

Transmission of paternal chloroplasts in *Nicotiana*

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Summary. Transmission of paternal chloroplasts was observed in *Nicotiana*, considered to inherit organelles in a strictly maternal way. Plants carrying streptomycin resistant plastids were used as pollen donors. Cell lines with paternal plastids in the offspring were selected as green (resistant) sectors on calli induced from the seedlings on streptomycin-containing media. The presence of paternal plastids in the regenerated plants was confirmed by restriction analysis. In the *Nicotiana plumbaginifolia* ♀ × *N. plumbaginifolia* Np(SR1)3 ♂ and the *N. plumbaginifolia* Np(gos)29 ♀ × *N. tabacum* SR1 ♂ crosses 2.5% and 0.07% of the offspring were found to contain paternal (*tabacum*) plastids, respectively. These plants, however, carried maternal mitochondria exclusively. This sexual cybridization method offers a simple way to transfer chloroplasts solely, a goal not accessible by protoplast fusion.

Key words: Cytoplasmic organelles – Maternal inheritance – *Nicotiana* – Plastid inheritance – Sexual cybridization

Uniparental-maternal inheritance of cytoplasmic organelles is a widespread phenomenon in the plant kingdom (Tilney-Bassett 1978; Sears 1980) which has been studied since the beginning of the century. The mechanism by which the male organelles (organelle DNA) are eventually excluded from or eliminated in the fertilized egg has been explained by various hypotheses (Sager 1975; Hagemann 1979, 1981; Vaughn et al. 1980; Day and Ellis 1984) and the evolutionary significance of maternal inheritance has also been discussed (Tilney-Bassett 1978; Sears 1980). One of the questions which has been raised is how strict is the maternal inheritance. Cytological identification of the presence or absence of organelles in the pollen generative cells does not exclude subsequent elimination or infrequent transmission of the paternal organelles, respectively (Hagemann 1976, 1979; Sears 1980). Genetic analyses were based generally on small numbers of progeny and exclusively on pigment-deficient mutations, a non-selectable trait (Tilney-Bassett 1978). There have been reports on plants showing traces of biparental plastid transmission, namely 0.01%–1% of the progeny were unexpected supposing strictly maternal inheritance (reviewed by Tilney-Bassett 1978). In these cases, however, it was not possible to exclude other explanations, such as spontaneous plastid mutation/

back mutation, restitution of mutant plastids, or layer alteration in chimaeral shoots (discussed by Tilney-Bassett 1978). In the experiments reported here transmission of paternal plastids was detected in *Nicotiana*, considered to inherit chloroplasts in a strictly maternal way (Tilney-Bassett 1978), using a cytoplasmic streptomycin resistance marker (Maliga et al. 1975). This plastid trait is easily selectable in cell cultures (Medgyesy et al. 1980).

In an intraspecific cross wild-type *Nicotiana plumbaginifolia* was pollinated with an *N. plumbaginifolia* line, Np(SR1)3, containing cytoplasm from the streptomycin-resistant *N. tabacum* SR1 mutant, produced by protoplast-cytoplasm fusion (Maliga et al. 1982). In an interspecific cross an *N. plumbaginifolia* line, Np(gos)29, containing cytoplasm from *N. gossei*, produced by protoplast fusion (Medgyesy et al. 1985b), was pollinated with the *N. tabacum* SR1 mutant. Cell lines with paternal plastids in the offspring were identified as green (resistant) sectors on calli initiated from the seedlings on streptomycin-containing media (Fig. 1). The paternal origin of the plastids among the resistant regenerates from the *N. plumbaginifolia* ♀ × Np(SR1)3 ♂ and the Np(gos)29 ♀ × *N. tabacum* SR1 ♂ crosses was confirmed using the *Sma*I (Fig. 2A) and *Eco*RI (not shown) restriction enzymes, respectively.

