SPATIAL PATTERNS OF MITOCHONDRIAL DNA VARIATION WITHIN JACK AND LODGEPOLE PINE POPULATIONS

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Abstract.--We have studied maternally inherited mitochondrial variation in a sample of 1655 individuals from six natural populations of jack and lodgepole pines *(Pinus banksiana* Lamb., *Pinus contorta* Doug).) in Alberta, Canada. Diversity was sufficient for analyses of within-population spatial distributions in one allopatric lodgepole pine population and three mixed-species populations. Surprisingly, no mitochondrial variants typical of jack pine were found in the three mixed populations. Spatial patterns were nonrandom in the three mixed populations, but random in the lodgepole pine population. These results provoke speculation that within-population mitochondrial spatial patterns may be restricted to hybridizing or introgressed populations. This conjecture is testable and has general implications for population genetic studies, as well as for germplasm improvement and conservation programs.

Keywords: *Pinus banksiana* Lamb., *Pinus contorta* Dougl., spatial autocorrelation, maternal inheritance

INTRODUCTION

Nonrandom spatial patterns of genetic variation within populations can result from the action of evolutionary forces. For example, effective gene flow tends to eliminate such patterns, while mating by proximity promotes their development (Epperson 1993). Similarly, natural selection can counteract dispersal and produce genotypic clusters within populations (Epperson and Allard 1989). However, interpretation of spatial patterns is complicated by many factors (Slatkin and Arter 1991).

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Regardless of the evolutionary cause(s) of spatial patterns, their presence or absence within populations is relevant to the exploration activities of germplasm improvement and conservation programs (e.g., Epperson 1992). Also, such patterns affect population genetic statistics that are sensitive to pooling of genetically subdivided samples, such as gametic-phase disequilibria (e.g., Prout 1973).

It is well established that organellar markers strengthen population genetic and systematic studies (e.g., Avise *et al.* 1987). Pines *(Pinus* L.) have special advantages for such studies, due to their paternal chloroplast yet predominantly maternal mitochondrial inheritance (Schnabel and Asmussen 1989; Dong *et al.* 1992; Dong and Wagner 1993). Consequently, we have been including organellar markers in our studies of natural hybridization and introgression in jack and lodgepole pines.

Organellar markers are no different than nuclear markers in requiring us to understand spatial patterns prior to interpretation of population genetic data. In preliminary work, we found that chloroplast DNA (cpDNA) variants, despite their paternal inheritance, form patches within populations in a sympatric region of natural hybridization between jack and lodgepole pines (Wagner *et al.* 1991). Here we show that mitochondrial genotypes can also form patches in mixed-species populations.

METHODS

Based on the distributional ranges of the two species (Critchfield 1985), we sampled two allopatric lodgepole pine populations, two allopatric jack pine populations, and two populations in a sympatric region of natural hybridization between the two species, all located in Alberta (Table 1). The two sympatric populations are those in which cpDNA spatial pattern was detected previously (Wagner *et al.* 1991). We reused the sympatric DNA samples, but DNA's from the other four populations were extracted from new foliage collections.

Within the sampled area of each population, we sampled all cone-bearing trees. Distances and compass bearings between trees were recorded, in order to map the locations of all sampled individuals.

A mitochondrial *coxll* restriction fragment length polymorphism (RFLP) was assayed as described by Dong and Wagner (1993), except that a 1:1 mix of two cloned white spruce *(Picea glauca* (Moench) Voss) coxll-associated restriction fragments (Sutton *et al.* 1991) was used to probe pine *Sstl* fragments from the four allopatric populations (instead of a maize cox// probe; Fox and Leaver 1981). The maize *coxII* probe was used to probe DNA of the two sympatric populations. Comparative assays showed that the maize and white spruce probes identified the same RFLP (T. Li and D.B. Wagner, unpublished data).

