A TECHNIQUE FOR ARTIFICIAL INSEMINATION OF *AEDES* MOSQUITOES [1]

RONALD E. WHEELER AND JACK COLVARD JONES
Department of Entomology, University of Maryland, College Park, Maryland

For many purposes the McDaniel-Horsfall technique (McDaniel and Horsfall, 1957) or some slight modification of it (Wheeler, 1962) is entirely adequate to inseminate aedine mosquitoes by the process of forced copulation. This technique, however, does not permit one to study many problems which are crucial to a more detailed understanding of the physiology of reproduction, e.g., the mechanism of sperm transference within the body of the female. It is the purpose of this paper to describe briefly an assembly of equipment which can be easily set up, is simple to operate, and allows one to place with great accuracy various substances into the reproductive system of the female mosquito *Aedes aegypti* (Linnaeus) (Diptera, Culicidae).

A sketch of the main details of the arrangement for artificial insemination is shown in Figure 1. The following items are essential: (1) two micromanipulators (Brinkman, model 30–50) or similar holding devices (Fig. 1, A and B); (2) one self-closing type micro-forceps (Aloe, catalogue item V 37392) (Fig. 1, H); (3) a suitable racking device (either the rack from a stereoscopic microscope or another micromanipulator) (Fig. 1, C); (4) a stereoscopic microscope (Fig. 1, D); (5) a suitably strong source of direct illumination with heat-absorbing filters; (6) a small lucite block (25 x 4 x 6 mm) (Fig. 1, E) having a sharp, knife-like upper ledge with a small central notch (less than 0.5 mm wide) (Fig. 1, N) cut out to just accommodate the terminalium of a mosquito; and a flat metal plate (25 x 50 mm) to which the lucite block is firmly glued (Fig. 1, F).

As shown in Figure 1, one or two attached corks are fitted into the rack (or into another micromanipulator). A previously chilled or otherwise anesthetized mosquito (Fig. 1, M) is placed with her dorsal side uppermost, and the head of a number 1 or 2 insect pin (Fig. 1, P), lightly loaded with a small drop of "Dek-adhese" (a non-toxic plastic cement manufactured by Donald Tulloch, Jr., Box 17, Chadds Ford, Pa.), is touched gently to the center of the mesothorax. We have recently found it better to attach the female to the pin with warm tackiwax (Central Scientific Company, C11444). The female-bearing pin is positioned in the cork and the rack placed on the left of the stage of a stereoscopic microscope. The terminalium of the immobile, horizontally-held female is inserted ventral side up into the small central slot in the lucite block (Fig. 1, E, N).

Unless the female is fully anesthetized her terminalium generally moves about so much that her cerci cannot be grasped suitably. We routinely place females in glass vials within an ice-filled bucket, but recently found nitrogen anesthesia much better. The self-closing forceps with needle-like tips, which are held in micromanipulator A (Fig. 1), are used to gently grasp the cerci of the female (Fig. 1, Cc). This delicate grasping manuever is made possible by first inserting a small can or probe in front of the crossing-site of the forceps, between the tips, so that the tips of the forceps are only very slightly opened. Once the cerci are firmly grasped, a manipulator screw is turned so as to pull the cerci slightly to the operator's right. This movement

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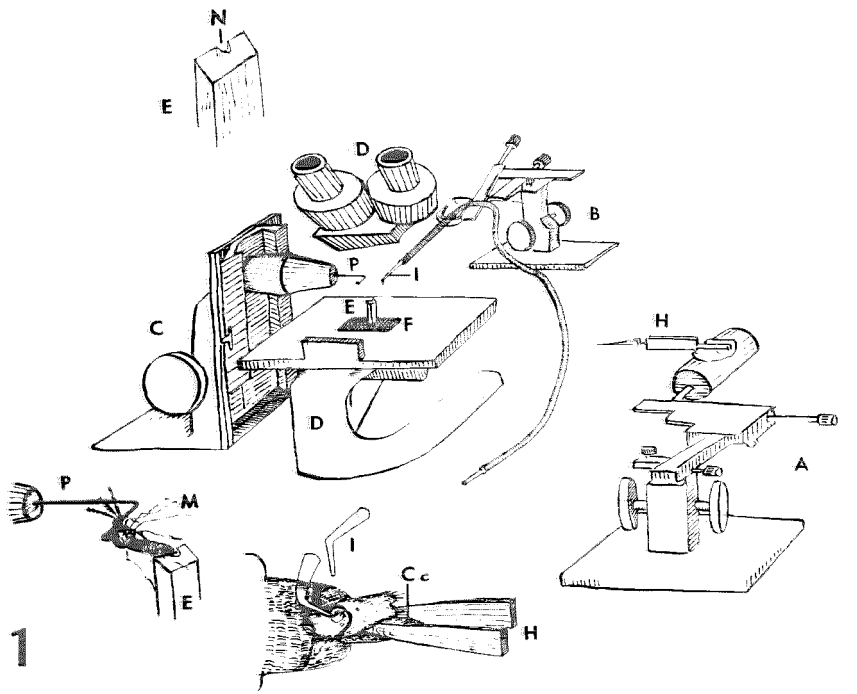

