American Journal of Botany 91(9): 1398–1408. 2004.

PHYLOGENY OF *POPULUS* (SALICACEAE) BASED ON NUCLEOTIDE SEQUENCES OF CHLOROPLAST *TRNT-TRNF* REGION AND NUCLEAR RDNA[1]

MONA HAMZEH AND SELVADURAI DAYANANDAN[2]

Forest and Evolutionary Genomics Laboratory, and the Centre for Structural and Functional Genomics, Biology Department, Concordia University, 7141 Sherbrooke St. West, Montreal, Quebec, Canada H4B 1R6

The species of the genus *Populus*, collectively known as poplars, are widely distributed over the northern hemisphere and well known for their ecological, economical, and evolutionary importance. The extensive interspecific hybridization and high morphological diversity in this group pose difficulties in identifying taxonomic units for comparative evolutionary studies and systematics. To understand the evolutionary relationships among poplars and to provide a framework for biosystematic classification, we reconstructed a phylogeny of the genus *Populus* based on nucleotide sequences of three noncoding regions of the chloroplast DNA (intron of *trnL* and intergenic regions of *trnT-trnL* and *trnL-trnF*) and ITS1 and ITS2 of the nuclear rDNA. The resulting phylogenetic trees showed polyphyletic relationships among species in the sections *Tacamahaca* and *Aigeiros*. Based on chloroplast DNA sequence data, *P. nigra* had a close affinity to species of section *Populus*, whereas nuclear DNA sequence data suggested a close relationship between *P. nigra* and species of the section *Aigeiros*, suggesting a possible hybrid origin for *P. nigra*. Similarly, the chloroplast DNA sequences of *P. tristis* and *P. szechuanica* were similar to that of the species of section *Aigeiros*, while the nuclear sequences revealed a close affinity to species of the section *Tacamahaca*, suggesting a hybrid origin for these two Asiatic balsam poplars. The incongruence between phylogenetic trees based on nuclear- and chloroplast-DNA sequence data suggests a reticulate evolution in the genus *Populus*.

Key words: cpDNA; phylogeny; *Populus*; rDNA; reticulate evolution; Salicaceae.

The species of the genus *Populus* (aspen, cottonwood, and poplars), collectively known as poplars, are one of the most commercially exploited groups of forest trees. They are widely distributed in the northern hemisphere from subtropical to boreal forests and play a significant ecological role as pioneer species in boreal forests and also as dominant species in the riparian forests that serve as rich wildlife habitats and watersheds (Braatne et al., 1992). Because of their fast growth rates, profuse vegetative propagation, adaptability to a variety of ecological sites, and the numerous uses of their wood (i.e., timber, paper pulp), *Populus* species have become one of the most economically important groups of forest trees (Stettler et al., 1996b). They have also become a subject of many interdisciplinary studies, including biotechnology and genetic engineering, and are becoming a model organism for the study of tree biology (Stettler et al., 1996a).

The extensive interspecific hybridization and the high levels of morphological variation among poplars have posed great difficulties in species delimitation for systematic and comparative evolutionary studies. The number of *Populus* species currently described in the literature ranges from 22 to 85 plus hundreds of hybrids, varieties, and cultivars (Eckenwalder, 1977b, 1996; Dickmann and Stuart, 1983). Discrepancies in the number of species could be attributed to the misinterpretation of some hybrids and to difficulties involved in delineating species boundaries. According to a recent classification (Eckenwalder, 1996), the genus *Populus* is classified into 29 species in six sections (*Abaso*, *Aigeiros*, *Leucoides*, *Populus*, *Tacamahaca*, *Turanga*). These sections are considered to be natural in most cases because they are delineated by the occurrence of major hybridization barriers (Zsuffa, 1975; Eckenwalder, 1996). However, the placement of several taxa within these sections remains controversial. For instance, *P. nigra* of section *Aigeiros* has a genetic affinity to species of section *Tacamahaca*. In *P. nigra*, cpDNA RFLP analysis showed similarity to species of the section *Populus*, but RFLP patterns of nuclear rDNA were distinct from the section *Populus*, suggesting a possible hybrid origin of *P. nigra* (Smith and Sytsma, 1990).

Recent phylogenetic analyses of the family Salicaceae using DNA sequence data from chloroplast *rbcL* (Azuma et al., 2000) and ITS of nuclear rDNA (Leskinen and Alstrom-Rapaport, 1999) strongly suggest that *Populus* is a monophyletic group sister to *Salix*. However, phylogenetic relationships within the genus remain controversial. A phylogenetic analysis of poplars using 76 morphological traits of buds, leaves, inflorescences, flowers, and fruits supported the monophyly of all sections except *Tacamahaca*, which resolved into two paraphyletic groups (Eckenwalder, 1996). However, relationships between sections as well as relationships among taxa within sections were only partially resolved.

Based on phylogenetic analysis of restriction fragment length polymorphism of chloroplast DNA, the section *Tacamahaca* has been suggested to be polyphyletic and the section *Populus* is considered as the terminal clade (Smith, 1988). On the other hand, a phylogenetic tree based on DNA sequences from the *ITS* region of the nuclear rDNA of four species of *Populus* showed an opposite trend with *P. alba* of section *Populus* as basal, followed by *P. lasiocarpa* of the section

[1] Manuscript received 14 October 2003; revision accepted 29 April 2004.

The authors thank Dr. Barbara Thomas (Alpac, and the University of Alberta), Mr. Alan Robertson (Alpac), Mr. David Kamelchuk (Alpac), Mr. Pierre Perinet (Le Ministere des Ressources Naturelles du Quebec), Mr. Normand Fleury and Ms. Edith Morin (Montreal Botanical Garden), Dr. Campbell Davidson (AAFC—Morden Research Station and the Arboretum, Manitoba), and Dr. Damase Khasa (Universite Laval) for their help in collecting leaf samples. This research was supported by grants from Concordia University, Natural Sciences and Engineering Research Council of Canada, Le Fonds Quebecois de la Recherche sur la Nature et les Technologies, and Canada Foundation for Innovation.

[2] E-mail: daya@alcor.concordia.ca.

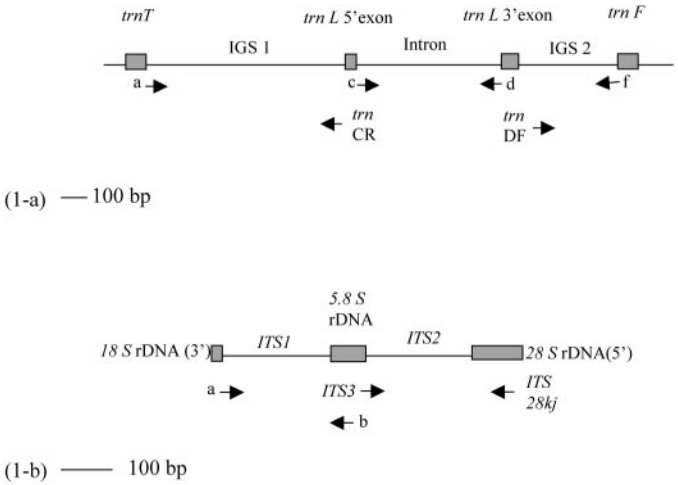

Fig. 1. Relative positions and directions of primers used for amplifying and sequencing chloroplast and nuclear genomic regions from *Populus* species. a) Three noncoding regions of chloroplast DNA. b) ITS regions of rDNA.

Leucoides and species of the sections *Aigeiros* and *Tacamahaca* as the terminal clade (Leskinen and Alstrom-Rapaport, 1999). Mitochondrial and chloroplast restriction site analysis of four *Populus* species suggests polyphyletic relationships for species in the section *Aigeiros* (Barrett et al., 1993; Rajora and Dancik, 1995).

In phylogenetic studies of groups in which hybridization between lineages has played a substantial role in their evolution (Smith, 1988), the combined use of cpDNA and nuclear DNA data is crucial to gain a comprehensive understanding of the evolutionary history. A striking feature of poplars that has received considerable attention from many researchers is the occurrence of interspecific hybrids (Eckenwalder, 1982, 1996; Dickmann and Stuart, 1983; Barnes and Pregitzer, 1985; Whitham et al., 1996). Hybrids are regularly found in regions where species of sections *Aigeiros* and *Tacamahaca* are sympatric, such as in the contact zones of *P. angustifolia, P. trichocarpa,* and *P. balsamifera* (Brayshaw, 1965). Similarly, species of section *Populus* are known to hybridize naturally with other members of the section, such as *P. alba* with *P. tremula* (Stettler et al., 1996b) and *P. grandidentata* with *P. tremuloides* (Barnes, 1961). Moreover, RFLP analysis of cpDNA and rDNA (Smith, 1988) has raised the possibility of ancient hybridization by which *P. nigra* appeared to be an introgressant of the *P. alba* (cpDNA) lineage and some other presently unknown paternal lineage of section *Populus*. Similarly, *P. tristis* (a central Asian member of section *Tacamahaca*) appeared to be an introgressant or a hybrid of the *P. nigra* (cpDNA) lineage and the lineage with Asian species of section *Tacamahaca*. Thus, it is not unreasonable to assume that there have been abundant opportunities for gene exchange among sympatric species, even between taxa of different sections (Stettler et al., 1996b).

