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Unequal distribution of plastids during generative cell formation in *Impatiens*

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Summary. This paper describes the unequal distribution of plastids in the developing microspores of *Impatiens walleriana* and *Impatiens glandulifera* which leads to the exclusion of plastids from the generative cell. During the development from young microspore to the onset of mitosis a change in the organization of the cytoplasm and distribution of organelles is gradually established. This includes the formation of vacuoles at the poles of the elongate-shaped microspores, the movement of the nucleus to a position near the microspore wall in the central part of the cell, and the accumulation of the plastids to a position near the wall at the opposite side of the cell. In *Impatiens walleriana*, the accumulated plastids are separated from each other by ER cisterns, and some mitochondria are also accumulated. In both *Impatiens* species, the portion of the microspore in which the generative cell will be formed is completely devoid of plastids at the time mitosis starts.

Key words: *Impatiens* – Microspore mitosis – Plastid distribution – Ultrastructure

Introduction

Plastids of higher plants are known to be inherited either by biparental or maternal uniparental means. Hagemann (1981 personal communication) distinguishes four types of plastid inheritance: a) the *Lycopersicon*-type, in which the generative cells lack plastids ab-initio; b) the *Solanum*-type, in which the generative cells contain plastids which are eliminated during subsequent cell development; c) the *Triticum*-type, in which plastids are present in both generative cells and sperm cells, whereas no plastids are transmitted to the zygote; d) the *Pelargonium*-type, in which sperm cell plastids are transmitted during fertilization. Functionally the former three types are the same, resulting in uniparental, maternal inheritance of plastids. Only type four results in biparental plastid inheritance. According to Richter-Landmann (1959), the sperm cells of *Impatiens glandulifera* contain plastids which are transmitted to the zygote. However, Dupuis (1972) showed that in *Impatiens balsamina* the generative cells lack plastids. The same was concluded for the sperm cells of *Impatiens walleriana* (Van Went 1974).

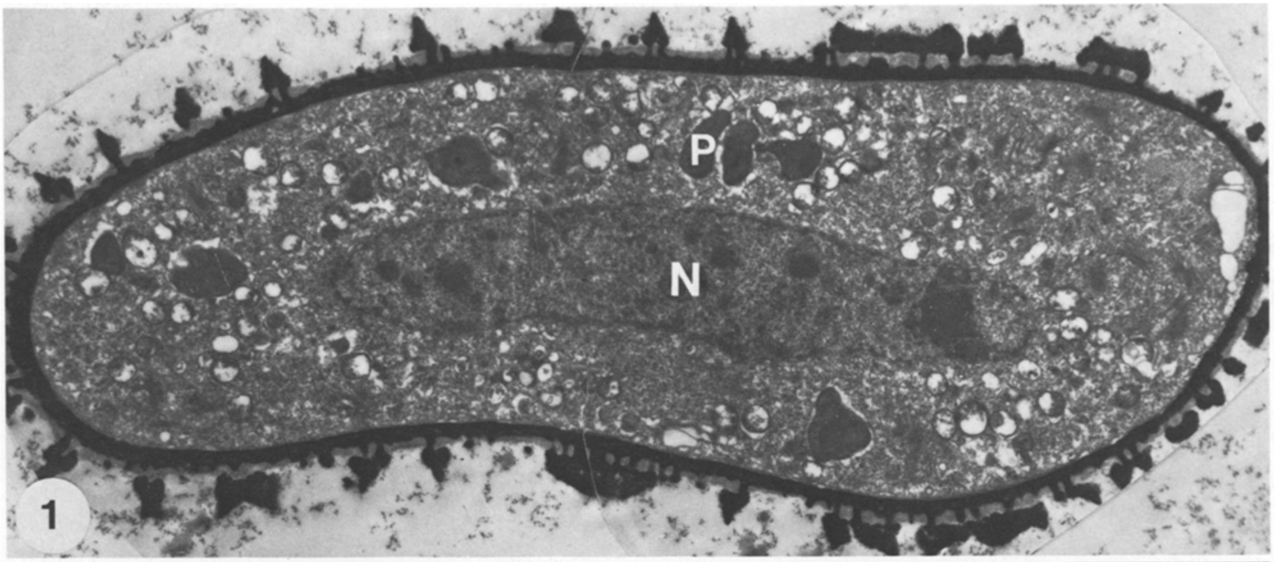
The present ultrastructural study describes the unequal distribution of plastids during generative cell formation in *Impatiens walleriana* and *Impatiens glandulifera* microspores.

