the vicinity of dwellings, as well as on the steps and in the open porches of houses.

Most of the females become active soon after dusk and the peak of biting activity is reached within two hours after dark. Few females seek a blood meal after 10 p.m. The great majority attempt to bite on the lower extremities and they prefer dark to light-skinned humans. While high population densities are only found near the swamps, this species tends to migrate during periods of heavy rains and at such times may be taken biting in fair numbers in well-drained forests at least 7 kilometers away from the nearest known breeding place. In Almirante, *C. taeniopus* has also been found infected with Venezuelan equine encephalitis virus (Grayson and Galindo, 1966) and in Trinidad it was found harboring EEE virus (Downs *et al.*, 1959).

SUMMARY. A strain of eastern equine encephalitis virus, the first to be isolated from mosquitoes in Panama, was obtained from *Culex (Melanoconion) taeniopus* Dyar and Knab collected in the Almirante area in September 1964.

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METHODS FOR DISSECTING MOSQUITOES

JACK COLVARD JONES
Department of Entomology, University of Maryland, College Park, Maryland

INTRODUCTION. Most of the following methods for dissecting mosquitoes have been employed in this laboratory for a number of years and have proved most useful. The various tools and equipment needed are included. A number of mosquito investigators have written to me about their various special techniques and I have included them here.

TOOLS

1. *Needles.* Sharpened Minuten Nadeln or other fine steel needles or larger insect pins may be inserted into freshly boiled, wooden, applicator sticks (Patton & Evans, 1929); after the sticks dry, the needles are firmly held. Pins vary in quality from brand to brand; many stainless steel pins are not useful. Larsen (personal communication) recommends the "Genuine Brill'd Eys Between" #10 made by James Smith & Sons, London. These are very stiff and sharp.

A large batch of needles in applicator-stick holders can be made up at one time and stored in suitable containers. Long applicator sticks with embedded needles

can be used directly as dissecting tools or the embedded needle and a small portion of the applicator stick can be cut off and inserted into a mechanical (drawing) pencil holder (e.g. "Koh-i-noor," "Eagle 3379" or "Chavoz 303"). Larsen recommends the use of pin vise model #94 A (General Tool Company). The needles can be sharpened into various cutting tools (points, scalpels, hooks, etc.) using an oil stone. Both straight and variously angled needles are useful (Kennedy, 1932). Barber and Rice (1936) use a medium-size sewing needle and grind the tip under the microscope. Lea cuts the tips off Dumont #5 microforceps and glues them into a pencil-size plastic rod and subsequently sharpens the tips.

Some workers use orange-wood sticks as needle holders. In this case a fine hole can be drilled into one end with a 1/64-inch drill, and the needle is bent about 1/3 of its length at an acute angle before inserting this end into the hole (the bent end firmly anchors the needle). A pair of sturdy forceps or long-nose pliers are used to jam the needle into position.

2. *Forceps.* Excellent microforceps of various types are available for dissecting mosquitoes. The needle-like Dumont #5 or Hamilton Bell #5 forceps (Ward's Natural Science Est., Rochester, N. Y.) are most valuable. The latter forceps are in plastic tubes with caps which reduces rusting and allows for easy safe storage. Aloe makes a series of very useful fine microforceps (e.g., V 37392, self-closing type; V 37380, microdissecting type with ultra fine points; and V 37384 A, with spear point tips). The tips must perfectly match. If the tips become damaged, an attempt should be made to straighten them with heavy forceps before sharpening and grinding. Grind first one side and then the other while the points are together.

3. *Scissors.* Iris, iridectomy, invertebrate microdissecting, accu-action, and fine cuticle scissors are not especially suitable for mosquitoes but Aloe makes particularly valuable clipper type microscissors (V 38556, V 38559 B). The latter has a 75μ blade tapering to a 3μ tip. Larsen recommends the Vannas scissor model 60 L-3587 (Lawton Company, New York).

4. *Scalpels.* New Gillette "super," Schick "Krona" or Wilkinson blades can be broken into fragments with diagonal ("Dykes") pliers and these fragments can be fitted into a mechanical (drawing) pencil holder. Fine tungsten wire (10μ tip) can be sheared and fitted into an orange-wood stick for very fine incisions (Hudson *et al.*, 1960).

5. *Special microneedles.* For injection or removal of material, fine glass capillary tubing (e.g. Aloe's V 23922P tubes which are 75 mm long x 1.4 to 1.5 mm diameter) is first drawn out in a microflame (a small hypodermic needle attached to gas line provides such a flame). Clean, sharp-edged tips can be made if the glass tubing is scored with a diamond pencil. Known size tips can be fashioned with a De Fonbruyne Microforge. Ephrussi and Beadle (1936) describe how to make transplantation needles: these have a special constriction which prevents the tissue from being sucked too far. Microneedles should be stored in saline which helps prevent tissue sticking to the glass sides. Aloe makes a microinjector and microsyringe (V 58091 A) which is most useful for transplantation work. Weathersby (1952) inserts injection needles into a 1/4-inch serum bottle stopper that is attached to another glass tube connected to a vacuum line.

