Scanning electron microscopic observations on egg surface structure of mosquitoes in Korea*

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一國文抄錄一

蚊卵表面像에 대한 走査電子顯微鏡的 觀察 所見

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韓國産 숲모가 및 집모기의 虫卵 表面를 走查電子顯微鏡으로 觀察하여 그 形態를 記述하였다. Outer chorionic reticulum tubercle 를 觀察할 수 있었으며, 種에 따라 그 크기와 分布狀이 特異하게 달랐다.

SEM 에 의한 虫卵 表面像의 觀察은 蚊의 種의 同定과 形態學의 研究에 有用함을 알았다.

Introduction

Recent investigations using scanning electron microscopy and transmission electron microscopy have revealed the ultrastructural feature of the insects and other arthropods of medical importance(Harbach and Knight, 1980; Kang and Jang, 1985a, b; Matsuo and Ochoa, 1979; Matsuo and Uemoto, 1975 and 1976; Matsuo et al., 1974). The morphology of the mosquitoes known as the most important vectors of the Japanese encephalitis, filariasis, and dengue fever, which were widely distributed in Korea, Japan, north and south China, and the islands of the Pacific Ocean, have been studied by many investigators(Hinton and Service, 1969; Sasa et al., 1971; Matsuo et al., 1972 and 1974a, b; Moriya et al., 1973; Matsuo, 1975).

However, the details of the fine structure

of vector mosquitoes have not yet been studied in Korea because of the lack of attention given to the problems of mosquito-borne diseases.

The present study attempts to describe and illustrate the egg surface structure of *Aedes albopictus*(Skuse) and *Culex pipiens* complex from Korea.

Materials and Methods

The specimens examined in this study were eggs obtained from females developed in our insectarium from wild larvae collected from the fields in the outskirts of Taegu city, Korea during the period from June to September in 1983.

They were preserved and fixed in MacGregor's solution. For scanning electron microscopic studies, specimens were washed three times in saline, fixed with 3% glutaraldehyde for 2

^{*} The results of this study were presented at the 25th annual meeting of the Korean Society for Parasitology in 1983.

hours in 0.1M phosphate buffer solution, pH 7.4, and post-fixed with 1% osmium tetroxide.

They were further dehydrated with a graded series of ethylalcohol and iso-amyl acetate, and critical pointed dried.

The specimens were coated with gold, using JFC-1100 ion sputtering apparatus, and then examined with a JEOL-U3 scanning electron microscope at an accelerating voltage of 15kv.

Results and Discussion

The shapes and surface structures of mosquito eggs have been studied by many investigators. These workers were limited to observations by light microscopy, which did not reveal the details of the surface structures and micropyle apparatus of eggs.

After the publication by Craig(1955) of a simple method of bleaching the shell of aedine mosquitoes so that the chorionic pattern could be examined easily with the light microscope, considerable attention was paid to distinguishing the eggs of North American species of *Aedes* by their chorionic pattern.

However, photographs taken with the light microscope at best give only a very poor impression of the structure and shape of the tubercles, or of the ridges that delimit the polygons (Craig and Horsfall, 1960).

From his review on respiratory systems of insect egg shells, Hinton (1969) presented the first time scanning electron microscopic pictures of eggs of various insects, including those of *Culex pipiens molestus* and some anophelines.

The mosquito eggs are mostly elongated oval shapes and are laid either individually such as in *Anopheles* and *Aedes* species, or in egg rafts as in *Culex* species. In most anopheline species, the eggs have a pair of floats along the lateral lines and a pole called micropyle surrounded by a ring-like structure at the anterior tip of eggs.

Fig. 2 is a picture of the micropyle and the outer chorionic reticulum of Aedes albopictus

egg at 650 magnification. The micropyle apparatus comprising the micropylar disc and micropylar collar is the volcano-like body with a central hole surrounded by irregular bosses.

The outer chorionic reticulum composed of a pattern of roughly pentagonal, hexagonal, or sometimes transversely elongated rhombic cells and has the outer chorionic tubercles surrounded by outer chorionic reticulum, and a number of small tubercles with rather pointed apex which are scattered between the large tubercles as shown in Fig. 3.

