

Fine Structure of the Egg of *Trichoprosopon digitatum* (Diptera: Culicidae) and Its Relationship to Egg Raft Formation

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ABSTRACT Eggs of *Trichoprosopon digitatum* (Rondani) occur in rafts; the anterior poles are submerged, and the posterior two-thirds float above the water surface. Each egg is approximately 1,060 μm long and 270 μm wide. The hydrophilic end is more curved dorsally than ventrally and is covered with flattened, anteriorly pointed, scalelike tubercles except for a small area around the micropyle. The hydrophobic portion is clad in a layer of closely arrayed, small, round tubercles among which are positioned much larger, flattened, anteriorly directed tubercles with deeply fissured surfaces. At three positions equidistant around the periphery of the egg, narrow, tongue-shaped extensions (the embrasures) from the anterior hydrophilic region project posteriorly into the hydrophobic region. Along these embrasures, progressing posteriorly, the tubercles change in form from flattened and bladelike to finely tapered, then, at the extension's posterior two-thirds, to long filaments with well-developed terminal hooks. Eggs in rafts are maintained in polygonal rosettes by the interlocking of these filaments and hooks and the surface tension of menisci between contiguous embrasures.

KEY WORDS Insecta, *Trichoprosopon digitatum*, egg structure, egg rafts

THE NEOTROPICAL SABETHINE MOSQUITO *Trichoprosopon digitatum* (Rondani) is one of 21 species in a genus studied taxonomically by Zavortink (1979a,b; 1981). *Tr. digitatum* is a widespread species, ranging throughout the entire distribution of the genus from Mexico to Ecuador and southern Brazil (Zavortink et al. 1983). Owing in large measure to its early colonization by Aitken et al. (1968), the biology of the species is reasonably well known. An unusual behavioral feature is that females that have oviposited remain many hours after completion of laying with the egg raft clutched between the mesothoracic legs (Aitken et al. 1968). Lounibos & Machado-Allison (1983) have confirmed recently that brooding the egg rafts occurs in the field and, on the basis of field and laboratory observations (Lounibos & Machado-Allison 1986), that the behavior substantially reduces the loss of eggs caused by rain.

Aitken et al. (1968) observed that eggs in rafts clustered to form rosettes, but unbrooded rafts in the field were observed to disintegrate to some extent (Lounibos 1983), suggesting that the female plays an important role in holding the eggs together. Several authors (Pawan 1922, Aitken et al. 1968, Mattingly 1974) have noted, however, that eggs in rafts appeared to be "glued" together, and Mattingly (1974), in providing the most complete description of the egg so far published, suggested that the "glue" itself was secreted in filaments.

Although several descriptions of the *Tr. digitatum* egg have been given previously (Pawan 1922, Aitken et al. 1968, Mattingly 1974), all were subject to the limitations of light microscopy. As we show in this paper, scanning electron microscopy (SEM) is required to describe this remarkable egg adequately. We then relate the fine structure of the individual egg to the mechanism of raft formation.

Materials and Methods

The 15 eggs of *Tr. digitatum* used for our study were collected from females brought to the United States from Panaquire, Venezuela (10°13'N, 66°14'W) and then fed on blood in the laboratory. The eggs were fixed for 1 h in 2.5% glutaraldehyde in 0.2 M sodium phosphate, 0.14 M NaCl buffer at room temperature, then postfixured for 1 h in 2.5% OsO₄ in 1.25% NaHCO₃ buffer. Eggs were dehydrated in ethanol, dried by the critical point method, and attached with Bakelite (Structure Probe, Westchester, Pa.) to small glass coverslips. These were glued in turn to stubs with Electrodag 191 (Acheson Colloids Company, Port Huron, Mich.) and coated with gold-palladium. A Hitachi S-510 scanning electron microscope was used to examine the specimens.

The terminology follows Harbach & Knight (1980), except for the term "embrasure," which was originated by Mattingly (1974) to describe each of the narrow, longitudinal hydrophilic strips that are equidistantly spaced around the egg (see below).

