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Genetic characterization and relationships of *Populus alba*, *P. tremula*, and *P.* × *canescens*, and their clones

O. P. Rajora and B. P. Dancik

Department of Forest Science, University of Alberta, Edmonton, Alberta, Canada T6G 2H1

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Summary. Isozyme analysis was conducted on individuals of *Populus alba* L., *P. tremula* L., and *P.* × *canescens* Smith to genetically characterize and differentiate species, hybrids, and individuals, and to determine genetic relationships among them. Thirty gene loci, with 71 alleles, coding for 15 enzymes were observed. Individuals could be identified on the basis of their multilocus genotypes. There were 21 unique multilocus genotypes among 23 *P. alba* clones. Five *P. alba* clones from Canada were genetically distinct from each other. Each of the 18 *P. tremula* and 15 *P.* × *canescens* clones had unique multilocus genotypes. Thirteen clones had a unique genotype at a single locus. Percentage of polymorphic loci, average number of alleles per locus, and mean observed heterozygosity were, respectively, 50.0, 1.86, and 0.085 in *P. alba*, 51.7, 1.66, and 0.096 in *P. tremula*, and 51.7, 1.86, and 0.157 in *P.* × *canescens*. *Populus alba* and *P. tremula* were genetically distinct from each other and could be distinguished by mutually exclusive alleles at *Aco-3*, *P. tremula*-specific gene *Mdh*-3, and allele frequency differences at 6 loci. *Populus* × *canescens* had allele contributions of *P. alba* and *P. tremula*. However, their allele frequencies were closer to those of *P. alba* than being truly intermediate. The mean genetic identity was 0.749 between *P. alba* and *P. tremula*, 0.987 between *P. alba* and *P.* × *canescens*, and 0.817 between *P. tremula* and *P.* × *canescens*. Canonical discriminant analysis of multilocus genotypes separated *P. alba*, *P. tremula*, and *P.* × *canescens* into three distinct groups and portrayed similar interspecific relationship as above. Our results suggested that the putative *P.* × *canescens* individuals consisted of a mixture of F_1 hybrids of *P. alba* and *P. tremula* and their backcrosses to *P. alba*.

Correspondence to: O. P. Rajora at the University of Alberta. Presently with the University of Alberta, and BioGenetica Inc., P.O. Box 8261, Edmonton, Alberta, Canada T6H 4P1

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Introduction

Populus alba L. (section *Leuce* Duby, subsection *Albidae* Dode), European white poplar, has wide natural distribution in river valleys over southern, central, and eastern Europe, western and central Asia, and northern Africa (Anonymous 1958, 1979). *Populus tremula* L. (section *Leuce*, subsection *Trepidae* Dode), European aspen, has a vast natural range extending almost over the whole of Europe with the exception of the southern plains, northwestern Asia, and North Africa (Anonymous 1958, 1979). *Populus* × *canescens* Smith, gray poplar, is generally recognized as a hybrid between *P. alba* and *P. tremula*, individuals of which have arisen spontaneously by natural hybridization. Gray poplar individuals are found in regions where the range of both parental species overlap in central and western Europe, as well as in northern Africa (Anonymous 1958, 1979). According to Muhle Larsen (1970), *P.* × *canescens* was first recognized as a separate species.

Populus alba and *P. tremula* are morphologically distinct (Anonymous 1958, 1979; Hyun et al. 1984). *Populus* × *canescens* trees have mixed characters; many are intermediate to *P. alba* and *P. tremula*, and some are similar to *P. alba* (Anonymous 1958, 1979; Hyun et al. 1984). Although *P.* × *canescens* is generally considered to be a hybrid between *P. alba* and *P. tremula*, its hybridity is not genetically proven. Relationships among *P. alba*, *P. tremula*, and *P.* × *canescens* are not known at the gene level. Peto (1938) studied the cytology of *P. alba* and *P.* × *canescens*; however, the cytological relationships of these poplars were not determined. Therefore, the understanding of genetic relationships among *P. alba*, *P. tremula*, and *P.* × *canescens* is of fundamental biological importance.

