ACTA BIOLOGICA CRACOVIENSIA Series Botanica 41: 177–183, 1999

PARTICIPATION OF ENDOPLASMIC RETICULUM IN THE UNEQUAL DISTRIBUTION OF PLASTIDS DURING GENERATIVE CELL FORMATION IN *GAGEA LUTEA* (L.) KER.-GAW. (LILIACEAE)

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Received December 20, 1998; revision accepted April 21, 1999

The distribution of plastids at the time of microspore and pollen grain development in *Gagea lutea* (L.) Ker.-Gaw. was analyzed using electron microscopy. It was shown that plastids are not transmitted to the forming generative cell of this species during microspore division. At the vacuolate microspore stage, preceding division, the microspore nucleus takes an acentric position and the plastids gather at the opposite side of the cell. In the highly polarized microspore at prophase of mitosis, all plastids are aggregated at one side of the nucleus, whereas mitochondria are dispersed throughout the cytoplasm. Numerous profiles of endoplasmic reticulum (ER) are present between the clustered plastids. Some of the ER profiles are attached by their ends to the outer membrane of plastid envelopes and join the distant plastids. The outer membrane of the microspore plastids may form long and thin evaginations contacting with other plastids. Microtubules are visible in plastid aggregations occasionally. In dividing microspores, long ER cisterns surround the area of the mitotic spindle and separate it from the region containing plastids. There are no plastids in the young generative cell: all plastids remain clustered in the region of the microspore that now forms the vegetative cell of the bicellular pollen grain. Later the connections between plastids and ER cisterns gradually disappear and plastids disperse in the cytoplasm of the whole vegetative cell. The results of our study are not sufficient to define the mechanism causing selective aggregation of plastids at the vegetative pole of the *Gagea* microspore, nor to say whether the microtubular cytoskeleton plays a role. However, the participation of ER in these processes, at least in holding the special arrangement of microspore plastids, seems certain.

Key words: *Gagea lutea*, endoplasmic reticulum, microspore mitosis, plastid distribution, ultrastructure.

INTRODUCTION

At the vacuolate microspore stage, the microspore nucleus undergoes the first mitotic division, followed by cytokinesis. After the completion of these processes, the male gametophyte consists of two cells: the intine-attached generative cell and the larger vegetative cell. The cell division is highly unequal, producing differences not only in cell size but also in cytoplasm contents. The vegetative cell contains most of the plastids and mitochondria; the smaller generative cell contains a few organelles, and plastids often are not observed in this cell. Plastids of angiosperms are known to be inherited by either biparental or maternal uniparental means (Kirk and Tilney-Bassett, 1978; Hagemann, 1979; Sears, 1980). Four types of distribution and transmission of paternal plastids have been distinguished (Hagemann, 1981; Hagemann and Schrder, 1989), but the cytological mechanisms and different cellular structures taking part in these processes are still not fully recognized.

We became interested in *Gagea lutea* firstly because according to Krupko (1926) proplastids are present in the generative cell of this species, and secondly because an analysis of 4', 6-diamidino-2-

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PL ISSN 0001-5296

phenylindole (DAPI) staining revealed the presence of numerous DNA-containing organelles throughout the whole generative cell (Zhang et al., 1995).

The present ultrastructural study describes the unequal distribution of plastids during generative cell formation in *Gagea lutea* microspores and the role played by the endoplasmic reticulum in this process.

MATERIALS AND METHODS

Young flower buds from wild plants of *Gagea lutea* (L.) Ker.-Gaw. (Liliaceae) growing in northern Poland were collected. The cytological stage of the microspores or pollen grains was determined by acetocarmine staining of one anther per flower. The remaining anthers were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0) for 2 h at room temperature. The samples were washed in the same buffer with four changes (15 min each) and postfixed in buffered 1% OsO_4 at 4°C overnight. After rinsing in distilled water the anthers were treated with 1% uranyl acetate in distilled water for 1 h at 4°C, dehydrated in a graded acetone series and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were cut with a Sorvall MT-2B ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Tesla BS 500 transmission electron microscope. Control semithin sections were poststained with 0.1% Toluidine Blue O in 1% sodium tetraborate.

