gypti distribution throughout the study area.

Larval surveys often have proved misleading because of discontinuous sampling and the varying skill among inspectors. Ovitraps in Waycross, Georgia, provided a continuous (weekly) monitoring of the existing *Ae. aegypti* population largely independent of entomological skills.

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GENETICALLY MARKED *AEDES AEGYPTI* IN STUDIES OF FIELD POPULATIONS

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Through the use of sterilized males of certain dipteran species, successful eradication of the target species over extensive areas has been obtained (Knipling, 1960; Steiner *et al.*, 1965). Attempts to apply the method to mosquitoes have not met with success (Weidhaas *et al.*, 1962; Morlan *et al.*, 1962). The success of an eradication campaign by this means depends upon several factors, such as: (a) the dispersion pattern of the released males in seeking out the females; (b) the acceptance of the released males by the females; (c) the normal number of effective matings of each female; (d) the competitiveness of the released males with the normal males; (e) the timing of the releases and the number of generations per season for the species; and (f) the population levels of the species in the field area.

The present paper describes preliminary trials to obtain information on the first two factors, (a) and (b), with respect to *Aedes aegypti* at Meridian, Mississippi, during the fall of 1967 through the release of genetically marked, nonsterile males reared in an insectary.

MARKER STRAIN. To obtain stock for genetic marking, eggs were collected from Meridian, Mississippi, by means of ovitraps during June 1967. After removal from the ovitraps, the eggs were conditioned on the paddles for 48 hours and then held in a refrigerator until sufficient numbers had accumulated. The eggs were then shipped to the University of Notre Dame for establishment of a laboratory strain, designated MERIDIAN. In addition, adults from field-collected eggs were crossed to a synthetic laboratory strain,

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MYS, which contained multiple genetic markers.

The genetic markers used included three autosomal mutants on linkage group 2, spot abdomen (*s*), yellow larva (*y*) and Silver mesonotum (*Si*) (Craig and Hickey, 1967). The most useful of these is Silver. Homozygotes, *Si/Si,* have the entire mesonotum covered with metallic silver scales (Figure 1). The well-known lyre-shaped

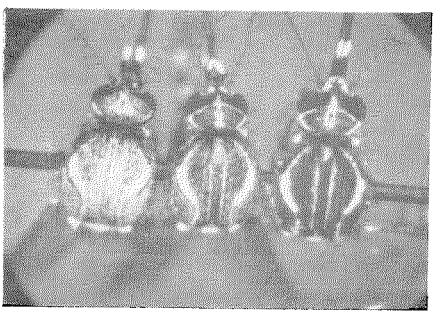

FIG. 1. *Aedes aegypti* with the genetic marker Silver. Left, MISS MARK strain homozygous or Silver mesonotum; center, F_1 heterozygote from cross between MISS MARK and MERIDIAN; and right, wild type from Meridian, Miss.

pattern is not visible and the median "lyre-strings" are obliterated. In the heterozygote, *Si/+*, there are silver scales scattered randomly through the brownish-black background, giving the appearance of dandruff. The lyre pattern is evident and the strings can be seen readily. Thus, whenever a male homozygous for Silver is crossed to a MERIDIAN female, the F_1 progeny have a distinctive mesonotal pattern and can be readily separated from wild type individuals.

The other markers, *y* and *s*, are recessive and cannot be detected in the heterozygote. The frequency of yellow larvae in the MERIDIAN strain is 27 percent of phenotypes. Neither spot nor Silver are present in MERIDIAN. These markers have the following arrangement on linkage group 2: *s* (6) *y* (6) *Si*. The figures in parentheses are approximate cross-over units. The three markers tend to be inherited as a block, thus providing a useful indicator system.

In order to synthesize a marked strain with maximum genetic material from the field population, females of the MYS strain were crossed to males of MERIDIAN from field-collected eggs. The resultant F_1 progeny were crossed again to obtain an F_2. The F_2 males were selected for the markers, spot, yellow and Silver; all other males and all females were discarded. Similarly, MERIDIAN females were crossed to MYS males and the homozygous marked females in the F_2 were selected. The selected males and females from the reciprocal F_2's were crossed and the resulting strain was designated MISS MARK.

