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CHORIONIC MORPHOLOGY OF EGGS OF THE *PSOROPHORA CONFINNIS* COMPLEX IN THE UNITED STATES. II. PRE- AND POSTDEPOSITION STUDIES OF *PSOROPHORA COLUMBIAE* (DYAR AND KNAB) EGGS[1]

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ABSTRACT. Scanning and transmission electron microscopic techniques were used to determine the morphology of the exochorion and endochorion of *Psorophora columbiae* eggs as found in the ovary and after deposition. In the ovary, the outer chorionic reticulations and tubercles of the eggs are ornately developed below the follicular epithelium. Expansion of the eggs after deposition causes the outer chorionic reticuli and sculpturing (i.e., minor and major tubercles) to form the characteristic spinose appearance of *Psorophora* eggs. Transmission electron microscopic micrographs of cross and sagittal sections of the chorionic layers of *Ps. columbiae* eggs indicate that the major outer chorionic tubercles have 3 distinct regions. The 3rd region was found only on the dorsum of the tubercle. Elevation of major tubercles appears to be regulated, at least in part, by ambient moisture conditions, and one function of these tubercles seems to be adhesion of the egg to an oviposition surface.

KEY WORDS Mosquito, *Psorophora columbiae,* egg, morphology, chorion

INTRODUCTION

In a previous study, we used scanning (SEM) and transmission (TEM) electron microscopy to compare the patterns of endochorionic sculpturing on embryonated eggs deposited by *Psorophora columbiae* (Dyar and Knab) females collected from different regions of the United States (Bosworth et al. 1983). Results of this previous study indicated that the species designated *Ps. columbiae* in California might be genetically different from the *Ps. columbiae* found elsewhere in the United States. In the current study, SEM and TEM were used to compare the chorionic morphology of *Ps. columbiae* eggs in the ovary (predeposition state) with that of oviposited eggs (postdeposition state) and to examine the exochorionic sculpturing of *Ps. columbiae* eggs in detail. With respect to this latter objective, the spinose exochorionic morphology of *Psorophora* eggs, as first described by Mitchell (1907), is considered a distinguishing characteristic of all species belonging to this genus of mosquitoes (Howard et al. 1912, 1917). However, detailed descriptions of the exochorionic structure of *Psorophora* eggs at the ultrastructural level are lacking.

MATERIALS AND METHODS

Predeposition Egg Study

In this study, fully developed oocytes (ova) were removed from primary follicles of *Ps. columbiae* ovaries, and their size, shape, and general appearance were determined under a stereo dissecting microscope equipped with a calibrated ocular micrometer. The surface morphologies of the outer and inner chorionic layers of these ova were then examined in detail using SEM techniques, while TEM was used to study the internal structures of these two layers.

Scanning electron microscopy procedures: Ovaries were dissected from anesthetized (with CO_2), uninseminated, gravid *Ps. columbiae* females that

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Fig. 1. Scanning electron micrograph of an ovariole dissected from the ovary of a *Psorophora columbiae* female showing the vitellarium containing a developing ovum (VT) and the germanium (GM).

Fig. 2. Scanning electron micrograph of a fully developed ovum of a *Psorophora columbiae* female covered by follicular epithelium. The letter A denotes the anterior end of the ovum, which is the end nearest to the germanium of the mosquito's ovariole.

Fig. 3. Scanning electron micrograph of the surface of a fully developed ovum of a *Psorophora columbiae* female

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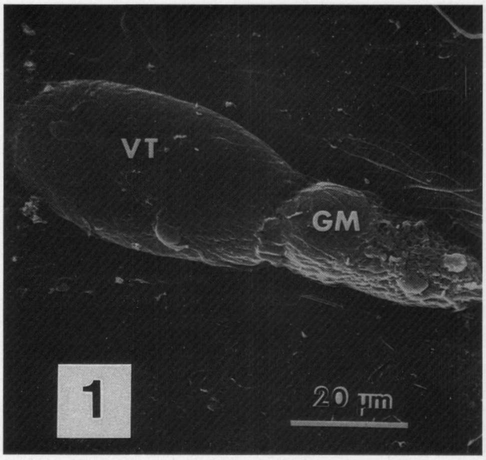
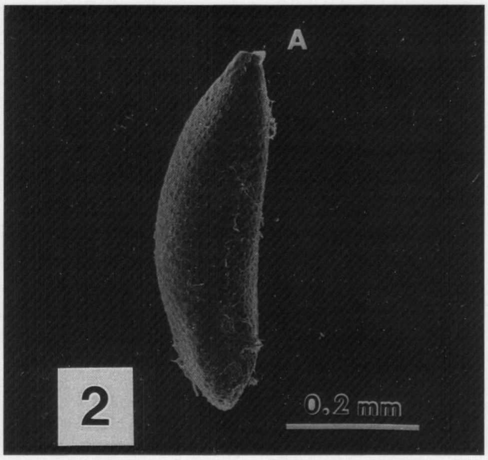
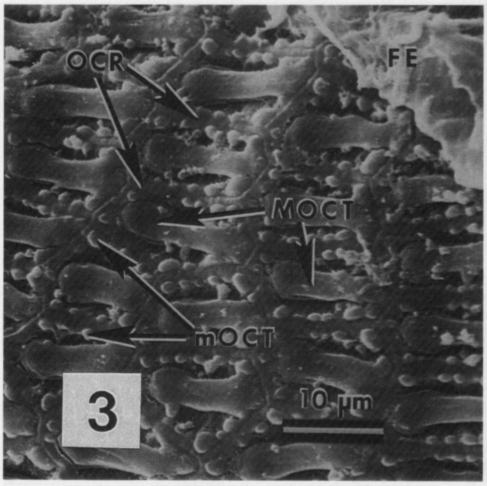
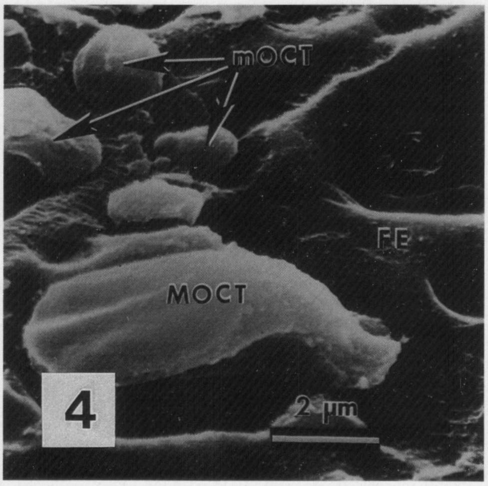
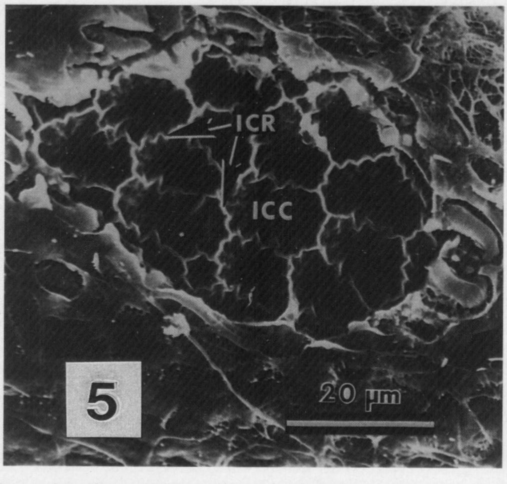






