THE EGGS OF *AEDES (FINLAYA) ALBOANNULATUS* AND *AEDES (FINLAYA) RUBRITHORAX* (DIPTERA: CULICIDAE)

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ABSTRACT. Scanning electron micrographs are used to provide descriptions of the eggs of *Aedes (Finlaya) alboannulatus* and *Ae. (Fin.) rubrithorax*. The structure in both eggs is complex and basically similar, with the outer chorionic cells differing completely between the ventral (upper) and dorsal (lower) surfaces. Among eggs of *Finlaya* species so far described, these two are unusual in having, in the mid-ventral line (upper surface), a strip of outer chorionic cells which differs substantially from those on the remainder of the ventral and lateral surfaces. Cells in this strip are wider in the anterior/posterior direction than circumferentially, and contain several medium-sized tubercles. Cells in the immediately adjacent ventrolateral and more lateral regions are proportionately wider circumferentially and contain a single large, central tubercle with several small, peripheral ones. The dorsal (lower) surface in each case is composed of cells covered with many small tubercles, which are elongated into filaments that together form a dense mat. In the subgenus *Finlaya*, such filaments are known only from *Ae. togoi* but are present also in two *Haemagogus* species recently described.

INTRODUCTION

Aedes (Finlaya) alboannulatus (Macquart) is both Oriental and Australasian in distribution and, in Australia, gives its name to a subgroup of *Finlaya* which comprises an important complex of sylvan mosquitoes. The biology of *Ae. alboannulatus* and its relationship to disease are reasonably well known (Lee et al. 1982), and its egg has been described briefly at the stereomicroscopic level by Dobrotworsky (1959). Pillai (1962) examined elements of the egg's structure relevant to its ability to resist desiccation, and illustrated structure of the ventral surface by means of a celloidin impression.

Also a species normally confined to woodlands, *Aedes (Finlaya) rubrithorax* (Macquart) is widely distributed in Australia and is known from all states except the Northern Territory (Lee et al. 1982). The only description of the egg is that given by Dobrotworsky (1959), with some additional detail provided by Pillai (1962). As part of an effort to improve knowledge of the eggs of Australasian mosquitoes, we examined eggs of both this species and *Ae. alboannulatus* with a scanning electron microscope. This paper presents the resulting more complete accounts of the eggs.

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MATERIALS AND METHODS

Eggs were collected from blood-fed females induced to oviposit on wet filter paper in the laboratory. The papers were folded around the inside of small plastic petri dishes (eggs on the inner surface), which were adequately moistened, sealed with tape and mailed to Vero Beach. Eggs were prepared for microscopy either by cutting out small pieces of the dried filter paper with attached eggs and affixing these to stubs with silver paint, or by lifting individual eggs with a fine artist's brush and repositioning them as required on stubs already covered with sticky tape. Specimens were dried completely over calcium chloride for 0.5 hr, then sputter coated with gold and examined in a Hitachi S-510 scanning electron microscope. More than 100 eggs of each species were mounted and available for study.

Outer chorionic cell lengths were measured across the two most widely separated cell corners aligned in the longitudinal (anterior/posterior) axis of the cell. Cell widths were measured similarly in the circumferential direction. Both large and small tubercles were measured across their tops at the widest point. All measurements given in the text, including means (± SE), were determined from examination of at least five eggs.

We have used the terminology proposed by Harbach and Knight (1980), except for "outer chorionic cell field" (Linley 1989) and "micropylar dome" (Linley et al. 1991).

DESCRIPTIONS

Aedes (Finlaya) alboannulatus (Figs. 1-4)

Size: As in Table 1. *Color:* Dull black. *Overall appearance:* Broadly cigar-shaped, widest at about anterior 1/3, anterior end somewhat conical, relatively little tapered posteriorly till posterior 1/3, then quite sharply tapered to pointed posterior end (Fig. 1). Outer chorionic cells on ventral (upper) surface uniform except for a longitudinal strip in the mid-ventral line, extending from just behind micropylar collar to about 90 μm from posterior end (Fig. 1). Most cells contain a single large tubercle, but cells in strip completely different, each instead contains several medium-sized tubercles (Fig. 1). Micropylar collar prominent.