Many *Populus* species are likely to have undergone complex reticulate evolution. In a hybrid lineage resulting from reticulate evolution, parental nuclear genes may become fixed for one of the parental types through homogenization by concerted evolution of gene families (Page and Holmes, 1998), segregation during sexual reproduction, or lineage sorting (Doyle, 1992). These events will generate discrepancies among phylogenetic trees based on uniparentally inherited organeller sequences (Sears, 1980; Neale and Wheeler, 1986) and biparentally inherited nuclear sequences or their phenotypic expression in morphology. Therefore, data from both nuclear and chloroplast genes are required to parse out reticulate evolutionary events (Page, 2000) and reconstruct robust phylogenetic trees.

The objective of our present study is to reconstruct the phylogeny of genus *Populus* based on DNA sequences of chloroplast and nuclear genomes to improve understanding of the evolutionary history of the genus and to provide a framework for biosystematic classification of the genus *Populus*.

TABLE 1. Sequences of primers used to amplify and sequence *trnT-trnF* three noncoding regions (see Fig. 1a) from *Populus* species.

Primer name	Sequence
a	5′-cattacaaatgcgatgctct-3′
c	5′-cgaaatcggtagacgctacg-3′
d	5′-ggggatagagggactttgaac-3′
f	5′-atttgaactggtgacacgag-3′
trnCR	5′-tgttagaacaacttccattgagtctc-3′
trnDF	5′-agtcccattctacatgtcaatatcg-3′

MATERIALS AND METHODS

Taxon sampling, DNA extraction, PCR amplification, and sequencing—Taxon sampling was based on the recent classification of genus *Populus* proposed by Eckenwalder (1977a, b, 1996) and Dickmann and Stuart (1983). Fresh leaves were obtained from 21 taxa including 17 species representing three sections of *Populus* [sections *Tacamahaca* Spach, *Aigeiros* Duby, and *Populus* (Leuce) Duby]. This sampling includes two varieties of *P. deltoides* and two putative species not included in Eckenwalder's (1977a, b) and Dickmann and Stuart's (1983) taxonomic treatments. DNA sequences from two species of *Salix* were used as outgroups. The list of species used in this study and the accession numbers are given in Appendix 1 (see Supplemental Data accompanying the online version of this article). The collected leaf samples were stored at −80°C prior to extraction of DNA. Total genomic DNA was extracted from frozen leaf tissue using the methods of Doyle and Doyle (1987) and Dayanandan et al. (1997).

The three noncoding regions of cpDNA: *trnT-trnL* intergenic spacer (IGS1), *trnL* intron, and *trnL-trnF* intergenic spacer (IGS2) (hereafter referred to as cpDNA) and ITS1, ITS2, and part of the 5.8S and the 5′ region of the 28S subunit of the rDNA (hereafter referred as rDNA) were amplified by the polymerase chain reaction (PCR). The oligonucleotide primers a, c, d, and f designed by Taberlet et al. (1991) for cpDNA, primers a and b modified from Leskinan and Alstrom-Rapaport (1999) for ITS1, and primers ITS3 (Becerra and Venable, 1999) and ITS 28kj modified from Culling (1992) for ITS2 were used for PCR amplification and sequencing. Two additional primers, trnCR and trnDF were designed for sequencing to cover some gaps in the cpDNA sequences (Fig. 1a, b; Tables 1, 2). Amplification reactions contained 230 μmol/L dNTP, 2.5 mmol/L $MgCl_2$, 5 μmol/L of each primer, 1 unit of *Taq* DNA polymerase, and 2.5 μL buffer (0.2 mol/L Tris pH 9.5; 0.25 mol/L KCl; 1 mg/mL BSA, 5 μL/mL Tween 20) in a total volume of 25 μL. The PCR amplification was performed in an Mastercycler gradient thermal cycler (Ep-

TABLE 2. Sequences of primers used to amplify and sequence ITS1 and ITS2 (see Fig. 1b) from *Populus* species.

Primer name	Sequence
a	5′-tcgtaacaaggtttccgtagg-3′
b	5′-gctacgttcttcatcgatg-3′
ITS3	5′-gcatcgatgaagaacgcagc-3′
ITS 28kj	5′-cttggacggaatttaccg-3′

pendorf, Westbury, New York, USA) at 94°C for 60 s, 55°C for 30 s, and 72°C for 60 s for 35 cycles. Amplified DNA was purified using a PCR purification kit (QIAGEN, Mississauga, Ontario, Canada) , electrophoresed on 1% agarose gel with ethidium bromide (0.33 μg/mL) at 3.5 V/cm for 90 min. Amplified fragments were visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene, Frederick, Maryland, USA). The digital image files were analyzed using Gene Tools software from Syngene. The quantity of DNA was estimated using a Mass Ruler DNA Ladder Mix (Fermentas, Burlington, Ontario, Canada). The purified, amplified DNA was directly sequenced using ABI Big Dye Terminator version 3.0 and 3.1 Cycle Sequencing Ready Reaction kit and an ABI310 automated genetic analyzer (Applied Biosystems, Foster City, California, USA). Each region was sequenced between two and seven times. Same primers were used for both PCR amplification and sequencing. The thermal cycling profile of sequencing reactions were: 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min for 25 cycles. The chromatograms of the DNA sequencing results were processed and analyzed using the Staden software package (Staden, 1979; http://staden.sourceforge.net). The assembled contigs of cpDNA and rDNA of each species were aligned using ClustalW (Thompson et al., 1994) multiple sequence alignment software. Aligned DNA sequences were imported to MacClade 4.0 software (Maddison and Maddison, 2001) for verification and manual editing of the sequence alignments. The 5′/3′ *trnL* exon (identified by comparing with the *Nicotiana* chloroplast sequence; Genebank accession number = NC001879). The final data set included three noncoding regions of the *trnT-trnF* of cpDNA (intergenic region of *trnT-trnL, trnL* intron, and intergenic region of *TrnL-trnF*) and ITS I, partial *5.8S* rRNA, ITS 2, and part of 28S subunit of the rDNA. The two data sets (cpDNA and rDNA) were analyzed separately.

Phylogenetic analysis—The phylogenetic analyses were conducted using PAUP* version 4.0 beta 8 (Swofford, 2001). For each data set, 10^6 random trees were analyzed to obtain the frequency distribution of tree lengths to assess the phylogenetic signal of the data matrix by calculating the skewness (g1) and kurtosis (g2). The nucleotide frequency distribution was investigated with a chi-square test of homogeneity of base distribution across sequences.

Parsimony analysis—Maximum parsimonious (MP) phylogenetic trees were reconstructed through heuristic search with equal character weights, gaps treated as missing, multistate taxa interpreted as uncertainty, starting tree obtained via stepwise addition, and sequence addition was as-is for the cpDNA data set (random addition was not possible because of computing limitations) and random addition of sequences with 1000 replicates for rDNA data set. Tree bisection-reconstruction (TBR) was used as the branch-swapping algorithm. Strict and 50% majority rule consensus trees were obtained. The phylogenetic trees were rooted using *Salix* species as outgroups. Bootstrap analysis with a fast heuristic search based on 1000 replicates was performed to assess the robustness of branches.

Maximum likelihood analysis—The software program Modeltest version 3.06 (Posada and Crandall, 1998) was used to find the best nucleotide substitution model that fits the data set using hierarchical likelihood ratio test. The maximum likelihood (ML) analyses of both cpDNA and rDNA were performed through heuristic search with TBR branch swapping, addition of sequences as-is, and the Tamura-Nei + I model (Tamura and Nei, 1993) as the nucleotide substitution model. The bootstrap analysis with fast heuristic search based on 100 replicates was performed to assess the robustness of branches.

Comparison of cpDNA with rDNA tree—The two consensus trees obtained through maximum parsimony analysis of two data sets were compared using nonparametric Templeton (Wilcoxon signed-ranks) and winning-sites tests (Templeton, 1993). To compare two maximum likelihood trees, we used the Kishino-Hasegawa (1989) test using bootstrap with full optimization, two-tailed test, and the Shimodaira-Hasegawa (1999) test using bootstrap with full optimization, one-tailed test. Each bootstrap analysis was performed with 1000 replicates.

