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Ultrastructural studies on plastids of generative and vegetative cells in *Liliaceae*

5. The behaviour of plastids during pollen development in *Chlorophytum comosum* (Thunb.) Jacques

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Summary. The behaviour of plastids and mitochondria during the formation and development of the male gametophyte of *Chlorophytum comosum* has been investigated using electron microscopy. During first pollen mitosis an intracellular polarization of plastids occurs in that the plastids are clustered in the centre of the microspore. The originating generative cell normally lacks plastids. Only in a small number of microspores have plastids been observed near the dividing nucleus of the microspore and later on in the generative cell. These observations agree with the genetic investigations of Collins (1922) on the mode of plastid inheritance which demonstrated a small amount of biparental plastid inheritance in *Chlorophytum*. The cytological mechanisms underlying plastid polarization during the first pollen mitosis are discussed.

Key words: *Chlorophytum comosum* – First pollen mitosis – Male plastid inheritance – Ultrastructure

Introduction

This paper is part of a continuing study of the plastid content of generative and vegetative cells in *Liliaceae* (Schröder 1984, 1985a, b, 1986; Schröder and Hagemann 1985). Using cytological data collected on plastid behaviour during pollen development in *Chlorophytum comosum* and other *Liliaceae*, we discuss the cytological mechanism underlying the maternal inheritance of plastids in angiosperms (for review, see Hagemann 1979, 1983).

Materials and methods

Plants of *Chlorophytum comosum* were cultivated in a greenhouse under natural conditions. The cytological stage of anthers was determined by aceto-carmine staining of one anther per flower; the remaining five anthers were prepared for electron microscopic studies as previously described (Schröder 1985a).

Results

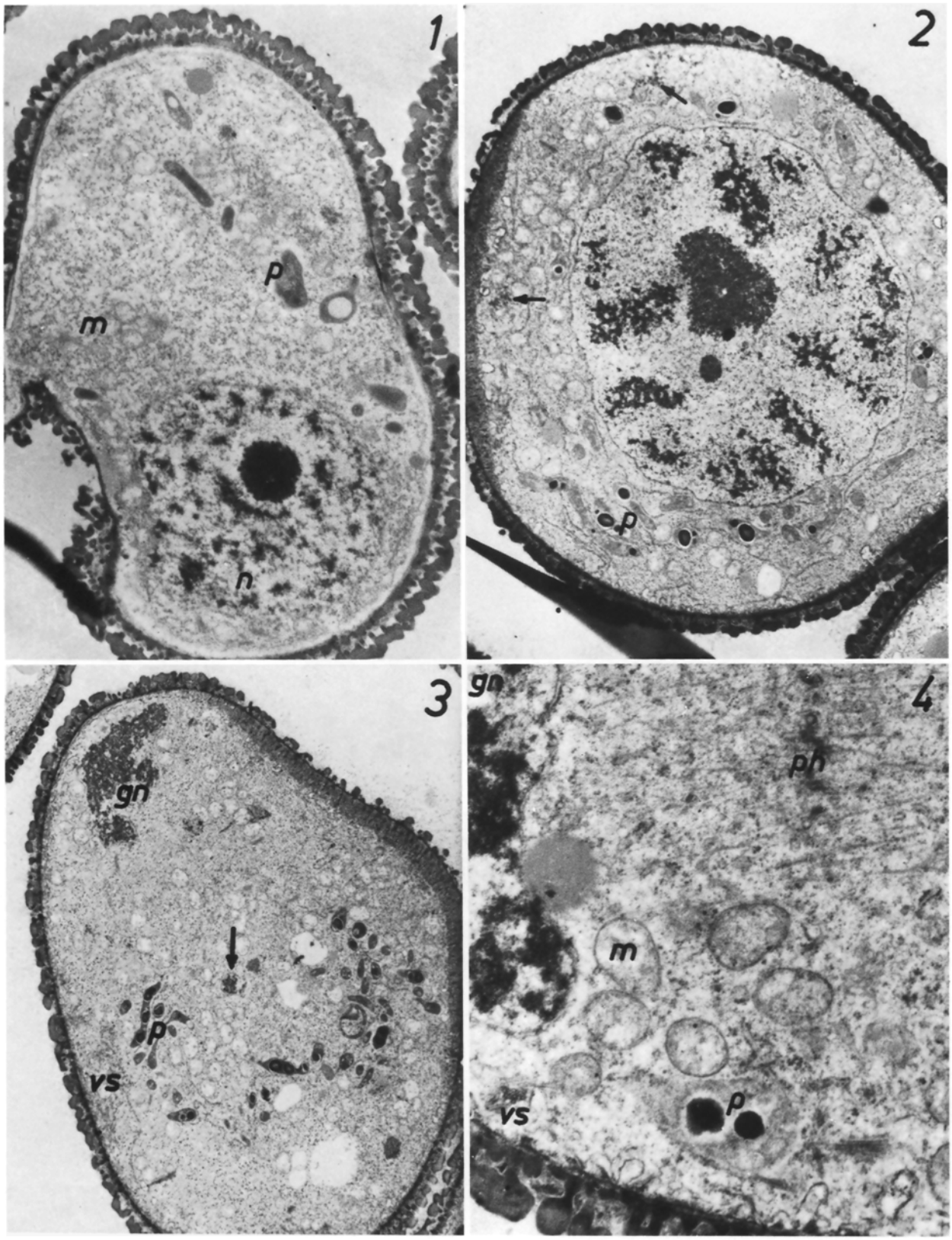
The microspores of *Chlorophytum comosum* contain a complete set of cytoplasmic organelles (Fig. 1). The nucleus of the microspore is located in a peripheral position at the distal pole of the microspore (on the

