Paternally Inherited Chloroplast Polymorphism in Pinus: Estimation of Diversity and Population Subdivision, and Tests of Disequilibrium With a Maternally Inherited Mitochondrial Polymorphism

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ABSTRACT

We have surveyed a chloroplast DNA restriction fragment length polymorphism in 745 individuals, distributed rangewide in eight allopatric natural populations of jack pine (*Pinus banksiana* Lamb.) and eight allopatric natural populations of lodgepole pine (*Pinus contorta* Dougl.). The polymorphic region of the chloroplast genome is located near duplicated *psbA* genes. Fourteen length variants were found in the survey, and these variants distinguished the two species qualitatively. Variant diversities were high in both species (h_{es} = 0.43 in jack pine; h_{es} = 0.44 in lodgepole pine). Population subdivision was weak within and among lodgepole pine subspecies and in jack pine (*i.e.*, θ values were less than 0.05). This weak subdivision is compatible with theoretical predictions for paternally inherited markers in wind-pollinated outcrossers, as well as for polymorphisms with high length mutation rates. If these populations are at a drift-migration equilibrium, the chloroplast DNA restriction fragment data and previous mitochondrial frequency data from the same individuals are consistent with gene flow that is differential through seeds and pollen. The new data have permitted the first empirical tests of disequilibrium between maternally and paternally inherited factors. As expected, these tests failed to detect convincing evidence of nonrandom association between chloroplast and mitochondrial variants.

AN understanding of the amounts, patterns and associations of genetic variation is a major goal of population genetics. Such knowledge is fundamental not only for tests of evolutionary hypotheses, but also for effective germplasm improvement and conservation strategies.

Plants, unlike animals, have three interdependent genomes which reside in nuclei, chloroplasts and mitochondria. Thus, plant population geneticists face the complexity that genetic variation occurs in as many as three, differently inherited, compartments.

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 populational differentiation at equilibrium than nuclear genes, as a consequence of (i) migration of maternally inherited organelles through seeds, but not pollen, and (ii) the diploid nuclear but haploid organellar composition of seeds (BIRKY 1988; PETIT *et al.* 1993a). In fact, maternally inherited chloroplast polymorphisms often do involve differences among populations [SOLTIS *et al.* (1992) and references therein]. Similarly, maternally inherited plant mitochondrial polymorphisms (DONG and WAGNER 1993; STRAUSS *et al.* 1993) have population subdivision statistics as high as 0.96.

Chloroplast DNA (cpDNA) in conifers and some other plants is paternally (or at least predominantly paternally) inherited (*e.g.*, SCHUMANN and HANCOCK 1989; BOBLENZ *et al.* 1990; WAGNER 1992). In contrast to maternally inherited loci, paternally inherited loci can migrate through both seeds and pollen [consider PROUT (1981)]. Consequently, population subdivision of neutral, paternally inherited factors is expected to be weaker than that of maternally inherited factors in outcrossing plants, when the mutation rate is lower than the migration rate, especially when pollen migrates more effectively than seeds (PETIT *et al.* 1993a). This expectation does not require that paternal inheritance be strict, but holds true even with considerable maternal leakage (PETIT *et al.* 1993a). Empirical population subdivision estimates are rare for paternally inherited polymorphisms, but subdivision can be substantial when migration is infrequent or absent (HONG *et al.* 1993; WANG and SZMIDT 1993).

Like population subdivision, genotypic associations among loci (disequilibria) may hinge on modes of inheritance. For example, nonrandom associations between paternally inherited and maternally inherited cytoplasmic genomes should disappear rapidly in outcrossing populations (SCHNABEL and ASMUSSEN 1989). Cytonuclear population genetic data, though uncommon in plants, have begun to address mitochondrial-nuclear and chloroplast-nuclear associations (PAIGE *et al.* 1991; SAGHAI MAROOF *et al.* 1992). However, the prediction of random

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TABLE 1

Sample site locations (abbreviations in parentheses), taxonomic classifications and sample sizes