RESULTS

Direct sequencing of purified PCR products of the *trnT-trnF* chloroplast region (cpDNA) and rDNA followed by ClustalW alignment of all sequences resulted in a data matrix with a length of 1414 characters (nucleotides) and 23 taxa with 71 parsimony informative ones for the cpDNA and 791 characters and 23 taxa with 43 parsimony informative ones for the rDNA genomic region, respectively. The corresponding DNA sequences of multiple individuals of the same species were identical.

The frequency distribution of the length of 10^6 random trees yielded g1 = −1.726, g2 = 4.396 for cpDNA and g1 = −1.313, g2 = 2.718 for the rDNA suggesting a strong phylogenetic signal in these data matrices. The chi-square test of homogeneity of base frequencies across taxa showed $P = 1.0$ in both cases, and the null hypothesis of homogeneous base distribution across sequences was accepted.

The length of ITS 1 in all *Populus* species included in our study was 224 base pairs (bp) except for *P. fremontii,* which was 225 bp. The length of ITS 2 was 212 bp for all *Populus* species examined, except for *P. davidiana* and *P. tremula* (214 bp) and *P. maximowiczii* and *P. simonii* (203 bp), which shared a deletion of 11 bp. The cpDNA data matrix comprised IGS1 (473 bp), *trnT-trnL* intron (628 bp), and IGS 2 (312 bp).

cpDNA trees—*Maximum parsimony analysis*—Maximum parsimony searches of chloroplast DNA yielded 30 939 equally parsimonious trees (tree length 118; consistency index [CI] = 0.924; retention index [RI] = 0.927; rescaled consistency index [RC] = 0.857). The 50% majority rule consensus tree (Fig. 2) and strict consensus tree differed only in the placement of *P. tremula* and *P. tremuloides* (with 53% occurrence). They appeared as sister taxa in a basal position to the other species of section *Populus* in the 50% majority rule tree, while in the strict consensus tree they grouped with other members of the section with an unresolved polytomy. The bootstrap value also did not support a basal position for these two aspens.

Based on MP analysis, all *Populus* species formed a strongly supported monophyletic group comprising three major clades. One clade comprised *P. simonii, P. maximowiczii, P. laurifolia,* and *P. songarica*. The other clade comprised species of section *Populus* (*P. grandidentata, P. alba, P. davidiana, P. tremula,* and *P. tremuloides*) and *P. nigra* of section *Aigeiros*. The phylogenetic relationships among taxa within this clade remained unresolved. The remaining clade comprised the remainder of the species. Within this clade, *P. angustifolia* clustered with *P. cathayana,* whereas *P. trichocarpa* clustered with *P. balsamifera*. *Populus szechuanica* occupied a position basal to a group of species comprising *P. tristis, P. fremontii, P. roegneriana, P. angulata, P. deltoides,* and *P. sargentii*. The phylogenetic relationships among species of this group remained unresolved.

Besides *P. nigra,* which did not group with the other *Aigeiros* group members, the cpDNA data of *P. tristis* and *P. szechuanica* (Asiatic balsam poplars) suggested that these were more closely related to North American cottonwoods of the *Aigeiros* group than to species of section *Tacamahaca*. The MP analysis placed the lineage consisting of North American cottonwoods (*P. fremontii, P. deltoides,* and its two varieties) and *P. tristis* of Asian balsam poplars (section *Tacamahaca*) in a terminal clade. However, based on the MP tree, the basal lineage could not be determined. Section *Populus* was mono-

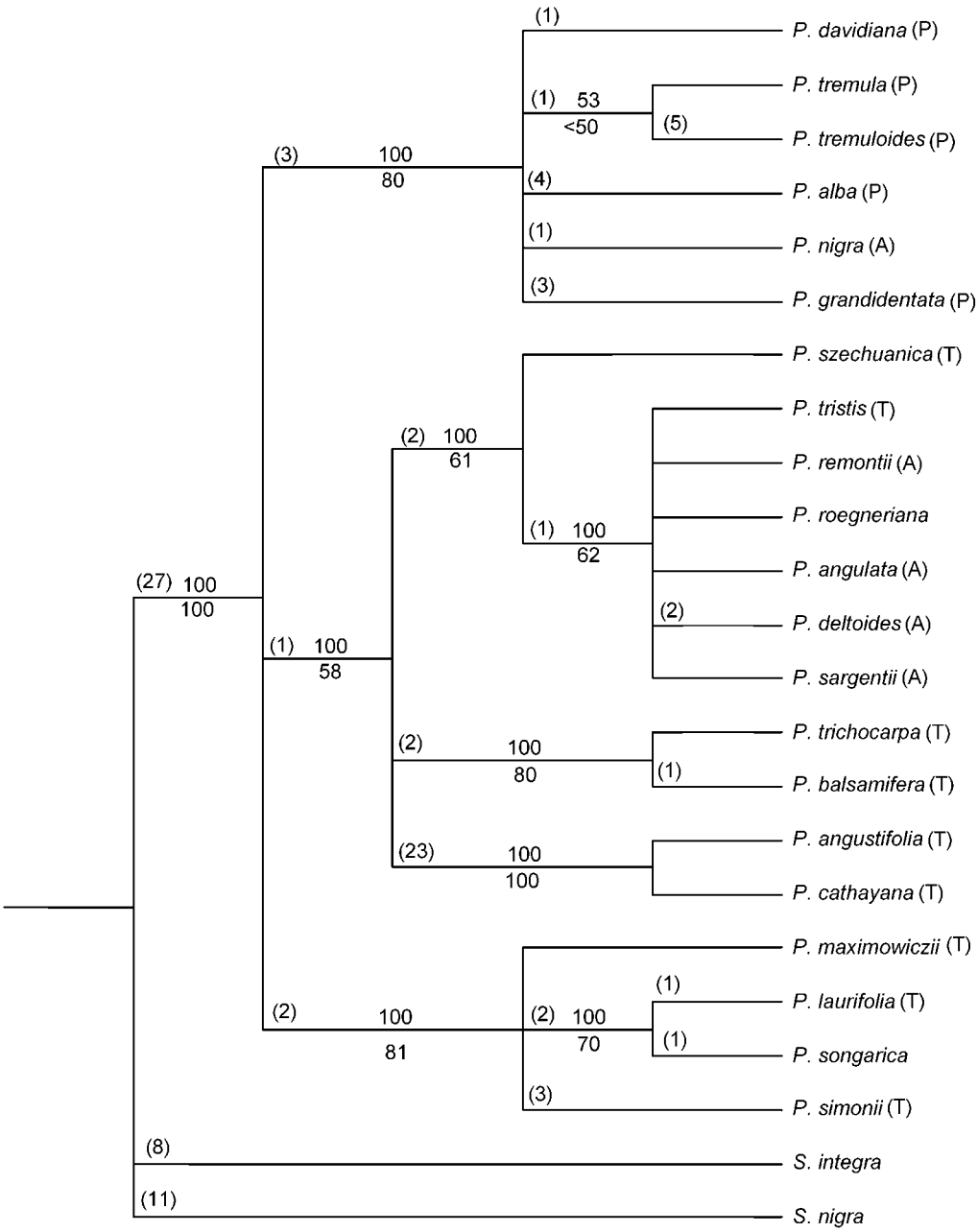

Fig. 2. The majority rule consensus tree of 30 939 equally parsimonious trees (tree length 118; consistency index = 0.924) based on three noncoding regions of *trnT-trnF* of cpDNA sequences from *Populus* species. Numbers above branches show the frequency of occurrence in 50% majority rule consensus tree, and numbers below branches indicate bootstrap percentage values. Numbers in brackets show branch lengths (number of nucleotide substitution). A, *Aigeiros*; P, *Populus*; T, *Tacamahaca*.

phyletic and sections *Tacamahaca* and *Aigeiros* were polyphyletic groups (Fig. 2).

Maximum likelihood analysis—The results of the Modeltest analysis showed that TrN + I (Tamura and Nei, 1993: equal rate for all transversions and different transition rates with unequal base frequencies) nucleotide substitution model was the most suitable model for the cpDNA. The parameters of the model were: base frequencies: A = 0.4175, C = 0.1336, G = 0.1467, T = 0.3023; rate matrix: (A–C) = (A–T) = (C–G) = (G–T) = 1.0; (A–G) = 1.7645; (C–T) = 2.5848; among-site rate variation: proportion of invariable sites, *I*, = 0.6953; equal rates of substitution for all variable sites.

The maximum likelihood analysis of cpDNA with the described model parameters retained a single tree (Fig. 3) with a topology identical to the 50% majority rule consensus tree obtained from parsimony analysis. Moreover, the bootstrap values for branch robustness under the maximum likelihood criterion were similar to the values obtained from the same test under the parsimony criterion.