With this crossing scheme, males of MISS MARK for release had at least 42 percent of their genetic material derived from MERIDIAN. Broken down according to linkage group, this would be 88 percent for group 1, 0 percent (plus cross-overs) for group 2 and 50 percent for group 3. Since crossing over is certainly occurring in group 2, one can estimate that about 50 percent of the genetic material in MISS MARK originated from MERIDIAN. Subsequently, this portion was raised markedly by six generations of back-crossing to MERIDIAN, followed by re-extraction of the multiple markers. Unfortunately, this improved strain was not available at the time of the release experiments.

Some biological attributes of MISS MARK and MERIDIAN were compared. For each strain, 10 groups of 200 newly-hatched larvae were established and reared. On the sixth day after hatching, 55.7 percent of MERIDIAN and 59.5 percent of MISS MARK had pupated. Of 2,000 initial individuals, 91.4 percent of MERIDIAN and 87.3 percent of MISS MARK emerged as adults. Both strains were close to 50 percent female. Adult longevity of the two strains was also similar.

Sexual capacity of males was tested by placing a single 7-day-old male of each strain with ten 7-day-old females, five of

each strain, for 24 hours. The females were then dissected and examined for insemination. For each strain, 40 males were tested. The MERIDIAN males inseminated a mean of 3.6 ± 1.2 females, whereas the MISS MARK males inseminated 3.1 ± 1.2 females, a difference that is not statistically significant. Neither strain showed any selectivity as to female; females of both strains were inseminated indiscriminately.

Mating competitiveness of males was assayed by placing 50 virgin MERIDIAN females in an 18 x 12 x 12-inch cage with 200 males, 100 MERIDIAN and 100 MISS MARK. All individuals were 4–6 days old and no anesthesia was used. After 24 hours with the males, females were blood-fed and removed to individual vials for oviposition. The eggs were hatched and the specimens reared to adulthood to determine paternity. Ten tests were made at different times, involving examination of 477 females. A mean of 45 percent of the females were inseminated by MISS MARK, compared to 52 percent inseminated by MERIDIAN and 3 percent inseminated by males of both strains. It should be noted that females of *Ae. aegypti* are basically monogamous (Craig, 1967). Multiple insemination occurs only when there is a very short interval between the first and the second males.

Therefore, the MISS MARK and MERIDIAN strains are comparable in a number of biological attributes and the MISS MARK males were only slightly less competitive in mating.

FIELD RELEASE. Operations for the field release of the marked males were initiated on September 5. Although sufficient numbers of eggs of the homozygous marked strain, MISS MARK, were not available at that time, the prospects of encountering cold weather during the terminal period if field tests were postponed precluded further delay. Eggs of the F_2 generation of the genetic crosses were used to supplement the numbers of MISS MARK eggs. Theoretically, one-fourth of the adults from this F_2 generation would be homozygous MISS MARK individuals, one-half would be *Si*/+ and therefore detectable, and one-quarter would be wild type. Since females usually mate only once, field females mated by heterozygotes would have progeny with 50 percent showing the marker.

The MISS MARK and the F_2 larvae were reared by mass production methods (Morlan *et al.*, 1963) using 5,000 larvae per tray. Pupae were separated from the larvae by means of a mechanical separator (Fay and Morlan, 1959) and subsequently separated into sexes using a second run with the same device. All samples of pupae were carefully examined to be sure that no larvae were inadvertently included. The male pupae were then allowed to emerge into gallon cardboard cartons covered with black nylon mesh. Before release in the field, the adults in each carton were inspected to insure that no adult females were present. Occasionally a female was found and removed from the carton with an aspirator. Collections of pupae from each tray were made for a 3-day period; the later separations of pupae required more time to insure accurate separation of the female pupae.

Since only a limited number of marked males were available, the area selected for the release was one which had shown moderate positivity for *Ae. aegypti* in ovitrap collections made earlier in the summer. Containers were found in this area with *Ae. aegypti* larvae just prior to the release period. All males were released at a single site. Some information on the general direction and extent of dispersal was obtained using eight black traps (Fay, 1968), one situated at 125 feet in each of the four cardinal directions from the release site and one located at 75 feet in the NE, SE, SW and NW directions from the release point. These traps were placed in operation daily, just before release of the males and were removed usually at 4:30 p.m. Collections of adult mosquitoes were identified for each trap.

