THE EGGS OF *AEDES AUSTRALIS* AND *AEDES CAMPTORHYNCHUS* (DIPTERA: CULICIDAE)

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ABSTRACT. Scanning electron micrographs are used to illustrate descriptions of the eggs of *Aedes* (*Halaedes*) *australis* and *Aedes* (*Ochlerotatus*) *camptorhynchus*. *Aedes australis* eggs are rhomboidal in ventral or dorsal view; *Ae. camptorhynchus* eggs are very broadly cigar-shaped. Both are more curved on the ventral surface. The ventral chorionic cells in *Ae. australis* have a distinct reticulum and many polygonal tubercles distributed over the cell fields. A clear boundary separates these cells from the dorsal type, where the reticulum is very poorly defined and the tubercles rounder and smoother. Ventral cells in *Ae. camptorhynchus* contain a single, large, central tubercle and many smaller, evenly spaced peripheral ones. This structure changes through a lateral transition zone to cells of the dorsal type, which, although differing in details of the reticulum, are similar to ventral surface cells in *Ae. australis*.

INTRODUCTION

Aedes (*Halaedes*) *australis* (Erichson), a strictly coastal species, is distributed along the eastern coastline of Australia, including southern Queensland, New South Wales, Victoria, Tasmania and the Bass Strait Islands, and South Australia. It is known also from Norfolk Island and New Zealand (South Island) and occurs in Western Australia, although there is some confusion with *Ae. ashworthi* Edwards in that region (Lee et al. 1984). The larvae typically live in rock pools above high tide level, almost invariably those periodically reached by the sea (Lee et al. 1984). Consequently, to cope with the effects of rain or evaporation, the larvae tolerate a very wide range in salinity (Woodhill 1936, as *Ae. concolor* Taylor). In many areas, the adults do not appear to feed readily on man and the species is generally not considered to be important as a potential vector of disease (Lee et al. 1984).

Aedes (*Ochlerotatus*) *camptorhynchus* (Thomson) is found in New South Wales, Victoria, South Australia, Western Australia and Tasmania (Lee et al. 1984). Larvae inhabit brackish water, mostly coastal swamps, and are the counterpart of *Ae.* (*Och.*) *vigilax* (Skuse) along the southern coastline of Australia. *Aedes camptorhynchus* also has been recorded inland, where brackish water exists (Lee et al. 1984), and wind-borne invasions of fresh water sites where the larvae apparently developed successfully, have been recorded (Dobrotworsky 1960, 1965). Once in the adult stage, female *Ae. camptorhynchus* readily attack man and animals. Ross River virus has been isolated from *Ae. camptorhynchus* in eastern Victoria (Campbell et al. 1989) and eastern Tasmania (R. C. Russell, unpublished data) and the species has been shown in the laboratory to be capable of carrying Murray Valley encephalitis virus (McLean 1953), and myxomatosis (Bull and Mules 1944) and almost certainly has been responsible for certain outbreaks of the latter disease along the Victorian coast (Fenner and Ratcliffe 1965).

A search of the literature, greatly facilitated by the comprehensive listings in Lee et al. (1984), revealed no information on the egg of either *Ae. australis* or *Ae. camptorhynchus*. Material was therefore collected in Australia and subsequently studied with the scanning electron microscope in Vero Beach to provide the following illustrated descriptions.

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MATERIALS AND METHODS

Live, fertile eggs of *Ae. australis* were obtained from a laboratory colony established briefly in Vero Beach from material collected in New South Wales. Eggs oviposited on wet filter paper were allowed to embryonate, then prepared for electron microscopy in two ways. Some still attached to the paper were cut out on small paper rectangles, air-dried and attached to stubs with sticky tape. Others were dislodged with a fine needle, picked up on a damp artist's brush and placed individually on stubs so as to permit examination of all surfaces.

Aedes camptorhynchus eggs were collected at Hollands Landing, Victoria, and shipped to Vero Beach on wet filter paper, whereupon they were prepared for study as already described. Once on stubs, eggs of both species were dried finally over calcium chloride (30 min), coated with gold, and immediately examined in a Hitachi S-510 scanning electron microscope.

Between 20 and 30 eggs of each species were inspected as a basis for the descriptions. All measurements were made from micrographs using a digitizing tablet and SigmaScan software (Jandel Scientific, Corte Madera, CA), with an equal number of determinations from five separate eggs of each species contributing to the means (±SE) cited in the text. In describing the outer chorionic cells, length was taken as the greatest dimension in the longitudinal axis of the egg, width as the circumferential dimension. Tubercles were measured across the widest point.

