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Chloroplast DNA restriction fragment length polymorphism in *Sequoia sempervirens* D. Don Endl., *Pseudotsuga menziesii* (Mirb.) Franco, *Calocedrus decurrens* (Torr.), and *Pinus taeda* L.

I.F. Ali, D.B. Neale* and K.A. Marshall

Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Box 245, Berkeley, CA 94701, USA

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Summary. The extent and type of chloroplast DNA restriction fragment length polymorphism was determined among individual tree samples of coast redwood, Douglas fir, incense-cedar, and loblolly pine. A total of 107 trees was surveyed for three restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII) and six chloroplast DNA probes from petunia (P3, P4, P6, P8, P10, S8). The probes comprise 64% of the petunia chloroplast genome. Polymorphisms were detected in all species but loblolly pine. Coast redwood and incense-cedar had a small number of rare variants, whereas Douglas fir had one highly polymorphic region of insertions/deletions in sequences revealed by the P6 probe from petunia. The mutation hotspot is currently being studied by DNA sequence analysis.

Key words: cpDNA RFLPs – *Sequoia sempervirens* D. Don Endl. – *Pseudotsuga menziesii* (Mirb.) Franco – *Calocedrus decurrens* (Torr.) Florin – *Pinus taeda* L.

Introduction

The application of molecular techniques has made it possible to study genetic variation encoded in the chloroplast and mitochondrial genomes of higher plants. Restriction site mapping, restriction fragment length polymorphisms (RFLPs), and DNA sequencing are the primary methods used. RFLPs are a simple and rapid approach for obtaining estimates of organelle DNA variability in population samples. Estimates of chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) variation based on RFLPs have been reported for numerous angiosperms [e.g., *Lupinus texensis* (Banks and Birky 1985), *Hordeum vulgare* and *H. spontaneum* (Holwerda et al. 1986; Neale et al. 1988), and *Tolmiea menziesii* (Soltis et al. 1989)]. For gymnosperms, however, the only extensive studies of organellar variation are those for cpDNA in a lodgepole pine-jack pine (*Pinus contorta* – *P. banksiana*) complex (Govindaraju et al. 1989; Wagner et al. 1987). The extent of cpDNA and mtDNA variation in conifers is largely unknown.

Chloroplast DNA is paternally inherited in all conifers that have been examined (Neale et al. 1986, 1989; Neale and Sederoff 1989; Stine et al. 1989; Szmidt et al. 1987; Wagner et al. 1987, 1989). Mitochondrial DNA is maternally inherited in loblolly pine (*Pinus taeda*) (Neale and Sederoff 1989), but is paternally inherited in coast redwood (Neale et al. 1989). Thus, some conifers have paternal inheritance of cpDNA and maternal inheritance of mtDNA, while other conifers have paternal inheritance of both organelle genomes.

New theory is emerging to interpret the increasing amount of data on organelle DNA variation (Birky et al. 1989). One unique opportunity is for the study of cytonuclear interactions in higher plants (Asmussen et al. 1987; Schnabel and Asmussen 1989). In pines, it might be possible to estimate nuclear-dicytoplasmic disequilibrium parameters where one genome is paternally inherited (chloroplast), one is maternally inherited (mitochondrial), and one has Mendelian inheritance (nuclear). However, a prerequisite to such estimates is to determine whether or not genetic variation exists within each of the three genomes. The purpose of this study is to estimate the extent and to determine the type of cpDNA variation in four species of conifers, representing three families of Coniferales. CpDNA RFLPs were detected in Douglas fir, coast redwood, and incense-cedar but not in loblolly pine. Most RFLPs resulted from small insertions or deletions; only one point mutation was detected among all samples.

* To whom correspondence should be addressed

Materials and methods

Population samples

Twenty-three coast redwood trees, ranging from Curry County/OR, to Monterey County/CA, were sampled from a redwood provenance test plantation in Lafayette/CA. Twenty-four Douglas fir trees, ranging from British Columbia to Arizona, were sampled from plantations in Placerville/CA, and Corvallis/OR. Thirty incense-cedar trees ranging from southern Oregon to Los Angeles County/CA, were sampled from a test plantation in Foresthill/CA. Thirty loblolly pine trees from eastern North Carolina were sampled from a Weyerhaeuser Co. seed orchard in Hot Springs/AR. No two trees for a given species were sampled from the same stand; the sampling strategy was to maximize the likelihood of detecting variation within a species, except for loblolly pine, for which sampling was restricted to one part of its range.

