POLLEN POOL HETEROGENEITY IN NATURAL STANDS OF UPLAND AND LOWLAND BLACK SPRUCE (*Picea mariana* (Mill.) B.S.P.)

by

Kevin B. Weaver ©

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ABSTRACT

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Keywords: upland and lowland black spruce, isozyme, pollen pool heterogeneity, genetic structure, mating systems, *Picea mariana*, ecological correlates.

One of the basic assumptions of the mixed-mating model is that the pollen pool of a population is homogeneous. However mounting evidence would suggest that a homogeneous pollen pool is not the norm in populations of forest tree species. Such a phenomenon may have major implications upon the mating systems and genetic structure of forest tree species. The present study was conducted in order to develop a better understanding of the nature and implications of pollen pool heterogeneity in natural populations of black spruce. Four natural stands of black spruce within 100 kilometers of Thunder Bay, Ontario were studied using isozyme markers from eight polymorphic loci. The stands studied represent the two basic ecological conditions under which black spruce is found, i.e. upland and lowland stands. Both log-likelihood G tests and multiple group discriminant analysis indicated the presence of heterogeneous pollen pools for all four sites. However, as indicated by the canonical R^2 for the first discriminant function of each site, the separation power of data from three of the four sites was quite small (R^2 of 0.063 to 0.127). Only for Raith 2 was the seperation power strong (R^2 of 0.379). Overall the heterogeneity tended to be random in nature. Due to the apparent random nature of the heterogeneity it was not possible to associate the examined site characteristics with the relative degree of pollen pool heterogeneity observed in the four sites. Possible agents which may have produced a heterogeneous pollen pool are considered as are potential implications of this phenomenon on the genetics of black spruce.

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Kevin B. Weaver

INTRODUCTION

Examination of the genetic structure of black spruce (*Picea mariana* (Mill.)B.S.P.) and many other tree species has shown that the greatest portion of genetic variation, as demonstrated by isoenzyme data, resides within individual populations (Hamrick *et al.*, 1979; Boyle, 1985; Yeh *et al.*, 1986). The apparent lack of population differentiation within a species has traditionally been attributed to the cohesive nature of long distance gene flow (Mitton *et al.*, 1977). Yet in recent years genetic examination of plant and animal populations has led to the conclusion that gene flow is far more restricted than previously believed. Furthermore, natural selection, not gene flow, would appear to be the key force controlling the differentiation of species (Ehrlich and Raven, 1969; Endler, 1973; Gleaves, 1973; Levin and Kerster, 1974; Slatkin, 1981). Concurrently it has been recognized that the genetic variability and evolutionary potential of plant populations are profoundly affected by the mating system (Clegg, 1980; Shea, 1987).

Recently a substantial amount of research has provided new insight in our understanding of plant mating systems and population structure. Much of this work has been stimulated by the availability and efficiency of isozyme markers which enable rapid inference of genetic characteristics (Brown and Moran, 1979; Brown *et al.*, 1985). Evaluation of plant populations' mating systems, including most conifer species, is often achieved through the use of the mixed-mating model (Clegg, 1980; Waller and Knight, 1989).

A principle assumption of the mixed-mating model is that the pollen gene frequency distribution be uniform over all maternal plants (Clegg, 1980; Brown *et al.*, 1985). Because conifers are wind pollinated, genetically highly variable, and can exhibit high levels of inbreeding depression it has been assumed that reproduction is essentially panmictic. Due to casual acceptance of the assumption of pollen pool homogeneity, the role of gene flow via pollen dispersal, has generally been examined in only a cursory fashion. Often such analyses have been treated as simply an aside to more comprehensive studies of mating systems and population structure. Yet mounting evidence would suggest that non-random gene frequency distribution of the pollen pool is common in many forest tree species (Cheliak *et al.*, 1985a;1985b; King *et al.*, 1984; Sproule, 1988; Hamrick and Schnabel, 1985; Yeh and Morgan, 1987; Merzeau *et al.*, 1989; Perry and Knowles, 1990).

Non-random pollen gene frequency distribution, or pollen pool heterogeneity, results in pollen genotype frequency differences among sub-populations throughout the population. Selective forces as well as various forms of isolation and chance events may be responsible for this phenomenon.

Black spruce is one of the most abundant conifers in North America with a range extending across the continent (Fowells, 1965; Hosie, 1979; Fowler and Mullin, 1977). The species is found in extensive pure stands on lowland sites and often in association with jack pine on uplands (Sims *et al.*, 1990). Because of its long wood fibres the species is of particular value to the newsprint

industry (Hearnden, 1975; Fowler and Mullin, 1977; Hosie, 1979). Research into the genetics of black spruce began in the mid-1960's. More recently, research has focused on the population structure and mating system of black spruce. Although generally considered an outcrosser the species is known to tolerate a significant degree of inbreeding which may provide it with an evolutionary advantage as a pioneering species (Park and Fowler, 1984). Beyond this point little is known about the evolutionary genetics of the species. Furthermore, little is known about the extent and nature of pollen pool heterogeneity in black spruce nor are the implications of such a phenomenon upon the mating system and evolutionary genetics of the species well understood. Conspicuous for its absence is our understanding of pollen pool heterogeneity in black spruce.

This study was designed to test two hypotheses:

1) The pollen pools of natural populations of black spruce are heterogeneous, and

2) The relative degree of heterogeneity exhibited for any population is dependent upon the ecological conditions under which stand development occurred.

LITERATURE REVIEW

ECOLOGY AND GENETICS OF BLACK SPRUCE

Black spruce is one of the most abundant conifers in North America. Its range extends across the continent from coast to coast and from 41° to 68° latitude (Fowells, 1965; Hosie, 1979; Fowler and Mullin, 1977). The species is of particular value to the forest industry and is a principal species used in newspaper production (Hearnden, 1975; Fowler and Mullin, 1977; Hosie, 1979). Black spruce is found on a wide range of sites and is often in continuous pure stands on lowland organic soils as a pioneer species (Sims *et al.*, 1990). On upland sites in northern Ontario the species is most commonly found in association with jack pine where it is considered to be an intermediate successional species (Sims *et al.*, 1990). Black spruce regenerates vegetatively on moist organic sites via layering of lower branches primarily (Stanek, 1961; Hosie, 1979). As well the species will readily reproduce sexually with frequent good seed years (Wilton, 1963; Groot, 1984).

Initial investigations into the genetics of black spruce began in the mid-1960's with a range-wide provenance study initiated by the Canadian Forestry Service, now Forestry Canada (Morgenstern, 1978). Results of this study indicated an overall clinal pattern for phenological and growth parameters based upon photoperiod and temperature patterns (Morgenstern, 1978; Park and Fowler, 1988). Similar north-south clinal trends have been observed for a variety of northern forest tree species Wright (1973). Based upon

morphological traits 81 to 94 percent of the total genetic variation in black spruce was associated with the geographic distribution of provenances, however, the six to nineteen percent of within-provenance variation was significant for within-provenance seed source delineation (Morgenstern, 1978).

Working at three range-wide black spruce provenance study locations Khalil (1984) observed high intra-provenance variation and significant differences between replicates. This work was important for two reasons: 1) intra-provenance variation of the better provenances should increase genetic gain through intense within-provenance family selection; and 2) differences between replicates demonstrated the sensitivity of black spruce to micro-environmental variation existing within the study locations.

While these provenance studies have concentrated upon growth parameters, selective forces can operate differently on reproductive and vegetative processes. Good growth does not necessarily ensure good reproductive capabilities (Wright, 1973; Harper, 1977; Barrett, 1985). Reflecting this view, research into the genetics of black spruce has taken essentially two approaches: examination of variation in growth, e.g. genotype-environment interactions, and examination of evolutionary and reproductive strategies, i.e. population structure/mating systems.

