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GENETICS OF THE ENCEPHALITIS VECTOR, 'CULEX TARSALIS' FOR POSSI--ETC(U)
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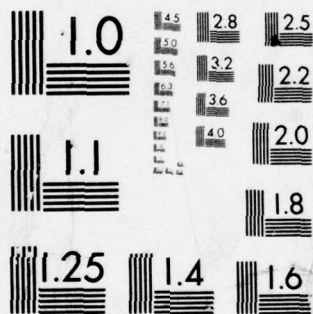
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The projects here reported represent part of an overall program designed to change <u>Culex tarsalis</u> genetically to inhibit its propagation in nature, and to render it less effective as a vector of disease. A resume of progress for the year 1975-76 is as follows: A. Laboratory strains from different geographic areas were increased to 10. B. Five multiple-marker stocks have been constructed with isolated mutants for genetic studies and identification of pseudolinkage.		

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20. Abstract continued

- The mutations are distributed across all 3 chromosomes
- C. Twenty-five new translocations have been identified with these marker stocks. Twenty-one are being maintained. Ten have been identified as to the involved linkage groups and are being assessed for their potential in a release study.
 - D. Additional reproductive studies with females were completed, primarily in relation to autogeny. There appears to be a stage in follicle development before which mating must have occurred if autogenous oviposition is to be stimulated.
 - E. Studies relating to diapause in Culex tarsalis demonstrated that the oviposition response to photoperiod shifts between 13 light-11 dark and 11 dark - 13 dark. Accumulate data suggest that the corpora cadaica has a role in mediating the oviposition response to photoperiod. All colonies were screened for inability to diapause.
 - F. Progress was made on the routine preparations of salivary-gland chromosomes for this species.
 - G. WEE vector-competence studies were continued in collaboration with personnel in the School of Public Health on this campus.
 - H. Five papers relating to the above were accepted for publication.

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

Annual Report, 1975-76
(Second Year)

Sister Monica Asman, Ph.D.

March 30, 1976

Supported by

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Annual Progress Report 1975-76

A. Culex tarsalis strains and "multiple-marker" lines.

In our continued search for inherited "markers" for genetic studies, we have increased to 15 the "wild-type" laboratory colonies maintained from different geographic areas (Table 1). Two colonies are from Canada, 3 are from other western states and 9 are from various California counties. Another laboratory strain, Berkeley, is a composite strain that holds some of the genotype of the several California lines, and consequently is a strong line that has considerable hybrid vigor.

Over 15 mutations have been isolated and 9 were successfully outcrossed and recovered in sufficient numbers to establish laboratory colonies (Table 2). With these mutants, 5 multiple-marker lines have been constructed to date (Table 3). The strains carry at least 1 marker on each of the 3 chromosomes. The weakness of these lines lies in the fact that all of them have the same autosomal markers--those on the 2 larger pairs, while the sex-linked markers on the smallest chromosome holds the only variables. Such multiple-marker lines limit the recovery of interchanges to those involving the single autosomal markers. Preliminary evidence does indicate that both "charcoal" and "white tarsomeres" are not sex-linked, and these can be incorporated into the lines as soon as conclusive data are accumulated.

The black eye (ble) and carmine (car) markers are definitely on different autosomes. Since it has not been possible to correlate either mutant with the middle-sized metacentric chromosome, designated II, or the largest, designated III (Asman, 1974) we have arbitrarily assigned black-eye to the number II and carmine to the number III linkage groups.

Phenotypically the 2 eye-color mutants have a unique relationship in that neither is epistatic over the other; both pigments express themselves "true" in the compound eye of the individual mosquito. Females and males homozygous for both car and ble show carmine eyes as larvae and pupa; however, when the adults emerge only the anterior portion of the compound eye, best seen ventrally, shows the red pigment while the more posterior part, best seen laterally, shows the black pigment. In older adults the eyes are almost totally ble, and it is difficult to distinguish them from individuals homozygous for ble alone. This loss of the carmine phenotype in older adults is also the case when the individuals have only the car genotype.

