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Inheritance of chloroplast DNA in *Populus*

M. Mejnartowicz

Department of Forest Genetics and Forest Plant Breeding, University of Göttingen, W-3400 Göttingen, FRG

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Summary. Restriction fragment length polymorphisms (RFLPs) were used as markers to determine the transmission of chloroplast DNA (cpDNA) in poplar crosses. The plant material studied included individual trees of *Populus trichocarpa, P. maximowiczii* × *trichocarpa, P. maximowiczii* × *nigra*, and offspring from controlled crosses between these trees. RFLPs were identified by direct observation of stained restriction fragments, as well as by molecular hybridization with heterologous cpDNA probes. Analysis of the restriction fragment patterns in the parents and their progeny showed only the patterns of the maternal tree in the progeny, while no paternal type was found. These results provide clear evidence of a maternal mode of chloroplast inheritance in the poplar clones studied.

Key words: cpDNA inheritance – cpDNA restriction analysis – Poplar

Introduction

Previous investigations in different angiospermous plant species usually showed a maternal inheritance of chloroplasts and, thus, cpDNA (Palmer 1987). However, in some genera, such as *Medicago* (Johnson and Palmer 1989; Masoud et al. 1990), *Pelargonium* (Metzlaff et al. 1981; Tilney-Basset and Almouslem 1989), and *Nicotiana* (Medgyesy et al. 1985), biparental inheritance was found. Paternal inheritance of plastids was found in *Daucus* (Boblenz et al. 1990) and predominantly paternal inheritance, in *Medicago sativa* progeny (Schumann and Hancock 1989). Occasional paternal inheritance of plastids has been reported, e.g., in rice (Dally and Second 1990).

Paternal inheritance was shown in investigations of gymnosperms: *Larix, Pseudotsuga, Picea, Sequoia, Pinus* (Neale et al. 1986; Szmidt et al. 1987; Szmidt et al. 1988; El-Kassaby et al. 1988; Neale et al. 1988; Neale et al. 1989). Biparental plastid inheritance has also been demonstrated in conifers (Szmidt et al. 1987; Wagner et al. 1988; Neale et al. 1989).

Comparison of restriction fragment patterns is a useful method in genetic research of chloroplast DNA. The use of cpDNA markers in combination with controlled crosses allows a clear determination of the transmission mode. However, only two genetic studies of cpDNA have been carried out previously in deciduous trees with this tool: in the genus *Coffea* (Berthou et al. 1983) and in the genus *Prunus* (Kaneko et al. 1986). The purpose of the present study is to determine the mode of inheritance of cpDNA in the genus *Populus*.

Materials and methods

Plant material

Controlled crosses were carried out between female *Populus trichocarpa* (clone 'Muhle Larsen') and male *P. maximowiczii* × *P. trichocarpa* (clone 'Androscoggin'), as well as between female *P. maximowiczii* × *P. berolinensis* (clone 'Oxford') and male *P. trichocarpa* (clone 'Columbia River') (Müller-Starck, personal communication). It was not possible to use intraspecific crosses to infer cpDNA inheritance.

Isolation of cpDNA

Chloroplast DNA was isolated from leaves following a modified method of White (1986). Chloroplasts were resuspended in a 50 m*M* TRIS, 10 m*M* EDTA buffer (equilibrated to pH 7.8, with 1 *N* HCl) with sodium sarkosylate and proteinase K, and incubated for 1 h at room temperature. They were then extracted with phenol/chloroform (1:1) treatments.

The cpDNA extraction procedure requires a relatively large (15 g) amount of leaf tissue. In most cases, it was therefore necessary to pool leaves of more than one plant. Leaves from the offspring were pooled from three to four different plants for each isolation; leaves from parent trees were pooled from two ramets from one and the same clone. In the case of clone 'Muhle Larsen' the extraction was made from one individual. Because no more than three to four plants were pooled, it should be possible to detect differing patterns among these progeny individuals.

The total DNA isolation

The DNA was isolated by a modified CTAB method, following Rogers and Benedich (1988).

