISOLATED SITE[1]

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ABSTRACT. Nearly 180,000 heterozygous translocated adult male *Culex tarsalis* Coquillett were released at an isolated field site in Kern County, California, in the spring of 1978. The release was an attempt to reduce the size of the native population, as egg rafts from females that mated with a translocated male would be about 70% sterile. The ratio of released laboratory males to field males exceeded 10:1 for 4 weeks. Introduction of the translocation was confirmed in male progeny of 22 females, 1% of those tested from field collections made during and after the release. Tests in large outdoor cages demonstrated that mating competitiveness of the release stock had declined from .75 to .24 since 1976.

INTRODUCTION

The first pilot release of genetically-altered *Culex tarsalis* Coquillett was carried out in 1977 at Poso West, in Kern County, California (Asman et al. 1979). Over 75,000 males heterozygous for a double male-linked translocation were released during April. The primary goal, reduction of the field population, was not achieved, nor was the translocation recovered from field progeny in subsequent generations. However, the study demonstrated that the translocated males could be mass-produced and could survive in the field as immatures and adults. The 1977 study also indicated the need for an improved release strategy and better techniques for monitoring of the field population during and after a release.

Characteristics of the release strain, T(1;2;3)*IA*, have been described in a previous publication (Asman et al. 1979). The translocation carried by the males causes approximately 70% sterility in egg

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rafts fathered by these males. Surviving female progeny are normal and male progeny carry the translocation. Competitiveness of the T(1;2;3)1*A* males against wild males for wild females was estimated to be .75 in outdoor cage tests conducted in 1976 (Terwedow et al. 1977). Males released in 1977 showed satisfactory performance in similar trials (McDonald et al. 1978).

The release site, Poso West, is an isolated canyon about 1 km long in barren foothills north of Bakersfield (Nelson et al. 1978, Asman et al. 1979). Intensive monitoring of the mosquito population, which is almost exclusively *Cx. tarsalis*, began in 1975. Observations on the dynamics of this population were utilized in the development of a computer model to simulate the impact of various genetic control strategies (Fine et al. 1979).

Computer simulation indicated that the release of 100,000 T(1;2;3)1*A* males during April should cause a reduction of 50% in the mid-summer peak population at Poso West. The actual number released in 1977 (76,313) fell short of this goal. The mosquitoes were introduced as pupae into artificial rearing ponds at the field site. Difficulties in separating sexes at the pupal stage led to the inadvertent release of nearly 11,000 normal female siblings of the T(1;2;3)1*A* males which increased the number of females present during the critical spring population buildup.

A second pilot release was made at Poso West in spring, 1978, using the same translocated male stock as in 1977. Release and monitoring procedures were modified to alleviate problems encountered in the 1977 trial. Objectives of the second release remained the same: reduction of the native population, recovery of the translocation in subsequent generations and improvement of release and monitoring techniques in programs to genetically control *Cx. tarsalis*.

METHODS

In the 1978 trial, mass rearing was done at the Bakersfield Arbovirus Laboratory as in the previous year (Asman et al. 1979), except that the release generation was held at the laboratory until after emergence to allow more accurate separation of sexes and counting. On 5 occasions during the release period, quality control tests were done in which samples of 50 T(1;2;3)1*A* males were outcrossed to 70–100 virgin females in the laboratory, and hatch rates of resultant rafts were determined to confirm the presence of the translocation sterility in the release stock.

Our goal was to release 5,000 T(1;2;3)1*A* male adults every other day for 2 months. Releases were made shortly after sunrise. One-third of each release cohort was marked in the field with fluorescent dust (Nelson et al. 1978). Seven distinct colors were used in rotation so that 14 days elapsed between consecutive use of any color. The mean daily survival rate of T(1;2;3)1*A* males released in 1977 was estimated to be 72%. Thus, only 1% (72^{14}) of any cohort was expected to be alive 14 days after release.

During the release period, shelter collections were made from 12 red box shelters (Goodwin 1942) and 20 "pipe traps" (Nelson, in preparation) located throughout the study area. Initially these collections were made only on mornings when releases were not scheduled. After 2 weeks, daily collections were made. Three CO_2/light traps (CDC light traps with dry ice) were operated 2 nights each week. The ratio of released to wild males was estimated for each day's collection as 3m : (n − 3m), where m = the number of marked males and n = the total number of males collected. Survival of each cohort was estimated on the basis of marked recaptures as described by Nelson et al. (1978). These survival estimates were later applied to the number of adults released to estimate the total number of T(1;2;3)1*A* males in the study area throughout the release period.

