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Taxonomic and population differentiation of mitochondrial diversity in *Pinus banksiana* and *Pinus contorta*

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Abstract. We have studied two mitochondrial DNA polymorphisms in 741 individuals from 16 allopatric populations of *Pinus banksiana* Lamb. and *Pinus contorta* Dougl. Restriction fragments of both polymorphisms distinguished the two species qualitatively, except in a *P. banksiana* population whose ancestors were involved in hybridization with *P. contorta*. *COXI*-associated restriction fragments were monomorphic within species, while *COXII*-associated restriction fragments were highly variable in *P. contorta* ($H_{es} = 0.68$). Population differentiation was substantial in *P. contorta* ($F_{st} = 0.31$ among subspecies; mean $F_{st} = 0.66$ within subspecies) and consistent with predictions for maternally inherited markers. Plant mitochondrial markers appear to be useful for the investigation of seed migration routes, hybridization and introgression, breeding zone designation, and the development of germ plasm conservation sampling strategies.

Key words: *COXI* – *COXII* – Maternal inerhitance – mtDNA – RFLP

Introduction

The non-recombining, maternal inheritance of animal mitochondrial DNA (mtDNA) confers great power for phylogeographic inference (Avise et al. 1987). Theory predicts that maternally inherited markers should also possess such power in plants (Birky 1988; Petit 1992). Indeed, chloroplast DNA (cpDNA) polymorphisms have been informative with regard to gene flow among species (Rieseberg et al. 1990). However, cpDNA is often insufficiently variable (Clegg 1989) for evolutionary analyses within species.

The plant mitochondrial genome is predominantly maternally inherited (Conde et al. 1979), and intraspecific mtDNA variation is not uncommon in plants (Palmer 1988). Therefore, plant mtDNA may contain more intraspecific geographic information than cpDNA. Unfortunately, sample sizes have generally been small, and thus little is known of the magnitude or distribution of plant mtDNA diversity in natural populations.

In this article we present a large population survey of maternally inherited mtDNA polymorphism in jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*Pinus contorta* Dougl.). These closely related species are each widely distributed in North America and hybridize in sympatric regions of western Canada (Fig. 1).

Since survey data have already been published for Mendelian-inherited isoenzyme loci and paternally inherited cpDNA polymorphisms of these two species (Wheeler and Guries 1987; Wagner et al. 1987), the new mtDNA data permit the first direct comparisons of geographic and taxonomic patterns of variation among three, differentially inherited, plant genomes. Consistent with theoretical predictions, mitochondrial polymorphism exhibits substantial population differentiation. Our data also provide evidence of previous unidirectional introgressive hybridization.

Materials and methods

Plant materials

Fresh foliage and seed cones were collected in 1990 from 741 individuals representing 16 allopatric populations of natural origin (Fig. 1, Table 1). Sampled individuals were well-spaced to

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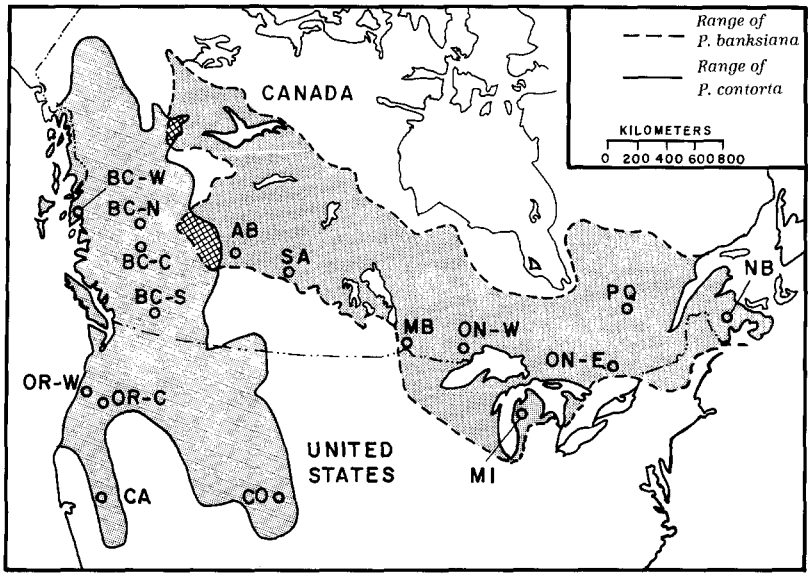

