THE EGGS OF *AEDES CASPIUS* AND *AEDES AFRICANUS* (DIPTERA: CULICIDAE)

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ABSTRACT. Scanning electron micrographs are used to illustrate descriptions of the eggs of *Aedes* (*Ochlerotatus*) *caspius* and *Ae.* (*Stegomyia*) *africanus*. The ventral surface of the egg in *Ae. caspius* is more curved than the dorsal and the outer chorionic cells are more elongate, smaller, and contain fewer tubercles than the larger, rounder, dorsal cells. The outer chorionic reticulum is unusually wide. In *Ae. africanus*, cells of the ventral surface contain a large, central tubercle, with many small peripheral tubercles almost invariably fused to the reticulum, which is narrow and elevated. Cell structure changes through a lateral transition zone to the completely different dorsal type, in which several small, low tubercles are scattered over the corrugated floor of each cell. The part of the micropylar disk surrounding the micropylar dome is vestigial or absent in *Ae. africanus*, exposing the dome to unusual prominence.

INTRODUCTION

Aedes (*Ochlerotatus*) *caspius* (Pallas) is a prominent pest widely distributed in the Palaearctic Region (Horsfall 1955), where its larvae are primarily halophilic, with occasional occurrences in fresh water (Horsfall 1955). Despite the widespread abundance of this species, its potential as a disease vector (e.g., Gad et al. 1987), and the fact that it is readily maintained in laboratory colonies, the eggs apparently have not been described save for small outline drawings showing no surface features (Marshall 1938). In the case of *Aedes* (*Stegomyia*) *africanus* (Theobald), no morphological information appears to have been recorded for the egg, yet this is an important sylvan mosquito in the Ethiopian Region. It is strongly anthropophilic and, particularly in West Africa, has been implicated in the transmission of yellow fever (Germain et al. 1976, Cordellier et al. 1977, Bang et al. 1980).

In a collaborative effort, we collected undamaged eggs suitable for electron microscopic study and here present illustrated descriptions of the eggs of these two *Aedes* species.

MATERIALS AND METHODS

Eggs of *Ae. caspius* were collected from laboratory colony females, the originating material having been obtained in Ismailia Governorate, Egypt. Eggs laid on filter paper by a number of females were washed off into alcoholic Bouin's fixative, sealed in small vials and mailed to Vero Beach. Once received, eggs were rinsed in three changes of 80% ethanol to remove picric acid, dehydrated completely through a continuing ethanol series (5% concentration increments) and dried by the critical point method. Eggs were set with a fine artist's brush on stubs coated with sticky tape, dried finally over calcium chloride (30 min), then coated with gold.

Aedes africanus eggs were oviposited on filter paper in the laboratory by blood-engorged females collected at human bait in Zika Forest, near Entebbe, Uganda. The papers were folded (eggs on inner surfaces) into small plastic Petri dishes, thoroughly moistened, and mailed. For electron microscopy, some eggs were left attached to the filter paper, which was cut into small pieces and stuck to stubs. Other eggs were loosened gently from the paper with a fine needle, then placed on stubs in various attitudes that would permit

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study of all surfaces. Eggs were dried and gold-coated as already described. Examination of specimens was done in a Hitachi S-510 scanning electron microscope.

At least 30 eggs of each species were examined for the descriptions. Measurements were made from micrographs with a digitizing tablet and Sigmascan software (Jandel Scientific, San Rafael, CA). Quantitative attributes of the chorionic cells and associated structures were derived from an equal number of measurements from three eggs of each species. Cell length was taken as the dimension in the longitudinal axis of the egg, width as the dimension in the circumferential direction. Tubercles were measured across their greatest diameters. Means are given ±SE in the text and tables; differences between them were tested by the *t*-test.

The terminology used is that of Harbach and Knight (1980), to which the terms "outer chorionic cell field" (Linley 1989) and "micropylar dome" (Linley et al. 1991) are additional.

