SCANNING ELECTRON MICROSCOPIC OBSERVATIONS AND DIFFERENTIATION OF EGGS OF THE *ANOPHELES DIRUS* COMPLEX

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ABSTRACT. Microscopic observations have revealed differences among the eggs of species A, B, C and D of the *Anopheles dirus* complex. The eggs of species A and C are similar in size and shape. They are intermediate in size between the eggs of species B, which is the largest, and that of species D, which is the smallest. The pattern of outer chorionic cells between the frill and the float is species specific. The pattern consists of rows of irregularly shaped cells in species D and different numbers of rows of regularly shaped cells in species A, B and C. Scanning electron microscopy revealed that the deck tubercles are arranged in aggregates which are more widely spaced in species A than in species B. The aggregates are large in species C, of moderate size in species A and B, and small in species D. The egg characters may be useful in separating species A, B, C and D of the *An. dirus* complex.

INTRODUCTION

Recent study on population cytogenetics of *Anopheles dirus* Peyton and Harrison, an important malaria vector in Thailand, has revealed that it is a species complex consisting of at least 5 isomorphic species (Baimai 1988). These species show different geographic distributions and biting cycles (Baimai et al. 1988). Members of the *An. dirus* complex are morphologically distinguishable in at least one of the post-egg stages, adult, pupa or fourth-instar larva (Peyton and Ramalingam 1988; E. L. Peyton, unpublished data). This report presents detailed microscopic observations on the surface structure of the eggs of 4 species within the complex.

MATERIALS AND METHODS

Members of the *An. dirus* complex were established from isofemale lines and maintained in laboratory colonies at 26°C and 80% RH by artificial mating (Ow Yang et al. 1963). The 4 isoline families investigated include species A (KS014 from Khon San, Chaiyaphum, July 1986), B (PT59 from Lojunka, Phatthalung, February 1985), C (KA70 from Ban Kang Rieng, Kanchanaburi, May 1987) and D (TY058 from Ku Raeh, Thung Yai, Nakhon Si Thammarat, November 1986) (Baimai et al. 1988). Three to 4 artificially inseminated females of each species were allowed to oviposit on moist filter paper in a plastic bowl. The eggs were collected using small pieces of filter paper and were placed on a glass slide for examination under a dissecting microscope. Egg length was measured from the anterior to the posterior end and width from the outer edge of the frill to the opposite convex side at the middle.

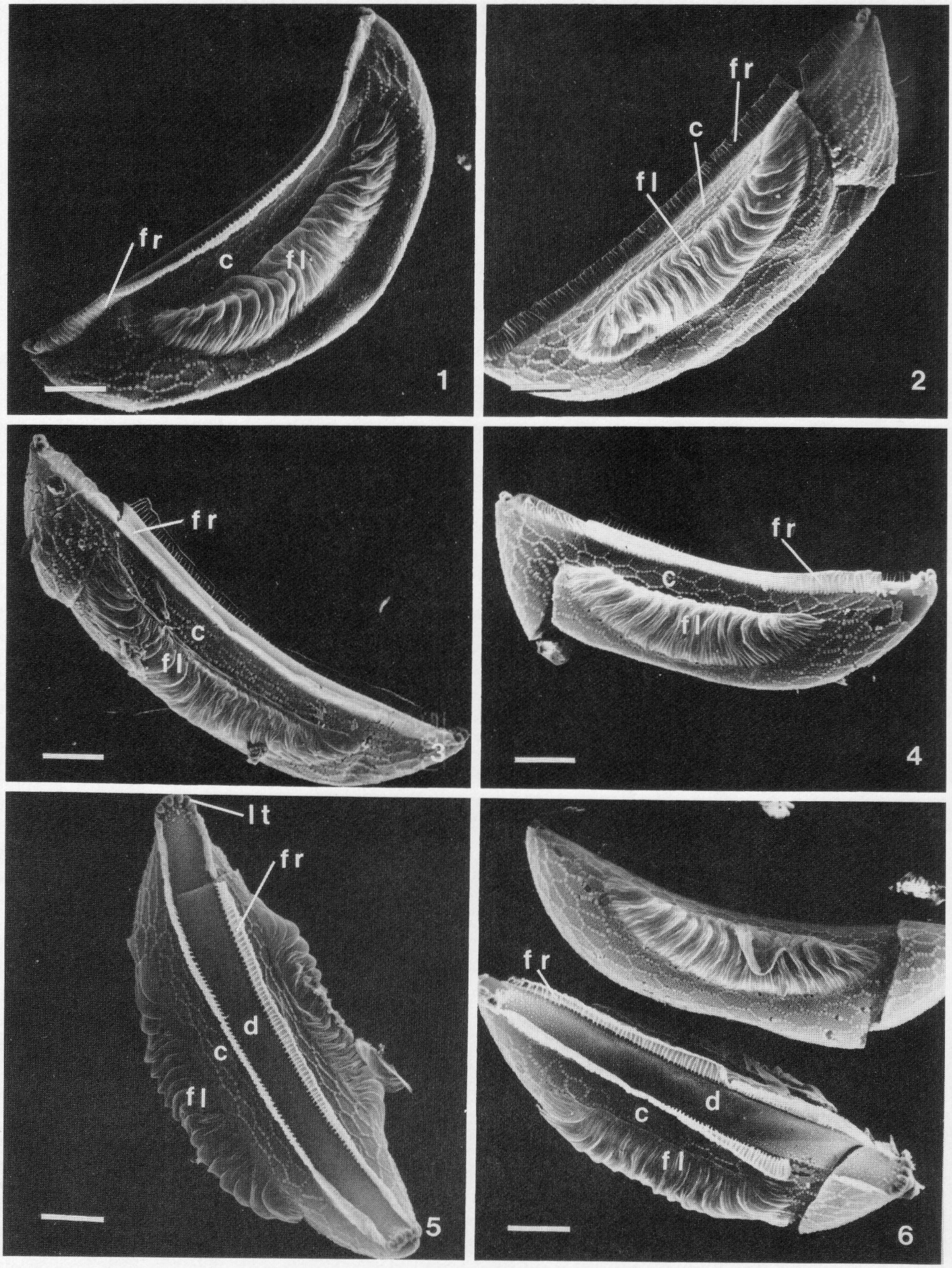
For scanning electron microscope preparations, the eggs were air-dried and mounted on aluminum stubs with double-stick tape. The specimens were then coated with gold in a sputter-coating apparatus (Polaron E5000) and examined in a Hitachi S430 scanning electron microscope.

