POLLEN POOL HETEROGENEITY IN JACK PINE (*PINUS BANKSIANA* LAMB.): A PROBLEM FOR ESTIMATING POPULATION OUTCROSSING RATE?

by

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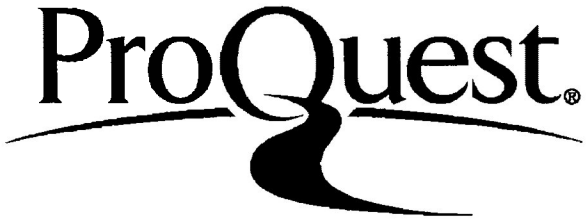

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ABSTRACT

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Key Words: isozyme, pollen pool heterogeneity, mating system, mixed-mating model, computer simulation, Pinus banksiana Lamb.

Pollen pool heterogeneity, which violates an assumption of the mixed-mating model, is one of the major problems facing population geneticists concerned with measuring plant mating systems. In the present study, isozyme markers were used to examine pollen pool heterogeneity in two natural populations of jack pine, Pinus banksiana Lamb.,in northwestern Ontario, Canada. Population multilocus estimates of outcrossing rate ranged from 0.829 to 0.952 and differed significantly between populations. Single-tree outcrossing rates were found to be homogeneous among trees in both populations. Computer simulation studies showed that the consanguineous mating pollen pool was a potentially important component of the pollen pool, capable of biasing population outcrossing estimates downward. In contrast, random heterogeneity of the pollen pool was found to have no effect on population estimates of outcrossing rates. Pollen pool heterogeneity existed in these two natural populations. However, it appeared to be random in nature and therefore did not affect the population outcrossing estimates.

TABLE OF CONTENTS

LIST OF TABLES

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INTRODUCTION

Reliable estimates of outcrossing are essential both for understanding the genetic structure of populations and for formulating breeding strategies and designing seed orchards (Brown 1990; Muona 1990). Plant mating system studies have greatly advanced since 1970 because of the use of isozyme markers and the development of appropriate statistical methods (Clegg 1980). The most commonly used procedure for estimating plant mating systems is the mixed-mating model (Brown and Allard 1970; Clegg 1980). This model assumes that mating takes place either by random outcrossing, at a rate t, or by selfing at a rate $s=(1-t)$ (Jones 1916; Fyfe and Bailey 1951). For simplicity, it also assumes that outcross pollen allele frequencies, p, are randomly distributed over all maternal plants and that the outcrossing rate is uniform for all maternal parents (Clegg 1980). However, two common findings in previous mating system studies based on this model are (1) heterogeneous pollen pool allele frequencies, and (2) heterogeneous outcrossing levels among plants within a population (Hamrick and Schnabel 1985). These findings violate two of the assumptions of the mixed-mating model. Have such violations of assumptions routinely biased the estimation

of the population outcrossing rate? This question remains neglected in the literature.

Violation of the assumptions may lead to inaccurate estimation of outcrossing rates (Schoen and Clegg 1984). Brown et al. (1975) proposed that the net effect of pollen pool heterogeneity is a downward bias in outcrossing, but Fripp et al. (1987) reported that temporal pollen pool heterogeneity did not have a major effect on population estimates of t. In fact, the principal difficulty in mating system studies is the accurate assessment of pollen pool heterogeneity because the observed pollen pool heterogeneity detected by the conventional heterogeneity G-test (G_H; Sokal and Rohlf 1981) or χ^2 test may arise from tree-to-tree variation in t or in p or both (Brown et al. 1975). Thus, most of the previous mating system studies (e.g., Brown et al. 1975; King et al. 1984; Cheliak et al. 1985; Merzeau et al. 1989) provided only tentative evidence that pollen pool heterogeneity really existed in the populations under study.

In this study, isozyme markers were used to examine pollen pool heterogeneity in two natural populations of jack pine (Pinus banksiana Lamb.) in northwestern Ontario, Canada. The specific objectives were (1) to determine whether pollen allele frequencies were homogeneous in the effective pollen pool, and (2) to assess the effect of tree-to-tree variation in p on the population estimates of

$\underline{t}$ by combining experimental and computer simulation procedures. For the second objective, the pollen pool (*i.e.*, pollen gametes effective in fertilizing viable seeds) must be clarified according to its component parts as follows: (1) selfing pollen pool (p_s), which consists of pollen alleles contributing to selfed progeny; (2) outcrossing pollen pool (p_o), which consists of pollen alleles contributing to outcrossed progeny; and (3) consanguineous mating pollen pool (p_c), which consists of pollen alleles contributing to progeny produced by consanguineous mating. The last component is included for two main reasons. First, evidence of consanguineous mating in plants has been found (Brown 1990; Ritland 1984, 1989; Waller and Knight 1989). Second, since the mixed-mating model does not specify consanguineous mating (Ritland 1984; Hamrick and Schnabel 1985; Waller and Knight 1989; Brown 1990), the p_o of this model includes p_c if p_c exists. Thus, the inclusion of p_c in the present analysis has the potential of clarifying the consequences of spatial heterogeneity of p_o more precisely.

LITERATURE REVIEW

This review deals with the mixed-mating model and its role in the development of measuring plant mating systems, pollen dispersal, and the current knowledge about the silvics, ecology and genetics of jack pine.

