Abundant Mitochondrial Genome Diversity, Population Differentiation and Convergent Evolution in Pines

Junyuan Wu, Konstantin V. Krutovskii' and Steven H. Strauss
Department of Forest Science, Oregon State University, Corvallis, Oregon 97331-7501
Manuscript received February 27, 1998
Accepted for publication August 19, 1998

ABSTRACT

We examined mitochondrial DNA polymorphisms via the analysis of restriction fragment length polymorphisms in three closely related species of pines from western North America: knobcone *(Pines attenuate* Lemur.), Monterey (*P. radiate* D. Don), and bishop (*P. muricata* D. Don). A total of 343 trees derived from 13 populations were analyzed using 13 homologous mitochondrial gene probes amplified from three species by polymerase chain reaction. Twenty-eight distinct mitochondrial DNA haplotypes were detected and no common haplotypes were found among the species. All three species showed limited variability within populations, but strong differentiation among populations. Based on haplotype frequencies, genetic diversity within populations (HS) averaged 0.22, and population differentiation (CST and 0) exceeded 0.78. Analysis of molecular variance also revealed that >90% of the variation resided among populations. For the purposes of genetic conservation and breeding programs, species and populations could be readily distinguished by unique haplotypes, often using the combination of only a few probes. Neighbor joining phenograms, however, strongly disagreed with those based on allozymes, chloroplast DNA, and morphological traits. Thus, despite its diagnostic haplotypes, the genome appears to evolve via the rearrangement of multiple, convergent subgenomic domains.

PLANT organelle genomes have been increasingly I applied to study population genetic structure and phylogenetic relationships in plants (see reviews in H1PK1NS *et al.* 1994; OLMSTEAD and PALMER 1994; DUMOLINLAPEGUE *et al.* 1997). The use of molecular markers derived from different genomes provides a more complete description of population structure *(e.g.,* HONG *et al.* 1993a; DONG and WAGNER 1994; MGGAUL EY *et al.* 1996) and, thus, aids in identification of species, races, and populations in breeding *(e.g.,* GRABAU *et al.* 1992) and conservation *(e.g.,* FURMAN *et al.* 1996) programs. For species such as woody perennials, nuclear genes *(e.g.,* allozymes) provide little power for discrimination among populations because the large majority of their diversity resides within populations (HAMRICK and GODT 1990; BROWN and SCHOEN 1992). In contrast, cytoplasmic organelle genomes (chloroplasts and mitochondria) are often strongly differentiated among populations. This difference may result from a low rate of sequence mutation, small effective population size, and limited gene flow for maternally inherited organelles (B1RKY 1988; DONG and WAGNER 1994). Plant mitochondrial DNA (mtDNA) has a very low rate of gene sequence evolution (WOLFS *et al.* 1987), suggesting a much lower rate of point mutation in plant mtDNA than in chloroplast DNA (cpDNA) and animal mtDNA (SEDEROFF 1987; PALMER 1992a,b). However, it is extremely variable in size and gene arrangement (PR1NG and LONSDALE 1985; PALMER 1992a), and it shows maternal inheritance in pines (NEALE and SEDEROFF 1989) and most other plant species. Taken together, these distinctive features of plant mtDNA make it a potentially powerful tool for the analysis of population differentiation. The few studies of mtDNA polymorphism in plants have revealed high variability within and/or among populations (BF.LHASSEN *et al.* 1993; DONG and WAGNER 1993; Luo *et al.* 1995), including a previous study of the California closed-cone pines (CCCP) using a single mtDNA gene probe (STRAUSS *et al.* 1993).

The aim of this study was to intensively assess the level and distribution of mtDNA genetic diversity in the CCCP via sampling of a number of regions of the genome. The CCCP contains three closely related species and includes several disjunct populations and distinctive taxonomic varieties, thus providing samples of several early stages of speciation. It is comprised of one interior species, *Pines attenuate* (knobcone pine), and two maritime species, *P. muricata* (bishop pine) and *P. radiata* (Monterey pine). Knobcone pine grows on interior sites of southern Oregon and California as disjunct populations. The two other species are distributed discontinuously along the California coast and on four islands (Figure 1; CRITCHFIFI.D and LITTLE 1966). Many characteristics of these species have been studied in previous population genetic analyses, including mor

Corresponding author: Steven H. Strauss, Department of Forest Science, Oregon State University, Corvallis, OR 97331-7501.
E-mail: strauss@fsl.orst.edu
' Permanent address: Laboratory of Population Genetics, N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, 117809 GSP-1, Moscow B-333, Russia.

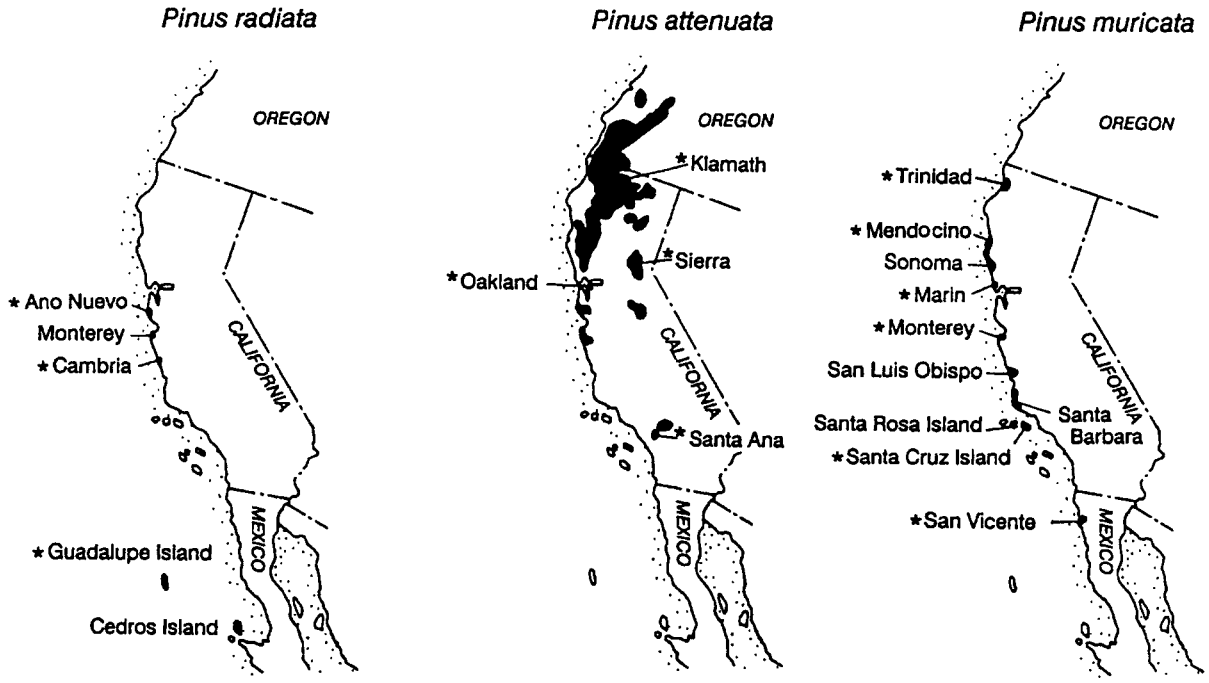

FIGURE 1.-Distribution of P. *radiata, P. attenuata,* and P. *muricata* (HONG *et al.* 1993a) and the origins of sampled populations (indicated by asterisks). All populations were studied for chloroplast DNA polymorphism by HONG *et al.* (1993a).

phology, secondary compound chemistry, allozyme and cpDNA (reviewed in MILLAR 1986; MILLAR et al. 1988; HONG et al. 1993a,b), providing reference points for comparison. The specific objectives of this study were to (1) document patterns of mtDNA diversity within and among populations, (2) compare these patterns with those of other genetic markers, (3) evaluate the phylogenetic value of mtDNA genome polymorphisms, and (4) assess the capability of mtDNA to quantitatively and qualitatively differentiate species and populations and, thus, assist in germplasm identification for conservation and breeding programs.

