in susceptibility between subpopulations within these populations.

Acknowledgements

The authors wis to thank Dr. K. G. Eldridge and the CSIRO Division of Forestry and Forest Products for providing the seedlings used in this study and B. M. Hine for carrying out the eight year assessment at Riamukka.

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Spatial Genetic Structure Within Two Natural Stands of Black Spruce (Picea mariana (Mill.) B.S.P.)

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(Received 11th January 1990)

Abstract

Needle tissue from approximately 500 adjacent trees in each of two lowland black spruce stands was electrophoretically analysed to resolve 5 and 8 loci respectively. All trees were mapped and clonal groups were identified on the basis on field evidence, identity of multilocus genotypes ,and map distances. Revised maps of all seed-origin trees were constructed to portray genotypes for each locus and examined visually for evidence of spatial genetic patterning. Genotype data matrices were statistically analysed for spatial autocorrelation by calculating Moran's I. The visual and statistical approaches yielded similar results indicating an overall distribution of genotypes that is nearly random with a few loci indicating a patchy substructure. Evidence for low levels of inbreeding in black spruce may be related to this spatial

genetic structure. It is proposed that isozyme determination of adjacent population members is a valuable approach for elucidating population structure by distinguishing sexual versus asexual origin and that spatial autocorrelation analysis can be a robust statistical procedure for assessing genetic patterning within forest tree populations.

Key words: Black spruce, *Picea mariana*, genetic structure, isozymes, spatial autocorrelation, family structure.

Introduction

It has been well established that levels of genetic variation in tree species are relatively high with most of it residing within populations (HAMRICK, 1979). To date, research emphasis has focused mainly on examining the pattern of variation over widespread geographic areas, that is, among populations (e. g., WHEELER and GURIES, 1982; LEDIG and CONKLE, 1983; DANCIK and YEH, 1983; FURNIER and ADAMS, 1986). Such work is crucial for elucidating evolutionary processes operating on a macrogeographical scale. However, an obvious question remains conspicuous for its neglect. Is there a spatial pattern to the substantial genetic variation residing within populations of forest trees? Such information would provide valuable progress in ascertaining the extent of microgeographical evolutionary processes such as family clustering or microsite selection effects.

Detection of spatial patterning or clustering of biological traits has traditionally involved a spatial autocorrelation analysis that was introduced into the scientific literature more that a decade ago (JUMARS *et al.*, 1977; SOKAL and ODEN, 1978a, 1978b). Methodological refinements and new statistical techniques since then have been numerous (CLIFF and ORD, 1981; SOKAL and WARTENBERG, 1981; 1983; ODEN, 1984; GETIS and FRANKLIN, 1987). Such procedures have proven useful for determining genetic and geographic associations in a variety of species such as salamanders (KARLIN *et al.*, 1984), aphids (BIRD *et al.*, 1981), beetles (KING, 1987), and perennial plants (WASER, 1987).

Clustering of genetic traits within stands of forest trees remains relatively obscure. Evidence of non-random distribution of genotypes within a population has been provided by SAKAI and PARK (1971) for *Cryptomeria japonica*, SAKAI and MIYAZAKI (1972) for *Thujopsis dolabrata*, LINHART *et al.* (1981) for *Pinus ponderosa*, KNOWLES (1984) for *Pinus contorta*, FURNIER *et al.* (1987) for *Pinus albicaulis*, and SPROULE (1988) for *Picea mariana*. On the other hand, a number of investigators failed to find evidence supporting genetic clustering such as TIGERSTEDT (1973), GURIES and LEDIG (1977), TIGERSTEDT *et al.* (1982), and ROBERDS and CONKLE (1984). EPPERSON and ALLARD (1989) found a nearly random distribution of genotypes over space within lodgepole pine populations with a few loci demonstrating a pattern.

The objective of the present study is to apply spatial autocorrelation procedures to assess whether electrophoretically determined genotypes are distributed randomly over space within each of two lowland stands of black spruce (*Picea mariana* (MILL.) B.S.P.). This species is a widely distributed boreal conifer that can reproduce sexually or asexually by layering of lower branches (STANEK, 1968). It is considered to have evolved with fire resulting in semiserotinous cones and the demographic characteristic of even-aged stands.

