# OPERATIONAL AND SCIENTIFIC NOTES

## PRESERVATION OF MOSQUITO PUPAE BY THE FREEZE-DRYING TECHNIQUE

JOHN M. WHITE
Dept. of Biology, Del Mar College,
Corpus Christi, Texas

Studies of the structures of the pupae of mosquitoes in many instances are made more difficult by the necessity for making slides. In many cases, the pupae must be separated between the cephalothorax and the abdomen before satisfactory mounts can be prepared. This has the disadvantage of mutilating specimens. Additionally, slides of the pupae are not always easy to study because not all surface aspects of the pupae can be readily observed beneath the microscope.

The following procedure for preparing specimens of mosquito pupae for study has proved to be useful to the author because the difficulties previously mentioned are eliminated.

First, living pupae are removed from the rearing pans or water from which they were collected, and placed for a short time in a stender dish containing tap water. This cleans the specimens. Each specimen is next removed from the stender dish with a pipette and placed in a drop of tap water on a one-half-inch-square piece of kitchen wax paper. The drop of water on the wax paper is reduced by means of a fine pipette until only a thin film of moisture covers the pupa. This prevents the pupa from curling the abdomen under the cephalothorax. Next, the individual squares of wax paper containing the pupae are placed on a glass slide and placed in the freezing compartment of a refrigerator at a temperature of minus ten degrees centrigrade. The pupae are allowed to freeze for a six- to eight-hour period. When the freezing process is first begun, the pupae should be checked periodically to see that they retain the desired position. At the end of the freezing period, the specimens are removed from the freezing compartment of the refrigerator and transferred to ten ml. serum bottles. The serum bottles are then connected to the vacuum drying attachment of a freeze-drying unit and dried for seventy-two hours. At the end of this period, the serum bottles are removed from the freeze-drying unit and the pupae may then be removed from the serum bottles and pinned on regular card points.

The author thanks Mr. W. C. Gilleland, Pres. Texas Biological Laboratories, Ft. Worth, Texas for use of freeze-drying equipment, and Cora Allen for technical assistance.

The author has preserved thirty pupae of *Culiseta inornata* (Williston) by this process. The specimens were placed on card points and stored in a regular Schmitt box. At this time the specimens are eight months old. They are in a good state of preservation and have an almost lifelike appearance. It has been observed that some of the specimens become slightly dark during the drying process. Specimens preserved by this process are excellent for study under the dissecting microscope. Adult structures can be seen clearly beneath the pupal skin. Caution should be exercised in handling the pupae during the preserving process and after they are pinned, since rough treatment will break off body hairs.

Meryman (1960), in a detailed study of the freeze-drying technique reports preserving in lifelike appearance, spiders, insects, salamanders, birds, toads, and small mammals.

### *Reference*

MERYMAN, H. T. 1960. The preparation of biological museum specimens by freeze-drying. Curator, III/1.

## AN ESCAPE-PROOF MOSQUITO EGG HARVESTER

DANIEL J. REYNOLDS, Fort Detrick, Md.

In the general laboratory procedure for producing *Aedes* eggs, moistened paper towelling material placed in beakers or other containers is frequently utilized as an oviposition site. This ordinarily would require inserting an arm into the cage through a cloth sleeve. Such an operational technique creates a two-fold problem of permitting escape of mosquitoes and exposing the person to mosquito bites.

A waterlock device using a water barrier has been fabricated to circumvent these usual cage manipulation problems. This system permits an operator to introduce paper towelling into a cage for oviposition without escapes or exposure to mosquito bites.

A mosquito egg harvester device, illustrated in the diagram (Fig. 1) has been designed and tested. It has three parts. The first part is a stainless steel pan which extends into the cage and acts as a waterlock when filled; this pan may be fitted with a draincock for draining and cleaning. The second component is a sheet metal baffle, which is constructed as an integral part of one wall of a mosquito cage, extends down into the waterlock pan and penetrates the water surface. The baffle and water combination effectively seal the interior of the cage from the outside and the water surface of the pan is divided into interior and exterior portions. Entrance to the interior of the cage can be made only by passage through the waterlock, under the baffle and up