THE EGGS OF *ANOPHELES ATROPOS* AND *ANOPHELES DARLINGI* (DIPTERA: CULICIDAE)

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ABSTRACT. The eggs of *Anopheles atropos* and *An. darlingi* are described by means of scanning electron micrographs. The egg of *An. atropos* is conventionally structured, with both ventral anterior and posterior lobed tubercles, a relatively large deck, and small floats. The plastron lattice of the dorsal and lateral surfaces is mostly of the more open type. The small *An. darlingi* egg possesses a prominent and distinctive anterior crown-like structure formed from a very much reduced but elevated frill. The floats are positioned more ventrally than laterally, are contiguous anteriorly and posteriorly and sometimes fused or nearly so in the ventral mid-line. The dorsal and lateral plastron is blister-like.

INTRODUCTION

Anopheles (Anopheles) atropos Dyar and Knab is a North American species restricted to coastal, brackish water habitats from New Jersey through Texas (Darsie and Ward 1981), with limited intrusion into the West Indies, including Cuba, Jamaica and the Cayman Islands (Belkin et al. 1970). Good descriptions of the larva, pupa and adults have been given (Carpenter and LaCasse 1955, Belkin et al. 1970), but no account has been published of the egg. In contrast, there are several early illustrated descriptions of the egg of *An. (Nyssorhynchus) darlingi* Root, undoubtedly because this species is an important malaria vector throughout its range from Argentina, Brazil, Peru, Colombia, Venezuela, Guianas, Honduras and Belize to Mexico (Knight and Stone 1977). Illustration of the structure has, however, been restricted to small drawings (Root 1926, Rozeboom 1942, Causey et al. 1944, Cova Garcia 1946), which represent the limitations of light microscopy. Descriptions provided here are based on the much greater detail and fidelity attainable with the scanning electron microscope.

MATERIALS AND METHODS

Eggs of three individual females of each species were used. The *An. atropos* females were collected with CDC traps in the vicinity of this laboratory; *An. darlingi* females were taken at human bait in Puerto Ayacucho, Amazonas, Venezuela. Embryonation was allowed to proceed to completion, then individual eggs were lifted from the oviposition paper with a very fine artist's brush and placed in required positions on sticky tape attached to stubs. Mounted eggs were dried completely over calcium chloride (20 min), then were gold-coated and examined immediately in a Hitachi S-510 scanning electron microscope. Based on limited experience with anopheline eggs, they do not resist desiccation well, but eggs of these particular species retained their natural form under vacuum for 1–2 h, long enough to yield good micrographs. Lengths and widths of living eggs were measured with a stereomicroscope and ocular micrometer. Perimeters and areas of tubercles on the anterior deck were measured from enlarged micrographs using a digitizing tablet and Sigmascan software (Jandel Scientific, Corte Madera, CA). Equal numbers of measurements were made from eggs of each of the three individuals of each species to yield means (±SE) cited in the text. Descriptive terminology follows Harbach and Knight (1980).

DESCRIPTIONS

Anopheles atropos (Figs. 1–4)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Boat-shaped in ventral (Fig. 1a)

Table 1. Dimensions of eggs of *An. atropos* (n = 15) and *An. darlingi* (n = 12).

Anopheles species	Length (μm)		Width (μm)[1]		L/W Ratio	
	$\bar{x} \pm SE$	Range	$\bar{x} \pm SE$	Range	$\bar{x} \pm SE$	Range
atropos	518.8 ± 2.8	502.5–530.3	180.3 ± 0.9	174.2–186.9	2.86 ± 0.02	2.74–3.00
darlingi	372.9 ± 2.4	360.4–385.5	132.6 ± 2.8	118.0–144.4	2.82 ± 0.04	2.58–2.82

[1] Including float.

Fig. 1. *Anopheles atropos*. (a) Entire egg, ventral (upper) view, anterior end at top; (b) entire egg, lateral view, ventral surface at left, anterior end at top. Scale = 100 μm.

