A TIMESAVING METHOD FOR THE REARING OF MOSQUITOES IN THE LABORATORY [1]

ROBERT C. LOWRIE, JR. AND DUANE J. GUBLER

Laboratories of Medical Entomology
Department of Pathobiology
School of Hygiene and Public Health
The Johns Hopkins University

The maintenance of mosquito colonies in the laboratory is frequently a time consuming effort. Especially tedious is the process of separating pupae from larvae using the old bulb-pipette method. In recent years separation techniques have been described which replace this with other more rapid methods (McKiel, 1957; Fay and Morlan, 1959; McCray, 1961; Bar-Zeev and Galun, 1961; Weathersby, 1963; and Hazard, 1967). We have constructed a device which eliminates the necessity of separating pupae from larvae.

[1] This investigation was supported in part by a research grant from the U. S. Public Health Service (5 RO1 AI 00351–15) and in part by fellowships from the National Institutes of Health, Bethesda, Maryland (5 SO1 FR 05445 and 5 F1 GM31853–02).

The larvae are reared to the pupal stage in a routine manner; however, best results are achieved with relatively synchronous pupal development. Approximately 24–36 hours after pupae first begin to emerge, the contents of each rearing pan are poured into a fine-mesh wire strainer. The larvae and pupae are then transferred to an enamel pan (Fig. 1a) of about 5-liter capacity (40 x 26 x 6.5 cm) containing approximately 2.0 liters of water. Usually not more than 3000 larvae and pupae are concentrated in one pan. A minimal amount of food is added for the remaining larvae. Hereafter this container shall be referred to as the emergence pan.

Windowpane glass measuring 43.5 x 27 cm, with a 5 cm diameter hole cut in the center, is used to cover the emergence pan. Care must be taken to select emergence pans which will allow this glass lid to rest flat on all edges. The bottom portion of a 100 mm diameter plastic petri dish, also with a 5 cm diameter hole in the center, is placed over the hole in the glass lid, and the two units are glued together (Fig. 1b). A No. 8 air pilot globe (Dietz Co., Syracuse, N. Y.) (Fig. 1c), having a base with an outside diameter of 8.7 cm, is then placed into the petri dish. The globe top is covered with bobbinet.

A black poster-board cover (Fig. 2a) is fitted

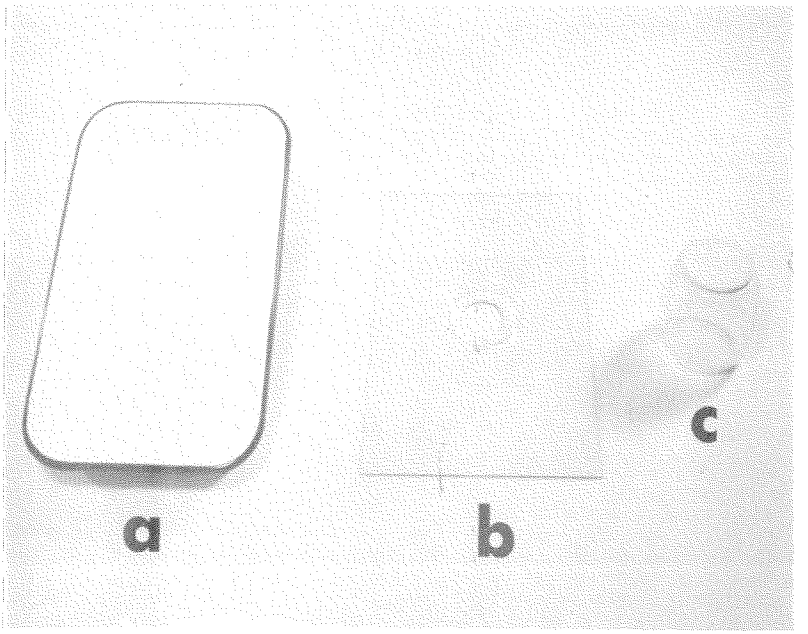

FIG. 1.—Showing the basic components which make up the emergence device: (a) enamel emergence pan, (b) glass lid with the petri dish glued in place, (c) air pilot globe covered with bobbinet.

