Atti Soc. Tosc. Sci. Nat., Mem., Serie B, **94** (1987)
pagg. 163-179, figg. 13

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MEGASPOROGENESIS IN GYMNOSPERMS: ASPECTS OF THE CITOPLASM AND THE CELL WALLS

Riassunto — *Megasporogenesi nelle Gimnosperme: aspetti del citoplasma e delle pareti cellulari.* Dalle numerose ricerche condotte principalmente al microscopio ottico sulla megasporogenesi delle Gimnosperme s.l. viventi, ricerche che risalgono anche a molti anni addietro, è ben noto che tra le cellule sporigene una o poche si differenziano come cellule madri delle megaspore. La meiosi porta alla formazione di tre o quattro megaspore. Solo in *Gnetum* e *Welwitschia* il prodotto della meiosi è una cenomegaspora tetranucleata. In questi due generi il gametofito femminile ha perciò un'origine tetramegasporiale; in *Stangeria paradoxa* è forse bimegasporiale, poiché è supposto che solo la cellula superiore della diade effettui entrambe le divisioni meiotiche; ma normalmente esso si sviluppa da una sola megaspora, quella calazale, mentre le altre degenerano e si esauriscono nella nutrizione del giovane gametofito. È stato proposto che le megaspore che si formano da un atto meiotico siano tutte potenzialmente equivalenti e quella calazale si sviluppa più rapidamente delle altre grazie alla sua vicinanza alle terminazioni vascolari dell'ovulo. Tuttavia varie ricerche successive all'ipotesi riportata indicano che la direzione del flusso trofico esplicherebbe la sua influenza anche prima della meiosi, inducendo nel citoplasma calazale del megasporocito una maggiore potenzialità metabolica rispetto alla regione micropilare. Tale polarizzazione della cellula madre delle megaspore risulta in molti casi espressa da una concentrazione calazale della maggior parte del corredo mitocondriale e/o plastidiale. La localizzazione differenziata di questi organuli si mantiene durante la meiosi. Ne consegue che la megaspora calazale ha una maggiore potenzialità di sviluppo che si manifesta precocemente, senza dubbio favorita dalla vicinanza al sito del rifornimento di nutrienti. L'influenza della direzione del flusso trofico appare atipica in *Taxus*, dove tutti i prodotti di un atto meiotico possono mostrare, anche per un periodo abbastanza lungo, una uguale capacità di sviluppo. È probabile che le cause di tale comportamento siano da ricercare in peculiari aspetti morfo-fisiologici della nucella. Un altro punto della megasporogenesi delle Gimnosperme che merita una discussione particolare è il comportamento parietale. In alcuni casi è stata evidenziata nel corso della meiosi la formazione di depositi callosici, destinati a scomparire precocemente dopo la meiosi stessa. È stato prospettato che questo polisaccaride svolga un ruolo nel separare le megaspore micropiliari di ridot-

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ta funzionalità dalla megaspora calazale più attiva. La generale presenza di ispessimenti che si appongono alla originaria parete del megasporocito, e che si mantengono o si accentuano attorno all'intera diade e tetrade o triade, sembra provare una tendenza del citoplasma sede della meiosi ad isolarsi nel suo complesso dalle cellule circostanti, con la deposizione di materiali parietali in alcuni casi sicuramente diversi dal callosio.

Certamente la conoscenza definitiva della natura, della funzione e di una possibile evoluzione degli ispessimenti parietali osservati richiede ulteriori ricerche ultrastrutturali e citochimiche.

Abstract — From the numerous studies carried out, over the years, using mostly the optic microscope (OM), on the megasporogenesis of living Gymnosperms s.l. it is well known that one or more sporogenous cells function as megaspore mother cells (MMC). Meiosis brings about the formation of three or four megaspores. Only in *Gnetum* and *Welwitschia* is the product of meiosis a tetranucleate coenomegaspore. Thus, in these two genera the female gametophyte has a tetramegasporic origin; in *Stangeria paradoxa* it is perhaps bimegasporic, since it is presumed that only the upper cell of the dyad undergoes both meiotic divisions. However, normally the female gametophyte develops from a single megaspore, i.e. the chalazal one, while the other megaspores degenerate and are utilized in the nutrition of the young gametophyte.

It has been suggested that the megaspores which are formed from a meiotic event are all potentially equivalent and that the chalazal megaspore develops more rapidly that the others because of its proximity to the vascular terminations of the ovule. Nonetheless, much research, subsequent to the hypothesis here mentioned, indicates that the direction of trophic flow would be effective even prior to meiosis, inducing a greater metabolic potentiality in the chalazal cytoplasma of the megasporocyte as compared to the micropylar region. The polarization of the MMC is, in many cases, expressed by a chalazal concentration of the greater part of the mitochondria and/or plastids. The polarized distribution of these organelles is maintained during meiosis. Thus it follows that the chalazal megaspore has a greater development potentiality, which is manifested almost immediately after meiosis, favoured, undoubtedly, by the proximity of the food supply. The influence of the direction of the trophic flow appears atypical in *Taxus*, where all the products of a meiotic act can show an equal development capacity, even for quite a long period. The causes of such behaviour may be found in some peculiar morpho-physiological aspects of the nucellus as yet not identified. Another topic worthy of particular note in the megasporogenesis of Gymnosperms is the behaviour of the cell walls. In some cases the formation of callose wall deposits during meiosis, which are then destined to disappear rapidly after meiosis, has been pointed out. It has been suggested that the callose is involved in separating the micropylar megaspores of reduced functionality from the more active chalazal megaspore. The presence of tickenings, which are added to the original wall of the megasporocyte, is a general feature. The thickenings are maintained or accentuated around the entire dyad and tetrad or tryad. These facts suggest a tendency of the cytoplasm which is the seat of meiosis to isolate itself entirely from the surrounding cells by depositing wall material. In some cases this material is certainly different from the callose. Undoubtedly definitive knowledge of the nature, function and possible evolution of the wall thickenings observed requires further ultrastructural and cytochemical research.

