effective in frequency of positivity than paddles resting at an angle. Hardboard paddles of standard width (3/4 inch) were more frequently positive than a narrower unit, but the difference was not statistically significant. The narrower paddles were found to be undesirable because of difficulty in handling and their tendency to warp.

Competitive tests under varying field conditions showed ovitraps with and without ethyl acetate attractant to be equally effective.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Elwood F. Hill for his aid in the statistical analysis of certain data used in this study.

References Cited

BANCROFT, HULDA. 1960. Introduction to Biostatistics. 210 pp. Paul B. Hoeber, Inc., New York.

FAY, R. W., and ELIASON, D. A. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosq. News 26(4):531–535.

FAY, R. W., and PERRY, A. S. 1965. Laboratory studies of ovipositional preferences of *Aedes aegypti*. Mosq. News 25(3):276–281.

SPIEGEL, MURRAY R. 1961. Theory and Problems of Statistics. 395 pp. Schaum Publishing Company, New York.

U. S. WEATHER BUREAU. 1966–1967. Climatological Data—Florida. Annual Summaries, Vol. 70–71. Weather Bureau, Asheville, N. C.

EVALUATION OF TECHNIQUES USED FOR MASS REARING *AEDES NIGROMACULIS* BY INDUCED MATING

TAKESHI MIURA [1]

The development of resistance to insecticides by the irrigated pasture mosquito, *Aedes nigromaculis* (Ludlow), in the Central Valley of California, has necessitated a search for long-range population-suppression methods as well as for new insecticides.

Since the report of Gjullin and Peters (1952) of mosquito resistance to insecticides in California, many attempts have been made to develop a self-sustaining colony of this species, but, in all cases, this mosquito has refused to reproduce in captivity.

At this laboratory, *A. nigromaculis* is reared routinely from eggs laid by wild-caught females. The progeny are used in testing new insecticides and for biological studies. However, this is a time-consuming and seasonal operation; furthermore, such mosquitoes are quite heterogeneous. To overcome these problems, a modified version of the induced mating technique of McDaniel and Horsfall (1957) has been used for maintaining laboratory colonies of *A. nigromaculis* at this laboratory (Miura 1967).

This paper describes further technical modifications for improving the production of eggs for use in laboratory investigations.

MATERIALS AND METHODS. Eggs were obtained from females maintained by induced copulation and from females collected in the vicinity of Fresno, California.

Eggs were collected on filter papers and stored in a chamber with a relative humidity of 100 percent. The chamber was kept in the laboratory at 24° C. Four to five hundred eggs at a time were hatched by flooding them with 200 ml of deoxygenated water in a pan (30 x 18 x 5 cm). After about 12 hours, the first instar larvae were transferred to a rearing pan (40 x 25 x 6 cm) containing grass and straw in 1000 ml of tap water; the water

[1] University of California, Mosquito Control Research, 5545 East Shields Avenue, Fresno, California 93727.

was aerated gently and held at 27° C. The larvae were fed several times daily with a pinch (0.1–0.2 g) of powdered mixture of rabbit and dog food (1:1 v/v) and 5 to 10 ml of 1 percent yeast suspension in a nutrient broth.

Most larvae pupated by the 7th day after hatching. Thirty to forty pupae were placed in a dixie cup (4 fl. oz.) containing 50 ml of fresh tap water; the cup was then placed in a holding cage (a 2-quart ice cream carton with a nylon net top). Identification of the strains and the date of pupation were labeled on each cage; cages were then held in an adult holding room at a temperature of approximately 24° C and a relative humidity between 75 and 85 percent. A few raisins were provided on the top of each cage for food. Most adults emerged within 2 days after pupation, and cages of newly emerged adults were held in the adult room for another 2 days for maturation. The holding cage was then covered with a wet paper towel and a sheet of black plastic and then held in an incubator (15° C).