Table 1. *Populus* species and the individuals thereof that were studied

Species and individuals[a]	Origin
Populus alba	
A33	var '*nivea*' Willd. Barres Arbres, France
A34, A63, A74, A549, and A551	A34, A63, and A74 planted in Ontario and Quebec, provinces of Canada; origin in Europe not known. A549 and A551 open-pollinated progeny of *P. alba* clone A10 (originally from Berlin, FRG), planted in Ontario
A208, A321, and A430	Progeny of controlled crosses: *P: alba*, Ontario × *P. alba*, FRG
A127	Kunpeszer, Hungary. Number 3 of Institute of Forest Science (IFS)
A418	Munchendorf, Austria
A473, A475, and A497	FRG; from Fasanengarten, Hann Munden, and Berlin, respectively
A478	VelikiBajar, Yugoslavia. Number 28/63 of Zavod za Topole, Novi Sad
A499	Cassale Monferrato, Italy. I-57/57
A558	cv 'Raket' of which one of the parents is *P. alba* var '*bolleana*' Lauche. Selected in Wageningen, The Netherlands. Their number: NL-1554
A562, A563, A565, A568, A569, and A570	Bulgaria
Populus tremula	
E16	Rubiano, Torino, Italy
E18	Helsinki, Finland
E25 and E26	France: Valloire, Savoie, and Hautes, Alpes, respectively.
E39	Sarajevo, Yugoslavia
E56, E57, and E58	Norway: E56 and E57 from Bable, Telemark. E58 from M. Haugberg. Numbers 6, 17, and 23, respectively, of M. Haugberg, Agricultural College Forest
E66	Szombathely, Hungary. H379#5 of IFS
E82 and E83	Russia Received from Bryant and May Ltd, Scotland
E100, E103, and E104	Lotbiniere and Mackintosh, England
E124	cv 'Bachofenii'. Dubovac, Yugoslavia. Number 4/63 of Zavod za Topole, Novi Sad
E147	Tiergarten, Beiwien, Austria
E152 and E155	Czechoslovakia
Populus × *canescens*	
AE36 and AE42	Progeny of a controlled cross *P. alba*, Ontario × *P. tremula*, Italy
C4 and C64	Wageningen, The Netherlands
C10	Oxford, England
C17	cv 'soviensis'. Kornik Gardens, Poland
C21, C32, C97, and C127	FRG: Frankfurt, Tannenhoft, Ingolstadt, and Munden, respectively
C165, C173, and C180	Yugoslavia: Zavod za Topole, Novi Sad
C179	Zidlochovice, Czechoslovakia
C185	Open-pollinated progeny of C18 (from Czechoslovakia) grown at Maple, Ontario

[a] Individuals are registered with these clonal accession codes at the Ontario Forestry Institute, Maple, Ontario

Populus alba was introduced to colonial North America as an ornamental and has remained popular for this purpose (Dickmann and Stuart 1983). According to Eckenwalder (1977), specimens of *P. alba* in the United States seem to be the members of a single pistillate clone. Spies and Barnes (1982) also found only female trees of *P. alba* in southeastern Michigan; however, in the western part of Michigan some male clones of *P. alba* have been located (B.V. Barnes, personal communication). *Populus alba* trees are also grown in Canada. However, it is not known whether the trees represent a single clone or different clones.

Populus alba, *P. tremula*, and *P.* × *canescens* are multipurpose trees and have significant regional economic importance (Anonymous 1958, 1979; Dickmann and Stuart 1983). In Canada these poplars are important in interspecific breeding (Heimburger 1968; Zsuffa 1975). *Populus* × *canescens* and *P. alba*'s hybrids with North American aspens, *P. tremuloides* Michx. and *P. grandidentata* Michx., are known for the vigorous growth and improved rooting ability of their shoot cuttings (Heimburger 1968; Zsuffa 1975). Many clones of *P. alba*, *P. tremula*, and *P.* × *canescens* have been assembled in arboreta of Ontario and are used in poplar breeding. However, an understanding of their genotypes, relationships, and identification, essential for an effective breeding program, is lacking. Also, an unambiguous differentiation of *Populus* species and hybrids and their clonal varieties would be useful in genetics and breeding studies.

In the study reported here we attempted to genetically characterize and differentiate *P. alba*, *P. tremula*, and *P.* × *canescens* and their individual clones, and to determine relationships among *P. alba*, *P. tremula*, and *P.* × *canescens* using isozymes as genetic markers. We also examined whether certain *P. alba* clones in Ontario's arboreta that were propagated from trees grown in Ontario and Quebec represent a single clone or different clones and whether buds of a tree show intratree isozyme