Fig. 2. *Anopheles atropos*. (a) Outer chorionic cells, lateral (frill at top) and dorsal surfaces, anterior end; (b) outer chorionic cell detail, lateral anterior surface, plastron meshwork of more closed type; (c) outer chorionic cell detail, dorsal surface, middle of egg, plastron meshwork of more open type; (d) inner edge of frill, anterior deck; (e) meshwork on ventral (upper) surface, middle of egg (adjacent to floats); (f) lateral surface, ventral margin of float. Scale = 20 μm (a,e,f), = 10 μm (b,c), = 5 μm (d).

or dorsal view, anterior end fairly blunt, posterior more tapered (Fig. 1a). Ventral surface more or less flat, or only slightly concave, particularly at posterior end, dorsal surface curved (Fig. 1b).

Dorsal (lower) and lateral surfaces: Uniformly covered with hexagonal or pentagonal outer chorionic cells (Figs. 1b,2a), each longer than wide, long dimension oriented in long axis of egg (Fig. 1b). These cells surround float

and in this region extend more onto ventral (upper) surface (Fig. 1b). Interior of each cell formed of a perforated meshwork supported on short columns (the plastron, Hinton 1968), surrounded by an elevated, palisade-like outer chorionic reticulum (Fig. 2b). Detailed structure somewhat variable; cells at anterior end particularly with plastron tending to be more closed, perforations round, palisade continuous, more elevated (Fig. 2a,b), with alternately slightly swollen and narrower portions, the latter occasionally perforated (Fig. 2b). Plastron in middle of dorsal surface and on posterior surface often more open, perforations larger, irregular, palisade formed by reticulum not as elevated, discontinuous (Fig. 2c). Plastron in 2 strips ventral to float also of open type, reticulum even less clearly defined (Fig. 2e). Floats fairly small, about half length of egg, number of ribs 13–20 (mean 15.5 ± 0.5, n = 30). Ribs tending not to extend to dorsal (lower) float margin, which is irregularly striated (Fig. 2f).

Ventral (upper) surface: Deck continuous, symmetrically narrowed at middle of egg, adjacent to floats (Fig. 1a), degree of narrowing variable. Frill continuous, outer edge supported by fairly thick, occasionally branched columns (Fig. 2a), inner edge with ridged columns (Fig. 2d). Boundaries of outer chorionic cells very indistinct, barely visible at low magnification (Fig. 1a). Outer chorionic tubercles uniform over whole ventral surface (Fig. 1a), similar in form on anterior, middle and posterior regions of deck (Fig. 3a,b,c, respectively), but somewhat smaller in middle region, as measured by perimeter and area (Table 2). Each tubercle irregular in outline, walls with fairly deep vertical ridges and clefts, tops of smaller tubercles usually domed, smooth, larger ones often with one to several conspicuous cavities (Fig. 3d).

Anterior end, micropyle: Tubercles surrounding large, lobed tubercles at anterior end (ventral surface) larger than those on remainder of anterior deck, forming distinct anterior patch (Fig. 4a,f). Lobed tubercles 8–12 in number (mean 9.3 ± 0.3, n = 15), usually oval, occasionally almost round (Fig. 4a), walls covered with tiny ridges (Fig. 4f). Number of lobes per tubercle 5–10 (mean 7.3 ±

Fig. 3. *Anopheles atropos*. (a) Outer chorionic tubercles, middle of anterior deck; (b) tubercles, middle deck; (c) tubercles, posterior deck; (d) detail, tubercles on anterior deck. Scale = 5 μm.

Fig. 4. *Anopheles atropos*. (a) Extreme anterior end, ventral (upper) surface, showing lobed tubercles; (b) anterior end, end-on view, with micropylar apparatus; (c) detail, micropylar apparatus; (d) extreme posterior end, ventral (upper) surface, showing lobed tubercles; (e) posterior end, end-on view; (f) detail, lobed and surrounding tubercles, posterior end. Scale = 20 μm (a,b,d,e), = 10 μm (c,f).

0.1, n = 87), each finger-like, often expanded at end. Micropylar collar hexagonal in outline (Fig. 4b), surface smooth, inner edge deeply and uniformly excavated, peaks between excavations tapering to form radial ridges extending about half way across micropylar disk, dividing disk into sectors (Fig. 4b). Number of sectors (also ridges) 7–9 (mean 7.7 ± 0.2, n = 15). Micropylar orifice 1.4 μm in diameter, surrounded by low mound (Fig. 4c).

Posterior end: Lobed tubercles again surrounded by patch of tubercles that are dis-

Table 2. Perimeters and areas of tubercles on the anterior, middle and posterior deck regions of eggs of *An. atropos* and *An. darlingi* (n = 30). Means followed by the same letter do not differ significantly.

