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Chloroplast DNA variation in *Populus*. I. Intraspecific restriction fragment diversity within *Populus deltoides*, *P. nigra* and *P. maximowiczii*

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Abstract We examined intraspecific chloroplast (cp) DNA variation within *Populus deltoides*, *P. nigra*, and *P. maximowiczii* by restriction fragment analysis using 16 restriction endonucleases and six heterologous probes of cloned *Petunia* cpDNA fragments. All three *Populus* species showed intraspecific cpDNA variation, which was intra- and inter-varietal in *P. deltoides*, intervarietal in *P. nigra*, and origin-specific in *P. maximowiczii*. Two varieties of *P. deltoides*, var *deltoides* and var *occidentalis*, showed distinct cp genomes/DNA. Three distinct cp genomes/DNA, separated by a loss or gain of 1 *Eco*RV restriction site and/or 1 restriction fragment length polymorphism (RFLP), were observed among the individuals of *P. deltoides* var *deltoides*. Within *P. nigra*, cpDNA of var *italica* was distinct from that of vars *nigra* and *plantierensis* by one RFLP and by a loss or gain of one *Bam*HI restriction site. *Populus maximowiczii* clones of Chinese origin were separated from those of Japanese origin by a gain or loss of one *Cla*I restriction site in their cpDNA. The estimate of nucleotide substitutions per site in cpDNA was 0.07% between two varieties of *P. deltoides*, 0.05% between var *italica* and var *nigra* or *plantierensis* of *P. nigra*, and 0.01% between Japanese and Chinese accessions of *P. maximowiczii*.

Key words Poplars · Plastid DNA · Intraspecific variation · Restriction fragment polymorphisms

Introduction

Chloroplast DNA (cpDNA) analysis, because of the many suitable features of the chloroplast (cp) genome, such as small size, evolutionary conservatism, predominant uniparental inheritance, and abundance of cpDNA in plant tissues, has been increasingly used for genetic, evolutionary, phylogenetic, and biosystematic studies, especially at the species or higher level, in plants, including forest trees (Palmer 1987; Palmer et al. 1988; Crawford 1990; Clegg and Zurawski 1992; Strauss et al. 1992; Wagner 1992). As the chloroplast genome displays evolutionary conservatism, little or no intraspecific cpDNA variability is generally assumed in plants, which has led many investigators to use one or only a few samples of each species, including *Populus* L. species (Smith and Sytsma 1990), to draw evolutionary and phylogenetic conclusions (reviews in Harris and Ingram 1991; Soltis et al. 1992). However, as intraspecific cpDNA variation has been reported for a wide taxonomic spectrum of plant species ranging from ferns to conifers to a diversity of angiosperms (reviews in Harris and Ingram 1991; Soltis et al. 1992), it may be more common and more extensive than initially believed. In forest trees, most studies that have attempted to identify intraspecific cpDNA variation have succeeded (Wagner 1992). The presence of intraspecific cpDNA variation has implications for various genetic, evolutionary, phylogenetic, and biosystematic studies. Thus, an assessment of intraspecific cpDNA diversity in plants is of fundamental importance.

Members of the genes *Populus* (Salicaceae) are fast-growing and multipurpose trees (FAO 1979; Dickmann and Stuart 1983). *Populus deltoides* Bartr. ex Marsh. (Section *Aigeiros* Duby), *P. nigra* L. (Section *Aigeiros*), and *P. maximowiczii* Henry (Section *Tacamahaca* Spach.) are important for the breeding hybrid poplar varieties for intensive culture programs (FAO 1979; Dickmann and Stuart 1983). *Populus deltoides*, cottonwood, with a wide natural range in the United States and southern parts of Canada, consists of two main varieties or subspecies: *P. deltoides* var *deltoides* (synonyms *P. deltoides* ssp. *deltoides*, *P. angulata* Ait.; eastern cottonwood) and *P. deltoides* var *occidentalis* Rydb. (synonyms *P. sargentii* Dode, *P. deltoides* ssp. *monilifera* Ait.; plains cottonwood) (Schreiner 1971; Eckenwalder 1977). These varieties can be distinguished by their

