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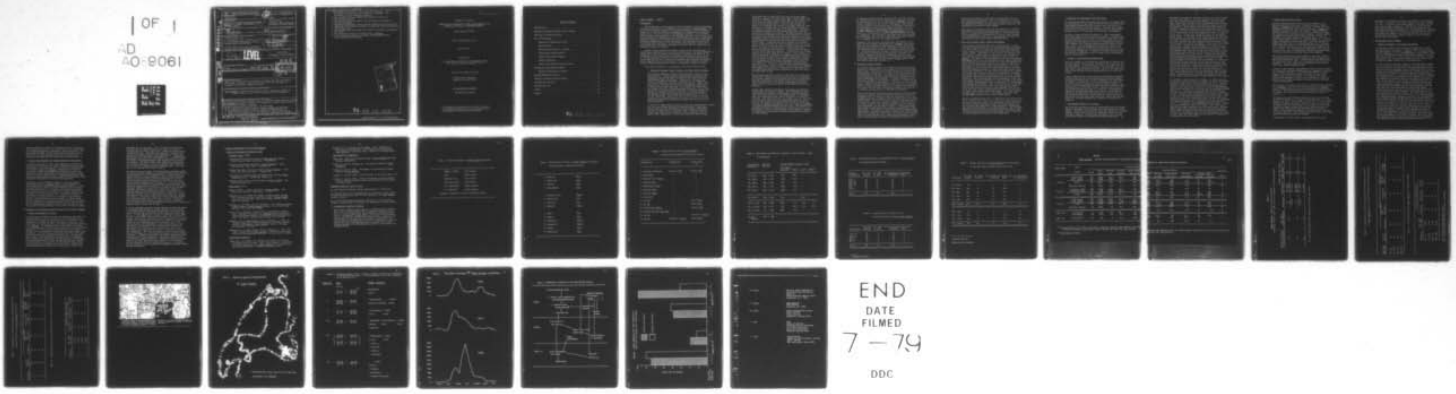

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: USA-A74-75

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): GENETICS OF THE ENCEPHALITIS VECTOR, CULEX TARSALIS FOR POSSIBLE APPLICATION IN INTEGRATED CONTROL.

5. TYPE OF REPORT & PERIOD COVERED: Annual 1978-79, 5th Year - Mar. 78-Feb. 79

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): Sr. Monica Asman Ph.D., Principal Investigator

8. CONTRACT OR GRANT NUMBER(s): DAMD17-74-C-4128

9. PERFORMING ORGANIZATION NAME AND ADDRESS:
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10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62760A, 3A762760A806 00 103

11. CONTROLLING OFFICE NAME AND ADDRESS:
U.S. Army Medical Research & Development Command, Fort Detrick, Frederick MD 21701

12. REPORT DATE: Feb. 1979

13. NUMBER OF PAGES: 29

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): 33p.

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

LEVEL III

16. DISTRIBUTION STATEMENT (of this Report)

Distribution unlimited

Annual rept. Mar 78-Feb 79,

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
Some of the enclosed data was presented at relevant meetings (see updated bibliography in report). Some has been and some will be published as indicated in updated bibliography of report.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Culex tarsalis; reciprocal translocations; vector competence; competition studies; mutants; pilot release studies; salivary chromosomes.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The projects here reported represent part of an overall program designed to change Culex tarsalis genetically to inhibit its propagation in nature, and to render it less effective as a vector of disease.
A resume of progress for the year 1978-79 is as follows:
A. The number of maintained strains for genetic studies was increased.
B. Multiple-marker strains for genetic studies and identification of translocations increased to 14. Two additional mutants were isolated.
C. Among the translocated strains that were given priorities for evaluation

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as release material are: 1 sex-linked, and 2 autosomal. The sex-linked is a multiple, involving all 3 chromosomes. The 2 autosomes are in the homozygous condition.

D. The sex-linked multiple translocation strain was used for 2 pilot-release studies.

E. Substantial progress was made in the preparation and mapping of salivary chromosomes.

F. Chromosome-linkage group correlation was established for this species.

G. Comparisons of reproductive behavior of laboratory and field-collected females were made.

H. Mark-release-recapture studies were carried on throughout the spring and summer.

I. The sterile-male method was developed for C. tarsalis.

J. A mass-production program successfully produced 180,000 males over a pre-determined period.

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REPORT NO. USA-A75-76

GENETICS OF THE ENCEPHALITIS VECTOR, *CULEX TARSALIS*, FOR POSSIBLE APPLICATION IN INTEGRATED CONTROL

Annual Report, 1978-79

Sister Monica Asman, Ph.D.

February 1979

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-74-C-4128

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Annual Progress - 1978-79

Introduction

The primary goal of our research is the development of genetic mechanisms that could contribute to an integrated control system for Culex tarsalis. Controlling this mosquito species is the principal approach to control Western Equine, St. Louis, and California encephalitis in California and the western United States. Since the vector is increasingly resistant to all currently known and accepted insecticides, we are pursuing autocidal control as an alternative method. More specifically, our primary efforts have been to genetically manipulate segments of this species so that self-destruction will follow when they are introduced into a native population. This destruction could be due to inherited semi-sterility or to the introduction of genetic factors that would either act as autocidal time-bombs or at least make the population less medically important or noxious.

Genetic control research is relevant because of the following facts: 1) increasingly constrained laws have all but banned some of the established pesticides, and the use of others is unacceptable or severely restricted; 2) the development of new chemical agents is becoming more difficult each year due to cost and regulatory regimes; 3) "natural" pest control mechanisms often are disrupted by the use of chemicals, especially when predators and parasites of the target species are also killed; 4) the autocidal (genetic) approach offers a highly species-specific means of controlling a mosquito species.

The 5 major categories of our research to date are described below:

A. Laboratory Colonies, Genetic Strains, and Basic Genetic Studies

Six wild type laboratory colonies are maintained for specific purposes (Table 1). These include 2 representative populations that originated from the Poso West field area in Kern County where our experimental stocks are released. The Poso West Colony was initiated in September of 1978 and is maintained in the laboratory. The second colony from that area is Poso West Quonset (PWQ). This population is maintained under natural environmental conditions in a large outdoor quonset-type cage. As in nature the females that are outdoors went into a diapausing stage this winter. We are confident that in early spring they will feed, and gravid females will oviposit to establish the next generation. In both populations, PWC and PWQ, we hope the genotypes are remaining close to that of the wild population. These lines will be used discreetly for outcrosses with our experimental material prior to release so that the genotypes introduced for competitive purposes will be close to the field population.

A total of 14 single mutation stocks are also maintained (Table 2) including the 3 new strains which will be described below. These mutations, which have been identified for their linkage relationships, are used for basic genetic experiments and to construct multiple-marker lines (Table 3) which carry at least one genetic marker on each of the 3 chromosomes. We now have 14 multiple marker stocks.