	An. atropos		*An. darlingi*	
Deck region[a]	Perimeter (μm)	Area (μm^2)	Perimeter (μm)	Area (μm^2)
Anterior	5.91 ± 0.19a	1.60 ± 0.07a	6.72 ± 0.28a	2.20 ± 0.10a
Middle	4.78 ± 0.18b	1.12 ± 0.08b	6.74 ± 0.20a	1.93 ± 0.08a
Posterior	5.91 ± 0.26a	1.44 ± 0.08a	7.20 ± 0.25a	2.10 ± 0.11a

[a] Refers in *An. darlingi* to deck region surrounded by float. True anterior deck is isolated within anterior crown (Fig. 1a).

tinctly larger than those on remainder of deck (Fig. 4d). Lobed tubercles similar in form to anterior ones (Fig. 4d,e), 8–11 in number (mean 9.1 ± 0.2, n = 15), this number not significantly different from anterior end, number of lobes 5–10 (mean 7.3 ± 0.1, n = 87), again not different from anterior end.

Anopheles darlingi (Figs. 5–8)

Size: As in Table 1. *Color*: Black. *Overall appearance*: Boat-shaped in ventral (Fig. 5a) or dorsal view, anterior end more rounded and with prominent crown-like structure, posterior end more tapered (Fig. 5a). Ventral surface more or less flat, dorsal surface curved (Fig. 5b).

Dorsal (lower) and lateral surfaces: Surfaces covered with blister-like outer chorionic cells (Figs. 5b;6d,e), but cell boundaries difficult to distinguish. Floats surrounded by these cells, which also extend over the ventral surface anteriorly and posteriorly (Fig. 5a,b). Cell interiors (plastron) covered with flat studs and with a central mound, perforated by 1–7 pores, 0.5–7.0 μm wide (Fig. 6e,f). Central mounds less apparent and pores fewer more laterally (Fig. 6d) and especially on anterior and posterior ventral surfaces (Fig. 8a,d). Plastron in areas immediately dorsal to floats with very few, small pores (Fig. 6c). Floats somewhat more than half length of egg (Figs. 5a,6a), often separated ventrally to expose narrow deck (Fig. 5a), but in some cases partly (Fig. 6a) or completely contiguous. Ribs 16–25 in number (mean 19.9 ± 0.5, n = 30), each rib almost reaching dorsal (lower) float margin (Fig. 6c).

Ventral (upper) surface: Deck in middle region of egg short, narrow (Fig. 5a), in some eggs partly or completely obliterated by floats. Frill absent except around anterior crown (see below). Chorionic cell boundaries not visible on deck. Tubercles irregular in outline, uniform over this middle deck region (surrounded by floats) in appearance (Fig.7a,b,c) and size (Table 2), upper surfaces rough and domed, sides undercut with wide grooves separated by crooked ridges (Fig. 7d).

Anterior end, micropyle: Anterior end with very prominent crown-like structure, formed of a much reduced but deep frill, outwardly flared at top (Figs. 5a,b;8a,b). Diameter (at top) 49–76 μm (mean 62.7 ± 2.4 μm, n = 15). Outer walls of crown columnar, inner walls deeply grooved (Fig. 8a,b,c). Small deck area within crown with larger surface tubercles than present on middle deck (compare Fig. 8a and d), crown tubercles less domed, tops more narrowed into ridges, tiny surrounding tubercles smaller and more numerous than on middle deck (Fig. 6b). Plastron immediately surrounding micropyle usually more open (Fig. 8c). Micropylar collar irregular in outline, surface slightly rough, inner edge deeply and uniformly excavated (Fig. 8f), fairly short radial ridges forming 6–8 sectors (mean 7.3 ± 0.2, n = 21). A faint continuous ring visible within sectors in some eggs (Fig. 8f). Micropyle surrounded by a low mound (Fig. 8f), orifice diameter 1.5 μm.

Posterior end: More pointed than anterior

Fig. 5. *Anopheles darlingi*. (a) Entire egg, ventral (upper) view, anterior end at top; (b) entire egg, lateral view, ventral surface at right, anterior end at top. Scale = 100 μm.

end (Fig. 5a), completely covered with plastron type outer chorion (Fig. 8d,e), blister-like mounds small and pores few in number, especially on ventral surface (Fig. 8d,e).