The terminology follows Harbach and Knight (1980), with the addition of "outer chorionic cell field" (Linley 1989) and "micropylar dome" (Linley et al. 1991).

DESCRIPTIONS

Aedes (*Halaedes*) *australis* (Figs. 1–4)

Size: As in Table 1. *Color*: Satiny black. *Shape, overall appearance*: Rhomboidal in ventral or dorsal view, widest just anterior to middle, anterior profile somewhat rounded, posterior more conical (Fig. 1), collar of micropyle very inapparent, conforming entirely with taper of egg. Lateral view shows ventral surface rounded, dorsal surface much flatter (Fig. 2A). Boundaries of outer chorionic cells visible but not conspicuous, cells wider than long, each containing many tubercles (Fig. 1).

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

Chorion, ventral (upper) surface: Outer chorionic cells pentagonal or hexagonal, irregular in shape and variable in size (Fig. 3A). Length 6.3–10.6 μm (mean 8.5 ± 0.4 μm, n = 12) not as great as width, 15.3–24.9 μm (mean 19.9 ± 0.9 μm), cell fields about 1.3 μm less in each dimension, cell floors rough (Fig. 3B, E). Tubercles in each cell 11–25 in number (mean 18.4 ± 1.1, n = 20), numbers increasing significantly ($P < 0.001$) with cell area (Fig. 4), distributed more or less evenly

Fig. 2. *Aedes australis*. (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, collar without fissures; (E) detail of micropylar apparatus, collar with fissures; (F) posterior end, lateral and partially end-on view; (G) posterior end, chorionic cell detail. Scale = 100 μm (A), = 20 μm (B,C,D,E,F,G).

over cell surface (Fig. 3A, B). Tubercles most typically tending to be polygonal, a few rounded or oval (Fig. 3A,B,E), diameter 0.6–2.6 μm (mean 1.7 ± 0.1 μm, n = 50), bases often slightly wider than tops, which are slightly domed and rough (Fig. 3B,E). Struc-

Fig. 3. *Aedes australis*. (A) Outer chorionic cells, ventral surface, middle of egg; (B) detail, chorionic cells, ventral surface; (C) variant type cell, ventral surface; (D) variant type cell, ventral surface; (E) detail, tubercles and reticulum, ventral surface; (F) lateral view, ventral/dorsal transition, ventral side at top, showing abrupt boundary between cell types; (G) detail of lateral boundary, ventral side at left and top, and dorsal type cells at right and bottom. Scale = 50 μm (F), = 10 μm (A,B,C,D,G), = 5 μm (E).

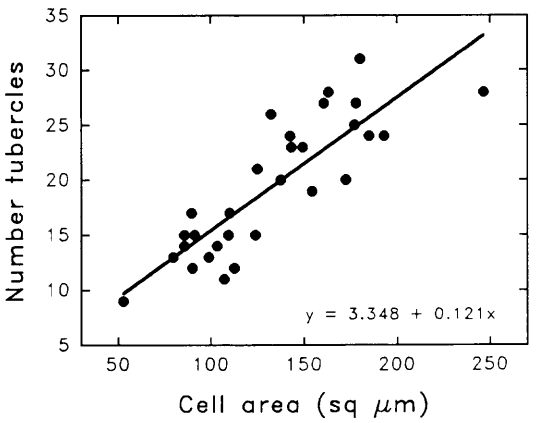

Fig. 4. *Aedes australis*. Regression of number of tubercles on ventral surface cell area.

ture of tubercles very variable between individual eggs, a few with tubercles more peaked, surfaces of some slightly nodular (Fig. 3C), or tubercles occasionally not as well formed, partially coalesced into confused groups (Fig. 3D). Chorionic reticulum narrow, 0.6–1.6 μm, slightly raised, surface scored transversely by shallow striations, tiny pores sometimes present (Fig. 3B,C,D,E).

Chorion, lateral surface (ventral/dorsal transition): Down sides of egg cell structure as on ventral surface until just below halfway, then abruptly changed to dorsal type at conspicuously defined boundary (Fig. 3F). Cells immediately ventral to boundary with reticulum much less distinct, surfaces with only a few or hardly any clearly formed tubercles, otherwise smoother, with irregular, small bumps (Fig. 3F,G), cells dorsal to these entirely differently structured, as below.

