FINE STRUCTURE OF THE EGGS OF Aedes (Ochlerotatus) Theobaldi, Ae. (Och.) Sagax and Ae. (Och.) Procax (Diptera: Culicidae)

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ABSTRACT. The fine structure of the eggs of Aedes (Ochlerotatus) theobaldi, Ae. (Och.) sagax and Ae. (Och.) procax is described with reference to scanning electron micrographs. In all three species, the ventral surface of the egg is more curved than the dorsal. Detailed structure is fairly uniform over all surfaces. Outer chorionic cell structure in the three species is basically similar, with each cell containing one or more large tubercles surrounded by several to many small ones positioned around the periphery of the cell. Large tubercles are single or fewer, and also larger in the anterior portion of the egg in Ae. theobaldi and Ae. sagax. Small tubercles are most numerous in Ae. theobaldi; least so in Ae. procax.

INTRODUCTION

The three Aedes species that form the subject of this paper, Ae. (Ochlerotatus) theobaldi (Taylor), Ae. (Och.) sagax (Skuse) and Ae. (Och.) procax (Skuse) are all floodwater species that bite man in varying degrees, and each can be at least a localized pest in southeast Australia. Their distributions in Australia are given by Lee et al. (1984). McLean (1953) showed that Ae. theobaldi was capable of carrying Murray Valley encephalitis virus (MVE), and Kay et al. (1989) proved Ae. sagax to be an efficient laboratory vector of MVE. Aedes procax has been considered a possible vector of myxomatosis virus (Fenner and Ratcliffe 1965), but several researchers have indicated that the other two species are unlikely to be vectors of this virus. Ross River virus has been isolated from Ae. procax in southeastern Australia (R. C. Russell, unpublished data).

Descriptions of the larval, pupal, and adult stages of these mosquitoes have, of course, been given previously, most recently for Ae. sagax and Ae. procax by Dobrotworsky (1965) and for Ae. theobaldi by Dobrotworsky (1965) and Marks (1967). The eggs, however, have never been described, although Pillai (1962), by means of celloidin impressions of the outer chorion, provided a surprisingly detailed view of the outer chorionic structure in Ae. theobaldi. In this paper we present complete descriptions of the eggs, based on scanning electron micrographs.

MATERIALS AND METHODS

Eggs were collected from wild-caught females given a blood meal and allowed to lay eggs on filter paper in the laboratory. Adequate time was allowed for embryonation, then the moist filter papers were shaped (eggs on inner surface) to line the insides of small petri dishes and mailed to Vero Beach. Groups of eggs were prepared for microscopy either by cutting out small circles of the paper and attaching them to stubs with silver paint, or by lifting individual eggs with a fine artist’s brush and placing them in required attitudes on sticky tape already attached to stubs. To ensure that eggs from several individual females were examined, eight stubs were prepared for each species. Each stub received eggs taken from widely separated positions on the egg papers, each of which had experienced oviposition from a number of gravid females. Once mounted, the specimens were completely desiccated over calcium chloride (0.5 hr), sputter coated with gold, and examined in a Hitachi S-510 scanning electron microscope.

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Dimensions of various structures given in the descriptions were determined from examination of at least 10 individual eggs in each case. Where mean (± SE) dimensions are given, they were calculated from an equal number of measurements from 5 individual eggs. Measurements of tubercles were taken across the widest point, as were the longitudinal and circumferential dimensions of the outer chorionic cells, with the following clarifications. The measurements were made from cells in the middle of the egg, on the ventral (most curved) surface. The Ae. theobaldi cells in this region are rounded at the anterior margin, but the complementary posterior margin has two of the cell corners forming pointed posterior extensions (Fig. 2b). The longitudinal dimension was therefore taken from the most anterior part of the curved front margin to the most posterior corner. In Ae. procax the longitudinal dimension is easily fixed, but the circumferential measurement was made across the two tongue-like extensions characteristically present in these cells (Fig. 12b). The Ae. sagax cells were more regular in form and were measured across the most widely separated corners in each direction.