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morphological characters and natural ranges (Maini 1968; Schreiner 1971; Eckenwalder 1977; FAO 1979). *Populus nigra*, European black poplar, has its natural range in Europe and western Asia, and consists of many varieties (Zsuffa 1974), including var *nigra* (the typical variety) and var *italica* Duroi (the oldest variety described). A summary, brief description, and the distinguishing features of *P. nigra* varieties and their synonyms are provided in Zsuffa (1974). No varieties have yet been described in *P. maximowiczii* (Japanese poplar), which is naturally distributed along the Pacific coast of Japan, China, and Korea (Chiba 1984).

Previous studies, have identified substantial intraspecific allozyme variation in *P. deltoides* (Rajora 1989a; Rajora et al. 1991), *P. nigra* (Rajora 1989b), and *P. maximowiczii* (Rajora 1988). Also, mitochondrial DNA has been found to exhibit intraspecific (intervarietal) variation within *P. deltoides* (Barrett et al. 1993). However, no intraspecific variation of cpDNA could be detected in *P. deltoides* and *P. nigra* by Smith and Sytsma (1990). On the other hand, substantial intraspecific cpDNA was observed in three species of the sister genus *Salix* L. (Brunsfeld et al. 1992). No information has yet been reported on cpDNA variation in *P. maximowiczii*.

The overall objective of the study described here was to determine intraspecific cpDNA variation within *P. deltoides*, *P. nigra*, and *P. maximowiczii*, and the interspecific cpDNA variation and genetic relationships of these *Populus* species and *P.* × *canadensis* Moench by restriction fragment analysis of cpDNA. Cloned cpDNA fragments from *Petunia hybrida* were used as heterologous probes for the cpDNA of *Populus*. In this paper, we report the methods of cpDNA analysis, and the results on intraspecific cpDNA variation, which was found to be intervarietal as well as intravarietal in *P. deltoides*, intervarietal in *P. nigra*, and geographic in *P. maximowiczii*. The results on interspecific cpDNA variation and genetic relationships are reported in an accompanying paper (Rajora and Dancik 1995).

Materials and methods

Poplar species and individuals

Thirty-five individuals of *P. deltoides*, *P. nigra*, and *P. maximowiczii* of diverse origins from three different continents (Table 1) were sampled. *Populus deltoides* samples consisted of 10 individuals belonging to var *deltoides* and 4 individuals belonging to var *occidentalis*. *Populus nigra* samples included 11 individuals of var *nigra* and 1 individual each of var *italica* and var *plantierensis*. Three *P. maximowiczii* individuals originated in the People's Republic of China, and 5 in Japan.

Dormant shoot cuttings of the sampled poplar individuals were collected in March 1989 from the Ontario Forest Research Institute (OFRI), Ontario Ministry of Natural Resources, Maple, Ontario. These shoot cuttings were rooted in a greenhouse at the University of Alberta.

DNA extractions, restriction, electrophoresis, and Southern blotting

Tissues of newly-emerged leaves from the shoot sprouts of the rooted cuttings were used for DNA extractions. Total cellular DNA was extracted from 1.0–1.5 g fresh weight of leaf tissue of each individual by the following modifications of the CTAB DNA isolation method of Doyle and Doyle (1987): (1) 10 ml of the extraction buffer was used instead of 8 ml per sample, (2) a higher concentration (25 m*M* in place of 20 m*M*) of EDTA was used in the extraction buffer, (3) extraction of lysate was done with chloroform: octanol (24:1) solution in place of chloroform: isoamyl alcohol (24:1) mixture, and (4) after extraction with chloroform: octanol solution, centrifugation was done at 9,000 rpm for 15 min at 15 °C.

