Theor Appl Genet (1998) 96: 950–956

S. Dayanandan · O. P. Rajora · K. S. Bawa

Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*)

Received: 14 November 1997 / Accepted: 17 November 1997

Abstract We have identified, isolated, and characterized microsatellite/simple sequence repeat (SSR) loci in trembling aspen (*Populus tremuloides*) by screening partial genomic libraries. We have also examined the compatibility and use of the *P. tremuloides* SSR primers to resolve microsatellites in other *Populus* species. Fourteen microsatellites were identified from 1600 clones screened. The TC/AG microsatellites were the most abundant. A total of 29 alleles were detected in 36 *P. tremuloides* individuals at the four SSR loci (two each of di- and tri-nucleotide repeats) characterized. The number of alleles at the SSR loci ranged from 5 to 11, with an average of 7.25 alleles per locus, and the observed heterozygosity ranged from 0.19 to 0.82, with a mean of 0.46 per locus. Although the highest polymorphism was observed for a dinucleotide SSR locus, the trinucleotide SSR loci showed substantial polymorphism. There were 34 unique multilocus genotypes among the 36 *P. tremuloides* individuals examined, and 89% of the individuals had unique multilocus genotypes. Two pairs of SSR primers were successful in PCR, amplifying genomic DNA and resolving microsatellites of comparable size from *Populus deltoides*, *P. nigra*, *P.* × *canadensis*, and *P. maximowiczii*. The microsatellite DNA markers developed could be used for clonal fingerprinting, certification of controlled crosses, genome mapping, marker-assisted early selection, genetic diversity assessments, and conservation and sustainable management of poplar genetic resources.

Key words Poplars (*Populus*) · Simple sequence repeats · Microsatellite loci · Polymorphism · Clone identification

Communicated by H. F. Linskens

S. Dayanandan[1] · K. S. Bawa
Biology Department, University of Massachusetts,
100 Morrisey Blvd, Boston, MA 02125, USA

O. P. Rajora (✉)
Department of Renewable Resources, 751 General Services Building, University of Alberta, Edmonton, Alberta, T6G 2H1 Canada
Fax: +1-403 492-4323
E-mail: om.rajora@ualberta.ca

Present address:
[1] Department of Renewable Resources, 751, General Services Building, University of Alberta, Edmonton, Alberta T6G 2H1

Introduction

Trembling aspen *(Populus tremuloides* Michx.) is a fast-growing, widespread tree species in North America, and an important source of wood for pulp and paper and oriented stranded board industries. In the last decade, its utilization in the Canadian province of Alberta alone has increased tenfold and is expected to increase significantly in the near future (Ondro 1991). This increasing commercial demand for trembling aspen wood may deplete its existing stock in natural forests unless the quality and productivity of regenerating stocks are improved (Rajora 1991). The precocious flowering, profuse vegetative reproduction through suckering, and relatively easy methods for controlled pollination and propagation through tissue culture techniques make *P. tremuloides* an ideal candidate for genetic improvement through breeding and genetic engineering. Highly informative genetic markers are essential for assisting genetic improvement processes, such as clonal identification, certification of controlled crosses, identification of species and hybrids, genome mapping, and marker-assisted early selection. Such markers are also valuable in assessing genetic diversity and structure and genetic effects of harvesting and natural disturbances, and in developing effective strategies for conservation and sustainable management of forest genetic resources.

A relatively new class of DNA markers with high allelic diversity, known as microsatellites or simple

sequence repeats (SSRs) (Weber and May 1989; Hearne et al. 1992) are now available for many animal and plant species. These DNA markers have also been developed for a small number of forest tree species (Smith and Devey 1994; Kostia et al. 1995; Echt et al. 1996; Chase et al. 1996; Van de Ven and McNicol 1996; Pfeiffer et al. 1997). However, there is no information on microsatellite markers in *Populus tremuloides* or any other *Populus* species. Their hypervariability, repeatability, and codominance make SSRs ideal for genome mapping as well as for population genetic studies. SSRs are being used in genome mapping of human (Weber 1990), trees (Devey et al. 1996; Echt and Nelson 1997) and many other organisms, as well as in population and conservation genetics of animals (e.g., Paetkau and Strobeck 1994) and plants (e.g., Chase et al. 1996). The utility of SSRs as genetic markers for tree species has been somewhat limited due to a paucity of available sequence data as well as the specificity of SSRs to a given taxon. However, it has been documented that SSR primers characterized for a given species could be used to detect polymorphism in related species, minimizing laborious steps of cloning and screening (Dayanandan et al. 1997).

