APPLICATION OF SSR MARKERS FOR PARENTAGE ANALYSIS OF *POPULUS* CLONES

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ABSTRACT

Seventeen microsatellite or simple sequence repeat (SSR) markers in the genus *Populus* were optimized for seven species of *Populus* (*P. balsamifera*, *P. tremuloides*, *P. deltoides*, *P. davidiana*, *P. alba*, *P. tremula* and *P. nigra*). The use of the touchdown protocol for annealing resulted in a high success rate for the optimization phase. Fourteen out of 17 primer pairs resulted in PCR products of the expected size for most species tested. The fourteen primer pairs amplified SSR loci exhibiting variable amounts of polymorphism across the species studied. Each of the 14 SSR loci was polymorphic in at least three species. Four diagnostic loci with large allele diversity (*PGMC 2060*, *PGMC 2163*, *PGMC 2571* and *PGMC 2679*) were used to determine the parentage of unknown or doubtful *Populus* clones. Based on the multilocus genetic structure, the identity of these clones could be verified. The presumed identity of two of the 15 clones tested was different from that obtained using the four diagnostic loci. The molecular identity of the remaining clones was congruent to the presumed ones. In some instances, intra-clonal variation was also observed. Given the ecological and economic importance of the genus *Populus* in the Northern Hemisphere, the set of SSR markers optimized in this study will be useful in selection and breeding programs, landscape ecology studies, and for the conservation and management of *Populus* genetic resources.

Key words: Parentage analysis, *Populus*, microsatellites, PCR, simple sequence repeats.

INTRODUCTION

The genus *Populus* L. comprises approximately 30 species of poplars, cottonwoods and aspens, which are widely distributed over the Northern Hemisphere (STETTLER *et al.* 1996). There are six sections in the genus (Abaso Eckenwalder, Turanga Bunge, Leucoides Spach, Aigeiros Duby, Tacamahaca Spach and *Populus*) and based on intercrossability studies, species of the sections Leucoides, Aigeiros, Tacamahaca and Populus are intra-sectionally compatible. Intersectional crosses between species of sections Leucoides, Aigeiros and Tacamahaca are also reported compatible, while crosses that involve species of section *Populus* are generally incompatible (ECKENWALDER 1996). Six species, *P. trichocarpa* Torr. & A. Gray (black cottonwood), *P. balsamifera* L. (balsam poplar), *P. angustifolia* James (narrowleaf cottonwood), *P. deltoides* Bartr. Ex Marsh. ssp. *deltoides* (eastern cottonwood), *P. deltoides* var. *occidentalis* Rydb. (plains cottonwood) and *P. tremuloides* Michx. (trembling aspen) are indigenous to Canada (FARRAR 1995).

Populus cultivars have been developed through selection and intensive breeding over the last 50 years and they are planted in many parts of the Northern Hemisphere (STETTLER *et al.* 1996, DICKMAN *et al.* 2001). Intensive culture of *Populus* trees, also known as populiculture, is being considered in several provinces in Canada. In the province of Alberta, forest companies and the Western Boreal Aspen Cooperative (WBAC) are considering to use genetically superior stock of hybrid poplars/aspens and/or native and exotic poplars/aspens to satisfy the demand for fibre in the near future. For instance, Alberta-Pacific Forest Industries (Al-Pac) is committed to sustainable fibre supply for the future through poplar plantations. To mitigate projected shortfalls in timber volumes and allow for an ecologically sustainable harvesting program, Al-Pac is implementing populiculture on private land close to its mill in Boyle, using genetically superior stock of hybrid

poplars/aspens and/or native and exotic poplars/aspens in combination with their specific mycosymbionts (KHASA *et al.* 2002). Genetic trials, including several hybrid poplar and aspen clones, have been established at the mill site in Boyle (THOMAS & ROBERTSON 1998). Preliminary results indicate that growth potential from genetically improved material is between 2 and 8 times greater than that of native poplar and aspen stands at rotation age (KHASA *et al.*, unpublished).

