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GENETICS OF THE ENCEPHALITIS VECTOR, 'CULEX TARSALIS' FOR POSSI--ETC(U)
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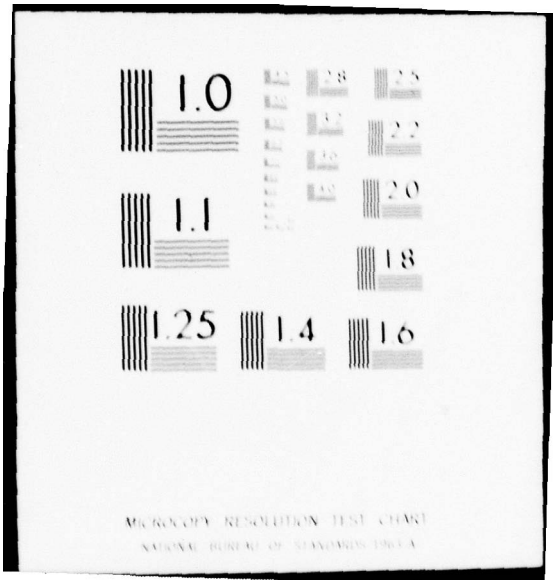
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18. SUPPLEMENTARY NOTES
Some of the enclosed data was presented at the Entomological Society of America annual conference and other relevant meetings (see updated bibliography in report). Some will also be published as indicated in updated bibliography of report.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Culex tarsalis: reciprocal translocations; vector competence; computer models; competition studies; mass rearing; mutants, population dynamics; pilot release study.

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 1977-78 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 8. Three new mutants were isolated,
C. Among the translocation strains that show promise are: 2 sex-linked,

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3 autosomal. Two sex-linked are multiples, involving all chromosomes. Two autosomes are in homozygous condition.

D. A sex-linked multiple translocation strain was studied for competitiveness in laboratory and large-outdoor cages.

E. A new method for isolating heterozygotes and homozygotes was devised.

F. A pilot release study was carried out at isolated sites in Kern County.

G. Population dynamics studies on native and release immatures were completed.

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

I. Computer models were used to determine number of males to be released this spring in 2nd pilot study.

J. A mass-production program successfully produced 84,000 males over a pre-determined time period. Another for this year's release is underway.

K. In vector competence studies, a refractory strain was successfully selected. Genetic studies determined that susceptibility to WEE virus was dominant and controlled by more than 1 gene.

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REPORT NO. USA-A75-76

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

Annual Report, 1977-78

Sister Monica Asman, Ph.D

February 1, 1978

Supported by

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University of California
Berkeley, California 94720

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Annual Progress Report - 1977-78

Introduction

(Since we have been requested to submit the annual progress report to coincide with the submission of the proposal for contract renewal, and our last Annual Progress Report was submitted in May, 1977, this Annual Progress Report covers approximately 8 months of research.)

Objectives during the 8-months time period were to pursue the ultimate goals expressed in the title of the project. More specifically, the primary efforts were to develop genetic systems which when introduced into a native population of Culex tarsalis would contribute to the self-destruction of the population and/or place characteristics in the population which would either act as autocidal time-bombs or make the population less noxious.

The relevance of genetic control research lies in the following facts: 1) increasingly constrained laws have all but banned some of the established pesticides, and the use of others is severely restricted; 2) the development of new chemical agents is becoming more and more difficult and expensive under the new regulatory regimes; 3) "natural" pest control mechanisms often are disrupted by the use of chemicals, especially when predators as well as target vectors and pests are killed; 4) the genetic or autocidal approach offers a highly species-specific means of mosquito control.

The 8 major categories of our research are described below:

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

The number of wild-type laboratory colonies maintained for specific purposes is 9; another strain, Berkeley, is a composite strain which holds some genotype of several California native lines (Table 1). Seven additional geographic strains have been held for several generations to observe genetic variability. A total of 15 single mutation lines are maintained (Table 2), and these include 4 new mutation stocks (ebony, gabled, divided eye, and melonotic). The new mutations are described below.

Multiple-marker mutation lines have been increased to 8 (Table 3) and 3 additional ones are currently being constructed.

With reference to the new mutants, ebony (eb) was shown to be sex-linked. Like char it has complete black legs and antennae; however the scales that are not black, as on the abdomen, are gray in color rather than white. The isolate was made from an irradiated line. Melonotic (mel), a spontaneous mutant, shows no evidence of being sex-linked; preliminary evidence places it on the second linkage group with blackeye (ble). Another newly-found mutant, gabled (gab), is autosomal like mel; preliminary evidence links it to carmine (car) which is linkage group III. The latter 2 autosomal mutations will provide raw material to construct a large number of additional multiple-marker lines, and these will be added to the current 7 sex-linked mutants on linkage group I and 4 autosomal mutants on the other 2 linkage groups. The most recently discovered mutation, divided eye (de), appears to be autosomal; however, we have not

determined its linkage relationship.

Work was also done to construct lines with at least 2 of the sex-linked mutations. These lines with sex as the third marker will then be used to identify the position of specific mutations and distances between them.

Mutations and marker stocks are essential to our work for various reasons: they are necessary tools for understanding the basic genetics of the species, they have application in the study of chromosome mapping, they serve to genetically identify the position of the chromosome interchanges (translocations), and can be used as possible tracer stocks for field experiments.

B. Isolation of Interchange Complexes

The most important advance made in the area of isolating potential autocidal systems was the development of a new system by which new heterozygous and homozygous translocations could be identified more rapidly. In the classical pseudolinkage method, a heterozygous translocation is induced in a wild type (unmarked phenotypically) by ionizing radiation and recovered by crossing potential translocation-carrying males with a multiple marker line. The F_1 are backcrossed to observe progeny. To obtain translocation homozygotes, the isolated heterozygotes are inbred and the resultant progeny are of 3 kinds: translocation homozygotes, heterozygotes (wild phenotypes) and normals (mutant phenotypes). From this mixture of homozygotes and heterozygotes, pairs are selected to start individual lines. Those which show no mutant progeny for 2 generations are tested to verify that they are indeed translocation homozygote lines (Figure 1).

By the use of this standard method a pure breeding homozygote line of an autosomal translocation was established in the 21st generation after irradiation. Great difficulties were experienced, most of which were ultimately related to the inability to distinguish homozygotes from heterozygotes among the progeny of inbred heterozygotes. To overcome these difficulties we devised an alternative method that involves the irradiation of a mutant-marked stock. When heterozygotes are isolated in this scheme and then crossed, the resultant progeny are: translocation homozygotes (mutant phenotype), translocation heterozygotes (wild) and normals (wild). Thus the translocation homozygotes are marked and easily recognized, and lines can be established immediately. Lines are also subsequently tested to verify that they are translocation homozygote lines (Figure 2).

Using this new method we recently isolated a pure breeding homozygote line for an autosomal translocation in the 5th generation after irradiation. This was significantly quicker than in the classical method. Additionally, the line is mutant in appearance, which may prove useful in laboratory and field experiments. Heterozygotes are more difficult to maintain with this new method, but homozygotes are easy to isolate and establish as pure lines. Our experience indicates there is a great saving in time and effort with the newer method.

C. Pilot Field Release Study

The top priority study during this summer of 1977 was to carry out a pilot-release of a double heterozygote translocation population at an isolated field site in Kern County within reasonable distance of our field station. The site, known as Poso West, is surrounded by an extensive arid area. The natural mosquito population is almost solely C. tarsalis (Figure 3). The creek water is stable as it is supplied by waste waters from oil wells. Earlier studies in 1976 provided detailed estimates of population densities throughout the summer as well as information that allowed us to project the numbers of genetically modified males that should be released in the pilot study. The background population density data also was incorporated into computer models to provide population densities (Figure 4).

1. Expansion of Experimental Stock for Release

Approximately 25 translocations have been induced and identified in our laboratory. The T(1;2;3)1A translocation had shown the most potential as an early candidate for controlling C. tarsalis in field situations. It is a multiple heterozygous translocation involving 2 separate interchanges of chromosome material, one between linkage groups 1 and 2, and the other between groups 1 and 3. The three chromosomes involved are transmitted as a set from males to male progeny because the interchanges are closely linked to the male-producing locus (Figure 5). The line is easily maintained by using genetic markers, and appears to be strong and viable.

