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Chloroplast DNA variation in *Populus*. III. Novel chloroplast DNA variants in natural *Populus* × *canadensis* hybrids

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Abstract A rare phenomenon of the occurrence of novel non-parental chloroplast DNA (cpDNA) variants in natural sexual interspecific hybrids between *Populus deltoides* var *deltoides* and *P. nigra*, *P.* × *canadensis* is described. Restriction fragment variation of cpDNA in 17 *P.* × *canadensis* cultivars was examined and compared with that of representative samples of *P. deltoides* and *P. nigra* using 83 combinations of 16 restriction enzymes and six *Petunia hybrida* cpDNA probes. Twelve cultivars had one to five novel non-parental cpDNA fragments in the chloroplast genome region homologous to the 9.0-kb *Pst*I cpDNA fragment of *Petunia* from the large single-copy region.

Key words Interspecific poplar hybrids · Non-parental chloroplast DNA fragments · Novel organelle DNA · RFLP · Chloroplast DNA recombination

Introduction

In angiosperms, the chloroplast (cp) genome is quite conserved in structure (Palmer 1985) and generally follows a uniparental-maternal mode of inheritance (Sears 1980; Smith 1989; Harris and Ingram 1991). Evidence for cpDNA recombination between parental genotypes is virtually non-existent in sexual controlled crosses of flowering plants, even in those plants ordinarily showing biparental cpDNA inheritance (Sears 1980; Rose et al. 1990; Harris and Ingram 1991).

In contrast, cpDNA recombination has been widely reported for different genetic lines of the unicellular algae *Chlamydomonas* (e.g., Lemieux et al. 1990). Intramolecular recombination of cpDNA, however, is well-documented in flowering plants (Palmer 1985). Also, there is evidence for intermolecular cpDNA recombination in interspecific and intergeneric somatic hybrids in angiosperms, although it is a very rare event (Rose et al. 1990). Recombination of cpDNA and the formation of novel non-parental cpDNA variants have been observed in interspecific somatic hybrids of *Nicotiana* (Medgyesy et al. 1985; Fejes et al. 1990) and intergeneric somatic hybrids between *Nicotiana* and *Solanum* (Thanh and Medgyesy 1989; Horvath et al. 1994). Among forest trees, non-parental novel cpDNA variants have been observed in sexual progeny of intraspecific controlled crosses of *Pseudotsuga* (Neale et al. 1986) and *Larix* (Szmidt et al. 1987), and in sympatric populations (natural interspecific hybrids) of two *Pinus* species (Govindaraju et al. 1989).

Populus × *canadensis* Moench [syn. *P.* × *euramericana* (Dode) Guinier] is an interspecific hybrid between *P. deltoides* and *P. nigra* (Zsuffa 1975; Rajora and Zsuffa 1989; Rajora 1990). The natural *P.* × *canadensis* hybrids are believed to have originated in France by the open interbreeding of *P. deltoides* var *deltoides* introduced from southeastern Canada with the indigenous *P. nigra* (Zsuffa 1975). A large number of natural and artificially developed *P.* × *canadensis* cultivars and clones are commercially available (FAO 1979; Dickmann and Stuart 1983). More than 90% of the cultivated poplars of the world belong to *P.* × *canadensis* and their parental species, *P. deltoides* and *P. nigra* (FAO 1979). *Populus* × *canadensis* hybrids are usually clonally propagated. In F_1 progeny of interspecific controlled crosses of *P. deltoides* × *P. nigra*, both cpDNA and mitochondrial DNA were found to follow the uniparental-maternal mode of inheritance (Rajora and Dancik 1992; Rajora et al. 1992).

In this paper, we document a very rare phenomenon of the occurrence of novel non-parental cpDNA variants in 12 out of the 17 natural *P.* × *canadensis* cultivars examined. Intraspecific and interspecific cpDNA variation of the parental species *P. deltoides* and *P. nigra* is described in accompanying papers (Rajora and Dancik 1995a, b).

