Gahan, James B., Carroll N. Smith and B. Michael Glancy. 1966. Resistance in Florida and countermeasures involving chemicals. Mosquito News 26(3):330–337.

Mount, G. A., D. A. Dame and C. S. Lofgren. 1971. Susceptibility of a Florida strain of *Aedes taeniorhynchus* (Wied.) to insecticides. Mosquito News 31(3):438–440.

Rathburn, Carlisle B., Jr. and Arthur H. Boike, Jr. 1967. Studies of insecticide resistance in Florida mosquitoes. Mosquito News 27(3):377–382.

Rogers, Andrew J. and Carlisle B. Rathburn, Jr. 1964. Present status of insecticides for mosquito control in Florida. Mosquito News 24(3):286–291.

LABORATORY OBSERVATIONS ON THE MATING BEHAVIOR OF *CULEX TRITAENIORHYNCHUS* GILES

TANWEER FATIMA SIDDIQUI, ABDUL QAYYUM KHAN[1] AND WILLIAM K. REISEN[2]
Pakistan Medical Research Center, 6 Birdwood Road, Lahore, Pakistan

ABSTRACT. Male insemination capacity and the incidence of multiple insemination was studied using a wild-type, laboratory strain and a recessive eye color mutant of *Cx. tritaeniorhynchus*. Sexually mature, wild-type males inseminated an average of 5.1 mature females during a 4-day test period with most inseminations occurring the 2nd night. Multiple insemination was detected in 8 of 389 fertile egg rafts and occurred most frequently in small cages having high pair densities. No preferential mating was observed among the mutant males and females, and progressively more wild-type matings occurred at lower pair densities and in larger cages.

Studies of *Culex tritaeniorhynchus* Giles mating behavior have recently been initiated to provide background information as a prelude to genetical control experiments in nature. Information on phenomena such as male insemination performance, the incidence of multiple insemination and preferential mating are critical in planning releases. Observations on *Cx. tritaeniorhynchus* mating behavior in nature (Kawai et al. 1967, Reisen et al. 1977) and during colonization attempts (Newson and Blakeslee 1957, Sasa et al. 1967; Shirasaka et al. 1968, Baker and Sakai 1974) have been restricted to the time and place of mating and the incidence of successful mating under laboratory conditions. In fact, there are relatively few detailed studies of many aspects of the mating behavior of *Culex* mosquitoes, in general, with the exception of *Cx. p. fatigans* (e.g. Kitzmiller and Laven 1958, Sebastian and de Meillon 1967) and to a lesser extent *Cx. tarsalis* (Asman 1975) and *Cx. nigripalpus* (Lea and Edman 1972).

The present paper describes male insemination performance, the incidence of multiple insemination, and preferential mating in *Cx. tritaeniorhynchus* under laboratory conditions.

METHODS AND MATERIALS

STRAINS. The Balloki, Pakistan, strain of *Cx. tritaeniorhynchus* was used throughout and has been under continuous culture at our laboratories for the past 6 years (Baker and Sakai 1974). A recessive, sex-linked, heat sensitive, eye color mutant, rose eye (Baker and Sakai 1973), was used in the multiple insemination and preferential mating experiments. This mutant, originally isolated from the Balloki strain, expresses itself well in the larval stage as red eye color at 28°C, becoming paler to

[1] Present address: Lecturer, Zoology Department, Forman Christian College, Lahore, Pakistan.

[2] Please address reprint requests to W. K. Reisen.

almost pure white at 32°C (Baker and Sakai 1973).

MALE INSEMINATION CAPABILITY. Thirty 4-day old virgin males were each offered ten 4-day old virgin females for 3 to 4 days while caged in individual cardboard cartons (volume = 4086 cm^3). The 30 groups of 10 females each were sacrificed daily and their spermathecae examined for the presence of spermatozoa.