B. Induction of reciprocal translocations.

Now that the above mentioned multiple-marker lines are available,

the isolation of translocated lines through the use of genetic crosses has been initiated on a large scale. In recent months 25 new translocations have been isolated, and 21 are being presently maintained. Ten of these interchanges have been further identified as to the involved linkage groups (Table 4). In 3 instances the female heterozygote has also been identified. The males of one interchange, T(1,2)a have already been re-irradiated in an attempt to induce a second re-arrangement in the same line. We also have established a radiation dose that yields a high percent of interchanges without decreasing the viability and vigor of irradiated males. Three irradiation doses have been used in the past--1000 r, 2000 r, and 3000 r. The exposures have provided 1,162 F₁ males for testing of induced translocations. While both the 2000 and 3000 r doses yielded re-arrangements, the latter was the most productive (Table 5).

C. Polytene chromosome preparations.

Techniques have not been worked out to produce suitable salivary-gland chromosome preparations for cytological study in C. tarsalis. The lack of such techniques is a great handicap when studying chromosomal abnormalities. Considerable time was given this past year to develop such procedures. Once developed we will be able to ascertain cytologically where the chromosomal breaks occurred in interchanges, and if other anomalies were contributing to zygotic inviability. Such preparations would also allow us to establish linkage group-chromosomal correlations. While good slide preparations still are not routine, we now are able for the first time to breakdown the nuclear membrane and the fine thread-like structures chemically which seem to bind the polytene chromosomes. Continued efforts in this area will hopefully allow such slide preparations to be prepared routinely in the near future.

D. Vector competence studies and the genetic ramifications.

One of the potential uses we envision for translocations is to transport desirable genotypes into a native population--e.g. to transport an insecticide susceptible gene, a male-producing mechanism, or a genotype that would interfere with the ability of C. tarsalis to be a vector of WEE or SL virus (Table 6). For the past 2 years we have been investigating 1 such genotype--refractoriness to WEE viral infection and/or transmission, in collaboration with Dr. James Hardy and Dr. William Reeves of the Department of Environmental and Biomedical Health Sciences. For several years it has been noted that individual females from different geographic field populations, as well as females within some strains that we already have colonized, vary considerably in their susceptibility to infection with WEE virus. To date we have been able to select several variant lines that are completely or highly resistant to infection with WEE virus after feeding on viremic chicks. The resistance to WEE infection appears to be

associated with a "gut" barrier, since susceptibility is 100% when the virus is introduced by intrathoracic inoculation. (Dr. Edward Houk, an insect physiologist associated with our program is currently studying the physical and/or biochemical basis of the phenomenon.)

Variability in the capacity to transmit WEE virus also has been observed with both colonized and field-collected C. tarsalis. For example, we have a colony of C. tarsalis in which only about 50% of the infected females can transmit WEE virus by bite after 14 days extrinsic incubation, and in each case where it has been examined, nontransmitting females contained about 100-to-1000 fold less virus than transmitting females. Thus it is possible that the abilities to become infected with and to transmit virus are under separate genetic control. Currently we are attempting to select a virus-susceptible but nontransmitting variant of C. tarsalis to determine where the virus is multiplying within the mosquito and if the ability to transmit virus is genetically controlled. There seems to be no question from available data that both factors are under genetic control.

It is also quite conclusive that susceptibility to infection is dominant over refractoriness to WEE infection. The selection data also indicate that inability to transmit virus is dominant over an inability to do so. The pattern of viral infection in females in subsequent generations suggests that the mode of inheritance is not monofactorial. The maximum refractoriness of WEE virus infection we have reached is 80%. We have recently made crosses between 2 lines highly opposed in ability to become infected. The F_1 progeny will be backcrossed and genetic data based on percent infection of females in the 3 generations involved should clarify conclusively whether or not the mode of inheritance is multifactorial.

E. Reproductive biology.

Genetic control and the laboratory handling of C. tarsalis depend upon the development of a knowledge of the basic reproductive biology of the species. Experiments have been undertaken to determine the effect of female age at the time of mating on the oviposition rate. When females are kept as virgins throughout the autogenous development of their follicles, they do not deposit the autogenously developed eggs (Table 7). Subsequent mating does not result in oviposition. However, subsequent mating and blood feeding, that resulted in secondary follicle development, did initiate oviposition of large rafts typical of the anautogenous size. One tentative interpretation is that there is a stage in follicle development before which mating must have occurred if oviposition is to be stimulated. For practical

purposes, females to be used in genetic crosses cannot be retained for 6-7 days as virgins and then be expected to provide autogenous rafts.

