ON THE MEGASPOROGENESIS IN *CHAMAECYPARIS LAWSONIANA* PARL.

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INTRODUCTION

In the context of embryological researches on some Gymnosperms (FRANCINI CORTI and MAUGINI 1964; MAUGINI and CECCHI FIORDI 1970), we observed that in the ovules of *Chamaecyparis lawsoniana* Parl. the chalazal functional megaspore of the triad formed through the meiosis shows, under the light microspore (LM), a cytoplasmic region with a peculiar feature below the nucleus (Fig. 1).

Various LM observations on the megasporogenesis of other Gymnosperms (COKER 1903, 1904; CAROTHERS 1907; OTTLEY 1909), together with the results of an ultrastructural research on the megasporocyte of *Ginkgo biloba* (STEWART and GIFFORD 1967), led us to observe at the electron microscope (EM) also the megaspore mother cell (MMC) of *C. lawsoniana*. In particular we have studied the behaviour, during meiosis, of its chalazal cytoplasm which will form the functional megaspore.

MATERIAL AND METHODS

For the EM observations ovules of *C. lawsoniana* Parl. have been collected from several female cones of a tree growing in the Florence botanical garden, at the beginning and at the end of March.

The material has been prefixed in a mixture of glutaraldehyde and paraformaldehyde in phosphate buffer at pH 7.4 (KARNOVSKY 1965); subsequently it has been fixed in a 2% solution of OsO_4 in the same buffer. After dehydration with ethyl alcohol, the ovules have been embedded in Epon (LUFT 1961).

The ultrathin sections, stained with uranyl acetate (GIBBONS and GRIMSTONE 1960) and lead citrate (VENABLE and COYGESHALL 1965), have been observed and photographed with a Philips EM 300 electron microscope.

Some semithin sections from the same ovules fixed and embedded for the ultrastructural investigation have been observed under the phase contrast LM or after staining with black Sudan B (Fig. 6).

Moreover, for the LM observations, some ovules collected in the same periods as the other ones, have been fixed in FAA, dehydrated with the tertiary butyl alcohol series, embedded in paraffin and finally stained with Heidenhein iron hematoxylin (Figs. 1, 2, 26).

OBSERVATIONS

In both the collections not every ovule from the same cone has reached the same development stage as far as the fertile cells are concerned. On the contrary, the growth of the vegetative parts looks more uniform.

Ovules collected at the beginning of March: megaspore mother cell and dyad.

The ovules collected in this period have such a small size that they are completely covered by the closely packed ovuliferous scales. The integument already surrounds completely the nucellus, except the apical region in which the micropyle is just being formed (Fig. 2). Several cell divisions occur both in the nucellus and in the integument.

As far as the fertile cells are concerned, we have only seldom observed two MMCs in contact with each other (Figs. 3, 7). Normally each ovule contains only one MMC, more or less differentiated (Figs. 2, 4), deep-seated in the middle part of the nucellus (Fig. 2).

In the youngest ovules seen the MMC has a size already much larger than the surrounding cells and an elongate shape. Under the EM the wall separating the MMC from the surrounding cells consists of a very thin incipient primary wall of tapetal cells, a light middle lamella and a thicker

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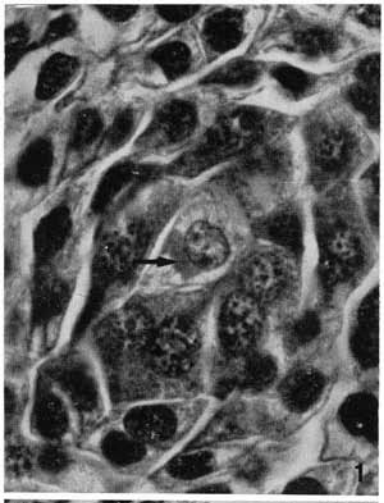
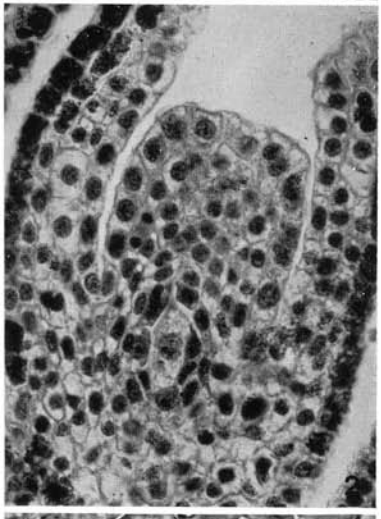
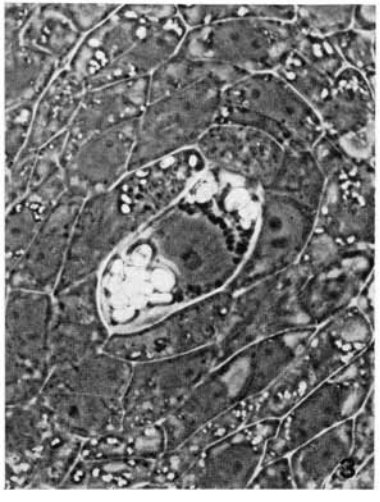
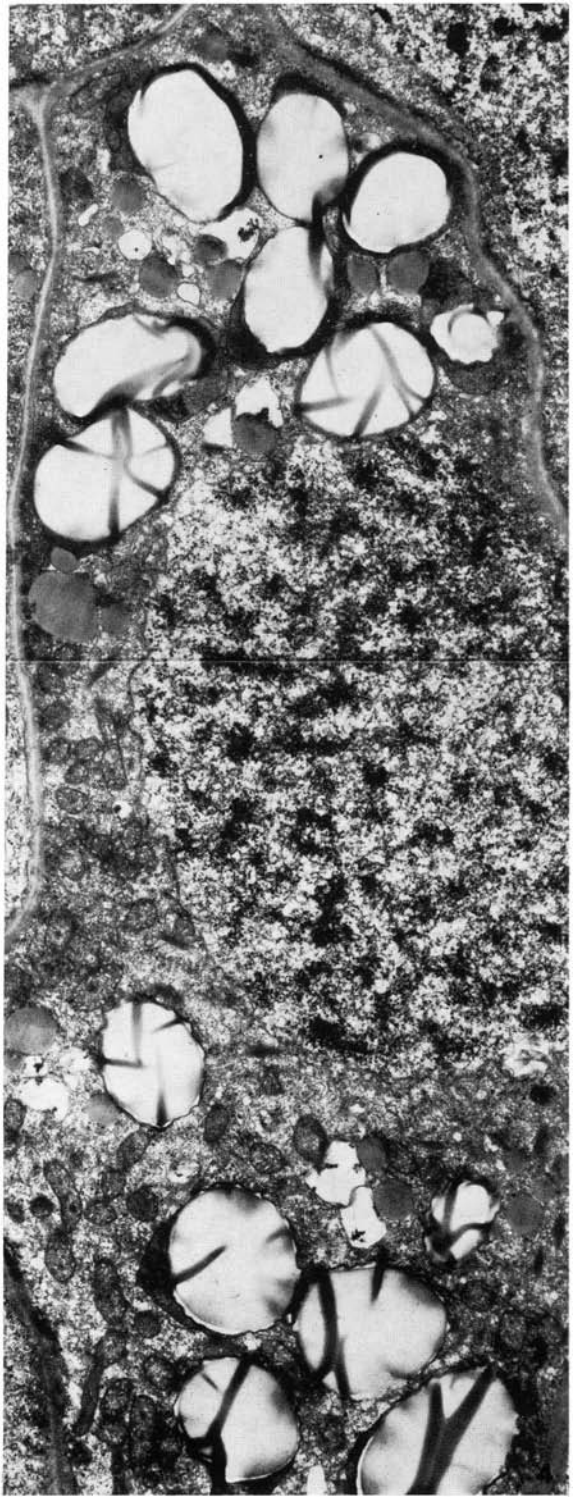
Figs. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13: specimens from ovules collected at the beginning of March.
Figs. 1, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25: specimens from ovules collected at the end of March.
Fig. 26: specimen from ovules collected at the beginning of April.