Genetic testing and clonal selection are an expensive and critical component of any tree improvement program. The use of materials for which parentage is unknown or uncertain can jeopardize much of the long-term breeding and selection efforts. Therefore, the accurate identification of parent stock used by Al-Pac or any other well planned breeding program, must be assured either by carefully tracing their origin or, if need be, by using robust genetic markers. Because of the importance of the genus *Populus* both ecologically and economically, several types of genetic markers have already been developed (see review by CERVERA *et al.* 1997). Recently, PCR-based DNA markers have been used to study the parentage or paternity of poplar seedlings (*e.g.*, HEINZE 1997, 1998, 2001, TABBENER & COTTRELL 2003). With the poplar genome sequencing by the US Department of Energy Joint Genome Institute in 2003, several thousands of microsatellite markers, also known as simple sequence repeats (SSRs), are now available for the genus *Populus* (*Populus* SSR Primer Sets at URL: http://www.ornl.gov/sci/ipgc/Links.htm; DAYANANDAN *et al.* 1998; RAHMAN *et al.* 2000a). The majority of these SSR DNA makers, however, have not yet been tested.

The objectives of this study were to optimize some SSR DNA markers in order to assess the level of polymorphism and transferability in seven economically and ecologically important species of *Populus* and to determine the parentage of Al-Pac hybrid poplar and aspen materials used in genetic tests, for which pedigree information is absent or doubtful. We first optimized SSR markers on seven pure *Populus* species in order to find diagnostic loci representative of the parents and used at least four of them to determine the parentage of 15 *Populus* clones.

Table 1. List of parental material and Al-Pac *Populus* clones for which parentage needed to be determined.

Putative parental species[1]	Parentage	Sample location[1]
Populus balsamifera (10)	-	Field I - HP trial
P. tremuloides (10)	-	Field I - HP trial
P. deltoides (8)	-	Field I - HP trial
P. davidiana (7)	-	Field I - HP trial
P. alba (4)	-	Field I - HP trial
P. tremula (3)	-	Field I - HP trial
P. nigra (3)	-	Field I - HP trial
Alpac clone number[2]		
10	*P. x jackii*[3]	Field I - HP trial
16	*P. balsamifera* × *P. nigra*	Field I - HP trial
24	*P. deltoides* × *P. nigra*	Field I - HP trial
25	*P. deltoides*	Field I - HP trial
26	*P. deltoides*	Field I - HP trial
27	*P. deltoides* × *P. balsamifera*	Field I - HP trial
36	Open-pollinated *P. deltoides*	Field I - HP trial
59	*P. balsamifera*	BP trial Field I
290 (other Nos. 17-2)	*P. alba* cv Bolleana	Took2 trial - Field 23
291 (9-2)	*P. alba*	Took2 trial - Field 23
295 (27-2)	*P. nigra*	Took2 trial - Field 23
306 (6007)	*P. tremuloides*	Field 23 - SACLONE
948	*P. tremula*	G × E - 41 - 99 Field 23
952 (293)	*P. tremuloides* × *P. tremula*	HYBAS Field 23
2217	*P. nigra*	Stoolbed

[1] In parentheses, number of trees for each species.
[2] According to Al-Pac codes.
[3] Corresponding to a natural hybrid between *P. balsamifera* and *P. deltoides*.

MATERIALS AND METHODS

Plant materials and DNA extraction

Foliage (min. 3 leaves/tree) from three to 10 putative parents per *Populus* species [*P. balsamifera, P. tremuloides, P. deltoides, P. davidiana* (Dode) Schneider, *P. alba* L., *P. tremula* L. and *P. nigra* L.] and foliage of two to three individuals per *Populus* clone for which parentage needed to be determined, were collected at the genetic trial site in Boyle, Alberta (Table 1). The leaves were stored at –80 °C prior to use.

