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Wall Structure During Stages in Development of *Selaginella pulcherrima* and *S. haematodes* Megaspores

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ABSTRACT: There are hundreds of exospore units that extend across the gap and into the mesospore in middle stages in megaspores of *Selaginella pulcherrima* Liebm.ex Fourn and *S. haematodes* (Kunze) Spring. The mesospore of these megaspores is resistant to acetolysis during middle stages suggesting the presence of sporopollenin. There are also spheroidal structures that are resistant to acetolysis on the surfaces of the outer and inner exospore in both species in middle and mature stages. In late stages the mesospore no longer exists and the inner exospore is a narrow stripe, i.e., there is no longer a space (gap) between outer and inner parts of the exospore. Our interpretation for the loss of the mesospore is that sporopollenin does not become polymerized under physiological conditions to its exceptional state of resistance to reduction, but does so when exposed to native or experimental oxidation; in our case, exposure to the atmosphere by cutting open the megaspore and plunging it into the acetolysis mixture.

KEY WORDS: Exospore unit structures, Megaspores, Mesospore resistance, Mesospore degrading, *Selaginella*.

INTRODUCTION

Morbelli and Rowley (1999) and Rowley and Morbelli (1995) found that a major gap separated distal and equatorial portions of the exospore of megaspores of *Selaginella argentea*, *S. bigellowii, S. kraussiana, S. diffusa, S. erythyropus* and *S. pulcherrima* early in their development. As a result the mesospore remains in contact with the new inner portion of the exospore and both structures are separated by the gap from the outer and main part of the exospore. The outer exospore is greatly enlarged in diameter by lateral growth of the exospore components (Rowley and Morbelli, 1995: Figs. 34-39). The mesospore and its covering of inner exospore become thickened by radial enlargement of their units, e.g., Morbelli and Rowley, 1999: Figs. 7E & 9C, but the mesospore does not enlarge in diameter to the extent of the outer exospore (Rowley and Morbelli, 1995: Table 1, Figs. 4A & 5D).

The results of Rowley and Morbelli (1995) and Morbelli and Rowley (1999) indicated that the mesospore is a normal part of megaspore development. The "wicks" described by Morbelli and Rowley (1993) extend from tapetal cells across the exospore, gap and mesospore to the megaspore protoplast; they considered wicks to be plasmodesmatal equivalents.

The mesospore is degraded during the pregermination stages in megaspore maturity as reported by Morbelli & Rowley (1999) so that in mature megaspores there is no mesospore.

Structures across the gap in the exospore of *Selaginella* megaspores during young and middle stages of development have been recognized at least as early as the publications of Mettenius in 1850 and Pfeffer in 1871. Since then they have been illustrated, mainly in

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camera lucida drawings, in works of Heinsen (1894), Campbell (1895, 1902), Arnoldi (1896), Bruchmann (1897, 1912), Fitting (1900), Denke (1902) and Lyon (1901,1905). Pettitt (1966) provided the start for ultrastructural studies of megasporogenesis. Since then, structures in the gap region of the exospore have been a part of illustrations based upon photomicrographs, SEM or TEM by Sievers and Buchen (1970, 1971), Buchen and Sievers (1978a, b, 1981), Pettitt (1971a, b), Minaki (1984), Taylor (1991, 1994), Rowley and Morbelli (1995), Morbelli and Rowley (1999), Morbelli *et al.* (2001) and many studies by Lugardon which are, to some extent, summarised in the book by Tryon and Lugardon (1991).

Many of the writers were of the opinion that both the gap and its contents were either an artefact or were only present in megaspores that had aborted. According to the results and interpretations of Morbelli and Rowley (1999) the idea of a abortion is to some extent correct in so far as finding a gap in fossil megaspores is concerned. It is in megaspores from sediments that this abortion interpretation has a clearly decisive relevance to the matter in hand. What Rowley and Morbelli (1995) and Morbelli and Rowley (1999) found is that the gap forms during early and middle development in association with the mesospore. In a megaspore that develops through to maturity the mesospore is degraded, becoming entirely absent as a structure, and the inner-exospore has become closely adjacent to the outer-exospore. Thus at the end of normal development there is neither a gap nor a mesospore while both may be preserved in members of a megaspore tetrad that have not completed their development (see notes about Heinsen's 1894 experiments below).