MULTIPLE INSEMINATION. In Experiment 1, ten 4-day old virgin females, homozygous for rose eye, were caged with 5 wild type and 5 rose eye, 4-day old virgin males in wire mesh cages (volume = 9027 cm^3) for a 4-day period. Females were subsequently offered mouse as a blood meal source for 3 successive days, held for 4 days, and then offered dilute rice straw infusion in a plastic cup as an oviposition substrate for 3 nights. Egg rafts were isolated daily in individual tubes, held overnight in the insectary at about 28°C and the resulting larvae examined for eye color. Egg rafts producing both wild and rose eye larvae implied that the female had received spermatozoa from both rose eye and wild type males, i.e., multiply inseminated. This procedure was repeated twice in larger cages (volume = 1 m^3) first using the same number of females and males, and then 30 rose eye females and 15 rose eye and 15 wild type males. Each mating in Experiment 1 was replicated 5 times. There were 3 possible sources of error in Experiment 1:1) contamination of egg rafts during isolation from the oviposition cup, 2) interruption of the female during oviposition resulting in the production of more than 1 small egg raft per night, and 3) failure to correctly separate rose eye larvae from the wild type larvae with brown eye color.

Experiment 2 was designed to study the effects of increasing density on the incidence of multiple insemination. Four-day old virgin rose eye females and equal numbers of 4-day old rose eye and wild eye males were used in the same proportions as Experiment 1. Seventy-two, 36 and 18 pairs were added to each small cage (volume = 9027 cm^3), and 14 and 8 pairs were added to larger cages (volume = 16374 cm^3) resulting in densities of 0.00790, 0.00400, 0.00200, 0.00085 and 0.00048 pairs/cm^3.One, 3, 6, 8 and 12 cages were used for each density, respectively, to yield approximately equal numbers of females per density. After release into the cages, females were offered a blood meal for 3 consecutive nights, held for 2 days and then all gravid or subgravid females were isolated individually in glass tubes each containing about 1 cm of dilute rice straw infusion as an oviposition substrate. This procedure prevented possible contamination between egg rafts during isolation or the separate isolation of 2 egg rafts laid by the same female. Tubes were checked daily and the resulting egg rafts transferred to test tubes, placed in an incubator at 32°C overnight, and the hatched larvae examined for eye color the following day. Incubation at 32°C simplified the scoring of the larvae eye-color phenotypes, i.e. rose eye larvae had white eyes, wild type had brown eyes.

RESULTS AND DISCUSSION

MALE INSEMINATION CAPABILITY. The mean (± standard error of the mean) number of females inseminated by 4-day old virgin *Cx. tritaeniorhynchus* males on each of 4 successive days was 1.63 ± 0.24, 3.06 ± 0.30, 0.40 ± 0.12, and 0.0 ± 0.0 females per male per day, respectively. The overall mean was 5.10 ± 0.32 females per male for the 4-day period. Although cohorts of male *Cx. tritaeniorhynchus* are 100% sexually mature at 4 days of age (Khan and Reisen 1977), maximum mating was not attained until the evening of the males 5th day of life, or possibly until after the males were offered females for at least 2 nights. The number of inseminations declined on day 3 and no inseminations were observed on day 4. Although depleted male mosquitoes may occasionally mate again (e.g. Hausermann and Nijhout 1975, Asman 1975), these events are considered to be

Table 1. Summary of mutliple insemination Experiments 1 and 2.

	No. pairs per cage	Cage size (cm^3)	Density ($prs./cm^3$)	No. Cages	Total egg rafts	Total hatching	Larval Phenotypes (No. families)			Chi-square[1]
							Rose eye	Wild eye	Mixed	
					Experiment 1					
a.	10	9.0×10^3	1.1×10^{-3}	5	40	40	18	18	4	0.10^{ns}
b.	10	1.0×10^6	1.0×10^{-5}	5	5	5	2	3	0	0.20^{ns}
c.	30	1.0×10^6	3.0×10^{-5}	5	95	95	29	66	0	14.41*
					Experiment 2					
a.	72	9.0×10^3	7.9×10^{-3}	1	32	26	16	10	0	1.38^{ns}
b.	36	9.0×10^3	4.0×10^{-3}	3	76	74	39	32	3	0.86^{ns}
c.	18	9.0×10^3	2.0×10^{-3}	6	53	46	24	21	1	0.09^{ns}
d.	14	16.4×10^3	8.5×10^{-4}	8	57	54	25	29	0	0.30^{ns}
e.	8	16.4×10^3	4.8×10^{-4}	12	52	49	23	26	0	0.18^{ns}