The following procedure was used to perform induced mating (Figure 1): 3 to 4 males, at a time, were gently aspirated from the holding cage and placed in a chamber where they were anesthetized by exposure to an atmosphere of CO_2 for a few seconds. The males were then quickly pinned, laterally through the thorax, by means of two minutens fixed to the end of a wooden stick. The pinned males were kept in a high-humidity chamber. Ten to twelve females were gently aspirated from the holding cage and anesthetized with CO_2, in the same manner as described for the males. Three to four females, lightly anesthetized with CO_2, were then re-anesthetized with

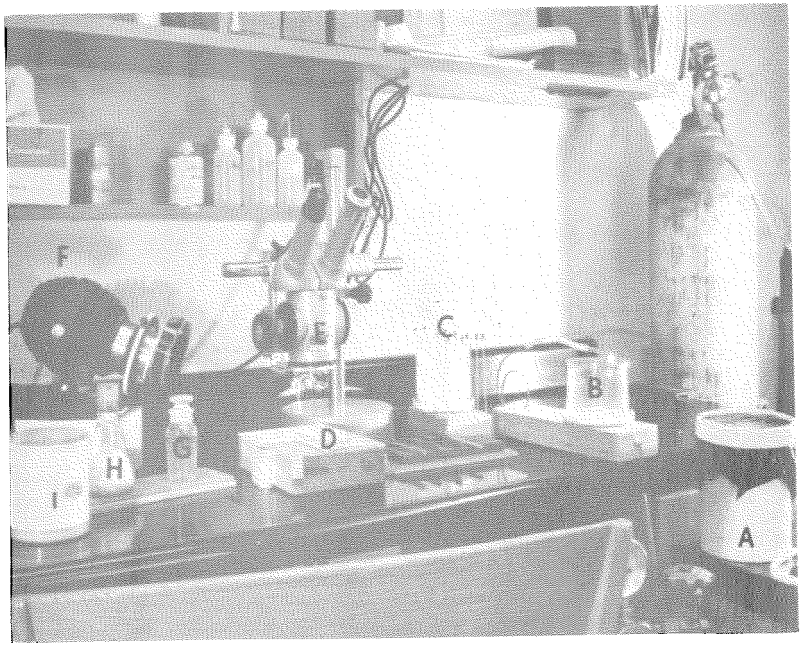

FIG. 1.—Laboratory set-up used for routine induced insemination, showing A, holding cage; B, gas chamber; C, humidity chamber; D, holding block; E, stereomicroscope; F, microscope lamp; G, methyl cellulose bottle; H, etherizer; I, recovery cage.

ether, for a few seconds, until relaxation of the legs and caudal end of the abdomen was apparent; the 8th abodminal segment is usually withdrawn into the 7th segment, but following the ether treatment, the 8th abdominal segment is extruded (Figure 2). At this stage the females were

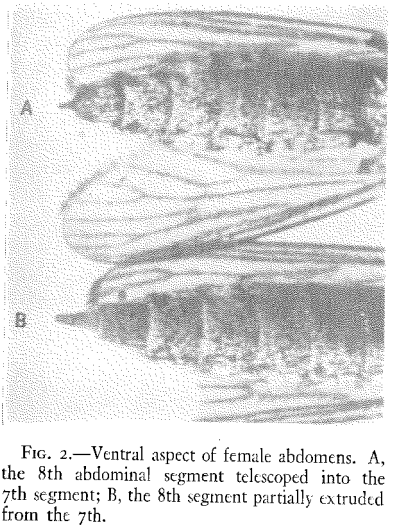

FIG. 2.—Ventral aspect of female abdomens. A, the 8th abdominal segment telescoped into the 7th segment; B, the 8th segment partially extruded from the 7th.

placed in a supine position on a holding block covered with a thin adhesive layer of methyl cellulose.

A pinned male, which had completely recovered from the anesthesia, was then taken from the humidity chamber, decapitated and the mid- and hind legs amputated.