Here we report the results of a study aimed at the identification, isolation, and characterization of SSR loci in *Populus tremuloides* by screening genomic libraries. We have estimated the genomic frequency of the identified SSRs and surveyed allelic diversity at four loci. Utility of the characterized SSRs in *P. tremuloides* clonal identification was assessed by constructing multilocus genotypes of each individual. We also examined the cross compatibility and possible use of the *P. tremuloides* SSR primers in resolving microsatellite loci in three other *Populus* species and an interspecific hybrid.

Materials and methods

Plant material and DNA extraction

Thirty-six individuals of *P. tremuloides* Michx. selected from 12 different locations distributed in the geographic range from 52°56′ to 57°46′ in latitude, and from 114°55′ to 119°53′ in longitude, in the province of Alberta, Canada, were used for the present study (Table 1). In addition, 12 individuals belonging to three *Populus* species (*P. deltoides* Marsh., *P. nigra* L., and *P. maximowiczii* Henry) and an interspecific *P. deltoides* × *P. nigra* hybrid (*P.* × *canadensis* Moench) were selected to test the cross compatibility of SSR primers developed from *P. tremuloides* in other *Populus* species. *Populus deltoides* was represented by both var '*deltoides*' and var '*occidentalis*' Rydb. Leaf samples of the 13 individuals of *P. tremuloides* from the Leddy Lake and DMI Plantation locations were collected from the trees growing in the forest or field. The leaf samples of the remaining 23 *P. tremuloides* individuals from 11 locations (Table 1) were collected from shoot sprouts of their root cuttings propagated in a greenhouse. The leaf material of 12 individuals of the other poplars was collected from shoot sprouts of their rooted shoot cuttings.

DNA was extracted from leaf tissues of each individual following a method described in Rajora and Dancik (1995a).

Table 1 Origin of trembling aspen individuals/clones studied in Alberta

Location	Number of individuals	Latitude (N)	Longitude (W)
Sand Creek	1	52°56′	115°14′
Brazeau Tower	4	53°03′	115°30′
Parker Lake	1	55°05′	114°36′
Island Creek	3	55°09′	115°20′
Swan River	3	55°10′	115°20′
Mooney Creek	3	55°18′	114°55′
Wagner	2	55°18′	114°55′
Boundary Lake	4	56°32′	119°53′
Carcajou	1	57°35′	117°08′
Keg River	1	57°46′	118°01′
Leddy Lake	7	56°28′	117°17′
DMI Plantation	6	56°22′	117°17′

Library construction and screening for SSR sequences

Fifty microliters (50–100 μg) DNA of an individual tree was digested with *Sau*3aI (New England Biolabs, Beverly, Mass.) in a total volume of 200 μl for 3 h at 37°C. The digested DNA was precipitated by adding 400 μl of 100% ethanol, washed with 70% ethanol, and dissolved in 30 μl of TE buffer (pH 7.4). One microgram of the M13 vector DNA (M13mp19; Life Technologies, Gaithersberg, Md.) was digested with *Bam*HI and dephosphorylated with 5 U of calf intestinal phosphatase (New England Biolabs, Beverly, Mass.) The phosphotase was deactivated by adding 0.25 μl of 0.5 *M* EDTA and heating to 75°C for 10 min. The digested DNA samples were electrophoresed on a 1% agarose gel with 1 × TBE buffer. DNA fragments between 250 and 1000 bp and M13 DNA were excised from the gel and purified with QIAquick gel extraction kit (Qiagen, Santa Clarita, Calif.).

The ligation of plant and vector DNA was performed in a total volume of 15 μl, with 4 μl of each of the gel-purified DNAs (approximately 80 ng of each DNA) and 1 μl of T4 DNA ligase (New England Biolabs, Beverly, Mass.), at 16°C for 16 h. Ligated DNA was transfected into XL1BlueMRF′-competent bacterial cells (Stratgene, Calif.) following the manufacturer's recommendations and plated on 150-mm culture plates with LB/tetracycline agar, Xgal, and IPTG. A library of about 1600 clones with a density of 200–300 plaques per plate was prepared.