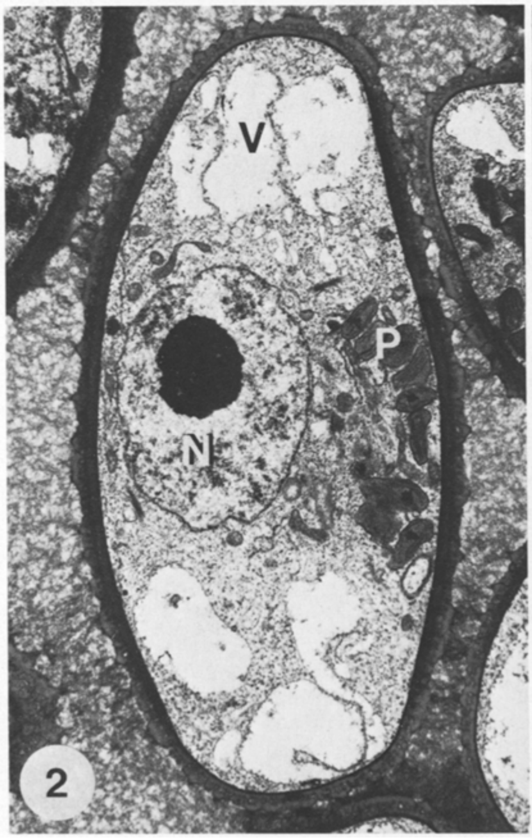
Materials and methods

Plants of *Impatiens walleriana* Hook f. and *Impatiens glandulifera* Royle, grown in the greenhouse, were used. Dissected androecia were fixed for 1 h in 3.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2), and, after rinsing in buffer, postfixed for 1 h in 2% osmium-tetroxide in the same buffer. The whole procedure was carried out at room temperature. Following dehydration in a graded ethanol series, the material was embedded in Epon. Ultra thin sections were poststained with uranyl acetate and lead citrate.

Results

In general the microspores of both *Impatiens walleriana* and *Impatiens glandulifera* show a similar organization and follow the same pattern of development. After their formation and release from the tetrads the micro-





