Amer. J. Bot. 77(9): 1176–1187. 1990.

EVOLUTION OF POPULUS NIGRA (SECT. AIGEIROS): INTROGRESSIVE HYBRIDIZATION AND THE CHLOROPLAST CONTRIBUTION OF POPULUS ALBA (SECT. POPULUS)[1]

RANDY L. SMITH AND KENNETH J. SYTSMA[2]

Botany Department, University of Wisconsin, Madison, Wisconsin 53706

Restriction site variation in chloroplast DNA and nuclear ribosomal DNA was examined in 16 accessions from the Salicaceae comprising ten species of *Populus* and one outgroup species of *Salix*. Forty-nine restriction site mutations in the chloroplast DNAs were used to generate one most parsimonious phylogenetic tree. This tree indicates that all varieties of *P. nigra* (black poplars of sect. *Aigeiros*) have a chloroplast genome, maternally inherited, derived from the clade including the white poplars (*P. alba* and segregate species of sect. *Populus*) and divergent from the American cottonwoods of their own section. Twenty-one restriction site mutations in the nuclear ribosomal DNAs generated a single most parsimonious phylogenetic tree that indicates that the nuclear genome of *P. nigra* is distinct from both the white poplars and American cottonwoods. The incongruity of these independent molecular phylogenies provides evidence for an unusual origin of the black poplars. *Populus alba* or its immediate ancestor acted as the maternal parent in a hybridization event with the paternal lineage of *P. nigra*. Subsequent backcrosses to the paternal species gave rise to the extant *P. nigra* with a chloroplast genome of *P. alba* and the nuclear genome of the paternal species. These hybridization and introgression events must have pre-dated the divergence of the black poplar varieties. The biphyletic nature of the *P. nigra* genomes suggests that dependency on one class of molecular or morphological markers or the merging of the two kinds of data sets to derive accurate estimates of true phylogenies could be misleading in plants.

Molecular systematics for the most part assumes that DNA-based phylogenies provide reliable estimates of the actual genealogical relationships of organisms. However, DNA phylogenies might not be congruent with species phylogenies due to a number of inherent biological phenomena (Sytsma, 1990). Most important for plants is the frequent occurrence of interspecific hybridization (Raven, 1980) and subsequent backcrosses with a parental species (Anderson, 1953). These events will generate phylogenetic discrepancies in comparisons of uniparentally inherited organeller sequences (Sears, 1980; Neale, Wheeler, and Allard, 1986) and biparentally inherited nuclear sequences or their phenotypic expression in morphology.

In plants these discrepancies are often first evidenced in routine chloroplast DNA (cpDNA) surveys (Palmer et al., 1983; Palmer, Jorgensen, and Thompson, 1985). Restriction site analysis of cpDNA has been particularly useful in phylogenetic analyses at the generic level in Angiosperms (Palmer and Zamir, 1982; Palmer et al., 1983, 1988; Palmer, Jorgensen, and Thompson, 1985; Sytsma and Gottlieb, 1986a, b; Sytsma and Smith, 1988; Sytsma, Smith, and Gottlieb, 1990). The chloroplast genome has a manageable size (e.g., 155 kb in *Populus*; Sytsma and Smith, unpublished data). Among congeneric species, cpDNA exhibits adequate levels of nucleotide divergence (to 1.5%) and low levels of convergences (less than 10%) to provide detailed and accurate phylogenetic relationships. Its maternal inheritance in most angiosperms, as in *Populus*, provides unambiguous documentation for the maternal parentage of hybrids, introgressants, and allopolyploids (Palmer et al., 1983; Palmer, Jorgensen, and Thompson, 1985; Hosaka and Hanneman, 1988; Wyatt et al., 1988; Wendel, 1989). The utilization of both cpDNA and nuclear rDNA (nrDNA) has permitted further

[1] Received for publication 24 October 1989; revision accepted 24 April 1990.

The authors thank James Eckenwalder and George Argus for determinations of *Populus* and *Salix*, respectively; Jeff Palmer, Bob Jansen, and Elizabeth Zimmer for probes; James Ambrose, Bruce Benz, Jan Pirzio-Biroli, Carl Mohn, Ray Schulenberg, Peter Vanderlinden, Betty Wullstein, and Gary Wycoff for supplying various poplar species; Antonio Vazquez for providing a preprint of the description for *Populus guzmanantlensis*; and Michael Donoghue for comments on the paper. This research was supported by NSF grant BSR-8516573 and a Wisconsin Alumni Research Foundation grant to KJS.

[2] Author for correspondence.

clarification of the hybrid or introgressant nature of suspected species or populations (Rieseberg, Soltis, and Palmer, 1988).

In this paper we use comparative restriction endonuclease site analysis of cpDNA and nrDNA to document the unusual origin of the European black poplars (*Populus nigra* var. *nigra,* var. *italica,* var. *betulifolia*). *Populus* (Salicaceae) comprises 30–40 species placed into six sections (*Populus, Aigeiros, Tacamahaca, Leucoides, Turanga,* and *Abaso*) (Eckenwalder, 1977a, b). The black poplars together with the American cottonwoods (*P. deltoides* and *P. fremontii*) form sect. *Aigeiros,* although the two groups are placed in separate subsections, *Euroasiaticae* and *Americanae,* respectively. Section *Populus* includes the white poplars of central Europe, the western Mediterranean basin, Asia minor, and Mexico (subsect. *Tomentosae*) and the aspens of North America and Eurasia (subsect. *Trepidae*). Our analysis shows that the black poplars of Europe have a chloroplast genome derived from the lineage encompassing the European white poplars of sect. *Populus* and have no cytoplasmic affinities to the cottonwoods of their own section. The nuclear rDNA results, however, provide alternative sets of relationships. These data suggest several conclusions regarding the peculiar origin and relationships of the European black poplars.

MATERIALS AND METHODS

As part of a larger study (Smith and Sytsma, unpublished data), fresh leaf material was obtained from one species of *Salix* L. and 15 accessions of *Populus* representative of ten species (plus one hybrid) of three currently recognized sections of the genus (Table 1). Chloroplast DNA intraspecific site variation is extremely low in *Populus* (Smith and Sytsma, unpublished data; C. S. Prakash, Z.-X. Sun, and D. B. Wagner, personal communication), suggesting that examination of one or two accessions per species would be appropriate for phylogenetic reconstruction among species. Total DNA was extracted by a described protocol (Zimmer, Rivin, and Walbot, 1981) modified to include a 2% polyvinylpolypyrrolidone grinding buffer and a buffer/leaf powder weight ratio of 20:1. The DNAs were digested with 23 restriction endonucleases recognizing six-base nucleotide sequences and were subjected to electrophoresis in 0.7% agarose/Tris/EDTA acetate gels. After denaturation and neutralization, the fragments were blotted onto BioTrans membranes in a bidirectional fashion. Membrane filters were sequentially probed with each of 12 Pst I and two Sal I clones representing nearly the entire cpDNA genome of *Petunia.* Also used was one clone from *Lactuca* representing the small single-copy region between the inverted repeats of the cpDNA molecule. Mapped positions of these clones are provided elsewhere (Sytsma and Gottlieb, 1986b). The collinearity of the cpDNA of *Salix, Populus, Petunia,* and the small single-copy region of *Lactuca* and the maternal inheritance of *Populus* cpDNA has been confirmed (Sytsma and Smith, unpublished data). In addition, nuclear rDNA restriction site and fragment length variation was assessed in *Populus* by re-probing filters used in the cpDNA analysis with heterologous probes from soybean as described elsewhere (Sytsma and Schaal, 1985).