Observations of egg rafts and the relationship of

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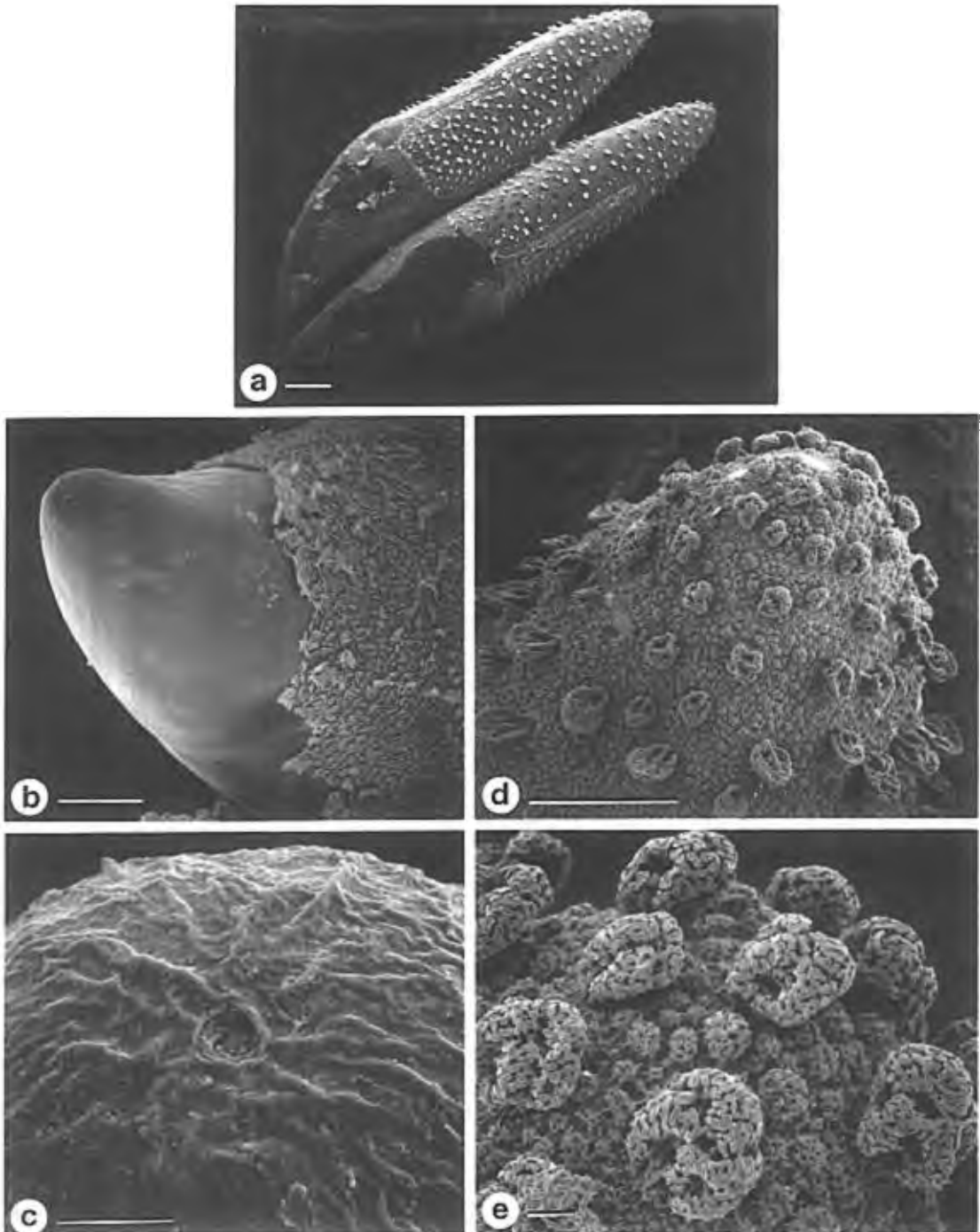


Fig. 1. (a) Two entire eggs, anterior pole at lower left. (b) Anterior pole with micropyle at tip. (c) Detail of micropyle and ruffled surrounding chorion. (d) Posterior pole. (e) Detail of outer chorionic tubercles at posterior pole. Scale: (a) 100 μm , (b and d) 50 μm , (c and e) 5 μm .

individual eggs to the water surface were made on material from a laboratory colony of *Tr. digitatum* maintained according to the methods of Aitken et al. (1968).

