Eggs of the stink bug *Acrosternum (Chinavia) marginatum* (Hemiptera: Pentatomidae): a scanning electron microscopy study

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SUMMARY - The morphology of the outer face of the eggshell, its appearance in cross fractures and the surface morphology of the inner face are described in the green stink bug *Acrosternum (Chinavia) marginatum* (Hemiptera: Pentatomidae) using scanning electron microscopy. Deposited eggs are barrel-shaped and the surface consists of hexagonally arranged, funnel-shaped pits that possess transverse diaphragms in their slender, basal portions. Only minor differences are detectable between the central portion of the anterior plate (the operculum) and the lateral wall together with the basal portion of the eggshell. The rim at the anterior pole of the egg carries processes with a bulbous end, the aero-microplar processes. A narrow band of the anterior plate bordering these processes shows hexagonally arranged elements. These differ from those of the operculum and the lateral wall, in that cylindrical pits are abundant. During hatching, the operculum is lifted precisely at the rim of this area. Inspection of the periphery of the inner face of the operculum reveals slender, radially arranged grooves. Their arrangement and spacing indicate that they bear a spatial relationship with the aero-microplar processes. Their role may lie in guiding the sperm to the site where fertilization occurs and in facilitating the gas exchange of the embryo. The remainder of the eggshell carries shallow elevations at its inner face. Exterior and interior features of the eggshell, as well as its appearance in cross-fractures, are discussed.

Key Words: aero-microplar processes · chorion · coarse surface pattern · egg-barrel · embryonic ovule

INTRODUCTION

The eggshell of insects consists of two major layers. The usually very prominent chorion forming the outer sheet, may be highly sculptured and carries various specializations. The thin vitelline membrane represents the innermost sheet. Both layers may be further subdivided. For a review on the eggshell of insects and the shapes of the chorion in different orders of the class, the reader is referred to Hinton (1981) and Margaritis (1985). It is obvious from light microscopy observations that the eggs of terrestrial bugs – placed in the order ‘Hemiptera’ by some (Borror et al., 1989) or the Hemipteran suborder ‘Heteroptera’ by other entomologists (Daly et al., 1998) – are reminiscent of barrels with tubular appendages, the so-called aero-microplar processes. They are attached to the anterior pole, where hatching takes place. The aero-microplar processes vary in number, architecture and length, and serve in the gas exchange of the developing embryo. In addition, they allow for the passage of the sperm through the eggshell prior to fertilization. The term ‘operculum’ refers to the portion at the anterior end of the eggshell that is lifted during hatching (Cobben, 1968), and this terminology is followed in the present article. It should be added that for some representatives of the Pentatomidae the term ‘pseudoperculum’ is used for the portion of the eggshell that detaches in the course of hatching (Southwood, 1956; for a broader discussion of this aspect, the reader is referred to Wolf and Reid, 2001). Scanning electron microscopy (SEM) has been used for the morphological characterization of the surface of insect eggs at high resolution. In the stink bugs, the

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apparently most abundant pattern consists of tapering, blunt or funnel-shaped spikes projecting from the surface. In face of a seedling feeding on cotton and soybeans, most showed this type, termed 'spinose' (Bundy and McPherson, 2000). Other Pentatomidae that have spinose eggs are Triostis luridus, Pimorimus bidens (Mayné and Breny, 1942), Carpocoris pucicus (Cobben, 1968), C. fasciatus (Candand and Suludere, 2000), Graphosoma lineatum (Candand and Suludere, 1999), G. semipunctatum (Candand, 1999), Palomena prasina (Candand, 1998) and Podisus maculiventris (Lambdín and Lu, 1984). The eggs of another stink bug, initially identified as Mormidea pictiventris, are of the spinose type as well. The species is, however, M. pama (Roulston, 1978), and its eggs have been analyzed in detail using SEM (Wolf and Reid, 2001). Less abundant are stink bugs with eggs showing a surface pattern that Bundy and McPherson (2000) describe as 'coarsely reticulate, foveate'. In other words, the egg surface is covered with pits. This type has so far been found in Rhaphigaster nebulosa (Candand and Suludere, 2001), Eurydema rugulosum (Suludere et al., 1999) and Acrosternum bilare (Javahey, 1994; Bundy and McPherson, 2000). These studies do not include information on the appearance of the eggshell in cross-fractures and do not refer to the inner face of the eggshell. In order to fill these gaps, we have carried out a detailed study of stink bug eggs of the coarse type.