Fig. 1. — Approximately longitudinal section of a triad. In the chalazal megaspore, below the nucleus, there is a region denser than the surrounding cytoplasm (arrow). (x 650).
Fig. 2. — Longitudinal section of an ovule. The integument begins to form the micropyle. In the middle and innermost region of the nucellus a single megaspore mother cell (MMC), surrounded by the tapetum, is already differentiated. (x 286).
Fig. 3. — Longitudinal section of an ovule showing two contiguous MMCs. The megasporocyte on the left-hand side appears tangentially sectioned. It contains very little starch and is surrounded by a wall thinner than that of the megasporocyte on the right-hand side. In the nucellus starch and vacuoles gradually increase from the tapetum to the outer regions. (x 647).
Fig. 4. — Longitudinal section of a MMC just at the beginning of the prophase I showing lipidic droplets, a few small vacuoles and amyloplasts containing large starch granules. The mitochondria are preferentially located in the perinuclear and chalazal cytoplasm. (x 6,703).









primary wall of the MMC (Fig. 5). The plasmodesmata are rather rare and almost always they are seen to cross only the very thin primary wall of tapetal cells and the middle lamella. The nucleus, with one or more nucleoli, is slightly shifted toward the micropylar region and is almost as wide as the whole cell. (Figs. 2, 3, 4).

It is immediately possible to distinguish the megasporocyte at a much advanced differentiation stage from the somatic cells not only because of its larger size, but also because of the presence, in both the micropylar and the chalazal cytoplasm, of a large amount of food-storage substances. These substances are represented by large starch granules (Figs. 3, 4, 5) and by rather electron-dense droplets which are isolated or only partially fused (Fig. 4). These droplets could be, at least partly, of lipidic nature as shown by their staining with black Sudan B when observed, in semithin sections, under the LM (Fig. 6).

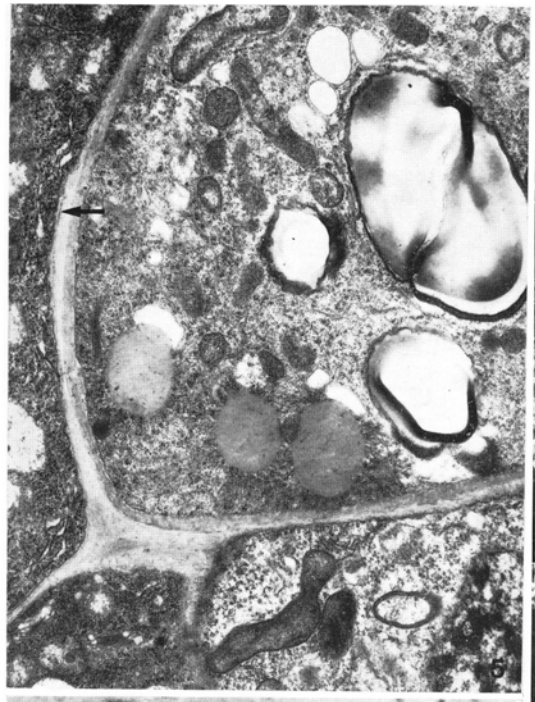
In the few cases in which we have observed two megasporocytes, one of them contains much less starch and is surrounded by a thin wall looking like that separating the neighbouring somatic cells (Figs. 3, 7). In such cases we are very likely seeing a MMC which will not undergo meiosis.

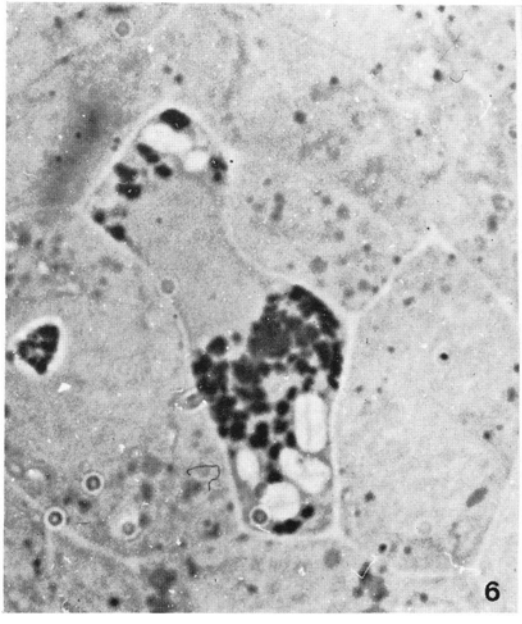
In the cytoplasm of the megasporocyte besides the reserve substances we observe mitochondria, dictyosomes, ribosomes, elements of endoplasmic reticulum and microtubules. The vacuoles are only seldom observed and are rather small (Figs. 4, 5). At early prophase I the mitochondria are localized mainly in the perinuclear and in the chalazal cytoplasm, though some of them are also observed in the micropylar region (Fig. 4). At late prophase I the MMC shows a noticeable cytoplasmic vacuolation and a smaller starch content (Figs. 8, 9). In our opinion, however, the most significant and interesting feature is that, at this stage, all the mitochondria are localized in the chalazal cytoplasm (Fig. 9). In this region the plasmalemma shows now lomasomes, small invaginations and vesicles (Fig. 10). The chalazal cytoplasm therefore appears to be the metabolically most active part of the MMC.

Fig. 5. — Portion of chalazal cytoplasm of the megasporocyte with ribosomes, mitochondria, small vacuoles, lipidic droplets and amyloplasts. The wall separating the MMC from the tapetum consists of a very thin primary wall of the tapetal cells (arrow), a light middle lamella and a thicker primary wall of the MMC. (x 7,524).

Fig. 6. — Approximately longitudinal section of the central region of the nucellus stained with Sudan black B. The megasporocyte contains numerous deeply stained droplets and some large, obviously colourless, starch granules. (x 1,600).

Fig. 7. — Longitudinal section of an ovule showing portions of two contiguous MMCs. The megasporocyte on the right-hand side has a thicker wall and contains a considerable amount of starch, while that one on the left-hand side is surrounded by thinner wall and contains very little starch. The latter could be a potential MMC. (x 3,182).

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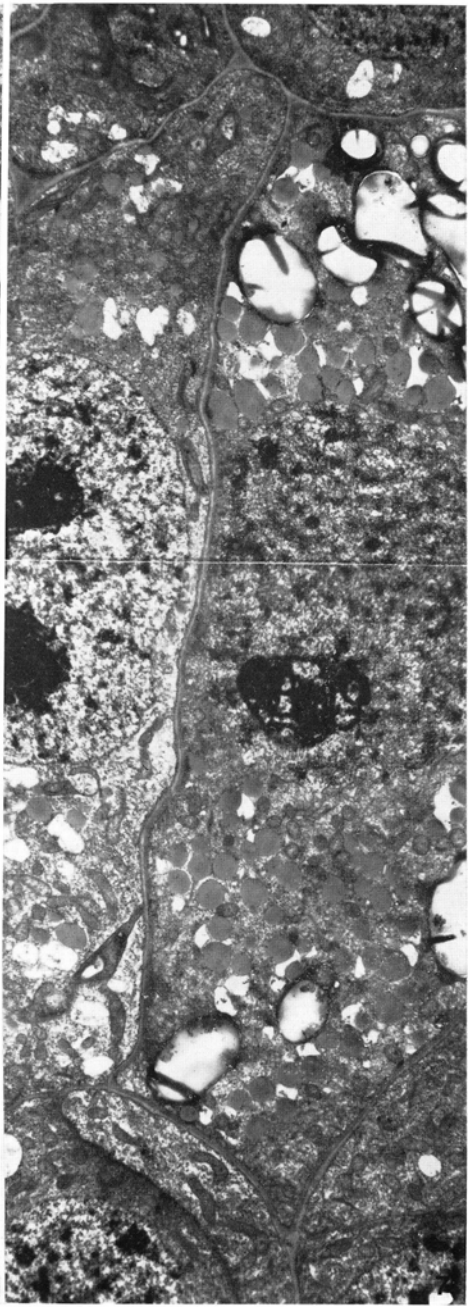


The first meiotic division gives rise to a dyad in which the food-storage substances are more or less uniformly distributed between the two cells (Fig. 11), while the chondriome is concentrated in the lower dyad cell (Fig. 12). This cell has a larger size than the upper one since in the megasporocyte the nucleus is slightly shifted toward the micropylar region.