Chorion, ventral (upper) surface: Mid-ventral strip 2-3 cells wide, cells mostly irregularly pentagonal in outline (Fig. 1), length (mean 20.8 ± 0.8 μm, n = 15) greater than width (mean 10.8 ± 0.5 μm, n = 15), cell fields about 2.9 μm less in each dimension. Each cell with 2-9 (mean 5.4 ± 0.3, n = 35) medium-sized tubercles around periphery of cell abutted against outer chorionic reticulum (Fig. 2a, c). Tubercles irregular in shape, mean diameter 4.4 ± 0.2 μm, n = 15, bases slightly larger than caps, which are often concave with indistinct nodular surfaces. Outer edges of caps usually with raised parapet, width about 0.4 μm, often bridging gaps between adjacent tubercles (Fig. 2a, c). Cell fields slightly rough (Fig. 2c). Outer chorionic reticulum 2.2-3.5 μm wide, consisting of a ragged lattice extending up outer edges of tubercles (Fig. 2c).

Chorion, lateral surface (ventral-dorsal transition): Transition from strip-type cells to adjacent ventrolateral type extremely rapid (Fig. 2a, c). Unlike those in central strip, lateral cells not as great in length (mean 13.3 ± 0.7 μm, n = 12) as width (mean 21.6 ± 0.9 μm, n = 12). Each cell invariably with a single large, central tubercle, mean diameter 9.3 ± 0.1 μm, n = 15, widest in anterior/posterior direction (Figs. 1, 2a). Each tubercle arising from a broad base, which spans floor of cell (Fig. 2a, b, d) and supports a large bulbous cap covered with close-packed more or less round nodules (Fig. 2a, b, d). Small tubercles confined to periphery of cell, mean number 5.6 ± 0.3, n = 25, intimately connected to reticulum (Fig. 2a, d), and often with small nodular caps (Fig. 2d). Reticulum a fine reticulate mesh in some places with tiny central protuberances along a very fine ridge (Fig. 2d). Proceeding down sides of egg (Fig. 2b), large tubercles become progressively smaller, caps flatter, nodular surface texture less pronounced (Fig. 2b, e), small tubercles larger, reticulum still a fine mesh but in some parts overlain by a second, porous mesh (Fig. 2b, e). Near transition to dorsal-type cells large tubercles disappear or are barely larger than small tubercles, which are themselves more numerous and larger (Fig. 2b, f). Meshwork of reticulum less clearly defined (Fig. 2f). Cells at transition often containing,

Table 1. Dimensions of eggs of *Ae. alboannulatus* and *Ae. rubrithorax* (n = 15).

Species	Length (μm) $\bar{x}$ ± SE	Length (μm) Range	Width (μm) $\bar{x}$ ± SE	Width (μm) Range	L/W ratio $\bar{x}$ ± SE	L/W ratio Range
Ae. alboannulatus	700.5 ± 5.9	669.6-762.0	187.8 ± 1.9	177.2-203.8	3.74 ± 0.05	3.46-4.05
Ae. rubrithorax	682.3 ± 6.1	632.9-727.8	179.6 ± 1.9	165.8-192.4	3.81 ± 0.06	3.49-4.37

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

ventrally, smooth-topped peripheral tubercles and, dorsally, tubercles elaborated into tangled filaments (Fig. 2b).

Chorion, dorsal (lower) surface: Length of outer chorionic cells greater than width, boundaries of individual cells often indistinguishable because of overlying filaments. Cells just dorsal to lateral/dorsal boundary containing many small tubercles, for the most part individually distinguishable (Fig. 3b, c), but tubercle surfaces appearing knotty, almost all with one or more filaments extending over adjacent tubercles. Masses of filaments occluding cell surface in some places (Fig. 3b, c). Outer chorionic reticulum slightly raised, width 1.2-2.1 μm, surface reticulated, in some sections with second overlying layer perforated by pores (Fig. 3b, c). Tubercles of more dorsal cells more completely extended to form filaments, which create a dense mat obscuring tubercles (Fig. 3d, e). Filament diameter 0.15-0.24 μm, lengths impossible to determine, individual elements intimately intertwined (Fig. 3f). Reticulated pattern of outer chorionic reticulum persisting, but less clearly defined (Fig. 3d), or almost absent, surface instead becoming somewhat rough with flat, scale-like nodules (Fig. 3e, f). Dorsal filament type cells present only in an oval area extending from about anterior 1/4 to posterior 1/3; chorionic cells in dorsal posterior and anterior region each containing several medium-sized, fairly smooth tubercles, but with no formation of filaments (Fig. 3a).