Phylogenetic analysis of cpDNA restriction site mutations was performed with the computer program "Phylogenetic Analysis Using Parsimony" (PAUP, version 2.4), developed by D. Swofford (Illinois Natural History Survey, Urbana), to reveal the shortest possible phylogenetic trees (i.e., those requiring the fewest convergent, parallel, or back-mutations). These trees were rooted (Watrous and Wheeler, 1981) by using a putative basal representative of *Salix* (*S. exigua*) as the outgroup (Dorn, 1976). The PAUP program is based on Wagner parsimony (Farris, 1970) and therefore weights equally both convergent site gains and convergent site losses. Also used was the computer program "Phylogeny Inference Package" (PHYLIP, version 2.7) with a Dollo parsimony option developed by J. Felsenstein. Dollo parsimony (Debry and Slade, 1985) discriminates against convergent site gains, invoking instead additional convergent site losses to account for the observed data. Bootstrap analysis (Felsenstein, 1985) in PHYLIP was used to place confidence levels on branches in a resulting majority rule tree. Finally, the distance algorithm of Fitch and Margoliash (1967) in PHYLIP was used to construct unrooted trees based on overall measures of cpDNA sequence divergence.

RESULTS

Phylogenetic results from cpDNA data—Approximately 342 restriction enzyme sites (six-base sequences) were surveyed in each of the cpDNAs, for a total sample of 1.3% of the *Populus* chloroplast genome (155 kbp). Of the 342 restriction sites, 49 (14.3%) were found to be variable among the species of *Populus* examined (Table 2). The lowest incidence of change occurred within the inverted repeat, an

TABLE 1. Populus *and* Salix *accessions assessed for cpDNA variation*

Species[a]	Accession[b]	Voucher
Salix sect. *Longifoliae* Pax.		
1. *Salix exigua* Nuttall	wild, Madison, WI	R. L. Smith 66, 82
Populus sect. *Populus* subsect. *Tomentosae*		
2. *Populus alba* var. *alba* L.	246-84ct	1637V87
3. *P.* ×*canescens* J. E. Smith = [*P. alba* × *P. tremula*]	720405	Ambrose 3368,3653
4. *P. tomentosa* Carr.	731176	Ambrose 3369,3642
5. *P. guzmanantlensis* Vazquez & Cuevas	wild, Mexico	Benz et al. 1184
sect. *Populus* subsect. *Trepidae*		
6. *P. tremula* L.	Ta-6-68	R. L. Smith 152
7. *P. tremuloides* Michx.	T-44-60	R. L. Smith 151
sect. *Aigeiros* subsect. *Euroasiaticae*		
8. *P. nigra* L. var. 'italica'	720436	
9. *P. nigra* var. *betulifolia* (Pursh) Torr	720964	
10. *P. nigra* L.	740551	Ambrose 3643
11. *P. nigra* L.	82-822	Ambrose 3649
sect. *Aigeiros* subsect. *Americanae*		
12. *P. deltoides* ssp. *monilifera* Eckenw.	720408	Ambrose 3654
13. *P. deltoides* ssp. *deltoides* Marsh	wild, Madison, WI	R. L. Smith 100
14. *P. fremontii* S. Watts	wild, Salt Lake City, UT	
sect. *Tacamahaca*		
15. *P. balsamifera* L.	wild, Guelph, ON	Ambrose 3640
16. *P. szechuanica* var. *tibetica* Schn.	531-57	R. L. Smith 153

[a] *Populus guzmanantlensis* is described as a new species in Vazquez and Cuevas (1989).

[b] Accessions obtained from: Dr. Ray Schulenberg and Dr. Peter Vanderlinden, The Morton Arboretum, Lisle, IL 60532 (#1); Dr. John Ambrose, The University of Guelph Arboretum, Ontario Agricultural College, Guleph, Ontario, N1G 2W1, Canada (#3, 4, 8–12, 15); Dr. Bruce Benz collected near Casimiro Castillo, Sierra de Manantlán, Mexico (#5); Dr. Gary Wyckoff, Institute of Paper Chemistry, Appleton, WI 54912 (#6, 7); Dr. Betty Wullstein, State Arboretum of Utah, University of Utah, Salt Lake City, UT 84112 (#14); Dr. Jan Pirzio-Biroli, Washington Park Arboretum, Center for Urban Horticulture, University of Washington, Seattle, WA 98195 (#16). Determinations of *Populus* (except #5) and *Salix* were made by Dr. James Eckenwalder and Dr. George Argus, respectively.

area well known for its high degree of conservatism (Palmer and Stein, 1986; Sytsma and Gottlieb, 1986b). No variation in cpDNA was seen among *P. alba, P.* ×*canescens,* and *P. tomentosa* (all white poplars), among the three varieties of *P. nigra,* or between the two subspecies of *P. deltoides.*

The proportion of base substitutions per nucleotide position, p (Nei and Li, 1979), was estimated from observed site differences for each of the possible pair-wise comparisons among the 15 accessions of *Populus.* These values ranged from 0 to 0.0137 (Table 3). Considering that not all members and sections of the genus are represented, the largest value is probably lower than would be found in a more comprehensive analysis. Nonetheless, the highest p value within *Populus* is intermediate in the range of infrageneric values currently known for angiosperms (Sytsma and Smith, 1988). Excluding *P. alba, P.* ×*canescens,* and *P. tomentosa* (often not segregated out of *P. alba*), the lowest sequence divergence value among species (0.0010) was between morphologically similar *Populus deltoides* and *P. fremontii* (sect. *Aigeiros*), and the greatest between *P. alba* and *P. balsamifera* (sects. *Populus* and *Tacamahaca,* respectively). *Populus nigra* and *P. alba* had a relatively low p value of 0.0020.

Cladistic analysis of the 49 restriction site mutations, polarized by *Salix exigua,* generated a single most parsimonious Wagner tree of 54 steps requiring five convergences (Fig. 1). This tree had a rate of homoplasy of 9.3% and a consistency index (Kluge and Farris, 1969) of 90.7%. This tree has an unresolved node affecting the relative placements of *Populus tremuloides, P. tremula,* and the white and black poplar lineage. The European white poplars (*Populus alba, P.* ×*canescens,* and *P. tomentosa*) form a sister group relationship with the black poplars, *P. nigra,* rather than with the American white poplar *P. guzmanantlensis* of their own subsection. A synapomorphic *Hind* III site mutation that aligns *P. nigra* with the white poplars and aspens is illustrated in Fig. 2. *Populus nigra* and *P. alba,* morphologically