I. The Mixed-Mating Model and Mating System

The pattern by which gametes unite to form the next generation is termed the mating system (Stern and Roche 1974). Plants exhibit a wide variety of mating systems such as random mating, inbreeding and assortative mating (Brown 1990). The random mating model is, therefore, not universally appropriate (Clegg 1980). Deviation from purely random mating in plant populations has been specified by the mixed-mating model. As previously described, all mating events in the mixed-mating model are assigned to one of two categories: random outcross or self fertilization. This model was first applied to predominantly self-pollinated crops (Jones 1916; Fyfe and Bailey 1951), and later used widely in agriculture and in natural populations of both inbreeding and outbreeding species including herbaceous plants and forest trees (Brown et al. 1985).

Traditional methods Traditional methods of mating

system assessment have been based on the analysis of floral morphology, greenhouse and field crossing experiments, and observation of pollination behavior (Clegg 1980). Forest geneticists have employed single gene morphological characters, such as seedling albinism to estimate selfing rate (Squillace 1974). Similar studies have been done in many coniferous species (notably Pinus sylvestris) (Brown et al. 1975). However, such methods do not provide direct measures of the success of matings in populations, and the information they produce is often inadequate for the analysis of genetic transmission at the population level (Shaw et al. 1981). Moreover, since forest trees have long generation intervals, it is too time-consuming to use dominant morphological markers, which require progeny tests to distinguish heterozygotes from the dominant homozygotes.

In 1916, Jones developed an approach to estimate outcrossing rate by using gene markers. In 1951, Fyfe and Bailey extended the use of the approach from experimental populations to natural or other populations in which allele frequencies were unknown, by combining statistical methods for estimating t and p jointly from population data. With the use of isozyme markers and the development of appropriate statistical methods, this approach to the study of plant mating systems has grown into a major area of research activity in plant population biology (Clegg 1980).

Protein electrophoresis Application of protein

electrophoresis to the study of populations has provided numerous genetic markers which are used to measure the transmission process in virtually any plant species (Clegg 1980). Electrophoresis separates enzymes on the basis of their molecular charge, size, and three-dimensional configuration. Different protein sequences resulting from differences in genetic code sometimes have different charges and structures (Conkle 1972). This leads to differential mobility in a gel medium when current is applied. Thus, loci and allelic products from those loci can be identified (Conkle 1972). This approach greatly simplifies studies of tree mating systems because any seed-bearing tree can be examined. In gymnosperms, in which double fertilization does not occur, isozyme techniques are especially powerful tools for estimating mating system parameters. The single fertilization process results in a haploid endosperm (megagametophyte). Thus, direct probabilistic inference of maternal genotypes can be made by analysis of haploid tissues from one plant (Morris and Speith 1978). Moreover, the paternal contribution can be unambiguously deduced when the maternal genotype and corresponding maternal haploid contribution can be definitively ascertained. Thus progeny arrays from seeds of conifers contain considerable information that can be used to study mating systems (Cheliak et al. 1983; Yeh and Morgan 1987).

Statistical models Many elaborate statistical models have been introduced since 1970 (Clegg 1980; Brown et al. 1985). Based on the theoretical mixed-mating model, Brown and Allard (1970) developed a two-step likelihood procedure for estimating outcrossing rate using a single locus. Clegg et al. (1978) later provided a one-step estimation procedure based on the method of Brown and Allard (1970). Several other single-locus procedures have been subsequently developed (summarized by Jain 1979). However, estimates of t obtained from a single sample have often differed significantly among the loci, even though the mating system must affect all loci identically (Shaw et al. 1981).

Therefore, more complex procedures using multilocus estimators have emerged to handle the problems resulting from single-locus estimates (Green et al. 1980; Shaw et al. 1981; Ritland and Jain 1981, Ritland 1984; Ritland and El-Kassaby 1985; Cheliak et al. 1983; Neale 1983; Brown et al. 1985). A multilocus estimator provides a standard of reference (null hypothesis) that can be useful in analyzing the effects of factors such as population heterogeneity and postmating zygotic selection on the transmission of genetic information at the population level (Shaw et al. 1981). The multilocus estimation procedure involves two steps: 1), detection of outcrosses through direct observation of the phenotypes of progeny that carry nonmaternal alleles; and

2), statistical compensation for outcrosses that are not directly observable (Shaw et al. 1981). As more loci are examined, the probability of identifying outcrosses in step 1 increases and the importance of compensation in step 2 decreases. Thus multilocus estimation is statistically more efficient than single-locus estimation. Another advantage of multilocus estimation is that it becomes increasingly insensitive to failure of assumptions that can have large effects on single-locus estimation as more and more outcrosses are detected by direct observation in the first step. However, independence among loci must be assumed in multilocus estimation.

Green et al. (1980) developed a procedure allowing different detection probabilitites for each maternal genotype in a three-locus analysis of outcrossing in Lupinus albus. The main problem with this method is that computing the detection probabilities requires making assumptions about the frequencies of pollen multilocus genotypes (Brown et al. 1985). Neale (1983) presented a single- and multi-locus estimation based on the maximum likelihood function developed by Green et al. (1980). A multilocus estimation procedure was also given to estimate outcrossing rate for individual trees.

