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A Simple Method for Determining the Identity of the Eggs of Aedes aegypti (Linnaeus) and Aedes albopictus (Skuse) by Dissection (Diptera: Culicidae) 【Research report】

簡易解剖法鑑定埃及斑蚊與白線斑蚊之卵(雙翅目: 蚊科)【研究報告】

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Abstract

A shaving blade was used to press slightly across the egg surface so as to make a small crack at a distance of 0.12-0.16 mm from the tip of the wider side of egg, and then pull out the embryo for examination under a microscope. The eggs laid on the papers inside ovitraps by two dengue vector mosquitoes, can be correctly identified to species, either Aedes aegypti or Aedes albopictus. The process of dissecting and a technique for the identity of the embryo larvae are presented in this paper.

摘要

以刮鬍用刀片於斑蚊卵較寬之部位，即距離頂端約0.12-0.16 mm之處，輕輕橫壓卵殼成一細縫，將成熟胚胎抽出後鏡檢，可正確鑑定兩種登革熱病媒蚊 - 埃及斑蚊與白線斑蚊產於紙上的卵。本文詳細介紹其解剖之步驟與鑑定成熟胚胎之技術。

Key words: dengue vectors, eggs, dissection, identification
關鍵詞: 登革熱病媒蚊、卵、解剖、鑑定。

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A Simple Method for Determining the Identity of the Eggs of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) by Dissection (Diptera: Culicidae)

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ABSTRACT

A shaving blade was used to press slightly across the egg surface so as to make a small crack at a distance of 0.12-0.16 mm from the tip of the wider side of egg, and then pull out the embryo for examination under a microscope. The eggs laid on the papers inside ovitraps by two dengue vector mosquitoes, can be correctly identified to species, either *Aedes aegypti* or *Aedes albopictus*. The process of dissecting and a technique for the identity of the embryo larvae are presented in this paper.

Key words: dengue vectors, eggs, dissection, identification

Introduction

The egg surface structure of the 13 species of *Aedes* from Taiwan by SEM was reported by Matsuo *et al.* (1974). Although the eggs of two dengue vector mosquitoes, *Ae. aegypti* and *Ae. albopictus*, can be distinguished by the characteristics of the exochorion, but the operation for SEM is time-consuming and the expense is high. Therefore, a simple method for separating the eggs of two medically important *Aedes* species by dissection was developed. Using this simple method, a large number of the eggs of both species can be identified in the laboratory in a short period of time.

Materials and Methods

Source of eggs

a. The eggs of *Ae. aegypti* and *Ae. albopictus* obtained from the colonized strains established in the insectary of the Division of Medical Entomology, National Institute of Preventive Medicine in Taipei, Taiwan.
b. 64 eggs of uncertain species of *Aedes* obtained from ovitraps set outdoors in the Putai District of Chia-I Hsien.

Treatment of eggs

a. Twenty-four hours after laboratory colonies of *Aedes* laid eggs on the wet

filter papers provided in each cage, the papers with eggs were removed and divided into several pieces for further operation. The pieces of paper were separately placed on an open glass petri-dish to let them dry under the room temperature for 9 days. By the end of 9 days, a little drop of fresh water was added to the eggs surface by a glass pipette to keep them moist for a day, and on the following day, the eggs were dissected under a stereoscopic microscope.

b. The field ovitraps were set up on 6 March 1996 and examined on 21 March 1996 by a member of the local health stations in the Putai district of Chia-I Hsien. On examination of the ovitraps, the pieces of oviposition paper were brought to the health station, and were spread out on the table to let dry for 4 days in the laboratory. The eggs were mailed to the Division of Medical Entomology on 26 March 1996. Upon the receipt of the pieces of egg paper, the above mentioned procedure was followed.

The process of dissection

a. A shaving blade was used to press slightly across the egg surface so as to make a small crack at a distance of 0.12-0.16 mm from the tip of the wider side of egg. (Fig. 1, 1-3)

b. Place the cracked eggs on a drop of fresh water on a microscopic slide. A pair of dissecting needles are necessary to perform the following process: (1) One needle was used to press firmly the distal part of the slender side of egg. (2) The other one was used to remove the cracked part of the shell from the wider side of egg, then the embryo larva can be easily