Materials and Methods

Two widely separated lowland black spruce stands were chosen, one within the city limits of Thunder Bay, Ontario, and the other 90 km west of the city. The fisrt site (Balmoral) had naturally regenerated after a clearcut in 1968 and was bordered to the north by an undisturbed mature black spruce stand. The second stand (Spruce River) was less dense, undisturbed and bordered to the north and west by a clearcut. The processes of sampling, mapping and isozyme analyses for the two sites were conducted in consecutive years; procedural differences reflect technical improvements over time or site-specific limitations.

Sampling for the Balmoral stand consisted of mapping all black spruce trees within a 30 meter radius of a randomly chosen central position and collecting needle tissue from each tree. Likelihood of clonal origin was noted for each tree based on field observation by tugging foliage

Table 1. — Enzyme systems assayed in needle tissue of Black Spruce.

Enzyme System	E.C. Number	Locus Abbreviation	Buffer System[1)]
Aspartate aminotransferase	2.6.1.1	*Aat*	B
Aldolase	4.1.2.13	*Ald*	A
Diaphorase	1.6.4.3	*Dia-3*	B
Glycerate-2-dehydrogenase	1.1.1.29	*G2d*	A
Malate Dehydrogenase	1.1.1.37	*Mdh-3*	A
Phosoglucose isomerase	5.3.1.9	*Pgi-2*	B
Phosphoglucomutase	2.7.5.1	*Pgm*	A
Shikimic acid dehydrogenase	1.1.1.25	*Skdh*	A

[1)] Buffer Systems:
A: Histidine-EDTA-tris pH 7.0 gel buffer; tris-citrate pH 7.0 electrode buffer (FLORENCE, 1981). Run at 200 volts.
B: Tris-citrate pH 8.5 gel buffer; lithium borate pH 8.1 electode buffer (RIDGEWAY *et al.*, 1970). Run at 300 volts.

of small trees to note movement in any adjacent stems. Such observation for clonal origin was inappropriate for the Spruce River census plot due to its more advanced maturity. This plot was rectangular in shape, approximately 42 m by 140 m. Trees were mapped and needle tissue was removed for laboratory analysis.

Isozyme analysis for the Balmoral site was conducted by grinding 0.5 g of needle tissue in liquid nitrogen with a buffer solution (extraction buffer #5: Cheliak and Pitel, 1984a). Five enzyme systems on four buffer systems were resolved as follows: aspartate aminotransferase (AAT) on tris-citrate (Mitton *et al.*, 1977), glycerate-2-dehydrogenase (G2D) and phosphoglucose isomerase (PGI) on citric acid (Ridgeway *et al.*, 1970), shikimic acid dehydrogenase (SKDH) on histidine-citric acid (Fildes and Harris, 1966), and phospholglucomutase (PGM) on histidine (Cheliak and Pitel, 1984a). The Spruce River site samples were ground in the same extraction buffer without liquid nitrogen. Running procedures are listed in *table 1*. Allele and genotype frequencies were tallied and an F-statistic (F_{IS}) was calculated using the procedures of Nei (1977).

Genotypes were plotted graphically for examination of clonal membership and spatial patterning of genetic markers using a two-step process. First, clonal membership was determined for the Balmoral site on the basis of map distances (only propinquitous trees could reproduce by layering), identity of multilocus genotype, and field observation data. For the Spruce River site, clonal identity was determined for each group of trees with identical multilocus genotypes by evaluating map distances with the calculated probability of that particular multilocus genotype. In other words, any two trees with identical multilocus genotypes that were close enough for layering were considered to be of clonal origin. The mapping data matrix was then revised for each site to represent individual seed-origin trees. A single representative for each clone was mapped at the Euclidean centre of the clonal group. Second, 14 computerized plots were constructed, one for each locus at each site with distinct symbols for each genotype.