Key words — Gymnosperms, megasporogenesis, meiosis, megaspore(s), plant embryology and cytology.

Our knowledge of megasporogenesis in Gymnosperms comes principally from research undertaken using the optic microscope (om): until now there have been very few specific and ultrastructural studies on this topic.

In living Gymnosperms s.l. megasporogenesis takes place in ovules which generally have a crassinucellate conformation; in fact a more or less developed parietal tissue is interposed between the epidermis of the nucellus and the sporogenous cells. One or more of these differentiate as megaspore mother cells (mmc) (SINGH, 1978). In the Ginkgoales (CAROTHERS, 1907; FAVRE-DUCHARTRE, 1956), in the majority of Coniferales, in *Ephedra* (MAHESHWARI, 1935), in Cycadales (DE SLOOVER, 1961) and *Welwitschia* (MARTENS, 1963) the normal condition is marked by the presence of one sole megasporocyte. Occasionally two meiocytes may appear in certain cases, as for example in *Ginkgo* (CAROTHERS, 1907), *Ephedra* (MAHESHWARI, 1935), *Chamaecyparis* (CECCHI FIORDI and MAUGINI, 1977); two or more megasporocytes, (perhaps not always due a correct interpretation), can develop in some *Taxaceae* (JÄGER, 1899; DUPLER, 1917; STERLING, 1948; personal observations), *Taxodiaceae* (LOOBY and DOYLE, 1942), *Cupressaceae* (COKER, 1904; LAWSON, 1907; MEHRA and SIRKAR, 1949; BAIRD, 1953), *Araucariaceae* (KONAR and OBEROI, 1969) ad in *Pinus roxeburghii* (KONAR, 1960). As many as 20 mmc have been found in *Gnetum* (VASIL, 1959; SANWAL, 1962). In the *Welwitschia* female cone axis some extra-floral megasporocytes, for the greater part destined to degenerate before meiosis, are sometimes present. Plurinucleate gametophytes have been observed in exceptional cases, but none were at the stage of maturity. However, the karyological phase of such structures has not been verified (MARTENS, 1963).

The morphologycal features that characterize the complete dfferentiation of a megasporocyte are multiple. The increase in the size of the nucleus, which generally assumes a more or less micropylar localization, is consistent and noteworthy. The entire cell as well enlarges until, usually, its roundish shape becomes clearly elongated (110 μ in *Ginkgo*). Quite frequently a thickening, visible even with the om, can be observed on the wall which is crossed by few plasmodesmata. Ultrastructural studies, which are still sporadic, have brought to light a pluristratified wall in the mmc

of *Ginkgo*. The thickening of this wall is due principally to the increase of the middle lamella and to the development of an additional wall layer interposed between the primary wall and the plasmalemma (Fig. 1, 2). This layer has a similar structure to that of the middle lamella (STEWART and GIFFORD, 1967). In *Chamaecyparis* the mature megasporocyte presents a thicker primary wall than that of the surrounding cells (Fig. 3) (CECCHI FIORDI and MAUGINI, 1977). In *Larix* it is surrounded by a double-layered wall.

The enlargement of the megasporocyte is always conspicuous and is due principally to the increase of the cytoplasmic matrix and the number of organelles. Notwithstanding the likely utilization of nutrients linked to such processes, the differentiation of the mmc is also characterized by a more or less early and, at the same time, considerable accumulation of reserves in the form of starch. In *Chamaecyparis* and *Ginkgo* lipidic droplets also appear; cytoplasmic inclusions of a similar ultrastructural aspect have been observed in *Larix* as well (unpublished personal observations). In some cases, for example in *Sciadopitys verticillata* (LAWSON, 1910), *Sequoia sempervirens* (LOOBY and DOYLE, 1942), *Encephalartos poggei* and other Cycadales (DE SLOOVER, 1961) the megasporocyte starch content is concentrated principally in the chalazal cytoplasm, or the starch is present at this localization during the early stages of the first meiotic division.

As has been revelaed by various studies using the om, including those of our earliest knowledge, a peculiar high density chalazal cytoplasmic zone, called the «kinoplasmic body» or «kinoplasmic mass» by some authors, characterizes the mature megasporocyte of some Gymnosperms, such as *Thuja* (LAND, 1902), *Taxodium* (COKER, 1904), *Ginkgo* (CAROTHERS, 1907), *Juniperus* (OTTLEY, 1909), *Chamaecyparis* (Fig. 4) (CECCHI FIORDI and MAUGINI, 1977) (for other bibliographical data v. STERLING, 1948). One or two of the above mentioned inclusions, found in proximity to the micropylar and chalazal poles of the nucleus, may be present in *Taxus baccata*. In *Stangeria paradoxa* (LANG, 1900) and *Taxus cuspidata* (STERLING, 1948) a similar cytological feature characterizes not only the functional megasporocyte but also the sporogenous cells that do not undergo meiosis.