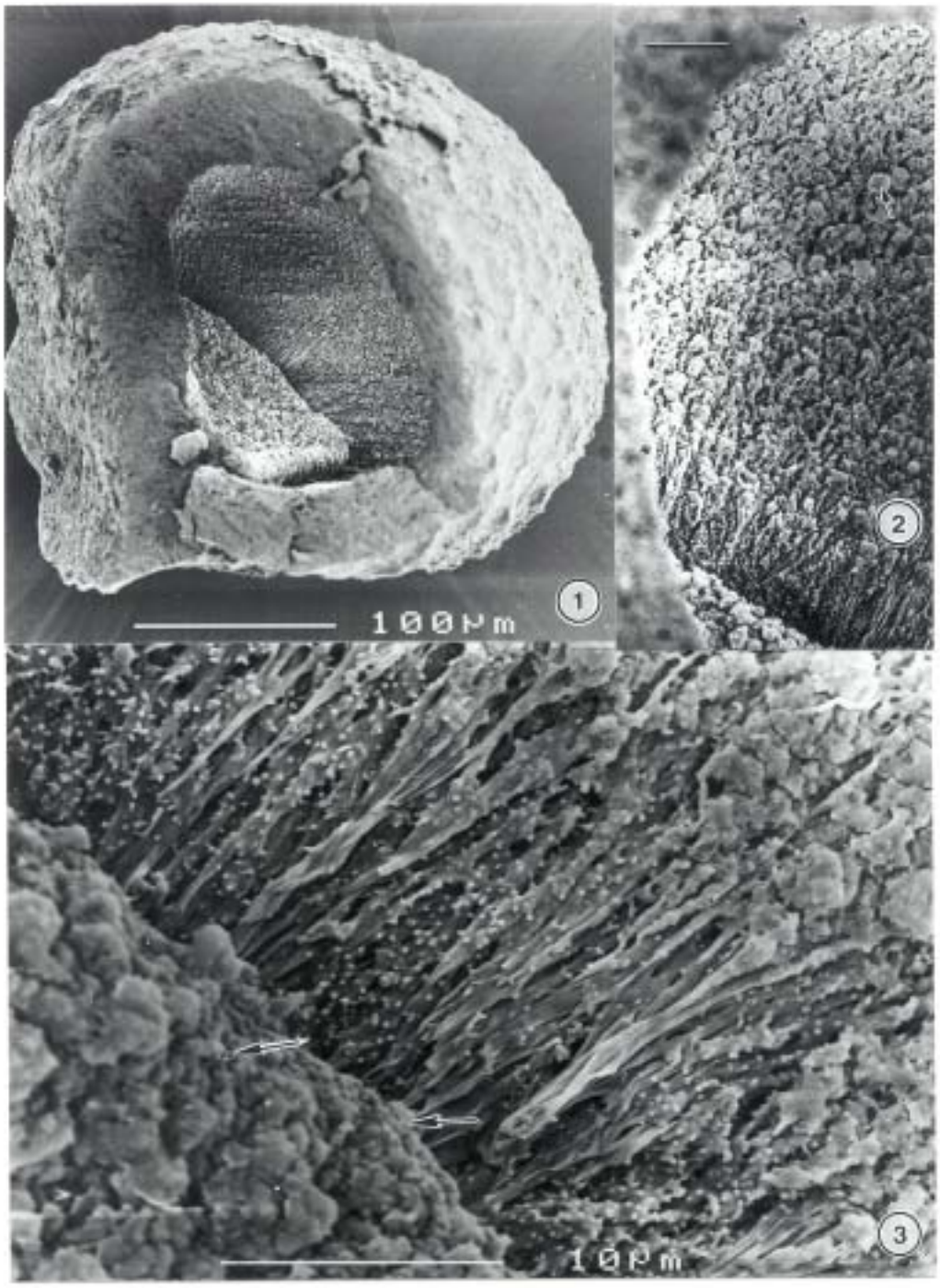
Our aim was to determine the resistance of the mesospore in young stages, during its development and presumed functional period, to the hot acid "acetolysis" reaction of Erdtman (1960). Acetolysis is credited with the destruction of most organic molecules except for sporopollenin. If the units of the mesospore resist acetolysis then presumedly they may consist of sporopollenin. The degradation of a sporopolleninous mesospore would represent an unexpected result with regard to the stability of sporopollenin.