Fig. 1. Mature microspore stage. The nucleus (n) is located in a peripheral position. Plastids (p); mitochondria (m). (×5,940)

Fig. 2. Peripheral section of a microspore at the the early prophase stage of the first pollen mitosis. The plastids (p) contain small starch grains. Virus-like structures (*arrows*). (×4,320)

Fig. 3. Late telophase stage of the first pollen mitosis. The irregularly-shaped plastids (p) contain starch and show a pronounced polarization in the centre of the microspore. Generative nucleus (gn); part of the vegetative nucleus (*arrow*); virus-like structures (vs). (×4,140)

Fig. 4. Late cytokinesis stage. Single plastid (p) is exceptionally located in the region of the phragmoplast (ph). Generative nucleus (gn); mitochondria (m); virus-like structures (vs). (×20,400)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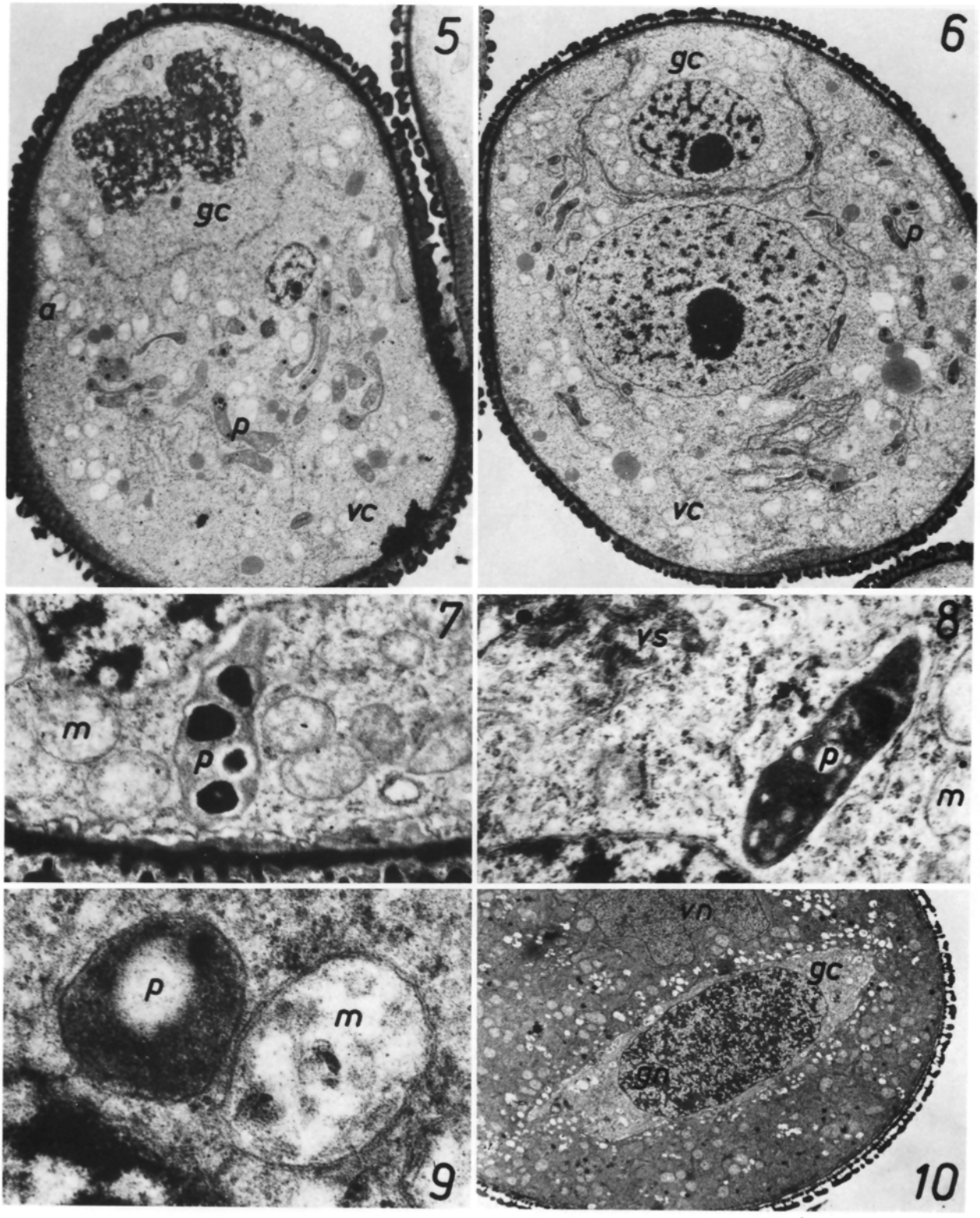
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opposite side of the aperture). The plastids are irregularly shaped (Fig. 1), contain only single tubular membranes within their electron-opaque stroma. Just before the first pollen mitosis the plastids contain small starch grains (Fig. 2; Dickinson and Wilson 1983). Up to this developmental stage, all of cytoplasmic organelles appear to be randomly distributed within the cytoplasm of the microspore.

