The Scanning Electron Microscopy of Mosquitoes (Diptera : Culicidae)

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The scanning electron microscope (hereafter SEM) has high resolving power and an advantage of a great depth of focus, so that with it, it becomes possible to obtain a fine three-dimension micrograph. Because of this benefit, the SEM has recently been utilized in a study of fine surface structure, from which taxonomy and physiology, of insects and mites. In the case of medically important mosquitoes, HINTON (1967 a, 1967 b) and HINTON and SERVICE (1969) describing the structure and function of the egg surfaces of *Anopheles*, *Culex* and *Aedes* in a study using the SEM, remarked that previous photographs taken with the light microscope at best give only a very poor impression of the structure and the shape of the tubercles and the ridges, and more often than not, such photographs are misleading. SASA et al. (1971) described the SEM micrographs of eggs, larvae and adults of some species of mosquitoes with special emphasis on showing structures which it was difficult or impossible to demonstrate by conventional equipment.

The present authors, using the SEM of the JSM-S1 type of Nihon Denshi Co. in Japan, are carrying out detailed observation of the surface structures of eggs, larvae, pupae and adults of mosquitoes. In this paper, the egg surface structures of five species of *Aedes* and one species of *Armigeres* as seen with the SEM are described and illustrated. A key is presented for the eggs of these species : *Aedes albopictus*, *Aedes japonicus*, *Aedes togoi*, *Aedes vexans nipponii* and *Armigeres subalbatus*, common in Japan, and *Aedes aegypti* common in tropical regions.

Materials and Methods

The specimens examined in this study were, (1) eggs produced in our insectarium by wild females collected from fields, (2) eggs obtained from laboratory colonies maintained in our insectarium, (3) egg shells of wild eggs collected with soil from fields, from which larvae hatched out in our insectarium and (4) eggs obtained from females developed in our insectarium from wild larvae collected from fields and mated by the forced copulation method. In our insectarium, eggs were deposited on moist filter paper and kept in that moist condition for two or three days in order to cause the embryo to develop. After that

the eggs were dried naturally on the filter paper under laboratory conditions. In the specimens of the egg shells, after larvae had hatched out from eggs reared individually, the egg shells were removed from the water to dry filter paper with a dissecting needle and were dried naturally. The dried eggs and egg shells, of which the natural contour had not been deformed because of loss of water, were examined with the SEM. The undeformed eggs and egg shells were removed from the filter paper with a dissecting needle and mounted on the specimen holders (bronze stubs, 10mm in diameter and 5mm in height) coated with silver paste to fix the specimen on the metal surface. Or, the eggs and egg shells were fixed on celophane adhesive tape (adhesive on both sides) fastened to the metal surface. Sometimes the eggs and egg shells with the filter paper were fixed on the metal surface with silver paste. The eggs and egg shells on the holder were coated first with carbon, and then with gold in a vacuum evaporater to prevent charging of the specimen. The surface structure of the specimen thus coated was viewed with the SEM, first at a low magnification of 30 times, to locate the specimen, and later at higher magnifications of 100 to 10,000 times, and from various angles.

Egg descriptions

The egg surfaces of the six species examined in this study are divided into polygonal fields delimited by a ridged network. Within the polygons there are various types of tubercles. The shapes and dimensions of these tubercles and the pattern of reticulation of the ridges differ enormously in the six species.

Aedes (*Stegomyia*) *albopictus* (SKUSE, 1894)

(Japanese name : Hitosuji-shimaka)

Figs. 1~4.

The ridges of this species form a roughly transversely elongated lozenge or an irregularly pentagonal and hexagonal reticulation. On one part of the egg surface, the lozenged pattern of reticulation can be seen close to the polygonal pattern. On the other part, only the lozenged pattern or only the polygonal one is seen. Two types of tubercles are found within each section of the ridge network. One of them is a larger and somewhat half-globular tubercle. One or sometimes two tubercles are located at the center of each section of the ridge network. The surface is seen at a magnification of more than 1,000 times. The other type of tubercle is smaller and a number of these which vary in dimension are found in rows on both sides of the ridge. The pattern of the tubercles and the ridge network are similar to those of *Aedes aegypti* which will be described later in this paper.

On a part of the egg surface where the outer layer is flayed, the inner layer can be seen directly and the ridge of the inner layer forms the same pattern of network as seen on the outer layer. But the tubercles mentioned above are not found.

In the egg shells of wild eggs from which larvae were hatched out in the insectarium, the peculiar apparatus mentioned above are occasionally not seen, because the outer layer of the egg shell is flayed or dust covers the egg surface (**Fig. 4**).