Location[a]	Taxonomic classification	Sample size
Doaktown, New Brunswick (NB)	*P.b.*[b]	50
Lac Mathieu, Quebec (PQ)	*P.b.*	50
Chalk River, Ontario (ON-E)	*P.b.*	49
Wellston, Michigan (MI)	*P.b.*	47
Raith, Ontario (ON-W)	*P.b.*	43
Hadashville, Manitoba (MB)	*P.b.*	46
Canwood, Saskatchewan (SA)	*P.b.*	47
Bellis, Alberta (AB)	*P.b.*	48
Mackenzie, British Columbia (BC-N)	*P.c.l.*[c]	44
Prince George, British Columbia (BC-C)	*P.c.l.*	43
Lumby, British Columbia (BC-S)	*P.c.l.*	43
Ward, Colorado (CO)	*P.c.l.*	49
Prince Rupert, British Columbia (BC-W)	*P.c.c.*[d]	43
Waconda Beach, Oregon (OR-W)	*P.c.c.*	46
Santiam Pass, Oregon (OR-C)	*P.c.m.*[e]	47
Wrights Lake, California (CA)	*P.c.m.*	50

[a] Most locations are in Canada, except MI, CO, OR-W, OR-C, and CA, which are in the United States. Map locations are shown in DONG and WAGNER (1993).

[b] *P. banksiana.*

[c] *P. contorta* var. *latifolia.*

[d] *P. contorta* var. *contorta.*

[e] *P. contorta* var. *murrayana* [for a discussion of taxonomic classification of OR-C population, see WHEELER and GURIES (1982)].

association between paternally and maternally inherited factors has not been tested.

Here we study natural populations of jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*Pinus contorta* Dougl.) (*i*) to estimate population subdivision parameters of a paternally inherited cpDNA polymorphism and (*ii*) to test statistical associations of chloroplast and mitochondrial DNA variants. These two much studied North American pines (*Pinus* L.) are widely distributed, closely related, wind-pollinated outcrossers, and they hybridize naturally in sympatric regions of western Canada (CRITCHFIELD 1985). They comprise an unusually valuable genetic system because of their paternal chloroplast, yet predominantly maternal mitochondrial, inheritance (WAGNER *et al.* 1987, 1989, 1991; DONG *et al.* 1992; DONG and WAGNER 1993). Because isoenzyme (*i.e.*, Mendelian) and mitochondrial surveys have already been reported in jack and lodgepole pines (WHEELER and GURIES 1982, 1987; DONG and WAGNER 1993), our new data allow immediate comparisons of population structure among three, differently inherited, plant genomes.

MATERIALS AND METHODS

Plant materials: The germplasm collections have been described in detail elsewhere (DONG and WAGNER 1993). Briefly, 745 individuals were sampled, representing eight allopatric natural populations of each species (Table 1). Sampled populations were distributed rangewide in both species and included three of the four lodgepole pine subspecies. The available samples provided approximately 95% power to detect variants that were present (*i*) with frequency ≥0.07 within a given population and (*ii*) with overall frequency ≥0.004.

Laboratory methods: We elected to examine a single highly variable chloroplast 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. After preliminary surveys, the particular polymorphism chosen for this study was selected because (*i*) it was the only cpDNA polymorphism known to vary intraspecifically in both study species and (*ii*) its paternal inheritance had been demonstrated (WAGNER *et al.* 1987, 1989; GOVINDARAJU *et al.* 1989; DONG *et al.* 1992).

Each sampled individual was classified by its variant of an *Sst*I restriction fragment length polymorphism (RFLP), as described previously (WAGNER *et al.* 1987; DONG *et al.* 1992). In the present study we found that a 7.4-kilobase-pair (kbp) *Hin*dIII fragment and a 700-base pair (bp) *Bam*HI-*Sma*I fragment from the lodgepole pine chloroplast genome (LIDHOLM and GUSTAFSSON 1991) could be used interchangeably as probes in molecular hybridizations. The *psbA* gene is duplicated in jack and lodgepole pines (LIDHOLM *et al.* 1991), and either of these probes reveals insertion/deletion polymorphism associated with the *psbAI-psbAII* genomic region (GOVINDARAJU *et al.* 1989; J. DONG and D. B. WAGNER, unpublished results). Although our focus here is not the molecular details of this polymorphism, it is relevant that at least some of its variability is due to copy-number variation of short tandem repeats (LIDHOLM and GUSTAFSSON 1991). This polymorphism is clearly a "hot spot" of chloroplast length variants in jack and lodgepole pines (WAGNER *et al.* 1987; GOVINDARAJU *et al.* 1989).

