OPERATIONAL AND SCIENTIFIC NOTES

Preservation of Mosquito Pupae by the Freeze-Drying Technique

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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.

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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

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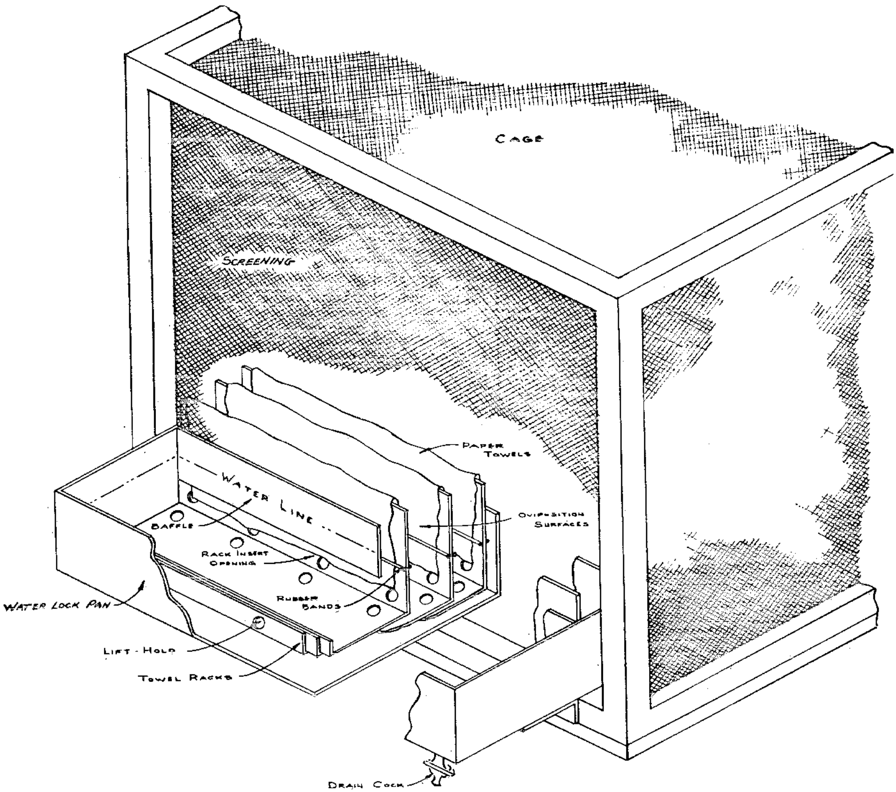
In the general laboratory procedure for producing *Aedes* eggs, moistened paper towelling material placed in beakers or other containers is frequently utilized as an oviposition site. This ordinarily would require inserting an arm into the cage through a cloth sleeve. Such an operational technique creates 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



MOSQUITO EGG HARVESTER

through the interior water surface. The third component of the egg harvester device consists of a metal structure used to hold the moistened paper towelling for oviposition. This structure is a J-shaped metal plate, the width of which is dependent upon individual requirements or the availability of proper width paper towels. The structure consists of a horizontal plate with two vertical plates. The higher vertical plate inside the cage has the paper towelling attached to it.

The paper towelling for oviposition surfaces is held in place on the higher vertical plate of the metal structure with a rubber band. A set of three metal structures with attached towelling is nested together for insertion into a cage.

The racks are introduced into the cage interior by rotating them through the pan waterlock under the baffle. The risers with affixed moistened towelling are brought to an upright position inside the cage. After the racks are in this position, they are drawn apart so that one covers the inner pan wall and another the inner wall of the baffle. With the exception of these two surfaces, each additional plate with overlapping towelling provides two oviposition surfaces.

After oviposition on the continually moistened towelling, the metal vertical plates are slid together and rotated out of the pan. Any adult mosquitoes clinging to the towelling are wetted and usually adhere to the racks when rotated through the waterlock. The towelling is inspected and the immobilized wetted adults are destroyed.

The egg papers may be left on the structure for conditioning or they can be removed and replaced with fresh paper towelling for additional oviposition.

With the harvester one can introduce paper towelling into a mosquito colony cage as an oviposition surface without the usual escape of adults or exposure to mosquito bites. The free water in the pan helps to humidify the cage. The oviposition surface is in constant contact with moisture.

ON THE DIFFERENT TYPES OF RESISTANCE

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At the Seventh Meeting of the World Health Organization Expert Committee on Insecticides the following definitions were made: "Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species. The term *behaviouristic resistance* describes the development of the ability to avoid a dose which would prove lethal."

This means that through selection there appeared an increase in the power to avoid irritants.

According to Busvine and Pal (1958), *physiological resistance,* which is specific to a particular type of poison, is inherited, apparently by normal Mendelian process, and in several cases through single genes.

The *vigor tolerance* of Hoskins and Gordon depends on non-specific defence mechanisms due to improved condition, greater weight. Many genes might be responsible for this condition. As improved vigor might be due solely to environmental effects, such as improved nutrition, it could be reversible.

Normal insects may show *protective behavior* and avoid certain stimulants. Through selection, they might show behavioristic resistance, and through selection in a suitable population might show physiological resistance. Referring to behavior a certain number of specimens, or a population, show either protective behavior or behavioristic resistance.

If we consider that under the heading of resistance there are four clearly defined entities, the theoretical combinations of them could lead to the finding, either in nature or in the laboratory, of seven types.

The literature mentions several instances of physiological resistance, vigor tolerance, protective behavior or behavioristic resistance as isolated entities but we are waiting for instances of their combinations.

Reference

BUSVINE, J. R. and PAL, R. 1958. Resistance of insects to insecticides. A note on terminology. Indian J. of Malariology 12(4):265–267.

Theoretical combinations of resistance entities

a.—Physiological resistance; b.—Vigor tolerance; c.—Protective behavior; d.—Behavioristic resistance

1.—		a	b		Physiological Resistance	+ Vigor tolerance [1]
2.—		a	c		Physiological Resistance	+ Protective Behavior [2]
3.—		a	d		Physiological Resistance	+ Behavioristic Resistance [1]
4.—		b	c		Vigor tolerance	+ Protective Behavior
5.—		b	d		Vigor tolerance	+ Behavioristic Resistance
6.— [3]		c	d		Protective Behavior	+ Behavioristic Resistance
7.—		a	b	c	Physiological Resistance	+ Vigor tolerance + Protective Behavior
8.—		a	b	d	Physiological Resistance	+ Vigor tolerance + Behavioristic Resistance
9.— [3]		a	c	d	Physiological Resistance	+ Protective Behavior + Behavioristic Resistance
10.— [3]		b	c	d	Vigor tolerance	+ Protective Behavior + Behavioristic Resistance
11.— [3]	a	b	c	d	Physiological Resistance	+ Vigor tolerance + Protective Behavior + Behavioristic Resistance

[1] Suggested by Busvine and Pal (1938).
[2] Suggested by Busvine and Pal (1938) and considered of "considerable importance."
[3] By definition this combination does not exist.