Results

Description of Egg. General Features. Mean length 1,060 μm (two intact and uncollapsed eggs measured under SEM), mean width 270 μm at wid-

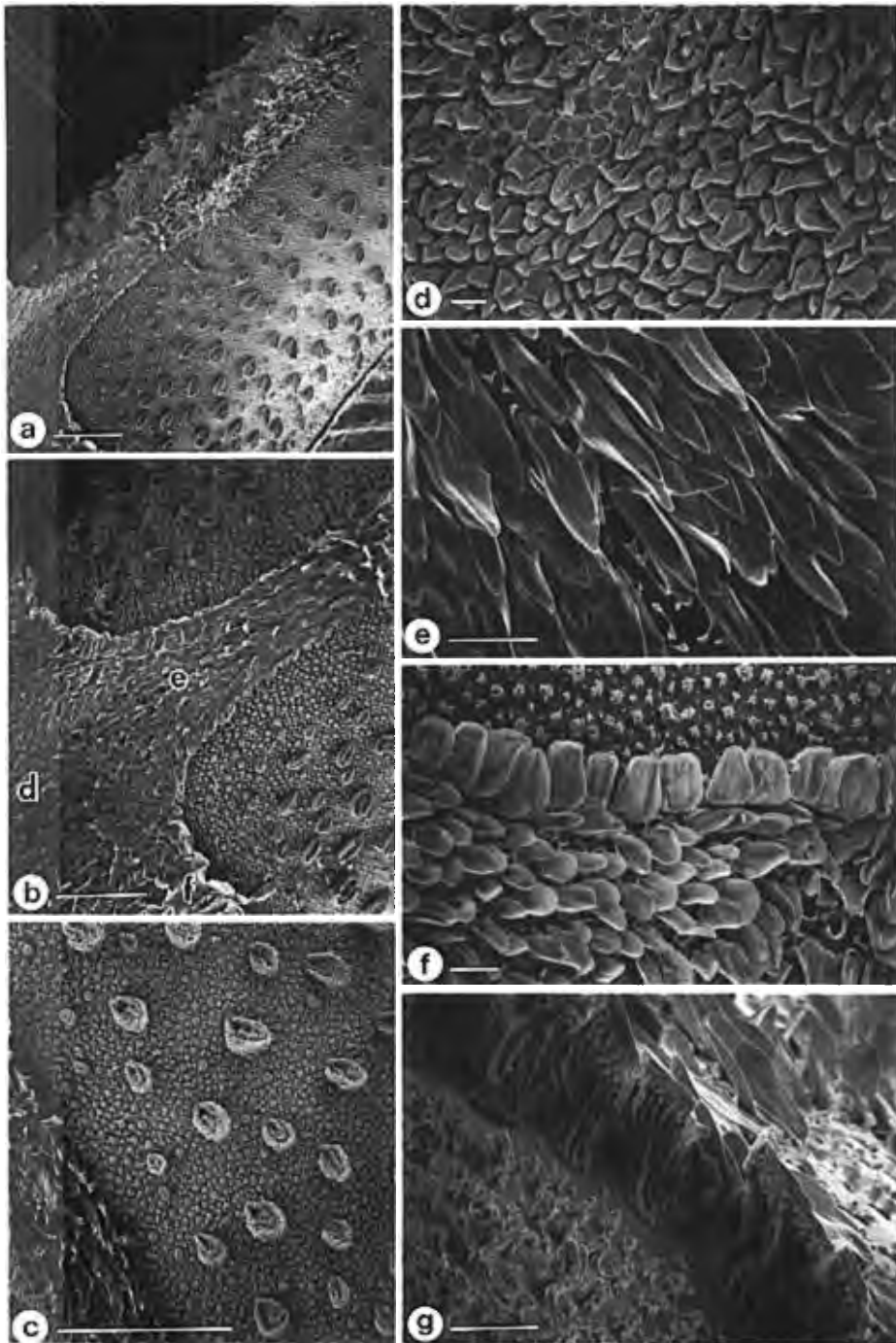


Fig. 2. (a) Middle region of egg, showing entire embasura. (b) Anterior origin of embasura and anterior limits and boundary of hydrophobe outer chorion; letters denote areas depicted by Fig. 2d-f. (c) Tubercle array on hydrophobe outer chorion, middle of egg. (d) Array of outer chorionic tubercles in anterior hydrophilic region. (e) Blade-like outer chorionic tubercles in anterior origin of embasura. (f) Palisade tubercles forming boundary between posterior hydrophobe region (upper) and anterior hydrophilic region (lower). (g) Undersides of palisade tubercles bounding hydrophobe region. Scale: (a-c) 50 μm , (d-g) 5 μm .

