

CHLOROPLAST DNA VARIATION IN THE SYMPATRIC
ZONE OF LODGEPOLE PINE (PINUS CONTORTA DOUGL.),
AND JACK PINE (P. BANKSIANA LAMB.).

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A chloroplast DNA restriction fragment length polymorphism (RFLP) and its relationship with cone morphology was studied in a sympatric population of lodgepole pine and jack pine. Novel cpDNA variants, found only in or near the sympatric region, occurred in approximately 5% of the individuals of this population. Chloroplast DNA typical of jack pine was associated with cone morphology typical of lodge pole pine and hybrids, suggesting that cpDNA of jack pine may be functional in the nuclear genetic background of lodgepole pine.

Additional keywords : Restriction fragment length polymorphism (RFLP), cpDNA polymorphism, sympatric zone.

An understanding of the levels of genetic variation in natural populations is fundamental to breeding programs. Patterns of genetic variation in forest trees traditionally have been described on the basis of provenance studies (Zobel and Talbert 1984), and also using biochemical genetic markers such as allozymes or monoterpenes (Ledig 1986, Kinloch et al. 1986). Recently, the availability of molecular technology has stimulated interest in studying the amount and distribution of genetic variation in forest trees at the molecular level (Neale et al. 1986, Wagner et al. 1987).

Genetic variation occurs in both nuclear and extranuclear (chloroplast and mitochondrial) genomes. It is important to understand extranuclear genetic variation because 1) it is yet unclear whether this variation has phenotypic implications in forest trees, 2) this variation may provide uniparentally inherited markers useful in monitoring pedigrees, and 3) if extranuclear variation is to be incorporated into tree improvement programs by traditional or biotechnological approaches, its interaction with nuclear variation must be understood.

The chloroplast genome has several interesting features: a) it is a relatively small circular molecule whose size varies from 120 kb to 180 kb (Palmer and Thompson 1982); b) a number of important genes, such as those responsible for RUBP carboxylase and herbicide resistance, have been mapped to the molecule; c) although it is considered to be evolutionarily conserved, portions of it appear to be highly polymorphic; and d) chloroplasts are

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maternally inherited in the majority of plants, but their inheritance in conifers may be paternally biased (Neale et al. 1986, Wagner et al. 1987). Paternal inheritance provides diagnostic markers for evolutionary and breeding studies, that may be unique to conifers.

We report here chloroplast DNA variation and its statistical relationship with cone morphology in a sympatric population formed by jack and lodgepole pines (figure 1). These two species originated from an ancestral complex and diverged into the present day species in the late Tertiary (Critchfield 1957, Yeatman 1967). The formerly geographically separated taxa have recently become sympatric in western Canada (Moss 1949).

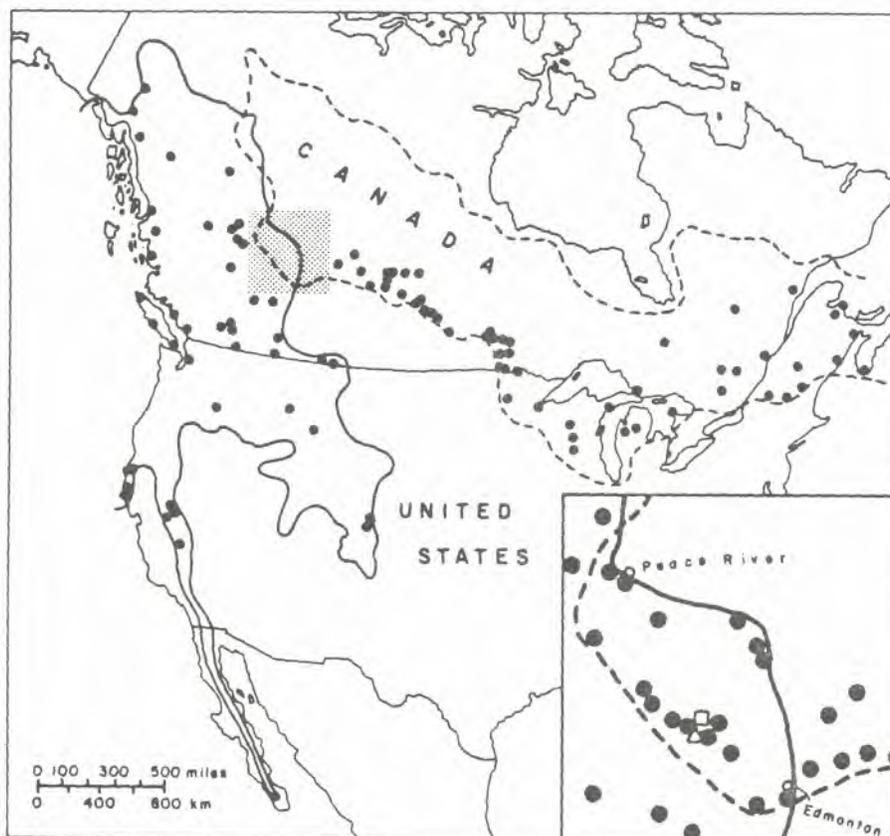


Figure 1. Distributional ranges of *P. banksiana* (broken line) and *P. contorta* (solid line). Filled circles represent populations from which cpDNA phenotypes of 363 individuals have been reported previously (Wagner et al. 1987.) Open square (Carson Creek, Alberta) indicates population of this study.