Plates were blotted with Nylon membranes (Hybond N^+, Amersham) for 1 min. Membranes were baked at 80°C for 2 h, soaked in 2 × SSC for 10 min and washed with 5 × SSC, 0.5% SDS for 1 h. Membranes were prehybridized overnight in a plastic bag (six membranes per bag) containing 150 ml of hybridization medium (150 ml 20 × SSC, 25 ml 100 × Denhardts, 5 g BSA, and 2 g SDS dissolved in a total volume of 500 ml with distilled water). Following prehybridization, the hybridization medium in the bag was replaced with 75 ml of fresh hybridization medium, and 80 μl of the probe (prepared as given below) denatured at 90°C was added and incubated in a shaking incubator for 4–12 h at 45°–55°C, depending on the probe used. Hybridized blots were washed twice with 150 ml of 0.1% SDS and 6 × SSC at room temperature, and then with 500 ml of 0.1% SDS and 6 × SSC for 10 min at 45°C. The washed, hybridized blots were exposed to autoradiographic film after blot drying.

We used oligonucleotides (AAAG)7, (AGAT)7, (AAAT)7, (AT)15, (AG)15, (ACG)10, (CA)15, (TC)15 and (TG)15 as probes. Each probe (20 pmol) was endlabelled with 1.11×10^6 Bq of γ-AT[^{32}P] (Amersham) using T4 Polynucleotide Kinase (Epicentre Technology) at 37°C for 30 min in a total volume of 30 μl, and the enzyme was inactivated by heating to 75°C for 10 min. The labelled probe was purified using Qiagen nucleotide removal columns (Qiagen, Calif.), following the addition of 50 μl distilled water.

Sequencing of positive clones, designing primers, and resolving SSR polymorphisms

Positive plaques were picked up with a sterile pipette tip and placed into 15-ml culture tubes containing 2 ml of LB/tetracycline and 100 µl of overnight-grown XL1BlueMRF′ bacterial cells. Tubes were incubated at 37°C with shaking for 6 h. A portion of each culture was transferred to 2-ml microfuge tubes and centrifuged at maximum speed (14000 rpm) for 5 min. Single-stranded DNA was isolated from the supernatant using the Wizard M13 DNA purification system (Promega, Madison, Wis). Purified DNA was sequenced using the ABI prism 377 automated DNA sequencer (Perkin Elmer – Applied Biosystems).

Oligonucleotide primers complementary to flanking regions of identified repeats were synthesized (Operon Technologies, Alameda, Calif.), and microsatellites in different *P. tremuloides* individuals were studied using the polymerase chain reaction (PCR). Amplification reactions were performed in a total volume of 15 µl, with 0.2 m*M* dNTP, 2 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* TRIS-HCl (pH 9.0), 0.1% Triton X-100, 2.5 pmol of each primer and 3.7×10^4 Bq of α-dCT [^{32}P]. After an initial denaturation for 1 min at 94°C, 5 cycles at 94°C (denaturation), 55°C (anealing), and 72°C (extension) were performed, each step for 1 min, followed by 30 cycles, with each step for 30 s. PCR reactions were repeated with different annealing temperatures (from 55°C to 66°C) to determine the optimal PCR conditions. After thermal cycling, 10 µl of stop solution (Xylene cyanol, Bromophenol blue in deionized formamide) was added. Amplified products (4 µl each) were electrophoresed on 6% denaturing polyacrylamide gel with 6 M urea and 1 × TBE. Electrophoresis was performed on a S2 electrophoretic apparatus (Owl Scientific, Woburn, Mass.) for about 4 h at 1750 V and 60 W. Radioactively labelled 10-bp ladder DNA (Research Genetics) was used as a size standard.