More recent studies using the om have identified the «kinoplasmic body» of *Ginkgo* with a chalazal aggregation of mitochondria (FAURE-DUCHARTRE, 1956). Ultrastructural analysis has

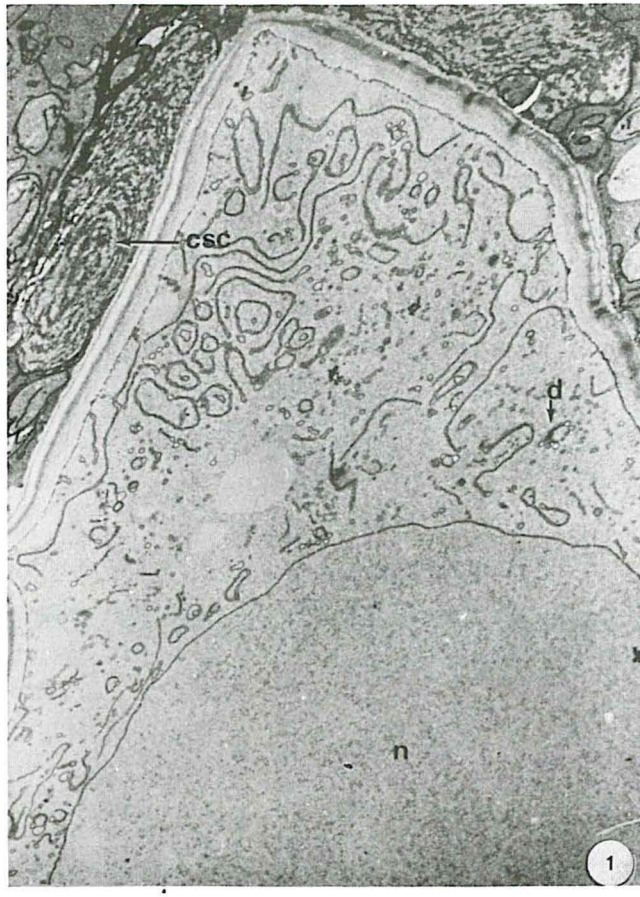
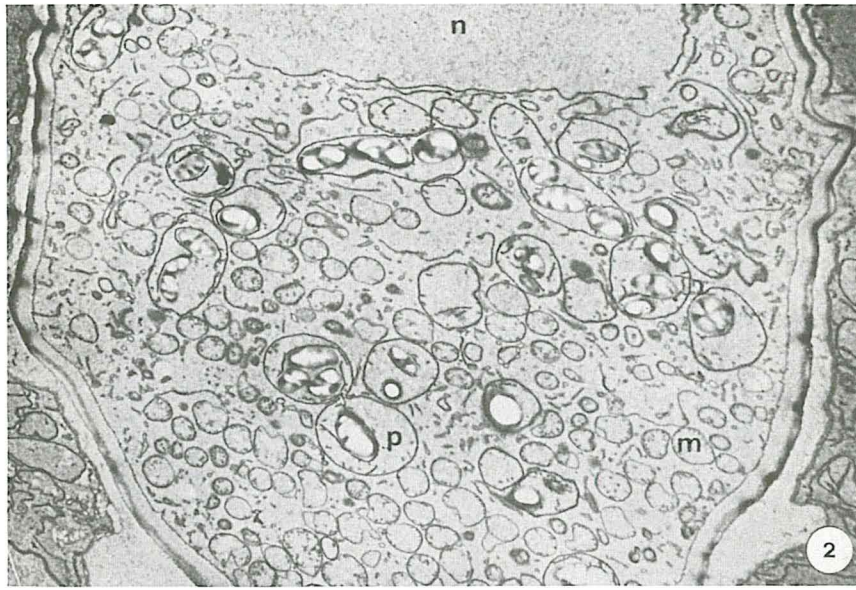
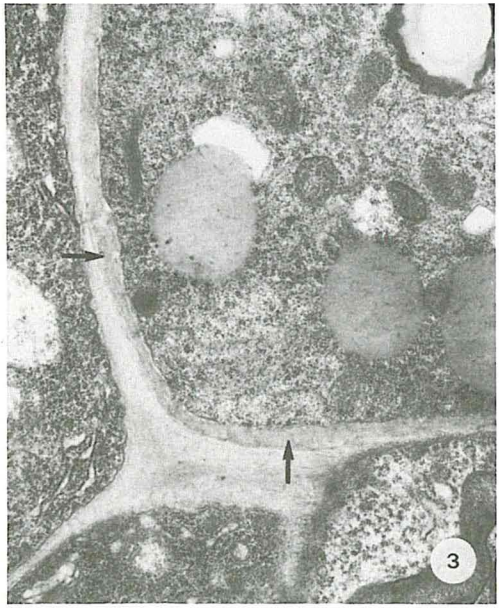


Fig. 1 — Megasporocyte of *Ginkgo biloba*. Micropylar cytoplasm with ample systems of endoplasmic reticulum. The megasporocyte wall consists of two layers. The middle lamella appears thickened. Csc, crushed spongy tissue cell; d, dictyosome; n, nucleus. (From STEWART and GIFFORD, 1967) X 4,800.