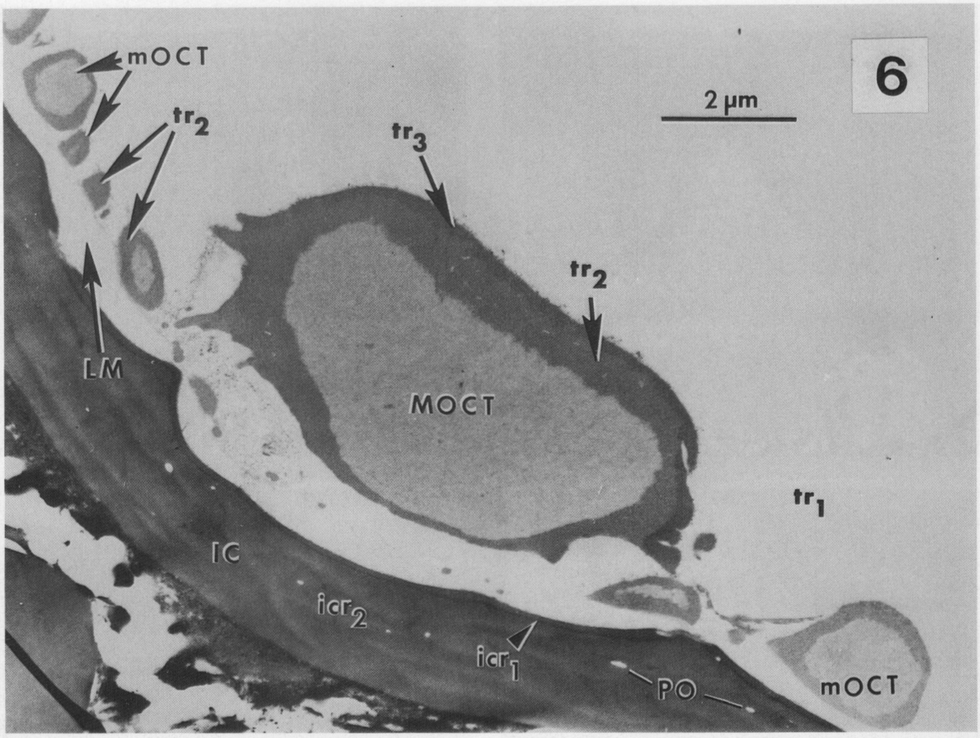




showing remnants of the follicular epithelium (FE), outer chorionic reticulations (OCRs), major outer chorionic tubercles (MOCTs), and minor outer chorionic tubercles (mOCTs).

Fig. 4. Scanning electron micrograph of the underside of the follicular epithelium (FE), which surrounds the ova of *Psorophora columbiae* females. Also shown are a major outer chorionic tubercle (MOCT) and some minor outer chorionic tubercles (mOCTs) that adhered to the follicular epithelium when it was removed from the ovum.

Fig. 5. Scanning electron micrograph of the inner chorionic cells (ICCs) and inner chorionic reticulations (ICRs) of a fully developed ovum of a *Psorophora columbiae* female.



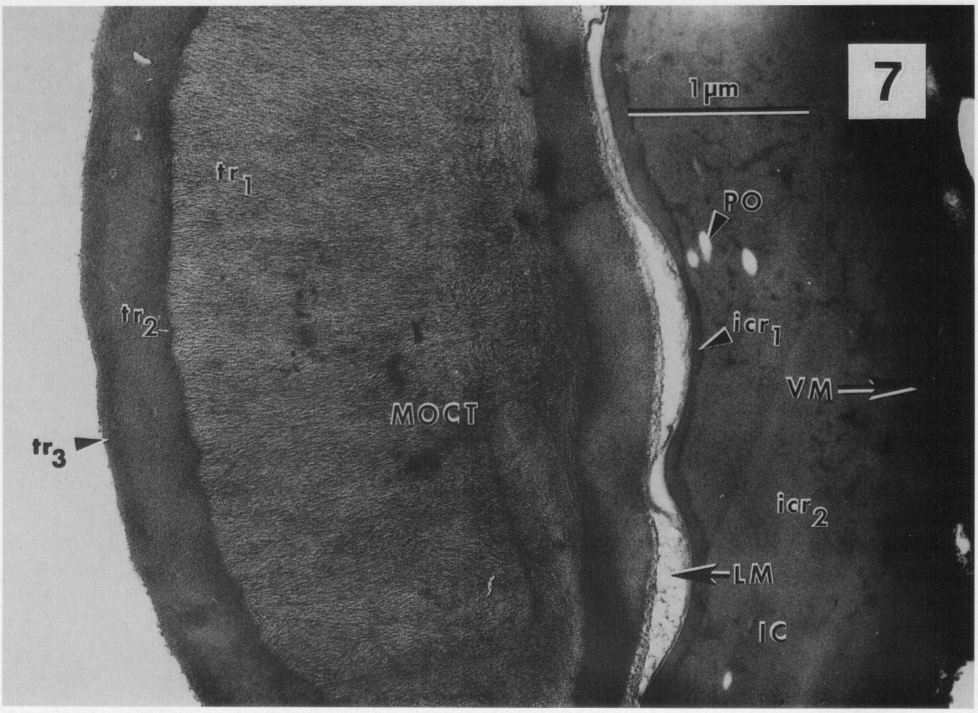


Fig. 6. Transmission electron micrograph of a cross section of the outer and inner chorionic layers of a fully developed ovum of *Psorophora columbiae.* Shown are the different regions (tr_1, tr_2, and tr_3) of the major and minor chorionic tubercles (MOCTs and mOCTs, respectively); the lamellate membrane (LM), which covers the surface of the inner chorion (IC); the two regions (icr_1 and icr_2) of the inner chorion (IC); and the pores (POs) in the IC.

Fig. 7. Transmission electron micrograph of a cross section of a major chorionic tubercle (MOCT) of a fully developed *Psorophora columbiae* ovum. Depicted are the inner, middle, and outer regions of the major tubercle (tr_1, tr_2, and tr_3, respectively); the lamellate membrane (LM), which covers the surface of the inner chorion (IC); the two regions of the inner chorion (icr_1 and icr_2); the pores (POs) in the inner chorion; and the vitelline membrane (VM).

had taken blood meals *ca.* 8 days prior to dissection. The females came from a population of *Ps. columbiae* reared in the laboratory from eggs deposited by wild-caught specimens of this species collected in Chambers County, TX. The dissected ovaries were placed in a fixative consisting of 3% gluteraldehyde, 2% paraformaldehyde, and 3 ml of saturated picric acid for every 21 ml of aldehyde dissolved in a 0.1 M phosphate buffer/0.1 M sucrose solution. Individual ovarioles and ova were teased from the ovaries while they were emersed in a bath of the fixative, and the follicular epithelium was removed. The ova were then pipetted into vials of the aldehyde fixative and stored in the fixative for 7 days at 4°C.