Table 2 SSR sequences identified in a partial genomic library of *P. tremuloides*

Repeat sequence
$(GGT)_5(N)_{45}(AGG)_9$
$(TGG)_8$
(TGG)5
(TC)11
(TC)17
(TC)8
(TG)7
(A)9CTG(A)6
(AAAG)3
(A)7TAC(A)9
(AT)8
(TC)5 A(TC)8
(A)11(TC)8
(AT)3(A)18(AG)5

Data analysis

The genomic distribution frequency of the SSRs was estimated by dividing the estimated total length of the cloned genomic library by the observed number of a given SSR. Informativeness of each locus was evaluated by calculating the number and frequency of individual alleles, and the observed heterozygosity. Single- and multilocus genotypes of each individual were determined to identify unique genotypes in order to assess the utility of the characterized SSRs in genetic fingerprinting of clones.

Results

The screening of a partial genomic library of *P. tremuloides* consisting of about 1600 clones with an average insert size of 600 bp with nine di-, tri- and tetranucleotide repeat oligonucleotide probes identified 20 positive clones. The sequencing of positive clones revealed SSRs with 3–18 repeat units in 14 clones (Table 2). The dinucleotide SSR, TC/AG, showed high abundance with 5 clones, and TG/AC showed low abundance with only 1 positive clone, suggesting respective frequencies of once in every 200 bp and 1000 bp. We isolated 3 clones with trinucleotide repeat sequences containing GGT/ACC, TGG/ACC and GGA/CCT using the dinucleotide probe (TG)15. Oligonucleotide primers were designed and PCR conditions were optimized for 4 SSR sequences (Table 3).

From 36 *P. tremuloides* individuals surveyed, a total of 29 alleles were identified at the four SSR loci. The highest diversity of 11 alleles was observed for the dinucleotide SSR locus *PTR3*. The loci *PTR1*, *PTR2*, and *PTR4* showed 5, 7 and 6 alleles, respectively (Fig. 1; Table 3). The frequency of a given allele varied from the lowest value of 0.01 at the most polymorphic *PTR3* locus to the highest value of 0.60 at the locus *PTR4* (Table 4). The most polymorphic locus, *PTR3,* showed the highest heterozygosity value of 0.82, and the locus *PTR4* showed the lowest heterozygosity value of 0.19 (Table 3). Among the 36 individuals examined using four SSR loci, a total of 34 multilocus genotypes (about 95% of the total) were observed. Thirty-two individuals, or 89% of the total individuals, had unique genotypes, and the remaining 2 genotypes, were shared by two individuals each.

Table 3 Repeat pattern, primer sequence, annealing temperature (T), number of alleles (A), and observed heterozygosity (Ho) for four SSR loci in *P. tremuloides*

Locus	Repeat	Primer sequence (5′ → 3′)	T (°C)	A	Ho
PTR1	$(GGT)_5N_{45}(AGG)_9$	AGCGCGTGCGGATTGCCATT TTAGTTTCCCGTCACCTCCTGTTAT	66	5	0.53
PTR2	$(TGG)_8$	AAGAAGAACTCGAAGATGAAGAACT ACTGACAAAACCCCTAATCTAACAA	63	7	0.31
PTR3	(TC)11	CACTCGTGTTGTCCTTTTCTTTTCT AGGATCCCTTCCCTTTAGTAT	60	11	0.82
PTR4	(TC)17	AATGTCGAGGCCTTTCTAAATGTCT GCTTGAGCAACAAACACACCAGATG	60	6	0.19