Next, the holding block, with 3 to 4 completely anesthetized females properly positioned, was placed on the stage of a stereoscopic microscope having a magnification of 16 diameters. The terminalia of the decapitated male were applied to the 8th abdominal segment of the female, at an angle of 60 to 90 degrees, in such a way that the 10th sternite of the male made contact and pressed into the female genital atrium. Copulation was achieved when the apex of the 8th abdominal segment of the female touched the base lobes of the male; the male showed a firm pressing movement of the bases of the claspers and the female responded by extruding the 8th abdominal segment; it was terminated with a movement of the claspers and the tip of the male abdomen. While in copula, both sexes were immobile.

As soon as contact was broken, the male was returned into the humidity chamber and the female was gently removed from the holding block and placed in a recovery cage (a 1-pint ice cream carton with net top).

Decapitated males remained functional for 30 minutes or more and they could repeatedly copulate with several females; however, no male was used more than four times; thus 3 to 4 males prepared at a given time could be used to inseminate 10 to 12 females.

At the end of the operation, the inseminated females were placed in a 1-ft.^{3} screen cage in the adult holding room. Raisins and citrated bovine blood were provided as food; the females were also permitted to take blood meals from a rabbit during a 1-hour period every day for about 1 week. A petri dish (9 x 1.5 cm) containing wet moss for oviposition was also placed inside each cage. Eggs deposited in the moss were collected by washing with ice-cold water onto filter paper and were then stored in a high-humidity chamber.

RESULTS AND DISCUSSION. The mechanics of copulation for *A. nigromaculis,* by induced mating, is probably identical to that of *Aedes aegypti,* as described by Spielman (1964). "The aedeagus of the *Aedes aegypti* male is placed superficially within the genital orifice of the female during copulation and is not a deep intromittent organ. Sexual union is accomplished through the junction of the everted aedeagus and the arterial membrane." Seminal material of *A. nigromaculis* is arranged in a rope-like fashion and transferred to the female. The average duration of copulation for 75 pairs of mosquitoes was 27 seconds, ranging from 5 to 158 seconds. Duration of the copu-

lations appeared to be largely dependent upon the ages of the males; usually young males were very responsive but remained in copula for shorter times and transferred the least amount of seminal material, while older males were least responsive, but remained in copula for longer times and transferred more seminal material. One 7-day-old male stayed in copula for 353 seconds.

Rates of insemination are dependent upon many variables. Table 1 shows the summary of 40 days' work by a half-time assistant. An average of 14.8 females were inseminated per hour during the 40 operating periods. The minimum number of females inseminated was 5.3, which occurred while utilizing 1-day-old males at room temperature of 25.6° C; the ratio of the number of inseminated females to the number of males used (response index of males) was 0.73. The maximum number inseminated was 25.3 females per one hour, and was accomplished while utiliz-

TABLE 1.—Effects of biological and physical factors on the rates of induced mating.

No. Mating/ hour	Response of Male[a]	Age of Male (days)	Duration of Operation (min.)	Room Temp (° F)
25.3	2.10	5	50	73
23.1	2.10	..[b]	55	70
22.4	2.67	3	150	69
20.6	2.63	1	150	69
20.0	3.33	1	60	70
19.9	..	3	100	70
19.7	2.71	1	140	72
19.3	2.12	1	165	75
19.2	2.00	2	150	70
18.7	2.10	4	135	69
16.9	2.00	1	50	73
16.4	1.95	..	155	75
15.6	2.05	4	165	71
15.3	1.83	2	165	71
14.5	1.90	1	165	70
14.4	1.50	2	150	70
14.0	1.26	3	90	77
13.8	1.58	1	165	72
13.4	1.68	4	165	74
13.1	1.28	4	165	79
13.0	1.62	..	60	71
13.0	1.62	1	120	70
13.0	1.86	6	180	68
13.0	1.25	2	135	75
12.7	1.36	5	180	70
12.6	1.52	5	195	69
12.6	1.29	1	105	71
12.5	1.81	4	225	71
11.6	1.52	2	165	79
11.6	1.52	4	165	69
10.9	1.67	4	165	..
10.9	1.36	3	165	72
10.4	1.13	2	150	71
9.7	1.42	4	165	77
9.6	1.00	3	150	78
9.4	1.30	5	165	72
8.5	1.15	1	160	74
8.4	1.23	4	330	70
7.7	1.82	3	240	78
5.3	0.73	1	90	78