F. Diapause in *Culex tarsalis*.

We are attempting to define the diapause response in *C. tarsalis* and establish conditions that will allow us to determine if there is a genetic basis of this phenomenon. We have studied the diapause response to several stimuli in the laboratory. Photoperiod was thought to be a most likely determining influence as it apparently plays an important role in nature, where *C. tarsalis* enters diapause in the fall even when temperatures are high. The Berkeley strain, a highly autogenous strain, was used for the study.

When females were subjected to a short day photoperiod regime (9 light/15 dark) they responded with reduced oviposition rates (Table 8). Controls were subjected to a 15 light/9 dark photoperiod regime. Subsequent dissection of the short day females revealed that they were retaining fully developed eggs in the ovaries. The short-day females were checked and were found to be inseminated. An unusual aspect of the experimental findings is that egg retention has been a rare phenomenon in overwintering females in nature. In natural populations autogeny is shut off and ovarian development stops in the resting stage. In further experiments we evaluated which stages of development were sensitive to a short photoperiod. The photo-sensitive period extended beyond the pupal stage to include adult females in the first 2 post-emergence days.

The length of photoperiod to which the mosquito would respond was determined in a series of experiments in which the photoperiod varied from 9 light/15 dark to 15 light/9 dark in two hour increments. The oviposition response to photoperiod shifted between 13 light and 11 light (Table 9). This shift could be particularly relevant for California mosquitoes as it encompasses the daylight changes that occur in the fall, 13.25 hours on September 15 to 10.75 hours on December 15.

The nature of the photoperiod-induced block of oviposition of virgins was investigated further. With the *Cecropia* moth, Truman and Riddiford (1971) found that mated oviposition was affected by removal of the corpora cadaica whereas virgin oviposition was not. In *C. tarsalis* virgin oviposition remained unaffected by photoperiod while mated oviposition was affected (Table 10). The data suggest that the corpora cadaica has a role in mediating the oviposition response to photoperiod.

We have begun a selection of lines of *C. tarsalis* for inability to diapause. Selection started with mosquitoes that did not retain eggs in the short day photoperiod. In addition, several strains that

represented various geographical areas were screened for inability to diapause. To date all the strains tested diapaused, as measured by egg retention after exposure to a short-day photoperiod.

G. Mass rearing for future release studies.

As reported last year our approach to mass production and release of translocated stocks will be to produce large numbers of eggs in the laboratory and to seed semi-natural breeding areas constructed in isolated areas that support a native population. Studies initiated in the first summer (1974) began to develop the methodology for this approach. As many as 1,000,000 eggs were produced in the laboratory in 1 week in a 4 cu ft cage, and over 10,000 adults were recovered from a 24 sq ft plastic-lined outdoor rearing pond in 1 generation. Studies this past summer (1975) included a determination of more optimal food concentrations and feeding schedules. Repeated observations were made on the time sequences of larval and pupal development under natural environmental conditions. Adjustments and improvements were made on the rearing ponds, methods to handle egg production and mass rearing of lab-produced eggs. These studies will be repeated again in 1976. Some will be done with our genetically-altered stocks to observe if these tailored genotypes can survive the environment in semi-natural breeding ponds.

H. Population dynamics of experimental field populations.

Over the past year data were collected at 3 projected release sites on the rise and decline of these isolated natural populations over a specific time period. The principal site is known as West Poso Creek and is in Kern County, California (Table 11). The collection of such data over several years prior to a release for control purposes will enable us to predict a precise time for release and the number of genetically altered specimens to release. The 3 areas are isolated by extensive surrounding arid desert, the water is from oil wells or springs, and all 3 are small in that they represent 1-2 acres or less of total water area. The mosquito population is almost solely *C. tarsalis*. In addition 2 semi-isolated areas have been identified in the Sacramento Valley that are potential sites for a second phase of pilot studies with translocated stocks. One of these areas includes rice fields as a significant water source.