TABLE 2. *Restriction site mutations within* Populus *cpDNA*

No.	Enzyme	Region[a]	Size fragments (Kb)[b]		Mutated species[c]
1	*Dra* I	S8	1.4 = 1.3 + 0.1		6
2	*Dra* I	S6/P16	3.4 = 2.1 + 1.3	*	12–14, 16
3	*Dra* I	S6/P16	3.4 = 2.6 + 0.8	*	6
4	*Dra* I	P8	8.0 + 0.3 = 8.3		6–16
5	*Dra* I	P10	5.5 = 4.8 + 0.7	*	2–4, 8–11
6	*Dra* I	P10	5.2 = 2.8 + 2.4	*	16
7	*Dra* I	P18	3.7 = 3.3 + 0.4	*	6
8	*Dra* I	P19/20	4.7 = 2.9 + 1.8		7
9[d]	*Dra* I	P19/20	4.7 = 2.5 + 2.2 2.9 = 2.5 + 0.4		15
10	*Kpn* I	P3	4.6 + 1.6 = 6.2		12–13
11	*Sma* I	P10	8.8 = 5.5 + 3.3		16
12	*EcoR* I	P3	5.5 = 4.6 + 0.9	*	2–11
13	*EcoR* I	P6	5.5 = 5.0 + 0.5	*	2–4
14	*Hind* III	S8	11.3 = 6.6 + 4.7		2–11
15	*Hind* III	P3	4.1 = 3.8 + 0.3		2–4
16	*Hind* III	S6	9.6 + 5.3 = 14.9		15
17	*Hind* III	P3	8.4 + 1.6 = 10.0		2–11, 16
18	*Hind* III	L1	7.2 = 5.3 + 1.9		2–11, 16
19	*Hind* III	L1	15.0 + 1.1 = 16.1		2–4, 8–11
20	*EcoR* V	L1	14.3 + 1.5 = 15.8		15
21	*EcoR* V	P1/4	2.3 + 0.5 = 2.8		16
22	*EcoR* V	P8/10/20	9.5 = 7.5 + 2.0		2–4, 6–14, 16
23	*Sst* II	P6	9.5 + 6.3 = 15.8	*	16
24	*Xba* I	P6	4.3 + 2.2 = 6.5		2–11, 16
25	*Sca* I	L1	9.0 = 4.6 + 4.4		12–15
26	*Sca* I	S8	13.5 = 11.3 + 2.2		15
27	*Apa* I	S8	18.5 + 14.5 = 33.0		15
28	*ApaL* I	S6/P16	8.7 + 0.9 = 9.6		7
29	*Nru* I	L1	20.9 + 13.9 = 34.8		16
30	*Nru* I	P3	16.4 + 4.4 = 20.8		2–11, 16
31	*Nru* I	S6/P16	9.5 + 1.3 = 10.8		12–15
32	*Nru* I	S6/P16	10.8 = 8.8 + 2.0		12–13
33	*Dra* I	L1	2.1 + 0.7 = 2.8	*	12–14
34	*Dra* I	L1	2.6 + 0.2 = 2.8		2–11, 16
35	*EcoR* V	P3	14.3 + 1.0 = 15.3	*	15
36	*Xba* I	L1	3.7 + 1.5 = 5.2		2–11
37	*EcoR* I	L1	3.2 + 1.3 = 4.5		16
38	*EcoR* I	L1	2.6 = 2.1 + 0.5	*	2–5, 8–11
39	*EcoR* I	SH	1.6 + 1.3 = 2.9		12–14
40	*Dra* I	P8/10/20	4.8 = 3.0 + 1.8		2–4
41	*Dra* I	P8/10/20	5.5 = 4.6 + 0.9		7
42	*Dra* I	P3	1.9 + 0.2 = 2.1		12–15
43	*Sst* II	P10	18.7 + 15.3 = 34		5
44	*BamH* I	P12	1.0 + 0.6 = 1.6		16
45	*BamH* I	L1	2.9 + 4.9 = 7.8	*	15
46	*Hind* III	P12	11.7 = 6.8 + 4.9		2–11
47	*Xba* I	P6	3.2 + 0.2 = 3.4		12–14
48	*Sma* I	P8	12.2 + 26 = 38.2		16
49	*Sma* I	P8	38.2 = 20 + 18.2		16

[a] Probe positions are described elsewhere (Sytsma and Gottlieb, 1986b).

[b] Where possible, mutations are listed with plesiomorphic state first followed by the apomorphic state. An asterisk indicates mutations that could not be polarized with the outgroup.

[c] Species are numbered as in Table 1.

[d] Restriction site mutation occurs at both ends of the inverted repeat flanking the large single copy region and subsequently reveals two contrasting restriction fragment patterns.

distinct species traditionally placed in separate sections of the genus (sects. *Aigeiros* and *Populus,* respectively) (Eckenwalder, 1977a, b), share a more recent common ancestral cytoplasm than either does with any of the remaining species. The close relationship of *P. nigra* and *P. alba* was also supported by the 87 trees one, two, and three steps longer than the most parsimonious trees. Dollo parsimony yielded a single most parsimonious tree requiring 55 steps. This tree was topologically congruent to the most parsimonious Wagner

TABLE 3. *Nucleotide divergence values (given as 100 × p) for chloroplast DNA (upper right matrix) and nuclear rDNA (bottom left matrix) in* Populus. *Accessions numbered as in Table 1. Chloroplast DNA divergence values between* Populus *and* Salix *were not determined*

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
2.	8.0	—	0.0	0.0	0.34	0.49	0.49	0.20	0.20	0.20	0.20	1.27	1.27	1.16	1.37	1.06
3.	8.0	0.0	—	0.0	0.34	0.49	0.49	0.20	0.20	0.20	0.20	1.27	1.27	1.16	1.37	1.06
4.	8.0	0.0	0.0	—	0.34	0.49	0.49	0.20	0.20	0.20	0.20	1.27	1.27	1.16	1.37	1.06
5.	8.0	0.0	0.0	0.0	—	0.34	0.34	0.25	0.25	0.25	0.25	0.95	0.95	0.85	1.06	0.90
6.	8.0	0.0	0.0	0.0	0.0	—	0.29	0.29	0.29	0.29	0.29	1.06	1.06	0.95	1.16	0.85
7.	8.3	1.0	1.0	1.0	1.0	1.0	—	0.29	0.29	0.29	0.29	1.06	1.06	0.95	1.16	0.85
8.	5.8	2.7	2.7	2.7	2.7	2.7	2.7	—	0.0	0.0	0.0	0.95	0.95	1.06	1.16	0.85
9.	5.8	2.7	2.7	2.7	2.7	2.7	2.7	0.0	—	0.0	0.0	0.95	0.95	1.06	1.16	0.85
10.	5.8	2.7	2.7	2.7	2.7	2.7	2.7	0.0	0.0	—	0.0	0.95	0.95	1.06	1.16	0.85
11.	5.8	2.7	2.7	2.7	2.7	2.7	2.7	0.0	0.0	0.0	—	0.95	0.95	1.06	1.16	0.85
12.	7.2	2.7	2.7	2.7	2.7	2.7	4.0	2.1	2.1	2.1	2.1	—	0.0	0.10	0.70	1.11
13.	7.2	2.7	2.7	2.7	2.7	2.7	4.0	2.1	2.1	2.1	2.1	0.0	—	0.10	0.70	1.11
14.	7.2	2.7	2.7	2.7	2.7	2.7	4.0	2.1	2.1	2.1	2.1	0.0	0.0	—	0.60	1.00
15.	7.7	4.2	4.2	4.2	4.2	4.2	3.7	2.4	2.4	2.4	2.4	2.4	2.4	2.4	—	1.32
16.	7.7	4.2	4.2	4.2	4.2	4.2	3.7	2.4	2.4	2.4	2.4	2.4	2.4	2.4	0.0	—

tree but additionally resolved the aspens, *P. tremuloides* and *P. tremula,* as a monophyletic group. At 56 steps, two additional Dollo trees were generated that were topologically congruent to the most parsimonious Wagner tree but differed in the relative placements of *P. tremuloides, P. tremula,* and the white and black poplar lineage.