RESULTS

The eggs of the 4 species are boat-shaped in general appearance. Viewed laterally, the contour of the entire egg is straight or slightly concave on the morphologically ventral surface (lower surface) and convex on the dorsal surface (upper surface). At the middle region, each of the lateral sides is fringed by a float, a folded longitudinal extension of the outer chorion. The ventral rim is bordered by a frill, a ridge of thin, folded outer chorion (Fig. 1–6). The deck, the area of outer chorion on the ventral surface enclosed by outer chorion on the ventral surface enclosed by the frill, bears a row of lobed tubercles at the anterior and posterior ends of the egg (Figs. 5 and 6). A crack is often noted at the anterior region (Figs. 1–6), which represents the exit for the hatching larva.

The size and shape of the eggs of these species are slightly different (Table 1). The eggs of species B and D viewed laterally appear to be considerably broader than that of species A and C. The egg of species A is moderately broad, whereas that of species C is relatively slender because of its longer length and relatively tapered ends (Figs. 1 and 3).

The float of *An. dirus* A is of moderate size (0.326 ± 0.023 × 0.073 ± 0.004 mm), consisting of 14–16 distinct ridges (Fig. 1, Table 1). The float of species B is larger than that of the other species (0.352 ± 0.020 × 0.081 ± 0.010 mm), exhibiting 15–19 well-defined ridges (Fig. 2, Table 1). A long float with pointed ends (0.330 ± 0.020 × 0.101 ± 0.011 mm) has been observed in species C (Fig. 3, Table 1). The float ridges of species C are not as sharply defined as in species A or B (Fig. 3). The float of species D is relatively small (0.307 ± 0.036 × 0.080 ± 0.007



Figs. 1–6. Eggs of the *An. dirus* complex: Figs. 1–4, lateral aspect of eggs of species A, B, C and D, respectively; Figs. 5 and 6, ventral aspect of eggs of species B and D, respectively. lt = lobed tubercle; fr = frill, d = deck; fl = float, c = cell, bar = 50 μm.

Table 1. Structure and dimensions of eggs of 4 members of the *Anopheles dirus* complex.