To obtain the eggs needed to ascertain any mating between the released males and

the field females, a grid of ovitraps (Fay and Eliason, 1966) at 120-foot intervals was established to cover a circular area of 540 feet in all directions from the release point. The ovitraps were serviced daily and all eggs were conditioned at a field laboratory prior to shipment. The eggs were then hatched and the resulting adults checked for genetic markers.

Adult males were released daily from September 15 to 28 inclusive. On 9 of the 13 days, at the time of release the wind was from the SE, 1 day it was from the S, and on the other 3 days no wind was evident. The black traps were operated for 16 days, *i.e.,* until 3 days after the last release.

Trap 1 (125 feet N) was placed by a tree trunk in an open location and failed to capture any *Ae. aegypti.* Trap 2 (75 feet NE) was on the N side of an abandoned auto and was positive on 4 days with a total of 11 *Ae. aegypti* males. Trap 3 (125 feet E) was on the N side of a house in a more protected location and was positive on 6 days with a total of 10 *Ae. aegypti.* Trap 4 (75 feet SE) was surrounded by a group of bushes and was positive on 3 days with a total of 3 *Ae. aegypti.* Trap 5 (125 feet S) was in an open position and was positive only 1 day with 1 *Ae. aegypti.* Trap 6 (75 feet SW) was placed by a tree trunk in an open location (similar to that of Trap 1) and was positive on 7 days with a total of 8 *Ae. aegypti.* Trap 7 (125 feet W) was located on the S side of a two-story building and was positive on 10 days with a total of 34 *Ae. aegypti.* Trap 8 (75 feet NW) was protected on the N and W by an L-shaped building and was positive on 12 days with a total of 519 *Ae. aegypti.*

From visual observations of the adult males at the times of release and from the subsequent black-trap catches, the general movement of the released males was downwind, and distances of 75 feet and 125 feet were reached within 1 day. The effectiveness of the black traps in recapturing the males was correlated to some extent with protection from the wind at specific locations. Data on the total daily catches are shown in Table 1. An average of 3.4 percent of the released males was recaptured each day. The trap-collection adults were classified as those with the MISS MARK genetic markings and those indistinguishable from the wild type. If one-fourth of the 12,000 released F_2 strain were

TABLE 1.—Adult male *Aedes aegypti* recaptured daily from September 15–30, 1967, by eight black traps, four located at 75 feet and 125 feet, respectively, from the release point.

Sept. 1967	Release Temp. (°F.)	Wind Dir.	Males Released		Trap Hours	Adult capture			Percent of Daily Release
			MM	F_2		MM	F_2	*Culex*	
15	72	SE	100	200	12–4	1	6	0	2.3
16	70	SE	200	2000	11–4:30	7	24	0	1.4
17	70	0	300	1000	9:30–4:30	12	41	5	4.1
18	72	0	300	2500	10:30–4:30	19	73	3	3.3
19	72	SE	200	1500	10:30–4:30	15	52	1	3.9
20	74	SE	80	1500	10:30–5:00	21	131	1	9.6
21	75	S	30	1500	10:30–4:30	0	12	1	0.8
22	71	SE	200	1000	10:30–4:30	8	20	1	2.3
23	65	SE	200	600	9:30–4:30	8	20	3	3.5
24	66	SE	600	200	9:30–4:30	14	20	6	4.2
25	68	0	1000	0	10:15–4:30	19	25	1	4.4
26	69	SE	1000	0	10:30–4:30	12	1	0	1.3
27	65	SE	600	0	10:15–2:30*	21	1	1	3.7
28	51	NW	...	..	9:00–4:30	0	1	2	...
29	50	0	...	..	8:45–4:30	1	0	14	...
30	54	0	...	..	8:30–4:30	0	1	43	...
Total	..	..	4810	12000		158	428	85	...

* Rain.

homozygous for the MISS MARK characters and were added to the 4,810 MISS MARK males, then the 158 recaptured individuals represent 2.1 percent of the 7,810 released while the other 428 males represent 4.8 percent of the 9,000 released. This may indicate the capture of wild males by the traps.

