

**SPOROGENESIS IN POLYPODIACEAE (FILICALES). I.
DRYNARIA SPARSISORA (DESV.) MOORE**

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SUMMARY

The development of the sporoderm in the fern *Drynaria sparsisora* (Desv.) Moore (Polypodiaceae) is described after application of different methods of electron microscopy. Scanning electron microscopy of freeze fractured sporangia in different stages has shown a succession of different patterns on the spore surface during exospore formation.

INTRODUCTION

For several years now, pteridologists from the Netherlands and various other countries have concentrated on the study of one family of ferns, the Polypodiaceae, by way of monographs and analysis of characters, such as those of the spores; this project is co-ordinated by Prof. Dr. E. Hennipman (State University of Utrecht, The Netherlands; Hennipman, 1984, 1985). The present study on sporogenesis was set up in order to further elucidate relationships within this family.

Study of sporoderm ontogeny may contribute to: 1) a better understanding of the variation encountered in mature spores; 2) the establishment of homologies between the various layers of which the sporoderm consists in different groups; 3) finding new ways of applying spore characters to the elucidation of phylogenetic relationships between species, groups of species on different taxonomic levels such as the genus or family level, and even between ferns and other groups of plants.

In order to study sporogenesis, transmission electron microscopy (TEM) is the technique most often applied (e. g., Lugardon, ref. div.). Recently, in a study of *Cosmos* pollen, Blackmore & Barnes (1985) described a freeze fracturing technique that allows observation of sporogenesis with the scanning electron microscope (SEM). The present study includes one of the first applications of this technique to sporogenesis in a fern, *Drynaria sparsisora*. It is the first in a series of studies on sporogenesis in the family Polypodiaceae.

Spores of Polypodiaceae (Hennipman, 1990) are bilateral and monolete. Their sporoderm consists of:

- a perispore, the outermost layer, which can be very thin and adhere closely to the exospore surface, or thicker and variously ornamented, and which is present in all species belonging to this family;

- a relatively thick exospore, the middle layer, which may be smooth or ornamented in different ways;
- an endospore, the innermost layer, which is not present until germination sets in.

Stages in sporogenesis

In an earlier paper (Van Uffelen, 1986) I distinguished six main stages during sporogenesis, mainly based on the study of TEM micrographs:

1. presence of spore mother cells (smc's) (see Plates I & II)
2. meiosis (see Plate III)
3. formation of the spore plasmalemma (see Plate III)
4. formation of the inner exospore (ie) (see Plates IV & V)
5. formation of the outer exospore (oe) (see Plates V–IX)
6. formation of the perispore (see Plate X)

Now that SEM micrographs of sporogenesis in *Drynaria sparsisora* have become available as well, in this species stage 5, the formation of the outer exospore, can be divided into different substages by the different surface patterns found during the deposition of this layer.

Previous studies

Many others have already studied sporogenesis of land plants, during the last two decades mainly with the TEM:

- *bryophytes*: Brown & Lemmon (1988, review), Denizot (1974, Hepaticae), Neidhart (1979, review);
- *ferns and fern allies*: Bell (1981, 1985, *Marsilea* megaspores), Brunkener (1973), Buchen & Sievers (1978a & b, *Selaginella* megaspores), Gabarajeva (1984, early stages in *Psilotum*), Hennipman (1970, 1977, *Bolbitis*), Herd, Cutter, & Watanabe (1985, *Azolla* microspores), Huang & Chiang (1983, *Isoetes*), Lehmann, Neidhart, & Schlenkermann (1984, *Equisetum*), Lugardon (1966, 1969, *Blechnum*; 1971, *Ophioglossum*, *Osmunda*, *Blechnum*; 1972, 1974, 1979, *Psilotum*), Mitui (1974, *Polystichum* perispore), Pettitt (1971, 1979), Schraudolf (1984, *Anemia*), Sheffield & Bell (1979, *Pteridium*, 1987), Singh & Devi (1989, *Pteris*), Surova (1981a & b, *Anemia*), Tilquin (1980, Hymenophyllaceae), Uehara & Kurita (1989, *Equisetum*), Van Uffelen (1985, 1986), Verma & Khullar (1976);
- *spermatophytes*: Bhandari, 1984 (review).

On sporogenesis in general work has been published by Blackmore & Crane (1985, 1988), Buchen & Sievers (1981), Heslop-Harrison (1964), and Locquin (1981). Some special aspects of sporogenesis have been treated by Bienfait & Waterkeyn (1976, sporocytic layers), Pacini et al. (1985, tapetum), and Pettitt & Jermy (1974, surface coats on spores).

MATERIAL AND METHODS

Among the Polypodiaceae available in the Leiden Botanic Garden, *Drynaria sparsisora* (Desv.) Moore from southeastern Asia, northern Australia, and islands in the southwestern Pacific (Roos, 1985) turned out to be the most convenient species for the study of sporogenesis. The plants produce fertile fronds almost the year round and usually have many developmental stages in one sorus (Plate II-1). I applied LM, SEM, and TEM to developing and mature sporangia of this species.

Four different plants have been studied.

From the Botanic Garden in Leiden, the Netherlands:

- *LEI 20339* (coll. Hennipman, Sulawesi) with which all TEM and some SEM work has been done; this plant has rather fragile sporangium stalks, which break very easily during SEM fixation.
- *LEI 22226* (coll. Franken and Roos, Sumatra) which was used for some LM and most of the SEM work.

From the Botanic Garden in Utrecht, the Netherlands:

- *81GR00119* (coll. Roy. Soc. Exped. nr. 4591, St. Isabel, Solomon Islands, via Kew Gardens) which was used for some SEM and TEM work.
- *84GR00187* (raised from spores from the Botanic Garden in Liberec, Czechoslovakia) which was used for some SEM work.

Table 1. Plants of *Drynaria sparsisora* studied.

	LM	SEM ¹	SEM ²	TEM ¹	TEM ³
<i>LEI 20339</i>	-	-	+	+	+
<i>LEI 22226</i>	+	+	+	-	-
<i>81GR00119</i>	-	-	+	-	+
<i>84GR00187</i>	-	-	+	-	-

1) fixed in 4% P-buffered paraformaldehyde, followed by 1% P-buffered OsO₄.

2) fixed in 1% P-buffered OsO₄.

3) fixed in (2–)3% P-buffered or cacodylate buffered glutaraldehyde, followed by 1% similarly buffered OsO₄.

Fixation

As buffers, 0.1M cacodylate buffer, or 0.134M P-buffer were used. For study with the SEM, small pieces of frond, each bearing one sorus, were treated in the following way (Blackmore & Barnes, 1985): with 1% P-buffered OsO₄ only (2–20

hrs), or 4% P-buffered paraformaldehyde (Pease, 1964) followed by 1% P-buffered OsO₄; freeze fracturing in 50% DMSO; long (14 days) postfixation in 0.1% P-buffered OsO₄, followed by 1% P-buffered OsO₄ (1 hr), 2% tannic acid (16 hrs), 1% P-buffered OsO₄ (1 hr); dehydration in an alcohol series and critical point drying in DMM.

After this treatment, the broken sori were mounted onto aluminum stubs (10 mm ϕ) with Scotch double-sided tape and coated with gold. For the study of mature spores, untreated spores from dried fronds were mounted with glue in acetone and coated with gold in the usual way (Van Uffelen & Hennipman, 1985). Micrographs were made on Kodak Panatomic-X FXP film (60 \times 60 mm negatives) with the JEOL JSM-35 scanning electron microscope at the Rijksherbarium, Leiden.

Some micrographs were made on the Hitachi S800 scanning microscope in the Natural History Museum (London).

For study with the TEM, sori, preferably without any part of the frond attached, were treated in the following way (Pease, 1964; Lugardon, 1971): with 4% P-buffered paraformaldehyde (2–4 months), or with 3% P-buffered or cacodylate-buffered glutaraldehyde (c. 5 or 24 hrs), or with 0.75% KmNO₄ in water (30 minutes); with 1% buffered OsO₄ (60–80 minutes or 24 hrs); dehydration in an alcohol series and embedding in Epon resin.

Sections were made with glass knives or a diamond knife on various microtomes. Sections were mounted on one hole or mesh grids and coloured with a 1–5% (almost saturated to saturated) solution of uranyl acetate in water and lead citrate (Reynolds, 1963). The sections were observed with a Hitachi HU 11B microscope (and photographed on Gevaert 'Scientia' 23-D-50 plates) or with a Philips EM-300 (and photographed on Kodak FRP 426 film).

Measurements

Measurements have been made of sporangium size, spore size, sporoderm thickness and some other features as seen on SEM and TEM micrographs. I am fully aware of the relative unreliability of these measurements by reason of the obliquity of sections (TEM) or spore position (SEM), and fluctuations in magnification of the apparatus used; moreover, the number of measurements is always rather small (see also Mogensen, 1981). Despite these difficulties in using SEM and TEM micrographs in order to make measurements of spores, LM could not be applied as it is not possible to assess accurately the stage of a sporangium using a light microscope.

Terminology

The following list contains only terms used in this publication and is not a complete list of terms as used in describing the morphology and ontogeny of fern spores. Such a list in English is still lacking for the spores of ferns and fern allies and is being prepared by the present author; a list of terms applicable to pollen, and partly to spores, is being prepared by S. Blackmore, A. Le Thomas, S. Nilsson, and W. Punt, and a list pertaining to fossil spores and pollen has recently been published (Traverse, 1988).

The terminology used in this publication is mainly based on the ultrastructural work of Lugardon (1971, 1975, 1978a, 1978b, 1981), who studied sporogenesis in several ferns with the transmission electron microscope. Very few terms are purely topographic or morphologic; most of the terms describing spore morphology have some ontogenetic connotations.

Fern terminology

Ferns and fern allies can be homosporous (Psilotales, Lycopodiales, Equisetales, Ophioglossales, Marattiales, and Filicales) or heterosporous (Selaginellales, Isoëtiales, Marsileales, and Salviniiales). Their life cycle consists of a diploid sporophyte producing haploid spores, and a haploid gametophyte producing gametes.

Fern — plant belonging to the order Ophioglossales, Marattiales, Filicales, Marsileales, or Salviniiales. It is a megaphyllous spore-bearing vascular plant.

Fern ally — plant belonging to the order Psilotales, Lycopodiales, Selaginellales, Isoëtiales, or Equisetales. It is a spore-bearing vascular plant with small leaves.

Frond — a megaphyllous fern leaf.

Gametophyte (adj. *gametophytic*) — The sexual generation of a plant that produces gametes, or an individual of this generation; e.g., the haploid generation of an embryophytic plant, produced by germination of the spores (see Traverse, 1988).

Leptosporangiate — of a fern with sporangia initiating from a single cell; the sporangium wall is only one cell layer thick (contr. eusporangiate, as in Ophioglossales and Marattiales).

Pteridophyte — a fern or fern ally (see Mabberley, 1987).

Sorus (pl. *sori*) — a group of sporangia (Plate II-1).

Sporangium (pl. *sporangia*) — an organ of the sporophyte in which meiosis takes place and haploid spores develop with the aid of a diploid tissue, the tapetum (Plates II-2, III-1).

Sporophyte (adj. *sporophytic*) — the diploid phase of the pteridophyte life cycle. In sporogenesis the term sporophytic applies to the diploid cells (spore mother cell, tapetum, sporangium wall) possibly involved in some stage of sporogenesis.

Spores and sporogenesis

Bilateral — of monolete spores, having two planes of symmetry perpendicular to each other, both passing through the polar axis (see Lugardon, 1971) (Plate XI-1).

Callose (adj. *callosic*) — a carbohydrate component of cell walls in certain plants; e.g., the amorphous cell wall substance that envelops the pollen mother cell during pollen grain development (see Traverse, 1988).

Channels — of the outer exospore: perforations that traverse the outer exospore and that connect the inner side of this layer with the exospore environment (see Lugardon, 1971) (Plate IX-2).

Colliculate — of a surface pattern: with rounded broad elevations, closely spaced, covering the surface (Van Uffelen & Hennipman, 1985) (Plate IX-5).

Cytokinesis — the formation of individual cells separated by a cell wall after nuclear division.

Distal — the part of a spore (or pollen grain) away from the centre of the tetrad, i.e. the part not bearing the laesura (see Traverse, 1988).