Species	Relative size	Entire egg		Cells		Floats	
		Shape	Length × width (mm)	No. of rows	Shape	Length × width (mm)	No. of ridges
A	Moderate	Long and moderately broad	0.524 ± 0.023 × 0.124 ± 0.015	2.5–3.5	Regular	0.326 ± 0.023 × 0.073 ± 0.004	14–16
B	Large	Long and broad	0.570 ± 0.003 × 0.146 ± 0.012	3.5–4.5	Regular	0.352 ± 0.020 × 0.081 ± 0.010	15–19
C	Moderate	Long with tapered ends	0.548 ± 0.016 × 0.143 ± 0.014	2.0–3.0	Regular	0.330 ± 0.020 × 0.101 ± 0.011	14–18
D	Small	Short and broad	0.515 ± 0.014 × 0.144 × 0.017	2.5–4.0	Irregular	0.307 ± 0.036 × 0.080 ± 0.007	15–18

mm) with moderately defined ridges (Fig. 4, Table 1).

The outer chorion of the dorsal and lateral surfaces of the eggs is sculptured in a polygonal pattern of cells. The cells are formed by rows of tubercles enclosing many smaller, less prominent tubercles (Figs. 7–10). Some cells have a more or less regular shape with 2 parallel sides of equal length (Fig. 9), while other cells are irregular in shape with unparallel sides of unequal length (Fig. 10). It is important to note that the pattern of cells in the area between the frill and the float is not the same in the 4 sibling species. The pattern in species D consists mostly of cells of irregular shape, while in the other species it consists of cells of regular shape (Fig. 8, Table 1). The space between the frill and the float also varies among the species. The space is widest in eggs of species B, comprised of 3.5–4.5 rows of long and narrow cells (Fig. 2). The egg of species C has the narrowest space with 2–3 rows of slightly long and narrow cells. The egg of species A has 2.5–3.5 rows of moderately broad cells, while the egg of species D has 2.5–4.0 rows of broad cells of irregular shape (cf. Figs. 1 and 4).

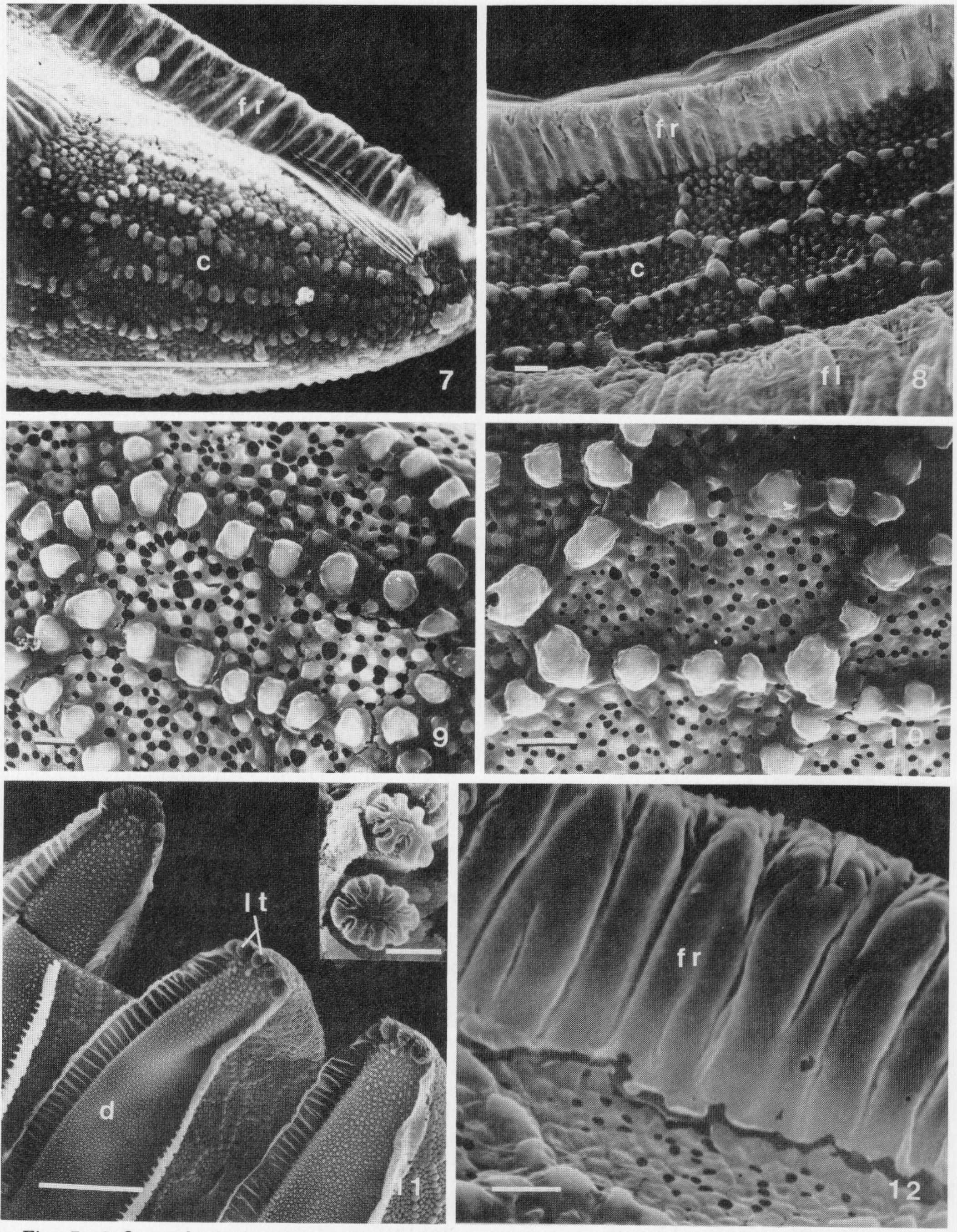
A row of 4–5 lobed tubercles occurs at each end of the deck in all of the species studied. The lobed tubercles are large deck tubercles with notched margins giving rise to 8 or 9 lateral lobes (Fig. 11).