At the end of the fixation period, the ova were processed and prepared for SEM using procedures described by Meola (1982). The fixed ova were subjected to postfixation in 1% osmium tetroxide and dehydration in a graded alcohol series, followed by acetone and critical-point drying. After dehydration, measurements of the length and width of 10 ova were taken using the stereo microscope set at 80× and equipped with the calibrated ocular micrometer. The ova were then mounted on stubs with either double-stick cellulose tape, silver conducting paint (SPI Supplies, West Chester, PA), or Television TubeKoat® (GC Electronics, Rockford, IL). The mounted ova were viewed with a JEOL-U3 SEM at 25 keV and photographed with Kodak® Tri-X pan and Plus-X pan sheet film.

Transmission electron microscopy procedures: The ova used in this phase of the predeposition egg study came from a single female selected at random from a colony of wild-caught *Ps. columbiae* females collected in Chambers County, TX. The female was taken from the colony 3 days after it had taken a blood meal in the laboratory and was isolated in a 10-ml beaker cage. On the following day, the female was anesthetized (with CO_2), and its head, wings, and legs were removed with scissors. The body was then stored at 4°C for a month in the same aldehyde fixative as was used in the SEM study. The body was subsequently postfixed in osmium tetroxide, dehydrated in ethanol and acetone, and embedded in Spurr's resin (Spurr 1969). Thin (90 nm) cross and sagittal sections were then made of the entire body of the mosquito, and those sections containing pieces of mature ova were observed with a Philips 300 TEM.

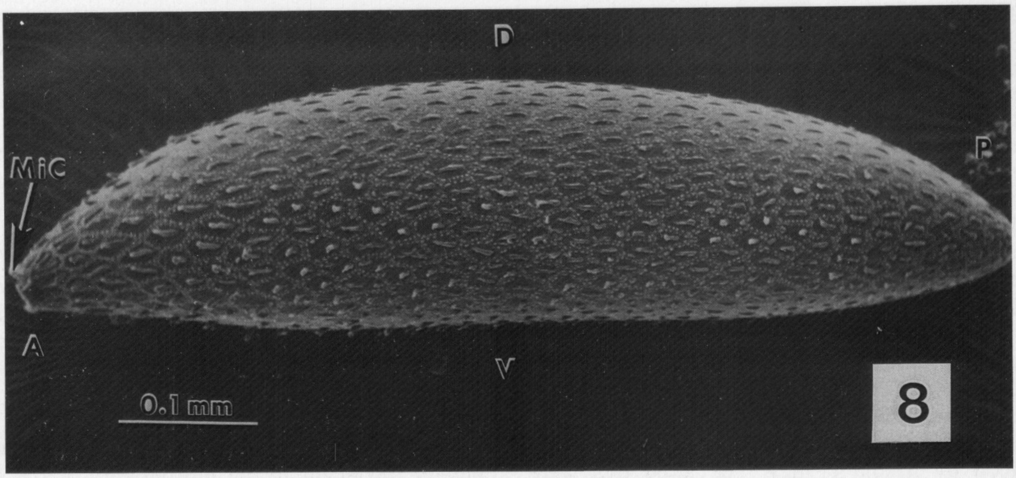
Postdeposition Egg Study

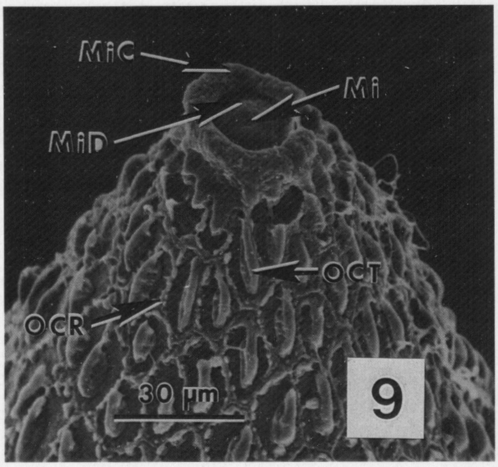
Egg size assessments: The stereo dissecting microscope set at 80× and equipped with a calibrated ocular micrometer was used to make egg size assessments. The sizes of fully embryonated *Ps. columbiae* eggs were determined by measuring the width and length of 10 1-week-old eggs from a batch of eggs deposited by a force-mated female taken from a colony of *Ps. columbiae* adults reared in the laboratory from eggs deposited by the wild-caught females collected in Chambers County, TX.

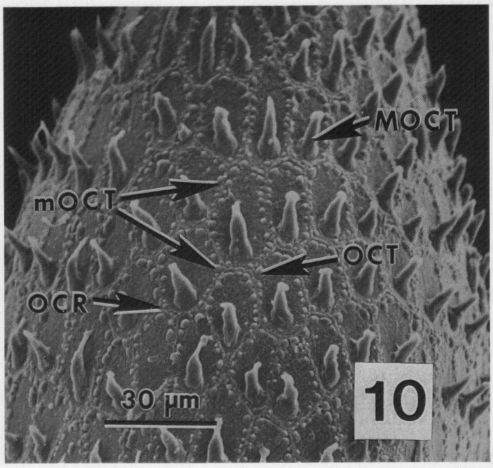
Ten eggs deposited by a decapitated *Ps. columbiae* specimen caught near Stuttgart (Arkansas County), AR, were used to assess changes in size of newly deposited eggs of this species over time. The length of each egg was initially measured just as it passed through the gonotreme. At this time, the chorion of the egg was untanned. Within an hour after its deposition, each egg had tanned, and its length was measured again. The eggs were observed continuously under the optics of the stereo dissecting microscope over the time between the initial and 1-h-postdeposition measurements to see whether or not any changes occurred in the morphology of the outer surfaces of the eggs.