RESULTS

After release from the tetrad the microspore of *Gagea lutea* is ellipsoidal and contains small vacuoles. The microspore nucleus is round and takes a central position in the cell; mitochondria and plastids are randomly distributed. During further microspore development a large vacuole forms at one of the cell poles, and the nucleus locates at the opposite pole simultaneously.

Microspores at prophase of mitosis are highly polarized; all plastids are aggregated near the thickened intine of the future aperture, whereas the cell nucleus is located distally to the aperture, close to the microspore wall (Fig. 1). At this time the mitochondria are randomly distributed in the microspore cytoplasm. Numerous profiles of rough endoplasmic reticulum (RER) are present between the clustered plastids (Fig. 2). Some of the RER profiles are attached by their ends to the outer membrane of plastid envelopes and join the distant plastids (Fig. 3). Frequently the envelopes of neighboring plastids are in contact. Moreover, the outer membrane of the microspore plastids may form long and thin evaginations (apart from short and oval ones), which are in touch with the opposite plastids (Fig. 3). Occasionally microtubules (MT) are visible in plastid aggregations (Fig. 4).

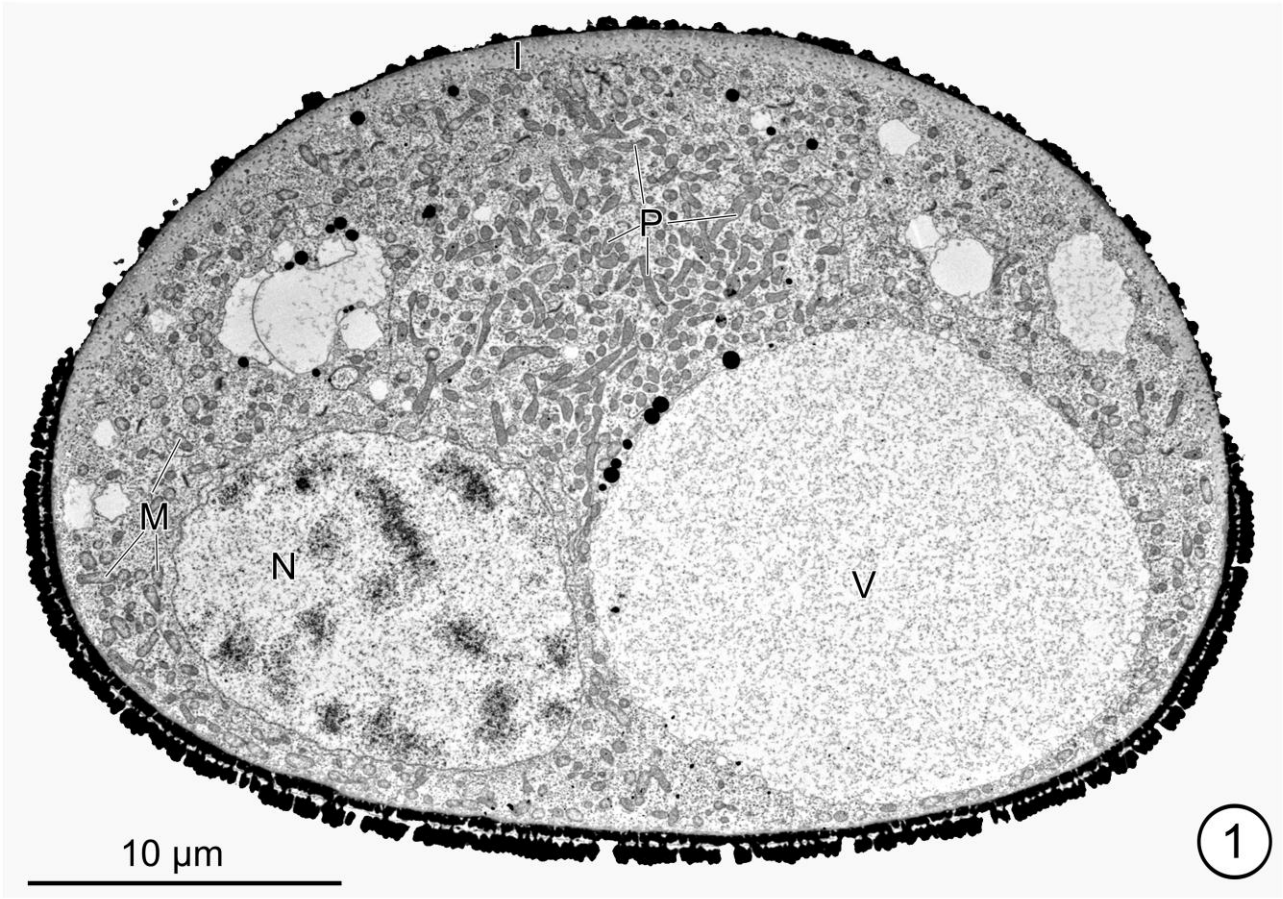
In dividing microspores, long endoplasmic reticulum cisterns surround the area of the mitotic spindle and separate it from the region containing plastids (Fig. 5). The male gametophyte is formed as a result of the first haploid mitosis. It consists of two cells: the wall-attached generative cell and the bigger vegetative cell. Now a thin wall separates these cells (Fig. 6).

