Unequal plastid distribution during the development of the male gametophyte of angiosperms

R. HAGEMANN

Department of Genetics, Section Biosciences, Martin-Luther-University,
DDR-402 Halle/s., Domplatz 1, GDR

Abstract

The difference between the uniparental maternal and biparental type of plastid inheritance is based upon a relatively simple mechanism. In the uniparental type plastids are excluded from the generative or sperm cells during the first pollen mitosis or during pollen development. In some angiosperms this exclusion is completely lacking or carried out partially.

In recent years the study of plastid inheritance in higher plants has given a new impetus for studies on gametogenesis and embryogenesis from a cytogenetic point of view. For decades it has been well known to geneticists that angiosperms form two groups regarding the inheritance of plastids: (a) In the majority of species there is a uniparental, purely maternal inheritance of genetic plastid characters, e.g. in *Antirrhinum*, *Beta*, *Hordeum*, *Mirabilis*, *Nicotiana*, *Petunia*, *Zea* and many others. (b) In contrast, in a minority of species biparental inheritance of genetic plastid differences is found; the best studied genera of this type are *Pelargonium*, *Oenothera* and *Hypericum* (Hagemann, 1964, 1965).

These genetic results led our research group in the Department of Genetics of the University in Halle to electron microscopical studies of male gametogenesis and embryogenesis of higher plant species, because the reason for these differences in the genetic behaviour of these species can only be found in electron microscopical studies of pollen development, fertilization process, zygote formation and embryogenesis.

On the female side no striking differences have been reported regarding the content of plasmatic organelles in the egg cells of angiosperms. In all angiospermous species studied so far, the egg cell of the embryo sac contains many plastids and mitochondria.

In contrast, the situation on the male side is much more complicated, and interesting differences have been found between different taxa. The first pollen mitosis, which leads to the formation of a gene-

rative and a vegetative cell, is a characteristic and very peculiar process. The mitotic spindle is oriented at right angles to the wall. After mitosis the daughter nucleus on the wallward side is always the generative nucleus, and that on the inside of the microspore is the vegetative nucleus. The following cell division leads to a hemispherical generative cell, attached against the intine of the microspore wall. Later on the generative cell is abstricted from the wall. As a spindle-shaped cell it moves into the vegetative cell. During the second pollen mitosis, the generative cell divides into two sperm cells, which after pollen tube growth are discharged into the embryo sac in the process of fertilization.

In all angiosperms the microspores, formed by meiosis, contain numerous plastids, mitochondria and other plasmatic organelles. Also the vegetative cell of the pollen always contains many plastids and mitochondria. The presence of plastids has convincingly been demonstrated in many electron microscopical studies, and was also proved by anther culture for several taxa (Heslop-Harrison, 1972; Nilsson-Tilgren, v. Wettstein-Knowles, 1970). However, the generative cells of different taxa significantly differ with regard to the content of plastids.

Our investigations lead to the conclusion that the first pollen mitosis of angiosperms is a very important, decisive event with regard to plastid distribution. We found this result in accordance with the observation of many other investigators. In angiosperms there are three possibilities for plastid distribution during the first pollen mitosis and further pollen development.

TYPE 1 — LYCOPERSICON TYPE

In many species pollen mitosis is extremely unequal for the plastids. All plastids of the microspore are distributed into the cytoplasm of the vegetative cell, and the generative cell does not get any plastids. Careful electron microscopical investigations have revealed that the generative cells of many species do not contain any plastids. We found this situation in *Antirrhinum majus* and *Lycopersicon esculentum* (Fig. 1); other examples are *Gossypium*, *Hordeum*, *Zea*, *Capsella*, *Mirabilis*, *Petunia*, and many others (Table 1). Plastid exclusion from the generative cell has the consequence that the two sperm cells do not contain plastids either and therefore cannot transmit plastids during fertilization into the egg cell.

The uniparental, purely maternal inheritance of the plastids in these species is the direct consequence of the exclusion of plastids from the generative cell during the first pollen mitosis.