Table 2. Enzymes assayed in *Populus alba*, *P. tremula*, and *P.* × *canescens*

Enzyme	EC number	Abbreviation	Buffer system[a]	Number of loci scored
Acid phosphatase	3.1.3.2	APH	D	3
Aconitase	4.2.1.3	ACO	A	3
Alcohol dehydrogenase	1.1.1.1	ADH	A	2
Aspartate aminotransferase	2.6.1.1	AAT	D	2
Colorimetric esterase	3.1.1.1	CE	B	1
Flourescent esterase	3.1.1.2	FLE	A, B	1
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6P	C	2
Isocitrate dehydrogenase	1.1.1.42	IDH	A	1
Leucine aminopeptidase	3.4.11.1	LAP	B	2
Malate dehydrogenase	1.1.1.37	MDH	A	3
Peroxidase	1.11.1.7	PER	B	2
Phosphoglucomutase	2.7.5.1	PGM	A	3
Phosphoglucose isomerase	5.3.1.9	PGI	B	2
6-Phosphogluconate dehydrogenase	1.1.1.44	6-PGD	C	2
Superoxide dismutase	1.15.1.1	SOD	B	1

[a] Buffer systems:
A: Electrode – 0.13 *M* TRIS and 0.043 *M* citric acid, pH 7.0; gel – 1:13 dilution of the electrode buffer (Siciliano and Shaw 1976)
B: Electrode – 0.06 *M* lithium hydroxide and 0.3 *M* boric acid, pH 8.1; gel – 1:10 dilution of the electrode buffer (Ridgeway et al. 1970)
C: Electrode – 0.125 *M* TRIS, pH 7.0 with 1.0 *M* critic acid; gel – 0.05 *M* L-histidine and 1.4 m*M* EDTA, pH 7.0 with 1.0 *M* TRIS; gels were made by using a 1:5 dilution of the gel buffer (modified from Namkoong et al. 1979)
D: Electrode – 0.31 *M* boric acid and 0.063 *M* sodium hydroxide, pH 8.1; gel – 0.08 *M* TRIS, pH 8.65 (Schaal and Anderson 1974)

variation. We used isozyme analysis because allozymes have been found to be powerful genetic markers for this type of study and other genetic, phylogenetic and breeding studies in poplars (Cheliak and Dancik 1982; Cheliak and Pitel 1984; Hyun et al. 1987a, b; Rajora 1988, 1989a, b, c, 1990a, b; Rajora and Zsuffa 1989, 1990).

Material and methods

Plant material

Fifty-six individuals of *P. alba*, *P. tremula*, and *P.* × *canescens* were sampled (Table 1) from a collection from an arboretum of the Ontario Forestry Institute (OFI), Ontario Ministry of Natural Resources, Maple, Ontario. Twenty-three clones of *P. alba* originating in eight European countries and Canada were sampled. The *P. tremula* sample had 18 individuals that originated in ten European countries (Table 1). One clone represented cv 'Bachofenii', whose relationship to *P. tremula* is controversial (Anonymous 1979). The *P.* × *canescens* sample consisted of 15 individuals: 13 natural hybrids that originated in six European countries and 2 individuals of progeny of a controlled cross made at OFI. Fifteen clones (A33, A34, A63, A74, A478, A497, E16, E18, E39, E100, E103, E124, C10, C64, and C127; Table 1) were females and 11 (A127, A321, A473, A475, E26, E56, E57, E58, E66, C17, and C32; Table 1) were males; the sex of the remaining 30 clones was not known.

Tissue collection and preparation, and enzyme extraction

Tissues of dormant vegetative buds were used for enzyme electrophoresis. Twigs with dormant buds were collected in November, 1987, the bud scales were removed, and bud tissues were collected in extraction buffer (Rajora et al. 1991). For 1 tissue sample of each individual, 1 or more buds were collected. However, to test within-tree interbud variation (bud mutations), 10 single buds each of 5 randomly chosen individuals each of *P. alba*, *P. tremula*, and *P.* × *canescens* were sampled individually. The crude enzyme extract was prepared by homogenizing the bud tissues in the extraction buffer with the help of a power-driven stirrer-type homogenizer on the same day that the electrophoretic analysis was done.

Enzyme electrophoresis and detection, and isozyme genotypes

Fifteen enzyme systems were assayed by horizontal starch gel electrophoresis using four buffer systems (Table 2). Starch gels, 12.5% w/v, were prepared from Connaught starch (Connaught Laboratories Willowdale, Ont., Canada). Zones of enzyme activity were detected by standard methods.

Genotypes of individual clones were inferred from the banding patterns. Gene loci and alleles were designated jointly for *P. alba*, *P. tremula*, and *P.* × *canescens* following the convention described in Rajora (1989a, b, 1990a) and Rajora and Zsuffa (1989). Due to complex MDH banding patterns, it was possible to interprete and score only three MDH gene loci. Genetic control of the scored isozyme loci was inferred from the known isozyme inheritance of peroxidase in *P. tremula* (Guzina 1978) and of some other enzymes in other *Populus* species (Hyun et al. 1987b; Rajora 1990b).