[1] Chi-square to test for departure from randomness (1:1) in the number of rose and wild eye matings occurring. Mixed rafts included based on the apparent first mating. * = $P < 0.01$, ns = $P > 0.01$.

infrequent, since the replenishment of both spermatozoa and/or male accessory gland fluid is generally considered inadequate to allow further inseminations (e.g. Jones 1973, Hausermann and Nijhout 1975). Thus maximum insemination activity in *Cx. tritaeniorhynchus* probably occurs during the evening of the 5th day of life and/or the 2nd evening after being offered virgin females. Every male observed inseminated at least 1 female and all males and females survived the experimental period. The maximum number of females inseminated by any one male was 8 and this total was attained by 3 of 30 males. These data agreed well with observations on the insemination ability of closely related *Cx. tarsalis* as reported by Asman (1975).

MULTIPLE INSEMINATION. Using morphological (Kitzmiller and Laven 1958) and biochemical (Bullini et al. 1976) markers in the *Cx. pipiens* complex, and morphological markers in *Cx. tarsalis* (Asman 1975), members of the genus *Culex* generally have been observed to be monogamous, although infrequent cases of multiple insemination have been reported (Kitzmiller and Laven 1958, Patterson and Lofgren 1968, Bullini et al. 1976). In the present experiments, 389 females laid fertile eggs of which 8 (2.06%) yielded progeny of mixed eye colors, i.e. were multiply inseminated (Table 1, Exp. la, 2b and c). Since females inseminated more than once by either wild or rose eye males could not be detected by the present system, the incidence of multiple insemination was considered to be 3X the number of mixed egg rafts observed or 6.17%, and thus was possibly as high as 30.0, 12.2 and 6.4% in Exp. la, 2b, and 2c., respectively (Table 1). In Exp. 2b all 3 cases of multiple insemination occurred in 1 of the 3 cages used and the incidence within this cage was considered as high as 32.1% (3 mixed rafts x 3/28 hatching egg rafts). In *Cx. tritaeniorhynchus* multiple insemination seems to be an event confined to small cages having high densities of sexually mature mating pairs and may even be a cage specific phenomenon. No explanation for the absence of multiple insemination at the most crowded density (7.9 x 10^{-3} pairs/cm^3) could be postulated at this time. The apparent relationship between the incidence of multiple insemination and pair density was attributed to the increased number of male and female contacts during peak mating activity. Increased contacts could possibly increase the incidence of partial inseminations resulting in the transference of inadequate quantities of male accessory gland substance (thereby preventing the formation of mechanical or humoral re-insemination barriers) or possibly increase the chances of females being seized and remated prior to the formation of the re-insemination barriers reviewed by Leopold (1976).

Our data seem to support the latter premise as within families having both eye-colored progeny, one of the phenotypes usually predominated (Table 2) and was considered to represent the genotype of the first male successfully mating (Bullini et al. 1976). In the 8 mixed rafts observed in the present experiment, 549 of the 662 successfully eclosed larvae (82.9%) were attributed to the male mating first. The percentage hatch of families 1 and 2 in Experiment 2 was very low; however, most of the un-

Table 2. Characteristics of egg rafts producing larvae with both wild and rose colored eyes.