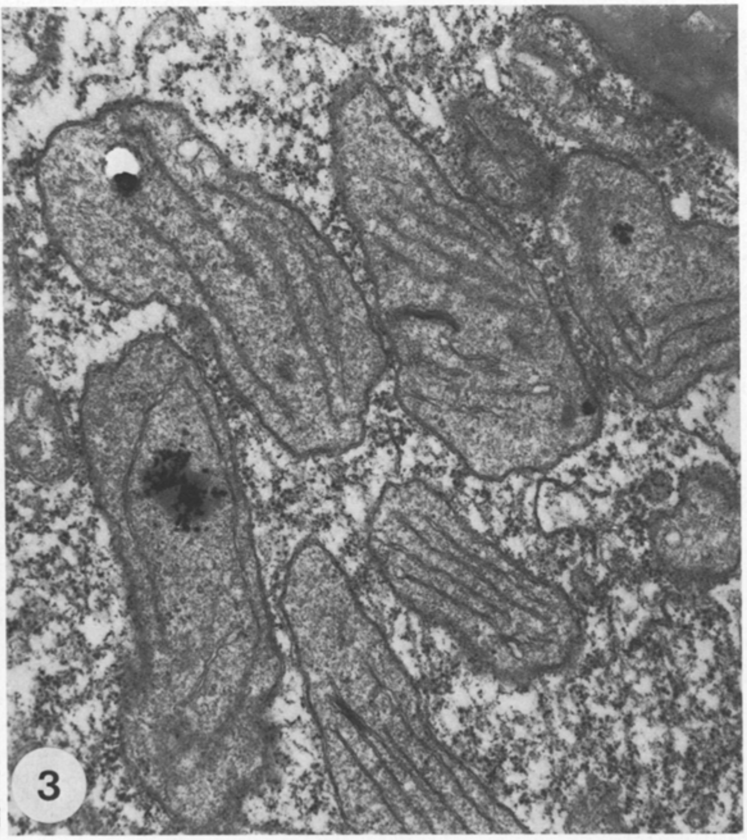
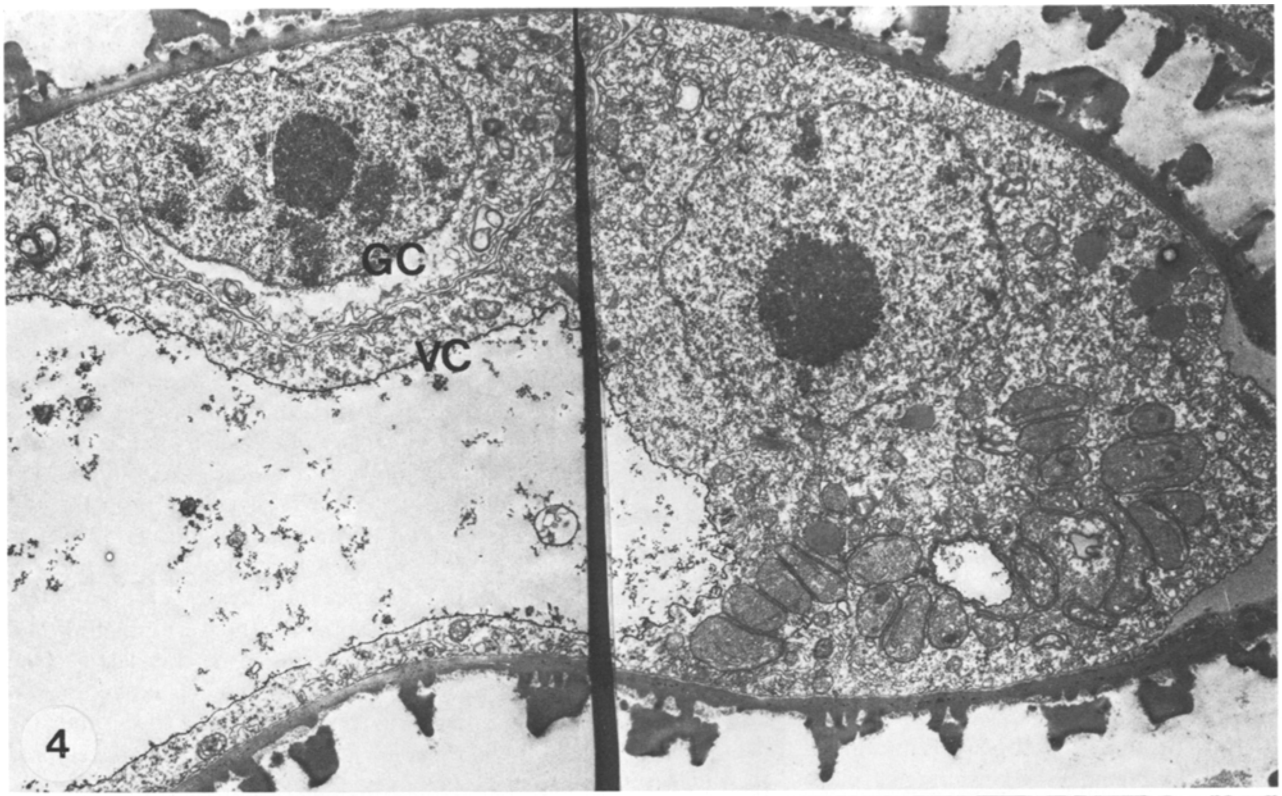


Fig. 1. Microspore of *Impatiens walleriana,* shortly after release from the tetrad, showing elongated shape of nucleus (*N*), and distribution of plastids (*P*). ×9,000