DNA isolation, Southern blots, and DNA hybridizations

Total DNA was isolated from 10 g of fresh needle tissue by following a modified CTAB (hexadecyltrimethylammonium bromide) procedure (Murray and Thompson 1980; Wagner et al. 1987). Approximately 1 μg of DNA was digested with each of three restriction endonucleases (*Bam*HI, *Eco*RI, and *Hin*dIII), and samples were fractionated on 1% agarose gels. DNA was transferred to Biotrans nylon membranes (ICN, Irvine/CA), and the DNA was cross-linked to these membranes by a 5-min exposure to UV light.

Blots were prehybridized for 16–24 h in 5 × SSC, 50 m*M* phosphate buffer, 0.4% SDS, 5 × Denhardt's solution, 2.5 m*M* EDTA (pH 8.0), and 100 μg/ml denatured herring sperm DNA. Blots were hybridized in fresh buffer for 12–24 h with ^{32}P-labelled chloroplast DNA probes from *Petunia hybrida* (Palmer et al. 1983), prepared by the random primer labeling procedure (Feinberg and Vogelstein 1983). The *Pst*I (P3, P4, P6, P8, P10) and *Sal*I (S8) (notation of Sytsma and Gottlieb 1986) chloroplast DNA clones used in this study included approximately 64% of the petunia chloroplast genome (Fig. 1). These clones were chosen as probes because: (1) they revealed variation in our preliminary studies with conifers or in published studies in angiosperms, and (2) they included clones from the inverted repeat, large single-copy, and small single-copy regions of the petunia chloroplast genome (Fig. 1).

Following hybridization, blots were washed three times in 2 × SSC, 0.1% SDS. The first wash was conducted at room temperature for 5 min, and the last two washes were at 65 °C for 30 min each. Blots were exposed to X-ray films with intensifier screens for 1–3 three days at −70 °C.

Results

Coast redwood

RFLPs were detected with the P3, P6, and P10 probes in the coast redwood samples; no polymorphisms were found with P4, P8, and S8 probes (Table 1). An insertion/deletion polymorphism was revealed in *Eco*RI, *Hin*dIII, and *Bam*HI digests hybridized with P6 (Fig. 2). For *Eco*RI, the 3.92 kb fragment was the common type, while the 4.04 kb fragment was found in only one tree and the 3.83 kb fragment was found in two trees (Table 1). The variant trees had approximately 100 bp insertions or deletions compared to the common type.

Coast redwood blots hybridized with P3 and P10 were monomorphic except for one tree from Mendocino County/CA. This tree had two extra fragments (12.46 and 9.66 kb), in addition to the four fragments (18.89, 5.71, 4.00 and 2.51 kb) found in all trees in *Bam*HI digests hybridized with P3. In hybridizations with P10, however, the unusual tree had fewer fragments than the common trees. *Bam*HI digests of the common type had four fragments (17.36, 5.67, 3.67 and 1.38 kb), and the unusual tree had three fragments (9.54, 5.67 and 3.67 kb) (Table 1). Similarly, the *Eco*RI digests of the common type had four fragments (9.06, 6.69, 4.47 and 1.75 kb), and the unusual tree had three fragments (8.42, 6.69 and 1.75 kb) (Table 1). The complex rearrangements in this one tree could not be attributed to a simple gain or loss of a restriction site or insertions/deletions. There were three cpDNA haplotypes among the 23 coast redwood samples based on the probes and enzymes used in this study (Table 1).