Restriction analyses of the resistant lines revealed the presence of paternal plastids in 2.5% and 0.07% of the offspring in the intraspecific and interspecific crosses, respectively (Table 1). This is the first definite proof of occasional paternal transmission in species typically exhibiting strict maternal plastid inheritance. The use of an efficient selection procedure in tissue culture seems to be the key to recovering cell lines with paternal plastids, because among 6,800 seedlings from the *N. plumbaginifolia* ♀ × Np(SR1)3 ♂ cross, germinated on filter papers wetted with a streptomycin solution (1 mg/ml), not one was found with green spots (resistant cells) on the cotyledons under a stereoscopic microscope. Probably the initial number of paternal plastids transmitted (as compared to that of maternal plastids) in a fertilized egg is so low, that paternal plastids are present only in some cells of the developing seedling and appear only sporadically in the plastid population of a cell. Direct selection in cell culture, therefore, seems to be essential for the sorting out of these plastids (and subsequently such cells) until a visible cell clump is formed (Fig. 1). The result suggests that using suitable markers and selection conditions a greatly unequal biparental plastid transmission may be detected in other plant species known



Fig. 1. Appearance of a green (resistant) sector on a seedling callus. Surface-sterilized (Maliga et al. 1982) seeds were germinated on RM salt solution (Murashige and Skoog 1962) containing 2% sucrose (RM medium) solidified with 0.5% Bacto-agar. Calli were induced from 1-week-old seedlings, slightly crushed with forceps, on RMOP medium (Cséplő and Maliga 1984) supplemented with 2% sucrose and 1 mg/ml streptomycin sulphate, in the light (1,000 lx; 16 h day). After 8 weeks green sectors were separated and grown for 4 weeks on the same medium, then for 4 weeks on streptomycin-free medium. Shoots developing from the regenerating calli were rooted on RM medium

to inherit organelles maternally. Rare phenomena such as genetic recombination of chloroplasts, previously only found after protoplast fusion (Medgyesy et al. 1985a), might also be observed after crosses using this sexual cybridization method.

Plants with paternal chloroplasts from the *N. plumbaginifolia* ♀ × Np(SR1)3 ♂ cross had diploid ($2n=20$) and tetraploid chromosome numbers (counted in root tips) in 17 and 15 lines, respectively. Tetraploidisation is a frequent phenomenon in tissue cultures. The ten diploid lines investigated from the intraspecific cross and the single line from the interspecific cross, carrying paternal chloroplasts, contained mitochondria exclusively from the maternal parents as revealed by *Bam*HI restriction analyses (not shown). This conclusion was further confirmed by hybridization of radiolabelled mitochondrial DNA (mtDNA) of *N. tabacum* (Fig. 2B) and *N. plumbaginifolia* (not shown) to *Bam*HI-digested mtDNA of four lines from the intraspecific cross.

This is the first case where chloroplasts and mitochondria could be separated in plants with maternal inheritance of organelles. In protoplast fusion experiments chloroplasts frequently segregated independently from mitochondrial traits (e.g., Belliard et al. 1978; Aviv and Galun 1980; Glielius et al. 1981), but in each case investigated the mitochondria had nonparental, recombinant-type DNA in the fusion-derived plants (e.g. Belliard et al. 1979; Nagy et al. 1981; Galun et al. 1982) suggesting the presence of (at least a part of) the mitochondrial genomes from both parents. Probably the use of a mitochondrial marker selectable in tissue culture would also allow demonstration of paternal transmission of these organelles. It is also remarkable that the single plant with paternal chloroplasts from the interspecific cross remained male sterile like the spontaneous