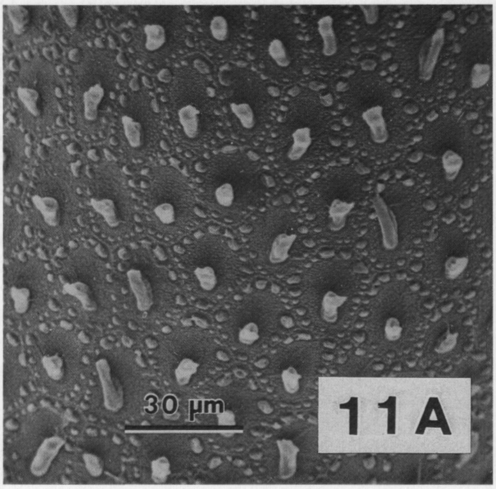
Outer chorion function assessments: Obvious changes did occur in the morphology of the outer chorion of the eggs described above, particularly with regard to the physical appearance and positioning of the exochorionic spines or tubercles on 1-h-old eggs as opposed to those on newly deposited eggs. Another study was initiated to gather more information on the factors involved in these observed changes and to gain some insight on the function of the tubercles. This study involved 100 embryonated eggs deposited by *Ps. columbiae* females collected in Chambers County, TX, and stored at 26°C for 1 month on damp filter paper pads in covered petri dishes. The eggs were removed from the storage pads using a camel hair brush and placed in a 10-ml beaker of cool (*ca.* 10°C), sterilized, deionized water. Cool water was used to prevent hatching. The eggs were subsequently passed through 4 additional rinses of cool water to remove surface debris and then were transferred to the surface of a 22-mm square coverslip using a sterile Pasteur pipette. The coverslip was then placed egg-side-up on a glass microscope slide, and during observation of the eggs with the stereo microscope, they were separated into 4 groups of 25 eggs each using the tip of a 26-gauge syringe needle attached to a 1-cc disposable syringe. The eggs in each group were positioned so that they did not touch each other and so that eggs lying either dorsally, ventrally, or laterally on the surface of the coverslip were represented in the group.

Following the grouping and positioning of the eggs, observations were made with both a stereo dissecting microscope and a compound microscope of the major positional changes of the chorionic tubercles on the eggs as the water evaporated from the coverslip. After the surfaces of all eggs appeared dry, small drops of sterilized water were applied to the surfaces of 3 of the egg groups, and the 4th group of eggs was allowed to remain dry. All groups of eggs were observed constantly at 100× while water was being added to the 3 groups of eggs and until all eggs in each group were once again dry.









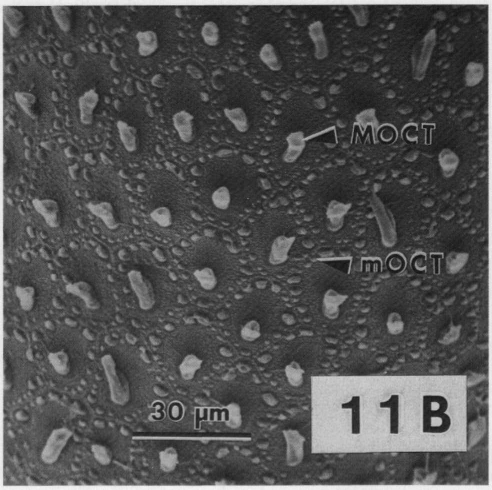


Fig. 8. Scanning electron micrograph of a deposited *Psorophora columbiae* egg with all chorionic layers intact. Anterior, dorsal, ventral, and posterior areas of the egg are denoted by A, D, V, and P, respectively. The micropylar area is the region near the micropylar collar (MiC).

Fig. 9. Scanning electron micrograph of outer chorionic sculpturing as it appears in the micropylar region of a deposited *Psorophora columbiae* egg. The micropyle is indicated as Mi, the micropylar disc as MiD, and the micropylar collar as MiC. Also shown are examples of the outer chorionic tubercles (OCTs) and outer chorionic reticulation (OCR).

Following the second drying of the eggs, they were all removed from the coverslip with a dry camel hair brush. The coverslip was then examined for rudiments of outer chorion in an effort to determine if the outer chorion adhered to the glass in the same manner as was described by Horsfall and Craig (1956) for the outer chorions of *Aedes* eggs.

Scanning electron microscopic assessments: Samples of embryonated eggs from all sources listed by Bosworth et al. (1983) were used to examine in detail the surface morphologies of the chorionic layers of postdeposited *Ps. columbiae* eggs. The structural appearances of deposited eggs with and without the outer chorionic layer were observed and recorded using both the JEOL-U3 and a Cambridge Stereo-scan SEM at 10–15 keV. Procedures followed in preparing the eggs for scanning were the same as those described by Bosworth et al. (1983) and Horsfall et al. (1970).

RESULTS AND DISCUSSION

Predeposition Egg Study

The ovariole as it appears in *Ps. columbiae* females while ova are developing is shown in Fig. 1. The formation of fully developed ova (Fig. 2) in this species occurs only after the female has taken one or more blood meals. The presence of a developing ovum in an ovariole is evidenced by an expansion and elongation of the vitellarium (VT, Fig. 1) in the region of the primary follicle. Each fully developed ovum is covered by a sheath of follicular epithelium (Fig. 2), which produces the egg chorion (Clements 1992). The anterior (micropylar) end of each ovum (A, Fig. 2) points toward the germanium of the ovariole (GM, Fig. 1) and in the general direction of the mosquito's head. In this position, the fully developed ova of *Ps. columbiae* were found to average 0.51 mm (±0.04 mm SE) in length and 0.14 mm (±0.01 mm SE) in width (n = 10).

Surface appearances of the chorionic layers of fully developed *Ps. columbiae* ova as viewed with SEM are shown in Figs. 3 and 4 (outer chorion) and Fig. 5 (inner chorion). The outer chorion is located directly beneath the follicular epithelium and appears as a network of small, widely marginated polygonal cells referred to as outer chorionic reticulations (OCRs, Fig. 3). A large major outer chorionic tubercle (MOCT, Fig. 3) appressed to the egg surface crosses the center of each polygonal cell parallel to the long axis of the egg. The posterior end of the MOCT can be differentiated from the anterior end by the former being round and knoblike in appearance. There are also a number of minor outer chorionic tubercles (mOCTs) within each reticular cell. The minor tubercles appear as small, roughly spherical structures located near the margins of each cell (mOCT, Figs. 3, 4). The structure and arrangement of the outer chorionic tubercles and reticulations on *Ps. columbiae* ova suggest that these items are products of the follicular epithelium (FE, Figs. 3, 4) as was described for other floodwater mosquitoes (e.g., Horsfall et al. 1970, Mathew and Rai 1975).

Removal of the outer chorionic layers of fully developed *Ps. columbiae* ova revealed a latticework of inner chorionic reticulations (ICRs, Fig. 5). The raised margins of each inner chorionic cell are strongly crenulated and appear to complement the margins of the outer chorionic reticulum that overlays the inner chorionic cell. Some of the crenatures of the inner chorionic cell (ICC) actually extend into the interior of the cell (Fig. 5), with the cell being roughly polygonal in form. Horsfall and Craig (1956) and Reinert (1972) described similar arrangements of marginal crenature for the inner chorionic cells of ova extracted from various species of *Aedes*.

