

HIGH DIVERSITY BUT LITTLE POPULATION SUBDIVISION OF A LODGEPOLE PINE CHLOROPLAST DNA POLYMORPHISM

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Abstract.--We have surveyed a chloroplast DNA restriction fragment length polymorphism in a total of 365 individuals, distributed rangewide in eight natural populations of lodgepole pine (*Pinus contorta* Dougl.). Six variants were found in the survey, and diversity was high ($H_{es} = 0.44$). Population subdivision was weak within and among subspecies (e.g., $F_{st} = 0.04$ among subspecies), which is consistent with theoretical predictions for paternally-inherited markers in wind-pollinated outcrossers. Comparison of the chloroplast data with mitochondrial frequency data from the same populations provides evidence that gene flow is differential through seeds and pollen.

Keywords: *Pinus contorta* Dougl., mitochondria, RFLP, paternal inheritance, differentiation, genetic variation.

INTRODUCTION

An understanding of the amounts and patterns of genetic variation is fundamental not only for tests of evolutionary hypotheses, but also for effective genetic improvement and germplasm conservation strategies. In plants, this requires consideration of variation in three genomes: chloroplast, mitochondrial, and nuclear.

Theoretical analyses have anticipated that genetic subdivision among populations is sensitive to mode of inheritance. For example, maternally inherited cytoplasmic variation in plants is expected to exhibit greater differentiation at equilibrium than nuclear genes, as a consequence of (i) organellar migration through seeds but not pollen, and (ii) the diploid nuclear but haploid organellar composition of seeds (Birky 1988; Petit 1992). In fact, chloroplast polymorphisms in *Quercus* L. (Whittemore and Schaal 1991; Petit et al. 1993) and *Datisca* L. (Liston et al. 1992), as well as mitochondrial polymorphisms in pines (*Pinus* L.) (Dong and Wagner 1993; Strauss et al. 1993), do indeed display substantial population differentiation (with F_{st} values as high as 0.895).

In contrast, population subdivision of paternally inherited loci, because of their migration through both seeds and pollen, is expected to be much weaker than that of maternally inherited factors in outcrossing plants (Petit 1992). Examples of paternally inherited chloroplast DNA (cpDNA) polymorphisms are known in several plant taxa including conifers (Schumann and Hancock 1989; Boblenz et al. 1990; Wagner 1992), but empirical estimates of population differentiation for these types of polymorphisms are not available in the primary literature. Here

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we report the estimation of population subdivision parameters of a paternally inherited cpDNA polymorphism in lodgepole pine (*Pinus contorta* Dougl.).

METHODS

Plant Materials

The germplasm collections have been described in detail elsewhere (Dong and Wagner 1993). Briefly, a total of 365 individuals were sampled from eight natural populations of lodgepole pine (Table 1). Sampled populations were distributed rangewide and included three of the four subspecies. The available samples provide approximately 95% power to detect variants that occur (*i*) with frequency > 0.07 within populations, and (*ii*) with frequency > 0.008 in the total survey.

Table 1. Sample site locations (abbreviations in parentheses), taxonomic classification, and sample sizes.

Location	Taxonomic Classification	Sample Size
Mackenzie, British Columbia (BC-N)	<i>P.c.l.</i>	44
Prince George, British Columbia (BC-C)	<i>P.c.l.</i>	43
Lumby, British Columbia (BC-S)	<i>P.c.l.</i>	43
Ward,  Colorado (CO)	<i>P</i>	49
Prince Rupert, British Columbia (BC-W)	<i>P.c.c.</i>	43
Waconda Beach, Oregon (OR-W)	<i>P.c.c.</i>	46
Santiam Pass, Oregon (OR-C)	<i>P.c.m.</i>	47
Wrights Lake, California (CA)	<i>P.c.m.</i>	50

^a Map locations are shown in Dong and Wagner (1993). Key to taxonomic classification: *P.c.l.* = *P. contorta* var. *latifolia*; *P.c.c.* = *P. contorta* var. *contorta*; *P.c.m.* = *P. contorta* var. *murrayana*. For discussion of taxonomic classification of OR-C population, see Wheeler and Guries (1982).