Cheliak et al. (1983) used the Expectation-Maximization (EM) Algorithm for obtaining the maximum likelihood estimates of mating system parameters. The

procedure involves a two-step algorithm; the expectation step and the maximization step. The advantage of this procedure is its facility in coping with a high number of alleles at the marker loci. This EM algorithm has been employed in several studies of forest trees (Yeh et al. 1983; King et al. 1984; Cheliak et al. 1983; Cheliak et al. 1985). However, this procedure does not provide values for the variances of the estimates, except in the case of a single parameter (Brown et al. 1985). Thus statistical testing of difference among mating system estimates is ruled out.

Ritland and Jain (1981) presented a complete multilocus estimation procedure based on many independent loci. They demonstrated numerically that the multilocus estimates were less affected by selection and non-random outcrossing than were single locus estimates. In order to estimate the unbiased "effective selfing" rate, Ritland (1984) developed a model that derives estimates of selfing rate of inbred parents as opposed to outbred parents. The estimation procedure includes maternal genotypic frequencies, apparently assumes equilibrium, and yields higher standard errors because of the contribution of parental sampling. In 1985, Ritland and El-Kassaby presented a multilocus procedure for estimating female outcrossing rates and outcross pollen gene frequencies for individual trees, in which two multilocus estimates of t

were made when outcrossing pollen gene frequency p was allowed, and not allowed, to vary among trees. To distinguish correlation of selfing from correlation of paternity, Ritland (1989) developed a "sibling pair" model. However, the estimates of correlated matings have high variance and lack statistical independence.

Paternity analysis There is a growing interest in the determination of paternity within and among plant populations (Ellstrand 1984; Hamrick and Schnabel 1985; Cheliak 1985; Meagher 1986; Levin 1988; Brown 1990). The use of paternity analysis to identify the paternal contributor to individual seeds or seedlings can remove many of the problems in measuring the mating system or gene flow (Hamrick and Schnabel 1985). If all paternal contributors are identified including the "maternal" plants, the actual number of progeny resulting from self-fertilization can be counted. Thus, it would be possible to separate different forms of inbreeding. If there are several seeds per flower or flowering head, it should be possible to determine whether selfing rates vary with the stage of flowering or flower position. The proportion of effective pollen originating outside of the study area can be determined, and exact distances of pollen movement within the study area can be measured. Plant-to-plant variation for incoming (female) or outgoing (male) pollen dispersal can be estimated.

Two general approaches can be used in paternity analysis. First, if the maternal and progeny genotypes are known for each polymorphic locus, a certain proportion of the potential paternal contributors can be excluded with each locus (Ellstrand 1984). Second, the individual that was most likely to have contributed the paternal gamete is identified using a maximum likelihood analysis. As with the exclusion analysis, the discriminating power of this technique improves with increasing numbers of polymorphic loci and alleles and with equal allele frequencies. An assumption necessary for this approach is that the loci are not linked.

Schoen and Clegg (1984) developed an one pollen parent model that assumes that successive outcross events within a family involve pollen drawn from a single male parent. They indicated that, besides providing a basis for the estimation of mating system parameters, application of the mixed-mating and one pollen parent models may help to provide preliminary estimates of the degree of multiple paternity in certain plant species. Schoen (1988) again presented a method for obtaining parameter estimates of the one pollen parent model using the progeny array as the unit of observation. This method is more direct and computationally simpler than that previously described by Schoen and Clegg (1984).

The unique power provided by the identification of

paternity affords new insights into the reproductive biology of natural populations. However, many plant species may not maintain levels of allozyme variation sufficient for the application of paternity analysis (Hamrick and Schnabel 1985).

II. The Mixed-Mating Model and Its Assumptions

Four important assumptions are necessary to specify the mixed-mating model (Brown and Allard 1970; Kahler et al. 1975; Clegg et al. 1978; Brown et al. 1985). First, gene frequency distribution among pollen is identical over all maternal plants. Second, the outcrossing rate is uniform for all maternal plants. Third, segregation of the alleles in heterozygous maternal plants is strictly Mendelian in a 1:1 ratio for both pollen and ovule production. Fourth, selection does not occur between fertilization and the assay of progeny genotypes.

If the mixed-mating model based on these four assumptions accurately reflects the reproductive biology of a population, estimates of outcrossing rates made from a given set of progeny arrays are expected to be the same for all loci, within sampling error, because the mating system must affect all loci identically. However, the previous studies(Shaw and Allard 1982; Cheliak 1985; Cheliak et al. 1985; Hamrick and Schnabel 1985; King et al. 1984; Snyder et al. 1985; Bijlsma et al. 1986; Barrett et al. 1987; El-

Kassaby et al. 1987) showed that estimates of t over loci were not always homogeneous, which indicates that one or more of its assumptions was invalid in the population under study.

Violation of these assumptions may be especially serious in insect-pollinated species and may lead to inaccurate estimates of outcrossing rates (Schoen and Clegg 1984). Brown et al. (1975) proposed that the net effect of pollen pool heterogeneity is an upward bias of inbreeding coefficients and a downward bias in outcrossing estimates. However, Fripp et al. (1987) reported that the temporal heterogeneity of allele frequencies did not have a major effect on single-locus, maximum likelihood estimates of outcrossing rates in the population. In fact, less attention has been paid to whether the routine estimate of outcrossing based on the mixed-mating model is a biased estimate of the population mean, when the assumption is invalid (Brown et al. 1985). The principal difficulty in the mating system studies is the accurate assessment of pollen pool heterogeneity because the observed pollen pool heterogeneity detected by the heterogeneity G-test may arise from tree-to-tree variations in t or in p or both (Brown et al. 1975; Brown et al. 1985).

