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# THE EGG OF *AEDES HENDERSONI* AND A COMPARISON OF ITS STRUCTURE WITH THE EGG OF *AEDES TRISERIATUS* (DIPTERA: CULICIDAE)

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**ABSTRACT.** The egg of *Aedes hendersoni* is described with the aid of scanning electron micrographs and its structure is compared with the egg of *Ae. triseriatus*. The egg of *Ae. hendersoni* is broadly cigar-shaped, with the dorsal surface (cemented to the substrate) distinctly more rounded in longitudinal profile than is the ventral surface. The micropylar collar is fairly conspicuous and substantially thicker on the ventral side. Ventral outer chorionic cells contain four to eight low, flat tubercles, the largest ones positioned in the cell corners. Cell structure changes transitionally down the lateral surfaces to the dorsal surface, where many small, more or less round tubercles are irregularly clustered around the periphery of each cell. Eggs of *Ae. hendersoni* differ significantly in several respects from those of a sympatric population of *Ae. triseriatus*. *Aedes hendersoni* eggs are wider and their length/width ratio is smaller; the cells of the ventral (upper) surface are larger in area and contain more tubercles which occupy a greater proportion of the cell field; the reticulum is narrower; the structure of the dorsal surface cells is distinctly different; the micropylar disk area takes up a smaller percentage of the area within the collar. Differences found between eggs of *Ae. triseriatus* from Michigan and Florida are discussed.

## INTRODUCTION

*Aedes* (*Protomacleaya*) *hendersoni* Cockerell is a widely distributed tree-hole breeding mosquito in the eastern and central United States (Darsie and Ward 1981). It occurs throughout much of its range with its somewhat less widespread sibling species *Ae.* (*Pro.*) *triseriatus* (Say) (Zavortink 1972). Although *Ae. hendersoni* has not been implicated in the transmission of disease, *Ae. triseriatus* is known to be the principal vector of LaCrosse encephalitis virus (Watts et al. 1972, DeFoliart 1983) and also is capable of transmitting dengue virus experimentally (Freier and Grimstad 1983). The differing vector status of these two species underlines the importance of correct identification, especially as the two are separable only as fourth-instar larvae or as fresh adults, in which scales are relatively undamaged. Recognizing the importance of a possible additional means of differentiation, Zaim et al. (1977) studied eggs of both species, with limited use of scanning electron microscopy (SEM), and considered the eggs to be quite easily separable when cleared, mounted, and viewed under a light microscope. In keeping with the objectives of their work, however, Zaim et al. (1977) did not provide a complete description of the *Ae. hendersoni* egg. The existence of no other information on the morphology of this species' egg prompted us to make the more complete examination presented here. Earlier descriptions of the egg of *Ae. triseriatus* exist (Horsfall and Craig 1956, Kalpage and Brust 1968, Horsfall et al. 1970), as well as a recent study with SEM (Linley 1989a), but it was worthwhile to compare directly eggs of sympatric populations (from Michigan) of the two species, and to look for possible differences between Michigan and Florida populations of *Ae. triseriatus*. Accordingly, we provide in the first part of this report a description of the *Ae. hendersoni* egg, followed by the comparisons mentioned.

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## MATERIALS AND METHODS

The eggs of *Ae. hendersoni* and *Ae. triseriatus* were alive when prepared for electron microscopy. Both Michigan strains were from laboratory colonies propagated by force-mating; the originating collections were from the University of Notre Dame Environmental Research Center (Gogebic County) at the far western end of the upper Michigan peninsula. Florida *Ae. triseriatus* material was from a colony started from females collected on the grounds of the Florida Medical Entomology Laboratory (Indian River County).

Most specimens used for electron microscopy were obtained by cutting small pieces of the oviposition paper, with eggs attached, and appressing these to stubs covered with sticky tape. However, to obtain views of the dorsal (lower) egg surfaces (glued to the substrate with copious cement), it was necessary to detach eggs, turn them over with an artist's brush, and reposition them on a separate area of the tape. Eggs selected for manipulation were those (relatively few) laid across small ridges in the paper so that only limited portions were attached by cement, leaving adequate areas of the dorsal surface undamaged and accessible to study. Five stubs of each species (or strain) were prepared, with eggs from widely separated positions on the oviposition paper, which in each case had received eggs from a number of females. Once prepared, stubs were dried completely over calcium chloride (20 min), sputter-coated with gold, and examined with a Hitachi S-510 scanning electron microscope.