It is interesting to note the marked drop in the numbers of captured *Ae. aegypti* on September 28, 29, and 30. This may be the effect of the lower temperatures and reduced flight or it may be the effect of the males leaving the trap area. The former assumption appears more valid. With the black trap, the catches are almost entirely limited to adult Diptera; insects of other orders appear to be accidental catches. The only other species of mosquito taken in the trap area was *Culex pipiens quinquefasciatus*. Although this species represented a minority in most of the catches, it became the predominant species in the last 3 days of the trapping period. This shift in the species catch distribution may arise from the temperature but it should be noted that a good correlation exists between the trapping hours and the capture of *Culex* adults. When the traps were placed in operation by 9:30 a.m. or earlier, *Culex* were taken more frequently, a time-of-day factor which will be checked in more detail.

Daily ovitrap collections were made from September 20, 5 days after the first male release, and continued through October 13, 16 days after the last male release. During the period the mean daily temperatures were in the lower 70's for the first 4 days, in the upper 60's for the next 5 days, in the lower 50's for 3 days, returning to the 60's for 8 days and then in the upper 50's for the last 4 days.

The numbers of positive ovitraps showed a correlation with the mean temperature of the preceding day (Fig. 2). With mean temperatures of 71°–75° F., 8 to 19 paddles were positive the following day (average of 13.7 positive); for the 8 days with mean temperatures of 66°–70° F., 1 to 15 paddles were positive (average of 7.5 positive); for the 4 days with mean temperatures of 61°–65° F., 1 to 6 paddles were positive (average of 4 positive); for the 4 days with mean temperatures 56°–60° F., 0 to 6 paddles were positive (average of 2.7 positive); and on the 4 days with mean temperatures of 50°–55° F., from 0 to 1 paddle was positive (average of 0.75 positive).

Since the ovitraps were normally serviced before 2:00 p.m. of each day, it is felt that the temperatures of the preceding

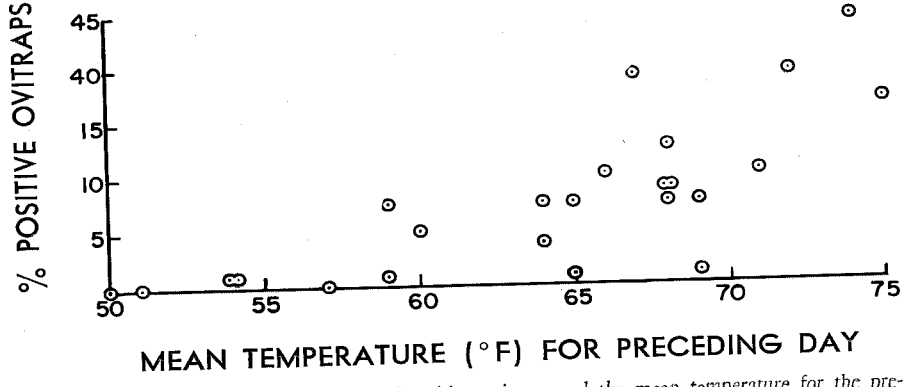

FIG. 2.—Relationship between the percent of positive ovitraps and the mean temperature for the preceding day based on values obtained at Meridian, Miss.

day had the greatest influence on the oviposition rate. Three days in the 66°–70° F. range preceded the 3-day cold spell and 5 days in the same temperature range followed the cold spell. The average number of positive ovitraps for the two periods were 7 and 8, respectively, indicating the same general population level for the entire sampling period. The decrease in positive ovitraps with lower temperatures appears then to be a factor of reduced oviposition rather than a decrease in the field population. The sensitivity of ovitraps in detecting the presence of *Ae. aegypti* in the field is lessened markedly on days with mean temperatures below 60° F.

One-fourth of the ovitraps were negative throughout the sampling period and one-half of the ovitraps were positive on 1 or 2 days only. The remaining one-fourth were positive on 3 to 8 days during the 24-day period. Since these traps showing higher positivity were contiguous in three areas of the grid, they may indicate centers of higher *Ae. aegypti* incidence. Based on the frequency of positive paddles at a given ovitrap, the presence of at least one ovipositing female each time is evident. If either the average number or the total number of eggs per paddle is used as a measure, the factors of preferred location of the trap or the lack of competitive sites must be considered.