MATERIALS AND METHODS

Plant materials: Trees were sampled from natural populations or from gene conservation and genetic test plantations, as described in HONG *et al.* (1993a). Two different collections contributed to this study. The following were primarily collected by HONG *et al.* (1993a): the Ano Nuevo, Cambria, and Guadalupe populations of Monterey pine; the Sierra Nevada and Santa Ana populations of knobcone pine; and the Santa Cruz population of bishop pine. The other populations were collected specifically for this study. For knobcone pine, the Klamath population was sampled over a 6.0-km transect adjacent to the Lakehead Exit on U.S. Interstate 5, California (latitude 40°55', longitude 122°30'), and the Oakland population was sampled over a 2.6-km transect along Flicker Ridge adjacent to the town of Moraga in the hills east of Oakland, CA (latitude 37°50', longitude 122°30'). For bishop pine, the San Vicente population was sampled in several small scattered populations along a road north of San Vicente that goes out to the town of Erendira, Mexico (latitude 31°15', longitude 116°30'); the Monterey population was sampled over one lin ear mile in the woods of Samuel F. B. Morse Botanical Reserve located south of Monterey, California (latitude 36°40', longitude 121°50'); the Marin population was sampled over a l.'7km transect -5.1 km southwest from the town of Inverness, California (latitude 38°08', longitude 122°45'); the Mendocino population was sampled for 2.6 km on both sides of U.S. Highway 101, 7.5 km south of the town of Point Arena, California (latitude 39°20', longitude 123°50'); and the Trinidad population was sampled over 1.7 linear kilometers up Fox Farm Road adjacent to the town of Trinidad, California (latitude 41°05', longitude 124°10').

Probe preparation and universal mtDNA primers: A total of 13 different probes were used in the restriction fragment length polymorphism (RFLP) analysis. Ten probes were specific for different single mtDNA genes: all, *atp6, cob, coxl,* cox2 (exon 1), *cox3, nadl* (exon 1), *uad3, nad4* (exons 1 and 2 including intron 1), and *rps14. Two* probes were specific to different parts of *nad5:* one probe *(uad5a)* hybridized to exons 1 and 2, including intron 1, and the other *(nad5d)* hybridized to exons 4 and 5, including intron 4. One probe hybridized to the intergenic region between *read3* and rps12.

Probes were amplified using universal mtDNA-specific primers (Table 1) via the polymerase chain reaction (PCR). To design universal mtDNA-specific primers, we retrived and aligned as many genes of fungal, algal, and higher plant mtDNA sequences as were available from international DNA sequence databases, including GenBank, EMBL, DDBJ, and others. Among plant species, we used monocots, dicots, and gymnosperms when available. GeneRunner (version 3.04; Hastings Software, Inc.) was used for multiple alignment, oligonudeotide analysis, and primer design. Our primary criteria for choosing primer sites and sequences were as follows: (1) high conservation of amino acid sequences across all available organisms; (2) exact or nearly exact matches of DNA sequences across seed plants; (3) avoidance of sites of likely C-to-Tediting when possible; (4) nearly perfect matches for the last seven to eight nucleotides and no mismatches for the last four to

TABLE 1

Nucleotide sequence, name, melting temperature (T), GC content, and expected size of amplified PCR products for universal primers used to amplify mitochondrial genes

Gene and probe'	End	Name	Sequence	Size (bp)	G + C (%)	*Tm*	PCR product (bp)
alp]	5'	atpain5l	TTTGCCAGCGGTGT(G = I°)AAAGG`	20	55	67	1039
	3'	atpain32	CTTCGCCiATATTGTGCCAATTC	22	46	70	
atp6	5'	atp6in51	GGAGG(A = I)GGAAA(C = I)TCAGT(A = I)CCAA	22	48	60	604
	3'	atp6in31	TAGCATC:ATTCAAGTAAATACA	22	27	56	
cob	5'	cob-in52	AGTTATTGGTGGGGGTTCGG	20	55	68	350
	3'	cob-in33	CC:CCAAAAGCTCATCTGACCCC;	22	59	74	
coxl`'	5'	coxlin5l	GGTGCCATTGC(T = I)GGAGTGATGG`	22	59	70	1485
	3'	coxlin32	TGGAAGTTCTT(:AAAAGTATG'	21	33	57	
	5'	coxlin53	GGCT(G = I)TTCTCCAC(T = I)AACCACAA	22	50	66	1507
	3'	coxlin33	GGAGGGCTTTGTACCA(A = I)CCATTC	23	52	69	
cox2	5'	cox2in51	GATGC(A = I)GC(G = I)GAACC(A = I)TGGCA`	20	55	58	340
(exon 1)	3'	cox2in32	TCCGATACCATTGATGTCC;	19	47	53	
cox3	5'	cox3in51	GTAGATCCAAGTCCATGGCCT'°	21	52	65	692
	3'	cox3in31	GCAGCTGCTTCAAAGCC'	17	59	61	
nadl	5'	nadlin52	CTAGCTGAACGTAAAGTAATGGC	23	44	64	306
(exon 1)	3'	nadlin32	CCAACC(T = I)GCTATAAT(A = I)ATTCC	21	38	54	
nad3	5'	nad3in51	AATTGTC;GGCCTACGAATC=TG'	21	48	67	215
	3'	nad3in31	TTCATAGAGAAATCCAATCGT	21	33	58	
nad3-	5'	nad3in51	AATTGTCGGCCTAGGAATGTG'	21	48	67	^370
rl)s12	3'	rps12o51	GCTCG(A = I)GTACGGTC(G = I)GTGGG	20	65	62	
nad4a	5'	nad4ai52	ATACGATTGATTCGGTC:TGTG (exon 1)	20	40	57	^•1500
(intron 1)	3'	nad4ai32	TGAACTGGTACCATAGGCACTTT (exon 2)	23	46	64	-2000
nad5a	5'	nad5in51	GAAATC=TTTGATGCTTCTTGGG (exon 1)	22	41	66	N1000
(intron 1)	3'	nad5in31	ACCAACATTGGCATAAAAAAAGT (exon 2)	23	30	64	
nad5d	5'	nad5in54	ATAAGTCAACTTCAAAGTGGA (exon 4)	21	33	56	^•1000
(intron 4)	3'	nad5in34	CATTGCAAAGGCATAATGATi (exon 5)	20	35	61	
rp.sl4	5'	rpsl4i51	ATACGAGATCACAAACGTAGA`	21	38	56	282
	3'	rps14i31	CCAAGACGATTT(C = I)TTTATGCC	21	38	61	

" atpl (or *atpA),* F,-adenosine triphosphatase (ATPase) subunit 1 (alpha) gene; *atp6* (or *atpF),* F,,-ATPase subunit 6 gene; *cob,* apocytochrome *b* gene; coxl (or coxl), cytochrome r oxidase subunit 1 gene; *cox2* (or coxll), cytochrome c oxidase subunit 2 gene; cox3 (or coxlll), eytochrome coxidase subunit3 gene; *nadl* (or *nadA, ndhA, ndhl,* or *nd1),* NADH-ubiquinone oxidoreductase subunit 1 gene; *nad3* (or *rcadC, rcdhC, ndh3,* or *nd3),* NADH-ubiquinone oxidoreductase subunit 3 gene; *nad4* (or *nadD, ndhD, ndh4,* or *nd4),* NADH-ubiquinone oxidoreductase subunit 4 gene; *nad5* (or *nadF; ndhl; ndh5,* or *nd5),* NADH-ubiquinone oxidoreductase subunit 5 gene; *rps12,* ribosomal protein subunit 12 gene; *rp.sl4,* ribosomal protein subunit 14 gene.

