

A MAGNETIC METHOD OF SEPARATING MOSQUITO PUPAE FROM LARVAE

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INTRODUCTION. Mosquito larvae, in rearing cultures, do not all pupate uniformly, and the time from first to final pupation usually lasts several days, depending on crowding, food, temperature and some intrinsic characteristics of the larvae (Shannon and Putnam 1934, Bar-Zeev 1956). In some laboratories, the pupae are transferred every day or so, by means of pipettes into emergence cages (Trembley 1955, Christophers 1960). This method is time-consuming, and practical only when relatively small numbers of mosquitoes are required. In other laboratories one places the container with the larvae and pupae in or below an emergence cage, so that the mosquitoes after emergence enter the cage (McKiel 1957, Bar-Zeev and Galun 1960). This method has the disadvantage that the larvae which did not pupate in time are lost. Furthermore, the adults thus obtained are not of uniform age. A method of separating mosquito pupae from larvae may also be useful for experiments on sterilization by irradiation, since the irradiated stage is the pupa.

A mechanical device for separating mosquito pupae from larvae has been described by Fay and Morlan (1959). The method consists of pouring the culture, containing pupae and larvae, through two glass plates, slightly separated and of adjustable distance so that most of the pupae, being a little larger than the larvae, are retained between the glass plates, while the rest of the culture drains into a receiving container below. According to the authors the separation of 1,000 pupae requires about 20 minutes.

The principle of the present method is as follows: When separation of pupae and larvae is to be carried out, a little iron dust is added to the culture. The larvae ingest some of the iron particles,

whereas the pupae (and prepupae) do not feed, and, therefore, do not contain any iron particles. Furthermore, larvae that feed, evacuate the contents of the alimentary canal before pupation, and thus free themselves again from the iron particles. The larvae and pupae are then exposed to a magnetic field, which will retain only the larvae.

MATERIALS AND METHODS. *Aedes aegypti* L. larvae were reared in jars containing each about 1,500 larvae in about 3½ litres of water, and kept at a temperature of 28° C. Pupation started on the 5th day and the first adults emerged on the 7th day. On the 6th day, therefore, separation of the pupae was carried out. Two-tenths gr. of iron dust (hydrogen reduced iron, Merck & Co. Inc., Rahway, N. J.) were added to each jar and the mixture stirred for a few seconds with a stick. The culture was then left for about 30 minutes, during which time the larvae ingested enough iron particles for an efficient separation to take place. The larvae and pupae were then filtered through cheesecloth, freed from adhering iron particles by washing with water and placed in a jar ("magnetic jar"), made of metal or any other material (Fig. 1), filled with water to about one-third and fastened on top of a specially built electromagnet that can magnetize the bottom of the jar. The latter can be made of metal or any other non-magnetic material, since the magnet acts through the bottom of the jar. The larvae adhered to the bottom of the jar. Then the water with the pupae and prepupae was poured out by turning the apparatus upside down with the help of a lever. The apparatus was returned to its former position, the electric switch was turned off; some water was added to the jar and the larvae

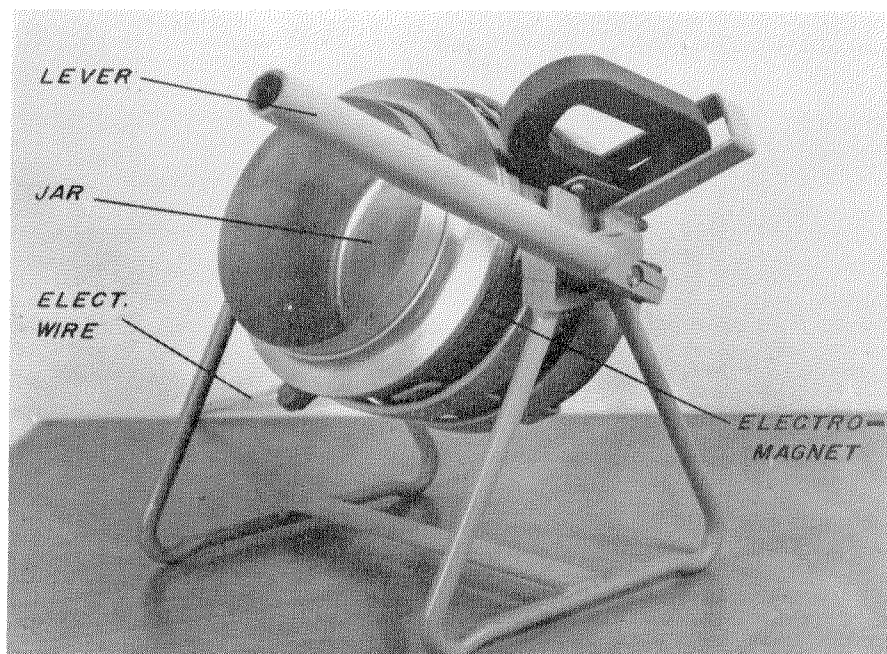


FIG. 1.—Apparatus for separating mosquito pupae from larvae

were poured into a prepared container.

A diagram of the apparatus is given in Fig. 2. The coil of the electromagnet is made of two parallel wires of 1 mm. in diameter and 820 turns. The current is 24 volts and 7 amperes. The larvae adhere to the bottom of the magnetic jar in two concentric circles, one corresponding to the circumference of the iron bar, and the other to the circumference of the metal plate (Fig. 2).

RESULTS. Most of the experiments were carried out with the mosquito *Aedes aegypti*, some with *Culex molestus* Forsk. Preliminary experiments with *A. aegypti* had shown that if too much iron is ingested by the larvae, their mortality and that of the resulting pupae is high, although those larvae and pupae which did survive produced healthy-appearing adults of normal life expectancy. Death

seemed to be due to the clogging of the alimentary canal with iron particles. The amount of iron dust per breeding jar (about 1,500 *Aedes* larvae in 3½ litres of water) finally adopted was 0.2 gr., and the larvae were allowed to feed for periods of 20, 30 and 40 minutes.