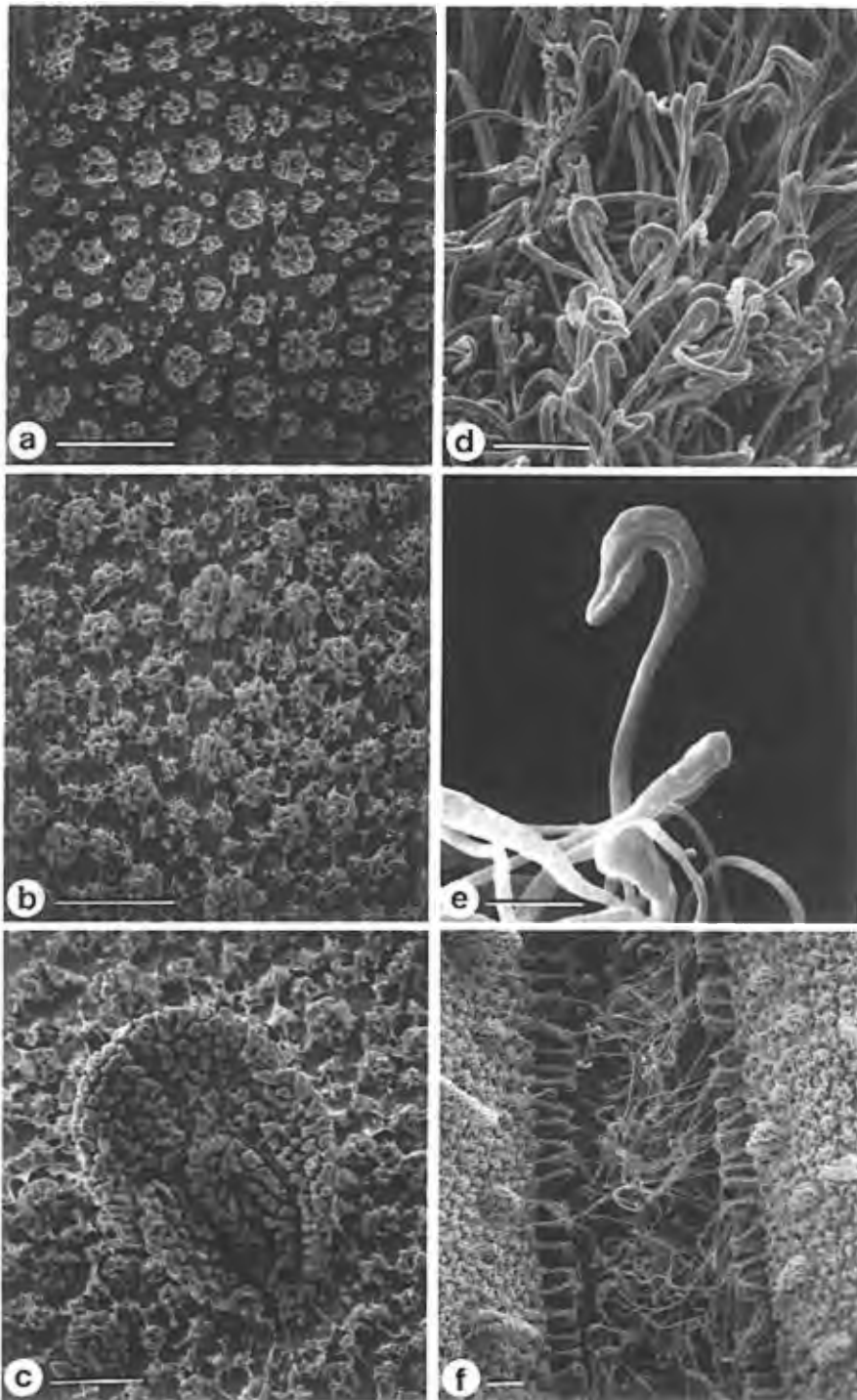


Fig. 3. (a) Less closely packed small outer chorionic tubercles on anterior areas of hydrophobe region. (b) More closely packed small outer chorionic tubercles on posterior areas of hydrophobe region. (c) Detail of large outer chorionic tubercle on hydrophobe region. (d) Detail of closely packed filaments and hooks, posterior part of embrasure. (e) Extreme detail, single hook. (f) Intertwined filaments and hooks of two adjacent embrasures on apposed eggs. Scale: (a-d, f) 5 μm , (e) 2 μm .