We studied spatial patterns by spatial autocorrelation analysis of genotypes, the computational methods of which have been described in detail by Sokal and Oden (1978). Briefly, an analysis of each genotype in each population leads to a plot of

Table 1. Mitochondrial Variant Frequencies in Samples from Six Natural Populations

	Population Names and Initial Classifications of Population Typea					
	Allopatric Lodgepole Pine		Sympatric		Allopatric Jack Pine	
Variant[b]	Coleman	Edson	Carson Creek	Windfall	Wandering Riverc	Bellis
2.9-7.6 (jack pine)						288
3.1-10.2 (lodgepole pine)	137	37	41	31	95	
5.2-10.2 (lodgepole pine)	2	202	232	422	161	
4.4-10.2			1			
6.8-10.2			1			
8.1-10.2					5	
Total	139	239	275	453	261	288

[a] Allopatric populations are named by nearest town. Additional information on sympatric populations available in Wagner *et al.* (1991); Bellis location shown in Dong and Wagner (1993). See also footnote "c" regarding classification of Wandering River population.

[b] Variants are named by sizes, in kilobase pairs, of *SstI* fragments hybridizing with *coxII* probes (this nomenclature includes only variable fragments). Species origin indicated parenthetically for each variant if established by surveys (Dong and Wagner 1993; T. Li and D.B. Wagner, unpublished data).

[c] The Wandering River location was reclassified as a mixed-species population after analysis (see text for details).

standard normal deviates (SND's) as a function of distance (i.e. a correlogram, e.g., Figure 1). Each SND was associated with a specific range of distances between trees and was based on observed and randomly expected numbers of pairs of trees in which the two trees of a pair both had the same genotype. One additional correlogram was constructed for each population, based on the total observed and expected numbers of pairs of all possible combinations of unlike genotypes (TU). **SND's** were computed by Pascal programs (Wagner *et al.* 1991).

Inspection of correlograms permits interpretation of spatial pattern (Figure 1). For example, organellar TU SND's are inversely related to gene identity probabilities (Epperson 1993). Thus, significant negative TU SND's in small distance classes, together with non-negative TU **SND's** in higher distance classes, imply the existence of genotypic patches.

We accepted an SND as a "valid" test of spatial pattern only if its expected number of pairs was greater than one (Cochran 1954). Because we computed many individual SND's, we used Sidak's probability *(ps=1-(1-m)k)* to evaluate overall statistical significance of mitochondria! spatial structure within each population (Oden 1984). Correlograms of different genotypes within a population are interdependent, because genotypic frequencies sum to one. Thus, for each population we conservatively took m as the minimum valid individual p value, and k as the total number of valid SND's, in all correlograms of the population.

RESULTS AND DISCUSSION

Population Subdivision and Hybridization/Introgression

Except for the Wandering River population, each allopatric population appeared monospecific, based on cone morphology, cpDNA, and mitochondria] DNA (mtDNA). However, the Wandering River (putatively jack pine) sample contained a mixture of jack and lodgepole pines, hybrids, and/or hybrid derivatives (T. Li and D.B. Wagner, unpublished data). Therefore, we treat Wandering River hereafter as a mixed-species population, rather than as a jack pine population.

Predictably (Petit *et al.* 1993), mitochondrial variant frequencies (Table 1) differed between the two allopatric lodgepole pine populations (x^2 =240.8, d.f. =1, p <0.001). Although variant frequencies also differed statistically among the three mixed populations (x^2 =115.2, d.f.=4, p<0.001), the magnitude of these frequency differences was less striking. Strong mitochondrial population subdivision has been reported previously in these pines (Dong and Wagner 1993).