The wall separating the dyad from the surrounding cells appears to be, on both sides, somewhat thickened when compared to the preceeding stage. On one side the somatic cells have thickened their own wall which is rather electron-dense and evenly thick, while on the other side the dyad has formed a thicker uneven layer. This layer lacks plasmodesmata and consists of an electron-transparent matrix and of fibrils which are either irregularly oriented or roughly parallel to the cell surface (Fig. 13). Therefore the wall between the dyad and the somatic cells seems to us rather similar to that observed in *Ginkgo biloba* (STEWART and GIFFORD 1967). In this species, however, the above mentioned stratification is formed at the megasporocyte stage and moreover the middle lamella « is irregularly thickened ».

Ovules collected at the end of March: triad.

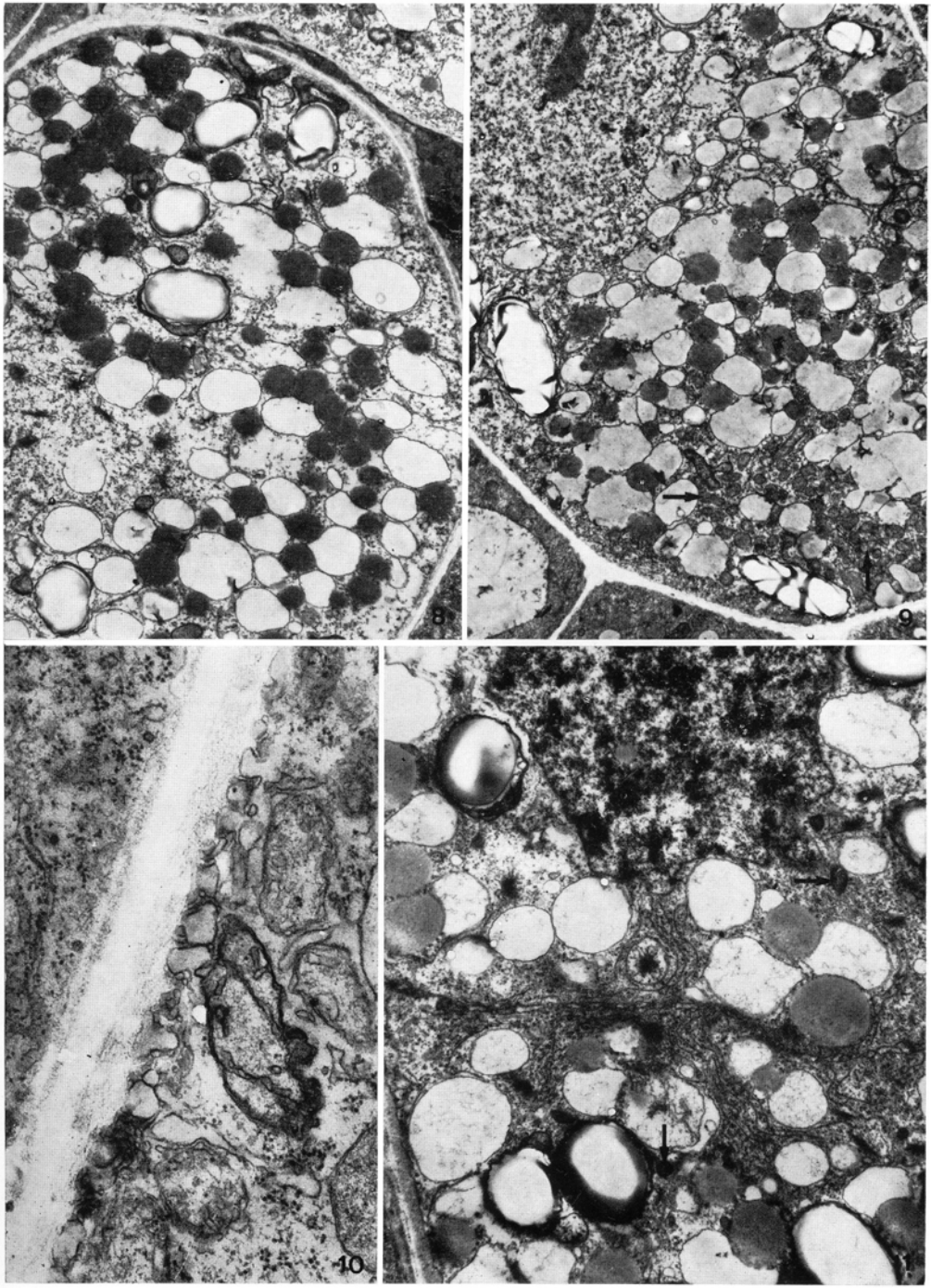
In the cones collected in this period, the micropyles of the ovules are clearly visible from the outside among the ovuliferous scales, which do not stick to each other any longer. The pollination drop shows up on all the micropyles and only in some of them it is possible to see pollen grains.

Inside the nucellus only seldom do we observe two linear triads of megaspores, one of which is degenerating. Normally there is a single triad.

In the youngest triads all the megaspores look normal, the micropylar two being smaller than the chalazal one (Fig. 14).

At a later stage the two micropylar megaspores degenerate without following always the same chronological sequence and only the chalazal megaspore remains functional (Fig. 15).

Fig. 8. — Micropylar cytoplasm of the MMC at late prophase I showing a high degree of vacuolation. This cytoplasmic region appears devoid of mitochondria. (x 3,499).
Fig. 9. — Chalazal cytoplasm of the MMC at late prophase I with the same degree of vacuolation as in Fig. 8. The mitochondria (arrows) appear to be restricted to the lower end of the cell. (x 3,499).
Fig. 10. — Portion of chalazal cytoplasm of the MMC at late prophase I. The plasmalemma shows numerous small invaginations and lomasomes. (x 28,638).
Fig. 11. — Portion of a young dyad. The micropylar cell and the upper region of the chalazal cell are devoid of mitochondria and almost equally supplied with food-storage substances and vacuoles. (The organelles marked with an arrow could represent peripheral sections of plastidal evaginations). (x 5,426).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Although we could not observe any intermediate stage, we believe that the upper dyad cell degenerates without dividing, all the more so because in this cell the mitochondria would be absent or at least only rarely present even if we admit that some sections could have escaped our observation. A triad of megaspores is produced also in *Biota orientalis* Endl. (MARTIN 1950 in MAHESWARI and SINGH 1967; SINGH and OBEROI 1962).

The degeneration of the upper dyad cell, judging from all the observed samples, would always begin after the reconstitution of the nucleus. However, already the telophase could possibly follow an anomalous sequence, since we have observed a young triad in which the nucleus of the micropylar megaspore shows a large evagination containing a dictyosome, some vesicles of endoplasmic reticulum and microtubules similar to the cytoplasmic ones (Fig. 16).