Data analysis

The allele frequencies at different loci, percentage of polymorphic loci (P), average number of alleles per locus (A), and mean observed heterozygosity per locus (H) were determined. Only 13 natural *P.* × *canescens* hybrids were used for these estimations. Genetic identities and distances were computed among *P. alba*, *P. tremula*, and *P.* × *canescens* following Nei's (1978) method for a small number of individuals. Sampling variances and standard errors of average heterozygosity and genetic distances were estimated (Nei and Roychoudhury 1974; Nei 1978).

Genotypes of individual clones at each of the 18 polymorphic loci were coded as described in Rajora (1988, 1989 a, b) and Rajora and Zsuffa (1989). Canonical discriminant analysis (CDA) and principal component analysis (PCA) were conducted using genotype codings of 56 clones at 18 loci. Scatter and grouping of *P. alba*, *P. tremula* and *P.* × *canescens* on canonical discriminant functions 1 and 2, and of individual clones on principal components 1 and 2 were determined.

The SOD locus was not included in any analysis as the genotypes and phenotypes of all individuals at *Sod* were identical to those at *Per-1* (Rajora et al. 1991).

Results

A total of 30 gene loci with 71 alleles, including *Sod* with 4 alleles, coding for 15 enzymes in *P. alba, P. tremula*, and *P.* × *canescens* were identified (Table 2). Eleven loci (*Acp-1*, *Acp-2*, *Acp-3*, *Ce-1*, *G6p-1*, *Lap-1*, *Lap-2*, *Mdh-1*, *Mdh-3*, and *Pgi-1*) were monomorphic in all of the *Populus* species and hybrid studied. The remaining 18 gene loci were polymorphic in at least one of *P. alba*, *P. tremula*, and *P.* × *canescens* (Table 3). Two to five alleles were detected at a polymorphic locus (Table 3). P, A, and H, respectively, were 50.0, 1.86, and 0.085 ± 0.027 in *P. alba*, 51.7, 1.66, and 0.096 ± 0.027 in *P. tremula*, and 51.7, 1.86 and 0.157 ± 0.039 in *P.* × *canescens*.

All of the buds of a tree had the same isozyme phenotypes and genotypes at the loci studied.

The interclonal isozyme variability was controlled by 15 polymorphic gene loci in *P. alba*, 16 gene loci in *P. tremula*, and 17 gene loci in *P.* × *canescens* (Table 3). PCA indicated that from all of the polymorphic loci, 8 loci (*6-Pgd-2*, *Aco-3*, *Adh-2*, *6-Pgd-1*, *Idh-1*, *Aco-2*, *Aco-1*, and *Per-1*) were the main contributors to the interclonal allelic variability. There were 21 unique multilocus genotypes among the 23 *P. alba* clones. Clones A33 and A34, and clones A63 and A418 shared the same multilocus genotypes. Each of the remaining 19 clones, including 5 clones from Ontario and Quebec, had unique 15-locus genotypes. Each of the 18 *P. tremula* and each of the 15 *P.* × *canescens* clones had unique multilocus genotypes. Thirteen clones had a unique genotype at a single locus as follows: A127 at *Per-1*, A208 at *Adh-1*, A430 at *Adh-2*, A565 at *Aco-3*, A570 at *6-Pgd-1*, E66 at *Pgm-2*, E83 at *Adh-1* as well as at *6-Pgd-1*, E104 at *Adh-1*, E152 at *Adh-2*, C17 at *Aco-2*, C64 at *G6P-2*, C127 at *Idh-1* and C180 at *Per-2* as well as at *Aco-1*. Significant interclonal variability was also observed for unscored MDH banding patterns (Fig. 1); clones A430, A478, E103, E152, E155, and C185 had unique MDH patterns.