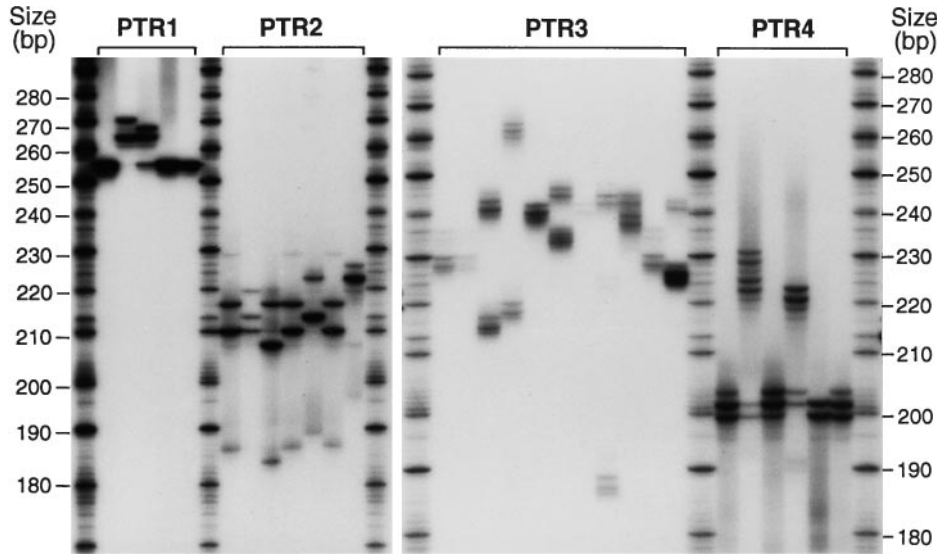

Fig. 1 Allelic variation at the four SSR loci (*PTR1*, *PTR2*, *PTR3*, and *PTR4*) in selected individuals of *P. tremuloides*

Table 4 Size and frequency of alleles for four SSR loci in 36 *P. tremuloides* individuals

Locus	Allele size	Frequency
PTR1	254	0.50
	260	0.07
	266	0.13
	269	0.25
	272	0.04
PTR2	204	0.02
	207	0.02
	210	0.22
	213	0.21
	216	0.31
	219	0.03
	225	0.19
PTR3	190	0.08
	216	0.05
	220	0.06
	228	0.15
	232	0.05
	236	0.24
	238	0.11
	242	0.11
	246	0.09
	248	0.06
	266	0.01
PTR4	200	0.60
	202	0.12
	210	0.03
	224	0.12
	226	0.07
	230	0.06

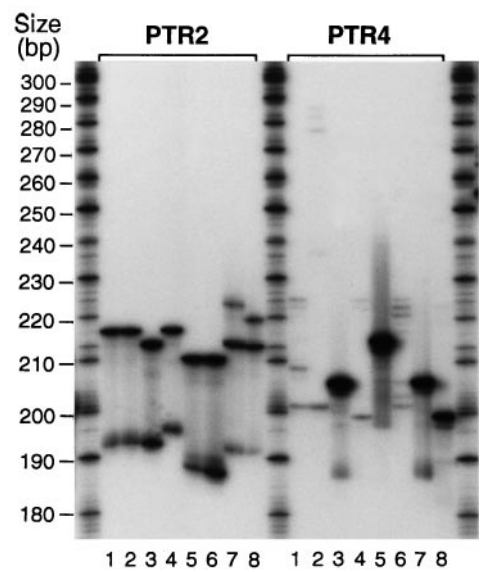

Fig. 2 PCR amplification products of genomic DNA from *Populus deltoides* (*lanes 1, 2*), *P. nigra* (*3, 4*), *P. maximowiczii* (*5, 6*), and *P.* × *canadensis* (*7, 8*) using SSR primers for the *PTR2* and *PTR4* loci developed for *P. tremuloides*

Two pairs of SSR primers developed from *P. tremuloides* showed PCR amplification products of a size comparable with that of genomic DNA from other *Populus* species (Fig. 2; Table 5). The locus *PTR2* showed amplification and resolution in both the hybrid and in all of the other three *Populus* species tested. The locus *PTR4* showed no amplification in *P. deltoides* but showed positive amplification with *P.* × *canadensis*, *P. nigra*, and *P. maximowiczii*.

Discussion

The estimated genomic frequency of TC/AG falls within the range of dinucleotide repeat frequency reported

Table 5 PCR amplification and resolution (+) of SSR loci in different species of *Populus* using primers developed for *P. tremuloides*

Species	Locus			
	PTR1	*PTR2*	*PTR3*	*PTR4*
P. deltoides	− [a]	+	−	−
P. nigra	−	+	−	+
P. maximowiczii	−	+	−	+
P. × *canadensis*	−	+	−	+

[a] −, depicts no PCR amplification and resolution of microsatellite loci

for other plant species, including forest trees (Condit and Hubbell 1991; Morgante and Olivieri 1993; Smith and Dewey 1994; Terauchi and Konuma 1994; Chase et al. 1996; Pfeiffer et al. 1997). However, TG/AC repeats were found to be about fivefold less abundant than TC/AG repeats in *P. tremuloides*. This is in agreement with the general finding that TC/AG repeats are more abundant than TG/AC repeats in plants (Wang et al. 1994; Jarret et al. 1997; Pfeiffer et al. 1997). An opposite trend with a higher abundance of TG/AC than TC/AG has been reported in primates (Jerka and Pethiyagoda 1995). Although trinucleotide SSRs are considered to be relatively less abundant (Wang et al. 1994), the detection of 3 trinucleotide SSRs using a dinucleotide probe indicates that trinucleotide SSRs may possibly be abundant in *P. tremuloides*.