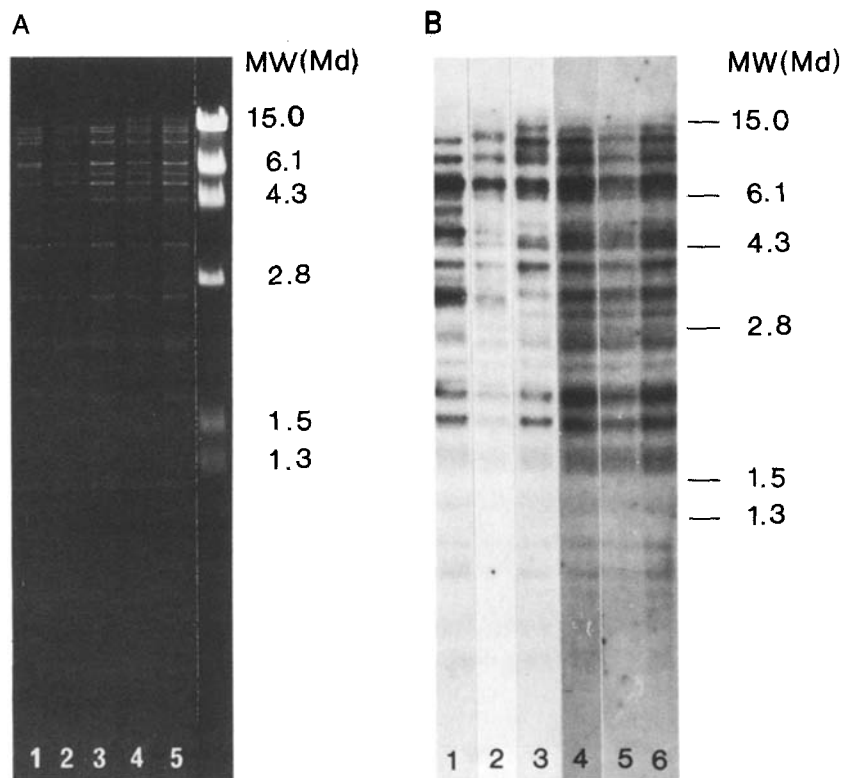


Fig. 2. A *Sma*I restriction patterns of chloroplast DNA of *Nicotiana plumbaginifolia*, lane 1; *N. tabacum*, lane 2 and some of the offspring, lanes 3-5; B autoradiogram showing hybridization of 32 P-labelled *N. tabacum* mitochondrial DNA (mtDNA) to *Bam*HI-digested mtDNA of *N. tabacum*, lane 1; Np(SR1)3, lane 2; *N. plumbaginifolia*, lane 3 and some of the offspring, lanes 4-6, from the *N. plumbaginifolia* ♀ × Np SR1 3 ♂ cross. Chloroplast and mitochondrial DNA restriction analyses were performed as previously described (Menczel et al. 1981; Nagy et al. 1983). Southern hybridization was performed according to Maniatis et al. (1982). A lambda *Hind*III digest is also shown (A, right-hand lane) or marked (B)

Table 1. Transmission of paternal plastids in *Nicotiana*

Cross	Number of seedlings		
	tested ^a	resistant ^b	paternal ^c
<i>Nicotiana plumbaginifolia</i> ♀ × Np(SR1)3 ♂	1,500	44	37
Np(gos)29 ♀ × <i>N. tabacum</i> SR1 ♂	1,500	15	1
<i>N. plumbaginifolia</i> self	1,200	9	—

^a Seeds from three capsules. Germination was near 100%

^b Seedlings which gave rise to green sectors on callus induced on RMOP medium with 1 mg/ml streptomycin

^c Paternal plastids were identified using *Sma*I and *Eco*RI restriction enzymes in the plants regenerated from the green sectors

streptomycin-resistant lines from this cross and the original mother line (cf. Medgyesy et al. 1985b). This is in agreement with the sole role of alien mitochondria in the alloplasmic male sterility in *Nicotiana* (e.g. Belliard et al. 1979; Galun et al. 1982).

Efficient transfer of chloroplasts by protoplast fusion requires selection for a plastid marker (Medgyesy et al. 1980; Menczel et al. 1982; Cséplő et al. 1984). This selection process, however, results in co-transfer of a considerable part of the mitochondrial genome from the cytoplasm donor, which, in the case of an incompatible nuclear background, makes 100% of the cybrids male sterile (Menczel et al. 1983; Medgyesy et al. 1985b). The sexual cybridization method described here, however, offers a simple way to transfer chloroplasts solely, a goal not accessible by protoplast fusion. A further agronomic importance of the method is raised by the discovery of conditions for the selection of plastids resistant to photosystem II herbicides in tissue cultures (Cséplő et al. 1985; Cséplő and Medgyesy 1986). It is also notable that this method can be used for chloroplast transfer in plants which have suitable callus cultures but which are recalcitrant from the viewpoint of protoplast techniques.

The selection advantage of maternal organelle inheritance in evolution has been queried (Tilney-Bassett 1978; Sears 1980) although higher plants show a tendency to loose biparental transmission of organelles. If the leakiness of maternal plastid inheritance in *Nicotiana* reported here is a general phenomenon, the spreading of maternal organelle inheritance can be considered rather as a secondary consequence of redundancy of organelles in the pollen (c.f. Tilney-Bassett 1978), than as a selective advantage of the heredity of pure maternal organelle populations.

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