Populus alba and *P. tremula* were genetically distinct. The *Mdh-3* locus was not detected in *P. alba* (Fig. 1). These two species had mutually exclusive alleles at *Aco-3* (Table 3; Fig. 2) and species-specific higher allele frequencies (> 0.77) at *Aco-1*, *Aco-2*, *Fle*, *G6p-2*, *Pgm-2*, and *6-Pgd-2* (Table 3). *Populus alba* and *P. tremula* also had

Table 3. Frequencies of different alleles at polymorphic loci in *Populus alba*, *P. tremula*, and *P.* × *canescens*

Locus	Allele	Rf of isozyme encoded	Allele frequency		
			P. alba	*P. tremula*	*P.* × *canescens*
Aco-1	*A*	0.380			0.039
	B	0.348	0.891	0.139	0.692
	C	0.308	0.109	0.861	0.269
Aco-2	*A*	0.289	0.109		0.115
	B	0.260	0.804		0.462
	C	0.215	0.087	0.056	0.423
	D	0.173		0.944	
Aco-3	*A*	0.236	0.043		
	B	0.215	0.370		0.308
	C	0.173	0.500		0.538
	D	0.155	0.087		
	E	0.141		1.000	0.154
Adh-1	*A*	0.491		0.028	
	B	0.459	0.087		
	C	0.432	0.913	0.944	1.000
	D	0.377		0.028	
Adh-2	*A*	0.300	0.022	0.028	
	B	0.238	0.935	0.944	0.962
	C	0.200		0.028	
	D	0.159	0.043		0.038
Fle	*A*	0.278	1.000	0.222	0.885
	B	0.222		0.778	0.115
G6P-2	*A*	0.188	1.000	0.083	0.885
	B	0.156		0.917	0.038
	O	0.156			0.077
Idh-1	*A*	0.512	0.978	0.944	0.923
	B	0.471	0.022	0.056	0.039
	O	0.471			0.038
Mdh-2	*A*	0.515	0.130		0.077
	B	0.488	0.870	1.000	0.923
Mdh-3	*A*	0.453		0.944	0.192
Per-1/ Sod	*A*	0.681	0.022		
	B	0.665	0.087	0.056	0.038
	C	0.624	0.891	0.889	0.962
	D	0.608		0.055	
Per-2	*A*	0.440	0.109	0.667	0.308
	B	0.403	0.891	0.333	0.538
	C	0.323			0.154
Pgm-1	*A*	0.619	0.457	0.972	0.615
	B	0.585	0.543	0.028	0.385
Pgm-2	*A*	0.550	0.152	0.778	0.231
	B	0.521	0.848	0.194	0.769
	C	0.490		0.028	
Pgm-3	*A*	0.463	0.022	0.500	0.077
	B	0.417	0.978	0.500	0.923
6-Pgd-1	*A*	0.452	0.044	0.333	0.115
	B	0.430	0.043		0.269
	C	0.408	0.848	0.667	0.500
	D	0.381	0.043		0.116
	E	0.354	0.022		
6-Pgd-2	*A*	0.229	0.978		0.846
	B	0.207	0.022	0.222	
	C	0.174		0.778	
Pgi-2	*A*	0.309	0.022		0.039
	B	0.226	0.956	0.972	0.923
	C	0.165	0.022	0.028	0.038

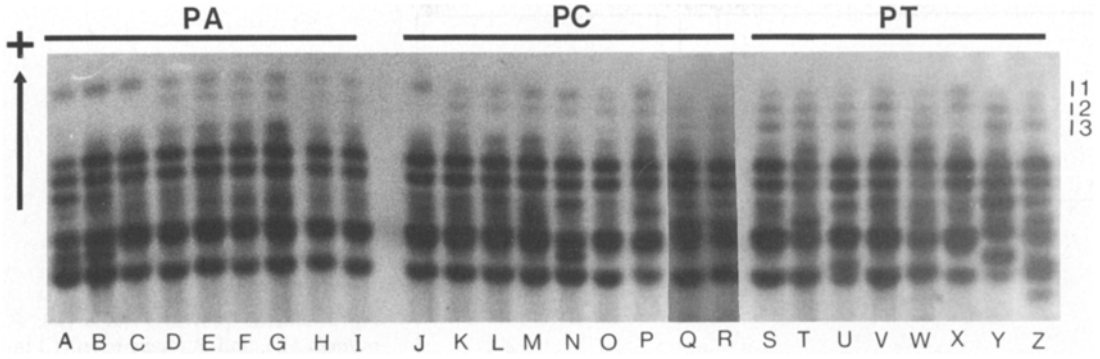

Fig. 1. MDH isozyme phenotypes and banding patterns of some clones of *P. alba* (*PA*), *P.* × *canescens* (*PC*), and *P. tremula* (*PT*). + anodal direction from the origin. The three zones of activity inferred to be controlled by three MDH loci are labelled on the *right side*. *A* A549, *B* A551, *C* A562, *D* A563, *E* A565, *F* A568, *G* A569, *H* A570, *I* A558, *J* C97, *K* C127, *L* C165, *M* C173, *N* C179, *O* C180, *P* C185, *Q* AE36, *R* AE42, *S* E83, *T* E100, *U* E103, *V* E104, *W* E124, *X* E147, *Y* E152, *Z* E155