For the study, we have selected A. marginatum, which in contrast to A. bilare does occur in the West Indies (Rider, 1987). It should be added that according to some entomologists, the new world representatives of the genus 'Acrosternum' should be named 'Chinavia' (Rider, 1987). A. marginatum is common on the castor-oil plant (Ricinus communis) and feeds on its fruits.

MATERIALS AND METHODS

Adults of both sexes of A. marginatum were collected from the leaves and fruits of the castor-oil plant in the months of January and February 2000 in a Botanical Garden located on the Mona Campus of the University of the West Indies (Kingston, Jamaica). The animals were kept in the laboratory at ca. 22°C in plastic containers together with immature fruits of the castor-oil plant, that were renewed once in two days. Eggs were found both on the plant material and the walls of the containers. Hatching occurred ca. two weeks later. Newly deposited and hatched eggs, intact as well as fractured, were prepared for SEM as described previously (Wolf and Reid, 2001). The article also described the analysis of the specimens. In brief, the material was fixed in 2% glutaraldehyde in Ringer's solution, dehydrated in an ascending series of ethanol, critically point dried, and sputter coated with gold-palladium. For viewing, a Philips SEM 505 operated at 8 to 10 kV was used.

RESULTS

Newly deposited eggs

The eggs are barrel-shaped with a maximum overall length of ca. 1.25 mm and a maximum diameter of ca. 1.0 mm. Besides the lateral walls, both the basal portion and the anterior plate bulge (Fig. 1a). The term 'anterior plate' refers to the area at the anterior pole that is surrounded by aeromicroplar processes (Wolf and Reid, 2001). The anterior plate consists of the operculum and a structurally different peripheral portion (see below). A. marginatum deposits eggs in batches with the lateral walls in close contact. At the roughly ovoid points of contact between neighboring eggs of

![Figure 1a-j SEM micrographs of deposited eggs (intact and hatched) of Acrosternum marginatum.](image-url)

- **Figure 1a-j** SEM micrographs of deposited eggs (intact and hatched) of Acrosternum marginatum. (a) Survey of an intact egg. At its basal pole (left hand side of the image), remnants of plant material, to which the egg has been attached during deposition, are detectable. The lateral wall (LW) shows a roughly ovoid area surrounded by a rim. (b) Detail of the lateral wall in an intact egg. Note irregularities in the form of wider (white asterisk) or smaller pits (black asterisk) in comparison to the surrounding area. Bar = 100 μm. (c) Basal pole of an intact egg. There are slender connections (arrowheads) between the lateral wall (LW) and a sheet of plant material, on which the egg rests. Bar = 50 μm. (d) End-on view of an intact egg with the anterior plate (AP) facing the viewer. The rim is studied with aeromicroplar processes (arrowheads). Lateral wall (LW). Bar = 200 μm. (e) Oblique view of the anterior plate (AP) and the lateral wall (LW) in an intact egg. It is apparent that there are thin sheets between the bases of the aeromicroplar processes. The sheets are missing locally (arrow). Note an example of irregular spacing between individual aeromicroplar processes (arrowheads). Bar = 100 μm. (f) Detail of the anterior plate in an end-on view of an intact egg. Between the rows of the aeromicroplar processes (arrowheads) and the central portion of the anterior plate (asterisk) with its cone-shaped pits, there is a narrow zone with cylindrical indentations. Bar = 20 μm. (g) Aeromicroplar process with apical indentation (large arrowhead) in an intact egg. The surface of the periiphery of the anterior plate shows rows of tightly juxtaposed columns (small arrowheads). Note also the jagged surface of the ridges. Bar = 20 μm. (h) Tangential view of the lateral wall in an intact egg. It is obvious at this extreme viewing angle that the ridges around the pits are not smooth, but possess dome-shaped elevations (arrowheads). Bar = 10 μm. (i) Extreme tangential view of the central portion of the anterior plate in an intact egg. The ridges around the pits are smooth. Bar = 10 μm. (j) End-on view of several hatched eggs. The central portion of the anterior plate is lost and in all five eggs fully displayed, the embryonic cuticle (E) is visible in the lumen. While the egg-busters with their Y-shaped specializations are preserved, the remains of the embryonic cuticles are usually shriveled. Lateral wall (LW). Bar = 500 μm.
one batch, the regular sculpturing of the egg surface (see below) is disturbed (Fig. 1a,b). Pits with larger or smaller apical openings than those covering the remainder of the lateral wall are found within the ovoid zones of contact. At the basal pole of eggs laid on plant material, slender threads extend between the substrate and the eggshell. The eggs appear to rest on numerous stilts (Fig. 1c).