OTHER MATERIALS AND EQUIPMENT

1. Binocular stereoscopic and compound microscopes, and illuminators. Lighting and magnification are particularly crucial to many types of dissection. Lamps with heat-absorbing filters may be used. American Optical, Wild, Leitz and Reichert stereomicroscopes can provide high magnification (up to about 200 times). Leitz makes a particularly useful paired lamp (COXEW type) which attaches to the dissecting microscope. A most useful vertical illumination device and gliding stage can be added to the Wild stereomicroscope.

2. Glassware. A variety of glassware may be used, e.g., dropping bottles for salines, sterilants, staining reagents, fixatives; medicine droppers or other pipettes, microscope slides, coverslips, stender dishes, Syracuse dishes, spot plates, and porcelain crucibles.

3. Wax, plasticine, or paraffin dissecting dishes of various types are particularly valuable. Larsen (personal communication) cuts lucite tubing of various sizes into rings; a bottom can be sealed on with plastic solvent.

4. Clay or plasticine is useful for special dissections and microsurgery.

5. Adhesives. "Dekadhese" plastic cement made by Donald Tulloch, Jr., Chadds Ford, Pa. is non-toxic and has many uses. Tacki wax is from Cenco.

6. Arkansas oil stone or Water of Ayr stone. Lea (personal communication) recommends a hard Arkansas bench stone (Behr-Manning Co., Division of Norton Co.).

7. Camel hair brush.

8. Salines. From 0.5 to 0.9 percent NaCl has been used (unbuffered) with mosquito dissections. Buffer tablets are now commercially available and perhaps should be used. Ephrussi and Beadle (1936) employed the following saline which is quite useful with mosquitoes:

NaCl	7.5 grams/liter (=128 mM/l)
KCl	0.35 grams/liter (=4.7 mM/l)
$CaCl_2$	0.21 grams/liter (=1.9 mM/l)

Bradford and Ramsey (1949) buffer the above saline with M/150 potassium phosphate to pH 6.8. In some cases it might be found advantageous to add a small crystal of either methylene blue, gentian violet, or neutral red.

DISSECTION TECHNIQUES

1. *General.* Generally, fresh living material is far superior to any fixed material for most dissections that are to be used for subsequent histological and cytological work. Two general methods are available, depending upon whether the mosquito is to be sacrificed or to be held for further study. With the "sacrificial" method, the organ is dissected into a small drop of saline on a glass slide using either needles and/or microforceps. With the "holding" method, the specimen is held in a plasticine mold or with thin plasticine strips or with tackiwax. Lavoipierre and Judson (1965) described a useful restraining table for implantation and transplantation experiments on mosquitoes. In many cases it is necessary to cover completely the specimen with saline. In all cases it is necessary to cover the operation site with saline and to work under it. Fine probes, needles, scalpels and/or forceps and/or microscissors are employed in surgical operations.

Ideally, specimens of known age, stage, sex, and physiological status should be used. A variety of methods are available to immobilize the specimen for handling prior to dissection. Larvae can be partially immobilized by withdrawing ambient water. Larvae and pupae are immobilized by placing them on an ice cube or on a cold stage. They may alternately be immobilized with ether vapors or 3 percent ether-water mixture or by placing them in 1 to 2 percent procaine hydrochloride. Adults are immobilized by chilling, or with nitrogen, ether, chloroform, or carbon dioxide vapors. If nitrogen gas is used, the specimens must be constantly held in a flowing stream of the gas, otherwise they can recover in seconds.

A few selected references giving directions for dissection of mosquitoes are: Patton and Evans (1929, see pages 708–714); Matheson (1950, see pages 595–597); Barber and Rice (1936); Mackie *et al.* (1945, see pages 678–679); Boyd *et al.* (1949, see pages 191–194); Hunter *et al.* (1950, see pages 837–840); Carpenter and LaCasse (1955, see pages 6–7); Belkin (1962, see pages 70, 73–77); Gordon and Lavoipierre (1962, see pages 316–310), and Russell *et al.* (1963, see pages 310–361).

2. *Sacrificial methods.* Needles are use-

ful for dissection of all stages. A sharp needle is held in each hand. Initially one needle (or fine forceps) is used to hold the specimen firmly and the other needle is used for puncturing, slitting or cutting. After the organ is located, both needles are used to help clean off extraneous tissues. The brain, imaginal discs, portions of the alimentary canal (Patton and Evans, 1929; Christophers, 1960), salivary glands (Patton and Evans, 1929; Jensen, 1955) and gonads (Polovodova, 1941; Detinova, 1959) can be often readily removed with needles alone. Microforceps may be substituted or used in conjunction with needles (Hodapp and Jones, 1961).