- Distal pole* — the centre of the distal surface of a spore or pollen grain (see Traverse, 1988).
- Elevation* — part of a surface that is higher than other parts of that same surface (Plate XI-2).
- Endospore* — the innermost spore wall layer, which is in contact with the spore cytoplasm and is continuous with the cell walls of the gametophyte after mitosis during spore germination (see Lugardon, 1971).
- Exine* — main layer of pollen grains lying outside the intine (Lugardon, 1971).
- Exospore* — main layer of the spore wall; it is continuous and acetolysis-resistant, and consists of several interconnected sublayers; it bears a differentiated aperture (the laesura) on the proximal side of the spore (see Lugardon, 1971) (Plate IX-5).
- Haplotypic* — of a feature of a spore or pollen grain that is a product of contact with other members of the tetrad in which it was formed; e. g., the laesura of spores (see Traverse, 1988).
- Heterosporous* — of a plant producing both microspores and megaspores (see Traverse, 1988).
- Homosporous* — of a plant producing only one kind of spore (see Traverse, 1988).
- Inner exospore (ie)* — innermost layer of the exospore. In the Filicales it is a compact and continuous layer; it forms the proximal fold of the laesura (Lugardon, 1971) (Plate V-1).
- Intine* — innermost layer of pollen grains, consisting of cellulosic compounds; it forms the outer layer of the pollen tube during germination (Lugardon, 1971).
- Laesura* — the trace on the proximal face of an embryophytic spore that marks the original contact with other members of the tetrad; it is a specialized area of the exo-spore, which is adapted to let out the gametophyte during germination; the term aperture is a much more general one, which also applies to certain pollen features (Plates IX-6, 7, X-1).
- Laesural fold* — (= Lugardon, 1978a: proximal fold) narrow projecting fold of the inner exospore layer, a first stage in the development of the laesura (Plate V-1, 7).
- Layer* — 1) well-defined part of the sporoderm, such as the endospore, exospore, perispore; 2) as a more general term, applied to any part of the spore wall (this is in conflict with APLF-recommendations, 1975).
- Meiocyte* — cell destined to go into meiosis, e. g., spore mother cell, pollen mother cell.
- Meiosis* — cell division with reduction of the chromosome number.
- Microspore* — one of the kinds of spores of a heterosporous embryophytic plant that germinates to produce a microgametophyte (Traverse, 1988).
- Monolete* — of a bilateral spore which has an oblong laesura; also applied to this type of laesura.
- Ornamentation* — any modification of the spore surface. Some authors prefer to use the term sculpture, but this term is linked to the controversy about the correct use of the terms structure and sculpture as applied to pollen exine morphology (Pragłowski, 1975).
- Outer exospore (oe)* — outermost exospore layer which covers the underlying exospore layer(s) entirely and constitutes the surface relief in ornamented exospores; it is perforated by channels or small fissures connecting the base of this layer with the exospore environment (Lugardon, 1971) (Plate IX-5).
- Periplasmodial tapetum* — tapetum of which the cell walls are broken down during intrusion between the smc's, after which the cytoplasm fuse, usually in late meiotic prophase (Pacini et al., 1985) (Plates II-2, III-3).

- Perispore* — spore wall layer deposited over the exospore surface, usually acetolysis-resistant; variable between species: thin or very thick, tightly or not connected to the exospore (Lugardon, 1971) (Plate XI-1).
- Pits* — round holes on the surface of a layer; if they are present, the surface is called foveolate (see Traverse, 1988) (Plate VII-4, 8).
- Plasmalemma* — (= plasma membrane, Gunning & Steer, 1986) the bounding membrane of the protoplast, normally in close contact with the inner face of the cell wall (Plate III-8).
- Plasmodesma* (pl. *plasmodesmata*) — narrow cytoplasmic channel, bounded by the plasma membrane, and interconnecting adjacent protoplasts through the intervening wall (Gunning & Steer, 1986) (Plate I-2).
- Polar axis* — imaginary line connecting the distal pole with the proximal pole.
- Proximal* — the part of a spore or pollen grain nearest or toward the centre of the original tetrad, i.e. the part bearing the laesura (see Traverse, 1988) (Plate X-7).
- Proximal pole* — the centre of the proximal surface of a spore or pollen grain (see Traverse, 1988).
- Rosette stage* — the stage during sporogenesis in which the spore mother cells lie close to each other, only separated by one layer of sporocyte coat and forming a rosette on cross section (Plate I-1).
- Rounded stage* — the stage during sporogenesis in which the spore mother cells have become globular in form and are lying further from each other, each surrounded by its own layer of sporocyte coat (Plates I-5, II-2).
- Sculpture* — see ornamentation.
- Smc* — see spore mother cell.
- Spherical bodies* — Small globular structures consisting of the same material as the outer exospore and finally covered with a layer of perispore; found on the exospore surface, sometimes contained in the perispore and in the mature sporangium of many pteridophytes; thought to be homologous to Ubisch bodies in pollen (see Lugardon, 1981; Traverse, 1988) (Plates X-6, XI-8).
- Spine* — a projection from the spore surface with a more or less sharp apex and a tapering trunk with a broad base (Harris, 1955) (Plate XI-7).
- Spore* — (Lugardon, 1971: only applied to ripe or almost ripe spores) haploid reproductive body produced by a sporophyte, germinating into a gametophyte, and surrounded by a resistant sporoderm (see Traverse, 1988).
- Spore coat* — the coat which covers the surface of the spore (the postmeiotic cell) (Pettitt & Jermy, 1974) (Plate III-7).
- Spore/sporocyte coat* — see surface coat.
- Spore height* — height measured along the polar axis.
- Spore length* — the maximum length in lateral view.
- Spore mother cell* (smc) — the mother cell in the sporangium of a spore-bearing plant, which, by reduction division, produces a tetrad of haploid spores (Traverse, 1988) (Plate I-4).
- Spore wall* — see sporoderm.
- Spore width* — the maximum width in polar view.
- Sporocyte* (adj. *sporocytic*) — see spore mother cell.
- Sporocyte coat* — the coat which covers the surface of the sporocyte (the premeiotic cell) (Pettitt & Jermy, 1974) (Plate I-6).

- Sporoderm* — the entire wall of a spore or pollen grain (Traverse, 1988); the sum of all layers surrounding a spore or pollen grain (see Lugardon, 1971).
- Sporogenesis* — the formation of spores from the differentiation of the spore mother cell to the final maturation of the spores. Spore wall formation is only part of the process of sporogenesis.
- Sporopollenin* — the very resistant organic substance of which the exine/exospore of spores and pollen is composed (see Traverse, 1988).
- Surface coat* — purely morphographic term, covers both the sporocyte and the spore coat (Pettitt & Jermy, 1974) (Plate III-7). I prefer the term spore/sporocyte coat as this is more specific than surface coat.
- Tapetum* (adj. *tapetal*) — tissue of nutritive cells in the sporangium of embryophytic plants, largely used up during development of the spores (Traverse, 1988).
- Tetrad* — product of one spore mother cell after meiosis; each tetrad consists of four spores and the enveloping coat (see Lugardon, 1971); a usually symmetric grouping of four embryophytic spores or pollen grains that result from meiotic division of one spore mother cell (Traverse, 1988) (Plate III-6, 7).
- Tetrahedral* — of a tetrad in which each grain rests atop three others (Traverse, 1988); it produces trilete spores (Lugardon, 1971).
- Tetraspore* — one of the four cells of a tetrad after cytokinesis. Towards the end of sporogenesis, each tetraspore becomes a spore (see Lugardon, 1971). I start using the term spore as soon as the laesural fold has been formed (Plates IV-2, V-1).
- Unit* — clearly distinguishable part of the spore surface separated from other units by grooves (Plate V-7).
- Verrucate* — bearing verrucae, i.e. broad projections with a trunk that is not constricted (see Harris, 1955) (Plate VIII-4).
- White line* — the centre of a tripartite lamella, on both sides of which sporopollenin has been deposited, occurring in the exine/exospore of many embryophytes (see Brown & Lemmon, 1988; Rowley, 1988) (Plate V-3).

RESULTS

In young sporangia, within the sporangial wall consisting of a single cell layer, differentiation in tapetal cells and spore mother cells takes place. After division of the single layer of tapetal cells, before smc prophase, the smc's are surrounded by a double layer of tapetum (Plate I-1).

1. The spore mother cell

Before meiosis usually 16 spore mother cells are present in each sporangium. At first, in what is called the rosette stage (Lugardon, pers. comm.), the smc's lie close to each other, separated only by a common layer of dark granulate sporocyte coat (Plate I-1, 2). Smc's are connected with each other by way of thin cytoplasmic strands (c. 0.06 μm wide), bounded by a plasmalemma, penetrating the sporocyte coat (Plate I-2).

Later on in the rosette stage, while the smc's are still in close contact with each other, the fluffy sporocyte coat splits into two layers (Plate I-3), so that each smc is surrounded by its own sporocyte coat.

As the sporocyte coat doubles, the smc's change shape and become more rounded, so that they gradually detach from each other (Plate I-4). When the smc's all have their own sporocyte coat (Plate II-5), they have entered the smc-rounded stage (Plate II-2, 3, 6); in this stage, the sporocyte coat may show a continuous growth, resulting in the formation of loops (Plate I-5). However, this may be due to a fixation artifact. Another such artifact may be found in smc's of very irregular outline during meiosis (Plate I-6). In many smc's, cytoplasmic protrusions in the nucleus can be observed (Plate I-5; Sheffield & Bell, 1979).

While the smc's change shape from angular to rounded, tapetal cell walls start to break down and the tapetum moves in between the smc's (Plates I-4, II-2). In this stage, smc's are about 15 μm in diameter.

On TEM the tapetal nuclei are the most conspicuous tapetal organelles (Plate I-4); on SEM, nuclei and vacuoles are most conspicuous (Plate II-3, 4).

2. Meiosis

In *Drynaria sparsisora* I found the onset of cytokinesis to take place after completion of both nuclear divisions and not after each of the nuclear divisions.

The position of the spores in their tetrads is pairwise (Plate III-6) and not tetrahedral, which determines their form as bilateral and monolete. The pairwise-90° configuration, where one pair of the tetrad lies in an angle of 90° with the other, has been found on TEM (Plate III-7); angles less than 90° have also been found between pairs (Plate III-5); some of these may be due to oblique cuts (see Huynh, 1973 and Verma & Khullar, 1976). The tapetum has further degenerated; the nuclei are lying close against the young tetrads (Plate III-2, 3).

3. Formation of the plasmalemma

In *Drynaria sparsisora* a cell plate is formed in association with aggregated mitochondria and vesicles (Plate III-3, 4, 5); on Plate III-4 the nuclear membrane is again (at least in part) present. As in smc's, invaginations between nucleus and cytoplasm are present in young tetraspores (cf. Bell, 1981) in order to increase the surface of contact between nucleus and cytoplasm (Plate III-6).

After cytokinesis, each tetrad is still enclosed in the sporocyte coat. Each tetraspore is surrounded by a plasmalemma, and tetraspores are separated from each other by the spore coat (Plate III-7, 8); they may be lying quite far apart (Plates III-7, IV-3). After conclusion of meiosis the tetrads start to increase in size. On TEM, the space between the tetraspores in the tetrad is occupied by loose, flaky material (Plate III-7, 8). On SEM, this material has been removed during fixation, and one tetrad consists of four tetraspores lying apart in an otherwise empty space, surrounded by tapetal nuclei (Plate IV-3, 5). On Plate IV-3 and IV-5 the young tetraspores are lying close to what is probably the spore/sporocyte coat, which is lying quite far away from the tapetum. Details (Plate IV-4, 6) show that this coat looks more massive than on TEM, and is densely set with granules; sometimes there are foldlike structures (Plate IV-4) to be seen. Plate IV-7 shows the rather smooth inner side of a sporocyte coat that has stayed in place after the spores have fallen out during fracturing, and is still

lying close to the tapetum. On SEM, tetrads and young tetraspores are rather variable in appearance, depending on the plane of fraction and subsequent rigorous postfixation (Plate IV-3, 5, 8). As all tetrads on Plate IV have been found in the same sporangium, they are approximately in the same stage of development.

4. *Formation of the inner exospore layer (ie)*

The inner exospore layer is deposited on the outside of the plasmalemma as a thin, opaque, sporopolleninous layer. During the formation of this layer a thin white line (Brown & Lemmon, 1988) may be visible (Plate V-3), showing that the material of which the inner exospore is formed is deposited by the tetraspore cytoplasm as well as from the outside of the tetraspore. Deposition of inner exospore material from the spore cytoplasm may continue during the first stages of outer exospore formation (Plate V-4). Later on in spore wall formation the white line disappears completely. The Golgi apparatus appears to play a role in the secretion of sporopollenin as seen on Plate V-4. The thickness of the complete inner exospore layer varies from 0.025 μm on the distal side to 0.040 μm on the proximal side.

While the inner exospore layer is being deposited, the laesural fold (Lugardon, 1969) is formed on the proximal side of the young tetraspore (Plate V-1, 2). Eventually this fold develops into the monolete laesura. During formation of the inner exospore layer and of the first part of the outer exospore, the inner surfaces of the fold move towards each other, the cytoplasm moving centripetally out of the fold, and the surfaces finally come to lie close to each other (Plate VI-1). Plate IV-2 depicts a laesural fold during its formation as seen with SEM. Here the fold is only c. 1 μm high, and thus still in formation, as the completed laesural fold usually measures up to 4 μm in its highest part.

5. *Formation of the outer exospore layer (oe)*

In *Drynaria sparsisora* almost all of the outer exospore layer is deposited in smaller or larger grains, and the exospore is not smooth until the very end of its formation. In the course of the formation of the outer exospore layer, several distinct surface types can be distinguished in this species.

5a. *Formation and presence of a surface consist of neatly defined units*

On the outside of the smooth thin inner exospore layer, the innermost part of the outer exospore layer is deposited in the form of irregular sporopolleninous lumps. Deposition begins near the laesural fold, so that in this stage the proximal side of the wall is always slightly further developed than the distal side (Lugardon, 1971). The first lumps to be deposited are very fine, usually less than 0.25 μm in diameter (Plate V-4–6). Gradually the surface becomes covered with neatly defined, roundish or angular units of c. 0.5 μm in diameter (Plate VI-4). On Plate V-7 and its detail, Plate V-8, different sizes of lumps are visible, especially on the laesural fold. Plate VI-1 shows a cross section of a spore early in this stage; both sides of the laesural fold are already lying close to each other; the irregular form of this spore is probably a fixation artifact. Plate VI-5 shows a section of collapsed spores in a slightly later

stage; it shows that the lower part of the laesural fold is already covered with exospore material; under the base of the laesural fold a thick deposition can be seen, the 'amas sous-apertural' (Lugardon, 1971). The inner surface of the spore wall (Plate VI-3) is rather smooth with small pits and clearly shows the closed and thickened base of the laesural fold.