The second meiotic division therefore would take place only in the chalazal dyad cell. It gives rise to a further segregation of the chondriome in the chalazal megaspore from which the female gametophyte will develop while the food-storage substances are approximately equally distributed between the two cells (Fig. 14). The chalazal megaspore has a larger size and an irregular polyhedral shape (Fig. 14). Its nucleus is shifted toward the upper cytoplasm and the mitochondria are concentrated in the chalazal region very close to the nucleus itself (Fig. 14). It seems to us that this localization of the chondriome explains the pattern of Fig. 1. Later on, the cytoplasm and the nucleus of the two degenerating micropylar megaspores become intensely electron-dense (Fig. 15). At the same time the chalazal megaspore increases in volume, crushes the overlying cell and becomes round. Besides, compared with that observed at earlier stages, the starch is now much less abundant, the vacuoles are larger and numerous so that they occupy almost the whole cytoplasm. The lipidic droplets, which are less electron-dense, seem to release their content into the vacuoles (Fig. 17). The mitochondria are distributed to the side of the nucleus which drifts toward the cell periphery (Fig. 17) and some of them are now present also in other cytoplasmic regions free from vacuoles. Moreover, at this stage, just at the outside of the plasmalemma, we observe a wall layer consisting of fibrils parallel to the cell surface. It completely surrounds

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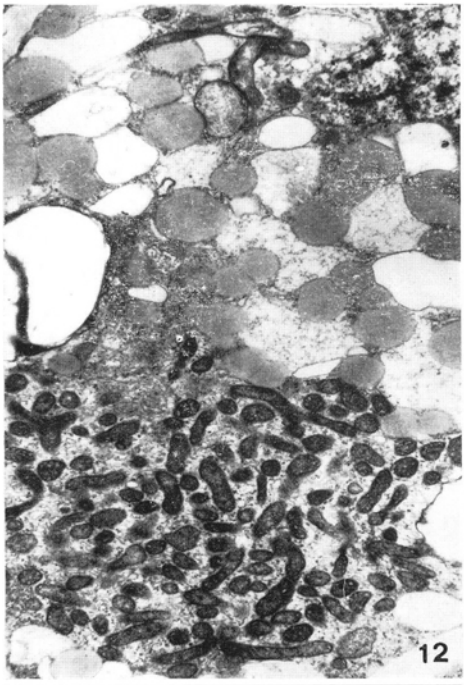
Fig. 12. — Lower end of the chalazal dyad cell which has inherited all the mitochondria of the MMC. (x 5,091).

Fig. 13. — Portion of the wall separating the dyad from the tapetum. The wall (p) of the tapetal cells appears thickened and the wall of the dyad shows a new lagyer (P) consisting of thin fibrils embedded in an electron-transparent matrix. (x 20,520).

Fig. 14. — Portion of a young triad. The three megaspores appear still to be functional and are almost equally provided with food-storage substances. The chalazal megaspore having a larger size and a polyhedral shape is the only one showing mitochondria clustered in the lower cytoplasm close to the nucleus. (x 4,435).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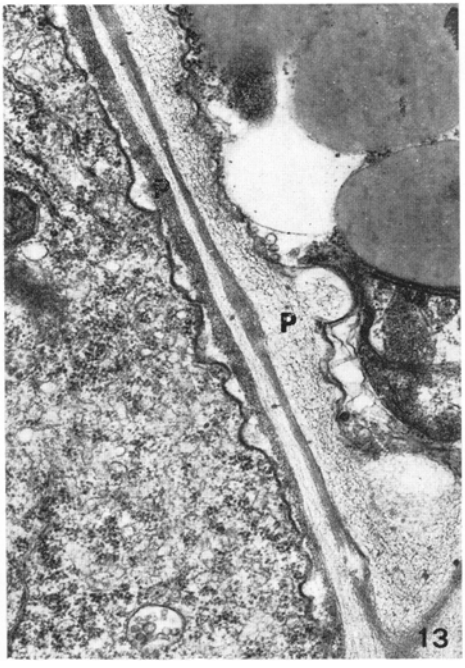
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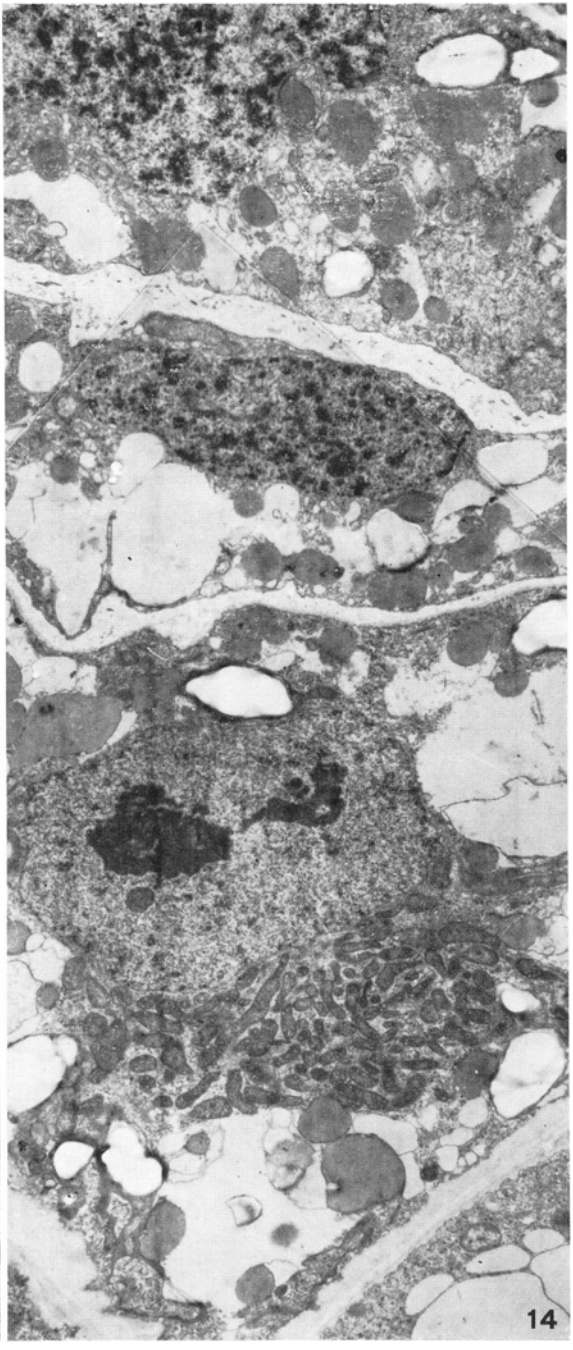
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the cytoplasm of the chalazal megaspore and appears to be thicker on the side bordering the overlying degenerating megaspore (Fig. 18). In the deposition of this layer an important role is probably played by the dictyosomes. In fact numerous dictyosomes are observed in the peripheral cytoplasm together with several small vesicles (Fig. 18). The appearance of this new wall material marks probably the beginning of the formation of that particular wall which in Gymnosperms is characteristic of the functional megaspore and, later on, of the female gametophyte.

Although numerous sections have been observed, this wall layer of the chalazal megaspore appears also to lack plasmodesmata (Figs. 19a and b), like the wall layers of the megasporocyte and of the dyad. Therefore we think that the early cytoplasmic connections are occluded by the successive wall thickenings laid down by the fertile cytoplasm. However, they are not modified by the new wall material formed, as already mentioned, by the somatic cells during the first meiotic division.

Nucellus.

Some large cells more undifferentiated than the others are observed in the neighbourhood of the megasporocyte. They have thin walls, a large nucleus and a few small vacuoles. Their cytoplasm is rich in helical polyribosomes either free or bound to the endoplasmic reticulum. Dictyosomes and mitochondria are also fairly numerous. The food-storage substances are very scarce. They consist of some lipidic droplets similar to those of the MMC and of a few small starch granules contained in differently shaped proplastids (Fig. 20).