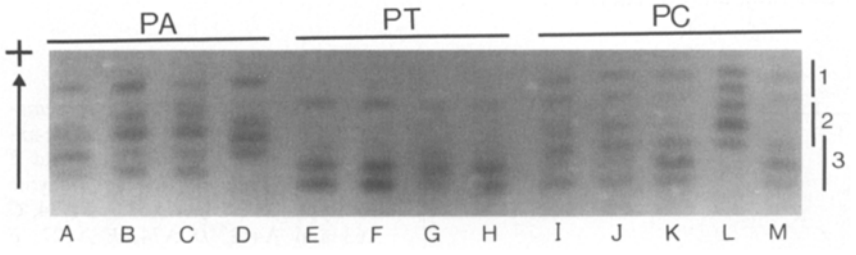

Fig. 2. Isozyme phenotypes of some individuals of *P. alba* (*PA*), *P. tremula* (*PT*), and *P.* × *canescens* (*PC*) at three ACO loci (numbered on *right side*). + anodal direction from the origin. *A* A34, *B* A569, *C* A473, *D* A478, *E* E16, *F* E56, *G* E57, *H* E58, *I* AE36, *J* AE42, *K* C127, *L* C165, *M* C17

Table 4. Genetic identities (I) and genetic distances (D) ± standard error among *Populus alba, P. tremula,* and *P.* × *canescens*

		P. alba	*P. tremula*
P. tremula	I	0.749 ± 0.071	
	D	0.289 ± 0.094	
P. × *canescens*	I	0.987 ± 0.006	0.817 ± 0.056
	D	0.013 ± 0.006	0.202 ± 0.068

species-specific MDH banding patterns (Fig. 1). Isozyme genotypes of AE36 and AE42 (individuals of F_1 progeny of a controlled cross of *P. alba* × *P. tremula*) were intermediate to the parental species. *Populus* × *canescens* had allele contributions from both *P. alba* and *P. tremula* (Table 3). However, their allele frequencies were closer to those of *P. alba* than being truly intermediate to *P. alba* and *P. tremula* (Table 3). The genotypes of the majority of the natural *P.* × *canescens* individuals were closer to *P. alba,* those of some were intermediate to *P. alba* and *P. tremula* (Fig. 2), and those of a very few individuals were similar to *P. tremula. Populus* × *canescens* also had certain alleles at *Aco-1*, *G6P-2*, *Idh-2*, and *Per-2* that were not detected in either of *P. alba* and *P. tremula* (Table 3).

The estimates of genetic identities and genetic distances among *P. alba*, *P. tremula*, and *P.* × *canescens* are given in Table 4. *Populus* × *canescens* showed a very high mean genetic identity (I) to *P. alba*, which was higher than that to *P. tremula.*

Canonical discriminant analysis portrayed the same genetic relationships among *P. alba*, *P. tremula,* and *P.* × *canescens* as displayed by the genetic distance analysis (Fig. 3). Canonical discriminant functions (CDF) 1 and 2 were highly significant ($P < 0.0003$) and accounted for 100% (98.4%, and 1.6%, respectively) of the variation in 18 polymorphic loci. CDF 1 and 2 separated *P. alba*, *P. tremula*, and *P.* × *canescens* into three distinct groups (Fig. 3). CDA correctly classified 22 out of the 23 *P. alba* clones into the *P. alba* group, all 18 *P. tremula* clones into the *P. tremula* group, and 13 out of the 15 *P.* × *canescens* clones into the *P.* × *canescens* group. The predicted group for the remaining 1 *P. alba* clone (A562) was *P.* × *canescens* and for 2 *P.* × *canescens* clones (C21 and C97) was *P. alba*. Cultivar 'Bachofenii' (clone E124) was classified into the *P. tremula* group.

The first two principal components (PCs) from PCA explained 36.9% (25.6% and 11.3%, respectively) of the total variation in 18 polymorphic loci. The relationships among the clones are portrayed by their ordination on the axes of PCs 1 and 2 (Fig. 4). PC 1 separated *P. alba* and *P. tremula* clones into two distinct groups. The two *P. alba* × *P. tremula*-controlled hybrids and 5 natural *P.* × *canescens* individuals formed an intermediate group between *P. alba* and *P. tremula,* while the remaining 8 *P.* × *canescens* individuals formed a group mixed in with the *P. alba* clones (Fig. 4). Clone E124 was grouped with other *P. tremula* clones. Certain *P. alba* clones of the same sex tended to group together on PCs 1 and 2