Total DNA (approximately 5 μg) of each individual plant was digested with 10–15 units of the following restriction enzymes: *Ava*I, *Bam*HI, *Bcl*I, *Bgl*II, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I for 4–5 h according to the

Table 1 *Populus* species and individuals analyzed

Species	Individual clones		Origin	Reference for details of origin
	Number	Accession code[a]		
P. deltoides	14			
var *deltoides*	10	D17, D32, D36, D37, D43, D56, D68, D70, D109, and D121	Canada, USA	Rajora 1989a
var *occidentalis*	4	D87[b], D155, D172, and D173	Canada, USA	Rajora 1989a
P. nigra	13			
var *nigra*	11	N13, N19, N20, N29, N40, N85, N96, N100, N102, N166, and N167	The Netherlands, Czechoslovakia, Hungary, France, Germany	Rajora 1989b
var *italica*	1	N84	Germany	Rajora 1989b
var *plantierensis*	1	N92	Germany	Rajora 1989b
P. maximowiczii	8			
	3	M2, M4, and M5	Peoples Republic of China	Rajora 1988
	5	M10, M11, M12, M13, and M15	Japan	Rajora 1988

[a] Clones were registered in these accession codes at the Ontario Forest Research Institute, Ontario Ministry of Natural Resources, Maple, Ontario

[b] D87 was originally classified in var *deltoides* but as this clone showed chloroplast and mitochondrial DNA restriction fragment patterns that are typical of var *occidentalis*, D87 is correctly classified here in var *occidentalis* of *P. deltoides*

manufacturer's recommendations (Boehringer Mannheim, Canada). Restriction DNA fragments were separated on 0.7% agarose gels and then transferred on to nylon membranes (Rajora and Dancik 1992).

Chloroplast DNA probes, hybridization, and autoradiography

The following six cloned cpDNA fragments of *Petunia hybrida* (Palmer et al. 1983) were used as hybridization probes for *Populus* cpDNA: P3, a 21-kb *Pst*I fragment from the large single-copy (LSC) region containing a part of the *rbc*L gene; P6, a 15.3-kb *Pst*I fragment from the LSC region; P8, a 9.2-kb *Pst*I fragment from the LSC region; P10, a 9.0-kb *Pst*I fragment from the LSC region containing a part of the *psb*A gene; P12, a 7.6-kb *Pst*I fragment from the inverted repeat; and S8, a 11.7-kb *Sal*I fragment from the LSC region. The fragment S8 was used as a probe only with *Bam*HI, *Eco*RI, and *Eco*RV digests, whereas all of the other five probes were used with each of the 16 restriction enzymes. The designations for the *Petunia* cpDNA fragments were according to Sytsma and Gottlieb (1986). Probe preparations, prehybridizations, hybridizations, washing of the hybridized blots, autoradiograpahy, removal of probes and reprobing of the blots were carried out as described in Rajora and Dancik (1992).

Data analysis

The restriction fragment similarities (F) and nucleotide substitutions per site (d) of cpDNA were calculated in a pair-wise fashion from the restriction fragment data (Nei 1987) as a measure of nucleotide identity or divergence among varieties and/or cp genome types within a species. Unbiased estimates of intraspecific cpDNA haplotype diversity were calculated (Nei 1987).

Results

Southern blots of the restricted *Populus* DNA hybridized very strongly with all of the six *Petunia* cpDNA probes, suggesting very high homologies between cpDNAs of *Populus* and *Petunia*. This observation led us to believe that the *Petunia* cpDNA probes hybridized with the cpDNA of *Populus* in the same region of the cp genome as the location of the *Petunia* cpDNA probes (fragments). Therefore, the observed restriction fragment polymorphisms in *Populus* were tentatively assigned to the same region as the cp genome location of the *Petunia* cpDNA probes. However, restriction site mapping of the cp genome in *Populus* species is required to confirm this tentative assignment of the restriction fragment polymorphisms.

Populus deltoides

Two hundred and seventy-eight restriction sites with a total of 281 different cpDNA restriction fragments were revealed by 83 restriction enzyme-probe combinations in *P. deltoides*. This species showed both intervarietal and intravarietal cpDNA variation (Table 2, Fig. 1).