Randomness of the distribution of genotypes was assessed visually from these single-locus genotype plots as well as statistically using a spatial autocorrelation statistic, Moran's I (Cliff and Ord, 1973) calculated with the Fortran program SAAP written by D. Wartenberg which uses the equations presented by Cliff and Ord (1973). Generally, this procedure calculates the distance between each pair of seed-origin trees within a site and assigns each pair to one of four distance classes, the ranges of which were determined by equalizing sample sizes among the classes. Binary weightings of 1 are assigned to pairs within each distance class and 0 to all other possible pairs. These weightings are then correlated using non-parametric techniques to genetic similarity with separate correlations for each distance class.

The assessment of genetic similarity between pairs of individual trees is made possible by the transformation of the raw data matrix of genotypes according to Smouse and Neel (1977) which acts to quantify the nominal genotype data, thus incorporating a portion of the meaning of an allele frequency into each genotype score. For each locus all alleles except the most common were analysed in this manner. This transformation has proven useful for delineating patterns over geographically widespread ares using multivariate procedures (e.g., Dancik and Yeh, 1983). The present application however, is a novel approach to the assessment of spatial patterning over microgeographical distances. Previous analyses of spatial autocorrelation within plant populations have used gene frequencies of sampling units of groups as small as 3 plants (Waser, 1987) or autocorrelation of the raw genotype scores (Epperson and Allard, 1989). The present transformation combines the advantages of both these approaches.

Finally, spatial correlograms were constructed by plotting the autocorrelation coefficients against the distance classes. These correlograms were tested for significance using the Bonferrroni procedure (Oden, 1984). This tests for significance in the trend of a series of non-independent Moran's I values.

Results

The two sampled stands differed markedly in age and number of clonal members (*Table 2*) with the older stand (Spruce River) showing a lower density and fewer clonal representatives. A description of the genetic data is presented in *table 3* and the calculated F_{IS} statistics for the loci in common are listed in *table 4*.

The computerized plots of genotypic distributions indicated a variety of patterns as detected by visual inspection. The spatial distributions of genotypes in the majority of plots (e. g. *Pgm-2, Skdh* both sites; *Pgm-3,* Balmoral; *Pgi, MdH, Aat-2, Ald, G2d,* Spruce River) were best interpreted as random. *Figure 1* illustrates this for the second allele of *Aat* at Spruce River with the third allele indicating a grouping trend. Similarly, patterns detected in alleles 2 and 3 of *Pgi* at Balmoral show

Table 2. — Stand Description Data.

	Balmoral	Spruce River
Mean age (approx)	9	110
No. of trees sampled	507	496
No. of seed-origin trees	244	411
No. of non-clonal trees	182	350
No. of clones	62	61
Mean: #trees/clone	5.24	2.39
Range: No. of trees/clone	2-44	2-4

Table 3 — Allele frequencies at the Balmoral and Spruce River sites.

		Balmoral	Spruce River Road
Aat	1	.920	.955
	2	.072	.039
	3	.008	.006
Ald	1	--	.982
	2	--	.018
Dia	1	--	.991
	2	--	.009
G2d	1	.969	.927
	2	.012	.033
	3	.040	.019
Mdh-3	1	--	.883
	2	--	.089
	3	--	.009
	4	--	.006
	5	--	.001
	6	--	.012
Pgi-2	1	.897	.904
	2	.076	.075
	3	.027	.021
Pgm	1	.479	.542
	2	.292	.276
	3	.224	.175
	4	.0	.007
Skdh	1	.636	.500
	2	.348	.499
	3	.016	.001

Table 4. — F_{IS} statistics for the black spruce populations at the Balmoral and Spruce River sites.

	F_{IS}	
Locus	Balmoral	Spruce River
Aat	-.042	-.013
G2d	.113	-.004
Pgi-2	.171	-.073
Pgm	.037	.063
Skdh	.159	-.010
Mean	.093	.011

evidence of tight and slightly looser clumping respectively (*Figure 2*). Of the 20 correlograms tested for significance using the Bonferroni procedure, 5 showed statistically significant trends.