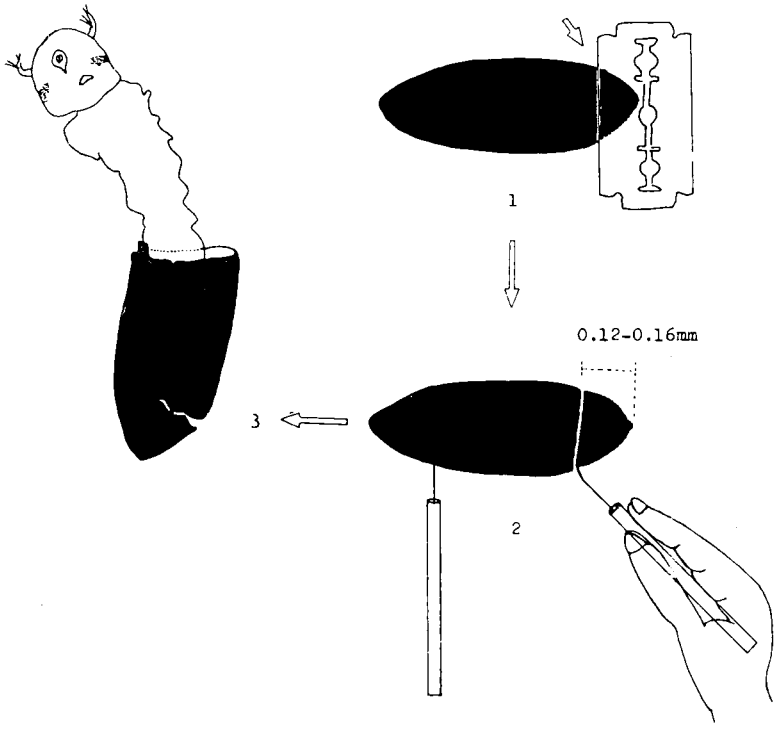

Fig 1. The process of dissecting.

pulled out from its shell proper.

c. After the embryo larvae were pulled out in the drop of water, they were then transferred into 70% alcohol to fix for 30 minutes. Subsequently, the embryo larvae were placed into a small drop of Gater's mounting medium (Gater's formula : gum arabic, 8 gms.; distilled water, 10 c.c.; chloral hydrate, 75 gms.; glucose syrup, 5 c.c.; acetic acid, 5 c.c.) on the center of a microscopic slide, and the specimens were arranged in a line so as to receive continuous exposure to the medium at room temperature overnight.

d. The surface of the drop of Gater's mounting medium will become somewhat dry and hard. A small drop of Gater's mounting medium was added with cover slip overlaid for examination.

The terminology used in the description follows that of Tanaka *et al.* (1979).

Results

A total of 130 embryo larvae from 140 eggs of *Ae. aegypti* and 145 from 154 of *Ae. albopictus* were successfully pulled out and examined in a short period of time in the laboratory. As a result, the larvae can be correctly identified to either *Ae. aegypti* or *Ae. albopictus* by the characteristic features of head hairs. Fig. 2 (A & B) shows that the postclypeal hairs are single for the former species, and 2-branched for the latter species. On the contrary, the outer sutural hairs of *Ae. aegypti* are 2-branched, while those of *Ae. albopictus* are single. In addition, the abdominal comb scales on segment VIII of *Ae. aegypti* carry a slender and pointed central denticle at distal portion, while those of *Ae. albopictus* do not carry such central denticle at distal portion. The different morphological features of comb scales for both species are shown in Fig. 4 (C & D).

With regard to the eggs from the ovitraps, 58 embryo larvae from 64 eggs were successfully identified. After comparing with the characteristics of the above findings with the embryo larvae from the laboratory colonies, 14 and 44 larvae were identified to be *Ae. aegypti* and *Ae. albopictus*, respectively.

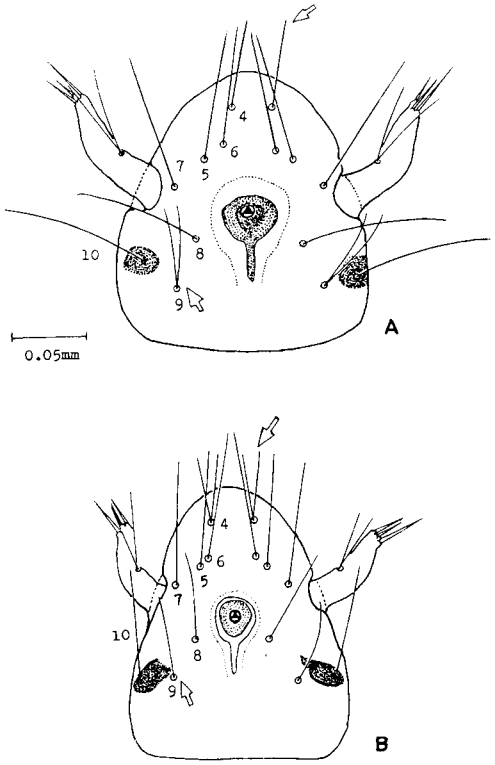

Fig 2. Head & Head chaetotaxy of immature larvae (dorsal view).
Head: A, *Aedes aegypti*, B, *Aedes albopictus*.
Head chaetotaxy:4. Postclypeal, 5. Inner Frontal, 6. Middle Frontal, 7. Outer Frontal, 8. Inner Sutural, 9. Outer Sutural, 10. Orbital.