Because of this difficulty, the previous studies provided only tentative evidence that pollen pool heterogeneity did exist. Bijlsma et al.(1986) reported

pollen pool heterogeneity in an open-pollinated maize population, and suggested that temporal variation in pollen pool and/or gametophytic selection, correlated with marker-locus genotype, were the major causes. Barrett et al. (1987) demonstrated that the pollen pool of a black spruce (Picea mariana) clonal seed orchard was heterogeneous. Pollen pool heterogeneity has also been reported to exist in the natural stands of white spruce (Picea glauca)(King et al. 1984) and jack pine (Cheliak et al. 1985; Snyder et al. 1985). Fripp et al. (1987) reported that allele frequencies in the outcross pollen pool of Eucalyptus regnans F. Muell. were found to vary with the flowering time. Merzeau et al. (1989) also reported pollen pool heterogeneity in beech (Fagus sylvatica L.).

Compared with the other assumptions, the first one is the most easily violated, especially in a natural population (Hamrick and Schnabel 1985). One possible basis for failure of this assumption is microhabitat selection causing similar genotypes to be clustered within populations (Shaw et al. 1981). Another possible cause of non-random distribution is the tendency of seeds to fall and grow near their maternal parent, causing relatives to be clustered. This type of nonrandom distribution of genotypes can lead to both pollen pool heterogeneity and inbreeding. Clustering in family groups thus causes single-locus estimates of t to be further biased downward (Shaw et

al. 1981). Ritland and Ganders (1985) reported that population substructure appears to be partly responsible for pollen gene frequency variation in three populations of Bidens menziesii.

III. Variation Of Mating Systems In Conifers

Coniferous tree species, like other plants, exhibit a wide variety of mating systems. They are wind-pollinated and genetically highly variable (Hamrick 1982). They often display strong inbreeding depression (Franklin 1970). Estimates of self-fertilization from studies using morphological markers in 12 coniferous species ranged from 2 to 20 percent (Squillace 1974). The incorporation of isozyme techniques and elaborate statistical models into forest genetics research has made the precise estimates of the mating systems of conifers possible. It is now recognized that the mating system parameters of conifers are far from being a fixed, constant value, but is rather a dynamic entity, varying at different levels (individual, population, and species) in time and space, and affected by a large number of biotic and abiotic factors.

An examination of published estimates of outcrossing levels in seed orchards of different species shows a range values from 0.837 to 0.977 (Muller-Starck 1979; Moran et al. 1980; Shen et al. 1981; Shaw and Allard 1982; Ritland and El-Kassaby 1985; Barrett et al. 1987), and in natural

stands from 0.635 to 1.00 (Shaw and Allard 1982; Farris and Mitton 1984; Epperson and Allard 1984; Cheliak et al. 1985; Boyle and Morgenstern 1986; Snyder et al. 1985; Perry and Knowles 1990; Perry and Dancik 1986; Furnier and Adams 1986; El-Kassaby et al. 1987; Yeh and Morgan 1987; Knowles et al. 1987).

Some studies have indicated that there is considerable variation in outcrossing among populations of certain species (Boyle and Morgenstern 1986; Furnier and Adams 1986; Knowles et al. 1987). Knowles et al.(1987) showed a range of outcrossing rates from 0.537 to 0.908 for five tamarack (Larix laricina) populations.

Estimates of outcrossing among individual trees also vary (Muller-Starck 1982; Shaw and Allard 1982; El-Kassaby et al. 1987; Denti and Schoen 1988; Xie 1989). El-Kassaby et al. (1987) reported that single-tree (Pinus monticola) estimates of outcrossing rates averaged over all loci varied among trees and ranged from 0.683 to 1.207. The latter value is a "biologically unrealistic" estimate (El-Kassaby et al. 1987). They suggested that the variation in t among trees might be due to variability in self-compatibility among trees and that violation of mixed-mating assumptions had occurred.

It has been shown that there are temporal and spatial variations of mating systems of conifers (Shen et al. 1981; Squillace and Goddard 1982; Snyder et al. 1985; Cheliak et

al. 1985). Cheliak et al. (1985) demonstrated an approximately linear increase in the apparent selfing rate with time since fertilization in jack pine. This increase could be explained by stochastic fluctuations and elimination of selfed embryos over time. Squillace and Goddard (1982) found that more selfing occurred in lower crowns than in upper crowns with higher estimated outcrossing rates for seed from the upper portion of the crowns.

Plant mating systems may change not only with the varying spatial structure of the population, but also with regard to environmental factors (Clegg 1980). Attempts to measure outcrossing rates in different habitats within a species have often revealed marked differences in mating systems (Levin and Kerster 1974; Farris and Mitton 1984; Shea 1987). Farris and Mitton (1984) reported that there was a marked difference between outcrossing rates from a low-density stand (t=0.81) in comparison to a normal density stand (t=0.91) of ponderosa pine.

