PRODUCTION OF *AEDES MEDIOVITTATUS* (COQUILLETT) AND ITS RESPONSE TO TEMPERATURE AND FOOD CONDITIONS[1]

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In Puerto Rico during operations of the Aedes aegypti Eradication Program, a second mosquito, *Aedes mediovittatus* (Coquillett), occurred in the same general ecological niches as the target species. To obtain specimens for training of operational personnel and for evaluation purposes, a rearing procedure for *Ae. mediovittatus* was developed. The responses of the two species to temperature and food regimens were compared in an effort to evaluate the potential of *Ae. mediovittatus* as a possible competitor for *Ae. aegypti*.

INSECTARY PRODUCTION. Eggs were collected at field sites on ovitrap paddles (Jakob and Bevier, 1969) by PHS operational personnel and were sent to the Technical Development Laboratories (TDL), Savannah, in March 1967. Eggs of *Ae. mediovittatus* and *Ae. aegypti* on the same paddles confirmed the use of the same ecologic niches by both species. All eggs were reared and the two species were separated in the adult stage. The *Ae. mediovittatus* adults were held in a 22-inch cubical cage (Morlan *et al.*, 1963), and when offered human blood, they alighted and fed readily on the underside of exposed areas. Little irritation was felt during the bite. After reaching repletion, the adults remained at the biting site for several minutes, unless they were purposely disturbed. Subsequently the adults were adapted to feed on rabbits offered for a 2-hour period on 3 days each week.

Gray or tan velour, ¾- by 5-inch paper strips provided an oviposition substrate. Two strips were clipped to the inside of a straight-sided, 1-pint glass jar which was coated on the outside with a glossy black ceramic finish. The lower end of the strips extended about three-fourths of an inch into water, and the eggs were laid, often several layers deep, above the water line. The strips were removed daily and placed, while moist, in a plastic container without any free water; the containers were covered tightly with aluminum foil to prevent evaporation. After 7 to 14 days of conditioning, the eggs hatched readily when placed in a 24-hour-old preparation of brewer's yeast in water. If free water was inadvertently sealed into the plastic container, many of the eggs hatched on the eighth day.

Larvae were reared in 2- by 9- by 13-inch rectangular, enamel pans at a density of 300 larvae per liter of tap water. Laboratory chow, ground to pass through a 40-mesh screen, was added daily at 0.15 mg. and 0.30 mg. per larva on days 1 and 2, and at 0.60 mg. per larva daily thereafter. With water at 25° to 27° C, the first stage larvae completed development on days 2 and 3, second stage on days 3 to 6, third stage on days 4 to 7, and fourth stage on days 6 to 12. The first pupae appeared on day 8. Adult males emerged after 2 to 3 days and adult females after 3 to 4 days. Rate of survival to the adult stage when this procedure was used ranged from 78 to 85 percent.

The average sex ratio was 54 male:46 female pupae. Separation of the two sexes with a mechanical separator (Fay and Morlan, 1959) was only partially successful. The pupae separated as males ended

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with an 82:18 male:female ratio, whereas the female pupal fraction had an 18:82 male:female ratio.

When less than 1 day old, 550 males and 300 females were placed in a colony cage and 50 adult females were removed daily for 4 days to determine the incidence and extent of mating. Each group of females was offered a rabbit as a blood source on 4 successive days. Only occasionally did a 1-day-old female feed, but essentially all of them had been successfully mated. Nearly all females took a blood meal at 48 hours, and eggs were laid 4 to 10 days after the first blood meal. Initial oviposition ranged from 44 to 55 eggs per female. Eggs conditioned for only 1 or 2 days before flooding failed to hatch; with 4 days of conditioning 39 percent of the eggs hatched; and with 7 to 14 days, an average of 73 percent hatched.

From a colony supplied daily with 50 adult males and 50 adult females, an average of 2,200 eggs were produced each day. From 8:00 a.m. to noon, 26 percent of the eggs were laid; from noon to 4:00 p.m., 14 percent; from 4:00 p.m. to 8:00 p.m., 41 percent; from 8:00 p.m. to midnight, 5 percent; and from midnight to 8:00 a.m., the remaining 14 percent.