Inosine was used in the syntheses instead of the corresponding nucleotide because of the nucleotide variation observed among plant sequences.

' Although unstable can form hairpins.

'Any of the four possible combinations-coxlin5l/coxlin32, coxlin5l/coxlin33, coxlin53/coxlin32, or coxlin53/coxlin33 can be used to produce practically identical coil-specific probes, but the coxlin5l/coxlin32 combination was used to obtain data.

° Overlaps with the primers designed by HnEar:L *et al.* (1994).

1 "Hot" PCR start is recommended because of a possibly stable hairpin.

five nucleotides in the primers' 3' ends; (5) avoidance of amino acids with highly degenerate codons, and preference of those encoding unique and low-degeneracy codons; (6) avoidance of internal repeats, hairpins, internal loops, and dimers; (7) selection of primers with a relatively high melting temperature, usually not less than 55°, and a high G:C ratio; and (8) no significant homologies to cpDNA sequences based on database searches. For these cpDNA homology searches, we used all published cpDNA genome sequences, including liverwort *Marchantia polymorpha,* maize *Zea mat's,* rice *Oryza sativa,* tobacco *Nicotiana tabacum,* and black pine *Pinus thunber*gzi, using the Organelle Genome Database (GOBASE: http://megasun.bch.umontreal.ca/gobase/content.html/).

Primers were synthesized in the Central Service Laboratory of the OSU Center for Gene Research and Biotechnology using the ABI 380B or 394 DNA synthesizers (Perkin Elmer Applied Biosystems Division, Foster City, CA). To test synthesized primers, we used DNA samples from a large variety of plant species and from enriched cpDNA and mtDNA samples provided by V. Hipkins and J. Aagaard (Aagaard *et al.* 1998), respectively. Primers amplified expected mtDNA fragments in almost all species tested (unpublished data). PCR products amplified from CCCP DNA samples were recovered from a 2% agarose gel under long-wave UV light, purified using the QIAquick gel extraction kit (Qiagen Inc., Chatsworth, CA) or GENECLEAN kit (BIO101 Inc., La Jolla, CA), and radioac

lively labeled with ^{32}P by primer extension using a random hexamer labeling kit (Boehringer Mannheim GmbH, Mannheim, Germany).

RFLP procedures: Total genomic DNA was extracted from needles using a CTAB-based DNA extraction protocol (WAGNER *et al.* 1987) followed by three phenol/ chloroform purifications and a final ethanol precipitation. Modifications of this protocol and procedures for restriction enzyme digestion, agarose electrophoresis, Southern blotting, hybridization, and washing and stripping of blots were as described in STRAUSS and DOERKSEN (1990) and HONG (1991). However, we added three high-stringency final washes (0.1 X SSC and 1% SDS solutions at 65°).

Preliminary detection of polymorphisms: For a preliminary survey, two trees were randomly chosen from each of the 13 study populations. Thirteen mtDNA probes and two restriction enzymes (BamHI and XbaI) previously identified as showing high polymorphism (STRAUSS *et al.* 1993) were used for the detection of mtDNA polymorphisms. Only those probeenzyme combinations that detected polymorphisms either within or between species in this preliminary survey, as well as those that did not give information redundant with other probes (see below), were retained for the full analysis of all 343 sampled trees *(atp6, cox1, cox2, nad3, nad4, nad5a, nad5d,* and *rps14).*

Data analysis: *Haplotype analysis:* Haplotypes were determined based on having unique restriction fragment patterns over the various combinations of restriction enzymes and probes. Haplotype frequency (where haplotypes are treated as alleles at a single genetic locus) in each population was used to estimate genetic diversity and population differentiation. Genetic diversity and NEi's (1986) population differentiation (G_{ST}) adjusted for sample size and population number were calculated using the GeneStat PC 3.3 program (LEWIS 1994). WEIR and COCKERHAM'S (1984) 0 value for population subdivision and the standard deviation derived by jackknifing over populations were calculated from individual haplotypes using the Genetic Data Analysis (GDA) program (LEWIS and ZAYKIN 1996) .

Probe-enzyme-based multilocus analysis: To better understand diversity in different parts of the mtDNA genome, we analyzed the data where each probe-enzyme combination was considered as a genetic locus and each restriction fragment profile variant was considered as an allele. Allele frequencies at each locus in each population were then used to estimate the genetic diversity parameters and NEI's (1986) GST using the GeneStat-PC 3.3 program. To give a more accurate estimate of gene diversity than the inflated value that would be obtained if only polymorphic combinations were used (see DISCUSSION), probe-enzyme combinations monomorphic in the preliminary sample were assumed to be monomorphic in all trees.

Analysis of molecular variance (AMOVA): AMOVA was used to partition molecular variance into different hierarchical levels. Each tree was scored by a vector of ones (presence of a band) and zeros (absence of a band) representing the components of their multibanded RFLP phenotypes. The proportion of shared fragments was calculated for each possible pairwise comparison according to NEI and Li (1985): S (similarity) = $2N_{AB}/(N_A + N_B)$, where NN, is the number of bands shared by individuals A and B; NA and NB are the number of bands in individuals A and B, respectively. The distance index was D = 1 - S. All similarity and distance indices were obtained using the RAPDPLOT program (BLACK 1996). An AMOVA was then performed on the resultant matrix for partitioning the total RFLP variation into the within- and among-group variance components and for producing (D-statistics, which are analogous to F-statistics. The significance of the variance component was computed using a nonparametric permutation test (Excoffier *et al.* 1992).

Phylogenetic analysis: To test the usefulness of mtDNA as a phylogenetic marker, pairwise Manhattan distances (Prevosti distance in WRIGHT 19'78) between populations were computed on the basis of the fragment pattern phenotypic similarities between haplotypes (presence or absence of a band as described above). A distance matrix, or a set of matrices via bootstrapping, was generated using the RAPDDIST program (BLACK 1996). The matrices were then subjected to the NJTREE and CONSENSE programs in the PHYLIP package (FELSENSTFIN 1995) to produce a neighbor joining consensus tree indicating the phylogenetic relationships between populations and species, and the results were compared to phylogenies based on other data.

RESULTS

Diversity: Of the 13 probes tested, three probes *(atpl,* cob, and *cox3)* failed to reveal any polymorphism, regardless of the restriction enzymes used. Four probes *(nad3, nad3-rps12, nad4,* and *nad5a)* detected polymorphism with only *Xbal,* whereas another 3 probes *(atp6, nadl,* and *rp.s14)* exhibited polymorphism with only *BamHI.* The other 3 probes (c*oxl, cox2,* and *nad5d)* showed polymorphism with both enzymes (e.g., Figure 2). However, 2 pairs of probes (r*cad3* and *nad3-rpsl2; uadl* and *rpsl4)* detected identical mtDNA RFLP patterns for the screened individuals. Thus, only one gene from each pair (e.g., *nad3* and *r(zs14)* was used in the full analysis. In sum, 22 probe-enzyme combinations were used to generate our data.