Total genomic DNA was extracted following a modified CTAB miniprep-method (BOUSQUET *et al.* 1990). Approximately 100 mg of tissue was ground with a mechanized homogenizer in 0.5 mL preheated CTAB extraction buffer (37 °C) [2 % (w/v) CTAB (Sigma), 1.4 M Nacl, 20 mM EDTA, 100 mM Tris-HCl, (pH 8.0), 1 % (w/v) PVP-40]. Two µL of RNase A (10 mg/mL) from bovine pancreas were added. The homogenate was incubated at 60 °C for 30 min, extracted with an equal volume of chloroform: isoamyl alcohol (24: 1, v:v), and then centrifuged (8,000 rpm, 5 min at 4 °C). DNA was precipitated by mixing with 0.5 mL of isopropanol, followed by centrifugation at 13,000 g for 30 min. The DNA pellet was rinsed with 0.5 mL of wash buffer [76 % (v/v) ethanol, 10 mM ammonium acetate buffer], and air-dried for 30 min at RT. The DNA was then dissolved in 100 µL of TE buffer pH 8.0 and stored at –20 °C prior to use.

PCR protocol and parentage analysis. The standard PCR reaction mix based on the recipe of KHASA *et al.* (2000) contained PCR buffer (50 mM KCl + 10 mM Tris-HCl, pH 8.3), 2 µg BSA, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 2.5 pmole of each of primer, approximately 20 ng of genomic DNA, and 1 unit of Amplitaq® DNA polymerase in a total volume of 10 µl. All reagents of the master mix were purchased from Perkin-Elmer Life Sciences Inc. (Boston, Massachusetts). A negative control (blank) containing all the components of a typical PCR reaction except template DNA was used in every experiment to test for the presence of nonspecific amplification fragments. PCR reactions were carried out on a GeneAmp 9700® thermal cycler (Perkin-Elmer/Cetus, Foster City, California) with a touchdown program: 3 min at 94 °C followed by 2 cycles with steps of 30 seconds for each of 94 °C (denaturation), 60 °C (annealing) and 72 °C (extension); 11 cycles with steps of 15 seconds for each of 94 °C, 60 °C and 72 °C with stepwise lowering of the annealing temperature from 60 °C or 58 °C to 54 °C by the 11th cycle; and 27 cycles with steps of 15 seconds for each of 94 °C, 54 °C and 72 °C, followed by incubation at 72 °C for 3 min as a final extension step (see Table 2 for the description of the SSR primers of *Populus* used). For the primers of the *PTR3* and *PTR4* loci, we followed a protocol described in DAYANANDAN *et al.* (1998).

The PCR products, along with a 20 base-pair DNA ladder standard (GenSura Laboratories, San Diego, California), were electrophoresed on 6 % denaturing polyacrylamide gels in 8 M urea and 1× TBE buffer run at 55 W constant power for 3 h. PCR products were denatured by adding 1 volume (10 µL) of fresh loading dye (10 mM NaOH, 95 % formamide, 0.05 % bromophenol blue and 0.05 % xylene cyanol) to 1 volume of PCR sample in a microtiter plate, mixed well, heated to 95 °C for 5 min and placed on ice. Gels were fixed and stained with silver nitrate using the protocol described in ECHT *et al.* (1996). The allele size was determined using a 1-bp ladder obtained from an M13 mp18 sequencing reaction.

Paternity exclusion analysis was conducted to determine the parentage. Paternity analysis techniques compare the mother and offspring genotypes to infer the paternal gametic contribution. Given the genotypes of a maternal parent and its progeny, the multilocus SSR genotype of the paternal parent can be reconstructed by inspection on a locus-by-locus basis. In this study, the multilocus genetic structure was useful to infer the molecular identity of the different clones by comparing multilocus genotypes between progeny and putative parents.

RESULTS AND DISCUSSION

Since PCR is sensitive to a number of parameters including magnesium, template DNA, primer concentration and annealing temperature during amplification, reaction conditions and DNA detection systems needed to be optimized to improve the efficiency of microsatellite analysis (RAHMAN *et al.* 2000b). In this study, we optimized 14 of these SSR primers for seven species of *Populus* using a touchdown protocol and specific PCR conditions as described above. Most of the 14 primer pairs optimized allowed reliable amplification of *Populus* species belonging to three sections of the genus (Aigeiros, Tacamahaca and Populus) (Table 3, Fig.1), suggesting homology in the genomic segments containing the studied SSR loci. Cross-taxa amplification for most SSR primer pairs appears to be common in the genus *Populus*, as has also been shown by RAHMAN and RAJORA (2002). It also appears to be widespread in other plant species at the subgenus level (THOMAS & SCOTT 1993, DAYANANDAN *et al.* 1997, KHASA *et al.* 2000). In some instances, spurious

Table 2. Description of poplar SSR markers tested.