The mass-rearing production program had been initiated in January of 1977. In order to assure a projected release of 10,000 translocated males every 3-4 days over a period of 1 month, a detailed breeding plan had to be followed. The actual number to be released was ascertained by computer simulations (Figure 6), based on the release of males that carried the specific sex-linked double heterozygous translocation, T(1;2;3)1A described above. Since the experimental stock had a low fertility (20-30%), and only males were to be used to expand the stock, it was difficult to obtain the projected number desired for release. Our main difficulty in the mass-producing study, other than the low fertility of egg rafts, was that a manual sexing process had to be carried out to differentiate the males that carried the translocations from possible recombinants among the males and from the non-carrying females. Culling with the use of genetic markers was necessary each generation except in the last, to be sure the translocation alone was carried into the field. We now know that very little recombination took place during the production of stocks for field release.

2. Release method

The last generation of males that were culled prior to release were outcrossed to the Knight's Landing colony, a vigorous laboratory stock for which we know its status of vector com-

petence, autogeny, susceptibility to insecticides, and mating competitiveness. This colony also had to be expanded to supply the females necessary for outcrossing. The progeny from the outcross were reared to pupa stage and sexed as males. The pupae were then transferred to holding ponds at Poso West so that adults would emerge at the release site. On emergence, the male adults escaped through an opening in the cover of the pond. In addition, on 2 occasions large numbers of males that had emerged in the laboratory were marked with fluorescent dusts and were released as adults. For 10 days after releases recovery recaptures were made to get survival data. We estimate that over a month's period we released 64,730 pupal-source males that emerged in the field, 11,583 marked adult males. Thus a total of 76,313 translocated males were released over a month's time. Unfortunately this number was approximately 24,000 less than we had planned to release. In addition, due to imperfections in the sexing mechanism it was estimated that 11,880 females were also released inadvertently. We now believe this can be prevented.

3. Capture-mark-recapture Studies at Poso West

In 1977, personnel with whom we collaborate repeated the monitoring of C. tarsalis populations at the release site. Over 44,000 captured mosquitoes were marked with fluorescent dusts and over 159,000 were collected in recovery efforts using CO_2/light traps. Population estimates based on these collections surpassed those of 1976 in the earlier months, May to July, but were lower in August and September. Data indicated that the survival estimates were similar for 1976 and 1977. Since the projected numbers of released males for the 1977 pilot release study were based on population estimates established by studies in 1975 and 1976, and the 1977 population was considerably higher than in the 2 previous years, the number of released males was proportionally too low to compete effectively with the native males. Even had we succeeded in releasing the projected number of 100,000 -- and no females -- the numbers released would have been inadequate. Analyses of the population data indicated that at no time did we have more than 13,000 translocated males in the field, while the native population reached as high as 41,000 (Figure 7). Thus our first genetic introduction did not establish itself sufficiently to produce the desired impact. The pilot field-release studies also revealed where more research was needed, and where improved techniques relevant to release strategies and field monitoring are required. A second pilot release is planned for the spring of 1978 using the same translocated stock but with modified techniques based on the experience of 1977. This study should add more conclusive data and further indicate where pertinent strategies need to be modified before the first release of new translocations. Meanwhile, while not available for the mass-production scheme now already in operation for this spring's release, a major effort is being directed at isolating a lethal genetic factor linked to females that will eliminate the tedious procedure for sexing in future

mass rearing if only males are to be released.

D. Controlled Large Outdoor Cage Studies

In 1977 we continued to run large outdoor cage experiments with C. tarsalis in 2 modified quonset huts. These structures are 11 by 6 meters each, and they are divided in half. Each is covered with a fine-mesh screen except for a solid section in the center of the roof which provides shade. Each section contains 2 artificial ponds for oviposition, cages that hold 3 chickens each to provide blood meals, and a walk-in "red box" resting site. A small cage in each sub-section allows for comparison studies relative to cage size (Figure 8). The major purposes of our experiment were: 1) to determine the effects of cage location and size on the performance of the females, primarily in terms of oviposition; 2) to test mutant markers for potential release into field situations, with emphasis on recovery of the mutants in the subsequent generations; 3) to test the competitiveness of the translocation T(1;2;3)1A in combination with various laboratory strains, or with a second translocation, T(2;3)5A, which is homozygous; and 4) to test for mating oviposition capacity of the semi-lethal fringe mutant strain.

In our 1976 quonset hut tests the data indicated that cage location might influence oviposition. Therefore, we investigated this effect with KL strain females and 2 strains of males -- KL and car/ble. In addition, the 2 kinds of males were used in half of the cages to see if a choice of males would be a factor. Each cage held approximately 725 females with twice that number of males in the diagonally opposite Cages 1 and 4. There were equal numbers of KL and car/ble males in the other 2 diagonally opposite Cages 2 and 3. Resultant data indicated a clear difference in oviposition rates per female among the 4 cages. Cages 2 and 4 to the north produced fewer rafts than cages 1 and 3 to the south (Table 4). The effect of differences in mating males where the females had a choice of 2 males did not appear to affect oviposition rates.

A second experiment involved a comparison of the field-collected RM strain and the KL laboratory strain to determine the effect of cage size on oviposition, insemination, and retention of eggs. Approximately 200 females and 200 males were introduced in each of the 4 large cages and in each of the smaller cages standing within these large cages. The data that ensued from the large cages revealed oviposition rates that were comparable in all cages and fairly representative of earlier data from these cages. However, data from small cages was significantly different. For the KL laboratory strain, oviposition was enhanced (13.6 X) in the smaller cage; and field-collected females had reduced oviposition (.44 X) compared to the larger cage (Table 5). At the termination of the experiment females were dissected to determine the insemination rates. The crosses involving RM (field) females had lower insemination rates than the laboratory-strain females. While this seemed logical in that the KL strain had been highly selected for small cage laboratory rearing, it indicated that this factor must be considered in using field material

and/or laboratory material for these types of experiments. The possibility of releasing genetic variants in the future also was investigated in large cage studies, primarily with reference to mating and oviposition. Males that were homozygous for car and ble were competed against field-collected males (McVans) for mating with field-collected McVans females. Likewise, males that were heterozygous for car and ble after an outcross with WPC field material were tested in the same manner. The recovery of mutants from the progeny of the competition cages was done by crossing the progeny with a laboratory car/ble stock. Data based on equal-mating competitiveness showed the mutants were highly successful in competing against normal males, as 30.9 percent car and 30.2 percent ble progeny were recovered as compared to expected values of 25 percent for each (Table 6). Likewise the release of the heterozygotes showed promise as there was a testcross recovery of 24.3 percent car and 17.7 percent ble, as compared with an expected value of 12.5 percent for each.

In another experiment the 2 south-facing cages (1 and 3) were used to determine if the wing mutant fringe (fr) would be a hindrance in the ability of males and females to mate in large exposed areas such as the field. The mutation, fringe (fr), affects the scales on the wing costa and those on the wing vein. In mutants the wing scales lie in the opposite direction to what would be normal. They appear to be brushed away from the wing apex and towards the thorax, and lie in disarray rather than in an orderly fashion. The mutant scales, especially those on the costal margin, prevent smooth emergence from pupal cases, and approximately 30 percent of adults drown during emergence.

KL adults were released in one cage and fr adults were placed in another, with a CO_2/light trap in each for night-time collecting. Over a 10 day period after the release of adults into the cage, the percent trapping for fr actually exceeded that of the KL strain. This indicated that at least in these large cages flight or the ability to mate was not impaired (Table 7), as even the insemination data was better for the fr than for KL.

One major purpose of our field-cage testing relates to the genetic control trials that will be conducted at Poso West. The quonset structures are considered as "half-way" duplications of field situations. During the time that we conducted our first pilot release in the field with the T(1;2;3)1A males we tested in the quonset cages 3 hybrids of these males, which had been crossed to KL, Chico laboratory strain, and car/ble. One cage held KL only as a control, and the other 3 held the hybrid males competing against KL males for KL females. Within the 3 competition cages the KL/translocation hybrid males demonstrated the most heterosis; in addition, all hybrids of the translocation males proved to be successfully competitive with the KL strain (Figure 9). The shift of egg-hatch values to the mid-fertility range rather than to the low range pointed to a new area that requires further investigation. We now believe that in the circumstances when egg hatch is only moderately low the translocation matings lead to

larval death particularly in the first instar.