In the 1977 pilot release, 3 methods were used in attempts to monitor the mating success of released males: collection of rafts from the field; collection of pupae, from which emerging males were crossed with laboratory females; and CO_2/light trap collections of (presumably) mated females which were placed in laboratory cages for blood-feeding and subsequently transferred to individual vials for oviposition.

Due to the difficulties of finding sufficient numbers of rafts in the field, and the poor ability of field-derived males to inseminate laboratory females (Asman et al. 1979), only the third method, collection of adult females, was used in 1978. Up to 200 females were collected each week for this purpose. Egg rafts were scored for hatch and embryonation, and in some cases for survival of first instar larvae. Males from rafts suspected to have been fathered by T(1;2;3)1*A* males (i.e., those with less than 70% hatch and/or poor L-1 survival) were crossed with laboratory females homozygous for 2 recessive mutant markers to begin a 2-generation pseudo-linkage test. When these also produced low hatch rafts, surviving males were again backcrossed to mutant females. Resulting progeny were examined for phenotype. If the parental male was normal, three-fourths of the progeny of each sex would be mutants. When most male progeny were wild phenotype and most female progeny were mutants, the presence of the translocation was confirmed. When testing did not extend through 2 laboratory generations, confirmation was based solely on persistent low hatch.

An ecologically similar field area about 6 km north of Poso West (McVan) was used as a control site. Up to 100 females were collected each week and processed as described above.

The impact of the release on the *Cx. tarsalis* population at Poso West was measured by 2 methods. Absolute population size was estimated by the mark-release-recapture method in June, July, August and September using procedures described by Nelson et al. (1978). During the intervals between mark-release studies, 3 CO_2/light traps were operated 1 night each week and shelter collections were made the following morning.

Competitive mating ability of the release stock was reassessed in large outdoor cages in late May, following procedures described by Terwedow et al. (1977). Wild-type males and females (PWW) were collected at Poso West as pupae and allowed to emerge in the laboratory. Translocated males were from the T(1;2;3)1*A* stock used for the field release. The competition cage contained 500 virgin PWW females, 500 PWW males and 500 T(1;2;3)1*A* males. Control cages contained 500 virgin PWW females and either 1000 PWW males or 1000 T(1;2;3)1*A* males. Rafts were collected for 10 days and scored for hatch and embryonation.

RESULTS

A total of 179,432 transolcated males was released between 19 March and 3 June (Table 1). These represented 39 cohorts whose size varied from 691 to 11,861 (average 4,601). One-third (59,828) were marked prior to release. Quality control data from 308 egg rafts indicated that 99.4% of the released males carried the translocation.

Recovery collections, which began on 20 March and continued through 9 June, provided 13,123 male and 17,931 female *Cx. tarsalis* (Table 1). During this 12 week period, 1,066 marked males were recaptured, 84% of them from shelters. Recapture rates for individual cohorts ranged from 0.2% to 7.7% (average 1.8%). Five marked females were collected, indicating an error rate of less than 0.5% in separating sexes prior to release. Released males outnumbered native males until early May.

Survival estimates for each release cohort were based only on recaptures from shelter collections in order to reflect uniform sampling effort. In some cases, it was necessary to combine data from 2 or more consecutive small cohorts. Mean daily survival was at least 90% for all cohorts released before the end of April,

Table 1. *Culex tarsalis* adults released and collected during genetic control pilot study at Poso West, Kern County, California, 1978.

Week of	T(1;2;3)1*A* males released			*Cx. tarsalis* collected[1]				Estimated ratio of T(1;2;3)1*A* ♂♂: native ♂♂
				Males		Females		
	No. of cohorts	Total males	Marked males	Total	Marked	Total	Marked	
3–19	4	16,151	5,386	150	40	22	3	4.0
3–26	3	15,415	5,136	106	36	57	0	—[2]
4–2	4	6,402	2,124	62	21	18	0	—[2]
4–9	3	2,847	951	162	50	37	0	12.5
4–16	4	22,561	7,529	188	61	103	0	36.6
4–23	3	26,131	8,716	433	102	345	1	2.4
4–30	4	16,800	5,599	387	94	164	1	2.7
5–7	3	18,166	6,061	609	102	301	0	1.0
5–14	4	31,134	10,387	1,587	175	1,149	0	0.5
5–21	3	16,890	5,627	2,241	242	1,929	0	0.5
5–28	4	6,935	2,312	3,517	136	4,899	0	0.1
6–4	0	0	0	3,681	7	8,907	0	<0.01
Total	39	179,432	59,828	13,123	1,066	17,931	5	

[1] From shelters and CO_2 light traps.
[2] Not estimable; estimate of released males exceeded total males.

then declined at a steady rate to 73% for males released during the final week. Using these survival rates, the estimated surviving T(1;2;3)1*A* population peaked at approximately 31,500 in late April (Figure 1).