Locus name	Primer sequences	Suggested annealing temperature (°C)[1]	Used annealing temperature (°C)[2]	Source[3]
PMGC 562	TTTTGGGAGGGGAGTCGAG (F) ACAACTCTCAACTTCCTAATC (R)	55 57	60↓54	PMGC
PMGC 571	CTGGTACCGATGGAGAAGAC (F) CAAACCAACAACTCACCGTAC (R)	57 57	60↓54	PMGC
PMGC 2020	TAAGGCTCTGTTTGTTAGTCAG (F) GAGATCTAATAAAGAAGGTCTTC (R)	57 57	60↓54	PMGC
PMGC 2060	CTCTCAAATGCTGATTTACCG (F) TCTTCAGTTGCAGTATTCAAAG (R)	55 55	58↓54	PMGC
PMGC 2140	GCTGTCAGAATCAAACACTTC (F) AAGCAGATAACTAAGACATGCC (R)	55 57	60↓54	PMGC
PMGC 2143	TCATCATCCATTACTCAACTTG (F) TCATCATCCATTACTCAACTTG (R)	55 55	58↓54	PMGC
PMGC 2163	CAATCGAAGGTAAGGTTAGTG (F) CGTTGGACATAGATCACACG (R)	55 55	58↓54	PMGC
PMGC 2328	CAAACCTCAACTTACAGTCAC (F) CCATTAGGCCATTATAGACAC (R)	55 55	58↓54	PMGC
PMGC 2408	TAGGTCACTAGAGTGGCGTG (F) CGAAAATGGTAGCTCTAATGCC (R)	57 59	60↓54	PMGC
PMGC 2419	TTTCCCTGTCATCGGCACTG (F) CATTGGAGACAGCTAATCAGC (R)	57 57	60↓54	PMGC
PMGC 2571	TCTCGCAGATTCATGTAACCC (F) GACTGTATGTTGACCATGCCC (R)	57 59	60↓54	PMGC
PMGC 2578	GAGAACTCGGTGACTGACTG (F) CAGCAACATCCACATATTAGC (R)	57 55	60↓54	PMGC
PMGC 2679	GGAATCCGTTTAGGGATCTG (F) CGTCTGGAGAACGTGATTAG (R)	55 55	58↓54	PMGC
PMGC 2731 A	CGTATAGTACTTGAAGAATCCC (F) CTGGTCAACAGCTACTGCAC (R)	57 57	60↓54	PMGC
PMGC 2731 B	CGTATAGTACTTGAAGAATCCC (F) CTGGTCAACAGCTACTGCAC (R)	57 57	60↓54	PMGC
PTR 3	CACTCGTGTTGTCCTTTTCTTTTCT (F) AGGATCCCTTCCCTTTAGTAT (R)	65 55	60	DAYANANDAN *et al.* 1998
PTR 4	AATGTCGAGGCCTTTCTAAATGTCT (F) GCTTGAGCAACAAACACACCAGATG (R)	65 69	60	DAYANANDAN *et al.* 1998

[1] Using the formula 2(A+T) + 4(C+G) - 5 (MULLIS *et al.* 1994).
[2] Touchdown temperature.
[3] PMGC, Poplar Molecular Genetics Cooperative at the University of Washington, Washington State, USA.

Table 3. Cross-species amplification and levels of polymorphism of SSR primers in poplar.