MATERIALS AND METHODS

The megaspores were dissected from living material grown in the greenhouses of the Botany Department of Stockholm University. Different stages in development were selected according to spore size and characteristics (shape, colour, ornamentation, etc.). Spores were cut in half and transferred to acetone for dehydration. One half was prepared for SEM soon after the living megaspores were sectioned; this half was transferred with a fine brush to a specimen stub. The other half was dried in acetone, placed in acetolysis mixture (Erdtman 1960) and heated to 100^{o}C for 12 min before washing and preparation for SEM. The preparations were sputter coated with gold-paladium and examined with a Cambridge Stereoscan 600 or JEOL JSM-6100 scanning electron microscope (the latter at 5-kV).

RESULTS

In a middle stage of development in *S. pulcherrima* megaspores the gap between the outer and inner exospore is crossed by hundreds of exospore unit-structures (Figs. 1-3). There are also many spheroidal structures that are 1 to 2 μm in diameter within the gap and these are located mainly on or near the surfaces of the inner and outer exospore. They are also located both on the surface of the units crossing the gap and on the surfaces of the inner and outer exospore (Fig. 3). All of these structures resisted acetolysis.



Figs. 1-3. Middle stage in megaspore development in *S. pulcherrima.* Material sectioned in half and acetolyzed for four minutes at 100 °C. Fig. 1. There is a big gap in the exospore in distal and equatorial regions. The mesospore and its cover of inner exospore are attached to the proximal pole. Because of the covering of inner exospore the mesospore can only be seen in sections (e.g., Figs. 6B and 9). At this stage the gap is crossed by unit-structures. Bar = 100 µm. Fig. 2. Enlargement of figure 1. At this magnification there is an enhancement of the 3-D appreciation of the unit-structures passing under the outer exospore (asterisks). There is a small portion of the inner exospore at the lower left. There are many spheroidal structures (arrows) associated with the upper parts of the unit-structures. Bar = 10 µm. Fig. 3. Thousands of exospore unit-structures (rods) cross the gap and contact the surface of the inner exospore (arrows) that covers the mesospore. There are spheroidal structures on the surface of the inner exospore and on the unit-structures that cross the gap. Bar = 10 µm.

In almost mature megaspores of *S. pulcherrima* (Fig. 4) there are spheroidal structures on the inner surface of the outer exospore and outer surface of the inner exospore (Fig. 5) and the mesospore no longer exists.

A megaspore of *S. haematodes* is shown in Fig. 14. It has very distinctive features, such as a pyramidal proximal area with high laesurae and a double equatorial pleated flange. The megaspores in Figs. 6A & B, 7 and 9 were cut in two. One half of each megaspore was prepared for SEM without any treatment (Figs 6A & B, 7 and spheres in Fig. 8) while the other half (Fig. 9) was kept in hot (100^{o}C) acetolysis mixture for 12 min. It can be appreciated in these micrographs and the enlargements of the treated half in Figs. 9-11, that the mesospore was retained throughout the acetolysis treatment.

Near maturity in *S. haematodes* (Figs. 12-13) most of the mesospore was degraded and spheres of variable size were evident on the inner surface of the outer exospore. At maturity (Figs. 15-16) the mesospore no longer exists (Fig. 15) and the inner exospore is a thin sheet-like structure which is folded in Fig. 15 and in contact with the outer exospore at the proximal pole. Both the inner portion of the exospore and the spheres (which are common structures in the gap between the outer and inner exospore, Fig. 16) on the gap surfaces resisted the acetolysis procedure.

