EUASTRUM VERRUCOSUM EHRENB., DIVISION UNDER SCANNING ELECTRON MICROSCOPE

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THE process of cell division in placoderm desmids is unique and quite intriguing. Some of the problems it raises may help in providing a better understanding of some of the principles of morphogenesis and the control of the shape of cells in general. With each division of cell, two daughter semicells are produced, which are extremely complex and symmetrical in shape. Hence, it was thought desirable to study the division under SEM.

Euastrum verrucosum Ehrenb., 624/1, was obtained from Cambridge culture collection, U.K. The clonal unialgal cultures were established in Chu's 10 and maintained at 18–22° C, receiving alternately 16 hr light and 8 hr dark conditions.

Cell division was mostly studied under light microscope. Pickett-Heaps[1] described cell division in the widely distributed desmid, *Cosmarium botrytis* by light microscopy and also at ultrastructural level by electron microscopy. Among placoderm desmids Pickett-Heaps[2] also studied cell division in *Micrasterias denticulata* and *Micrasterias thomasiana*. However, the division was rarely studied and followed under scanning electron microscopy (SEM). The cells were studied not only for their morphology but also their division process.

Euastrum verrucosum shows markedly granulate cell-wall with prominent verrucose protuberances across the broadest part of each semicell above the isthmus[3-5]. Semicells are quite separated from each other. The ornamentation is more prominent at the protuberances. It is interesting that prominent mucilage ring is seen at the isthmus region.

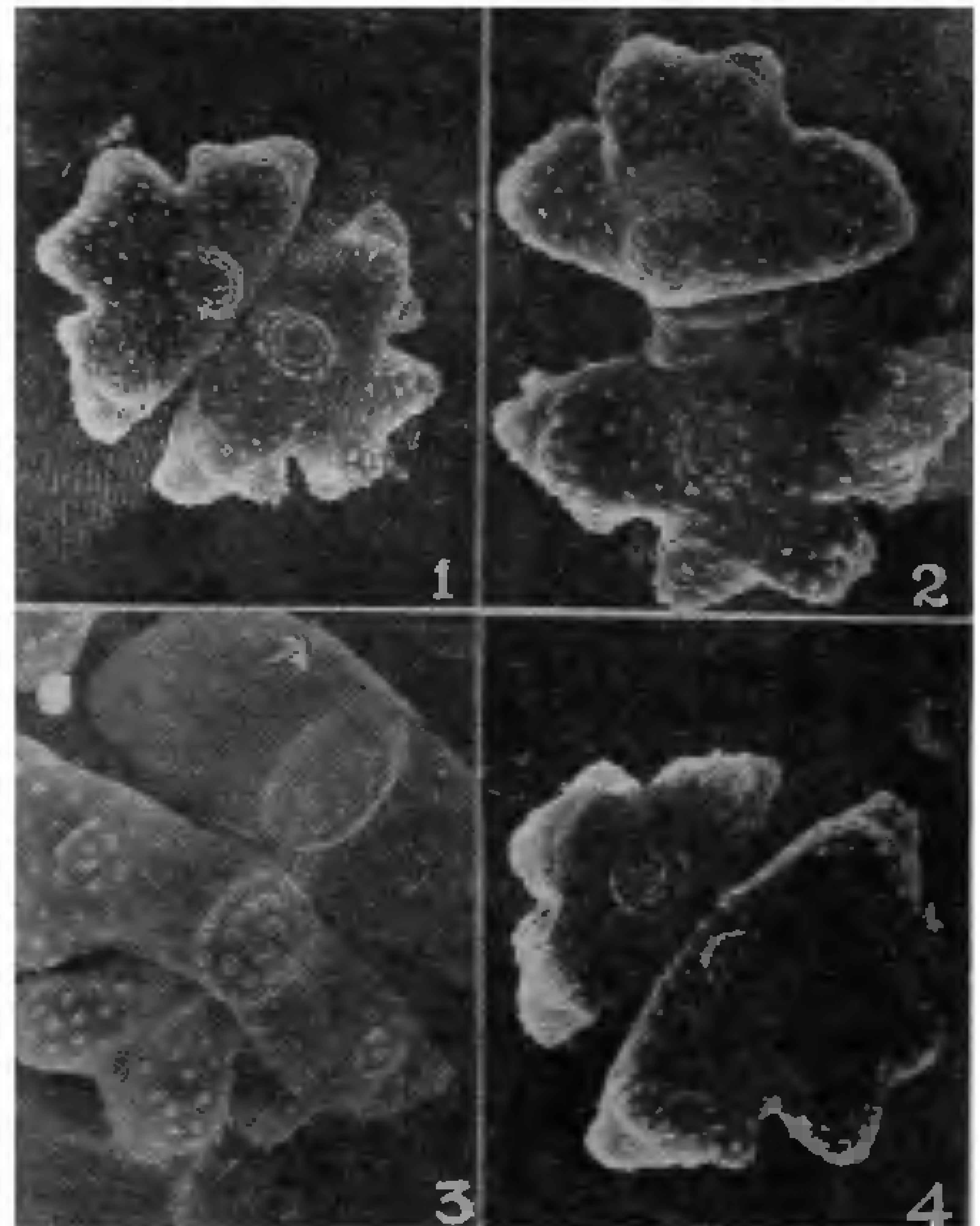
To study the development of newly-formed semicells during division, the cells from actively growing cultures were fixed in glutaraldehyde and osmium tetroxide for scanning electron microscopy with an hourly interval of 24 hr. These samples were then dehydrated with acetone, dried in critical point drying apparatus, coated with gold and carbon and studied under SEM at 15 kV (*Joel-JSM*-25S).