Chorion, dorsal (lower) surface: Cells of this surface with very indistinct boundaries, chorionic reticulum barely or not discernible (Fig. 3F,G), each cell with many more or less round, occasionally oblong, smooth-surfaced tubercles, cell floors with no roughness (Fig. 3F,G),

Anterior end, micropyle: Chorionic cells smaller toward anterior end, boundaries becoming indistinct, reticulum no longer raised, especially just posterior to collar of micropyle (Fig. 2B). Tubercles also becoming progressively less elevated and discreet, increasingly replaced by confused, rough texture (Fig. 2C). Micropylar collar not clearly demarcated in ventral or lateral view (Figs. 1,2A), but easily discerned when viewed end-on (Fig. 2D,E), almost always continuous or sometimes with fissures (Fig. 2E), which occasionally may separate inner margin. Collar height (when visible) 4–11 μm, diameter 48–62 μm, wall width usually 9–22 μm, collar internal diameter 22–26 μm. Inner collar edge only slightly raised, with shallow excavations (Fig. 2D,E), disk diameter 14–19 μm, area around orifice slightly domed, but outer edge of dome not visible (diameter not measured). Orifice indistinctly trilobed (Fig. 2E), diameter 2.8 μm.

Posterior end: Cells approaching posterior end smaller, boundaries more distinct than at anterior end, reticulum clearly visible, tubercles smaller, not as elevated, but individually distinct even in cells at tip of egg (Fig. 2F,G).

Aedes (*Ochlerotatus*) *camptorhynchus*
(Figs. 5–9)

Size: As in Table 1. *Color*: Matte black. *Shape, overall appearance*: Very broadly cigar-shaped in ventral or dorsal view (Fig. 5), widest at about anterior 0.3, anterior end slightly conical, posteriorly little tapered until posterior 0.25, then rapidly so. Ventral surface more curved in lateral view, dorsal surface flatter (Fig. 6A). Boundaries of outer chorionic cells not clearly defined, each cell of ventral surface with single large, central tubercle, many smaller surrounding ones (Fig. 5). Micropylar collar fairly distinct (Fig. 5).

Chorion, ventral (more curved) surface: Outer chorionic cells irregular in shape, pen-

Table 1. Dimensions of eggs of *Ae. australis* (n = 10) and *Ae. camptorhynchus* (n = 7).

Aedes species	Length		Width	
	x̄ ± SE	Range	x̄ ± SE	Range
australis	444.7 ± 4.8	419.2 ± 462.1	208.6 ± 2.9	194.4–227.3
camptorhynchus	485.9 ± 7.6	458.7 ± 520.3	290.7 ± 4.8	275.7–306.8

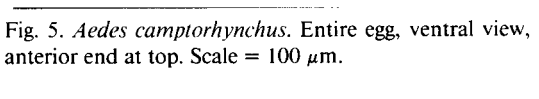

Fig. 5. *Aedes camptorhynchus*. Entire egg, ventral view, anterior end at top. Scale = 100 μm.

tagonal, quadrilateral, or even triangular (Fig. 7A), length 15.3–27.2 μm (mean 19.7 ± 0.9 μm, n = 15) less than width, 25.7–37.9 μm (mean 33.6 ± 0.9 μm), cell fields about 2 μm less in each dimension, cell floors smooth (Fig. 7A,B). Complement of tubercles in each cell consisting of a single large, more or less round central one (rarely with a smaller, secondary tubercle), diameter 5.2–6.4 μm (mean 5.9 ± 0.1 μm, n = 25), in most eggs surrounded by 16–28 (mean 21.5 ± 0.6, n = 15) small tubercles, diameter 1.1–3.2 μm (mean 2.0 ± 0.1 μm, n = 50), regularly spaced around perimeter of cell adjacent to reticulum, a few occasionally within outer rows (Fig. 7A). Number of small tubercles increasing significantly ($P < 0.01$) with length of cell perimeter (Fig. 8A). Large tubercles made up of smooth bases with vertical walls, evenly and slightly excavated to form short pillars (Fig. 7B,D), tops domed with very faint, flat, nodular sculpturing (Fig. 7D). Small tubercles peaked, tops of larger ones flat, slightly nodular (Fig. 7B,D), outer edges touching or overlain by reticulum (Fig. 7D). Reticulum low, appressed to cell surface, 2.0–2.5 μm wide, made up of a fine but rather indistinct mesh with a central line of tiny but prominent papillae (Fig. 7B,D). Most eggs with ventral chorion as described above, but a few structured somewhat differently. In these, cells still with large, central tubercle, but small tubercles not evenly spaced around cell, arrangement more haphazard, with tubercles bunched into groups, reticulum less uniform, in some places indistinct (Fig. 7C).