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Table 1 Novel (non-parental) chloroplast DNA (cpDNA) restriction fragment variants observed in *P.* × *canadensis* cultivars

Enzyme-Probe	Novel cpDNA variants (size in kb)	Cultivars
*Bam*HI-P10[a]	4.3, 1.0	Gelrica, Grandis, I-214, Regenerata, and Steckby
	4.3	Blanc du Poitou, Canada Blanc, I-55/56, I-132/56, Jacometti, Ostia, and Zurich 03/3
*Bcl*I-P10[a]	8.1, 6.3, 4.3, 3.0	Ostia

[a] 9.0 kb *Pst*I *Petunia hybrida* cpDNA fragment

Materials and methods

Chloroplast DNA variation was examined in 17 *P.* × *canadensis* cultivars ('Baden 431', 'Blanc du Poitou', 'Canada Blanc', 'Dorskamp 925', 'Eugenei', 'Gelrica', 'Grandis', 'Heidemij', 'I-55/56', 'I-132/56', 'I-214', 'Jacometti', 'Ostia', 'Regenerata', 'Robusta', 'Steckby', and 'Zurich 03/3') in relation to samples from *P. deltoides*, *P. nigra*, and *P. maximowiczii* using 83 combinations of 16 restriction enzymes and six *Petunia hybrida* cpDNA probes, as described in the accompanying papers (Rajora and Dancik 1995a, b).

Results and discussion

All sampled *P.* × *canadensis* cultivars shared the same 280 restriction fragments as *P. deltoides* var *deltoides* (Rajora and Dancik 1995b). None of the *P.* × *canadensis* samples had cpDNA fragments that were characteristic of *P. deltoides* var *occidentalis* or of its paternal parent *P. nigra*. However, 12 of the 17 cultivars showed non-parental novel cpDNA fragments when *Bam*HI and *Bcl*I individual restriction digests of their DNA were hybridized with the 9.0-kb *Pst*I *Petunia* cpDNA fragment P10 from the large single-copy (LSC) region. Cultivars 'Blanc du Poitou', 'Canada Blanc', 'I-55/56', 'I-132/56', 'Jacometti', and 'Zurich 03/3' had one *Bam*HI-P10 novel cpDNA fragment; cvs 'Gelrica', 'Grandis', 'I-214', 'Regenerata', and 'Steckby', two novel cpDNA fragments;