In examining genotype-environment interactions Maliondo and Krause (1985) and Mullin (1985) identified significant interactions between families and soil nutrient status. In particular Maliondo and Krause (1985) noted that variation in family performance for

black spruce increased with increased soil nutrient availability.

On a broader scale, genotype-environment studies have attempted to determine if upland/lowland edaphic ecotypes of black spruce exits. However, studies employing mensurational characteristics, isozymes, and flavonoid analysis have found no conclusive evidence to support the concept of such ecotypes for black spruce (Fowler and Mullin, 1977; Parker *et al.*, 1983; O'Reilly *et al.*, 1985). O'Reilly *et al.* (1985) note that the extremes in environments associated with upland and lowland sites result in entirely different sets of selective pressures working on black spruce gene pools at the two site types. It is possible that black spruce, as a pioneering species, may maintain a high level of flexibility in its genetic structure so that a wide range of sites might be utilized without a change in the species' gene pool (Mullin, 1985).

O'Reilly *et al.* (1985) found a slight excess of heterozygotes in lowland stands while heterozygotes were slightly deficient in upland stands. Due to differential selection lowland sites, being more favourable for the seedling establishment, may exhibit a wider range of genotypes and thus greater levels of heterozygosity while genotypes of upland spruce represent a more restricted subset of the regional gene pool (Mullin, 1985; O'Reilly *et al.*, 1985).

In recent years efforts have been placed upon understanding the mating system and population structure of black spruce. Examination of inbreeding in black spruce has shown that while

controlled crosses can produce relatively high levels of self-fertility (47%) such levels are not exhibited under natural conditions (Park and Fowler, 1984). Indeed, allozyme studies for natural populations indicate that black spruce is predominantly an outcrosser (Boyle and Morgenstern, 1986; Sproule, 1988). Although the influence of inbreeding varied considerably between families, in general inbreeding resulted in fewer filled seed, increased embryonic death due to genetic load, and poorer seedling performance (Park and Fowler, 1984). However the ability of black spruce to tolerate high levels of inbreeding may be an adaptive strategy when pioneering a site and for advanced generation populations which exhibit increased intra-population relations i.e. layering on bog sites (Park and Fowler, 1984).

In terms of population structure, allozyme studies for natural populations of black spruce have attributed over 94% of observed genetic diversity to within-population variation (Yeh *et al.*, 1986; Boyle, 1985). In general, conifers existing in large continuous stands can be broken up into smaller breeding units based on mating among relatives, limited pollen and seed dispersal and other such factors (Yeh *et al.*, 1986). Such may be the case for black spruce.

Although Boyle (1985) and Knowles (1990) observed little family structuring in black spruce, significant near neighbour inter-relations and spatially heterogeneous allelic frequencies have been identified for both black and white spruce (O'Reilly *et al.*, 1985; Cheliak *et al.*, 1985a; Yeh *et al.*, 1986). From these

results it would seem that discrepancies exist in our understanding of the role of genotypic heterogeneity in black spruce population structure. Some studies favour the idea of clusters of related individuals making up a population while other reports support the concept of a homogeneous population of randomly distributed individuals.

For white spruce significant levels of pollen pool heterogeneity as well as the existence of clusters of related individuals within populations has been observed (Cheliak *et al.*, 1985a; King *et al.*, 1984). In a study by Coles and Fowler (1976) increased genetic load, observed following controlled pollinations of white spruce from two natural populations of white spruce, was associated with a clustering of related individuals within these two populations. While these results may support the notion of heterogeneity in black spruce population structure it should be remembered that the two species grow under different conditions with white spruce rarely forming pure stands.

Similar to findings for white spruce, studies in a black spruce clonal orchard demonstrated the existence of a spatially heterogeneous pollen pool as a result of imbalances in effective male to female ratios and clonal variation in fecundity (O'Reilly *et al.*, 1982; Barrett, 1985). Sproule's (1988) work indicates that similar pollen pool heterogeneity may also be found in natural populations of black spruce. However, to date, no studies have taken an in-depth look into the nature of pollen pool heterogeneity in natural populations of black spruce.

MATING SYSTEMS AND POPULATION STRUCTURE

According to Clegg (1980), in the study of evolutionary genetics there are three basic areas to consider:

1. adaptive processes,
2. quantifying observed levels of genetic variation, and
3. processes of genetic transmission at the population level.

Jain (1975) considers the examination of the mating systems of species to be a key feature of plant population biology. In terms of population structure, mating system is of importance for determining both genetic structure and the manner in which genetic variation will be transferred from generation to generation (Clegg, 1980; Boyle and Morgenstern, 1986; Brown *et al.*, 1975; Brown *et al.*, 1985; Cheliak *et al.*, 1983; Hamrick and Schnabel, 1985). Because of the influence of mating systems on population structure most studies of forest tree species population genetics examine structure and mating systems simultaneously (Mitton *et al.*, 1977; Cheliak, 1985; Boyle, 1985, Cheliak *et al.*, 1985a; King *et al.*, 1984; Brown *et al.*, 1975; Yeh and Morgan, 1987; Bannister, 1965; Boyle and Morgenstern, 1986).

A knowledge of tree species mating system has practical applications both in terms of plant breeding and genetic conservation. Examination of mating systems in seed orchards has concentrated on the implications of orchard design on genetic gain (Park and Fowler, 1984; Ritland and El-Kassaby, 1985; Cheliak,

1985; Muller-Starck, 1982; Hamrick and Schnabel, 1985; O'Reilly *et al.*, 1982). While studies of natural populations aid in our understanding of evolutionary processes and in the development of gene conservation strategies (Jain, 1975; Brown *et al.*, 1985; Hedrick, 1985a; Mitton *et al.*, 1977).

There are three basic types of mating systems: predominantly outbreeding, predominantly inbreeding, and mixed self-fertilizing and outcrossing (Hedrick, 1985b). In addition Jain (1976) identified apomixis and vegetative reproduction as "mating systems".

Inbreeding has often been associated with negative connotations such as reduced vigour, and increased genetic load (Jain, 1976; Park and Fowler, 1984). However inbreeding species generally exhibit an excess of heterozygotes relative to outbreeders (the *heterozygosity paradox*, Brown (1979)). For an inbreeding species, variation in selective pressures, due to a patchy environment, may be more important for the maintenance of an overall global polymorphism than heterozygote advantage (Jain, 1975;1976; Hedrick, 1985b). Furthermore inbreeding may be an important reproductive method for colonization, reproduction under conditions of low population densities, and rapid adaption to changes in selective forces (Jain, 1975; Jain, 1976; Bannister, 1965; Hedrick, 1985a; Mitton *et al.*, 1977).

Most studies of plant mating systems are based on a "mixed-mating" model (Brown *et al.*, 1985; Hamrick and Schnabel, 1985; Ritland and El-Kassaby, 1985; Boyle and Moregenstern, 1986; Clegg,

1980; Hedrick, 1985b). Because most inbreeders exhibit some level of outcrossing and vice versa a mixed-mating model is often most appropriate (Clegg, 1980; Hedrick, 1985a). The maintenance of some plasticity in reproductive strategy allows a species to respond to variations in environmental factors (Bannister, 1965; Jain, 1975; Jain, 1976; Clegg, 1980). As discussed by Clegg (1980) and Brown *et al.* (1985), the basic assumptions of the mixed-mating model are:

1. Mating events are due to random outcrossing (t) or self-fertilization (s);
2. Uniform pollen gene frequency distribution over all maternals;
3. Rate of outcrossing is independent of maternal genotype.

