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Scanning electron microscopy of egg-surface sculpturing of two common Indian short-horn grasshoppers (Orthoptera, Acrididae)

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Abstract

Surfaces of the eggs of acridids show chorionic sculpturing — distinct features that can be used to identify species. With a scanning electron microscope, we document dimensions and surface detail for eggs of two common Indian acridids: *Hieroglyphus banian* and *Acrida exaltata*. Their eggs are characterized by distinguishing features: *A. exaltata* by many small tubercles scattered almost uniformly, *H. banian* by thick-ridged hexagonal polygons. The similarities and dissimilarities of their egg-surfaces to those of other acridid species are not consistent with subfamilial classification.

Key words

Acridid, egg surface, SEM, chorion, sculpturing

Introduction

Uvarov (1928) apparently was the first to suggest that the eggs of grasshoppers might be identified on the basis of chorionic sculpturing. He pointed out that the reticulations on the eggs of many of the Locustidae (Acrididae) might be of systematic importance. Katiyar (1957) remarks that in shorthorn grasshoppers, egg-surface sculpturing shows interesting characteristic structures which help in distinguishing eggs of different species of acridids.

The first attempt to identify acridid eggs on the basis of chorionic sculpturing was a detailed study of 48 grasshopper species of midwestern North America by Tuck and Smith (1939). They built upon the unpublished M.S. Thesis of Raymond Bushland (1934) who had described the eggs of 18 South Dakota Grasshoppers. Hartley (1961) describes patterning of acridid eggshells for three locust species and six grasshopper species of England.

However, very little is known about the sculpturing of the egg-shell surfaces of Indian acridids and a study of this has practical value. In this context Katiyar (1957) undertook a light microscopic study of eggshell surfaces of ten common Indian acridids. But as Hinton (1968a, 1968b) and Hinton & Service (1969) point out, photographs of egg-shell surfaces taken with the light microscope, can give at best, only a poor impression of the structure and the shape of the tubercles and ridges.

In the present study a scanning electron microscope was used to carry out detailed observations of the eggshell surface structures of two common Indian acridids: *Hieroglyphus banian* (Fabricius) and *Acrida exaltata* (Walker).

Materials and methods

Adult males and females of *H. banian* and *A. exaltata* were collected from Santiniketan and its vicinity (lat 23°39′ N, long 87°42′ E, eastern India). They were reared, keeping single females each to a jar, in the laboratory of the Entomology research unit, department of Zoology, Visva-Bharati University, Santiniketan, West Bengal, India. We used the rearing procedures of Ewen and Hinks (1986) and Hinks and Erlandson (1994) slightly modified. Identities of the acridid species were confirmed by the Zoological Survey of India (ZSI), Kolkata.

After the eggs were laid, for each species four egg pods were collected from four different females and five eggs separated from each of these pods.

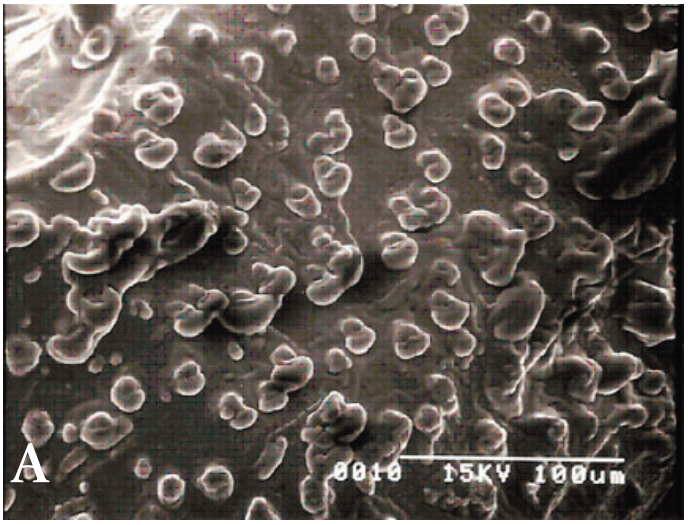
To prepare the eggs for viewing with the SEM, they were washed several times in saline solution. The specimens were then fixed with 2.5% glutaraldehyde, mixed in phosphate-buffered solution (PBS) at pH 7.4 at 4°C for 24 h. Then they were rinsed twice with PBS at 10-min intervals. The rinsed eggs were treated with 1% osmium tetroxide at room temperature for one day postfixation. This treatment was followed by rinsing the eggs twice with PBS and dehydrating with alcohol. To replace water in the eggs with ethyl alcohol, they were subjected to increasing concentrations of alcohol in five steps of a dehydration series: 30, 50, 70, 80 and 90%. The eggs remained in each concentration of alcohol for 12 h during each step of the dehydration process. They were then placed in absolute alcohol for two 12-h periods, followed by acetone for two 12-h periods. Finally the eggs were subjected to critical point drying to complete the dehydration process. They were attached with carbon double-stick tape to aluminium stubs and coated with gold in a sputter-coating apparatus, before being viewed with an Hitachi scanning electron microscope. Photographs were taken with a Mamia camera.