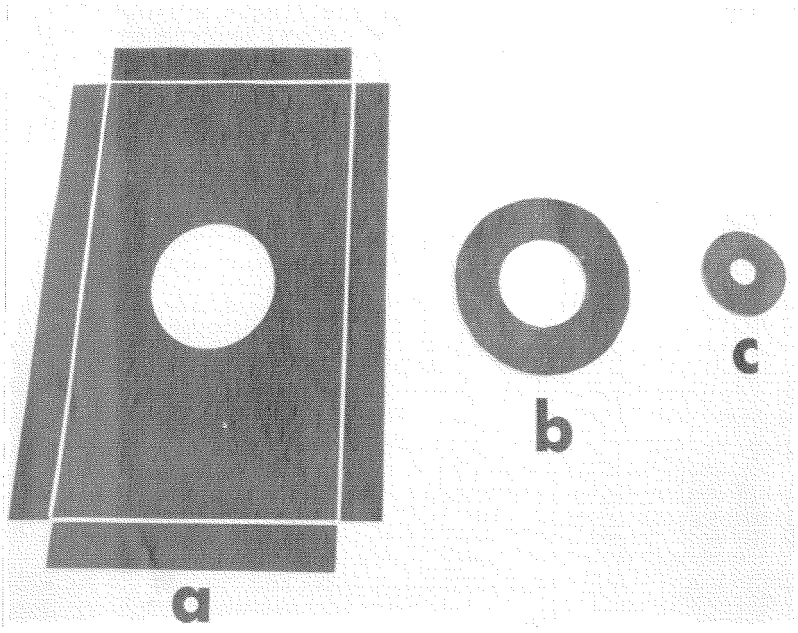

FIG. 2.—Showing the overlaying poster board components: (a) the cover with attached flaps, (b) the sleeve, (c) the insert.

over the glass lid. This cover is provided with 3.75 cm. flaps fastened on all sides with adhesive tape. A 12.5 cm diameter hole is cut in the center, thus allowing the cover to be removed while the globe remains in place. This permits one to observe the progress of emergence without removal of the glass lid. Beneath this cover a 17.5 cm diameter poster-board sleeve is fitted around the base of the petri dish (Fig. 2b). A poster-board insert with a 2.5 cm diameter hole is placed inside the petri dish (Fig. 2c). Consequently, when all three poster-board components are in place, the only source of light into the emergence pan is through the small hole in this insert. Since most adult mosquitoes exhibit a positive phototaxis or a negative geotaxis, or both, they will be attracted upward into the globe (Fig. 3). Certain mosquito species seem to have difficulty resting on the glass surface; therefore it may be desirable to place a loose roll of coarse nylon bobbinet inside the globe. At night, artificial light is directed at the globe.

To transfer the mosquitoes from the globe to a cage, two thin rigid plastic discs are inserted consecutively between the petri dish and the globe. Then the upper disc is removed with the globe while the lower one remains in place, covering the petri dish. For convenience in handling during transfer, the disc covering the base of the globe is replaced with the bottom portion of a 100 mm plastic petri dish. The mosquitoes are liberated into the cage, after which the globe is rejoined to the petri dish on the glass lid by removing the lower disc which separates them.

When emergence is complete, the few adults that may remain under the glass lid are killed in the following manner. The lower plastic disc described above is replaced with the cover of a 100 mm plastic petri dish in which a hole has been drilled and plugged with cotton. The cotton is saturated with chloroform, thus killing the adults, after which the glass lid can be removed.

This device has been used exclusively to maintain colonies of *Aedes polynesiensis* and *Aedes albopictus* in our laboratory since February 1967. Also it has been utilized successfully on the following species: *Aedes aegypti, A. pseudoscutellaris, A. taeniorhynchus, A. togoi; Anopheles albimanus; Armigeres subalbatus; Culex pipiens molestus, C. p. quinquefasciatus;* and *Eretmapodites chrysogaster.*

In our experience, adult mortality in the emergence pan is generally less than that encountered when pupal emergence bowls are placed in cages. The exception occurs when scum forms on the surface of the water in the emergence pan when

FIG. 3.—The device with all components in place showing, in this case, *Aedes albopictus* in the globe.

food is added in excess. It is imperative, therefore, that the amount of food put into the emergence pan be kept to an absolute minimum. If reasonably synchronous pupation has been achieved, it may not be necessary to add food beyond the amount retained during the process of concentrating the larvae and pupae.

Employment of this device saves considerable time, since the tedious process of separating pupae from larvae is eliminated. The efficiency of the procedure is not related to the quantity of mosquitoes reared. Furthermore, the globe need not be emptied daily, since sugar pads may be placed on the bobbinet covering it.

Further modifications are being made at the present time. Plexiglas, ¼ inch in thickness, can be substituted for the glass lid. The petri dish may then be attached to the lid with screws rather than with glue, and the poster board cover can be replaced with a more permanent material.

References

BAR-ZEEV, M., and GALUN, R. 1961. A magnetic method of separating mosquito pupae from larvae. Mosq. News, 21(3):225–228.

FAY, R. W., and MORLAN, H. B. 1959. A mechanical device for separating the developmental stages, sexes and species of mosquitoes. Mosq. News, 19(3):144–147.

HAZARD, E. I. 1967. Modification of the ice water method for harvesting *Anopheles* and *Culex* pupae. Mosq. News, 27(1):115–116.

MCCRAY, E. M., JR. 1961. A mechanical device for rapid sexing of *Aedes aegypti* pupae. J. Econ. Entomol., 54(4):819.

MCKIEL, J. A. 1957. A simplified method for large-scale laboratory rearing of *Aedes aegypti* (L.). Mosq. News, 17(1):25–29.

WEATHERSBY, A. B. 1963. Harvesting mosquito pupae with cold water. Mosq. News, 23 (3):249–251.