Cross sections of the outer and inner chorionic layers of fully developed *Ps. columbiae* ova as viewed by TEM are shown in Figs. 6 and 7. The MOCTs (Figs. 6, 7) appear to be composed of 3 distinct regions. The innermost region, or central core, of each tubercle appears in micrographs as a light gray fibrous matrix of material (tr_1, Fig. 7). The second region, surrounding the central core, is a medium gray layer of homogeneous material (tr_2, Fig. 7). The third and outermost region occurs only on the dorsum of each major tubercle and is the darkest of the three regions as viewed in electron micrographs (tr_3, Fig. 7).

The mOCTs seem to have only one or two regions or layers in their structure (Fig. 6). All minor tubercles, regardless of size, have regions that appear similar in composition to the second region in major tubercles. The larger minor tubercles also have a central core of fibrous material like that observed in major tubercles. The central core of fibrous material and the homogeneous layer surrounding this core appear to correspond in composition to the regions of the outer chorionic tubercles of *Ae. aegypti* (Linnaeus) eggs described by Mathew and Rai (1975). However, the outermost

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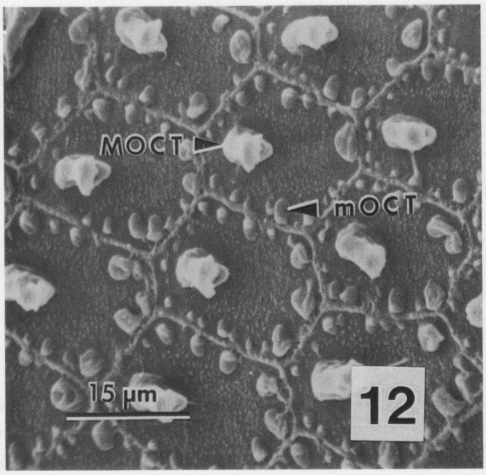
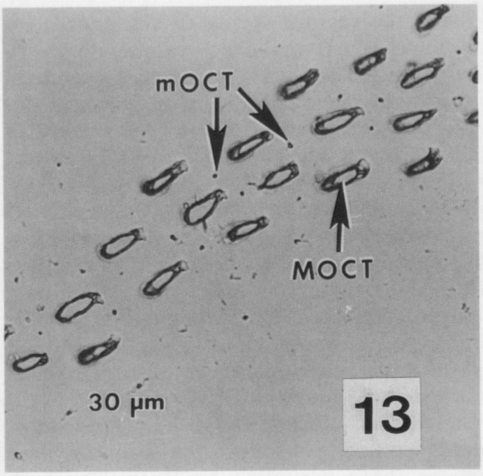
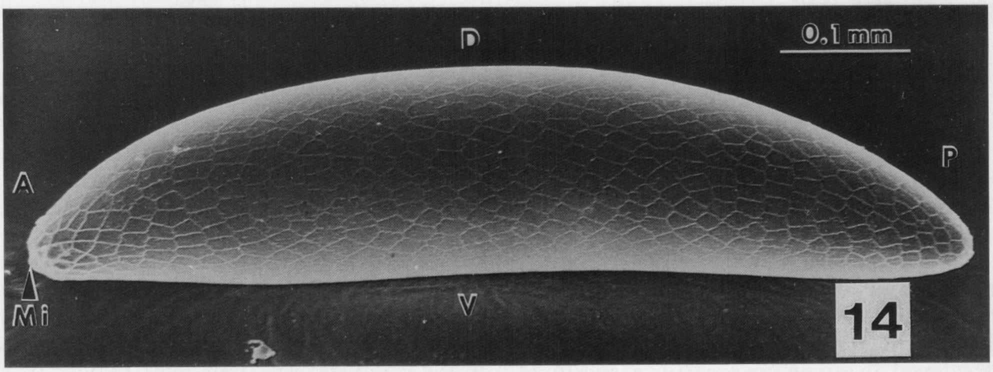
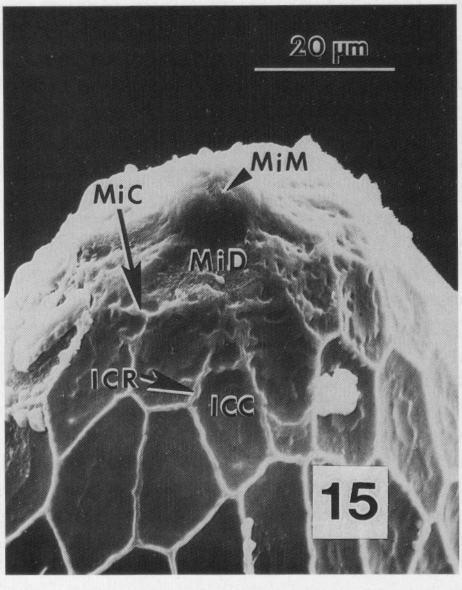
Fig. 10. Scanning electron micrograph of the outer chorionic sculpturing as it appears on the anteriolateral surface of a deposited *Psorophora columbiae* egg. The outer chorionic tubercles (OCTs) are divided into major outer chorionic tubercles (MOCTs) and minor outer chorionic tubercles (mOCTs). The outer chorionic reticulum is labeled OCR.

Fig. 11. Stereopaired scanning electron micrographs of the outer chorionic sculpturing as it appears on the anteriolateral surface of a deposited egg from the California species (*Psorophora confinnis* complex). The major and minor outer chorionic tubercles are labeled MOCT and mOCT, respectively.









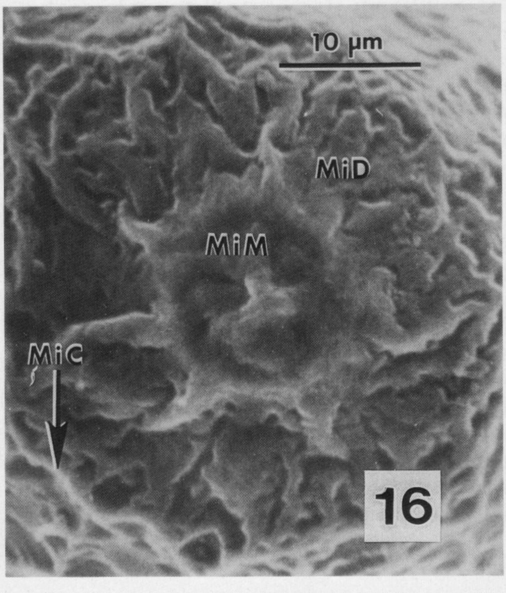


Fig. 12. Scanning electron micrograph of the major and minor chorionic tubercles (MOCTs and mOCTs, respectively) as they appear on the anteriolateral surface of a deposited *Psorophora columbiae* egg.

Fig. 13. Remnants of major and minor outer chorionic tubercles (MOCTs and mOCTs, respectively) that remained attached to a glass coverslip after a *Psorophora columbiae* egg was removed.