IV. Gene Flow

Gene flow is a collective term that includes all the mechanisms resulting in the movement of genes from one population to another (Slatkin 1985b). It is now recognized that gene flow is influenced by many factors. These include life history characteristics, pollen dispersal,

seed dispersal, mating system, species' ecology and the action of selection (Sluder 1969; Levin and Kerster 1974; Guries and Ledig 1981; Hamrick 1982; Loveless and Hamrick 1984; Slatkin 1985b). Two important consequences of gene flow are (1) that higher levels of gene flow can counteract the action of selection by reducing genetic differentiation among populations, and (2) that lower levels of gene flow can affect polymorphism within populations by the introduction and maintenance of novel alleles (Hamrick 1982).

Some aspects of gene flow in forest trees have been investigated, particularly those most closely related to commercial use of the resource (e.g., pollen flow and seed dispersal) (Wang et al. 1960; Sarvas 1967; Sluder 1969; Greenwood 1986). Sluder (1969) noted that gene flow in forest stands seemed to be limited enough to promote differentiation among stands and geographic areas.

The results of many studies on pollen dispersal in tree species indicate that pollen dispersal patterns generally are leptokurtic (Levin and Kerster 1974; Bradshaw 1972). Pollen dispersal may be by wind or insects or both, and the relationship of these mechanisms to dispersal patterns is unclear (Slatkin 1985b). The factors that affect pollen dispersal may be terminal velocity of pollen grains, nature of the source, distance between populations, nature of the target, prevailing wind, topography,

temperature and humidity, and characteristics of surrounding vegetation (Levin and Kerster 1974; O'Reilly et al. 1982).

The successful dispersal of genes by pollen depends on the occurrence of fertilization. Thus, actual pollen dispersal must be distinguished from effective gene flow via pollen. Effective gene dispersal via pollen may be affected by local versus long-distance pollen proportions in the pollen pool, phenological differences between trees, differential selection, and reduced viability of long-distance pollen (Sluder 1969).

Several methods have been developed to obtain estimates of gene flow (Hamrick 1982). The first type is to compare juvenile gene frequencies or means with that of the adults. It appears to be a straightforward approach. However, adult frequencies may not be representative of effective pollen frequencies and other factors (e.g. selection) may affect the estimates of gene flow.

The second most common approach is to track the movement of dispersal units, pollen and seed. Although this is the most common method used, it has a number of limitations since little is known about the fertilization, germination or reproductive ability of dispersal units relative to their locally derived counterparts. Wang et al. (1960) used this method to conduct research on pollen dispersal of pines.

The third procedure is to follow the dispersal of pollen or seed derived from an individual with a unique allele. Use of markers gives a more accurate picture of the effective movement of genes within and among populations. Muller (1977) used a unique LAP allele to measure gene flow in Pinus sylvestris. He observed that the marker allele occurred in progeny of trees up to 80 m from the source. Since it is very difficult to find individuals with unique alleles, this method is not applicable to all species or populations.

Wehrhahn and Powell (1987) presented a maximum likelihood (ML) model to quantify gene flow using isozyme data. For hypothesis testing, the ML estimator has the advantage over the estimator based on rare alleles because the standard error of ML estimate can be obtained for any allele of a gene (Yeh 1988; Perry et al. 1990). However, their model needs to assume that a steady-state equilibrium between migration and random drift has been attained (Wehrhahn and Powell 1987).

Slatkin and Barton (1989) compared three indirect methods for estimating the average level of gene flow in natural population: F_{st} (Wright 1951), rare alleles (Slatkin 1985a), and maximum likelihood (Wehrhahn and Powell 1987), and concluded that F_{st} is more useful under realistic conditions.

In summary, most of the currently used measures of

gene flow in plant populations do not provide detailed or dependable estimates of gene movement. Paternity analysis (mentioned above) may provide improved insights into phenomena such as pollen dispersal in the future.

V. Silvics And Ecology Of Jack Pine

Jack pine is an important species for planting and direct seeding, and is a major source of pulpwood, lumber, and round timber (Rudolph and Yeatman 1982). Its range extends latitudinally from 42°N to 60°N and longitudinally from 60°W to 127°W (Schoenike 1976). Schoenike (1976) reported that jack pine was found at elevations from near sea level to 2,600 ft (790 m) on river flood plains, broad level uplands, coarse-textured glacial and lake deposits, rocky seacoasts, mountain slopes, and other formations. It grows not only on sandy, loose-textured soils, but also on loamy soils, on thin soils over the granites and metamorphosed rocks of the Canadian shield, over limestones, on peats and raw humus, and in soil over permafrost (Rudolf 1958; Schoenike 1976).

Jack pine usually grows in diverse continental climates with mean annual temperatures between -5°C and 9.5°C and mean minima between -20°C and -45°C or lower (Rudolf 1958; Schoenike 1976). The precipitation varies between 25 and 140 cm per year with 13 to 58 cm of this coming during the 60- to 170-day growing season (Schoenike

1976).

Jack pine is a monoecious species with staminate cones occurring in the lower tree crown and ovulate cones generally in the upper crown. Staminate cone primodia are initiated in early or mid-July, and ovulate cone primodia in August. The ovulate cones usually begin to emerge in mid-May of the second year and pollination occurs soon after. Jack pine is a wind-pollinated, cross-fertilizing species. Wind-pollination time varies greatly from year to year, and location to location, occurring within the period from mid-May to mid-June. The pollinated ovulate cones need two growing seasons to mature. Jack pine in the boreal forest usually has serotinous, persistent cones, allowing seed to be stored on the tree for many years while retaining viability (Rudolph and Yeatman 1982).