Quantitative measurements or counts were made from micrographs placed over a digitizing tablet used with SigmaScan software (Jandel Scientific, San Rafael, CA). Equal numbers of determinations were made from eggs on each of the five stubs to contribute to means (± SE) cited in the text and tables. In the comparisons involving structures in the outer chorionic cells of the ventral (upper) surface, all data except reticulum width were taken from seven marked cells on a micrograph (1,000×) of an area in the middle of one selected egg on each stub. Reticulum measurements were digitized in the middle of each cell side of the seven marked cells, but additional determinations from other cells were made until 60 measurements had been made for each population. Cell dimensions (at widest points) were taken such that lengths were along the longitudinal axis of the egg and widths were in the circumferential direction. "Form factor" (see Table 2) was used as a quantitative index of cell shape (roundness); its maximum value of 1.0 corresponds to a perfect circle.

Measurements of areas of several structures in the micropylar apparatus were made from two eggs (micrographs of 1,500×) on each stub. The populations also were compared in terms of the radial width of the micropylar collar by measuring its thickness at radii drawn at 20° increments from the collar center (0° being at the most dorsal point).

Data were analyzed using Statgraphics software (STSC Inc., Rockville, MD). The terminology is that of Harbach and Knight (1980), supplemented by "outer chorionic cell field" (Linley 1989b) and "micropylar dome" (Linley et al. 1991).

## RESULTS

### Egg of *Aedes* (*Protomacleaya*) *hendersoni*

*Size*: As in Table 1. *Color*: Matte black. *Shape, overall appearance*: Broadly cigar-shaped in ventral (Fig. 1a) or dorsal view, ventral (upper) surface somewhat flatter than dorsal in lateral view (Fig. 2a); widest at about anterior 0.3, anterior profile consequently more rounded than posterior (Fig. 1a). Outer chorionic cells clearly demarcated, wider than long, large tubercles in corners of adjacent cells positioned to form ridges along surface of egg (Fig. 1a). Micropylar collar conspicuous, rounded anteriorly.

*Chorion, ventral (upper) surface*: Outer chorionic cells usually pentagonal, occasionally hexagonal (Fig. 3b), length 20–37 μm (mean 25.9 ± 0.5 μm, n = 35), width 14–23 μm (mean 18.8 ± 0.4 μm). Cell fields about 1.3 μm less in each dimension. Tubercles 4–8 in number (mean 5.20 ± 0.19, n = 35), low and very irregular in outline, variable in size

**Table 1.** Mean dimensions[1] of eggs of *Ae. hendersoni* and two populations of *Ae. triseriatus* (n = 20).

| Species | Length (μm) | | Width (μm) | | L/W ratio | |
|---|---|---|---|---|---|---|
| | x̄ ± SE | Range | x̄ ± SE | Range | x̄ ± SE | Range |
| *Ae. hendersoni* (Michigan) | 722.0 ± 5.6a | 672.0–760.5 | 243.2 ± 2.0a | 223.9–263.7 | 2.97 ± 0.03a | 2.76–3.22 |
| *Ae. triseriatus* (Michigan) | 750.9 ± 5.9b | 692.0–806.5 | 230.5 ± 2.8b | 210.2–253.1 | 3.27 ± 0.04b | 2.94–3.51 |
| *Ae. triseriatus* (Florida) | 736.6 ± 7.0ab | 635.9–772.4 | 229.4 ± 4.3b | 188.3–265.3 | 3.23 ± 0.06b | 2.71–3.81 |

[1] Means followed by same letter do not differ significantly.

(Figs. 3b; 4a), largest ones in cell corners and almost all, except occasional small ones, touching reticulum. Total tubercle area (y) increases significantly ($P < 0.001$) with cell area (x) according to $y = -9.626 + 0.390x$. Tops of tubercles flat, with rather faintly defined irregular nodules (Fig. 4a,b). Cell fields fairly smooth or partially or completely (Fig. 4a,b) covered by a porous meshwork. Outer chorionic reticulum 1.2–4.3 μm in width (mean 2.65 ± 0.07 μm, n = 60), edges raised, especially adjacent to tubercles, surface covered with an intricate mesh, sometimes perforated (Fig. 4a,b).