confirmed this finding and has furthermore shown a preferential chalazal localization even of plastids (Fig. 2) and the presence of abundant endoplasmic reticulum complexes in the micropylar cytoplasm (Fig. 1) (STEWART and GIFFORD, 1977). JUEL (1900) observed an accumulation of starch around and below the nucleus, and a formation which he indicated as reticular plasma in the micropylar region, in the megasporocyte of *Larix sibirica* at the beginning of meiosis. An ultrastructural analysis has not been carried out on this species. Instead, the use of this method has revealed in the mmc





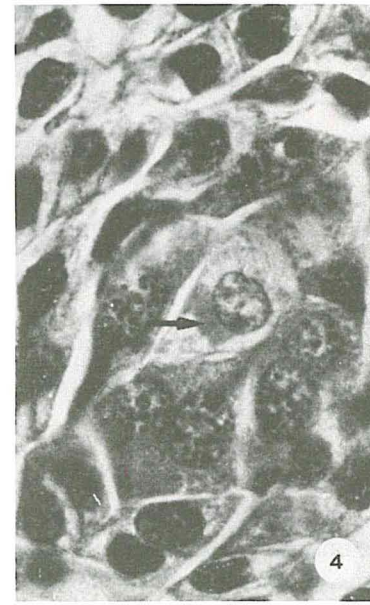


Fig. 2 — Megasporocyte of *Ginkgo biloba*. Chalazal cytoplasm in which the plastids and the mitochondria are concentrated. The wall shows the same structure as in Fig. 1. n, nucleus; p, plastid; m, mitochondrion. (From STEWART and GIFFORD, 1967) X 3,570.

Fig. 3 — Megasporocyte of *Chamaecyparis lawsoniana* showing a tickened primary wall (arrows). (From CECCHI FIORDI and MAUGINI, 1977) X 11,300.

Fig. 4 — Megasporocyte of *Chamaecyparis lawsoniana* with a deeply stained cytoplasmic region (arrow) below the nucleus. (From CECCHI FIORDI and MAUGINI, 1977) X 850.

of *Larix leptolepis* a distribution of the mitochondria, plastids and endoplasmic reticulum which is somewhat similar to that of *Ginkgo* (Cecchi Fiordi et al., 1984). In *Chamaecyparis* the presence of a «kinoplasmic body» is due to a marked concentration of the chondriome in the chalazal cytoplasm where particularly wavy profile of the plasmalemma is evident (Cecchi Fiordi and Maugini, 1977). In the mature megasporocyte of *Ephedra* a prominent chalazal accumulation of plastids can be seen (Moussel, 1981).

Even if several mmc differentiate in a nucellus, generally only one of them undergoes meiosis. Nevertheless, more than one megasporocyte can be functional in *Ginkgo* (Carothers, 1907), in some *Taxaceae (*Jäger, 1899; Dupler, 1917), in *Ephedra* (Maheshwari, 1935) and in some *Taxodiaceae (*Looby and Doyle, 1942); several megasporocytes are functional in *Gnetum* (Waterkeyn, 1954; Vasil, 1959; Sanwal, 1962).

Usually meiosis follows the classical pattern: the first division leads to the formation of a dyad whose lower cell is normally of a larger size. When the plastids and/or other organelles have a polarized localization in the megasporocyte, they are segregated according to their polarization (Fig. 5, 6). The second division takes place to a greater or lesser extent in both cells, even if it is accompanied by a slight delay in the upper cell. The four megaspores are arranged in tetrads which, depending on the orientation of the meiotic spindles, may be linear, oblique, T-shaped, bilateral or tetrahedral of various types. The simultaneous presence of tetrads having different configurations has also been described; e.g. in *Ginkgo biloba* (Carothers, 1907), *Sequoia sempervirens* (Looby and Doyle, 1942), *Ephedra distachya* (Moussel, 1981) and, according to some authors, *Cupressus funebris* (Mehra and Sirkar, 1949). The triad formation is also very frequent. In *Stangeria* it is thought that only the upper cell of the dyad undergoes both divisions (Lang, 1900), but in all other cases, where triads are formed, the second divsion occurs exclusively in the lower cell. This latter pattern of meiosis is known, for example, in *Macrozamia spiralis, Cycas rumphii, Pinus wallichiana, Biota orientalis, Chamaecyparis lawsoniana, Agathis brownii, Saxegothaea conspicua*, various species of *Podocarpus* and *Pherosphaera* (Maheshwari and Singh, 1967; Konar and Oberoi, 1969; Cecchi Fiordi and Maugini, 1977). In some rare cases, e.g. *Sciadopitys verticillata*, the triad present a binucleate central cell, since only the second meiotic division is followed by the formation of the cell walls (Lawson, 1910; Gianordoli, 1964).

Both triads and tetrads, the latter of similar and different types, have been observed not only in the same genus but also in the same species; examples include: *Taxus baccata* (JÄGER, 1899), *Ginkgo biloba* (CAROTHERS, 1907), *Larix europaea* (SAXTON, 1930), *Taxus cuspidata* (STERLING, 1948), *Cedrus deodara* (ROY CHOWDHURI, 1961), *Encephalartos poggei* (DE SLOOVER, 1961) and some species of *Ephedra* (MAHESHWARI, 1935; MOUSSEL, 1981). An arrangement of the products of meiosis which appears half-way between that of a tetrad and that of a triad is found occasionally in *Ginkgo biloba* (CAROTHERS, 1907) and *Encephalartos poggei* (DE SLOOVER, 1961), where the second division of the upper cell of the dyad, with horizontal or oblique spindle, may or may not be followed by cytokinesis (Fig. 7). In *Gnetum* (VASIL, 1959) and *Welwitschia* (MARTENS, 1963) it has been definitively found that neither of the two meiotic karyocineses is followed by the division of the cytoplasm. What results is the formation of tetranucleate coenomegaspores.