The mutants newly isolated this past year are bronze (brz), melonotic-2 (mel-2), and spaced eyes (spe). Bronze affects the color of all the black scales in both sexes of the adults. The scales not only appear brown in color but also have a sheen which gives the bronze effect. Tests to date indicate the mutant to be autosomal and linked to the carmine mutation, thus placing it on linkage group 3. Bronze was first observed in the carmine stock and is thought to be spontaneous. Melanotic-2 appeared spontaneously in the carmine/black line which was being used in outdoor-pond studies. The dark melonotic patterns of this mutation appeared to be expressed differently in larvae than mel-1 which we had maintained in pure strain for some time. Reciprocal crosses between the 2 melonotic strains gave wild-type progeny supporting our observations that these were indeed different mutants. Spaced-eye was first observed in our mutant colony, ebony, and since the latter originally came from a line exposed to Co-60, spaced-eye could be either induced or spontaneous. The mutant is expressed by a relatively broad space between the compound eyes ventrally. In the normal-type adult the eyes border each other directly. "Spaced-eyes" was isolated recently and to date found only in females. This suggests it is either sex-linked or autosomal with expression only in females. Further tests will establish its linkage relationship and potential for genetic studies. Since bronze and melonotic-2 appear to be autosomal and on different chromosomes they provide the raw material to construct several additional multiple-marker lines that will identify pseudolinkage in isolating chromosomal interchanges (translocations). In the past, 27 chromosomal interchanges were genetically recognized and 3 autosomal and 9 sex-linked translocations were established as lines with these multiple marker stocks.

Both mutations and marker stocks are essential to our work for various other reasons: they are necessary tools for understanding the basic genetics of the species, they serve to genetically identify the position of chromosome breaks and re-attachments, they have application in the study of chromosome mapping of genetic factors, and can be used as tracer stocks for field experiments.

Studies were completed to determine the fitness of the mutant carmine (car), an eye-color mutant. This assessment was necessary because this marker is incorporated in the translocation lines for several purposes: their identification, to determine where the interchange occurs, and to learn what new linkage is involved. Both laboratory tests and outdoor-pond tests indicated that carmine eye-color does not significantly decrease mating ability. The study also indicated that the fitness of the car stock can be improved substantially if it is outcrossed frequently to recently colonized material from the field. This requires the subsequent recovery of a marked mutant in backcrosses of F_1 hybrid crosses. Such a scheme would also allow efficient isolation of translocations with greater fitness for competitive purposes in field releases.

An important breakthrough was made this past year that provides an understanding of the basic genetics of C. tarsalis. This was the determination that the sex-determining chromosomes are the longest chromosomes of the 3 pairs. Both genetic and cytogenetic data support the conclusion which is different from the pattern reported for other Culicine mosquitoes (Table 4, Fig.1). In Aedes aegypti and Culex pipiens sex determination is on the shortest chromosome, while in Culex tritaeniorhynchus one sex locus is on the shortest and another has been found on the longest chromosome. The evidence we have might prove very important in establishing there has been a different evolutionary history for C. tarsalis than for other Culex mosquitoes.

Progress in the isolation, identification, and mapping of the large salivary polytene chromosomes of C. tarsalis will contribute much to our program in basic genetics. The 3 chromosomes have been identified as distinct and separate entities and specific banding patterns can now be recognized and described (Fig. 2). Within the next year we expect to publish the first paper on the salivary chromosomes of this species. Hopefully, the modified techniques that we have established will also help solve the problems encountered in the preparation and spreading of polytene chromosomes in other Culex and Aedes species where past efforts have been fruitless.

B. Isolating Autocidal Systems

An important advance made in the isolation of translocation systems was our development of a new scheme to select for new homozygote lines. This again was possible because we had developed multiple-marker stock (Fig. 3). Males of a multiple-marker stock are irradiated at 2000 rads. Any heterozygous translocation is easily isolated by genetic analysis of the F_1 testcross to identify pseudolinkage of the markers. To produce homozygotes the latter are crossed to wild normals to produce a 1:1 ratio of normals to translocation heterozygotes. Sib crosses of the outcross progeny produce 4 possible mating combinations, with 3 phenotypic ratios for their descendant families. In families that consist of 83.3 percent wild to 16.6 percent mutant progeny the latter can be isolated and identified as translocation homozygotes.

Among the colonized translocation lines that show some potential as autocidal systems for the release evaluation, we now have 2 homozygote lines, 1 isolated in the 21st generation by the older standard method and 1 isolated in the 5th generation by the newly-devised method. Since both of these lines are autosomal they can be carried by either sex. Another advantage of these specific autocidal systems is that they can transport desirable genotypes into a population and the impact could be to reduce populations and/or make the population more tolerable by replacement of specific genes. This concept is based on the theory from population genetics that 2 genetic forms cannot coexist if the hybrid between them is less viable than either form. If fitness is the same for both, the least frequent will be displaced in subsequent generations. Thus we hope to utilize this replacement mechanism as a

bonus when we introduce a homozygotic translocation that also carries autocidal properties. Both of our homozygote lines have been undergoing expansion for the past 6 months so that they may be evaluated this spring in large outdoor quonset-type huts, and subsequently utilized for field releases.

Males from a line selected last year for resistance to the WEE virus infection have been irradiated during the past year in an effort to isolate additional translocations. The resistance-to-infection genotype is a desirable one to use in future releases since combining this factor with a newly-induced autocidal system would serve the double purpose described above.

C. Pilot Field Studies Using a Double Heterozygote

A top priority study during the past 2 years has been to carry out pilot-release studies with the double heterozygote translocation, T(1:2:3)1A, at a release site (Poso West) near Bakersfield in Kern County, California.The site has been described in detail in past reports. Advantages of this site are its isolation; the water source is stable since it comes from an oil refinery process; and the natural mosquito population is almost solely C. tarsalis. The study in 1977 indicated that the sex-linked double heterozygous males could be mass-produced, were highly competitive with wild-type males, could survive in the field as immatures and adults, and the development time of their progeny in the field was similar to that of the native field population. The first pilot study also showed where more research was needed, and where improved techniques relevant to release strategies and field monitoring were necessary. In that year the population was considerably larger than in previous years, and consequently the number of released males (76,000) was proportionally too low to compete effectively with the native males and have an impact on the wild population. The 76,000 specimens were approximately 24,000 less than planned, and never reached a 1:1 ratio of males in the field. An estimated 10,880 females, which did not carry the interchanges, were inadvertently released. One of the difficulties in the mass-rearing phase of this pilot study, other than the low percent hatch of egg rafts and the need to cull expanding lines for eye color, was that a manual sexing process had to be used that did not always remove small-sized female pupae. And in that year we released pupae into the field. The complete 1977 pilot study was described in detail in last year's Annual Report.

Based on information from 1977, a second release was initiated in 1978. Since we met all but 2 of our objectives the previous year, our main concern was to reduce the population significantly with an insert of males carrying the double sex-linked interchange, and to recover the translocation from egg-rafts or progeny that were derived from matings in the field population. The isolation hopefully would be verified genetically in the laboratory. Monitoring the population would indicate whether or not a reduction of the population had occurred at the release site.