References cited in report

- Asman, S.M. 1974. Cytogenetic observations in *Culex tarsalis*: Mitosis and meiosis. *J. Med. Ent.* 11:375-382.
- Truman, J. W. and L. M. Riddiford. 1971. Role of the corpora cadauca in the behavior of saturnid moths. II Oviposition. *Biol. Bull.* 140:8-14.

Table 1. Laboratory maintained colonies
of Culex tarsalis

California strains	Frink (Imperial Valley)
	Poso Creek (Kern County)
	Bakersfield-BFS (Kern County)
	Knights Landing (Yolo County)
	Owen's Valley (Inyo County)
	BFS-Ball and Chao (Kern County)
	Sacramento Valley (Butte County)
	Ralston (Yuba County)
	Dewarts (Placer County)
	Berkeley (Hybrid of several strains)
Other	Yuma (Arizona)
	BFS-Winnipeg (Canada)
	Fort Collins (Colorado)
	Presidio (Texas)
	Manitoba (Canada)

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

Mutant (symbol)	Mutagenic agent and colony source	Description	Linkage*
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 or III recessive +
Mulberry (mul)	Ethyl methane sulfonate (EMS) Berkeley Strain	Facets of compd. eye irregular in shape--giving convex	sex-linked (I) recessive +
Microcephalic (mic)	EMS Berkeley Strain	Many individual facets of compd. eye completely absent--	sex-linked (I) recessive +
Carminc (car)	Spontaneous Yuma Strain	Dark red pigmented eye, seen in larvae, pupae and adults	II or III recessive +
Setaceous palps (sp)	Spontaneous Dewarts Strain	♀♀ have 1 or 2 setae on each apical sement 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	II or III recessive +
White tarsomere (wt)	Spontaneous West Poso Creek	Distal segment of hind tarsi with white scales only	II or III recessive +

* + or - value for linkage studies

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

TABLE 4. Confirmed translocations in Culex tarsalis as of 12/31/75

Translocation ^a	Pseudo-linkage crossover units (no. scored)		Egg rafts fathered by ♂♂ selected for translocation		♀♀ tested ^b
	M - ble	M - car	ble - car	Normal	
T(1:2)a	0.9 (996)		91	0	(-)
T(1:2)b	22.9 (428)		30	3	+
T(1:2)c	0.0 (15)		9	0	(-)
T(1:2)d	8.7 (23)		4	0	(-)
T(1:2)e	25.0 (20)		2	3	(-)
T(1:2)f	24.5 (17)		4	8	(-)
T(1:2)g	31.5 (155)		7	1	+
Control	43.5		-	-	
T(1:3)a		17.8 (73)	6	3	(-)
T(2:3)a		3.0 (25)	3	6	(-)
T(1:2:3)a	- ^c	-	5	0	+

^a An identifying code name is assigned to each translocation. "T" stands for translocation. The numbers in parenthesis indicate the linkage groups involved in the interchange. The last letter distinguishes translocation involving the same combination of linkage groups.

^b - The translocation has been identified in the females and the appropriate crosses for creation of the homozygote translocation is in progress.

^c Due to the complexity of the arrangement and the small numbers scored (N = 27), pseudo-linkage data is presently incomplete.

TABLE 5. Relation of increasing radiation dose with capture of translocations

Radiation dose	No. ♂♂ tested ^a	No. ♂♂ fathering egg rafts	Percent rafts identified as translocations
1000r	228	104	0.0
2000r	630	308	4.9
3000r	269	119	8.4

^a Males from F₁ (normal ♀♀ X irradiated ♂♂) backcrossed to normal ♀♀.

Table 6. Potential applications of "tailor-made" genotypes to encephalitis control programs.