Anterior end, micropyle: Chorionic cells smaller towards anterior end, cells of type in mid-ventral strip not extending quite to micropylar collar (Fig. 4a, b), large tubercles in adjacent cells remaining prominent to boundary of collar, surface sculpturing distinct. Small tubercles fewer in number, absent in some cells next to collar. Reticulum as elsewhere on ventral surface, but in some places raised, with perforations (Fig. 4b). Micropylar collar conspicuous, mostly discontinuous, with 1-3 relatively narrow notches (Fig. 4a, d), rarely complete (Fig. 4c). Height of micropyle 8-15 μm, outer diameter 25-30 μm, wall width 3.5-8.5 μm, anterior surface rough (Fig. 4d), internal diameter 16-20 μm, inner wall with shallow excavations, (Fig. 4c, d), disk distinct, diameter 14-16 μm, micro-

Fig. 2. *Aedes alboannulatus.* (a) Transition from chorionic cells of mid-ventral strip (left) to adjacent more lateral cells (right); (b) lateral view, middle of egg, showing entire ventral-dorsal transition, ventral side at top; (c) detail, mid-ventral strip and adjacent transitional cells (d) cell detail, ventrolateral portion of transition; (e) cell detail, lateral portion of transition; (f) cell detail, dorsolateral portion of transition. Scale = 10 μm.

Fig. 3. *Aedes alboannulatus.* (a) Chorionic cells on dorsal surface, near posterior end of egg; (b) detail of dorsal filament cell at dorsolateral boundary; (c) extreme detail of knotty, filament-bearing tubercles in cell at boundary; (d) filament-type cell, dorsal surface; (e) variant of filament-type cell, dorsal surface; (f) extreme detail, intertwined filaments. Scale = 20 μm (a), = 5 μm (b,c,d,e,f).

pylar dome present but boundary not sharply demarcated, diameter about 11 μm (Fig. 4d), micropylar orifice trilobed, diameter 3.2 μm.

Posterior end: Chorionic cells smaller approaching posterior end, mid-ventral strip cells not persisting to end of egg, replaced by cells with large central tubercles (Fig. 4e). These large tubercles remaining distinct, with visible sculpturing, almost to end of egg, when surfaces become smooth (Fig. 4f).

Fig. 4. *Aedes alboannulatus.* (a) Anterior end, ventrolateral view; (b) anterior end, outer chorionic cell detail; (c) anterior end and micropylar apparatus; (d) detail, micropylar apparatus; (e) posterior end, ventrolateral view; (f) posterior end, outer chorionic cell detail. Scale = 20 μm (a), = 10 μm (b,c,d,e,f).

Aedes (Finlaya) rubrithorax (Figs. 5-9)

Size: As in Table 1. *Color:* Dull black. *Overall appearance:* Broadly cigar-shaped, widest at about anterior 1/4, then tapering anteriorly, little tapered posteriorly until about posterior 1/4, then rapidly so (Fig. 5). Ends of egg less conical than in *Ae. alboannulatus.* Outer chorionic cells on ventral surface of 2 types, as in *Ae. alboannulatus;* first type present in a longitudinal mid-ventral strip, several cells wide, extending from just behind micropylar collar to about

100 μm from posterior pole. Boundaries of these cells indistinct and each containing several medium-sized tubercles. Remaining ventral surface cells each with single large, central tubercle, imparting a bumpy appearance (Fig. 5). Micropylar collar fairly prominent.

Chorion, ventral (upper) surface: Mid-ventral strip 4-5 cells wide, cells mostly pentagonal or quadrilateral in outline, but boundaries sometimes very difficult to distinguish. Length (mean 19.2 ± 1.0 μm, n = 10) greater than width (mean 10.5 ± 0.5 μm, n = 10), cell fields about 3.5 μm less in each dimension. Number of tubercles per cell 2-6 (mean 4.1 ± 0.1, n = 35), positioned around edges of cell and almost always in cell corners, mean diameter 4.1 ± 0.3 μm. Bases of tubercles considerably wider than tops (Fig. 6c), tops with low nodular sculpturing, and surrounded on outer edges by a raised crest, which may be partially folded over top of tubercle. Crest almost always connected (Fig. 6c) to adjacent tubercles by thin bridges, diameter 0.3-0.6 μm. Outer chorionic reticulum difficult to distinguish, consisting of faint striations or reticulations overlain by low ridges or raised connections between adjacent tubercles of different cells (Fig. 6c). Striations present also on sides of tubercles.