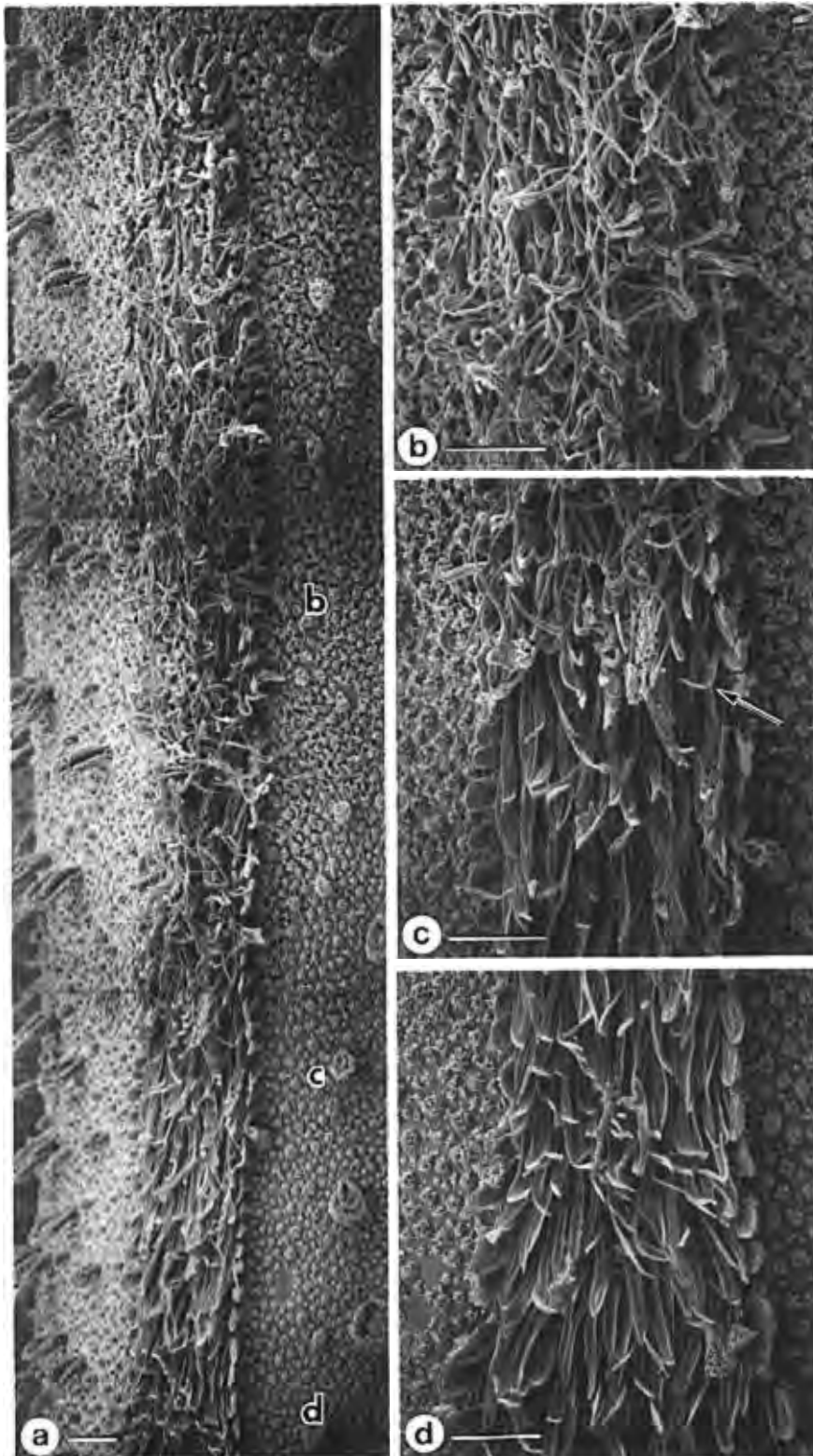


Fig. 4. (a) Top view, entire embasura. (b-d) Detail of embasura at positions indicated by corresponding letters in (a). Arrow (in c) indicates transitional type of tubercle. Scale: 10 μm .

est point (see Fig. 1a). Color of living eggs dark grey, appearing frosted, especially when viewed from above. Egg widest at anterior $\frac{1}{3}$ (Fig. 1a), where a circumferential boundary separates anterior hydrophilic region from posterior hydrophobic region. At three positions equidistant around egg, boundary extends posteriorly to form narrow, hydrophilic strips (embrasures) that project into hydrophobe region to about posterior $\frac{1}{3}$. Anterior portion of egg quite sharply tapered and curved, owing to much greater curvature of dorsal surface. Posterior portion gradually tapered, taper increasing at posterior end (Fig. 1a). Areas corresponding to individual outer chorionic cells impossible to distinguish on any part of egg.

Anterior Pole, Micropyle. Anterior pole devoid of outer chorion, curved and quite sharply pointed (Fig. 1b), micropyle at tip. Inner chorion at tip and around micropyle rumpled (Fig. 1b and c), micropyle circular, diameter about $2.4 \mu\text{m}$, not surrounded by any specialized structures.

Anterior Hydrophilic Region. Uniformly covered with flattened, pointed, anteriorly directed, scalelike tubercles $3\text{--}7 \mu\text{m}$ long (ranges determined from examination of 5 eggs), $1.6\text{--}4 \mu\text{m}$ wide (Fig. 1b and 2d). Each tubercle arises from smooth floor surrounded by low wall (Fig. 2d). Walls usually without gaps and contiguous with those of adjacent tubercles. Tubercles at base (anterior end) of embrasure (Fig. 2b) longer, bladelike, and