The rim at the anterior pole of the egg is studded with aeromicropylar processes. At that level, the eggs have a diameter of ca. 0.78 mm. The aeromicropylar processes consist of a roughly cylindrical basal portion with considerably decreasing diameter towards the basal end and a bulbous apical piece, which carries an opening (Fig. 1g). The entire surface of the aeromicropylar processes shows a coarse, irregular
sculpturing, which is different from the other portions of the eggshell. The distance between individual aero-micropylar processes varies between 31 and 62 μm and up to 54 such processes have been counted per egg. Thin sheets extend between the cylindrical basal portions of the aero-micropylar processes at this stage (Fig. 1e); these sheets do not encroach on their bulbous apical portions. The sheets are missing occasionally between the aero-micropylar processes and get entirely lost as the eggs age (Fig. 1d,e; compare Fig. 2a,d). Based on differences in surface ornamentation, the anterior plate is subdivided into two zones. Bordering the aero-micropylar processes is the so-called periphery of the anterior plate. It is up to 50 μm wide and characterized by pits whose walls appear to be composed of aligned columns projecting at right angles from the surface (Fig. 1f,g). The central portion of the anterior plate, which corresponds to the operculum, shows a different sculpturing, which is described in greater detail below. Some researchers use the terms ‘hatching line’ or ‘eclosion line’ (Candan et al., 2001) when a narrow transition zone is detectable between the periphery and the center of the anterior plate. In A. marginatum, the transition is abrupt.

The operculum has cone-shaped pits with a smooth surface (Fig. 1e). Both, there and in the periphery of the anterior plate, the outer opening of the pits are ca. 19 μm wide. The lateral wall of the eggs of A. marginatum principally shows the same type of sculpturing as the operculum and the pits are extensively arranged in a hexagonal pattern. Only a closer comparison of this area with the lateral wall using extreme tangential views reveals that the ridges are uneven in the latter and smooth in the former (Fig. 1h,i).

Hatched eggs

During hatching, the operculum is lifted and smoothly detached from the remainder of the eggshell. End-on views of the anterior pole of hatched eggs show the embryonic cuticle, an envelope that surrounds the embryo during its

![Figure 2a-f SEM micrographs of hatched eggs of Arthrostomum marginatum. (a) View of the anterior pole. During hatching, a smooth fracture plane (white asterisks) is produced. It is located at the boundary of the zone with cylindrical pits (black asterisks) and the central portion of the anterior plate. The aero-micropylar processes (arrowheads) are intact, but thin sheets between their bases are missing. Bar = 20 μm. (b) View of an eggshell manually fractured at the level of the periphery of the anterior plate. At the left hand side is a pit that extends into a slender tube with three transverse diaphragms (small arrowheads). At the right hand side, the basal portion of an aero-micropylar process is visible (asterisk). Underneath, a slender channel traverses the eggshell (large arrowheads). Bar = 20 μm. (c) Pit extending into a slender tube from a fractured lateral wall. Transverse diaphragms are marked (arrowheads). Bar = 10 μm. (d) The inner face of the periphery of the anterior pole and the relationship of its structural details to those visible at the outer face are documented. Below the smooth fracture plane through the eggshell (asterisks), there are faint, straight grooves of the inner face (arrowheads). Their origins correspond in number and location to the aero-micropylar processes at the outer surface. Bar = 20 μm. (e) Inner face of the central portion of the anterior plate. Radially arranged faint, straight grooves (arrowheads) are visible. The fracture plane (asterisks) is smooth at the resolution used. Bar = 20 μm. (f) Inner face of the eggshell at the lateral wall. Shallow elevations (asterisks) are visible. Bar = 10 μm.](image-url)
development (Southwood, 1956), sitting in the lumen. The portion of the embryonic cuticle carrying the egg-burster with its Y-shaped, sclerotized elevation is usually flat while the remainder has collapsed (Fig. 1f). It is detectable, even at low resolution, that the empty eggshell has retained the periphery of the anterior plate and the aero-micropylar processes (Fig. 1f). This view is confirmed when eggshells are viewed at higher resolution. There is a regular and smooth fracture plane precisely at the transition between the periphery of the anterior plate and the operculum (Fig. 2a). The aero-micropylar processes are intact, but the thin sheets extending between them in earlier stages of egg development are no longer observed after hatching. It is conceivable that these thin sheets are brittle and fragment over time.