Microscissors are most valuable in complicated dissections, e.g., where one wishes to study the body musculature or the ventral nervous system or the circulatory system (Jones, 1954). Microscissors, however, can be used to great advantage in examining the internal anatomy of the adult in the following way. The thorax of the mosquito is held with microforceps and Aloe clipper microscissors are used to make a single lateral slice along as large a portion of the abdomen as possible (only one cut is usually possible). Still holding the thorax, the cut region is thrust into a drop of saline on a glass microscope slide. The cut region will separate or spread out due to the hydrofuge nature of the cuticle. The scissors are then used to sever the abdomen from the thorax. The various organs within the spread out abdomen are now obscured by the overlying cuticle, and it is therefore necessary to invert the microscope slide over a deep well-slide or concavity slide in order to see such organs as the dorsal vessel, alimentary canal and reproductive system (Curtin and Jones, 1961).

Microscissors are used in dissecting specimens which are pinned in a plasticine or paraffin dish. All dissections are done under a layer of saline. It is usually best to have the specimen ventral side uppermost, and to begin cutting at the posterior end and proceed laterally along one side first. The cut portions are then pinned out and then the other side is cut and subsequently pinned.

3. *Holding methods.* Where it is desirable to observe the specimen over a long period of time, surgical procedures must of necessity be done with much greater care and attention. Sterilized dissecting tools and saline are generally advisable for best survival. Use of antibiotics may help (e.g. streptomycin). Chao (1955) works within a sterile chamber and dips mosquitoes in 1:1000 hexylresorcinol to surface sterilize. If mosquitoes are fasted 2 to 4 days before surgery this makes the operations much easier due to the reduction of fat body (technique of Arden Lea). Weathersby (1952) recommends fasting the mosquitoes one day before injections. Larvae and pupae can be manipulated, injected or operated upon if they are placed on a small piece of cotton (or filter paper) moistened with saline, being held down with a strand or two of cotton in the manner of Ephrussi and Beadle (1935, 1936) (Larsen and Bodenstein, 1959).

Injected or operated larvae and pupae should not be returned to water if the wound is still open; the wound may be closed with paraffin or celloidin. Lightly anesthetized specimens can be arranged very gently upon a previously sculpted plasticine mould and the plasticine molded around the animal (also thin strips of plasticine can be used). The site of the operation is covered with sterile saline (Lea, 1963). Although Lea (1963) inserted streptomycin crystals in wounds after operating, he has subsequently observed that this step can be eliminated without affecting the survival (Lea, personal communication). Unusual care and experience are required to prevent breaking of legs and wings of the adults. But "delegged" and "dewinged" mosquitoes will survive quite well (Larsen and Bodenstein, 1959).

One site for injecting adults is the lateral thorax in the membranous area posterior to the mesothoracic spiracle (Weathersby, 1952), or in the metathoracic

coxa (Chao and Ball, 1956). Injection can also be made by inserting a fine needle in the intersegmental membrane between the third and fourth abdominal segments (Larsen and Lea, personal communications). Weathersby (1952) uses a pipette with a tip 40 to 50 microns; Chao and Ball (1956) use 10 to 90 micron tips.

WHOLE MOUNT TECHNIQUES

Whole mounts are particularly valuable for observations of many systems. Both live and fixed whole mounts should be used.

1. *Live whole mounts.* Undarkened eggs can be examined in water whole mounts. Darkened eggs can be cleared without injuring the embryo by the method of Hokama and Judson (1963). Rosay (1959) places *Culex* eggs in peanut oil for observing embryonic development.

Larvae can be placed in a small drop of clean water and gently covered with a thin coverglass. If done properly, the larva is not crushed but is greatly or entirely restricted in its movements. In some cases, a paper clip or strip of wire can be placed beside the drop of water to prevent crushing of large larvae; the water drop must be adjusted to prevent excessive larval movements. With this technique, it is possible to make detailed observations on nearly every tissue in the insect under various magnifications (10 to 480 times).

Pupae can be examined laterally in glass chambers fashioned from glass microscope slides and coverslips held together with glass cement. The chambers are arranged on a movable arm attached to a stand with clamps. The binocular microscope must be mounted parallel to the chamber on a rod stand.

Adults may be mounted in the center of a glass slide by placing their mesonotum and/or wings in a small drop of Dekadhese (or alternately wings may be cut off). The slide containing the adult is then inverted and placed on the stage of a compound microscope with the condenser lowered to accommodate the adult. Adults may also be glued with Dekadhese in various positions to the head of an insect pin and can be examined under the binocular microscope. With suitable specimens one can thus observe the beating of the heart, contractions of the midgut and the ventral diaphragm in the intact adult mosquito.