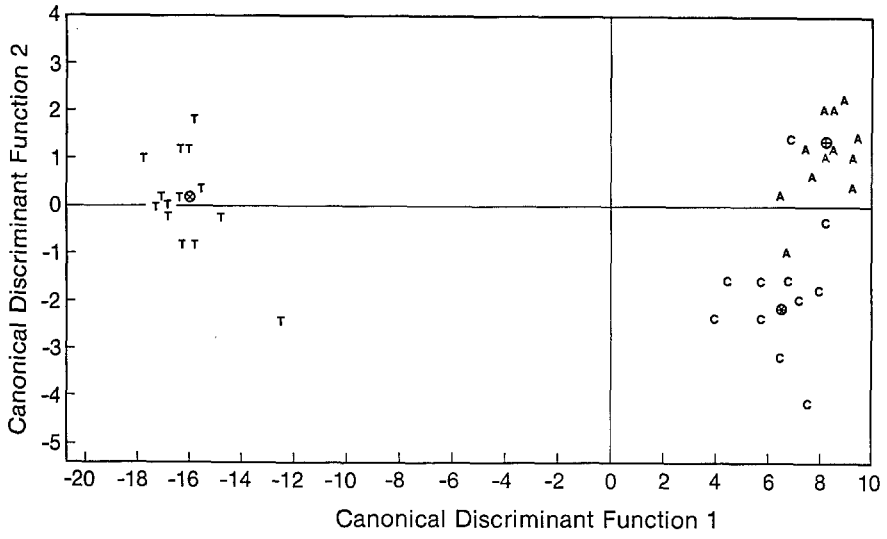

Fig. 3. Scatter plot of *P. alba* (*A*), *P. tremula* (*T*), and *P.* × *canescens* (*C*) individuals on the axes of the first two canonical discriminant functions. ⊕ centroid for *P. alba,* ⊗ centroid for *P. tremula,* ⊛ centroid for *P.* × *canescens*

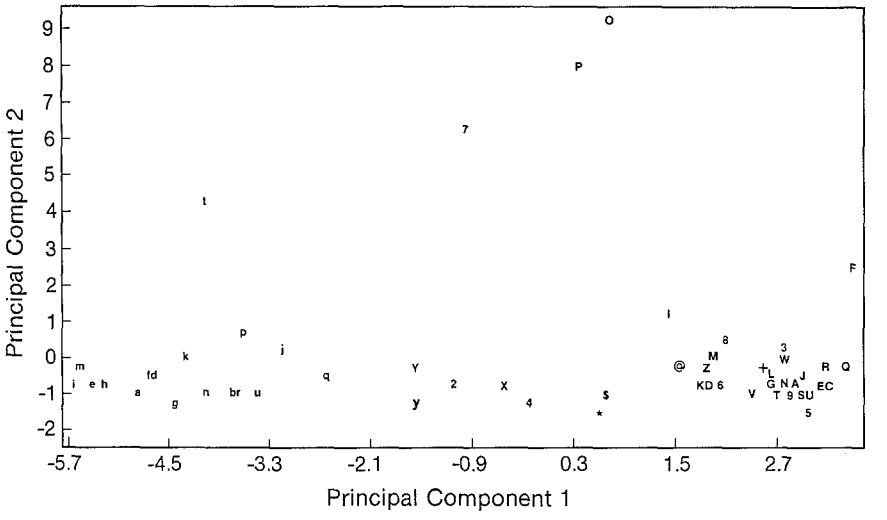

Fig. 4. Ordination of *P. alba*, *P. tremula*, and *P.* × *canescens* clones on the axes of principal components 1 and 2 based on their multilocus isozyme genotypes. *P. alba: A* A33 and A34, *C* A63 and A418, *D* A74, *E* A127, *F* A208, *G* A321, *I* A430, *J* A473, *K* A475, *L* A478, *M* A497, *N* A499, *O* A549, *P* A551, *Q* A562, *R* A563, *S* A565, *T* A568, *U* A569, *V* A570, *W* A558. *P. tremula*: *a* E16, *b* E18, *d* E25, *e* E26, *f* E39, *g* E56, *h* E57, *i* E58, *j* E66, *k* E82, *m* E83, *n* E100, *p* E103, *q* E104, *r* E124, *y* E147, *t* E152, *u* E155. *P.* × *canescens: X* AE36, *Y* AE42, *Z* C4, *@* C10, *$* C17, + C21, * C32, *2* C64, *3* C97, *4* C127, *5* C165, *6* C173, *7* C179, *8* C180, *9* C185

(Fig. 4). There was no evidence of such grouping in *P. tremula* and *P.* × *canescens*.