Table 2 Intraspecific chloroplast DNA (cpDNA) restriction fragment variants observed in *P. deltoides*, *P. nigra*, and *P. maximowiczii* (*LSC* large single copy, *IR* inverted repeat, – absent)

	Restriction enzyme	Probe	Cp genome region	CpDNA fragment variants (size in kb)	Variety/clone
Populus deltoides					
(A) Intervarietal polymorphisms					
	*Cla*I	P6	LSC	3.2	var *occidentalis*
				3.1	var *deltoides*
	*Eco*RI	P6	LSC	1.0	var *occidentalis*
				0.9	var *deltoides*
	*Kpn*I	P3	LSC	7.0	var *occidentalis*
				5.2, 1.8	var *deltoides*
(B) Intravarietal polymorphisms in var *deltoides*					
	*Bam*HI	P10	LSC	1.7	Clone D121
				1.6	Clones D17, D32, D36, D37, D43, D56, D68, D70, and D109
	*Eco*RV	P10	LSC	13.4	Clones D37, D68, D70, D109, and D121
				–	Clones D17, D32, D36, D43, and D56
Populus nigra					
	*Bam*HI	P12	IR	4.3	var *italica*
				3.2, 1.1	var *nigra*, and var *plantierensis*
	*Sma*I	P6	LSC	26.5	var *nigra*, and var *plantierensis*
				21.0	var *italica*
Populus maximowiczii					
	*Cla*I	P6	LSC	40.5	M2, M4, and M5
				–	M10, M11, M12, M13, and M15

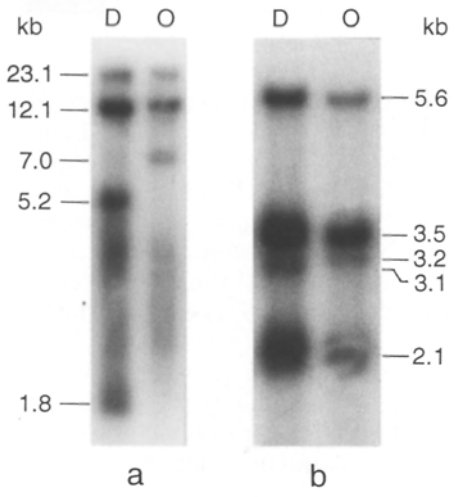


Fig. 1a, b Restriction fragment patterns demonstrating intraspecific intervarietal cpDNA variation in *Populus deltoides*. *D P. deltoides* var *deltoides*, *O P. deltoides* var *occidentalis*. **a** *Populus deltoides* DNA was restricted with *Kpn*I, and Southern blots of the restriction digests were hybridized with the 21.0-kb *Petunia* cpDNA fragment P3. **b** DNA was restricted with *Cla*I and hybridized with the 15.3-kb *Petunia* cpDNA fragment P6

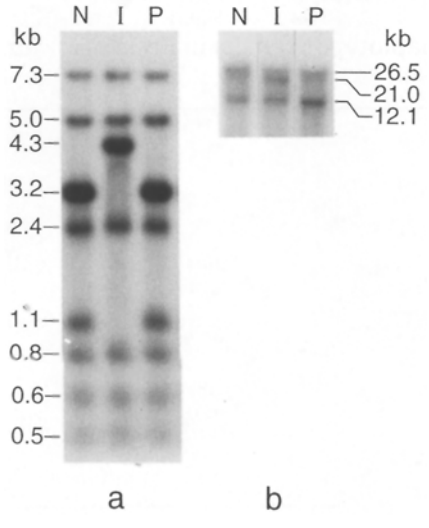
There were 278 restriction sites with 280 different restriction fragments of cpDNA in *P. deltoides* var *deltoides* individuals, and 278 restriction sites with 279 different restriction fragments of cpDNA in *P. deltoides* var *occidentalis* individuals. Of these, 276 restriction sites/fragments were common between the two varieties. The cp genomes of these two *P. deltoides* varieties (*deltoides* and *occidentalis*) were distinct from each other by the gain or loss of one *Kpn*I site in the LSC region and 1 RFLP each identified by *Cla*I and *Eco*RI restriction enzymes (Table 2, Fig. 1). As the same RFLP of 100 bp between var *deltoides* and var *occidentalis* was evident with *Cla*I or *Eco*RI, this may represent only a single fragment length mutation of a deletion or gain of 100 bp. Restriction site mapping will be necessary to confirm this assumption. Clone D87 representing cv 'Lydick', originally classified with var *deltoides*, showed cpDNA restriction fragment patterns typical of var *occidentalis*. Thus, this clone was correctly classified with var *occidentalis*. The cpDNA restriction fragment similarity and nucleotide substitutions per site (nucleotide diversity) between var *deltoides* and var *occidentalis* were 0.9875, and 0.0007, respectively.