The first pollen mitosis

The dividing nucleus is located in a peripheral position at the distal pole of the microspore (Fig. 3). Serial sections made of microspores at the telophase stage (Fig. 3) show that the plastids are located mainly in the centre of the microspore although in some cases they are also located at the distal pole (Fig. 4). Cisterns of the endoplasmic reticulum are also clustered in the centre of the microspore.

The large vacuole of the microspore is located at the proximal pole. The mitochondria appear to be randomly distributed in the microspore cytoplasm (Fig. 3). At cytokinesis the polarization of the cytoplasmic organelles appears to be stronger than that seen in earlier mitotic stages. The starch grains of plastids are electron-opaque (Figs. 2 and 3), and seem to be comparable with the starch in plastids of maturing microspores in *Lilium* (cf. Dickinson and Wilson 1983). Occasionally plastids have also been observed in the region of the phragmoplast (Fig. 4). In contrast to the observations reported by Vaughn et al. (1981), degenerating mitochondria were not been observed in the microspores of *Chlorophytum*.

The cytoplasm of microspores in the investigated *Chlorophytum comosum* plants contains a large amount of virus-like structures. The single virus-like structure is tubular (with a diameter of 30 to 35 nm), and forms aggregates. These aggregates could be observed at all developmental stages of the microspores and pollen grains. They are preferentially visible in the peripheral area of the cytoplasm as well as in the region of the growing cell wall at the time of cytokinesis (Figs. 3, 4, 5).

The two-cellular pollen grain

The cytoplasm of the young (wall-attached) generative cell regularly contains mitochondria, endoplasmic reticulum, dictyosomes, ribosomes, lipid droplets and vacuoles. Plastids have only been observed in a small percentage of generative cells (Figs. 7 and 8). The examination of ultrathin sections of 99 different generative cells (6 to 10 sections per cell) led to the detection of 6 generative cells containing a single plastid (per section) and one generative cell containing two plastids (per section).

During the maturation of the generative cell, the starch grains of plastids exceptionally transmitted to the generative cell disappear (Figs. 7 and 8). Just before the detachment of the generative cell from the pollen wall the plastids have an electron-opaque stroma (Fig. 8). We could not find signs of degeneration of plastids within generative cells.