*Chorion, lateral surface (ventral–dorsal transition)*: Cells in ventrolateral region similar to ventral cells (Fig. 5a), except that field surfaces smooth or with little overlying meshwork. Tubercles as already described, but appearing more completely to fill cells, reticulum less easily distinguished, smooth or with only faint striations (Fig. 4c). Midlateral cells without much further structural change (Fig. 5a) other than that tubercles tend to be more mounded, rounder (Fig. 4d). Transition to dorsal type cells rapid once dorsolateral area is reached (Figs. 4e; 5a). Tubercles appear smaller, as low mounds (Fig. 5a), then rapidly become partly or completely coalesced, and ultimately smaller, more numerous, and clumped, to form a low peripheral wall (Fig. 4e).

*Chorion, dorsal (lower) surface*: Cells with peripheral tubercles more clearly separated, beadlike, but surfaces rough (Figs. 3c; 4f). Cell fields rough or with poorly defined meshwork (Fig. 4f), devoid of tubercles except, occasionally, for 1 or 2 some distance from edge (Fig. 3c). Reticulum tending to form cracks (artifact of drying?); surface similar to that of cells (Fig. 4f).

*Anterior end, micropyle*: Chorionic cells diminish in size toward anterior end (Fig. 2b), field surfaces slightly rough, not covered with porous mesh as in middle of egg, tubercles clearly defined even to posterior margin of micropylar collar (Fig. 2b,c), but surface nodules indistinct (Fig. 2c). Micropylar collar conspicuous (n = 10 for all measurements of micropylar apparatus), continuous (no gaps), anterior surface rounded, texture rough (Fig. 2b,c,d). Collar height 9–17 μm, diameter 37–47 μm (mean 41.8 ± 0.8 μm), wall width (Fig. 2d) least on dorsal side, 5–8 μm (mean 6.7 ± 0.3 μm), and incrementing radially as in Fig. 6 to 11–14 μm (mean 13.0 ± 0.4 μm) ventrally. Collar internal diameter 19–25 μm (mean 21.9 ± 0.4 μm), edge slightly excavated (Fig. 2d), disk edge irregular in outline, slightly raised, disk diameter 14–21 μm (mean 17.1 ± 0.5 μm). Micropylar dome barely perceptible in some specimens (Fig. 2d), about 9 μm in diameter, invisible in others, orifice diameter 1.3 μm.

*Posterior end*: Chorionic cells smaller approaching posterior end, reticulum less easily visible (Fig. 2e), smooth or with surface striations barely visible (Fig. 2f). Tubercles less clearly defined than in mid-ventral cells, sometimes partly fused, with smoother surfaces, becoming rounded and domed in cells at very end of egg (Fig. 2f).

## Comparison of *Ae. hendersoni* and *Ae. triseriatus*

On the basis of eggs from sympatric Michigan populations of these two species, egg

Fig. 1. a, *Aedes hendersoni* (Michigan); b, *Aedes triseriatus* (Michigan). Entire eggs, ventral views, anterior ends at top. Scale = 100 μm.