Three distinct cp genomes were observed among 10 individuals within var *deltoides* of *P. deltoides* (Table 2). These cp genomes differed from each other by the gain or loss of one *Eco*RV restriction site and/or one *Bam*HI-P10 RFLP in the cp genome region homologous to the 9.0-kb *Petunia* cpDNA fragment P10 (Table 2). Clone D121 from Illinois had a distinct cp genome. The second cp genome type was shared by clones D37 (Ontario), D68 (Indiana), D70 (Illinois), and D109 (Mississippi). The third cp genome type was shared by clones D17, D32, D36, D43, and D56, all from Ontario. CpDNA variation was not associated with the sex of the clone. Among the three cpDNA types within var *deltoides*, cpDNA restriction fragment similarities ranged from 0.9946 to 0.9982 with an average of 0.9964, and nucleotide substitutions per site ranged from 0.0001 to 0.0003 with an average of 0.0002. The unbiased estimate of cpDNA haplotype diversity within *P. deltoides* var *deltoides* was 0.61.

Populus nigra

In *P. nigra* cpDNA, 272 restriction sites with a total of 274 different restriction fragments were yielded by 83 restriction enzyme-probe combinations. Two distinct cp genome types were observed among 13 individuals of this species. Variety *italica*, represented by clone N84, had a cp genome distinct from that of the other two varieties, var *nigra* and var *plantierensis*, represented by 12 individuals (Table 1 and 2, Fig. 2). No cpDNA variation was detected between var *nigra* and var *plantierensis*, and among 11 individuals of var *nigra*. The cpDNA of var *italica* differed from that of var *nigra* or var *plantierensis* by the gain or loss of one *Bam*HI restriction site (Table 2, Fig. 2a) in the inverted repeat (IR) region and 1 RFLP revealed by the *Sma*I-P6 enzyme-probe combination in the LSC region (Table 2, Fig. 2b). Whether this RFLP was the result of an insertion/deletion event or genome rearrangement is unclear. Eighty-three restriction enzyme-probe combinations revealed 271 restriction sites/fragments in the cpDNA of var *italica* and 272 in the cpDNA of var *nigra* or *plantierensis*. Of these, 269 restriction sites/fragments were shared by all three *P. nigra* varieties. The cpDNA restriction fragment identity and nucleotide substitutions per site

Fig. 2a, b Restriction fragment patterns demonstrating intraspecific intervarietal cpDNA variation in *Populus nigra*. *N P. nigra* var *nigra*, *I P. nigra* var *italica*, *P P. nigra* var *plantierensis*. *P. nigra* DNA was restricted with *Bam*HI (**a**) and *Sma*I (**b**) and hybridized with the 7.6-kb *Petunia* cpDNA fragment P12 (**a**) and the 15.3-kb *Petunia* cpDNA fragment P6 (**b**)



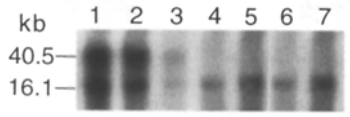


Fig. 3 Chloroplast DNA restriction fragment variation in *P. maximowiczii* revealed by hybridization of *Sma*I restriction digests of its DNA with the 15.3-kb *Petunia* cpDNA fragment P6

between var *italica* and var *nigra* or var *plantierensis* were 0.9907, and 0.0005, respectively.