Restriction fragment and haplotype polymorphisms: The 22 probe-enzyme combinations produced a total of 76 scored fragments (detailed data on restriction fragment phenotypes are available at http://www.fsl.orst.edu/tgerc/: "Protocols/Laboratory Data"). The number of fragments produced per combination varied from 1 to 5 for each haplotype, suggesting that several genes have multiple copies in many of the populations. The multiple fragments usually had identical relative hybridization intensities among individuals, which would result from simple duplications and deletions.

A total of 28 haplotypes were identified on the basis of RFLP patterns of all probe-enzyme combinations. The number of fragments for each haplotype ranged from 25 to 38 out of a total of 76 fragments. Only 13 (22%) of the fragments were present among all haplotypes. Sixteen (27%) of the fragments were unique to a single haplotype, and 60 fragments (73%) were shared by 2 or more haplotypes. There were 6 haplotypes for Monterey pine, 11 for knobcone pine, and 11 for bishop pine. There was no haplotype common among any of the populations from the different species (Table 2).

Genetic diversity within populations: Eight population samples had two or more haplotypes, and five samples had only a single haplotype. The frequency of common haplotypes in the polymorphic population samples varied from 52 to 96% (Table 2). The Oakland population of knobcone pine and the Santa Cruz population of bishop pine each contained five different haplotypes,

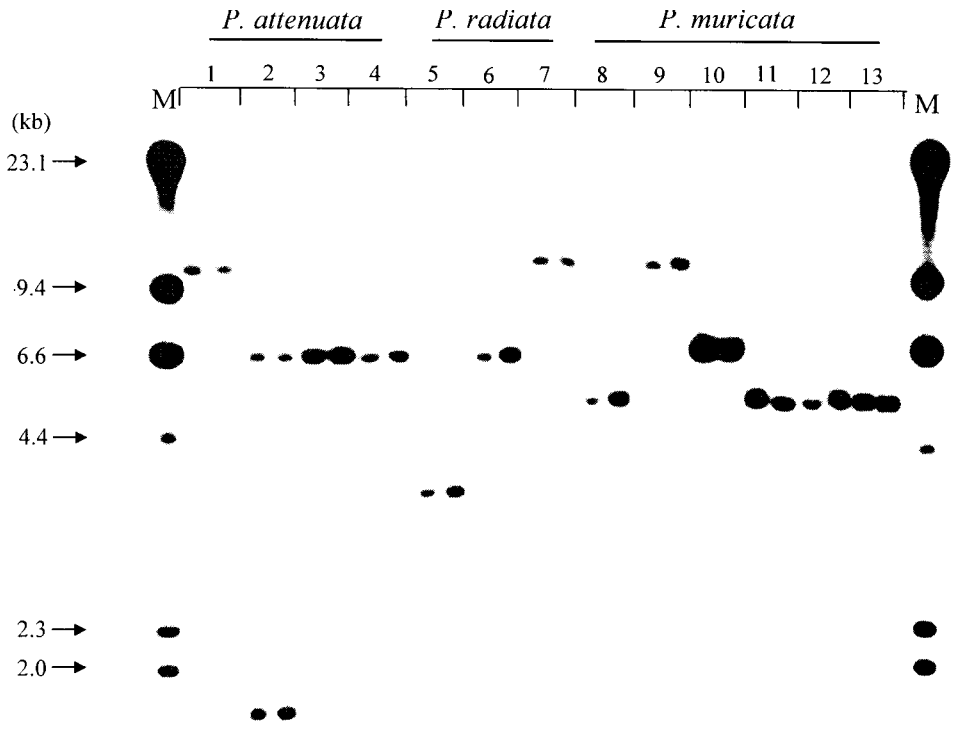

FIGURE 2.-An example of autoradiograms showing interspecific and interpopulation mtDNA diversity using two different individual trees per population. DNA was digested with BamHI and probed with the cox2 gene. M, DNA size markers (lambda/HindIII digest DNA). Lanes 1-4 represent *P. attenuates (1,* Santa Ana; 2, Sierra Nevada; 3, Oakland; 4, Klamath). Lanes 5-7 represent P. radiates (5, Ano Nuevo; 6, Cambria; 7, Guadalupe). Lanes 8-13 represent P. muricata (8, Mendocino; 9, San Vicente; 10, Santa Cruz; 11, Marin; 12, Monterey; 13, Trinidad).

while four haplotypes each were detected for the Cambria population of Monterey pine and the Sierra Nevada population of bishop pine.

On average, the gene diversity within populations based on haplotype frequencies was 0.22 (Table 3), ranging from 0.21 to 0.23 between species. As expected, diversity was substantially lower (0.03; Table 4) when each probe-enzyme combination was considered as a genetic locus and each fragment profile variant was designated as an allele; averaged over populations, the number of effective alleles per locus was only slightly higher than one. The percentage of polymorphic loci ranged from a relatively high value of 22.7% in *P. attenuate* to a low of 11.4% in *P. muricata*.

Levels of diversity differed greatly for some populations, depending on whether multilocus or haplotype analysis was used. For example, the Cambria and Mendocino populations each had two main haplotypes occurring in roughly equal frequencies, but the haplotypes differed because of fragment changes at four loci in the Cambria population and at only one locus in the Mendocino population (Table 2; data on web site). As a result, the haplotype diversity of the Mendocino population was similar to that of the Cumbria population

TABLE 2

Haplotype frequencies in sampled populations and number of trees assayed (*N*)

Species	Population	Haplotype frequencies	*N*
P. radiata	Año Nuevo	a: 1.0[a]	26
	Cambria	a: 0.04, b: 0.64, c: 0.08, d: 0.24	25
	Guadalupe	e: 0.96, f: 0.04	25
P. attenuata	Klamath	g: 1.0	25
	Sierra Nevada	h: 0.05, i: 0.85, j: 0.05, k: 0.05	22
	Oakland	l: 0.54, m: 0.04, n: 0.04, o: 0.34, p: 0.04	26
	Santa Ana	q: 1.0	23
P. muricata	Trinidad	r: 1.0	25
	Mendocino	s: 0.27, t: 0.73	29
	Marin	t: 0.48, u: 0.52	25
	Monterey	r: 0.92, v: 0.08	25
	Santa Cruz	w: 0.04, x: 0.04, y: 0.04, z: 0.04 aa: 0.84	24
	San Vicente	bb: 1.0	43

° Haplotype phenotypes are given in "Protocols/ Laboratory Data" at http://www.fsl.orst.edu/tgerc/.