Family	No. Eggs	No. Larvae	Progeny Eye Color: Wild	Rose
		Experiment[1]		
1	110	110	95	15
2	119	114	29	85
3	120	120	102	18
4	103	97	82	15
		Experiment[2]		
1	88	29	21	8
2	70	32	8	24
3	92	74	48	26
4	104	98	92	6

hatched eggs were embryonated suggesting the females were fully inseminated.

PREFERENTIAL MATING. The possibility that highly inbred laboratory genetic stocks have evolved abberrant or even preferential mating behavior is of great importance in the application of any method of genetical control. This phenomenon has been observed in an eye-color mutant of *An. stephensi* at our laboratory (S. Khan and M. Aslamkhan, unpublished). In the present experiment, there was no significant departure from randomness (1 rose eye: 1 wild-type) in the matings of the females in small cages (Exp. la and Exp. 2); however, in the large cages used in Exp. 1c, significantly more wild-type males successfully mated (Table 1). In these analyses, mixed matings were also included based on the male considered to have mated first (Table 2). In Experiment 2, even though there was no significant departure from randomness among the matings of rose and wild-eye males, the incidence of rose eye matings was found to increase significantly as a linear function of pair density ($\ln y = 20.218 + 0.463x, r^2 = 0.949, P < 0.01$, y = rose eye/total matings, x = no. pairs/cm^3 of cage volume). Thus, in the present experiment, there was no preferential mating among the rose eye females and males. The decrease in the incidence of rose eye matings as a function of decreasing density suggested that the wild eye males were more competitive at reduced densities and/or in larger cages.

ACKNOWLEDGMENTS

We thank Mr. Sarfraz Hussain Chishti, Ms. Yasmin Aslam and Ms. Rukhsana Mukhtar for their technical assistance; and Drs. R. K. Sakai and R. H. Baker for their valuable suggestions and for critically reading the manuscript. This research was supported by Grant No. AI-10049 from the National Institute of Allergy and Infectious Diseases, N.I.H. The administrative assistance of the United States Agency for International Development in Pakistan is gratefully acknowledged.

Literature Cited

Asman, M. 1975. Observations on the mating behavior of *Culex tarsalis*. Ann. Entomol. Soc. Amer. 68:777–778.

Baker, R. H. and R. K. Sakai, 1973. Genetics of rose, an allele of the white locus in a mosquito. J. Hered. 64:19–23.

Baker, R. H. and R. K. Sakai. 1974. Genetic studies on *Culex tritaeniorhynchus*. In: The use of genetics in insect control, R. Pal and M. J. Whitten (eds.) Elsevier North-Holland Publ. Co. Amsterdam, pgs. 133–182.

Bullini, L., M. Coluzzi and B. Bullini. 1976. Biochemical variants in the study of multiple insemination in *Culex pipiens*. L. (Diptera, Culicidae). Bull. Ent. Res. 65:683–687.

Hausermann, W. and H. F. Nijhout, 1975. Permanent loss of male fecundity following sperm depletion in *Aedes aegypti* (L.). J. Med. Ent. 11:707–715.

Jones, J. C. 1973. A study on the fecundity of male *Aedes aegypti*. J. Ins. Physiol. 19:435–439.

Kawai, S.,. Y. Wada and N. Omori. 1967. Preliminary note of the swarming of *Culex tritaeniorhynchus*. Trop. Med. (Nagasaki) 9:58–64.

Kitzmiller, J. B. and H. Laven. 1958. Tests for multiple fertilization in *Culex* mosquitoes. Amer. J. Hyg. 67:207–213.

Khan, A. Q. and W. K. Reisen. 1977. Laboratory observations on developmental rhythms in *Culex tritaeniorhynchus* Giles. Mosquito News 37:637–645.

Lea, A. O. and J. D. Edman. 1972. Sexual behavior of mosquitoes. 3. Age dependence of insemination of *Culex nigripalpus* and *C. pipiens quinquefasciatus* in nature. Ann. Entomol. Soc. Amer. 65:290–293.