Data analysis: Frequency data from the 16 populations (Table 2) were used to estimate: numbers of variants in species (A_s) and populations (A_p) (HAMRICK and GODT 1990), unbiased variant diversities in species (h_{es}) and populations (h_{ep}) (NEI 1978), and population subdivision (WRIGHT 1951). Population subdivision was estimated as θ by the method described by WEIR (1990, p. 150), and the statistical significance of differentiation among sampled populations was evaluated by chi-square tests (WEIR 1990, p. 137).

Mitochondrial data from a *COXII*-associated length polymorphism were available for 740 of the same individuals studied here (DONG and WAGNER 1993). Therefore, we used chi-square analyses of two-factor contingency tables to test statistical associations between chloroplast and mitochondrial variants. These tests were performed (*i*) within each of the seven populations (PQ, ON-E, SA, BC-N, BC-C, OR-W, OR-C) that were variable for *both* chloroplast *and* mitochondrial polymorphisms [see Table 1 of DONG and WAGNER (1993); Table 2 of this study], and (*ii*) at the species and subspecies levels by pooling data from populations within species and within lodgepole pine subspecies.

RESULTS

Diversity: By any standard, levels of variation were high in both species (Tables 2 and 3). Fourteen variants were found in the total survey, eight in jack pine and six in lodgepole pine. The number of variants per population ranged from 2 to 6, averaging 4.5 in jack pine and 4.0 in lodgepole pine.

Variant diversities at the species level (h_{es}) were similar in jack and lodgepole pines (0.43 and 0.44, respectively). Within-population diversities (h_{ep}) also were comparable in the two species, ranging over all populations from 0.22 to 0.66. Notably, no population was fixed for a single cpDNA variant (Tables 2 and 3).

TABLE 2

Chloroplast variant frequencies in 16 populations of *P. banksiana* and *P. contorta*

	P. banksiana populations								*P. contorta* populations							
									var. *latifolia*				var. *contorta*		var. *murrayana*	
Variant[a]	NB	PQ	ON-E	MI	ON-W	MB	SA	AB	BC-N	BC-C	BC-S	CO	BC-W	OR-W	OR-C[b]	CA
4.4/5.7	0.02	0.10	0.02		0.02		0.02	0.06								
4.7/5.7	0.10	0.14	0.16	0.17	0.07	0.26	0.09	0.13								
4.8/5.7	0.76	0.62	0.71	0.66	0.88	0.70	0.81	0.77								
5.0/5.7		0.04		0.15			0.02									
4.3/4.8/5.7	0.06	0.10	0.06	0.02	0.02	0.04	0.06	0.04								
4.7/4.9/5.7	0.06															
5.0/5.5/6.2			0.02													
5.5/6.2/6.7			0.02													
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				

Location abbreviations are defined in Table 1 [see also DONG and WAGNER (1993)].

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

[b] See WHEELER and GURIES (1982) for discussion of subspecies taxonomic classification in this geographic region.

TABLE 3

Population genetic statistics for a chloroplast polymorphism in *P. banksiana* and *P. contorta*

	P. banksiana populations								*P. contorta* populations							
									var. *latifolia*				var. *contorta*		var. *murrayana*	
Statistic[a]	NB	PQ	ON-E	MI	ON-W	MB	SA	AB	BC-N	BC-C	BC-S	CO	BC-W	OR-W	OR-C[b]	CA
A_p	5	5	6	4	4	3	5	4	4	4	2	4	5	5	4	4
h_{ep}	0.41	0.59	0.47	0.52	0.22	0.46	0.34	0.39	0.46	0.55	0.28	0.26	0.66	0.60	0.37	0.26
Mean A_p				4.50							4.00					
Mean h_{ep}				0.42							0.43					
A_s				8							6					
h_{es}				0.43							0.44					
θ				0.02; $P < 0.01$[c]							0.04 (among subspecies); $P < 0.001$[c]					
											0.02 (within var. *latifolia*); $P < 0.05$[c]					
											0.00 (within var. *contorta*); NS[c]					
											0.00 (within var. *murrayana*); NS[c]					

Populations as described in Table 1.