The somatic cells farther away from the MMC are characterized by more vacuolation and by a larger amount of starch (Figs. 3, 21). The intercellular spaces are absent in the whole nucellus. We can certainly claim that a tapetum is present in spite of the fact that the above mentioned cytological characteristics change gradually from the innermost to the outer-

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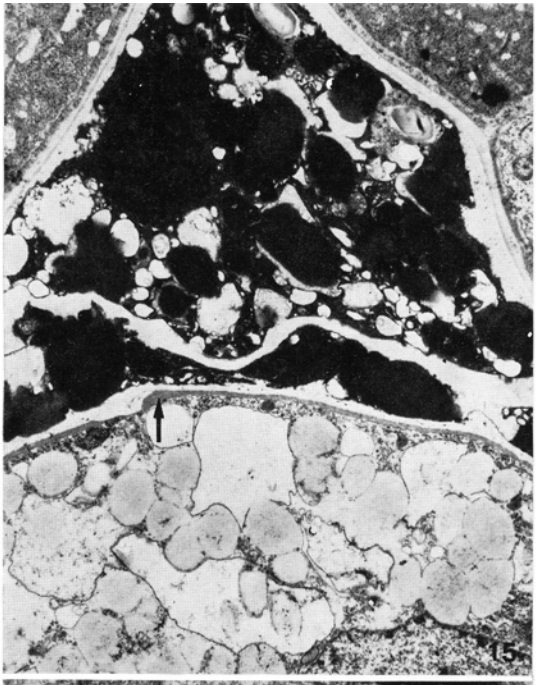
Fig. 15. — Portion of a triad older than that shown in Fig. 14. The two micropylar megaspores are clearly degenerated while the chalazal one is functioning. The latter shows a peculiar wall layer (arrow) which appears to be thicker at the upper side contiguous to the second degenerated megaspore. (x 4,651).
Fig. 16. — Cytoplasm and nucleus of the micropylar megaspore from a young triad. Some vesicles of endoplasmic reticulum, a dictyosome and several microtubules (arrows), similar to the cytoplasmic ones, can be observed in the large nuclear evagination. (x 28,728).
Fig. 17. — Approximately longitudinal section of the functional megaspore. The nucleus is shifted toward the peripheral cytoplasm and the mitochondria spread along its sides. The starch granules are fewer than in the preceeding stages and the lipidic droplets seem to discharge their contents into large vacuoles. (x 3,659).
Fig. 18. — Portion of peripheral cytoplasm of the functional megaspore showing active dictyosomes. The wall contiguous to the second degenerated megaspore shows a peculiar layer with tangentially oriented fibrils. (x 39,900).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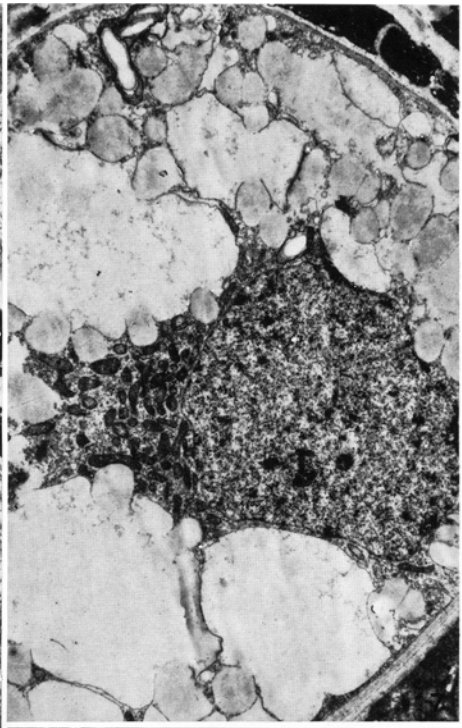

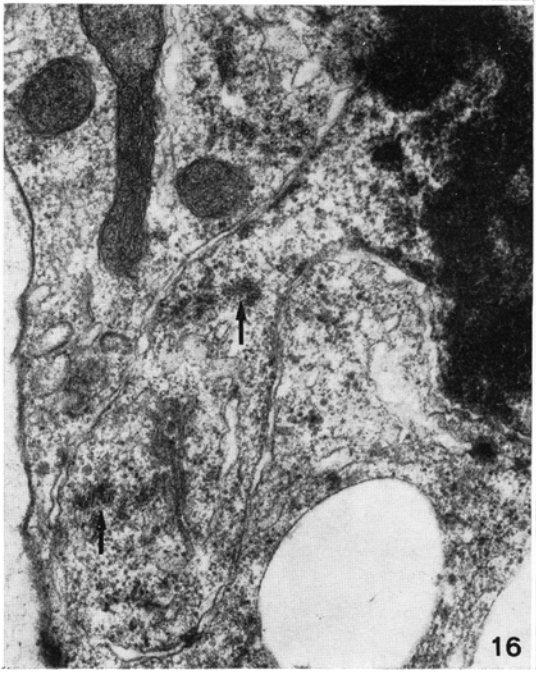
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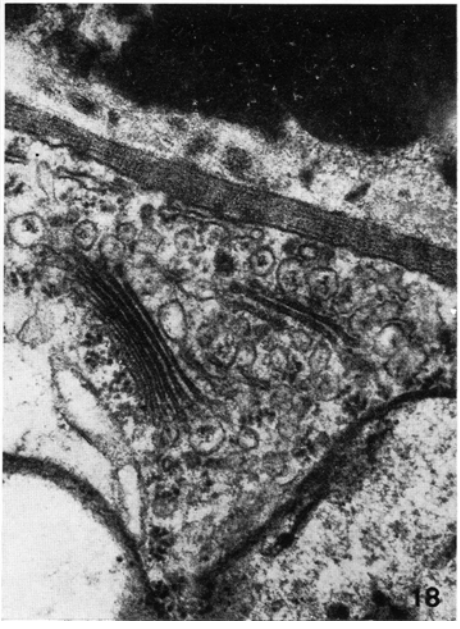
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most nucellar cells. In the sections observed under the LM (Figs. 2, 3) the tapetum is morphologically similar to that of *C. nootkatensis* (OWENS and MOLDER 1975). However we did not observe cell divisions more frequently than in the outermost nucellar cells as is the case for the species just mentioned. Moreover in *C. nootkatensis* the tapetal cells do not accumulate any starch (OWENS and MOLDER 1975), whereas in *C. lawsoniana* small starch granules are visible also under the LM (Fig. 3).

Various features of the tapetum have been observed in other Cupressaceae. It is « conspicuous » in *Thuja orientalis* (COKER 1904) and « poorly developed » in *Libocedrus decurrens* (LAWSON 1907). In *Callitris* the tapetum has a short life (BAIRD 1953) and consists of only two or three sporogenous cells which don't undergo meiosis, while in *Biota orientalis* Endl. it consists of many potential MMCs (SINGH and OBEROI 1962). Finally, in *Cupressus funebris* Endl. the tapetum includes 6-10 cells (KONAR and BANERJEE 1963).

At each stage some nucellar cells, as said before, divide and some of them occasionally degenerate. The degeneration process is more frequent in the tapetum during meiosis or soon after.

The apical nucellar cells of the ovules collected at the end of March show a peculiar feature: both the radial and the tangential walls are rather thick and contain some elongate or lens-shaped electron-transparent inclusions (Figs. 21, 25). These appear localized sometimes also in the middle lamella region (Fig. 21).

OWENS and MOLDER (1975) in their study on the reproductive cycle of *C. nootkatensis* observe a « poor natural regeneration » of this species and LIU (1970 in LI 1972) reports the low germination percentage of the seeds in *C. formosensis* and *C. obtusa* var. *formosana*, due probably to an « unsuccessful pollination, imperfect fertilization and deficiency of nutrients » (LI 1972).