Additional assumptions for studies employing electrophoretic data include: segregation of alleles according to Mendelian inheritance, and no selection between the time of fertilization and the time at which progeny are assayed (Brown *et al.*, 1985).

There are of course some problems with these assumptions. The influence of structured populations (on microgeographic scale) and restricted pollen dispersal may limit the validity of a uniform pollen gene frequency over all maternals (Clegg, 1980; Ennos and Clegg, 1982; Brown *et al.*, 1985; Cheliak *et al.*, 1985a; King *et al.*, 1984; Cheliak, 1985; Mitton *et al.*, 1977; Yeh and Morgan, 1987). As well the assumption that inbreeding is due solely to self-fertilization ignores the potential for consanguineous matings. Such an assumption may result in under-estimating

outcrossing particularly in populations where seed dispersal is restricted and/or vegetative reproduction has produced a family structure (Brown *et al.*, 1985; Park and Fowler, 1984). Finally, the influence of embryonic selection, identified for many conifers (Brown *et al.*, 1985; Park and Fowler, 1984; Cheliak *et al.*, 1985b; Yeh and Morgan, 1987), may upwardly bias outcrossing estimates.

A population can be considered structured if localized sub-populations exhibit genetic drift; mating is not random throughout the population; or the probabilities for migration are not equal throughout the population (Heddrick, 1985a). In essence population structure is primarily a function of the ability to disperse genes via pollen and seed (Hamrick and Schnabel, 1985). Low population density or restriction of pollen/seed dispersal will result in small neighbourhood size and thus produce a structured population (Sewall Wright cited in Wright, 1976). In contrast Gleaves (1973) felt that localized clinal patterns were the result of barriers to gene flow and selective forces as a result of a heterogeneous environment. This apparent discrepancy actually highlights two groups of evolutionary processes which influence a population's genetic structure. The first group considers the interaction of various evolutionary forces in association with population structure i.e. genetic drift and migration, while the second group considers spatial and temporal distributions of selection intensities, i.e. genotype-environment interactions and heterogeneous environments (Dickson and Antonovics, 1973; Park and

Fowler, 1984; Parker *et al.*,1983; Wright, 1973; Mullin, 1985; Maliondo and Krause, 1985; Fowler and Mullin, 1977; O'Reilly *et al.*, 1985).

As stated by Jain (1975) "*the extremes of population structure are represented by the species with one large and continuous random mating population (perhaps hypothetical) and those with one or more small discontinuous populations (isolates)*". All factors except selection and mutation contribute to a structured population (Hedrick, 1985a). Within any structured population the genetic relatedness of sub-populations depends on the level of effective gene flow between sub-populations (Hedrick, 1985a).

Species can be distributed in a linear habitat i.e along a river bank, or in a two dimensional arrangement (Jain, 1975). Likewise, gene flow or migration can be in two modes: 1) stepping-stone model where each sub-population exchanges genes only with neighbouring sub-populations, and 2) island model, where each sub-population exchanges genes with each other sub-population (Jain, 1975; Hedrick, 1985a). A simpler model of population structure, presented by Hedrick (1985a) is the continent-island model in which migration is unidirectional from a large "continental" population to a smaller "island" population. However this model is less applicable to the real world. Such models measure the joint effects of genect drift and migration.

One of the key features of a subdivided population is that such a structure encourages the retention of variability for a species because of "*increased number of and wider conditions for*

genotypic frequency equilibria" (Jain, 1975). When outcrossing is high, effective neighbourhood size small, and migration low, genetic drift encourages homozygosity. In contrast high levels of selfing, migration, and sub-division allow a higher level of heterozygosis than expected in a single large population (Jain, 1975).

A key feature to consider then is neighbourhood size which is limited primarily by pollen and seed dispersal (distance dependent), and breeding system. This brings into consideration the role of gene flow, particularly pollen dynamics, in population genetics.

GENE FLOW IN FOREST TREES

Gene flow is a collective term which includes all mechanisms resulting in the movement of genetic material from one population to another, (Slatkin, 1985a). There are two basic forms of gene flow; *Potential*, the deposition of pollen on a receptive surface as a function of distance, and *Actual*, the fertilization of an ovule and/or the establishment of reproducing individuals as a function of distance (Levin and Kerster, 1974).

The perceived role of gene flow in terms of evolutionary processes has changed over the past three decades (Slatkin, 1985a). Traditionally, gene flow was considered the principle cohesive force preventing the differentiation of populations within a species (Mayr, 1963; Stanley, 1979; Wallace 1968 cited in Hedrick, 1985a). This view identified the species as the principle

evolutionary unit (Levin and Kerster, 1974). However, today it is commonly accepted that gene flow is far more restricted than previously believed and that natural selection is the key force controlling the differentiation of species (Ehrlich and Raven, 1969; Endler, 1973; Gleaves, 1973; Levin and Kerster, 1974; Slatkin, 1981). Yet, the degree of gene flow varies by and within species (Ehrlich and Raven, 1969; Endler, 1973; Slatkin, 1985a). Furthermore observed dispersal distances of most species do not account for the apparent level of gene flow Slatkin (1989). Such discrepancies suggest that there is no single role for gene flow.

In general the examination of gene flow is concentrated in two areas: 1) determining the level of gene flow for a species under various conditions, and 2) determining the role of gene flow under a given set of circumstances. Our inability to clearly define the role of gene flow is associated with limitations in the methods used to estimate the level of gene flow in natural populations. Although computer modelling has been widely used particularly when examining the role of gene flow in structured populations and clines validation using data from natural populations is required before such models can be used for evaluating evolutionary implications (Jackson and Pounds, 1979).

Direct and indirect methods exist for estimating gene flow. Direct measures indicate conditions existing at the time of assessment while indirect measures estimate average levels of gene flow over time (Slatkin, 1985a). Observation of dispersing individuals and mark/recapture techniques are useful in determining

potential gene flow however examination of the spread of unique alleles permits the evaluation of actual gene flow (Slatkin, 1985a; Gleaves, 1973; Bateman, 1974). As well observation of pollinating and dispersal agents are often used to infer dispersal. In contrast, indirect methods use such features as the spatial distribution of alleles, phenotypic traits, or chromosal segments to draw inferences about the level or pattern of gene flow in a population. Wright's inbreeding coefficient, F, is often used for this type of evaluation. This technique is particularly useful because of the ability to partition F to evaluate population and sub-population levels of inbreeding (Slatkin, 1985a).

Another indirect method employed by Slatkin (1981, 1985b) is the use of rare alleles which are particularly sensitive to gene flow. Based on the properties of conditional average frequencies the method gives qualitative results of gene flow. Furthermore, through the use of private alleles (alleles which are unique to one individual within a population) quantitative estimates of neighbourhood size and quantitative estimates of gene flow can be obtained (Slatkin, 1981; 1985b). However possible bias associated with selection favouring a specific allele for a given site and inadequate sampling of adjacent demes may under-estimate gene flow using private alleles (Slatkin, 1985b).

Two additional indirect methods have also been employed. The first method, spatial autocorrelation, examines the correlation of different alleles in relation to spatial distribution, while the second method, genetic distance, provides a quatitative measure of

differences in two or more sets of allele frequencies (Slatkin, 1985a). Nei (1972) suggests that genetic distance can be used to estimate migration and while there is no formal justification it is generally assumed that the smaller the genetic distance the greater the gene flow. It is possible that genetic distance may be a useful means of estimating the elements of migration within structured populations (Slatkin 1985a).