Laboratory Methods

In general, chloroplast sequences evolve slowly (Clegg et al. 1991), and interparental chloroplast recombination is very rare or nonexistent (Chiu and Sears 1985). Thus, many chloroplast haplotypes (plastomes) should be identifiable by the genotypes of any sufficiently variable cpDNA polymorphism. With this in mind, we elected to examine a single highly variable polymorphism, rather than several markers of lower variability. This choice maximized the numbers of individuals and populations that could be surveyed, given the available resources. The particular polymorphism chosen for this study was selected because (i) it was the only cpDNA polymorphism known to vary within lodgepole pine, and (ii) its paternal inheritance has been demonstrated (Dong et al. 1992).

Each sampled individual was classified by genotype (variant) of an *SstI* restriction fragment length polymorphism (RFLP), as described previously (Wagner et al. 1987; Dong et al. 1992). However, in the present study a 7.4-kilobase-pair (kbp) *HindIII* fragment and a 700-base-pair *BamHI-SmaI* fragment from the lodgepole pine chloroplast genome (Lidholm and Gustafsson 1991) were used interchangeably as probes in molecular hybridizations. The *psbA* gene is duplicated in lodgepole pine (Lidholm et al. 1991), and either of these probes reveal insertion/deletion polymorphism associated with the *psbA1* - *psbAII* genomic region. This polymorphism may be due to copy-number variation of short tandem repeats (Lidholm and Gustafsson 1991) and is a "hot spot" of chloroplast variation in lodgepole pine (Wagner et al. 1987; Govindaraju et al. 1989).

Data analysis

Frequency data from the eight populations (Table 2) were used to estimate: numbers of variants in species (A_s) and populations (A_p), unbiased variant diversities in species (H_s) and populations (H_{ep}), and population differentiation (F_{st}) (Wright 1951; Nei 1978; Hamrick and Godt 1990; Weir 1990, p. 150). The statistical significance of differentiation was evaluated by chi-square (Weir 1990, p. 137).

RESULTS AND DISCUSSION

Diversity

Six variants were found in the total survey. The number of variants per population ranged from two to five with an average of four. Diversities within populations ranged from 0.26 to 0.66. Notably, no population was fixed for a single cpDNA variant (Tables 2, 3).

Chloroplast DNA variability in lodgepole pine, whether measured in terms of variant number or diversity (Table 3), is greater than that of typical isoenzyme polymorphisms in this and other plants (Wheeler and Guries 1982; Hamrick and Godt 1990). This may appear remarkable but does not contradict the generally slow rate of chloroplast sequence evolution (Clegg et al. 1991), for at least two reasons.

Table 2. Chloroplast genotypic frequencies in 8 populations of *P. contorta*.

Variants ^b	Populations								
	var. <i>latifolia</i>				var. <i>contorta</i>		var. <i>murrayana</i>		
	BC-N	BC-C	BC-S	CO	BC-W	OR-W	OR-Cc	CA	
4.3/5.0	0.07	0.12	--	0.04	0.19	0.11	0.02	0.02	
4.4/5.0	0.09	0.14	0.16	0.08	0.09	0.13	0.09	0.04	
4.5/5.0	0.73	0.65	0.84	0.86	0.53	0.61	0.79	0.86	
4.7/5.0	0.11	0.09	--		0.14	0.13	0.11	0.08	
4.3/4.5/5.0					0.05	0.02			
4.3/4.7/5.0				0.02					

^a Location abbreviations are defined in Table 1 (see also Dong and Wagner 1993).

^b Variants are denoted by restriction fragment sizes (in kilobase pairs); only the variable fragments are listed, separated by slashes within each variant.

See Wheeler and Guries (1982) for discussion of subspecies taxonomic classification in this geographic region.

First, the *psbA*-associated polymorphism results from insertions/deletions, possibly due to the presence of short tandem repeats (Govindaraju et al. 1989; Ali et al. 1991; Lidholm and Gustafsson 1991). Such repeats have been implicated in generating high levels of cpDNA variation in other plants (Palmer et al. 1987; Aldrich et al. 1988; Blasko et al. 1988; Ogihara et al. 1988). Clearly, data from polymorphisms that arise through length mutation are unrelated to conclusions about chloroplast base-pair substitution rates.