A total of 1,084 *Ae. aegypti* eggs were taken on the ovitrap paddles during the sampling period. In addition, occasional paddles contained eggs of *Aedes triseriatus* or *Orthopodomyia signifera*. The eggs were conditioned for 48 hours and at the termination of the sampling period half of the paddles were shipped to the University of Notre Dame and the remainder to Technical Development Laboratories.

The adults from eggs of each paddle were examined for genetic markers and two paddles produced genetically marked adults. The first paddle taken on September 27 from Station 19W (approximately 360 feet west of the release point) contained 51 eggs and produced 18 male and 22 female adults. Of the 40 adults, 21 contained the Silver mesonotum marker in a heterozygous state indicating the probable mating of a heterozygous F_2 male with a field female. The second paddle, taken on October 8 from Station 16E (approximately 75 feet NE of the release point), contained three eggs and produced one male and one female adult. The female carried the Silver mesonotum marker in heterozygous state.

Although the tests described were of a preliminary nature, they demonstrated the feasibility of using the black traps to determine the dispersion pattern of released males, as well as the utility of obtaining eggs by means of the ovitraps for confirming the mating of the released males with the field females.

SUMMARY. The genetic markers, Silver mesonotum and spot abdomen, were introduced in homozygous condition into a field strain of *Ae. aegypti* without affecting the vigor or mating competitiveness of the marked males. Release of the males in an area at Meridian, Mississippi, showed downwind dispersion with recapture by black traps at distances of 75 and 125 feet. Mating with field females was demonstrated in two instances by marked progeny from eggs collected in ovitraps.

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GYNANDROMORPHISM IN *CULEX TRITAENIORHYNCHUS*

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INTRODUCTION. Gynandromorphs have been found in fair numbers in laboratory strains of mosquitoes: e.g., in the hundreds in *Culex pipiens* (Laven, 1967) and *Aedes aegypti*, (Craig and Hickey, 1967). In natural populations they are comparatively rare; only 91 specimens belonging to 23 species have been described in the genera *Toxorhynchites, Trichoprosopon, Mansonia, Orthopodomyia, Aedes, Haemagogus, Culiseta,* and *Culex,* (for references to reported gynandromorphs see Kitzmiller, 1953, Brust, 1966, and Lee, 1967). All of these have occured in the subfamilies Culicinae and Toxorhynchitinae but surprisingly none in the subfamily Anophelinae. This report describes five gynandromorphs, the first reported in *Culex (Culex) tritaeniorhynchus* Giles.

MATERIALS AND RESULTS. The first gynandromorph was found in October 1967, in the F_1 offspring of a cross between two strains of *Culex tritaeniorhynchus* maintained in our laboratory. The mother was from the Dacca strain collected originally from Dacca, East Pakistan, and the father was from the Rattipindi strain, collected from a village of the same name near Mangowal District Gujrat, West Pakistan. The Dacca mother laid a raft of 75 eggs from which 43 larvae hatched. Of the remaining 32 eggs, 22 embryonated eggs did not hatch and 10 appeared to be non-embryonated. Only 18 of the larvae reached adult stage (4 normal females and 13 normal males and the 1 gynandromorph). The F_2 crosses using these males and females did not succeed. All other crosses between these two strains resulted in nearly 100 percent hatch. The F_1 males and females from these latter crosses all appeared to be fertile in subsequent F_2 crosses.

Gynandromorph No. 2 was found in May 1968, in the Karachi strain, originally collected near Karachi in November 1967, and maintained in a laboratory strain since that time. In the course of inbreeding for the recovery of mutants, one gynandromorph was found in the F_2 from an original brother-sister mating. No other abnormal mosquitoes were found in the egg raft, nor in the F_3 nor F_4 from the sibs.

However, the third gynandromorph was also found in the Karachi strain also in May 1968. A mutant, *hairy palpi,* had been discovered in the Karachi strain, and the sibs from the egg raft in which this mutant had occurred were inbred and selected for it. During the inbreeding the gynandromorph was discovered in the course of routine examination of adults.

The fourth gynandromorph occurred in May 1968 in a strain which was actually a hybrid between the Dacca and Karachi strains. These populations were being tested for possible incompatibility; the inbreeding had already produced a white-eyed mutant, and further selection for

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