Chorion, lateral surface (ventral-dorsal transition): Strip-type cell structure changes very rapidly to ventrolateral type (Fig. 6a, c). The latter cells not as long (mean 13.6 ± 0.4 μm, n = 10) as wide (mean 22.7 ± 0.5 μm, n = 10) and each containing a single large tubercle (Fig. 6a, d), mean diameter 8.3 ± 0.2 μm, n = 15, aligned in the anterior/posterior direction (Fig. 6a). The broad base of each tubercle slightly tapered upward to a large, bulbous cap ornamented with conspicuous nodules (Fig. 6d). Small tubercles present, but often low and indistinct (Fig. 6a, d), diameters not measured, more prominent ones with distinct nodular caps (Fig. 6d), but many appearing only as low, smooth bumps on the surface (Fig. 6a). Outer chorionic reticulum wide (diameter 4.5-5.2 μm) and distinct, a fine reticulated mesh with very small central protuberances and extending laterally up sides of small and large tubercles (Fig. 6c, d). Further down sides of egg (Fig. 6a) large tubercles become smaller, nodular surface of caps less pronounced (Fig. 6a, e), small tubercles become

Fig. 5. *Aedes rubrithorax.* Entire egg, ventral view, anterior end at top. Scale = 100 μm.

Fig. 6. *Aedes rubrithorax.* (a) Lateral view, middle of egg, showing entire ventral-dorsal transition, ventral side at top; (b) detail of cells at lateral-dorsal boundary; (c) cell detail, mid-ventral strip and adjacent transitional cells; (d) cell detail, ventrolateral portion of transition; (e) cell detail, lateral portion of transition; (f) cell detail, dorsolateral portion of transition. Scale = 10 μm.

Fig. 7. *Aedes rubrithorax.* (a) Detail of transition at lateral-dorsal boundary; (b) extreme detail, tubercles elaborated into filaments in dorsal filament cells; (c) detail, filaments of dorsal filament cells forming dense mat. Scale = 10 μm.

Fig. 8. *Aedes rubrithorax.* (a) Entire egg, dorsal view, anterior end at top. Scale = 100 μm.

somewhat more prominent, reticulum a distinct fine mesh in some places with overlying porous layer (Fig. 6e). Just before transition to dorsal-type cells, large tubercles disappear (Figs. 6a, 7a), cells instead contain several medium-sized tubercles, usually but not always peripherally located (Figs. 6f, 7a), with fairly smooth or faintly nodular surfaces, meshwork of reticu-

Fig. 9. *Aedes rubrithorax.* (a) anterior end, ventrolateral view; (b) anterior end and micropylar apparatus; (c) detail, micropylar apparatus; (d) posterior end, ventral view; (e) posterior end, outer chorionic cell detail; (f) dorsal surface, middle of egg, showing filamentous mat. Scale = 20 μm.

lum still distinct (Fig. 6f). At transition boundary (Figs. 6b, 7a), some cells containing, ventrally, tubercles with nodular surfaces and, dorsally, tubercles formed into knotty masses of filaments.

Chorion, dorsal (lower) surface: Chorionic cells longer than wide, but measurement not possible. In more dorsally positioned cells each tubercle elongated apparently into a single long filament (Fig. 7b), diameter 0.2-0.3 μm, often entangled with other filaments to form sheets (Fig. 7c), which cover most of the dorsal side of the egg with a dense mat (Figs. 8, 9f). Where visible (Fig. 7b, arrow), outer chorionic reticulum seems to consist of a somewhat coarser meshwork than in more ventral cells.

Anterior end, micropyle: Chorionic cells diminish in size towards anterior end, mid-ventral strip cells not extending to micropylar collar (Figs. 5, 9a). Large tubercles in some cells much elongated, small tubercles very few or absent near anterior end (Fig. 9a). Layer overlying outer chorionic reticulum more complete, with ragged holes (Fig. 9a). Micropylar collar fairly conspicuous, usually continuous, with no gaps (Fig. 9a, c), but occasionally 1 or 2 (Fig. 9b). Collar height 11-13 μm, diameter 32-38 μm, wall width 4.5-6.5 μm, anterior surface rough (Fig. 9b, c), internal diameter 19-22 μm, inner wall with clear excavations (Fig. 9c). Micropylar disk distinct, edge clearly demarcated and raised (Fig. 9b, c), diameter 11-13 μm, no micropylar dome visible, micropylar orifice trilobed, diameter 3.3 μm.