Premitotic cells were very dense as compared with cells following division. The two daughter cells became highly vacuolated during cell expansion, and as might be expected, their cytoplasm was much less dense than in the single premitotic cell.

Division was initiated by a cylindrical strip of membrane on the inside wall at the level of the junction of the two semicells. From this strip the septum arises as an annular growth, and when full closed cross the cell; it splits into two, so that each original semicell acquires a new daughter semicell which gradually extends longitudinally until it reaches its maturity. The semicells concurrently separate slightly, a sure sign of imminent division.

The secondary wall secreted just before the cells were fully expanded. This wall was totally different in appearance from the primary wall, being even and dense. During formation, it matches and then attains the pattern of ornamentation already established by the primary wall.

The daughter cells remain joined to one another, apex to apex, until the casting off or shedding of the primary wall, which can often be seen in cultures of desmids, near recently divided cells. Brandham's[4] time-lapse films show that the daughter cells may then move apart quite vigorously, presumably due to



Figures 1–4. *Euastrum verrucosum* Ehrenb. **1.** Cell in front view showing ornamentation (×560). **2.** Cell showing isthmus elongation and formation of cylindrical strip of membrane (×840). **3.** Cell in surface-view showing one of the semicell smooth and a mucilage ring at the isthmus region (×840). **4.** Young semicell showing lobe formation and ornamentation (×560).

extrusion of mucilage. All these stages of cell division were completed within a period 20–22 hr, and the various stages of division are shown in figures 1–4.

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INDUCED MUTATIONS FOR SEED PROTEIN IMPROVEMENT IN *HORDEUM VULGARE* L.

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An assemblage of short-awned mutants was induced by ethyl methane sulfonate (EMS) in diploid, Himalayan hull-less barley. These mutants were classified into triple, double and single short-awn types. Their awn lengths being 1.5, 2.7 and 4.2 cm, respectively in comparison with 12 cm of the control. Seeds of these short-awn types were analysed quantitatively for protein by macro-Kjeldahl on Kjeltec System II and for DBC (mg dye bound per gram of sample) by Udy Protein Analyser. All the three categories of short-awned mutations exhibited significantly higher protein levels and were around 20% richer in protein content (table 1).

Negative correlationship between protein content, grain weight and yield is already well documented. Bansal *et al.*[1] have however reported successful positive alterations in the relationship between grain weight and protein content. The degree of adversity in our studies varied in all the three types. In the single short-awn, the weight was less than that of the control; however, the protein content was higher. While, in the double short-awn, the weight was a little on the lower side and the protein content was higher than that of the single short-awn as well as of the control. Whereas in the triple short-awn, the weight as well as the protein cotent was almost similar to that of the double short-awn mutants. Hence, it is apparent that the shortening of the awns had adversely affected the weight of the seeds, perhaps through reduced photosynthetic area[2,3].

Although the reduced weight of the seed was the reflection of the mutation in the gene controlling the awn length, this alteration also affected the protein synthesising potentials of the genotype in almost all the cases. In all the three mutants, the yield per plant remained almost the same and if the quantity of protein per gram of seeds per spike is taken into consideration, the higher protein synthetic potentials of the double and triple short-awn mutations were apparent. Thus, this report for the first time demonstrates the positive alterations in the adverse relationship between the grain weight and protein quantity in the double and triple short-awn mutants through induced mutations.

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Table 1

Changes in the association between protein quantity and grain weight in control and advanced induced mutant progenies.

Sample	Protein(%)		DBC (mg dye bound per gm of sample)		1000 grain weight (g)	
	Range	Mean	Range	Mean	Range	Mean
Control	16.9–17.0	16.9	41.4–41.7	41.5	3.7–3.9	3.8
Single short-awn	18.6–20.8	19.7	43.5–44.6	44.5	2.6–2.7	2.6
Double short-awn	20.3–20.8	20.5	46.3–46.3	46.3	2.2–2.4	2.3
Triple short-awn	19.8–20.3	20.0	46.0–46.2	46.1	2.3–2.3	2.3