In Ae. theobaldi the shape of the outer chorionic cells, as well as the numbers and sizes of tubercles they contain, change along the length of the egg. Similar changes in the tubercles occur also in Ae. sagax. Cells in the mid-ventral region were therefore selected as representative for these species and are described incorporating data from 5 eggs. However, changes along the length of these eggs are illustrated for both Ae. theobaldi and Ae. sagax by making measurements along the ventral surface of a single egg in each case. To do this, a composite micrograph was made showing almost the entire length (ventral surface) of the selected egg at 800x (Ae. theobaldi) and 600x (Ae. sagax). The mid-points of selected cells in the longitudinal axis, on or near the mid-ventral line along the length of the egg, were marked and the following data recorded for each cell: distance (μm) of cell mid-point from anterior margin of micropylar collar, numbers of large and small tubercles, diameter (μm) of all large tubercles, diameter (μm) of eight small tubercles in each cell. The small tubercles measured were the first eight positioned along the upper cell margin (egg viewed with anterior end at left) starting from the cell's anterior apex.

The terminology is that proposed by Harbach and Knight (1980). Additional terms are "outer chorionic cell field" (Linley 1989a) and "micropylar dome" (Linley et al. 1991).

DESCRIPTIONS

*Aedes (Ochlerotatus) theobaldi* (Figs. 1-5)

**Size:** As in Table 1. **Color:** Matte black. **Overall appearance:** Shape asymmetrical in lateral view, ventral surface more curved (Fig. 1), fairly broad in relation to length, broadly cigar-shaped in ventral view (Fig. 2a), anterior end somewhat conical, posterior end rounded (Figs. 1, 2a). Outer chorionic cells elongated in longitudinal axis, large tubercles conspicuously larger at anterior end, increasing in number but diminishing in size posteriorly (Figs. 1, 3). Micropylar collar rather indistinct.

**Chorion, ventral, lateral and dorsal surfaces:** Surfaces similar (Fig. 1), except as indicated. Representative cells in mid-ventral region with rounded anterior margin, lateral margins fairly straight, more or less parallel to longitudinal axis, posterior margins usually with 2 (sometimes 1) corners narrowing posteriorly (Figs. 2b,
Mean cell length 33.5±0.9 µm (n = 15), width 11.9±0.3 µm. Large tubercles variable in size, sometimes partly fused (Fig. 2b), mean number 7.5±0.4 (n=15), diameter 3.4±0.2 µm, dome-shaped with faint peripheral striations (Fig. 2c, d). Small tubercles numerous, mean number 38.1±1.6 (n=15), diameter 1.5±0.1 µm, mostly arranged around periphery of cell (Fig. 2b, e), round, smooth-surfaced and often partly fused (Fig. 2e). Surface of cell fields smooth, sometimes with scattered tiny tubercles. Outer chorionic reticulum clearly visible, width 1.4-1.7 µm, faintly striated with a central prominent, irregular and mostly continuous ridge (Fig. 2h), width about 0.6 µm.

Progressive changes in numbers and dimensions of large and small tubercles along length of an egg illustrated in Fig. 4. Large tubercles in most anterior cells occasionally single, or 2 or 3 in number (Figs. 1, 3), numbers increasing posteriorly, diameters decreasing, except at extreme end of egg (Fig. 4). Small tubercle numbers increase out to about 100 µm from anterior end, then decrease gradually towards posterior end, diameters correspondingly decrease initially, then increase and remain more or less constant in size towards posterior end (Fig. 4).

Outer chorionic cells at anterior end often pentagonal or hexagonal (Figs. 2c, d; 3). Large tubercles prominent, domed, with clearly visible striations (Fig. 2c). Raised central ridge of reticulum very prominent, almost always continuous (Fig. 2c). Slightly more posteriorly, cells become larger, large tubercles smaller with fainter top surface markings, ridge in reticulum less raised (Fig. 2d). Towards posterior end, large tubercles much less prominent, some with tops slanting directly into contact with cell floor (Fig. 2f), reticulum less conspicuous, central ridge mostly discontinuous, reduced in most places to a row of bead-like prominences (Fig. 2f), but flanking striations still visible.