Table 1
Plastid content in male gametophytes and gametes of angiosperms

Object	Veg. cell	Gen. cell	Sperm cell	Gross	Authors of el. micr. stud.*
Type 1					
<i>Gossypium hirsutum</i>	P+	P—	P—	P—	Jensen, 1972
<i>Hordeum vulgare</i>	P+	P—	P—	P—	Cass, Karas, 1975
<i>Beta vulgaris</i>	P+	P—	P—	P—	Hoefert, 1969
<i>Antirrhinum majus</i>	P+	P—	P	P—	Knoth (Halle lab.)
<i>Capsella bursa pastoris</i>	P+	P—			Schulz, Jensen, 1968
<i>Castilleja foliosa</i>	P+	P—			Jensen et al., 1974
<i>Lycopersicon esculentum</i>	P+	P—		P—	Krahnert (Halle lab.)
<i>Mirabilis jalapa</i>	P+	P—		P—	Lombardo, Gerola, 1968
<i>Petunia hybrida</i>	P+	P—		P—	Sassen, 1964; van Went, 1970
<i>Triticum aestivum</i>	P+	P—		P—	Knoth (Halle lab.)
<i>Zea mays</i>	P+	P—		P—	Larson, 1965
Type 2					
<i>Solanum chacoense</i>	P+	P+	P—		Clauhs Grun, 1977
<i>Solanum tuberosum</i>		young			
<i>Hyoscyamus niger</i>	P+	P+	P—		Knoth (Halle lab.)
		young			
Type 3					
<i>Pelargonium zonale</i>	P+	P+	P+	P+	Khera, 1975; Knoth (Halle lab.); Lombardo, Gerola, 1968
<i>Oenothera hookeri</i>	P+	P+	P+		Diers, 1963
<i>Oenothera erythrosepala</i>				P+	Meyer, Stubbe, 1974
<i>Castilleja wightii</i>	P+	P+	P+		Jensen et al., 1974
<i>Linum usitatissimum</i>	P+	P+	P+		Vazart, 1970
<i>Fritillaria imperialis</i>	P+	P+			Bopp-Hassenkamp, 1960
<i>Secale cereale</i>	P+	P+			Laub (Halle, lab.)

* All references are given in: Hagemann, 1964, 1976, 1979

TYPE 2 — SOLANUM TYPE

In *Solanum tuberosum*, *S. chacoense* (Clauhs, Grun, 1977) and *Hyoscyamus niger* the first pollen mitosis is not so extremely unequal. Most plastids are distributed into the vegetative cell, but the generative cell also gets some plastids. Therefore young generative cells contain some plastids. But in the course of further pollen development the plastids are lost (or eliminated) from the generative cell. Thus the sperm cells no longer contain plastids. In this type, the absence of plastids in sperm cells is due to the elimination of these organelles during pollen maturation. Thus Type 1 and Type 2 have the same genetic effects: no transmission of paternal plastids into the zygote. But the cytological mechanisms are different.

Fig. 1. Pollen of *Lycopersicon esculentum*

The generative cell contains no plastids, but some mitochondria and dictyosomes. In the vegetative cell there are many plastids with starch grains. Potassium permanganate. The bar is 1 μm

Electron micrograph: S. Krahnert, Dept. Genetics Halle

Fig. 2. Pollen of *Pelargonium zonale*

The generative cell contains many electron-dense plastids, sometimes with small starch grains. Glutaraldehyde. The bar is 1 μm