Posterior end: Chorionic cells diminishing in size towards posterior end, mid-ventral strip cells not reaching end of egg (Figs. 5, 9d), replaced by large tubercle cells, the large tubercles in these tending to be more irregular in shape, nodular surfaces becoming smoother (Fig. 9d, e), completely so at very end of egg (Fig. 9e). Small tubercles becoming fewer, absent in cells at posterior apex (Fig. 9e), meshwork of outer chorionic reticulum becomes less conspicuous, almost completely smooth at end of egg (Fig. 9e).

DISCUSSION

Aedes alboannulatus and *Ae. rubrithorax* both belong to the Alboannulatus Group of subgenus *Finlaya* and their close relationship is reflected in the structural similarity of their eggs. Both eggs, however, differ considerably from those of other *Finlaya* species that have been examined with the electron microscope. Matsuo et al. (1972, 1974) described five species, unfortunately only with respect to the ventral (upper) surface, but none shows any indication of the presence of a mid-ventral strip of cells markedly different in structure from the adjacent ones. Such differences are very slightly apparent in the egg of *Ae. notoscriptus* (Skuse) (Notoscriptus Group), which we have recently described (Linley et al. 1991). The chorionic cells in the mid-ventral line are not different with respect to their relative dimensions, but the caps of their large central tubercles are smaller, and there are no spoke-like bridges connecting the small tubercles to the large one (see Linley et al. 1991).

The dorsal (lower) surfaces of *Ae. alboannulatus* and *Ae. rubrithorax* eggs provide additional evidence that filament cells in some degree of differentiation may be the typical structure in subgenus *Finlaya*. Filaments as such are not present in *Ae. notoscriptus*, but we have noted (Linley et al. 1991) that the many small tubercles in each cell plausibly represent an early stage from which filaments could have been elaborated. *Aedes (Fin.) togoi* (Theobald) eggs have highly developed filaments on the dorsal surface (J.R. Linley and K.L. Chan, personal observations), and so also do the eggs of two species of *Haemagogus* (Linley and Chadee 1991), a genus probably closely related to *Aedes*, and specifically the subgenus *Finlaya* (Arnell 1973). *Finlaya* is a subgenus containing many species in several groups and subgroups. Much more work will be required for a comprehensive understanding of how the eggs are related structurally, how this may affect current concepts of species groups and how structure may be adapted to the particular habitats and oviposition substrates of each species.

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REFERENCES CITED

Arnell, H. 1973. Mosquito studies (Diptera: Culicidae). XXXII. A revision of the genus *Haemagogus*. Contr. Am. Entomol. Inst., (Ann Arbor) 10(2):1-174.

Dobrotworsky, N.V. 1959. Notes on Australian mosquitoes (Diptera: Culicidae). IV. *Aedes alboannulatus* complex in Victoria. Proc. Linn. Soc. N.S.W. 84:131-145.

Harbach, R.E. and K.L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Plexus

Publishing, Marlton, New Jersey.

Lee, D.J., M.M. Hicks, M. Griffiths, R.C. Russell and E.N. Marks. 1982. The Culicidae of the Australasian Region. Volume II. Entomology Monograph No. 2, Commonwealth Department of Health, Australian Government Publishing Service.

Linley, J.R. 1989. Comparative fine structure of the eggs of *Ae. albopictus, Ae. aegypti,* and *Ae. bahamensis* (Diptera: Culicidae). J. Med. Entomol. 26:510-521.

Linley, J.R. and D.D. Chadee. 1991. Fine structure of the eggs of *Haemagogus equinus* and *Haemagogus janthinomys* (Diptera: Culicidae). J. Med. Entomol. 28:434-445.

Linley, J.R., M.J. Geary and R.C. Russell. 1991. The eggs of *Aedes (Verrallina) funereus, Aedes (Finlaya) notoscriptus* and *Aedes (Mucidus) alternans.* Proc. Entomol. Soc. Wash. 93:592-612.

Matsuo, K., Y. Yoshida and I. Kunou. 1972. The scanning electron microscopy of mosquitoes (Diptera: Culicidae). I. The egg surfaces of five species of *Aedes* and *Armigeres subalbatus*. J. Kyoto Pref. Univ. Med. 81:358-364.

Matsuo, K., Y. Yoshida and J.C. Lien. 1974. Scanning electron microscopy of mosquitoes. II. The egg surface structure of 13 species of *Aedes* from Taiwan. J. Med. Entomol. 11:179-188.

Pillai, J.S. 1962. Factors influencing egg survival in *Aedes* eggs with special reference to some Victorian species (Diptera: Culicidae). Ph.D. thesis, Dept. of Zoology, University of Melbourne.