Cuttings taken from young trees and needle fascicles can be rooted. Grafting is highly successful with some clones, but graft incompatibility is evident in others. Cell culture and tissue culture have not yet resulted in successful propagation of a complete jack pine tree (summarized by Rudolph and Yeatman 1982).

VI. Genetics Of Jack Pine

Three basic methods for studying genetic varation in forest tree species are provenance tests, progeny tests and isozyme analysis (Farmer et al. 1983). Provenance tests

examine variation between geographically widespread populations. Progeny tests evaluate parental genetics based on offspring performance in a common environment. Isozyme analysis uses resolved enzymes to infer genetic structure. All of these methods have been used in the study of jack pine genetics.

Studies of jack pine in natural stands have provided valuable information on the great variation among and within populations in growth, tree form, wood and bark properties, cold hardiness, fall coloration, seed yield, disease and insect resistance, the phenotypic plasticity of the species, and its adaptability to various environments (summarized by Rudolph and Yeatman 1982). Schoenike (1962, 1976) suggested that environmental factors are the dominant selection forces responsible for the natural variation within the current jack pine range.

Studies of provenance variations in jack pine have been conducted since 1942 (Rudolph and Yeatman 1982; Magnussen and Yeatman 1988). Some important conclusions obtained are that growth of jack pine provenances is related to environmental gradients associated with latitude (photoperiod) and length and temperature of the growing season; that such growth shows clinal variation over these gradients; and that migration in the postglacial period combined with natural selection has led to clear patterns of adaptive variation over the nearly transcontinental

range of the species.

Genetic variation within seed zones of jack pine has also been investigated in various types of one- and two-parent progeny tests since 1959 (Rudolph and Yeatman 1982). Some important conclusions obtained are: (1) that trees from "local" sources usually grow as well as or better than the average of those from all sources; (2) that provenances moved slightly northward within the same climatic zone usually outgrow the local provenance; and (3) that evaluation of growth at about 10 years is reliable for predicting later growth and wood production. This information from both clonal and open-pollinated progeny tests of the selected trees has played an important role in the establishment and management of seed orchards of jack pine. The flowering at an early age in jack pine and the early predictability of mature performance make possible rapid generation turnover.

Since 1979, isozyme techniques have been used for studying various aspects of genetic variation of jack pine (Tobolski 1979; Dancik and Yeh 1983; Knowles 1985; Cheliak et al. 1985; Snyder et al. 1985; Ross and Hawkins 1986; Wheeler and Guries 1987; Govindaraju and Dancik 1987). Studies have shown that jack pine is genetically less variable than most other conifers (Danick and Yeh 1983; Cheliak et al. 1985; Ross and Hawkins 1986). The enzyme data were concordant with earlier hypotheses that lodgepole

and jack pine evolved from a common progenitor (Dancik and Yeh 1983; Wheeler and Guries 1987). Two studies on the mating system of jack pine have indicated that the mean estimate of outcrossing over years and loci is 88 per cent in the studied stands and that a very low level of consanguineous mating exists in natural stands (Cheliak et al. 1985; Snyder et al. 1985).

Estimates of selfing rate in jack pine populations using morphological markers (Fowler 1965; Sittman and Tyson 1971; Rudolph 1979) are 19, 10, and 7 percent respectively. These estimates are similar to those derived from enzyme markers (Cheliak et al. 1985; Snyder et al. 1985). According to Rudolph (1981), jack pine is generally self-compatible, at least through the S_2 generation. However, he also reported that there was severe inbreeding depression associated with selfs in jack pine.

MATERIALS AND METHODS

(i) Experimental Studies

Study sites included two natural populations of jack pine, GLFP and CP11 near Thunder Bay, Ontario, Canada. GLFP, with a density of 5939 stems/ha and an average tree height of 8 m, was a young stand (16 years old) originating via direct seeding following logging. CP11 was a mature, fire-origin stand (104 years old) with a density of 3750 stems/ha and an average tree height of 22 m. Twelve trees were randomly selected from each of the two populations. In both populations, most of the sampled trees were located at least 10 m apart, within an area of 60 m by 60 m. The seeds from cones collected from the sampled trees were extracted by hand, stored below $0^{o}C$, and maintained by individual mother-tree identity throughout storage and processing.

Seeds were germinated on moist filter paper until germinants grew a 3-5 mm radicle (approximately 4 to 5 days). Each emerging embryo was excised from its surrounding megagametophyte and both tissues were homogenized separately in 20 and 30 ul of extraction buffer (Yeh and O'Malley 1980) respectively. Eighty such embryo-megagametophyte pairs were assayed for each tree.

