SCIENTIFIC NOTES

IMPROVED TECHNIQUES FOR THE LABORATORY REARING OF *Aedes aegypti* (LINN.)

JOHN E. PORTER, GEORGE KOZUCHI AND MARTIN J. KUCK [1]

Various methods have been utilized in rearing *Aedes aegypti* in the laboratory. Optimum temperature and humidity conditions have been amply defined by Horsfall (1955), Trembley (1955), the United States Department of Agriculture (1955) and Christophers (1960). Conditions necessary to achieve maximum egg deposition and to maintain healthy colonies of larvae have been well described by these authors.

Rearing containers and cages for adults show considerable variation in shape, size and complexity depending upon the ultimate purpose for which the specimens are being reared. Trembley, Chao (1959) and Christophers propose the use of various sizes and shapes of cages covered with screen netting and/or clear plastic; Pollard (1960) adds a built-in animal restrainer to a cage of the type described by Trembley while Micha Bar-Zeev and Galun (1960) suggest a more intricately designed cage to prevent the escape of infected adults.

Many investigators have indicated satisfaction with the feeding devices and nutritional ingredients used for maintaining colonies of adult *aegypti* and these have become more or less standardized. It is, however, understandable that each investigator has the right to choose, modify or develop those procedures most satisfactory to his particular needs. It was because of problems peculiar to the investigations undertaken by the authors that necessity dictated the development of certain modifications in these standard procedures which we hope may be of value to other workers.

Adult female *aegypti* require both blood meals and a sugar or honey-water solution as an alternate food while the male mosquito will survive on the sugar or honey-water solution alone. In the warm and humid conditions often characteristic of an insectary, preparations of sugar or honey solution very quickly ferment and mold. Pads or wicks of cotton soaked in this solution need to be frequently turned over, remoistened or replaced. Then too, these cotton pads or wicks are also attractive oviposition sites for the gravid females.

It is evident that we need (1) a means of preventing fermentation and molding of the honey solution, (2) a container which will prevent rapid evaporation of the honey solution, and (3) a container which will be impervious to egg deposition.

Pharmacists are known to add parabens solution to certain preparations to inhibit mold and fermentation. A stock solution of this material is composed of:

methyl paraben, USP ...... 1.0 gm.
propyl paraben, USP ...... 1.5 gm.
and water to make 1000 ml.

Taking this lead, we diluted 25 ml. of the stock paraben solution with 75 ml. water. Ten ml. of this was then replaced with an equal amount of honey to make a 10 percent honey-paraben-water solution. Tests over a considerable period of time indicate that the addition of paraben to the honey-water does not repel the mosquitoes. It is non-toxic. Now we can prepare in advance a quantity of the honey-paraben-water solution and keep it indefinitely in the refrigerator. The material when added to a cotton pad, wick, or the new type feeder, does not mold or ferment.

Encouraging results were noted in preliminary tests using parabens-treated egg deposition papers. However, more extensive tests indicate that these chemicals crystallize out on the papers where they are then repellent to the adult female and also exert an ovicidal action on the eggs.

The problems relating to evaporation and egg deposition were answered by finely perforating, in a ½-inch-wide band, the sides of a 3-inch-high, 10 dram plastic capsule vial (Fig. 1). These perforations were made by punching the vial with a hot dissecting needle which created a roughened surface to which the adults can easily cling when feeding. A small roll of blotting paper is inserted to act as a wick and the vial is filled up to the perforations with honey-paraben-water solution. The tight fitting plastic cap through which an insect pin is inserted and bent over in hook fashion is then replaced on the vial.

This new type feeding device can be hung from the top of any cage being used for the adults. Eggs are not deposited on it and it is not necessary to refill the containers more than once every 2 or 3 weeks.

Attempting to establish colonies of mosquitoes for resistance studies from small numbers (approximately 10 to 15) of larvae or adults taken in the field often is difficult or impossible when the adults are released in an 18 x 18 x 18 inch cage. Whether this failure is due to unfavorable

[1] Senior Scientist, Entomologist and Biologist, respectively, Quarantine Station, Division of Foreign Quarantine, Public Health Service, U. S. Department of Health, Education, and Welfare, Miami Beach, Florida.

air currents, the vastness of the cage in comparison with the relatively small number of mosquitoes or some other factor, is not known. Believing the large size of the cage to be a detrimental factor, we reduced the sizes of our cages and struck upon the use of the following sleeve-type cage (Fig. 2) which is made of ½-inch mesh hardware cloth and tubular gauze bandage. The ½-inch hardware cloth is cut and rolled so as to make an open cylinder 1 foot long by 6½ to 7 inches