The spatial autocorrelation statistic (Moran's I) for the four distance classes at each site (*Tables 5* and *6*) confirms the visual interpretation from the graphical plots. The significant positive correlations in the shortest distance classes indicate that like genotypes are more often associated than unlike genotypes. Most of the significant Moran's I values occur as negative correlations in the longest distance classes which is interpreted as the tendency for data pairs or joins to have dissimilar genotypes in these classes. Finally, an example correlogram representing a summary graphical portrayal of the Moran's I values for one locus is presented in *figure 3.*

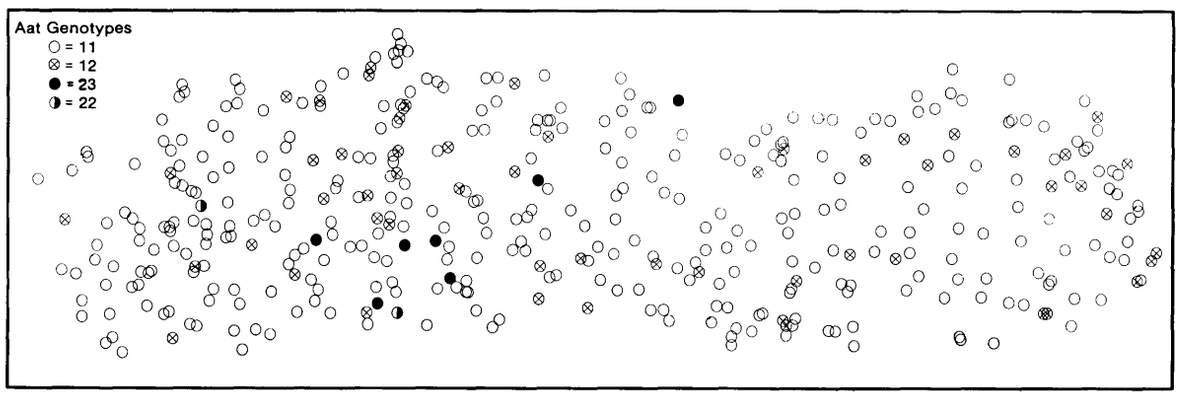

Figure 1. — Spatial distribution of *Aat* alleles in the Spruce River site. Symbol locations designate mapped tree locations.