The majority rule bootstrap tree utilizing 500 replicates was also identical to the most parsimonious Wagner tree (bootstrap confidence levels are provided in Fig. 1). Confidence levels are sensitive to and dependent on the fineness of resolution of the tree (Sanderson, 1989); i.e., the greater the number of closely related species added to a study and defined within a specific clade, the lower the confidence levels for each branch within this clade. Therefore, an iterative bootstrapping analysis was done to place confidence levels on the lineage comprising *Populus nigra* and the *P. alba* group at more inclusive levels. The confidence level of this lineage increased from 66% to 96% with the exclusion of the *P. guzmanantlensis* (Fig. 1a) and to 100% with the additional exclusion of the aspens (Fig. 1b). Although bootstrap analysis does not place *P. nigra* as the sister group to *P. alba* with statistical confidence, the analysis does place *P. nigra* within the white poplar subsection with 96% confidence and within sect. *Populus* with 100% confidence. Thus, the close relationship of *P. nigra* and *P. alba* (and other members of sect. *Populus*) relative to the cottonwoods are maintained by Wagner and Dollo parsimony and bootstrap analysis.

Phylogenetic analysis based on sequence divergence gave results exactly concordant to those based on parsimony. This was demonstrated by utilizing p values in the Fitch and Margoliash algorithm of PHYLIP to generate one unrooted tree (Fig. 3) that minimized the average percentage standard deviation (% S.D. = 5.70) between calculated and observed values of p. This unrooted tree is topologically identical to the most parsimonious Wagner tree (Fig. 1). Thus, a tight congruence of results exists between Wagner parsimony, Dollo parsimony, bootstrap analysis, and distance analyses which strengthens our preliminary maternal or cytoplasmic phylogeny for *Populus.*

Alternative less parsimonious topologies were examined by utilizing both Wagner par-

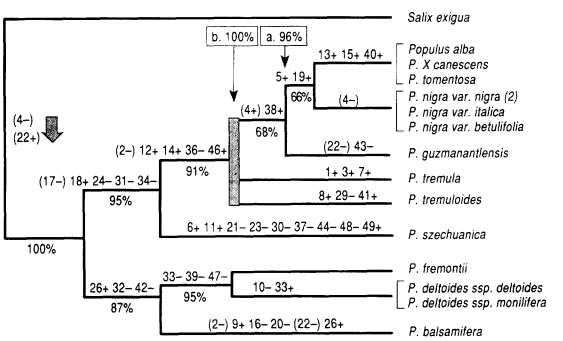

Fig. 1. The shortest Wagner parsimony tree of 54 steps using 49 chloroplast DNA restriction site mutations. Gray area represents the unresolved trichotomy. Mutations are numbered according to Table 2. Gains and losses of sites are indicated by + and −, respectively. Parentheses indicate convergences. A large number of other site mutations (many of which could not be precisely inferred in side-by-side comparisons) separated *Salix exigua* from all other *Populus* species and are not shown at the base of the tree. Two convergent site mutations occur between *Salix* and specific lineages within *Populus.* These mutations are arbitrarily ordered relative to *Salix.* Confidence levels for each lineage from bootstrap analysis are shown as percents. This Wagner tree is topologically congruent to the shortest Dollo tree.

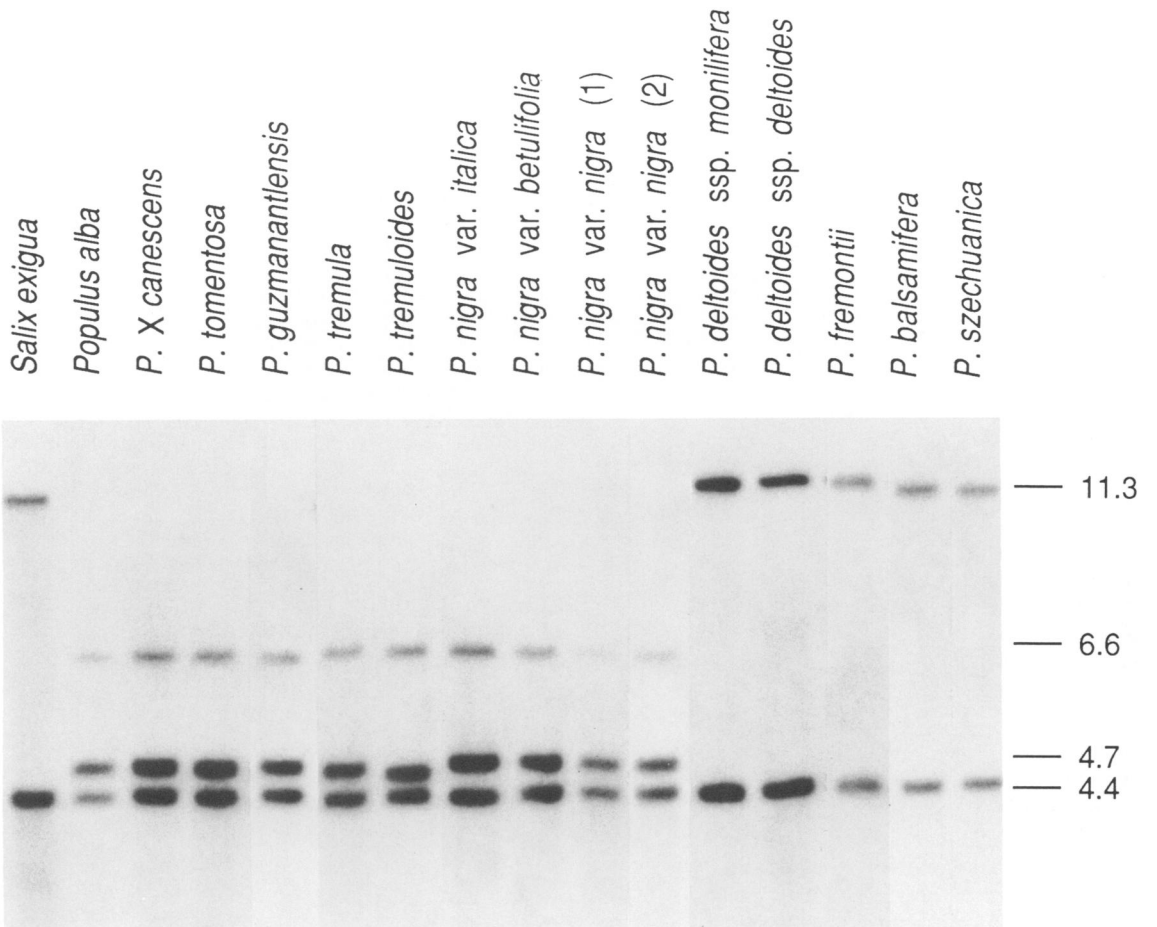

Fig. 2. Restriction fragment pattern for *Salix* and *Populus* illustrating a synapomorphic site mutation placing all varieties of *P. nigra* (black poplars of sect. *Aigeiros*) with the white poplars and aspens of sect. *Populus*. The autoradiogram depicts *Hind* III fragments probed with *Petunia* clone S8. A restriction site gain (relative to *Salix* and other species of sects. *Aigeiros* and *Tacamahaca*) within the 11.3-kb fragment generates two fragments of 6.6 and 4.7 kb.