Electron micrograph: R. Knoth, Dept. Genetics Halle

TYPE 3 — PELARGONIUM TYPE

In striking contrast to the processes of formation of generative and sperm cells in Types 1 and 2 is pollen development in the third group of species. In the genera *Pelargonium* and *Oenothera* and in some other species during the first pollen mitosis thus obviously is an equal distribution of plastids to the generative and the vegetative cell. Our investigations of the pollen development showed that the generative and sperm cells of *Pelargonium* contain numerous plastids (Fig. 2). The plastids in the generative cells are smaller than those in the vegetative cells; they are frequently without starch grains, but sometimes have small starch grains (and mitochondria). The plastids in the vegetative cell are amyloplasts full of big starch grains. Interestingly the plastids in the generative cells are electron-dense and contain accumulations of phyto-ferritin (Knoth et al., 1979). The sperm cells of *Pelargonium* contain plastids, too. Their regular transmission into the egg cell has been proved by the demonstration of paternal plastids in developing embryos (Khera, 1975; Knoth, personal communication). Moreover, it has been well known for a long time from genetic experiments that in *Pelargonium zonale* transmission of paternal (green, yellow or white) plastids into the egg cell takes place regularly (Hagemann, 1964, 1965; Sager, 1973; Gillham, 1978; Kirk, Tilney-Bassett, 1978). My coworker R. Laub has recently shown that *Secale cereale* also belongs to Type 3, i.e. it contains plastids in the generative cell. This is in contrast to the behavior of other *Gramineae*, like wheat and barley, which seem to belong to Type 1, as does maize.

Summarizing these findings we come to the conclusion that the difference between the uniparental maternal and the biparental type of plastids inheritance is based upon a cytologically relatively simple mechanism: the exclusion of plastids from the generative or sperm cells during the first pollen mitosis or during pollen development in most species of angiosperms (Type 1 and 2), and the absence of this exclusion mechanism in a small group of species (Type 3).

In this connection one point should be mentioned: almost all biological processes can be disturbed occasionally. It is therefore not surprising that the strict exclusion of plastids from the generative and sperm cells resp. does not always work with a 100% efficiency. I expect that the Types 1 and 2 are not always absolutely separated. And moreover, some plastids may in exceptional cases get into the generative cell and even the sperm cells. This is obviously the reason that three genetic experiments with *Antirrhinum majus* (which normally belongs to Type 1) have demonstrated the exceptional transmission of some paternal plastids into the egg cell with such a low frequency as 0.03% of hybrid seedlings (Diers, 1971). However, such exceptional cases cannot disturb the general pattern of plastid distribution described.

The findings reported so far lead to three more general aspects.

(1) How usual or unusual is unequal plastid distribution in higher plants?

Studies of the development of the female gametophyte of *Epilobium palustre* (Bednara, Rodkiewicz, 1974) and of orchids (Corti, Cecchi, 1970) have shown that after meiosis the haploid cell, which later develops into the embryo sac, contains the majority of plastids, whereas the other 3 haploid cells, that degenerate later, have almost no plastids. In contrast, in some gymnosperms, e.g. *Cryptomeria japonica*, the active megaspore gets only of a few plastids, whereas the spermatogenic and sperm cells contain many of them. This causes a distinct paternal bias of plastid inheritance in these gymnosperms (Ohba et al., 1971). Thus, in general, unequal plastid distribution during cell division in ontogenetic development is not as seldom as is usually thought.

(2) Do green angiosperms contain cells without plastids?

For a long time it was considered a truism that every cell of a green higher plant contains plastids. But the careful study of male gametophytes has shown that the generative and the sperm cell of many angiospermous taxa regularly do not contain plastids, and thus there is no transmission of paternal plastids into the next generation. Therefore one should not hold too conservative viewpoints (Butterfass, 1979). But many more cytological investigations are necessary to work out the details of the exclusion of plastids from the generative and sperm cells of many angiosperm taxa.

(3) By what cytological/physiological means are the plastids excluded from the generative and sperm cells?