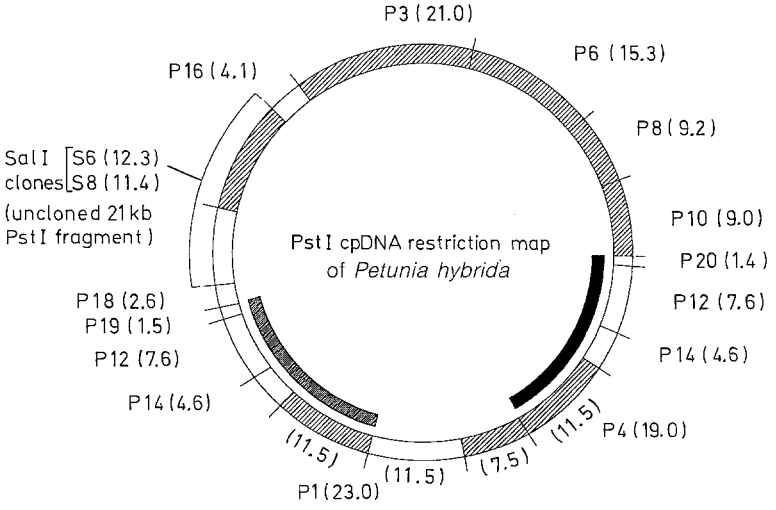

Fig. 1. The chloroplast genome of *Petunia hybrida* shown with the *Pst*I and *Sal*I clones. Hatch-marked clones indicate probes used to survey the conifer chloroplast genomes (Sytsma and Gottlieb 1986). (#) length of the fragment in kb

Table 1. Restriction fragment length polymorphisms (kb) in chloroplast DNA of coast redwood. Only variant fragments are listed

Source	Probe/restriction enzyme					Haplotype no.
	P3 *Bam*HI	P6 *Eco*RI	P6 *Hin*dIII	P10 *Bam*HI	P10 *Eco*RI	
1. Curry County, Oregon	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
2. Curry County, Oregon	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
3. Curry County, Oregon	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
4. Del Norte County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
5. Del Norte County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
6. Del Norte County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
7. Del Norte County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
8. Humboldt County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
9. Humboldt County, California	18.89, 12.46, 9.66	4.04	4.74	9.54	8.42	3
10. Humboldt County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
11. Humboldt County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
12. Humboldt County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
13. Humboldt County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
14. Mendocino County, California	18.89	3.83	4.50	17.36, 1.38	9.06, 4.47	2
15. Mendocino County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
16. Mendocino County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
17. Napa County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
18. Marin County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
19. San Mateo County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
20. Santa Cruz County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
21. Monterey County, California	18.89	3.83	4.50	17.36, 1.38	9.06, 4.47	2
22. Monterey County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1
23. Monterey County, California	18.89	3.92	4.61	17.36, 1.38	9.06, 4.47	1

Table 2. Restriction fragment length polymorphisms (kb) in chloroplast DNA of Douglas fir. Only variant fragments are listed

Source	Probe/restriction enzyme	Haplotype no.
	P6 *Bam*HI	
1. Nanaimo, British Columbia	3.10	3
2. Siscamous, British Columbia	2.93	4
3. Alta Lake, British Columbia	3.06	1
4. Alta Lake, British Columbia	3.01	2
5. Quesnel, British Columbia	3.10	3
6. Chelan County, Washington	3.06	1
7. Chelan County, Washington	3.06	1
8. Chelan County, Washington	3.25	6
9. Benewah County, Idaho	3.25	6
10. Boundary County, Idaho	3.01	2
11. Boundary County, Idaho	3.19	5
12. Lincoln County, Montana	3.01	2
13. Lincoln County, Montana	3.19	5
14. Lewis and Clark County, Montana	2.93	4
15. Mountain View, Wyoming	3.19	5
16. Trinity County, California	2.93	4
17. Eldorado County, California	3.06	1
18. Eldorado County, California	3.10	3
19. Boulder County, Colorado	2.93	4
20. Valencia County, New Mexico	3.01	2
21. Valencia County, New Mexico	2.87	7
22. Valencia County, New Mexico	3.01	2
23. Pinal County, Arizona	3.06	1
24. Graham County, Arizona	3.10	3