Discussion

The results demonstrate that *P. alba* and *P. tremula* are genetically distinct having interspecific genic and allelic differentiation. The species can be distinguished by mutually exclusive alleles at *Aco-3* and the species-specific locus *Mdh-3*, and also by allele frequency differences at 6 loci. Genetic distance analysis suggests that, on an average, 0.29 electrophoretically detectable allele substitutions per locus have taken place during the course of the evolution of *P. alba* and *P. tremula*. Although *P. alba* and *P. tremula* are genetically distinct, allelic data also suggest that these species are closely related as they have not accumulated many unique/mutually exclusive genes and alleles. The mean genetic identity observed between *P. alba* and *P. tremula* is within the upper range of mean genetic identities observed among congeneric species. The genetic distance value observed between these species is lower than those observed among geographically isolated *P. deltoides* Marsh., *P. nigra* L., and *P. maximowiczii* Henry (Rajora and Zsuffa 1990).

The results confirm the assumptions that *P.* × *canescens* clones are hybrids between *P. alba* and *P. tremula* because they have allelic contributions from both *P. alba* and *P. tremula*. Also, the mean observed heterozygosity in *P.* × *canescens* (0.157) was markedly higher than that was observed in *P. alba* (0.085) and *P. tremula* (0.096). Relatively high genetic identities of *P.* × *canescens* to the parental species, especially *P. alba,* and the occurrence of only a few unique alleles in *P.* × *canescens* suggest that

the hybrid event was recent relative to the divergence of the parental species. Our results show closer genetic relationships of *P.* × *canescens* to *P. alba* than to *P. tremula*. Such a high genetic identity, as was observed between *P. alba* and *P.* × *canescens*, is typical of the intraspecific level. Based on our isozyme genotypes, allele frequency, and genetic distance data, we think that the sampled *P.* × *canescens* clones consisted of a mixture of F_1 hybrids of *P. alba* and *P. tremula* and their backcrosses to *P. alba*. The *P.* × *canescens* clones C17, C32, C64, C127, and C179, that showed genotypes close to those of controlled *P. alba* × *P. tremula* clones AE36 and AE42 and grouped with them on PC1, may represent F_1 hybrids. The remaining 8 *P.* × *canescens* clones most likely represent the progeny of *P.* × *canescens* backcrosses to *P. alba*. It has been noted that *P.* × *canescens* extends broadly to the northwest area in Europe that is currently occupied by *P. alba* var *'nivea'* Willd. (Anonymous 1958). We think that the backcrossing of *P.* × *canescens to P. alba* may be a contributing factor for such extension. Our study was based on the selected individuals of *P. alba*, *P. tremula*, and *P.* × *canescens*. The results suggest a need for further population genetic studies by sampling natural populations of *P. alba, P. tremula*, and *P.* × *canescens*.

On the basis of the rooting behavior of shoot cuttings of cv 'Bachofenii' grown in Yugoslavia it has been suggested that this cultivar should be grouped with *P.* × *canescens* (Anonymous 1979). However, our results clearly suggest that this cultivar, represented by clone E124, should be grouped with *P. tremula*.

The results clearly demonstrate that individual clones of *P. alba*, *P. tremula*, and *P.* × *canescens* could be identified by their multilocus isozyme genotypes. Also, some clones could be identified by their unique genotype at a single locus. The same multilocus genotypes shared by *P. alba* clones A33 and A34, and A63 and A418 seem to be coincidental as these clones had distant origins. However, these clones may be differentiated by exploring additional isozyme variability.

Populus alba individuals A34, A63, A74, A549, and A551 are genetically distinct from each other. Thus, not all *P. alba* trees grown in Canada are members of a single clone.

In the genus *Populus*, the unit of breeding, propagation, and cultivation is the clone. Isozyme genotyping technique can be of significance in clonal identification, certification, and varietal control in *P. alba*, *P. tremula*, and *P.* × *canescens*. Isozyme markers and multilocus allozyme genotypes have been found to be very effective for clone and cultivar identification in other *Populus* species and hybrids (Cheliak and Pitel 1984; Rajora 1988, 1989a, b, c; Rajora and Zsuffa 1989).

The isozyme gene markers established in this study could be used in *P. alba*, *P. tremula*, and *P.* × *canescens* for species and hybrid differentiation, clonal identification, certification and registration, and for other genetics and breeding studies by themselves or in combination with morphological and phenological traits.

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