The empty eggs offer themselves for an analysis of, firstly, the eggshell in cross-fractures at various levels and, secondly, the inner face of the eggshell. The analysis of cross-fractured anterior plates and lateral walls reveals relatively regular tubes (diameter ca. 3 μm; length 13 to 17 μm) apparently extending from the bases of the individual pits visible at the egg surface. Taken together, the pits are interpreted as funnel-shaped indentations of the egg surface. The tubular portion is not continuous but possesses two to three transverse diaphragms. They are found at varying levels in the tube, but one diaphragm is usually located close to the upper end (Fig. 2b,c). The basal tubes end ca. 10 μm short of the inner surface of the eggshell, which has an overall thickness of ca. 40 μm.

Eggshells cross-fractured at the level of the rim of the anterior plate, where the aero-micropylar processes are mounted, occasionally show a straight channel that traverses the entire width of the chorion (Fig. 2b). Apparently each channel is continuous with an aero-micropylar process, that is supposedly hollow. In cross-fractures through the periphery of the anterior plate, the slender, tubular indentations seen in the operculum are not detected. Thus, it can be considered that the pits decorating the periphery of the anterior plate do not penetrate deeper into the chorion.

The appearance of the inner face of the eggshell differs between the anterior plate and the lateral wall. Some probably artificial wrinkles excepted, the surface of the former appears smooth at the resolution applied. There are, however, faint, straight grooves at the inner face of the anterior plate. They originate under the aero-micropylar processes and continue through the periphery of the anterior plate (Fig. 2d) into its central portion, where they gradually become less conspicuous (Fig. 2e). The grooves are radially arranged and spaced 32 to 50 μm from one another at their origins. This is very similar to the distances between the aero-micropylar processes at the outer surface. Therefore, and in light of the spatial arrangement of the grooves in relation to the aero-micropylar processes, there can be little doubt that we are dealing with their extensions at the inner face of the egg. The inner face of the lateral wall shows shallow elevations (Fig. 2f).

DISCUSSION

Gross morphology and outer surface

Egg morphology in representatives of the Pentatomidae has been surveyed by Southwood (1956). The eggs are barrel-shaped and studded with aero-micropylar processes at their anterior pole. The gross morphology of deposited eggs of A. marginatum, examined in detail in the present SEM study, is fully in keeping with the previous concept. The eggs of another Acrosternum species studied, A. bilare, though slightly larger, have a very similar gross surface sculpturing (Bundy and McPherson, 2000). Also the eggs of R. nebulosa (Candane and Suludere, 2001) are very similar to those of A. marginatum. Unfortunately these authors did not report structural details of the egg surface, and this lack of information precludes a closer comparison. Among the Eurydema species studied by Suludere and co-workers (1999), the pits on the surface of the eggs of E. regulorum appear to be very similar to those in the two Acrosternum species, but the surface morphology of the periphery of the anterior plate is different in that polymorphic tubercles occur. It has to be kept in mind that a coarse surface pattern used in reference to stink bug eggs applies to the bulk of the eggshell, and there may be localized variations on the egg surface. The periphery of the anterior plate may, for example, differ in its sculpturing from the operculum and the lateral wall. Eggs of other Eurydema species do not show pits at their surface, but polygons and tubercles with sunken apical surfaces abound (Suludere et al., 1999). Thus, a coarse surface pattern is not too common in eggs of stink bugs and other families of the Hemiptera. Persuing the available literature on Hemiptera (Hinton, 1961; Hartley, 1965; Salkeld, 1972; Chauvin et al., 1973; Baker and Ma, 1994; Simiczew, 1994; Chiappini and Reguzzi, 1998; Sites and Nichols, 1999; Wolf and Reid, 2000) and Homoptera eggs (Guglielmino et al., 1997) revealed no cases of a surface ornamentation similar to that seen in A. marginatum.