METHODS

A detailed account of both DNA extraction and the analysis of cpDNA variation in jack and lodgepole pines has been presented elsewhere (Wagner et al. 1987). The following, therefore, is a very brief description of the methods. Total cellular DNA was extracted from 10 g of fresh needle tissue. Approximately 1-2 ug of the total DNA was completely digested with the restriction endonuclease Sst I (BRL laboratories) under conditions recommended by the supplier. Restriction fragments were separated by electrophoresis and transferred to Biotrans membranes. Pine chloroplast DNA fragments were visualized by autoradiograph, following hybridization (Southern 1975) of membrane-bound pine DNA to ³²P-labeled (Rigby et al. 1977) Petunia hybrida cpDNA (Palmer et al. 1983). Cone morphology was used to classify individuals as putative jack pine, lodgepole pine, or hybrid (Moss 1949). The association between cpDNA variation and cone morphology was tested by X² analysis of the contingency table (table 2).

RESULTS AND DISCUSSION

Sst I cpDNA restriction fragment banding patterns (variants) in the allopatric ranges of jack and lodgepole pines provided an unambiguous distinction between the two species in a previous allopatric sample of 202 individuals (Wagner et al. 1987). However, in this study we have detected a number of novel cpDNA variants that apparently occur only in or near the sympatric region (table 1).

Table 1.--Frequencies of cpDNA variants in the Carson Creek population.

cpDNA Variant	Frequency	Species
4.4/5.0*	42	L
4.5/5.0	230	L
4.7/5.0	23	L
4.5/4.8	1	L
4.4/5.7	2	J
4.7/5.7	6	J
4.8/5.7	15	J
5.0/5.7	0	J
<u>Sympatric</u>	16	
Total	335	

*Denotes Sst I fragment sizes in kilobase pairs.

These novel variants occur in approximately 5 percent of the individuals of this population, a significantly greater frequency than in the allopatric ranges ($t = 58.3, P < 0.0005$). The new variants might have arisen due to "bursts of transposition and mutation" (Gerasimova et al. 1984) or intragenic recombination (Golding and Strobeck 1982) in genetically unstable hybrids in the sympatric zone (Barton and Hewitt 1985). In addition, although cpDNA inheritance seems predominantly paternal in P. contorta X P. banksiana hybridizations, the novel variants may result from a low frequency of

"maternal leakage" of cpDNA. This would be the converse of the "paternal leakage" of mitochondrial genomes that has been documented in animal taxa (Powell 1983, Takahata 1985) .

Using cone morphology as an indicator of the taxonomic status of each individual in the zone of sympatry, 19 individuals had cpDNA typical of Pinus banksiana with cone morphology typical of P. contorta or hybrids. Only four trees had cone morphology and cpDNA both typical of P. banksiana (table 2).

Table 2.--Association between cpDNA and cone morphology in lodgepole pine, jack pine and their hybrids in the Carson Creek population of the sympatric zone.

cpDNA	Cone Morphology		TOTAL
	CONCORDANT	DISCORDANT	
LODGEPOLE	143 (136.4)	153 (159.6)	296
JACK	4 (10.6)	19 (12.4)	23
TOTAL	147	172	319

$\chi^2 [1] = 7.01, P \cong 0.01$

P. contorta cpDNA, however, was associated in excess of expectations with P. contorta cone morphology. These results suggest that unidirectionally biased interspecific chloroplast gene flow may occur in the sympatric zone from jack pine into lodgepole pine.

The fact that the novel variants reported here and the variants typical of jack pine or lodgepole pine (Wagner et al. 1987) do not extend beyond the zone of sympatry into the "wrong" species may be due to one of the following three factors: a) the sympatric region between jack and lodgepole pine is relatively recent and the time required for diffusion of alien chloroplast genomes across species boundaries may be long, b) the sympatric region may have been "trapped" by both genetic and environmental factors (Barton and Hewitt 1985), or c) there may be nucleo-cytoplasmic incompatibility between jack and lodgepole pines which is preventing the efficient invasion of chloroplast genomes across the species boundary.

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