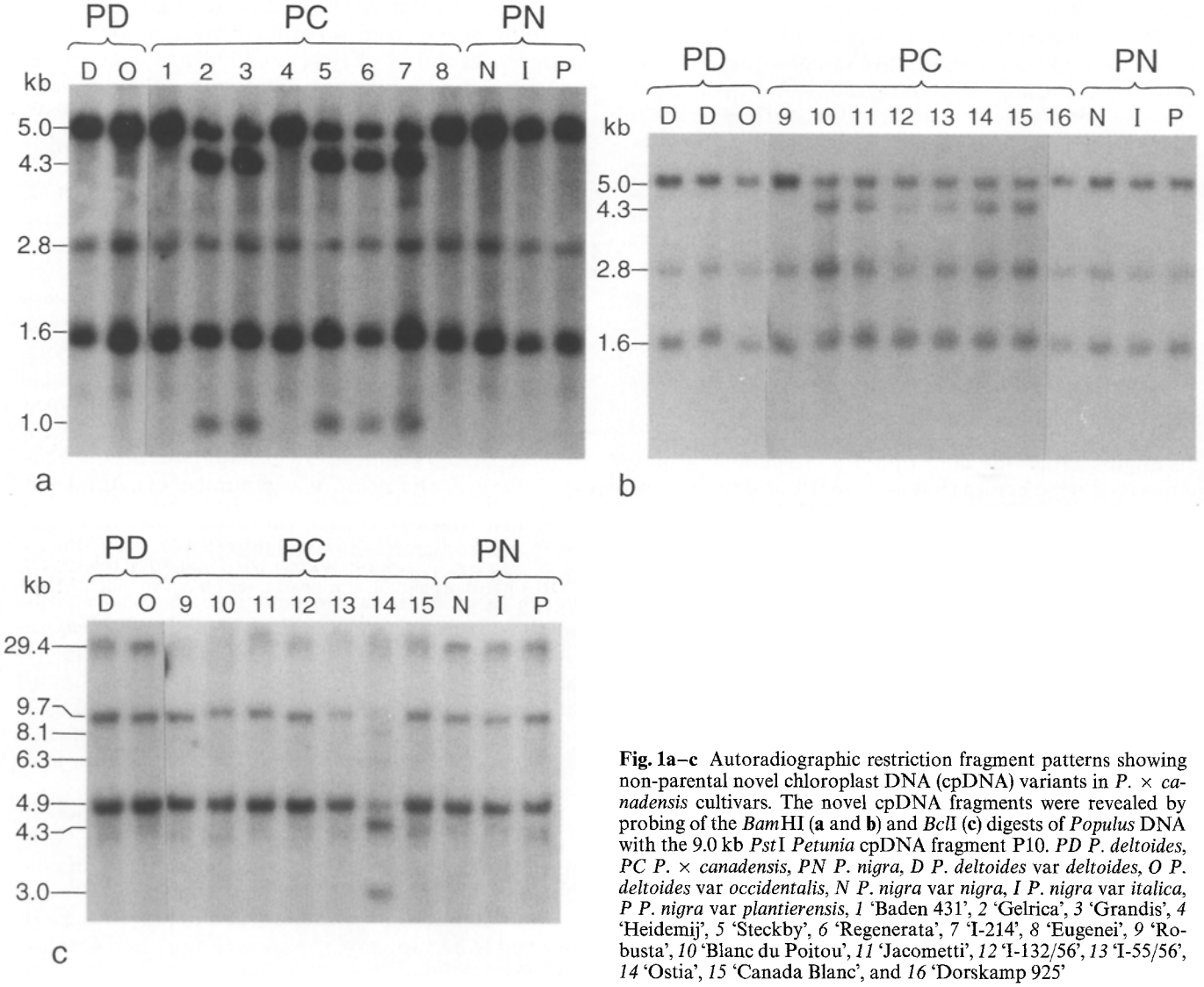

Fig. 1a–c Autoradiographic restriction fragment patterns showing non-parental novel chloroplast DNA (cpDNA) variants in *P.* × *canadensis* cultivars. The novel cpDNA fragments were revealed by probing of the *Bam*HI (**a** and **b**) and *Bcl*I (**c**) digests of *Populus* DNA with the 9.0 kb *Pst*I *Petunia* cpDNA fragment P10. *PD P. deltoides*, *PC P.* × *canadensis*, *PN P. nigra*, *D P. deltoides* var *deltoides*, *O P. deltoides* var *occidentalis*, *N P. nigra* var *nigra*, *I P. nigra* var *italica*, *P P. nigra* var *plantierensis*, *1* 'Baden 431', *2* 'Gelrica', *3* 'Grandis', *4* 'Heidemij', *5* 'Steckby', *6* 'Regenerata', *7* 'I-214', *8* 'Eugenei', *9* 'Robusta', *10* 'Blanc du Poitou', *11* 'Jacometti', *12* 'I-132/56', *13* 'I-55/56', *14* 'Ostia', *15* 'Canada Blanc', and *16* 'Dorskamp 925'

and cv 'Ostia', one *Bam*HI-P10 and four *Bc*/I-P10 novel cpDNA fragments (Table 1, Fig. 1). *Bam*HI restriction digests of poplar DNA hybridized with the *Petunia* cpDNA fragment P10 revealed one restriction fragment length polymorphism that characteristically distinguished between *P. deltoides* and *P. nigra* (Rajora and Dancik 1995b). No cpDNA variation was detected between *P. deltoides* and *P. nigra* for *Bc*/I digests probed with the *Petunia* cpDNA fragment P10 (Fig. 1).