The competition trials with males that carried both T(1;2;3)1A and the homozygous T(2;3)5A were disappointing. The data indicated that these males were not effective for genetic-control purposes (Figure 10). In the last quonset study a "genetic time bomb" was released using the male hybrids from a cross between fr and the T(1;2;3)1A translocation. Since the fr mutant contributes to mortality of emerging adults, the experiment was to measure the effect that mutant had in subsequent generations. One cage held fr females and males only; the other the hybrid males and fr females. The results were as follows: the translocation carried the marked reduction in fertility (egg hatch) that was expected; in addition 33 percent of the females were fr, of which 41 percent drowned as they were trying to emerge. Thus the additive effect of female lethality was demonstrated in the outdoor cage situation, more than the expected extent (Figure 11). Such a mutant could, however, be one of several that if introduced would reduce female viability in subsequent generations.

E. Polytene Chromosome Preparations

Progress has been made in developing techniques for preparing polytene chromosomes of C. tarsalis. Salivary-gland polytenes were spread to the extent that pictures of sections and whole chromosomes could be photographed. Centromeres were recognized and individual patterns of banding will be established for specific chromosomes as these are studied in the next months. Once normal chromosomes are recognized, we will be able to ascertain cytologically where the chromosomal breaks occur in the induced translocations, and if other anomalies are contributing to zygotic inviability. Good preparations also will allow us to establish linkage-group chromosomal correlations.

F. Vector-Competence Studies and the Genetics Involved

Data from studies to select a strain of C. tarsalis highly resistant to oral infection with Western Equine Encephalitis virus can be seen in Figure 12. These studies entailed 17 generations and demonstrated susceptibility profiles obtained with the highly susceptible and refractory strains when fed on viremic chicks. A completely refractory strain of C. tarsalis could not be selected since 15-20 percent of the females that fed on high concentrations of virus were found to be infected after an intrinsic incubation of 10-12 days. Also it was found that the resistant and susceptible strains were equally susceptible when the midgut was bypassed by intrathoracic inoculation. This suggested that some females might become infected by means other than those under genetic control. Genetic backcrossing studies between susceptible and refractory lines supported this contention. The "leaky gut" concept derived from studies of other viruses in insects was considered to be involved, since it is conceivable that ingested virus might enter the hemocoel through a leaky midgut without first having multiplied in midgut epithelial cells. If this occurred, then infection in a small proportion of the population would be

similar to intra-thoracic inoculation. Further studies will hopefully clarify these findings. In genetic studies the exact mode of inheritance was not established for susceptibility or refractoriness; however, susceptibility was found to be dominant over resistance and to be controlled by more than one gene. Definitive genetic studies cannot be done until we establish the significance of females that contain intermediate concentrations of virus after feeding on high viral challenges.

G. Sterile-Male Technique Study

Last spring (April 1977) we initiated a new project to study the feasibility of using the sterile-male control method as an additional tool to control C. tarsalis. It was felt that the so-called early "failures" with applying this method to mosquitoes should not necessarily be ascribed to a defective principle but perhaps to the lack of sufficient information, relative to a particular species. It was evident from earlier studies that radiation exposures sufficient to produce sterility tended to reduce mating frequency, competitiveness, and life-span in irradiated males. In recent years, however, it has been shown that there is a more favorable balance between these 2 effects when males are irradiated under an atmosphere of nitrogen rather than air. Survival time and mating activity can also be greatly extended by use of nitrogen, so it is important to recognize that protection afforded by anoxia was not a factor in the earlier studies. We have now established preliminary sterility curves for C. tarsalis under both air and nitrogen, using doses of 5, 7, 9, and 11 krads. Preliminary data suggest that the sterility curve for irradiation in nitrogen is higher than in air. Thus if we wish to attain equivalent sterility in the 2 atmospheres it will be necessary to irradiate at higher doses in nitrogen than in air. We will continue to test other irradiation doses to expand the sterility curves from 3 to 13 krad to determine the dose in nitrogen that is necessary to produce approximately a 5 percent hatch. Our ultimate goal is to determine the optimal dose/atmosphere and to evaluate the mating competitiveness of these irradiated males. The best combination could then be recommended for a sterile-male program.

H. A Pilot Study on Mating Preferences in C. tarsalis

The purposes of this project were: to determine if laboratory females discriminate between males of different phenotypes, to determine if there was a relationship between mutant eye phenotypes and male competitiveness, and to develop effective experimental designs suitable for quonset-hut cage studies in the field area. The general technique called for tagging one of the competing male types with carmine (car), and using car/+ females as mating recipients so the parentage of every family could be scored among first-instar larvae. This procedure would greatly simplify the analysis of data since the probabilities of one or the other mating would be determined directly. In addition a complete analysis could be done in a single cage, thus eliminating

the bias between cages that has plagued almost all of our quonset competition studies in Bakersfield.

The first study involved the Chico and car stocks. In 4 replicate tests in small laboratory cages (60 X 60 X 45) the males heterozygous for car competed more successfully for the Chico females than did the Chico males. In tests where car was in the homozygous condition in the competing males, Chico strain males were more successful in competing for the car females since only 43 percent of the resultant egg rafts carried the carmine eye color. In another study in small gallon cages Chico males competed with heterozygous Chico/car males for Chico/car females. Here only 22 percent of the egg rafts were fathered by heterozygous males, suggesting that females might be discriminating male types in this size cage. Additional studies in small and large cages will clarify this issue. In another test to ascertain what kind of selection disfavored the car eye mutant, females and males heterozygous for car were mated. Of 1278 pupae observed for eye color, 301 or 23.5 percent were carmine. Based on an expected fitness value of .95, the data indicated that there was only a 5 percent selection against it.

I. Updated Biographical Sketch and Bibliography

Principal Investigator and Genetic Staff

Presented papers (1977)

"Laboratory and Field Cage Competition Studies of Translocation-Carrying Males in Culex tarsalis." California Mosquito Control Ass'n., Palm Springs, CA, January 1977.

"Isolation of Translocation Homozygotes for Genetic Control of C. tarsalis," CMCA, Palm Springs, CA, January 1977.

"Pilot Field Study for Control of Culex tarsalis by Sex-linked Translocations," Entomological Society of America Meeting, Washington, D.C., November 1977.

"Translocation Homozygotes for Genetic Replacement in Culex tarsalis," ESA Meeting, Washington, D.C., November 1977.

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Publications Submitted

McDonald, P.T., S.M. Asman, and H.A. Terwedow. 1977. Sex-linked Translocations in Culex tarsalis: Abnormal Segregation and A Sex-linkage Switch Mechanism. (Submitted to J. of Heredity)

McDonald, P.T., S.M. Asman, and H.A. Terwedow. 1977. Alternative Methods for Isolating Homozygotes of Autosomal Translocations in the Mosquito Culex tarsalis. (In preparation)

Hardy, J.L., G. Apperson, S.M. Asman, and W.C. Reeves. 1977. Selection of a strain of Culex tarsalis highly resistant to oral infection with Western Equine Encephalomyelitis virus. Amer. J. of Trop. Med. and Hyg. (In press)

In Preparation

Asman, S., P.T. McDonald, R.L. Nelson, W.C. Reeves, M.M. Milby. First pilot field release study using genetically altered Culex tarsalis. (To be submitted to Mosquito News)

Asman, S. Genetically-determined Variations in Culex tarsalis. (To be submitted to the Proceedings of the CMCA)

McDonald, P.T., S.M. Asman, and R.W. Ainsley. Outdoor cage tests of genetic strains of Culex tarsalis. (To be submitted to Proceedings of CMCA)

Ainsley, R.W., S.M. Asman, and P.T. McDonald. Laboratory Mating Competitiveness of Culex tarsalis colonies. (To be submitted to Proceedings of CMCA)

Personnel receiving contract support

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

Dr. McDonald has been on the program 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.

