Paternal Chloroplast DNA Inheritance in *Pinus contorta* and *Pinus banksiana*: Independence of Parental Species or Cross Direction

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We studied chloroplast DNA inheritance in 133 *Pinus contorta* seedlings and in 88 seedlings of interspecific matings between *P. contorta* and *P. banksiana*, to determine if the mode of inheritance is consistent in matings within and between these two species. Segregation data from matings of 14 *P. contorta* parents and five *P. banksiana* parents, representing a diversity of chloroplast DNA genotypes and geographic sources, were consistent with paternal chloroplast DNA inheritance. Nonetheless, nine nonpaternal seedling genotypes were observed, which may have resulted from contamination, parental chimerism, maternal leakage, or recombination. Our results, taken together with earlier reports, suggest that the paternal predominance of chloroplast DNA inheritance in *P. contorta* and *P. banksiana* is independent of parental genotype, geographic source, species, or mating direction. This apparent consistency will be useful for interpretation of cytonuclear data from sympatric populations of these two species.

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Natural hybridization between jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*P. contorta* Dougl.) has been studied for many years (Critchfield 1985; Moss 1949), not only because of its potential evolutionary significance (e.g., Wheeler and Guries 1987), but also because of practical concerns in germplasm deployment and improvement programs (Critchfield 1980; Rudolph and Yeatman 1982). For example, *P. contorta* introductions from recognized areas of introgression are prohibited in Sweden, in order to avoid *P. banksiana* influence in breeding populations (Fries 1987).

Investigators of hybridization and introgression in *P. banksiana* and *P. contorta* have traditionally employed morphological, biochemical, and quantitative characteristics as parental species markers (e.g., Mirov 1956; Moss 1949; Pollack and Dancik 1985; Wheeler and Guries 1987; Zavarin et al. 1969), but chloroplast DNA (cpDNA) polymorphisms also distinguish these two species (Govindaraju et al. 1989; Wagner et al. 1987). The cpDNA polymorphisms, in concert with nuclear markers, may be unusually informative in sympatric *P. banksiana–P. contorta* populations, because cytonuclear analyses permit powerful evolutionary inference and unique insights in regions of natural hybridization (Asmussen et al. 1987; Schnabel and Asmussen 1989). However, complete interpretation of organellar and cytonuclear population data requires that the mode of organellar inheritance be understood for species of interest.

Unlike most angiosperms, cpDNA inheritance appears paternal in at least three taxonomic families of conifers, including Pinaceae (e.g., Neale et al. 1989, 1991; Sears 1980; Sutton et al. 1991b). However, most studies of coniferous cpDNA inheritance have been limited in both parental and progeny sample sizes, and few of these investigations have examined reciprocal matings. In particular, in *P. banksiana* and *P. contorta*, cpDNA inheritance data have come only from unidirectional species hybrids (*P. contorta* females × *P. banksiana* males) and from crosses within *P. banksiana* (Wagner et al. 1987, 1989). Because cpDNA inheritance is genotype-dependent in some plants (e.g., Chiu et al. 1988), it is clearly important to examine cpDNA inheritance in *P. contorta* and reciprocal species hybrids before employing cpDNA markers to study hybridization and introgression in sympatric populations of *P. banksiana–P. contorta*. Here we generalize previous inferences of predominant paternal cpDNA inheritance in these two species, with data from *P. contorta* and from reciprocal species crosses.

Table 1. Parental sources and cpDNA genotypes

Parent[a]	Species	Geographic source[b]	Genotype[c]
61	*P. banksiana*	unknown	4.8/5.7 (6)
65	*P. banksiana*	Somerset County, Maine (U.S.A.)	4.8/5.7 (1)
101	*P. banksiana*	Smoky Lake, Alberta	4.5/5.7 (4)
102	*P. banksiana*	Smoky Lake, Alberta	4.5/5.7 (1)
104	*P. banksiana*	Smoky Lake, Alberta	4.8/5.7 (1)
14-6-3	*P. contorta*	Kamloops, British Columbia	4.3/5.0 (1)
14-13-6	*P. contorta*	Kamloops, British Columbia	4.3/5.0 (12)
55-9-6	*P. contorta*	Smithers, British Columbia	4.1/4.5/5.0 (8)
55-14-4	*P. contorta*	Smithers, British Columbia	4.3/5.0 (1)
72-10-6	*P. contorta*	Salmon Arm, British Columbia	4.3/5.0 (1)
72-15-1	*P. contorta*	Salmon Arm, British Columbia	4.3/5.0 (1)
SCA47	*P. contorta*	Lower Post, British Columbia	2.8 (1)
SCA56	*P. contorta*	Watson Lake, Yukon	3.0 (1)
SCA68	*P. contorta*	Whitehorse, Yukon	3.0 (1)
SCA71	*P. contorta*	Takhini River, Yukon	3.0 (1)
SCA75	*P. contorta*	Watson Lake, Yukon	3.0 (1)
SCA81	*P. contorta*	Faro, Yukon	2.8 (1)
SCA83	*P. contorta*	Little Salmon, Yukon	3.1 (1)
SCA90	*P. contorta*	McCabe Creek, Yukon	3.0 (1)