Normally a single female gametophyte develops from a triad or a tetrad, sometimes after a period of quiescence. Except for the gametophyte of *Stangeria*, which may be bisporic, the female gametophyte originates from a single megaspore, the most chalazal one, even if other megaspores may begin to develop. This happens for example in *Sequoia* (LOOBY and DOYLE, 1942), *Callitris, Cupressus, Pherosphaera* (MAHESHWARI and SINGH, 1967). The development, (which may even be complete) of several gametophytes is frequent in species of Taxodiaceae and Taxaceae; it is occasional in the Pinaceae and Cupressaceae (STERLING, 1948a), but the origin of these gametophytes from one same meiotic act is not alway ascertained. In *Gnetum* several coenomegaspores, sometimes relatively numerous, undergo a certain development, but only one gametophyte reaches maturity. The above mentioned genus and *Welwitschia* are the only phylogenetically significant examples of Gymnosperms s.l. which have tetramegasporic gametophytes. A parallel has been drawn between this mode of development and the non-differentiation of archegonia (VASIL, 1959; MARTENS, 1963).

Fig. 5 — *Chamaecyparis lawsoniana*: chalazal cytoplasm of the dyad lower cell in which all the mitochondria of the megasporocyte have been segregated. (From CECCHI FIORDI and MAUGINI, 1977) X 12,300.

Fig. 6 — Triad of *Chamaecyparis lawsoniana*. Only the chalazal megaspore has mitochondria. (From CECCHI FIORDI and MAUGINI, 1977) X 4,430.

Fig. 7 — Triad of *Encephalartos poggei*. The second meiotic division has not been followed by cytokinesis in the dyad upper cell. (From DE SLOOVER, 1961) X 760.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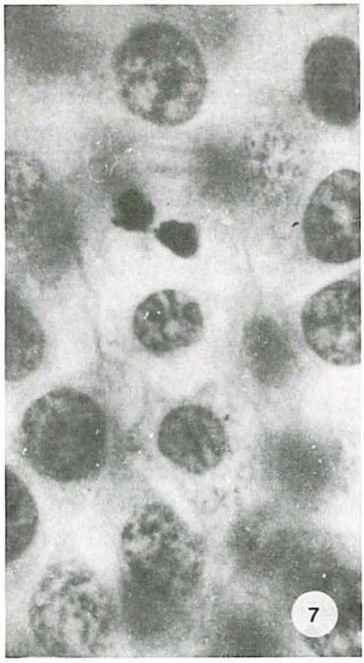
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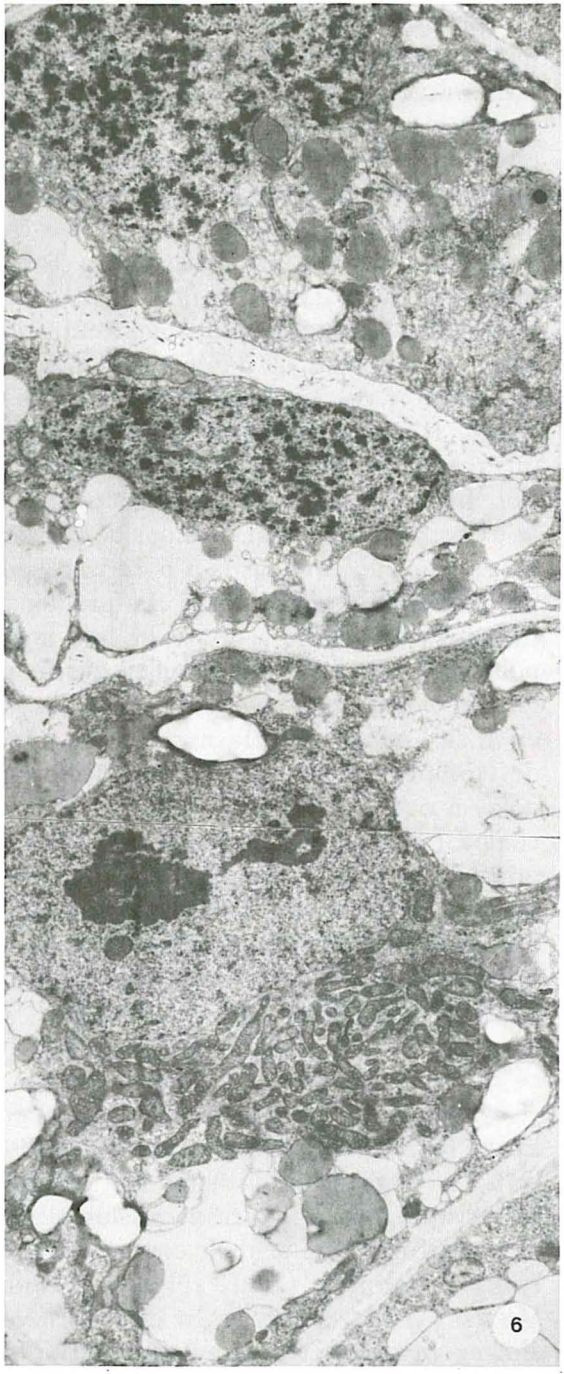
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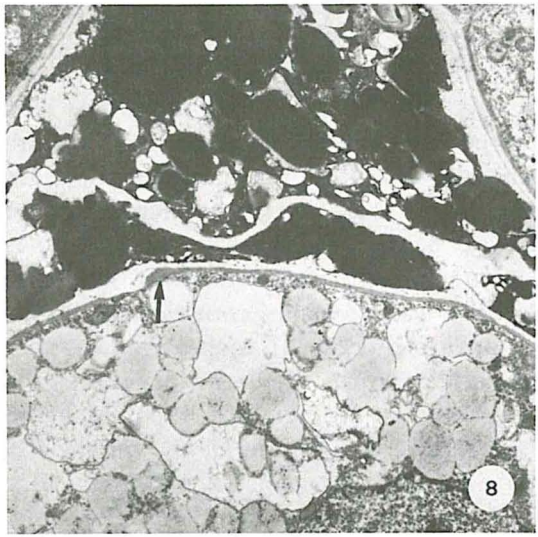
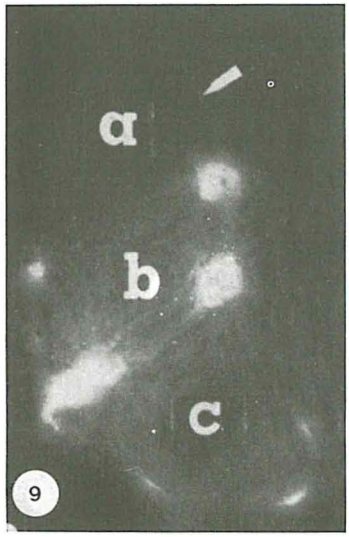
According to CHAMBERLAIN (1935) the chalazal megaspore develops more rapidly than other potentially equivalent ones because of its proximity to the vascular terminations of the ovule, i.e. it is in a trophically privileged position. Several authors now admit that the effects of the nutrient flow direction are operative even prior to meiosis, conferring a particular metabolic potientiality on the chalazal cytoplasm of the megasporocyte (FAURE-DUCHARTRE, 1956; CECCHI FIORDI and MAUGINI, 1977; MOUSSEL, 1981). This fact can be morphologically expressed by a concentration of plastids, which have a greater or lesser starch content, or by a concentration of plastids and mitochondria, or only that of mitochondria. In *Chamaecyparis* even the appearance of the chalazal plasmalemma is indicative of greater activity. It is very likely that the «kinoplasmic bodies» (whose ultrastructure is not known) are also the expression of a polarization in the megasporocyte physiology.