DISCUSSION

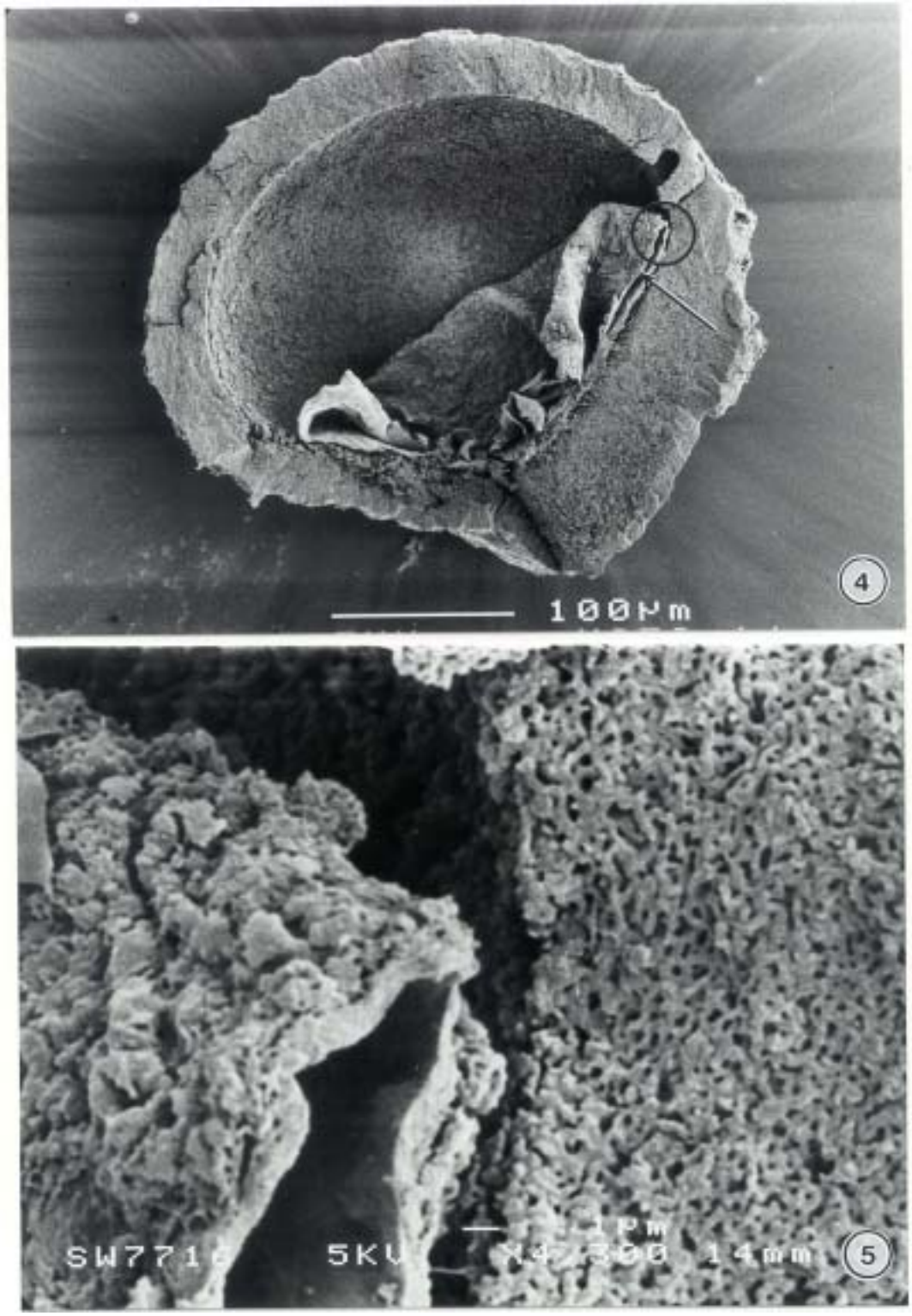
In the megaspores we have exposed to the Erdtman (1960) acetolysis procedure the mesospore has been found to be resistant.

Our interpretation of the acetolysis resistance of the mesospore and its loss late in maturation is that while the mesospore is sporopolleninous its sporopollenin is not polymerized to the legendary resistance to reduction while it is under physiological (living and nonoxidative) conditions. The great resistance of sporopollenin only occurs after exposure to the atmosphere (oxidative conditions). In our experiments the megaspores and their mesospores were exposed to the atmosphere and to the acetolysis mixture.