[a] Locations of parent trees were as follows: 61 (which originated from an unknown source and was provided by the Massachusetts Department of Conservation) and 65 (which is now dead) were in arboreta of the Institute of Forest Genetics, Placerville, California, U.S.A.; 101, 102, and 104 occurred in a natural population at the Alberta Forest Service's Pine Ridge Forest Nursery, Smoky Lake, Alberta, Canada; 14-6-3, 14-13-6, 55-9-6, 55-14-4, 72-10-6, and 72-15-1 were in a provenance test plantation of the British Columbia Ministry of Forests, Prince George, British Columbia, Canada; and SCA47, SCA56, SCA68, SCA71, SCA75, SCA81, SCA83, and SCA90 were in a clonal seed orchard of the Swedish Cellulose Company, north of Sundsvall, Sweden (at latitude 62°30'N; longitude 17°30'E).

[b] All geographic sources are in Canada, except for parents 61 and 65.

[c] For the North American parents, genotypes are denoted by sizes, in kilobase pairs (kbp), of *polymorphic Sst*I fragments, as described by Wagner et al. (1987). For the Swedish parents, genotypes are denoted by sizes, in kbp, of *polymorphic Bam*HI fragments (see text for details). The number of DNA samples analyzed per parent tree is indicated in parentheses.

Materials and Methods

Genetic Materials

In 1985 and 1986 we constructed 21 matings, some of which were reciprocal, among North American *P. contorta* and *P. banksiana* individuals (Tables 1–3). Prior knowledge of parental cpDNA genotypes (Wagner et al. 1987) ensured that maternal and paternal genotypes differed in each mating. Four different cpDNA genotypes (two of each species) and at least five geographic sources were represented among these parents. A total of 187 seedlings from these matings was available for inference of cpDNA inheritance.

We also examined cpDNA inheritance in 34 seedlings of 13 controlled crosses among eight additional *P. contorta* individuals. These eight individuals were growing in Sweden, and they represented three cpDNA genotypes from seven sources in northern British Columbia and Yukon Territory, Canada (Tables 1 and 2). The Swedish crosses were made during 1982 and 1983 (Fries et al. 1986).

DNA Analyses

For analyses of the North American crosses, cpDNA genotypes of an *Sst*I cpDNA polymorphism were assayed as described previously (Wagner et al. 1987), except that a 700-base-pair *Bam*HI-*Sma*I fragment from the chloroplast genome of *P. contorta* (Lidholm and Gustafsson 1991) was used as the probe in molecular hybridizations (Southern 1975). This probe hybridized with the same polymorphic *Sst*I restriction fragments as the 9.0-kilobase-pair, *Pst*I, *Petunia hybrida*, cpDNA fragment (Palmer and Stein 1986) used previously, but the *P. contorta* probe produced superior signals on autoradiograms. The *P. contorta* clone, designated pPCB121BS0.7, was constructed and kindly provided by J. Lidholm and P. Gustafsson (Umeå University, Sweden).

In the Swedish crosses, a *Bam*HI cpDNA polymorphism was assayed. Chloroplast DNA was purified from each individual as described by Szmidt et al. (1986), and *Bam*HI restriction fragments were fractionated electrophoretically through agarose gels. Restriction fragments were transferred to nylon membranes and hybridized (Southern 1975) with a radiolabeled, 769-base-pair, *Pst*I-*Xba*I, cpDNA fragment from the *Spinacia oleracea psbA* clone pPSII32/1 (equivalent to amino acids 87–341 of the D1 polypeptide). The *Bam*HI polymorphism was then visualized by autoradiography. The *S. oleracea* clone was kindly provided by R. G. Herrmann (Munich, Germany).