On TEM, tapetal nuclei are still recognizable (Plate VI-1); on SEM, the tapetum is more of an amorphous mass (Plate VI-2).

5b. Grains cover the surface units

The sharply defined units gradually get covered by a layer of grains, which are also deposited in between the units, but in such a way that the original units remain visible (Plate VII-1–3). Hereby the relief of the spore surface becomes less pronounced. Plate VII-2 shows grains to be c. 0.1–0.3 μm in diameter. On Plate VII-4, which shows the spore surface magnified 45,000 times, the grains can be seen to be even smaller, c. 0.05 μm in diameter; many small (0.25 μm diameter) round holes – channels connecting the surface with the inner parts of the exospore – are left open. Plate VII-7 shows a TEM section of approximately the same stage; the exospore is 0.4–0.8 μm thick, and the laesural fold is rather high, 4.2 μm ; channels connecting the inner exospore layer with depressions on the exospore surface are visible as dark lines. Spherical bodies (Plate VII-3) may be present in the tapetum and consist of sporopollenin secreted by the tapetum; their surface is much more smooth than the exospore surface in this stage; they are c. 1–5 μm in diameter.

5c. Surface becomes almost smooth, pitted

Gradually, the granular surface as seen on Plate VII-1–4 becomes covered with a layer of fine grains, obscuring the units that were present just after the onset of the outer exospore deposition (Plate VII-5). Numerous small pits, indicating the presence of channels, occur very frequently (Plate VII-6); they are larger and more numerous around the laesura (Plate VII-8). The openings of these channels form an important part of the surface pattern at this stage. The spore surface gets more and more smooth; deposition of material does not start around the laesura any more, but on the distal side of the spores, as seen on Plate VIII-1. The tapetum, although rather desorganized, still seems to play a role in the deposition of exospore material (Plate VIII-3). During this stage most of the laesural fold becomes covered with exospore material (Plate VIII-2).

5d. Pits disappear, surface becomes almost smooth, sometimes slightly verrucate

During the last stage of outer exospore formation, the exospore surface becomes covered with a very fine-grained material which forms low rounded or angular verrucae of c. 1.5–2.5 μm in diameter (Plate VIII-4–7). Tiny holes, especially around the laesura, show the position of channels (Plate VIII-5). These channels are well visible on TEM cross sections, as on Plate IX-1, 2; on Plate IX-2, former surfaces of the exospore during its formation can be traced by the presence of dark granules halfway the present outer exospore layer which is 1.1 μm thick. Plate IX-3 and 4 show similar sections with SEM: channels are not visible; even the inner exospore layer in the part of the laesural fold already covered with exospore material is not

visible; on these sections, the exospore is little more than 1 μm thick. Plate IX-4 shows that the inner surface of the exospore is rather smooth, more so than on Plate VI-3, which suggests that either deposition of additional material or consolidation of the existing material takes place on the inner surface of the exospore during exospore formation.

The tapetum may lie close to the spore surface and leave tiny fragments of material (Plate VIII-5, 6). It sometimes contains spherical bodies (Plate IX-4).

Later a smooth or almost smooth, slightly colliculate outer surface is formed (Plate IX-5, 8).

The laesural fold becomes completely covered with exospore material only at the end of this last stage of exospore formation (Plate IX-6, 7). Its height depends on the site of section: on Plate IX-6, 7, it is 2.4 and 4.6 μm high, respectively.

5e. The mature exospore

The mature exospore is about 1.5 μm thick, being only slightly thicker on the proximal than on the distal side (Plate IX-5). Its surface is smooth or very slightly verrucate, the verrucae are colliculate, 2–4 μm in diameter (Plate X-1). The laesura extends over the greater part of the length of the proximal side of the spore (Plate X-1).

6. Perispore formation

After the exospore formation is completed, the perispore is formed. Perispore formation, usually described as the quick precipitation of tapetal residues, is a very elusive process, probably mainly because it takes such an extremely short time.

At the base of the developing perispore, lamellae are often to be found (Plate X-3–5); they are not always forming a single layer, but are sometimes present in slightly overlapping pieces of 0.2 μm long (Plate X-4); similar strips of lamellae are also observed in the tapetum at the end of exospore maturation (Plate X-2). Angular blobs of dark material, in size varying from 0.05 to 0.2 μm , are present in the tapetum (Plate X-2) and in the vicinity of the spores (Plate X-3) and are eventually deposited on the spore surface over the lamellae (Plate X-3–5). They may end up covering most of the spore surface.

In *Drynaria sparsisora* the perispore consists of a rather thick slightly verrucate layer on which spines are formed later on during perispore formation. It is not clear whether the spines are formed out of these blobs, as is suggested by the presence of the small spine on Plate X-5, or are assembled from other building blocks formed by the tapetum. Plate X-7 and 8 show SEM pictures of the spore surface of which the scale of the surface structures seems to correspond to the TEM pictures of Plate X-3–6.

The formation of the basal layer is very elusive. The resemblance between the ornamentation of this layer (Plate XI-2) and the pattern found after condensation of glycerin jelly on a glass slide (Plate XI-5) suggests the possibility of a condensation process being involved in perispore formation (Van Uffelen, in press). TEM-pictures of the mature perispore (Plate XI-6) are not very instructive with respect to the structure of the basal layer and the spines.

Spines are deposited perpendicular to the perispore surface. This is best to be seen on the laesura (Plate XI-7) or on detailed micrographs taken exactly from above (Plate XI-2).

7. The mature perispore

The mature perispore (Plate XI-1, 3) consists of a 0.07–0.4 μm thick basal layer which is slightly and irregularly verrucate; elevations usually colliculate (Plate XI-6, 7), but sometimes not touching (Plate XI-2), flat and round or longish, irregular in outline; basal layer set with many tapering short spines; spines 1–1.5 or 2(–5, rarely up to 10) μm long, 0.2–1.5, usually around 0.6 μm in diameter, usually straight and pointed, sometimes slightly bent, or ending in more than one point (Plate XI-2, 4), one or more globules (Plate XI-4, 7) or abruptly thinner cones (Plate XI-2), or in a very long point (Plate XI-3); the longer the spines, the more irregular their distribution over the spore surface, the shorter they are, the closer and more evenly spaced (Plate XI-1 vs. Plate XI-3). Spines are solid, as shown in TEM pictures (Plate XI-6) and on Plate XI-4. The position of the spines is not independent of the basal perispore pattern: spines tend to be deposited where the basal pattern is highest, not in between the elevations (Plate XI-2, 4, 7).

Mature spores of *LEI 20339* (Plate XI-1) and *84GR00187* are most alike, their perispores very densely set with short spines; spores of *LEI 22226* are more or less intermediate between *LEI 20339* and *84GR00187* on the one hand and *81GR00119* (Plate XI-3) on the other, with a less neat perispore not very densely set with longer spines; spores of *81GR00119* have much less but longer spines on their perispores and are rather different from those of the other three specimens.

Mature spores of *Drynaria sparsisora* (Plate XI) are about 50 μm long and c. 0.7 times as high and broad. The laesura is c. 0.4 times as long as the spore.

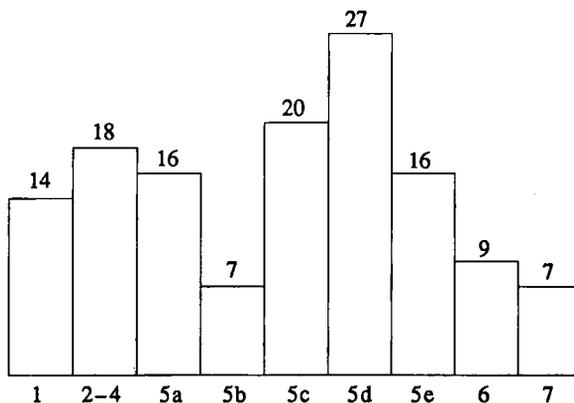


Fig. 1. Frequency diagram of the different stages in 134 sporangia studied with SEM (1. Presence of spore mother cells; 2–4. meiosis, young tetrads; 5a. formation of the outer exospore (a); 5b. formation of the outer exospore (b); 5c. formation of the outer exospore (c); 5d. formation of the outer exospore (d); 5e. mature exospore; 6. perispore formation; 7. mature spores).

Stage length

The relative length of the different stages in sporogenesis is difficult to assess, but it is probably correlated with the frequency of their occurrence in the sporangia studied. After observation of 134 broken sporangia of *Drynaria sparsisora* with the SEM, the frequency with which the different stages were found, has been calculated; some tentative conclusions may be drawn as to their duration in this species (fig. 1).

The last stages of exospore formation (5c: surface becomes almost smooth, pitted; 5d: pits disappear, surface becomes almost smooth, sometimes slightly verrucate) are most frequently found, which indicates that it takes a long time to deposit the fine-grained outermost part of the exospore. Young tetrads and the first stage of exospore formation have been found fairly often too, but apparently stages 5b (grains cover the surface units) and 6 (perispore formation) take up very little time.

Measurements

Some features of the sporangia and spores have been measured on as many SEM micrographs as possible:

- sporangium size, measured as the height (without stalk) of the 134 sporangia studied with the SEM (fig. 2); sporangium growth is largest before and during the first stages of outer exospore formation (1–5b) and after exospore formation has been completed (5d–7); there is no significant increase in size during formation of the main part of the outer exospore.
- spore length (fig. 3); there is a large increase in spore length before the start of outer exospore deposition; during the formation of the outer exospore and the perispore (5a–7), spore length increases about the same amount in each stage.
- height of the part of the laesural fold still uncovered with exospore material (fig. 4); during formation of the outer exospore layer the part of the laesural fold covered with exospore material increases. There is much overlap between the stages.
- unit size during outer exospore formation (fig. 5) increases tenfold between the deposition of the inner exospore layer and the final deposition of the outermost exospore layer. However, this increase in size is not linear, and the range of observations is sometimes very wide. The largest increase in size occurs when the pitted surface of stage 5c is covered with a layer of slight verrucae as found in the mature exospore.

DISCUSSION

Technical comments

Fixatives are generally chosen on account of their fixation of membranes and other subcellular structures, not on how they react with viscous liquids and colloidal solutions. This should be taken into account when interpreting some of the structures found in SEM micrographs of sporangia that have undergone freeze fracturing. Most of the SEM micrographs of mature spores in this study, which have not undergone any artificial fixation process save drying, do not have this problem (Plate XI-1).

Fixation of young sporangia which probably contain a solution of high osmolarity (with many fairly large organic molecules, e.g. wall precursors) in a solution of low osmolarity (the fixative contains only small organic and sometimes also small inorganic molecules), may cause the sporangium to imbibe water osmotically, and individual spores to collapse.

Paraformaldehyde fixation

In this study a combination of two techniques has been used for the first time: freeze-fracturing (Tanaka, 1981; Blackmore & Barnes, 1985) has been applied to sori that have been fixed in buffered paraformaldehyde (Pease, 1964) for quite a long time – from one week to over a year.

Paraformaldehyde fixation over a long period seems especially to promote fixation of the cytoplasm and some amorphous cell components in such a way that freeze fracturing with subsequent postfixation and etching with OsO₄ has not the same effect as after a short fixation in 1% OsO₄ only (see e.g. Plates II-6, V-5, 7).

The main advantage of paraformaldehyde as a fixative is that it is very easy to use: it needs no refrigeration and the material can stay in the P-buffered fixative for any period between one week and many months; this also provides the opportunity to have material collected by others and/or in far-away places, which greatly enlarges the range of material that may be studied.

Division of the process into stages

Although it is very difficult to capture a process in the series of snapshots resulting from the study of fixed dead material (see Lugardon, 1971: 9), I have tried to describe the process of sporogenesis and define stages using morphological criteria.

In indicating stages, the main problem is that a stage may be roughly described by a process (meiosis, formation of ...), or by a situation (presence of ...) – during which undoubtedly numerous processes are taking place as well. When dividing a process such as sporogenesis into stages, it is obviously more appropriate to define each of the stages by a process. However, I was forced to include some stages defined by a situation as I found many sporangia in exactly the same stage, indicating a prolonged more or less static situation.

The six main stages in sporogenesis as distinguished in Van Uffelen (1986) are mainly based on the study of TEM micrographs. They result from a study of sporogenesis in several Polypodiaceae, and probably apply to sporogenesis in most other homosporous ferns as well (Lugardon, 1971).

In *Drynaria sparsisora*, stage 5, the formation of the outer exospore, can be divided into substages by using the consecutive surface patterns as found by a detailed SEM study as a criterion.

These consecutive exospore patterns seem to be characteristic on the specific, generic, or even suprageneric level (e.g. in *Belvisia*, Van Uffelen, in prep.). Therefore the subdivision of stage 5 is supposed to be characteristic of sporogenesis in *Drynaria sparsisora*:

- 5a = formation of the outer exospore (a): formation and presence of a surface consist of neatly defined units;
- 5b = formation of the outer exospore (b): grains cover the surface units;
- 5c = formation of the outer exospore (c); surface becomes almost smooth, pitted;
- 5d = formation of the outer exospore (d); pits disappear, surface becomes almost smooth, sometimes slightly verrucate;
- (— 5e = the mature exospore: exospore finished, deposition of perispore not yet started).

Blackmore & Crane (1985, 1988) distinguished five stages in microspore ontogeny in bryophytes, pteridophytes, and spermatophytes: 1) the meiotic division, 2) the tetrad stage, 3) the free microspore stage, 4) microspore mitosis, 5) final maturation. They suggested that a relatively small number of variations in the early stages of development (meiosis and tetrad stage) may account for the diversity as found in mature pollen and spores.