RESPONSE TO TEMPERATURE AND FOOD CONDITIONS. In pretreatment surveys in urban areas in Puerto Rico, the incidence of *Ae. mediovittatus* was only slightly lower than that of *Ae. aegypti*; and after larvicide applications, *Ae. mediovittatus* appeared less affected (Regnier *et al.*, 1971). Although the chances for dispersion from Puerto Rico would seem to be essentially equal for the two species, *Ae. mediovittatus* apparently has been unable to establish itself permanently in the southern United States. Some limiting factors to permanent dispersion must exist. One may be temperature. The mean temperatures for January and July in Puerto Rico are recorded as 73° F and 79° F, respectively; the highest afternoon temperature as 86° F; and the lowest night temperature as 68° F. To clarify their influence on the larval development of the two species, several temperatures and food levels were tested.

The methods followed in checking the effects of food and temperature have been described by Keirans and Fay (1968). Twenty-five first stage larvae in a liter of tap water were fed finely ground, standard laboratory chow at three levels: full rations of 0.15 mg and 0.30 mg per larva on days 0 and 1, respectively, and 0.60 mg per larva daily thereafter; and one-half and one-quarter rations of proportionately less food. Replicate tests were made with each food regimen in control cabinets set at constant temperatures of 60°, 70°, 80° and 90° F, and fluctuating temperatures of 50°–70° F, 60°–80° F, 70°–90° F, and 80°–100° F, respectively. The fluctuating temperatures were programmed so that with the 50°–70° F range, for example, the temperature changed at a uniform rate from 60°–50° F and back to 60° F in 12 hours and then from 60°–70° F and back to 60° F in the succeeding 12 hours. Relative humidity was held at 80 percent for all temperature conditions.

The results (Table 1) show that: (1) at all comparable food regimens and temperature conditions the periods for larval development were usually longer for *Ae. mediovittatus*; (2) only at 80° F and 70°–90° F with full food were the days to completion of pupation equivalent for the two species; and (3) the temperatures giving essentially complete pupation with *Ae. mediovittatus* were limited to the 80° F and 70°–90° F temperatures at all food levels whereas *Ae. aegypti* with full food had essentially complete pupation at constant temperatures of 70°, 80° and 90° F and with the fluctuating temperature patterns of 60°–80° F, 70°–90° F, and 80°–100° F.

The limitations of *Ae. mediovittatus* development imposed by temperatures of 60° F and 50°–70° F may well explain the failure of the species to establish in southern United States since more severe temperatures are often encountered even in southern Florida. At the higher tem-

TABLE 1.—Rate of development of *Ae. aegypti* and *Ae. mediovittatus* with various food and temperature conditions. Values represent mean of two replicates.

	Days to 1st Pupa			Days to Last Pupa			Percent Pupation		
Chamber Air Temp. ° F.	¼ Food	½ Food	Full Food	¼ Food	½ Food	Full Food	¼ Food	½ Food	Full Food
Aedes aegypti									
60	32	25	26	38	45	43	26	51	76
50–70	22	21	21	38	28	38	83	84	60
70	11	10	10	18	16	14	76	98	100
60–80	11	11	9	24	16	13	100	100	100
80	9	8	7	9	12	13	100	82	100
70–90	9	8	7	9	12	13	66	98	100
90	7	6	5	14	10	8	100	100	98
80–100	8	6	5	15	11	8	82	100	96

At 46° F. larvae were dead in 5 days; at 54° F. larval growth was not complete; at 102° F. larvae were dead in 5 days.

Aedes mediovittatus									
60	52	52	45	78	100	63	48	48	22
50–70	41	37	42	56	64	60	16	32	8
70	16	17	16	27	28	25	60	82	74
60–80	18	16	16	22	26	33	86	94	70
80	10	9	9	36	20	13	100	100	100
70–90	11	9	9	34	20	13	100	100	96
90	9	8	7	26	18	14	84	88	90
80–100	12	10	9	28	20	18	86	78	84

At 50° F. no larval growth occurred in 11 days; at 92° F. and 94° F. larvae were dead in 9 and 15 days; and at 96° and 98° F. larvae were dead in 3 days.

peratures of 90° F and 80°–100° F, the time interval to completion of pupation for *Ae. mediovittatus* was almost twice as long as that for *Ae. aegypti*. Therefore, in many cases the breeding sites might dry up before *Ae. mediovittatus* pupation was complete. *Ae. aegypti*, in contrast, showed faster pupation at these temperatures provided adequate food was available. Furthermore, the *Ae. aegypti* larvae apparently withstand the higher temperatures more successfully.