A rangewide survey of jack and lodgepole pines (Dong and Wagner 1993) permitted us to ascertain the species origin of mtDNA variants (Table 1). We encountered only jack pine mtDNA in the Bellis population, but, surprisingly, we found no mtDNA variant typical of jack pine in any mixed-species population. In contrast, chloroplast genotypes and cone morphologies typical of both species were

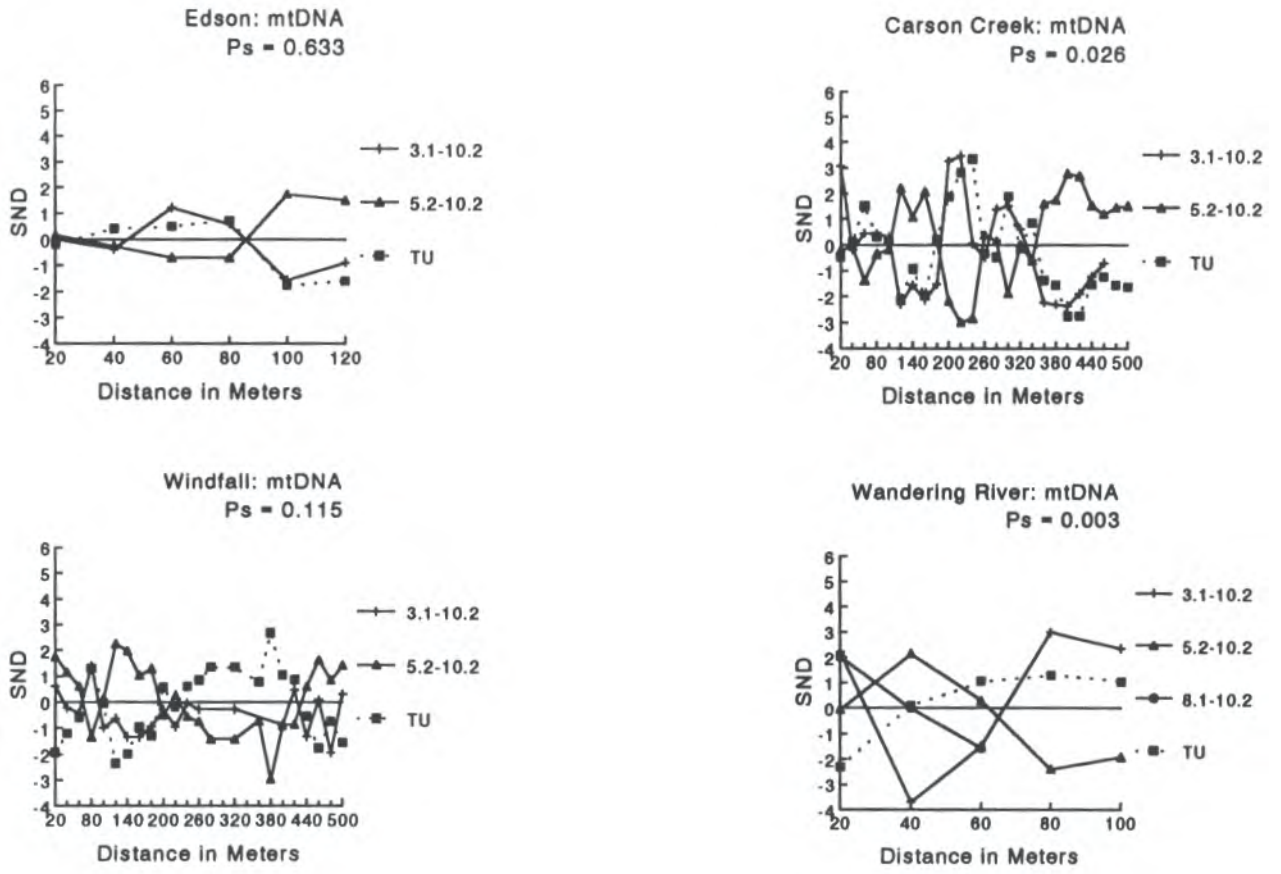

Figure 1. Mitochondrial correlograms, depicting only "valid" **SND's (i.e.,** those with expected numbers of pairs greater than 1). Edson is an allopatric lodgepole pine population; Carson Creek, Windfall, and Wandering River are mixed-species populations. Sidak's overall probability (p_s) is indicated for each population.

found in all three mixed populations (Wagner *et al.* 1991; T. Li and D.B. Wagner, unpublished data). Due to the mode of organellar inheritance in pines, these results indicate unidirectional hybridization in which lodgepole pine tends to serve as the female parent. The replacement of jack pine mtDNA by lodgepole pine mtDNA is compatible with phenological differences between the two species (Critchfield 1980) and has also occurred in a jack pine population located in an ancient sympatric region, hundreds of kilometers east of current areas of hybridization (Dong and Wagner 1993).