Table 1. Laboratory maintained colonies of *Culex tarsalis*

California strains	RM (Kern County)
	Poso Creek (Kern County)
	Bakersfield-BFS (Kern County)
	Knights Landing (Yolo County)
	Sacramento Valley (Butte County)
	Berkeley (Hybrid of several strains)
Other	Yuma (Arizona)
	BFS-Winnipeg (Canada)
	Fort Collins (Colorado)
	Manitoba (Canada)

Table 2. Monofactorial mutations that have been established as laboratory colonies.

Mutant (symbol)	Mutagenic agent and colony source	Description	Linkage Group
Black eye (ble)	Spontaneous Hart Park Strain	Black pigment--actually dark green under high magnification but black to naked eye--good penetrance and in both sexes	II recessive +
Mulberry (mul)	Ethyl methane sulfonate (EMS) Berkeley Strain	Facets of compd. eye irregular in shape--giving convex impression	sex-linked (I) recessive +
Microcephalic (mic)	EMS Berkeley Strain	Many individual facets of compd. eye completely absent--	sex-linked (I) recessive +
Carmine (car)	Spontaneous Yuma Strain	Dark red pigmented eye, seen in larvae, pupae and adults	III recessive +
Setaceous palps (sp)	Spontaneous Dewarts Strain	♀♀ have 1 or 2 setae on each apical segment of palps, parallel to prob.	linkage (?) recessive +
Bleached ocelli (bloc)	Spontaneous Presidio Strain	Ocelli of larvae and pupae light pink	sex-linked (I) recessive
Fringe wing (fr)	Co-60 irradiation Berkeley Strain	Wing scales heavy and ruffled giving fringe appearance	sex-linked (I) +
Charcoal (char)	Co-60 irradiation Berkeley Strain	White scales on proboscis, legs and antennal pedicel missing--also reduced white on abdomen	sex-linked (I) recessive
Wide wing (ww)	Co-60 irradiation Berkeley Strain	Wing wider than usual due to lobe on posterior margin	sex-linked (I) +
Clubbed palp (cp)	EMS treated Berkeley Strain	1 or both palps clubbed at distal segment	Not determined
Orange (orb)	Spontaneous Sacramento Strain	Fat bodies light orange	Not determined
Ebony (eb)	Co-60 irradiation Berkeley Strain	Black scales only on proboscis, legs, and antennal pedicel; black and grey scales on abdomen	sex-linked (II) recessive +
gabled (gab)	Spontaneous Porters Strain	Wings in gabled position when at rest; antennae eagle-spread	III recessive +
melonotic (mel)	Spontaneous Hybrid of K.L. X Ft. Collins	Fat bodies of larvae, pupae, adults dark grey to black	II recessive +
divided eye (di)	EMS treated car/ble strain	Eye of pupae and adult divided by tissue without ommatidia	II or III recessive

Table 3. Multiple-marker lines now available for genetic studies.

	Chromosomes		
	I	II	III
1.	sex (gene determined)	black eye	carmine eye
2.	fringe (fr)	black eye	carmine eye
3.	bleached ocelli (bloc)	black eye	carmine eye
4.	mulberry (mul)	black eye	carmine eye
5.	microcephalon (mic)	black eye	carmine eye
6.	wide wing (ww)	black eye	carmine eye
7.	charcoal (char)	black eye	carmine eye
8.	ebony (eb)	black eye	carmine eye

Table 4. Effects of cage location on oviposition in Culex tarsalis

Cage	Location	♀♀	♂♂	Oviposition Total rafts	Egg rafts/♀
1	SE	725 KL	1676 KL	260	0.355
2	NE	746 KL	833 KL	73	0.098
			955 car ble		
3	SW	723 KL	751 KL	206	0.285
4	NW	705 KL	1747 KL	143	0.203
			947 car ble		

KL: Knights Landing, laboratory strain

car ble: carmine and black eye, laboratory strain

Table 5. Effects of cage size on lab and field strains of Culex tarsalis

Cage	♀♀	♂♂	Oviposition: egg rafts ♀[1] Large cage	Small cage	Captured ♀♀[2] Inseminated	Gravid
1	197 RM	197 KL	0.101			
1.1	198	185 KL		0.025	12/25	24/25
2	199 KL	192 KL	0.075			
2.1	194 KL	195 KL		1.021	20/25	6/25
3	197 KL	190 RM	0.076			
3.1	197 KL	185 RM		1.046	22/25	9/25
4	197 RM	191 RM	0.117			
4.1	198 RM	190 RM		0.051	17/25	23/25

RM: Rawlee-Merced, field collected

KL: Knights Landing, laboratory strain

1 Large cage: 6m x 5.5m x 3m

Small cage: 60 cm x 60 cm x 60 cm

2 At termination of experiment

Table 6. Outdoor cage release of eye color mutants in Culex tarsalis

Cage	♀♀	♂♂	First generation No. egg rafts hatching	Second generation[1] No. sampled	% car OBS	% car EXP	% ble OBS	% ble EXP
1	739 McV	739 McV	50	5911	24.3	12.5	17.7	12.5
		739 car ble/WPC						
3	566 McV	566 McV	29	6329	30.9	25	30.2	25
		566 car ble						

McV: McVan, field collected

car ble: carmine and black eye, laboratory strain

car ble/WPC: Progeny of car ble X field collected West Poso Creek

1 From cross of car ble ♀♀ X first generation ♂♂

Table 7. Outdoor cage release of fringe wing mutant in Culex tarsalis

Cage	♀♀	♂♂	Trapped in 10 days No. ♀♀	Trapped in 10 days No. ♂♂	Trapped ♀♀ inseminated
2	985 KL	979 KL	87	29	55/61
4	269 fr	234 fr	90	105	60/62

KL: Knights Landing, laboratory strain

fr: fringe (wing), laboratory strain

Figure 1. Sequential steps in the isolation of autosomal translocation homozygotes by standard pseudolinkage method. In 4th generation, single pair cross may be of type A, B, or C.

GENERATION	CROSS	PROGENY (PHENOTYPE)
	♀♀ x ♂♂	
1	$\frac{car\ ble}{car\ ble} \times \frac{+\ +}{+\ +}$ ↯	heterozygote (wild)
2	$\frac{car\ ble}{car\ ble} \times \frac{+\ +}{car\ ble}$	1 heterozygote: 1 normal (wild) (carmine, black-eye)
3	$\frac{+\ +}{car\ ble} \times \frac{+\ +}{car\ ble}$	1 homozygote: 4 heterozygotes: 1 normal (wild) (wild) (carmine, black-eye)
A 4	$\frac{+\ +}{+\ +} \times \frac{+\ +}{+\ +}$	homozygotes (wild)
A 5	$\frac{+\ +}{+\ +} \times \frac{+\ +}{+\ +}$	homozygotes (wild)
B 4	$\frac{+\ +}{+\ +} \times \frac{+\ +}{car\ ble}$ $\frac{+\ +}{car\ ble} \times \frac{+\ +}{+\ +}$	1 homozygote: 1 heterozygote (wild) (wild)
B 5	$\frac{+\ +}{+\ +} \times \frac{+\ +}{+\ +}$	As A4
	$\frac{+\ +}{+\ +} \times \frac{+\ +}{car\ ble}$ $\frac{+\ +}{car\ ble} \times \frac{+\ +}{+\ +}$	As B4
	$\frac{+\ +}{car\ ble} \times \frac{+\ +}{car\ ble}$	As 3
C 4	$\frac{+\ +}{car\ ble} \times \frac{+\ +}{car\ ble}$	As 3