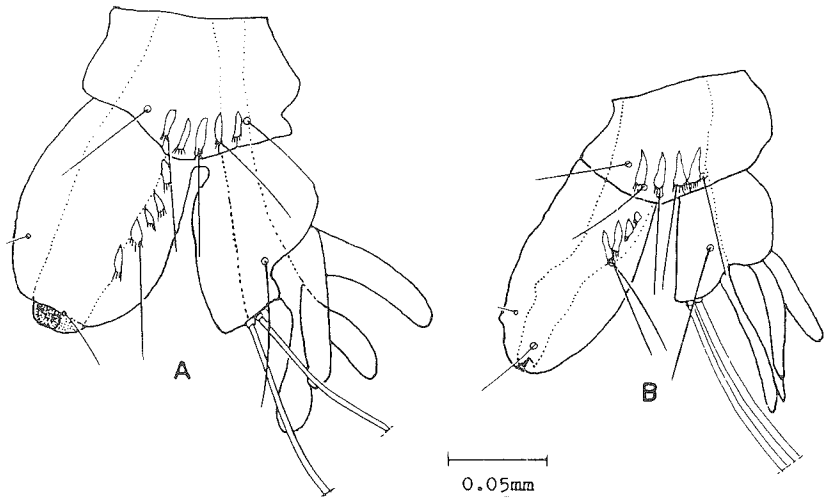

Fig 3. VIII segment of immature larvae (in side view). A, *Aedes aegypti*, B, *Aedes albopictus*.

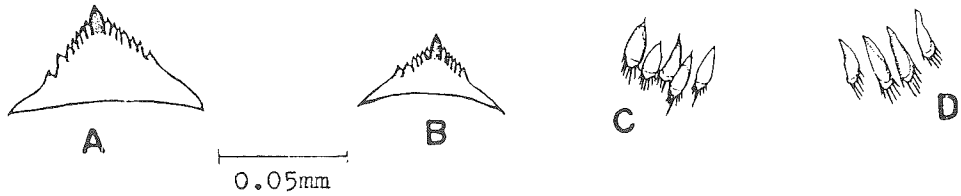

Fig 4. Mentum & Comb-scale. A, C, *Aedes aegypti*, B, D, *Aedes albopictus*.

The number of mentum teeth on each side of the large central tooth is 7-8 with *Ae. aegypti* and 6-7 with *Ae. albopictus* Fig. 3. Moreover, the embryo larvae either from the colony strains or from the field, the siphon hairs are single in *Ae. aegypti* and 2-branched in *Ae. albopictus* Fig. 4.

Discussion

Gander (1951) found that the chorion of *Ae. aegypti* eggs kept continuously moist, the embryo became mature in 90 hours at a temperature range of 25-30 ℃. At a temperature range of 21-23 ℃ the embryos may mature within 100 hours (Johonson, 1937). According to Bhattacharya and Dey (1969) the incubation period of *Ae. albopictus* eggs was 3-4 days with favorable conditions. During the course of this study, the eggs of both *Aedes* species kept continuously wet on the filter paper for 5 days, were treated with the method mentioned above. Unfortunately, the results were unsatisfactory, because the egg-shells became so soft that the serosa cuticle of embryo became ruptured easily as the blade touched the surface of eggs. The ruptures made the serosity fluid leak out in two directions both anteriorly and posteriorly. Moreover it also creates difficulty in pulling out whole embryo for identification. After unsuccessful trials, the

author finally found that the *Aedes* eggs kept moist for 24 hours after oviposition, and then kept dry for 9 days at room temperature, were better materials for use. During this period of time, the embryos became mature and the egg-shell became much more elastic. The eggs treated in such a way made subsequent operation much easier. However, this technique has its defect. The specimens treated in Gater's mouting medium become very transparent, and identification of the specimens becomes much more difficult.

The use of ovitrap provide a sensitive and economical way of detecting the presence of *Ae. aegypti* or *Ae. albopictus* when the population density is low, and general larval surveys produce unsatisfactory results (Wld. Hlth. Org., 1995). The eggs laid inside the ovitrap can not be positively identified either to *Ae. aegypti* or to *Ae. albopictus* in a short period of time with conventional microscopic examination. For this reason, the effectiveness against the respective species could not be analyzed. This simple method can solve the problem, and may be used to promote detailed analysis of the function of ovitraps for the dengue vector mosquitoes surveillance in the area where the integrated control of dengue vectors are being undertaken.

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簡易解剖法鑑定埃及斑蚊與白線斑蚊之卵（雙翅目：蚊科）

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摘　要

以刮鬍用刀片於斑蚊卵較寬之部位，即距離頂端約 0.12-0.16 mm 之處，輕輕橫壓卵殼成一細縫，將成熟胚胎抽出後鏡檢，可正確鑑定兩種登革熱病媒蚊——埃及斑蚊與白線斑蚊產於紙上的卵。本文詳細介紹其解剖之步驟與鑑定成熟胚胎之技術。

關鍵詞：登革熱病媒蚊、卵、解剖、鑑定