Sources of eggs: 1. The eggs were obtained from a laboratory colony maintained at

our insectarium. 2. The wild eggs were collected with soil from Kyoto City. The egg shells were obtained from the wild eggs, from which larvae of this species hatched out at our insectarium.

Aedes (*Stegomyia*) *aegypti* (LINNAEUS, 1762)

(Japanese name: Nettai-shimaka)

Figs. 5~7.

The ridges of this species form a roughly pentagonal and hexagonal shape or a transversely elongated lozenge. Where the outer layer is flayed, the ridges of the inner layer, which form the same pattern of network seen on the outer layer, can be found. One larger tubercle, roughly half-globular, can be seen at the center of each section of the ridge network. Sometimes, there are two tubercles within one section. The surface is rough when seen at a magnification of more than 1,000 times. Some smaller tubercles are found on each side of the ridge and frequently connect with the larger tubercle. But there are no tubercles on the inner layer.

The egg surface structure is similar to that of *Aedes albopictus* and it is difficult to distinguish it from *Aedes albopictus*.

The egg surface structure described above is identical with the SEM micrographs of the species, strain not noted, described by HINTON & SERVICE (1969) and those, probably a Southeast Asian strain, by SASA et al. (1971).

Source of eggs: The eggs were obtained from a laboratory colony, Malaysian strain, maintained at our insectarium.

Aedes (*Finlaya*) *japonicus* (THEOBALD, 1901)

(Japanese name: Yamato-yabuka)

Figs. 8~10.

The ridges of this species form a roughly pentagonal or hexagonal shape when seen at a magnification of 1,000 times. Fine lines, close together, extend from the top of the ridges to the bases of the tubercles. There are some larger and some smaller tubercles within each section of the ridge network. The larger tubercles are like mountains and their tops are somewhat flat. The tops are various shapes, roughly circular to polygonal. The bases extend like a skirt. Conjunct tubercles can be seen frequently within the ridge network. The smaller tubercles are irregular in shape and frequently connect with larger or smaller tubercles. This structure of the tubercles can be seen at a magnification of 1,000 times.

Source of eggs: Adults developed from wild larvae collected from stone basins in Kyoto City. The eggs were obtained from the females mated with the males by the forced copulation method.

Aedes (*Finlaya*) *togoi* (THEOBALD, 1907)

(Japanese name: Togo-yabuka)

Figs. 11~12.

The conspicuous ridges of this species form a roughly pentagonal or hexagonal shape. So the network of the ridges can be seen at the magnification of 200 times. There are a number of somewhat globular tubercles within most sections of the ridge network, but

sections without tubercles are also seen. Some tubercles connect with each other and some tubercles connect with ridges. This structure of the tubercles can be seen at a magnification of 1,000 times.

The SEM micrographs of the egg surface structure described above are identical with those described by HINTON (1969). However, there are some differences in the shape of tubercles between the SEM micrographs described by the present authors and HINTON, and those described in an abstract by MORIYA et al. (1972).

Sources of eggs: 1. Adults developed, in our insectarium, from wild larvae collected from rock pools along the coast in Kyoto Prefecture. The eggs were obtained from the females mated by the forced copulation method. 2. The eggs were obtained from a laboratory colony maintained at the Osaka Prefectural Institute of Public Health.

Aedes (*Aedimorphus*) *vexans nipponii* (THEOBALD, 1907)

(Japanese name: Kin-iro-yabuka)

Figs. 13~15.

The ridges of this species form a distinct axially-polygonal shape when seen at a magnification of more than 500 times. There are three to seven tubercles in a row within each section of the ridge network. These are roughly hexahedron and the tops are somewhat uneven and more limited than the bases. Where the outer layer is flayed, the network of the ridges on the inner layer can be seen to have the same shape as those seen on the outer layer, but the tubercles can not be seen.

HORSFALL & CRAIG (1956), MYERS (1967) and KALPAGE & BRUST (1968) described the egg surface structure of this species as seen with reflected incandescent light through a stereoscopic dissecting microscope and a phase contrast microscope, respectively. However, the photographs taken with those microscopes gave only a poor impression of the ridge network although it is generally similar in shape to the present network as seen with the SEM.

Source of eggs: The eggs were obtained from the wild females collected from Osaka Prefecture.

Armigeres (*Armigeres*) *subalbatus* (COQUILLETT, 1898)

(Japanese name: Ookuro-yabuka)

Figs. 16~18.

The ridges of this species form a roughly quadrilateral to hexagonal shape when seen at a magnification of 1,000 times. The one tubercle within each section of the ridge network is like a mountain with one to four summits. From some parts of these tubercles, lines run toward the ridge.