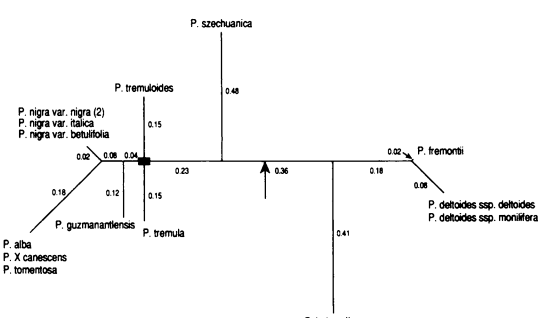

Fig. 3. Unrooted Fitch and Margoliash tree for *Populus* using nucleotide divergence values (p) for chloroplast DNA. Numbers represent calculated distances. This is the unrooted tree with the smallest average percent standard deviation of calculated distances from observed distances. Gray area represents the unresolved trichotomy. This unrooted tree is topologically congruent to the Wagner parsimony tree in Fig. 1. Arrow indicates the position of the root from both midpoint rooting and parsimony analysis.

simony and analyses of overall measures of sequence divergence. When *Populus nigra* is placed basal to the members of sect. *Populus,* rather than coupled with *P. alba,* two additional steps must be invoked by Wagner parsimony, and the average % S.D. of the corresponding unrooted Fitch and Margoliash tree increases to 10.3. Furthermore, when *P. nigra* is realigned to the base of its own section (*Aigeiros*), 14 additional steps are required by Wagner parsimony, and the average % S.D. of the Fitch and Margoliash tree increases to 27.5, nearly five times the minimum value.

Phylogenetic results from nuclear rDNA—Restriction site variation was screened for 22 of the 23 enzymes used in the cpDNA analysis; the *Pst* I site variations were too difficult to interpret due to poor cutting of nrDNA. Five of 22 enzymes did not cut within the nrDNA

TABLE 4. *Restriction site mutations in nuclear rDNA of* Populus *relative to* Salix exigua

No.	Restriction enzyme	Site mutation	Mutated[a] species
1	*Dra* I	Loss	2–16
2	*EcoR* V	Gain	12–14
3	*Xba* I	Gain	2–6, 12–14
4	*ApaL* I	Loss	2–16
5	*Sph* I	Loss	2–16
6	*Sph* I	Gain	2–7
7	*Sst* I	Loss	2–7
8	*Sst* I	Loss	2–16
9	*Bgl* I	Gain	2–16
10	*Bgl* I	Gain	2–16
11	*Hind* III	Loss	2–16
12	*Hind* III	Loss	2–15
13	*EcoR* I	Loss	2–16
14	*EcoR* I	Gain	8–11
15	*EcoR* I	Gain	15–16
16	*Pvu* II	Gain	2–7
17	*Pvu* II	Gain	12–16
18	*Pvu* II	Loss	2–16
19	*Xho* I	Loss	2–16
20	*Xho* I	Loss	7, 15–16
21	*BstE* II	Gain	16

[a] Species are listed by number as shown in Table 1.

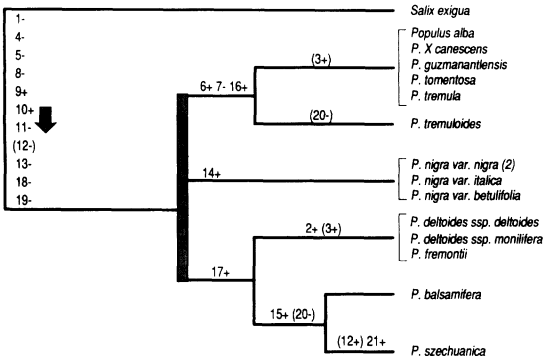

Fig. 4. The shortest Wagner parsimony tree of 24 steps using 21 nuclear rDNA restriction site mutations. Gray area represents the unresolved trichotomy. Mutations are numbered according to Table 4. Gains and losses of sites are indicated by + and −, respectively. Parentheses indicate convergences. Eleven site changes separating *Salix exigua* and all species of *Populus* are arbitrarily ordered relative to *Salix*. The consensus tree of three equally short Dollo trees places the lineage of *P. balsamifera* and *P. szechuanica* as a fourth radiation from the unresolved polychotomy.

repeat of *Populus* and *Salix*. A total of 32–37 restriction sites were surveyed in the nrDNA of each accession. From these, 21 restriction site mutations were scored using 12 enzymes that exhibited variation (Table 4). Length variation in the intergenic spacer was observed within and among accessions, but this variation was not used in the phylogenetic analysis nor did it affect the interpretation of site variation. Nuclear rDNA p values ranged from 0 to 0.083 (Table 3). *Salix* to *Populus* divergence averaged 0.072 (0.058–0.083), and within *Populus* divergence averaged 0.022 (0–0.042). The *Populus* nrDNA divergence values are two to three times larger than those reported for nrDNA within one lineage of *Lisianthius* (Sytsma and Schaal, 1985).

Phylogenetic analysis of the 21 nrDNA restriction site mutations using Wagner parsimony (in PAUP) generated a single shortest tree (Fig. 4) of 24 steps (CI = 0.875). This tree has an unresolved trichotomy of three main lineages: 1) sect. *Populus*; 2) sects. *Tacamahaca* and *Aigeiros* (minus *P. nigra*); and 3) *P. nigra*. Three equally short trees of 26 steps were obtained with Dollo parsimony. A consensus tree of these three trees is similar to the Wagner parsimony tree (Fig. 4). The Dollo consensus tree places the lineage composing *P. balsamifera* and *P. szechuanica* as a fourth lineage radiating out from the unresolved basal node in *Populus* rather than as the sister group to the American cottonwoods.

The major discrepancies apparent between the nuclear rDNA and cpDNA phylogenies are the placements of *P. nigra* and *P. szechuanica*. The latter is examined in more detail in a broader survey of *Populus* (Smith and Sytsma, unpublished data). In contrast to the cpDNA results, *P. nigra* does not have a nuclear genome similar to that found in the *P. alba* lineage. All the white poplars and aspens of sect. *Populus* are united by three site mutations indicating that they form a monophyletic lineage. *Populus nigra* does not share these mutations and also contains its own unique site mutation. The American cottonwoods (*P. deltoides* and *P. fremontii*) form their own lineage by sharing three site mutations. The nuclear genome (and chloroplast genome) of *Populus nigra* is thus distinct from that found in these other species placed in its own section. Although the exact alignment of the three main lineages (sect. *Populus,* sect. *Aigeiros* subsect. *Americanae* and sect. *Tacamahaca,* and *P. nigra*) is unresolved, *P. nigra* is distinct in its nuclear genome from sect. *Populus* from which its chloroplast genome is derived and from sect. *Aigeiros* subsect. *Americanae* to which it has morphological resemblance and with which it has been taxonomically placed (as subsect. *Euroasiaticae*).