Figure 2. 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	$\frac{+}{+}\ \frac{+}{+}$ x $\frac{car}{car}\ \frac{ble}{ble}$	heterozygote (wild)
2	$\frac{car}{car}\ \frac{ble}{ble}$ x $\frac{car}{+}\ \frac{ble}{+}$	1 heterozygote: (carmine, black-eye) 1 normal (wild)
3	$\frac{+}{+}\ \frac{+}{+}$ x $\frac{car}{car}\ \frac{ble}{ble}$	1 heterozygote: (wild) 1 normal (wild)
A 4	$\frac{car}{+}\ \frac{ble}{+}$ x $\frac{car}{+}\ \frac{ble}{+}$	1 homozygote: (carmine, black-eye) 4 heterozygotes: (wild) 1 normal (wild)
B 4	$\left\{ \frac{car}{+}\ \frac{ble}{+} \text{ x } \frac{car}{+}\ \frac{ble}{+} \right.$ $\left. \frac{car}{+}\ \frac{ble}{+} \text{ x } \frac{car}{+}\ \frac{ble}{+} \right\}$	1 heterozygote: (1 wild: 1 carmine: 1 black-eye: 1 carmine, black-eye) 1 normal (wild)
C 4	$\frac{car}{+}\ \frac{ble}{+}$ x $\frac{car}{+}\ \frac{ble}{+}$	normal (9 wild: 3 carmine: 3 black-eye: 1 carmine, black-eye)

Figure 3. West Poso Creek release site

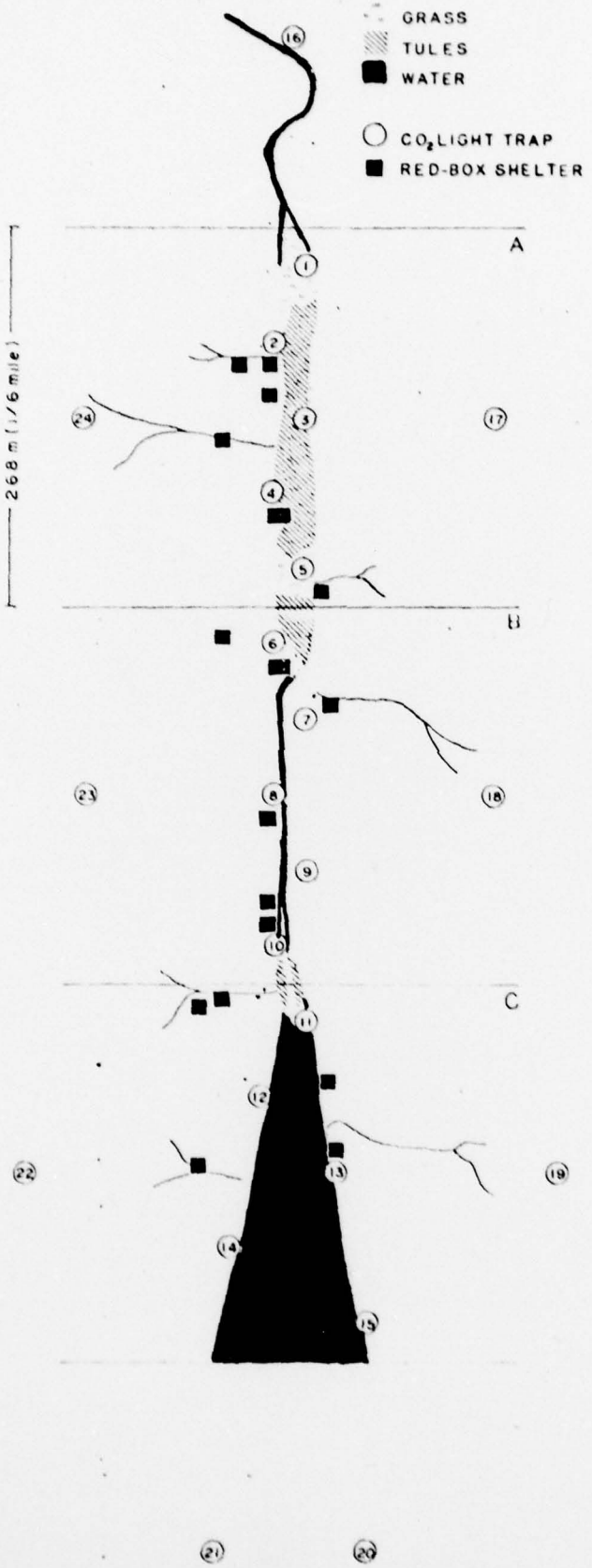


Figure 4. CDC - CO_2 light trap indices and population estimates, female *C. tarsalis*, Poso West, 1976.