TABLE 3

Estimates of population subdivision based on haplotype frequencies and 4)-statistics from AMOVA

Level of analysis	*Hs'*	PST'	Gsr`	e`'	")ST'	P/
Pooled populations	0.22 (0.06)	0.77	0.78	0.78 (0.07)	0.93	0.01
Species	0.78 (0.03)	0.22	0.22	0.21 (0.02)	0.26	0.22
Populations in species						
P. radiates	0.21 (0.17)	0.78	0.79	0.79 (0.14)	0.91	0.01
P. attenuates	0.21 (0.14)	0.79	0.79	0.78 (0.13)	0.90	0.01
P. muricata	0.23 (0.09)	0.69	0.75	0.77 (0.06)	0.95	0.01
RegionalX	0.60 (0.05)	0.30	0.34	0.35 (0.01)	0.66	0.33

° *H,* haplotype diversity within populations; standard errors in parentheses.
°*DsT,* haplotype diversity among populations (HI - Hs).
` C~T, Nrt's (1986) (;ST unbiased for sample size and population number.
8, subdivision estimate of WEIR and Cockerham (1984) with jackknife-derived standard deviation over populations.
A)s., = V(A)/ [V(A) + V(B)], where V(A) = variance among a hierarchical level (e.g., among populations), and V(B) = variance within a hierarchical level (e.g., within populations).
/ P, probability of obtaining a larger V(A) and *(DsT* by chance under permutation test.
A Regional analyses investigate the diversity among three groups of populations within *P. muricata*: Trinidad and Mendocino (northern), Marin and Monterey (intermediate), and Santa Cruz and San Vicente (south).

(0.41 us. 0.54), but its multilocus diversity was much lower (0.02 vs. 0.08, data not shown). AMOVA analysis showed very low molecular variance within populations (7% of total variance), comparable with our estimate of multilocus diversity (Table 4; Hs = 0.03).

Population differentiation: Haplotype frequencies differed substantially among populations (Table 2). With the exception of one individual of the Cambria population that showed the same haplotype as the Ano Nuevo population, every population of Monterey pine and knobcone pine had a distinctive haplotype. The southern populations of bishop pine, San Vicente and Santa Cruz, each had unique haplotypes. No haplotype was shared among species.

Based on haplotype frequencies, Nm's (1986) *C3T* and WEIR and Cockerham (1984) B values were very similar (Table 3) . Differentiation among populations within species was 0.79 for Monterey pine, 0.78-0.79 for knobcone pine, and 0.75-0.77 for bishop pine. Differentiation among species (0.21-0.22) and among northern, intermediate, and southern regions of bishop pine (0.34-0.35) were substantially lower than differentiation among populations in the total species complex (0.78) and in bishop pine as a whole (0.75-0.77). Thus, the strong population differentiation observed does not accumulate linearly at higher phyletic levels.

Gs,- was considerably higher when the probe-enzyme multilocus analysis was used. Population differentiation varied from 0.87 to 0.93 for three species. AMOVA analysis also demonstrated that the total mtDNA RFLP polymorphism was mainly attributed to the variance among populations within species (87.3% of total variance; P G

TABLE 4

Population genetic statistics based on 22 individual probe-enzyme combinations

Level of analysis	AH,P°	A91 *M*	*Hs'*	PST"	~ ` '
Pooled populations	1.04 (0.02)	16.78 (6.05)	0.03 (0.01)	0.31	0.91
Species	1.64 (0.05)	50.00 (0.00)	0.25 (0.06)	0.11	0.31
Populations in species					
1'. radiates	1.04 (0.04)	19.70 (15.38)	0.03 (0.01)	0.37	0.93
P. attenuates	1.06 (0.05)	22.73 (13.25)	0.04 (0.01)	0.25	0.87
P. muric:ata	1.04 (0.02)	11.36 (7.85)	0.02 (0.01)	0.23	0.91
Regional	1.27 (0.18)	22.73 (13.89)	0.09 (0.03)	0.17	0.65

° ASP, effective number of alleles per locus. Each restriction fragment profile variant was counted as an allele, and each probe-enzyme combination was counted as a genetic locus; standard errors in parentheses. 'Pg, = percentage of loci polymorphic, where the frequency of the most common allele was <0.99. ` Hs, unbiased average gene diversity within populations. 'Ds,, unbiased gene diversity among populations. e GsT, NFt's (1986) Gs,, unbiased for sample size and population number. I Regional groups defined in Table 2.

0.01). Variance among species and within populations each accounted for <7% of total variance. The (D value (AT analog), like (~,, and 0, can be interpreted as the fraction of among group variance compared to the total amount ofvariance in the reference group. The (1) values for populations were all >0.90 within the three species (Table 3). Although the (D values among species and among regions of bishop pine appeared to be high (0.22 and 0.66, respectively), they were not statistically significant (P > 0.20).

Phylogenetic analysis: The neighbor joining phylogenetic tree indicated that mitochondrial genomes representing the species and populations were often polyphyletic (Figure 3). The phenogram topology had three main clusters. One cluster (at bottom) contained four populations from two species and had low bootstrapping support. The four northern populations of bishop pine were grouped into a second paraphyletic cluster with strong bootstrapping support (98%). The third cluster (at the top) included populations from all three species, yet it had very high bootstrapping support (100%).

DISCUSSION

Diversity: We used 22 probe-enzyme combinations (11 independent probes and 2 enzymes) to detect mtDNA RFLP polymorphisms in 343 individuals from 13 populations. This appears to be the most intensive genome sample used in plant population genetic studies of mtDNA to date, providing a window on polymorphism and microevolution of the entire genome. By contrast, BELHASSEN *et al.* (1993) used one heterologous probe in their study of 52 individuals from 3 populations *of Thymus vulgaris.* By hybridization with 2 probes, Dong and WAGNER (1993) surveyed 741 individuals from 16 allopatric populations of *P. banksiana* and *P. contorta.* STttAUSS *et al.* (1993) examined RFLP polymorphisms associated with one gene sequence in 268 trees derived from 19 CCCP populations. HONG *et at.* (1995) applied 3 gene probes when analyzing 72 trees from 18 populations of Douglas fir.

Based on our preliminary survey, probes *nad3* and *nad3-rpsl2, nadl* and *rpsl~* provided identical mtDNA polymorphisms for both enzymes, confirming that the *nad3* and *rps12* genes and the *nadl* and *rpsl4* genes are very closely located in the pine mitochondrial genome, similarly to angiosperms (PEBBOTTA *et al.* 1996). Thus, only 22 out of 26 probe-enzyme combinations were used in our studies. Eleven of these 22 combinations detected intraspecific and/or interspecific polymorphism and were used for the full survey of all collected samples. Two of the remaining 11 monomorphic combinations were used in an extended study (100 trees), but no additional polymorphism was detected; therefore, these 11 combinations were not studied further. Because of the strong population differentiation for mtDNA, it is unlikely that significant additional polymorphisms existed that were missed by the preliminary surveys.

The level of mtDNA haplotype diversity is often high in plants. Similar to our results, total gene diversity ranges from 0.68 for lodgepole pine (*P. contorta* Dougl., DONG and WAGNER 1993) to 0.78 for Douglas fir *[Pseudotsuga menziesii* (Mirb) Franco, HONG *et al.* 1995]. STRAUSS *et al.* (1993) found earlier that intrapopulation haplotype diversities over 19 CCCP populations averaged only 0.07 for mtDNA, less than half of that for allozymes and approximately one-third of the present estimate of 0.22. However, only a single gene probe was used and, thus, only a small portion of the mtDNA genome was surveyed in that study. In contrast, we used 11 probes from 11 different genes, allowing us to resolve 28 haplotypes in 13 CCCP populations, while only 9 haplotypes were detected in 19 CCCP populations studied earlier (Strauss *et al.* 1993). Haplotype diversity within populations of lodgepole pine (0.21) is very similar to that for the CCCP, although it is very low for jack pine (*P. banksiana* Lamb.) (0.03) when a species-hybrid population was excluded (DONG and WAGNER 1993). In Douglas fir, the mtDNA genetic diversity within populations is 0.33, which is higher than the values in most other species (HONG *et al.* 1995).