It is considered that the size and distribution of tubercles were different between species of mosquitoes. A study of Hinton and Service (1969) reported that within the polygons on the surface of the shell were tubercles of various kinds, and he claimed that the shapes and dimensions of these tubercles differed enormously between species. Such consideration was recognized also by Sasa et al. (1971) and Matsuo et al. (1974).

In the present study inner chorion pictures are omitted, but Matsuo et al. (1974) reported in their paper that the reticulation of inner chorion were composed of the same pattern as outer chorionic reticulum, but tubercle was absent.

Fig. 4 is a picture of the outer chorionic tubercle of *Aedes albopictus* eggs at high magnification(2,300×); one is a larger somewhat half-globular, roughly hemispherical and present at the center of each chorionic cell, and the others are smaller and a number of tubercles which vary in dimension are formed in a row parallel to outer chorionic reticulum, often confluent with large tubercle and outer chorionic reticulum.

Simiar details of morphological features have been presented by Hinton and Service(1969), Sasa et al. (1971), Matsuo et al. (1972), and Matsuo(1975).

In Culex species the eggs are deposited in boat-shaped rafts on the surface of water.

The smaller end is bluntly pointed, and the

longer rounded end at the lower surface of the raft has a cup-shaped corolla, as seen in Fig. 5, and also enlarged in Fig. 6.

The corolla is a delicate frill-like collar surrounding the micropyle(Fig. 6).

The surface of outer chorionic cell is somewhat poorly delimited, and has numerous globular tubercles connected with peculiar networks, as seen in Fig.7, and also more enlarged in Fig.8.

Similar details of morphological features were also reported by Moriya et al. (1973), and Harbach and Knight(1980).

The results of this study generally indicate clear evidence that the pictures of mosquito eggs obtained by scanning electron microscopy are extremely useful in morphology, because they visualize the stereoscopic figures of the fine surface structures in a way that no other optical microscope can do.

Summary

The egg surface structure of *Aedes* and *Culex* species collected in Korea is described and illustrated with the aid of a scanning electron microscope.

Outer chorionic reticulum and tubercle can be seen on the shells and these features are characteristics for the species of mosquitoes.

It was found that the size and distribution outer chorionic tubercles differ enormously between species of mosquitoes, and that the pictures of mosquito eggs obtained by scanning electron microscope are extremely useful in morphology and taxonomy.

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 The egg surface structure of 13 species

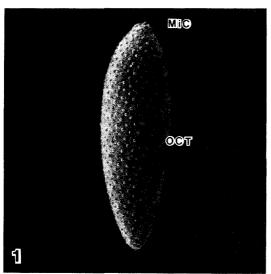
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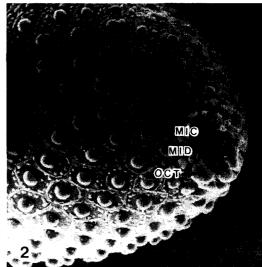
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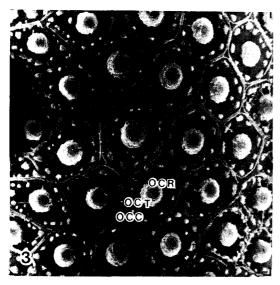
Explanation of photographs

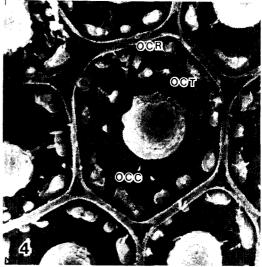
- Fig. 1. Ventral surface of outer chorion with blow-up showing outer chorionic sculpture of *Aedes albopictus*(×150).
- Fig. 2. Anterior pole and the micropyle apparatus of the same $egg(\times650)$.
- Fig. 3. The egg shell surface of the same egg $(\times 1,000)$.
- Fig. 4. The enlarged view of outer chorionic tubercles of the same $egg(\times 2,300)$.
- Fig. 5. Ventral surface of outer chorion with cup-shaped corolla of *Culex pipiens* complex(×150).
- Fig. 6. The enlarged view of frill-like corolla surrounding the micropyle (> 1,500).
- Fig. 7. The egg shell surface of the same egg $(\times 5,000)$.
- Fig. 8. The enlarged view of outer chorionic cell, numerous globular tubercles connected with peculiar networks (> 10,000).

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