DISCUSSION

Hybridization in Populus—Numerous examples of natural or spontaneous hybridization in *Populus* are documented or proposed (Eckenwalder, 1977a, b, 1982, 1984a, b, c; Spies

and Barnes, 1982; Barnes and Pregitzer, 1985); however, widespread interspecific hybridization, although commonly associated with *Populus,* is generally limited to infrasectional crosses or intersectional crosses between sects. *Aigeiros* and *Tacamahaca* (Zsuffa, 1973; Ronald, 1982). *Populus* exhibits strong intersectional incompatibility barriers, the cellular and molecular bases of which have not yet been fully characterized (Villar, Gaget, and Dumas, 1986). A variety of artificial manipulations have been developed which successfully bypass these barriers and greatly expand the number of possible crosses. These include applications of mentor pollen, compatible pollen extracts, and treatments of incompatible pollen and nonreceptive stigmas with solvents (Knox, Willing, and Pryor, 1972; Zsuffa, 1973; Whitecross and Willing, 1975; Guries and Stettler, 1976; Willing and Pryor, 1976; Stettler, Koster, and Steenackers, 1980). It is an underlying presumption in the taxonomic literature that the relative crossability of poplars (especially where these crosses are spontaneous) is a reflection of their genetic similarity and, therefore, can form a basis on which to infer taxonomic affinities. As introgression/hybridization are likely to have played a significant role in the evolution of *Populus,* phylogenies based exclusively on maternally inherited DNA (e.g., cpDNA) or on biparentally inherited DNA (e.g., nrDNA) may not, individually, adequately reflect the true genealogy of the species (Sytsma, 1990).

Such a discrepancy is apparently involved with *Populus alba* and *P. nigra,* species with very similar chloroplast genomes but distinctive nuclear rDNAs. A pronounced sexual incompatibility exists between these two species. This incompatibility of sect. *Populus* with *P. nigra* extends as well to all members of sects. *Aigeiros* and *Tacamahaca* (Ronald, 1982). In contrast, *P. nigra* readily produces viable crosses with *P. deltoides* and *P. fremontii* of its own section and with *P. trichocarpa* of sect. *Tacamahaca* (Stout and Schreiner, 1933; Pauley, 1949; Zsuffa, 1973; Eckenwalder, 1982), the latter a section closely related to sect. *Aigeiros* (Eckenwalder, 1977b). Therefore, the most problematic crosses for *P. nigra* appear to be with the immediate members of its own cpDNA-based lineage, especially *P. alba.* Only with solvent treatments and with *P. alba* as the maternal parent can *P. nigra* and *P. alba* be crossed today (Willing and Pryor, 1976; Ronald, 1982; Villar et al., 1987).

Relationship of Populus nigra and P. alba—Prior systematic studies in *Populus* have included a diverse array of technical approaches: hybrid indices, portrayals of morphological characters (vegetative or reproductive) via bivariate scatter diagrams, analyses of secondary compounds (flavonoids and phenolic glycosides), ecological factors (growth habit, habitat, phenology), experimental genetics (hybridization studies, progeny testing), and both phenetic and cladistic analyses of morphological and chemical characters (Eckenwalder, 1977a, b, 1984b, c). Such studies have overwhelmingly placed *P. nigra* near the American cottonwoods and separate from the white poplars and aspens of sect. *Populus,* a segregation maintained by every infrageneric treatment of the genus (Duby, 1828; Spach, 1841; Hartig, 1851; Bugała, 1967; Eckenwalder, 1977b).

Members of sect. *Populus* are distinguished from *P. nigra* by a relative immunity to certain leaf rust organisms; e.g., *Melampsora medusae* (North America) and *M. larici-populina* (Europe), both known to infect members of sects. *Aigeiros* (including *P. nigra*) and *Tacamahaca,* but only rarely attacking the aspens and white poplars of sect. *Populus* (Bingham, Hoff, and McDonald, 1972; Ostry and McNabb, 1986). A phenetic analysis of leaf and stem characters in four of the *Populus* sections generated a set of relationships congruent with traditional schemes based on floral, fruit, and crossability characters (Hu, Crovello, and Sokal, 1985). In that study, sect. *Aigeiros* stands as a distinct group with the black poplars of subsect. *Euroasiaticae* situated in the midst of subsect. *Americanae.* A 3-D principal components ordination of this same data set places *Populus nigra* intermediate to the American cottonwoods (subsect. *Americanae*) and sect. *Tacamahaca,* relationships previously hypothesized for these groups based on floral characteristics and crossing relationships (Eckenwalder, 1977b). Only with flavonoids has there been a suggestion of a relationship between the black and white poplars. In an extensive chemotaxonomic study of *Populus,* a dendrogram generated by cluster analysis of flavonoid similarity values revealed that *P. alba* var. *alba* is most similar to *P. nigra* (Eckenwalder, 1977b). Furthermore, among species of sects. *Aigeiros, Tacamahaca,* and *Populus,* only *P. alba* and *P. nigra* share the phenolic glycoside isolariciresinol (Thieme and Benecke, 1970a, b).

Evolution of Populus nigra—Our phylogenetic results for maternally (cpDNA) and biparentally (nrDNA) inherited molecules reveal a sister group relationship of the cytoplasm of all European black poplars to the European

(but not American) white poplars and not to the American cottonwoods, yet a divergent relationship of the nuclear genome of the black poplars to both the white poplars and cottonwoods. The results suggest that the genomes of *Populus nigra* are a combination of at least two different species. Two scenarios for the evolution of *P. nigra* are possible. The first scenario would have *P. nigra* misplaced taxonomically and as actually derived from within sect. *Populus* subsect. *Tomentosae.* But, subsequent to its split in Eurasia from the white poplars or their immediate ancestor, *P. nigra* hybridized with a species outside sect. *Populus* that contributed the nuclear genome via introgression. This scenario is unlikely for a number of reasons. It would predict the presence of a species with a *P. nigra*-like nuclear genome (as seen by nrDNA analysis) distinct from those seen in sects. *Populus, Aigeiros* (exclusive of *P. nigra*), and *Tacamahaca.* In a larger preliminary analysis of the genus *Populus,* the nrDNA pattern of *P. nigra* is still distinctive and occupies an isolated position in the genus (Smith and Sytsma, unpublished data). The placement of *P. nigra* within one clade of subsect. *Tomentosae* is also not supported by the weight of classical morphological, chemical, and crossing studies. Morphological characters of fertile parts (stigma dilation and lobing, disk shape, capsule shape, stamen number) and of vegetative parts (bracts of pistillate aments, compression of petioles, viscidness of overwintering terminal buds) provide no evidence of a relationship of *P. nigra* to *P. alba,* but rather a relationship to the cottonwoods. Although all these characters have not been analyzed in a cladistic manner (Eckenwalder, 1984b, c), placement of *P. nigra* as the sister taxa to *P. alba,* or other white poplars, would necessitate invoking a considerable amount of morphological convergence of *P. nigra* to the cottonwoods.

The second scenario would have *Populus nigra,* as it now exists, as derived from an ancient hybridization event in Eurasia, involving an ancestor or relative of *P. alba* as the maternal (cpDNA) donor, and the immediate ancestor of *P. nigra* ("pre" *P. nigra*) as the paternal donor. The discrepancy between the maternal and nuclear phylogenies involving the placement of *P. nigra* strongly implicates subsequent backcrossing (introgression) of the hybrid to its paternal ancestor. The weight of earlier studies using morphology and other characteristics might argue that this "pre" *P. nigra* had a nuclear genome related to the American cottonwoods. In this case, the unusual nature of present-day *P. nigra* rDNA (relative to the white poplars and cottonwoods) would have to be explained by rapid evolution of rDNA by some molecular phenomena operating to promote rapid turnover and subsequent homogenization of the gene family (Zimmer et al., 1980; Dover, 1982). Alternatively, because the nuclear rDNAs of *P. nigra* accessions do not fall within any of the examined lineages, it could be argued that "pre" *P. nigra* might not be related to either the American cottonwoods or the white poplars (nor to any extant lineage so far examined). The exact relationships of the hypothesized paternal ancestor of the black poplars to extant species within *Populus* must await additional information obtained from other nuclear encoded DNAs, such as restriction fragment length polymorphisms (RFLPs) (see Keim et al., 1989). In any case, these putative hybridization and introgression events must have predated the divergence of the black poplar varieties, as all varieties of *Populus nigra* have the same chloroplast genome and nuclear rDNA array.