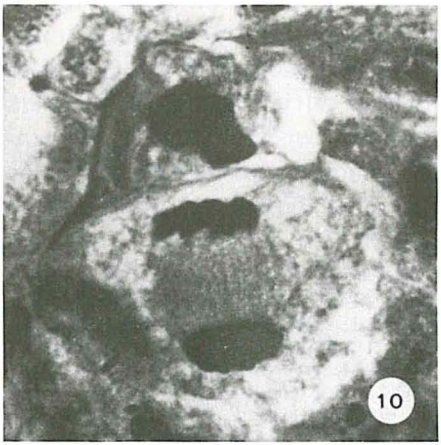
On the other hand, it must be remembered that, in general, the mature megasporocyte is a polarized cell, thanks to the micropylar position of the nucleus and the greater abundance of cytoplasm in the chalazal region. A more or less accentuated micrphyle-chalaza polarization, which is maintened during meiosis, determines the formation of megaspores, among which the more chalazal one is privileged in size and/or cytoplasmic content (Fig. 6). The consequent greater development potientiality is realized immediately, or after a short delay if other megaspores begin to develop. The development of the chalazal megaspore is certainly favoured by its proximity to the nutrient supply. However, the influence of trophic flow seems atypical in *Taxus*. Here, even the potential megasporocytes may have one or two high density cytoplasmic zones and all the products of meiosis show, at times, an equal development capacity which lasts even for quite long periods (DUPLER, 1917). Such behaviour could be associated with some peculiar aspects of the nucellus which should be studied by ultrastructural analysis.

At germination the chalazal megaspore organelles, if polarized in the localization, assume a uniform distribution. The other aploid cells, having reduced metabolic potientiality, degenerate rapidly. Observations using the transmission electron microscope (TEM) show the transformation of their contents into electron-dense material probably of a lipidic nature (Fig. 8) (CECCHI FIORDI and MAUGINI, 1977; MOUSSEL, 1981). In *Laryx* the degenerative process, which is perhaps slower, involves evident activity of the endoplasmic reticulum. This

organelle becomes suitably localized during the course of maturation and during two meiotic divisions of the megasporocyte (CECCHI FIORDI, et Al., 1984).







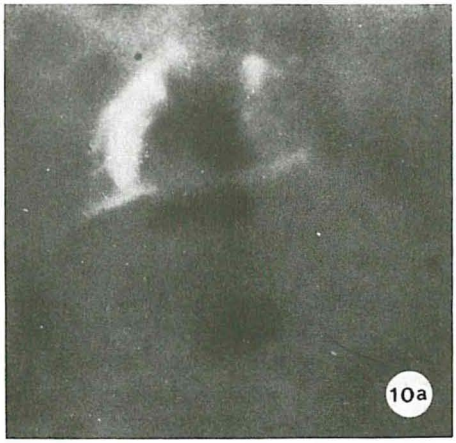


Fig. 8 — Triad of *Chamaecyparis lawsoniana*. The two micropylar megaspores are degenerated; the chalazal one has deposited a new wall layer (arrow). (From CECCHI FIORDI and MAUGINI, 1977) X 4,260.