Lateral and dorsal surfaces of egg much the same as ventral surface, except that cells in middle and posterior regions generally tend to have more and smaller large tubercles, with almost smooth surfaces (Fig. 2g), ridge of reticulum broken.

Anterior end, micropyle: Chorionic cells immediately behind collar small with several large tubercles almost completely fused longi-
Fig. 2. *Aedes theobaldi*. (a) Whole egg, ventral view; (b) chorionic cells, mid-ventral region of egg; (c) ventral surface cells just posterior to micropylar collar; (d) cells at anterior 1/5; (e) mid-ventral cells; (f) cells at posterior 1/4; (g) lateral surface cells, middle of egg; (h) detail, large and small tubercles and chorionic reticulum. Scale = 100 μm (a), = 20 μm (b), = 10 μm (c,d,e,f,g), = 5 μm (h).
tudinally (Fig. 5a), slightly more posterior cells with one or two large tubercles, small tubercles almost all fused into groups (Fig. 5b). Micropylar collar well developed, but not pronounced, conforming to overall taper of egg, usually discontinuous, with 1 or 2 gaps (Fig. 5c, d), but sometimes continuous. Collar height 7-12 µm, outer diameter 29-38 µm, wall width 3-7 µm, surface slightly rough (Fig. 5d), internal diameter 19-23 µm, inner wall with shallow excavations (Fig. 5c, d). Micropylar disk slightly raised, diameter 12-14 µm, surface nodular, central part domed, but dome not clearly demarcated within disk, micropylar orifice trilobed, diameter 2.6 µm.

Posterior end: Chorionic cells become smaller approaching posterior end, large tubercles somewhat fewer, slightly larger (Figs. 4; 5e, f), tending to be fused, surfaces slightly rough. Small tubercles become less distinct at very end of egg, ridge of reticulum visible but not prominent (Fig. 5f).

**Aedes (Ochlerotatus) sagax (Figs. 6-10)**

Size: As in Table 1. Color: Satiny matte black. Overall appearance: Asymmetrical in lateral view, ventral surface more curved, dorsal surface relatively flat (Fig. 6), anterior end conical, as visible also in ventral view (Fig. 7a), width of egg greatest at about anterior 1/3, egg then tapered steadily to rounded posterior end (Figs. 6, 7a). Boundaries of outer chorionic cells not easily distinguished, in some places detectable only from peripheral rings of small tubercles surrounding large ones (Figs. 6, 8). Large tubercles conspicuously larger at anterior end of egg, diminishing in size towards middle, but becoming larger again at posterior end (Figs. 6, 8). Micropylar collar not prominent.

Chorion, ventral, lateral and dorsal surfaces: All surfaces similar except for small differences as noted. Representative cells in mid-ventral region irregularly pentagonal or hexagonal (Fig. 7b, c), cell boundaries visible more by arrangement of small tubercles (especially Fig. 7c) than reticulum, which is not easily visible. Mean cell length 24.1±1.0 µm (n=10), width 25.9±1.4 µm. One or 2 prominent large tubercles present, mean diameter 5.5±0.1 µm (n=15), very round in shape (Fig. 7b, c), each consisting of a
Fig. 4. *Aedes theobaldi*. Numbers and diameters of small and large tubercles in cells along ventral surface of egg (from Fig. 3). Distances measured from anterior end.

smooth-walled base supporting a round cap ornamented with many small, low nodules (Fig. 7e). Small tubercles also round, structure similar to large tubercles (Fig. 7b, c, e), mean number 14.3±0.8 (n=15), mean diameter 1.9±0.2 μm (n=15). Small tubercles arranged around boundary of cell somewhat loosely in some eggs (Fig. 7b), very clearly so in others (Fig. 7c). Cell fields fairly smooth. Outer chorionic reticulum difficult to detect, width 1.7-2.2 μm, made up of irregularly spaced minute protuberances, diameter 0.2-0.35 μm, often with low, wavy ridges oriented predominantly across width of reticulum (Fig. 7e).