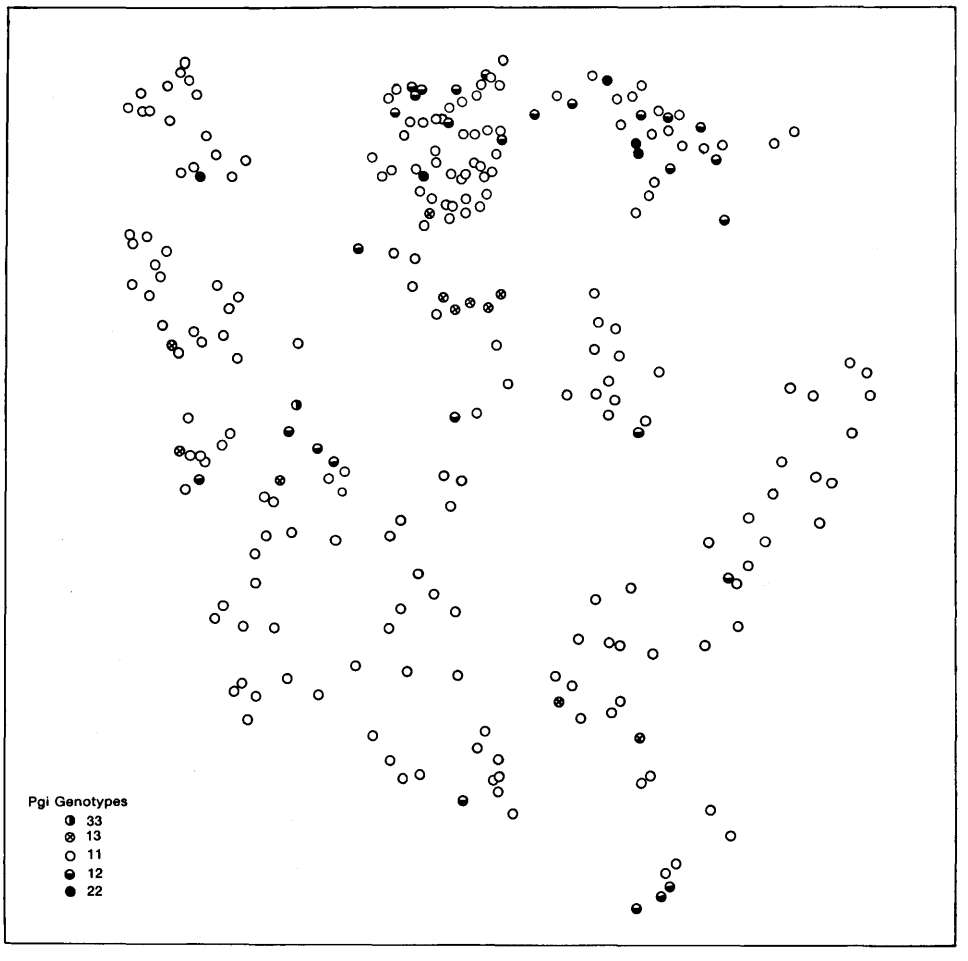

Figure 2. — Spatial distribution of *Pgi* alleles in the Balmoral site. Symbol locations designate mapped tree locations.

Table 5. — Spatial autocorrelation coefficients (MORAN'S I) for seven alleles and four distance classes in Black Spruce at the Balmoral site. Only those alleles with a minimum absolute occurence of 6 were included.

		Distance Class (meters)				
Locus	Allele	0-7.5	7.5-12	12-16.50	16.50-30.0	Significance of Correlogram
Aat	2	.004	-.009	.016	-.027**	*
G2d	1	.007	-.001	.005	-.028**	n.s.
Pgi	2	.035**	.001	-.017	-.037**	**
	3	.016	-.035**	.002	.001	**
Pgm	2	-.006	.002	-.007	-.005	n.s.
	3	-.007	-.002	-.011	+.004	n.s.
Skdh	2	+.009	-.018	-.004	-.003	n.s.

*) $p < 0.05$
**) $p < 0.01$

Discussion

These results indicate that the distribution of alleles over space within two lowland black spruce stands is almost random with evidence of a small amount of genetic patterning. However, the extent of this substructuring might best be described as patchy and subtle. In other words, clumping of genotypes is detectable but is not necessarily consistent among loci, nor does it impose a strong pattern to the distribution of genotypes over space. These findings are consistent with those of EPPERSON and ALLARD (1989) who proposed that the absence of strong evidence of patch structure in their

Table 6. — Spatial autocorrelation coefficients (MORAN's I) for thirteen alleles and four distance classes in Black Spruce at the Spruce River Road site. Only those alleles with a minimum absolute occurence of 6 were included.

Locus	Allele	Distance Class (meters) 0-24	24-43.5	43.5-73	73-147	Significance of Correlogram
Pgm	2	.006	-.002	-.003	-.011	n.s.
	3	.002	.009	-.005	-.015**	n.s.
Skdh	2	.000	-.005	-.010	.006	n.s.
Pgi	2	-.009	-.002	.000	.001	n.s.
	3	-.009	.002	-.002	-.001	n.s.
Mdh-3	2	-.006	-.006	-.001	.004	n.s.
	3	-.006	.000	-.004	.000	n.s.
Aat	2	-.001	-.010	.002	.000	n.s.
	3	.017**	-.013	-.012	-.001	**
Ald	2	.000	.009	-.002	-.017**	*
Dia	2	-.008	-.001	-.001	.000	n.s.
G2d	2	.006	-.005	-.012	.002	n.s.
	3	-.001	-.005	-.003	.000	n.s.

**) $p < 0.01$
*) $p < 0.05$

lodgepole pine study sites may result from high levels of gene flow.

Interpretation of the significant positive Moran's I coefficients in the shorter distance classes of this study is straightforward and taken as evidence of clumping of individuals caused by undetermined forces such as assortative mating, restricted dispersal of pollen and seed, or microsite selection. The significant negative coefficients in the longest distance classes is potentially caused by similar forces. Alternatively, it may be evidence of "genealogic clusters" (SPROULE, 1988), which are distantly related trees (descendants of a common, distant generation) distributed more widely throughout a stand.