overlapping (Fig. 2e), $8\text{--}11 \mu\text{m}$ long, $1.6\text{--}2.8 \mu\text{m}$ wide, tips broadly pointed or rounded. Flattened tubercles along posterior boundary of hydrophilic region between embrasures (Fig. 2f) and also along embrasure edges (Fig. 4a–d) larger ($8\text{--}12 \mu\text{m}$ long, $5\text{--}8 \mu\text{m}$ wide), more erect, forming palisade. Each palisade tubercle supported on a spreading, fluted column arising from a distinct, disklike base (Fig. 2g).

Posterior Pole. Broadly rounded, covered with more or less evenly spaced, large, cauliflower-like outer chorionic tubercles, diameter $7\text{--}16 \mu\text{m}$ (Fig. 1d and e). Large tubercles surrounded (Fig. 1e) by tightly packed, smaller, predominantly round tubercles of very variable diameter ($1\text{--}5 \mu\text{m}$). Bases of tubercles connected by complex system of low ridges (Fig. 1e). A short distance from posterior pole, large tubercles become more flattened, inclined anteriorly (Fig. 1d). Surfaces of all tubercles deeply cleft with narrow fissures (Fig. 1e).

Posterior Hydrophobic Region. Uniformly covered with large, flattened, earlike, anteriorly inclined tubercles (Fig. 2a–c), length $8\text{--}18 \mu\text{m}$, width $5\text{--}13 \mu\text{m}$, becoming smaller in areas adjacent to embrasure or anterior boundary (Fig. 2b). Large tubercles surrounded by fairly densely packed, smaller, round tubercles, diameter $0.3\text{--}3 \mu\text{m}$, which are joined by fewer basal connecting ridges in areas adjacent to embrasure (Fig. 3a) than away from embrasure or toward posterior pole (Fig. 3b). Surfaces of small tubercles made up of irregularly shaped bumps separated by clefts and holes (Fig. 3a, b). Large tubercle surfaces consisting of raised,