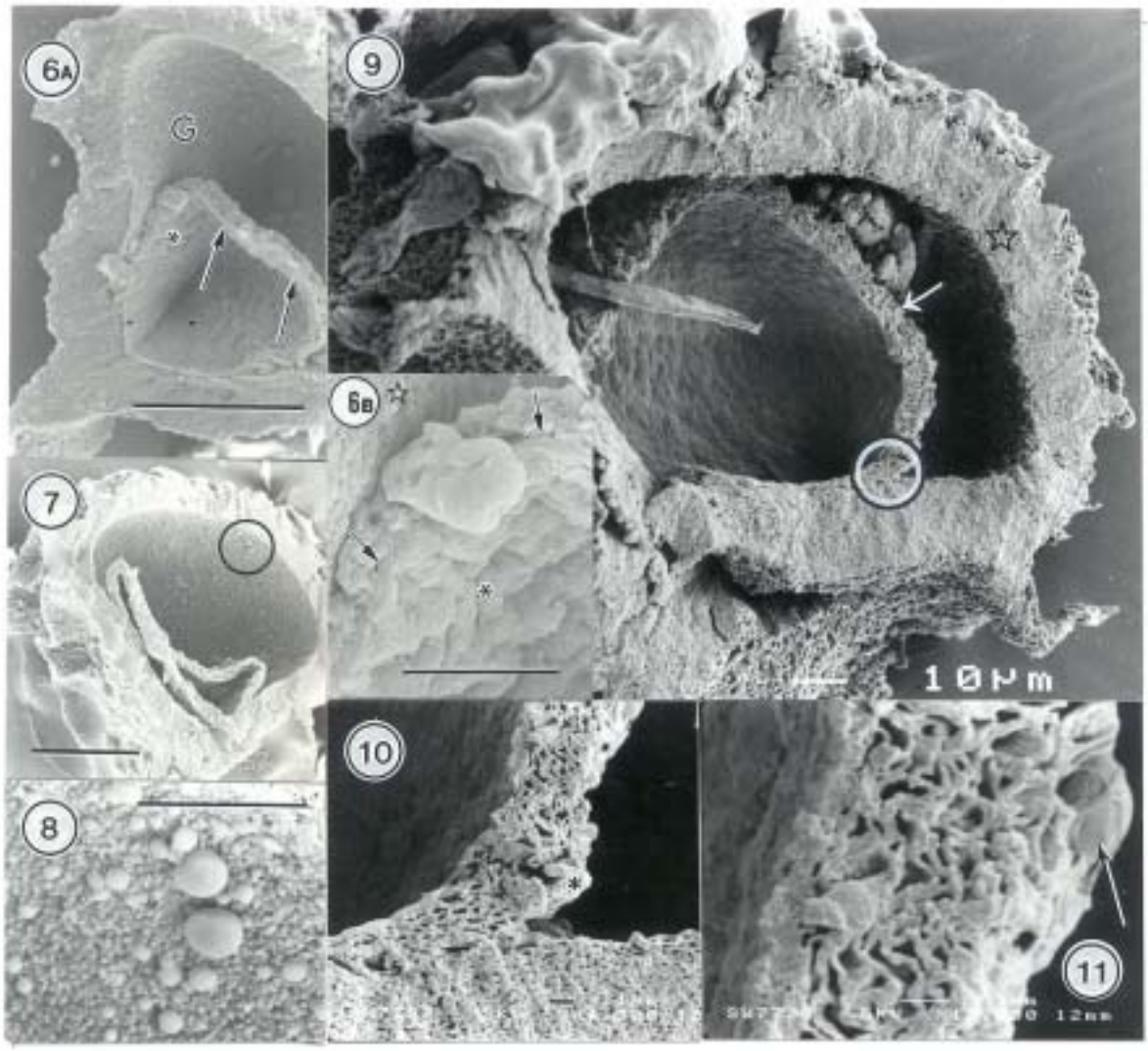
Pettitt (1966) noted that the mesospore could resist fossilization. This was in agreement with results of Taylor and Taylor (1988), Archangelsky and Villar de Seoane (1990) and Taylor (1994). These authors had interpreted the presence of a mesospore in fossilized material as due to abortion. The abortion idea is based upon the common observation that one, two or even three of the megaspores in tetrads, in many species of *Selaginella*, remain small (immature). These have generally been assumed to have aborted.