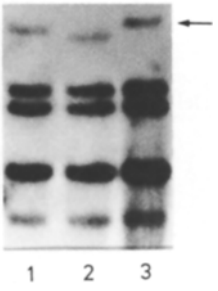

Fig. 2. *Lanes 1–3* are coast redwood DNAs digested with *Eco*RI and probed with P6 *P. hybrida* clone. *Arrow* indicates variant fragments from three different haplotypes

Douglas fir

RFLPs were found among the 24 Douglas fir DNAs that were probed with P6 and P10 clones; no polymorphisms were found when these DNAs were probed with P3, P4, P8, and S8 clones. The P6 hybridizations revealed seven distinct fragments ranging from 2.87 to 3.25 kb in *Bam*HI digests (Table 2; Fig. 3). These polymorphic fragments likely resulted from insertions or deletions based on corresponding fragment length variants also found in *Hin*dIII and *Eco*RI digests. There were seven haplotypes among the 24 Douglas fir trees sampled based on the polymorphism revealed by P6 (Table 2). Fragments of variable lengths were also detected in *Eco*RI digests hybridized with P10, but because of some unknown factor,

Table 3. Restriction fragment length polymorphisms (kb) in chloroplast DNA of incense-cedar. Only variant fragments are listed

Source	Probe/restriction enzyme					Haplotype no.
	P4 *Bam*HI	P4 *Eco*RI	P10 *Bam*HI	P10 *Hin*dIII	S8 *Eco*RI	
1. Southern Oregon[a]	4.03	3.38	4.68	3.21	1.19, 0.94	1
2. Southern Oregon	3.97	3.33	4.68	3.21	1.19, 0.94	3
3. Southern Oregon	4.03	3.38	4.68	3.21	1.19, 0.94	1
4. Southern Oregon	4.03	3.38	4.68	3.21	1.19, 0.94	1
5. Southern Oregon	4.03	3.38	4.68	3.21	1.19, 0.94	1
6. Northern California[b]	4.13	3.45	4.68	3.21	1.19, 0.94	2
7. Northern California	4.03	3.38	4.59	3.11	1.19, 0.94	5
8. Northern California	4.03	3.38	4.68	3.21	1.19, 0.94	1
9. Northern California	4.03	3.38	4.68	3.21	1.19, 0.94	1
10. Northern California	4.03	3.38	4.68	3.21	1.19, 0.94	1
11. Shasta County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
13. Shasta County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
14. Shasta County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
15. Shasta County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
16. Calaveras County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
17. Calaveras County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
18. Calaveras County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
19. Calaveras County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
20. Calaveras County, California	4.13	3.45	4.68	3.21	1.19, 0.94	2
21. Kern County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
22. Kern County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
23. Kern County, California	3.90	3.28	4.68	3.21	1.19, 0.94	4
24. Kern County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
25. Kern County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
27. Los Angeles County, California	3.97	3.33	4.68	3.21	1.90	6
28. Los Angeles County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1
30. Los Angeles County, California	4.03	3.38	4.68	3.21	1.19, 0.94	1

[a] Southern Oregon samples are from Jackson and Klamath counties
[b] Northern California samples are from Del Norte, Siskiyou, and Shasta counties

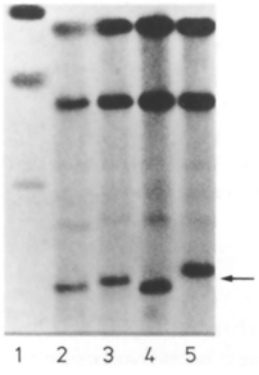

Fig. 3. *Lane 1* is lambda/*Hin*dIII size marker. *Lanes 2–5* are Douglas fir DNAs digested with *Bam*HI and probed with P6 *P. hybrida* clone. *Arrow* indicates variant fragments from four different haplotypes

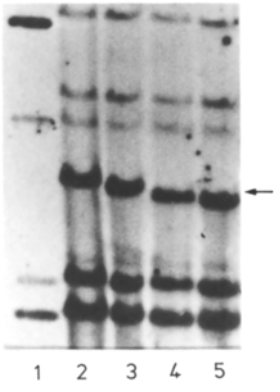

Fig. 4. *Lane 1* is lambda/*Hin*dIII size marker. *Lanes 2–5* are incense-cedar DNAs digested with *Bam*HI and probed with P4 *P. hybrida* clone. *Arrow* indicates variant fragments from four different haplotypes

these patterns were not reproducible from one experiment to another.