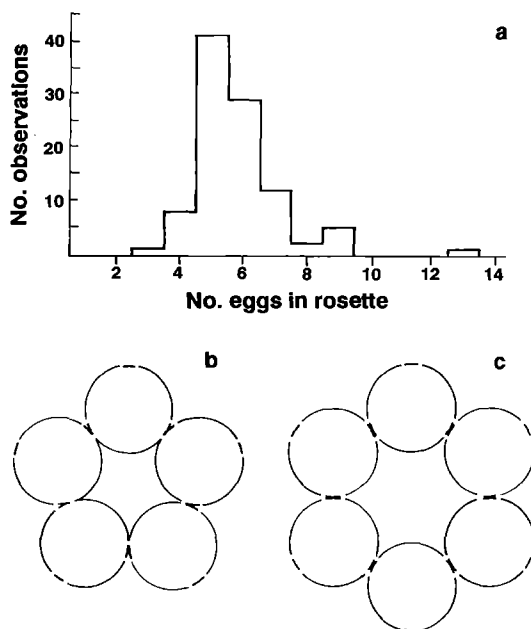


Fig. 5. (a) Frequency distribution of eggs in rosettes among nine rafts removed from brooding females. (b and c) Diagrammatic top views of eggs arranged in pentagonal and hexagonal rosettes. Location of embrasures indicated by the short line segments.

flat-topped, fairly closely packed projections with deep separating spaces (Fig. 3c) and most with a clear, wider, and continuous loop-shaped cleft arising close to the tubercle base (Fig. 2c and 3c).

Embrasures. Three in number, equidistantly spaced around circumference of egg. Each about $300 \mu\text{m}$ long, $30 \mu\text{m}$ wide, posterior $\frac{1}{2}$ perhaps tending to be slightly wider (Fig. 4a). Embrasure originating in a tapering posterior extension of the anterior hydrophilic region (Fig. 2b), covered with overlapping, bladelike tubercles (Fig. 2e), which rapidly change shape in anterior $\frac{1}{3}$ (Fig. 4a), becoming longer ($12\text{--}17 \mu\text{m}$), narrower ($0.8\text{--}1.5 \mu\text{m}$), some with pointed tips, others becoming more tapered and filamentous (Fig. 4d). At about anterior $\frac{1}{3}$, tubercles changing form (Fig. 4a) to long filaments with terminal hooks, maintained along remaining $\frac{2}{3}$ of embrasure (Fig. 4a). Transition of tubercles to hook shape sudden, with very few apparently intermediate types (Fig. 4c, arrow). Hook tubercles forming a tangled mass along posterior part (about $250 \mu\text{m}$) of embrasure (Fig. 3d and 4b). Individual tubercles $20\text{--}25 \mu\text{m}$ long, filaments $0.2\text{--}0.6 \mu\text{m}$ wide, tending to be thinner basally, thicker just below hooks (Fig. 3d). Hooks well developed, thicker than filaments, often shaped like shepherd's crook, with grooved sides (Fig. 3e).

Egg Raft Structure. Nine rafts were removed from brooding females, care being taken to preserve the shape of the egg arrays. These rafts were composed of from 27 to 76 eggs (median, 43) clus-

tered in from 6 to 20 connected polygons. The commonest polygons contained five or six eggs, these two shapes accounting for 70.7% of all types (Fig. 5a). Pentagons were more common than hexagons, even though the latter should, with three embrasures spaced at 120°, ensure more contact (Fig. 5b, c). All points of contact in polygons were examined by teasing eggs apart under a dissecting microscope. Contacts at embrasures accounted for 462 of 471 connections (98.1%) between adjacent eggs.

Discussion

In describing mosquito eggs, it is usual to make frequent reference to the outer chorionic cells because these are the fundamental units normally recognizable on the egg surface. In the *Tr. digitatum* egg, however, the areas making up individual cells cannot be distinguished with certainty on any part of the egg. The large, anteriorly directed tubercles on the hydrophobic (posterior) part of the egg probably form the centers of individual cells, but no discernible boundaries or repeating patterns in size distribution are visible in the surrounding small tubercles. Each tubercle on the wettable (anterior) part of the egg is surrounded by a low wall, suggestive of a single cell, but it is more likely that the cells here actually include several of these tubercles, but again with no clear boundaries between them. Whatever the organization of individual cells, stereomicroscopic observations of living eggs show that there are two types of outer chorionic surface on the egg: wettable over the anterior $\frac{1}{2}$ and in the embrasures, and non-wettable over the posterior $\frac{1}{2}$. Both these areas have characteristic tubercles, the wettable, scalelike tubercles of the anterior region having become extremely modified in the embrasures to form filaments with terminal hooks.