After separation, the pupae include also some larvae, mostly prepupae, i.e. larvae which have stopped feeding and will pupate within 24 hours. Only a very small percentage of larvae (not prepupae) (Table 1), does not adhere strongly enough to the magnetic field and is found in the pupal group. The larval group contains very few pupae that remain stuck to the wall of the magnetic jar when pouring out the water containing the pupae; these pupae can, if desired, be removed by adding water once more to the jar and pouring it out.

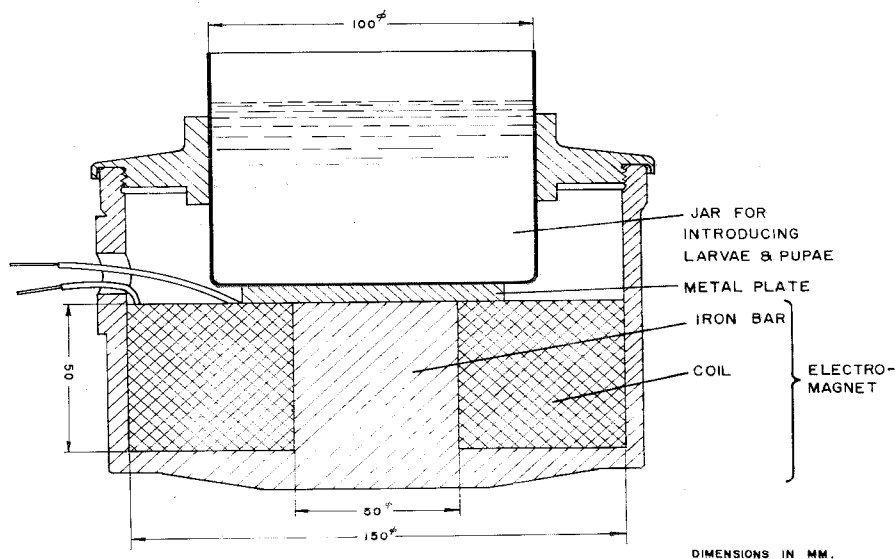


FIG. 2.—Diagram of the apparatus

Immediately after separation, the number of pupae and larvae in the pupal group and the number of pupae and larvae in the larval group was counted. Twenty-four hours after separation the number of larvae remaining in the pupal group (therefore not having been prepupae) was counted and the mortality of larvae and pupae in both groups recorded.

(Larvae and pupae that died, as a result of the iron dust, did so within 24 hours.) Results are given in Table 1.

The separation of pupae was practically complete and the larvae in the pupal group were mainly prepupae; the mortality of larvae and pupae was negligible. In the larval groups, some food is added, and the separations of new pupae on the

TABLE 1.—Results of separating *A. aegypti* pupae from larvae*

	Duration of feeding (in min.)		
	20	30	40
Percent pupae separated \pm S.E.	98.4 \pm 0.4	99.1 \pm 0.1	98.6 \pm 0.4
Percent of total larvae remaining in the pupal group after separation	15.3 \pm 1.0	11.8 \pm 3.6	4.2 \pm 0.4
Percent of total larvae remaining in the pupal group 24 hours after separation	1.1 \pm 0.4	1.9 \pm 0.3	0.7 \pm 0.2
Percent mortality of total larvae and pupae	2.1 \pm 0.5	1.7 \pm 0.6	2.8 \pm 0.7

* About 1,500 larvae and pupae per breeding jar of 3/2 litres containing 0.2 gr. of iron dust (5 replicates).

following days are carried out in a similar manner, always with the same results.

Some separation experiments were also carried out with *Culex molestus*. It was found that under similar conditions of rearing, the amount of iron dust required to produce an effective separation was twice as much as for *A. aegypti*, viz. 0.4 gr. for the same period of time (30 min.). At this level, there was practically no mortality of *Culex* larvae and pupae; this mosquito seems, therefore, to be more resistant to the iron feeding. The reason for the larger amount of iron required by *Culex* may be the larger size of the larva.

In the method described, an ordinary horseshoe magnet of about 3 kg. strength may also be used, if relatively small numbers of mosquitoes are required. The magnet is covered with cheesecloth, and then submerged into the mixture of the larvae and pupae which had fed on the iron and had been filtered and placed in a small container with water. The magnet is moved back and forth in the water. The cheesecloth with the larvae adhering will be removed from the magnet and submerged in water to release the larvae. The same procedure is repeated several times until no more larvae adhere to the magnet.

DISCUSSION. The method used was found efficient with the two species of mosquitoes tested. It will most probably be effective also in other species of mosquitoes, since various species of mosquito larvae tested (Coggeshall 1926, Howland 1930a, 1930b) did not discriminate in the selection of food, and, therefore, will ingest the iron particles added to the culture. The amount of iron dust, and the time allowed to feed on it, will have to be determined for each species; it will also vary with the amount of water and larvae used per rearing container.

If too many larvae are separated at one time, the separation may not be as ef-

fective, since the whole magnetic surface at the bottom of the magnetic jar may be covered with adhering larvae. In such a case, the separation of the pupae (still containing larvae) should be repeated. It would be preferable to build the electromagnet so that the whole surface of the bottom of the magnetic jar will be strongly magnetized.

SUMMARY. A method is described by which mosquito pupae can be quickly separated from the larvae. Iron dust is added and the culture exposed to a magnetic field. Only the larvae which have ingested iron, will be affected by the magnetic field.

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