The SEM micrographs of the egg surface structure mentioned above are similar to those reported in an abstract by MORIYA et al. (1972).

Source of eggs: The eggs were obtained from a laboratory colony maintained in our insectarium.

Key to eggs of one species of *Armigeres* and five species of *Aedes*.

1. Ridges form an axially-polygonal shape. ..... *Aedes vexans nipponii*

Ridges form a polygonal shape. ·····2.

2. There is one tubercle like a mountain with two to four summits within each section of the ridge network. ·····*Armigeres subalbatus*

   There are usually more than two tubercles within each section of the ridge network.···3.

3. Similarly shaped tubercles, somewhat globular, can be seen within each section of the ridge network.·····*Aedes togoi*

   Two types of tubercles, larger and smaller, can be seen within each section of the ridge network.·····4.

4. One, sometimes two, large half-globular tubercles can be seen at the center of each section of the ridge network.·····*Aedes albopictus*, *Aedes aegypti*

   Two to four large tubercles like mountains with a flat top can be seen within each section of the ridge network.·····*Aedes japonicus*

Summary

The egg surface structure of *Aedes albopictus*, *A. aegypti*, *A. japonicus*, *A. togoi*, *A. vexans nipponii* and *Armigeres subalbatus* were described and illustrated with the aid of the scanning electron microscope. The scanning electron micrographs of the six species can useful in distinguishing among these mosquitoes. A key is presented for the eggs of the six species.

Acknowledgement

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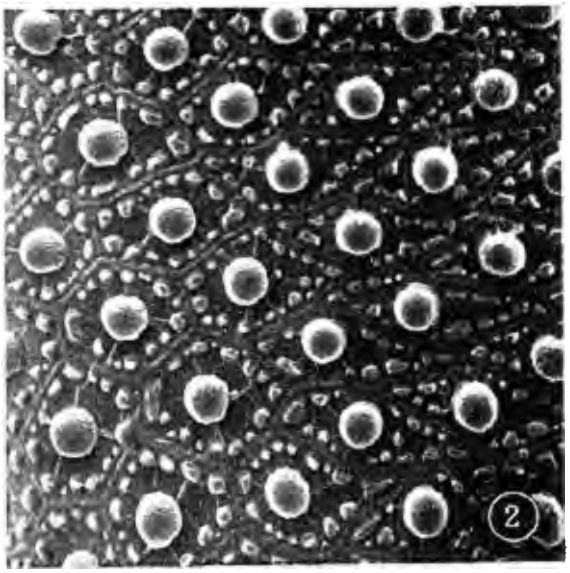
Explanation of scanning electron micrographs of mosquito eggs.

Fig. 1. *Aedes albopictus* : ×150
Fig. 2. *Aedes albopictus* : ×1,000
Fig. 3. *Aedes albopictus* : ×3,000
Fig. 4. *Aedes albopictus* : ×1,000, wild egg.
Fig. 5. *Aedes aegypti* : ×150
Fig. 6. *Aedes aegypti* : ×1,500
Fig. 7. *Aedes aegypti* : ×3,000
Fig. 8. *Aedes japonicus* : ×150, deformed egg.
Fig. 9. *Aedes japonicus* : ×1,000
Fig. 10. *Aedes japonicus* : ×2,500
Fig. 11. *Aedes togoi* : ×150
Fig. 12. *Aedes togoi* : ×1,000
Fig. 13. *Aedes vexans nipponii* : ×150
Fig. 14. *Aedes vexans nipponii* : ×1,000
Fig. 15. *Aedes vexans nipponii* : ×1,000, a part where the outer layer is flayed.
Fig. 16. *Armigeres subalbatus* : ×170, deformed egg.
Fig. 17. *Armigeres subalbatus* : ×1,000
Fig. 18. *Armigeres subalbatus* : ×3,000