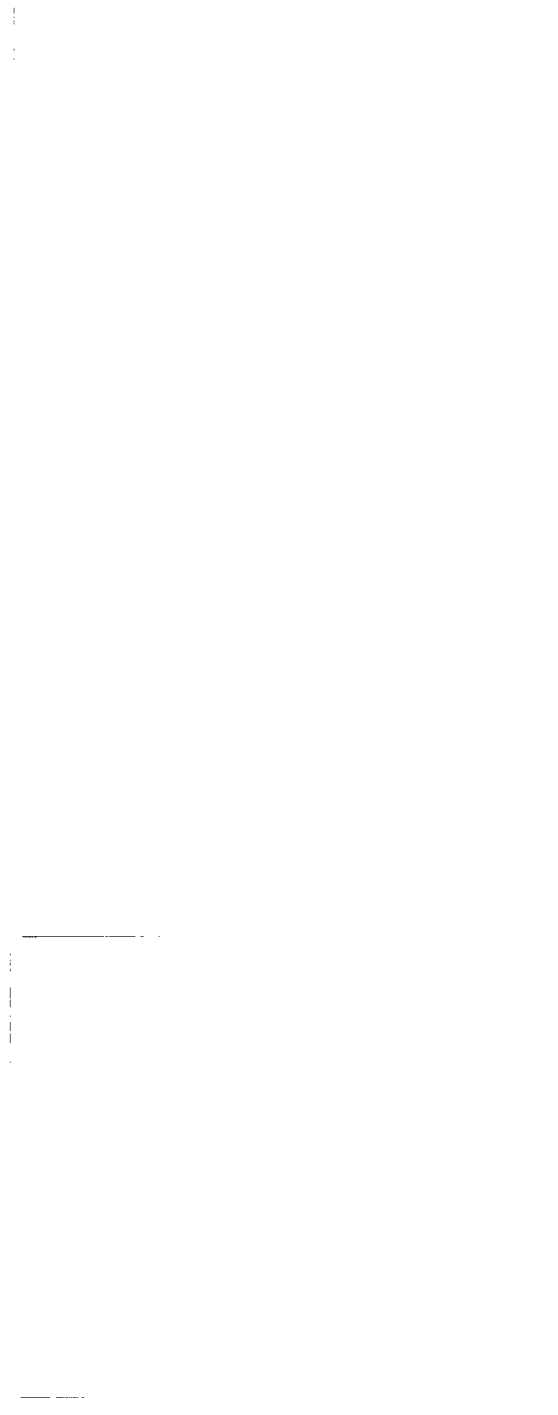

Fig. 2. *Aedes hendersoni* (Michigan). a, Entire egg, lateral view, anterior end at left; b, anterior end, lateral view; c, anterior end, chorionic cell detail; d, detail, micropylar apparatus, dorsal side at top; e, posterior end, lateral view; f, posterior end, chorionic cell detail. Scales = 200 μm (a), 50 μm (b,c,e,f), 20 μm (d).

Fig. 3. a–c, *Aedes hendersoni* (Michigan); d–f, *Aedes triseriatus* (Michigan). a,d, Whole eggs, ventral views; b,e, outer chorionic cells, ventral surfaces, middle of eggs; c,e, chorionic cells, dorsal surfaces, middle of eggs. Scales = 500 μm (a,d), 20 μm (b,c,e,f).