In *C. lawsoniana* we have observed degeneration processes at very early stages. In fact, in some ovules collected at the beginning of March

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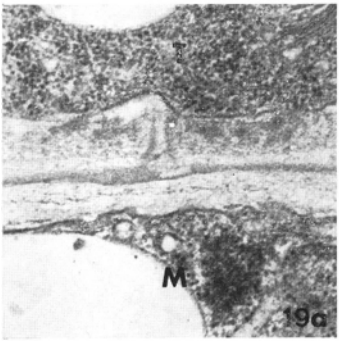
Figs. 19a and 19b. — Portions of the wall separating the functional megaspore (M) from the tapetal cells (T). The plasmodesmata do not appear as continuous cytoplasmic connections. (a x 30,780; b x 30,492).
Fig. 20. — Portion of a tapetal cell with ribosomes, proplastids containing very little starch, some lipidic droplets, small vacuoles, mitochondria, dictyosomes and elements of endoplasmic reticulum. The cytoplasm is surrounded by a thin wall (x 11,880).
Fig. 21. — Portions of two apical nucellar cells. The intercellular wall is rather thick and shows elongated or lens-shaped electron-trasparent inclusions both in the peripheral and in the middle region. These nucellar cells are more vacuolated and contain more starch than the internal ones. (x 9,576).
Fig. 22. — Chalazal cytoplasm of a degenerating MMC. The lipidic droplets are very abundant and the mitochondria do not show any internal structure. (x 3,500).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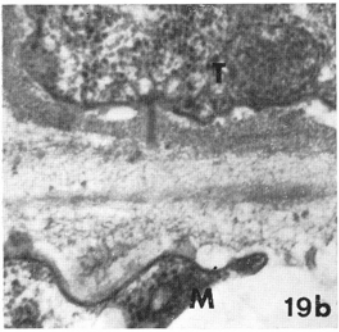
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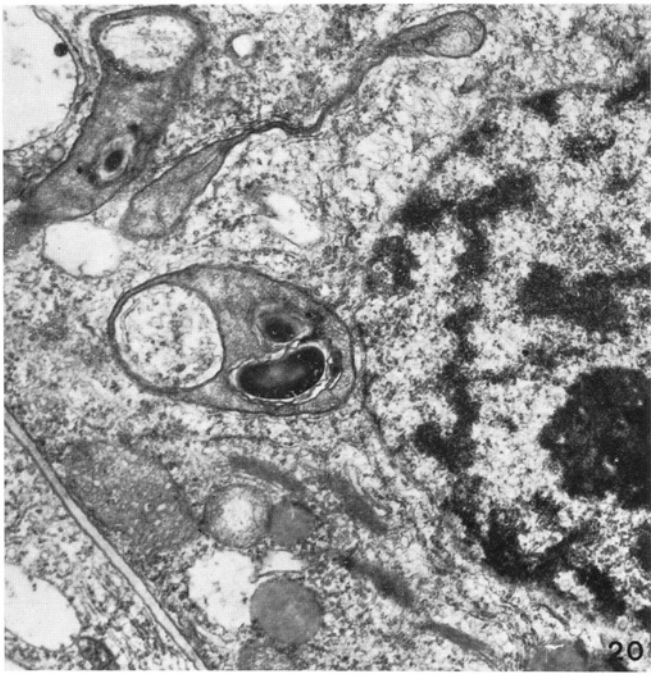
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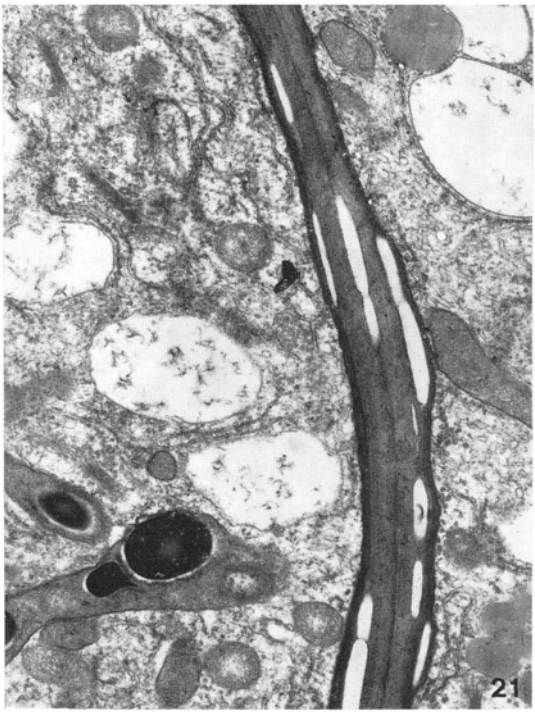
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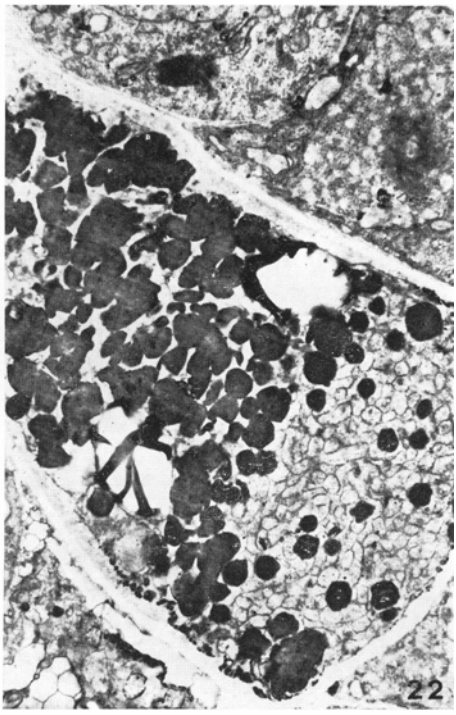
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we found megasporocytes easily recognizable by the large size, elongate shape, the amount of food-storage substances and by the characteristic polarization of the chondriome. However, they are clearly degenerating since the cytoplasm and the nucleus are intensely osmiophilic, the mitochondria do not show cristae and the lipidic droplets are more numerous and more electron-dense (Fig. 22). The surrounding nucellar cells appear quite normal in some specimens and, to a great extent, degenerating in others. In the material collected at the end of March, more numerous are the ovules in which the degeneration process involves the whole triad of megaspores. Such a process could begin more or less early after meiosis, because in some of these ovules the chalazal megaspore already appears surrounded by that peculiar wall layer characteristic of the normal development (Fig. 23). In other degenerating triads this wall layer of the chalazal megaspore is absent (Fig. 24). In each case the most remarkable modification is the increase of the lipidic substances in the cytoplasm (Figs. 23, 24), as for the degenerating megasporocyte.

DISCUSSION AND CONCLUSIONS

In the ovules of *C. lawsoniana* Parl. only rarely two megasporocytes differentiate and undergo meiosis. Normally there is only one MMC surrounded by a tapetum consisting of large almost undifferentiated cells.

A single MMC has been observed also in *Cupressus funebris* Endl. (KONAR and BANERJEE 1963) and in *Thuja orientalis*. In the latter species it is also possible to observe occasionally two or three MMCs (COKER 1904). More frequently in the Cupressaceae several sporogenous cells have been observed. However only one or two of them undergo meiosis (BAIRD 1953; SINGH and OBEROI 1962; KONAR and BANERJEE 1963; MAHESHWARI and SINGH 1967). On the contrary, LAWSON (1907), in his work on the Cupressaceae, reports that the number of MMCs ranges from one to three and that each of them undergoes meiosis.