A. marginatum deposits its eggs in batches, and the walls of neighboring eggs of each batch are in intimate contact with one another. When the eggs are separated for preparation, disturbances in the regular surface pattern of the lateral walls become visible. There are three views to explain how these patches on the egg surface arise. Firstly, there is the possibility that the chorion is still soft, when the eggs are deposited. The variations in the lateral wall would then be mechanically introduced during the juxtaposition of the eggs. Secondly, additional material that is secreted by the female to stably glue the eggs of each batch to one another.
leads to the variations. Thirdly, a combination of the preceding operations could result in the observed patches. At present, we cannot distinguish between these alternative explanations, but the close physical contact between neighboring eggs of one batch may be of biological significance and play a role in synchronizing the hatching. It has been observed in the Southern green stink bug, Nezara viridula, that emerging nymphs stimulate hatching in adjacent eggs (Todd, 1989). It is likely that extra secreted material composes the slender threads that connect the basal pole of the egg to the substrate; these threads stabilize the eggs in an upright orientation.

The eggshell in cross-fractures

Our SEM study in A. marginatum revealed that the egg surface is covered with hexagonally arranged pits that extend into basal tubes. These extensions do not perforate the eggshell, but end some distance short of its inner face. Each tube possesses a series of transverse diaphragms. As the basal portions of the pits end blindly, they cannot be interpreted as aeropyle. Instead, as common in eggs of stink bugs (Cobben, 1968), when the gas exchange is mediated by the aero-micropolar processes. Our observation of slender channels through the chorion in the rim area underneath the aero-micropolar processes substantiates this notion.

Dotting the egg surface with indentation saves building material. The pits are small and have rounded contours. It is a principle in mechanical engineering that small omissions with rounded contours can be introduced into so-called Hookean solids without significantly compromising the mechanical strength of a given element. Biological materials such as the eggshell that are intended to fracture can be treated as Hookean solids (Atkins and Mai, 1988). It is, therefore, assumed that the pits in the eggshell of A. marginatum have only minor effects on its mechanical strength. The elaborate design of the basal tube with its diaphragms and the subtle differences in surface morphology between the central portion of the anterior plate and the lateral wall hint that there are in all likelihood biological and/or physical reasons beyond material economy that lead to the particular design of the eggshell in A. marginatum. The precise reason for this design is yet to be determined.

The anterior plate of the eggs of A. marginatum is not uniformly textured. The central portion, the operculum, with the hexagonally arranged cone-shaped pits can be clearly distinguished from the narrow peripheral zone with its cylindrical pits. During hatching, the operculum detaches precisely at the boundary between the areas showing these two forms of surface texture, and is lifted for the nymph to escape. This indicates that the structural differences visible throughout the surface of the anterior plate are not superficial but reach deeper. We have to assume that there is a structural line of weakness that facilitates hatching. Otherwise a clean and smooth fracture plane, as observed in the present study, would probably not form.

The inner surface

The analysis of the inner surface of the eggshell of A. marginatum revealed differences between the anterior plate and the remainder of the eggshell. The latter shows sculping in the form of shallow elevations. The former is smooth at the resolution used with the exception of radially arranged grooves. Their positioning highly suggests a relationship if not continuity with the aero-micropolar processes mounted on the outside of the eggshell. Principally the same spatial relationship between aero-micropolar processes and grooves was previously found in another stick bug, M. pana, which produces eggs of the spinose type (Wolf and Reid, 2001). Thus, while the external morphology varies, spinose and coarse, the appearance of the inner face of the anterior plate in stink bugs is conserved. Further work involving different species is needed in order to determine whether radially arranged grooves, dotting the inner face of the anterior portion of the eggshell, are common in eggs of representatives of the family.

Where could the function of the newly detected grooves lie? There are two interpretations derived from the roles of the aero-micropolar processes, which are in all likelihood continuous with the superficial grooves on the inner face. First, the grooves serve in dissipating gases over the apical surface of the developing embryo. Second, the grooves assist in fertilization by providing canals for the entering sperm and direct it towards an appropriate site of the plasma membrane of the egg cell. If so, this would be a central position at the apical pole. The sperm entry site controls the development of body axes in ascidians and nematodes but not in Drosophila melanogaster (Gilbert, 1997). It remains to be seen, whether the sperm entry site plays a role in specifying the body axes in representatives of the Pentatomidae.

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