Restriction enzyme analysis

The cpDNA was digested with restriction endonucleases *Bam*HI, *Eco*RI, *Xba*I, *Pst*I, *Hin*dIII, with 20 units per 5 µg DNA, for 4 h at 37 °C. The restriction fragments were separated by horizontal slab gel electrophoresis, stained with an ethidium bromide solution, and photographed under UV (302 nm) illumination. The DNA fragment length was determined according to the method of Schafer and Sederoff (1981), using a BASIC computer program designed by A.E. Szmidt.

DNA transfer

The transfer (alkali transfer) to Hybond Nylon+ filters (Amersham) was performed by a modified method of Southern (1975).

Hybridization

After testing the heterologous probes for homology by molecular hybridization to poplar cpDNA, filters with total DNA were hybridized to the probes. These are labelled non-radioactively with digoxigenin-dUTP (Boehringer, Mannheim).

The following cloned chloroplast DNA probes were used in this study: *Nicotiana tabacum* – pTB1, 1.2 kb, – rbcL coding region and 3′untranslated sequence; *Nicotiana tabacum* – pTB8, 4.8 kb, – psbA, trnH, rpl2 and trnI coding region, both kindly provided by Prof. M. Sugiura; *Spinacia oleraceae* – pSoC 1080, 8.2 kb, including the psaA coding region, kindly provided by Dr. E.M. Orozco, Jr.

Results and discussion

A fragment length polymorphism with a unique restriction pattern for each parental individual was sought. As Figs. 1 and 2 show, the restriction patterns differ between the parental clones.

Pattern from *Pst*I digests differ as follows: 'Oxford' (Ox) and 'Androscoggin' (An) do not have the 14.7-kb (no. 4) and 5.8-kb (no. 6) fragments that appear in electropherograms of 'Columbia River' (CR) and 'Muhle Larsen' (ML) after digests, but they appear to have two copies of fragment no. 3 (20.8 kb). It is possible that fragments no. 4 and no. 6 originate from one no. 3 fragment, because the sum of their molecular sizes is approx. equal to the size of no. 3.

Ox and An do not have fragments no. 2 (6.6 kb), no. 3 (5.1 kb), no. 7 (4.1 kb), or no. 10 (3.7 kb) from the

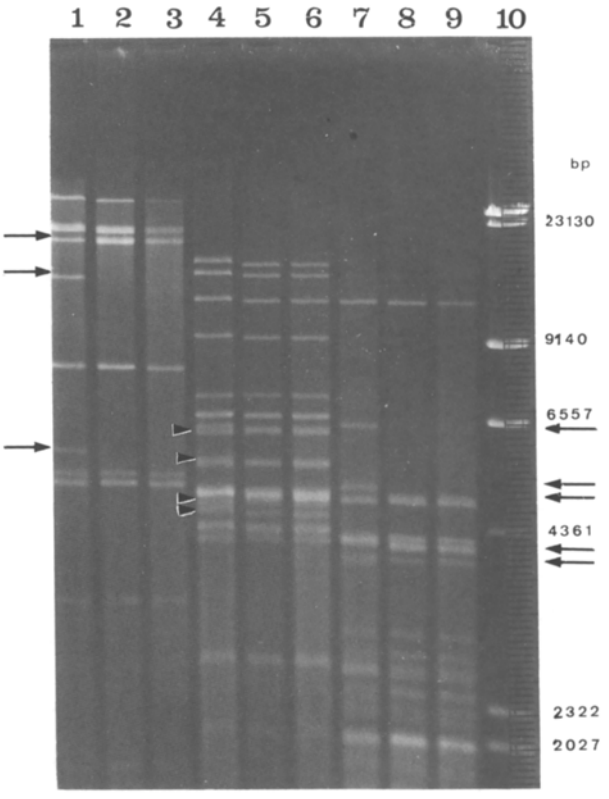

Fig. 1. Restriction analysis of cpDNA from 'Oxford' × 'Columbia River'. From *left* to *right*: male parent (Columbia River), offspring sample (Oxford × Columbia River), female parent (Oxford). Lanes 1–3: *Pst*I digest; lanes *4–6*: *Hin*dIII digest; lanes 7–9: *Eco*RI digest; lane *10*: length marker – DNA with *Hin*dIII digested