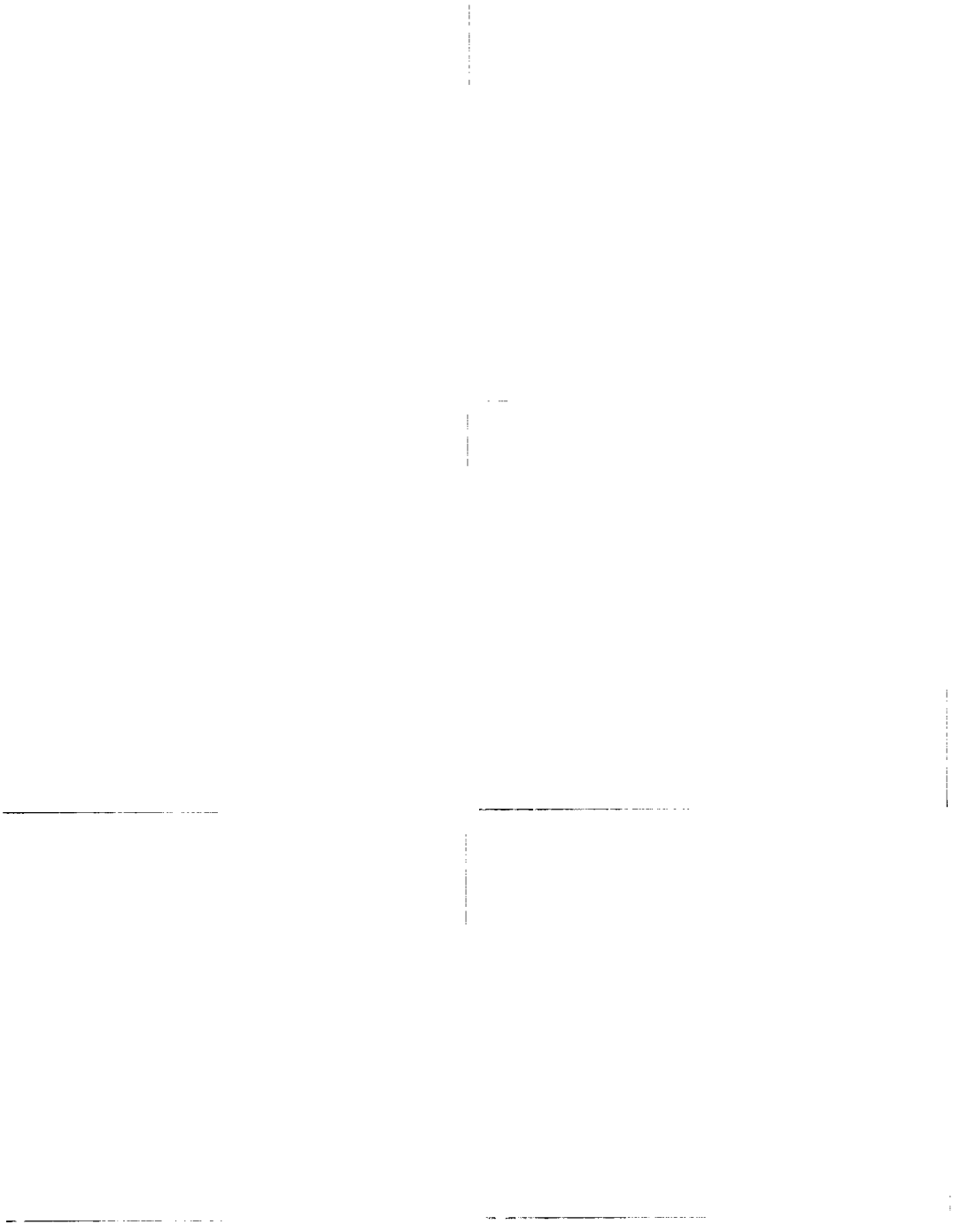

Fig. 4. *Aedes hendersoni* (Michigan). a, Chorionic cell detail, ventral surface, middle of egg; b, extreme detail, tubercles, cell floor and reticulum, ventral surface; c, cell detail, ventrolateral area of transition; d, cell detail, midlateral area of transition; e, cell detail, dorsolateral area of transition; f, detail of dorsal surface cells. Scales = 10 $\mu$m.

Fig. 5. a,b, *Aedes hendersoni* (Michigan); c–f, *Aedes triseriatus* (Michigan). a,d, Lateral aspects, middle of eggs, showing almost entire ventral–dorsal transition, ventral sides at top; b,e, more dorsal portion of transition, ventral sides at top; c,f, cell detail, more typical and variant forms, respectively, of dorsal cell structure, *Ae. triseriatus* (Michigan). Scales = 50 μm (a,b,d,e), 10 μm (c,f).

**Table 2.** Attributes (means ± SE)[1] of outer chorionic cells (n = 35) and micropyle (n = 10) of *Ae. hendersoni* and *Ae. triseriatus* populations from Michigan and Florida. Linear measurements in μm, areas in sq μm.

| Attribute | Population | | |
|---|---|---|---|
| | *A. h.* (M) | *A. t.* (M) | *A. t.* (F) |
| Cell area | 355.1 ± 6.1a | 316.3 ± 8.4b | 374.6 ± 9.1a |
| Cell form factor[2] | 0.79 ± 0.01a | 0.84 ± 0.01b | 0.77 ± 0.01a |
| Reticulum width[3] | 2.65 ± 0.07a | 3.47 ± 0.09b | 3.46 ± 0.08b |
| Number tubercles/cell | 5.20 ± 0.19a | 2.29 ± 0.17b | 2.91 ± 0.17c |
| Tubercle area percent[4] | 36.2 ± 0.6a | 21.4 ± 0.7b | 23.7 ± 1.2b |
| Micropylar area[5] | 1,402.3 ± 38.2a | 1,443.5 ± 52.2a | 1,155.8 ± 19.1b |
| Collar area | 1,019.5 ± 32.9a | 1,058.4 ± 43.6a | 798.2 ± 24.6b |
| Area within collar[6] | 382.9 ± 13.9a | 385.1 ± 21.9a | 358.2 ± 12.6a |
| Disk area | 237.4 ± 9.3a | 273.5 ± 14.2a | 246.1 ± 10.3a |
| Disk area percent[7] | 62.2 ± 2.2a | 71.7 ± 3.5b | 68.7 ± 1.7ab |