Sampling within Individuals

Chloroplast DNA genotypes are occasionally variable within single individuals in sympatric populations of *P. banksiana–P. contorta* (Govindaraju et al. 1988). Therefore, we prepared multiple DNA samples from individuals of the present study when sufficient foliage samples were available (Tables 1–3), and we also extracted multiple DNA samples from three parents and 17 progeny of an earlier *P. banksiana* cpDNA inheritance test (Wagner et al. 1989). DNA was isolated independently from at least two branch tips of each of these individuals, with samples spaced as widely as possible within each individual.

Results and Discussion

Chloroplast DNA was inherited from the paternal parent by most seedlings, in all types of crosses, crosses within and among geographic sources, interspecific crosses, and reciprocal crosses (Tables 2 and 3). This was true despite the diversity of parental cpDNA genotypes and geographic sources. Nonetheless, one maternal and eight nonparental genotypes occurred in the progeny arrays.

The unusual seedlings may have been introduced by contamination, especially of pollen, during construction of the experimental material (e.g., Adams et al. 1988), as in a previous *P. banksiana* cpDNA inheritance test (Wagner et al. 1989). Two observations are consistent with contamination of the crosses. First, several nonpaternal seedlings carried genotypes that we have observed in the neighborhoods of their maternal parents. For example, in the cross 104 × 14-13-6 the cpDNA genotypes of both unusual seedlings are known to occur in the maternal parent's natural population (and thus presumably in the pollen cloud) at Smoky Lake, Alberta (Dong J, Wagner DB, and Yanchuk AD, unpublished data). Also, all Swedish nonpaternal progeny carried cpDNA genotypes that are present in the clonal seed orchard where the crosses were made (Wang X-R and Szmidt AE, unpublished data).

Second, the only seedling from self-pollination (SCA47 × SCA47) in the present study had a nonparental cpDNA genotype. This is again consistent with pollen contamination, because selfed progeny are at a disadvantage in conifers (Ledig 1986). Note that in the previous cpDNA inheritance study of *P. banksiana* four of six nonparental progeny appeared in self-pollinations, and four of those six nonparental

Table 2. Chloroplast DNA segregations in *P. contorta*

Cross	Progeny genotypic frequencies[a]			
	Paternal	Maternal	Nonparental	Total
14-6-3 × 55-9-6	20 (6)	0	0	20 (6)
55-9-6 × 14-6-3	10 (3)	0	0	10 (3)
14-13-6 × 55-9-6	15 (7)	0	0	15 (7)
55-9-6 × 14-13-6	19 (13)	0	0	19 (13)
55-9-6 × 55-14-4	16 (0)	0	0	16 (0)
55-14-4 × 55-9-6	19 (0)	0	0	19 (0)
SCA47 × SCA47	0	0	1 (0)	1 (0)
SCA47 × SCA56	4 (0)	0	0	4 (0)
SCA56 × SCA47	1 (0)	1 (0)	0	2 (0)
SCA47 × SCA68	0	0	1 (0)	1 (0)
SCA68 × SCA47	4 (0)	0	0	4 (0)
SCA47 × SCA75	4 (0)	0	0	4 (0)
SCA75 × SCA47	4 (0)	0	0	4 (0)
SCA47 × SCA90	2 (0)	0	0	2 (0)
SCA68 × SCA75	1 (0)	0	1 (0)	2 (0)
SCA71 × SCA90	1 (0)	0	0	1 (0)
SCA90 × SCA71	1 (0)	0	0	1 (0)
SCA81 × SCA68	4 (0)	0	0	4 (0)
SCA83 × SCA68	4 (0)	0	0	4 (0)
Total	129 (29)	1 (0)	3 (0)	133 (29)

[a] In each cross, the female parent is listed first. The number of seedlings in each cell of the table for which two, independently purified DNA samples were assayed per seedling is indicated in parentheses.

Table 3. Chloroplast DNA segregations in interspecific matings

Cross	Progeny genotypic frequencies[a]			
	Paternal	Maternal	Nonparental	Total
61 × 72-10-6	1 (0)	0	0	1 (0)
101 × 14-6-3	3 (0)	0	0	3 (0)
14-6-3 × 101	4 (0)	0	0	4 (0)
104 × 14-6-3	6 (5)	0	0	6 (5)
14-6-3 × 104	2 (2)	0	0	2 (2)
104 × 14-13-6	11 (5)	0	2 (2)	13 (7)
14-13-6 × 104	7 (4)	0	2 (2)	9 (6)
14-6-3 × 61	1 (0)	0	0	1 (0)
14-6-3 × 65	4 (0)	0	0	4 (0)
14-6-3 × 102	2 (0)	0	0	2 (0)
14-13-6 × 61	5 (2)	0	0	5 (2)
14-13-6 × 65	14 (1)	0	0	14 (1)
14-13-6 × 101	13 (7)	0	0	13 (7)
72-10-6 × 65	1 (0)	0	1 (1)	2 (1)
72-15-1 × 65	9 (0)	0	0	9 (0)
Total	83 (26)	0	5 (5)	88 (31)

[a] As in Table 2.

genotypes were shown by isoenzyme analysis to arise from contamination (Wagner et al. 1989).