1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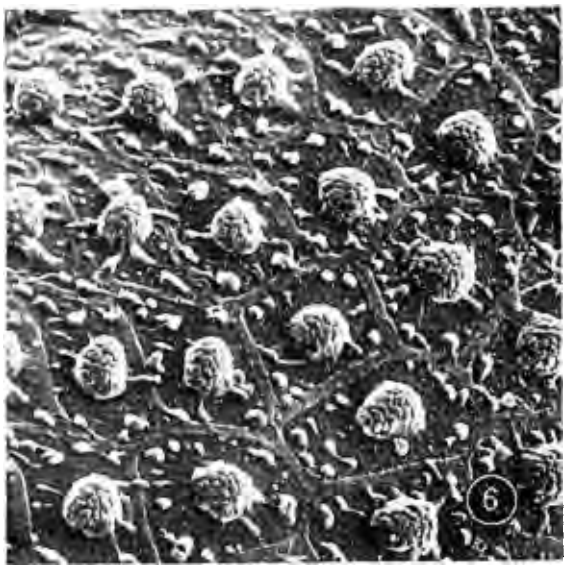
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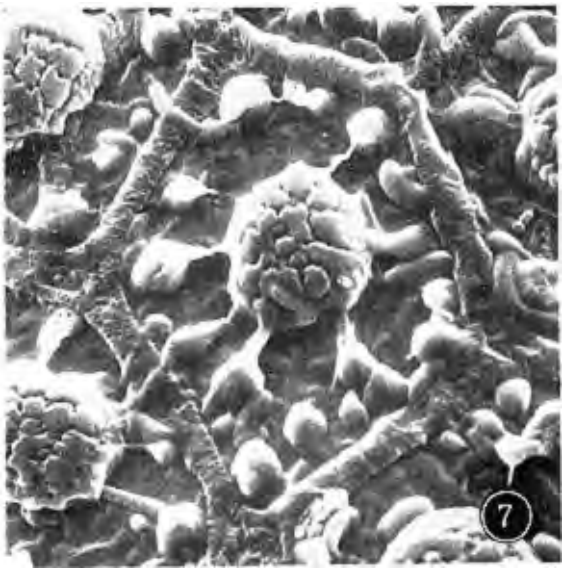
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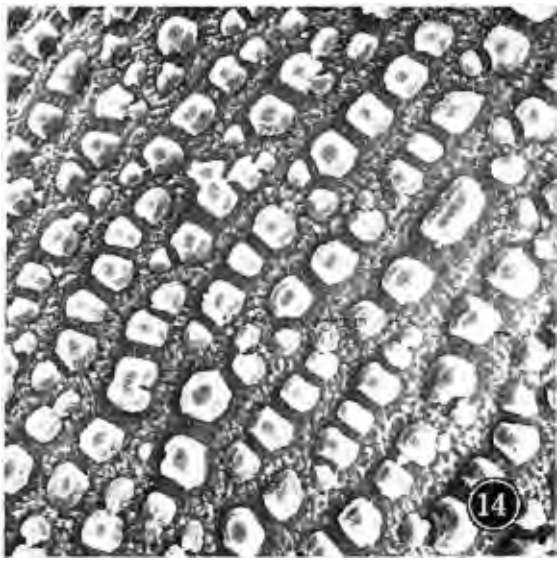
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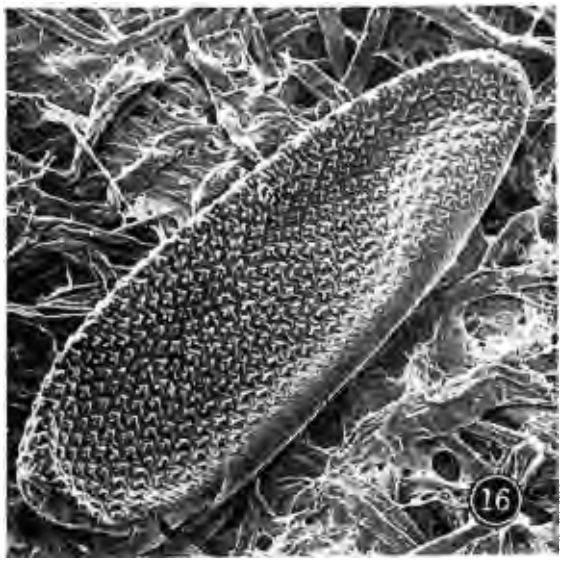
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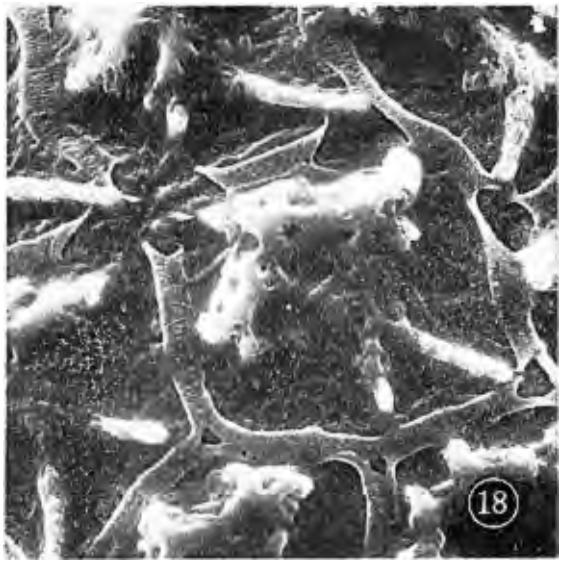
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