[a] Abbreviations for population genetic statistics are defined in the text.

[b] As in Table 2.

[c] Chi-square probabilities.

Differentiation: Chloroplast DNA variants unambiguously distinguished jack pine from lodgepole pine (Table 2), as in previous work (WAGNER *et al.* 1987). However, the earlier work sampled few individuals per population, precluding formal analysis of population subdivision.

Several of the chi-square tests indicate statistically significant ($P < 0.05$) frequency differences among sampled populations and subspecies (Table 3). Nonetheless, differentiation among conspecific populations and subspecies appears weak: all θ values are 0.04 or less, and pairwise genetic identities (NEI 1978) range upwards to 1.00, from 0.97 in jack pine and from 0.94 in lodgepole pine (genetic identity matrices are available upon request from the authors).

Chloroplast-mitochondrial associations: Only one (PQ) of the seven tests of association within individual populations was statistically significant (Table 4). This significance ($P = 0.01$) in the PQ population is trivial, because it was due to a single cell in its contingency table with expected frequency less than one. The significance disappeared when we pooled the four least frequent cpDNA variants of this population into a "synthetic" variant and repeated the test.

Similarly, we found little evidence of association between chloroplast and mitochondrial variants at the species or subspecies levels (Table 4). The species-level significance in lodgepole pine ($p = 0.02$) is questionable, because many cells in the contingency table had

TABLE 4

Tests of association between chloroplast and mitochondrial variants

Population(s)	Chi-square value	d.f.	Probability
PQ	13.56[a]	4	0.01
ON-E	0.41	5	0.99
SA	5.00	8	0.76
BC-N	1.31	3	0.73
BC-C	0.55	3	0.91
OR-W	7.08	8	0.53
OR-C	2.67	3	0.45
P. banksiana[b]	14.33	21	0.85
P. contorta[b]	34.90[c]	20	0.02
P. contorta var. *latifolia*[b]	11.02	8	0.20
P. contorta var. *contorta*[b]	5.79	8	0.67
P. contorta var. *murrayana*[b]	2.36	3	0.50

This table includes populations that were polymorphic for chloroplast and mitochondrial markers. Location abbreviations are defined in Table 1.

[a] This chi-square value was reduced to 2.53 (with one degree of freedom (d.f.), $P = 0.11$), when a cell with expected frequency less than one was removed from the contingency table by pooling rare cpDNA variants into a synthetic variant.

[b] Data were pooled within species or subspecies prior to tests indicated by this superscript.

[c] This chi-square value was reduced to 21.76 (with 12 degrees of freedom, $P = 0.04$), when cells with expected frequencies less than one were removed from the contingency table by pooling rare cpDNA variants into a synthetic variant.

expected frequencies less than one. These low expected frequencies were removed by pooling cells with rare variants, which increased the chi-square probability to 0.04.

DISCUSSION

Diversity: Two of the cpDNA variants found in this survey (*5.0/5.5/6.2* and *5.5/6.2/6.7*) have not been reported previously. These bring the total number of *psbA*-associated variants now known in jack and lodgepole pines to 36, in a total sample size of nearly 2300 individuals (WAGNER *et al.* 1987; GOVINDARAJU *et al.* 1988; GOVINDARAJU *et al.* 1989; WAGNER *et al.* 1989; DONG *et al.* 1992). This is clearly a minimum estimate of the total number of variants, because small differences (of about 50 bp or less) in restriction fragment sizes would have been unresolved on autoradiograms.