The megasporocyte of *C. lawsoniana* differentiates by a considerable increase in volume and a remarkable storage of starch and of another reserve

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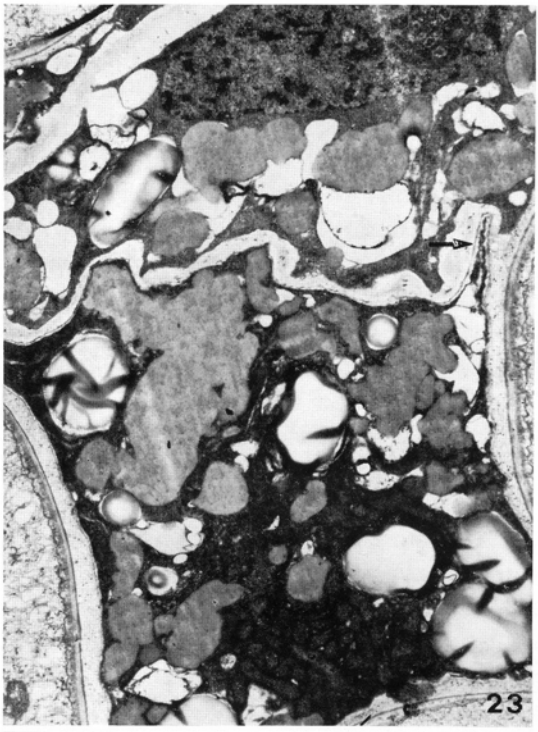
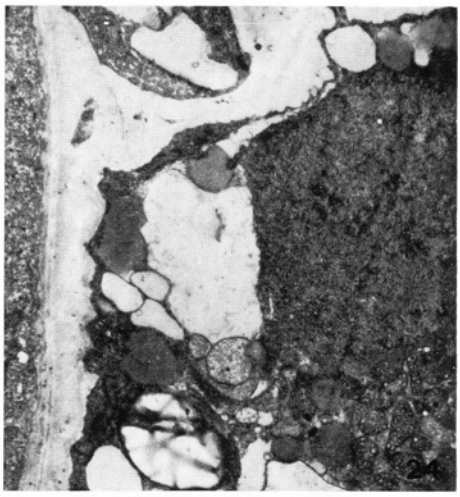
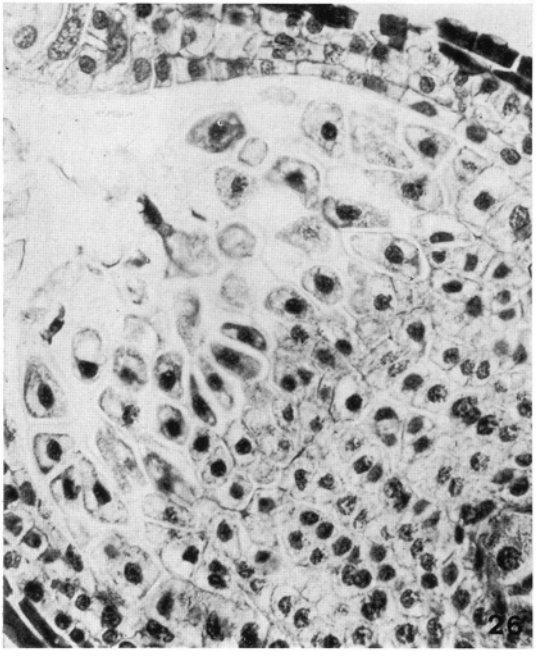
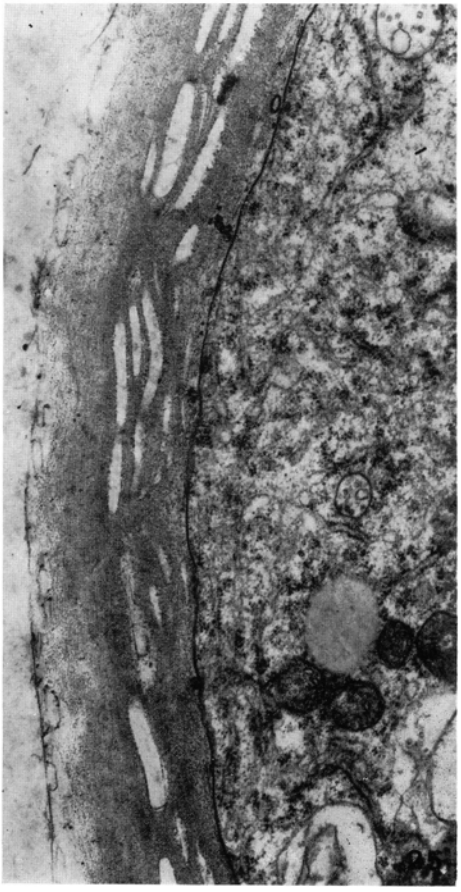
Fig. 23. — Portion of a degenerating triad. The wall thickening (arrow) characteristic of the functional megaspore can be observed at the outside of the chalazal megaspore cytoplasm. (x 5,016).
Fig. 24. — Portion of the chalazal megaspore in a degenerating triad. The wall thickening observed in Fig. 23 his here absent. (x 5,161).
Fig. 25. — Portion of an apical nucellar cell. Electron-transparent inclusions can be observed in the outer tangential wall, from which they seem to release the content to the outside of the cell. (x 17,955).
Fig. 26. — Longitudinal section of an ovule showing the degenerating distal end of the nucellus. (x 271).


23


24


26



substance which could be, at least partly, of lipidic nature. We remark that in *C. nootkatensis* only starch has been observed under the LM (OWENS and MOLDER 1975).

During prophase I the megasporocyte of *C. lawsoniana* appears as a fully polarized cell, due to the localization of all, or almost all, the mitochondria in the chalazal cytoplasm. This polarization leads to the segregation of the chondriome in the dyad chalazal and, later on, in the chalazal functional megaspore of the triad. Moreover the special potentiality of the megasporocyte chalazal cytoplasm is early demonstrated.

As above mentioned, some LM investigations on other Gymnosperms, including Cupressaceae, and also on *Ginkgo biloba*, have shown cytoplasmic structures not clearly identified or resolved in the MMC chalazal region (COKER 1903, 1904; CAROTHER 1907; OTTLEY 1909). In *Taxus baccata* such a structure will be eventually inherited, through meiosis, only by the chalazal functional megaspore (COKER 1904, Fig. 21).

In *Encephalartos poggei* Asch. the whole of the starch of the mature megasporocyte is localized in the chalazal cytoplasm of the cell and subsequently, after meiosis, in the chalazal functional megaspore (DE SLOOVER 1961).

The development of the megasporocyte of *Ginkgo biloba* has been studied also at the EM by STEWART and GIFFORD (1967). They found that all the plastids and mitochondria are « restricted » to the chalazal cytoplasm of the mature MMC and, later on, to the chalazal dyad cell.

The same authors mention that the functionality of the chalazal megaspore of a tetrad would be determined, according to CHAMBERLAIN (1935), by its proximity to the vascular supply of the ovule. Moreover they remark that « in *Ginkgo*, however, the position of the plastids and mitochondria before the nuclear division of meiosis I favours the chalazal dyad cell in the production of the functional megaspore ».

In our opinion, if the development of the chalazal megaspore is really due to its trophically privileged position, this circumstance would be effective not only after meiosis and on a particular cell, but also earlier and on a particular cytoplasmic region of the megasporocyte. As a consequence there would be a preferential localization of the food-storage substances only, as in *Encephalartos* (DE SLOOVER 1961), or of the plastids and mitochondria, which play an extremely important role in the cell metabolism, as in *Ginkgo* (STEWART and GIFFORD 1967), or of the mitochondria only, as in *C. lawsoniana.*

In such cases therefore, the three or four megaspores produced by meiosis would not be potentially equivalent. On the other hand that occurs also in *Taxus baccata* (COKER 1904, Fig. 21).