The structure of the frill does not differ among the eggs of the species studied. The outer surface of the frill is smooth with parallel grooves along the entire length (Figs. 12 and 13). In contrast, the inner surface is minutely sculptured and marked by picketlike ridges with wrinkled margins. The outer edge of the inner surface is extensively wrinkled (Fig. 14).

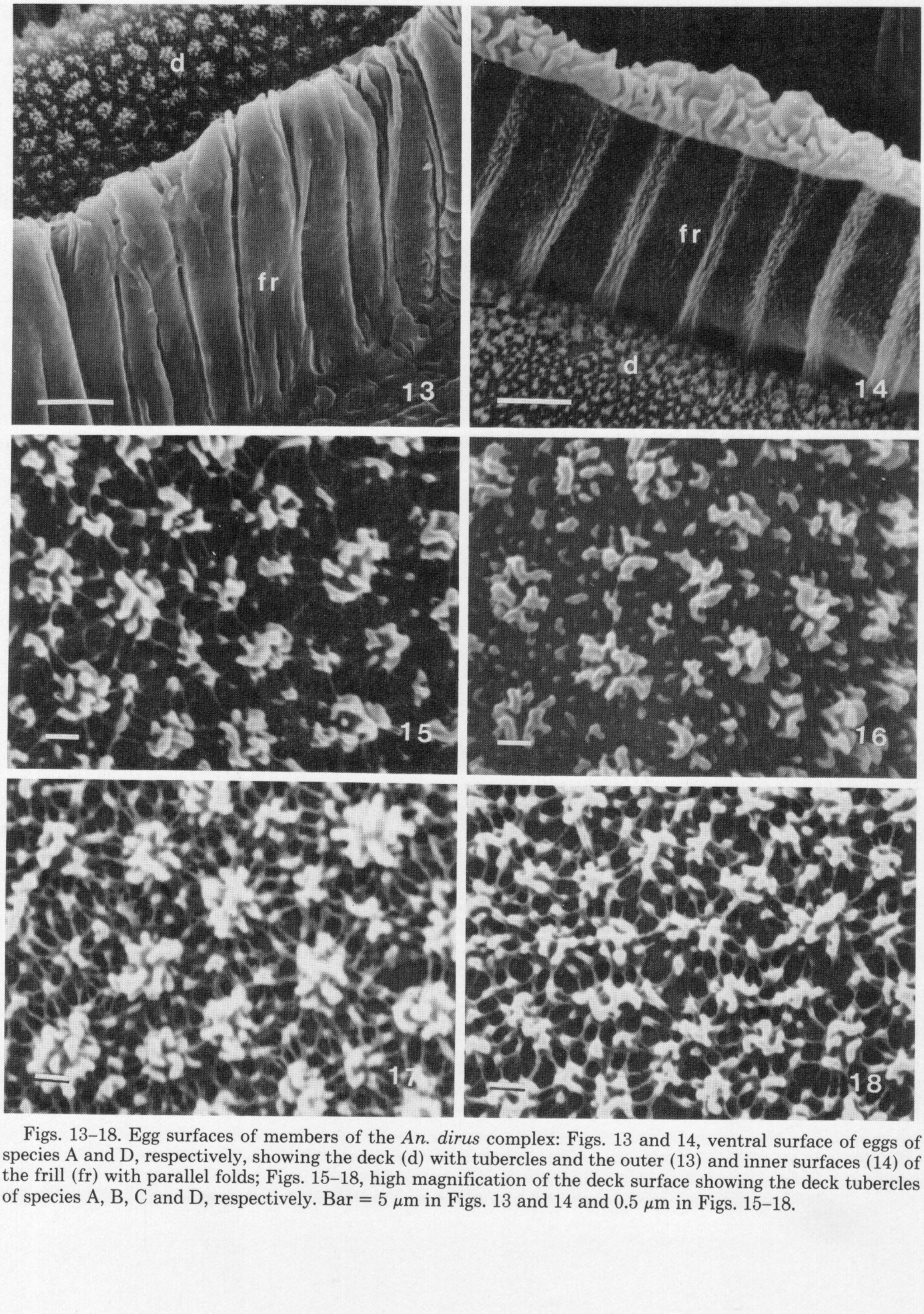
The outer chorion of the deck surface is comprised of an interconnected system of fine tubercles. These tubercles form aggregates which are species specific. The aggregates observed in species A and B are formed by relatively large tubercles. Species A has moderately large aggregates which are widely spaced, while species B has aggregates of similar size that are more closely spaced (cf. Figs. 15 and 16). The pattern of aggregates in species C and D are formed by small tubercles. Species C has large aggregates which are widely spaced, while species D is characterized by small aggregates (cf. Figs. 17 and 18).