In terms of forest tree species one of the principle means of gene flow is through pollen dispersal. A thorough understanding of gene flow via pollen is essential in both tree improvement (Lanner, 1966) and our understanding of evolutionary genetics. In the past, dispersal of pollen over great distances was believed to be both common and necessary to prevent the differentiation of a species (Polunin, 1951). While many instances of long distance pollen dispersal have been reported such events are not the norm nor are they considered effective as a form of gene flow (Polunin, 1951; Stanley and Liskens, 1974; Wright, 1953). In terms of realistic pollen dispersal most pollen is dispersed along a concentration gradient with the greatest portion of pollen deposited near the source (Silen, 1962; Boyer, 1966; Wang *et al.*, 1960; Wright, 1952; 1953; Stanley and Liskens, 1974). For a variety of angiosperms the activities of the pollinating vectors (primarily insects) limit the dispersal of pollen to near neighbours (Grant, 1949; Schall, 1975; Ennos and Clegg, 1982). It is likely that such restrictions of pollen dispersal exist in wind pollinated species as well (Wright, 1976). As a result the majority of seed in natural stands results

from pollination by close neighbours (Wright, 1976; Levin, 1988).

In considering the mechanisms of wind pollination it is important to have an appreciation of the theory behind the dispersal of light particles (aerosols) (Strand, 1956; Green and Lane, 1964). As with other aerosols the rate of deposition of wind dispersed pollen is a function of terminal velocity accoriding to Stoke's law (Sutton, 1947; Green and Lane, 1964). However under natural conditions such factors as mechanical and thermal turbulence, meteorological conditions, topography, ground cover, and source type will modify pollen dispersal (Strand, 1957; Ebell and Schmidt, 1964; Green and Lane, 1964; Raynor, 1967; Raynor *et al.*, 1970; Moore, 1976; Fægri and van der Pijl, 1971; Sutton, 1932; Wright, 1976; Stanley and Liskens, 1974). Furthermore, under controlled conditions, Niklas (1984) has demonstrated a non-stochastic phenomena for three coniferous species in which ovulate cone morphology modifies airflow so as to promote entrapment of wind-borne pollen by the ovulate cones. Perhaps future research in this area will aid in our understanding of the implications of wind as a pollinating vector on the mating systems and population genetics of boreal forest tree species.

MATERIALS AND METHODOLOGY

FIELD PROCEDURES

Four natural stands of black spruce within a 100 kilometer radius from Thunder Bay, Ontario were examined. Within each stand the study area was centrally located to avoid border effect and provide as homogeneous an environment as possible. Two sites, Hick's Lake (HL) and William's Bog (WB), demonstrated properties characteristic of lowland spruce bogs while the remaining two sites, Raith 1 and 2 (R1 and R2), exhibited the superior growth commonly associated with upland black spruce (Table 1). These two stand types represent the ecological extremes under which black spruce is found (Fowells, 1965). Lowland sites are typically herb poor with a continuous ground cover of sphagnum and feathermoss. *Chamaedaphne calyculata* ((L.) Moench) and *Ledum groenlandicum* (Oeder) dominate the shrub layer and trees exist as widely spaced stunted clumps of black spruce (Sims *et al.*, 1989). In contrast upland sites are productive well-stocked stands with a diversity of flora.

Table 1: General characteristics of the four study sites.[a]

Site	Study Area (ha)	Ave. Age (yrs)	Height (m)	Basal Area (m^2/ha)	Percent[b] Stocking	Site[c] Class
R1	0.65	65	11.8	19.8	58	1
R2	0.69	65	13.1	20.4	60	1
HL	0.43	110	4.9	5.4	17	3
WB	0.86	109	5.9	8.6	27	3

[a] Additional site information is provided in Appendix 1.

[b] Stocking is an indication of stand density relative to the theoretical maximum density of a fully stocked pure stand (as defined in "Normal Yield Tables for major forest tree species of Ontario" (Plonski, 1981)).

[c] Site Class is an indication of site productivity by tree species. It is based on the relationship between average tree age and height and can be obtained from (Plonski, 1981).

Field collections were conducted between November of 1986 and March of 1987. Thirteen to fifteen trees were randomly selected from each stand. A straight line running the length of each stand was established as an X-axis. Using a table of random numbers X,Y coordinates were selected. The X coordinate was measured along the X-axis with the associated Y coordinate being measured out from the X coordinate at a 90° angle to the X-axis. The tree located nearest this location was selected for assessment. Exact location and basic mensurational data were then recorded for each sampled tree. All closed cones were collected for each sampled tree (approximately one litre of cones per tree). Due to the semi-serotinous nature of black spruce cones some seeds greater than one year in age were inevitably collected. However since the majority of black spruce seed is dispersed during the first year following cone maturation (Fowells, 1965) potential temporal influence from different seed years was considered to be minimal. Only those trees that provided a minimum of 26 assayed seeds were included in the analysis. The average number of seeds assayed per tree was 67 with as many as 109 seeds assayed for a single tree.

ELECTROPHORETIC PROCEDURES

Seed was extracted manually according to procedures described in the manual *Seeds of Woody Plants in the United States* (USDA, 1974) with seed lot integrity maintained by maternal tree from collection through to analysis. Germinants were grown to a minimum radicle length of 2mm on moist filter paper. Germination was

conducted in a growth chamber under a daily regime of 16 hours light, at 30°C, followed by 8 hours of darkness, at 20°C. Embryo and megagametophyte were separated and individually homogenized with two micro-litres of extraction buffer (Yeh and O'Malley, 1980) following which the tissues were electrophoresed according to Cheliak and Pitel (1984).

Eight polymorphic loci were used in this study (Table 2). Mendelian inheritance of the loci has been confirmed by Boyle and Morgenstern (1985) and Pitel *et al.* (1987). Pollen genotypes were determined by inference after comparing embryo genotypes with the corresponding megagametophytes.

Table 2: Enzyme systems assayed.

Enzyme system	EC No.	Abbreviation	No.of Loci
Aspartate aminotransferase	EC 2.6.1.1	AAT	1
Fumarase	EC 4.2.1.2	FUM	1
Glutamate dehydrogenase	EC 1.4.1.3	GDH	1
Isocitrate dehydrogenase	EC 1.1.1.42	IDH	1
Malate dehydrogenase	EC 1.1.1.37	MDH	1
Phosphoglucomutase	EC 2.7.5.1	PGM	1
Phosphoglucose isomerase	EC 5.3.1.9	PGI	1
6-Phosphogluconate	EC 1.1.1.44	6-PG	1

STATISTICAL ANALYSIS OF POLLEN POOL HOMOGENEITY

Two procedures were used for evaluating pollen pool homogeneity. The first procedure was a test of independence using a log-likelihood G-test (Sokal and Rohlf, 1981). Using a modification of the method described by Brown *et al.* (1975), the frequencies of pollen genotypes at a given locus were compared between trees with the same maternal genotype, i.e. trees with the maternal genotype

"11" at *Pgi* were examined together using one G-test while trees with a maternal genotype of "12" at *Pgi* were examined together in a seperate G-test. The test of independence was conducted for all four natural stands separately. In turn the data were pooled for stands of a similar type, i.e. lowland stands and upland stands. The pooled data sets were then analyzed using the log-likelihood G-test of independence. This allowed evaluation of overall differences between similar types of stands. For cases in which a significant G value was found, a Stepwise-Testing-Procedure (STP) test (Sokal and Rohlf, 1981) was employed to identify the source of significant heterogeneity. The STP test was conducted for both the individual and pooled stand data sets.

The second procedure, a multiple group discriminant analysis (MDA), tested for differences in pollen allele frequencies (Green, 1978). Individual and combined stand analysis was conducted using a canonical correlation approach for MDA. An F approximation, calculated using the MDA procedure, tested the equality of group centroids. Plotting of the canonical scores for group centroids allowed a graphical depiction of the discriminant space.