[1] Means followed by same letter do not differ significantly.
[2] $(4 \times \pi \times \text{area})/\text{perimeter}^2$.
[3] n = 60.
[4] Total tubercle area as percent cell area.
[5] Area of entire micropylar apparatus (within outer edge of collar).
[6] Bounded by inner edge of collar.
[7] Disk area as percent of area within collar.

length was significantly greater in *Ae. triseriatus,* although the ranges overlapped considerably (Table 1). Egg width was significantly greater in *Ae. hendersoni,* which consequently had a lower length/width ratio (Table 1). These measurements are consistent with simple visual impressions (Fig. 1a,b) that the egg of *Ae. triseriatus* is somewhat narrower in relation to its length and its sides are straighter. Especially when seen in groups (Fig. 3a,d), the eggs of *Ae. triseriatus* seem longer and somewhat less rotund than those of *Ae. hendersoni.*

When magnification is sufficient to reveal the structure of the ventral outer chorion, clear differences immediately become apparent (Fig. 3b,e). The cells are significantly larger in *Ae. hendersoni,* the reticulum is narrower, and the shapes of the cells are not as round as the smaller cells in *Ae. triseriatus* (Table 2). *Aedes hendersoni* cells have significantly more tubercles, which are flatter compared to the domed form in *Ae. triseriatus* (Fig. 3b,e) and occupy a greater proportion of the total cell area (Table 2). In *Ae. triseriatus,* cells are almost always dominated by a single large, domed tubercle (Fig. 3e) and the alignment of these creates the appearance of longitudinal ridges on the whole egg (Fig. 1b; Linley 1989a), whereas large tubercles in adjacent cells are seen in *Ae. hendersoni* (Fig. 1a). In lateral transitional areas, the large tubercles in *Ae. triseriatus* remain very distinct and prominent down the side of the egg (Fig. 5d), causing the appearance to be quite different from *Ae. hendersoni,* where the tubercles become rather difficult to distinguish individually (Fig. 5a). Also in the latter, the zone of change to cells of the dorsal type appears less distinct (Fig. 5b) than the abrupt structural transformation in *Ae. triseriatus* (Fig. 5e). The dorsal surfaces, although normally cemented to the substrate and difficult to observe, are quite different in the two species. Small, more or less round tubercles that are clumped around the cell periphery in *Ae. hendersoni* (Figs. 3c; 4f) contrast with partially fused finger-like outgrowths in *Ae. triseriatus* (Figs. 3f; 5c).

In terms of micropylar structure, the two species are very similar (Table 2), differing only in that the disk occupies a somewhat smaller proportion of the area within the collar in *Ae. hendersoni.* There is a difference also in the radial change in collar width, which in *Ae. hendersoni* increments linearly from

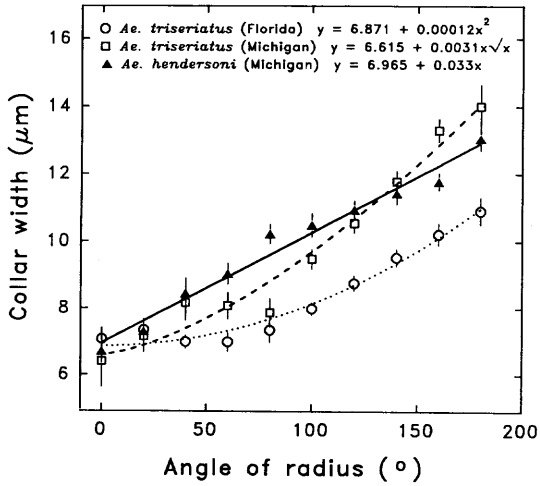


Fig. 6. Relationship between mean collar width (vertical lines indicate standard errors) and radial angle from collar center (0° is at most dorsal position).

the dorsal to ventral side, but disproportionately more toward the ventral side in *Ae. triseriatus* (Fig. 6). The collar in *Ae. triseriatus* is slightly thinner dorsally than *Ae. hendersoni* and thicker ventrally. However, this difference was not detectable from inspection of individual micrographs.