Alternatively, nonpaternal seedlings may have resulted from within-parent cpDNA polymorphism and/or from maternal cpDNA leakage (Govindaraju et al. 1988; Sutton et al. 1991a; White 1990). However, we failed to detect cpDNA variability within any individual, despite assaying multiple (as many as 12) DNA samples from each of four of the parents, including a parent (14-13-6) of four nonpaternal seedlings (Tables 1 and 3). Similarly, we found no evidence of cpDNA variability within any of the three *P. banksiana* parents (assaying 4–6 independently purified DNA samples per parent; Dong J, Wagner DB, and Magnussen S, unpublished data) of six nonparental cpDNA genotypes detected in the previous *P. banksiana* inheritance study (Wagner et al. 1989).

In principle, maternal leakage could produce seedlings with mixed restriction fragments from both parents. However, analyses of two independent DNA samples from each of five aberrant seedlings (Table 3) failed to identify any evidence of within-seedling mixtures of parental genotypes. Neither could we detect mixed genotypes in any of 17 progeny (assaying four independently purified DNA samples per seedling, including six nonpaternal progeny; Dong J, Wagner DB, and Magnussen S, unpublished data) of the previous *P. banksiana* inheritance study (Wagner et al. 1989). The apparent lack of within-tree cpDNA polymorphism in these controlled crosses contrasts with a previous observation of chimerism in the *P. banksiana–P. contorta* sympatric region (Govindaraju et al. 1988).

Nonparental (as opposed to mixed or maternal) progeny might be observed if maternal leakage leads to recombination between parental chloroplast genomes; putative recombinants have been identified previously in sympatric *P. banksiana–P. contorta* populations (Govindaraju et al. 1989). In this regard, it is intriguing that in the present study several of the nonparental seedlings were produced by interspecific pollinations (Table 3). In fact, the unusual genotype (4.3/4.8/5.7) from 72-10-6 × 65 has been observed only once previously, in a population near the sympatric region (Wagner et al. 1987; Dong J, Wagner DB, and Carlson MR, unpublished data).

In addition to the cpDNA data, mitochondrial genotypic data are available for the four nonparental seedlings from the reciprocal cross of parents 104 and 14-13-6; each of these seedlings has its putative maternal parent's mitochondrial genotype (Wagner et al. 1991). Because of the predominant maternal inheritance of mitochondrial DNA in *P. banksiana* and *P. contorta* (Wagner et al. 1991), this result appears inconsistent with contamination of the maternal experimental germplasm but is uninformative regarding pollen contamination.

With present data it is premature to reject rigorously any of the hypothetical causes of the nonpaternal seedling genotypes. However, in view of the fact that occasional pollen contamination does occur in attempted controlled matings of these and other conifers (Adams et al. 1988; Wagner et al. 1989), pollen contamination seems the most parsimonious explanation. Moreover, regardless of the cause of the few nonpaternal seedlings, the predominance of paternal cpDNA inheritance is clear in *P. banksiana* and *P. contorta.*

This predominance is not surprising in view of the concordance of available genetic (e.g., Neale et al. 1991; Ohba et al. 1971; Sutton et al. 1991b) and ultrastructural (Chesnoy 1987; Owens and Morris 1990, 1991) evidence. However, the present data (Tables 2 and 3), together with previous reports (Wagner et al. 1987, 1989), suggest that the paternal predominance of cpDNA inheritance in *P. banksiana* and *P. contorta* is independent of parental species, source, or cross direction. This generalization and the recent verification of predominantly maternal mitochondrial inheritance in *P. banksiana* and *P. contorta* (Wagner et al. 1991) place these two spe-

cies into an unusual group of organisms. In such organisms, which include *Chlamydomonas* and at least three genera of Pinaceae, *Pinus, Picea,* and *Pseudotsuga* (Boynton et al. 1987; Marshall and Neale 1992; Neale et al. 1986; Neale and Sederoff 1989; Sutton et al. 1991b), biochemical markers of three differentially inherited genomes are now available for population analyses. These taxa, therefore, represent extraordinary model systems for nuclear-dicytoplasmic analyses (Schnabel and Asmussen 1989).

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