Heinsen's (1894: 474-475) ingenious germination experiments cast doubt on the idea that the presence of the mesospore in fossil megaspores results from abortion, as we noted previously (Morbelli and Rowley, 1999). He put mega-and micro-spores of eight speicies on peat-soil and blotting-paper. He removed all germinating megaspores every two days and after four weeks when there were no new germinations, he added new microspores. These procedures were repeated and megaspores of all species were still germinating after one and a half year.

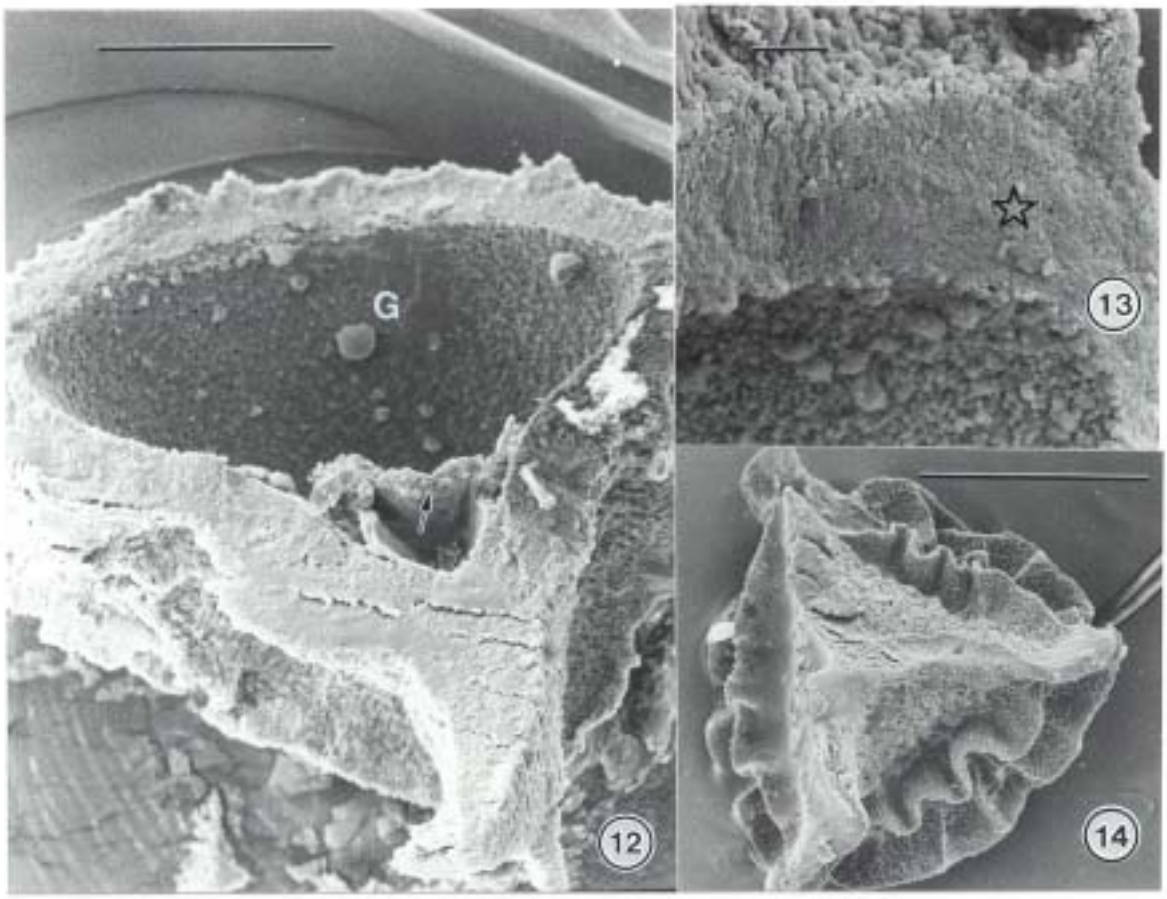
The experiments of Heinsen (1894) have not been repeated, so far as we know; but his results, if confirmed, would indicate that the genus *Selaginella* "holds back" members of a tetrad for continued later development when conditions may be favourable. This can be one reason for the very long success of this genus in environments from the far north to the far south.