Locus		*P. balsamifera* (*N* = 10)	*P. tremuloides* (*N* = 10)	*P. deltoides* (*N* = 8)	*P. davidiana* (*N* = 7)	*P. alba* (*N* = 4)	*P. tremula* (*N* = 3)	*P. nigra* (*N* = 3)
PMGC 562	Ap	++	+	++	+	–	–	nt
	Mna	4	6	1	3	na	na	nt
	Sr	222–240	234–254	126·	212–238	na	na	nt
PMGC 571	Ap	++	++	++	++	++	–	nt
	Mna	4158–176	5	4	5	2	na	nt
	Sr		152–164	140–186	162–174	158–162	na	nt
PMGC 2020	Ap	++	++	++	++	++	++	nt
	Mna	8	7	6	6	3	2	nt
	Sr	132–168	144–166	135–151	140–157	143–153	147–149	nt
PMGC 2060	Ap	++	++	++	++	++	++	++
	Mna	13	9	6	3	3	1	1
	Sr	142–195	136–154	158–186	140–152	142–150	143·	142·
PMGC 2140	Ap	++	++	++	++	++	++	nt
	Mna	7	2	3	2	2	3	nt
	Sr	146–174	138–164	142–152	138–156	156–158	142–150	nt
PMGC 2163	Ap	++	++	++	++	++	++	++
	Mna	11	11	1	3	3	1	2
	Sr	195–231	189–229	185·	189–195	195–203	191·	219–235
PMGC 2328	Ap	++	++	++	++	++	–	nt
	Mna	6	7	9	7	2	na	nt
	Sr	79–96	70–89	75–117	70–89	75–107	na	nt
PMGC 2419	Ap	++	++	++	++	++	++	nt
	Mna	8	7	8	7	2	1	nt
	Sr	86–108	70–102	75–118	70–96	92–94	92·	nt
PMGC 2571	Ap	++	++	++	++	++	++	++
	Mna	7	11	1	4	1	1	1
	Sr	92–108	82–122	79·	82–92	88·	84·	80·
PMGC 2679	Ap	++	++	++	++	++	++	++
	Mna	7	5	6	3	2	1	2
	Sr	102–112	101–109	108–128	105–109	101–103	103·	102–110
PMGC 2731	Ap	++	++	++	++	–	–	nt
	Mna	6	8	6	6	na	na	nt
	Sr	154–212	152–190	168–208	158–206	na	na	nt
PMGC 2818	Ap	++	++	++	++	++	++	nt
	Mna	3	7	3	6	mb	2	nt
	Sr	121–133	116–138	115–125	113–121	mb	117–121	nt
PTR 3	Ap	??	++	+	++	++	–	nt
	Mna		10	3	5	1	na	nt
	Sr		180–246	200–228	212–238	232·	na	nt
PTR 4	Ap	+	++	–	++	++	–	nt
	Mna	2	7	na	3	2	na	nt
	Sr	198–204	188–216	na	192–212	204–210	na	nt
Average polymorphism	Mna	6.21±0.88	7.29±0.66	4.38±0.74	4.5±0.45	2.09±0.21	1.25±0.16	1.5±0.29

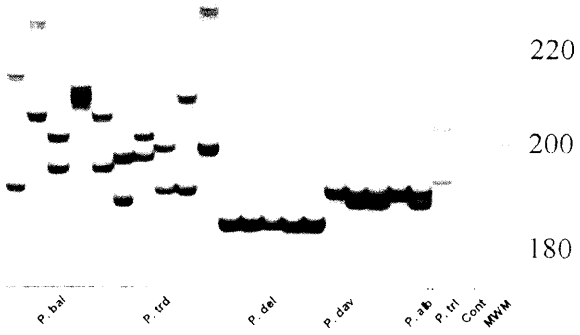

Figure 1. Single-locus amplification products with the primer pair *PGMC 2163* showing allelic variation in six *Populus* species (*P. bal* = *P. balsamifera*, *P. trd* = *P. tremuloides*, *P. del* = *P. deltoides*, *P. dav* = *P. davidiana*, *P. alb* = *P. alba*, *P. trl* = *P. tremula*, Cont = control). MWM is the molecular weight marker (20 base-pair DNA ladder standard).