Fig. 1. Sampled locations (*circles*) in the distributional ranges (*shaded*) of *P. banksiana* and *P. contorta* (after Critchfield 1985). Key to location abbreviations (*P. contorta* subspecies in parentheses): NB, Doaktown, New Brunswick; PQ, Lac Mathieu, Quebec; ON-E, Chalk River, Ontario; MI, Wellston, Michigan; ON-W, Raith, Ontario; MB, Hadashville, Manitoba; SA, Canwood, Saskatchewan; AB, Bellis, Alberta; BC-N, Mackenzie, British Columbia (var *'latifolia'*); BC-C, Prince George, British Columbia (var. *'latifolia'*); BC-S, Lumby, British Columbia (var. *'latifolia'*); CO, Ward, Colorado (var *'latifolia'*); BC-W, Prince Rupert, British Columbia (var *'contorta'*) OR-W, Waconda Beach, Oregon (var *'contorta'*); OR-C, Santiam Pass, Oregon (var *'murrayana'*; but see Wheeler and Guries 1982); CA, Wrights Lake, California (var *'murrayana'*). Additional details available from the authors upon request

Table 1. Mitochondrial genotypic frequencies in 16 populations of *P. banksiana* and *P. contorta*[a]

Endonuclease/ probe combination and variant[b]	*P. banksiana* populations								*P. contorta* populations							
									var *'latifolia'*				var *'contorta'*		var *'murrayana'*	
	NB (50)	PQ (50)	ON-E (49)	MI (47)	ON-W (43)	MB (46)	SA (46)	AB (47)	BC-N (44)	BC-C (43)	BC-S (43)	CO[c] (48)	BC-W (42)	OR-W (46)	OR-C[d] (47)	CA (50)
SstI/COXII																
2.9/7.6	1.00	0.92	0.98	1.00	1.00	1.00	0.37	1.00	–	–	–	–	–	–	–	–
2.9/10.2	–	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–
2.9/4.5/7.6	–	0.08	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–
3.1/7.6	–	–	–	–	–	–	–	–	0.64	0.02	–	–	1.00	0.24	0.40	1.00
5.2/10.2	–	–	–	–	–	–	0.59	–	0.36	0.98	1.00	–	–	–	0.60	–
7.6/10.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.28	–	–
5.2/7.6/10.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.48	–	–
5.9/10.2/14.2	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–	–
PstI/COXI																
2.6	1.00	1.00	1.00	1.00	1.00	1.00	0.37	1.00	–	–	–	–	–	–	–	–
2.4/4.4	–	–	–	–	–	–	0.63	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] Location abbreviations are defined in Fig. 1; sample sizes are indicated in parentheses below each location abbreviation
[b] Variants are denoted by restriction fragment sizes (kbp); only variable fragments are listed, separated by slashes within each variant. Note that in the Saskatchewan population all individuals with the *PstI/COXI* 2.6 variant also had the 2.9/7.6 *SstI/COXII* variant; thus restriction fragments of the two endonuclease/probe combinations are in complete disequilibrium in the survey
[c] For the *PstI/COXI* polymorphism in this population, $n=45$
[d] See Wheeler and Guries (1982) for a discussion of subspecies in this geographic region

avoid over-representation of maternal half-sibs, and each sampled tree was permanently labeled. Fifteen populations were sampled in situ, but we obtained the Lac Mathieu, Quebec samples from a grafted seed orchard at the Harrington Forestry Centre, Calumet, Quebec. Three of the four *P. contorta* subspecies (var *'latifolia'*, var *'contorta'* and var *'murrayana'*) were represented.