Although mtDNA has the lowest sequence mutation rate among the three plant genomes, its presumed high rate of structural rearrangement is likely to be the cause of its high level of diversity. All the polymorphisms that we detected appeared to result from structural rearrangements, particularly large duplications and deletions, rather than point mutations. This is in agreement both with results from previous studies in CCCP (Strauss *et al.* 1993), jack, and lodgepole pines (Dong and WAGNER 1993), as well as Douglas fir (HONG *et* al. 1995), and with most observations of plant mtDNA polymorphisms (PALMER 1990, 1992a).

Differentiation: More than three-quarters of mtDNA diversity was distributed among populations in all three species, in contrast to the low population differentiation typical in nuclear genes of long-lived woody species *(Gsr = 0.10;* HAMRICK and GovT 1990) and allozyme studies for the CCCP (G*sr = 0*.12-0.22; MILLAR *et* al. 1988). The few other surveys of mtDNA polymorphisms have also found high levels of population differentiation. For lodgepole pine, *Fsr = 0*.31 among subspecies and was up to 0.82 among populations within subspecies (DONG and WAGNER 1993), whereas *Fs,* at a single allozyme locus was rarely larger than 0.06 in this species (WHEELER and GUBIES 1982). Of the two organelle genomes and the nuclear genome in Douglas fir, mtDNA also showed the highest degree of genetic differentiation (G*sr)* among populations (0.45) and geographic regions (0.29), while differentiation among populations was 0.20 for cpDNA and 0.14 for nuclear RAPD markers (HONG *et* al. 1995). Mitochondrial coxl-associated

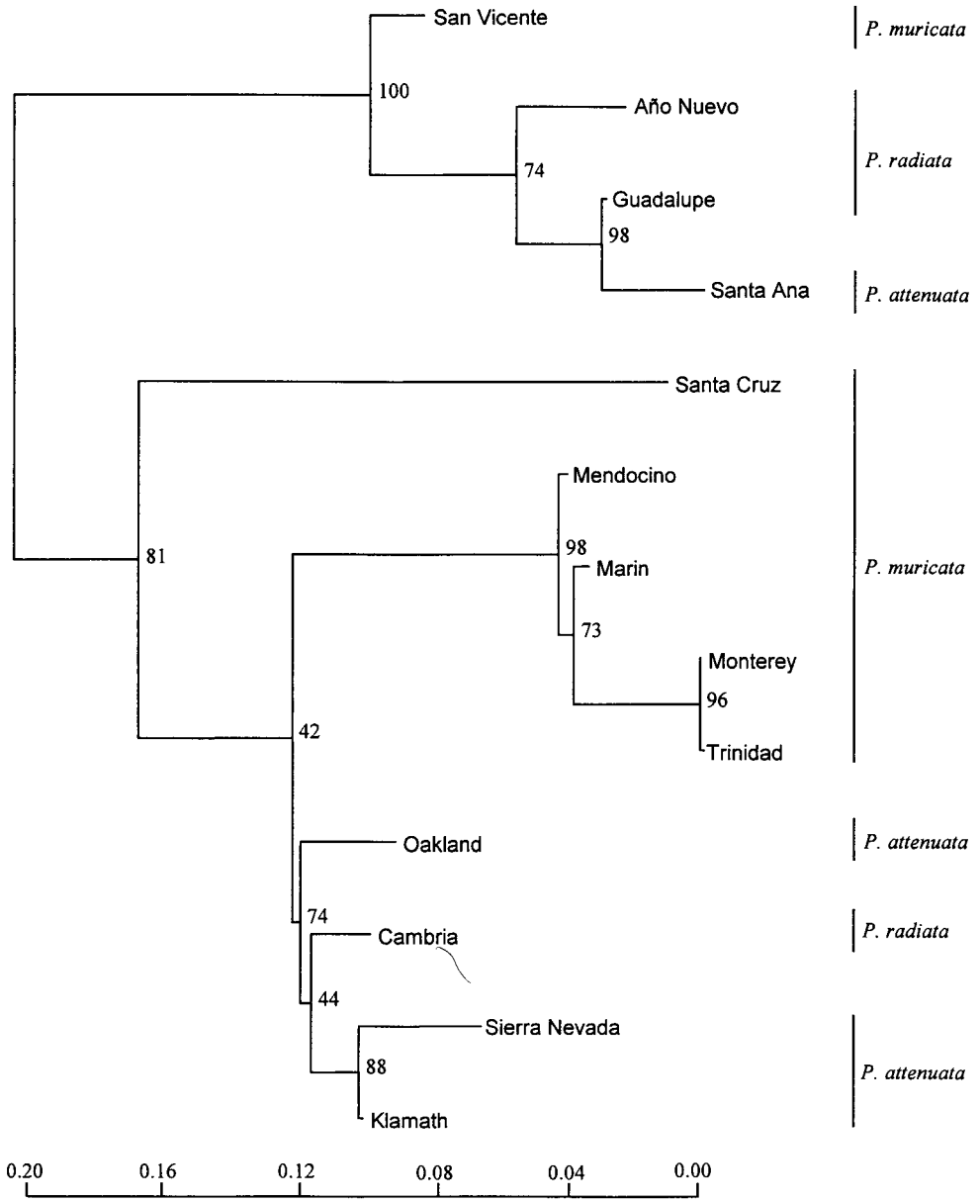

FIGURE 3.—Neighbor-joining phenogram derived from Manhattan distances on the basis of mtDNA phenotypes. The tree was rooted at midpoint between the pair of taxa with the greatest patristic distance. The numbers to the right of relevant nodes are the percentages of 200 bootstrap replicates.

values were also as high as 0.88 in the previous study of mtDNA variation in the CCCP (STRAUSS *et al.* 1993).

Maternally inherited cytoplasmic polymorphisms in plants are expected to exhibit greater population differentiation at equilibrium than nuclear polymorphisms. This is because of the influence of maternal inheritance on both gene flow and effective population size, and it is a consequence of the lower effective population size of haploid vs. diploid genomes (BIRKY 1988; PETIT *et al.* 1993a) . As a result, maternally inherited cpDNA, like mtDNA, also can show strong population subdivision. PETIT *et al.* (1993b) reported that >85% of cpDNA diversity resided among populations within the Quercus species, while the value for allozymes was <5% (KREMER and PETIT 1993). Similar results have been obtained in *Eucalyptus nitens* (BYRNE and MORAN 1994), where the majority of cpDNA variation was distributed among populations as a result of population isolation and genetic drift (*NSr* and CST = 0.78).

However, cpDNA in conifers shows predominant paternal inheritance (NEALE and SEDEBOFF 1989; WAGNER *et al.* 1992), which allows cpDNA to migrate through both seeds and pollen. Consistent with drift-migration equilibrium predictions for paternally inherited markers, population differentiation for cpDNA in Douglas fir is less than half of that for maternally inherited mtDNA (HONG *et al.* 1995). In bishop pine, however, mtDNA subdivision is similar to that for cpDNA restriction site mutations, where strong differences among populations (Gs1 >8'7%) were observed (HONG *et al.* 1993a). However, most bishop pine populations are geographically and reproductively isolated (CRITCHFIELD and LITTLE 1966; MILLAR and CRITGHFIELD 1988), and gene flow among populations is likely to be infrequent. As a result,

the proportion of allozyme variation attributable to population differentiation in bishop pine (22%) is also much greater than what is typical for other conifers. The low effective population size of haploid organelle genomes, as well as the possibility of periodic selection (BIRKY 1988; MARUYAMA and BIBKY 1991), could also contribute to the high subdivision of organelle DNA compared with nuclear gene markers.