The stages recognized by these authors apparently are mainly based on pollen ontogeny. In pteridophytes no separate tetrad stage and free microspore stage can be distinguished as there is no thick callose wall present around the tetrads and tetraspores during the early stages of spore formation.

Blackmore & Crane (1988) mentioned that the last two stages are usually found in this order in pollen, but not in spores, as "in developing spores mitotic division of the microspore nucleus is often delayed until germination." Therefore, in pteridophytes the end of perispore formation should be taken as the moment of final maturation; in this group, microspore mitosis usually takes place during germination and after maturation, i. e. after the perispore has been completed.

Lugardon (1978b) stated that in both pollen and spores formation of the intine/endospore may occur only after the mitosis that turns the unicellular microspore contents into a multicellular gametophyte; only in those cases, and if formation of the endospore is taken as the moment of final maturation, the order of the last two stages as proposed by Blackmore and Crane would be correct. In my opinion, in pteridophytes it is not.

Lugardon (1971) distinguished no formal stages in sporogenesis of each of the three rather distantly related fern species studied in his thesis [*Ophioglossum vulgatum* L., *Osmunda regalis* L., and *Blechnum spicant* (L.) Roth.], indicating that the different stages in sporogenesis cannot easily be placed in a rigid system that applies to species not closely related to each other. He merely denoted the different phases in sporoderm formation in each species by a caption above each paragraph. However, as he defined the different sporoderm layers very precisely by the order in which they are formed, the formation of each layer can be regarded as a well-defined stage.

Assessing the stage and its duration

It may be difficult to place a series of micrographs in exact chronological order, although there are many clues in sporangium contents and in comparison with publications on sporogenesis in other plants.

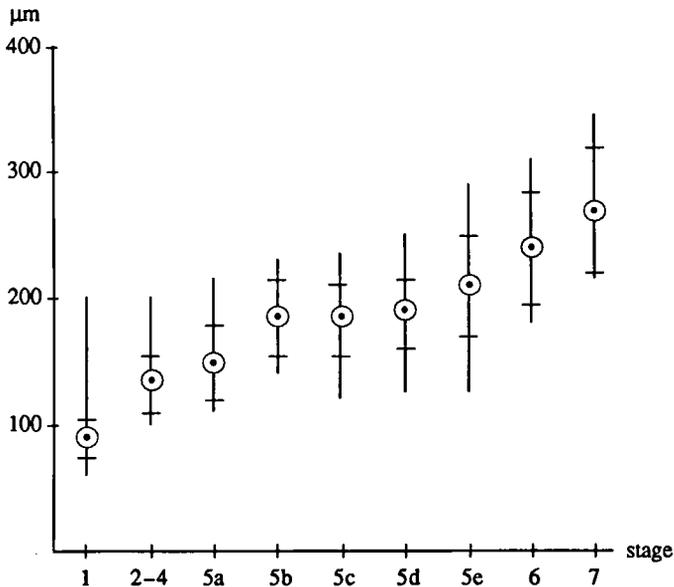


Fig. 2. Sporangium height in 134 sporangia studied with SEM — mean, standard deviation (includes 68% of observations) and range. For stage numbers, see figure 1.

Stage assessment

Some dimensions of sporangia and spores are indicative of stage, though poorly so, as measurements after stage assessment in *Drynaria sparsisora* have shown:

- sporangium height is not very clearly indicative of stage, as sporangium size does not increase linearly with it. Sources of error may be sporangia that are not broken right through the middle or sections that are oblique (fig. 2).
- spore size, measured as the most commonly observable spore length, not spore height or width (fig. 3); spore length increases more or less linearly during the stages 5a–7, but there is much overlap between stages.
- height of the part of the laesural fold not yet covered with exospore material (fig. 4) is roughly correlated with the stage of outer exospore formation; it decreases with outer exospore formation. However, there is much overlap between the stages; therefore, it is not suitable as an indicator of stage. Measurement is also complicated by the fact that the height of the laesural fold depends on the site of section in TEM micrographs, and that in SEM micrographs the base of the fold is not always visible. Finally, all of the laesural fold gets covered with exospore material, although this layer may be extremely thin over the top of the laesural fold (Plate IX-6).
- exospore thickness should correlate neatly with stage. As the mature exospore is about 1.5 µm thick, and this layer is always less than 1.5 µm thick during its de

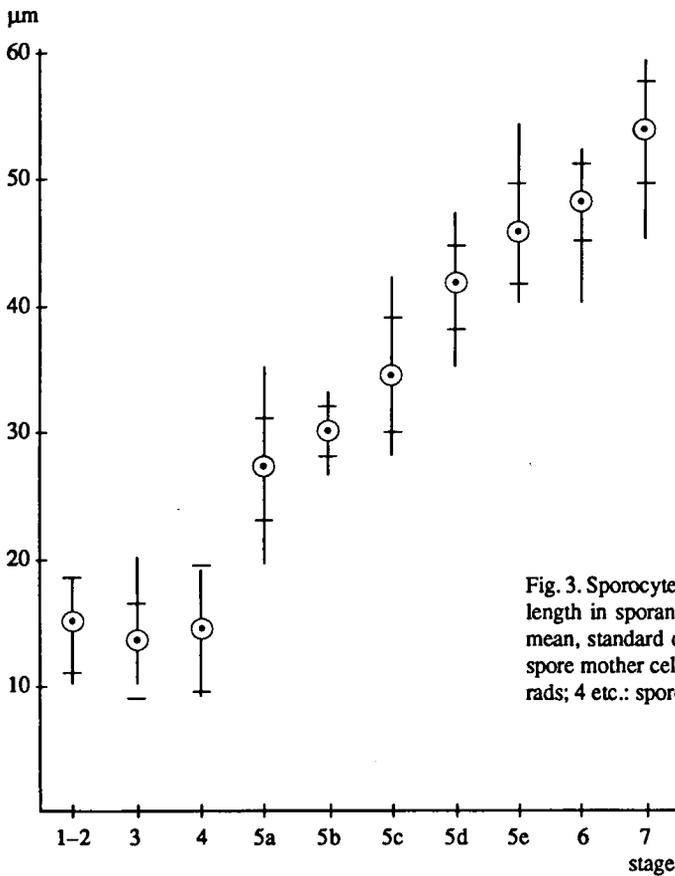


Fig. 3. Sporocyte/tetrad diameter and spore length in sporangia studied with SEM — mean, standard deviation and range (1, 2: spore mother cells or young tetrads; 3. tetrads; 4 etc.: spore length).

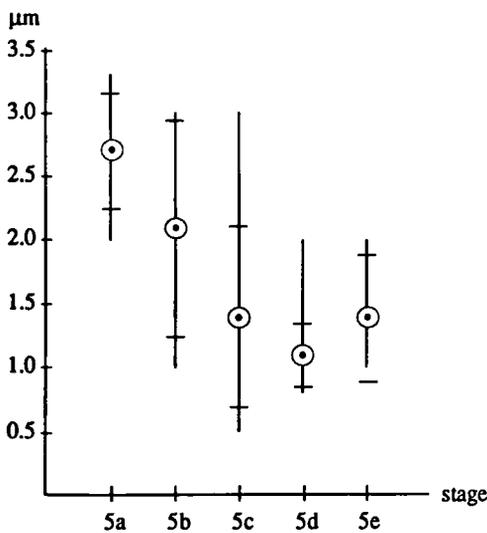


Fig. 4. Height of laesural fold raised above the exospore surface in spores studied with SEM — mean, standard deviation and range. For stage numbers, see figure 1.

position, the exospore does not get demonstrably thinner in the process of maturation. However, it is very difficult to measure, since there are not enough SEM micrographs of broken spores, and not enough TEM measurements that can be correlated with a surface pattern to go with a stage of outer exospore formation. Moreover, exospore thickness is never uniform throughout the whole spore: near the laesura the exospore is always thicker than elsewhere on the spore.

- unit size of the exospore surface (fig. 5); its increase is not linear, and there is much overlap between stages. The increase in unit size is partly explainable as a change in surface pattern, and partly due to stretching of the exospore to accommodate increase in spore size.
- spore length divided by unit size: the number of units along the length of one spore (fig. 6) decreases with the progress of exospore formation. This can be explained by the changes in surface pattern that occur during exospore formation.

Stage length

Assessment of stage length (fig. 1) by way of counting the frequency of occurrence of the different stages in the sporangia studied may have been influenced by the following factors:

- age of the sori studied; sori may contain mainly young or mainly old sporangia; this bias can be reduced by the study of a large number of sori (in this study: c. 40 sori);
- small (young) sporangia are more easily missed in fracturing the sporangia of a sorus; this leads to the occurrence of more large, old, broken sporangia and therefore to too long estimates of the later stages; as late stages may also be found in small sporangia, this bias is rather low.
- with freeze fracturing the frequency of encountering a stage could be positively influenced by the fragility of the sporangia in that particular stage, negatively by the fragility of their stalks.

Stages within one sporangium

Another point is whether all smc's, tetrads, or spores in one sporangium are in exactly the same stage.

Before meiosis, connections between the cells of the sporogenous tissue may synchronize development. Heslop-Harrison (1964: 41–42) found plasmodesmata between pollen mother cells (pmc's), between pmc's and tapetum and between tapetum cells in *Cannabis*. These plasmodesmata are eliminated by the growth of the callosic special mother cell wall. Later during prophase, channels up to 1.5 μm wide develop between pmc's; they are severed during the first meiotic division. These cytomictic channels are thought to serve synchronization of part of the process of meiosis.

In *Pteridium aquilinum* Sheffield & Bell (1979: 399) found that these channels are blocked during the metaphase/anaphase period and that development of the smc's becomes increasingly asynchronous afterwards.

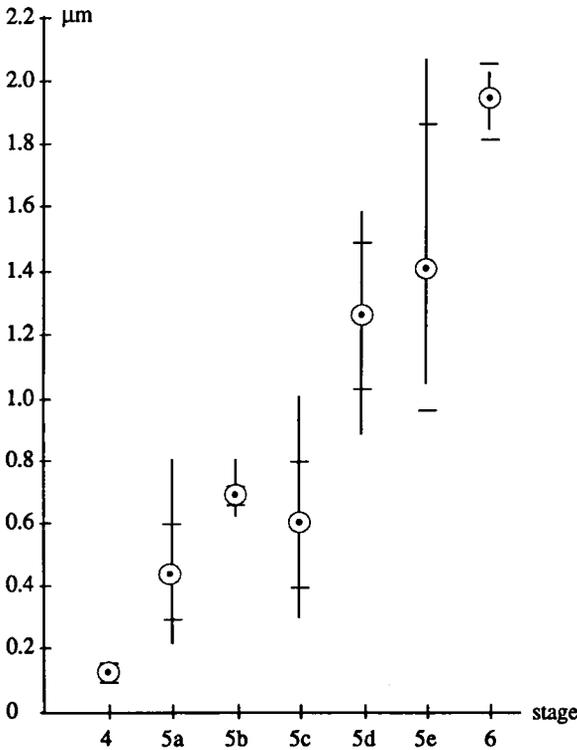


Fig. 5. Unit size in spores studied with SEM — mean, standard deviation and range. For stage numbers, see figure 1.

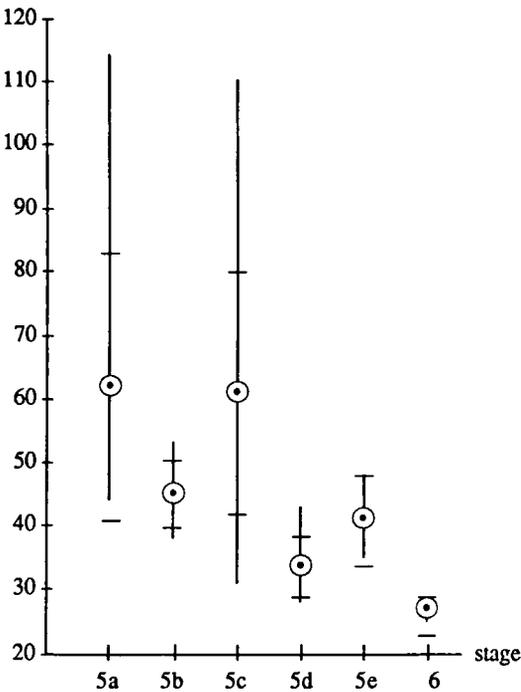


Fig. 6. Number of units per spore length — mean, standard deviation and range. For stage numbers, see figure 1.

On the other hand, Lugardon (1971: 106) found that in *Blechnum spicant* smc's may differ in stage during meiosis, but that from the end of meiosis till maturity all spores in one sporangium are in exactly the same stage. On p. 67 (op. cit.) he stated that in *Osmunda regalis* all tetrads in a sporangium are in exactly the same stage until spore maturity. However, on p. 40, in his description of the early stages of sporogenesis in *Ophioglossum vulgatum*, he stated that tetrads may differ very slightly in stage, and that even within spores there may be a difference in stage between the proximal and distal pole. Development of the spore wall seems to take place from the centre of the tetrad outwards, so that the proximal pole, the region around the laesura, usually is slightly further in development than the distal region.

I found that tetrads in one sporangium may differ slightly in stage (e. g. Plate IV). Assessment of the exact stage of these tetrads is difficult because they are all broken along a different plane, so that they all offer a different view of the young spores.

With the TEM (Plate V-1) I found that in young spores in the very first stage of exospore formation the proximal side is always slightly further developed than the distal side, thus confirming Lugardon's observations on *Ophioglossum vulgatum*.