This second scenario would predict the presence (extant or extinct) of a species or populations ("pre" *P. nigra*) with a "lost" *P. nigra* chloroplast genome. Two central Asian poplars, *Populus sosnowskyi* Grossh. and *P. usbekistanica* Kom., while perhaps mere taxonomic segregates of *P. nigra,* could conceivably still harbor the cpDNA-type "lost" following an ancient *P. alba* × "pre" *P. nigra* cross. It is important to note that *P. alba* × *P. nigra* crosses can now only be performed (using solvent washes) with *P. alba* as the maternal source and thus also as the contributor of the chloroplast genome for the hybrid.

In summary, the very similar nature of the chloroplast DNAs in *Populus nigra* and *P. alba,* the very distinctive nuclear rDNA of *P. nigra,* and the weight of previous classical morphological, chemical, and crossing studies strongly support the second scenario as the most probable for the evolution of the black poplars. The phylogenetic analysis would thus indicate that: 1) the immediate ancestor of *P. nigra* ("pre" *P. nigra*) occupied a position either within sect. *Aigeiros* or possibly outside sects. *Aigeiros, Tacamahaca,* and *Populus*; 2) it served as the male parent in a cross with *P. alba* or a related species in Eurasia thereby gaining the *P. alba* chloroplast genome; 3) the resulting hybrid progeny, acting as the female parent, backcrossed with "pre" *P. nigra* thereby effectively losing or minimizing the expression of the *P. alba* nuclear genome; and 4) subsequent progeny, bearing the *P. alba* cytoplasmic genome, ultimately diverged into the various extant va-

rieties of black poplar in Europe and Asia. The demonstration with nuclear-encoded RFLPs that unidirectional introgression has occurred between *P. fremontii* and *P. angustifolia* in overlap zones (Keim et al., 1989) strengthens the proposed model for the evolution of the black poplars.

The continued presence of *Populus nigra* in Europe has come into question. Recently, in Europe, "pure" *P. nigra* has been displaced from its native habitat by spontaneous hybridization with numerous cultivars of *P.* ×*canadensis* extensively utilized in forestry (Bialobok, 1973; Zsuffa, 1973; Mohrdiek, 1983). *Populus* ×*canadensis* is the progeny of *P. deltoides* × *P. nigra* crosses (known also as *P.* ×*euramericana*) and is produced only unilaterally, with *P. deltoides* always acting as the maternal (cpDNA) donor (Villar et al., 1987). It remains to be seen, owing to both the unilateral hybridization phenomena between these species and the maternal inheritance of chloroplasts within the genus, whether the cpDNA genome of *P. nigra* is currently being extirpated from sexually reproducing populations in Europe. If so, this could serve as a working model for the hypothesized ancient introgressive origin of the black poplars outlined above.

Systematic implications—Phylogenetic discrepancies between maternally inherited genomes and biparentally inherited genomes or with biological species boundaries have been noted for both plants and animals (Ferris et al., 1983; Palmer et al., 1983; Powell, 1983; DeSalle and Giddings, 1986; Sytsma, 1990). These studies have postulated hybridization and unilateral introgression to account for such discrepancies, although matriarchal lineage sorting (Neigel and Avise, 1986) has not been ruled out. The confounding effect of these events on estimates of phylogenetic relationships is particularly great in plants and suggests that exclusive use of characters from just morphology, chloroplast DNA, or the nuclear genome can give rise to errors in phylogenetic reconstruction. Moreover, this study emphasizes the need for caution when analyzing phylogenetically informative characters when they are derived from different sources (e.g., morphology, chloroplast DNA, and nuclear DNA). These results argue against the combination of distinct sets of characters as suggested by Hillis (1987) for morphological and molecular data sets. Instead, this study argues for preliminary separate analyses for each character set, with subsequent examination and explanations of congruence or discordance (Sytsma, 1990).

LITERATURE CITED

ANDERSON, E. 1953. Introgressive hybridization. *Biological Review and Biological Proceedings of the Cambridge Philosophical Society* 28: 280–307.

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.

BIALOBOK, S. [ed.] 1973. The poplars (*Populus* L.). (Translated from Polish, 1976.) U.S. National Technical Information Service, Springfield, VA.

BINGHAM, R. T., R. J. HOFF, AND G. I. MCDONALD. 1972. Biology of rust resistance in forest trees: proceedings of NATO-IUFRO Advanced Study Institute. Miscellaneous Publication 1221. U.S.D.A. Forest Service, Washington, D.C.

BUGAŁA, W. 1967. Systematyka Euroazjatyckich topoli z grupy *Populus nigra* L. *Arboretum Kórnickie* 12: 45–219. [English summary.]

DEBRY, R. W., AND N. A. SLADE. 1985. Cladistic analysis of restriction endonuclease cleavage maps within a maximum-likelihood framework. *Systematic Zoology* 34: 21–34.

DESALLE, R., AND V. GIDDINGS. 1986. Discordance of nuclear and mitochondrial DNA phylogenies in Hawaiian *Drosophila*. *Proceedings of the National Academy of Sciences USA* 83: 6902–6906.

DORN, R. D. 1976. A synopsis of American *Salix*. *Canadian Journal of Botany* 54: 2769–2789.

DOVER, G. 1982. Molecular drive: a cohesive mode of species evolution. *Nature* 299: 111–117.

DUBY, J. E. 1828. Botanicon Gallicum, Synopsis plantarum in flora Gallica descriptarum, vol. 1, 2d ed. Bouchard-Huzard, Paris.

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

———. 1977b. Systematics of *Populus* L. (Salicaceae) in Southwestern North America with special reference to sect. *Aigeiros* Duby. Ph.D. Thesis, University of California–Berkeley.

———. 1982. *Populus* × *inopina* hybr. nov. (Salicaceae), a natural hybrid between the native North American *P. fremontii* S. Watts and the introduced Eurasian *P. nigra* L. *Madrono* 29(2): 67–78.

———. 1984a. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections *Aigeiros* and *Tacamahaca*. I. Population studies of *P.* × *parryi*. *Canadian Journal of Botany* 62: 317–324.

———. 1984b. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections *Aigeiros* and *Tacamahaca*. II. Taxonomy. *Canadian Journal of Botany* 62: 325–335.

———. 1984c. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections *Aigeiros* and *Tacamahaca*. III. Paleobotany and evolution. *Canadian Journal of Botany* 62: 336–342.

FARRIS, J. S. 1970. Methods for computing Wagner trees. *Systematic Zoology* 19: 83–92.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FERRIS, S., S. D. SAGE, C. HUANG, J. T. NIELSEN, U. RITTE, AND A. C. WILSON. 1983. Flow of mitochondrial DNA across a species boundary. *Proceedings of the National Academy of Sciences USA* 80: 2290–2294.

FITCH, W. M., AND E. MARGOLIASH. 1967. Construction of phylogenetic trees. *Science* 155: 279–284.

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

HARTIG, T. 1851. Vollständige Naturgeschichte der forstlichen Kulturpflanzen Deutschlands. Förstner, Berlin. 580 pp.

