Female gametophyte and pollen tube of *Epilobium palustre* L.

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Abstract

The monosporic, tetranucleate embryo sac of *Epilobium palustre* (*Onagraceae*) develops from the micropylar megaspore in a linear tetrad. In mononucleate embryo sacs a peculiar chromatic structure associated with a nucleolus appears in the nucleus. This structure seems to be formed by fibrillar material and is not visible in the subsequent stages of development.

A large amount of rough ER cisternae occurs in the late mononucleate stage, during the binucleate stage their contents become optically dense. It the early tetranucleate stage the amount of ER is small, it increases again in the developing synergids and central cell.

Numerous amyloplasts present in the mononucleate embryo sac loose their starch grains and some are transformed into cup-shaped plastids or proplastids. They are passed on to each of the embryo sac cells.

The growth of the pollen tube ceases immediately after the penetration through the filiform apparatus of a synergid. At the apex of the tube a pore is formed. At the last stages of the penetration the apical part of the pollen tube becomes separated by a transverse partition from the distal part of the tube. The contents of the both parts differ in their internal structure. The distal part contains cytoplasm with numerous organoids, while the apical part is mainly filled with spherical bodies.

INTRODUCTION

The embryo sac of *Epilobium* develops from a micropylar megaspore of a linear tetrad. After two nuclear divisions a four nucleate embryo sac of *Oenothera* developmental type is formed. This type of development has only been described in the *Onagraceae* family (Geerts 1908, Modilewski 1909). On the micropylar pole of the embryo sac an egg apparatus composed of two synergids and one egg cell differentiates. The bulk of the volume of the embryo sac is taken up by a mono-
nucleate central cell. In the synergids a filiform apparatus is formed, in the cytoplasm a large amount of endoplasmic reticulum occurs. On the chalazal side the synergids do not have a cell wall \cite{Bednara1975}. Similar synergids were found in the embryo sac of \textit{Oenothera lamarckiana} \cite{Jalouzot1975}, \textit{Gossypium} \cite{Jensen1965a}, \textit{Petunia} \cite{VanWent1970a}, \textit{Quercus} \cite{Mogensena1972} and several other species examined under the electron microscope \cite{Rokiewicz1974}.

\textbf{MATERIAL AND METHODS}

The ovules of \textit{Epilobium palustre} (Onagraceae) were fixed in 4\% glutarate aldehyde and 1.5\% OsO$_4$ at pH 7.4 or in 2\% unbuffered KMnO$_4$. The material was dehydrated with ethanol, passed through propylene oxide and embedded in Epon 812. The sections were contrasted using uranyl acetate and lead citrate. For investigations in the light microscope the material was fixed in a mixture of ethanol with glacial acetic acid (3:1). Callose was detected with an aniline blue fluorescence method. Other insoluble polysaccharides were detected by the PAS reaction.

\textbf{OBSERVATIONS}

\textbf{I. Mononucleate embryo sac}

During the transformation of functional megaspore into an embryo sac, some changes in plastid distribution and cytoplasm structure take place. It is, however, difficult to determine a strict boundary between these two phases of development. Initially a mononucleate embryo sac is an elongated cell with a distribution of organoids similar to that in a megaspore (Fig. 1A).

The nucleus occupies a central position, and fairly dense cytoplasm begins to vacuolize at both poles of the cell. Amyloplasts are found in the micropylar part, dictyosomes mainly in the chalazal part, whereas mitochondria and polyribosomes are distributed throughout the cytoplasm. Amyloplasts which contain starch grains have irregular shapes, some after they lose their starch have the shape of cup-bodies (Pl. I. Fig. 1).

The small amount of ER cisternae is remarkable. In the cytoplasm there are small vacuoles with remains of cytoplasm, they may be called autophagic vacuoles.

The cell wall maintains the appearance that it had in the megaspore stage, it is much thicker in the chalazal than in the micropylar part (Pl. I. Fig. 2, 3). The wall is PAS positive, but in contrast to the
previous stage does not give an aniline blue fluorescence reaction for callose.

The older, much larger mononucleate embryo sac is strongly vacuolated (Fig. 1B). The cell nucleus is still in the middle part of the cell, amyloplasts, dictyosomes and mitochondria are dispersed in the cytoplasm, autophagic vacuoles are not longer visible, there is a large amount of ER cisternae, with which the bulk of the ribosomes is associated.