Second, recall that we chose to study a single hot spot of cpDNA polymorphism, precisely because of *a priori* knowledge of its intraspecific variability (Wagner et al. 1987). Thus, this polymorphism hardly portrays the situation for "typical" chloroplast base pairs. Several other population surveys of cpDNA diversity, including those that have examined point mutations, have also focused on polymorphic hot spots (e.g., Whittemore and Schaal 1991; Petit et al. 1993). Consequently, much of the accumulating cpDNA population data is not representative of random base pairs.

Despite these caveats, intraspecific cpDNA hot spots carry useful information. When such markers occur, their high diversity permits efficient assay of chloroplast haplotypes. This diversity, combined with uniparental inheritance (either maternal or paternal), empowers new fields of inquiry, such as cytonuclear population genetics (Asmussen et al. 1987).

Table 3. Population genetic statistics in *P. contorta*.

Statistics ^b	Populations							
	var. <i>latifolia</i>				var. <i>contorta</i>		var. <i>murrayana</i>	
	BC-N	BC-C	BC-S	CO	BC-W	OR-W	OR-C ^c	CA
A _p	4	4	2	4	5	5	4	4
H _{ep}	0.46	0.55	0.28	0.26	0.66	0.60	0.37	0.26
Mean A _p	4							
Mean H _{ep}	0.43							
A _s	6							
H _{es}	0.44							
F _{st}	0.04 (among subspecies); p < 0.001 ^d							
	0.02 (within var. <i>latifolia</i>); p < 0.05 ^d							
	-0.01 (within var. <i>contorta</i>); N.S. ^d							
	-0.01 (within var. <i>murrayana</i>); N.S. ^d							

^a As in Table 2.

^b Abbreviations for population genetic statistics are defined in the text.

As in Table 2.

^c Chi-square probabilities.

Differentiation

Two of the chi-square tests indicate statistically significant (p<0.05) frequency differences among sampled populations and subspecies (Table 3). Nonetheless, the plastome differentiation among populations and subspecies is weak: all F_{st} values are 0.04 or less, and pairwise genetic identities (Nei 1978) range from 0.94 to 1.00 (the genetic identity matrix is available upon request from the authors). Weak differentiation is consistent with theoretical expectations for paternally inherited polymorphisms in outcrossers (Petit 1992).

In contrast, population subdivision of maternally inherited mitochondrial polymorphism, estimated from the same DNA samples that we used for the chloroplast analysis, is much higher (e.g., F_{st} = 0.31 among subspecies, and F_{st} is as high as 0.82 among populations within subspecies, Dong and Wagner 1993). We note that, given sufficient intraspecific variability, the maternally inherited cpDNA polymorphisms typical of other plants also generally exhibit considerable subdivision among populations (reviewed by Soltis et al. 1992).

Interestingly, the CO population was fixed for a "private" (Slatkin 1985) mitochondrial variant (Dong and Wagner 1993), yet all but one individual in this population had cpDNA variants typical of other lodgepole pine populations (Table 2). A Colorado population also differed from more central populations in an isoenzyme study (Wheeler and Guries 1982). Taken together, this information is consistent with a dearth of seed migration but occasional pollen migration involving Colorado populations, which are located at the periphery of lodgepole pine's current distributional range. Similarly, the high frequency of two private mitochondrial variants in the OR-W population (Dong and Wagner 1993) contrasts with the cpDNA data (Table 2), again compatible with differential gene flow through pollen and seeds.

CONCLUSIONS

Pines clearly represent an unusual model system for population and evolutionary genetic investigations, because of their opposite chloroplast and mitochondrial inheritances (Neale and Sederoff 1989). In lodgepole pine, population subdivision of the three major eukaryotic genomes conforms with theoretical predictions for outcrossers. Specifically, Mendelian allozymes and paternally inherited plastomes display little differentiation among populations (Wheeler and Guries 1982; this study, Table 3), while maternally inherited mitochondrial polymorphisms feature abundant population subdivision (Dong and Wagner 1993). Knowledge of the population genetic architectures of differentially-inherited plant genomes may be useful when biologists choose genetic markers for specific purposes.

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