As with the MP analysis, the ML analysis also showed the monophyletic origin of section *Populus* and the polyphyletic

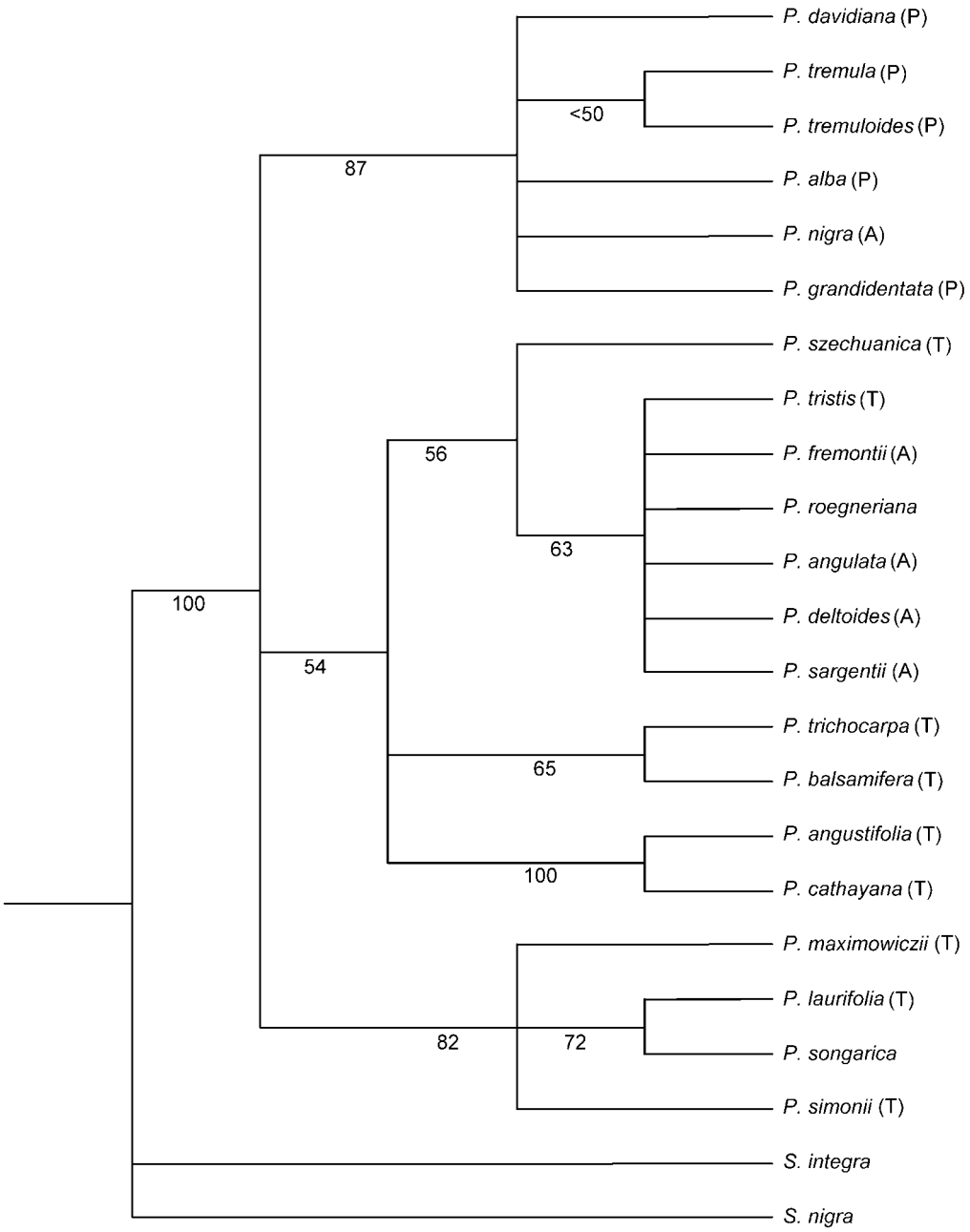

Fig. 3. Maximum likelihood tree based on three noncoding regions of *trnT-trnF* of cpDNA sequences from *Populus* species. Numbers below branches show bootstrap percentage values. A, *Aigeiros*; P, *Populus*; T, *Tacamahaca*.

origin of sections *Tacamahaca* and *Aigeiros*. *Populus nigra* clustered with members of the section *Populus*. *Populus tristis* and *P. szechuanica* grouped with the lineage comprising the North American cottonwoods of section *Aigeiros*.

rDNA trees—*Maximum parsimony analysis*—The maximum parsimony analysis based on nuclear rDNA yielded 497 equally parsimonious trees (tree length = 94; CI = 0.851; RI = 0.888; RC = 0.756). In the 50% majority rule consensus tree (Fig. 4), two North American aspens, *P. tremuloides* and *P. grandidentata*, grouped as sister taxa in the lineage consisting of other *Populus* species, but their placement in the strict consensus tree remained unresolved. In the 50% majority rule consensus tree, a group of balsam poplars of section *Tacamahaca*, namely *P. angustifolia, P. cathayana, P. trichocarpa, P. balsamifera, P. tristis*, and *P. szechuanica* clustered as a sister group to the lineage comprising members of section *Aigeiros*. Although the branch representing *Tacamahaca* occurred in 75% and *Aigeiros* 100% of 497 most parsimonious trees, these branches were not supported by bootstrap analysis. Moreover, in the 50% majority rule consensus tree, within the balsam poplar lineage, *P. trichocarpa, P. balsamifera, P. tristis*, and *P. szechuanica* clustered together as a sister group to *P. angustifolia* and *P. cathayana*. However, none of these internal nodes and relationships was supported by the bootstrap analysis, even though they occurred with a high percentage in

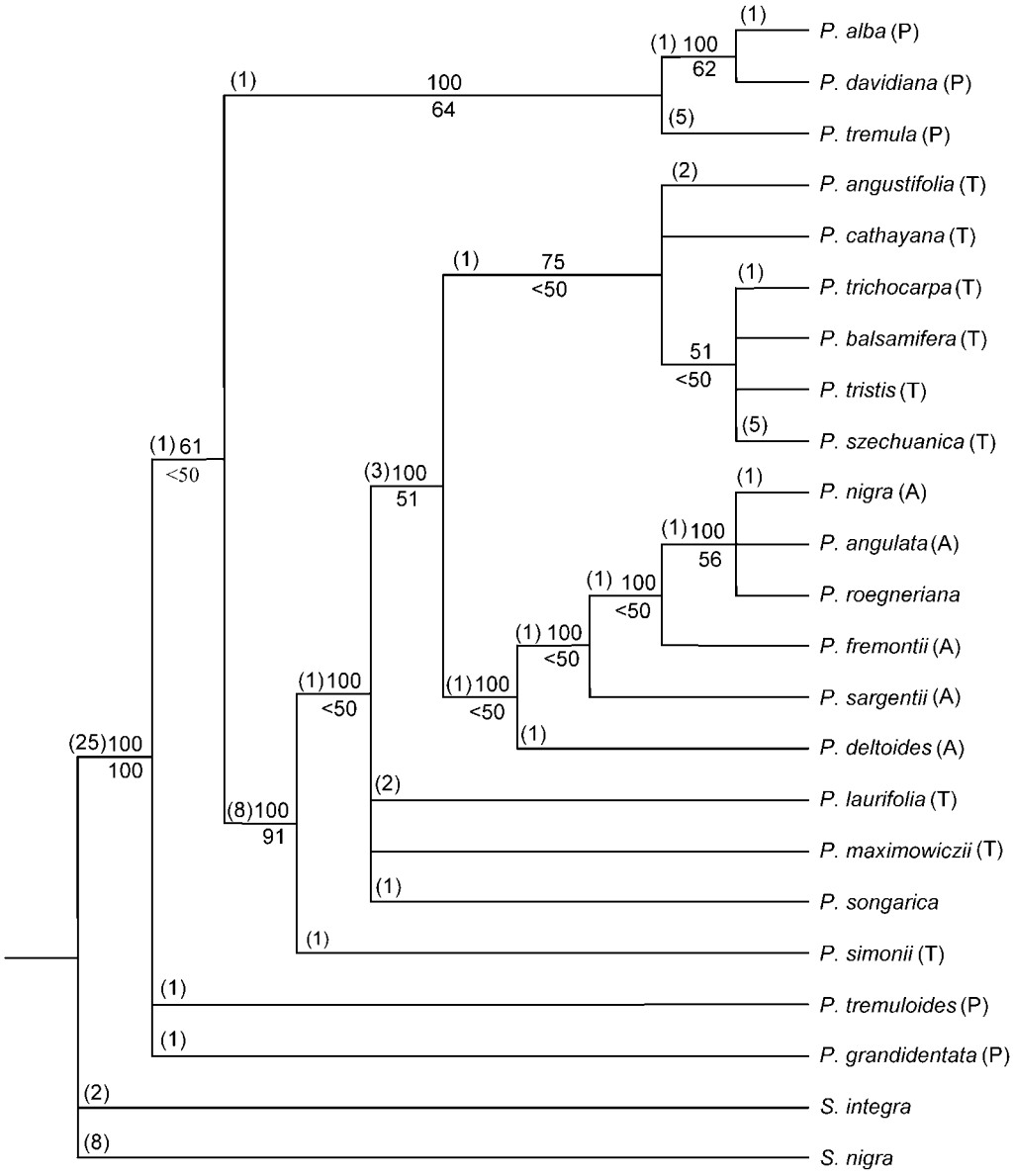

Fig. 4. The majority rule consensus tree of 497 equally parsimonious trees (tree length 94; consistency index = 0.851) based on partial 5.8S RNA gene, ITS1 and ITS2 and part of 28S subunit sequences from *Populus* species. Numbers above branches show frequency of occurrence in 50% majority rule consensus tree, and numbers below branches indicate bootstrap percentage values. Numbers in brackets show branch lengths (number of nucleotide substitution). A, *Aigeiros*; P, *Populus*; T, *Tacamahaca*.