In the cell nucleus near the nucleolus a fibrillar structure which is difficult to identify can be observed. It was found in four out of six examined embryo sacs. A similar structure was seen twice in synergids and once in a somatic cell. This structure is composed of felted fibers 200–300 Å in diameter, placed parallel to the surface of the nucleolus. In some places the fibrils adhere to the nucleolus. From serial preparations this structure is known to be saucer-shaped, it is slightly less optically dense than the nucleolus and has a density similar to chromatin in chromocenters. Chromatin structures do not, however, have similar fibrillar elements (Pl. II. Fig. 5, 6). The location of these structures in the nucleus was identical in all cases. The nucleolus was near the nuclear envelope, and the described structure was always on the side opposed to the proximal surface of the nucleus (Pl. I. Fig. 4).

II. Binucleate embryo sac

A binucleate embryo sac is distinctly larger than a mononucleate one. Nuclei lie in the cytoplasm initially in the middle part of the cell, both poles are taken up by two vacuoles (Fig. 2A). In the cytoplasm there are many cisternae of rough ER filled with contents denser than basal cytoplasm (Pl. II. Fig. 8). Mitochondria, plastids and a few dictyosomes are dispersed throughout the cytoplasm. Plastids with oval or elongated profiles often do not have starch, dictyosomes are not numerous, poorly structured and do not form vesicles.

A binucleate cell grows in the direction of the micropyle where the adjoining cells undergo degeneration. Their contents become homogenous and electron translucent, only some remains of chromatin are maintained longer. The cell walls between the adjoining micropylar cells and the embryo sac are dissolved, then the cells are only separated by a cytoplasmic membrane (Pl. II. Fig. 7).

III. Embryo sac composed of four cells

Two nuclei of the binucleate embryo sac move to the micropylar pole and undergo division. The nucleus closer to the apex divides in the plane parallel to the long axis of the cell, the lower nucleus di-
vides in a plane perpendicular to the long axis. After the division cell plates are formed. First two synergids are delimited (Fig. 2b), later the egg cell is separated from the central cell.

Fig. 1. A) Mononucleate embryo sac (embryo sac mother cell) in an early stage of development. The chalazal part is covered by a thickened cell wall containing PAS-positive materials and callose. The distribution of plastids and dictyosomes is shown. Particular zones of the cell are presented in the electron micrographs (Pl. I). B) The embryo sac mother cell in the vacuolization stage. Cell wall is PAS-positive but without callose. In the whole cell there are amyloplasts and short ER cisternae. Parts of the cell are shown in electron micrographs (Plates I, II. Figs. 3–6)

A four cell embryo sac is formed, in which three cells of the egg apparatus do not distinctly differ in structure. Young synergids take up the micropylar part of the embryo sac and the egg cell is located under them (Fig. 2C).

All cells are separated from each other by cell plates through which plasmodesmata pass. Cells of the egg apparatus contain mitochondria, proplastids, amyloplasts with starch grains, long ER cisternae, whereas dictyosomes are not very numerous (Pl. II. Fig. 9).

In various places of all cells of the egg apparatus small vacuoles can be seen. The central cell is different in this respect. Most of it is taken up by an enormous vacuole, the cytoplasm is mainly grouped by the egg apparatus forming the zone surrounding the cell nucleus. Cytoplasm contains the same, very similar organoids as other cells of the embryo sac.

The configuration of the cells of the egg apparatus changes during the development of the embryo sac. Initially the egg cell is under synergids which occupy the micropylar pole of the embryo sac (Pl. II.
Fig. 9), afterwards a growth of all these cells takes place in the direction of the chalaza and then the synergids surround the egg cell on three sides. At this time the protoplasm of the synergids (Pl. III. Fig. 10, 11), of the egg cell (Pl. IV. Fig. 14) and the central cell (Pl. IV. Fig. 15) remains still very similar. The cytoplasm contains numerous polyribsomes, amyloplasts, leuoplasts, mitochondria, active dictyosomes and some short ER cisternae. In the cells of the egg apparatus there are

![Diagram of developing embryo sac](image)

**Fig. 2. The developing embryo sac:** A) After the first nuclear division. B) Shortly after the second nuclear division, when cell plates separate two micropylar cells — the future synergids. C) A young four cellular embryo sac with an incompletely developed egg apparatus. The micropylar part is filled by two synergids, under them a diagonally slanted egg cell and a fragment of the central cell. Parts of the embryo sac are presented in electron micrographs (Pl. II. Fig. 7—9)

small vacuoles, and the central cell has a large chalazal vacuole. All cells are separated by cell plates.

