SIMPLIFIED METHOD FOR MAINTAINING *CULEX PIPIENS* LINNAEUS IN THE LABORATORY (DIPTERA: CULICIDAE)

J. McLINTOCK [1]

Entomology Research Institute, Research Branch, Canada Department of Agriculture, Central Experimental Farm, Ottawa, Canada

When a laboratory colony of *Aedes aegypti* is not being used for experimental purposes, it can be preserved for about six months by storing the eggs, after appropriate conditioning that involves drying (Shannon and Putnam, 1934). On the other hand, the eggs of *Culex pipiens* will not withstand drying, and the colony must be reared continuously even when it is not in use. The larvae are usually reared in trays, and this involves the expenditure of considerable time and labor in measuring out rearing media, transferring pupae to cages, harvesting of eggs, and cleaning utensils. For some years this laboratory has had a colony of *C. pipiens;* at first the colony was reared by the usual method, but in 1954 I began attempts to find a less time-consuming procedure for maintaining this species. The following procedure was in use at the end of 1955, but the colony was destroyed early in 1956. Our present colony was established in October, 1957, and from the start has been maintained continuously by the method that follows.

All stages of the mosquito are kept in one cage. The only labor is in adding larval food to the dishes three times a week, replacing the rearing dishes for the larvae once or twice a year, placing a chicken in the cage twice a week as a source of blood for the females, and keeping two small bottles of sugar solution in the cage.

Our rearing room is maintained with a relative humidity of 75 to 80 percent and a temperature of 70° to 72° F. These temperatures are below the optimum for *C. pipiens,* but we are obliged to use them because *Culiseta inornata* is reared in the same room, and its temperature optimum is near 68° F.; both species survive well at the compromise temperatures. The light in the room is controlled automatically to give an artificial daytime of 17 hours and a six-hour period of darkness preceded by a half-hour sunset period and followed by a half-hour sunrise period. *C. pipiens* swarms and mates in the cage during the sunset and sunrise periods.

The cage containing the colony is 2 x 2 x 2 feet with screen on two sides, back, and top, and a "Masonite" bottom; the front is of quarter-inch "Plexiglas" in a wooden frame hinged at the top. A cotton sleeve, eight and one-half inches in diameter, is inserted in the center of the Plexiglas panel. The floor of the cage is covered with green blotting paper.

Two pyrex crystallizing dishes, 190 mm. in diameter and 100 mm. deep, each containing about 1500 ml. of Bates' "Medium S" (Bates, 1941), are placed in the cage for the eggs, larvae, and pupae. On the day that the dishes are prepared, the level of the surface of the medium in each dish is marked with a grease pencil on the outside of the dish, and this level is maintained by the addition of distilled water when necessary. In addition to the Bates' Medium, each dish is started with 100 mg. dry "Difco" Brain Heart Infusion; 50 mg. dry, powdered baker's yeast; and 240 mg. dry, powdered, whole wheat bread crumbs. Three times a week thereafter, 50 mg. of the yeast and 240 mg. of the bread are added to each dish. The bread crumbs are prepared by drying slices of whole wheat bread in an oven at 40° C. for several days then passing the dried bread through a grinder with a 40-mesh (.025 inch) sieve. The yeast is "Fleischmann's, Active Dry" passed through a

[1] Entomologist.

grinder with a 60-mesh (.016 inch) sieve. A continuous stream of compressed air is bubbled slowly through the medium in each dish to prevent formation of a scum that would smother the larvae and pupae.

The females deposit their egg rafts on the surface of the medium during the period of darkness. The eggs hatch and the larvae and pupae complete their development in the dishes. The dishes appear to be crowded, but this apparently does not harm the colony other than to give a greater variation and smaller mean size in the adults. One hundred dead females picked at random from the bottom of the cage had a maximum wing length of 4.4 mm., a minimum of 3.2 mm. and a mean length of 3.8 ± 0.02 mm.[2] One hundred females reared in trays containing 250 larvae in 2½ litres of medium had a maximum wing length of 4.3 mm., a minimum of 3.9 mm. and a mean length of 4.0 ± 0.003 mm. The probability of this difference in the means occurring by chance was less than 1/1000 so it seems safe to assume that the method here recommended for maintaining the colony produces smaller and less uniform adults. In the tray-reared specimens, only four percent of the larvae died, one percent of the pupae, and the ratio of males to females in the emerged adults was 1:1.06.