Incense-cedar

RFLPs were found in incense-cedar when probed with P4, P10, and S8 clones; no other polymorphisms were found in hybridizations with P3, P6, and P8 (Table 3). Probe P4 revealed an insertion/deletion polymorphism in *Bam*HI and *Eco*RI digestions (Fig. 4). The common genotype had a 4.03 kb fragment in *Bam*HI digests, whereas two trees had a 4.13 kb fragment, two other trees had a 3.97 kb fragment, and one tree had a 3.90 kb

fragment (Table 3). Although the absolute sizes of the insertions/deletions differed in *Eco*RI digests, the relative size differences between fragments was the same. One tree had a small (100 bp) deletion when blots were hybridized with P10 (Table 3). This deletion was detectable in *Bam*HI and *Hin*dIII digests. Hybridizations with S8 revealed a point mutation at an *Eco*RI site (Table 3). The common type had two fragments of 1.19 and 0.94 kb, whereas the tree lacking the *Eco*RI site had a single 1.90-kb fragment. The 230 bp discrepancy between the sum of the estimated fragment sizes in trees with the site versus the tree without it could have been due to an additional deletion within the 1.90 kb fragment or simply due to a measurement error. This was the only point mutation we detected in the entire study. Six haplotypes among the 30 samples were detected in incense-cedar (Table 3).

Loblolly pine

The 30 loblolly pine trees examined revealed no polymorphisms when probed with the six clones.

Discussion

One-hundred seven individual trees were assayed to determine the extent and type of cpDNA variation within each of four species of conifers (coast redwood, Douglas fir, incense-cedar, and loblolly pine). The number of cpDNA haplotypes detected per species was three in redwood, seven in Douglas fir, six in incense-cedar, and one in loblolly pine (Tables 1–3). The proportion of the total species range sampled in loblolly pine was much less than for the other three conifers, which could account for the lack of observed variation in loblolly pine. For nuclear-encoded isozyme loci, however, the within-population component of variability is generally very high in conifers (Hamrick and Godt 1990); thus, if the same were true for cpDNA variability, we might have expected to detect variability among the loblolly pine samples. The variant restriction fragments detected in coast redwood and incense-cedar were rare, whereas in Douglas fir there was a more uniform distribution of the frequency of variants (Tables 1–3).

Almost all RFLPs were insertions/deletions; the two exceptions were the one tree with the complex rearrangement in redwood and the one tree which lacked a restriction site in incense-cedar. Only three restriction enzymes were used in this study; therefore, our data are biased towards the detection of insertions/deletions, as opposed to point mutations, relative to other studies in which more enzymes have been used. In a preliminary study, however, we surveyed 16 widely distributed Douglas fir trees with 14 enzymes and the same six cpDNA probes, and not a single restriction site change was detected. On the basis of these results, we concentrated our experimental effort on more trees and fewer enzymes in the present study. RFLPs were detected with five of the six petunia probes used; only P8 failed to reveal variation. No one probe consistently revealed variation across species; thus, no apparent mutation hotspots were common to the four conifers. The one notable observation was that the highly polymorphic region detected with P6 in Douglas fir was also polymorphic in redwood.

Lodgepole pine (*Pinus contorta*) and jack pine (*Pinus banksiana*) are the only conifers for which extensive, intraspecific cpDNA variation studies have been conducted. Wagner et al. (1987) sampled a total of 363 trees from zones of sympatry and allopatry. Thirteen variants were detected in *Sst*I digests hybridized with the P10 petunia probe. In a much larger sample from the zone of sympatry (902 trees), many of these variants plus some novel types were detected with the same probe and enzyme combination (Govindaraju et al. 1989). These variants appeared to be due to insertions/deletions. The amount of variability detected in allopatric populations of lodgepole pine and jack pine is comparable to that found in the three species of the present study, but is in contrast to the high level of variability found in sympatric populations. It would be valuable to clone the polymorphic sequences from trees in the lodgepole pine – jack pine sympatric region and to determine whether homologous sequences exist in the conifers used in this study.