Data for this procedure were prepared in a manner similar to that employed by She *et al.* (1987), and Smouse and Neel (1977). Each allele was treated as a single dummy variable. If the pollen contribution for a given locus corresponded to allele "1" it was given a value of 1 otherwise it was given a value of 0. This procedure was conducted for each of the alleles in developing the restructured data set.

RESULTS

GENETIC CHARACTERISTICS OF STANDS

Based on the marker loci examined, 25 of the 51 maternal trees studied had a unique multilocus genotpe. However within any one of the four sites, over 62% of the trees examined had unique genotypes. Table 3 presents the maternal tree genotypes by site based on the eight marker loci employed. Allelic frequencies for filial and parental populations are presented in Table 4.

Table 3: Maternal tree genotypes by site.

Site: Raith 1

Tree	*AAT*	*PGI*	*GDH*	*PGM*	*FUM*	*6PG*	*MDH*	*IDH*
02,04	11	11	11	11	11	11	11	11
03,05	11	11	12	11	12	11	11	11
06	11	11	12	11	13	11	11	11
07	11	11	11	11	11	11	15	11
08	11	12	12	11	11	11	17	11
09	12	11	11	11	11	12	11	11
11	11	11	22	33	12	13	11	11
12	11	11	12	11	11	11	16	11
13	11	11	11	22	11	12	16	11
14	11	11	12	11	11	11	11	11
15	11	11	12	23	11	11	16	11

Site: Raith 2

Tree	*AAT*	*PGI*	*GDH*	*PGM*	*FUM*	*6PG*	*MDH*	*IDH*
01	14	11	11	11	11	11	11	11
02	11	11	11	12	11	11	15	11
03,05	11	11	11	12	11	11	11	11
04,11, 13	11	11	11	11	11	11	11	11
06	11	11	11	13	11	11	11	11
07	11	12	11	11	11	11	26	11
08	11	11	11	22	11	12	12	11
09	11	11	11	12	11	11	16	11
10	11	11	12	22	11	11	23	12
12	11	13	11	11	11	11	11	11

Site: Hick's Lake

Tree	*AAT*	*PGI*	*GDH*	*PGM*	*FUM*	*6PG*	*MDH*	*IDH*
01	11	11	11	13	11	11	12	11
02,03, 12	11	11	11	12	11	11	11	11
04	12	11	11	12	12	11	11	11
05	11	11	11	11	11	11	11	11
06	12	11	11	12	11	11	12	11
07	11	12	11	13	11	11	11	11
08	11	11	11	13	11	11	11	11
09	11	12	11	11	11	12	11	11
10	11	11	11	22	11	11	12	11
11	11	12	11	22	11	11	15	11

Site: William's Bog

Tree	*AAT*	*PGI*	*GDH*	*PGM*	*FUM*	*6PG*	*MDH*	*IDH*
01	11	11	12	13	11	11	11	11
03,09, 13	11	11	11	11	11	11	11	11
04	11	11	11	22	11	11	15	11
05	11	11	11	12	11	11	11	11
06	11	11	11	13	11	11	11	11
07	11	12	11	12	11	13	55	11
08	11	11	11	22	11	11	11	11
10	11	11	11	11	11	11	15	11
11	11	11	11	12	11	11	15	11
12	11	12	11	11	12	11	15	11
14	11	11	11	11	12	12	11	11

Table 4: Allelic frequencies for parental and progeny populations.

	Raith 1			Raith 2			Hick's Lake			William's Bog		
	Parent	Filial	Pollen	Parent	Filial	Pollen	Parent	Filial	Pollen	Parent	Filial	Pollen
AAT												
1	0.964	0.945	0.928	0.962	0.916	0.887	0.917	0.907	0.911	1.0	0.959	0.918
2	0.036	0.048	0.058		0.020	0.041	0.083	0.068	0.039		0.023	0.047
3		0.002	0.042		0.016	0.033		0.025	0.050		0.009	0.016
4		0.005	0.095	0.385	0.048	0.039					0.009	0.019
FUM												
1	0.964	0.959	0.982	1.0	0.988	0.975	0.958	0.965	0.983	0.923	0.950	0.968
2	0.036	0.041	0.018		0.012	0.025	0.042	0.035	0.017	0.077	0.050	0.032
GDH												
1	0.679	0.786	0.917	0.962	0.956	0.936	1.0	0.974	0.949	0.962	0.970	0.944
2	0.321	0.213	0.081	0.038	0.042	0.060		0.025	0.050	0.038	0.029	0.054
3		0.001	0.002		0.002	0.004		0.001	0.001		0.001	0.002
IDH												
1	1.0	0.998	0.996	0.962	0.979	0.986	1.0	0.999	0.999	1.0	1.0	1.0
2		0.001	0.002	0.038	0.021	0.014		0.001	0.001			
3		0.001	0.002									
MDH												
1	0.821	0.851	0.850	0.731	0.826	0.845	0.833	0.867	0.886	0.769	0.794	0.865
2		0.037	0.074	0.115	0.089	0.100	0.125	0.102	0.085		0.028	0.059
3		0.018	0.036	0.038	0.026	0.026		0.010	0.019		0.007	0.014
4		0.002	0.004		0.001	0.001		0.004	0.007		0.007	0.012
5	0.036	0.021	0.002	0.038	0.019	0.004	0.042	0.016	0.0	0.231	0.156	0.032
6	0.107	0.058	0.029	0.077	0.038	0.024		0.001	0.002		0.008	0.016
7	0.036	0.012	0.005						0.0		0.001	0.002
PGM												
1	0.643	0.573	0.567	0.654	0.678	0.639	0.500	0.542	0.582	0.654	0.610	0.592
2	0.214	0.268	0.284	0.308	0.242	0.229	0.375	0.340	0.281	0.269	0.292	0.247
3	0.143	0.158	0.148	0.038	0.080	0.132	0.125	0.117	0.136	0.077	0.097	0.160
4		0.001	0.001					0.001	0.001		0.001	0.001
PGI												
1	0.964	0.938	0.889	0.923	0.914	0.897	0.875	0.891	0.893	0.923	0.883	0.876
2	0.036	0.051	0.090	0.038	0.060	0.086	0.125	0.097	0.083	0.077	0.105	0.101
3		0.010	0.021	0.038	0.025	0.016		0.011	0.022		0.012	0.023
4					0.001	0.001		0.001	0.002			
6PG												
1	0.893	0.909	0.923	0.962	0.949	0.932	0.958	0.961	0.948	0.923	0.907	0.925
2	0.071	0.065	0.062	0.038	0.046	0.060	0.042	0.032	0.038	0.038	0.053	0.065
3	0.036	0.027	0.015		0.004	0.009		0.007	0.013	0.038	0.040	0.011

POLLEN POOL HOMOGENEITY

Log-likelihood G-test results

Examination of pollen pool homogeneity using the G statistic revealed heterogeneity of pollen allele frequencies in all four stands. Significant G_h tests are presented in Table 5. Six of the eight loci exhibited significant differences in pollen frequencies in at least one population. Results of STP tests conducted for locus/genotype combinations exhibiting a significant G_h are presented in Table 6.

Table 5: Loci exhibiting significant heterogeneity G values.