Numbers and dimensions of large and small tubercles along length of eggs as in Fig. 9. Al-
Fig. 5. *Aedes theobaldi*. (a) Anterior end, lateral view; (b) anterior end, chorionic cell detail; (c) anterior end and micropylar apparatus; (d) detail, micropylar apparatus; (e) posterior end, lateral view; (f) posterior end, chorionic cell detail. Scale = 20 μm (a,b,c,e,f), = 10 μm (d).
tubercle diameters diminish steadily from anterior end to about middle of egg, then continue to decrease slightly before increasing at posterior end (Figs. 6, 9). Comparable changes in numbers and diameters of small tubercles also shown in Fig. 9.

Some chorionic cells in anterior ventral region of several eggs examined had low, short and thick ridges arranged seemingly at random both in cell fields and in the area occupied by reticulum (Fig. 7d). In all eggs cells on dorsal (least curved) surface had considerably fewer small tubercles and were bounded by a reticulum visible only as a low ridge (Fig. 7f).

**Anterior end, micropyle:** Chorionic cells immediately posterior to micropylar collar small, each with single large usually elongated tubercle, few small tubercles, reticulum distinctly raised (Fig. 10a, b). Micropylar collar with slightly rounded outer wall, but not conspicuous (Fig. 10a). Wall almost always interrupted, with 1-3 gaps (Fig. 10c, d), rarely continuous (Fig. 10e), surface slightly rough. Collar height 9-13 µm, outer diameter 38-41 µm, wall width 2-8 µm and highly variable, interior diameter 24-32 µm, inner wall with shallow excavations and deeper radial notches (Fig. 10d, e). Micropylar disk clearly defined, usually with elevated ridge around outer edge (Fig. 10c, d), but not always so (Fig. 10e). Disk diameter 16-22 µm, micropylar dome sometimes visible (Fig. 10d), but sometimes not (Fig. 10e), diameter 10-14 µm when present. Micropylar orifice indistinctly trilobed, diameter 2.2 µm.

**Posterior end:** Chorionic cells smaller, almost invariably a single large tubercle of greater diameter than in slightly more anterior cells (Fig. 10f, g). Small tubercles fewer in number.

*Aedes (Ochlerotatus) procax* (Figs. 11-13)

**Size:** As in Table 1. **Color:** matte black. **Overall appearance:** Asymmetrical in lateral view, ventral surface more curved than dorsal (Fig. 11), widest at about anterior 1/3, slightly conical anteriorly in both lateral and ventral view (Fig. 12a), gradually tapered posteriorly to rounded end (Figs. 11, 12a). Outer chorionic cells visible by groupings of tubercles, but cell boundaries not easily visible at low magnification (Fig. 11). Each cell with one to several large

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Fig. 6. *Aedes sagax*. Entire egg, lateral view, ventral side at right, anterior end at top. Scale = 100 µm.

most invariably a single large tubercle in the anterior and posterior portions of the egg, but some cells in the middle part with 2. Large
Fig. 7. *Aedes sugax*. (a) Whole egg, ventral view; (b) chorionic cells, mid-ventral region of egg, small tubercles more randomly arranged; (c) cells, mid-ventral region, small tubercles arranged in lines along cell boundaries; (d) chorionic cells in anterior ventral region, showing low, thick ridges occasionally present; (e) detail, large and small tubercles and chorionic reticulum; (f) chorionic cells, dorsal surface, middle of egg. Scale = 100 μm (a), = 20 μm (b,c,d,f), = 5 μm (e).
tubercles, many small ones.