During their growth the cells of the egg apparatus undergo a typical vacuolization and in the micropylar part along cell plates cell walls with plasmodesmata are formed (Pl. III. Fig. 11, 12). At this stage some dictyosomes characteristically placed between the new built wall and amyloplasts can be observed (Pl. III. Fig. 11).

The chalazal part of the egg apparatus cells does not have walls and protoplasts of the synergids, egg cell and central cell touch each other by their plasmalemma (Fig. 3A). In some places plasmalemmae of adjoining
cells separate forming vesicle-like distensions (Pl. IV. Fig. 15). In a mature egg apparatus large, almost optically empty areas can be seen between the cells (Pl. IV. Fig. 16, 18).

In the not fully developed synergid having a large vacuole but without a filiform apparatus a large number of cisternae of the rough ER filled with thick contents can be seen. The cisternae have tendency to form parallel systems (Pl. III. Fig. 13). During the formation of the filiform apparatus a large number of cisternae of the rough ER with very irregular profiles are present in the cytoplasm and the mitochondria assume a condensed form.

![Fig. 3. Diagrams of the micropylar part of the embryo sac: A) Before formation of the filiform apparatus in synergids. One of the two synergids and the egg cell are shown. There is no cell wall on the chalazal side. B) Just before fertilization, with the pollen tube at the filiform apparatus. C) After discharging of part of the pollen tube contents into the synergid and the space between the egg and central cell. Electron micrographs (Plates III — VI) ]

In a mature synergid with a developed filiform apparatus (Pl. V. Fig. 20) there is still a large number of cisternae of the rough ER (Pl. V. Fig. 19).

The egg cell is almost completely vacuolized so that in the mature stage a thin layer of cytoplasm adheres to the cell wall only at the chalazal apex there is a slightly larger amount of cytoplasm with a cell nucleus (Pl. IV. Fig. 16, 18). In the cytoplasm all cell organoids are present, mitochondria, amyloplasts, but only the small amount of short ER cisternae is visible (Pl. IV. Fig. 16, 18).
Nuclear chromatin of the egg cell has a fairly loose structure; the nucleus is surrounded by a smooth nuclear envelope containing numerous pores. (Pl. IV. Fig. 17).

In the central cell the nucleus grows larger, the nucleolus develops, the nuclear envelope becomes wavy, in the cytoplasm a large amount of rough ER occurs (Pl. VI. Fig. 24).

IV. Pollen tube and embryo sac

A fully formed embryo sac is ready to receive the pollen tube (Fig. 3B). Most commonly the tip of the pollen tube flattens out on the top of the embryo sac (Pl. V. Fig. 21), then the micropylar part of the synergid is pushed into the embryo sac, the filiform apparatus is partially destroyed and the contents of the pollen tube enter the synergid (Pl. V. Fig. 21).

The pollen tube opens by a pore and ceases to elongate right after crossing through the filiform apparatus (Fig. 3C). The pore is situated on the terminal wall of the pollen tube; slightly higher in the pollen tube a partition is formed separating the apical part from the distal one (Pl. V. Fig. 21, 22, Pl. VI. Fig. 23). The content of the separated tip is composed of a great number of irregularly shaped bodies similar to those which are in the cytoplasm of the pollen tube which fills the synergid. Neither in the synergid nor in the tip of the pollen tube any typical cell organoids can be distinguished, the main component are dark spherical bodies.

A similar material originated from the pollen tube can also be found in the zone between the central cell and the egg cell. Over the described apical compartment the cytoplasm of the pollen tube appears normal it contains numerous distyosomes, ER cisternae and vacuoles and osmophilic bodies (Pl. V. Fig. 22).

DISCUSSION

The embryo sac mother cell in Epilobium develops into a four cellular embryo sac of the Oenothera type, only exceptionally an eight nucleate (seven cells) embryo sac of the Polygonum type was found (Gaczecziadze 1975).

While the embryo sac grows and develops it cytoplasm undergoes vacuolization and the nucleus divides twice. Four daughter nuclei with large amounts of cytoplasm are placed in the micropylar part and large vacuoles occupy the chalazal part of the embryo sac.

In successive stages of development of the embryo sac distinct differences in ultrastructure were observed; from some of these one may
assume that changes of physiological processes are taking place. The functional megaspore (the mother cell of the embryo sac) undergoes a rearrangement of its structure during its transformation into the embryo sac. First the character of the cell wall and the distribution of organoids is altered, then changes in the amount and structure of the ER and changes in the appearance of the organoids can be observed.