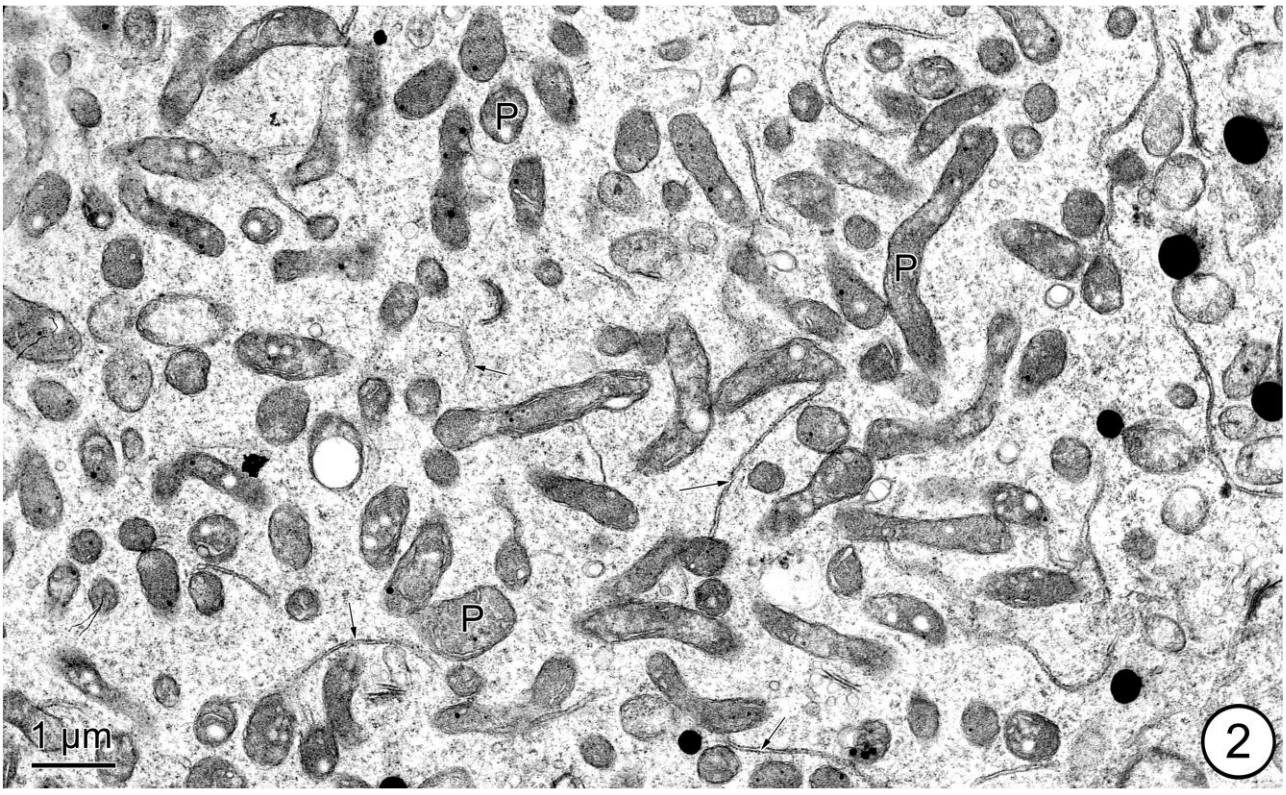
There are no plastids in the young generative cell: all plastids are located in the vegetative cell. The connections between plastids and RER cisterns gradually disappear and the plastids disperse throughout the cytoplasm of the whole vegetative cell (Fig. 6).