With 42 to 50 individuals sampled per population (Table 1), we have for each polymorphism at least a 95% power of detecting variants that occur with frequency ≥ 0.07 in each population. For each polymorphism at the species level, the survey provides approximately a 95% power for detecting variants that occur with an overall frequency ≥ 0.004.

Laboratory analyses

We surveyed two mitochondrial restriction fragment length polymorphisms (RFLPs). One polymorphism, assayed as described previously, involved molecular hybridization of pine *SstI* restriction fragments with a [^{32}P]-labeled *COXII* sequence from maize (*Zea mays* L.) mitochondria (Wagner et al. 1991). The second polymorphism was studied by molecular hybridization of pine *PstI* restriction fragments with a [^{32}P]-labeled (Feinberg and Vogelstein 1983), 712-bp *COXI* sequence from knobcone pine (*Pinus attenuata* Lemm.). The *P. attenuata COXI* probe was amplified by the polymerase chain reaction (PCR) by S. Strauss, Y.-P. Hong and V. Hipkins (Strauss et al. 1993) using

Table 2. Population genetic statistics for two mitochondrial polymorphisms in *P. banksiana* and *P. contorta*[a]

Endonuclease/ probe combination and statistic[b]	*P. banksiana* populations								*P. contorta* populations							
									var *'latifolia'*				var *'contorta'*		var *'murrayana'*	
	NB	PQ	ON-E	MI	ON-W	MB	SA	AB	BC-N	BC-C	BC-S	CO[e]	BC-W	OR-W	OR-C	CA
SstI/COXII																
A_p	1	2	2	1	1	1	3	1	2	2	1	1	1	3	2	1
H_{ep}	–	0.15	0.04	–	–	–	0.53	–	0.47	0.05	–	–	–	0.65	0.49	–
Mean A_p		1.50								1.62						
Mean H_{ep}		0.09								0.21						
A_s		4 (including SA) 2 (excluding SA)								5						
H_{es}		0.17 (including SA) 0.03 (excluding SA)								0.68						
F_{st}		0.50 (including SA); $P<0.001$[c] 0.04 (excluding SA); $P<0.01$[d]								0.31 (among subspecies); $P<0.001$ 0.82 (within var *'latifolia'*); $P<0.001$ 0.56 (within var *'contorta'*); $P<0.001$ 0.59 (within var *'murrayana'*); $P<0.001$						
PstI/COXI																
A_p	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1
H_{ep}	–	–	–	–	–	–	0.48	–	–	–	–	–	–	–	–	–

[a] Population location abbreviations are defined in Fig. 1
[b] Abbreviations for population genetic statistics are defined in text; species-level statistics are not reported for *PstI/COXI* because this endonuclease/probe combination was monomorphic within each species (excluding the unusual Saskatchewan *P. banksiana* population)
[c] Chi-square probabilities
[d] This significance may be spurious; see text for details
[e] As in Table 1

western red cedar (*Thuja plicata* Donn) primer sequences (Glaubitz and Carlson 1992). Following the hybridizations, restriction fragments were visualized on autoradiograms.

In *P. banksiana* and *P. contorta* nuclear loci are inherited biparentally, cpDNA is inherited predominantly paternally and mtDNA is inherited predominantly maternally (Wagner et al. 1991). Because the *PstI/COXI* polymorphism had not been studied previously, we tested its mitochondrial basis with inheritance data. Five seedlings were studied in each of two controlled crosses among three parents. Both matings (14-13-6 × 101 and 14-13-6 × 61) had a *P. contorta* female parent and a *P. banksiana* male parent and have been described previously (Wagner et al. 1991).

Mitochondrial data from the *P. banksiana* population in Saskatchewan were suggestive of introgression with *P. contorta* (see Results). Therefore, we also assayed nuclear, chloroplast, and morphological species markers. Standard assays were used for nuclear-encoded malate dehydrogenase (MDH) isoenzymes in a single haploid megagametophyte of each sampled individual (Dancik and Yeh 1983; Wheeler and Guries 1987). Two chloroplast RFLPs, one assayed with the restriction endonuclease *SalI* and the other with *SstI* (Wagner et al. 1987), were studied in the unusual Saskatchewan population. Finally, we examined classical taxonomic features of the seed cones (Moss 1949) in this population.