The highest level of polymorphism, with 11 alleles, was observed for the locus *PTR3* with dinucleotide repeats and the lowest level of polymorphism, with 5 alleles, was detected for *PTR1*, a locus with trinucleotide imperfect repeats. Although clones with long repeats are considered to potentially contain many alleles (Weber 1990; Terauchi and Konuma 1994; Pfeiffer et al. 1997), locus *PTR3* with 11 repeat units showed higher polymorphism than the locus derived from 17 units of TC/AG repeats. Similar results where longer repeats do not necessarily show high polymorphism have been reported for *Pinus* (Echt et al. 1996). Contrary to the general view that trinucleotide repeats are less polymorphic than dinucleotide repeats, two trinucleotide repeat loci in *P. tremuloides* (*PTR1* and *PTR2*) showed a considerable level of polymorphism. The locus *PTR2*, which had only 8 repeat units in the clone, showed a relatively high level of polymorphism with a total of 7 alleles. Thus, trinucleotide repeat SSR loci are quite highly polymorphic in *P. tremuloides*.

The presence of high levels of unique multilocus genotypes makes SSRs a valuable molecular tool in the fingerprinting and identification of *P. tremuloides* clones. Isozymes have been used to identify clones in several poplar species (Rajora 1988, 1989a, b; Rajora and Zsuffa 1989; Rajora and Dancik 1992a), where 77–100% of the clones could be uniquely identified based on their multilocus allozyme genotypes. However, the allozyme multilocus genotypes in these cases were based on 9–17 polymorphic loci. In the present study, 89% of the *P. tremuloides* individuals could be uniquely identified using just only four SSR loci. The 2 clones that shared one multilocus genotype were from the same population (Island Creek), whereas the 2 other clones which shared another multilocus genotypes were from 2 distant populations (Island Creek and Boundary Lake). The 2 individuals from the same Island Creek population, sharing the same four-locus genotype, are evidently highly genetically related. The same multilocus genotype shared by 1 individual each from the Island Creek and Boundary Lake seems to be coincidental because these populations are geographically distant . We believe that these clones could also be uniquely identified using additional SSR loci which we are at present isolating and characterizing. Trembling aspen is highly genetically variable in Alberta (Cheliak and Dancik 1982; Rajora and Dancik 1995b). Each of the 36 individuals included in our study was genetically distinct based on random amplified polymorphic DNA (RAPD) analysis, where a total of 564 RAPD markers/loci were studied using 31 primers (Rajora and Dancik 1995b). All the individuals could be distinguished by a combination of 2 or more RAPD primers. However, each primer produced 7–28 products, each assumed to be controlled by a single locus, with an average of 18.2 loci per primer. Therefore, compared to allozyme or RAPD markers for the same *P. tremuloides* clones studied, SSR markers show higher polymorphism and, thus, are more informative per locus basis for clonal identification.

The positive amplification of DNA and resolution of microsatellite loci from different species of *Populus* using SSR primers developed for trembling aspen indicates that these primers could potentially be used for genetic studies of related *Populus* species. It has been shown that SSRs developed for a given species could be used with related species of animals (Moore et al. 1991; Schlotterer et al. 1991; Levine et al. 1995) and plants (Wu and Tanksley 1993; Kijas et al. 1995; Dayanandan et al. 1997). However, the success of cross-species amplification and resolution of microsatellite loci depends on the evolutionary relatedness of the taxa being sampled (Dayanandan et al. 1997). Although locus *PTR2* showed amplification in all of the *Populus* species tested, locus *PTR4* showed amplification in all but *P. deltoides*, indicating that *P. nigra* and *P. maximowiczii* are more closely related to *P. tremuloides* than the evolutionary relatedness between *P. tremuloides* and *P. deltoides*. This is in agreement with the genetic distances based on mitochondrial DNA restriction fragment data (Barrett et al. 1993), which suggested that the evolutionary relatedness of *P. nigra* and *P. maximowiczii* to *P. tremuloides* is closer than the evolutionary relatedness between *P. deltoides* and *P. tremuloides*. *Populus* × *canadensis* is a hybrid between *P. deltoides* and *P. nigra* and has nuclear genomic compliments