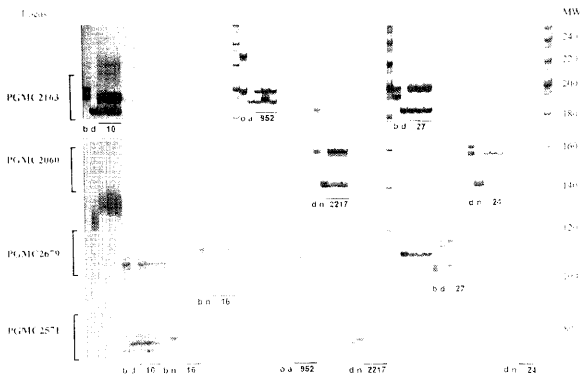

Figure 2. Parentage analysis of some *Populus* clones (10, 16, 952, 2217, 27, and 24) at four marker loci (*PGMC 2163, PGMC 2060, PGMC 2679* and *PGMC 2571*); **b** = *P. balsamifera*, **d** = *P. deltoides*, **n** = *P. nigra*, **o** = *P. tremuloides*, **a** = *P. tremula* and MW is the molecular weight (20 base-pair DNA ladder standard).

amplification products were produced, but they were outside the expected size range of alleles. Stutter bands usually ascribed to DNA polymerase slippage (TAUTZ 1989), were also observed for a few primer pairs (*e.g.*, locus *PMGC 2818*, Table 3).

No amplification was observed for *P. alba* or *P. tremula* using the primer pairs for the *PGMC 562* and *PGMC 2731* loci, nor for *P. tremula* using the primer pairs for the *PGMC 562, PGMC 571, PGMC 2328, PGMC 2731, PTR 3* and *PTR 4* loci. In a previous study (DAYANANDAN *et al.* 1998), primer pairs for the locus *PTR 3* showed no amplification in *P. deltoides* and *P. nigra*, while the *PTR 4* locus amplified in *P. deltoides* but not in *P. nigra*. The minimum number of alleles found at all 14 loci and their size range for the trees studied are shown in Table 3. Each of the 14 SSR loci was polymorphic in at least three species. The number of alleles per locus in our small sample of trees ranged from 1 to 13. Even though this study was not intended as a thorough population survey for each species investigated, the numbers of alleles per locus were comparable between *P. tremuloides* and *P. basalmifera,* but greater than that observed in *P. deltoides* and *P. davidiana* (Table 3). Caution should be exercised in the interpretation of the small number of alleles observed for *P. alba, P tremula* and *P. nigra* because of the small sample sizes used for these species our SSR markers *(PGMC 2060, PGMC 2163, PGMC 2571* and *PGMC 2679*) were found useful for the parentage analysis of *Populus* clones. The parentage analysis involves identification of genetically compatible individuals that are putative parents of specific seeds or seedlings. Genotypic exclusion requires markers with very high exclusion power, which is determined by the number of loci and their level of polymorphism (EVETT & WEIR 1998). These four diagnostic loci were retained because they showed high numbers of alleles, from which it was possible to exclude a tree as being a parent of a given offspring. The multilocus genotypes of the putative parents at the four diagnostic loci are presented in Table 4. Figure 2 shows the products of the four loci amplified in the parents and some clones for which parentage needed to be determined. In this study, the presumed Al-Pac parentage for two clones (2217 and 36) did not conform with the parentage identification using SSR diagnostic markers (Table 5). The clone 2217, presumed to be pure *P. nigra*, turned out to be a hybrid between *P. nigra* and *P. deltoides*. The clone 36, presumed to be an open-pollinated *P. deltoides*, showed allelic composi tion referring to *P. nigra* at three *(PGMC 2060, PGMC 2571* and *PGMC 2679*) out of four loci. This clone or its DNA may have been misclassified during laboratory handling. The clone 10 (*P.* × *jackii* Sarg.) was confirmed as a natural hybrid between *P. balsamifera* and *P. deltoides*. All crosses involving *P. deltoides* are more fertile when the mother is *P. deltoides*, reciprocals are either infertile (*e.g.*, with *P. nigra*) or much less fertile

← [1] Abbreviations: *N*, sample size; *Ap*, amplification pattern; *Mna*, minimum number of alleles detected; *Sr*, size range detected in base pairs; ++, strong amplification; +, weak amplification; -, no amplification; mb, multiple bands; na, not available; nt, not tested; Since some of the results are based on small sample sizes, the presence of one allele does not necessarily imply a monomorphic locus.