Figs. 4-5. A megaspore of *S. pulcherrima* at a mature stage, sectioned in half. Fig. 4. The mesospore has already been degraded and the inner exospore is a thin layer (arrow) folded on the proximal part of the spore showing the exospore units. The location of figure 5 is circled. Bar = 100 µm. Fig. 5. Enlargement of a portion of Fig. 4. The outer exospore is at the right. The inner exospore to the left is folded and covered by many spheroidal structures. In living megaspores at this stage the inner exospore is pressed against the outer exospore by nutrients filling the central space, when these are lost during preperation the inner exospore commonly becomes folded. Bar = 1 µm.



Figs. 6-11. Sections of a middle stage in development in a *S. haematodes* megaspore. The sectioned mesospore in figures 6-7 was not acetolyzed. The megaspore in figures 9-11 was acetolyzed for 12 minutes. Fig. 6A. The section shows a thin mesospore (arrows), see figure 6B. The inner surface of the mesospore is rugulated (asterisk). There is a large gap (G) in distal and equatorial regions at this stage. Bar = 50 μm. Fig. 6B. An enlarged portion around the asterisk in figure 6A. The asterisk marks the rugulated inner surface of the mesospore. The outer edge of the mesospore is marked by arrows. The arrows themselves are over the inner exospore. The surface of the outer exospore is marked by a star. Bar = 10 μm. Fig. 7. The thin (<10 μm) mesospore is attached to the outer exospore at the proximal pole. There are spheres (circled) on the outer surface of the gap. Bar = 50 μm. Fig. 8. The spheres on the circled inner surface of the outer exospore in figure 7. Bar = 1 μm. Fig. 9. In this section of the acetolyzed megaspore there are contents in the gap between the outer exospore (star) and inner exospore and mesospore (arrow). The arrow is in contact with the inner exospore. The junction between the inner and outer exospore is circled. Bar = 10 μm. Fig. 10. An enlargement of the circled region in figure 9 showing that the structure of the exospore and mesospore have different patterns as seen in section. Inner part of the outer exospore at the bottom and inner exospore (asterisk) covering the mesospore. Bar = 1 μm. Fig. 11. Section of the mesospore showing unordered exospore units. A sphere (arrow) is seen as part of the inner exospore covering the mesospore. Bar = 1 μm.



Figs. 12-14. Megaspores of *S. haematodes* near maturity; they were not acetolyzed. Fig. 12. A megaspore cut in half. The gap (G) is large. Most of the mesospore (arrow) has been degraded. Most of the inner exospore and remnant of the mesospore were cut away in sectioning the megaspore. There are many spheres on the inner surface of the exospore (see figure 13). Bar = 50 μm. Fig. 13. An enlargement of the outer exospore in Fig. 12. The exospore surface is at the top. It is perforated. The sectioned exospore is marked by a star. Large and small spheres are on the inner surface of the outer exospore. Bar = 5 μm. Fig. 14. A proximal view of a megaspore. The three proximal faces are pyramidal and the complex structures of the equatorial zone (collar, flange) are evident. The aperture is high. Bar = 100 μm.