**Chorion, ventral, lateral and dorsal surfaces:**
All surfaces similar. Outer chorionic cells distinctly shaped, length less than width owing to presence of tongue-shaped extensions of each cell in the circumferential direction (Fig. 12b, c). Mean length of representative mid-ventral cells $17.7 \pm 0.4 \mu m$ ($n=10$), width $35.4 \pm 1.5 \mu m$. Cell boundaries easily distinguished owing to easily visible reticulum (Fig. 12b, c). Large tubercles single on many eggs (Fig. 12b, c), but structure variable, some eggs with more than one large tubercle, or several, which may barely be distinct from small tubercles (Fig. 12d). Single large tubercles more or less round, mean diameter $4.6 \mu m$ ($n=10$), consisting of a smooth-walled base with more or less round cap covered with small, low nodules (Fig. 12g). Shape of multiple large tubercles much more irregular, top surfaces slightly rough or with poorly defined nodules (Fig. 12d). Small tubercles numerous, mean number $23.7 \pm 0.8$ ($n=10$), shapes very variable (Fig. 12d, g), some barely raised above floor of cell, some much more prominent, formed into small domes or short, wide ridges (Fig. 12d, g). Outer chorionic reticulum fairly distinct, width $2.1-2.4 \mu m$, formed of a very indistinct meshwork with a central row of tiny tubercles, diameter $0.30-0.33 \mu m$, spaced at short intervals (Fig. 12d, e).

Cells on mid-lateral parts of egg only rarely with single large tubercle, usually several present (Fig. 12f), but otherwise similar to ventral surface cells. Dorsal surface cells essentially same as those on ventral surface (Fig. 12g).

**Anterior end, micropyle:** Cells at anterior end smaller, almost invariably with one large tubercle, small tubercles fewer and less distinct, cell boundaries very clearly delineated by continuous raised central ridge in reticulum (Fig. 13a, b). Cells immediately posterior to micropylar collar more irregular, large tubercles often low, smooth and poorly defined, small tubercles indistinct (Fig. 13b). Micropylar collar not prominent, conforming to overall taper of egg, rounded anteriorly, surface slightly rough (Fig. 13d). Collar height $9-11 \mu m$, outer diameter $27-35 \mu m$, sometimes continuous (Fig. 13d), but amount of collar present extremely variable, some with small gaps (Fig. 13c), with large

Fig. 8. *Aedes sagax*. Whole egg, ventral surface, enlarged view. Scale = $50 \mu m.$
Fig. 9. *Aedes sagax*. Numbers and diameters of small and large tubercles in cells along ventral surface of egg (from Fig. 8). Distances measured from anterior end.

pieces of collar missing (Fig. 13e), or almost all missing (Fig. 13f). Collar wall width 2-9 μm, extremely variable, inner diameter 20-23 μm, interior surface with shallow excavations (Fig. 13c, d). Boundary of micropylar disk raised, clearly delimited, also tending to have shallow excavations (Fig. 13d), diameter 14-18 μm, micropylar dome clearly visible in some eggs (Fig. 13d), less so in others (Fig. 13e), diameter when present 12-13 μm. Orifice of micropyle 2.1 μm in diameter.

*Posterior end:* Cells smaller towards posterior end, tongue-like extensions not present in cells very close to end of egg (Fig. 13g, h). Numbers of tubercles fewer, large and small tubercles becoming crowded and partly fused, reticulum with continuous, prominent central ridge, as at anterior end (Fig. 13h).
Fig. 10. *Aedes sagax*. (a) Anterior end, lateral view; (b) anterior end, chorionic cell detail; (c) anterior end and micropylar apparatus, collar with 3 gaps; (d) micropylar apparatus, collar with single small gap, disk with peripheral ridge; (e) detail, micropylar apparatus, disk without peripheral ridge; (f) posterior end, lateral view; (g) posterior end, chorionic cell detail. Scale = 20 μm (a,b,c,f,g), = 10 μm (d,e).

