ARTICLES

EGGS OF MOSQUITOES FOUND IN *AEDES AEGYPTI* OVIPOSITION TRAPS

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During 1966, 1967, and 1968 the oviposition trap, or ovitrap, was used on an increasing scale in surveying areas for the yellow fever mosquito, *Aedes aegypti* (Linnaeus) on the cooperative Public Health Service-State Health Department Aedes aegypti Eradication Program. Research of many Public Health Service entomologists indicated that *Aedes aegypti* females were attracted to shiny black jars holding an inch or more of water and would lay their eggs on a ¾-inch-wide hardboard paddle 5 inches long. The paddle is clipped inside the jar in a verticle position with the rough side turned toward the center of the jar and the lower end standing in the water. (Fay and Perry, 1965; Fay and Eliason, 1966; Pratt and Jakob, 1967).

In order to train technicians to identify the eggs of *Aedes aegypti* correctly, photographs were made of the eggs of this mosquito and of other species which lay their eggs on the hardboard paddles in these ovitraps: *Aedes triseriatus* (Say) in eastern and southern United States, *Aedes atropalpus* (Coquillett) in Texas, *Aedes mediovittatus* (Coquillett) in Puerto Rico and the Virgin Islands, and *Aedes albopictus* (Skuse) in Hawaii. Eggs of *Orthopodomyia signifera* (Coquillett) and several other species were occasionally found on these paddles.

Eggs of *Aedes* have been studied intensively by Horsfall and Craig (1956). Using dissecting microscopes having powers between 10X and 150X, technicians identified the eggs by means of the following characters:

Aedes aegypti (Figure 1). The eggs were found attached to the hardboard paddle, generally one or two per paddle depression, less commonly on the edges of the paddle, particularly the rough portions. These eggs are relatively shiny, smooth, cigar-shaped with moderately parallel sides. Eggs on paddles have been seen from the Virgin Islands, Puerto Rico, Florida, Georgia, South Carolina, Alabama, Louisiana, Texas, and Hawaii.

Aedes triseriatus (Figure 2). These eggs also were attached to the hardboard paddle, usually one or two per depression. They were relatively duller, rougher, with definite elevations, and with more convex sides than those of *Ae. aegypti*. Eggs have been seen from Florida, Georgia, South Carolina, Alabama, Louisiana, and Texas.

Aedes atropalpus (Figures 3 and 4). These eggs were not glued firmly to the hardboard paddles. They were similar to those of *Ae. triseriatus* in being relatively rough and with moderately convex sides. The key character for identifying *Ae. atropalpus* was that the *dry* eggs had whitish waxy tips on the elevations best

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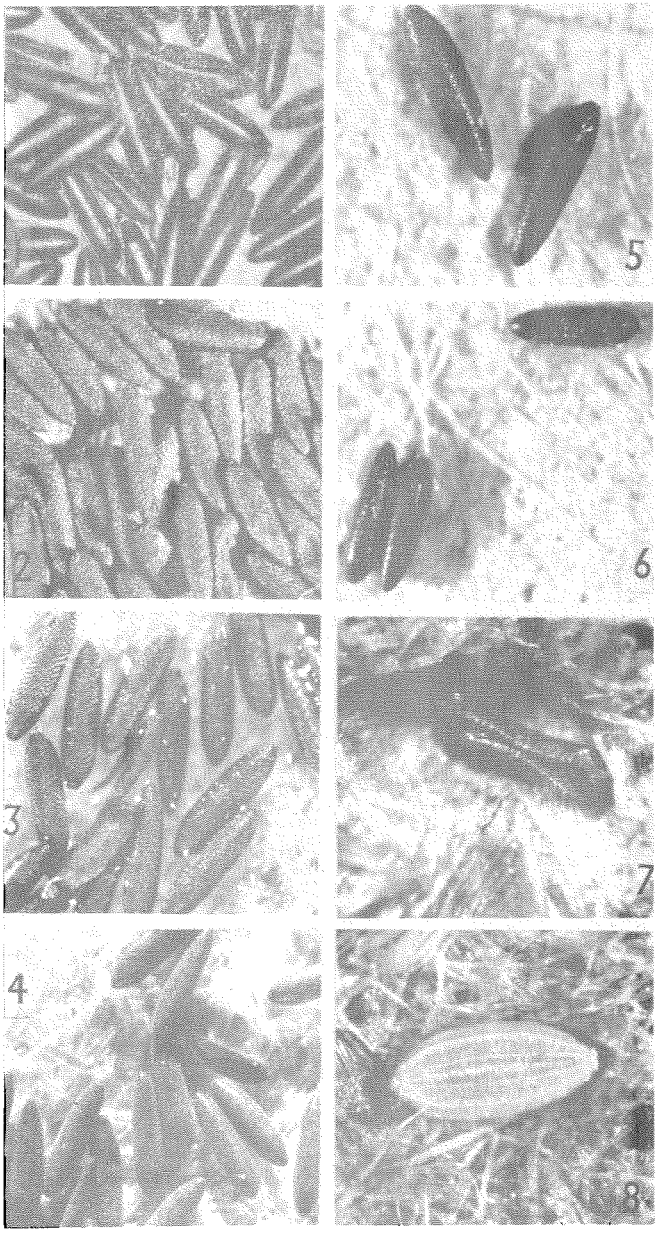

FIGS. 1–8.—Eggs of mosquitoes found in oviposition traps.
1. *Aedes aegypti;* 2. *Ae. triseriatus;* 3. *Ae. atropalpus;*
4. *Ae. atropalpus;* 5. *Ae. mediovittatus;* 6. *Ae. albopictus;*
7. *Orthopodomyia signifera;* 8. Unknown.

seen with high illumination. The importance of this character was first appreciated by Elmo Williams of the Texas Aedes aegypti Project. Eggs have been seen from Texas.

