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PREPARATION OF THE CHORION OF EGGS OF AEDINE MOSQUITOES FOR MICROSCOPY [1, 2]

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During recent years, there has been an increased interest in the bionomics of eggs of the *Aedes* group of mosquitoes. Mosquitoes of *Aedes, Psorophora* and related genera spend most of their lives in the egg stage. Survey procedures for locating eggs are being standardized, prehatching treatments for destroying eggs are being devised and physiological and embryological studies are being made. Workers have been hindered in these activities by inability to recognize the eggs that have been obtained in the field.

In connection with the general problem of identifying eggs of aedine mosquitoes, a method that permits rapid and accurate examination of egg shells has now been developed. This depends on the fact that the layers that comprise the shell of the egg of the mosquito bear distinctive surface features which are specific in many instances. Descriptions of eggs given by Goeldi (1905) and Mitchell (1907) called attention to some of these features. Later work on *Anopheles* showed differences even at subspecific levels (cf. Horsfall, 1955 for ref.). Howard *et al.* (1912) and James (1922) made further observations on the surface structures seen on whole eggs of aedine mosquitoes. Marshall (1938) noted that the eggs of *Aedes* could be determined by gross shape but did not study surface features. Horsfall *et al.* (1952) found that eggs of *Psorophora* bear distinctive markings that are visible for the most part by reflected light and are very distinct when fragments of chorion are examined by phase microscopy.

Former methods for preparing fragments of chorion for mounting on microscope slides have met with certain difficulties that have now been overcome to a large extent. The natural color of eggs of many species of *Aedes* and *Psorophora* is so dark that light can not pass through the shell. The untreated chorion is hard and brittle, shattering readily when pressed by the coverslip. Crushing the whole egg

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causes membranes and fat globules from the larva to adhere to the shell, even after considerable washing, and photomicrography is hindered by any foreign material. Also, former methods do not allow permanent mounting and storage. Lastly, the former methods required preparation of slides of a great many eggs in order to obtain a few slides that could be used. The present method avoids these difficulties.

METHOD. The gelatinous exochorion and any adherent debris are removed by rolling the egg between two moist pieces of filter paper. The pattern of the exochorion usually approximates that of the endochorion. However, in some species where the exochorion is particularly adhesive, the pattern of the endochorion may be obscured by sheets of exochorion. Most of the exochorion will come off in alcohol.

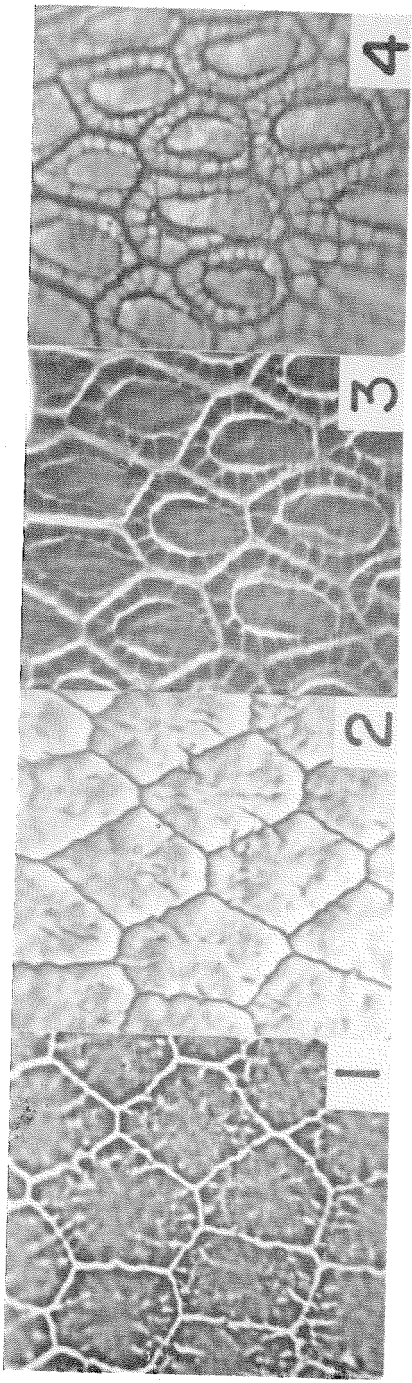
After the shell has been cleaned, the egg is placed in water in a deep flat-bottomed depression slide where preparations prior to mounting are carried out. The cap is removed from the anterior end of the shell and the larva and vitelline membrane are teased out with dissecting needles. If the bleaching operation is carried out while the larva is still in the shell, the bleaching proceeds very slowly and it becomes impossible to remove the shell contents. In living, freshly killed, or preserved eggs, the contents will tease out readily but when the egg has been dead for some time, most of the decomposed material must be squeezed out. This operation should not be carried out under alcohol because the vitelline membrane may then adhere to the inner surface of the endochorion. After dissection, the egg is placed in absolute alcohol and washed several times.

In order to remove the color and soften the rigid shell, the chorion must be bleached. This is done by a modification of Mayer's Chlorine Bleach (Gatenby and Beams, 1950). The Mayer method was used by Beckel, 1953, to study the embryology of eggs of *Aedes hexodontus*. Many other bleaching agents were tried, including aqua regia (De Coursey and Webster, 1952) and sodium hypochlorite (Mortenson, 1950). These were unsatisfactory because they removed the entire chorion or made it transparent so that the surface sculpturing was no longer visible.