Stand	Locus	G_h	d.f.	Prob.	
Raith 1	*GDH*	39.282	20	0.01> $P(G_h)$	>0.005**
Raith 1	*MDH*	64.561	43	0.025> $P(G_h)$	>0.01 *
Raith 2	*GDH*	37.890	22	0.025> $P(G_h)$	>0.01 *
Raith 2	*PGM*	42.750	18	0.001> $P(G_h)$	***
Raith 2	*6PG*	35.796	22	0.05> $P(G_h)$	>0.025*
Raith 2	*MDH*	46.457	28	0.025> $P(G_h)$	>0.01 *
William's Bog	*AAT*	53.628	30	0.010> $P(G_h)$	>0.005**
William's Bog	*FUM*	19.406	9	0.025> $P(G_h)$	>0.01 *
Hick's Lake	*FUM*	22.817	10	0.025> $P(G_h)$	>0.01 *
Hick's Lake	*6PG*	37.169	20	0.025> $P(G_h)$	>0.01 *
Hick's Lake	*MDH*	56.991	34	0.01> $P(G_h)$	>0.005**

ns Not significant
* Significant at 0.05
** Very significant at 0.01
*** Highly significant at 0.001

Pooled data: Examination of pooled data identified significant heterogeneity of pollen allele frequencies at five loci for upland stands and four loci for lowland stands (Table 7). Review of STP results indicated that, in six of twelve cases, those trees which

Table 6: Step-wise Testing Procedure results for loci exhibiting a significant difference in pollen allele frequencies.

Stand	Locus	Geno	STP Ranking
Raith 1	*GDH*	11	Tree R113 R109 R107 R104 R102
			Freq.[a] .969 .968 .905 .850 .770
			Group[b] ----------
Raith 1	*MDH*	11	Tree R109 R106 R111 R103 R104 R105 R114 R102
			Freq. .968 .943 .927 .900 .850 .850 .821 .770
			Group ----------
Raith 2	*GDH*	11	Tree R207 R205 R201 R208 R209 R213 R211 R202 R204 R203 R212 R206
			Freq. .983 .980 .971 .965 .955 .946 .945 .944 .939 .930 .889 .826
			Group ----------
Raith 2	*PGM*	11	Tree R201 R207 R212 R213 R211 R204
			Freq. .879 .717 .692 .663 .618 .570
			Group ----
Raith 2	*6PG*	11	Tree R203 R211 R210 R205 R206 R212 R213 R207 R201 R202 R209 R204
			Freq. .983 .982 .960 .959 .955 .954 .948 .932 .922 .897 .889 .840
			Group ----------
Raith 2	*MDH*	11	Tree R212 R213 R205 R206 R203 R204 R211 R201
			Freq. .923 .918 .910 .870 .868 .862 .852 .755
			Group ----------
Hick's Lake	*FUM*	11	Tree HL02 HL05 HL07 HL09 HL10 HL03 HL12 HL01 HL08 HL06 HL11
			Freq. 1.00 1.00 1.00 1.00 1.00 .988 .986 .983 .983 .956 .919
			Group ----------
Hick's Lake	*6PG*	11	Tree HL12 HL08 HL10 HL07 HL03 HL11 HL02 HL05 HL04 HL06 HL01
			Freq. .957 .983 .972 .970 .964 .960 .954 .941 .915 .914 .873
			Group ----------
Hick's Lake	*MDH*	11	Tree HL02 HL04 HL08 HL09 HL07 HL12 HL03 HL05
			Freq. .868 .868 .862 .884 .954 .966 .965 .971
			Group ----------
William's Bog	*AAT*	11	Tree WB05 WB14 WB06 WB08 WB07 WB11 WB12 WB13 WB03 WB09 WB04
			Freq. .976 .956 .953 .945 .912 .895 .889 .867 .923 .902 .906
			Group ----------
William's Bog	*FUM*	11	Tree WB04 WB12 WB05 WB06 WB11 WB07 WB09 WB03 WB14 WB13 WB08
			Freq. 1.00 1.00 1.00 1.00 .981 .976 .976 .962 .932 .935 .904
			Group ----------

[a] Freq. is the frequency for genotype "1".
[b] Lines indicate grouping of trees.

accounted for heterogeneity in tests of individual stand data also accounted for heterogeneity in the pooled data. Overall the pollen allelic frequencies appear similar within the pooled upland and lowland data sets.

Table 7: Pollen heterogeneity among upland and lowland stands.

Stand	Locus	G_h	d.f.	Prob.	
Upland	*AAT*	90.973	69	0.05> $P(G_h)$ >0.025	*
Upland	*GDH*	73.305	32	0.001> $P(G_h)$	***
Upland	*PGM*	77.973	45	0.001> $P(G_h)$	***
Upland	*6Pg*	65.381	46	0.025> $P(G_h)$ >0.01	*
Upland	*MDH*	146.982	91	0.005> $P(G_h)$ >0.001	**
Lowland	*AAT*	99.783	62	0.005> $P(G_h)$ >0.001	**
Lowland	*FUM*	45.282	21	0.005> $P(G_h)$ >0.001	**
Lowland	*6Pg*	63.802	39	0.025> $P(G_h)$ >0.01	*
Lowland	*MDH*	133.862	77	0.005> $P(G_h)$ >0.001	**

Multiple Group Discriminant Analysis (MDA) results

MDA procedures were used to examine pollen allele frequencies for 11 to 14 trees within each site using up to 16 restructured allelic variables (Tables 8a & b). Tests of overall discrimination were highly significant for all four sites (Table 8a). Total variation (% trace, Table 8b) accounted for by the first two discriminant functions of each site was large. However the amount of variation attributable to differences among groups (based on the canonical R^2, Table 8b) was in general quite small. The exception to this was the data of Raith Site 2 which exhibited the greatest separation power. For Raith 2 the first discriminant function alone accounted for 52.8% of the total variation of which 37.9% (canonical R^2 of 0.379) was attributed to differences among trees (Table 8b).

Figure 1 depicts pollen pool heterogeneity in two dimensional canonical space for Raith 2. As can be seen, three sample trees (R201, R207, and R210) account for the heterogeneity exhibited in the Raith 2 data.

Pooled data: As with the univariate G-test the data were pooled to examine differences between the grouped upland and lowland sites.

Table 8a: Multiple Discriminant Analysis results: Equality of group centroids.

Site	No. Trees	No. Var.	F	Degrees of Freedom	Prob. > F	
William's Bog	11	16	1.478	160 & 4169	0.001> P(F)	***
Hick's Lake	12	13	1.375	143 & 4914	0.005> P(F) >0.001	**
Raith Site1	14	14	1.251	182 & 7622	0.025> P(F) >0.01	**
Raith Site2	13	15	4.562	180 & 7411	0.001> P(F)	***

Table 8b: Multiple Discriminant Analysis results: Canonical discriminant functions.

Site	Dis.[a] Fun.	Can.[b] R^2	χ^2	d.f.	Prob. > χ^2		%[c] trace
William's	1	0.127	234.372	160	0.001> P(χ^2)	***	29.73
Bog	2	0.097	166.512	135	0.05> P(χ^2) >0.025	*	21.87
Hick's	1	0.094	195.576	143	0.005> P(χ^2) >0.001	**	30.27
Lake	2	0.084	137.392	120	0.150> P(χ^2) >0.100	ns	25.37
Raith	1	0.063	226.929	182	0.025> P(χ^2) >0.01	*	23.17
Site 1	2	0.051	174.964	156	0.200> P(χ^2) >0.150	ns	18.68
Raith	1	0.379	788.069	180	0.001> P(χ^2)	***	52.80
Site 2	2	0.159	408.867	154	0.001> P(χ^2)	***	16.32
	3	0.144	271.345	130	0.001> P(χ^2)	***	14.56
	4	0.055	147.559	108	0.01> P(χ^2) >0.005	**	5.06

[a] "Dis. Fun." refers to the canonical discriminant function.
[b] "Can. R^2" refers to the canonical correlation coefficient.
[c] "% trace" refers to the percent of total variation accounted for by the discriminant function.