1. Expansion of Experimental Stock for Release

The mass-rearing production program was initiated in January, 1978. The experimental line was again T(1;2;3)1A, the sex-linked double-heterozygous translocation that was described in detail as to origin and behavior in previous reports. This line has a stable low fertility of between 20 and 30 percent, and all viable male progeny inherit the interchange from generation to generation.

In order to assure a projected release of 5,000 translocated males every other day over a period of 2 months, a detailed plan had to be followed. The process was similar to that used in 1977 and described last year in detail. The last generation of each expansion line represented an outcross of translocation males to virgin females from a vigorous laboratory colony (Knight's Landing). Prior to the last-generation outcross the purity of the experimental stock was maintained by crossing the males with carmine-black females, and by culling pupae and adults to eliminate possible recombinants. In addition, periodic monitoring was done during the expansion process to sample rafts and determine percent hatch (Table 5).

2. Release of Translocation-carrying Males

The actual number to be released was ascertained by computer simulations based on the population estimates at the field site over the past summers. It was decided to release male adults and to guard against the insertion of females. To accomplish this, male pupae were picked and adults allowed to emerge in the laboratory. Females that had been included inadvertently were observed and removed.

A major current effort is being directed at developing a genetic sexing mechanism to be used in future mass-production programs. Alternative possibilities are fully described in the application for contract continuation. In this second release study, the projected number for release was reached without incidence. In total, 150,000 translocation-carrying males were released between mid-March and the first week in June. Quality-control tests of the release material were made on 5 occasions. Representative samples of the experimental males that were to be released were removed and crossed with virgin females that had been taken as pupae from the field site. Genetic analysis proved that only 2 egg rafts of the 308 tested gave high hatch, indicating that 99.4 percent of the males released were carrying the translocation (Table 6).

3. Monitoring the Effect of a Release

The following monitoring system was established during the release period to determine if the released males were mating with native females. Each week (when possible) up to 200 females were collected from the release site, caged in the laboratory, blood fed, and allowed to oviposit. The egg rafts were given 3 days to hatch, and then were scored for percent hatch and/or embryonation. Ap-

proximately 30 medium or low hatch rafts were set aside to determine the larval survival. The male progeny from 10 low-hatch rafts were crossed to carmine-black females. Egg-rafts from these crosses that had high hatch were discarded, and the low to medium hatch rafts were sent to the Berkeley laboratory for further analysis. Males derived from those rafts were then backcrossed to confirm the presence of the translocation (Table 7). In April, 13 percent of the rafts collected at the field site were low or medium hatch, in May this figure was reduced to 8 percent, and in June 12.5 percent of the egg rafts were low and medium percent hatch. Of the rafts chosen for analysis, some were discarded because of high hatch in the second generation, some families died out before the tests were completed, and in 17 cases the tests confirmed that the translocation males released at the site had mated with the native females. Thus we met 1 more objective in 1978 -- namely, we ascertained that our genetically-altered males had mated with field females in a field situation. Unfortunately, parallel tests in which the release males competed against field males for mating with field females in large quonset-type cages, revealed that the release stock had become less competitive than it was in 1977. We feel that selection factors related to laboratory rearing conditions had changed the T(1;2;3)1A genetically-altered stock over a period of time. Thus the impact of our release was not sufficient to cause a significant or on-going reduction of the field population. From mark-recapture studies we also learned that the released males did not move within the study area as readily as did males from the natural populations. While data are not inclusive, the release may have had some impact on the field population. In 1978, the population in June at the release site was 40 percent less than in 1976 and 1977, although the drought had long been ended. Although the water source at the release site is relatively stable, 1978 was a year of excess rainfall in this region.

The previous 2 years, there was a drought. In 1978, there was an obvious 4-week delay before the population reached a peak comparable to those in the 2 previous years (Fig. 5). In 1978 the population peaked in July when there was an estimated 450,000 females in the study area. This peak in 1978 was approximately twice that observed in 1977, which in turn was considerably higher than in 1976. Initially we were surprised to experience this increase as flash floods had inundated the area on 2 occasions in 1978. Vegetation was stripped from the stream bottom, a layer of oil, tar, and sand was deposited, and the water level in the reservoir at the creek terminal was raised to very high levels. These developments gave our Field Station personnel an opportunity to observe the capacity of C. tarsalis to quickly populate an area. It is apparent that flooding enriched the aquatic environment and increased the larval food supply. There also was a decrease in predator populations in the early season. Combined, these changes would partially decrease the density dependence effects which we reported last year, and explain the steep increase of the population; however, since our releases also ended in early June, the insert might have contributed to reduction in the population up to that point.

4. Mark-release-recovery studies

The mark-release-recovery studies that were conducted by our associates provided estimates of the daily survival of adult female C. tarsalis under natural conditions. Between June and September of 1978 29,217 male and 50,164 female C. tarsalis were marked with fluorescent dusts and released. These mosquitoes had been collected as adults in the field or collected as pupae and emerged in the laboratory prior to marking. Recovery efforts included collections of 429,598 females and 55,039 males and included 5,520 marked females and 81 marked males. This type of study though tedious allowed us to estimate accurately the actual number of mosquitoes in the study area. From June through September mean daily survival fluctuated between 54 to 67 percent. These rates were similar to those in 1977 when they ranged from 54 to 67 percent and less than in 1976 when they ranged from 64 to 77 percent. This illustrated the importance, if an autocidal system is to be used, of introducing a female conditional lethal to further increase the mortality rate of adult females.

When the translocated stock was released at Poso West 59,811 of the 180,000 genetically-modified males that were released were marked with fluorescent dusts. Collection of 9,095 males during the period March 19 to June 3 led to recovery of 1,055 marked specimens. The daily survival rate declined from 90 to 73 percent as the average temperature rose.

Release of equal numbers (approximately 6,000) of marked translocated male and laboratory derived female C. tarsalis in mid-summer was followed by recovery of 108 marked males and 2,150 marked females. The mean daily survival rates were 38 percent for females and 71 percent for males. As stated earlier, adults derived from laboratory colonies did not disperse as rapidly or widely as field-collected females or females that hatched from field-collected pupae.

We know very little about the survival of overwintering populations of C. tarsalis. This past fall over 40,000 adult C. tarsalis equally divided between the 2 sexes were marked and released at Poso West. Recovery collections are continuing through the winter and spring, and 5 females have been recovered, the latest on January 3.

One problem that must still be resolved is that we do not have an effective system for the study of male populations of C. tarsalis in the field. Large numbers of males can be collected from shelters or reared from pupae; however the success in recovery of marked males from such sources is very poor. We cannot make effective estimates of population size or survival rates. We surmise that marking males with dusts has had a significant effect on their survival, movement, and probably their capacity to mate in the field. New procedures will be tested in the coming year and hopefully resolve this problem.