However, later in exospore deposition, the opposite may occur: there are marked differences in surface pattern between proximal and distal poles, but exactly the other way round, as the distal surface is further developed than the proximal surface; in these cases all spores in a sporangium look exactly alike, so their exospores are in exactly the same stage of development [see Plate VIII-1, oe in form. (c), where the surface of the distal side is further advanced than that of the proximal side]. This indicates that exospore material is added from outside the tetrad on those surfaces that are most exposed while the spores are still lying in the tetrad formation. This may be interpreted as the result of a change in control of exospore deposition from sporocytic/gametophytic to sporophytic early in exospore formation (Van Uffelen, in press).

Some aspects of sporogenesis

LM studies indicate that in leptosporangiate ferns like the Polypodiaceae, where each sporangium initiates from a single cell, the development of sporangia is very similar till after completion of meiosis of the spore mother cells. All ferns, Equisetales and Psilotales have a similar type of tapetum, a periplasmodial tapetum, in which the tapetal cells lose their walls, fuse, and intrude between the smc's (Pacini et al., 1985).

In *Drynaria sparsisora* I found cytokinesis to be simultaneous, in agreement with Verma & Khullar (1976) who observed that "In pteridophytes cytokinesis is predominantly (if not exclusively) simultaneous." In *Drynaria sparsisora* cell plates are formed as the first part of the process of cell wall formation. Heslop-Harrison (1964) found no cell plates after meiosis of pollen mother cells in *Cannabis*, but furrowing.

The origin of the spore coat is difficult to assess. I assume that the sporocyte coat and spore coat both originate from the same source, even though they are not formed at the same time. The sporocyte coat is formed as a granular layer between smc's during the rosette stage; it doubles, so that each smc is surrounded by its own granular layer. It is formed in the very tight space between smc's, therefore sporocytic origin is most probable as the tapetum is nowhere near the doubling granular layer.

In SEM pictures it is very difficult to establish whether the granular layer covering young tetrads or spores is the spore wall in the course of formation/consolidation, tapetum, or the spore or sporocyte coat. Plate IV-3 shows such a young tetrad observed with SEM. The spore/sporocyte coat may have disappeared during fixation, but on the other hand, the granular layer that is now visible where one spore has gone from this tetrad may be the sporocyte/spore coat lying against the plasmalemma of the spores that are still present. In some places the granular layer is more or less continuous with the tapetum. The folds on Plate IV-4 can be no shrinkage artifact (W. Linnemans, pers. comm.), so they must either belong to the inner surface of the spore/sporocyte coat or allow the coat to expand when necessary. Plate III-7 shows three young tetraspores with large open spaces between them, especially in the centre of the tetrad; these spaces probably contain a liquid out of which all kinds of material may precipitate during SEM fixation after freeze fracturing, as may be the case on Plate IV. On Plate V-1, around the spore covered with an inner exospore layer (ie), there is a large open space with flaky material around the spore; on TEM, all contact between young tetraspores seems to be absent, also during later stages of exospore formation – so the observation of spores lying far apart on SEM pictures is no specific SEM fixation artifact.

The different layers of the outer exospore are all deposited on top of each other, i.e. centrifugally, as Lugardon stressed in his 1978 (b) publication. This type of exospore or exine deposition does not occur in gymnosperms or angiosperms (Lugardon, 1978b), nor in bryophytes (Brown & Lemmon, 1988). Traces of the succession of different exospore patterns are difficult to find, but may be visible in TEM sections as series of darker granules that once were lying on the unfinished spore surface (Plate IX-2).

In *Drynaria sparsisora* intercalary growth is not evident; at no stage of exospore formation does the surface pattern show any signs of new units interposed between existing ones; with TEM, radial differences in staining properties of the exospore caused by a different degree of polymerization of sporopollenin, secreted at different moments, have not been found. Probably the growing exospore is elastic enough to provide for an increase in spore size by stretching; this may be gradual but is more likely to be stepwise (see Mogensen, 1981: 195, about size increase in bryophytes), enlargement mainly taking place between the deposition of the different exospore layers. However, this is not verifiable with spore length measurements, as spore lengths in the different stages of exospore formation rather tend to overlap. The increase in spore length during exospore development is shown in figure 3.

The lateral grooves that are seen during formation of the outer exospore (see Plate V-5, 7) can be explained by pinching of the lateral sides of the spore along the proximal part during the formation of the laesural fold, early in spore wall formation.

CONCLUSIONS

The replacement of osmium fixation by subsequent fixation in paraformaldehyde and osmium makes it possible to study material that has been collected far afield or by others.

It is evident that this relatively new method of freeze fracturing as applied to sporogenesis has yielded an entirely new range of observations on the succession of different patterns during exospore formation. In the study of sporogenesis SEM has therefore become far more important than used to be the case.

The occurrence of a succession of different surface patterns during formation of the exospore should lead to further research into the occurrence of such successions in other plant groups. It should yield an important set of characters with which phylogenetic relationships can be further elucidated.

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I also want to thank the staff of the botanical gardens in Leiden and Utrecht for cultivating the plants studied.

Laura Cobb made the TEM micrograph depicted on Plate VIII-7.

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LEGENDS OF THE PLATES

Fixation data — SEM: no fixation, i.e. dried only (nofix.); standard fixation with osmiumtetroxide (osfix.); paraformaldehyde fixation (parafix.) — TEM: glutaraldehyde fixation (glufix.); paraformaldehyde fixation (parafix.)

[c = channel; ie = inner exospore; n = nucleus; oe = outer exospore; sc = spore/sporocyte coat; smc = spore mother cell; sw = sporangium wall; t = tapetum; te = tetrad; tn = tapetal nucleus; v = vacuole]

Plate I: Spore mother cells — TEM (LEI 20339, parafix.)

- 1: sporangium with smc's in the rosette stage; from left to right: sporangium wall, the double layer of tapetum cells, rosette of smc's; 4000 ×
- 2: detail of Plate I-1, showing connections between smc's (arrow); 20000 ×
- 3: sporocyte coat in the course of doubling (arrow); 4000 ×
- 4: one smc in between rosette stage and rounded stage; sporocyte coat completely doubled; 4000 ×
- 5: sporocyte coat with loops, and nucleus with cytoplasmic intrusions (arrow); 4000 ×
- 6: smc, late in meiosis, irregularly shaped; 3000 ×

Plate II: Sorus and spore mother cells — SEM (LEI 22226; osfix., unless indicated otherwise)

- 1: cross section of a sorus; 65 ×
- 2: sporangium with rounded smc's, surrounded by periplasmodial tapetum (arrow); 700 ×
- 3: detail of one smc from Plate II-2; 3000 ×
- 4: see Plate II-2; detail of vacuolized tapetum; 7000 ×
- 5: see Plate II-2; detail of the sporocyte coat (arrow); 17000 ×
- 6: smc-rounded phase, smc's not broken (arrow); parafix.; 650 ×

Plate III: Tetrads during and after meiosis — TEM (LEI 20339, parafix.) and SEM (LEI 22226, osfix.)

- 1: sporangium with tetrads in meiosis; SEM; 500 ×
- 2: detail of one tetrad from Plate III-1; note smc-nucleus (arrow); 2450 ×
- 3: tetrad just after meiosis; TEM; 2300 ×
- 4: detail of plasmalemma formation in another such tetrad of the same sporangium; note plasmalemma (arrow); TEM; 14000 ×
- 5: young tetrad, showing position of spores; TEM; 2250 ×
- 6: tetrad showing one pair of young tetraspores; note invaginations between nucleus and cytoplasm (arrow); TEM; 2300 ×
- 7: tetrad showing three young tetraspores; TEM; 2400 ×
- 8: same sporangium, other tetrad; detail of the area between two tetraspores; note spore coat (arrow) and sporocyte coat (sc); TEM; 16000 ×

Plate IV: One sporangium after meiosis — SEM (*LEI 22226*, osfix.)

- 1: sporangium containing young tetrads; 370 ×
- 2: one spore of this sporangium; note laesural fold (arrow); 5000 ×
- 3: one tetrad of the sporangium; note spore/sporocyte coat (arrow); 2300 ×
- 4: see Plate IV-3; detail of surface coat; note foldlike structures (arrow); 11500 ×
- 5: same sporangium, other tetrad; note tapetal nuclei (tn) and spore/sporocyte coat (sc); 2300 ×
- 6: see Plate IV-5; detail of spore/sporocyte coat (arrow); 20000 ×
- 7: see Plate IV-1; detail of smooth inner side of the sporocyte coat; 4000 ×
- 8: same sporangium, other tetrad, one spore in cross section, note the nucleus (n); 3500 ×

Plate V: Formation of the inner and the first outer exospore layer — TEM (*LEI 20339*, parafix.) and SEM (*LEI 22226*, parafix.)

- 1: spore with only the inner exospore layer present, formation of the laesural fold (arrow) in progress; TEM; 4000 ×
- 2: proximal detail of the spore on Plate V-1; 30000 ×
- 3: formation of the inner exospore layer from without and from within the spore, note white line (arrow); TEM; 30000 ×
- 4: first stage of outer exospore formation: deposition of lumps on the inner exospore layer; note addition of material to the inner exospore layer from within (arrow); TEM; 20000 ×
- 5: spore with the very first beginning of the outer exospore, laesural fold present; SEM; 2200 ×
- 6: detail of Plate V-5; note granules of outer exospore material (arrow); 13000 ×
- 7: slightly later stage of outer exospore formation (a), laesural fold also bearing outer exospore material (arrow); SEM; 2200 ×
- 8: detail of Plate V-7; note different sizes of lumps; 13000 ×

Plate VI: Formation of the outer exospore layer (a) — TEM (*LEI 20339*, parafix.) and SEM (*LEI 22226*, parafix.)

- 1: formation of the outer exospore, cross section of one spore; note closed laesural fold (arrow); TEM; 3500 ×
- 2: formation of the outer exospore, tetrad with one spore in proximal view and two halved spores; SEM; 1550 ×
- 3: same sporangium, other spore – inside of the proximal spore face; note base of laesural fold (arrow); SEM; 8000 ×
- 4: detail of Plate VI-2; note cross section of the laesura (arrow); 8500 ×
- 5: outer exospore in formation, laesural zone and more distal parts of the spore; TEM; 16000 ×

Plate VII: Formation of the outer exospore layer (b-c) — TEM (*LEI 20339*, parafix.) and SEM (*LEI 22226*; osfix., unless stated otherwise)

- 1: one spore in distal view; SEM; 2500 ×
- 2: detail of Plate VII-1, spore surface; 11000 ×
- 3: one spore with tapetum and many spherical bodies (arrow), which lie embedded in the tapetum by which they are secreted; SEM; 1800 ×
- 4: detail of Plate VII-3; spore surface with small pits (arrow); 45000 ×
- 5: lateral view of a spore; SEM; 1750 ×
- 6: surface detail of another spore from the same sporangium; SEM; 8000 ×
- 7: proximal section of a spore with developing outer exospore (b), laesural fold not yet covered; note channels (arrow); TEM; 10000 ×
- 8: detail of a laesura with many channels; SEM (parafix.) 8000 ×

Plate VIII: Formation of the outer exospore (c-d)

- 1: one spore in lateral/proximal view; note smooth distal side; SEM (*LEI 22226*, osfix.); 1550 ×
- 2: proximal detail of the spore on Plate VIII-1; note uncovered part of the laesural fold (arrow); 7000 ×
- 3: same sporangium, detail of tapetum (t) against another spore; SEM (*LEI 22226*, osfix.); 7500 ×
- 4: lateral view of one spore, covered with broad verrucae; SEM (*84GR00187*, parafix.); 1400 ×
- 5: same sporangium, proximal detail of another spore; note tiny channels (arrow); SEM (*84GR00187*, parafix.); 3500 ×
- 6: same stage, surface detail of a spore with tapetal remains (arrow); SEM (*84GR00187*, parafix.); 7500 ×
- 7: cross section of an entire spore, covered with large verrucae; TEM (*81GR00119*, glufix. with DMSO); 6000 ×

Plate IX: The last stage of exospore formation; the mature exospore — TEM (*LEI 20339*, parafix.) and SEM (*LEI 22226*, osfix.)

