ARTICLES

COLONIZATION OF *CULEX SALINARIUS* IN THE LABORATORY [1]

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Mosquito-rearing in the laboratory has become of increasing importance in recent years because of studies of arbovirus-vector relationships. In spite of expanding needs, however, only a few colonies of vector species are available. Some species that have been successfully reared in captivity are no longer maintained for studies in the laboratory. Such has been the case with *Culex salinarius,* originally established by Wallis and Spielman (1953). While Chapman (1966) has intermittently reared the southern form of this species in Louisiana, no colony exists of the northern form. Its recolonization for arbovirus-vector studies was therefore attempted, and the purpose of this communication is to report the success of this undertaking.

PROCEDURE AND RESULTS. Colonization attempts were initiated in this laboratory during the summers of 1964–1966, but because of a decline of the mosquito population during these years, due to unusually dry weather, the field material collected was meager. During the period from 27 June through 20 July 1967, however, approximately 50 biting female mosquitoes were hand-captured at Indian River, Milford, Connecticut. These were transported alive to the laboratory, where the *C. salinarius* females were allowed to engorge on blood from man and were then placed in cages containing water for oviposition. They were maintained in the insectary at 80° F. and 80 percent relative humidity. As egg rafts were desposited by the females, each raft was put out to hatch in white enameled pans containing starting media (50 mg. of dry powdered Brewer's yeast and 50 mg. of Bacto Brain Heart infusion powder per 1 liter of distilled water) as described by Heal and Pergrin (1945). After the eggs were hatched, Gaines dog food pellets were added as nutrient for the larvae. During the development of the larvae, each pan was kept covered with a sheet-metal plate except at feeding time. Following 10 to 14 days of larval development, 4th instar larvae were examined for taxonomic verification of species. As pupae developed, they were transferred to half-pint ice-cream cartons containing water and allowed to emerge as adults in a screened 24 x 24 x 24-inch cage. Ten percent sucrose solution was provided for food, and daily blood meals (from humans) were offered during daylight hours.

From the succession of egg rafts produced by the original wild females in captivity, larvae and pupae were reared to provide a continuous supply of young adults in the first laboratory-reared generation. From 27 July through 6 September, 7,809 pupae were harvested and added to the population. Blood meals (from humans) were given five times each week during a 4-week period; as a result, 269 egg rafts were produced by the first (F_1) laboratory-reared generation. Approximately 21 percent of these hatched, and larvae were then reared in the same manner as before, except that they were established in a separate series of larval rearing pans to provide pupae for stocking

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a new cage (identical to the F_1 cage) housing the F_2 generation of adults. From 20 August through 6 September, 2,091 pupae were obtained and allowed to emerge as adults in the F_2 generation cage. To offset the diminishing hours of natural daylight, early in September an automatically regulated 100-watt electric light bulb was set to maintain 16-hour photoperiods. F_2 generation adults were provided blood meals by being allowed to feed on a young chicken twice each week. Water in a pan was left in the cage for oviposition, and from 3 through 27 September 148 egg rafts were obtained. Fifty-nine percent of these hatched, and larvae from them were again maintained in a separate series of rearing pans to provide pupae for stocking an F_3 generation cage (identical to the F_1 and F_2 generation cages). After viable egg rafts were obtained from the F_2 generation of laboratory-reared *C. salinarius,* blood-feeding of the original wild-caught females and of the F_1 generation population was discontinued.

Another cage was established 15 September to house adults in the F_3 generation. It was identical in size to the F_1 and F_2 cages and was maintained in the same insectary room. From 15 through 29 September, 3,371 pupae were allowed to emerge into the cage after they were harvested from larval material produced by the F_2 generation. On 22 September biweekly blood meals from chickens were offered to the females, but little feeding activity occurred until 26 September. On 1 October the first F_3 generation egg rafts were produced.

During the first week of October, 38 egg rafts were produced from the F_3 generation but only five hatched. Larvae from these exhibited considerable variability in rate of development. While a few progressed rapidly to the 4th instar within 14 days, others were delayed in the 2nd and 3rd instars. First pupae appeared in most pans after 2 weeks of larval development, but the peak of pupal production did not occur until after 3 weeks, and some larvae in each of the pans did not pupate for as long as 5 weeks after hatching. During the second week of October, 78 additional egg rafts were obtained, of which seven hatched. From these, larvae were reared for the next generation, and the pupae produced were added to the F_3 generation cage for routine rearing of *C. salinarius* in the laboratory.

Throughout the winter months the percentage of fertile egg rafts remained quite variable. During this time, large numbers of rafts were put out to hatch to compensate for the low fertility, and it was possible to maintain the experimental cages well stocked with adult mosquitoes. The percentage of egg rafts that hatched tended to increase after January 1968, and is well over 50 percent at the present time.

DISCUSSION AND SUMMARY. Initial attempts to re-establish a laboratory colony of *C. salinarius* during the summers of 1964–1966 were unsuccessful because of prolonged below-average rainfall that resulted in drastic reduction in the populations of this species in Connecticut. The numbers of larvae that could be collected at one time were insufficient to supply an adequate stock of adults for colonization work during those years. With the return of normal amounts of rainfall in the spring and early summer of 1967, adults reappeared in reasonable abundance by late June. From wildcaught females, egg rafts were obtained to provide an abundant stock of laboratory-reared larvae that developed to pupation all about the same time. These provided a population of adults of nearly uniform age. These adults fed on blood and in turn produced fertile egg rafts to supply stock for the next generation of laboratory-reared material. The percentage of fertile egg rafts was variable in the early generations but after several months of continuous rearing, viability increased and thereafter remained consistently over 50 percent.

Although uniform production of fertile eggs has been achieved, *C. salinarius* will be less easily maintained in the laboratory than are closely related species such as *C. pipiens* and *C. quinquefasciatus* because

of its long period of larval development. The first pupae produced are predominantly males, and the peak of production of female pupae occurs as much as 2 weeks later. Since the males are less hardy than the females in confinement, many of them die before the majority of the females can be added to the cage in a single generation. Therefore, to insure continuous laboratory rearing of *C. salinarius* it is essential to produce overlapping generations to provide the necessary numbers of males in the cage for sustained fertility.

As rearing of the initial generations progressed into August and September, there was concern about the possibility of the onset of pre-hibernation activity of the adults. Wallis (1959) showed that *Culex restuans* females could not be induced to feed on blood during the late summer; adult females of this species utilized sugar solutions for fat body formation in preparation for winter hibernation. The same was reported for *Culex tarsalis* (Bennington *et al.*, 1958). Although the factors responsible for initiating pre-hibernation activity of *Culex* are not known, it was considered important to maintain uniform lengths of daily photoperiods in the adult *C. salinarius* colony. It was hoped that utilization of the larval rearing method of Heal and Pergrin (1945) would be helpful in preventing such activity since the larvae, in covered rearing pans, were isolated from changing photoperiods. With this treatment of the larvae and with maintenance of the adults in a standard photoperiod at constant laboratory temperature, no inherent tendency for diminished blood-feeding was detected.

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