Fig. 14. Scanning electron micrograph of a deposited *Psorophora columbiae* egg with its outer chorion removed. The anterior, posterior, dorsal, and ventral areas of the egg are denoted by A, P, D, and V, respectively. The micropyle of the egg occurs in the area labeled Mi.

region of material (i.e., tr_3, Fig. 7) covering the dorsum of the major chorionic tubercles of *Ps. columbiae* appears unique, if not to the species itself, then perhaps to the genus *Psorophora.*

Postdeposition Study

An SEM view of the general appearance of a deposited *Ps. columbiae* egg with all chorionic layers intact is shown in Fig. 8. For purposes of defining the location of various structural features, each egg examined was divided into four general surface regions, i.e., the anterior (A), posterior (P), dorsal (D), and ventral (V) regions. These regions were based on the final position and orientation of the mature embryo housed in the egg. Thus, the anterior end of a deposited egg includes the micropylar area at its tip (Figs. 8, 9). The micropylar area includes the micropyle (Mi), the micropylar disc (MiD), and the micropylar collar (MiC), as shown in Fig. 9. The outer chorionic sculpturing (OCS) over the rest of the egg surface differs from that in the micropylar area and consists basically of outer chorionic tubercles (OCTs) bounded by OCR, as shown in Figs. 9 and 10. The reticuli form and delimit polygonal cells in the same manner as was observed for undeposited ova. The deposited egg also displayed two types of tubercles; i.e., MOCTs and mOCTs, as shown in Figs. 10 and 11. The tip of each major tubercle is expanded into a liplike or flanged structure that appears to take form while the egg is still in the ovary (Fig. 12).

A 3-dimensional SEM view of the relative positions and appearances of the tubercles and other structures of the outer chorion can be obtained by using a stereoviewer to observe the pair of micrographs shown in Fig. 11. Although this particular pair of micrographs depicts the surface of an egg of the California species of the *Ps. confinnis* complex (see Bosworth et al. 1983), the general appearances of the various structures composing the outer chorion of the eggs of this species are basically the same as those of comparable structures on the eggs of *Ps. columbiae.*

Deposited, fully embryonated *Ps. columbiae* eggs averaged 0.74 mm (±0.01 mm SE) in length and 0.18 mm (±0.01 mm SE) in width ($n = 10$). A comparison of these dimensions with those recorded for undeposited, fully developed ova indicates that deposited eggs of this species may be up to *ca.* 31% longer and *ca.* 22% wider than undeposited ova. Although these comparisons were made using ova and eggs from different parents, the female mosquitoes used as sources of these ova and eggs were similar in size and came from the same laboratory population.

Further evidence as to the degree that embryonated *Ps. columbiae* eggs increase in size once they are deposited was reflected by measurements taken of eggs just after they were deposited as compared to those taken of the same eggs 1 h later. The average length of these eggs increased from 0.58 mm (±0.01 mm SE) at the time of deposition to 0.73 mm (±0.03 mm SE) 1 h after deposition ($n = 10$), for an overall increase in egg length of *ca.* 21%. Christophers (1960) compared the length of *Ae. aegypti* eggs in the ovaries with that of eggs that had just been deposited and found similar increases in length. It should be noted that Bosworth et al. (1983) observed no noticeable increases in the length or width of unembryonated *Ps. columbiae* eggs after they were deposited, and this is why the inner chorionic patterns of these particular eggs remained wrinkled.

Coincident with the changes in size of a deposited embryonated *Ps. columbiae* egg over time, there are several changes in the appearance of the egg's outer chorion. As the egg is deposited, the posterior end leaves the genital opening of the female first with the exochorionic tubercles lying flat on the egg surface. Within an hour after the egg is deposited (i.e., during the time when the egg is increasing in size and tanning of the shell is taking place), the major tubercles separate at their anterior ends from the reticuli and lift up and away from the surface of the egg. The degree to which the major tubercles lift up from the egg surface appears to be regulated, at least in part, by ambient moisture conditions. For example, major tubercles subjected to drying conditions in this study lifted to an almost upright position, as shown in Figs. 10–12. However, when water was placed on eggs whose tubercles were nearly upright, the tubercles would immediately return to an almost horizontal position, although they never completely went back to being as appressed to the egg surface as they were when the egg was first deposited.

When wet eggs were left to dry on the surface of a glass coverslip, major and minor outer chorionic tubercles were found to hold the eggs to the glass surface. Upon brushing the attached eggs off the coverslip, it was found that the chorionic tubercles remained attached to the glass surface (Fig. 13). Thus, it appears that at least one function of the tubercles is adhesion, i.e., the same function performed by the "sticky pads" described by

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Fig. 15. Scanning electron micrograph of inner chorionic sculpturing as it appears in the micropylar region of a deposited *Psorophora columbiae* egg. The micropylar mound, disc, and collar are labeled MiM, MiD, and MiC, respectively. The inner chorionic cells and reticulation are denoted as ICC and ICR, respectively.

Fig. 16. Scanning electron micrograph of the micropylar region of a deposited *Psorophora columbiae* egg whose outer chorion has been removed. The micropylar mound, disc, and collar are labeled MiM, MiD, and MiC, respectively.