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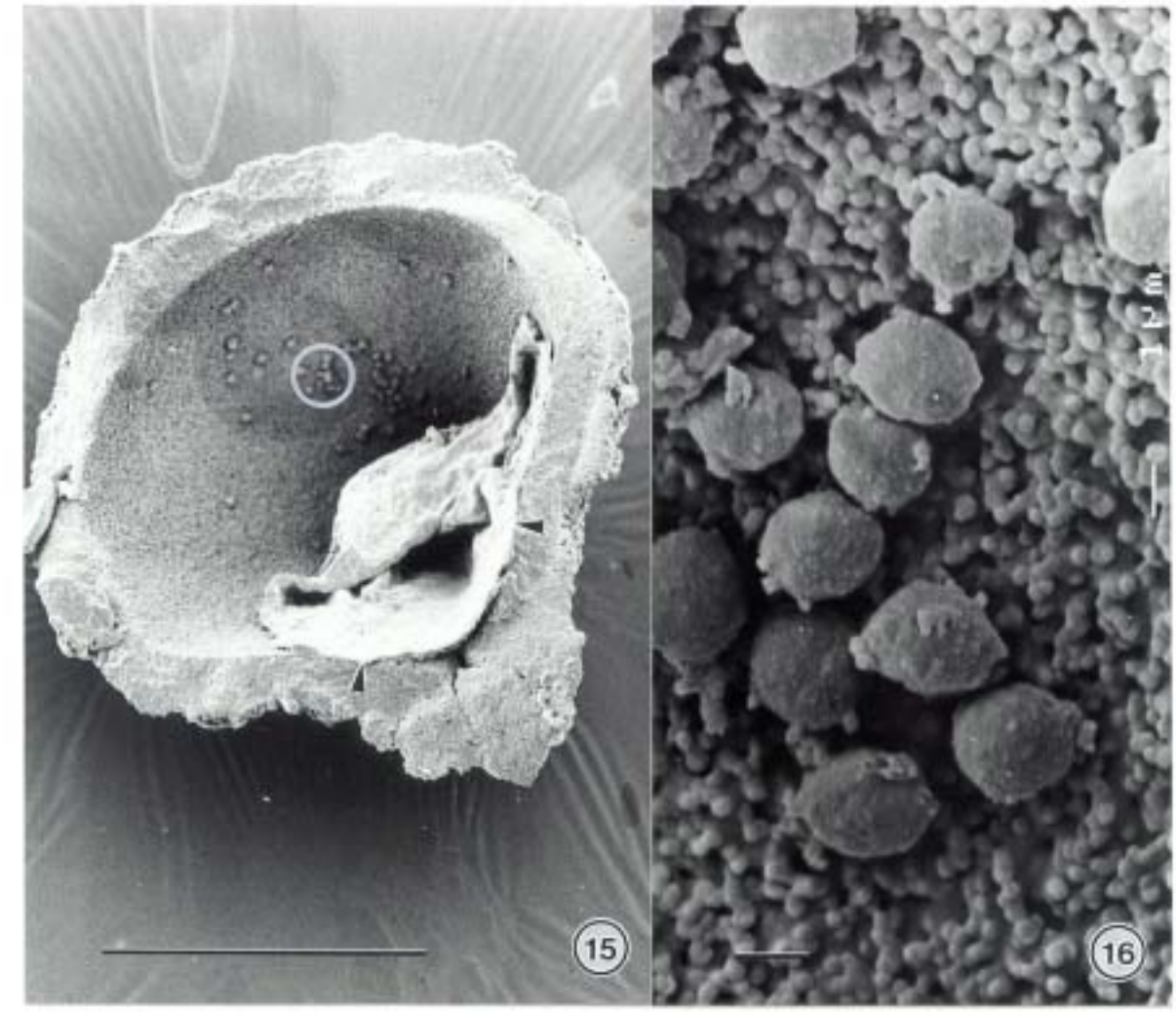

Figs. 15-16. A mature megaspore of *S. haematodes* cut in half and acetolized. Fig. 15. The mesospore has been degraded. The inner exospore is folded and is in contact (arrowheads) with the outer exospore only in the proximal zone. The region of figure 16 is circled. Bar = 100 μm. Fig. 16. Some of the spheroidal structures that are 1-2 μm in diameter and small spheres can be seen on the inner surface of the outer exospore. Bar = 1 μm.

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美麗卷柏及紅色卷柏大孢子之發育

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摘　　要

美麗卷柏及紅色卷柏之大孢子在發育中期，會有數以百計的外壁單元穿過間隙(gap)進入中壁層。這些發育中期的中壁層可抵抗酸化分解，顯示其中已有孢粉素存在。此兩種卷柏之大孢子發育中期及成熟時期，大孢子的外壁之內、外表面上皆具有球狀構造，此構造亦能抵抗酸化分解。發育晚期，中壁層消失，外壁內層僅呈一條細帶，亦即外壁層的內、外層之間的空隙已不再存在。我們推論：中壁層的消失並非其在抗還原期(特殊的發育期)之生理作用使然，而是大孢子由於本身特性而暴露於大氣中，或在實驗操作中氧化，而使得孢粉素聚合化並使中壁層消失。在我們的例子中，由於切開大孢子使其暴露於大氣中，又加以酸化處理才獲至此結果。

關鍵詞：外壁層單元結構、大孢子、中壁層抗性、中壁層降解、卷柏。

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