- 1: section of the laesura; note channels (arrow); TEM; 12000 ×
- 2: same sporangium, different spore; proximal section with large channels (c); note darker granules marking former spore surfaces (arrow); 30000 ×
- 3: cross section of a spore, detail of the laesura; SEM; 4500 ×
- 4: lateral/distal detail of cross section of the same spore; note smooth inner surface of the exospore; SEM; 4500 ×
- 5: broken spore, exospore finished; SEM; 1800 ×
- 6: detail of Plate IX-5; cross section of the laesura; 7500 ×
- 7: cross section of a laesura; TEM; 16000 ×
- 8: same sporangium, proximal cross section of another spore; TEM; 22000 ×

Plate X: Perispore formation, the mature perispore — TEM (*LEI 20339*, parafix.) and SEM (*LEI 20339*)

- 1: mature spore; almost all of the perispore abraded, mature exospore surface visible; SEM (nofix.); 1200 ×
- 2: tapetal residue with spherical bodies; note angular blobs (arrow); TEM; 20000 ×
- 3: exospore; note angular blobs (arrow); TEM; 50000 ×
- 4: same sporangium as Plate X-3, exospore covered with lamellae (arrow) and blobs; TEM; 70000 ×
- 5: same sporangium as Plate X-3, exospore with lamellae, blobs and small spine (arrow); TEM; 70000 ×
- 6: exospore with blobs and spherical bodies (arrow); TEM; 27000 ×
- 7: perispore in formation; proximal view of a spore; SEM (osfix.); 1200 ×
- 8: detail of the laesura on Plate X-7; 5000 ×

Plate XI: The mature spore

- 1: proximal/lateral view of a mature spore; SEM (*LEI 20339*, nofix.); 1100 ×
- 2: detail of the perispore surface; note the position of the spines with respect to the surface pattern; SEM (*LEI 20339*, nofix.); 8000 ×
- 3: mature spore with longer and sparser spines; SEM (*81GR00119*, nofix.); 1000 ×
- 4: detail of Plate XI-3, showing different types of spines; 4500 ×
- 5: pattern on an LM-slide, probably caused by evaporation and subsequent precipitation of the glycerin jelly in which the objects were embedded; note resemblance to the basal pattern of the perispore on Plate XI-2; 90 ×
- 6: detail of a loose perispore fragment; TEM (*LEI 20339*, parafix.); 16500 ×
- 7: detail of laesura with spines perpendicular to the perispore surface; pattern of the basal layer smaller than in Plate XI-2; SEM (*84GR00187*, nofix.); 4500 ×
- 8: detail of a perispore with spines perpendicular to the surface, and of the sporangium wall with a perispore-coated spherical body and other perispore-like particles; SEM (*81GR00119*, parafix.); 1900 ×

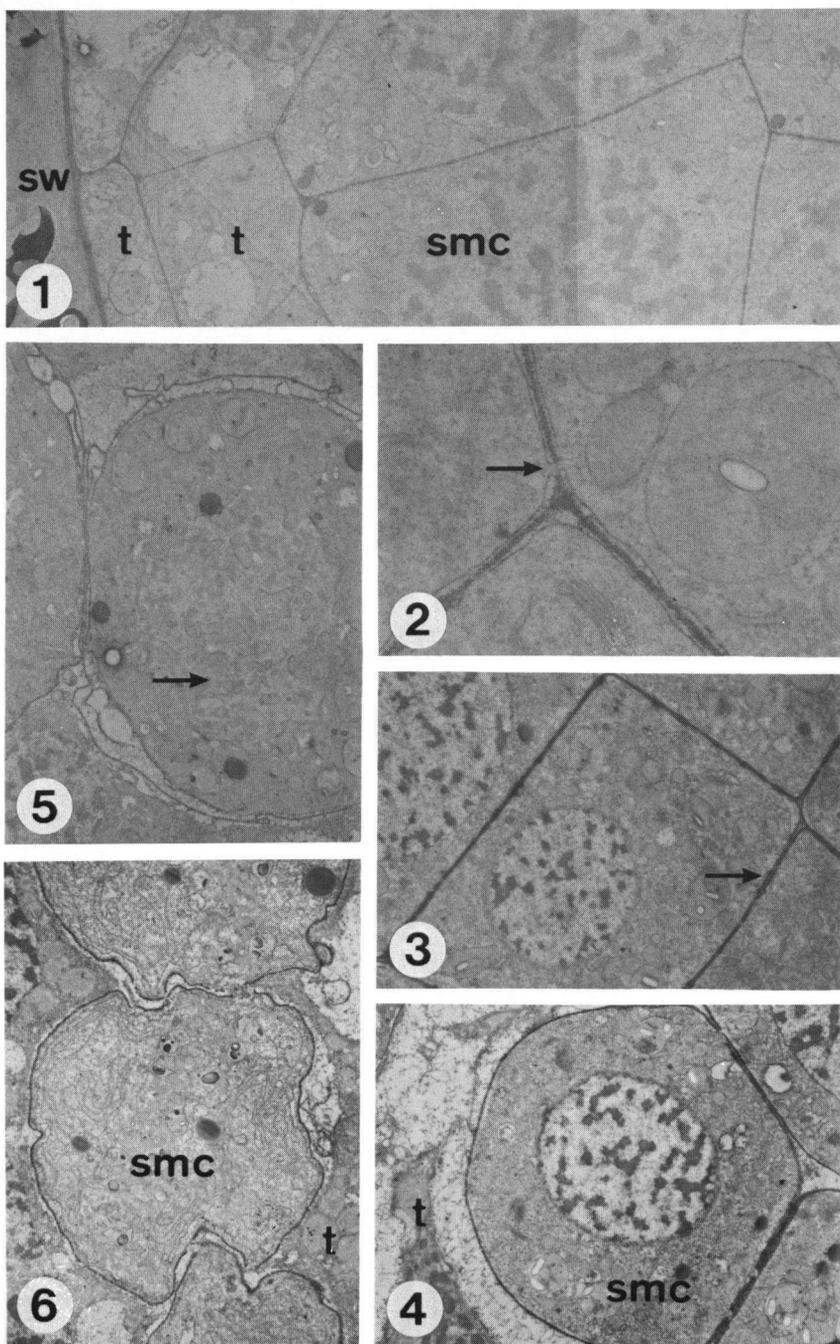


Plate I

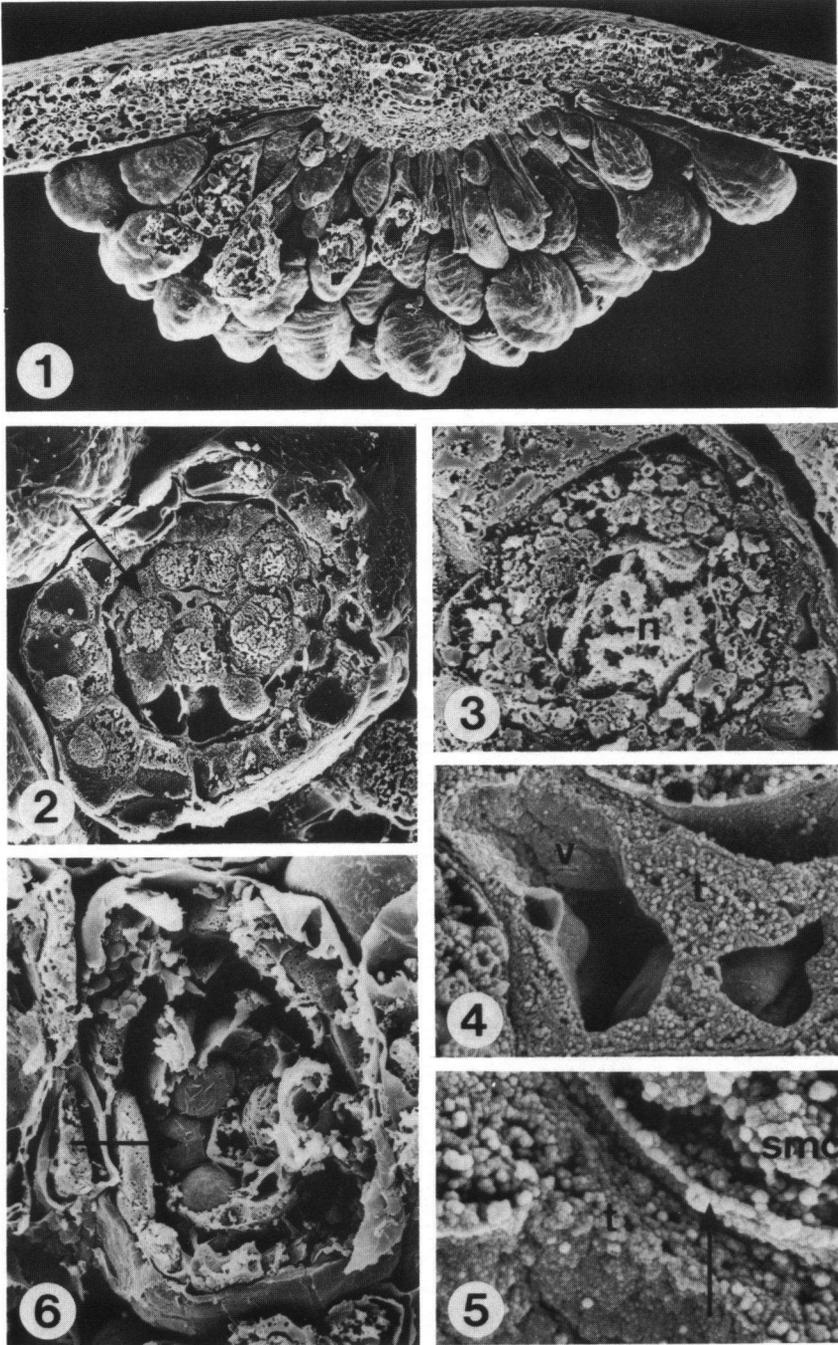


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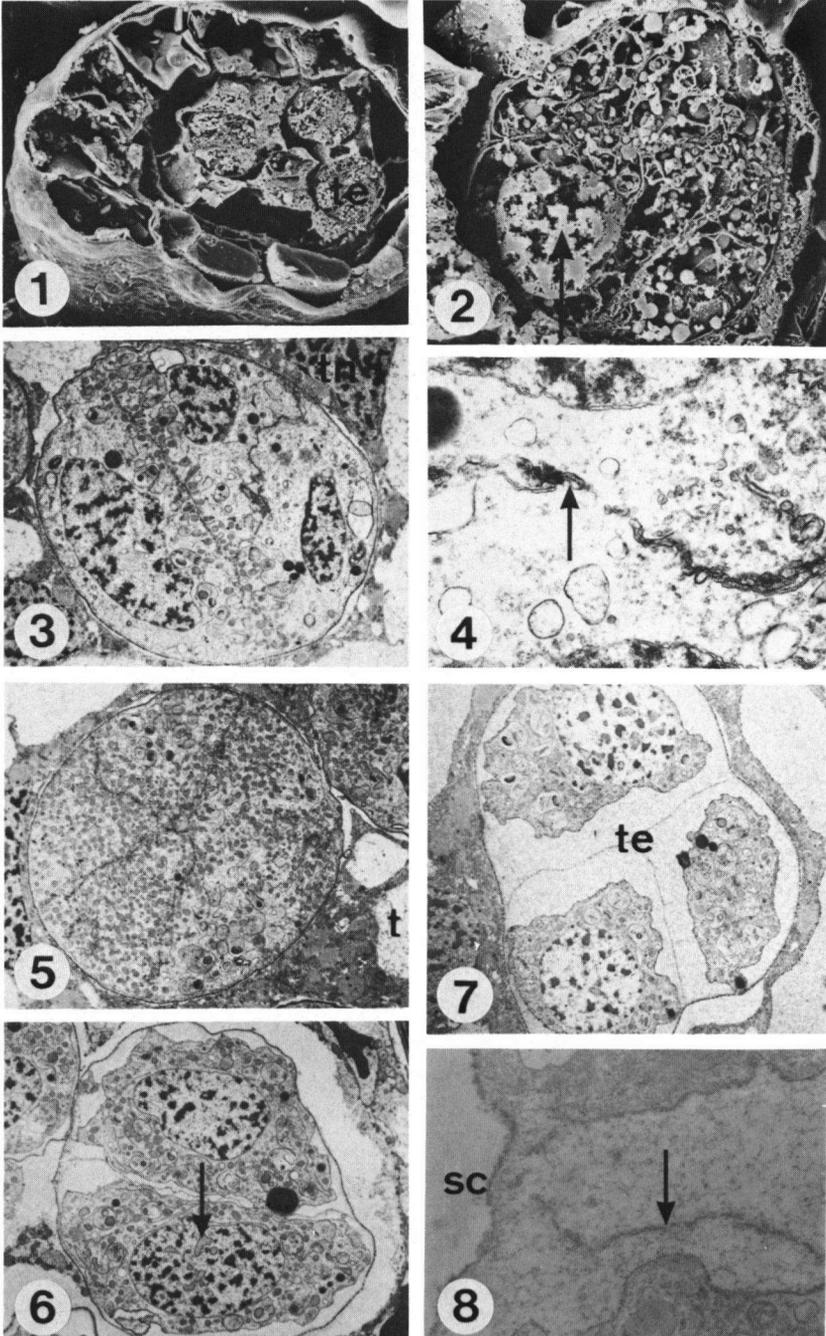