Population-level genetic variation in cpDNA has been studied in several species of angiosperms [e.g., *Lupinus texensis* (Banks and Birky 1985); *Hordeum vulgare* and *H. spontaneum* (Holwerda et al. 1986; Neale et al. 1988); *Zea* species (Doebley et al. 1987); *Helianthus* species (Rieseberg et al. 1988); *Solanum* species (Hosaka and Hanneman 1988); *Tolmiea menziesii* (Soltis et al. 1989); and *Glycine* species (Close et al. 1989)]. It is very difficult to compare the relative amounts of cpDNA variation among angiosperms or between angiosperms and gymnosperms because of the large differences in sample sizes, the number of restriction enzymes, and the number of probes used among studies. In general, the amount of variation detected in the four conifers of this study is roughly equivalent to that found in angiosperms. Mutations occur in either the large or small single-copy regions of angiosperm cpDNA. Although the conifers studied to date lack the large inverted repeat (Lidholm et al. 1988; Strauss et al. 1988), all but one of the RFLPs we detected mapped to single-copy regions. Because P4 contains sequences from both the inverted repeat and the small single-copy region of petunia, we do not know, without more accurate mapping, whether the polymorphism detected in incense-cedar is within this region. One possible difference in restriction fragment polymorphism between angiosperms and conifers is the relative abundance of restriction site changes in angiosperms and the near ab-

sence of them in conifers. Small insertions and deletions appear to be more abundant in conifers than in angiosperms.

The most interesting region of polymorphism observed in this study was the large number of insertions/deletions detected with the P6 probe in Douglas fir. This region was also highly polymorphic among trees sampled from two Douglas fir seed orchards (Neale et al. 1986; Neale, unpublished results). The presence of a mutation hotspot in Douglas fir might be related to either of two features of Douglas fir cpDNA, which similarly have been implicated with high levels of rearrangement in some angiosperms. The first is the absence of the large inverted repeat in Douglas fir (Strauss et al. 1988) which has been found in only a few members of the legume family (Palmer and Thompson 1982; Palmer 1985; Palmer et al. 1987) and in other pines (Lidholm et al. 1988; Strauss et al. 1988). The second feature associated with high levels of cpDNA rearrangement in angiosperms is the presence of small to medium (50–1,500 bp) dispersed repeats (Palmer 1985). Tsai and Strauss (1989) found six families of dispersed repeats in Douglas fir cpDNA. One member of their repeat family 1 maps close to the P6 hotspot. We plan to sequence fragments of the polymorphic region from several trees and search for sequences that might reveal a mechanism by which the rearrangements occurred.

The primary objective of this study was to make a preliminary assessment of how much genetic variation exists in the chloroplast genomes of the four conifers. The number of haplotypes observed among the rangewide samples of coast redwood, Douglas fir, and incense-cedar indicates that population-level variation is likely and that population genetic parameters such as cytonuclear disequilibrium estimates could be made. Only in loblolly pine were we unable to detect variation. One factor, however, that may complicate the estimation and analysis of cpDNA variation in conifers is that most variation appears to be in the form of insertions and deletions. Unlike point mutations, it is not possible to determine from restriction fragment lengths whether two trees are genetically the same or different on the basis of the insertion or deletion of a piece of DNA somewhere in the length of a fragment revealed by the probe. This problem could be overcome by sequencing the fragments, including the insertions or deletions. If genomic sequencing of polymerase chain amplified fragments could be employed, it would be feasible to extend genetic analyses to the population level.