A new device, the "pipe trap", was developed by one of our

colleagues in an effort to increase the success of male-trapping. This trap is constructed of a meter length of pipe with a diameter of 14 cm. A rod with a foam rubber plunger is inserted into the pipe, and the pipe is buried in a bank along a stream bed with the opening exposed. Mosquitoes enter the pipe seeking refuge from light and excessive temperatures. The mosquitoes are collected by placing the sleeve of a cardboard-carton cage over the orifice and pulling the plunger up to force the insects into the cage. The new device provides a valuable adjunct to the old red-box shelter collecting method.

D. Sterile-male Control Program

1. Method of Approach to Successful Application

For the past 2 years we have studied the feasibility of using the sterile-male technique as a support system to the insertion of translocations to control C. tarsalis. The procedures followed were suggested by the International Atomic Energy Agency (IAEA), which has co-ordinated several international programs to control insect pests (Fig. 6). We have completed much of Phase I on that diagram, and will continue to do research in this phase as well as initiate studies of Phase II in the coming year.

We have irradiated young males (0-24 hr post-emergence) by means of a Co-60 source to produce sterility. The dose rate of this source is approximately 200 r/min. We have established the sterility curves for C. tarsalis. Mating competitive tests in laboratory cages consistently indicated that 5 krads from this source delivered in approximately 33 minutes produced highly sterile and competitive males. We found a 43 percent egg hatch resulted when irradiated and non-irradiated males competed in a 1:1 ratio, as compared to a 92 percent hatch in the control unirradiated cage, and 3.0 percent in the control irradiated-male cage. In 1978, 2 competitive mating experiments were completed in large outdoor quonset-type cages. The first consisted of 1000 PWC (Poso West Colony) irradiated males competing against 1000 PWW (Poso West Wild) untreated males for 1000 virgin females. A sterile control test was run in an adjacent cage with 500 sterilized males and 500 virgin females. Data from earlier tests with untreated males had to serve as the control data since we had a shortage of such males at this time. In a second competitive mating experiment in quonset type cages 750 sterilized PWC males competed against 750 PWW males for 1500 virgin females. The sex ratio was changed from the first experiment because wild females were thought to be entering diapause and about 50 percent were not expected to oviposit this late in the summer. A sterile male control test was run simultaneously in a smaller cage placed inside the larger quonset-hut cage that held the experimental adults. The results are summarized in Table 8. In the first experiment 51 percent of the egg rafts had low hatch, an average of 3.1 percent hatch/raft. The control cage with sterile males only was also 3.1 percent hatch/raft, while the untreated control results when males were not irradiated had consistently had an average 94 percent hatch/raft. In the

second experiment 33 percent of rafts had low hatch, an average of 0.9 percent hatch/raft. The percent hatch in the control cage with irradiated males only averaged 0.7 percent hatch/raft. In both tests the number of sterile and fertile rafts was similar for the first 4 days; however, after that time there was a decline in the proportion of sterile rafts oviposited (Fig. 7).

2. Factors that Determine the Competitiveness of Sterile Males

A study was completed in 1978 in the laboratory to determine if the sex ratios and ratios of sterile to fertile males were important factors to be considered if the sterile-male technique was to be used to control C. tarsalis. Releases of sterile males for control necessarily increases the proportion of males to females in a target population, and for success requires a high ratio of sterile to fertile males. Abnormal mating behavior may result when large numbers of sterile males are introduced in field situations and compete with the wild male population.

The results of this experiment showed, as in previous mating trials with sterile C. tarsalis males, a clear separation in the distribution of high and low hatch egg rafts (Table 9). When the sex ratio was 2 males to 1 female and the ratio of sterile to fertile males was 1:1, 41 percent of the rafts were sterile. When the sex ratio was 1:1 and the ratio of males remained 1:1, 49 percent of the rafts were sterile. And lastly when the ratio of sterile to fertile males was 3:1, in favor of the sterile males, and the sex ratio was 2:1, 63 percent of the rafts were sterile. While there was an obvious increase in sterile rafts when the frequency of the sterile males was increased, the ability of the sterile male to inseminate females and give sterile rafts was not significantly different when the ratio of males was the same and the only variant factor was the ratio of males to females.

E. Biological and Ecological Studies Relevant to Our Control Program

1. Mating and Reproductive Studies

It has become an accepted fact that the single most important contributing factor to lack of success in the sterile-male or any other genetic programs for insect control is a deficiency in information on the basic biology of the species in question. For this reason we continue to carry out studies on the mating behavior of C. tarsalis. We completed a study on the effects of laboratory colonization on the reproductive abilities of a field-collected population. We still do not know the best way to bring field material into the laboratory and maintain the desired vigor of that population. In this study we compared Poso West Wild (PWW) taken from the release site, to Knight's Landing (KL), a strong laboratory strain. The reproductive characters we were documenting were: insemination, oviposition, and egg hatching. The PWW material was collected from the field as pupae and allowed to emerge in the laboratory to ensure that the females were virgin, and to control the age of the adults used in the study. Generally 200 newly-emerged adults of each sex were placed into a colony cage

measuring 60 cm on each side. After 3-5 days a blood meal was provided, and 100 engorged females were placed in a cotton-stopped shell vial with a sugar wick. A layer of water provided an oviposition site. Egg rafts were examined 3 days after oviposition to determine egg hatch and embryonation. Females were frozen after a 6-7 day oviposition period and spermathecal examinations were made later to determine the insemination status. The number of Christopher-stage-V eggs remaining in the ovaries was recorded. The field line was also inbred for 4 generations for further observations. Nearly all engorged females, whether from the field or laboratory colony, developed eggs. Those that didn't were excluded from further analysis.

The results were as follows: a) the insemination rate was highest for inbred laboratory females and lowest for inbred field-collected females (Table 10); b) the oviposition rate of inbred field-collected females was reduced compared to that of inbred laboratory females; c) only 12 percent of the developed eggs from inbred field-collected females hatched while 77 percent of those from inbred laboratory females hatched (Table 11). In the field population that was retained for 4 generations, the insemination rate gradually increased from 28 percent to 76 percent; the oviposition rates were reduced in the second and third generations and then increased substantially (Table 12); finally, the percent of developed eggs that hatched remained low for 3 generations and appeared to improve in the fourth generation (Table 13). It was also observed that while the laboratory strain completed oviposition within 3-4 days after feeding, the field population extended their oviposition time over to as long as 7 days. In addition, the laboratory strain oviposited equally well when confined to vials or colony cages, while surprisingly the inbred field females oviposited better in vials than in cages.

A second experiment related to mating behavior was initiated based on an earlier observation that marked laboratory females tended to be trapped earlier than marked field females even though they were of the same age and had been treated identically after emergence. It was suggested that perhaps the laboratory females might be ready to take their first blood meal sooner than the field females, and thus were attracted to the CO_2-baited traps in greater numbers on the initial days of collection.[2] PWW and KL pupae were placed in 1 gal. emergence cartons at a specific time. All emerged adults were transferred to 1 gal holding cartons, and 3 days later in the morning at the time when field releases were made, adults from each group were released into larger cages. Ten females were removed from each cage to ascertain if they had been inseminated. They were all negative. After taking a blood meal the following day, the females were examined for physiological conditions. A sample of both empty and engorged females from each group was checked for insemination. The results indicated that a significantly higher proportion of laboratory females took blood meals on the first night following "release". This appeared to be true regardless of their insemination status; i.e., approximately 10 percent of both engorged and unengorged laboratory females had been inseminated.