### Comparison of Two Populations of *Ae. triseriatus*

Although eggs from the Michigan and Florida *Ae. triseriatus* populations did not differ in dimensions (Table 1), there were differences in the outer chorionic cells and, surprisingly, a considerable difference in the micropylar collar. Cells of the ventral surface were significantly smaller and rounder in form in the Michigan population (Table 2), and contained slightly but significantly fewer tubercles. On the dorsal surface, in relatively limited areas where detail was not obscured by cement, Michigan eggs revealed a complex structure with finger-like tubercles arising from cells surrounded by a wide reticulum having a meshlike (Fig. 5c) or, less commonly, a papillate (Fig. 5f) surface. Florida eggs, in contrast, had cells with smooth, somewhat domed surfaces (Linley 1989a) or low, smooth ridges. A more surprising difference than any in the chorion was the much smaller area of the micropylar apparatus in the Florida population (Table 2). The area within the collar was not significantly smaller in Florida eggs, but the area of the collar itself was considerably so, indicating this structure to be substantially less massive in this population, as illustrated by radial measurements of width (Fig. 6). Dorsal collar width is about the same in both groups, but in the Florida population it increments progressively less toward the ventral side.

## DISCUSSION

For differentiating eggs of *Ae. hendersoni* and *Ae. triseriatus* in field or ovitrap collections, criteria of shape or size are of little value. Statistically significant differences exist between the two (Table 1), but the limits of variation overlap considerably. Although one's impression when looking at groups of eggs of the two species side by side is that *Ae. hendersoni* eggs are wider in relation to length (compare Fig. 3a and b), any momentary confidence about identifying individual eggs evaporates when a single unknown egg is presented. Given that practicable criteria for differentiation should be visible in eggs as laid, without the need for further manipulation or preparative work, then chorionic differences are the ones that merit attention. These are very obvious at sufficient magnification, when surface relief is clearly delineated (Fig. 1a,b), and the identity of the two species becomes immediately apparent. Cells are easily seen to be larger in *Ae. hendersoni* and to contain more tubercles, which form less elevated and more widely spaced longitudinal ridges along the egg (Fig. 1a,b). But the extent to which magnification and perception of cell topography are important becomes very apparent under a stereomicroscope, where considerably less of both are obtainable, and where the unfortunate reality of poor stereomicroscopic image quality at higher magnifications becomes limiting. Zaim et al. (1977) showed, reasonably convincingly, that the eggs could be differentiated under a compound microscope after being cleared and mounted (Craig

1955). This method requires preparative time, which in some applications might not be practicable, and its success depends on a fortuitous difference in the optical behavior of the two types of chorion in transmitted light. Under reflected light and a stereomicroscope, it is certainly more difficult to distinguish between the two structural patterns, but perhaps not prohibitively so. The image in both is of very closely packed groups of nodules arranged in longitudinal or often diagonal lines, with separating gaps. The nodule groups in both cases are composed of adjacent large tubercles in the raised circumferential corners of the chorionic cells (Fig. 3b,e). The apparent gaps are the more sunken central portions of each cell, as can be seen in Fig. 1a,b. Gaps do not correspond, as might be thought initially, to the outer chorionic reticulum. When sufficient eggs have been examined, it is possible to distinguish that the nodular groups in *Ae. hendersoni* are larger and more widely separated but not as prominent as in *Ae. triseriatus,* where the large, domed tubercle in each cell creates more relief on the egg surface. Another impression gained from examination of many eggs is that those of *Ae. hendersoni* tend to be slightly grayer in tone, rather than quite black as in *Ae. triseriatus.* With strong, diffuse illumination and an optically good stereomicroscope, therefore, we believe it would be possible to learn to differentiate living eggs of the two species and that this could be done consistently, provided that familiarity with the material was maintained by constant practice.

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