DISCUSSION

Within the admittedly limited amount of material studied, the structure of the *An. atro-*

Fig. 6. *Anopheles darlingi*. (a) Entire egg, ventral (top) view, showing contiguous floats almost obscuring deck; (b) detail, tubercles within anterior "crown"; (c) lateral surface, dorsal margin of float; d) outer chorionic cells, lateral and dorsal surfaces, anterior end; (e) chorionic cells, middle of dorsal surface; f) chorionic cell detail, middle of dorsal surface. Scale = 100 μm (a), = 20 μm (d,e), = 10 μm (b,c,f).

pos egg was quite consistent. There was some variation in the degree of narrowing of the central region of the deck. Some eggs were only slightly narrowed, others were constricted to as little as half the gap depicted in Fig. 1a. The proportion of open pore area in

the plastron of the dorsal chorionic cells also varied, particularly in the mid-dorsal region. Cells in the anterior and anterior lateral areas were almost invariably of the more closed type (Fig. 2b), but while mid-dorsal cells were consistently more open, they varied from somewhat less so than Fig. 2c to appreciably more so. Given these variations, the egg of *An. atropos* was considered conventional in form overall, with no extreme or distinctive modifications. Detailed structural comparisons with other North American species must await further work as no scanning electron microscopic studies of their eggs exist.

The ultrastructural view of the *An. darlingi* egg conforms with but adds considerable detail to light microscopic descriptions already available (Cova Garcia 1946, Causey et al. 1944). The distinctive feature of this egg is the prominent anterior crown, a much reduced but deep frill surrounding a very small, isolated anterior deck supporting larger tubercles than are found on the middle deck region. Among eggs of the three females examined, the crown was quite consistent in structure and diameter, but it may be smaller and anteriorly tapered (Root 1926, Causey et al. 1944). Among South American *Nyssorhynchus*, only *An. rangeli* Gabaldon, Cova Garcia and Lopez apparently is similar, the anterior crown usually being isolated from the floats and having very high walls (see Causey et al. 1944). In the *An. rangeli* egg, however, the floats extend to the posterior end and appear to have substantially more ridges.

Fig. 7. *Anopheles darlingi*. (a) Outer chorionic tubercles, middle of anterior deck; (b) tubercles, middle deck; (c) tubercles, posterior deck; (d) detail, tubercles on anterior deck. Scale = 5 μm.

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Fig. 8. *Anopheles darlingi*. (a) Extreme anterior end, ventral (upper) surface; (b) anterior end, end-on view; (c) anterior end, dorsal view, showing micropyle; d) extreme posterior end, ventral (upper) surface; e) posterior end, end-on view; (f) detail, micropylar apparatus. Scale = 20 μm (a,b,c,d,e), = 10 μm (f).

REFERENCES CITED

Belkin, J.N., S.J. Heinemann and W.A. Page. 1970. Mosquito studies (Diptera, Culicidae) XXI. The Culicidae of Jamaica. Contrib. Am. Entomol. Inst. (Ann Arbor) 6(1):1–458.

Carpenter, S.J. and W.J. LaCasse. 1955. Mosquitoes of North America (north of Mexico). University of California Press, Berkeley.

Causey, O.R., L.M. Deane and M.P. Deane. 1944. An illustrated key to the eggs of thirty species of Brazilian anophelines, with several new descriptions. Am. J. Hyg. 39:1–7.

Cova Garcia, P. 1946. Notas sobre los anofelinos de Venezuela y su identificacion. Caud. Amar. Conf. Sanit. Panam. 12:1–208.

Darsie, R.F. and R.A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Mosq. Syst. Suppl. 1:1–313.

Harbach, R.E. and K.L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Plexus Publishing, Inc., Marlton, New Jersey.

Hinton, H.E. 1968. Observations on the biology and taxonomy of the eggs of *Anopheles* mosquitoes. Bull. Entomol. Res. 57:495–508.

Knight, K.L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae). Thomas Say Found. 6:xi–611.

Root, F.M. 1926. Studies on Brazilian mosquitoes. I. The anophelines of the *Nyssorhynchus* group. Am. J. Hyg. 6:684–717.

Rozeboom, L.E. 1942. Subspecific variations among Neotropical *Anopheles* mosquitoes, and their importance in the transmission of malaria. Am. J. Trop. Med. 22:235–255.