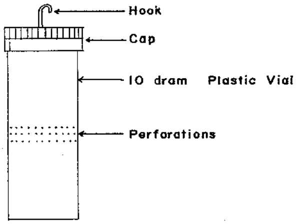

Fig. 1— SOLUTION FEEDER for ADULT MOSQUITOES

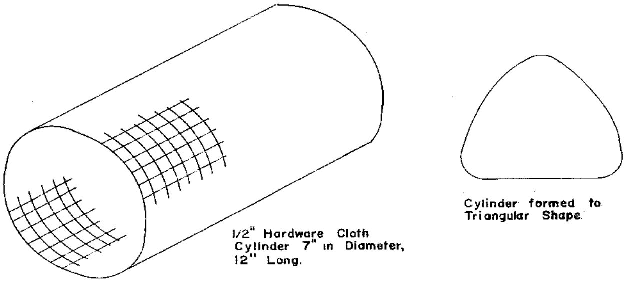

Fig. 2— SLEEVE CAGE

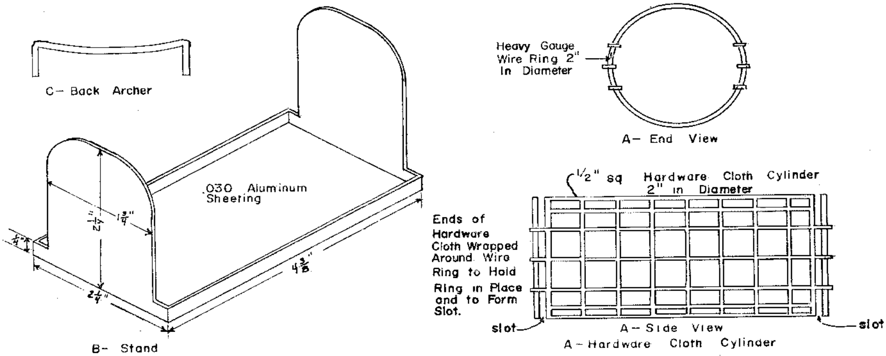

Fig. 3— HAMSTER RETAINER

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in diameter. By bending the cut end wires alternately one way and the other it is possible to fasten the cylinder without need of solder. The rough cut ends of the cylinder can be covered with masking tape to prevent tearing of the 2-inch-wide tubular gauze bandage which is now slipped over the hardware cloth. Enough gauze is allowed over each end to permit closing the cylinder with a rubber band and/or a snap clothes pin. A cage of this type is smaller, is cheaply and quickly constructed and can be easily cleaned and decontaminated if necessary.

The compactness of the cage is an added feature. With slight pressure one can bend the shape of the cage—making it more or less triangular in cross-section. In such fashion there is no rolling from side to side and the cages can be easily stacked two or three high if needed.

With a large number of these small cages it was soon apparent to us that rabbits provide a rather inconvenient and time-consuming means of providing the small numbers of adults per cage with a blood meal. To eliminate this problem we shaved the backs of hamsters and found that the *aegypti* females were readily attracted to them. A small retaining device for the hamsters was made (Fig. 3) in which the animals can be easily placed in each mosquito cage for the feeding period. This device consists of three parts: the hardware cloth cylinder and two end rings (A). These rings are fastened in such a fashion as to form slots into which the end pieces of the stand (B) will slide. A short length of wire (C) is slipped under the caged hamster to arch its back and make it more vulnerable to the mosquitoes and also prevent excessive movement.

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VARIATION IN THE STRUCTURES OF HAIRS FOUND IN ANOPHELINE LARVAE FROM EAST PAKISTAN

S. R. HAIDER *

Some remarkable diversities in the structures of larval hairs were observed by the author during routine examinations of larval collections made available from different parts of East Pakistan. In routine examination of thousands of anophelines for species identification, those with variation in structures of their hairs were kept separate for detailed study. All the larvae under observation appeared in the 4th instar. They were mounted in chloral gum and then examined under a compound microscope.

The variations observed in the specimens are described below in detail.

Anopheles annularis Van Der Wulp 1884. (a) In one of the specimens of *A. annularis* variation was noticed in the posterior clypeal hair which was trifid at the right side while at the left it was simple (Fig. 1).

(b) In another specimen of *A. annularis* variation was again noticed, but this time it was in the sutural hair. A pair of inner sutural hairs were placed quite approximately at the right side while only a single unbranched hair was found at the left (Fig. 2).

Anopheles pallidus Theo, 1901. Variation in the character of larval hair was observed in one of the specimens of *A. pallidus*. The posterior clypeal hair had seven branches and not 2 to 5 as is usually present in *A. pallidus*. The presence of such character sometimes causes confusion and leads to incorrect identification. Puri (1954) in his identification table for anopheline larvae has also pointed out the large range of variation in the number of branches of posterior clypeal hairs in case of *A. pallidus* which sometimes creates difficulties in their correct identification.

The filaments of abdominal palmate hairs were, however, more than half as long as blades of leaflets. (Figs. 3 and 4).

Anopheles subpictus Grassi. Theo, 1902. Variation in the character of post spiracular hair was observed in a single specimen of *A. subpictus* larvae. The post spiracular hair at the right side had seven branches while the hair at the left consisted of four branches as usually found in case of *A. subpictus*. This is shown in figure 5. This naturally may cause confusion because post spiracular hairs with seven or eight branches are found in *A. sundaicus* which is a vector species in East Pakistan.

Variations in the larval chaetotaxy of *Anopheles* mosquitoes have been reported by previous workers in other countries too. Yates (1943), during the study of the 4th instar larvae of *Culex tarsalis*,

* Malaria Institute of Pakistan, DACCA/East Pakistan.