Chorion, lateral surface (ventral/dorsal transition): Progressing dorsally, single central tubercle in each cell replaced by one or two medium-sized tubercles, which then become more numerous and smaller, small peripheral tubercles tending to become larger (Fig. 7E), tops wider and flatter (Fig. 7F), central and peripheral tubercles ultimately becoming uniform (Fig. 7E). Reticulum as on ventral surface (Fig. 7E). Lateral transition in variant type of chorion essentially as already described (Fig. 9A), reticulum unchanged from ventral surface.

Chorion, dorsal (flatter) surface: Cells slightly smaller than on ventral surface, length 10.8–14.8 μm (mean 12.8 ± 0.4 μm, n = 15), width 23.5–34.3 μm (mean 27.5 ± 0.9 μm), shapes irregular, similar to ventral surface (Fig. 9B), cell floors fairly smooth (Fig. 9C). Tubercles 13–25 in number (mean 19.3 ±

Fig. 6. *Aedes camptorhynchus*. (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, collar with large gaps, disk barely larger than dome; (E) micropylar apparatus, disk almost invisible; (F) detail, micropylar apparatus, disk distinctly larger than dome; (G) posterior end, lateral view; (H) posterior end, chorionic cell detail. Scale = 200 μm (A), = 20 μm (B,C,D,E,F,G,H).

Fig. 7. *Aedes camptorhynchus*. (A) Outer chorionic cells, ventral surface, middle of egg; (B) detail, chorionic cells, ventral surface; (C) variant, chorionic cells, ventral surface; (D) detail, tubercles and reticulum, ventral surface; (E) lateral view, ventral/dorsal transition, ventral side at bottom; (F) transitional cell detail, lateral surface. Scale = 50 μm (A,E), = 10 μm (B,C,D,F).

0.8, n = 15), significantly ($P < 0.001$) more numerous with increasing cell area (Fig. 8B), distributed more or less evenly over cell fields, some partly fused (Fig. 9B). Diameter of tubercles 1.1–3.2 μm (mean 2.1 ± 0.1 μm, n = 50), form rather squat, tops only slightly

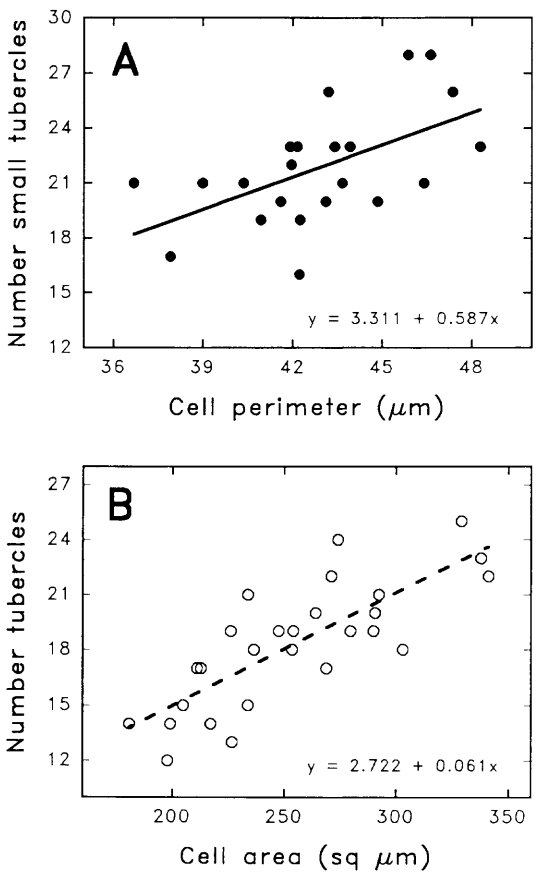

Fig. 8. *Aedes camptorhynchus*. Regressions of (A) number of small tubercles on length of cell perimeter, ventral surface and, (B) number of tubercles on cell area, dorsal surface.

domed, nodular sculpturing very faint (Fig. 7C,E). Reticulum similar to ventral surface, but meshwork very faint, in places undetectable, central papillae not quite as prominent (Fig. 9C). In variant type of chorion, tubercles in dorsal cells not as even in size as more usual type, tending to be more bunched into fused groups (Fig. 9D), reticulum irregular, meshwork visible but variable in width, papillae sometimes fused to edge rather than centrally placed (Fig. 9E).