In contrast to the generative cell, the vegetative cell contains a normal set of cytoplasmic organelles (Fig. 6). The plastids (2.5 μm in their greatest length) have an electron-dense stroma containing several starch grains and tubule-shaped thylakoids. At the young generative cell stage the plastids of the vegetative cell are irregularly shaped and are associated with cisterns of the rough endoplasmic reticulum (Fig. 6). Until the mature generative cell stage the plastids become more spherical. However, the association between plastids and endoplasmic reticulum disappears during further pollen maturation and, simultaneously, the starch grains of plastids disappear. The number of mitochondria seems to increase during the development of the two-cellular pollen grain. The mitochondria (approximately 1 μm in diameter) have an electron-transparent matrix containing well-developed cristae. Degenerating mitochondria have not been observed within the vegetative cytoplasm during the development of the two-cellular pollen grain. The mature generative cell is spindle-shaped and surrounded by the cytoplasm of the vegetative cell (Fig. 10). Normally, the mature generative cell does not contain plastids. Plastids could only be observed in a very low percentage of generative cells as

Fig. 5. Cytokinesis stage immediately before the completion of the cell wall between the generative cell (gc) and the vegetative cell (vc). The originating generative cell does not contain plastids. The vegetative cell contains plastids (p) with starch inclusions. The plastids are associated with cisterns of the endoplasmic reticulum. Aperture (a). (×4,860)

Fig. 6. Two-cellular pollen grain just after the first pollen mitosis. The generative cell (gc) is wall-attached, and does not contain plastids. The cytoplasmic organelles of the vegetative cell (vc) appear to be randomly distributed. Plastids (p). (×3,780)

Fig. 7. Plastid (p) with starch grains and mitochondrion (m) within the cytoplasm of a young generative cell. (×13,000)

Fig. 8. Part of a wall-attached generative cell of a later developmental stage as shown in **Fig. 7.** The plastid (p) does not contain starch, and has an electron-opaque stroma. Mitochondria (m); virus-like structures (vs). (×34,000)

Fig. 9. Part of a mature generative cell. The plastid (p) has an electron-opaque stroma. Mitochondrion (m). (×81,600)

Fig. 10. Two-cellular pollen grain at the mature generative cell stage. The generative cell (gc) is spindle-shaped, and does not normally contain plastids. The vegetative nucleus (vn) is irregularly shaped and shows a lower degree of chromatin condensation than the generative nucleus (gn). (×3,300)

already described. These plastids have a simple structure (Fig. 9): they do not contain starch and are obviously intact organelles. The mitochondria of the mature generative cell as well as the mitochondria of the vegetative cell do not show any sign of degeneration (Fig. 9).

Discussion

The majority of angiosperms shows a purely maternal inheritance of plastids (Hagemann 1964; Kirk and Tilney-Bassett 1978). Three different cytological mechanisms have been revealed, which cause this behaviour: 1. plastid polarization during the first pollen mitosis (Van Went 1984; Schröder 1985a) causes the formation of generative cells without plastids ab-initio (*Lycopersicon* type, Hagemann 1983); 2. plastid degeneration during the maturation of the generative cell (Clauhs and Grun 1977; Schröder 1985b, 1986) results in the lack of plastids within mature generative cells (*Solanum* type, Hagemann 1983); 3. plastid exclusion during the fertilization process (Mogensen 1982) prevents the male plastids from being transmitted into the egg cell via sperm cells (*Triticum* type, Hagemann 1983).

Chlorophytum comosum is a species of the *Lycopersicon* type. Our ultrastructural observations demonstrate that the plastids are clustered in the centre of the microspore at the first pollen mitosis. Therefore, plastids are *not* regularly transmitted into the generative cells. However, we could observe exceptional single plastids in generative cells. These findings are in accordance with genetic investigations on the mode of plastid inheritance in *Chlorophytum* (Collins 1922) demonstrating a small amount of biparental plastid inheritance in this species.

The intracellular arrangement of plastids (and other cell organelles) is mediated by the cytoskeleton (Menzel 1985) and probably controlled by intracellular gradients (Schröder 1985a). In *Chlorophytum* the plastid polarization during the first pollen mitosis might be disturbed by the virus-like structures within the pollen grains in that they influence the exact action of the intracellular gradients responsible for the plastid polarization. Reynolds (1984) also reported a change in the mode of plastid distribution during the first pollen mitosis in *Hyoscyamus* caused by anther culture. We do not consider the presence of plastids in some generative cells in *Chlorophytum* as a normal event. In *Fritillaria* (Schröder 1985b) and *Convallaria* (Schröder 1986) plastids are also transmitted into the generative cell, but in contrast to *Chlorophytum*, the plastids of these species degenerate during the maturation of the generative cell to anthesis. Vaughn et al. (1981) reported on the organelle transmission in *Chlorophytum* and concluded that the mitochondria of the microspore degenerate. Our own observations do not confirm this report. In *Chlorophytum* the generative cell as well as the vegetative cell contain intact mitochondria during the whole pollen development.

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