Populus maximowiczii

A total of 282 restriction sites/fragments was observed in cpDNA of *P. maximowiczii* with 83 restriction enzyme-probe combinations. Two distinct cpDNA types were observed among 8 individuals of this species (Table 2, Fig. 3), indicating regional geographic variation. Five *P. maximowiczii* individuals of Japanese origin had a cp genome distinct from that of the 3 *P. maximowiczii* individuals of Chinese origin by a loss or gain of a *Cla*I restriction site in the cp genome region homologous to the 15.3-kb *Petunia* cpDNA fragment P6 (Table 2, Fig. 3). To verify that the 40.5-kb *Sma*I-P6 cpDNA fragment in *P. maximowiczii* clones of Chinese origin was actually representing a restriction site difference and was not an artifact due to partially- or undigested DNA, we restricted DNAs of all *P. maximowiczii* individuals with two-fold (2–4 units/μg of DNA), five-fold, (5–10 units/μg of DNA), and 10-fold (10–20 units/μg of DNA) concentrations of *Sma*I in two independent replications. Southern blots of these restriction digests were then hybridized to *Petunia* cpDNA probe P6. In each case, identical results were obtained, showing the same restriction site difference. No cpDNA variation was detected among individuals of the same origin. Of the total 282 restriction sites/fragments, 281 were shared by all 8 individuals of *P. maximowiczii*. CpDNA restriction fragment identity and nucleotide substitutions per site between Japanese and Chinese accessions were 0.9982, and 0.0001, respectively. An unbiased estimate of cpDNA haplotype diversity in *P. maximowiczii* was 0.50.

Discussion

The existence of intraspecific cpDNA was clearly evident in all three *Populus* species, even though a relatively small number of individuals per species was examined. Variation in cpDNA was found to be intervarietal as well as intravarietal in *P. deltoides*, intervarietal in *P. nigra*, and geographic in *P. maximowiczii*. Our results suggest that the cp genome of *P. deltoides* var *deltoides* is distinct from that of *P. deltoides* var *occidentalis*, the cp genome of var *italica* of *P. nigra* is distinct from that of the other two *P. nigra* varieties (var *nigra* and var *plantierensis*, which share the same cp genome), and the cp genome of *P. maximowiczii* accessions from the Peoples Republic of China is distinct from that of *P. maximowiczii* accessions from Japan. Without knowledge of the intraspecific cpDNA variation reported here, in particular that observed between var *deltoides* and var *occidentalis* of *P. deltoides* with *Cla*I-P6 and *Eco*RI-P6 enzyme-probe combinations, interspecific variation among the *Populus* species (Rajora and Dancik 1995) could have been misinterpreted. Two varieties of *P. deltoides* have also been found to have distinct mitochondrial genomes in and around the *coxI* region (Barrett et. al. 1993), and Chinese and Japanese accessions of *P. maximowiczii* are also distinct for nuclear allozyme genes (Rajora 1988). The results are in contrast with those of Smith and Sytsma (1990) for *P. deltoides* and *P. nigra*. No variation in cpDNA was detected by Smith and Sytsma (1990) among three varieties [vars *nigra*, *italica* and *betulifolia* (Pursh) Torr.] of *P. nigra*, and between two varieties (*deltoides* and *occidentalis*) of *P. deltoides*.

In *P. deltoides* and *P. nigra*, restriction site and restriction fragment length mutations accounted for about one-third to one-half of all intraspecific cpDNA variation. This is in agreement with the nature of intraspecific variation observed in other plants (Soltis et al. 1992). However, in *Salix* species, higher proportions of restriction site to restriction fragment length mutations have been observed (Brunsfeld et al. 1992). Intraspecific nucleotide diversity estimates of 0.01% in *P. maximowiczii*, 0.05% in *P. nigra*, and 0.07% in *P. deltoides* are in agreement with the magnitude of intraspecific cpDNA sequence diversity in *Salix* (Brunsfeld et al. 1992) and other plants (Soltis et al. 1992).