the 497 retained equally parsimonious trees (Fig. 4). Moreover, a sister relationship of *P. maximowiczii* and *P. laurifolia* to the clade comprising two lineages of the *Aigeiros* and group of *Tacamahaca* species (as mentioned earlier) was weakly supported. The basal position of *P. simonii* was not supported by the bootstrap analysis.

In the MP analysis of rDNA with respect to the results of the bootstrap analysis, all *Populus* species studied formed a strongly supported monophyletic group comprising two major clades. One clade comprised all species of the section *Populus* with *P. tremuloides* and *P. grandidentata* occupying a position sister to the clade comprising Eurasian species of section *Populus* (*P. tremula, P. alba,* and *P. davidiana*), suggesting a monophyletic origin for this section. The other major clade included all of the remaining species studied.

The relationships among species within section *Tacamahaca* were unresolved, and they grouped as a polytomy. However, this section could be divided into two distinct groups of taxa: *P. maximowiczii, P. simonii,* and *P. laurifolia* in one group and the other members of the section in another group. The relationships among species within section *Aigeiros* were resolved, and the MP analysis of rDNA suggested a monophyletic origin for this section. However, this was not supported by bootstrap analysis (<50%). A close relationship among *P. nigra, P. deltoides* var. *angulata,* and *P. roegneriana* was evident. In contrast to the cpDNA-based MP tree, the rDNA-based tree did not have a close affinity between *P. nigra* and members of section *Populus*. *Populus tristis* and *P. szechuanica* clustered as an unresolved polytomy with the remaining species of sections *Tacamahaca* and *Aigeiros*.

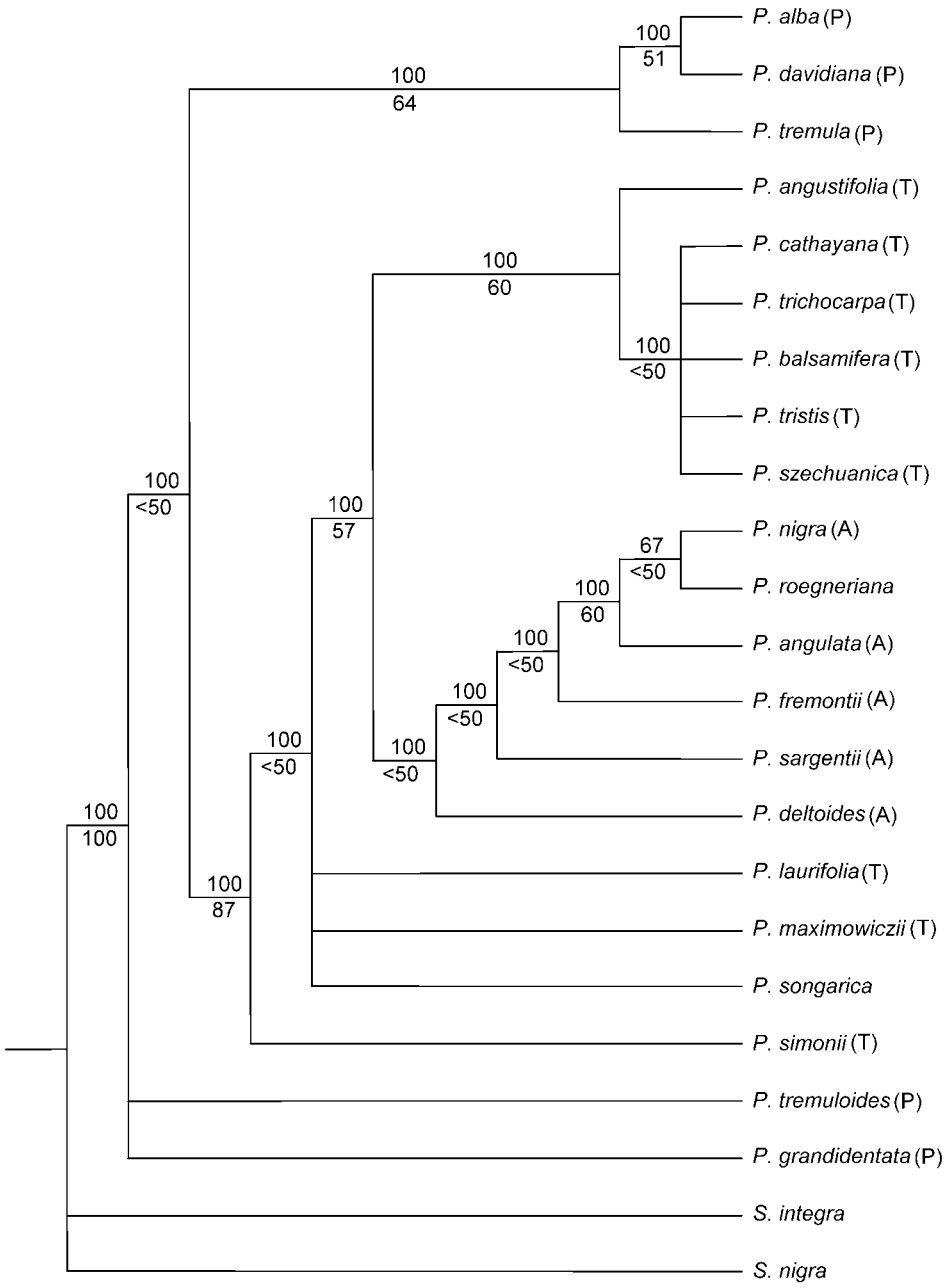

Fig. 5. The majority rule consensus tree of three maximum likelihood trees based on partial 5.8S RNA gene, ITS1 and ITS2, and part of the 28S subunit sequences from *Populus* species. Numbers above branches show frequency of occurrence in 50% majority rule consensus tree, and numbers below branches show bootstrap percentage values. A, *Aigeiros*; P, *Populus*; T, *Tacamahaca*.

Maximum likelihood analysis—The result of the Modeltest analysis showed that TrN + I (Tamura and Nei, 1993: equal transversion rate with variable transition substitution rate and unequal base frequencies) was the most suitable model for the rDNA. The parameters of the model were: base frequencies: A = 0.1841, C = 0.3205, G = 0.3242, T = 0.1711; rate matrix: (A–C) = (A–T) = (C–G) = (G–T) = 1.0, (A–G) = 3.5870, (C–T) = 5.6386; and among-site rate variation: proportion of invariable sites, *I*, = 0.8085, equal rates of substitution for all variable sites.

The ML analysis of rDNA sequences with the described model parameters yielded three trees. The 50% majority rule consensus tree (Fig. 5) and the strict consensus tree differed only at the placement of *P. deltoids* var. *angulata*. In the 50% majority rule consensus tree, it occupied the basal position to the clade, comprising *P. nigra* and *P. roegneriana*, whereas in strict consensus they all were sister to one another.

The 50% majority rule consensus ML tree had a topology similar to the 50% majority rule consensus MP tree except (1) in the placement of *P. deltoides* var. *angulata* as given earlier and (2) in clustering *P. catahyana* with *P. trichocarpa, P. balsamifera, P. tristis,* and *P. szechuanica*. Likewise, bootstrap analysis of rDNA under the maximum likelihood criterion resulted in a tree with a topology compatible with the MP con-

sensus tree after collapsing branches with low bootstrap values (<50%). However, the clade comprising *P. balsamifera, P. trichocarpa, P. cathayana, P. angustofolia, P. szechuanica,* and *P. tristis* was supported by bootstrap analysis (Figs. 4, 5).