Phylogenetic relationships: Despite high haplotype differentiation, genetic distances between populations were often low. Although nearly every population had unique haplotypes, most of the fragments were shared by other populations, including those of the other species. For example, the Guadalupe population of Monterey pine and the San Vicente population of bishop pine shared no haplotypes, yet they had 24 fragments in common out of 31 total fragments; their genetic distance was only 0.06 (data not shown).

The phylogenetic trees based on our mtDNA analyses roughly agree with those reported in the mtDNA study of STRAUSS *et al.* (1993), but both disagree strongly with those based on morphology, allozymes, and RAPDs. Allozymes have strongly confirmed the monophylly of the three species (MILLAR *et al.* 1988) and the close relationships of three mainland populations of Monterey pine. Allozymes, terpenes (MIROV *et al.* 1966), and cpDNA (HONG *et al.* 1993b) have recognized the strong divergence of the northern vs. southern populations of bishop pine. In contrast, our phylogenetic trees would suggest that the species are all polyphylefic. Similar results have been found in other studies of conifers. DONG and WAGNER (1993) found that populations of lodgepole pine did not generally cluster by subspecies, discordant with traditional taxonomy. HONG *et al.* (1995) observed that Douglas fir populations in each of three geographic regions of British Columbia failed to cluster on the basis of geographic affinity.

The complex nature of mtDNA evolution is probably the cause of its poor performance as a phylogenetic marker. The assumption that the presence or absence of a mtDNA fragment is caused by the same mutational event, and that the phenotypes reflect the underlying mutational events in mtDNA, is likely violated. RFLP polymorphisms of plant mtDNA are mostly length mutations and complex rearrangements rather than site mutations. It is therefore difficult to infer the evolutionary homology among different haplotypes (or fragments) because the complex and overlapping nature of structural changes (PALMER 1992a) cause apparent homoplasies and convergent evolution. For example, the Guadalupe population of Monterey pine, the Santa Ana population of knobcone pine, and the San Vicente population of bishop pine shared most of their restriction fragments (phenotypes of e, q, and bb haplotypes; data at web site), although they are widely separated geographically. We hoped that because of our large sample of the genome, we might be able to "average over" individual homoplasious rearrangements reported earlier (STRAUSS *et al.* 1993); however, this clearly was not the case.

The high frequency of convergent evolution is likely to be associated with the repetitive nature of mtDNA. mtDNA structural rearrangements are associated with recombination among major repeat elements (PALMER 1992a); if there are a finite number of genome sections that recombine in predictable ways across these hotspots, then similar genome structures could evolve repeatedly (STRAUSS *et al.* 1993).

Although mtDNA rearrangements do not appear to be of value for phylogenetic interpretations in pines, they may be of use in other taxa for grouping closely related genomes (PALMER 1992a). For example, most of 345 rubber *(Hevea brasiliensis)* accessions can be grouped according to their geographical distributions and hydrographical origin (Luo *et al.* 1995). DEU *et al.* (1995) also successfully used mtDNA to cluster several races in wild and cultivated sorghum *(Sorghum bicolor ssp. arundinaceum* and *S. bicolor ssp. bicolor).* The value of mtDNA for phylogenetic inferences is likely to vary widely, depending on genome size, repetitive structure, and, thus, modes of evolutionary rearrangement.

We thank Tony Gario for sampling the San Vicente population of bishop pine, Jan Aagaard and Nathan Strauss for their help in field collections, Bill Libby for his advice and help in accessing the study populations, Steve DiFazio for help with editing the manuscript, and the National Science Foundation (NSF Conservation and Restoration Biology, DEB-9300083) for grant support.

LITERATURE CITED

AAGAARD, J. E., K. V. KRUTOVSKII and S. H. STRAUSS, 1998 RAPD markers of mitochondria) origin exhibit lower population diversity and higher differentiation than RAPDs of nuclear origin in Douglas fir. Mol. Ecol. 7: 801-812.

BELHASSEN, E., A. A'T'LAN, D. COUVETI', P. H. GOUYON and F. QUETIER, 1993 Mitochondria) genome of *Thymus vulgaris L. is* highly polymorphic between and among natural populations. Heredity 71: 462-472.

BIRKY, C. W., JR., 1988 Evolution and variation in plant chloroplast and mitochondria) genomes, pp. 23-53 in *Plant* Evolutionary *Biol*ogy, edited by L. D. GOTTLIEB and S. K. JAIN. Chapman & Hall, London.

BLACK, W. C., IV, 1996 RAPDDIST 1.0, RAPDFST 4.0, RAPDPLOT 2.4. Department of Microbiology, Colorado State University, Fort Collins, CO.

BROWN, A. H. D., and D. J. SCHOEN, 1992 Plant population genetic structure and genetic conservation, pp. 88-104 in *Biodiversity for Sustainable Development,* edited by O. T. SANDLUND, K. HINDAR and A. H. D. BROWN. Scandinavian University Press, Oslo.

BYRNE, M., and G. F. MORAN, 1994 Population divergence in the chloroplast genome of *Eucalyptus niten.r.* Heredity 73: 18-28.

CRITCHFEILD, W. B., and E. L. LITTLE, 1966 *Geographic Distribution, of Pines of the World.* USDA Misc. Publ. 991.

DEFT, M., P. HAMON, P. DUFOUR, A. D'HONT and C. LANAUD, 1995 Mitochondria) DNA diversity in wild and cultivated sorghum. Genome 38: 635-645.

DONG, J., and D. B. WAGNER, 1993 Taxonomic and population differentiation of mitochondria) diversity in *Pinus bauksianrc* and *Pinus contorta.* Theor. Appl. Genet. 86: 573-578.

DONG, J., and D. B. WAGNER, 1994 Paternally inherited chloroplast polymorphism in *Pinus:* estimation of diversity and population

subdivision, and tests of disequilibrium with a maternally inherited mitochondria) polymorphism. Genetics 136: 1187-1194.

DUMOLIN-LAYEGUK, S., B. DEMESURE, S. FINESCHI, V. LE COKRr, and R. J. PETIT, 1997 Phylogenetic structure of white oaks throughout the European continent. Genetics 146: 1475-1487.

EXCOFFIER, L., P. E. SMOUSE and J. M. QUATTRO, 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondria) DNA restriction data. Genetics 131: 479-491.

FELSENSTEIN, J., 7995 PHYLIP (Phylogeny Inference Package) version 3.57c. Department of Genetics, University of Washington, Seattle, WA.

FURMAN, B. J., W. S.,DVORAK, R. R. SEDEROFF and D. M. O'MALLKY, 1996 Mole~ar markers as diagnostic tools to identify species, hybrids and mtrogression: a study ofC:entral American and Mexican pines, pp. 485-491 in *Tree Improvement for Sustainable Tropical FOreStly,* wilted by M. J. DIETERS, A. C. MA'1'tIESON, D. G. NIKLKS, C. E. HnRWOOD and S. M. WALKER. Proceedings of the OFRIIUFRO Conference, Caloundra, Queensland, Australia.

GRArIAU, E. A., W. H. DAMS, N. D. PHr:Lrs and B. G. GENGENBACH, 1992 Classification of soybean culfivars based on mitochondria) DNA restriction fragment length polymorphisms. Crop Sci. 32: 271-274.

HAMRICK, _J. L., and M. J. W. GonT, 1990 Allozyme diversity in plant species, pp. 43-63 in *Plant Population Genetics, Breeding and Genetic Resources* edited by A. H. D. BROWN, M. T. CLEGG, A. L. KAHLr:R and B. S. WEIR. Sinauer Associates, Sunderland, MA.