Data analyses

The number of restriction fragment size-class arrays (variants) per species (A_s), number of variants per population (A_p), unbiased gene diversity within species (H_{es}) and unbiased gene diversity within populations (H_{ep}) (Nei 1978; Hamrick and Godt 1990) were estimated. Measures of differentiation among populations and subspecies (i.e. F_{st} values) were also estimated (Weir 1990, p 150). The significance of frequency differentiation among sampled populations and subspecies was evaluated by chi-square (Weir 1990, p 137). Phenetic relationships among populations were studied by UPGMA analysis (Sneath and Sokal 1973) of unbiased genetic identities (Nei 1978), treating the two mtDNA polymorphisms as independent genetic markers.

Results

PstI/COXI inheritance

The female *P. contorta* parent of both crosses had *PstI/COXI* variant 2.4/4.4, but both male *P. banksiana* parents had variant 2.6 (see Table 1 for variant nomenclature). All 10 seedlings from the controlled crosses had the 2.4/4.4 variant of their maternal parent. This result is consistent with other mitochondrial inheritance data from the pine genus (*Pinus* L.), including *P. banksiana* and *P. contorta* (Neale and Sederoff 1989; Wagner et al. 1991).

Amount of mtDNA diversity

We found eight variants of the *SstI/COXII* polymorphism (Table 1). Variability was substantial in *P. contorta* (e.g. $H_{es} = 0.68$), but markedly lower in *P. banksiana* (Table 2). With the exception of the Saskatchewan *P. banksiana* population, *SstI/COXII* variants differed

qualitatively between the two species. Excluding Saskatchewan, *P. banksiana* escapes complete monomorphism only by the presence of a rare variant (2.9/4.5/7.6) in the Quebec and eastern Ontario samples.

Only two *Pst*I/*COXI* variants were detected (Table 1). Both species were monomorphic, if we exclude the Saskatchewan *P. banksiana* population in which 29 trees had restriction fragment sizes exactly like those of *P. contorta*.

Each of the *Sst*I/*COXII* variants was strictly associated in our sample with only one of the *Pst*I/*COXI* variants, i.e. linkage disequilibrium was maximal. Thus, each individual's two-marker haplotype is uniquely specified by that individual's *Sst*I/*COXII* restriction fragment sizes.

The Saskatchewan P. banksiana population

Of the individuals sampled from the allopatric *P. banksiana* population in Saskatchewan 17 had the 2.9/7.6 haplotype typical of this species. However 27 individuals in this population had 1 of *P. contorta*'s frequent haplotypes (5.2/10.2). The remaining 2 individuals had an haplotype (2.9/10.2) not seen elsewhere in our survey, but had *Pst*I/*COXI* restriction fragment sizes (2.4/4.4) identical to those of *P. contorta* (Table 1).

We verified the validity of these results for the aberrant Saskatchewan individuals in a second, independent set of foliage samples collected in 1991. The second round of laboratory assays confirmed all of our initial results.

In contrast to their mtDNA haplotypes, all individuals sampled in Saskatchewan had *Sal*I and *Sst*I cpDNA restriction fragment profiles (data not shown) like those of *P. banksiana* but unlike those of *P. contorta*. Similarly, the MDH isoenzymes and the morphological characteristics of all individuals sampled in the Saskatchewan population were typical of *P. banksiana* (data not shown).

Population differentiation

The *Sst*I/*COXII* polymorphism is substantially differentiated among sampled *P. contorta* populations ($F_{st}=0.31$ among subspecies; F_{st} averages 0.66 within subspecies). Nonetheless, 3 populations are quite variable, with A_p as high as 3 and H_{ep} as high as 0.65. If the atypical Saskatchewan population is excluded, F_{st} was only 0.04 in *P. banksiana* and would be zero if not for the rare haplotype in Quebec and eastern Ontario (Table 2).