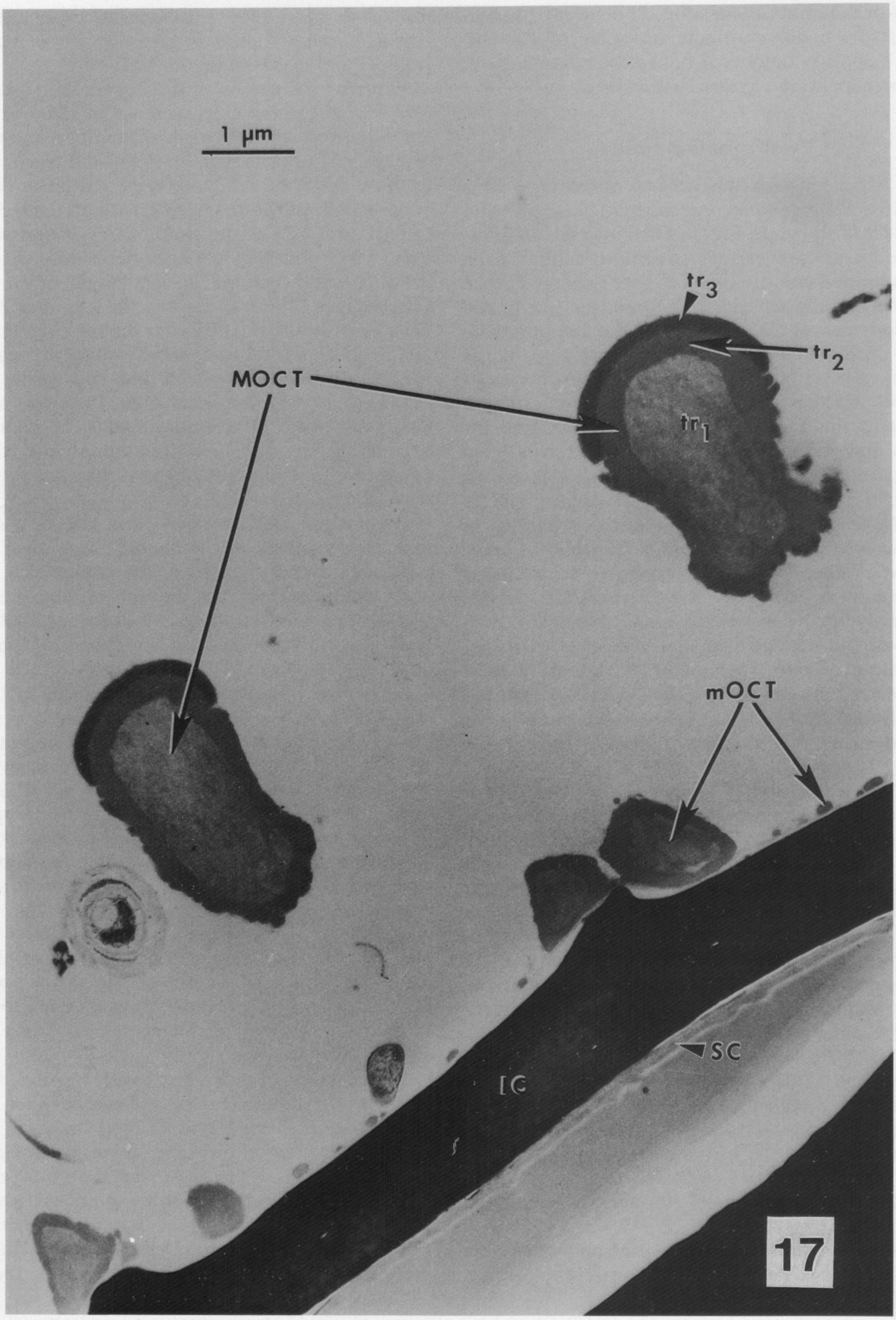


Fig. 17. Transmission electron micrograph of a cross section of the outer and inner chorionic layers of an embryonated egg deposited by a *Psorophora columbiae* female. Examples of the 3 regions of the major outer chorionic tubercles (MOCTs) are labeled tr_1, tr_2, and tr_3, respectively. Also shown are minor chorionic tubercles (mOCTs), the inner chorion (IC), and the serosal cuticle (SC).

Christophers (1960) on *Ae. aegypti* eggs. Padmaja and Rajulu (1981) indicated that the chorionic pad of *Ae. aegypti* eggs was made of a weak acid mucopolysaccharide that has properties of swelling and adhering to surfaces in water. The chemical composition of the tubercles on *Ps. columbiae* eggs was not determined in the current study.

The typical appearance of a deposited *Ps. columbiae* egg whose outer chorion has been removed is shown in Fig. 14. The pattern of inner chorionic sculpturing depicted in the micrograph agrees with descriptions given for eggs of this species by Horsfall et al. (1952). The entire surface of the inner chorion consists of a network of polygonal ICCs (Fig. 15) formed by the ICRs (Fig. 15). The only exception to this general pattern of inner chorionic sculpturing is in the vicinity of the micropylar area at the anterior tip of the egg (Mi, Fig. 14).

The micropylar area of a deposited *Ps. columbiae* egg with the outer chorion removed is shown in Figs. 15 and 16. Nomenclature for some of the inner chorionic structures located in this area of a mosquito's egg is not as clearly defined in the literature as it is for structures of the outer chorion. However, in keeping with the taxonomic glossary developed for mosquito eggs by Harbach and Knight (1980), the inner chorionic micropylar area of *Ps. columbiae* eggs appears to include a micropylar mound (MiM), an MiD, and an MiC (Figs. 15, 16). The MiM is the easiest structure to identify because of its domed shape. In contrast, the MiC is often seen as a slightly raised ridged line around the anterior of the egg and is occasionally difficult to differentiate from the inner chorionic reticuli in this area of the egg. The MiD is the surface area extending from the MiM to the MiC encircling the MiM.

Cross sections of the chorion of a *Ps. columbiae* egg after deposition are shown in Figs. 17–21. These figures represent a sample of the TEM micrographs taken of thin sections of the chorion located in the anteriolateral region of the egg. An analysis of all micrographs taken indicated that the internal morphology of MOCTs (Fig. 17), mOCTs (Figs. 17, 18) and the lamellate membrane (LM, Figs. 18–21) of deposited eggs are similar to those of the undeposited *Ps. columbiae* ova. In the case of deposited eggs of this species, the MOCTs have 3 regions (tr_1, tr_2, and tr_3, Fig. 17), with tr_3 again occurring only on the dorsum of the tubercle. The other 2 regions, i.e., the central core of fibrous matrix (tr_1) and the homogeneous region surrounding the matrix (tr_2), are common to all MOCTs and most mOCTs (Figs. 17, 18). Some minor tubercles may have only one region, with this region having the appearance of the tr_2 region of the other tubercles (mOCT, Fig. 19).

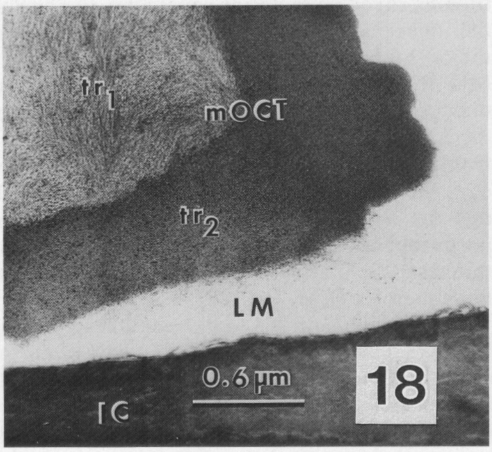
The LM (Figs. 20, 21) covers most of the surface of a *Ps. columbiae* egg, with breaking points occurring only at locations near ICRs (Fig. 21). In cross section, this membrane can be seen as a continuous layer of material separating the surface of the inner chorion from the bases of both minor and major tubercles (LM, mOCT, Fig. 19; and LM, MOCT, Fig. 20, respectively). As was the case for undeposited ova of *Ps. columbiae,* the thickness of the LM of a deposited egg of this species can vary from one point to another over the expanse of the egg. Measurements taken during this study indicate that the membrane is thickest (*ca.* 0.6 μm) at the posteriobasal portion of the major tubercles and thinnest (*ca.* 0.2 μm) beneath the bases of the minor tubercles (LM, Fig. 20).