Results

Acrida exaltata (Walker)
(Fig. 1 A, B, C)

General appearance.— Length 4.81 ± 0.28 (mm ± $s_{\bar{x}}$, n = 20), width 0.63 ± 0.09 (mm ± $s_{\bar{x}}$, n = 20); yellowish brown to dark brown in color. Eggs of this species are thin, slender, rounded at the anterior pole, subconical at the posterior pole. Katiyar (1957) reported the presence of 41-52 pipette-shaped micropylar canals, narrowing down abruptly to form a fine tubular portion. Though seen, micropyles are not visible in the presented figures.

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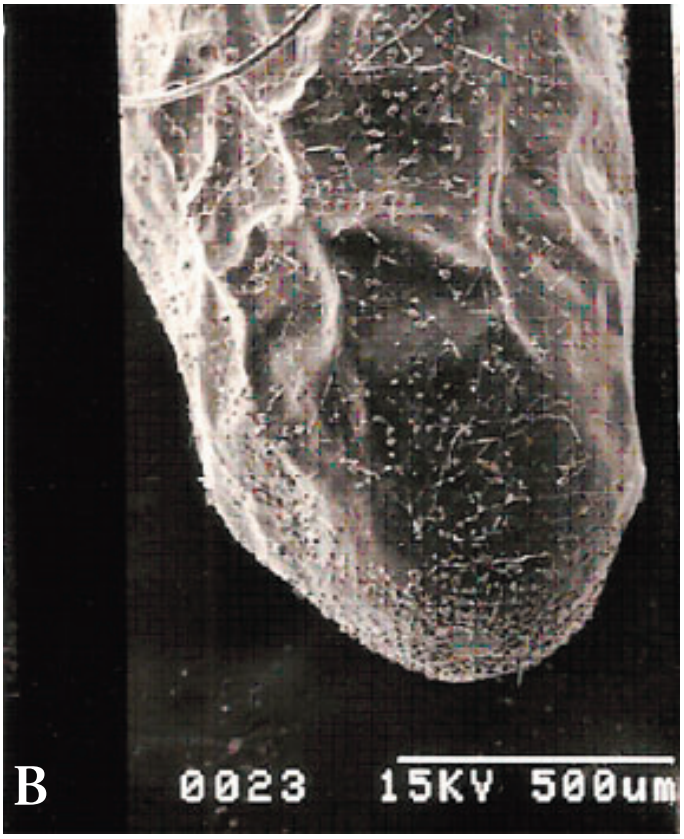


Fig. 1A. Middle egg surface of *A. exaltata* with tubercles of diverse sizes and shapes scattered almost uniformly. **B.** Anterior pole of the egg of *A. exaltata* with tubercle density increased at the extremity. **C.** Posterior pole of the egg of *A. exaltata*; at this end of the egg tubercles are arranged in pentagonal or hexagonal patterns.



Surface sculpture.— The chorion was found to have a large number of small tubercles varying greatly in shape and size. Tubercles were scattered throughout most of the surface almost uniformly (Fig. 1A) and no network of polygonal sculpturing was evident. Tubercles were more densely packed at both extremities, and at the posterior pole they were arranged in somewhat pentagonal or hexagonal patterns (Fig. 1B, C).

Hieroglyphus banian (Fabricius)
(Fig. 2 A, B, C)

General appearance.— Length 3.87 ± 0.17 (mm ± $s_{\bar{x}}$, n = 20), width 0.98 ± 0.05 (mm ± $s_{\bar{x}}$, n = 20); yellowish brown to dark brown in color. Eggs of this species are thin, subcylindrical, straight, rarely bent in the middle. Anterior pole is round, the posterior one tapers from the micropylar ring (Katiyar 1957). In this case also the micropyles are not visible in the presented figures. Eggs are slightly round or flat at the extremity.