F. Updated Biographical Sketch and Bibliography

Principal Investigator and Genetic Staff

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"Outdoor Cage Tests of Genetic Strains of Culex tarsalis." Calif. Mosq. and Vector Cont. Ass'n. Yosemite, CA.

"Laboratory and Outdoor Competition Studies with Sterilized Aedes sierrensis males." ESA Meeting, Houston, TX.

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Asman, S.M., R.L. Nelson, P.T. McDonald, M.M. Milby, W.C. Reeves, K.D. White, and P.E.M. Fine. 1978. Pilot release of a sex-linked multiple translocation into a Culex tarsalis field population in Kern County, California. (Submitted to Mosquito News)

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Publications in preparation

Asman, S.M. Three new sex-linked mutants in Culex tarsalis and their linkage relationships.

Asman, S.M. and H.A. Terwedow, Jr. Two eye-color mutants in Aedes sierrensis.

Ainsley, R. and S.M. Asman. Development of a sterile-male release method for Culex tarsalis.

Ainsley, R. and S.M. Asman. Genetic fitness in the mutant carmine eye.

Ainsley, R. and S.M. Asman. Factors determining success of sterile males in laboratory mating trials: sex ratio and ratio of sterile to fertile males.

Personnel receiving contract support

Dr. Paul McDonald (Assistant Research Entomologist II) (100% time)

Dr. McDonald has been on theprogram from its conception in 1974. Prior to that time he had three years of field experience with Aedes aegypti control in Africa.

Mr. Arvin Krueger, Research Assistant (50% time), is a pre-doctoral student in the Division of Entomology and Parasitology, Berkeley Campus.

G. Collaboration Involved in Project

This overall research project requires a multidisciplinary approach, and one of the greatest assets for a successful conclusion of our work lies in the collaborative efforts among the participating personnel. Collaborators include members of the staff from the Department of Environmental and Biomedical Health Sciences, Berkeley, and the personnel at the Arbovirus Field Station at Bakersfield. We believe that the multidisciplinary team that contributes to both laboratory and field investigations gives us a unique ability and a rational approach to the investigations relative to the specific objectives we have.

Table 1. Wild-type colonies of Culex tarsalis maintained

Knight's Landing	(Yolo County)
Chico	(Butte County)
Poso West Wild	(Kern County)
Poso West Colony	(Kern County)
O.P. Susceptible	(Fresno County)
O.P. Resistant	(Fresno County)
Berkeley	(composite of several California strains)

Table 2. Monofactorial mutations of Culex tarsalis established and maintained as laboratory colonies.

1. black eye	(ble)
2. carmine eye	(car)
3. mulberry	(mul)
4. microcephalic	(mic)
5. bleached ocelli	(bloc)
6. fringe wing	(fr)
7. wide wing	(ww)
8. charcoal	(char)
9. ebony	(eb)
10. gabled	(gab)
11. melonotic-1	(mel-1)
12. melonotic-2	(mel-2)
13. bronze	(brz)
14. spaced eyes	(spe)

Table 3. Multiple-marker lines of *Culex tarsalis* established and maintained for genetic studies.

Linkage group I	Linkage group II	Linkage group III
1. sex (gene determined)	black eye (*ble*)	carmine (*car*)
2. fringe (*fr*)	"	"
3. bleached ocelli (*bloc*)	"	"
4. mulberry (*mul*)	"	"
5. microcephalon (*mic*)	"	"
6. wide wing (*ww*)	"	"
7. charcoal (*char*)	"	"
8. ebony (*eb*)	"	"
9. sex (*M*)	"	gabled (*gab*)
10. sex (*M*)	"	bronze (*brz*)
11. ebony/fringe (*eb*/*fr*)	"	carmine (*car*)
12. mulberry/charcoal (*mul char*)	"	"
13. sex (*M*)	"	melonotic-2 (*mel-2*)
14. sex (*M*)	melonotic-2 (*mel-1*)	bronze (*brz*)

Table 4. Sex-linked translocations isolated in *Culex tarsalis* after δ-irradiation

Translocation designation	Fertility ($\bar{X} \pm SE$)	Pseudolinkage (crossover units)			
		No. progeny examined	*M-ble*$^+$	*M-car*$^+$	*car*$^+$*-ble*$^+$
T(1; 2)2 *A*	.452 ± .013	1131	18.7	-	-
T(1; 2)9 *A*	.443 ± .021	648	25.2	-	-
T(1; 2)10*A*	.458 ± .015	1671	6.3	-	-
T(1; 2)11 *A*	.522 ± .014	1453	9.9	-	-
T(1; 2)12 *A*	.442 ± .012	1698	5.4	-	-
T(1; 3)13 *A*	.529 ± .027	719	-	26.3	-
T(1; 2; 3)1*A*	.284 ± .005	1183	7.2	8.9	1.7
T(1; 2; 3)4*A*	.167 ± .011	337	10.3	5.3	5.0
T(1; 2; 3)6 *A*	.212 ± .009	1049	4.2	-	-
car ble (marker)	.837 ± .006				

Table 5. Monitoring of hatch of experimental stocks of Culex tarsalis, the generation before release.

Proposed release date	No. rafts sampled	No. rafts no hatch	No. of rafts with hatch rate low/medium	high*
April 1	50	11	39	0
April 13	50	16	34	0
April 19	50	5	45	0
April 25	100	5	95	0
May 7	50	24	25	1
May 19	50	5	44	1
Totals	350	66	282	2

Table 6. Quality control of hatch of rafts from PWC ♀♀ mated to release Culex tarsalis.

Release date	No. rafts sampled	No. rafts no hatch	No. of rafts with hatch rate low/medium	high*
March 22	42	5	37	0
April 9	84	5	79	0
April 21	66	2	64	0
May 3	77	4	73	0
May 15	59	4	53	2
Totals	358	20	306	2

*greater than 70%.

Table 7. Genetic monitoring of Culex tarsalis field populations at Poso West (PW) and McVann (McV) sites, 1978.

Site-month	No. rafts sampled	No. rafts no hatch	No. of rafts with hatch rate low/medium	high*	No. recoveries of translocation
PW - March	20	0	0	20	0
PW - April	260	8	24	228	4
PW - May	631	28	44	559	11
PW - June	560	47	34	479	2
PW - July	649	24	25	600	0
WPC - Totals	2120	107	127	1886	17
McV - May	12	3	0	9	**
McV - June	168	57	7	104	**
McV - July	156	45	4	107	**
McV - Totals	336	105	11	220	**

*greater than 70%.

**recovery not attempted

Table 8.