from both the parental species (Rajora and Zsuffa 1989), and its organellar genomic compliment from its maternal parent *P. deltoides* (Barrett et al. 1993; Rajora and Dancik 1995c). As the SSR locus *PTR4* was amplified in *P. nigra*, and not in *P. deltoides*, its amplification in *P.* × *canadensis* was not unexpected because the *PTR4* primers could have amplified the *P. nigra* genomic compliment in this hybrid. This would only be possible if the *PTR4* SSR was located in the nuclear genome of *P. nigra* because the organellar genomes are maternally inherited in *P. deltoides* × *P. nigra* hybrids (Rajora and Dancik 1992b; Rajora et al. 1992).

In conclusion, we have for the first time isolated and characterized microsatellite loci in *P. tremuloides*. This may be the first report of microsatellites in any *Populus* species. Certain SSR primers developed for *P. tremuloides* could also be used for resolving microsatellite loci in *P. deltoides*, *P. nigra*, *P.* × *canadensis*, and *P. maximowiczii*. The microsatellite markers developed could be used in *Populus* for clonal fingerprinting, as demonstrated in this study, and also for certification of controlled crosses, identification of species and hybrids, genome mapping, marker-assisted early selection, genetic diversity assessment, and the conservation and sustainable management of genetic resources. We are continuing to isolate and characterize additional microsatellite loci in *P. tremuloides* and other *Populus* species.

Acknowledgements The authors thank Kristen Long and Amy Tucker for technical assistance. The research was funded by a Natural Sciences and Engineering Research Council of Canada Operating (Individual) Research Grant (OGP 0170651) to O. P. Rajora, and in part by grants from US National Science Foundation, and International Plant Genetic Resources, Rome, to K. S. Bawa.

References

Barrett JW, Rajora OP, Yeh FC, Dancik BP, Strobeck C (1993) Mitochondrial DNA variation and genetic relationships of *Populus* species. Genome 36 : 87–93

Chase MR, Kesseli RV, Bawa KS (1996) Microsatellite markers for population and conservation genetics of tropical trees. Am J Bot 83 : 51–57

Cheliak WM, Dancik BP (1982) Genetic diversity of natural populations of a clone-forming tree *Populus tremuloides*. Can J Genet Cytol 24 : 611–616

Condit R, Hubbell S (1991) Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome 34 : 66–71

Dayanandan S, Bawa, KS, Kesseli RV (1997) Conservation of microsatellites among tropical trees (*Leguminosae*). Am J Bot 84(12) : 1658–1663

Dewey ME, Bell JC, Smith DN, Neale DB, Moran GF (1996) A genetic linkage map for *Pinus radiata* based on RFLP, RAPD, and microsatellite markers. Theor Appl Genet 92 : 673–679

Echt CS, Nelson CD (1997) Linkage mapping and genome length in eastern white opine (*Pinus strobus* L). Theor Appl Genet 94 : 1031–1037

Echt CS, May-Marquardt P, Hseih M, Zahorchak R (1996) Characterization of microsatellite markers in eastern white pine. Genome 39 : 1102–1108

Hearne C, Ghosh S, Todd J (1992) Microsatellites for linkage analysis of genetic traits. Trends Genet 8 : 288–294

Jarret RL, Merrick LC, Holms T, Evans J, Aradhya MK (1997) Simple sequence repeats in watermelon (*Citrullus lantus* (Thumb.) Matsum. and Nakai). Genome 40 : 433–441

Jerka J, Pethiyagoda C (1995) Simple repetitive DNA sequences from primates: compilation and analysis. J Mol Evol 40 : 120–126