DISCUSSION

The general features of eggs of the *An. dirus* complex appear rather similar under the dissecting microscope. However, differences in size and shape and the fine structure of some specific



Figs. 7–12. Outer chorionic patterns of eggs of the *An. dirus* complex: Figs. 7 and 8, posterior end of egg of species A and middle portion of egg of species D, respectively, showing the frill (fr), cell pattern (c) and float (fl); Figs. 9 and 10, the regular and irregular shapes of cells of eggs of species A and C, respectively; Fig. 11, deck (d) of egg of species C with high magnification (inset) of the lobed tubercles (lt); Fig. 12, lateral aspect showing smooth outer surface of frill with parallel grooves. Bar = 50 μm in Figs. 7 and 11, and 5 μm in Figs. 8–10, and 12.



Figs. 13–18. Egg surfaces of members of the *An. dirus* complex: Figs. 13 and 14, ventral surface of eggs of species A and D, respectively, showing the deck (d) with tubercles and the outer (13) and inner surfaces (14) of the frill (fr) with parallel folds; Figs. 15–18, high magnification of the deck surface showing the deck tubercles of species A, B, C and D, respectively. Bar = 5 μm in Figs. 13 and 14 and 0.5 μm in Figs. 15–18.

details of the outer chorion, e.g., cells, floats and aggregatess of deck tubercles, can be observed. These characters may be useful in differentiating species A, B, C and D. The data indicate that the egg of *An. dirus* B is the largest, while that of species D is the smallest. The eggs of species A and C resemble one another in their moderate size and relatively slender shape. The egg of species C is slightly more slender than the egg of species A because of the length and tha tapered ends.

It may be noted that these closely related species exhibit intraspecific variation in size and shape of the eggs. Thus, these parameters are not completely reliable for separating the species. However, the space and the pattern of cells between the frill and the float appear to be species specific. Species B shows the largest space with 3.5–4.5 rows of long, narrow, regularly shaped cells. In comparison, species A shows a moderate space with 2.5–3.5 rows of fairly broad, regularly shaped cells, while species C has the least space with 2–3 rows of long, narrow, regularly-shaped cells. The egg of species D is unique for this particular character in having 2.5–4.0 rows of broad cells of irregular shape.

The character of the float can also be used in differentiating the eggs of the different species. The float of species B is slightly larger than that of species A, although this structure has well-defined ridges in both species. The egg of species C bears floats with markedly pointed ends. The float of species D is shorter than those of the other species.

No significant differences in the form of the frill and the lobed tubercles were observed. However, minor differences in the sculpturing of the deck were observed in the scanning electron microscope.

Careful examination of various egg structures may be useful in distinguishing members of the *An. dirus* complex. The present data clearly indicate that differences exist between the eggs of species B and D, while species A and C are generally similar in egg morphology. These observations seem to support the cytogenetic data of Baimai et al. (1987), which suggest that *An. dirus* A and C are more closely related than species B and D.

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