Aedes mediovittatus (Figure 5). The eggs were similar to those of *Ae. aegypti* but were generally dull, slightly more convex, not shiny, and with finer reticulation than those of *Ae. aegypti,* as determined by Arthur Regnier, of the Puerto Rico Aedes aegypti Project. Eggs have been seen from Puerto Rico and the Virgin Islands.

Aedes albopictus (Figure 6). The eggs were similar to those of *Ae. aegypti* but were slightly more convex. Eggs have been seen from Hawaii and from a colony in Savannah, Ga.

Orthopodomyia signifera (Figure 7). The eggs were glued firmly to the hardboard paddles and were much more difficult to dislodge than those of *Ae. aegypti* or *triseriatus*. These *Orthopodomyia* eggs showed the longitudinal flanges mentioned by Ross and Horsfall (1965) or Marshall (1938), and looked somewhat like an elm seed. Eggs have been seen from Alabama and Florida.

Unknown egg A (Figure 8). This yellowish-white egg with definite longitudinal ridges was seen on paddles collected in Alabama. Attempts to hatch these eggs were repeatedly unsuccessful. This may not be a mosquito egg.

The Training & Consultation Section of the Aedes aegypti Eradication Program provided technicians in the field with reference specimens of known mosquito eggs. Small pieces of paper toweling with eggs glued to them with fingernail polish were attached to a microscope slide, placed in cells made of cardboard or washers, and sealed with a cover glass. Some of these eggs remained in good condition for 3 to 6 months before collapsing due to dehydration. Even at the end of 6 months the eggs of the two commonest species, *Ae. aegypti* and *Ae. triseriatus,* still showed the key characters, the shiny, smooth egg of *Ae. aegypti* and the dull, rough egg of *Ae. triseriatus*. Eggs on hardboard paddles were kept in 2 percent formalin for months. When needed for teaching, the paddles were air dried for an hour before class and the key characters of the eggs showed up well, even a year after being placed in formalin.

The determination of eggs of these container-breeding *Aedes* is more difficult than of the larval, pupal, or adult stages. It requires experience, good photographs of known eggs or reliably identified reference specimens, and a good microscope and microscope light. In cases where the determination of eggs was in doubt, they were placed in water and allowed to hatch so that final identification could be made in the larval stage.

Only two species were commonly found in southeastern United States. Here it was generally possible to identify the relatively smoother, shiny, parallel-sided eggs of *Ae. aegypti* from the rougher, duller, more convex eggs of *Ae. triseriatus*. In Puerto Rico and the Virgin Islands, with experience it was usually possible to distinguish the eggs of *Ae. mediovittatus,* with their slightly duller color and finer reticulation, from those of *Ae. aegypti,* with the shinier color and slightly coarser reticulation. In Texas the shiny, relatively smooth eggs of *Ae. aegypti* could generally be distinguished from those of *Ae. triseriatus* and *Ae. atropalpus* which were duller and rougher. However, the separation of these last two could be reliably made only with *dry* eggs and good lighting. In general, the eggs of *Ae. atropalpus* had slightly pale, waxy-appearing tips on the rough projections on the egg. The authors have had only limited experience identifying eggs of *Ae. aegypti* and *Ae. albopictus* from Hawaii and do not feel competent to distinguish these two species on ovitrap paddles from the Pacific at this time.

The authors acknowledge gratefully the advice, and particularly the assistance in providing known laboratory specimens, of Dr. H. F. Schoof, Dr. R. W. Fay, and Mr. W. L. Jakob; of Dr. B. R. Evans and H. Akins; of E. Williams and B. L. Hoffman, particularly with *Ae. atropalpus;* and of A. J. Regnier with *Ae. medio-*

vittatus. Field personnel on the Aedes aegypti Eradication Program submitted thousands of eggs for identification or confirmation, and hatched "problem" eggs to help confirm identifications using larval characters. The photomicrographs were made by Jack Gust of the National Medical Audiovisual Center.

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A COMPARATIVE STUDY OF EGG HATCHING TECHNIQUES FOR *AEDES AEGYPTI* (L.)[1]

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The existence of a relationship between the concentration of dissolved oxygen and the percent hatch of mosquito eggs has been established (Gjullin *et al.*, 1941; Borg and Horsfall, 1953 and Horsfall, 1956). Lowering the concentration of oxygen stimulates hatching. Borg and Horsfall (1953) and Burgess (1959) adequately reviewed previous work on hatching stimulants.

A great deal of work has been performed on different types of oxygen removers, and tests have been performed to determine the effectiveness of these methods as possible hatching agents. These include vegetable extracts, acids, sugars, vitamins, salts, microorganisms, gases and evacuation. A great diversity in the efficiency of hatching agents has been reported. Percent hatch and the time required for complete hatch using a particular agent have been rated differently by various workers. But evaluations and comparisons are difficult to make because of the incompleteness of the data reported in many studies. Time required for complete hatch was not always mentioned. Other workers state only the time elapsed before initiation of hatch. Borg and Horsfall (1953) suggest that the diversity in percent hatch and hatching time reported by various workers was due to improper conditioning of eggs.

Burgess (1959), Judson (1960), Gander (1951) and others failed to mention the total time required for hatch. In addition, a high percentage hatch was achieved in only a few of the above studies. The agents used in these studies varied greatly. Among the substances tried were boiled distilled water (Burgess, 1959), nitrogen gas and ascorbic acid (Judson, 1960) and wheat germ (Gander, 1951). In some cases other authors have reported hatch time. Borg and Horsfall (1953) indi-

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