DESCRIPTIONS

Aedes (Ochlerotatus) caspius (Figs. 1–3)

Size: As in Table 1. *Color*: Matte black. *Shape, overall appearance*: Boat-shaped in ventral or dorsal view, width greatest just anterior to middle, posterior end slightly more pointed (Fig. 1). Ventral side more arched in lateral view, dorsal side flatter (Fig. 2A). Micropylar collar inconspicuous, boundaries of outer chorionic cell fields rounded, not angular, each containing several tubercles (Fig. 1).

Chorion, ventral (more curved) surface: Outer chorionic cells usually hexagonal, sometimes pentagonal (Figs. 1;3A), length greater (mean 27.8 ± 0.7 μm, n = 15) than width (mean 16.7 ± 0.5 μm), as indicated in length/width ratio (mean 1.69 ± 0.07). Cell fields 4–6 μm less in each dimension, floors fairly smooth (Fig. 3B). Each cell with 4–10 (mean 7.7 ± 0.3, n = 21) medium-sized tubercles (Figs. 1;3A), diameter 0.7–5.3 μm (mean 2.8 ± 0.2 μm, n = 42), mostly arranged close to periphery of cell field (Fig. 1). Tubercles quite elevated, basal portions smooth, tops domed, slightly nodular (Fig. 3B). Some cells with filamentous strands adhering to or between tubercles (Fig. 3A,B). Outer chorionic reticulum wide (4.0–6.0 μm), widest near and at cell corners, elevated at edges, slightly concave, with a central line of tiny papillae connected to low, transverse ridges. Remainder of surface perforated with minute pores (Fig. 3A,B).

Chorion, lateral surface (ventral/dorsal transition): Progressive changes from ventral to dorsal surface not great (Fig. 3C), cells tending to become wider in relation to length, tubercles somewhat more numerous and smaller, especially near dorsal surface, reticulum becoming narrower (Fig. 3C), but structure of these elements very similar to ventral surface (Fig. 3D).

Chorion, dorsal (flatter) surface: Cell length less (mean 20.5 ± 0.4 μm, n = 15) than on ventral surface, width greater (mean 20.5 ± 0.6 μm), length/width ratio (mean 1.01 ± 0.04) therefore significantly ($P < 0.001$) smaller. Cell fields 2–4 μm less in each dimension, floors smooth (Fig. 3F). Tubercles scattered over cell floors (Fig. 3E,F), numbers 11–22 (mean 16.0 ± 0.7, n = 21) diameter 0.4–3.3 μm (mean 2.0 ± 0.1, n = 42). Mean number significantly ($P < 0.001$) greater than on ventral surface, mean diameter signifi-

Table 1. Dimensions of eggs of *Ae. caspius* (n = 7) and *Ae. africanus* (n = 12).

Species	Length (μm)		Width (μm)		L/W ratio	
	x̄ ± SE	Range	x̄ ± SE	Range	x̄ ± SE	Range
Ae. caspius	631.8 ± 7.8	591.3–676.4	215.2 ± 2.6	206.9–235.2	2.94 ± 0.04	2.69–3.11
Ae. africanus	448.4 ± 5.1	429.6–486.4	136.2 ± 2.0	117.5–145.9	3.29 ± 0.05	3.03–3.70

cantly ($P < 0.001$) smaller. Structure of tubercles similar to ventral surface, except that some very small and nodular top surface structure on all tubercles absent or barely visible (Fig. 3F). Outer chorionic reticulum structurally similar to ventral surface, but not as wide (2–4 μm).

Anterior end, micropyle: Chorionic cells smaller approaching anterior end, tubercles fewer but not reduced in size (Fig. 2B,C). Micropylar collar inconspicuous, conforming to taper of egg, almost always continuous (Fig. 2D,E), gaps rarely present. Posterior edge ragged (Fig. 2C,D,E), height consequently variable, 1.5–12 μm, collar diameter very variable, 25–42 μm, wall width 2.5–11 μm, surface rough (Fig. 2D,E). Collar internal diameter 18–24 μm, inner edge sometimes irregularly (Fig. 2D) but more often regularly excavated (Fig. 2E). Micropylar disk very inapparent, edge in some places very slightly raised (Fig. 2E), diameter 17–21 μm, surface rough. Micropylar dome fairly conspicuous, diameter about 12 μm, orifice round (Fig. 2E), diameter 2.1 μm.