Differentiation among sampled populations is statistically significant in both species (Table 2). However, the 2.9/4.5/7.6 variant is responsible for the low chi-square probability in *P. banksiana* (excluding Saskatchewan) while having an "expected" frequency of less than 1 in each population. Consequently, the significance in *P. banksiana* (excluding Saskatchewan) may be spurious.

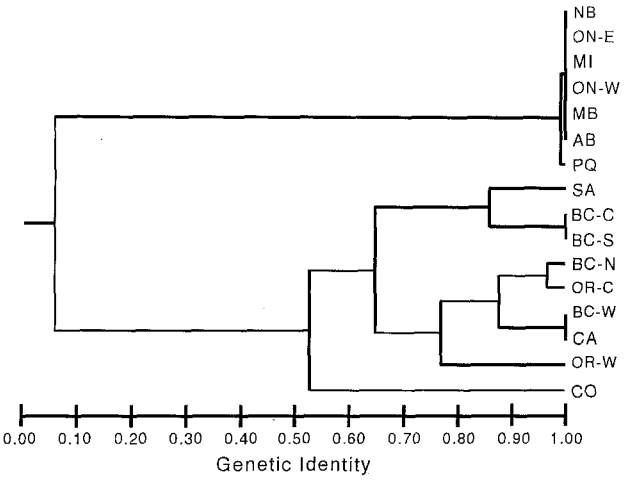

Fig. 2. UPGMA dendrogram of unbiased genetic indentities (Sneath and Sokal 1973; Nei 1978); see Fig. 1 for location abbreviations

Phenetic analysis

The Saskatchewan *P. banksiana* population's mtDNA is phenetically most similar to that of two British Columbia *P. contorta* var '*latifolia*' populations. All other *P. banksiana* populations share pairwise genetic identities of 1.00 or near 1.00 and have genetic identities of zero with any *P. contorta* population (Fig. 2).

In contrast, genetic identities are quite variable in *P. contorta*. Despite significant differentiation (Table 2), populations do not necessarily cluster by subspecies (Fig. 2).

Discussion

Basis of the polymorphisms

The maternal inheritance of the *Pst*I/*COXI* restriction fragments is convincing evidence that their locus is mitochondrial since nuclear loci are biparentally inherited, cpDNA is paternally inherited and mtDNA is predominantly maternally inherited in *P. banksiana* and *P. contorta*. A mitochondrial basis for the *Sst*I/*COXII* polymorphism has been demonstrated previously (Wagner et al. 1991).

Both polymorphisms are detectable with many restriction endonucleases (data not shown). Thus, they are likely to be due to insertions/deletions or rearrangements, which is consistent with the structural lability and slow rate of base sequence evolution of the plant mitochondrial genome (Palmer 1990).

Mitochondrial variants as species markers

In principle, the 29 atypical haplotypes in Saskatchewan (Table 1) may have arisen from mutation within *P.*

banksiana and/or hybridization with *P. contorta*. However, we reject mutation-based hypotheses for two reasons.

First, all 29 atypical haplotypes are deviant for both polymorphisms, which contradicts the expectation that single-locus mutants should be more frequent than two-locus mutants. If mutation produced the aberrant haplotypes, two independent mutations must have occurred in a single *P. banksiana* mitochondrial lineage, or two mutations must have recombined after their appearances in separate lineages.

Second, it seems unlikely that mutation could have produced restriction fragments of sizes exactly like those of *P. contorta* in one of eight allopatric *P. banksiana* populations, yet this was the case for all 29 unusual *Pst*I/*COXI* variants and for 27 of the atypical *Sst*I/*COXII* variants. Note also that the most frequent haplotype (5.2/10.2) of *P. contorta* var *'latifolia'* occurs in 27 of the aberrant Saskatchewan individuals. Because of its geographic proximity to *P. banksiana*, *'latifolia'* is the most likely of the four *P. contorta* subspecies to have hybridized with *P. banksiana*.