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References

Asmussen MA, Arnold J, Avise JC (1987) Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. Genetics 115:755–768

Banks JA, Birky CW (1985) Chloroplast DNA diversity is low in a wild plant, *Lupinus texensis*. Proc Natl Acad Sci USA 82:6950–6954

Birky CW, Fuerst P, Maruyama T (1989) Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121:613–627

Close PS, Shoemaker RC, Keim P (1989) Distribution of restriction site polymorphism within the chloroplast genome of the genus *Glycine*, subgenus *Soja*. Theor Appl Genet 77:768–776

Doebley J, Renfroe W, Blanton A (1987) Restriction site variation in the *Zea* chloroplast genome. Genetics 117:139–147

Feinberg AP, Vogelstein S (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Govindaraju DR, Dancik BP, Wagner DB (1989) Novel chloroplast DNA polymorphism in a sympatric region of two pines. J Evol Biol 2:49–59

Hamrick JL, Godt MJW (1990) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources., Sinauer Associates Inc. Sunderland, MA pp 43–63

Holwerda BC, Jana S, Crosby WL (1986) Chloroplast and mitochondrial DNA variation in *Hordeum vulgare* and *Hordeum spontaneum*. Genetics 114:1271–1291

Hosaka K, Hanneman RE (1988) Origin of chloroplast DNA diversity in the Andean potatoes. Theor Appl Genet 76: 333–340

Lidholm J, Szmidt AE, Hällgren J-E (1988) The chloroplast genomes of conifers lack one of the rRNA-encoding inverted repeats. Mol Gen Genet 212:6–10

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Neale DB, Sederoff RR (1989) Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor Appl Genet 77:212–216

Neale DB, Wheeler NC, Allard RW (1986) Paternal inheritance of chloroplast DNA in Douglas-fir. Can J For Res 16:1152–1154

Neale DB, Saghai-Maroof MA, Allard RW, Zhang Q, Jorgensen RA (1988) Chloroplast DNA diversity in populations of wild and cultivated barley. Genetics 120:1105–1110

Neale DB, Marshall KA, Sederoff RR (1989) Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don. Endl. Proc Natl Acad Sci USA 86:9347–9349

Palmer JD (1985) Comparative organization of chloroplast genomes. Annu Rev Genet 19:325–354

Palmer JD, Thompson WF (1982) Chloroplast rearrangements are more frequent when a large inverted repeat sequence is lost. Cell 29:537–550

Palmer JD, Shields CR, Cohen DB, Orton TJ (1983) Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. Theor Appl Genet 66:181–189

Palmer JD, Osorio B, Aldrich J, Thompson WF (1987) Chloroplast DNA evolution among legumes. Loss of a large inverted repeat occurred prior to other sequence rearrangements. Curr Genet 11:275–286

Rieseberg LH, Soltis DE, Palmer JD (1988) A molecular reexamination of introgression between *Helianthus annus* and *H. bolanderi* (Compositae). Evolution 42:227–238

Schnabel A, Asmussen MA (1989) Definition and properties of disequilibria within nuclear-mitochondrial-chloroplast and other nuclear-dicytoplasmic systems. Genetics 123:199–215

Soltis DE, Soltis PS, Ranker TA, Ness BD (1989) Chloroplast DNA variation in a wild plant, *Tolmiea menziesii*. Genetics 121:819–826

Stine M, Sears BB, Keathley DE (1989) Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. Theor Appl Genet 78:768–774

Strauss SH, Palmer JD, Howe GT, Doerksen AH (1988) Chloroplast genomes of two conifers lack a large inverted repeat and are extensively rearranged. Proc Natl Acad Sci USA 85:3898–3902

Sytsma KJ, Gottlieb LD (1986) Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* Sect. *Peripetasma* (Onagraceae). Evolution 40:1248–1261

Szmidt AE, Aldén T, Hällgren JE (1987) Paternal inheritance of chloroplast DNA in *Larix*. Plant Mol Biol 9:59–64

Tsai C-H, Strauss SH (1989) Dispersed repetitive sequences in the chloroplast genome of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Curr Genet 16:211–218

Wagner DB, Furnier GR, Saghai-Maroof MA, Williams SM, Dancik BP, Allard RW (1987) Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc Natl Acad Sci USA 84:2097–2100

Wagner DB, Govindaraju DR, Yeatman CW, Pitel JA (1989) Paternal chloroplast DNA inheritance in a diallel cross of jack pine (*Pinus banksiana* Lamb.). J Hered 80:483–485