The lateral and chalazal walls of a functional megaspore contained callose, and the whole wall was built of PAS positive material. In the mononucleate stage callose disappears from the lateral walls and later from the chalazal wall. In the two nucleate stage the hitherto thick wall becomes thinner. On the micropylar pole the wall of the embryo sac and of the adjacent degenerating cell becomes almost completely dissolved. It seems that the protoplast of the embryo sac is separated from the somatic micropylar cell by two cytoplasmic membranes. The outer one is adjacent to the remains of the micropylar wall. The building up of the micropylar wall into a filiform apparatus begins much later in a four-celled embryo sac.

Amyloplasts which seem to be the only plastids of the functional megaspore, are situated in the micropylar part of the cell after the second meiotic division. Afterwards they undergo distribution throughout the cell, similarly dictyosomes at first mainly located in the chalazal part undergo gradual distribution. Almost all amyloplasts are filled with

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**Plates I–VI**

**Key to labelling:**

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<thead>
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<th>Symbol</th>
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<td>CC</td>
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<td>cell wall</td>
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<td>D</td>
<td>dictyosome</td>
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<td>endoplasmic reticulum</td>
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<td>FA</td>
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**Plate I**

- Fig. 1. Micropylar part of the embryo sac mother cell with polymorphic amyloplasts containing starch grains. Some plastids deformed into so-called cup-bodies. Mitochondria visible between amyloplasts. In the middle of the cell a large nucleus with a well developed nucleolus. \( \times 3200 \)
- Fig. 2. Chalazal part of the embryo sac mother cell with multivesicular bodies, mitochondria and dictyosomes. Some dictyosomes are arranged in pairs. \( \times 13000 \)
- Fig. 3. Zone of narrowing cell wall at the micropylar part of the embryo sac mother cell. The nucleus with a large nucleolus and amyloplasts with starch grains are visible. \( \times 7500 \)
- Fig. 4. Mononucleate embryo sac. Cell nucleus with a large nucleolus, by which a dense fibrillar structure is visible. In the cytoplasm vacuoles, rough ER, mitochondria and amyloplasts with starch grains. \( \times 7500 \)
starch grains some, however, after partial or total loss of starch assume a cup-shaped form. In the two or four cellular embryo sac besides amyloplasts with starch and a few cup-shaped plastids with undifferentiated thick stroma can be seen. It should be assumed that they were formed from amyloplasts and cup-shaped plastids.

The stage of a mononucleate embryo sac is characterized by the occurrence of active dictyosomes and autophagic vacuoles, which may play a part in the intensive vacuolization at both poles of the cell. Afterwards abundant ER composed of rough cisternae with folded profiles appears. Rough ER elements are also in the two nucleate embryo sac but cisternae look different, they become shorter, wider and are filled with a basic component which is optically denser than the cytoplasm. In a four nucleate and four celled embryo sac only narrow elongated cisternae of the ER are visible. One can thus consider that intensive protein synthesis takes place mainly in the one and two nucleate stages of the embryo sac development.

The structure and size of the nucleolus in these stages may also be considered as an indication of intensive protein synthesis.

In the one and two nucleate stages nucleoli are large and with attached clearly fibrillar and granular structures of a character which is difficult to define. Moreover, apart from this stage these associated structures have been observed only exceptionally. They may be heterochromatin segments or else fibrills which emerged from the nucleolus. It seems that the second possibility is more plausible as these structures are composed of fibrills similar to those found in the nucleolus. The fibrills of the described structures seem to be attached to the nucleolus or even to emerge from it.

From serial section the perinucleolar structures are seen to be saucer shaped, thus have a different shape than heterochromatin segments. The
formation of such perinucleolar structures may be related to high activity of the nucleoli.


In all species in synergids a filiform apparatus is formed, an abundant endoplasmic reticulum and other cellular organoids occur. It is generally considered that synergids are very active cells.

The egg apparatus in most of the examined species had only incomplete cell walls. Chalazal regions of cells of the egg apparatus are naked and their plasmalemmae come into contact with a plasmalemma of the central cell (Jalouzot 1975, Jensen and Fisher 1967, Mogensen 1972, Pluijm 1964, Van Went 1970). In Epilobium adjacent plasmalemmae in some places of this zone come apart forming rows of vesicular almost optically empty spaces similar to those described in Oenothera (Jalouzot 1975).