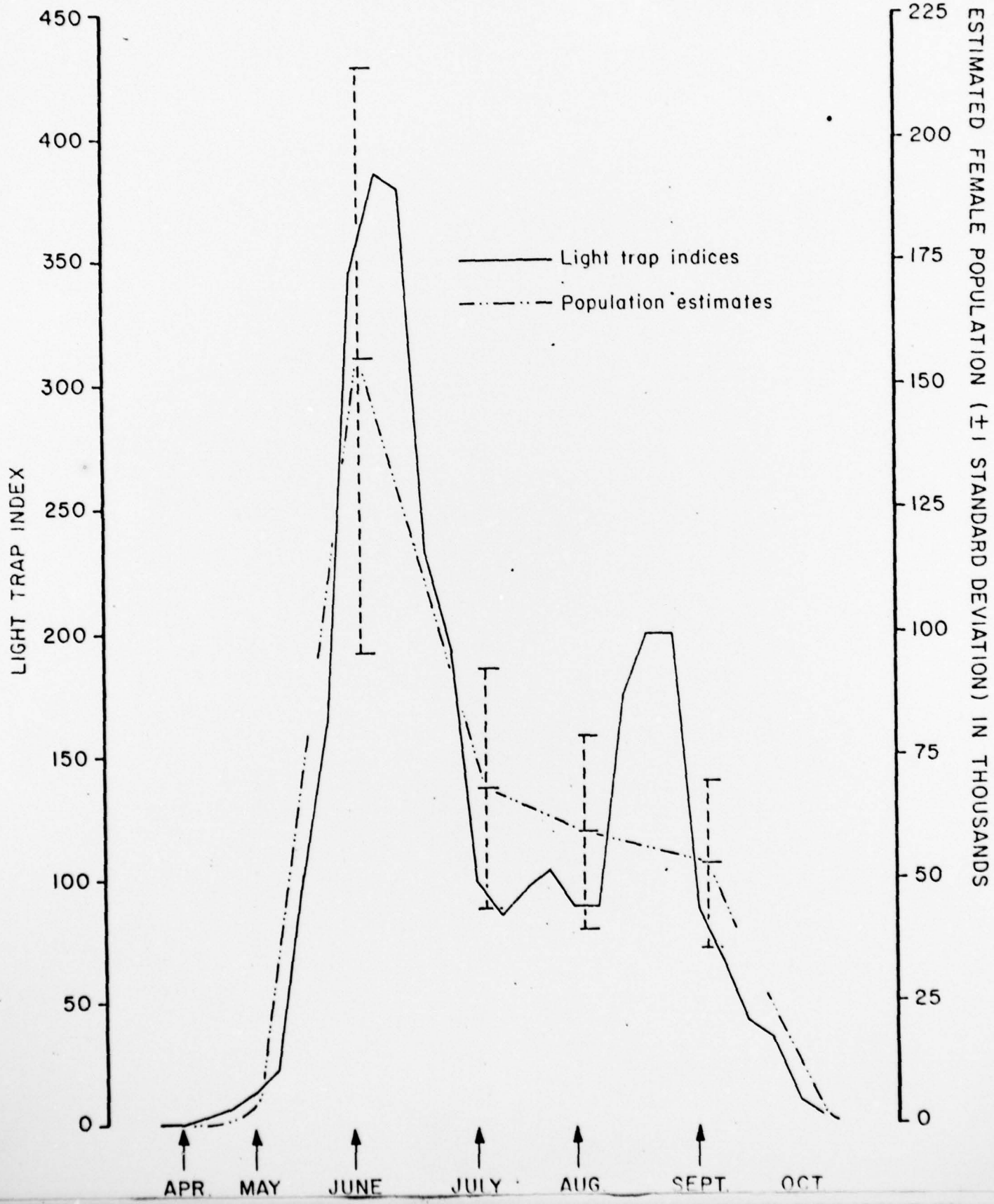


Figure 5. SEX-LINKED MULTIPLE TRANSLOCATION, T(1-2-3)a

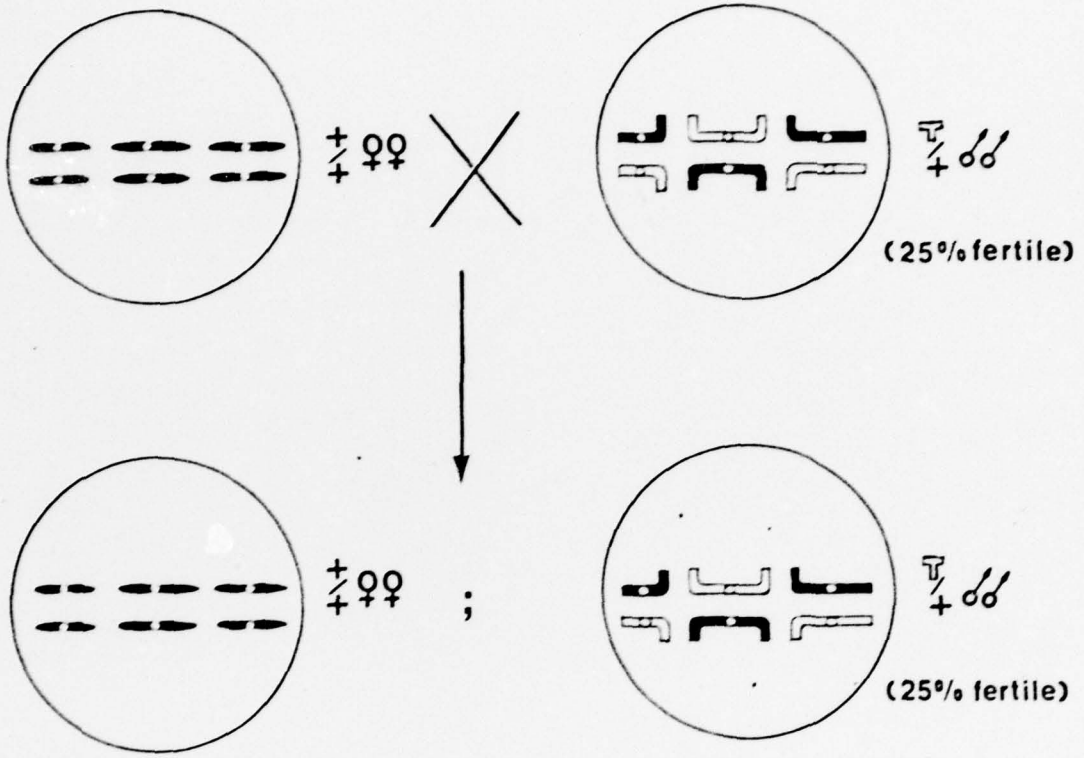
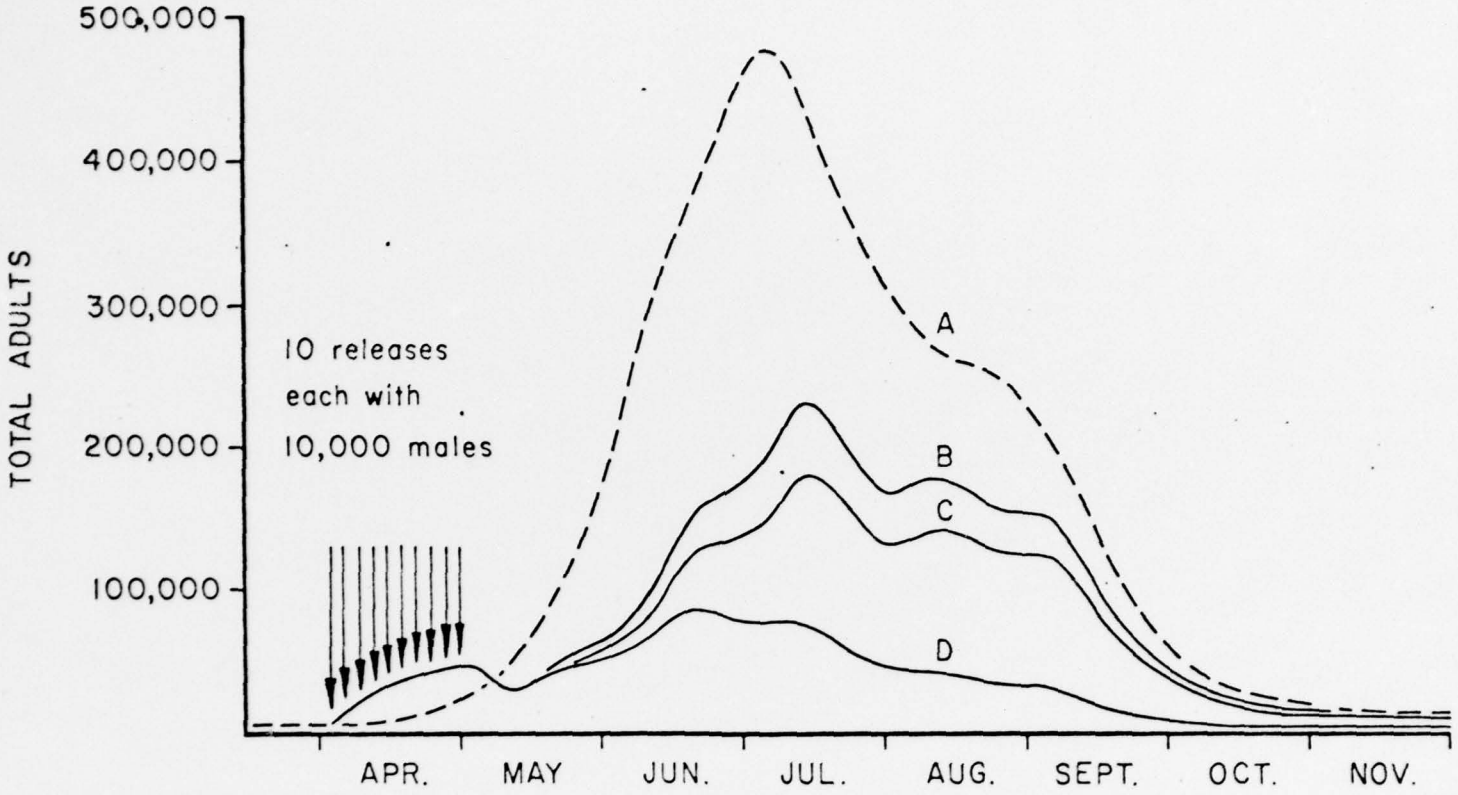


Figure 6. Computer predictions when a heterozygote double translocation is inserted into a simulated *Culex tarsalis* population at Poso West.



A. Normal population.
B. Predicted : mating competitiveness ratio 0.75, adjusted for density dependence.
C. Predicted : mating competitiveness ratio 1.0, adjusted for density dependence.
D. Predicted : mating competitiveness ratio 1.0, no density dependence adjustment.

Figure 7. Estimated adults in 1977 pilot field release study.