Differences between upland and lowland sites accounted for 59.2% (based upon canonical R^2) of the variation accounted for by the single discriminant function. However the single function was found to be non-significant. Furthermore, an overall test of the equality of group centroids found no significant difference between upland and lowland pooled data. In this test the calculated F

equalled 1.651 with 22 and 25 degrees of freedom (P>0.05). These results suggest that the pollen pools of the two site types are similar.

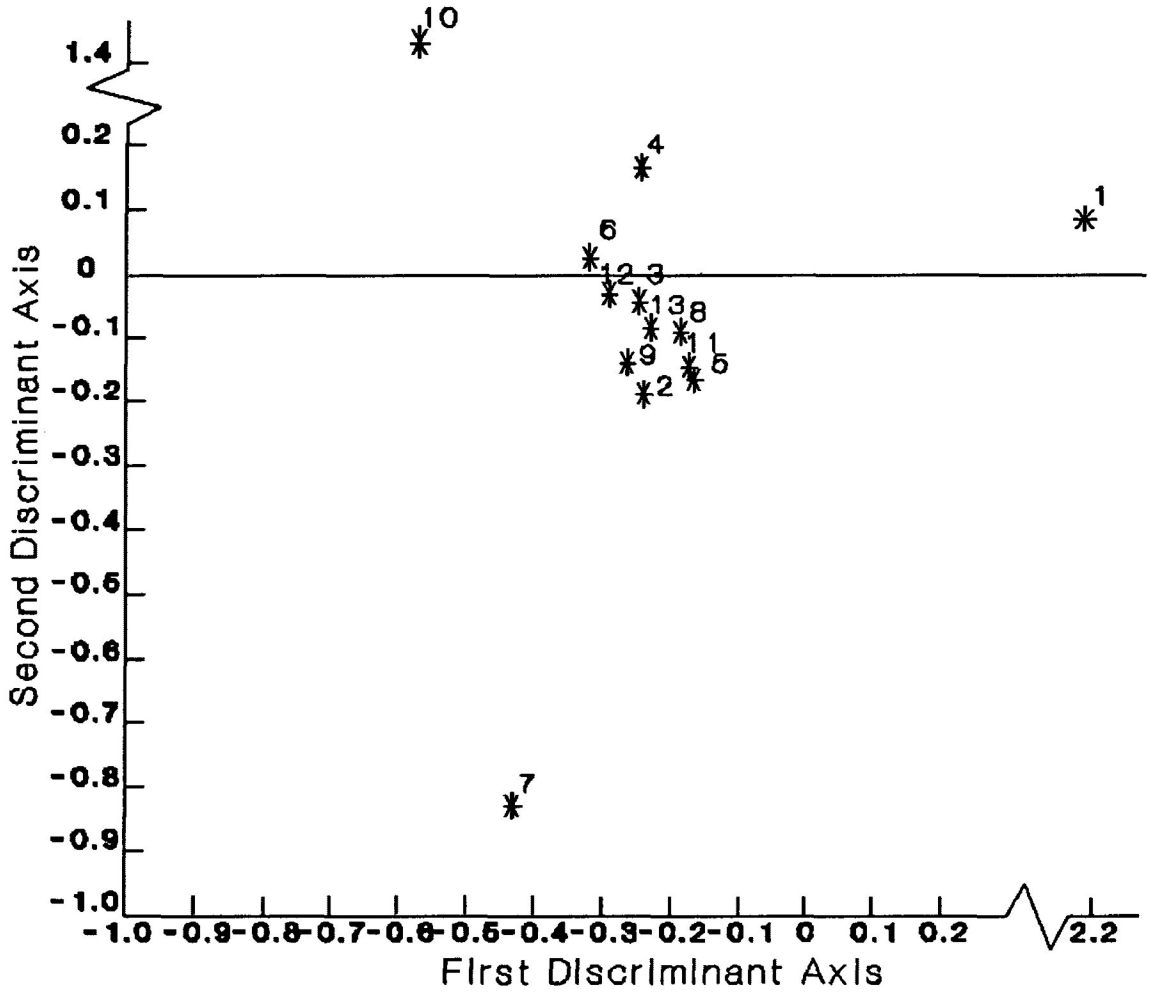

Figure 1: Distribution of sub-populations from Raith Site 2 in two dimensional canonical space. (Sub-populations indicated by *).

DISCUSSION

The findings of the present study indicate that the uneven distributions of pollen alleles in the four populations are due, at least in part, to spatially heterogeneous pollen pools. A basic assumption of the mixed mating model is a homogeneous pollen pool (Clegg, 1980). This study adds to the mounting evidence that a spatially homogeneous pollen pool is an exception rather than the norm for wind-pollinated forest tree species (Brown *et al.*, 1975; Cheliak, 1985; Cheliak *et al.*, 1985a; Cheliak *et al.*, 1985b; King *et al.*, 1984; Knowles *et al.*, 1987; Shea, 1987; Perry and Knowles, 1990; Muller-Starck, 1982; Shen *et al.*, 1981; Merzeau *et al.*, 1989; Sproule, 1988).

Significant heterogeneity of pollen allele frequencies may be indicative of tree-to-tree variation in either the outcrossed pollen pool or outcrossing rates (Brown *et al.*, 1975). Black spruce can exhibit relatively high levels of inbreeding (Park and Fowler, 1984; Sproule, 1988). In his examination of black spruce Sproule (1988) associated elevated levels of inbreeding with a family structuring exhibited by some populations of upland spruce. In contrast, results of research for natural populations of black spruce in New Brunswick have shown black spruce to be predominantly an outcrosser exhibiting no family structure (Boyle and Morgenstern ,1986). Furthermore examination of the Hick's Lake population has determined that no family structuring is present (Knowles, 1990). Because of the lack of family structure in the Hick's Lake population and the similar nature of Hick's Lake with the remaining

three populations examined in this study it is felt that outcrossing heterogeneity has had little influence upon the results of this study. In this study the argument supporting the view of a heterogeneous outcrossed pollen pool is based on the following three pieces of evidence.

1) Significant differences between the maternal and pollen allele frequencies were observed in all four populations.

Significant differences between ovule and pollen allele frequencies can be indicative of a finite parent population from which samples were drawn (Ritland and El-Kassaby, 1985). Although Boyle and Morgenstern (1987) observed significant heterogeneity of pollen allele frequencies in natural stands of black spruce the lack of significant differences between maternal and pollen allele frequencies led the authors to conclude that heterogeneity of pollen allele frequencies was the product of tree-to-tree variation in outcrossing rates rather than spatial heterogeneity of the pollen pool. In contrast, significant differences in this study between maternal and pollen allele frequencies in 28 of 32 comparisons (Table 4) indicate the presence of a spatially heterogeneous paternal pool. The sampling intensity for maternal trees in this study was comparable to that of Boyle and Morgenstern (1987) in which 40 maternal trees were sampled from an area of one to two hectares in size. Even so it should be recognized that the small sample size of the maternal allelic pools in this study may have somewhat biased the results in favour of significant differences between maternal and pollen allelic frequencies.

2) Rare/less common pollen alleles were not randomly distributed throughout the populations.

Under a simple model of random mating, rare pollen alleles should be uniformly distributed across the sampled maternal trees (Cheliak, 1985). Review of the data has shown that less common and rare pollen alleles were unevenly distributed across the sampled trees. Assuming that selective forces are not at work upon the examined loci or closely linked loci, such a phenomenon is indicative of restricted pollen dispersal and a spatially heterogeneous outcrossed pollen pool.

3) The degree of heterogeneity, as indicated by the G_h statistic, varies among loci within a given population.