Experiments with mixed adult *Ae. aegypti* and *Ae. mediovittatus* introduced another factor which might limit the establishment of *Ae. mediovittatus* in new areas already occupied by *Ae. aegypti*. Interspecific mating was demonstrated when 40 virgin *Ae. mediovittatus* females were caged with 50 newly emerged *Ae. aegypti* males for 5 days and then 50 newly emerged *Ae. mediovittatus* males were added. Starting on day 2 the females were offered a blood meal every other day. No eggs were laid, however, until 10 days after the *Ae. mediovittatus* males were added. Only 33 eggs were obtained in a single oviposition, and all of them hatched to give *Ae. mediovittatus* larvae.

As a control, a second group of 40 *Ae. mediovittatus* females were held in a colony cage for 5 days and then 50 *Ae. mediovittatus* males were added. Blood meal scheduling was the same as above, and eggs were laid 2 days after the adult males were added. During 6 nights of a 10-day period 1,477 eggs were obtained. After conditioning of the eggs, a total of 884 *Ae. mediovittatus* larvae were obtained.

When 40 virgin *Ae. aegypti* females were placed with 50 *Ae. mediovittatus* males for a 17-day period prior to the introduction of *Ae. aegypti* males, the first eggs were obtained on the 6th day. During a 12-day period, 910 eggs were

collected, conditioned, but failed to hatch. Three days after the *Ae. aegypti* males were introduced, thousands of eggs were obtained in a 2-day period and after conditioning showed good hatch.

These results indicate that the *Ae. aegypti* males prevented the subsequent insemination of the *Ae. mediovittatus* females by the *Ae. mediovittatus* males, with the exception of possibly a single female that laid the 33 eggs. The *Ae. mediovittatus* males, however, did not prevent subsequent insemination of *Ae. aegypti* females by males of the latter species.

Similar results (Gubler, 1970a) occurred with mixed populations of *Aedes albopictus* and *Aedes polynesiensis*: (1) virgin *Ae. albopictus* females did not oviposit; (2) *Ae. albopictus* females combined with *Ae. polynesiensis* males were not inseminated and did not lay eggs; (3) virgin *Ae. polynesiensis* oviposited readily and on the same schedule as inseminated females but had infertile eggs only; (4) *Ae. polynesiensis* females mated readily with *Ae. albopictus* males but laid only sterile eggs; and (5) *Ae. polynesiensis* females inseminated by *Ae. albopictus* males were not subsequently inseminated by *Ae. polynesiensis* males. Some *Ae. polynesiensis* females were not inseminated by the *Ae. albopictus* males and subsequently successfully mated with the *Ae. polynesiensis* males.

Although *Ae. albopictus* was able to eliminate *Ae. polynesiensis* when the two species were reared together in small cages (Gubler, 1970b), in a large cage test (Rozeboom, 1971) after a marked reduction in numbers, a small proportion of *Ae. polynesiensis* persisted. The large cage test was made using regulated insectary conditions so that the effects of limiting environmental conditions were not exerted.

The combined effects of temperature and potential interspecific mating of *Ae. mediovittatus* and *Ae. aegypti* may explain the failure of *Ae. mediovittatus to* establish itself in southern United States.

SUMMARY. An insectary method for *Ae. mediovittatus* production with an efficiency of approximately 80 percent has been developed. Comparisons in the development rate of *Ae. mediovittatus* and of *Ae. aegypti* at constant temperatures of 60°, 70°, 80° and 90° F and at fluctuating temperatures of 50°–70°, 60°–80°, 70°–90° and 80°–100° F showed *Ae. mediovittatus* to be more susceptible to the lower temperatures. In experiments with mixed adult populations the *Ae. mediovittatus* females mated with *Ae. aegypti* males failed to lay eggs and did not subsequently mate with their own males. The reciprocal cross of *Ae. aegypti* females with *Ae. mediovittatus* males apparently did not occur.

ACKNOWLEDGMENTS. The authors wish to thank Mr. William Prince and Mr. Norman Johnson for their efforts in the experimental work.

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