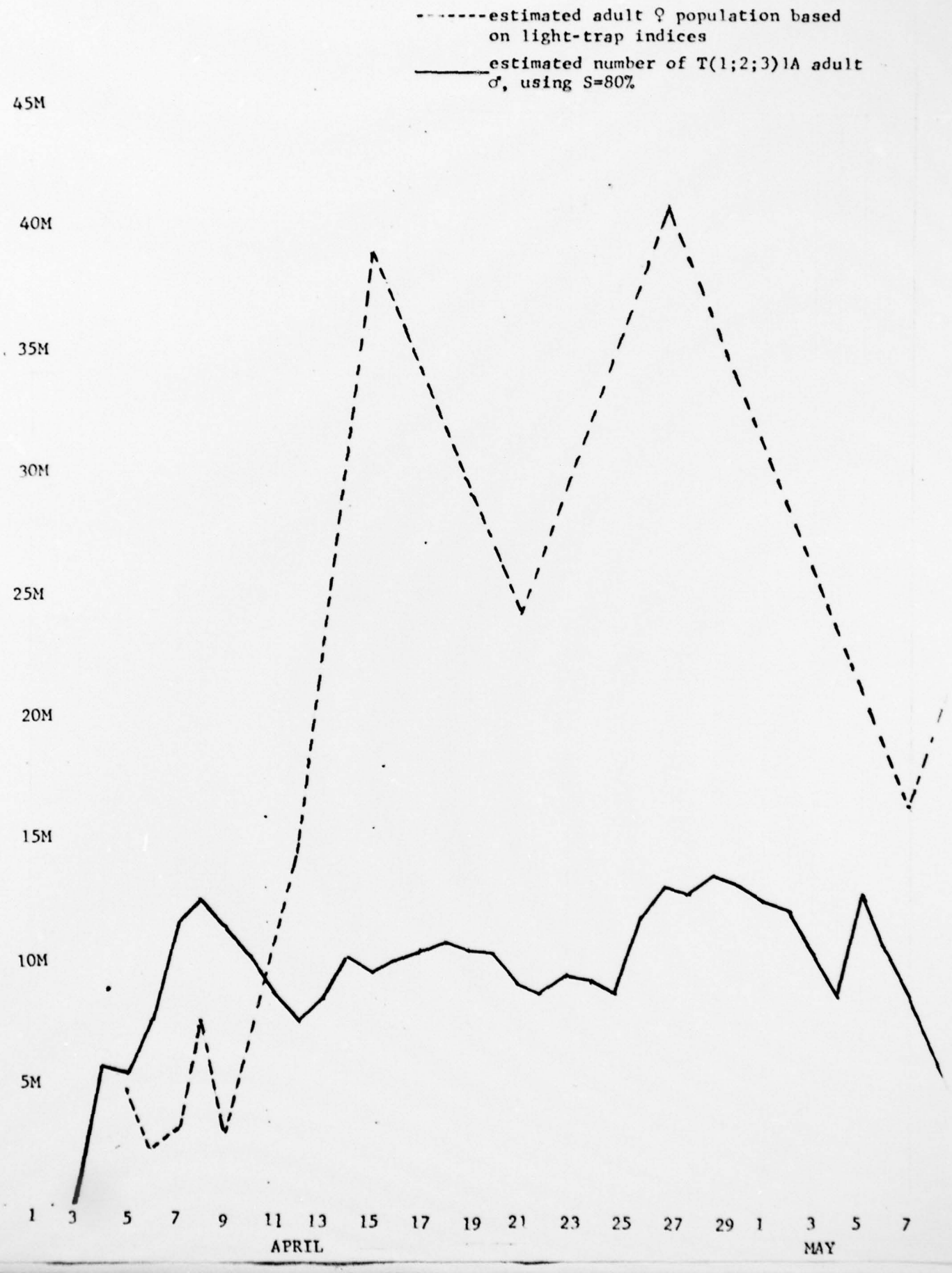


Figure 8. Modified quonset-hut outdoor cages.

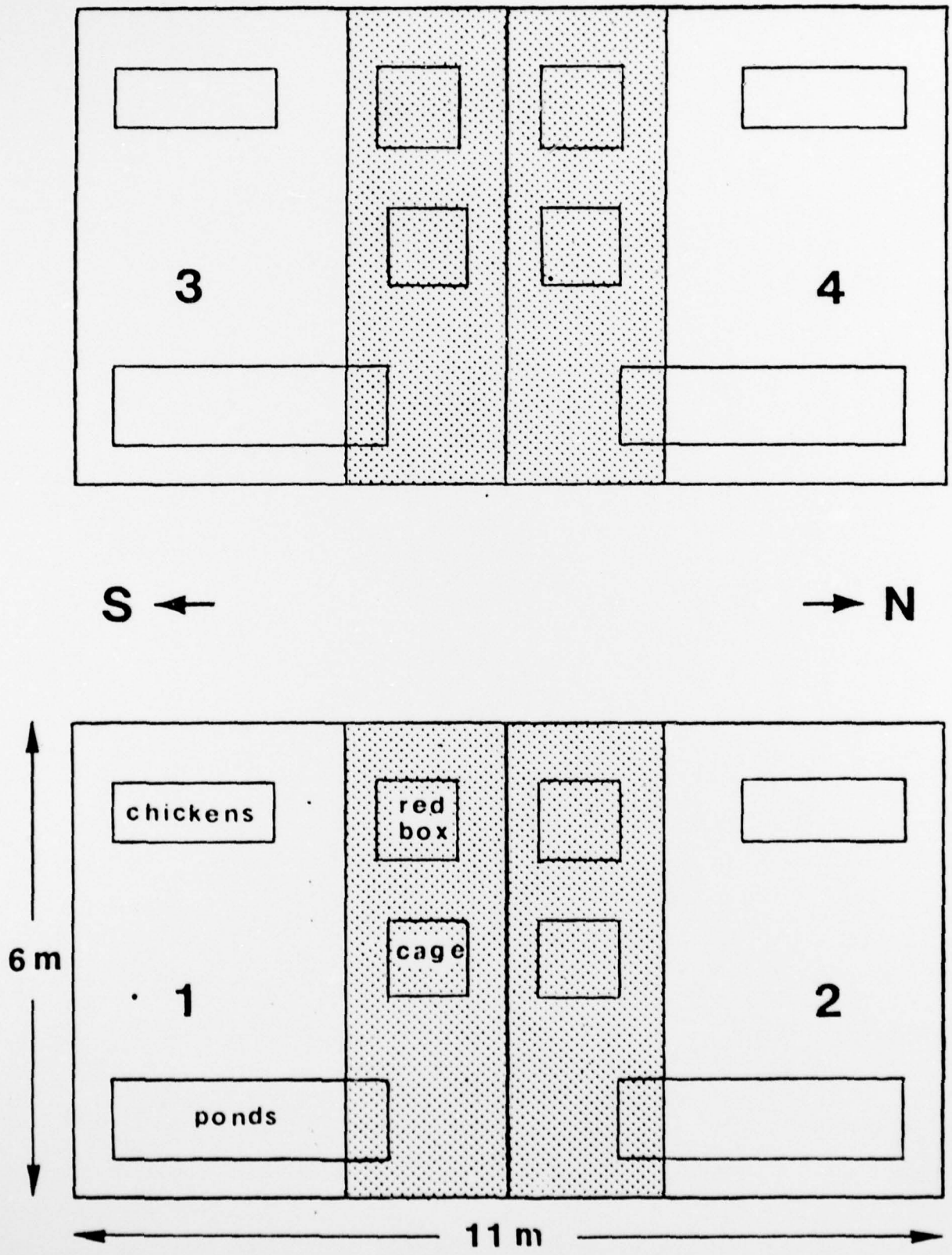


Figure 9. Competition data of hybrid KL males competing against KL males for KL females.