Electrophoretic procedures, staining recipes and enzyme nomenclature used follow those of Cheliak and Pitel (1984). The following enzyme systems were assayed: aconitase (ACO; E.C.4.2.1.3); esterase (fluorescent) (F-EST; E.C.3.1.1.1); glutamate dehydrogenase (GDH; E.C.1.4.1.3); malate dehydrogenase (MDH; E.C.1.1.1.37); malic enzyme (ME; E.C.1.1.1.40); 6-phosphogluconate dehydrogenase (6PGD; E.C.1.1.1.44); phosphoglucose isomerase (PGI; E.C.5.3.1.9); and phosphoglucomutase (PGM; E.C.2.7.5.1). These eight systems yielded nine polymorphic loci (Aco, F-est3, Gdh, Mdh1, Me, 6pgd1, 6pgd2, Pgi, Pgm) that could be clearly scored for both haploid megagametophyte and diploid embryo tissues. The structure of coniferous seeds (haploid megagametophyte and diploid embryo) allows inference of bands from pollen parents in the embryo. Examination of the available data in this study and comparison with related references (Cheliak et al. 1984; Knowles 1985; Snyder et al. 1985; Tobolski 1979) showed that the deviation from a 1:1 segregation ratio in megagametophytes and linkage of the employed loci were not significant ($P>0.05$).

Single- (t_s) and multilocus (t_m) estimates of the population outcrossing rate and pollen allele frequencies were calculated for both populations, using the maximum likelihood procedures of Ritland and El-Kassaby (1985). Tests of the proportions (Zar 1974) of allele frequencies

were conducted to examine the differences in allele frequencies between outcross pollen and maternal parents for each of the populations. Tests for both heterogeneity of $\underline{t}_s$ among loci and heterogeneity of $\underline{t}_m$ among populations, and tests of the hypothesis Ho: $\underline{t}_{(s \text{ or } m)} = 1$ were conducted using the Neyman-Pearson likelihood ratio criterion (Rao 1973).

Single-tree estimates of outcrossing rate ($\underline{t}_f$) and outcross pollen gene frequencies ($\underline{p}_f$) for both populations were calculated using the maximum likelihood procedures of Ritland and El-Kassaby (1985). Two methods for $\underline{t}_f$ estimation were used as follows: one ($\underline{t}_{f1}$) with $\underline{p}$ held constant, equal to the population pollen gene frequencies obtained by calculating $\underline{t}$ and $\underline{p}$ jointly with the combined data of the population, and the other ($\underline{t}_{f2}$) with joint estimation of $\underline{t}$ and $\underline{p}$ for each tree. Both were done for two purposes. (1), $\underline{t}_{f2}$ allowed for the determination of heterogeneity of $\underline{t}_f$ among trees using a likelihood ratio test (Rao 1973), and (2), the $\underline{t}_{f2}$ estimation obviates the need to assume homogeneity of pollen allele frequencies since in this method ($\underline{t}_{f2}$) the outcross pollen allele frequencies which include the consanguineous mating component can be estimated for single trees (Ritland and El-Kassaby 1985). Therefore, the differences in estimates obtained using these two methods ($\underline{t}_{f1}$ and $\underline{t}_{f2}$) are due to the manner in which $\underline{p}$ is estimated (population vs. single-

tree). I used the Wilcoxon signed-rank test (Zar 1974) to assess this difference. Any significant differences between $\underline{t}_{f1}$ and $\underline{t}_{f2}$ detected by this test indicate a bias of $\underline{t}_{f1}$ resulting from the tree-to-tree variation in $\underline{p}$. The Wilcoxon signed-rank test was chosen because both the nature of the distribution of estimated values per tree and the correlation of two estimates ($\underline{t}_{f1}$, $\underline{t}_{f2}$) per tree are unknown.

To test for heterogeneity of the observed pollen pool among maternal trees in each population, observed pollen allele frequencies (which include $\underline{p}_o$, $\underline{p}_c$, and $\underline{p}_s$) in progeny arrays of maternal trees with like genotypes were compared in contingency tables using a heterogeneity $\underline{G}$-test. One $\underline{G}_H$ value per locus was obtained by summation over genotypes. This test is a modification of the method described by Brown *et al.* (1975). To determine the relationships between the observed pollen pool heterogeneity and variation in $\underline{t}$ and between the observed pollen pool heterogeneity and variation in outcross pollen, the following Spearman's rank correlation coefficients were obtained: 1) $\underline{r}_{s(t)}$, the correlation between $\underline{t}_{f2}$ and the frequency of alternate alleles (alleles other than the $\underline{1}$ allele) observed in progeny arrays of maternal trees homozygous for the $\underline{1}$ allele; and 2) $\underline{r}_{s(p)}$, the correlation between the alternate allele frequencies estimated jointly with $\underline{t}_{f2}$ and the frequencies of these alternate alleles

observed in progeny of 11 homozygous maternal parents. Since sample sizes were small, this test should be conservative (Perry and Knowles 1990).

(ii) Simulation Studies

To determine what aspects of the pollen pool potentially affect population estimates of t, three different scenarios of pollen pool composition which may occur in natural stands were simulated, (1) homogeneous pollen pool (p_{ho}), i.e., $p=p_s+p_o$, (2) random pollen pool heterogeneity (p_{her}), i.e., p_o is altered by random factors other than p_c, and (3) pollen pool heterogeneity with consanguineous mating (p_{hec}), i.e., p_o is biased by the presence of p_c. To imitate conditions as closely as possible to those which might be experienced in one of the studied stands, the genotypes of the 12 parental trees for the CP11 site as well as the estimated population outcross pollen allele frequencies for the nine assayed loci were used. Thus, the actual simulations of these three scenarios had outcross pollen pools constructed as follows:

(1) p_{ho}: p_o was not biased by either neighbour tree or maternal tree. Hence, the p_o was simply that estimated for CP11, and did not vary among trees.