HILLIS, D. M. 1987. Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics* 18: 23–42.

HOSAKA, K., AND R. E. HANNEMAN, JR. 1988. Origin of chloroplast DNA diversity in the Andean potatoes. *Theoretical and Applied Genetics* 76: 333–340.

HU, C., T. J. CROVELLO, AND R. R. SOKAL. 1985. The numerical taxonomy of some species of *Populus* based only on vegetative characters. *Taxon* 34: 197–206.

KEIM, P., K. N. PAIGE, T. G. WHITHAM, AND K. G. LARK. 1989. Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. *Genetics* 123: 557–565.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18: 1–32.

KNOX, R. B., R. R. WILLING, AND L. D. PRYOR. 1972. Interspecific hybridization in poplars using recognition pollen. *Silvae Genetica* 21: 65–69.

MOHRDIEK, O. 1983. Discussion: future possibilities for poplar breeding. *Canadian Journal of Forest Research* 13: 465–471.

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

NEI, M., AND W. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of National Academy of Sciences USA* 76: 5269–5273.

NEIGEL, J. E., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. *In* S. Karlin and E. Nevo [eds.], Evolutionary processes and theory, 515–534. Academic Press, Orlando.

OSTRY, M. E., AND H. S. MCNABB, JR. 1986. *Populus* species and hybrid clones resistant to *Melampsora, Marssonia* and *Septoria*. U.S.D.A. Forest Service, N. Central Experiment Station, Research Paper #NC-272.

PALMER, J. D., R. K. JANSEN, H. J. MICHAELS, M. W. CHASE, AND J. R. MANHART. 1988. Chloroplast DNA variation and plant phylogeny. *Annals of the Missouri Botanical Garden* 75: 1180–1206.

———, R. A. JORGENSEN, AND W. F. THOMPSON. 1985. Chloroplast DNA variation and evolution in *Pisum*: patterns of change and phylogenetic analysis. *Genetics* 109: 195–213.

———, C. R. SHIELDS, D. B. COHEN, AND T. J. ORTON. 1983. Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. *Theoretical and Applied Genetics* 65: 181–189.

———, AND D. B. STEIN. 1986. Conservation of chloroplast genome structure among vascular plants. *Current Genetics* 10: 823–833.

———, AND D. ZAMIR. 1982. Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. *Proceedings of the National Academy of Sciences USA* 79: 5006–5010.

PAULEY, S. 1949. Forest-tree genetics research: *Populus* L. *Economic Botany* 3: 299–330.

POWELL, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila*. *Proceedings of the National Academy of Sciences USA* 80: 492–494.

RAVEN, P. H. 1980. Hybridization and the nature of species in higher plants. *Canadian Botanical Association Bulletin* 13: 3–10 (Supplement).

RIESEBERG, L. H., D. E. SOLTIS, AND J. D. PALMER. 1988. A reexamination of introgression between *Helianthus annuus* and *H. bolanderi* (Compositae). *Evolution* 42: 227–238.

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

SANDERSON, M. J. 1989. Confidence limits on phylogenies: the bootstrap revisited. *Cladistics* 5: 113–129.

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

SPACH, E. 1841. Revisio populorum. *Annales de Sciences Naturelles Botanique* 15: 28 (Series 2).

SPIES, T. A., AND B. V. BARNES. 1982. Natural hybridization between *Populus alba* and the native aspens in southeastern Michigan. *Canadian Journal of Forest Research* 12: 653–660.

STETTLER, R. F., R. KOSTER, AND V. STEENACKERS. 1980. Interspecific crossability studies in poplars. *Theoretical and Applied Genetics* 58: 273–282.

STOUT, A. B., AND E. J. SCHREINER. 1933. Results of a project in hybridizing poplars. *Journal of Heredity* 24: 217–229.

SYTSMA, K. J. 1990. DNA and morphology: inference of plant phylogeny. *Trends in Ecology and Evolution* 5: 104–110.

———, AND L. D. GOTTLIEB. 1986a. Chloroplast DNA evidence for the origin of the genus *Heterogaura* from a species of *Clarkia* (Onagraceae). *Proceedings of the National Academy of Sciences USA* 83: 5554–5557.

———, AND ———. 1986b. Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (Onagraceae). *Evolution* 40: 1248–1261.

———, AND B. A. SCHAAL. 1985. Phylogenetics of the *Lisianthius skinneri* (Gentianaceae) complex in Panama utilizing DNA restriction fragment analysis. *Evolution* 39: 594–608.

———, AND J. F. SMITH. 1988. DNA and morphology: comparisons in the Onagraceae. *Annals of the Missouri Botanical Garden* 75: 1217–1237.

———, ———, AND L. D. GOTTLIEB. 1990. Phylogenetics in *Clarkia* (Onagraceae): restriction site mapping of chloroplast DNA. *Systematic Botany* 15: 280–295.

THIEME, H., AND R. BENECKE. 1970a. Über die Identität der Glucoside Xylomosid und Poliothyrsosid mit Nigracin. *Pharmazie* (Berlin) 25: 492.

———, AND ———. 1970b. Die Phenolglykoside der Salicaceen, 7. über die Glykosidführung einheimischer bzw. in Mitteleuropa kultivierter *Populus*-Arten. *Pharmazie* (Berlin) 25: 780–789.

VAZQUEZ, A., AND R. CUEVAS. 1989. Una nueva especie tropical de *Populus* (Salicaceae) de la Sierra de Manantlán, Jalisco, México. *Acta Botanica Mexicana* 8: 39–45.

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

———, ———, C. SAID, R. B. KNOX, AND C. DUMAS. 1987. Incompatibility in *Populus*: structural and cy-

tochemical characteristics of the receptive stigmas of *Populus alba* and *Populus nigra*. *Journal of Cell Science* 87: 483–490.

WATROUS, L. E., AND Q. D. WHEELER. 1981. The outgroup comparison method of character analysis. *Systematic Zoology* 30: 1–11.

WENDEL, J. F. 1989. New World tetraploid cottons contain Old World cytoplasm. *Proceedings of the National Academy of Sciences USA* 86: 4132–4136.

WHITECROSS, M. I., AND R. R. WILLING. 1975. Hybridization in incompatible poplars following solvent treatment of stigmas. *Experientia* 31: 651–653.

WILLING, R. R., AND L. D. PRYOR. 1976. Interspecific hybridization in poplars. *Theoretical and Applied Genetics* 47: 141–151.

WYATT, R., I. J. ODRZYKOSKI, A. STONEBURNER, H. W. BASS, AND G. A. GALAU. 1988. Allopolyploidy in bryophytes: recurring origins of *Plagiomnium medium*. *Proceedings of the National Academy of Sciences USA* 85: 5601–5604.

ZIMMER, E. A., S. MARTIN, S. BEVERLEY, Y. W. KAN, AND A. C. WILSON. 1980. Rapid duplications and loss of the genes coding for the chains of hemoglobin. *Proceedings of the National Academy of Sciences USA* 77: 2158–2162.

———, C. RIVIN, AND V. WALBOT. 1981. A DNA isolation procedure suitable for most higher plant species. *Plant Molecular Biology Newsletter* 2: 93–96.

ZSUFFA, L. 1973. A summary review of interspecific breeding in the genus *Populus*. Proceedings of the 14th Meeting of the Canadian Tree Improvement Association, part II, pp. 107–123. Fredericton, New Brunswick, Canada.