Fig. 9 — Triad of *Encephalartos poggei*. The megaspores show fluorescent callose which envelops in very different ways the micropylar megaspore (a), the central megaspore (b), the chalazal megaspore (c). (From DE SLOOVER, 1961) X 860.

Fig. 10 — Dyad of *Biota orientalis*. (From FABBRI TARCHI, 1969) X 1,320.

Fig. 10a — Dyad of *Biota orientalis*. Only the wall of the upper cell appears fluorescent due to the presence of callose. (From FABBRI TARCHI, 1969) X 1,100.

Even the original wall of the megasporocyte, which is frequently thicker than that of the surrounding cells, is subject to ulterior modifications which are destined to disappear precociously after meiosis. The behaviour of the cell-wall is indeed another key-topic that emerges from the analysis of available data on the megasporogenesis of Gymnosperms.

In *Encelphalartos* during meiosis, beginning with the dyad stage, two new wall layers are deposited which surround every single cell. The outer layer is more developed and is composed, at least partially, of callose. In the sections of the triad this polysaccaride appears more abundant in the middle megaspore, and in particular at the four peripheral points of contact with the other megaspores. It is less abundant in the chalazal megaspore and extremely scarse in the micropylar one (Fig. 9). This wall layer is considered similar to the «special wall» which appears during microsporogenesis and which, on disintegration, finally allows the separation of the microspores inside the microsporangium (De Sloover, 1961). Martens (1966) verified the presence of callose in *Encephalartos*, but he did not find it in either *Ginkgo* or *Larix*. He considers its presence primitive and sees it as a trace of the ancestral mechanism of isolation which the heterosporous pteridophytes carry out so as to obtain an isolated dispersion of their megaspores. Some preliminary observations made by Fabbri Tarchi (1969) show that a deposit of callose forms around the dyad upper cell during megasporogenesis in *Biota orientalis* (Fig. 10, 10a). In the light of Marten's interpretation the above mentioned author underlines the primitive character of other embryological events of the species considered. Meiotic wall

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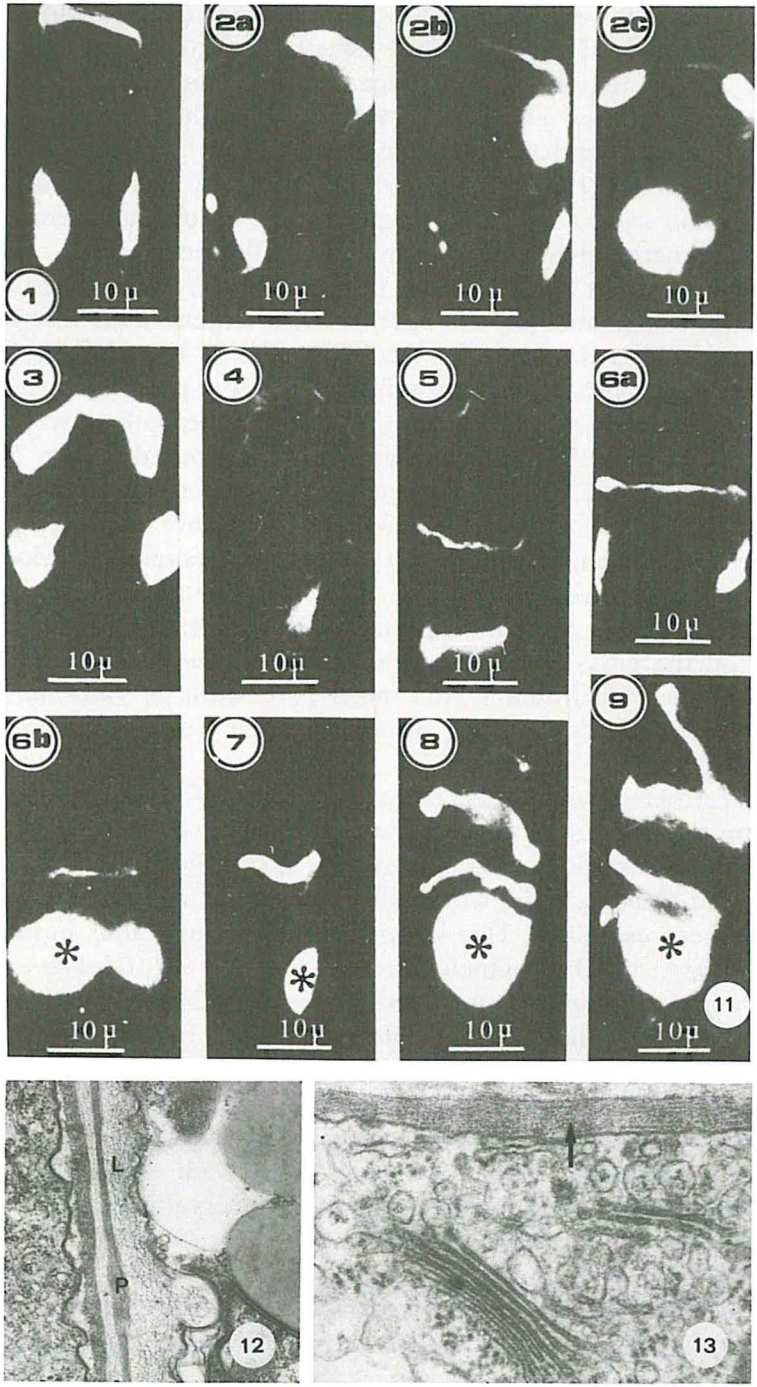
Fig. 11 — *Ephedra distachya*. Successive stages in the formation and distribution of the callose thickenings (recognizable because they appear fluorescent) in the wall of the megasporocyte (micrographs 1; 2a, 2b, 2c; 3; 4), in the walls of the dyad (micrographs 5; 6a, 6b; 7), and in those of the tetrads (micrograph 8, linear tetrad; micrograph 9, oblique tetrad). 2a, 2b, 2c: serial sections of the same megasporocyte; 6a, 6b: serial sections of the same dyad. The asterisk marks a prominent callose thickening at the height of the equatorial girdle in the wall of the dyad lower cell and of the chalazal functional megaspore. (From Mousset, 1981).