Posterior end: Chorionic cells diminish in size toward posterior end, tubercles fewer (Fig. 2F,G), fields of most posterior cells extremely small, often obliterated by reticulum, which fuses with tubercles (Fig. 2G).

Aedes (Stegomyia) africanus (Figs. 4–7)

Size: As in Table 1. *Color*: Black. *Shape, overall appearance*: Broadly cigar-shaped in ventral (Fig. 4) or dorsal view, dorsal side often slightly flatter in lateral view (not shown). In ventral aspect, egg is widest at about anterior 0.3 and from this point roundly tapered anteriorly, relatively little tapered posteriorly until posterior 0.25, then rapidly so (Fig. 4). Collar of micropyle fairly prominent, outer chorionic cells distinct and very regular in outline, each with one, rarely 2 large tubercles (Fig. 4).

Chorion, ventral (upper) surface: Cells hexagonal or pentagonal, outlines uniform (Fig. 5A), length (mean 22.8 ± 0.4 μm, $n = 15$) greater than width (mean 15.2 ± 0.4 μm),

Fig. 1. *Aedes caspius*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

Fig. 2. *Aedes caspius*. A, Entire egg, lateral view, ventral side at top, anterior end at left; B, anterior end, lateral view; C, anterior end, chorionic cell detail; D, micropylar apparatus, excavations of inner collar wall irregular, disk surface rough; E, micropylar apparatus, excavations of inner wall regular, disk surface very rough; F, posterior end, lateral view; G, posterior end, chorionic cell detail. Scale = 200 μm (A), = 20 μm (B–G).

Fig. 3. *Aedes caspius*. A, Outer chorionic cells, ventral surface, middle of egg; B, detail, single cell, showing tubercles and outer chorionic reticulum; C, lateral view of egg showing ventral/dorsal transition, ventral surface at top; D, detail, single lateral cell; E, chorionic cells, dorsal surface, middle of egg; F, detail, single dorsal surface cell. Scale = 20 μm (A,C,E), = 10 μm (B,D,F).

length/width ratio mean 1.52 ± 0.06. Cell fields 0.8–1.2 μm less in each dimension. Cell floors smooth except for several tiny, short ridges (Fig. 6A). Almost all cells containing a single large, prominent, centrally positioned tubercle 7.4–10.3 μm in diameter (mean 8.6 ± 0.2 μm, $n = 15$), but 2 tubercles rarely present (Fig. 5A). Bases of tubercles slightly rough (Fig. 6A,F), tops ornamented with flat, irregular nodules, separated by narrow fissures (Fig. 6A,F). Small tubercles 11–17 in number (mean 14.7 ± 0.4, $n = 15$), quite evenly spaced around periphery of cell and fused to reticulum (Figs. 5A;6A), top surfaces peaked or, in larger ones, slightly nodular (Fig. 6A). Outer chorionic reticulum conspicuously raised, but narrow (width 0.8–1.2 μm), often overlain by small tubercles, surface faintly striated, occasionally perforated (Fig. 6A,F).

Chorion, lateral surface (ventral/dorsal transition): Proceeding dorsally, chorionic cells quickly become wider, width soon equal to or greater than length (Fig. 5B). Large tubercles somewhat smaller, many small tubercles joined by spoke-like bridges to large tubercles, reticulum wider, more perforated, and occasionally extended to fuse with large tubercle (Figs. 5B; 6B). Close to dorsal surface large tubercles become smaller, nodular top surfaces less distinct, cell floors lumpy or corrugated (Figs. 5B; 6C,D). Transition at boundary to dorsal type chorion quite abrupt, large tubercles disappear or are much smaller and occasionally multiple, cells now considerably wider than long, small tubercles absent and reticulum flatter, much less distinct, and not perforated (Fig. 6D).

Chorion, dorsal (lower) surface: Cells much wider than long, floors corrugated, peripheral small tubercles replaced by 9–12 (mean 10.1 ± 0.5, $n = 12$) low, smooth tubercles scattered over cell floors (Fig. 6E). Reticulum low, featureless, rather smooth, wider (1.4–2.0 μm) than on ventral surface.