**DISCUSSION**

In order to gain complete familiarity with the eggs and also to check for consistency in the structure, at least 70 eggs of each species were examined from various aspects under the electron microscope. Structural uniformity was generally high, especially in *Ae. theobaldi* and
the inner edge of the reticulum, rather than more scattered within the cell field. However, individual eggs did not differ greatly in appearance. In *Ae. proca*, there were differences in the numbers and sizes of the large tubercles. Some eggs had more cells with a single large tubercle, especially towards the anterior end, while others had only very few such cells, or ventral surface cells with several large tubercles of smaller size rather than one or two larger ones (earlier discussion). The appearance of the whole egg was not much affected by these differences, however, and was as shown in the whole egg micrograph.

Consistency in structure and appearance was checked not only for its morphological importance, but because we were concerned with practical insights that might devolve from an intimate appreciation of structure. For example, it was not immediately evident from stereomicroscopic examination alone that eggs of *Ae. aegypti* (L.) could be distinguished from those of *Ae. albopictus* Skuse solely on the basis of the micropylar collar (Linley 1989a). Considerably more confidence exists in the interpretation of a stereomicroscopic image when it is viewed against a clear mental image of the fine structure. We therefore examined the eggs of all three species for possible stereomicroscopic differentiation.

Eggs of all three species considered in this paper are distinctive under the light microscope. *Aedes sagax* is the easiest to recognize; the single round large tubercle in almost all cells stands out clearly against the rather smooth, shiny cell field, giving the egg a spiny appearance. *Aedes theobaldi* eggs can be separated from the other two because the shape of the longitudinally elongated cells can be distinguished and the predominantly longitudinal alignment of the large tubercles also is evident, although the tubercles are not individually as prominent as in *Ae. sagax*. *Aedes proca* is the most difficult and is less easily separable from *Ae. theobaldi* than *Ae. sagax*. It lacks, however, the clear longitudinal cell elongation of *Ae. theobaldi*, and the several large tubercles can be seen clustered together in a more or less compact, round group. As is invariably the case when training the eye to detect small differences, practice and familiarity with the material progressively facilitates the

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**Fig. 11.** *Ae. proca*. Entire egg, lateral view, ventral surface at left, anterior end at top. Scale = 100 μm.

*Ae. sagax*. Some variation in the distribution of small tubercles in ventral surface cells occurred in *Ae. sagax*, inasmuch as the outer chorionic cell boundaries were more easily discernible when the small tubercles were arranged in lines along
Fig. 12. *Aedes procax*. (a) Whole egg, ventral view; (b) chorionic cells, mid-ventral region of egg; (c) detail, cells in mid-ventral region of egg, type with single large tubercle; (d) variant type of mid-ventral cell with multiple large tubercles of smaller size; (e) detail, large and small tubercles and chorionic reticulum; (f) cells in lateral region, middle of egg; (g) cells on dorsal surface, middle of egg. Scale = 100 μm (a), = 20 μm (b,c), = 10 μm (d,f,g), = 5 μm (e).

ability to distinguish accurately. As eggs are described for other floodwater species that share the habitats of the three here considered, it will be of interest to see if stereomicroscopic recognition of their eggs is practicable.

As a final point it is worth noting that, although nothing is known of the oviposition behavior of these three species, the overall sur-
Fig. 13. *Aedes procax*. (a) Anterior end, lateral view; (b) anterior end, chorionic cell detail; (c) anterior end and micropylar apparatus, collar with single gap; (d) micropylar apparatus, collar complete; (e) micropylar apparatus, large section of collar absent; (f) micropylar apparatus, collar almost completely absent; (g) posterior end, lateral view; (h) posterior end, chorionic cell detail. Scale = 20 μm.
face uniformity of their eggs indicates that they probably do not cement their eggs to the oviposition substrate. In species that do, the glued surface tends to be quite different in structure (Linley 1989a, 1989b), or apparently is adapted to enhance adhesion (Linley and Chadee 1991). When glue is present, it is easily detected under the electron microscope and none was present on any of the many eggs here examined.

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