*Eco*RI digest, but they do have no. 4 (4.8 kb), no. 6 (4.2 kb), and no. 8 (4.0 kb). Fragment no. 4 could originate from no. 2 after a small deletion (see hybridization results). *Hin*dIII digests show differences between fragments no. 7 (6.7 kb), no. 10 (5.77 kb), no. 15 (4.7 kb), and no. 17 (4.6 kb), which are present in the CR and ML digests but not in An/Ox patterns. An/Ox patterns possess fragment 11 (5.5 kb) in two copies and, additionally, fragments 8, 14, 16, and 18 (6.5, 4.88, 4.7, and 4.5 kb); see Fig. 1.

*Bam*HI An/Ox patterns lack the 5.7-kb fragment no. 6, one from no. 8 (5.14 kb) and one from no. 11 (4.5 kb) – in CR/ML both twice – but they have, additionally, no. 7 (5.3 kb) and no. 10 (4.6 kb); see Fig. 2.

In the *Xba*I digest patterns only one fragment, no. 8 (4.4 kb), is lacking in An/Ox (data not shown).

In every case the progeny show digest patterns identical to that of the female parent; see Fig. 1, lanes 2, 5, and 8; Fig. 2, lanes 2, 4, and 7. These results provide clear evidence for maternal inheritance of chloroplast DNA in the *Populus* material studied. They are in accordance with the results for other deciduous trees (Berthou 1983;

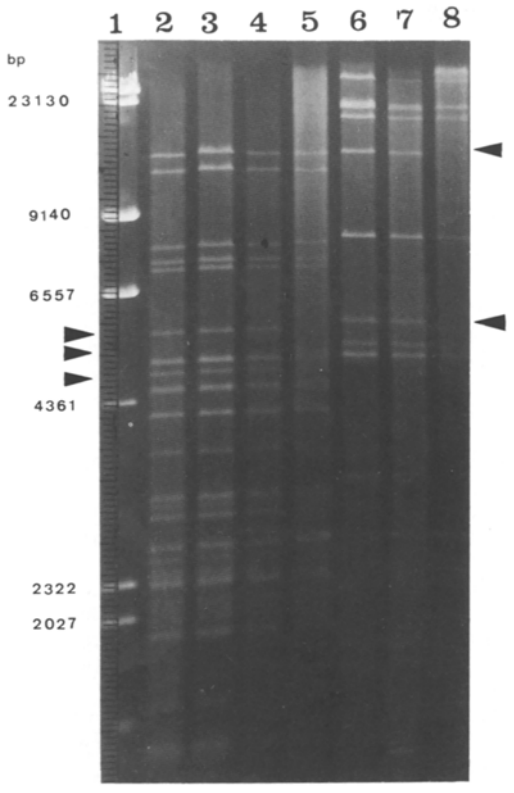

Fig. 2. Restriction analysis of cpDNA from 'Muhle Larsen' × 'Androscoggin'. *Bam*HI digest: lanes 2–5 and *Pst*I digest: lanes 6–8. Lanes 2, 4, 7: 'Muhle L.' × 'Androscoggin'; lanes 3, 6: 'Muhle L.'; lanes 5, 8: 'Androscoggin'

