

siderable experience. Manual dexterity is necessary for orientation of parts of the specimen which are comparatively fragile. Various mounting media other than those mentioned herein have been used by entomologists, and may be tested by the

newcomer to slide-making. The methods presented here have been time-tested, and, if followed faithfully, should result in permanent slides of mosquito eggs, larvae, pupae, and adults, of which the entomologist can be proud.

A TECHNIQUE FOR MICROMANIPULATION OF MOSQUITOES¹

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The importance of insects in basic research has become more generally recognized in recent years. Many possible lines of investigation have been pointed out in physiological research by Roeder (1952). In one of these, using the mosquito as an experimental animal, Roth (1952) describes physiological experiments requiring microsurgery of the adult mosquito. Shambaugh (1952) points out the importance of microinjection techniques for studies in toxicology and physiology, and describes a microinjection technique for *Aedes aegypti*. However, in studies involving mosquito microsurgery and microinjection, extreme difficulty is encountered in the handling of the mosquito due to its very fragile structure and small size. Physical damage of mechanically holding the mosquito, surgical shock, heat desiccation, and prolonged anesthesia in such operations all tend to produce a high operative mortality rate. Studies in this laboratory requiring micromanipulation of living mosquitoes for microsurgery have resulted in an improved, simplified technique that has effectively lowered post-operative mortality, and reduced preparation time for experiments that require operations on large numbers of mosquitoes.

This success is due principally to the introduction of a suction pipette as a holding device to replace pinching or other friction holding instruments for securing the anesthetized mosquito while operating under a dissecting microscope. The suction pipette, pictured in Fig. 1, was made by drawing out a quarter-inch glass tube to a small oval tip sized 1 by 2 millimeters. This tip was then covered with grid by cementing on a piece of 51 gauge nylon cloth material. The pipette was attached with rubber tubing to a water pump suction device, and sufficient suction maintained to securely hold an adult mosquito to the grid tip of the pipette.

In use, the adult mosquitoes were exposed briefly to CO₂ gas in small cartons, and when anesthetized, the rubber tubing to the pipette was pinched to shut off the suction, the tip of the pipette placed close to the scutum of the mosquito, the tubing released to start suction in the pipette so that it firmly secured the mosquito against the grid tip, as shown in Figure 1. In this manner the mosquito is held in place by gentle pressure against the relatively strong thick integument of the scutum, with little damage to the mosquito. Also, as shown in Figure 1, there is a minimum surface area occluded by the holding device, and the legs and other appendages are left freely accessible. Holding a mosquito thus, it could be picked up and manipulated freely under the field of the dissecting microscope without physical damage. Also, held securely in this man-

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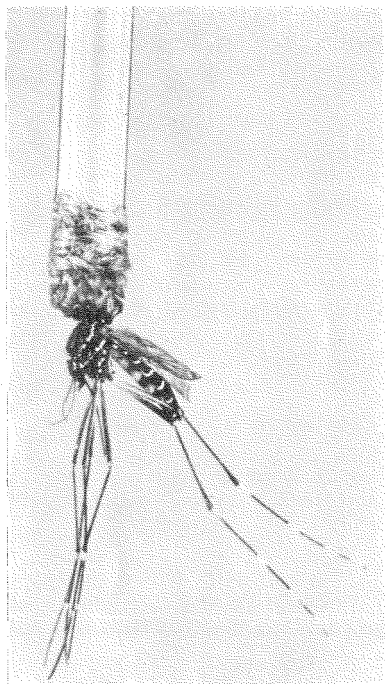


FIG. 1. Female *Aedes polynesiensis* held by suction to the tip of a nylon covered pipette for manipulation under a dissecting microscope.

ner it was not necessary to maintain full deep anesthesia and operative mortality due to overanesthetization was avoided.

The operative field under a dissecting

microscope was covered with a transparent plastic hood. Within this hood cool moist conditions were maintained to protect the mosquito from the effects of heat desiccation. Heating and drying were also reduced by supplying a light source from outside the plastic hood. Instruments were standard for such techniques, including fine glass rod points and hooks, iris scissors, and small scalpels made from bits of razor blades (Roth, 1952).

By using the suction grid tip pipette method of holding mosquitoes for microsurgery, operation time, operative and post-operative mortality have been greatly reduced. Such operations, in numbers, as single and multiple leg amputations, tarsiectomies, palpectomies, and antennectomies have been performed on *Aedes aegypti*, *A. polynesiensis*, *Anopheles quadrimaculatus*, *An. freeborni*, *Culex pipiens*, and *C. fatigans*. It is believed that the use of the suction pipette may be of use in the micromanipulation of mosquitoes for other operations as well as for the handling of other delicate insects.

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