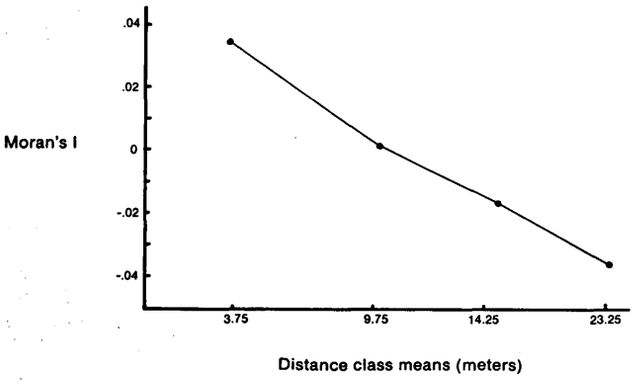

Figure 3. — Correlogram of spatial autocorrelation coefficients (MORAN's I) for *Pgi-2* allele at the Balmoral site.

Is this weak pattern of substructuring consistent with slightly restricted gene flow or assortative mating in these stands? The F_{IS} statistic would support such an association. A substantial excess of homozygotes as indicated by the sizeable positive mean for the youngest stand (Balmoral) coincides with the larger proportion of statistically significant correlograms. The older Spruce River site, on the other hand, shows less evidence of both inbreeding with the quantitatively lower F_{IS} value, and substructuring, with the smaller proportion of significant correlograms. Note that alternative or additional explanations such as heterogeneous selection regimes over space or time cannot be ruled out due to lack of salient evidence.

Further, these results supplement those of previous studies (CHELIAK and PITEL, 1984b; HUENNEKE, 1985) in underscoring the value of the electrophoretic technique for identifying clonally reproduced individuals within a population. These two populations differ markedly in their clonal structure with proportions of seed-origin trees varying from 48% in the Balmoral site to 83% in the Spruce River population. The major ecological factors differentiating these two stands include age, density, and type of disturbance before stand establishment. Is there any biological foundation to the association of anthropogenic disturbance such as clear-cutting and increased dependence on vegetative reproduction as seen in the Balmoral stand? This question awaits a closer scrutiny with a greater selection of stand types.

Finally, the results of this study validate the use of the statistical procedure, spatial autocorrelation analysis,

for delineating genetic substructure using data matrices comprised of transformed individual genotypes. A comparison between the computer maps of genotypes over space with the spatial autocorrelation coefficients, Moran's I, for each site and locus indicates excellent agreement. All of the relatively tight clusters that were visually identified were also detected statistically and vice versa. The negative significant correlations in the longer distance classes were easily identifiable on the computer maps.

This statistical approach to delineating spatial genetic structure warrants caution and a knowledge of the population biology of the species under investigation. Dependence on the calculation of Moran's I correlation coefficients without visual mapping procedures risks misinterpretation of rare clustering configurations over space. For example, tight clusters of infrequent alleles separated by relatively long distances can escape detection by this correlation coefficient depending on cluster number, size, and allele frequency. Furthermore, the establishment of biologically reasonable distance classes would be optimally based on knowledge of neighbourhood sizes such as the inbreeding-effective genetic neighbourhood value of WRIGHT (1969). Such information is unfortunately limited, particularly for forest tree systems. In the absence of actual metric distances associated with neighbourhood sizes for this species, I have used the statistical justification of equalizing sample sizes as criterion for distance class establishment. However, given the magnitude of the data matrix (>82,000 joins or data pairs at the Spruce River site), the analysis would remain robust with slightly unequal class sizes. A biological justification for determination of sizes of distance classes should take precedence. It is hoped that the present objective of introducing a method to quantify spatial genetic structuring within populations will provide a useful statistical tool for accelerating the understanding about microevolutionary forces operating in forest tree populations.

Acknowledgements

I am grateful to DAN PERRY for analytic assistance, and for field and laboratory help I thank THERESA HOLLA, SUSAN SAMPSON, LEA TARNOWSKY, and KEVIN WEAVER. KAREN SPINNEY-HELMER provided graphics assistance. Access to computer programs provided by DAN WARTENBERG is appreciated. This project was supported financially by NSERC operational grant A7652.

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