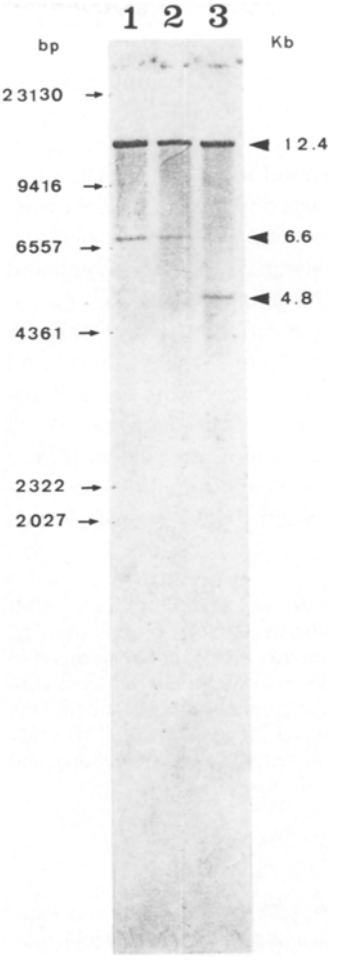

Fig. 3. Southern blot analysis of cpDNA from *Populus*. DNA, digested with *Eco*RI, was fractionated on a 0.8% agarose gel and transferred to a Hybond Nylon+ filter. The filter was hybridized with digoxigenin-dUTP-labelled pSoC probe. Lane 1: 'Muhle Larsen'; lane *2*: 'Muhle L.' × 'Androscoggin'; lane *3*: 'Androscoggin'

Kaneko et al. 1986) as well as with those of the majority of angiosperm studies.

This hypothesis of maternal inheritance was checked by employment of molecular hybridization using three heterologous chloroplast gene probes (see 'Material and methods'). One of them, pSoC1080, showed a difference in hybridization pattern (see Fig. 3). It hybridized with 12.4- and 4.8-kb fragments from Ox/An, and 12.4- and 6.6-kb fragments from CR/ML DNA, treated with *Eco*RI. Analysis of a total of 28 offspring of the Muhle Larsen × Androscoggin cross supported the assumption of the maternal source of their cpDNA. Total DNA from all progeny of this cross hybridized with the pSoC 1080 probe, just as the female parent did (M. Mejnartowicz, unpublished data).

Paternal or mixed patterns were not observed. There is also no evidence for mutation in the F_1 progeny.

Various reasons for the absence or exclusion of paternal plastids are conceivable. It is a common phenomenon in most cytologically examined angiosperm species that a pollen generative cell either contains no plastids or that the plastids degenerate during pollen maturation (Whatley 1982; Connet 1987; Corriveau and Coleman 1988; Hagemann and Schröder 1989).

Cases of predominantly maternal inheritance and biparental transmission of plastids have been thoroughly investigated in the genus *Oenothera* (Chiu et al. 1988). Plastid-dependent differences in the multiplication of cpDNA were found to be the reason for this. For *Medicago*, a high paternal plastid transmission was reported to be a result of the effects of the maternal nuclear genome and possibly the paternal nuclear and/or plastid genomes (Schumann and Hancock 1989). In the genus *Pelargonium*, an allele (*Pr*-allele) responsible for the control of plastid inheritance was found (Kirk and Tilney-Basset 1978). The pollen generative cells from the studied species of this genera contain plastids, and these plants exhibit biparental inheritance.

This report supports the hypothesis that poplar pollen does not contribute to the transmission of plastids and that the paternal plastids are not excluded during

plant ontogenesis. The plant material used for crosses allows the exclusion of plastome-genome incompatibilities or competition between plastids types in the zygote. The results of performing defined, quasi-reciprocal interspecific crosses shows that the plastid type is not dependent on the nuclear background in the plant. The same (maternal) type of cpDNA was observed in the following clones: 'Oxford' (*P. maximowiczii* × *P. berolinensis*) and 'Androscoggin' (*P. maximowiczii* × *P. trichocarpa*), and in progeny from cross (*P. maximowiczii* × *P. trichocarpa*) × *P. trichocarpa*. A different type of cpDNA was found in *P. trichocarpa* clones 'Muhle Larsen' and 'Colombia River,' and in the progeny from cross *P. trichocarpa* × (*P. maximowiczii* × *P. trichocarpa*). If the nuclear background plays an important part in plastid transmission, then in the progeny from the last cross (Muhle Larsen × Androscoggin) both types of plastids should be observed.