DISCUSSION

The results of this study clearly show that during the first pollen mitosis the generative cell of *Gagea lutea* does not receive any plastids: there is a very unequal distribution of the plastids into the vegetative cell only. Therefore, using the criteria of Hagemann (1981), this species belongs to the *Lycopersicon*-type with respect to plastid inheritance. The absence of plastids in the generative cell *ab initio*, characteristic of the *Lycopersicon*-type, has been reported for various other species (for review: Van Went, 1984; Hagemann and Schröder, 1989). On the basis of our results, the numerous DNA-containing organelles observed by Zhang et al. (1995) after DAPI staining in the generative cell of *G. lutea* can be recognized as mitochondria but not plastids;

Fig. 1. Microspore of *Gagea lutea* at prophase of mitosis, showing polarity in cell organization. All plastids (P) are aggregated at one side of the nucleus (N) near the thickened intine (I) of the future aperture, whereas mitochondria (M) are randomly distributed in the microspore cytoplasm. V – vacuole. **Fig. 2**. Numerous profiles of rough endoplasmic reticulum (arrows) are present between the clustered plastids (P).


I
P
M
N
V
10 µm
1


P
P
P
1 µm
2

this is also confirmed by an analysis of ultrastructure of the generative cell inside the pollen tube (Bohdanowicz, unpublished).

In *G. lutea* during prophase of the first haploid mitosis the plastids become polarized and clustered at the proximal pole of the microspore, whereas the dividing nucleus is located at the distal pole. Similar behavior of these organelles has been observed in microspores of, for example, *Impatiens walleriana* and *I. glandulifera* (Van Went, 1984), *Gasteria verrucosa* (Schröder, 1985) and *Chlorophytum comosum* (Schröder, 1986). Various mechanisms leading to plastid polarization and exclusion from the generative cell have been postulated. Hagemann (1981) supposed a partly mechanical reason: the mitotic spindle may mechanically push the plasmatic organelles, especially the larger plastids, to the outside and into the cytoplasm of the vegetative cell. This supposition has been rejected (e.g., Van Went, 1984) because the plastids are already unequally distributed before the mitotic spindle is formed. According to Van Went (1984) the accumulation and clustering of the plastids can be correlated with the changing shape of the nucleus and its movement to an acentral position in the cell and the formation of vacuoles at the poles of the developing microspore of *Impatiens*. However, that does not explain why mitochondria do not (in *Gagea*) or do not strictly (in *Impatiens*) submit to these processes.