The different behaviour of the MMC of *C. lawsoniana* in female gametophyte development in comparison with *Encephalartos poggei* (DE SLOOVER 1961) and *Ginkgo biloba* (STEWART and GIFFORD 1967) is believed to be particularly significant. In fact the chalazal cytoplasm of the MMC of *Chamaecyparis* does not show any particular tendency to accumulate food-storage substances, but rather it tends to concentrate the cell organelles necessary to utilize the reserve substances, which will arrive later on, during the female gametophyte development, through the neighbouring vascular system of the ovule. To be timely is advantageous to avoid waste.

We still have to investigate the nature of the thickenings occurring in the wall of the megasporocyte of the dyad and of the functional chalazal megaspore. They could have the same functional significance as the callose layers which, as is now well known, often appear in plant meiocytes and post-meiotic cells, both in micro- and in megasporogenesis. Namely they would isolate the cytoplasm which is about to follow a special path of development from the cytoplasm of the normal somatic cells, thus avoiding possible interactions (DE SLOOVER 1961; RODKIEWICZ 1967, 1970; FABBRI TARCHI 1969). From this point of view, the partial occlusion of the plasmodesmata connecting the fertile and the somatic cytoplasm at early stages seems to us very significant.

The apical nucellar cells deserve now a brief discussion. As said above, in the walls of these cells we observe several very electron-transparent inclusions. These occur sometimes also in the middle lamella region. They can be regarded as the sites where an enzymatic hydrolysis of amorphous polysaccharide components of the wall would take place. One could perhaps hypothesize that the products of such hydrolysis, because of their tendency to hydratation, could form the pollination drop. In this respect it is useful to remember that these electron-transparent inclusions are also observed in the outer tangential walls from where they seem to release the contents to the outside of the cells (Fig. 25). Besides, the hydrolysis of some components of the cell wall and specifically of the middle lamella, would lead to the separation of the cells from each other. On the other hand, the water absorption by the products of the enzymatic hydrolysis mentioned before, would lead to the degeneration of the apical nucellar cells. Both processes would be connected with the entry of the pollen tube inside the nucellus and with its nutrition. In fact the degeneration of the apical nucellar cells is clearly apparent in ovules collected at the beginning of April (Fig. 26), i.e. when pollination is fully underway. According to OWENS and MOLDER (1975), the apical nucellar cells could participate in the production of the pollination drop also in *C. nootkatensis*, in which, however, they degenerate before pollination.

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SUMMARY

In *Chamaecyparis lawsoniana* Parl. the megaspore mother cell (MMC) differentiates in the middle and innermost region of the nucellus as is generally the case in Gymnosperms. During its development the MMC attains a size much larger than that of the surrounding

somatic cells and accumulates a considerable amount of food-storage substances. These consist of starch and droplets which could be, at least partly, of lipidic nature. During prophase I the MMC becomes a fully polarized cell since all or almost all the mitochondria are restricted to the chalazal cytoplasm. In the authors' opinion this is determined by the trophically privileged position of the chalazal cytoplasm. The polarization of the chondriome determines the course of the subsequent events in such a way that the meiotic divisions give rise to a linear triad, in which the two micropylar megaspores soon degenerate while the chalazal one inherits all of the mithochondria from the megasporocyte and remains functional. On the other hand the food-storage substances appear more or less uniformly distributed at all megasporogenesis stages.

The preparation for the female gametophyte development should therefore be accomplished via an accumulation of the cell organelles necessary for the consumption of the food-storage substances, rater than via an accumulation of the food-storage substances themselves. The latter may in fact be supplied later by the nearby vascular system. This behaviour of the megasporocyte of *C. lawsoniana* is discussed in connection with that previously observed in *Encephalartos poggei* and in *Ginkgo biloba.*

In the EM preparations the primary wall of the megasporocyte appears thicker than that of the adjacent cells. After the first meiotic division the wall separating the dyad from the tapetum shows different thickenings on the two sides. Finally, new wall material is laid down by the cytoplasm of the chalazal functional megaspore. These thickenings seem to occlude, at least partially, the plasmodesmata connecting at early stages the fertile cytoplasm to the somatic.

The ultrastructural features of the nucellus are briefly described. We observe a gradual morphological change from the innermost nucellar cells, which form the tapetum and are less differentiated, to the external ones showing thicker walls, greater vacuolation and more starch.

Some nucellar cells divide, while some others, mainly in the tapetum region, degenerate.

The walls of the apical nucellar cells show very electron-transparent inclusions. These are considered to be the sites of enzymatic activities which should initially produce the pollination drop, and then the separation and degeneration of the above cells.

Ovules with megasporocytes or whole triads degenerating have been frequently observed.

RIASSUNTO

In *Chamaecyparis lawsoniana* Parl. la cellula madre delle megaspore (CMM) si differenzia nella parte assile e più profonda della nucella, come generalmente avviene nelle Gimnosperme. Durante il suo sviluppo essa raggiunge dimensioni molto maggiori delle circostanti cellule somatiche ed accumula una notevole quantità di sostanze di riserva, sotto forma di amido e di gocciole di natura, almeno in parte, lipidica. Durante la profase I tale cellula acquisisce una netta polarizzazione, in quanto tutto, o quasi tutto, il condrioma si localizza nel citoplasma calazale. Ciò sarebbe determinato, secondo gli AA., dalla vicinanza di tale citoplasma all'apparato vascolare dell'ovulo. La polarizzazione del megasporocito condiziona lo svolgersi degli eventi successivi, così che le due divisioni meiotiche portano alla formazione di una triade lineare, costituita da due megaspore micropilari, che ben presto degenerano, e da una megaspora calazale funzionante, la sola che ha ereditato tutti, o quasi tutti, i mitocondri del megasporocito.

Le sostanze di riserva invece appaiono più o meno uniformemente distribuite a tutti gli stadi della megasporogenesi.

La predisposizione per lo sviluppo del gametofito femminile si realizzerebbe quindi non con un immagazzinamento di sostanze nutritive — queste potranno infatti affluire anche più tardi dal vicino apparato conduttore — ma con un accentramento di quegli organuli cellulari indispensabili alla utilizzazione delle sostanze nutritive stesse. Tale comportamento del megasporocito di *C. lawsoniana* viene discusso in rapporto a quanto è stato precedentemente osservato in *Encephalartos poggei* e *Ginkgo biloba.*

La parete che separa il megasporocito dalle cellule del tappeto appare, al ME, costituita da una sottilissima parete primaria delle cellule somatiche, da una lamella mediana e da una più spessa parete primaria della CMM. Dopo la prima divisione meiotica si osserva un ispessimento della parete delle cellule del tappeto e la formazione di un secondo strato parietale da parte delle cellule della diade. Nuovo materiale parietale viene elaborato, infine, dal citoplasma della megaspora calazale funzionante. I suddetti ispessimenti sembrano occludere, almeno in parte, gli originari rapporti di continuità tra il citoplasma fertile e quello somatico.

Sono brevemente descritte le caratteristiche ultrastrutturali della nucella. In questa, dalle cellule più interne più spiccatamente giovanili e costituenti il tappeto, si passa gradatamente a quelle più esterne che sono relativamente più differenziate; esse hanno infatti pareti più spesse, vacuoma più sviluppato ed una maggiore quantità di amido.

Varie cellule della nucella appaiono in mitosi e, soprattutto al livello del tappeto, alcune in degenerazione.

Le pareti delle cellule nucellari apicali mostrano delle inclusioni molto trasparenti agli elettroni: esse vengono interpretate come la sede di attività enzimatiche che porterebbero, in un primo tempo, alla produzione della goccia d'impollinazione e, successivamente, allo scollamento e alla degenerazione delle cellule stesse.

Sono stati osservati frequentemente ovuli con megasporociti o intere triadi degeneranti.