Many of the *psbA*-associated variants are individually rare and are concentrated in or near a geographic region of sympatry between the two species. A previous report discussed several possible causes for these rare variants, including natural hybridization and evolution in marginal populations (GOVINDARAJU *et al.* 1989). Three rare variants of the present study (*4.3/4.5/5.0*; *4.3/4.7/5.0*; *4.7/4.9/5.7*) were previously detected only in or near regions of natural hybridization. But these three variants, as well as the two newly discovered variants, occurred here only in marginal allopatric populations (NB, ON-E, CO, BC-W, OR-W) that are far-removed from regions of sympatry. Thus, although natural hybridization may be involved in the production of certain rare cpDNA variants, it cannot be the only cause. Thorough understanding of mechanisms responsible for generating all these variants will require detailed investigation at the molecular level.

The *psbA*-associated cpDNA variability in jack and lodgepole pines, whether measured in terms of variant number or diversity (Table 3), appears remarkable. Nonetheless, the high level of variability does not contradict the generally slow rate of chloroplast sequence evolution (WOLFE *et al.* 1987; CLEGG *et al.* 1991), for at least two reasons.

First, although we have not elucidated the molecular basis of all the variants, it is known from several lines of evidence that the *psbA*-associated polymorphism results primarily from insertion/deletion mutation (GOVINDARAJU *et al.* 1989; LIDHOLM and GUSTAFSSON 1991). Short tandem repeats have been implicated here and in other systems in generating high levels of cpDNA length variation (PALMER *et al.* 1987; ALDRICH *et al.* 1988; BLASKO *et al.* 1988; OGIHARA *et al.* 1988; ALI *et al.* 1991; LIDHOLM and GUSTAFSSON 1991). Clearly, data from polymorphisms that arise through length mutation are unrelated to general 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, the diversity of this polymorphism should not be viewed as "typical." Several but not all other investigators of cpDNA diversity, including those who have examined point mutations, have also emphasized polymorphic regions of the chloroplast genome in their surveys (*e.g.*, compare methodologies of WHITTEMORE and SCHAAL 1991; HONG *et al.* 1993; WANG and SZMIDT 1993; PETIT *et al.* 1993b). Consequently, the cpDNA population data that is accumulating in the literature must be treated cautiously and appropriately when inferring generalities regarding diversity in the chloroplast genome.

Despite these caveats, intraspecific cpDNA hot spots carry useful information. Their high diversities, combined with uniparental inheritance (usually maternal in angiosperms but usually paternal in conifers), empower new fields of inquiry, such as cytonuclear population genetics (ASMUSSEN *et al.* 1987). In this regard, it is promising that the high diversities we report here are not unique; examples of intraspecific cpDNA diversity exist even for site mutations (*e.g.*, SOLTIS *et al.* 1992 and references therein; WANG and SZMIDT 1993; PETIT *et al.* 1993b).

Differentiation: At the species level, it is not surprising that cpDNA polymorphism distinguishes jack from lodgepole pine. Chloroplast variation occurs commonly among plant species, even among closely related ones, and has been much-used in phylogenetic analyses (PALMER 1987; SOLTIS *et al.* 1992). Although intraspecific cpDNA variation and introgression can potentially confound phylogenetic investigations (DOYLE 1992; SOLTIS

et al. 1992; RIESEBERG and BRUNSFELD 1992), it is encouraging for biosystematists that the *psbA*-associated cpDNA variants of jack and lodgepole pines show no species ambiguity (despite substantial intraspecific variability). However, note that some individual restriction fragment sizes (*e.g.*, the 4.4-kbp size class) do occur in both species, which indicates the importance of screening a large number of restriction fragments when seeking cpDNA species markers.

Consistent with drift-migration equilibrium predictions for paternally inherited neutral polymorphisms in outcrossers (PETIT *et al.* 1993a), *psbA*-associated differentiation among conspecific jack and lodgepole pine populations is weak and roughly similar to nuclear subdivision in these species. For example, *psbA*-associated θ is 0.04 among lodgepole pine subspecies (Table 3), while a corresponding isoenzyme statistic (G_{st}) is 0.03 (WHEELER and GURIES 1982).