Kijas JMH, Fowler JCS, Thomas MR (1995) An evaluation of sequence-tagged microsatellite site markers for genetic analysis within Citrus and related species. Genome 38 : 349–355

Kostia S, Sirkaa-Liisa V, Vakkari P, Pulkkinen P (1995) Microsatellite sequences in a conifer, *Pinus sylvestris*. Genome 38 : 1244–1248

Levine I, Cheng HH, Bacter JC, Hillel J (1995) Turkey microsatellite DNA loci amplified by chicken specific primers. Anim Genet 26 : 107–110

Moore SS, Sargeant LL, King TJ, Matick JS, Georges M, Hetzel DJS (1991) The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10 : 654–660

Morgante M, Olivieri AM (1993) PCR amplified microsatellites as markers in plant genetics. Plant J 3 : 175–182

Ondro WJ (1991) Present trends and future prospects for poplar utilization in Alberta. For Chron 67 : 271–274

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. Mol Ecol 3 : 489–495

Pfeiffer A, Olivieri AM, Morgante M (1997) Identification and characterization of microsatellites in Norway spruce (*Picea abies* K). Genome 40 : 411–419

Rajora OP (1988) Allozymes as aids for identification and differentiation of some *Populus maximowiczii* Henry clonal varieties. Biochem Syst Ecol 16 : 635–640

Rajora OP (1989a) Genetic structure and identification of *Populus deltoides* Marsh. clones based on allozymes. Genome 32 : 440–448

Rajora OP (1989b) Characterization of 43 *Populus nigra* L. clones representing selections, cultivars and botanical varieties based on their multilocus allozyme genotypes. Euphytica 43 : 197–206

Rajora OP (1991) A long-term program plan for genetic improvement of aspen and other poplar hardwoods in Alberta. Alberta Forest Service, Edmonton, Alberta

Rajora OP, Dancik BP (1992a) Genetic characterization and relationships of *Populus alba*, *P. tremula* and *P.* × *canescens*, and their clones. Theor Appl Genet 84 : 291–298

Rajora, OP, Dancik BP (1992b) Chloroplast DNA inheritance in *Populus*. Theor Appl Genet 84 : 280–285

Rajora OP, Dancik BP (1995a) Chloroplast DNA variation in *Populus*. I. Intraspecific restriction fragment diversity within *Populus deltoides*, *P. nigra*, and *P. maximowiczii*. Theor Appl Genet 90 : 317–323

Rajora OP, Dancik, BP (1995b) Aspen decay and stain and genetic fingerprinting. Research Report, Canada-Alberta Partnership Agreement in Forestry, Edmonton, Alberta

Rajora OP, Dancik BP (1995c) Chloroplast DNA variation in *Populus*. II. Interspecific restriction fragment polymorphisms and genetic relationships among *Populus deltoides*, *P. nigra*, *P.* × *canadensis*, and *P. maximowiczii*. Theor Appl Genet 90 : 324–330

Rajora OP, Zsuffa L (1989) Multilocus genetic structure, characterization and relationships of *Populus* × *canadensis* cultivars. Genome 32 : 99–108

Rajora OP, Barrett JW, Dancik BP, Strobeck C (1992) Maternal transmission of mitochondrial DNA in interspecific hybrids of *Populus*. Curr Genet 22 : 141–145

Schlotterer C, Amos B, Tautz D (1991) Conservation of polymorphic simple sequence loci in cetecean species. Nature 354 : 63–65

Smith DN, Devey ME (1994) Occurrence and inheritance of microsatellites in *Pinus radiata*. Genome 37 : 977–983

Terauchi R, Konuma A (1994) Microsatellite polymorphism in *Dioscorea tokora*, a wild yam species. Genome 37 : 794–801

Van de Ven WTG, McNicol RJ (1996) Microsatellites as DNA markers in Sitka spruce. Theor Appl Genet 93 : 613–617

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem repeats. Theor Appl Genet: 88 : 1–6

Weber JL (1990) Informativeness of human (dC-dA)n-(dG-dT)n polymorphisms. Genomics 7 : 524–530

Weber JL, May PE (1989) Abundant class of human DNA polymorphysms which can be typed using the polymerase chain reaction. Am J Hum Genet 44 : 388–396

Wu K, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241 : 225–235