Hatching rafts were obtained from 2,034 of 2,810 females in attempts to recover the translocation (Table 2). Of these, 22 were confirmed (16 by pseudolinkage and 6 by low hatch) to have been fathered by T(1;2;3)1*A* males. After analyzing hatch and embryonation data from the outdoor cage test, new criteria were developed to distinguish translocated from normal rafts (see below). On this basis, an additional 45 rafts from which no F_2 progeny were obtained were presumptively designated as translocated. The last of these was from a female col-

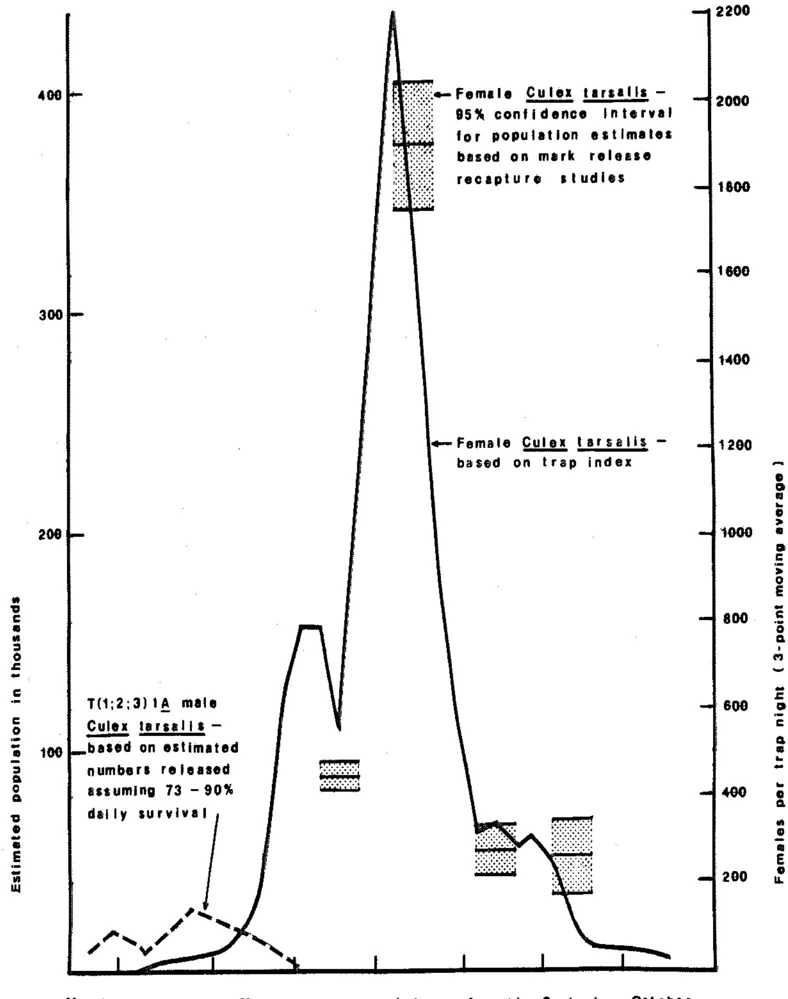

Figure 1. *Culex tarsalis* population estimates, Poso West, Kern County, California, 1978.

Table 2. Attempts to recover T(1;2;3)1*A* progeny from *Culex tarsalis* females collected at Poso West, Kern County, California, 1978.

	Release Period	Post-release	Total
Number of females tested	1,189	1,621	2,810
Number of hatching rafts	875	1,159	2,034
Number (%) of rafts with <70% hatch	68 (7.8)	61 (5.3)	129 (6.3)
Number (%) of confirmed recoveries	18 (2.1)	4 (0.3)	22 (1.1)
Number (%) of presumed recoveries	27 (3.1)	18 (1.6)	45 (2.2)
Number (%) of total estimated recoveries	45 (5.1)	22 (1.9)	67 (3.3)

lected 6 weeks after the final T(1;2;3)1*A* males were released.

None of 242 hatching rafts from females collected at McVan was confirmed or presumed to have been carrying the translocation.

Four monthly mark-release-recapture studies were done to provide a basis for population estimates at Poso West. In these studies, 81 of 29,217 marked males and 5,520 of 50,164 marked females were recaptured. Recovery collections totaled 55,039 males and 429,598 females. Estimates of mean daily loss rates of marked females from the population varied from 31 to 41% (Table 3) and the population peaked at over 400,000 females in early July (Figure 1).