In contrast, population subdivision of maternally inherited mitochondrial DNA length variants, *estimated from the same DNA samples that we used for the chloroplast analyses*, is much higher (*e.g.*, $\theta = 0.31$ among lodgepole pine subspecies, and θ is as high as 0.82 among populations within subspecies; DONG and WAGNER 1993). Recall that, given sufficient intraspecific variability, maternally inherited polymorphisms in other plants also generally exhibit considerable subdivision among populations (SOLTIS *et al.* 1992; STRAUSS *et al.* 1993; PETIT *et al.* 1993b).

As in jack and lodgepole pines, population subdivision may be weak for paternally inherited slash pine (*Pinus elliottii* Engelm.) cpDNA length variants (WAGNER *et al.* 1992). However, WANG and SZMIDT (1993) recently found that population subdivision of cpDNA haplotypic diversity in *Pinus densata* ($G_{st} = 0.181$) is relatively high in comparison with *psbA*-associated subdivision in jack and lodgepole pines. This result for *P. densata* appears to contrast with neutral equilibrium predictions under drift and migration (PETIT *et al.* 1993a), possibly due to the hybrid origin of this species (WANG and SZMIDT 1990).

Again in apparent contrast to drift-migration predictions and our results in jack and lodgepole pines, subdivision of paternally inherited cpDNA site mutation haplotypes in bishop pine (*Pinus muricata* D. Don) is strong ($G_{st} = 0.842$) among geographic regions (HONG *et al.* 1993). There are at least two, possibly interacting, potential causes of the discrepancy.

First, jack and lodgepole pines occupy vast, frequently continuous, geographical ranges in North America, with ample opportunities for gene flow (CRITCHFIELD 1985; GOVINDARAJU 1988). Bishop pine populations can be geographically and reproductively isolated (CRITCHFIELD and LITTLE 1966; MILLAR and CRITCHFIELD 1988), and genetic exchanges among populations from different geographic groups may be exceedingly rare even through pollen. Indeed, the proportion of isoenzyme variation that is attributed to population differences in bishop pine (22%) (MILLAR *et al.* 1988) is much greater than is typical for other conifers including jack and lodgepole pines. For these and other reasons HONG *et al.* (1993) consider the southern and north-intermediate geographic groups of bishop pine populations to be separate species. In this connection, it is important to note that population subdivision of cpDNA site mutation haplotypes in bishop pine is lower within geographic groups than among geographic groups. For example, subdivision among populations of the southern group does not differ significantly from zero.

Second, cpDNA mutational mechanisms may influence the apportionment of variation within and among populations. Equation (21) of BIRKY *et al.* (1989) indicates that population subdivision at equilibrium is inversely related to mutation rate. Chloroplast site mutation rates may be considerably lower than rates of length mutation (CLEGG *et al.* 1991; ALI *et al.* 1991). Hence, in the absence of migration, site mutations might be expected to exhibit greater population subdivision than insertion/deletion polymorphisms.

Indeed, there may be a relationship between subdivision and mutational mechanism in the species complex that includes bishop pine (HONG *et al.* 1993). The relationship is unclear, however, because population subdivision of a cpDNA length polymorphism varied from $G_{st} = 0.073$ in Monterey pine (*Pinus radiata* D. Don) to $G_{st} = 0.682$ in knobcone pine (*Pinus attenuata* Lemm.).

Since the *psbA*-associated polymorphism of jack and lodgepole pines is due primarily to length mutation, the low θ values within each of these two species could be due to a high length mutation rate. If population subdivision is at a mutation-drift equilibrium, we can estimate the number of new mutants ($N_{eo}u$) each generation. For example, assuming a neutral equilibrium, no migration, a large number of demes, and substituting our *P. contorta* var. *latifolia* θ estimate (0.02) as G_{st} into Equation 21 of BIRKY *et al.* (1989), we obtain $N_{eo}u = 24.5$. Twenty-four new mutants each generation implies a very high mutation rate, even with large population sizes.

In fact, the *psbA*-associated length mutation rate must be greater than the migration rate if mutation is predominantly responsible for the low population subdivision of this polymorphism. This is because mutation and migration enter in exactly the same way into the equilibrium formula for organellar population subdivision [Equation 21 of BIRKY *et al.* (1989)].