Van Went (1984) and Schröder (1985) assumed that the plastid distribution in microspores is mediated by the cytoskeleton and probably controlled by biochemical gradients. This assumption was partly supported by the detection of a three-dimensional network of actin filaments in pollen grain of *Gasteria verrucosa*, in which plastids and other cytoplasmic organelles seem to be embedded (Schröder et. al., 1988). According to Schröder et al. (1988) and Hagemann and Schröder (1989), the actin cytoskeleton is responsible for the intracellular movement or positioning of the plastids and other organelles during microspore division. However, Tanaka (1991) concluded from his studies on plastid distribution during microsporogenesis in *Lilium longiflorum* that actin filaments do not function in the distribution of plastids.

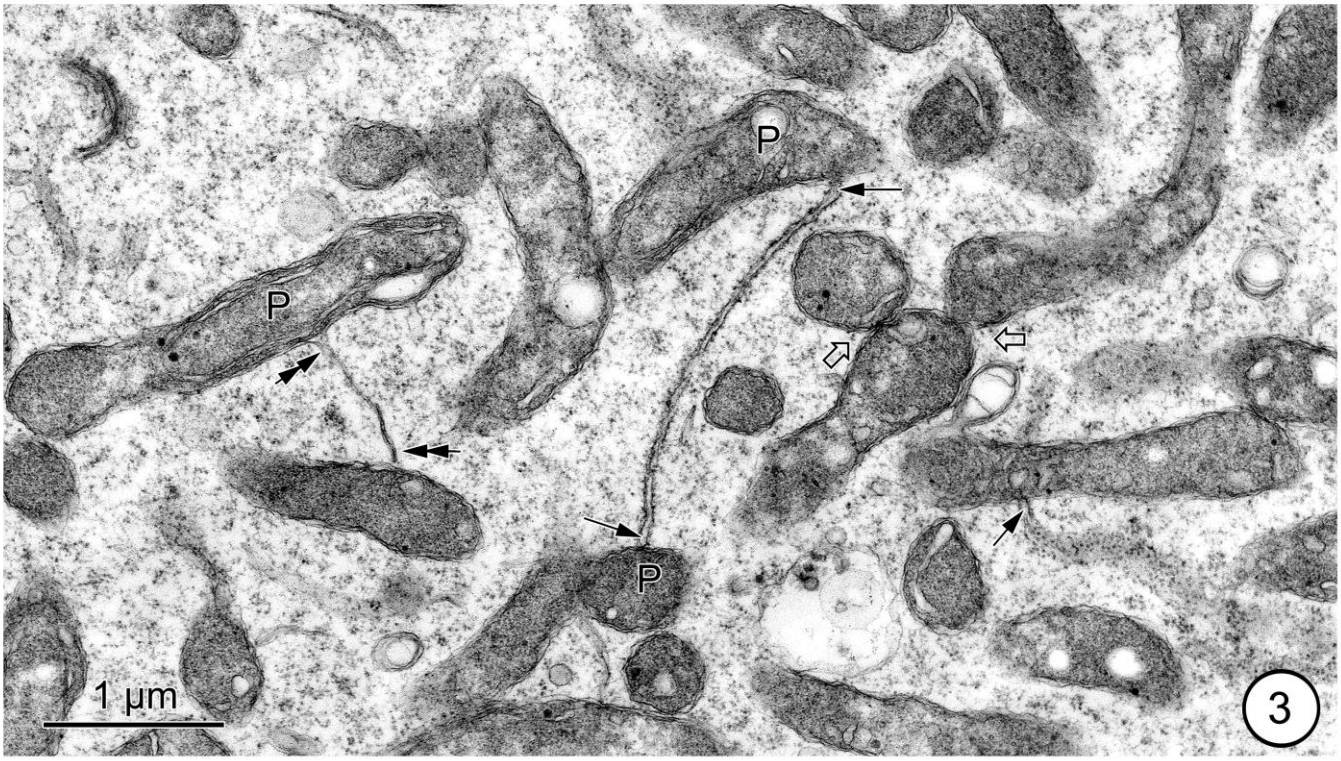
The role of the microtubular cytoskeleton in the ordered arrangement of microspore plastids is also not clear. Hagemann and Schröder (1989) found no correlation between the distribution pattern of microtubules (Van Lammeren et al., 1985) and the clustering of plastids in microspores (Schröder, 1985) during the first haploid mitosis in *Gasteria*, and concluded from this that microtubules are not involved in these processes. On the other hand, microtubules that radiate from the cell nuclei and exclude the plastids from around the nuclei have been observed during microsporogenesis in *Lilium longiflorum* (Tanaka, 1991). Tanaka has assumed that the system of radiating microtubules also controls the distribution of plastids during the subsequent formation of male gametes, which are deficient in plastids in many angiosperms.