Therefore, we conclude that mtDNA variants are valid species markers and that the Saskatchewan *P. banksiana* population has received *P. contorta* mtDNA through past hybridization. Indeed, hybridization between *P. banksiana* and *P. contorta* is well known. For example, Rudolph and Yeatman (1982, p 11) have summarized the postglacial influence of *P. contorta* in Saskatchewan:

"... in northern Saskatchewan ... small, isolated stands of lodgepole pine remained when jack pine arrived ... to ... dominate the regional pollen cloud. ... Now, some 40 to 80 generations later, only traces of the lodgepole pine remain ..."

Despite numerous studies of hybridization and introgression between *P. banksiana* and *P. contorta*, the determination of its directionality has been elusive (Critchfield 1985). Because of differential mitochondrial, chloroplast and nuclear inheritance in these two species (Wagner et al. 1991), our data indicate that *P. contorta* ancestors of the Saskatchewan population were predominantly successful as females. This conclusion is consistent with species differences in reproductive phenology (Critchfield 1980) and was implied by Rudolph and Yeatman (1982).

Amount of mtDNA diversity

Extant populations of *P. contorta* encounter wider latitudinal and elevational extremes and likely originated from more refugia than *P. banksiana* (Critchfield 1985), which may lead to greater diversity in *P. contorta*. Indeed, *Sst*I/*COXII* species-level diversity in *P. contorta* ($H_{es}=0.68$) is greater than that of most of the polymorphisms in this and other plants (Hamrick and Godt 1990). For example, in *P. contorta* species-level isoenzyme gene diversities range between 0.00 and 0.66 (Wheeler and Guries 1982), while the most variable known chloroplast polymorphism has $H_{es}=0.50$ (based on data from Wagner et al. 1987).

In sharp contrast, mitochondrial diversity in *P. banksiana* appears to be almost non-existent outside the introgressed Saskatchewan population and is lower than isoenzyme or cpDNA diversity in this species (Dancik and Yeh 1983; Wheeler and Guries 1987; Wagner et al. 1987). We note, however, that this apparent difference in mtDNA diversities between *P. banksiana* and *P. contorta* is due solely to the *Sst*I/*COXII* polymorphism – excluding Saskatchewan, *Pst*I/*COXI* is monomorphic in both species (Table 1).

Population structure of mtDNA diversity

The degree of mtDNA differentiation among sampled *P. contorta* populations is extraordinary for a single genetic marker in wind-pollinated plants (Hamrick and Godt 1990). Although multi-locus analyses reveal differentiation (Westfall and Conkle 1992), single-locus F_{st} values typically average only 0.10 or less in conifers (Hamrick and Godt 1990). In particular, allozymic F_{st} rarely exceeds 0.06 in *P. contorta* (Wheeler and Guries 1982). Neither are geographic patterns evident for paternally inherited cpDNA polymorphisms (Wagner et al. 1987).

The few other surveys in plants of maternally inherited polymorphisms have also found high levels of population differentiation. Mitochondrial *COXI*-associated F_{st} values are as large as 0.88 in three other *Pinus* species of western North America (Strauss et al. 1993). F_{st} values of oak (*Quercus* L.) chloroplast polymorphisms are similarly large (Whittemore and Schaal 1991; Petit 1992). The prediction that maternally inherited genetic markers would be geographically informative in plants (Birky 1988; Petit 1992) appears to have been validated.

Interestingly, the Santiam Pass, Oregon population aligned most closely in our mitochondrial dendrogram with a var *'latifolia'* population, yet approximately 40% of the Santiam Pass individuals shared a two-marker haplotype that was fixed in the California var *'murrayana'* population (Fig. 2, Table 1). These results support previous isoenzyme and morphological evidence of influence in the Oregon Cascades from both subspecies (Wheeler and Guries 1982).

Despite significant differentiation among *P. contorta* populations (Table 2), several phenetic relationships (Fig. 2) contradict traditional taxonomy. For example, *P. contorta* populations did not generally cluster within subspecies. A mitochondrial phenogram also differed from traditional taxonomies in the only other mtDNA survey of conifers (Strauss et al. 1993). This type of discordance may be due to rapid mitochondrial evolution

(Palmer 1990) and/or to the limited number of mitochondrial markers studied.