Anterior end, micropyle: Chorionic cells markedly diminished in size nearer and immediately posterior to micropyle (Fig. 6A), large central tubercles remain distinct, but small tubercles fewer, becoming fused in cells close to collar (Fig. 6B). Collar itself fairly prominent (Figs. 5,6B), rarely continuous, gaps almost always present (Fig. 6D,E), height 7.5–10.5 μm, diameter 30–42 μm, wall width 3.5–8.0 μm, outer wall rounded anteriorly, surface slightly rough (Fig. 6E,F). Inner collar diameter 25–30 μm, inner wall quite deep, with shallow excavations (Fig. 6D,E). Disk in many eggs barely wider than micropylar dome (Fig. 6D,E), more visibly wider in others (Fig. 6F), diameter 18–22 μm, surface rough, outer edge distinct, vertical. Dome quite prominent, diameter about 16 μm, its outer edge difficult to distinguish when disk only slightly wider (Fig. 6D), quite obvious when disk distinctly wider (Fig. 6F). Micropylar orifice trilobed, diameter 2.0 μm.

Posterior end: Chorionic cells smaller toward posterior end, small tubercles fewer (Fig. 6G), but cell structure remains unchanged until most terminal cells, where small tubercles become less distinct and progressively more fused with large tubercles (Fig. 6H).

DISCUSSION

Aedes australis is one of only two species in the subgenus *Halaedes*. The other, *Ae. (Hal.) ashworthi*, known only from coastal rock pool habitats in Western Australia, is very closely related to *Ae. australis* but is considered distinct from it (see Lee et al. 1984) despite Belkin's (1962) view that the two are synonymous. It was not possible to obtain eggs of *Ae. ashworthi* for comparison, but the egg must presumably be very similar to that of *Ae. australis*, particularly as the habitats are the same. The lateral transition between distinct ventral and dorsal cell types in *Ae. australis* is characteristic of species that glue their eggs to the oviposition substrate, as for example in *Ae. albopictus* Skuse, *Ae. aegypti* (L.) and *Ae. bahamensis* Berlin (Linley 1989). As expected, the presence of glue was easily confirmed under the scanning electron microscope, where it could be seen beneath the dorsal surface of eggs anchored to filter paper. Observations by one of us (RCR) of eggs attached to the sloping sides of depressions in rocky (sandstone) littoral shelves above the high tide mark indicate that firm attachment is essential in view of the flushing action of waves at high tide. We noted during

Fig. 9. *Aedes camptorhynchus*. (A) Lateral view, ventral/dorsal transition, cells of variant type, ventral side at top; (B) chorionic cells, dorsal surface, middle of egg; (C) detail, chorionic cells, dorsal surface; (D) variant type chorionic cells, dorsal surface; (E) detail, tubercles and chorionic reticulum, dorsal surface. Scale = 20 μm (A,B), = 10 μm (C,D,E).

preparation for microscopy that the outer chorion of *Ae. australis* eggs was particularly susceptible to the formation of cracks, much more so than other *Aedes* species that have been studied. It should be borne in mind that cracks seen on the whole egg (Fig. 1), in micrographs of the anterior and posterior ends (Fig. 2B,C,F,G), and near the transition boundary (Fig. 3F), are not present in living eggs, although it cannot be said that chorionic cracks never form under natural conditions.

In many respects the egg of *Ae. camptor-*

hynchus resembles that of *Ae. vigilax* (Linley et al. 1992). The length of the *Ae. vigilax* egg (627.7 ± 4.7 μm) is significantly greater ($t = 15.797$, $df = 12$, $P < 0.001$), but the ventral outer chorionic cells are similar in shape and in both species contain a single large, central tubercle surrounded by many small peripheral ones. There is a difference in that almost all the small tubercles closest to the large one in *Ae. vigilax* are connected to it by bridges (Linley et al. 1992), which were not seen in any of the *Ae. camptorhynchus* eggs. Around each cell, the chorionic reticulum also is extremely similar in the two species. There are differences on the dorsal surface, although cell structure is fundamentally similar. Many small tubercles replace the ventral pattern in both instances, but those in *Ae. vigilax* remain mostly peripheral and loosely connected, while in the commonest form of *Ae. camptorhynchus*, tubercles are much more evenly distributed over the entire cell field. There is, on the other hand, much resemblance between *Ae. vigilax* dorsal cells and the variant form (Fig. 9D) in *Ae. camptorhynchus*. We found no material on the *Ae. camptorhynchus* eggs to suggest that they are cemented in any way to the oviposition surface, although there is little accurate information on oviposition sites. *Aedes camptorhynchus* larvae are typically found in earthen ground pools, often with marginal vegetation, and not in rock pools or containers where cement may be more appropriate.

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