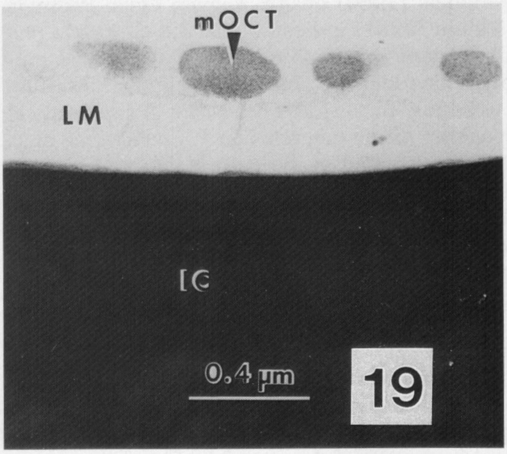
Micrographs of a sagittal section of an unembryonated deposited egg of *Ps. columbiae* and a cross section of an embryonated deposited egg of this species are shown in Figs. 20 and 21, respectively. The inner chorionic regions (icr_1 and icr_2) previously described for fully developed ova are not as distinct on micrographs of the deposited eggs. A slightly darker surface layer (possibly icr_1) can be distinguished from the rest of the inner chorion in Fig. 20, but no such distinction can be made in Fig. 21. Streaks of light and dark areas and pores (POs) are present in the inner chorionic region 2 (icr_2, Fig. 20). The oval POs are unevenly distributed within this region and are frequently found to be blocked or partially blocked with dark material in the case of deposited *Ps. columbiae* eggs. In contrast, these POs are always found open in the mature undeposited ova of this species (Fig. 7).

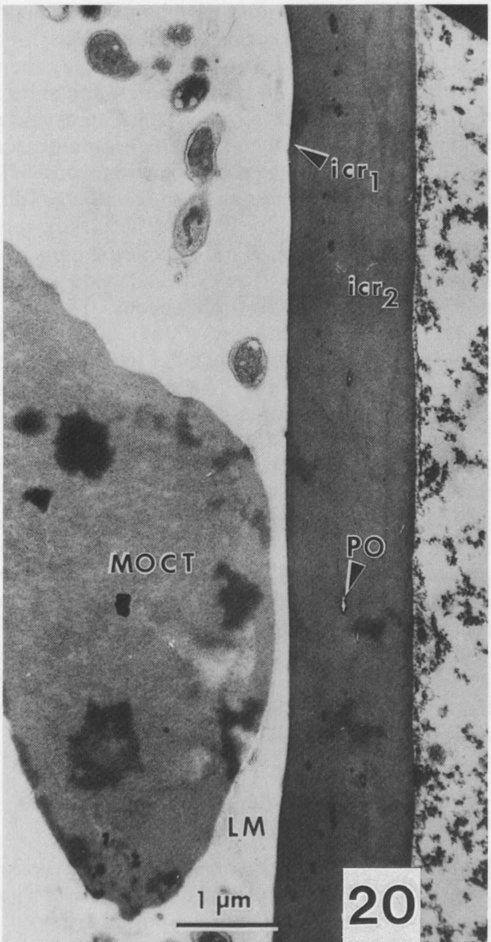
The vitelline membrane (VM) of embryonated deposited *Ps. columbiae* eggs is located on the surface of the serosal cuticle (SC), which in turn is directly below the inner chorion (IC, Fig. 21). The SC, which is *ca.* 0.32–0.43 μm thick, appears to be attached to the thin (*ca.* 0.01 μm thick) VM. Fully developed ova (Fig. 7) and unembryonated deposited eggs (Fig. 20) of *Ps. columbiae* do not have a distinctly formed serosal cuticle. As a result, the presence of the VM is uncertain in these cases because it may appear to be part of the underside of the inner chorion (Fig. 20). In some instances, the vitelline membrane may also be difficult to locate in embryonated *Ps. columbiae* eggs, especially if the serosal cuticle does not pull away from the inner chorion (Fig. 17).

SUMMARY AND CONCLUSIONS

As a result of this study, detailed descriptions of the exo- and endochorionic morphology of undeposited ova and deposited eggs of *Ps. columbiae* are now available and are supported by both SEM and TEM imagery data. The degree to which embryonated eggs of this species expand and elongate shortly after they are deposited has been documented. Also, at least one function of the MOCTs, which give the characteristic spinose appearance to eggs of *Ps. columbiae* and other species of *Psorophora,* has been determined: adhesion of the eggs to surfaces on which they are deposited. It was fur-







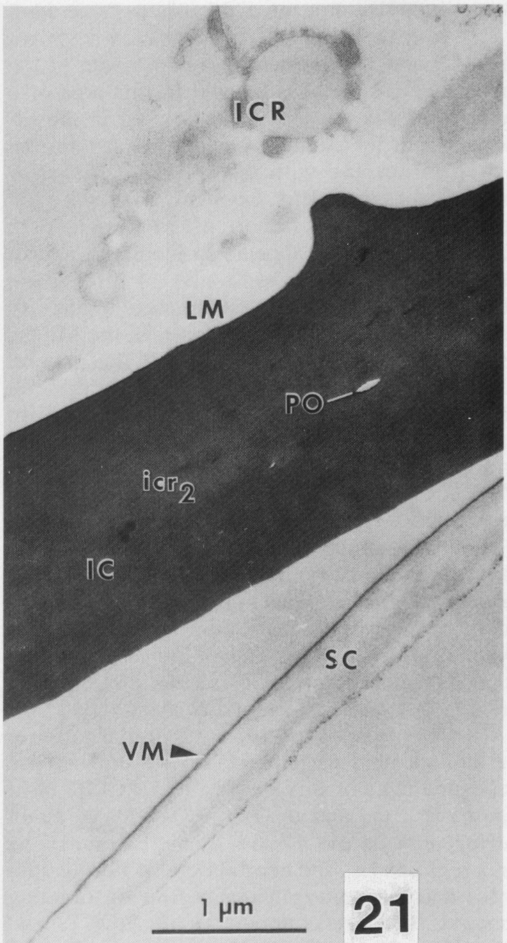


Fig. 18. Transmission electron micrograph of a cross section of a deposited *Psorophora columbiae* egg showing a minor outer chorionic tubercle (mOCT), a lamellate membrane (LM), and the inner chorion (IC). Also shown are the 2 regions of the minor outer chorionic tubercles (mOCTs): outer chorionic tubercle regions 1 (tr_1) and 2 (tr_2).

Fig. 19. Transmission electron micrograph of a cross section of a deposited *Psorophora columbiae* egg showing small minor outer chorionic tubercles (mOCTs), the lamellate membrane (LM), and part of the inner chorion (IC).

Fig. 20. Transmission electron micrograph of a sagittal section of the inner and outer chorion of an unembryonated

ther determined that the raising and lowering of these exochorionic tubercles appears to be regulated, at least to some degree, by ambient moisture conditions existing in the immediate vicinity of where the eggs are deposited.

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deposited *Psorophora columbiae* egg. Shown are a major outer chorionic tubercle (MOCT), the lamellate membrane (LM), the inner chorion (IC) with 2 regions (icr_1 and icr_2), and pores (POs) that occur in inner chorionic region 2.

Fig. 21. Transmission electron micrograph of a cross section of the lamellate membrane (LM), inner chorion (IC), and serosal cuticle (SC) of an embryonated deposited *Psorophora columbiae* egg. Shown also are region 2 (icr_2) of the IC, pores (POs) in the IC, and an inner chorionic reticulation (ICR).