Waxy tubercles, which serve for egg flotation, have been described for another sabethine mosquito, *Wyeomyia vanduzeei* Dyar & Knab (Frank et al. 1981). Unfortunately, no useful comparison of the *Tr. digitatum* egg can be made with other species in the genus. Busck (1908) described the egg of *Tr. compressum* Lutz; he mentioned fringes of white hairs which apparently assist flotation. Mattingly (1974) described (as a still unnamed species) the egg of a species very closely related to *Tr. digitatum*, but the description and illustration offer only the sketchiest account of structural details, and there is no specific statement that this egg has embrasures. *Tr. espinii* Martini also is mentioned in this paper, but this species is now assigned to the genus *Runchomyia* (Zavortink 1979a).

The embrasures on the *Tr. digitatum* egg appear to be structures that evolved along with the egg-brooding habit as a means of enhancing cohesion of the egg raft. It may well be that, primitively, eggs were laid singly or, if deposited and held briefly in a group, tended to drift apart when the

female departed after laying eggs. However, in the container habitats favored by this species, brooding greatly enhances egg survival during rain (Lounibos & Machado-Allison 1986). Thus, it may be supposed that if some females interrupted by rain during laying did not immediately fly away (as might happen if laying were incomplete), survival of any eggs retained between their legs would be improved. This would be so even if additional rain fell before the eggs hatched, but especially so if there was none. With selection pressure favoring females that remained and protected even a few of their eggs for progressively longer periods, the fully developed brooding habit, with eggs guarded until hatching, would gradually become established. Only if the average lifetime reproductive potential of females were to be reduced by some factor associated with brooding (e.g., predation) would this progression not occur. SEM views (Fig. 3 and 4) provide no support for Mattingly's (1974) suggestion that a glue affixes eggs at embrasures. Instead, the interlocking of filaments and hooks physically connects eggs in a manner similar to the synthetic fastener known commercially as "Velcro" (Fig. 3f).

Given the equidistant spacing of embrasures around the eggs, one might expect to find rosettes formed of six eggs, as this arrangement would allow perfect contact between embrasures (Fig. 5c). In fact, pentagons were somewhat more common than hexagons (Fig. 5a), even though a polygon with five eggs provides less linkage at embrasures (Fig. 5b). It may be significant that a pentagonal polygon is more compact, wasting less "central space" (compare Fig. 5b and c). The codominance of six- and five-sided polygons may represent a trade-off between raft security (enhanced by hexagons) and compactness (increased by pentagons).

Given the selective advantage of brooding, then any structural change in the eggs that would allow the female to hold and brood larger batches or keep eggs together more securely would be advantageous and would selectively reinforce the brooding habit. The *Tr. digitatum* egg floats vertically in the water with the hydrophilic end immersed. Assuming that eggs initially lacked embrasures, then the weight of each egg would distort the surface downward around its entire circumference. Because like menisci attract (Hinton 1981), the eggs would have some tendency to remain together. Adhesion would be weak, however, because it would depend on the length of the attracting menisci, which are limited by egg curvature. The only means of increasing adhesion of menisci is to increase the interacting distances. Such an increase would occur if even small upward extensions of the wettable surface developed around the egg's circumference, producing increased lengths of meniscus but of curvature opposite to that of the intervening areas. Eggs would tend at first to adhere in these areas, which evidently were three in *Tr. digitatum*, and cohesion would improve as further modification

increased the lengths of the extensions (protoembrasures). At first, eggs may well have adhered and formed more coherent rafts by this means. Subsequently, any tendency of the embrasure tubercles to elongate would probably have been advantageous because of their tendency to become entangled or interlocked, leading eventually to development of filaments with terminal hooks. Further lengthening of the embrasures would ultimately bring larger areas of hooks and filaments into contact with each other.

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