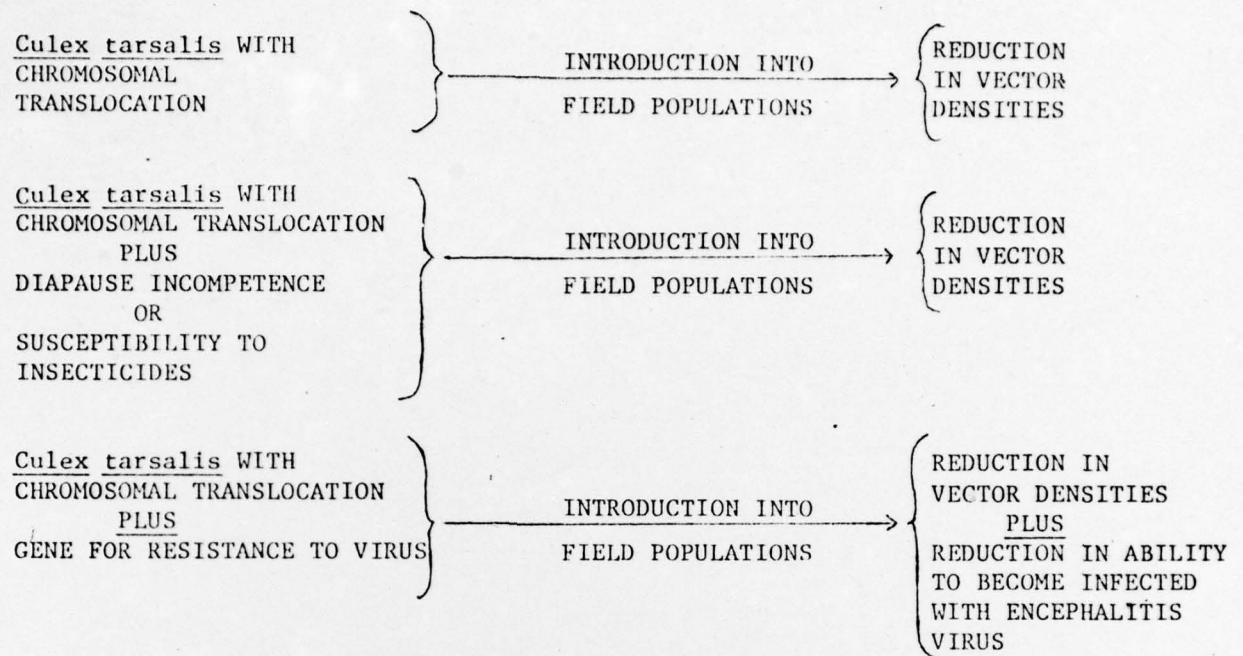


Table 7. Oviposition by females mated either before or after autogenous development was completed. Autogenous development complete by 7 days post emergence.

State	No. ♀♀	No. autogenous rafts before 7 days	No. ♀♀ after 7 days (no bloodmeal)	Subsequent autogenous rafts	No. ♀♀ after 7 days (with bloodmeal)	Subsequent oviposition
Mated before autogenous egg development	50 50 50	33 39 27	49 50 40	0 2 1		
Mated after autogenous egg development	50 50 50	2 1 3	50 49 44	0 0 0		
Mated before autogenous egg development	40 40 40	19 23 22			33 35 32	31 32 31
Mated after autogenous egg development	40 40 40	2 0 0			39 33 33	35 32 33

Table 8. Egg retention of autogenous *Culex tarsalis* transferred to short day.

Transfer	No. ♀♀	Percent Oviposition	Percent Retention
Larvae	138	40	43
Larvae	75	28	24
Pupae	135	36	38
Pupae	75	24	51
Control	134	66	8
Control	75	64	0

Table 9. Oviposition response of autogenous Culex tarsalis at various photoperiods.

Photoperiod	No. ♀♀	Percent oviposition
9L/15D	49	55
	61	49
11L/13D	44	48
	55	44
13L/11D	22	73
	79	62
15L/9D	67	73
	50	66

Table 10. Oviposition response of virgins (-) and mated (+) autogenous Culex tarsalis.

Photoperiod	Mating state	No. ♀♀	Percent oviposition
9L/15D	-	50	2
	-	50	6
	-	50	6
9L/15D	+	50	46
	+	50	32
	+	50	44
15L/9D	-	50	4
	-	50	2
	-	50	6
15L/9D	+	50	66
	+	50	82
	+	50	56

Table 11. *Culex tarsalis* collected per night, West Poso Creek, Kern County, California, 1975.