Plate III

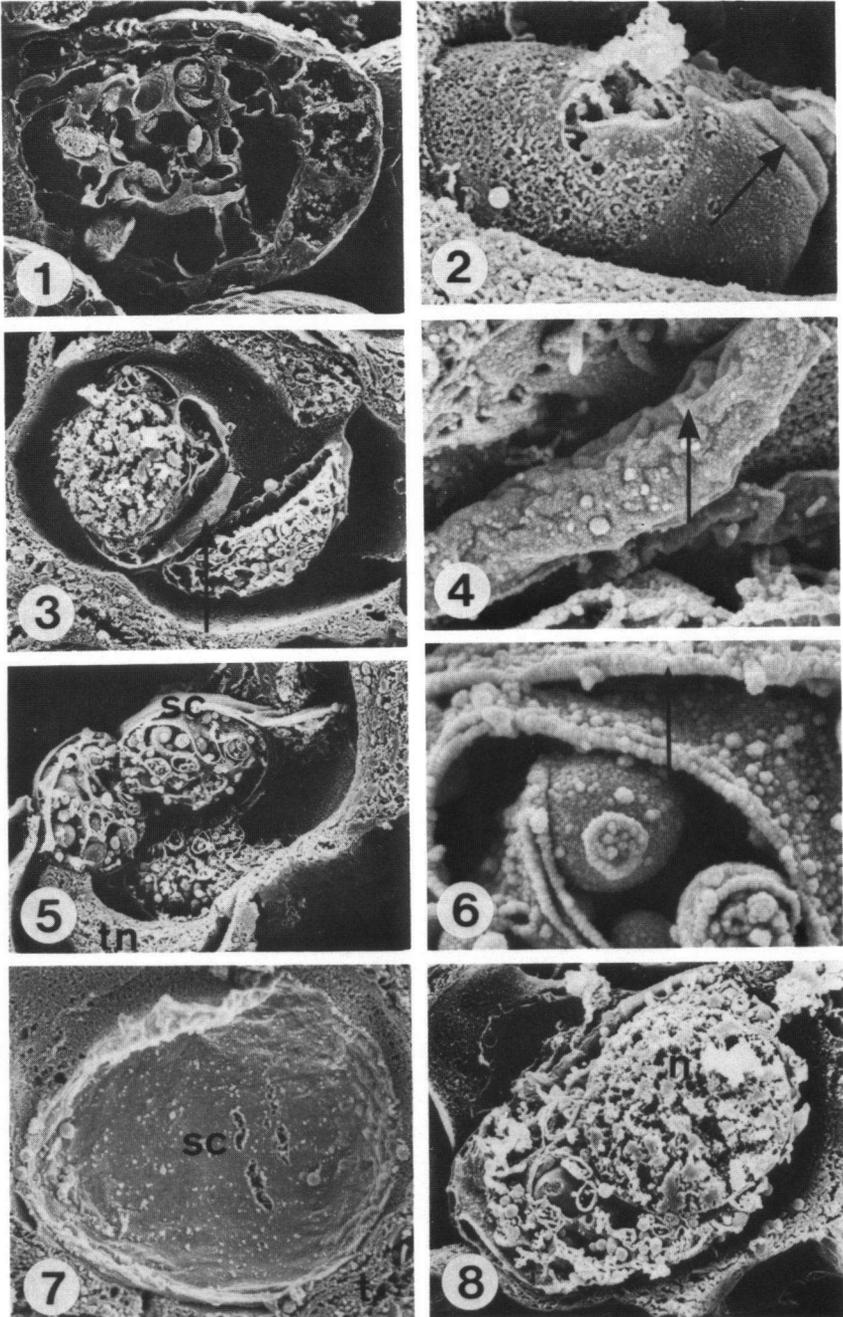


Plate IV

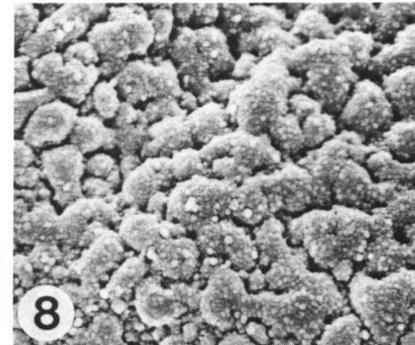
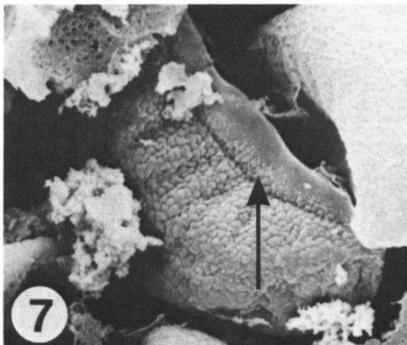
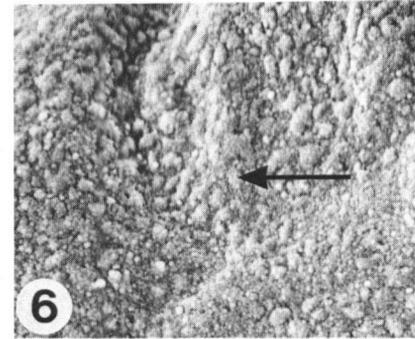
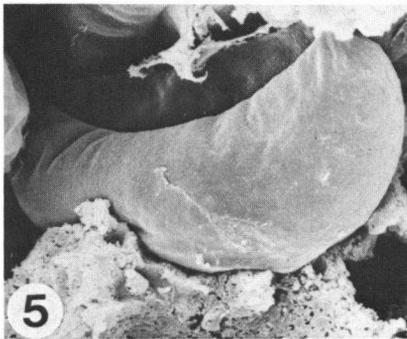
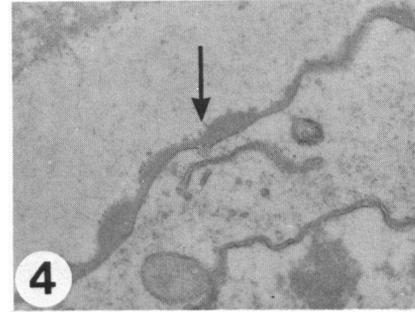
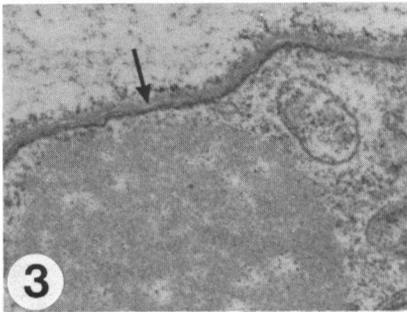
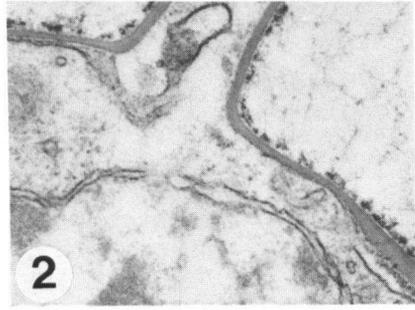
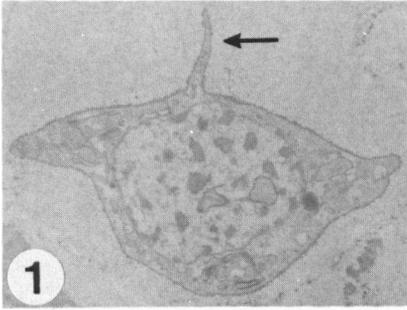


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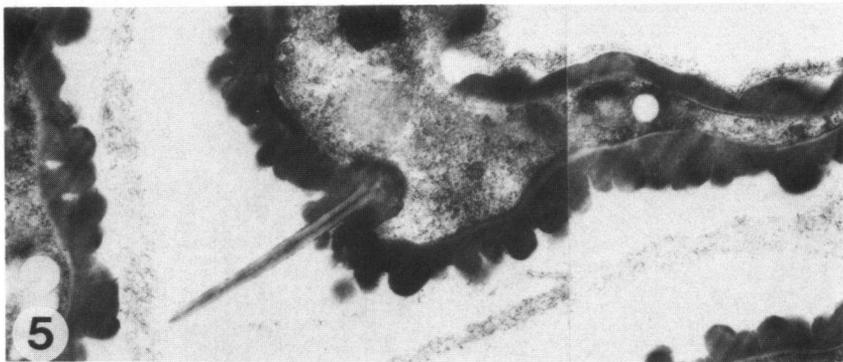
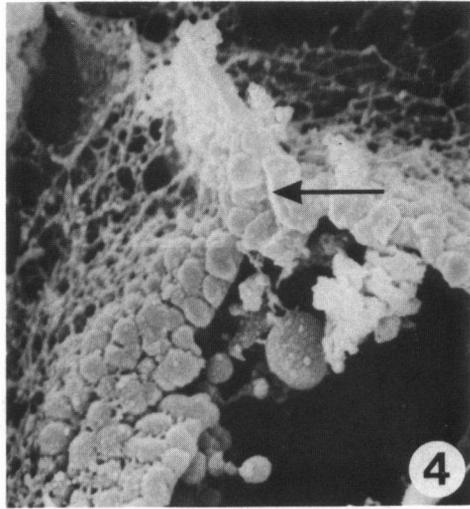
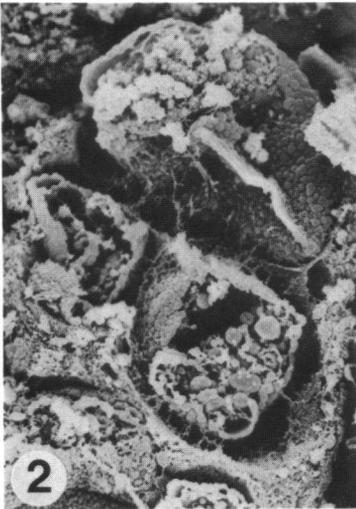
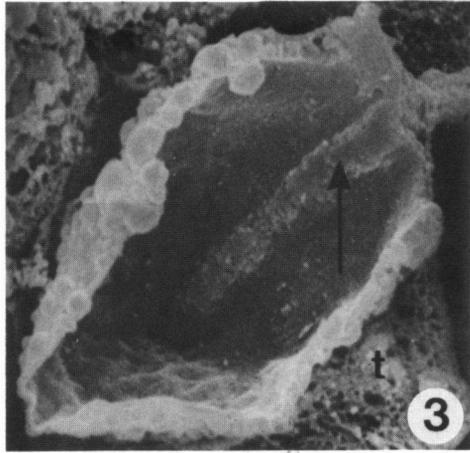
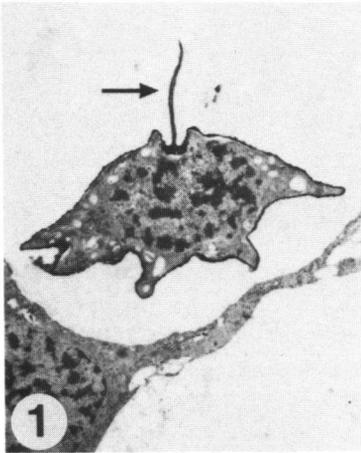