FIG. 1.—Arrangement for artificial insemination of mosquitoes showing the micromanipulator (A) holding the microforceps (H), the micromanipulator (B) holding the injection micropipette (I), the racking device (C) which holds the pin (P) to which the female (M) is glued. The stereoscopic microscope is shown at D; in the center of the microscope stage is positioned the lucite block (E) glued to the metal plate (F). The upper ledge of the lucite block (E) has a central slot (N) through which the terminalium of the female is placed. The cerci (Cc) of the female are grasped by needle-forceps (H) and the micropipette (I) is then placed into the atrium of the female.

causes the female's genital lips to open (Fig. 1, 2 and 3).

Micromanipulator B holds a fine glass micropipette (Fig. 1, I) at the end of a rubber or polyethylene tube. The open end of the tubing is held in the operator's mouth. Micromanipulator B is positioned in front of the stage of the stereoscopic microscope in such a way (Fig. 1) that the micropipette can be quickly brought into position with respect to the female's genital opening (Fig. 2 and 3).

Micropipettes are fashioned from Pyrex capillary tubing only with the aid of an Aloe De Fonbruyne microforge (Aloe, Series A, No. 168). We have found that a pipette with a 90° angled tip is most suitable. The end of the pipette should be of a size only slightly larger than the distended opening of the bursa copulatrix.

During actual injection of the female's reproductive system, it is necessary to keep up a gentle slight pressure within the pipette so that the fluids being delivered to the female will not be automatically drawn back into the pipette by capillary

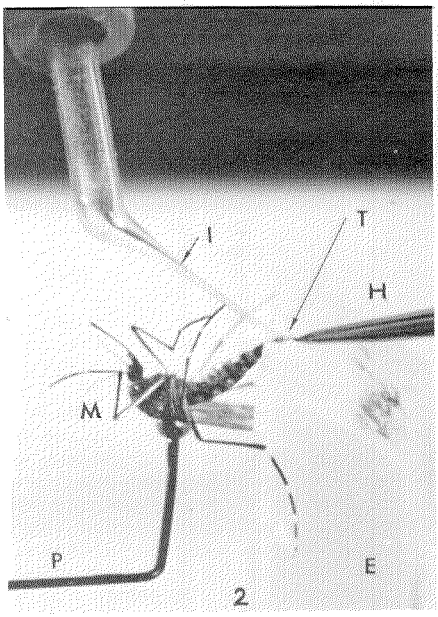

FIG. 2.—Photomicrograph showing the position of the mosquito during artificial insemination. The terminalium of the female is shown at T, being held by forceps (H). The mosquito (M) is pinned to the head of a number one insect pin (P). The micropipette (I) is immediately over the genital opening of the female. The lucite block is shown at E.

action. After injecting the genital system of the female, the cam or probe is placed in the self-closing forceps in front of the crossing-site to release the cerci. The injected females may be kept on the pins or released into a cage by breaking the cement or wax seal with a fine sharp needle or with a razor edge.

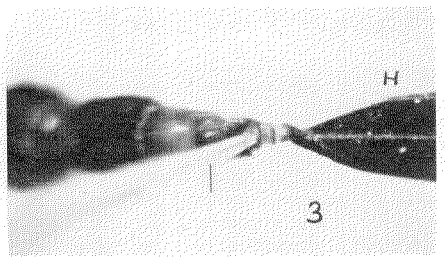

FIG. 3.—Photomicrograph showing the terminalium stretched with the forceps (H) by pulling on the cerci, and the placement of the needle over the opened genital lips.

With practice, the bursa copulatrix of a single *Aedes* female can be injected with great accuracy within less than 2 minutes. Mortality of injected females is less than 10 percent. With some experience many mosquitoes can be processed quickly. We are convinced that anyone who has used the artificial insemination techniques of either Burcham (1957) or Hayes (1953) will find the very simple arrangement described in this paper well worth the cost of the equipment. This arrangement for artificial insemination of mosquitoes is definitely *not* considered to be a substitute for the McDaniel-Horsfall technique.

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