Anterior end, micropyle: Chorionic cells slightly smaller near anterior end, but struc-

Fig. 4. *Aedes africanus*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

of micropyle quite prominent, only rarely continuous, 1–3 gaps usually present (Fig. 7C,D), outer edge rounded, surface rough (Fig. 7D). Collar height 5–7 μm, diameter 30–36 μm, wall width 3.5–8 μm, internal diameter 15–21 μm and very variable owing to irregularities associated with gaps, inner wall high with deep, scoop-like excavations (Fig. 7D,E). That part of the micropylar disk surrounding the micropylar dome absent (Fig. 7C), very small with irregular boundary (Fig. 7D), or fragmentary (Fig. 7E), thus exposing micropylar dome (diameter 7.5–8.0 μm) to unusual prominence (Fig. 7C,D,E). Orifice of micropyle tri-lobed, diameter 2.5 μm.

Posterior end: Chorionic cells smaller near posterior end (Fig. 7F), but structure of large tubercles little changed (Fig. 7G). Small tubercles fewer or absent in cells at extreme end of egg.

DISCUSSION

A striking feature of the egg of *Ae. caspius* is the width of the chorionic reticulum. In other species of *Ochlerotatus* it ranges on the ventral surface from 1.4–2.4 μm in diameter in *Ae. theobaldi* (Taylor), *Ae. sagax* (Skuse) and *Ae. procax* (Skuse) (Linley et al. 1992a), to 0.9–3.2 μm in *Ae. infirmatus* Dyar and Knab (Linley 1990) and 3.0–3.3 μm in *Ae. vigilax* (Skuse) (Linley et al. 1992b). The *Ae. caspius* reticulum (4.0–6.0 μm wide) exceeds all these so that the cell fields appear unusually small (Fig. 5). As a proportion of the total cell area in nine ventral cells in the middle of the egg, the field comprised a mean of only 46.9 ± 1.2% in *Ae. caspius*, as compared with 69.4 ± 1.3% in *Ae. procax* (measured from file micrographs).

According to Marshall (1938), eggs of *Ae. caspius* are laid in vegetation covering the larval habitats. Under the electron microscope, we found no material adhering to the chorion, which might suggest that the eggs are glued to the oviposition substrate. They are probably thrust into small crevices and interstices sought out by the probing female abdomen. In contrast, the egg of *Ae. africanus* is firmly cemented to the surface on which it

Fig. 5. *Aedes africanus*. A, Outer chorionic cells, ventral surface, middle of egg; B, lateral view, ventral/dorsal transition, ventral side at top. Scale = 20 μm.

turally little changed except immediately posterior to micropylar collar, where large tubercles almost fill the very small cells, small tubercles no longer present (Fig. 7A,B). Collar

Fig. 6. *Aedes africanus*. A, Cell detail, ventral surface; B, cell detail, ventro-lateral part of transition; C, cell detail, dorso-lateral part of transition; D, detail, cells at boundary of dorsal zone (right); E, cell detail, dorsal surface, middle of egg. F, detail of tubercles and outer chorionic reticulum, ventral surface. Scale = 10 μm (A–E), = 5 μm (F).

Fig. 7. *Aedes africanus*. A, Anterior end, lateral view; B, anterior end, chorionic cell detail; C, micropylar apparatus, part of disk surrounding dome absent; D, micropylar apparatus, disk around dome partially present; E, micropylar apparatus, dome with attached disk fragments only; F, posterior end, lateral view; G, posterior end, chorionic cell detail. Scale = 20 μm (A,B,C,F,G), = 10 μm (D,E).

is laid and the dorsal chorion, to which cement is applied, is structurally much less protrusive and ornate than the ventral. This pattern is similar to that in other *Stegomyia* species such as *Ae. aegypti* (L.) and *Ae. albopictus* Skuse (Linley 1989), which also attach their eggs with glue. The egg of *Ae. africanus* is, in fact, very similar overall to these two species, though quite distinct in its structural details. The micropylar region in *Ae. africanus* is unusual. All or most of the outer parts of the micropylar disk, which usually surround the micropylar dome, are absent or fragmentary (Fig. 7C,D,E). The surrounding surface is instead conspicuously wrinkled and the dome uncommonly prominent, with abrupt, steep edges.

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