Culex tarsalis: MATING COMPETIVENESS OF RADIATION STERILIZ

Experiment	Raft Class	Fertile Rafts				
		Total	Total eggs	Hatched eggs	Percent hatched	Embryonated eggs only
Q.H.14	High hatch	83	8063	7612	94.4	100
	Low hatch	82	7984	260	3.3	502
	Total	165	16047	7872	49.1	602
	Sterile control	72	8232	259	3.1	791
Q.H.13	+ Control (13a)	88	8532	8132	95.3	131
	+ Control (13b)	14	1765	1622	92.0	15
	Total	102	10297	9754	94.7	146
Q.H.15	High hatch	116	13793	13459	97.6	245
	Low hatch	71	7497	104	1.4	829
	Total	186	21290	13563	66.3	1074
	Sterile control	72	8831	63	0.7	441
LAB 5 Kr	+ Control*	5	337	231	69	79
	Low hatch*	19	1713	12	0.7	184
LAB 7 Kr	Low hatch*	11	781	1	0.1	140

* Radiated males were CHICO wildtypes competing against NEW CARMINE males fo
scored by progeny phenotype, only a subset was held for egg counts.

+ Percentage hatched.

VENESS OF RADIATION STERILIZED MALES

hed gs	Fertile Rafts: Percent hatched	Embryonated eggs only	Percent embryonated	Hatch range+: Minimum	Hatch range+: Maximum	Infertile rafts
512	94.4	100	1.2	55	100	-
260	3.3	502	6.3	0	38.6	-
172	49.1	602	3.8	0	100	10
259	3.1	791	9.6	0	26	3
132	95.3	131	1.5	50	100	3
522	92.0	15	0.9	48.8	100	0
754	94.7	146	1.4	50	100	3
159	97.6	245	1.8	77	100	-
104	1.4	829	11.0	0	23	-
563	66.3	1074	5.1	0	100	19
63	0.7	441	5.0	0	3.8	12
231	69	79	23	57	83	-
12	0.7	184	10.7	0	3	-
1	0.1	140	17.9	0	1.1	-

gainst NEW CARMINE males for heterozygote females. Since mating outcome was
as held for egg counts.

Table 3.

Culex tarsalis Laboratory Mating Trials:
Effects of sex ratio and ratio of sterile to fertile males

Ratio of sterile to fertile males	Sex ratio (males:females)	Replicate	Number of egg rafts <50% hatch	>50% hatch	Not sired
1:1	2:1	A	23 (38%)	38	2
		B	36 (43%)	47	0
		Totals	59 (41%)	85	2
1:1	1:1	A	33 (47%)	37	2
		B	20 (53%)	18	1
		Totals	53 (49%)	55	3
3:1	2:1		27 (63%)	16	8

* % low hatch egg rafts among total sired egg rafts.

Table 10. Observations on ovarian development and fertilization of gravid ♀♀ Culex tarsalis from laboratory colonies and field populations.

Population (♀♀ x ♂♂)	No. ♀♀ observed	Percent of females				
		inseminated	inseminated that oviposited	laid hatching rafts	laid non-hatching rafts	retained all eggs
KL x KL	98	96	98	92	5	3
KL x PWW	98	78	96	65	23	12
PWW x KL	96	47	73	34	15	51
PWW x PWW	100	28	71	18	27	55

Table 11. Developed eggs from Culex tarsalis in laboratory cages.

Population	No. eggs developed	Percent of eggs developed		
		oviposited	embryonated	hatched
KL x KL	18,213	96	80	77
KL x PWW	19,740	87	59	57
PWW x KL	14,407	45	28	28
PWW x PWW	15,425	40	13	12

KL = Knights Landing laboratory colony
PWW = field-collected Poso West, Kern County, California.

Table 12. Observations on ovarian development and fertilization of gravid ♀♀ from the new Culex tarsalis PWW colony.

Generation	No. ♀♀ observed	Percent of females				
		inseminated	inseminated that oviposited	laid hatching rafts	laid non-hatching rafts	retained all eggs
1	100	28	71	18	27	55
2	50	54	22	12	8	80
3	62	48	57	21	29	50
4	17	76	77	53	18	29

Table 13. Developed eggs from newly-colonized PWW Culex tarsalis ♀♀ in laboratory cages.

Generation	No. eggs developed	Percent of eggs developed		
		oviposited	embryonated	hatched
1	15,425	40	13	12
2	9,191	19	11	10
3	8,586	37	10	9
4	3,442	78	57	55

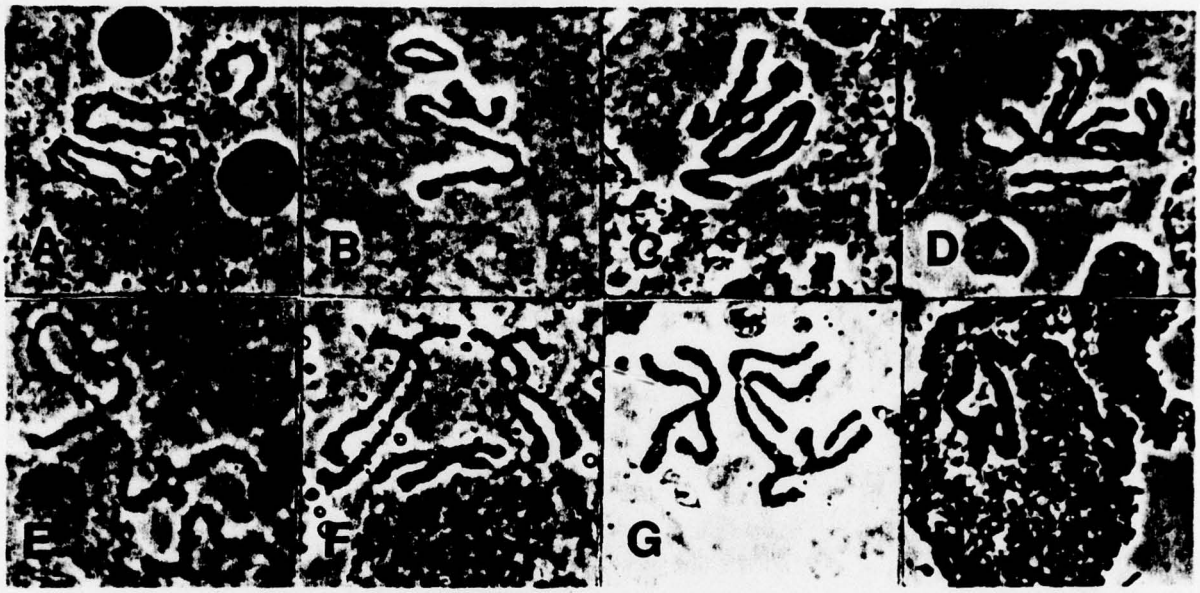


FIGURE 1—Cytology of sex-linked translocation heterozygotes in *Culex tarsalis*. *A*—*T(1;2)2A*, oogonial prophase. *B*—*T(1;2)10A*, spermatocyte. *C*—*T(1;2)11A*, spermatocyte. *D*—*T(1;2)11A*, spermatogonial metaphase. *E*—*T(1;2)12A*, spermatocyte. *F*—*T(1;2)1A*, spermatogonial metaphase. *G*—*T(1;3)13A*, spermatogonial metaphase. *H*—*T(1;2;3)6A*, spermatocyte.