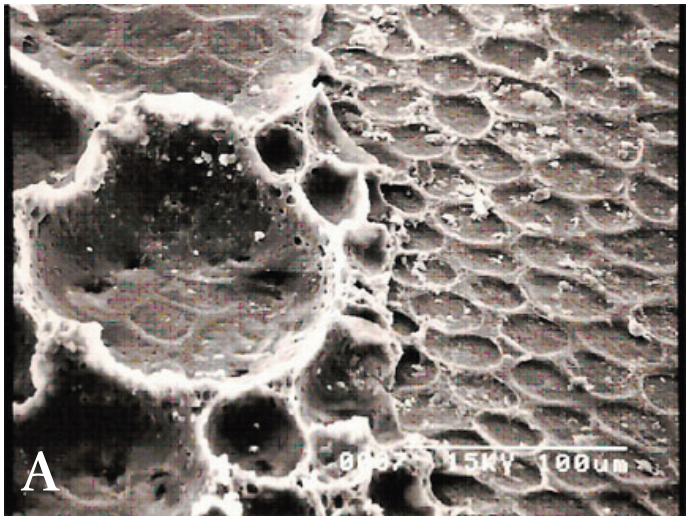
Surface sculpture.— Sculpturing of the chorion was very sharply defined. Most of the surface showed hexagonal polygons outlined by thick ridges (Fig. 2A). The thickening of these ridges made the cells appear almost oval, but still six points of a polygon can usually be identified.

Along with these hexagonal networks occurred a number of bigger cells without such definite patterns (Fig. 2A). The floors of those bigger cells were found to have a network of hexagons similar to the rest of the surface. Sculpturing at both anterior and posterior poles included these two networks: smaller hexagons and bigger irregular cells -- but the latter structures were packed at the extremity (Fig. 2B, C). The irregular shape and size of these bigger cells reveals they are not a part of egg-surface sculpture; they may be foam-like protective cement that enjoins the eggs. Granules were sometimes found scattered upon the surface, but were mostly present on the margins of the cell ridges. Perhaps these are corner tubercles that had been displaced? In any case, no central tubercle was observed.

Discussion

According to Snodgrass (1935) the follicular epithelium secretes the chorion and so molds a surface, often marked with minute lines and ridges which form more or less regular pentagonal and hexagonal cells. These 'cells' vary widely among species, but are relatively constant within a species (Tuck & Smith 1939). In some eggs the boundaries of these cells are sharp, in others they are quite vague. In some species the corners of the cells contain thickenings and in others there are thickenings in the centers of the cells or the cells

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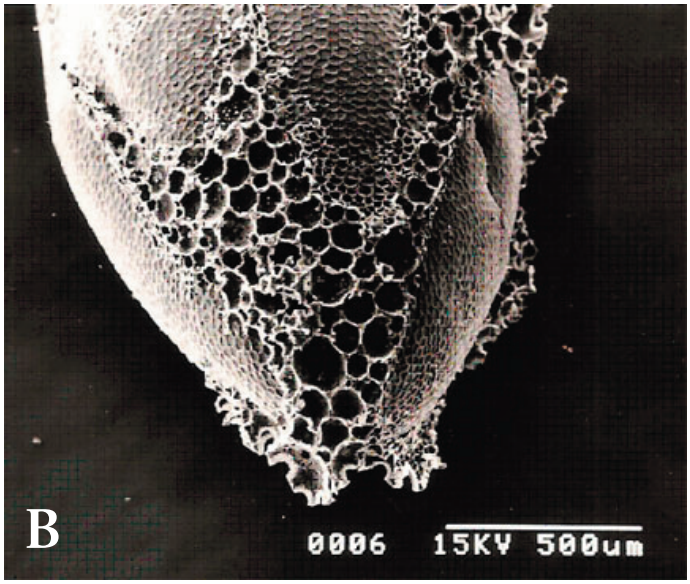
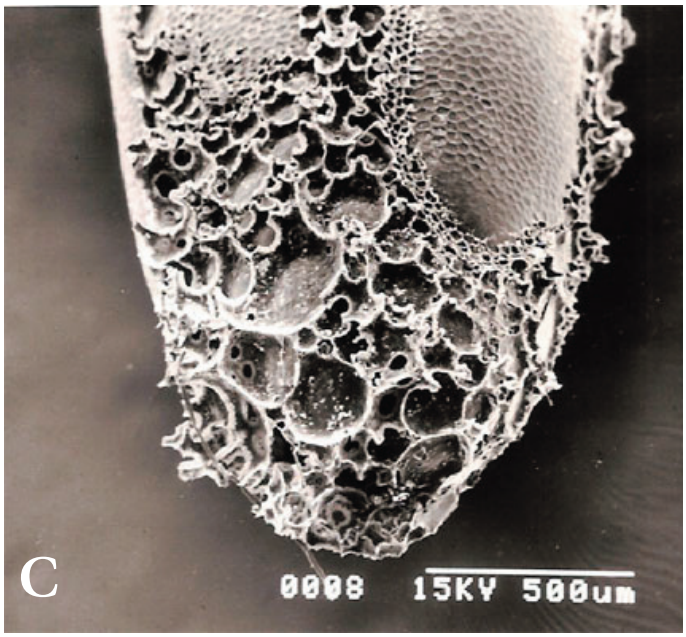