Plate VI

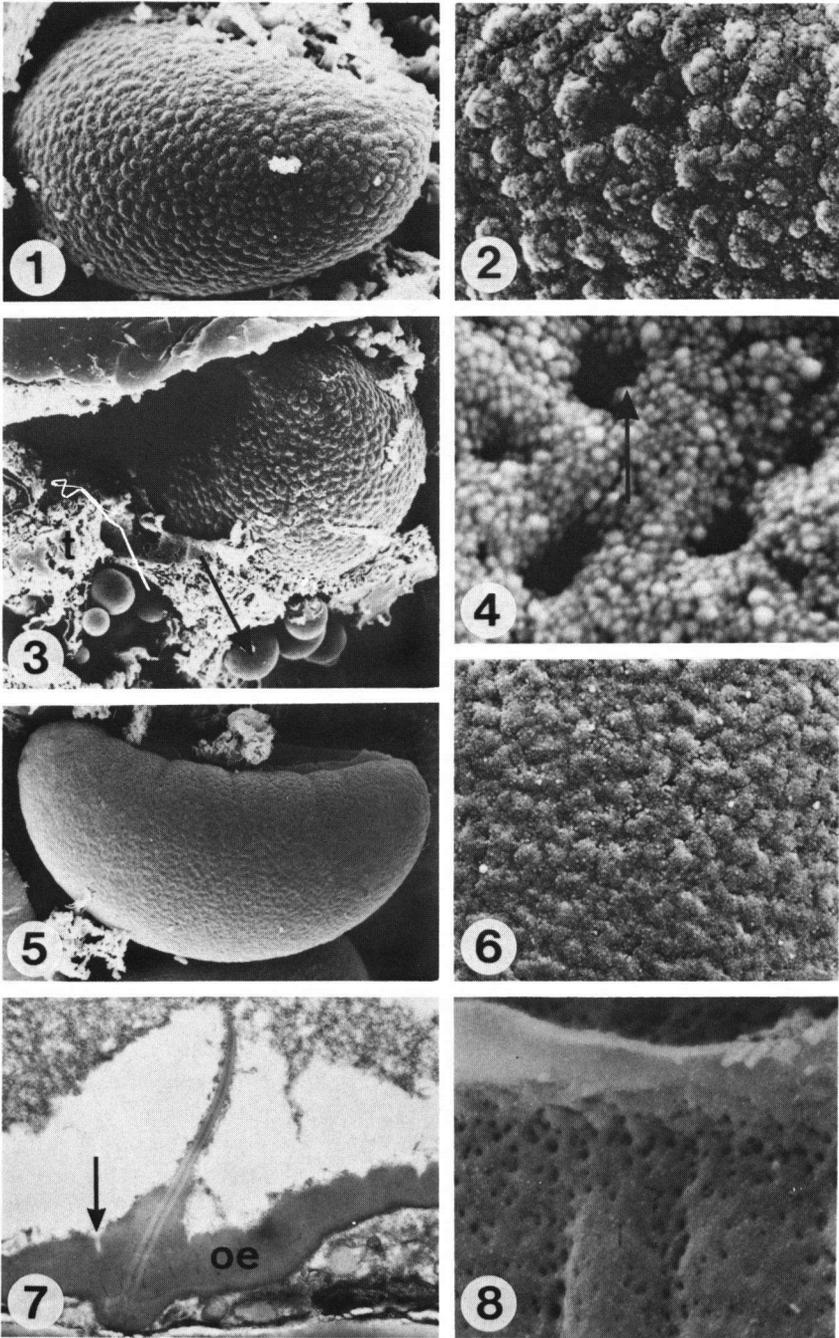


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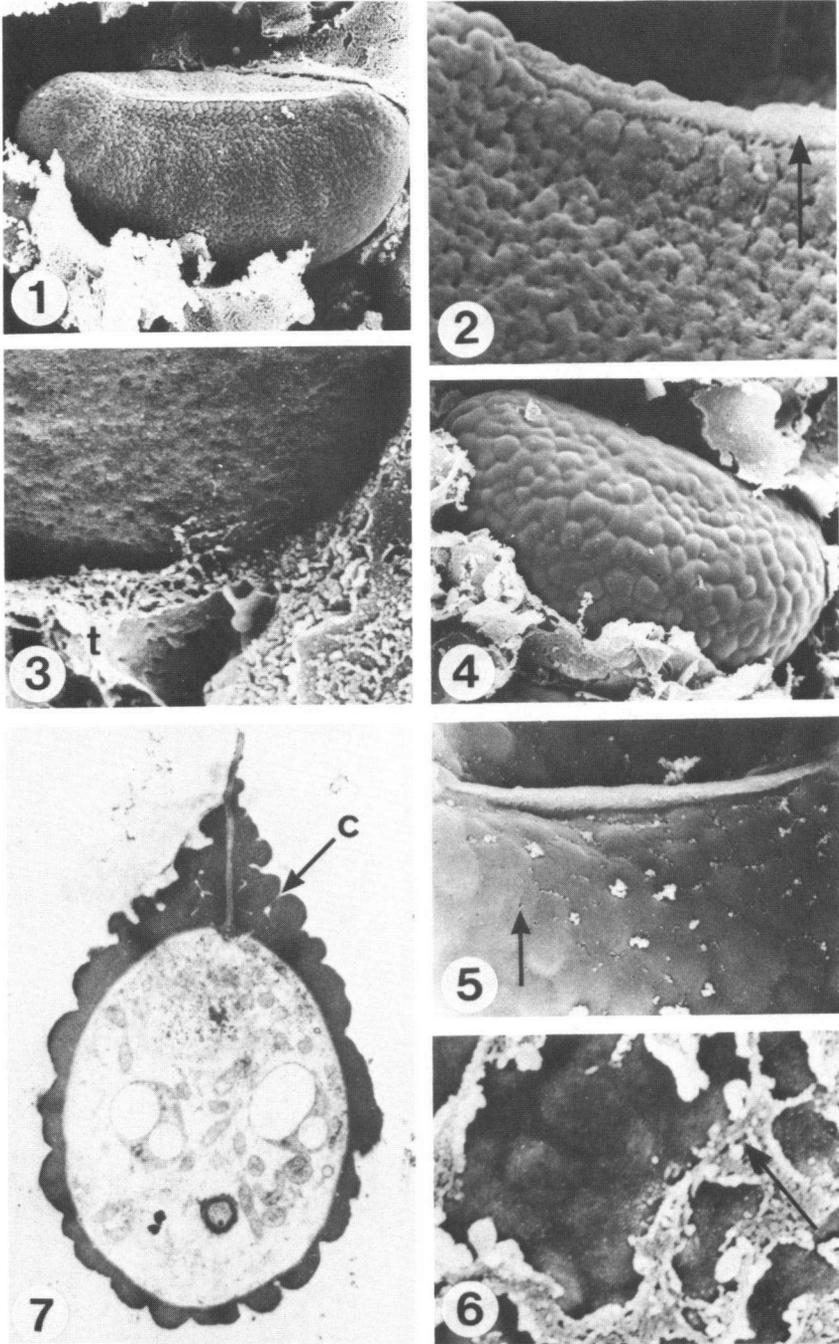


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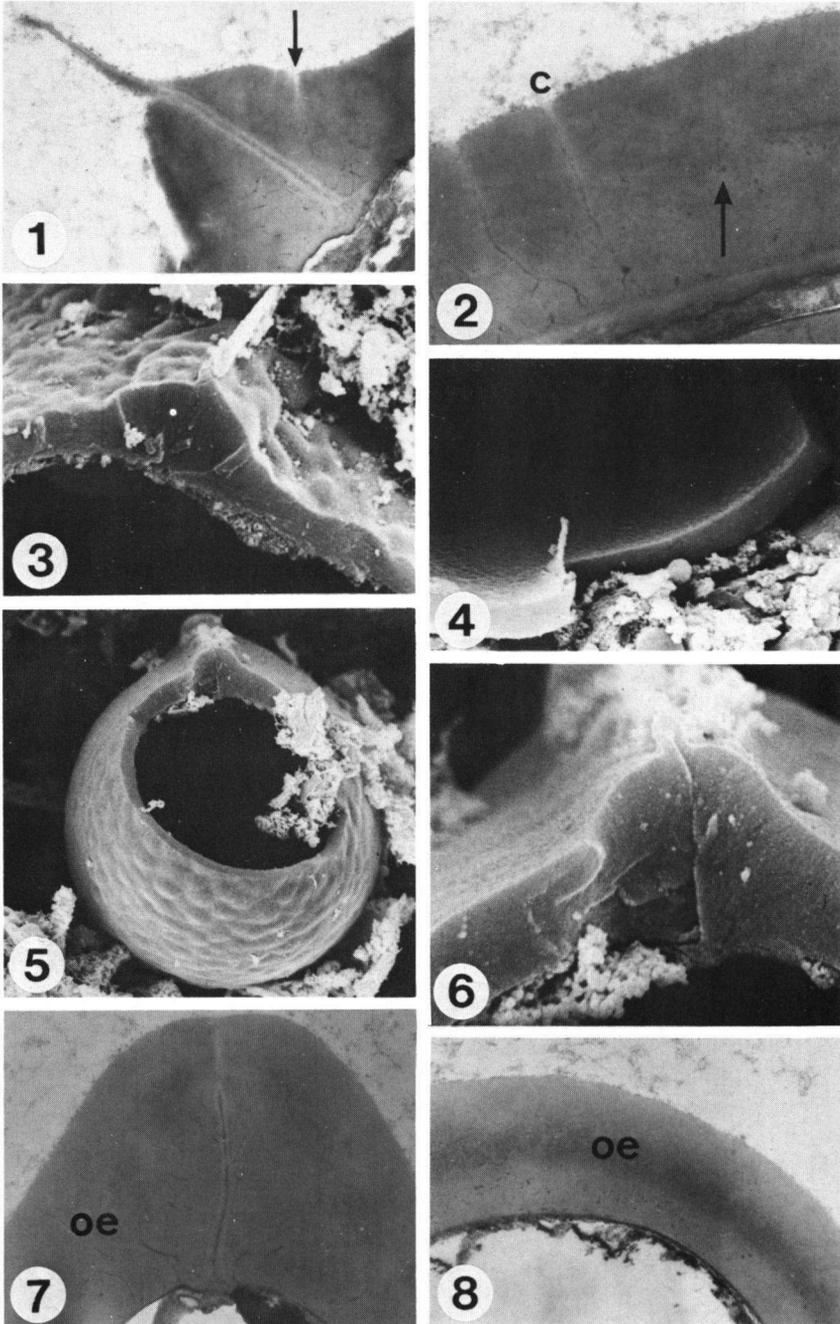


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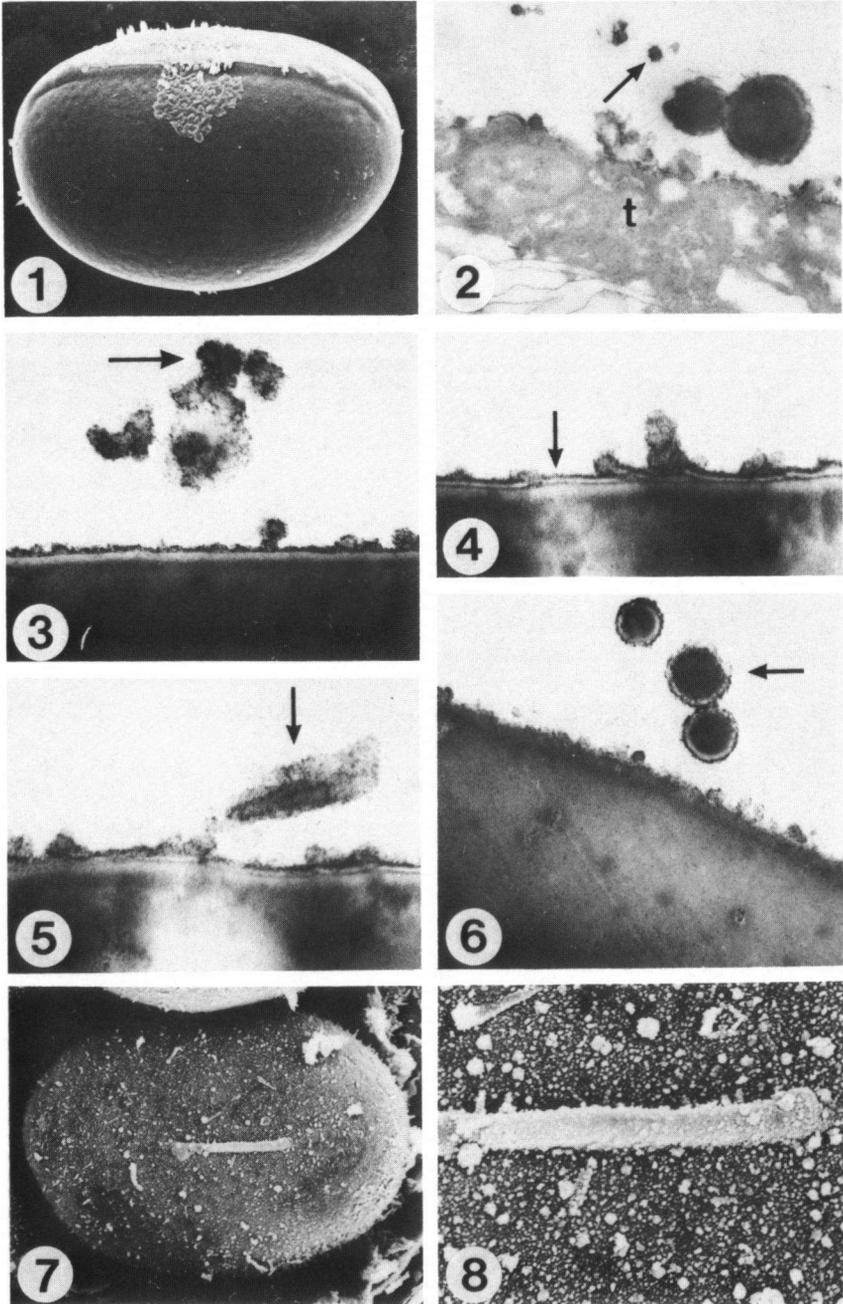


Plate X

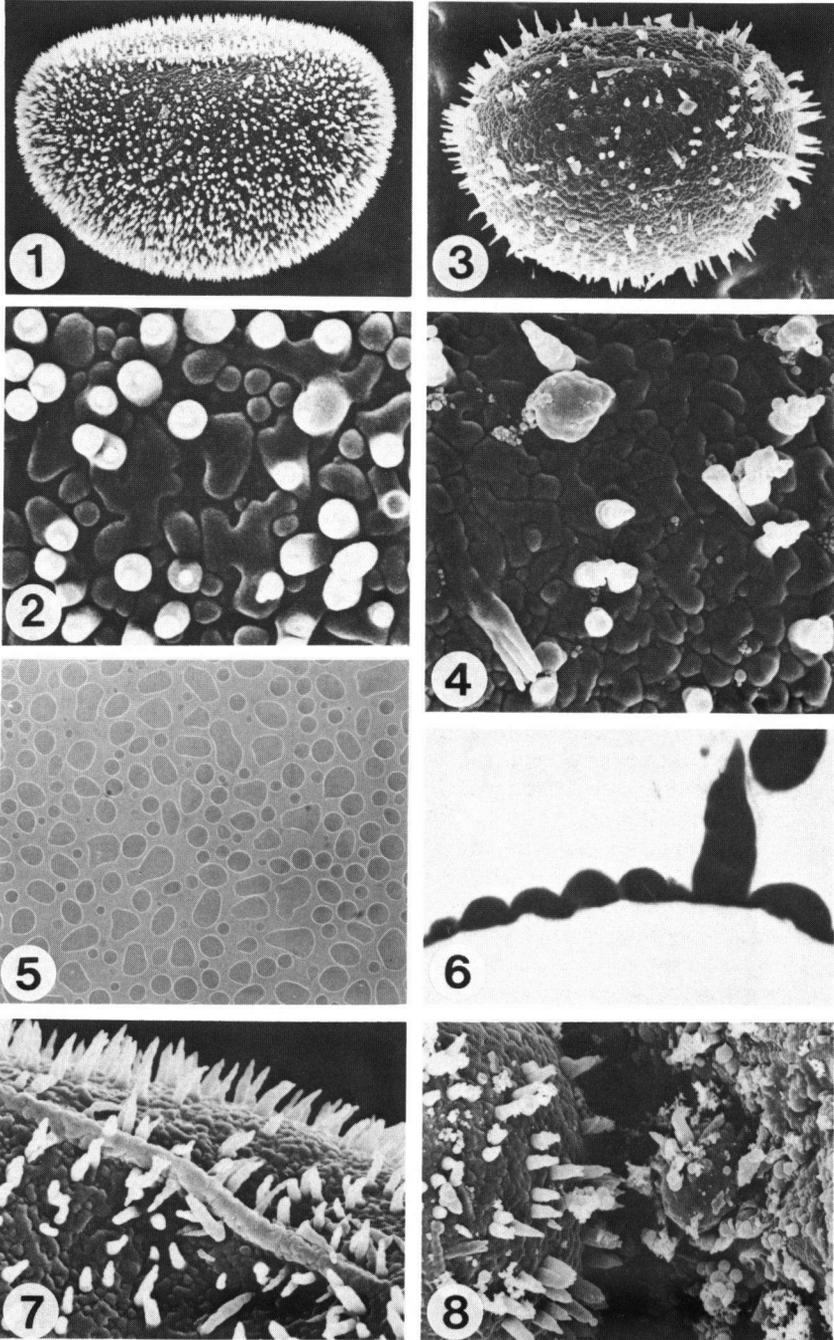


Plate XI