Results from the outdoor cage test are shown in Table 4. Based on mean hatch rates in the 3 cages the T(1;2;3)1*A* males were estimated to have been only .24 as competitive as PWW males. Unhatched eggs in all but 2 rafts from the translocation control cage contained embryos. (These 2 low hatch/low embryonation rafts were attributed to poor or partial insemination; the same phenomenon was observed in 4 rafts from the PWW control cage.) On this basis (i.e., low hatch/high embryonation) it was possible to classify 19 of 101 rafts from the competition cage as translocated (7 rafts had low embryonation and could not be classified). This implies a competitiveness value of .23 (19:82) which is almost identical to the value estimated on the basis of hatch rates alone.

Table 3. Estimates of mean daily loss rate and population size for female *Culex tarsalis*, Poso West, Kern County, California, 1978.

Month	Mean daily loss rate	Population size
June	34%	90,547
July	36%	380,239
August	41%	56,229
September	31%	52,730

DISCUSSION AND CONCLUSIONS

The high survival rate of cohorts released during the first 6 weeks probably led to some overlap of identically marked

Table 4. Large outdoor cage test to determine the mating competitiveness of T(1;2;3)1*A* *Culex tarsalis* males, 1978.

Cage	Females	Males	No. of hatching rafts	Mean hatch	Mean embryonation
Competition	500 PWW[1]	500PWW and 500 T(1;2;3)1*A*	108	83.6%	95.9
Translocation control	500 PWW	1000 T(1;2;3)1*A*	25	35.4%	92.7%
Wild-type control	500 PWW	1000 PWW	88	95.3%	96.8%

[1] Reared from pupae collected at Paso West.

males released 2 weeks apart. If mean daily survival was in fact 90%, then 23% ($.90^{14}$) would remain alive after 14 days. The assignment of marked recaptures to the most recently released cohort may have biased estimated survival rates and, consequently, estimates of the number of released males remaining in the population.

The decreased competitiveness of the T(1;2;3)*1A* males in the outdoor cage test suggested that this stock had become less fit for field release since 1976. In Fine et. al. (1979), the proportion of females which mated with translocated males was given by $N_tC/(N_tC + N_w)$, where N_t and N_w are the numbers of translocated and wild-type males, respectively, in the population, and C is the competitiveness of the translocated males. In the present study, this proportion was 5.1% during the release period (Table 2). Using estimates of N_t and N_w for this period, derived from data in Table 1, we calculated a C value of .11, which is even lower than that observed in the outdoor cage test.

This decrease in fitness was further substantiated by data on dispersal of T(1;2;3)*1A* males at Poso West, which will be reported separately (Nelson and Milby, in preparation). Additional evidence of poor dispersal was provided by the relatively high recapture rate of the marked T(1;2;3)*1A* males (1.8%) which is double that observed for marked males derived from field-collected pupae in other studies at Poso West (Milby 1979). The effect of long-term colonization on behavior has been discussed at length (Bush 1978, Knipling 1967, Mackauer 1976). These observations have led to a reevaluation of laboratory rearing procedures in an effort to improve the behavioral characteristics of this and other stocks under consideration for genetic control experiments. One approach will be to rear such stocks in large outdoor cages, thus providing environmental conditions which more closely resemble those they will encounter when released into a field area.

The observation that rafts sired by T(1;2;3)*1A* males tend to have high embryonation rates despite reduced hatch provides a more efficient method than selection based on low hatch alone to screen rafts for recovery of the translocation. Confirmation of such recoveries remains difficult, as it requires mating of field-derived males with laboratory females. Nevertheless, our failure to recover the translocation from field progeny in 1977 is puzzling. During the 1977 release period, 29% of the rafts obtained from field-collected females had less than 70% hatch, as compared to 8% in 1978. Embryonation rates were not determined for rafts in 1977, so it is impossible to estimate the proportion of low hatch rafts which resulted from poor insemination. Many of the females collected during the 1977 monitoring could have been those which were inadvertently released along with the translocated males. It is also possible that those colony females were more likely than the native females to have mated with the T(1;2;3)*1A* males. Mating preferences have been demonstrated for field-released laboratory mosquitoes (Dame, et. al. 1964), and will be investigated at Poso West in future studies.

Despite the apparent poor fitness of the T(1;2;3)*1A* males, their ability to survive and mate in the field was demonstrated by the confirmed recovery of the translocation from progeny of females collected more than 2 weeks after the termination of releases. The observations lend credence to the feasibility of using translocated heterozygote males to control population size. Improved release and monitoring techniques facilitated both the implementation of this pilot study and the evaluation of its effectiveness. However, future genetic control efforts must await development of rearing methods designed to enhance mating behavior of release stocks.

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