But migration is a potent evolutionary force in jack and lodgepole pines (*e.g.*, N_em based on isoenzymes averages 6.23 in *P. contorta* var. *latifolia*) (GOVINDARAJU 1988), and migration can virtually eliminate population subdivision for paternally inherited plant genomes (PETIT *et al.* 1993a). Thus, it seems likely that differential

migration through seed and pollen is at least partly responsible (probably in concert with a high cpDNA insertion/deletion rate) for the simultaneous occurrence in jack and lodgepole pines of (*i*) weak population subdivision of *psbA*-associated cpDNA length variants (Table 3) and (*ii*) strong population subdivision of mitochondrial variants (DONG and WAGNER 1993).

Quantitative assessment of the relative importance of mutation and migration for homogenizing cpDNA variant frequencies among jack and lodgepole pine populations must await surveys of cpDNA point mutations and estimation of *psbA*-associated length mutation rates in these two species. Unfortunately at present, cpDNA point mutations are unknown to us within either species and *in vivo* estimation of intraspecific cpDNA length mutation rates is a formidable, if not impossible, task.

Interestingly, all but one individual in the CO population had cpDNA variants typical of lodgepole pine (Table 2). Yet this same population is fixed for a "private" (SLATKIN 1985) mitochondrial variant (DONG and Wagner 1993). A Colorado population also differed from more central populations in an isoenzyme study (WHEELER and GURIES 1982). If migration and drift are responsible for these patterns, this information is consistent with a dearth of seed migration but more frequent or more recent pollen migration involving Colorado populations, which are 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), which again may be consistent with differential gene flow through pollen and seeds.

Chloroplast-mitochondrial associations: Statistical associations (disequilibria) between two organellar loci decay rapidly within populations when one locus is paternally inherited while the other locus is maternally inherited (SCHNABEL and ASMUSSEN 1989). Furthermore, a sufficiently high cpDNA insertion/deletion rate intuitively should lead to low or zero chloroplast-mitochondrial disequilibrium (but we are unaware of any formal reports regarding the effects of mutation rates on disequilibria among cytoplasmic genomes; M. A. ASMUSSEN, personal communication). For these reasons, a general lack of significant associations between chloroplast and mitochondrial variants was to be expected in most jack and lodgepole pine populations.

Despite these general expectations, the random associations observed in the SA jack pine population may be a bit surprising because of the high proportion of lodgepole pine mitochondrial genotypes that remain in this unusual population after past introgressive hybridization (DONG and WAGNER 1993). The decoupled nature of chloroplast and mitochondrial inheritance, and/or a high cpDNA insertion/deletion rate, appear sufficient in this population to obscure epistatic fitness effects (if such effects exist) of interspecific physiological interactions between the chloroplast and mitochondrial genomes (HUSIC *et al.* 1987).

We note, however, that the available sample sizes would be too small to detect weak associations within populations [*e.g.*, BROWN (1975)]. In this connection, it is interesting that associations were also nonsignificant in the larger sample sizes of the species-level test in jack pine (with $N = 378$) and the subspecies-level tests in lodgepole pine (Table 4). The significance of the lodgepole pine species-level association ($P = 0.04$) may be a stochastic consequence of performing 14 statistical tests of association (Table 4) or of pooling data from populations and subspecies with statistically heterogeneous variant frequencies (PROUT 1973).

Conclusions: Pines represent an unusual model system for population and evolutionary genetic investigations because of their opposite chloroplast and mitochondrial inheritances. In the case of jack and lodgepole pines, patterns of population subdivision of variants in the three major eukaryotic genomes, together with predominantly random associations between chloroplast and mitochondrial variants, conform with theoretical predictions for outcrossers. Specifically, a maternally inherited mitochondrial polymorphism features abundant population subdivision (DONG and WAGNER 1993), while Mendelian allozymes and a paternally inherited cpDNA insertion/deletion polymorphism display relatively little differentiation among conspecific populations (WHEELER and GURIES 1982) (this study, Table 3). Plant biologists, by considering the mode of inheritance of genetic markers, would appear to have the luxury of selecting characters with appropriate levels and patterns of variation for investigation of a wide array of unresolved population and evolutionary genetic questions.

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