In time, a sediment forms at the bottom of the dishes and occasionally the surface of the medium has to be skimmed with filter paper if a mold begins to form on the floating debris. One dish has been left in the cage for a year and when removed was still teeming with larvae and pupae. Usually the dishes are changed every six or nine months, for aesthetic reasons.

Two 30-ml. serum bottles with wicks of absorbent dental rolls and containing 10 percent sucrose solution are kept in the cage for the adults. A chicken in a restraining cage is placed inside the mosquito cage twice a week to provide the females with blood. The chicken is placed in the cage at 9:00 a.m. and removed at 4:30 p.m., and, because *C. pipiens* feed more readily in the dark, the mosquito cage is covered with a black cotton shroud while the chicken is in the cage; this also serves to keep the chicken quiet. The blotting paper on the floor of the cage is replaced from time to time when it becomes heavily soiled with dead mosquitoes and feces.

In the above method, all stages are present in the cage at the same time, and, so long as withdrawals are not too great, the colony, without further modification, can provide material for experimental purposes. If greater numbers, or larger and more uniform specimens are required, eggs can be removed from the cage for tray rearing.

Our present colony of *C. pipiens* has been maintained in one cage for 20 months. The colony was started from hibernating females from Guelph, Ontario, which were placed in the cage when the temperature of the rearing room ranged from 74° to 79° F. and relative humidity and light were as above. The first blood meal was taken 10 days after the mosquitoes were brought into the laboratory, the first eggs were laid three days after the first blood meal, and adults began to emerge 11 days after the first eggs were laid. The temperature was gradually lowered later to accommodate the *Culiseta inornata,* and it is estimated that the time from egg to egg for the *C. pipiens* in the cage is now about 25 days. No evidence of autogeny has yet been found in our strain of *C. pipiens,* it is homodynamous and, judging from the frequency with which my hand has been bitten, it is anthropophilous as well as ornithophilous.

It should be noted that we have not yet been successful in adapting *C. inornata* to this method of rearing. The larvae of this species cannot develop in as wide a range of habitats as *C. pipiens,* and mortality among the larvae is always high after the first generation in the cage. On the other hand, *Aedes aegypti* thrives in the cage when a guinea pig supplies the blood instead of a chicken, and it is not necessary to shroud the cage when the guinea pig

[2] Mean ± standard error.

is present. *A. aegypti* females lay their eggs on the surfaces of the media in the dishes and around the insides of the dishes just above the level of the surfaces of the media. To collect the eggs of this species, it is only necessary to hang strips of paper toweling down the inside wall of a dish with the bottom edges of the papers dipping into the medium.

It is likely that other stenogamous species with larvae that can tolerate a wide range of habitats could also be reared by this method.

SUMMARY. A method, requiring a minimum of time and labor, is described for maintaining a colony of *Culex pipiens* in the laboratory. All stages of the mosquito are kept in one cage, and the only attention required is the addition of larval food three times a week, provision of a blood meal for the females twice a week, and occasional cleaning of the cage and replacement of the two larval dishes. The adults are less uniform in size and have a smaller mean size than those produced from tray-reared larvae, but the colony appears to be vigorous and has been providing material for experimental purposes for almost two years.

References

BATES, M. 1941. Studies in the technique of raising anopheline larvae. Amer. Jour. Trop. Med. 21:103–122.

SHANNON, R. C., and PUTNAM, P. 1934. The biology of *Stegomyia* under laboratory conditions: I. The analysis of factors which influence larval development. Proc. Ent. Soc. Wash. 36:185–216.