HIESEL, R., B. COMBETTES and A. BRENNICKE, 1994 Evidence for RNA editing in mitochondria of all major groups of land plants except the Bryophyte. Yroc. Natl. Aced. Sci. USA 91: 629-633.

HII'KLNS, V. D., K. V. KRUTOVSKn and S. H. S~rxnuss, 1994 Organelle genomes in conifers: structure, evolution, and diversity. Forest Genet. 4: 179-189.

HONG, Y. P., 1991 Chloroplast DNA variability and phylogeny in the California closed-cone pines, Ph.D. thesis, Oregon State University, Corvallis, OR.

HONG, Y. P., V. D. HIYKINS and S. H. STRAUSS, 1993a Chloroplast DNA diversity among trees, populations and species in the California closed-cone pines. Genetics 135: 1187-1196.

HONG, Y. P., A. B. KRUPKIN and S. H. STRAUSS, 1993b Chloroplast DNA transgresses species boundaries and evolves at variable rates in the California closed-cone pines *(Pines radiate, P. muricata,* and P. *attenuate).* Mol. Phylog. Evol. 2: 322-329.

HONG, Y. P., B. PONOY and J. E. CARLSON, 1995 Genetic diversity and phylogeny in Douglas-fir based on RFLP and RAPD (DAF) analysis of nuclear, chloroplast, and mitochondria) genomes, pp. 247-266 in *Population Genetics arid Genetic Conservation of Forest Trees,* edited by PH. BARAnA r, W. T. ADAMS and G. MLJLLERSrnuc:K. SYB Academic Publishing, Amsterdam.

KREMER, A., and R._J. Pr°rrr, 1993 Gene diversity in natural populations of oak species. Ann. Sci. Forest. 50 (Suppl. 1): 186s-202s.

LEWIS, P. O., 1994 (ieneStat-PC 3.3. Department of Statistics, North Carolina State University, Raleigh, NC.

LEWIS, P. O., and D. ZAYKrN, 1996 Genetic Data Analysis (GDA). Department of Statistics, North Carolina State University, Raleigh, NC.

Luo, H., B. V. COI'PKNOr,LE, M. SEQUIN and M. Bou rrxv, 7995 Mitochondria) DNA polymorphism and phylogenetic relationships in *Hevea brasiliensis.* Mol. Breed. 1: 51-63.

MARUYAMA, T., and C. W. BIRKY, JR., 1991 Effect of periodic selection on gene diversity in organelle genomes and other systems without recombination. Genetics 127: 449-451.

McCAULr:Y, D. E., J. E. 3rr:vr:NS, P. A. Pr:RONI and J. A. RAVr:It.L, 7996 The spatial distribution of chloroplast DNA and allozyme polymorphism within a population of *Silene alba* (Caryophyllaceae). Am. J. Bet. 83: 727-731.

MILLAR, C. L, 1986 The California Closed-Cone Pines (Subsection *Oocarpae* Little and Critchfield): a taxonomic history and review. Taxon 35: 657-670.

MILLAR, C. L, and W. B. CRITCHEn:LI), 1988 Crossability and relationships of bishop pine. Madrono 35(1): 39-53.

MILLAR, C. L, S. H. STRAUSS, M. T. CONKLE: and R. WESTFALL, 1988 Allozyme differentiation and biosystematics of the California Closed-Cone Pines. Syst. Bot. 13: 351-370.

MtROV, N. T., E. ZAVARIN, K. SNAJsERx and K. COSTELLO, 1966 Further studies of *Pines muricata* in relation to its taxonomy. Phytochemistry 5: 343-355.

NEALE, D. B., and R. R. Sr;OEROFE, 1989 Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondria) DNA in loblolly pine. Theor. Appl. Genet. 77: 212-216.

NEI, M., 1986 Definition and estimation of fixation indices. Evolution 40: 643-645.

NEI, M., and W. H. Lt, 1985 Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Aced. Sci. USA 76: 5269-5273.

OI,MSTEAD, R. G., and J. D. PALMER, 1994 Chloroplast DNA systematics: a review of methods and data analysis. Am. J. Bot. 81: 12051224.

PALMER, J. D., 1990 Contrasting modes and tempos of genomc evolution in land plant organelles. Trends Genet. 6: 115-120.

PALMER, J. D., 7992a Mitochondria) DNA in plant systematics: applications and limitations, pp. 36-39 in *Molecular-Systematics* of *Plants,* edited by P. S. Sourrs, D. E. SOLTIS and J.,J. DOYLE. Chapman & Hall, London.

PALMER, J. D., 1992b Comparison of chloroplast and mitochondria) genome evolution in plants, pp. 100-133 in Cell *Organelles,* edited by R. G. HERRMANN. Springer-Verlag, New York.

PERRO'I'TA, G., T. M. R. REGINA, I,. R. CECI and C:. QUAGLIARIELLO, 1996 Conservation of the organization of the mitochondria) *nad3* and rpS12 genes in evolutionary distant angiosperms. Mot. Gen. Genet. 251: 326-337.

PETIT, R. J., A. KREMER and D. B. WAGNER, 1993a Finite island model for organelle and nuclear genes in plants. Heredity 71: 630-641.

PETIT, R. J., A. KREMER and D. B. WAGNER, 1993b Geographic structure of chloroplast DNA polymorphisms in Europe oaks. Theor. Appl. Genet. 87: 122-128.

PRING, D. R., and D. M. LONSDALE, 1985 Molecular biology of higher plant mitochondria) DNA. lot. Rev. Cytol. 97: 1-46.

SEDEROFF, R. R., 1987 Molecular mechanisms of mitochondria) genome evolution in higher plants. Am. Nat. 130: S30-S45.

STRAUSS, S. H., and A. H. DOERKSEN, 1990 Restriction fragment analysis of pine phylogeny. Evolution 44: 1081-1096.

STRAUSS, S. H., Y. P. HONG and V. D. HIPKINS, 1993 High levels of population differentiation for mitochondria) DNA haplotypes in *Pines radiates, muricata,* and *atlenuala.* Theor. Appl. Genet. 86: 605-611.

WAGNER, D. B., G. R. FURNII?K, M. A. SAGHAI-MAROOE, S. M. WILLIAMS, B. P. DANCIK *et al.,* 1987 Chloroplast DNA polymorphisms in lodgepole pines and their hybrids. Proc. Natl. Aced. Sci. USA 84: 2097-2100.

WAGNER, D. B., W. L. NANCE, C. D. NELSON, T. LI, N. PATEL *et al.,* 1992 Taxonomic pattern and inheritance of chloroplast DNA variation in a survey of Pines *eclainata, P. elliottii, P. palastri.s* and *P. taeda.* Can. J. Forest Res. 22: 683-689.

WEIR, B. S., and C. C. COCKERHAM, 1984 Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

WHEELER, N. C., and R. P. GURIES, 1982 Population structure, genie diversity, and morphological variation in *Pinus contorta* Doug]. Can. J. Forest Res. 12: 595-606.

WOLFE, K. H., W. H. Lt and P. M. SHARP, 1987 Rates of nucleotide substitution vary greatly among plant mitochondria), chloroplast, and nuclear DNAs. Proc. Natl. Aced. Sci. USA 84: 9054-9058.

WRIGHT, S., 1978 Evolution and genetics of populations, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

Communicating editor: A. H. D. BROWN