For the bleaching operation, the alcohol is drawn off and some crystals of potassium chlorate (quantity is not important) are placed in the depression with the shell. Then concentrated hydrochloric acid is dropped on the crystals. The solution turns yellow and bubbles of free chlorine are given off. After a short time, the dark color is removed from the shell, which now appears translucent and light tan. Moreover, the shell loses its brittleness and inflexibility and becomes softer and more pliable. The shell can remain in this solution indefinitely without further change. If any dark color remains, more hydrochloric acid or potassium chlorate is added. Eggs differ according to species in the speed at which they are bleached. This is probably due to differences in the thickness of the shell. *Aedes atropalpus* and *A. aegypti* will bleach in 30 seconds or less while *Aedes triseriatus, A. stimulans* and *Psorophora ciliata* may take many minutes. Shells tend to float on top of the liquid. These will bleach but care must be taken to prevent them from being pushed out of solution by the bubbles.

Finally, the shell is cleaned of bleach by removing it from the bleaching solution to absolute alcohol. Transfers into or out of acid are made with a small wooden spatula or pointed wooden stick rather than a pipette. Fragments of crystals that may cling to the shell may be removed by immersing the shell in concentrated hydrochloric acid. Washing in several changes of absolute alcohol removes all acid.

The shell is mounted in euparal. The shell is transferred from the absolute alcohol with a drop of euparal on a dissecting needle and is placed in a drop of euparal on a slide. A No. 2 circular coverslip is placed on the drop and the coverslip is allowed to settle without pressure. Flat, single layers of chorion are obtained by sliding the coverslip back and forth with pressure around the egg



from the curved side of curved forceps. With practice, it is possible to flatten the shell and spread it into two single sheets.

MICROSCOPY. After the shell fragments have been mounted, the surface features may be examined with a standard or phase contrast microscope. The patterns on the eggs of most species of *Aedes* and *Psorophora* are entirely visible with a standard microscope under magnifications of 100× or less. However, for those species with faint patterns, the phase microscope is preferable, since it gives the impression of added depth and dimension to the surface features.

This method has been used for efficient identification of eggs of 15 species of *Aedes*. It has also been applied to eggs of 6 species of *Psorophora* with satisfactory results.

Plate I shows pieces of shell of eggs of *Psorophora* as observed by the phase contrast microscope. These pictures were taken with a Zeiss-Winkel attachment camera on Kodak M plates, using fine-grain developer. The prints were enlargements on high contrast paper. Magnification on the film was 420× through a 1.8 mm. objective and 10× occular.

SUMMARY. A method has been developed to examine the chorion of eggs of aedine mosquitoes by transmitted light. This entails removal of the larva from the shell, bleaching and softening the shell with potassium chlorate and hydrochloric acid, washing, mounting in euparal and breaking and separating the shell into flat, single sheets. The surface features of

PLATE I. Portions of bleached chorion of eggs of mosquitoes showing surface features by transmitted light as photographed with the phase contrast microscope. Position of fragments near anterior end of egg. Magnification 520 diameters.

Figure	Species	Phase contrast lighting
1	*Psorophora confinnis*	bright medium
2	*Psorophora confinnis*	dark low
3	*Psorophora varipes*	bright medium
4	*Psorophora varipes*	dark low

the shell of eggs of *Aedes* and *Psorophora* as observed by high power and transmitted light may indicate characters for classification of the eggs of these genera.

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NOTES ON THE INFLUENCE OF SALT-MARSH TOPOGRAPHY ON TIDAL ACTION[1]

W. L. BIDLINGMAYER AND J. W. KLOCK

Field observations of the effect of tidal inundation of several breeding areas of salt-marsh mosquitoes, *Aedes taeniorhynchus* (Wied.) and *Aedes sollicitans* (Walk.) in the Savannah, Georgia, area in 1952 suggested that tidal flooding was not always determinable by terrain elevation *per se* in relation to tidal levels. As a result, detailed observations were made on two salt-marsh areas on Little Tybee Island, Georgia, to observe the influence of a spring tide upon their inundation.

Little Tybee Island is a barrier island 2 miles long and less than 0.5 mile in width that lies about 1.5 miles behind the present coastline and is surrounded by extensive salt marshes. The island consists of a number of parallel sandy ridges which are separated by narrow sloughs, some of which extend the full length of the island. The vegetation of the ridges is composed primarily of pine and scrub palmetto associated with cabbage palms, cedar, and live oak. The lower reaches of the sloughs are dominated by a dense stand of *Spartina alterniflora* Lois, these plants being subjected to the daily ebb and flow of the tides. Farther up the sloughs dense stands of saltgrass, *Distichlis spicata* (L.) Beauv. frequently occur, while in the upper reaches the vegetation consists of freshwater species (e.g. *Mariscus jamaicensis* Crantz; *Hibiscus* sp.) which can probably tolerate a slight degree of salinity. The areas of the sloughs containing *D. spicata* frequently are good breeding sites for salt-marsh mosquitoes, particularly *A. taeniorhynchus* (Wied.). Two sloughs on this island were selected as sites for the tidal observations. The August spring tide studied was not as high as those that occur at other times of the year and thus may be considered more representative of the general tidal flows than would a spring tide

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