Fig. 2. Vacuolated microspore of *Impatiens glandulifera,* shortly before mitosis, showing polarity in cell organization. Vacuoles (*V*) are located in the peripheral parts of the cell. The nucleus (*N*) is situated along the wall in the cell centre, and the plastids (*P*) are accumulated along the opposite part of the cell wall. ×3,600

Fig. 3. Clustering of plastids in the cytoplasm of *Impatiens glandulifera* microspore, before mitosis. ×29,000



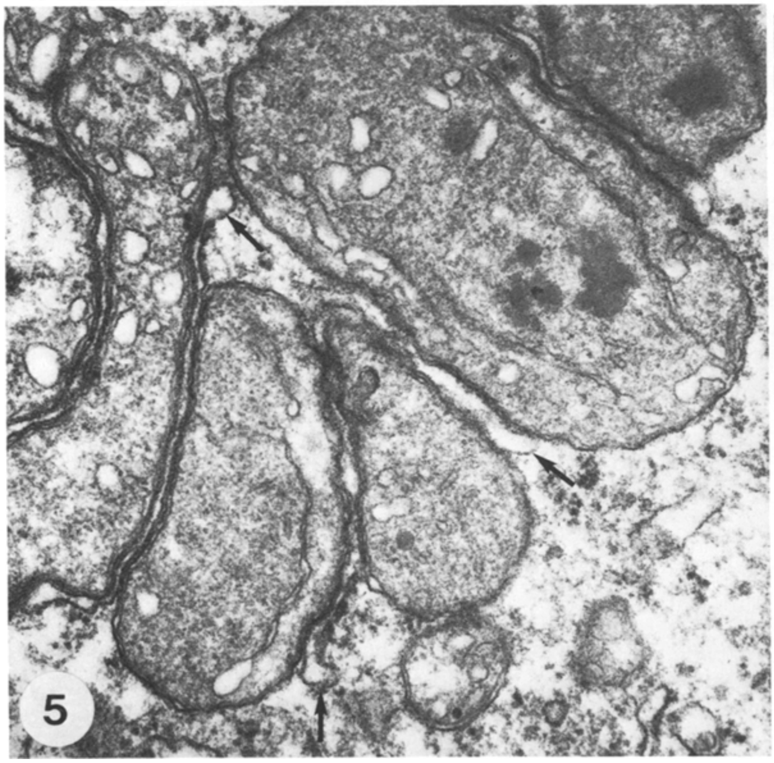


Fig. 4. *Impatiens walleriana* pollen after completion of mitosis. The generative cell (*GC*) does not contain plastids. In the vegetative cell (*VC*) the plastids are still clustered in large aggregates. × 10,500

Fig. 5. Enlarged portion of plastid cluster in *Impatiens walleriana* microspore. Arrows indicate the presence and position of ER cisterns. × 40,000

Fig. 6. Cluster of mitochondria in the cytoplasm of *Impatiens walleriana* microspore. × 40,000

spores have an elongated shape, and contain only small vacuoles. The nuclei also are elongated in shape and located in the centre of the cells. All cell organelles appear to be randomly distributed (Fig. 1).

Next in microspore development is the formation of large vacuoles at the cell poles. Simultaneously, the cell nucleus becomes spherical in shape and takes a position near the wall in the centre of the cell where the future generative cell will be formed.

At the same time the plastids also become located in the cell centre, taking a position opposite to the future site of cell division (Fig. 2). Here they become closely crowded together, forming large clusters (Fig. 3). In *Impatiens walleriana* some of the mitochondria also become clustered at the same place, forming complex aggregates (Fig. 6). Most of the mitochondria, however, remain randomly distributed in the cytoplasm. In *Impatiens glandulifera,* clustering of mitochondria does not occur at all. In *Impatiens walleriana,* ER cisterns are frequently observed in between the clustered plastids (Fig. 5). The ER cisterns run parallel to the plastid surface and separate the plastids from each other. In *Impatiens glandulifera* such a structural relationship of clustered plastids and ER cisterns is not present. In general, the clustering of plastids in *Impatiens glandulifera* is not as tight and absolute as in *Impatiens walleriana.* In both species, however, no plastids are present in the cell region where the generative cell will be formed. In both species microspore mitosis results in a large vacuolated vegetative cell and a small lens-like generative cell which is attached to the microspore wall. The generative cell contains every type of organelle except plastids. All plastids are located in the vegetative cell (Fig. 4).