The results of our study are not sufficient to define the mechanism causing selective aggregation of plastids at the vegetative pole of the *Gagea lutea* microspore. The microtubules observed between the aggregated plastids of the prophasic microspore are not very common, but their role in this process cannot be ruled out. The overall structure and function of the cytoskeleton in the polarization of *Gagea* microspore will require further immunofluorescence study.

To the best of our knowledge, the long, thin evaginations of the outer membrane of the plastid envelope, by which the distant plastids presumably hold together (or are in contact), has not been reported earlier for microspore plastids. Their relationship with the thin tubular projections emanating from individual plastids and sometimes interconnected with other plastids, which have been recently observed in leaves of transgenic plants (Köhler et al., 1997), remains unknown.

RER profiles clearly joining distant plastids are frequently observed in dividing microspores of *Gagea*; presumably their function is to hold together these plastids at the vegetative pole of the microspore. A structural association of microspore plastids and endoplasmic reticulum cisterns has also been described by Pacini et al. (Pacini and Cresti, 1976; Pacini and Juniper, 1984) for *Lycopersicon peruvianum*, and by Van Went (1984) for *Impatiens*

Fig. 3. Some of the RER profiles are attached (black arrows) to the outer membrane of plastid envelopes and join the distant plastids (P). The envelopes of neighboring plastids are in contact (open arrows). Double arrows show long evaginations of the outer membrane of plastids. **Fig. 4**. Microtubules (arrows) are visible in the plastid aggregation occasionally. **Fig. 5**. Microspore at anaphase. Long endoplasmic reticulum cisterns (arrows) surround the area of the mitotic spindle and separate it from the region containing plastids (P).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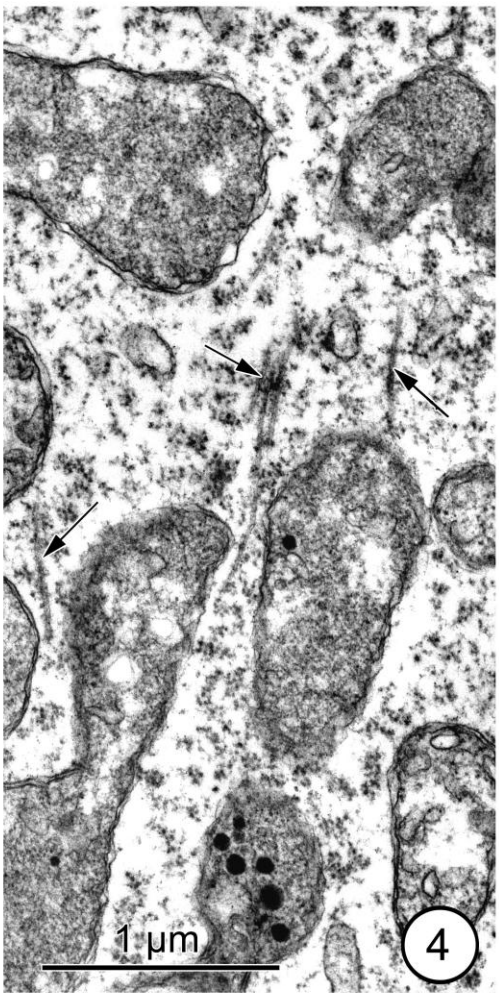
P
P
1 µm
3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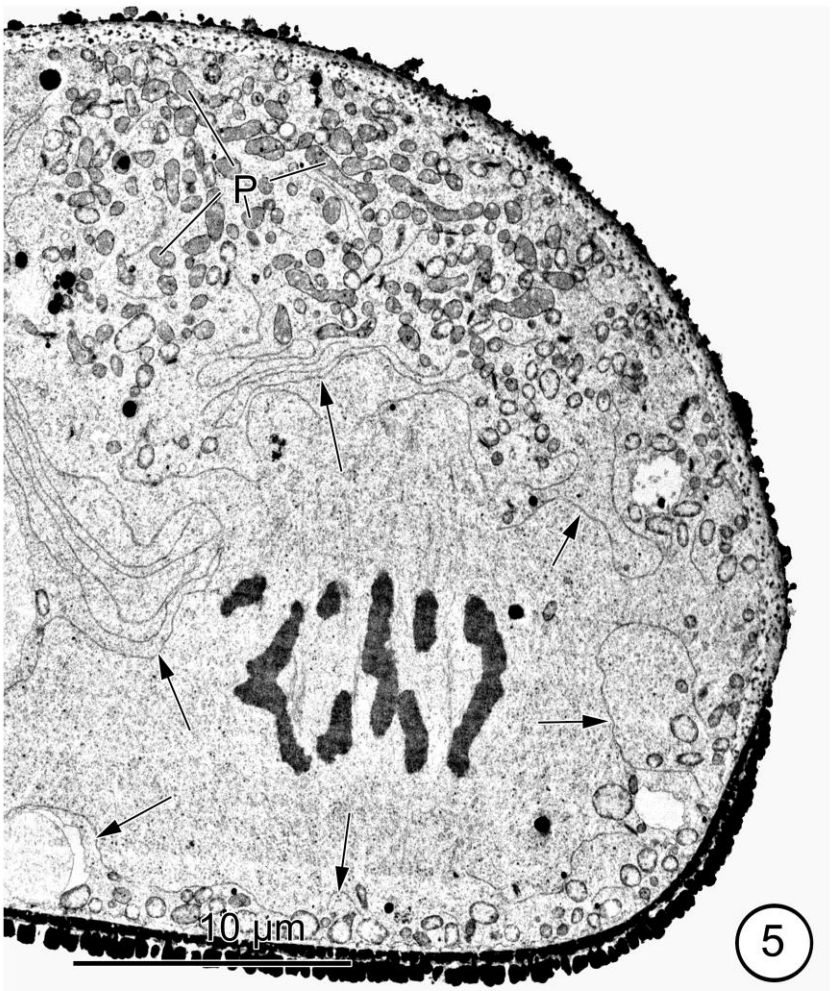
P
10 µm
5