Topology comparison test results—The Templeton (Wilcoxon signed-ranks) test statistic $Z = -5.9244$ was smaller than the absolute values of the rank sums. The Winning-site test with $P < 0.0001$ indicates a significant difference at $P < 0.05$ under the null hypothesis of no difference between two trees. Both the Kishino-Hasegawa and Shimodaira-Hasegawa tests rejected the null hypothesis ($P = 0.000$), suggesting that there was no difference between the two trees.

DISCUSSION

Phylogenetic relationships of Populus and hybridization—Trees derived from maximum likelihood analysis were compatible with the tree topology obtained through maximum parsimony analysis. Therefore, in order to discuss the evolutionary patterns in *Populus,* we will consider the tree topologies similar to the maximum parsimony trees to be the most plausible and conservative hypotheses for the phylogenetic relationships within the genus *Populus* based upon cpDNA and nuclear rDNA sequence data (Figs. 2, 4).

Compared to the willows (*Salix* spp.), there are relatively few species of poplars, and they fall into a number of groups that are traditionally recognized as sections. With few exceptions, there is a reasonable agreement in the literature on the characteristics and species composition of these sections, and major barriers to hybridization are known to lie between the sections (Zsuffa, 1975). However, the relationships between sections and among species within each section and the placement of some controversial species or taxa of purported hybrid origin have been subjects of controversy for a long time.

The species of section *Populus* are monophyletic with respect to their maternal lineage (cpDNA), with the exception of *P. nigra,* a taxon considered to be a member of section *Aigeiros,* which clusters with species in section *Populus*. In terms of rDNA (nuclear) lineage section *Populus* is monophyletic, and *P. nigra* clusters with the other species of section *Aigeiros*. The phylogenetic relationships among the species within section *Populus* based on cpDNA and rDNA sequence data are not in agreement with one another. Based on maternal chloroplast sequence data, European aspen (*P. tremula*) and the American trembling aspen (*P. tremuloides*) cluster together as sister taxa and occupy the terminal position in the *Populus* clade. However, this relationship is weak and has less than 50% bootstrap support. In the rDNA-based phylogenetic tree, white poplar (*P. alba*) and Korean aspen (*P. davidiana*) cluster together as sister species and occupy the terminal position. These two species along with European aspen (*P. tremula*) form a monophyletic group within section *Populus* with sister relationships to the North American aspens, *P. tremuloides* and *P. grandidentata*.

The relationships between sections *Aigeiros* and *Tacamahaca* have been controversial. The species of these two sections are known to be freely interfertile (Zsuffa, 1975; Eckenwalder, 1984a). Both cpDNA and rDNA sequence data suggest a polyphyletic origin of section *Tacamahaca*. The polyphyly of section *Tacamahaca* was also suggested by morphology-based phylogenenetic analysis (Eckenwalder, 1996). The rDNA-based phylogenetic tree suggests a monophyletic origin of section *Aigeiros*. Similarly, the cpDNA-based phylogeny suggests a monophyletic origin, with the exception of *P. nigra,* which shares its chloroplast ancestor with the section *Populus* and *P. tristis* from section *Tacamahaca,* which clustered with North American cottonwoods of section *Aigeiros*. In other words, North American cottonwoods of section *Aigeiros* are monophyletic with respect to their chloroplast lineage with the exception of *P. tristis* of section *Tacamahaca,* which clusters within this group.

The phylogenetic relationships among species within sections, based on both cpDNA and rDNA, are not well resolved. However, a few congruencies between the phylogenetic trees based on cpDNA and rDNA (with a low confidence level in the case of rDNA) can be recognized. The three Asian balsam poplars, *P. simonii, P. maxomowiczii,* and *P. laurifolia,* form a clade distinct from other members of the section *Tacamahaca*. Section *Aigeiros* has a closer affinity to the lineages of balsam poplars that includes *P. tristis* and *P. szechuanica* (Figs. 2, 4). Interspecific hybridization within *Populus* is generally limited to intrasectional crosses or intersectional crosses between species of *Aigeiros* and *Tacamahaca* (Ronald, 1982). Intersectional crosses between *Tacamahaca* and *Aigeiros* are generally compatible in most combinations (Zsuffa, 1975) and have given rise to many vigorous clones used in plantations (Dickmann and Stuart, 1983).

The polyphyletic origin of section *Tacamahaca* and *Aigeiros* and the unresolved relationships among species within and between sections suggest relatively close evolutionary relationships among the species. This finding is consistent with observations of spontaneous hybridization among these species in nature and in cultivation. For instance, spontaneous hybridization between European *P. nigra* and introduced North American *P. deltoides* resulted in a dominant and widespread hybrid *P.* × *euramericana* (Lefevre et al., 2001).

Inter- and intra-sectional hybridization is common in regions of sympatry (Eckenwalder, 1996). Only section *Populus* is strongly reproductively isolated from the others and has strong intersectional incompatibility barriers. However, the cellular and molecular bases of this have not yet been characterized (Villar et al., 1986). Nevertheless, this significant reproductive isolation reflects the evolutionary divergence of species of section *Populus* from those in other sections including the species of two closest sections, *Aigeiros* and *Tacamahaca* (Eckenwalder, 1996; Figs. 2, 4). This is consistent with the manipulation required to succeed in the artificial crossing of aspens with other poplars (Guries and Stettler, 1976; Ronald, 1982).

It is generally assumed in the taxonomic literature that the relative interfertility of poplars (especially where these crosses are spontaneous) is a reflection of their genetic similarity (Stettler et al., 1996b; Dickmann et al., 2001) and therefore can form a basis on which to infer taxonomic affinities. According to Eckenwalder (1977b), *P. trichocarpa* and *P. balsamifera* of section *Tacamahaca* have clear signs of introgression (Viereck and Foote, 1970) in their regions of overlap (e.g., the Rocky Mountains and Alaska), probably because of the affinity of their genomes, which permits natural hybridization and repeated back-crossing with parental species. Sections *Tacamahaca* and *Aigeiros* are broadly sympatric in North America (Little, 1971), with overlapping ecological preferences (Fowells, 1965). The North American representatives of sections *Aigeiros* and *Tacamahaca* have such strong similarities in floral traits that they can hardly be distinguished on the basis of floral morphology (Eckenwalder, 1977b, 1984b). In fact, based

on an extensive study of New World poplars, Eckenwalder (1977b) predicts the eventual merger of these two sections. The sister relationship of section *Aigeiros* (with North American cottonwoods in the basal position) with the rDNA-based clade containing three North American balsam poplars of section *Tacamahaca* (*P. balsamifera, P. trichocarpa,* and *P. angustifolia*; Fig. 4) and the close affinities of *P. deltoides* and *P. fremontii* with North American balsam poplars in the cpDNA-based phylogenetic tree (Fig. 2) suggest a close evolutionary relatedness between these species and support the merger proposed by Eckenwalder (1977b).

This trend of extensive hybridization in *Populus* is a major cause of disagreements on the total number of poplar species and their classification. In fact, some poplars such as *P. Balm-of-Gilead* (a variety of *P.* × *jackii*) and *P.* × *tomentosa,* which were originally described as species and cultivated for centuries, have later been identified as natural or near-natural hybrids (Eckenwalder, 1996). In addition to the long-term commercial benefit of hybridization in *Populus* including exploitation for increased variability and novel gene combinations, hybridization may also have played a significant role in the evolution of sections and their rapid allopatric speciation (Eckenwalder, 1996).

Discrepancies between phylogenetic trees based on chloroplast and nuclear nucleotide sequence data—The major discrepancies between the nuclear rDNA and cpDNA phylogenetic trees involve the placement of *P. nigra, P. tristis,* and *P. szechuanica* and may suggest an ancient hybridization event between ancestors of paternal and maternal lineages of these extant species.

Similar to the results of chloroplast RFLP analysis (Smith and Sytsma, 1990), the cpDNA sequence data indicate that *P. nigra* has a chloroplast genome derived from the clade of section *Populus* and divergent from the American cottonwoods of section *Aigeiros*. However, based on the number of nucleotide substitutions mapped on the maximum parsimonious tree (Fig. 2), *P. nigra, P. tremula,* and *P. davidiana,* with a single substitution from the common ancestral node, are more closely related to each other than to *P. alba,* which has four base substitutions. This indicates that the chloroplasts of *P. nigra* may have originated from ancestors of *P. tremula* or *P. davidiana* rather than from *P. alba,* as suggested by Smith and Sytsma (1990). Alternatively, we can conclude that either an extinct ancestor of section *Populus* or any extant species within this section but not included in this study may have played the maternal role in this hybridization event. Moreover, similarity between data from rDNA sequences and rDNA restriction site variation (Smith and Sytsma, 1990) indicate that the nuclear genome of black poplar (*P. nigra*) is distinct from species in section *Populus* and, contrary to Smith and Sytsma (1990), very closely related to *P. deltoides* var. *angulata* from section *Aigeiros* (Fig. 4). It also shows a sister relationship with *P. fremontii*. *Populus deltoides* and *P. sargentii* (*P. deltoides* var. *occidentalis*) occupied a basal position. However, these relationships were not strongly supported by bootstrap analysis (<50%). Thus, the extant *P. nigra* may have derived from an ancient hybridization event involving an ancestor or extant species of section *Populus* as the maternal (cpDNA) donor and the ancestor of the cottonwoods (probably the immediate ancestor of *P. deltoides*) of section *Aigeiros* as the paternal (rDNA) donor. The possible geographic location of this ancient hybridization event and its likely geographic isolation from the paternal species are discussed later.