It was not possible to uniquely distinguish the two varieties of *P. deltoides* and three varieties of *P. nigra* examined on the basis of variation in allozyme genes that could be assigned as varietal-specific (Rajora 1989a,b). Variation in the restriction fragment patterns of cpDNA not only allowed a clear distinction between var *deltoides* and var *occidentalis* of *P. deltoides*, and that of var *italica* from the other two *P. nigra* varieties, but also allowed correct classification of the clone D87 representing cv 'Lydick' with *P. deltoides* var *occidentalis*. This cultivar also showed restriction fragment patterns of mitochondrial DNA that were typical of *P. deltoides* var *occidentalis* (Barrett et al. 1993). 'Lydick' had been classified with *P. deltoides* var *deltoides*, presumably on the basis of morphological characters. The two *P. deltoides* varieties (*deltoides* and *occidentalis*) can be distinguished by morphological characters of leaves and winter buds (FAO 1958; Maini 1968; Eckenwalder 1977). However, intervarietal variation in these characters sometimes may not be unambiguous. Evidently, variation in cpDNA provides more reliable molecular markers for the unambiguous distinction of *P. deltoides* and *P. nigra* varieties.

Populus nigra var *italica*, which is considered to have originated in central Asia (Zsuffa 1974), has a distinct cp genome (this study) and is also distinct morphologically

from the other *P. nigra* varieties by its fastigiate form, branching habit, and bark and leaf characters (FAO 1958; Zsuffa 1974). *Populus nigra* var *plantierensis* of fastigiate form is considered to have originated in the Simon-Louis Nurseries in France in 1868 as a result of a cross between var *italica* and var *betulifolia* (Zsuffa 1974). Based on our cpDNA variation data in *P. nigra* (this study), and uniparental-maternal inheritance of the cp genome in *P. nigra* crosses (Rajora and Dancik 1992), we believe that var *italica* is not likely the female parent of var *plantierensis*.

Variation of cpDNA within *P. maximowiczii* clearly followed a geographic pattern. Within *P. deltoides* var *deltoides*, with the exception of one clone (D37), all clones from Ontario shared the same distinct cp genome. In *Salix*, most cpDNA variability represented geographic patterns of divergence (Brunsfeld et al. 1992). These observations suggest that cpDNA variation could potentially be used for population genetic analysis in *Populus*.

Populus deltoides var *occidentalis* is distinct from *P. deltoides* var *deltoides* for morphological characters and natural range (FAO 1958, 1979; Maini 1968; Schreiner 1971; Eckenwalder 1977), mitochondrial genome (Barrett et al. 1993), and even for the evolutionary conservative chloroplast genome (this study). *Populus deltoides* var *occidentalis* has been treated taxonomically in various ways. In addition to being known as a variety, it has also been described as a separate species, *P. sargentii* (FAO 1958, 1979; Schreiner 1971) and a subspecies *P. deltoides* ssp. *monilifera* (Eckenwalder 1977). However, the nomenclature of var *occidentalis* is the most commonly used (FAO 1958, 1979; Maini 1968; Schreiner 1971; Dickmann and Stuart 1983). Subspecies *monilifera* also refers to the forms of *P. deltoides* other than var *occidentalis* (FAO 1979; Dickmann and Stuart 1983). Therefore, we suggest that the two *P. deltoides* varieties should be recognized as subspecies as previously suggested by Eckenwalder (1977) but that var *occidentalis* should be named as *P. deltoides* ssp. *occidentalis*.

In conclusion, our study identified the existence of substantial intraspecific variation of cpDNA in *Populus* species and highlights the importance of examining intraspecific cpDNA variation in multiple samples of a taxon.

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