Leopold, R. A. 1976. The role of male glands in insect reproduction. Ann. Rev. Entomol. 21:199–221.

Newson, H. D. and T. E. Blakeslee. 1957. Observations of a laboratory colony of the mosquito *Culex tritaeniorhynchus* Giles. Mosquito News. 17:308–311.

Patterson, R. S. and C. S. Lofgen. 1968. The potential use of sterile males to control *Culex pipiens quinquefasciatus* Say. Proc. N. J. Mosq. Exterm. Assoc. 55:170–175.

Reisen, W. K., Y. Aslam and T. F. Siddiqui. 1977. Observations on the swarming and mating of Pakistan mosquitoes in nature. Ann. Entomol. Soc. Amer. 70:988–995.

Sasa, M., A. Shirasaka and Y. Wada. 1967. Comparative studies on breeding habits of a laboratory adapted variant and wild colonies

of *Culex tritaeniorhynchus summorosus*, the principal vector of Japanese encephalities. Jap. J. Exp. Med. 37:257–264.
Sebastian, A. and B. de Meillon, 1967. Experiment on the mating of *Culex pipiens fatigans* in the laboratory. Bull. Wld. Hlth. Org. 36:47–52.
Shirasaka, A., M. Sasa, Y. Wada, M. Watanabe and I. Yamashita. 1968. Notes on the laboratory colonization of *Culex tritaeniorhynchus summorosus*, the principal vector of Japanese encephalities in Japan. Jap. J. Exp. Med. 39:423–435.

MOSQUITO VECTORS OF DOG HEARTWORM, *DIROFILARIA IMMITIS*, IN WESTERN MASSACHUSETTS[1]

J. J. ARNOTT[2] AND J. D. EDMAN
Department of Entomology, University of Massachusetts, Amherst, MA 01003

ABSTRACT. A total of 3445 female mosquitoes representing 23 species were collected in the field, held for 10 days and dissected for the presence of infective stage larvae of *Dirofilaria immitis*. Eleven naturally infected individuals of 3 species of *Aedes* mosquitoes were found. In the laboratory, 1451 females representing 19 species were fed on a heartworm infected dog and subsequently dissected for evidence of larval development. Ten species were found to support development of at least some filariae to the infective stage.

Based on these data and available biologic information, *Aedes canadensis* and *Ae. excrucians* appear to have the greatest potential as vectors of dog heartworm in western Massachusetts.

INTRODUCTION

Dirofilaria immitis was first isolated from Massachusetts dogs in 1937 (Augustine 1938) but has not been considered a general problem in New England until very recently. Although no systematic records exist, voluntary case reporting by veterinarians in western Massachusetts from 1973 to 1975 showed that about 10% of dogs tested were positive for *D. immitis* microfilaria (Downhill, Edmonds, Hilt, O'Conner, Ruder, and Roy, 1975, per commun.). Recognizing the common, widespread occurrence of heartworm within the last 5 yrs, area veterinarians now routinely test for infection and recommend prophylactic drugs for all dogs during the warm season.

It is not clear whether the current picture reflects new geographic and numeric spread or if greater awareness and better diagnosis have merely brought into true focus a long extant situation.

At least 36 nearctic mosquitoes have been indicated as possible vectors of dog heartworm (Ludlam et al. 1970) and the primary vector, when known, appears to vary greatly with geography (Schlotthauer et al. 1969). Research reported here was directed toward identifying potential vectors in western Massachusetts since effective vector control appears to be an important ingredient in arresting local transmission of this disease. Two approaches were employed: 1) attempted isolation of naturally infected mosquitoes, and 2) comparison of laboratory infection

[1] Paper No. 2178, Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst. This research supported (in part) from Experiment Station Project No. 320. Part of a thesis submitted by the senior author to the Graduate School of the University of Massachusetts in partial fulfillment of M.S. degree requirements.

[2] Present address: 714th Medical Detachment, Fort Bragg, N.C. 28307.