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References

Berthou F, Mathieu C, Vedel F (1983) Chloroplast and mitochondrial DNA variation as indicator of phylogenetic relationships in the genus *Coffea*. Theor Appl Genet 65:77–84

Boblenz K, Nothnagel T, Metzlaff M (1990) Paternal inheritance of plastids in the genus *Daucus*. Mol Gen Genet 220:489–491

Chiu W-L, Stubbe We, Sears B (1988) Plastid inheritance in *Oenothera* organelle genome modifies the extent of biparental plastid transmission. Curr Genet 13:181–189

Connet MB (1987) Mechanism of maternal inheritance of plastids and mitochondria: Developmental and ultrastructural evidence. Plant Mol Biol Rep 4:193–205

Corriveau JL, Coleman AW (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am J Bot 75:1443–1458

Dally AM, Second G (1990) Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza*, section Oryza). Cladistic mutation and genetic-distance analysis. Theor Appl Genet 80:209–222

El-Kassaby AY, Sigurgeirsson A, Szmidt AE (1988) The use of restriction analysis of chloroplast DNA in classifying hybrid spruce seedlots. In: Molecular genetics of forest trees. Proc Frans Kempe symp, Umeå, Sweden, June 14–16, 1988, pp 67–89

Hagemann R, Schröder MB (1989) The cytological basis of the plastid inheritance in angiosperms. Protoplasma 152:57–64

Johnson LB, Palmer JD (1989) Heteroplasmy of chloroplast DNA in *Medicago*. Plant Mol Biol 12:3–11

Kaneko T, Terachi T, Tsunewaki K (1986) Studies on the origin of crop species by restriction endonuclease analysis of organellar DNA. II. Restriction analysis of ctDNA of 11 *Prunus* species. Jpn J Genet 61:157–168

Kirk JTO, Tilney-Basset RAE (1978) The plastids. Elsevier/North Holland, Amsterdam, pp 251–254

Masoud SA, Johnson LB, Sorensen EL (1990) High transmission of paternal plastid DNA in alfalfa plants demonstrated by restriction fragment polymorphic analysis. Theor Appl Genet 79:49–55

Medgyesy P, Pay A, Marton L (1985) Transmission of paternal chloroplasts in *Nicotiana*. Mol Gen Genet 204:195–198

Metzlaff M, Börner T, Hagemann R (1981) Variations of chloroplast DNAs in the genus *Pelargonium* and their biparental inheritance. Theor Appl Genet 60:37–41

Neale DB, Wheeler NC, Allard RW (1986) Paternal inheritance of chloroplast DNA in Douglas fir. Can J For Res 16:1152–1154

Neale DB, Marshall KA, Sederoff RR (1988) Inheritance of chloroplast and mitochondrial DNA in conifers. In: Hällgren J-E (ed) Molecular genetics of forest trees. Proc Frans Kempe symp, Umeå, Sweden, June 14–16, 1988, pp 89–101

Neale DB, Marshall KA, Sederoff RR (1989) Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens*. PNAS USA 86:9347–9349

Palmer JD (1987) Chloroplast DNA evolution and biosystematic uses of cpDNA variation. Am Nat 130:6–29

Rogers SO, Benedich AJ (1988) Extraction of DNA from plant tissues. Plant Mol Biol Manual A6:1–10

Schafer HE, Sederoff R (1981) Improved estimation of DNA fragment lengths from agarose gels. Anal Biochem 115:113–122

Schuman CM, Hancock JF (1989) Paternal inheritance of plastids in *Medicago sativa*. Theor Appl Genet 78:863–866

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503

Szmidt AE, Alden T, Hällgren J-E (1987) Paternal inheritance of ctDNA in *Larix*. Plant Mol Biol 9:59–64

Szmidt AE, El-Kassaby YA, Sigurgeirsson A, Alden T, Lindgren D, Hällgren J-E (1988) Classifying seedlots of *Picea sitchensis* and *P. glauca* in zones of introgression using restriction analysis of cpDNA. Theor Appl Genet 76:841–845

Tilney-Basset RAE, Almouslem AB (1989) Variation in plastid inheritance between *Pelargonium* cultivars and their hybrids. Heredity 63:145–153

Wagner DB, Govindaraju DR, Dancik BP (1988) Chloroplast DNA polymorphism in a sympatric region. In: Cheliak WM, Yapa AC (eds) Molecular genetics of forest trees. Proc 2nd IUFRO Working Party Mol Genet, Chalk River, Ontario, pp 75–79

Whatley JM (1982) Ultrastructure of plastid ineritance: green algae to angiosperms. Biol Rev 57:527–569

White EE (1986) A method for extraction of a chloroplast DNA from conifers. Plant Mol Biol Rep 4:98–101