Figure 2. Salivary-gland chromosomes of Culex tarsalis

* Identification of the 4 ends in two of the three chromosomes in C. tarsalis.

Figure 3. Sequential steps in the isolation of autosomal translocation homozygotes by new pseudolinkage method. In 4th generation, single pair cross may be of type A, B, or C.

GENERATION	CROSS	PROGENY (PHENOTYPE)
	♀♀ x ♂♂	
1	$\frac{+\ +}{+\ +}$ X $\frac{car\ ble}{car\ ble}$ ↯	heterozygote (wild)
2	$\frac{car\ ble}{car\ ble}$ X $\frac{car\ ble}{+\ +}$	1 heterozygote: 1 normal (carmine, black-eye) (wild)
3	$\frac{+\ +}{+\ +}$ X $\frac{car\ ble}{car\ ble}$	1 heterozygote: 1 normal (wild) (wild)
A 4	$\frac{car\ ble}{+\ +}$ X $\frac{car\ ble}{+\ +}$	1 homozygote: 4 heterozygotes: 1 normal (carmine, black-eye) (wild) (wild)
B 4	$\left\{ \frac{car\ ble}{+\ +} \text{ X } \frac{car\ ble}{+\ +} \right.$ $\left. \frac{car\ ble}{+\ +} \text{ X } \frac{car\ ble}{+\ +} \right\}$	1 heterozygote: 1 normal (1 wild: (wild) 1 carmine: 1 black-eye: 1 carmine, black-eye)
C 4	$\frac{car\ ble}{+\ +}$ X $\frac{car\ ble}{+\ +}$	normal (9 wild: 3 carmine: 3 black-eye: 1 carmine, black-eye)