The evidence from classical morphological, chemical, crossing, and pathogenic studies do not consistently favor the placement of *P. nigra* in either section *Aigeiros* or section *Populus* (Smith, 1988), both of which have been proposed as the origin of *P. nigra*. In the cladistic analysis of 76 morphological characters of buds, leaves, inflorescences, and male and female flowers and fruits performed by Eckenwalder (1996), *P. nigra* had a sister relationship with the clade comprising *P. fremontii* and *P. deltoides* from section *Aigeiros*. Thus, the placement of *P. nigra* within section *Populus* may necessitate invoking a considerable amount of morphological convergence of *P. nigra* to the cottonwoods. In addition, of all possible crosses involving *P. nigra* as one parent (Dickmann et al., 2001), the most problematic crosses are among members of its own maternal lineage, section *Populus*. Successful crosses of section *Populus* with *P. nigra* apparently are only rarely achieved. Moreover, section *Populus* is also distinguished from *P. nigra* by its relative immunity to certain leaf rust organisms. *Melampsora medusae* (American) and *M. populina* (European) infect members of section *Aigeiros* including *P. nigra* and *Tacamahaca,* but they rarely attack the aspens and white poplars of section *Populus* (Smith, 1988; Newcombe, 1996; Lefevre et al., 2001). Based on this evidence, the placement of *P. nigra* in either section *Populus* or section *Aigeiros* remains controversial.

Another major incongruence between the two data sets is the status of *P. tristis,* which has a chloroplast affinity to section *Aigeiros* (cottonwoods) and a nuclear genome related to *Tacamahaca* (balsam poplars; Figs. 2, 4). This suggests that *P. tristis* (Himalayan balsam poplar) may have derived from an ancient hybridization event with an ancestor of North American cottonwoods as the maternal (cpDNA) donor and probably the immediate ancestor of the lineage comprising species of section *Tacamahaca* (North American *P. angustifolia, P. balsamifera, P. trichocarpa,* or Chinese *P. cathayana* and *P. szechuanica*) as a paternal donor. The introgressant status of *P. tristis* was also suggested earlier by Smith (1988). Based on the results of chloroplast RFLP studies and a preliminary analysis of nuclear rDNA RFLP, he concluded that *P. tristis* is an introgressant or hybrid of the *P. nigra* (cpDNA) lineage and the Asian portion of section *Tacamahaca*. Again, however, the cpDNA sequence data in our study links *P. tristis* with the North American cottonwood (*P. deltoides* and *P. fremontii*) lineage. The nuclear sequence data suggest an affinity to species of the section *Tacamahaca,* especially to North American *P. balsamifera* and *P. trichocarpa*. The high morphological similarity of *P. tristis* to North American *P. balsamifera* and the recent development of the highly valued hybrid clone *P. tristis* × *P. balsamifera* (Dickmann and Stuart, 1983) strengthen the proposed model for the evolution of the Himalayan poplar.

Populus szechuanica, a species native to China, is another source for discrepancies in the *Populus* taxonomy, and its placement in section *Tacamahaca* is controversial. The cpDNA RFLP study clustered this species with species of section *Populus* and *P. nigra*. Conversely, rDNA RFLP analysis showed a close affinity between balsam poplars (Section *Tacamahaca*) and *P. szechuanica* (Smith and Sytsma, 1990). Our nuclear rDNA data confirmed the rDNA-based RFLP analysis results and clustered *P. szechuanica* in the monophyletic clade that consisted of certain *Tacamahaca* species, including *P.*

tristis (Fig. 4). The cpDNA, however, had a different pattern. In the cpDNA-based most parsimonious tree, *P. szechuanica* occupied a basal position to the lineage comprising members of section *Aigeiros* and *P. tristis* (Fig. 2). However, the cladistic analysis of 76 morphological characters of buds, leaves, inflorescences, and male and female flowers and fruits put *P. szechuanica* in the same clade as other *Tacamahaca* species, which is paraphyletic to the *Aigeiros* lineage (Eckenwalder, 1996). Therefore, the evolutionary history of *P. szechuanica* may be similar to that of *P. tristis* as discussed earlier.

Eckenwalder (1996) proposed that natural hybridization, while common in genus *Populus* over at least the last several million years, has had little effect on speciation in this group, relative to factors promoting divergence. Eckenwalder's model of evolution for *Populus* (Eckenwalder, 1996) is based on cladistic analysis of morphological characters. He proposed an evolutionary pattern involving phases of ecological radiation and geographical vicariance. Nevertheless, our data and phylogenetic trees suggest ancient intersectional hybridization or reticulate evolution for at least three of the taxa, namely *P. nigra, P. tristis,* and *P. szechuanica*. Hence, natural hybridization in poplars, particularly intersectional hybridization, merits further investigation at the molecular level for a better understanding of the evolutionary history of genus *Populus*.

It could be speculated that the putative hybridization and introgression events, mentioned earlier, must have predated the beginning of the Miocene about 23.5 million years ago (Parrish, 1987), when the northern land mass, Laurasia, had broken apart, opening up the Atlantic Ocean and consequently separating Eurasia from North America. In the putative hybrids studied, at least one of the parental lineages (*Aigeiros* cottonwoods) is native to North America and the other putative parental species is native to Eurasia with no geographical sympatry. Because of the short viability periods of poplar pollen and seeds, as well as unfavorable chemical and physical conditions, cross-oceanic hybridization is unlikely (but cannot be ruled out). Thus, hybridization likely occurred while North America and Europe/Asia were contiguous within the Laurasian land mass. Following hybridization, introgression to one of the parental lineage or geographical isolation of the hybrid population through long-distance seed dispersal followed by local adaptation could have led to significant phenotypic divergence.

Finally, for the two taxa collected from the Montreal Botanical Garden, identified as *P. songarica* and *P. roegneriana*, we were unable to find any information about their history or the geographic source of the trees. The botanical garden had obtained them from the Sheridan Nursery in Montreal in 1973 and the Boyce Thompson Arboretum, USA, in 1937, respectively, and no further information was available. However, the cpDNA data of *P. songarica* had a close affinity to *P. laurifolia* from section *Tacamahaca,* and its rDNA data placed the species with *P. maximowiczii* and *P. laurifolia*. Thus, even though the relationships among these species are not resolved, they occupied the basal position to a clade comprising species of section *Aigeiros* and other members of *Tacamahaca* (with less than 50% bootstrap confidence value; Figs. 2, 4). *Populus songarica,* therefore, could be a variety or cultivar of *P. laurifolia*. Both cpDNA and rDNA sequence data showed close relationships between *P. roegneriana* and species in section *Aigeiros*.

The overall conclusions of this study are that, although *Populus* is a readily defined genus consisting of well-marked sections, at least two such sections, *Tacamahaca* and *Aigeiros*, are not monophyletic in origin. Species of section *Aigeiros* are monophyletic based on rDNA data, but polyphyletic with respect to their chloroplast lineage. Both cpDNA and rDNA sequences data suggest a polyphyletic origin for section *Tacamahaca*. The lineage comprising species of section *Populus* is distinct from the two other sections and the lineage of Asiatic balsam poplars (*P. simonii, P. laurifolia,* and *P. maximowiczii*) of section *Tacamahaca* is diverged from other members of the section. The incongruence between phylogenetic trees based on nuclear- and chloroplast-DNA sequence data suggests a reticulate evolution in the genus *Populus*. The fossil evidence and current biogeographic distribution patterns of poplars suggest that the probable hybridization underlying the origin of *P. nigra, P. tristis,* and *P. szechuanica* likely predated the break up of the Laurasian land mass in the Miocene.

LITERATURE CITED

AZUMA, T., T. KAJITA, J. YOKOYAMA, AND H. OHASHI. 2000. Phylogenetic relationships of *Salix* based on *rbcL* sequence data. *American Journal of Botany* 87: 67–75.

BARNES, B. V. 1961. Hybrid aspens in the lower Peninsula of Michigan. *Rhodora* 63: 311–324.