There are three groups among the 12 *P.* × *canadensis* cultivars based on their sharing of the novel cpDNA variants (Table 1, Fig. 1). The fourth group consisted of those *P.* × *canadensis* cultivars ('Baden 431', 'Dorskamp 925', 'Eugenei', 'Heidemij' and 'Robusta') that did not have any novel cpDNA variants and whose cpDNA fragment patterns were identical to those of *P. deltoides* var *deltoides*. 'Ostia' was unique in having four *Bc*/I-P10 novel cpDNA fragments. This grouping indicates intercultivaral chloroplast genome relationships and, with few exceptions, is similar to that observed based on isozyme genotypes (Rajora and Zsuffa 1989). 'Canada Blanc' was suspected to be a renamed ramet of 'Ostia' (Zufa 1960), and these 2 cultivars share the same 31-locus isozyme genotypes (Rajora and Zsuffa 1989) and random amplified polymorphic DNA fingerprints (unpublished data). Our cpDNA analysis results, however, suggest that 'Canada Blanc' and 'Ostia' have distinct chloroplast genomes.

Based on nuclear isozyme (Rajora and Zsuffa 1989; Rajora 1990) and DNA (unpublished data) markers, it is well-established that *P.* × *canadensis* clones and cultivars, including those studied here, are sexual interspecific hybrids between *P. deltoides* and *P. nigra*. Mitochondrial DNA and cpDNA analyses have suggested that *P. deltoides* var *deltoides* is the maternal progenitor of natural *P.* × *canadensis* (Barrett et al. 1993; Rajora and Dancik 1995b). We provide evidence in this study for the existence of one to five novel non-parental cpDNA variants in 12 out of the 17 *P.* × *canadensis* cultivars. This suggests that interspecific hybridization in *Populus* could lead to novel polymorphisms in the chloroplast genome. Novel cpDNA variants also have been detected in putative natural interspecific hybrids between *Pinus banksiana* and *Pinus contorta* (Govindaraju et al. 1989), interspecific somatic hybrids of *Nicotiana* (Medgyesy et al. 1985; Fejes et al. 1990), and intergeneric somatic hybrids between *Nicotiana* and *Solanum* (Thanh and Medgyesy 1989; Horvath et al. 1994). One of the primary mechanisms for generation of novel cpDNA variants in these interspecific and intergeneric hybrids has been considered to be the recombination of the cpDNA of parental genotypes. In 12 *P.* × *canadensis* cultivars, novel cpDNA variants could have primarily been formed by the recombination of cpDNA of progenitor genotypes of *P. deltoides* and *P. nigra* by itself or in combination with deletion/insertion events. However, other mechanisms such as higher mutation rate and sequence instability in hybrids (Barton and Hewitt 1985) cannot be ruled out. Additional work is needed to elucidate the mechanisms for the creation of novel cpDNA variants. Nonetheless, we, for the first time, document a rare event of the existence of novel non-parental cpDNA variants in sexual interspecific hybrids of a tree angiosperm.

All novel non-parental cpDNA variants were detected only when *Petunia* cpDNA fragment P10 from the LSC region was used as a hybridization probe (Table 1, Fig. 1). No novel cpDNA fragment was observed with any of the other five *Petunia* cpDNA probes used. This suggests that the novel cpDNA variants may be located in the LSC region of the chloroplast genome of *P.* × *canadensis* that is homologous to the 9.0-kb *Pst*I *Petunia* cpDNA fragment P10. If these novel cpDNA fragments in *P.* × *canadensis* were formed as a result of the recombination of cpDNA of *P. deltoides* and *P. nigra* progenitors, the cpDNA recombination site is presumably located in this LSC region. Comparative restriction site mapping and sequencing of the chloroplast genomes of *P. deltoides*, *P. nigra*, and *P.* × *canadensis* are necessary to determine the location and possible causes of the formation of the novel cpDNA variants observed.

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