2. *Fixed whole mounts.* Fixed eggs can be cleared with aqua regia (DeCoursey and Webster, 1952) or with bleaching agents (see Mortenson, 1950; and Craig, 1955).

All stages may be fixed in a variety of substances (usually and preferably with alcoholic fixatives). They may be quick frozen or heat prefixed (Kennedy, 1932) before placing them in a suitable fixative. Specimens are placed in 95 percent ethanol in a test tube immersed in dry ice and alcohol bath (Spielman, personal communication) for quick freezing or are placed in a beaker of hot water (55 to 60° C. for one minute). After fixation, they may be transferred to 95 percent ethanol, thence to beechwood creosote for half an hour, and then in damar-balsam and coverslipped for a day (Spielman, personal communications).

Material can be placed directly into cellosolve and left until cleared and then mounted in balsam.

Portions of the body (e.g., genitalia) can be dissected in saline and then transferred to 70 percent alcohol in a porcelain spot plate and then transferred to xylene (or potassium hydroxide). The genitalia may then be placed in a drop of fresh balsam on a slide and oriented into a series of positions by periodically dipping microneedles in xylene. The brittle portions can be fractured off to reveal structural relationships. Using this balsam-xylene technique (Wheeler, personal communication), genitalia may be oriented into any desired position for subsequent permanent mounting. Capillary glass rods can be especially valuable as spacers arranged around the object to prevent crushing or distortion. Structures may also be oriented into any desired position by placing them in a tiny drop of copal gum (Fairchild and Hertig, 1948). Such structures

do not change position when mounted in balsam.

Live or fixed material may be mounted in Hoyer's medium and kept level in an oven or on a hot plate at 50° C. for 2–3 days. The preparation is ringed with clear fingernail polish. Belkin (1962) wets whole mosquitoes with 95 percent ethanol; dips in 5 percent hypochlorite for 30 seconds to 2 minutes; washes repeatedly; dips in ethanol; places in hot 10 percent KOH containing 0.2 percent of trisodium phosphate (the trisodium phosphate increases penetration); washes in hot water; brings up through alcohols, and dissects and mounts in euparal. Belkin (1962) leaves KOH macerated material in clove oil overnight and dissects in clove oil before mounting in euparal.

KOH macerated material can be stained after washing by placing 4 to 6 drops of stock acid fuchsin (0.5 g. acid fuchsin; 25 ml. of 10 percent HCl; 300 ml. H_20) to 10 ml. of water (Belkin, 1962).

General Biological Supply House, Inc., makes a particularly valuable non-resinous stain mountant (CMC-S) which allows one to make a permanent and well-stained mount of tissues directly from water or saline.

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EFFECT OF MATING SEQUENCE ON EGG-HATCH FROM FEMALE *AEDES AEGYPTI* (L.) MATED WITH IRRADIATED AND NORMAL MALES[1]

J. A. GEORGE

Canada Department of Agriculture, Vineland Station, Ontario, Canada.

INTRODUCTION. It has been suggested that the release of sterile males would be an effective means of controlling insect populations even when the target species is polygamous (Knipling, 1955, von Borstel, 1960) or, more accurately, polyandrous, for if the spermatozoa from treated males are equally competitive with those from normal males, the proportion of sterile to fertile eggs obtained should depend exactly on the proportion of sterile to fertile males competing for the females.

Both sexes of the yellow fever mosquito, *Aedes aegypti* (L.), mate repeatedly. A single female placed with 11 males copulated 50 times in one hour, and a single male caged with 16 virgin females copulated 30 times in 30 minutes (Roth, 1948). Radiosterilized males, caged with normal males and females in the ratio of 20:1:1, reduced the egg-hatch of the females from the normal 76 percent to 1.5 percent (McCray *et al.*, 1961). But field releases of irradiated males failed to effect any reduction in the population (Morlan *et al.*, 1962). Males sterilized by chemosterilants have since been found to be more effective than radiosterilized males in reducing the egg-hatch of virgin females placed with treated and normal males (Weidhaas and Schmidt, 1963).

Reported here are the results of an investigation of the relative competitiveness of spermatozoa from normal males and those from males sterilized by gamma-irradiation in the pupal stage.

MATERIALS AND METHOD. The Penang strain of *A. aegypti* was employed; it was obtained from Penang, Malaya and had been cultured for 7 years at the University of Western Ontario, London, Ontario. Larvae were fed on a 5:2:1 by weight mixture of brewer's yeast powder, blood albumin and ribonucleic acid. Unmated adults were obtained by separating the sexes by pupal size and taking the emergents within 12 hours of eclosion.

A group of 300 male pupae due to emerge within approximately 8 hours was divided into two equal lots, one of which was exposed to a dose of 8400 R. of

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