Spatial Autocorrelation

Two of the sampled populations were each fixed or nearly fixed for a single variant (Table 1). Thus, spatial analyses are restricted to the Edson lodgepole pine population and the three mixed populations (Figure 1).

At Edson, we detected no deviation of mtDNA variants from a random distribution (p_s=0.633). However, mitochondrial spatial patterns were significantly nonrandom in two of the mixed populations, Carson Creek and Wandering River (Figure 1). Although p_s=0.115 for the Windfall population, this probability is conservative (Oden 1984) and two features of this third mixed population's correlograms suggest spatial pattern. First, each variant's SND is positive in the lowest distance class (the 5.2-10.2 variant's SND's are, in fact, positive in the first three distance classes). Second, the TU SND is negative in its lowest three distance classes (significantly so in the 0-20 meter distance class).

Previous allopatric isoenzyme studies in these two species detected little spatial pattern, except for loci on chromosome segments that may be subject to selection (Epperson and Allard 1989; Xie and Knowles 1991). Studying mtDNA variants, we too failed to detect spatial pattern in an allopatric lodgepole pine population (Edson). Thus, the assumption that genotypes are usually randomized spatially within populations of conifers (and other wind-pollinated, outcrossing plants with efficient dispersal mechanisms) may not depend on the mode of inheritance. However, it would be premature indeed to advance this notion as more than conjecture, after study of mitochondria! diversity in only one allopatric population and in the face of prediction that spatial patterns could be strong for maternally inherited markers (Petit *et al.* 1993).

The nonrandom distributions of mtDNA variants found in all three mixed populations may represent an effect of natural hybridization. This effect could arise through any of several mechanisms, including reproductive and genomic incompatibilities between jack and lodgepole pines (e.g., Critchfield 1980).

Note that the three mixed populations' mtDNA variants were mostly typical of lodgepole pine (Table 1). It is intriguing that the mitochondrial spatial pattern observed in these populations involved variants of only one of the two hybridizing species. We speculate that physiological effects of the lodgepole pine mitochondrial

genotypes may be variable and dependent on the genetic backgrounds in which they occur. Ongoing studies of chloroplast and nuclear genetic markers in these same populations may permit tests of hypotheses arising from this speculation.

CONCLUSIONS

1. Maternally inherited mitochondrial genotypes can form patches within sympatric populations of jack and lodgepole pines.

2. Mitochondrial spatial patterns are population specific; *a priori,* such patterns may not be predictable for a population of interest.

3. Limited seed dispersal may not be the most important factor responsible for mitochondrial spatial patterns; mechanisms associated with natural hybridization may be equally or more influential.

4. This report of cytoplasmic spatial patterns within populations is not isolated (van Damme 1986; Wagner *et al.* 1991); thus, failure to account for spatial structure may lead to serious artifacts (e.g., Prout 1973).

ACKNOWLEDGEMENTS

We thank M. Depper, N.K. Dhir, A. Evans, W.E. Gladstone, L. Graham, C. Hansen, A.J. Sikora, and R. Thomson (Alberta Forestry, Lands and Wildlife) for locating allopatric sampling sites and for authorizations; B.C.S. Sutton for white spruce *coxll* clones; and M. Dusenberry, C.H. Hamilton, S. Magruder, M. Prince, D. Talbot, and L. Taylor for field and laboratory assistance. We especially thank Canada Immigration for its considerable interest and involvement in this project upon our field crew's arrival at the U.S.-Canada border in North Portal, Saskatchewan. This work was supported in part by USDA grants 85-FSTY-9-0149, 90-37290-5681, and KY00640 and by the Kentucky Agricultural Experiment Station. The investigation reported in this paper (no. 95-09-087) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the director.

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