Research Note

Ultrastructural comparison of egg surface structure of seven *Culicoides* species (Diptera: Ceratopogonidae) from Japan

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Morphological studies of *Culicoides* biting midges have been reported on the larvae, pupae, and adults of various species (Jones, 1961; Kettle and Elson, 1975 a, b; Kitaoka and Wada, 1977). However, no morphological observations have been made on the eggs of *Culicoides* biting midges. This report describes the egg surface structures observed by scanning electron microscopy (SEM) of 7 species of *Culicoides* collected from Japan, and a discussion of the functional significance of the morphological difference among the egg surfaces.

The eggs were obtained from blood-engorged females collected in the field or developed in an insectarium, as shown in Table 1. These midges were anesthetized with chloroform, and then selected females were decapitated on a wet filter paper to make them lay eggs (Morii and Kitaoka, 1968). Newly laid eggs were immediately fixed in 1% (v/v) glutaraldehyde solution buffered with 0.1 M phosphate buffer (pH 7.2) at 4°C overnight. After rinsing in 3 changes of 0.1 M phosphate buffer for 30

min each, they were refixed in Párducz's fixative (6:1 mixture of 2% (w/v) osmic acid aqueous solution and saturated mercury chloride aqueous solution) for 2 hr at room temperature. Then the samples were dehydrated in ethanol series, and dried in a critical point dryer with liquid CO₂. The dried eggs were mounted on the specimen holder by double sided mending tape. The eggs on the holder were coated with gold in an ion-sputter to prevent electron charging of the specimens during observation. The surface structures of the specimens were examined with a JSM 25S-III type (JEOL) SEM.

In this paper, curvature minor is referred to as "ventral," and curvature major as "dorsal" for the orientation of eggs.

The eggs of *Culicoides* spp. were generally banana-shaped with a size of 0.3 to 0.5 mm. The color of newly laid eggs was milk white, but changed to black within several hours. SEM observation revealed that the eggs of biting midges had many papillae on their surface (Figs. 1-14). The patterns of the papillae were divided into two types, granular and hair-like. The egg surface of 5 species (*C. arakawae*, *C. oxystoma*, *C. punctatus*, *C. sumatrae*, and *C. actoni*) had granular papillae, and the other 2 species (*C. brevitaris* and *C. maculatus*) had hair-like ones.

The pattern of the egg surface structure in each species examined was as follows;

Culicoides arakawae (Figs. 1 and 2): The egg of this species had many granular papillae arranged lengthwise. Each line consisted of 2-4 rows of granular structures arranged irregularly transversely. An egg surface had 13 or 14 of these rows, and the diameter of each granule was 1.6 µm on average.

Culicoides oxystoma (Figs. 3 and 4): A smaller number of granular papillae than shown by *C. arakawae* was arranged in 14-16 lengthwise lines. Each line consisted of a single row of granules, and the size of each granule was small with an average diameter of 1.25 µm.

Culicoides punctatus (Figs. 5 and 6): Thirteen to 14 lines of granular structures were arranged lengthwise, and each line consisted of a single row of granules as in *C. oxystoma*. However, granules were larger, 2.7 µm in diameter on average, than those

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Table 1 *Culicoides* eggs examined and the area in which collected.

Species	Collection area
<i>C. arakawae</i>	Bred in Kyorin Univ.
<i>C. oxystoma</i>	Kagoshima Pref.
<i>C. punctatus</i>	Kagoshima Pref.
<i>C. sumatrae</i>	Kagoshima Pref.
<i>C. actoni</i>	Kagoshima Pref.
<i>C. brevitarsis</i>	Kagoshima Pref.
<i>C. maculatus</i>	Kagoshima Pref.

of the former species.

Culicoides sumatrae (Figs. 7 and 8): The egg surface of this species had a different pattern dorso-ventrally. On the dorsal side, relatively large granular papillae were arranged lengthwise and formed 8–9 lines, each of which consisted of a single row of granules. Average diameter of the granules was 3.0 μm . Ventrally, fairly small granules, averaging 1.5 μm in diameter, were scattered irregularly. At the border between the dorsal and ventral sides ran a lengthwise row of oval-shaped papillae averaging $5.5 \times 3.6 \mu\text{m}$.

Culicoides actoni (Figs. 9 and 10): Ten to 11 lines of very small granular papillae were present on the surface of an egg. Granules were the smallest of all species examined, 0.9 μm in diameter on average. Each line consisted of single or double rows of granules arranged transversely.

Culicoides brevitarsis (Figs. 11 and 12): Unlike the former 5 species, the egg had hair-like papillae which were scattered irregularly on the egg surface.

Culicoides maculatus (Figs. 13 and 14): The papillae on the egg surface varied in shape. On three-fourths of the area at the dorsal side of egg, there were short hair-like papillae surrounded by shorter spines arranged in a hexagonal pattern. Hair-like papillae were scattered in each hexagonal block. At the remaining one-fourth of the ventral area, the anterior of hair-like papillae expanded and fused to make frills.

Each species examined thus had a specific pattern on its egg surface. These exochorionic sculpturing patterns can be useful for the identification of different *Culicoides* species.

As to the functional significance of these

structures, species having granular papillae on their egg surface are known to lay eggs on moist ground such as paddy field, and eggs with hair-like papillae are laid in areas not as moist such as in cow dung (Kitaoka, 1977). Such difference of egg surface structures as observed in the present examination may be related with the adherence of eggs to the media in which they are laid.

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摘 要

走査電子顕微鏡による日本産ヌカカ 7 種の卵表面の観察

Culicoides 属ヌカカの卵の表面構造を走査電子顕微鏡を用いて観察した。

その結果、種により特徴的な卵表面像が認められた。表面構造は大きく 2 型に区分され、観察した 7 種のうち 5 種 (*C. arakawae*, *C. oxystoma*, *C. punctatus*, *C. sumatrae*, *C. actoni*) は、表面に粒状突起をも

Figs. 1-8

Figs. 9-14

Figs. 1-14 Scanning electron micrographs of the eggs of *Culicoides* spp.

1 and 2, *C. arakawae*: 1, whole egg, $\times 290$; 2, center area showing papillae plurally arranged in a line, $\times 1,400$. 3 and 4, *C. oxystoma*: 3, whole egg, $\times 280$; 4, center area showing papillae singularly arranged in a line, $\times 1,600$. 5 and 6, *C. punctatus*: 5, whole egg, $\times 180$; 6, center area, $\times 1,300$. 7 and 8, *C. sumatrae*: 7, whole egg, $\times 220$; 8, center area showing papillae which shape and size differ on the dorsal and ventral sides, $\times 1,100$. 9 and 10, *C. actoni*: 9, whole egg, $\times 260$; 10, center area showing small granular papillae, $\times 1,200$. 11 and 12, *C. brevitarsis*: 11, whole egg, $\times 320$; 12, center area showing hair-like papillae, $\times 1,100$. 13 and 14, *C. maculatus*: 13, whole egg, $\times 320$; 14, center area showing short hair-like papillae on the dorsal side and longer papillae making frills on the ventral side, $\times 840$.

ち、2種 (*C. brevitarsis*, *C. maculatus*) は、毛状突起を有していた。粒状突起をもつ5種の卵表面構造は、互いに類似していたが、粒状突起の大きさおよび

配列が、おのおのの種により異なっていた。また、これらの構造的特徴から、ヌカカの産卵場所について考察した。