These spaces increase in size and remain optically empty in a mature embryo sac. It cannot be excluded that these spaces are to some extent artefacts, even though their real existence has been postulated for example in Crepis (Gerasimova-Navashina and Guljaev 1973). Subsequently, after pollination the contents of the pollen tube which fill one of the synergids enter also into these spaces.

Authors which have examined fertilization under the electron microscope agree that the pollen tube grows through the filiform apparatus into one of the synergids (Deschamps 1974, Godineau 1969, Jensen 1965a, b, Schultz and Jensen 1968). In the synergid the pollen tube grows for a certain time and then breaks or, according to
Coccuci and Jensen (1969b), in the wall of the pollen tube a pore is formed through which the contents pass out into the synergid.

In Capsella bursa-pastoris the pore was found to be plugged after the material from the pollen tube was discharged (Schultz and Jensen 1968). In many species, however, examined under the light microscope the pollen tube has been described to go directly into the embryo sac by passing the synergids (ref. Steffen 1963).

It may be assumed that in Epilobium palustre the pollen tube enters the embryo sac in the following way: the apical part of the pollen tube after coming into contact with the micropylar top of the embryo sac flattens out and swells. Subsequently it squeezes the apices of the synergids and pierces the filiform apparatus of one of them. After passing through the apparatus the growth of the pollen tube immediately comes to an end and in the apical wall a pore is formed through which the contents of the apical compartment of the pollen tube are discharged into cell. Later a part of the contents enters the space between the central cell and the egg cell. The apical part of the pollen tube entering an embryo sac is separated by a continuous partition from the distal part of the tube. The contents of the separated zones differ considerably.

The apical part of the pollen tube and the synergid connected to it by a pore are filled with densely clustered bodies spherical or irregular in shape and of a polysaccharide nature (Schultz and Jensen 1968).

The distal part of the pollen tube contains cytoplasm with numerous organoids. It may be assumed that the separating of the apical part of the pollen tube whose contents are prepared for discharge prevents the cytoplasm of the whole pollen tube from passing into the synergid.

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REFERENCES


PLATE V

Fig. 19. Cytoplasm of a mature synergid with cisternae of rough ER. ×15000

Fig. 20. Fragment of filliform apparatus in a synergid and an adjacent pollen tube. ×8000

Fig. 21. Egg apparatus after entrance of the pollen tube contents into the synergid, the egg cell on the right side. ×2400

Fig. 22. A wall separating the distal part of the pollen tube from the apical part. The distal part contains cytoplasm with organeloids, the apical part contains mainly spherical bodies. ×18500
Fig. 23. Apical part of the pollen tube in the embryo sac. A pore in the tube wall and the remnants of the filiform apparatus. ×10000

Fig. 24. Fragment of the protoplast of the central cell in a differentiated embryo sac. Extensive rough ER and a large nucleus with nucleolus. ×8000
Streszczenie

U Epilobium w czasie rozwoju czterokomórkowego woreczka załączkowego z komórki macierzystej następuje szereg zmian ultrastrukturalnych. W stadium jednojądrowym w jądrze tworzy się niezidentyfikowana struktura o talerzykowatym kształcie zbudowana z fibryli wykazujących ciągłość z jąderkiem. Pod koniec fazy jednojądrowej rozwija się szorstkie ER, jego cysterny w stadium dwujądrowym wypełniają się gęstą zawartością. Liczne amyloplasty przekształcają się w plastyny kubkowate i proplastydy. Amyloplasty trafiają do wszystkich komórek woreczka załączkowego. Ilość ER zmniejsza się we wczesnej fazie 4-komórkowego woreczka, wtedy trzy komórki aparatu jajowego oddzielone są od siebie przegrodami pierwotnymi i nie różnią się od siebie ultrastruktury. W czasie dojrzewania woreczka w synergidach i komórce centralnej tworzy się obfite szorstkie ER. Chałalazalne części komórek aparatu jajowego nie mają ścian komórkowych.

Łagiewka wrasta do aparatu włókienkowego i natychmiast po jego przebieciu otwiera się terminalnie położoną porą. Wierzchołkowa część łagiewki oddzielona jest przegrodą od części dystalnej gdzie jest cytoplazma z licznymi wyraźnymi organoidami. Przegroda zapobiega przechodzeniu cytoplazmy z organoidami z łagiewki do woreczka załączkowego.