BARNES, B. V., AND K. S. PREGITZER. 1985. Occurrence of hybrids between bigtooth and trembling aspen in Michigan. *Canadian Journal of Botany* 63: 1888–1890.

BARRETT, J. W., O. P. RAJORA, F. C. H. YEH, B. P. DANCIK, AND C. STROBECK. 1993. Mitochondrial DNA variation and genetic relationships of *Populus* species. *Genome* 36: 87–93.

BECCERRA, J., AND D. L. VENABLE. 1999. Nuclear ribosomal DNA phylogeny and its implications for evolutionary trends in Mexican *Bursera*. *American Journal of Botany* 86: 1047–1057.

BRAATNE, J. H., T. M. HINCKLY, AND R. F. STETTLER. 1992. Influence of soil water supply on the physiological and morphological components of plant water balance in *Populus trichocarpa, Populus deltoides* and their F1 hybrids. *Tree Physiology* 11: 325–340.

BRAYSHAW, T. C. 1965. Native poplars of southern Alberta and their hybrids. Canadian Forest Service Publication 1109, Ottawa, Ontario, Canada.

CULLING, K. W. 1992. Design and testing of plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology* 1: 223–240.

DAYANANDAN, S., K. S. BAWA, AND R. V. KESSELI. 1997. Conservation of microsatellite among tropical trees (*Leguminosae*). *American Journal of Botany* 84: 1658–1663.

DICKMANN, D. I., J. G. ISEBRANDS, J. E. ECKENWALDER, AND J. RICHARDSON. 2001. Poplar culture in North America. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.

DICKMANN, D. I., AND K. STUART. 1983. The culture of poplars in Eastern North America. Department of Forestry, Michigan State University, East Lansing, Michigan, USA.

DOYLE, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany* 17: 144–163.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

ECKENWALDER, J. E. 1977a. North American cottonwoods (*Populus*, Salicaceae) of sections *Abaso* and *Aigeiros*. *Journal of the Arnold Arboretum* 58: 193–208.

ECKENWALDER, J. E. 1977b. Systematics of *Populus* L. in southwestern North America with special reference to sect. *Aigeiros* Duby. Ph.D. dissertation, University of California, Berkeley, California, USA.

ECKENWALDER, J. E. 1982. *Populus xinopia* hybr. nov. (Salicaceae), a natural hybrid between the native North American *P. fremontii* S. Watts and the introduced Eurasian *P. nigra* L. *Madroño* 29: 67–78.

ECKENWALDER, J. E. 1984a. Natural intersectional hybridization between North American species of *Populus* in sections *Aigeiros* and *Tacamahaca*. I. Population studies of *P. parryi*. *Canadian Journal of Botany* 62: 317–324.

ECKENWALDER, J. E. 1984b. Natural intersectional hybridization between North American species of *Populus* in sections *Aigeiros* and *Tacamahaca*. II. Taxonomy. *Canadian Journal of Botany* 62: 325–335.

ECKENWALDER, J. E. 1996. Systematics and evolution of *Populus*. *In* R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley [eds.], Biology of *Populus* and its implications for management and conservation, 7–32. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.

FOWELLS, H. A. 1965. Silvics of forest trees of the United States. U.S. Forestry Service Agricultural Handbook 271. U.S. Department of Agriculture, Washington, D.C., USA.

GURIES, R. P., AND R. F. STETTLER. 1976. Pre-fertilization barriers to hybridization in the poplars. *Silvae Genetics* 25: 37–44.

KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Homonoidea. *Journal of Molecular Evolution* 29: 170–179.

LEFEVRE, F., D. KAJBA, B. HEINZE, AND J. TUROK. 2001. Black poplar: a model for gene resource conservation in forest ecosystems. *Forestry Chronicle* 77: 239–244.

LESKINEN, E., AND C. ALSTROM-RAPAPORT. 1999. Molecular phylogeny of Salicaceae and closely related Flacourtiaceae: evidence from *5.8S, ITS1* and *ITS2* of the rDNA. *Plant Systematics and Evolution* 215: 209–227.

LITTLE, E. L. 1971. Atlas of United States trees, vol. 1, Conifers and important hardwoods. U.S. Department of Agriculture Miscellaneous Publication 1146. U.S. Department of Agriculture, Washington, D.C., USA.

MADDISON, D. R., AND W. P. MADDISON. 2001. MacClade 4. Sinauer, Sunderland, Massachusetts, USA.

NEALE, D. B., AND N. C. WHEELER. 1986. Paternal inheritance of chloroplast DNA in Douglas fir. *Canadian Journal of Forest Research* 16: 1152–1154.

NEWCOMBE, G. 1996. The specificity of fungal pathogens of *Populus*. *In* R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley [eds.], Biology of *Populus* and its implications for management and conservation, 223–246. NRC Research Press, National Research Council of Canada, Ottawa, Ontario Canada.

PAGE, R. D. 2000. Extracting species trees from complex gene trees: reconciled trees and vertebrate phylogeny. *Molecular Phylogenetics and Evolution* 14: 89–160.

PAGE, R. D., AND E. C. HOLMES. 1998. Molecular evolution, a phylogenetic approach. Blackwell, Oxford, UK.

PARRISH, J. T. 1987. Global palaeogeography and palaeoclimate of the Late Cretaceous and Early Tertiary. *In* E. M. Friis, W. G. Chaloner, and P. R. Crane [eds.], The origins of angiosperms and their biological consequences, 51–73. Cambridge University Press, Cambridge, UK.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

RAJORA, O. P., AND B. P. DANCIK. 1995. Chloroplast DNA variation in *Populus*. Interspecific restriction fragment polymorphisms and genetic relationships among *P. deltoides, P. nigra, P. maximowiczii, P.* × *canadensis*. *Theoretical and Applied Genetics* 90: 324–330.

RONALD, W. G. 1982. Intersectional hybridization of *Populus* sections, *Leuce-Aigeiros* and *Leuce-Tacamahaca*. *Silvae Genetica* 31: 94–99.

SEARS, B. B. 1980. The elimination of plastids during spermatogenesis and fertilization in the plant kingdom. *Plasmid* 4: 233–255.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparison of log-likelihoods with applications to the phylogenetic inference. *Molecular Biology and Evolution* 16: 1114–1116.

SMITH, R. L. 1988. Phylogenetics of *Populus* L. (Salicaceae) based on restriction site fragment analysis of cpDNA. M.S. thesis, University of Wisconsin, Madison, Wisconsin, USA.

SMITH, R. L., AND K. J. SYTSMA. 1990. Evolution of *Populus nigra* (sect. *Aigeiros*): introgressive hybridization and the chloroplast contribution of *Populus alba* (sect. *Populus*). *American Journal of Botany* 77: 1176–1187.

STADEN, R. 1979. A strategy of DNA sequencing, employing computer programs. *Nucleic Acids Research* 7: 2601–2610.

STETTLER, R. F., H. D. BRADSHAW, JR., P. E. HEILMAN, AND T. M. HINCKLEY. 1996a. Biology of *Populus* and its implications for management and conservation. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.

STETTLER, R. F., L. ZSUFFA, AND R. WU. 1996b. The role of hybridization in the genetic manipulation of *Populus*. *In* R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley [eds.], Biology of *Populus* and its implications for management and conservation, 87–112. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.

SWOFFORD, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (* and other methods). Version 4.0b8 for Macintosh. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TAMURA, K., AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526.

TEMPLETON, A. R. 1993. Convergent evolution and non–parametric inferences from restriction fragment and DNA sequence data. *In* B. Weir [eds.], Statistical analysis of DNA sequence data. Marcel Dekker, New York, New York, USA.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL W, improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position, specific gap penalties and matrix choice. *Nucleic Acids Research* 22: 4673–4680.

VIERECK, L. A., AND J. M. FOOTE. 1970. The status of *Populus balsamifera* and *P. trichocarpa* in Alaska. *Canadian Field Naturalist* 84: 169–173.

VILLAR, M., M. GAGET, AND C. DUMAS. 1986. Sexual reproduction biology in *Populus*, compatibility and incompatibility. *In* D. L. Mulcahy, G. B. Mulcahy, and G. Ottaviano [eds.], Biotechnology and ecology of pollen. Springer-Verlag, New York, New York, USA.

WHITHAM, T. G., K. D. FLOATE, G. D. MARTINSEN, E. M. DRIEBE, AND P. KEIM. 1996. Ecological and evolutionary implications of hybridization: *Populus*–herbivore interactions. *In* R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley [eds.], Biology of *Populus* and its implications for management and conservation, 247–275. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.

ZSUFFA, L. 1975. A summary review of interspecific breeding in the genus *Populus*. *In* Proceedings of the 14th annual meeting of the Canadian Tree Improvement Association, part 2, 107–23. Canadian Forest Service, Ottawa, Ontario, Canada.