Table 4. Genotyping (in base pairs) of putative parents (1–10) for seven species of *Populus* at the four diagnostic SSR markers used to confirm Al-Pac collection of poplar pedigrees.

Species	Locus	Parent number									
		1	2	3	4	5	6	7	8	9	10
P. balsamifera	*PMGC 2060*	172/168	176/176	152/160	180/158	166/150	176/176	178/164	195/150	nt	160/144
	PMGC 2163	243/191	223/205	201/195	211/209	205/195	205/199	201/195	231/231	nt	223/219
	PMGC 2571	108/101	101/101	108/92	101/96	92/92	102/102	105/94	105/105	102/102	92/92
	PMGC 2679	112/106	104/104	112/106	112/102	106/104	110/106	110/106	104/104	nt	112/106
P. tremuloides	*PMGC 2060*	154/142	148/140	148/142	148/142	142/142	150/136	142/142	152/138	138/138	142/140
	PMGC 2163	197/189	201/199	199/191	209/191	229/199	209/191	225/195	191/191	191/191	200/193
	PMGC 2571	110/101	101/84	99/88	122/88	94/88	116/88	96/88	88/88	88/82	86/86
	PMGC 2679	103/101	105/102	101/101	101/101	101/101	101/101	103/101	107/107	107/107	103/101
P. deltoides	*PMGC 2060*	166/166	166/166	160/152	166/158	162/162	160/160	168/160	186/186	na	na
	PMGC 2163	185/185	185/185	185/185	185/185	185/185	185/185	185/185	185/191	na	na
	PMGC 2571	79/79	79/79	79/79	79/79	79/79	79/79	79/79	79/79	na	na
	PMGC 2679	116/114	116/112	116/102	128/110	116/114	108/108	116/108	128/108	na	na
P. davidiana	*PMGC 2060*	146/140	140/140	146/140	152/140	140/140	146/140	146/140	na	na	na
	PMGC 2163	191/191	191/189	191/189	191/191	191/189	191/191	195/189	na	na	na
	PMGC 2571	92/88	92/82	92/92	92/88	90/88	88/82	92/88	na	na	na
	PMGC 2679	109/105	105/105	109/105	109/109	107/105	107/107	107/105	na	na	na
P. alba	*PMGC 2060*	150/143	150/148	150/143	150/150	na	na	na	na	na	na
	PMGC 2163	193/191	195/195	203/193	195/195	na	na	na	na	na	na
	PMGC 2571	86/86	86/86	86/86	86/86	na	na	na	na	na	na
	PMGC 2679	101/101	103/101	103/101	103/101	na	na	na	na	na	na
P. tremula	*PMGC 2060*	143/143	143/143	143/143	na	na	na	na	na	na	na
	PMGC 2163	191/191	191/191	191/191	na	na	na	na	na	na	na
	PMGC 2571	82/82	84/84	nt	na	na	na	na	na	na	na
	PMGC 2679	102/102	nt	nt	na	na	na	na	na	na	na
P. nigra	*PMGC 2060*	142/142	142/142	142/142	na	na	na	na	na	na	na
	PMGC 2163	235/205	235/219	227/219	na	na	na	na	na	na	na
	PMGC 2571	80/80	82/80	80/80	na	na	na	na	na	na	na
	PMGC 2679	110/102	102/102	110/102	na	na	na	na	na	na	na

Table 5. Multilocus genotypes (in base pairs) using four discriminatory SSR markers developed for the poplar pedigree material from Al-Pac collection.