The number of available polymorphisms is certainly an important factor because UPGMA analysis of *SstI/COXII* genetic identities failed to place the Colorado population within *P. contorta* (analysis not shown). In contrast, treatment of *PstI/COXI* and *SstI/COXII* as independent markers correctly grouped the Colorado population within *P. contorta* (Fig. 2). It will be interesting to learn why *COXI*-associated restriction fragments so conclusively distinguish *P. banksiana* from *P. contorta* (Table 1), yet lack such resolution in several other pines (Strauss et al. 1993). Clearly, populational relationships of mtDNA diversity must be viewed tentatively at present.

Nonetheless, the substantial population differentiation exhibited by maternally inherited genetic markers in plants is remarkable. This differentiation indicates potentially powerful roles for such markers in intraspecific biogeography, including the designation of breeding zones and the development of germ plasm conservation strategies.

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References

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu Rev Ecol Syst 18:489–522

Birky CW, Jr (1988) Evolution and variation in plant chloroplast and mitochondrial genomes. In: Gottlieb LD, Jain SK (eds) Plant evolutionary biology. Chapman & Hall, London, pp 23–53

Clegg MT (1989) Molecular diversity in plant populations. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer Assoc, Sunderland, Mass., pp 98–115

Conde MF, Pring DR, Levings CS III (1979) Maternal inheritance of organelle DNAs in *Zea mays – Zea perennis* reciprocal crosses. J Hered 70:2–4

Critchfield WB (1980) Genetics of lodgepole pine. Research paper WO-37, US Dep Agric, Washington, D.C.

Critchfield WB (1985) The late Quaternary history of lodgepole and jack pines. Can J For Res 15:749–772

Dancik BP, Yeh FC (1983) Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var *'latifolia'*) and jack pine (*P. banksiana*) in Alberta. Can J Genet Cytol 25:57–64

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

Glaubitz JC, Carlson JE (1992) RNA editing in the mitochondria of a conifer. Curr Genet 22:163–165

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 Assoc, Sunderland, Mass., pp 43–63

Moss EH (1949) Natural pine hybrids in Alberta. Can J Res Sect C 27:218–229

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

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Palmer JD (1988) Intraspecific variation and multicircularity in Brassica mitochondrial DNAs. Genetics 118:341–351

Palmer JD (1990) Contrasting modes and tempos of genome evolution in land plant organelles. Trends Genet 6:115–120

Petit RJ (1992) Polymorphisme de l'ADN chloroplastique dans un complexe d'especes: les chenes blancs Europeens. Subdivision de la diversité des genes cytoplasmiques chez les plantes. PhD thesis, University of Paris XI

Rieseberg LH, Beckstrom-Sternberg S, Doan K (1990) *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. Proc Natl Acad Sci USA 87:593–597

Rudolph TD, Yeatman CD (1982) Genetics of jack pine. Research paper WO-38, US Dep Agric, Washington, D.C.

Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman and Co., San Francisco

Strauss SH, Hong Y-P, Hipkins VD (1993) High levels of population differentiation for *coxI*-associated mitochondrial DNA haplotypes in *Pinus radiata*, *muricata*, and *attenuata*. Theor Appl Genet 86:605–611

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, Dong J, Carlson MR, Yanchuk AD (1991) Paternal leakage of mitochondrial DNA in *Pinus*. Theor Appl Genet 82:510–514

Weir BS (1990) Genetic data analysis: methods for discrete population genetic data. Sinauer Assoc, Sunderland, Mass.

Westfall RD, Conkle MT (1992) Allozyme markers in breeding zone designation. New For 6:279–309

Wheeler NC, Guries RP (1982) Population structure, genic diversity, and morphological variation in *Pinus contorta* Dougl. Can J For Res 12:595–606

Wheeler NC, Guries RP (1987) A quantitative measure of introgression between lodgepole and jack pines. Can J Bot 65:1876–1885

Whittemore AT, Schaal BA (1991) Interspecific gene flow in sympatric oaks. Proc Natl Acad Sci USA 88:2540–2544