DATE	No. of female/male	CO ₂ BAIT CANS								G.M.**
		SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6	SITE 7	SITE 8	
5-13	8	3	2	4	1	1	3	1	2.9	
5-19	4	0	16	1	3	3	19	3	2.5	
5-27	50/27	20	22	3	27	8	31	32	12.5	
6-3	16.5/25	23	86	46	49	43	48	43	23.7	
6-10	97/235*	8	33	48	43	44	43	44	46.2	
6-18	16/17	47/1	104	43	44	44	43	44	24.4	
6-24	27/14	63/1	79	73	34	34	34	34	51.5	
7-1	71/42	30	26	61	45	45	45	45	47.7	
7-8	27/11	115	36	16	39	39	39	39	38.0	
7-15	24/23	40	20	203	32	32	32	32	46.4	
7-22	34/10	45	22	43	34	34	34	34	37.6	
7-29	14/11	6	16	38	39	39	39	39	22.6	
8-5	14/5	26	33	42	31	31	31	31	17.7	
8-12	9/5	7	1	5	4	4	4	4	33.9	
8-19	11/2	15	34	9	33	33	33	33	2.4 (wind, rain)	
8-26	18/7	53	18	16	47	47	47	47	27.4	
9-2	15/10	5	20	11	51	51	51	51	23.2	
9-9	14/23	24	23	19	23	23	23	23	21.7	
9-16	5/6	8	6	5	6	6	6	6	21.4	
9-23	4/1	0	6	14	22	22	22	22	6.0	
9-30	0/1	0	0	0	1	1	1	1	11.6	
10-7	3/0	0	0	0	0	0	0	0	0.1	
10-14	2/0	5	1	0	0	0	0	0	0.2	
10-21	0/0	1	1	0	0	0	0	0	0.6	
10-28	0/0	1	1	0	0	0	0	0	0.2	

* Week of maximum collections at this site
 ** Geometric mean of females collected in bait cans.

Bibliography of publications and papers presented

A. Principal investigator

Presented papers (1975)

"Genetics of new mutants in Culex tarsalis" 1975 Calif. Mosq. Cont. Ass'n., Redding, Calif.

"The use of genetics in control programs" 1975 Guest Lecturer, U. of California, Davis Campus

"Linkage relationships of three eye mutants" 1975 ESA Meeting, New Orleans, La.

Publications (1975)

Asman, S. M. 1975. Reduced temperature and embryonation delay in Culex tarsalis. Mosquito News 35:230-31.

Asman, S. M. 1975. Observations of mating behavior in Culex tarsalis. Ann. Entomol. Soc. Am. 68:777-778.

Asman, S. M. 1975. The genetics of two new eye-color mutants in Culex tarsalis. J. Heredity 66:297-300.

Hardy, J. L., W. C. Reeves, S. M. Asman. 1975. Arbovirus research program at the University of California, Berkeley. Proc. Calif. Mosq. Cont. Ass'n. 42:15-18.

Asman, S. M. 1975. Genetics of new mutants in Culex tarsalis. Proc. Calif. Mosq. Cont. Ass'n. 42:96 (abstract)

In press

Asman, S. M. A preliminary study on inducing reciprocal translocations and other chromosomal anomalies in Culex tarsalis. Mosquito News (March '76 Issue)

B. Dr. Paul McDonald (Post-doctoral Research Entomologist VI)

Presented papers (1975)

"Genetic methods of mosquito control." Province of Alberta, Canada, Mosq. Abatement Symposium, Edmonton (by invitation).

"Photoperiodic determination of egg retention in Culex tarsalis". 1975 ESA meeting, New Orleans, La.

"Factors influencing diapause in Culex tarsalis". 1975 Calif. Mosq. Cont. Ass'n., Redding, Calif.

Personnel receiving contract support

Dr. Paul McDonald, (Post-doctoral research Entomologist VI)
(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 Kreuger, Research Assistant (50% time), who is a pre-doctoral student in the Division of Entomology and Parasitology, Berkeley Campus.

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