Clone number	10	16	24	27	2217	952	36	25	26	59	290	291	295	306	948
Presumed Al-Pac parentage	*P. x jackii*	*P. balsami-fera* × *P. nigra*	*P. deltoides* × *P. nigra*	*P. deltoides* × *P. balsa-mifera*	*P. nigra*	*P. tremulo-ides* × *P. tremula*	*Open-pollinated P. deltoides*	*P. deltoides*	*P. deltoides*	*P. balsami-fera*	*P. alba*	*P. alba*	*P. nigra*	*P. tremulo-ides*	*P. tremula*
PMGC 2060	160/160	168/142	158/142	152/152	160/142	143/140	142/142	162/158	168/158	178/150	150/143	150/143	142/142	142/142	143/143
	160/160	168/142	158/142	152/152	160/142	143/140	142/142	162/158	168/158	154/150	150/143	150/143	142/142	142/142	143/143
	160/160	168/142	158/142	152/152	160/142	143/140	142/142	162/158	168/158	168/154	150/143	150/143	142/142	142/142	143/143
PMGC 2163	195/185	243/205	191/185	201/185	227/185	199/191	243/191	191/185	185/185	209/209	193/191	193/191	235/219	209/191	191/191
	195/185	243/205	191/185	201/185	227/185	199/195	243/191	191/185	185/185	205/200	193/191	203/193	235/219	205/205	191/191
	195/185	243/205	191/185	201/185	227/185	199/191	243/191	191/185	185/185	199/197	193/191	nt	235/219	nt	191/191
PMGC 2679	112/116	112/102	110/102	116/106	110/102	103/98	102/102	110/110	114/110	112/112	101/101	103/101	102/110	101/101	103/103
	112/116	112/102	110/102	116/106	110/102	102/102	102/102	110/110	114/110	108/102	101/101	103/101	102/110	101/101	103/103
	112/116	112/102	110/102	116/106	110/102	nt	102/102	110/110	114/110	102/102	101/101	103/101	102/110	101/101	103/103
PMGC 2571	108/79	108/80	82/79	112/79	80/79	84/84	82/80	79/79	79/79	108/108	86/86	86/86	80/80	94/88	84/84
	108/79	108/80	82/79	112/79	80/79	110/84	82/80	79/79	79/79	102/102	86/86	86/86	80/80	116/88	84/84
	108/79	108/80	82/79	112/79	80/79	nt	82/80	79/79	79/79	nt	86/86	86/86	80/80	nt	84/84
Confirmed parentage using SSRs	*P. balsami-fera* × *P. deltoides*	*P. balsami-fera* × *P. nigra*	*P. deltoides* × *P. nigra*	*P. deltoides* × *P. balsa-mifera*	*P. nigra* × *P. deltoides*	*P. tremulo-ides* × *P. tremula*	*P. nigra?*	*P. deltoides*	*P. deltoides*	*P. balsami-fera*	*P. alba*	*P. alba*	*P. nigra*	*P, tremulo-ides*	*P. tremula*

(*P. simonii, P. trichocarpa, P. maximowiczii*). For the remaining 13 clones, the pre sumed and molecular-based parentages were congruent. In some instances, intraclonal variation was also observed (*e.g.*, clones 952, 59, 291 and 306, see Table 5). This may be attributable to mutation events. Microsatellite markers are known to show high levels of mutation rates of one or two steps, observed at molecu lar loci (GOLDSTEIN & POL LOCK 1997; LI *et al.* 2002). Given that *Populus* clones might contain significant intra-genet polymorphism (CHELIAK & DANCIK 1982), a phenomenon that has not been thoroughly investigated, the variation observed may also be due to a putative clone mixture. A much more detailed sampling procedure is therefore needed to investigate the intra-clonal polymorphism of microsatellites in *Populus*. Because of such possible mutation events, at least two diagnostic microsatellite loci showing unambiguous parental contributions to progeny should be sought to diagnose the parentage.

The diagnostic SSR markers described in this study are useful for typing the various *Populus* clones analysed and confirmed their parentage. This could not be achieved using leaf morphology alone (ECKENWALDER 1996, KEMPERMAN 1977). In addition, these SSR markers will be useful for an array of other uses: (i) for mating system studies, (ii) for depicting patterns of population differentiation and landscape ecology studies, (iii) for fingerprinting purposes in selection and breeding programs, and (iv) for the conservation and management of *Populus* genetic resources in populicul ture.

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