Fig. 12 — *Chamaecyparis lawsoniana*. Detail of the wall between the dyad and tapetum cells. An unevenly thickened layer (L), which has a fibrillar ultrastructure, can be seen inside the megasporocyte primary wall (P). (From Cecchi Fiordi and Maugini, 1977) X 18,470.

Fig. 13 — *Chamaecyparis lawsoniana*. Portion of the functional megaspore with its own peculiar wall (arrow). Note the active dictyosomes in the peripheral cytoplasm. (From Cecchi Fiordi and Maugini, 1977) X 41,500.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12
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callose deposits were observed in *Ephedra distachya* by MOUSSEL in 1981. Their behaviour, and more especially their constant absence in the lower region of the megasporocyte and of the chalazal megaspore and their abundant development in the transversal septa (Fig. 11), were found to be in agreement with the theory of NAHER DE HALAC et Al. (1977). These authors propose that the callose is deposited in such a way as to separate the cytoplasmic entities of reduced functionality from the metabolically more active one. The callose could then be utilized by the megaspore, which gives origin to the female gametophyte, together with degenerated micropylar megaspores.This degeneration could be due to the ulterior reduction of the trophic supply caused by the scarse permeability of the callose walls. Thus the isolation function of the callose would not be that of a genetic character, generally attributed to the callose itself, in micro and macrosporogenesis by various authors (HESLOP HARRISON, 1964; RODKIEWICZ, 1973). These authors maintain that a callose envelope could be necessary for the autonomous development of the reproductive aploid cells. Such a necessity, at least in the megasporogenesis of Gymnosperms, had been excluded by PETTITT (1977) on the basis of the localization and of the small quantity of the polysaccaride found in the megaspore walls of *Encephalartos*, and because of its constant absence in the walls of *Ginkgo* and various Cycads.

Nevertheless some ultrastructural aspects of the megasporocyte wall must be considered: in *Chamaecyparis lawsoniana* this wall shows maximum thickening around the entire dyad (Fig. 12) (CECCHI FIORDI and MAUGINI, 1977) with a stratification whose chemical nature has not been examined. This stratification is comparable, in its basic morphology, to that which already appears in *Ginkgo* at the megasporocyte stage. The new wall material in *Chamaecyparis* tends, moreover, to occlude the few plasmodesmata initially present. In *Larix*, another genus for which the non-production of callose during female meiosis is known, an increasing ultrastructural complexity of the megasporocyte wall up to the triad stage has been found; this is a tickening which has never been seen to extend to the transversal walls (unpublished personal observations).

Observations at TEM also show that the cytoplasm of the sole chalazal megaspore lays down a wall stratum almost immediately after meiosis, with the active contribution of the Golgi apparatus (Fig. 13) (CECCHI FIORDI and MAUGINI, 1977; MOUSSEL, 1981; unpublish-

ed personal observations). This new layer probably represents the beginning of that elaborate wall which generally is characteristic of the female gametophyte of Gymnosperms. The inconspicuous wall stratum, which appears also in the micropylar megaspores in *Encephalartos*, inside the callose envelope, may be the manifestation of an unsuccessful attempt at germination. PETTITT (1977) emphasized that the female gametophyte wall reaches maximum complexity in the zooidogamous forms.

In conclusion, the formation of wall callose deposits during megasporogenesis in Gymnosperms s.l. has been verified by little research until now. The localization of callose in the single aploid cells is often irregular and is more abundant in the transversal septa than in the lateral walls. The quantity of this polysaccharide is not uniform in the various meiotic products. All these aspects seem to uphold the point of view that in Gymnosperms the parietal callose is not an indispensable requirement for the development of megaspores. The presence, around the mature megasporocyte, of a thickened wall is constant. It can further modify itself around the complex of aploid cells by the deposition of materials which in some cases are certainly different from callose. Moreover, the plasmodesmata can be occluded. Thus, conditions aimed at reducing or selecting metabolite exchange between the megasporocyte cytoplasm on the whole and surrounding cells actually occur, to enable the onset and the course of the meiotic event. Undoubtedly, in order to add to our knowledge of the nature, function and the possible evolution of the parietal thickenings observed, wider use of ultrastructural investigations and more cytochemical studies are necessary.

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(ms. pres. il 15 dicembre 1986; ult. bozze il 23 aprile 1987)