Figure 4. Poso West estimated ♀♀ *Culex tarsalis* population

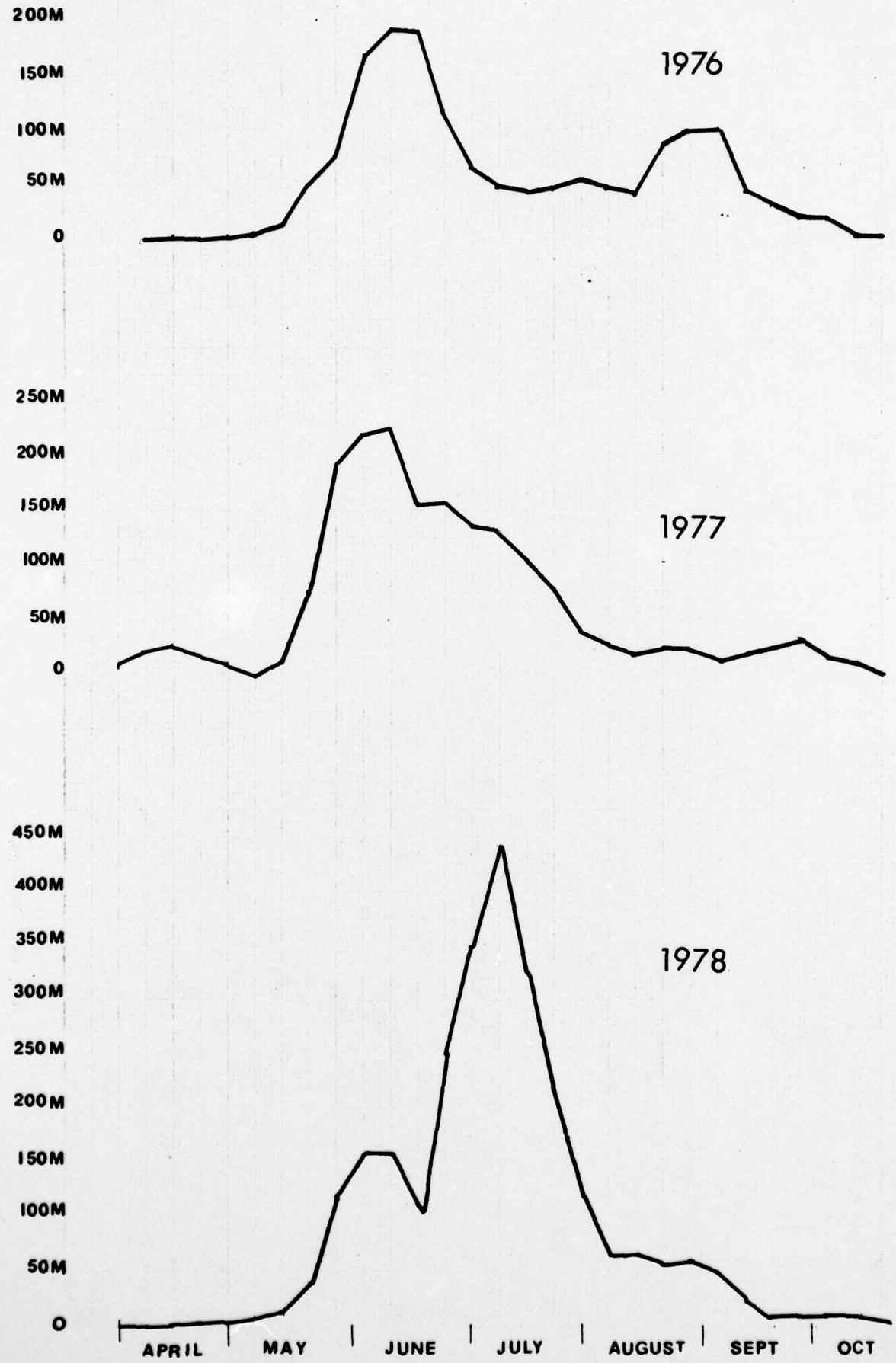


Figure 5. SEQUENTIAL APPROACH TO THE SMR CONTROL METHOD

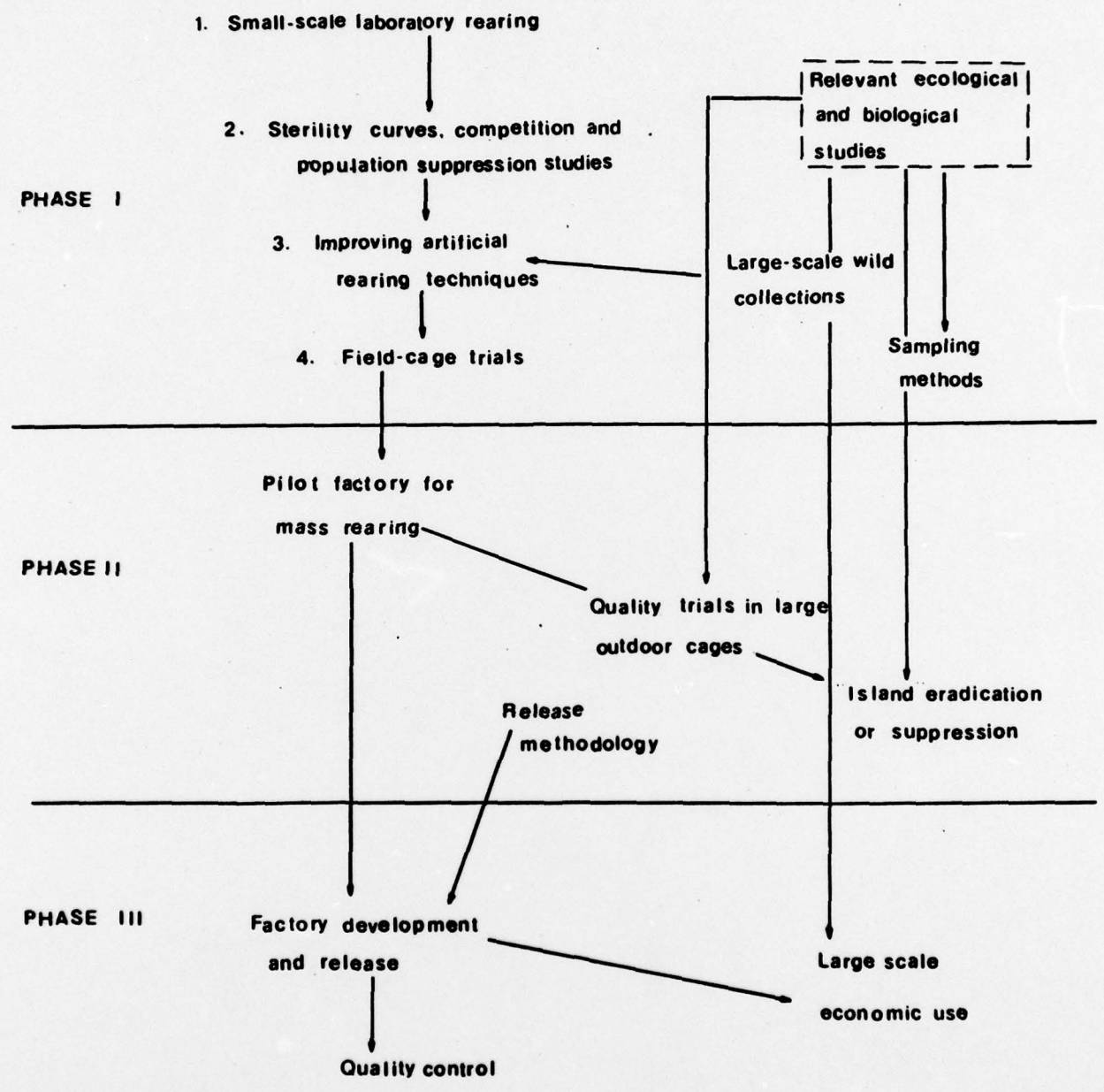
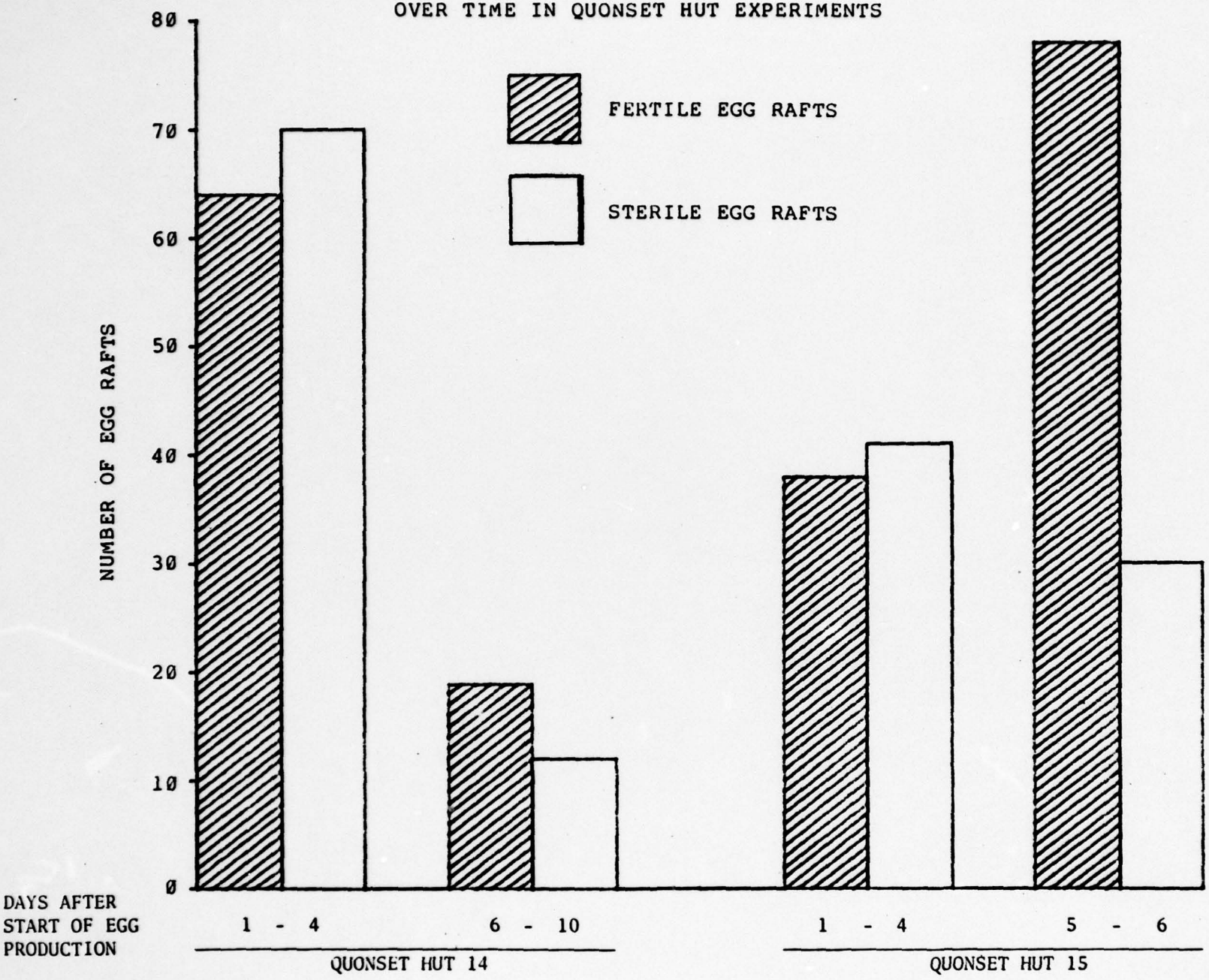


Figure 6. CULEX TARSALIS EGG RAFT PRODUCTION OVER TIME IN QUONSET HUT EXPERIMENTS



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