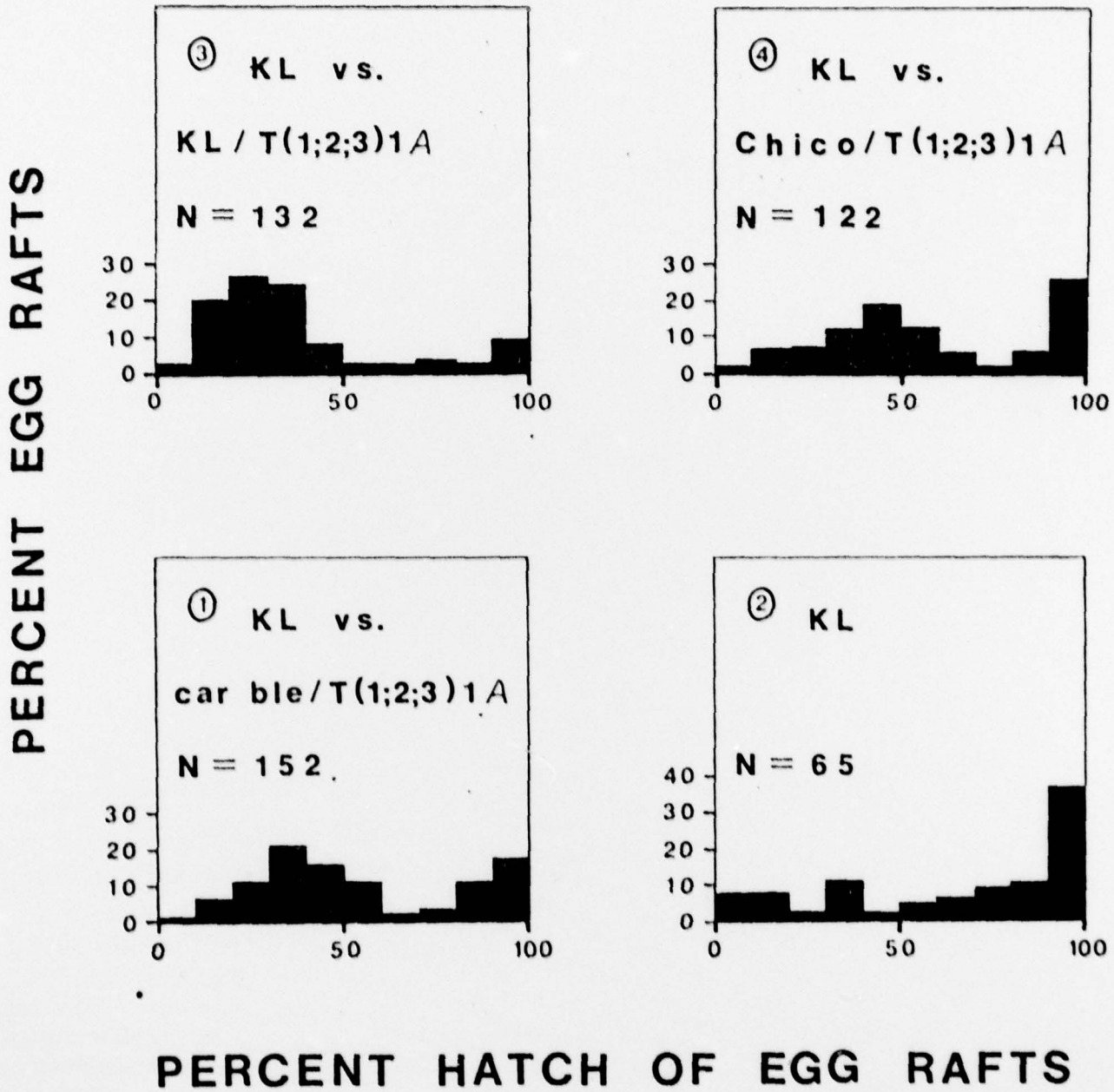


Figure 10. Competition data of males carrying 2 different translocations against WPC males for WPC females.

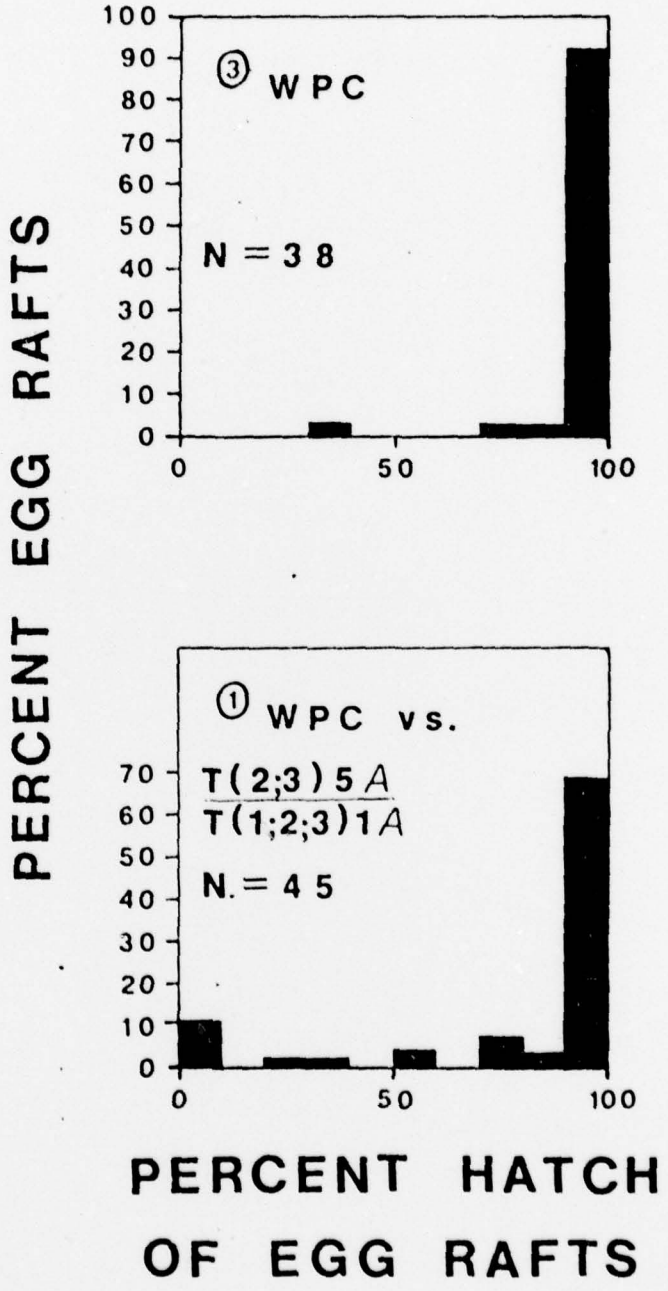


Figure 11. Percent egg hatch from females crossed to hybrid fringe males and to hybrid fringe males carrying T(1;2;3)1A translocation.

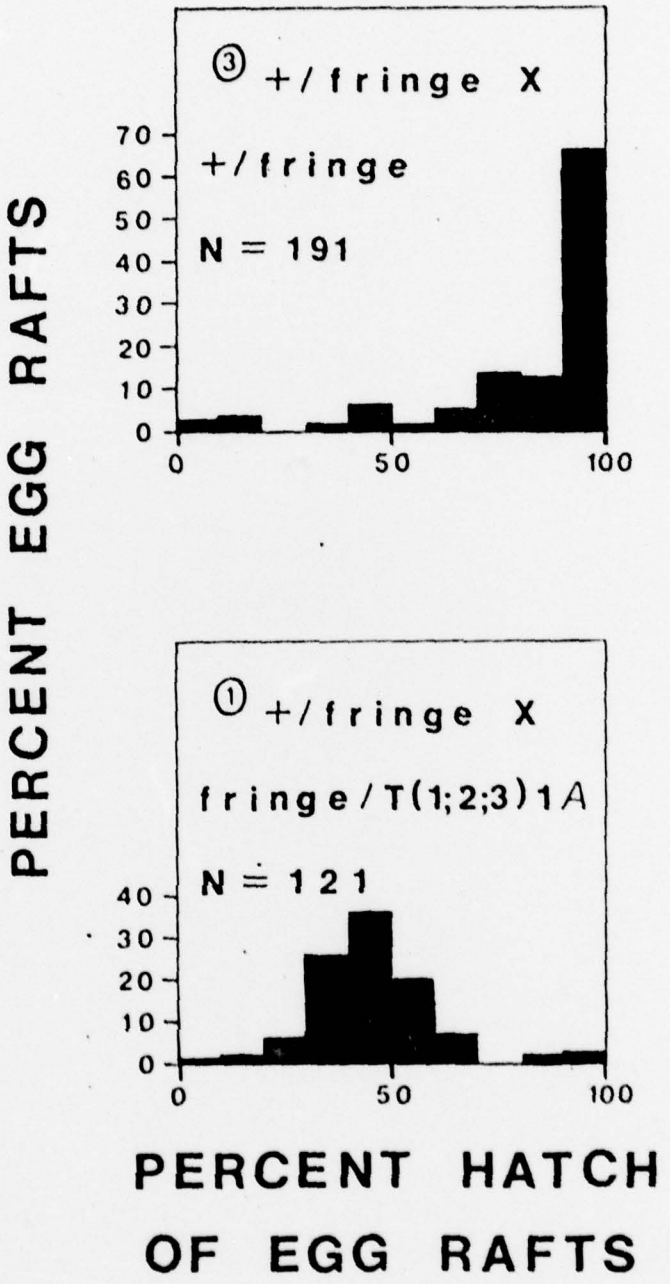


Figure 12. Selection for refractoriness to oral infection with WEE virus