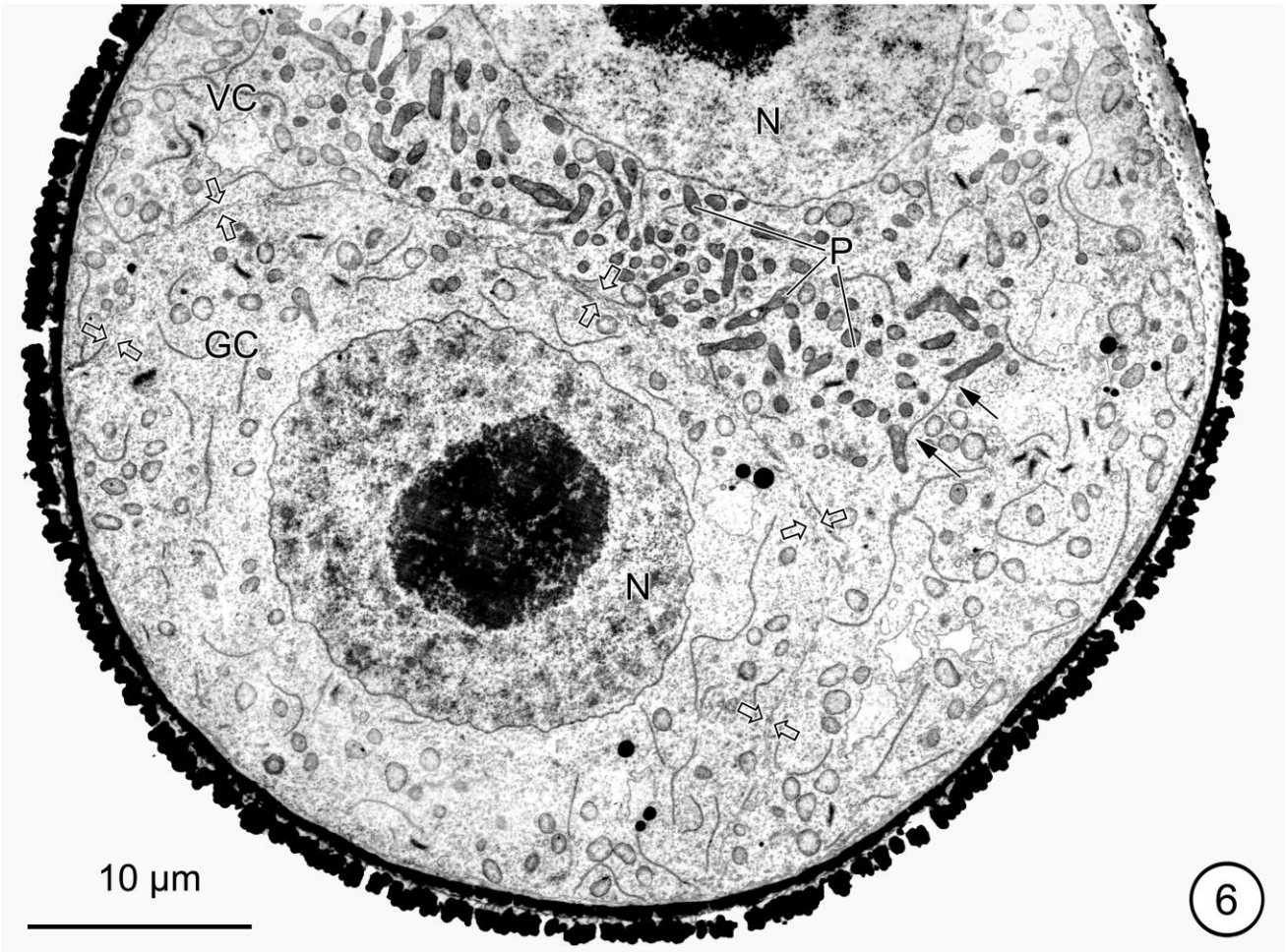

Fig. 6. Young, bicellular pollen grain. Open arrows show the thin wall separating the generative cell (GC) from the vegetative cell (VC). The GC does not contain plastids. Nearly all connections (black arrows) between plastids and RER cisterns have disappeared, and the plastids disperse in the cytoplasm of the whole VC. N – nucleus.

walleriana. However, both the arrangement of ER cisterns (pressed between the clustered plastids) and the assumed function of the associations (exchange of metabolites) differ from the situation observed in *Gagea* microspores. ER is known to be intimately associated with the cytoskeleton in plant cells (for review: Hepler et al., 1990), and may serve to anchor and facilitate the application of force for movement of organelles (Brown and Lemmon, 1991a,b).

The process of selective exclusion of plastids from the generative pole of the microspore in *Lycopersicon*-type angiosperms seems to be a complex phenomenon involving the concerted action of the cytoskeleton and endoplasmic reticulum. Participation of ER in these processes, at least in holding the special arrangement of microspore plastids during microspore division in *Gagea lutea*, seems certain.

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