As seen in Table 5 the significance of calculated G_h values varied among loci within any one of the four populations. Theoretically, in the absence of such forces as selection and mutation, mating system affects all loci equally (Brown *et al.*, 1975; Knowles *et al.*, 1987). The fact that G_h values vary substantially among loci within any one of the four populations tends to support the view that tree-to-tree variation in mating system is an unlikely cause of the allelic heterogeneity.

Recently multivariate statistics have gained popularity as a technique for analysing allozyme data from conifer genetics studies (Yeh *et al.*, 1985; Knowles, 1985; O'Reilly *et al.*, 1985; Yeh *et al.*, 1986; Epperson and Allard, 1988; Knowles *et al.*, (in press)). In terms of the present study MDA is particularly useful for isolating the source of the heterogeneity for Raith 2 (Figure 1).

In contrast, attempts to isolate the source of heterogeneity using the step-wise testing procedure (Table 6) varied depending upon the locus examined.

Although pollen pool heterogeneity was observed in all four populations no apparent eccological correlate could be identified. The fact that ecological correlates were not identified in this study may be due to other factors swamping the effect of stand ecology or simply that the ecological factors considered in this study were not appropriate. Overall the evidence tends to support the conclusion that chance random events have produced the observed phenomenon. Noting the results provided in Table 8b we see that the first two discriminant functions of each site account for a substantial amount of the total variation in the data. However the proportion of variation attributed to differences between sub-populations, as indicated by the canonical R^2, was low for all but Raith 2. This suggests a larger component of variation within as opposed to among groups. Review of the within and among group sum of squares cross products matrices confirmed this result. It is important at this point to recognize that low allelic frequencies may artifically produce significant differences in the pollen pools. However through the use of an efficient sampling scheme and by assaying a large number of seeds per tree (on average 67 seeds per tree for this study) the probability of detecting less common alleles is increased. In this manner the influence of low allelic frequencies upon the results were limited. Review of the results

from both univariate and multivariate procedures confirm that less common and rare alleles had little influence on the results.

When plants of any given population are widely scattered or in small isolated clumps the number of potential fathers is small (Levin, 1988). Due to the clumped distribution and low densities of the Hick's Lake and William's Bog populations a higher degree of pollen pool heterogeneity, relative to the two Raith populations, was expected. Examination of a similar relationship has demonstrated a positive correlation between outcrossing rate and stand density in tamarack (Knowles *et al.*, 1987), Englemann spruce, and sub-alpine fir (Shea, 1987). Yet in studies of black spruce (Sproule, 1988) and eastern white cedar (Perry and Knowles, 1990) no evidence of such a relationship was observed. In contrast, because of the uniform, well-stocked nature of the two Raith sites it was expected that no significant heterogeneity of the pollen pool would be observed. In spite of the fact that the causal agents for this phenomenon cannot be isolated, the most likely explanation would appear to be stochastic events.

So what may have caused the heterogeneity? Within the four sites, chance factors such as tree-to-tree variation in male fecundity and floral asynchrony may be the most likely causes of the observed heterogeneity. As demonstrated in a number of studies, clonal variation in pollen production can produce a heterogeneous pollen pool (O'Reilly *et al.*, 1982; Barrett, 1985; Frampton *et al.*, 1982; Erickson and Adams, 1989; Schoen and Stewart, 1987; Denti and Schoen, 1988). As well, timing and

duration of pollen release and female receptivity may significantly affect cross pollination (Erickson and Adams, 1989). As little as six to twenty-four hours between the first and subsequent pollination events may be adequate to limit the success of subsequent pollination events (Webber and Yeh, 1987). Furthermore it is important to recognize that pollen dispersal can be modified by many factors including source type and forest cover (Moore, 1976; Stanley & Liskens, 1974; Raynor, 1967). In particular, Raynor (1967) has shown that pollen dispersal is dramatically restricted by a forest's canopy. Isolation due to low stand density may have played a major role in the heterogeneity observed at Hick's Lake and William's Bog while the full crown closure of the Raith sites is speculated as an additional mechanism restricting pollen dispersal in the dense stands.

In terms of our basic knowledge of population genetics homogeneity of the pollen pool is a key assumption of the mixed-mating model. Deviation from this assumption reduces estimates of outcrossing (Ennos and Clegg, 1982). Recognizing the possibility for biased estimates of outcrossing, King *et al.*(1984) recommend that mating system analysis be based on material sampled over a number of years. However further studies may be required to determine the evolutionary implications of pollen pool heterogeneity. Of particular interest is the question posed by the comments of King *et al.*, (1984) in which they suggest that temporal variation in combination with spatial heterogeneity of the pollen

pool and natural selection may produce a final population structure approximating that found in a strictly random mating situation.

Relative to large scale reforestation, pollen pool heterogeneity should be considered in any seed procurement program (King *et al.*, 1984; Cheliak *et al.*, 1985a; Sproule, 1988). In particular it is important to recognize that the development of sound tree improvement strategies are influenced by both our understanding of the patterns of genetic variation and our underlying knowledge of mating systems (Cheliak *et al.*, 1985a). An incorrect assumption regarding pollen pool homogeneity may result in over-estimates of additive genetic variance and hence genetic gain from the open-pollinated families comprising a breeding program (King *et al.*, 1984). Particularly, factors such as floral asynchrony and disproportionate fecundity, as well as, inbreeding and incompatibility, have been associated with pollen pool heterogeneity in forest tree seed orchards and may limit the efficiency of seed orchards (Webber and Yeh, 1987; Muller-Starck, 1982; Shen *et al.*, 1981; O'Reilly *et al.*, 1982; Cheliak, 1984; Barrett, 1985; Schoen and Stewart, 1987; Denti and Schoen, 1989, Erickson and Adams, 1989). As a result new orchards should be designed so as to maximize the genetic contribution of all seed and pollen parents by managing for families with synchronous phenologies (Muller-Starck, 1982; Cheliak, 1985).

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APPENDIX

Table A1: Additional characteristics of the four study sites.

Site	Latitude	Longitude	FEC Type[a]	
			Vegetation	Soils
R1	48°51'	89°56'	V34	S11
R2	48°50'	89°57'	V37	S12S
HL	48°47'	89°09'	V38	S12S
WB	48°18'	89°19'	V38	S12S

[a] Vegetation and soil types were classified using the *Field Guide to the Forest Ecosystem Classification for Northwestern Ontario*, (Sims, 1990).

Table A2: List of lesser vegetation by stratum for Raith 1 & 2.

Stratum	Raith 1		Raith 2	
Trees	*P. mariana*	95%	*P. mariana*	89%
	Larix laricina	5%	*Abies balsamea*	10%
			P. glauca	1%
Shrubs	*P. mariana*	10%	*P. mariana*	10%
	Salix spp.	10%	*Salix spp.*	5%
	Ledum groenlandicum	30%	*L. groenlandicum*	50%
	Vaccinium myrtilloides	20%	*Chamaedaphne calyculata*	15%
	V. angustifolium	10%	*V. angustifolium*	10%
Herbs	*Gaultheria hispulada*	10%	*G. hispulada*	30%
	Carex spp.	10%	*Carex spp.*	15%
	Equisetum sylvaticum	5%	*Smilacina trifolia*	5%
	Cornus canadensis	10%	*Lycopodium annotinum*	15%
Mosses & Lichens	*Pleurozium schreberi*	80%	*P. schreberi*	15%
	Dicranum spp.	5%	*Dicranum spp.*	5%
	Sphagnum spp.	10%	*Sphagnum spp.*	80%
	Cladina spp.	3%	*Cladina spp.*	3%
	Hylocomium splendens	5%		