In both species the degree of plastid clustering in the vegetative cell gradually diminishes after completion of mitosis. At the time that the vacuoles are disappearing and starch formation begins, the plastids are randomly distributed.

Discussion

The results of this study clearly show that the generative cells of both *Impatiens walleriana* and *Impatiens glandulifera* lack plastids ab-initio. Therefore, using the criteria of Hagemann (1981), both species belong to the *Lycopersicon*-type with respect to plastid inheritance.

Our results are in contrast to the observations of Richter-Landmann (1959) who described the presence of plastids in the generative cells and sperm cells of *Impatiens glandulifera.* Since Dupuis (1972) reported the absence of plastids in the generative cells of *Impatiens balsamina* also, it is likely that uniparental maternal plastid inheritance according to the *Lycopersicon*-type is common for the genus *Impatiens.* The absence of plastids in the generative cell ab-initio has been reported for various other species: *Epidendrum scutella* (Cocucci and Jensen 1969), *Haemanthus katherinae* (Sanger and Jackson 1971), *Tillandsia caput-medusa* (Brighigna et al. 1981), *Beta* (Hoefert 1969), *Hordeum* (Cass 1973), *Parkinsonia* (Larson 1965), *Secale* (Karas and Cass 1976), *Endymion non-scriptus* (Burgess 1970), *Heleocharis* (Carniel 1972), and *Prunus avius* (Cresti et al. 1979). However, the cellular organization and processes leading to this absence of plastids in the generative cell remain unclear.

Hagemann (1981) postulated that plastid exclusion from the generative cell may partly have a mechanical reason. The mitotic spindle may mechanically push the plasmatic organelles, especially the larger plastids, to the outside, and thereby into the cytoplasm of the vegetative cell. In *Impatiens walleriana* and *Impatiens glandulifera,* it is obvious that the exclusion of the plastids from the generative cell results from the unequal distribution of plastids during mitosis. However, this unequal distribution is not caused by any activity of the mitotic spindle since it is already achieved before the mitotic spindle is formed. The accumulation and clustering of the plastids seems to be correlated to the changing shape of the nucleus and the latters' movement to an acentral position in the cell, and the formation of vacuoles at the poles of the developing microspore.

At the time mitosis begins, the plastids are positioned in the cytoplasm of the future vegetative cell far away from the site of cytokinesis. Although the general pattern of plastid clustering in both *Impatiens* species appears to be the same, there are also some differences. In *Impatiens glandulifera* only the plastids exhibit clustering, whereas in *Impatiens walleriana* the plastids and many ER cisterns cluster, as do some of the mitochondria. A structural association of plastids and ER cisterns has also been described by Pacini and Cresti (1976) for the microspore of *Lycopersicon peruvianum* during its development until the completion of microspore mitosis. Pacini and Cresti suppose that the plastid-ER association has something to do with the rapid transfer of materials for the formation of the cell wall, especially nexine development. It is possible that in *Impatiens walleriana,* the association of plastids and ER cisterns also have a specific metabolic function. On the other hand, the phenomena in *Impatiens walleriana* indicate that clustering is not organelle specific, or based on a specific plastid character, since mitochondria are also accumulated. The clustering of plastids may involve the activity of a more general cell organizing principle, responsible for the localization of all cell constituents and the site of cytokinesis as well. Possibly the cytoskeleton, consisting of cytoplasmic microtubules, may be the basis for this cell organizing principle.

Work is now being directed to establish the organization of the cytoskeleton and the possible role of cytoplasmic microtubules in relation to the unequal distribution of plastids in the dividing microspore.

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