The plastid exclusion from the generative cell may partly have a mechanical reason. During the first pollen mitosis the wallward pole of the spindle is truncated and the spindle fibers seem to be almost parallel. The generative nucleus, moving toward the wall, enters a cytoplasm,

largely derived from spindle material. The mitotic spindle may mechanically push the plasmatic organelles, especially the large plastids, to the outside and thus into the cytoplasm of the vegetative cell. On the other hand, an efficient physiological gradient may cause the unequal plastid distribution. In *Equisetum* spores and *Fucus* zygotes illumination from one side builds up a gradient for plastid distribution. The plastids move to the light side. Spore division leads to a rhizoid cell (at the averted side) with no (or almost no) plastids, whereas the other daughter cell at the light side has received almost all plastids. Natural electric currents or fields can have similar effects. These cytological and physiological findings can be stimulating models for further studies about the cellular mechanism of plastid exclusion during the development of the male gametophytes of some angiosperms.

REFERENCES

- Bednara J., Rodkiewicz B., 1974. Megasporeocyte and megaspore ultrastructure in *Epilobium*. Bull. Acad. Polon. Sci. Cl. II 22: 847-850.
- Butterfass T., 1979. Patterns of chloroplast reproduction. A developmental approach to protoplasmic plant anatomy. Wien, New York, Springer-Verlag.
- Clauchs R. P., Grun P., 1977. Changes in plastid and mitochondria content during maturation of generative cells of *Solanum* (Solanaceae). Amer. J. Bot. 64: 377-383.
- Corti E. F., Cecchi A. F., 1970. The behaviour of the cytoplasm during the megasporogenesis in *Paphiopedilum spicerianum* (RcWb. f). Pfitzer. Caryologie 23: 715.
- Diers L., 1971. Übertragung von Plastiden durch den Pollen bei *Antirrhinum majus*. II. Molec. Gen. Genetics 113: 150-153.
- Gillham N. W., 1978. Organelle heredity. New York: Raven Press.
- Hagemann R., 1964. Plasmatische Vererbung. Jena: VEB G. Fischer.
- Hagemann R., 1965. Advances in the field of plastid inheritance in higher plants. Proc. XI. Internat. Congr. Genetics, The Hague. Genetics Today, vol. 3: 613-625.
- Hagemann R., 1976. Plastid distribution and plastid competition in higher plants and the induction of plastom mutations by Nitroso-urea-compounds. Pp. 331-338. Genetics and Biogenesis of Chloroplasts and Mitochondria (Bücher F. et al., Ed.). Amsterdam, North Holland.
- Hagemann R., 1979. Genetics and Molecular Biology of Plastids of Higher Plants. Stadler Genetics Sympos. (Univ. Missouri, Colombia) vol. 11: 91-116.
- Heslop-Harrison J., 1972. Sexuality of angiosperms. Pp. 133-289. Plant Physiology. A Treatise. Vol. VIC (Steward F. C., Ed.). New York, London, Academic Press.
- Khera P. K., 1975. Plastid development in zonal pelargoniums. Ph. D. Thesis, University College of Swansea, Wales.
- Kirk J. T. O., Tilney-Bassett R. A. E., 1978. The Plastids. Their chemistry, structure, growth and inheritance. Amsterdam, Elsevier, North Holland.
- Knoth R., Wrischer M., Vetter J., 1979. The cytoplasmic organelles in male gametophytes and gametes of higher plants. II. Phytoferritin accumulat-

- ing plastids in the male generative cell of *Pelargonium zonale* hort., Protoplasma (Wien), (in press).
- Nilsson-Tillgren T., v. Wettstein-Knowles P., When is the male plastome eliminated? Nature 227: 1265-1266.
- Ohba K., Iwakawa M., Ohada Y., Murai M., 1971. Paternal transmission of a plastid anomaly in some reciprocal crosses of Suzi, *Cryptomeria japonica* D. Don. Silvae Genetica 20: 101-107.
- Sager R., 1972. Cytoplasmic Genes and Organelles. New York, London, Academic Press.

Note added during proof-reading:

- Further examples of Type 2 — *Solanum*-Type — are described and discussed by Vaughn, K. C., De Bonte L. R., Wilson K. G., Schaffer G. W., 1980. Organelle alteration as a mechanism for maternal inheritance. Science 208: 196-198.