[a] Ratio of number of females inseminated to number of males used in an operation.
[b] Denotes no data

ing 5-day-old males at room temperature of 22.8° C; the response index was 2.10. The rate of insemination is probably proportional to the response index of males. In order to inseminate 16 or more females per hour, a single male must be utilized for insemination of 2 or more females. The duration of the operation is another important factor. During a short period of operation, more females were inseminated. The room temperature may also influence the rate of insemination; more females were inseminated when the temperature was between 20.6° to 23.9° C.

TABLE 2.—Fecundity and mortality of females inseminated by induced mating.

	At the beginning of project (November 1967)		At the end of project (March 1968)	
	Mean	Range	Mean	Range
No. of eggs/♀	22.1	16.1 – 29.0	46.4	14.7 – 88.8
Viable eggs (%)	55.49	40.81– 70.11	60.38	50.99– 69.36
Mortality (%)	18.69	12.33– 29.73	9.39	0. – 21.88
Longevity (days)	16.4	15. – 18.	21.3	18. – 25.
No. of females/cage	102.	73. –139.	110.	74. –163.

The fecundity of *A. nigromaculis* is not well understood. According to Husbands and Rosay (1952), field-caught females, fed on human blood, produced 0 to 110 eggs per female in the laboratory. Barr and Al-Azawi (1958), reported that a female of this species laid 156 eggs. The results of this study are shown in Table 2. The average egg production per female was 46.4 and ranged from 14.7 to 88.8. Table 2 also compares the yield between the beginning and end of 5 months of experience. At the end of the 5-month period, the egg production per female was doubled; the percentage of viable eggs obtained was increased about 5 percent and the longevity of the females was also increased by 5 days. Furthermore, the mortality of females, caused by handling during insemination, was reduced by one-half. Increased egg production and longevity of mosquitoes at the end of the 5-month period probably was due to improved handling of the mosquitoes by the operator as well as the adaptation of the mosquitoes to the new environment and food source.

SUMMARY. Mass production of *Aedes nigromaculis* eggs was attempted from the fall of 1967 to the spring of 1968. The average egg production of inseminated females was about 46; with a little experience, an assistant was able to inseminate about 15 to 20 females per hour; thus, with two part-time assistants, 80 to 100 females could be inseminated daily. About 60 percent of the total eggs obtained were viable. This technique now provides this laboratory with a sufficient number of eggs to investigate the biology of *Aedes nigromaculis* as well as to allow small scale insecticidal tests.

ACKNOWLEDGMENT. The author wishes to express his thanks to Drs. C. H. Schaefer, and J. R. Anderson for comments on the manuscript.

References Cited

BARR, A. R., and AL-AZAWI, A. 1958. Notes on oviposition and the hatching of eggs of *Aedes* and *Psorophora* mosquitoes. Univ. Kansas Sci. Bull. 39:263–273.

GJULLIN, C. M., and PETERS, R. F. 1952. Abstract of recent studies on mosquito resistance to insecticides in California. Proc. Calif. Mosq. Cont. Assoc. 20:44–45.

HUSBANDS, R. C., and ROSAY, B. 1952. A cooperative ecological study of mosquitoes of irrigated pastures. Proc. Calif. Mosq. Cont. Assoc. 20:17–26.

MCDANIEL, I. N., and HORSFALL, W. R. 1957. Induced copulation of aedine mosquitoes. Science 125:745.

MIURA, T. 1967. Establishment and maintenance of a laboratory colony of *Aedes nigromaculis* (Ludlow) (Diptera:Culicidae) by induced mating. Calif. Vector Views 14:57–58.

SPIELMAN, A. 1964. The mechanics of copulation in *Aedes aegypti*. Biol. Bull. 127:324–344.