Fig. 2. A. Egg surface of *H. banian* showing distinct hexagonal sculpturing along with a larger cell. Some tubercles are visible on the margins of ridges. **B.** Anterior pole of egg of *H. banian* with an aggregate of larger cells at the extremity, taking in this instance roughly the shape of Ψ (psi). **C.** Posterior pole of the egg of *H. banian*, again with clumping of larger cells toward the extremity.



lose their pentagonal and hexagonal shape becoming circular.

Chapman and Robertson (1958) suggest a classification of types of acridid eggshell sculpturing which was presented in a modified form by Uvarov (1966). According to that, eggshell sculpturing can be of six types: 1) surface smooth 2) irregular tubercles 3) tubercles arranged in hexagons 4) hexagonal pentagonal or oval cells without tubercles 5) cells with corner tubercles and 6) cells with corner and central tubercles.

The present study deals with the egg-surface sculpturing of two acridids from two different subfamilies: Acridinae and Oxyinae. In the case of *A. exaltata* (Acridinae), the surface has tubercles of different shapes and sizes scattered irregularly throughout. Sculpturing of a similar type is described by Zimin (1938) for most of the egg surface of *Pyrgodera armata* (Fischer-Waldheim), which is classified within the subfamily Oedipodinae. But the eggs of these two species (*A. exaltata* and *P. armata*) differ from each other at the posterior pole, where *P. armata* has dot-shaped tubercles arranged irregularly and *A exaltata* has densely packed tubercles arranged in polygonal patterns.

Katiyar (1957) found irregularly shaped tubercles on the egg surface of *Acrida gigantia* (Herbst.) (Subfamily Acridinae), but arranged in both pentagonal and hexagonal form. He also noticed dot-like tubercles in *Parahieroglyphus bilineatus* (Bolivar) (subfamily Hemiacridinae) forming concentric or intersecting polygons. Although *A gigantia* and *P. bilineatus* have similar arrangements of egg-surface tubercles, a distinction between the species is fubercle shape.

The second species of the present study, *H. banian* belongs to subfamily Oxyinae. It shows distinct hexagonal networks throughout the egg surface, along with corner tubercles. According to Zimin (1938) this pattern is very similar to that of *Celes variabilis* (Pallas) (Subfamily Oedipodinae). Definite polygonal cells are also found in the egg surfaces of *Schistocerca gregaria* (Forskal) (Subfamily Cyrtacanthacridinae), *Romalea microptera* (Beauvois) (Subfamily Romaleinae) (Roonwal 1954) and *Chorthippus parallelus* (Zetterstedt) (Subfamily Gomphocerinae) (Waloff 1950); but in those cases the presence of tubercles was not reported.

If the above-mentioned eight acridid (and one romaleid species) are grouped according to similarities of their egg surface sculpture (applying the types suggested by Chapman & Robertson), the groups will be: 1) *A. exaltata* and *P. armata* (irregular tubercles), 2) *A. gigantia* and *P. bilineatus* (tubercles arranged in polygons), 3) *H. banian* and *C. variabilis* (cells with corner tubercles) and 4) *S. gregaria*, *R. microptera* and *C. parallelus* (polygonal cells without tubercles). Three of these four groupings involve different subfamilies and one group, different families. It is clearly evident that chorionic patterns will not be diagnostically useful at such an inclusive taxonomic level.

Egg surface morphology separates taxa into groups which do not coincide with present taxonomic arrangements based on adult characteristics. Uvarov (1966) pointed out that the taxonomic distribution of the types of acridid chorionic sculpturing is not clear. However he also held the opinion that eggs of Pamphagidae, Pyrgomorphidae, Catantopinae, Calliptaminae and Cyrtacanthacridinae usually have a well-developed cellular pattern; those of Oedipodinae, have an irregularly tuberculate surface, while in Gomphocerinae the

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surface is frequently smooth, or with a weak cellular pattern. Since the latter is the most advanced group, Uvarov speculated that the evolution of patterning has followed a gradual simplification, from heavily sculptured to smooth; however only a few representatives of each group have been studied.

Egg-surface sculpture, while not indicative of higher taxonomic groupings, does also vary from species to species in a manner causing no confusion in identification of their eggs. Hence the present study offers key information about the egg surface sculpture of the chosen acridids by which their eggs could be easily recognized.

Acknowledgement

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