(2) p_{her}: p_o was biased by a simulated nearest neighbour tree. The frequency of an allele in this pollen pool was equal to 0.7 times its frequency in the

population outcross pollen pool plus 0.3 times its frequencies (0, 0.5 or 1.0) in a neighbour tree which was randomly drawn from a hypothetical population with allele frequencies identical to those estimated for CP11. For each allele, the additive values 0, 0.5 or 1.0 were assigned to the genotypes aa, Aa or AA, where "A" was the allele in question and "a" was the class of other alleles at that locus. A different neighbour tree was randomly drawn for each sample tree.

(3) p_{hec}: p_o was biased by maternal genotypes. The frequency of an allele in this pollen pool was equal to 0.7 times its frequency in the population outcross pollen pool plus 0.3 times its frequency in the maternal tree (0, 0.5 or 1.0).

In all three situations, the self pollen pool had the same allele frequencies as the sample tree (0, 0.5 or 1.0).

Each scenario of simulation consisted of three replications with p adjusted as above for the simulation. In each replication, a simulated sample of 80 seeds/tree was randomly drawn. Each seed was determined to be outcrossed or selfed by drawing a pseudorandom number, R, uniformly distributed between 0 and 1. An outcrossing rate of 90% was arbitrarily chosen from the potential values based on empirical work. Therefore, if $R \leq 0.9$, a seed was assigned as outcrossed.

The pollen allele of the seed at each locus was determined by comparing a pseudorandom number, r, again uniformly distributed between 0 and 1, to the cumulative pollen allele frequency level. At a locus with three alleles with frequencies of 0.5, 0.25, and 0.25 respectively, for example, the allele would be assigned as 1 if $r \leq 0.5$; if $0.5 < r \leq 0.75$ then the allele would be 2; the allele would be 3 if $r > 0.75$. For outcrossed seed, p was adjusted for the simulated conditions and, consequently, the corresponding cumulative frequency level would change accordingly. For selfed seed, p was determined by the genotype of the sample tree (0, 0.5 or 1.0). This process was repeated for each of the nine loci. Selection of a pollen genotype at one locus was independent of its genotype determined at other loci. The simulated data were analysed by the same procedures as given in the experimental studies. The bias of the population estimates of t against the sampled t due to the variation in p was calculated as: $(t_{estimated} - t_{sample})/t_{sample}$.

RESULTS

With an exception at Aco (allele 3) in CP11, no significant differences in allele frequencies between the ovule and outcross pollen pools within the two populations were observed (Table 1), indicating that the effective pollen pool was a random sample of the adult trees, represented by the maternal trees. The cause for the difference at Aco (allele 3) in CP11 is unknown.

Population estimates of t_m ranged from 0.824 to 0.952 with a mean of 0.891 and were heterogeneous ($P<0.01$) over populations (Table 2). The estimates of t_m for CP11 were significantly lower than for GLFP (Table 2). All t_m estimates were significantly less than 1. Population estimates of t_s ranged from 0.446 to 0.997, and were heterogeneous ($P<0.05$) over loci within each population (Table 2). Estimates of t_s showed a significant departure from complete outcrossing ($t=1.0$) at Aco in GLFP and at Aco and 6pgd1 in CP11 (Table 2). The differences between t_m and mean t_s were not large. Since the arithmetic means over loci were unrealistic estimates due to the low estimates of t_s for the Aco locus, minimum variance estimates of t were calculated (El-Kassaby et al. 1985) and used in the analysis.

Table 1. Allele frequencies for ovule gene pool and outcross pollen pool for GLFP and CP11 populations (S.E. in parentheses).

Locus/Allele		GLFP		CP11	
		Ovule	Outcross Pollen	Ovule	Outcross Pollen
Aco	1	0.417(0.101)	0.346	0.375(0.099)	0.322
	2	0.583(0.101)	0.645	0.542(0.102)	0.666
	3	0.0 (0.0)	0.010	0.083(0.056)	0.012*
F-est3	1	0.0 (0.0)	0.004	0.0 (0.0)	0.009
	2	1.0 (0.0)	0.994	1.0 (0.0)	0.991
	3	0.0 (0.0)	0.002	0.0 (0.0)	0.0
Gdh	1	0.0 (0.0)	0.019	0.0 (0.0)	0.048
	2	1.0 (0.0)	0.981	1.0 (0.0)	0.952
Mdh1	1	0.875(0.068)	0.920	1.0 (0.0)	0.951
	2	0.125(0.068)	0.080	0.0 (0.0)	0.049
Me	1	0.0 (0.0)	0.003	0.0 (0.0)	0.003
	2	1.0 (0.0)	0.997	1.0 (0.0)	0.997
6pgd1	1	0.375(0.099)	0.504	0.583(0.101)	0.472
	2	0.625(0.099)	0.496	0.417(0.101)	0.528
6pgd2	1	0.167(0.076)	0.111	0.042(0.041)	0.130
	2	0.042(0.041)	0.009	0.0 (0.0)	0.016
	3	0.792(0.083)	0.843	0.917(0.056)	0.814
	4	0.0 (0.0)	0.037	0.042(0.041)	0.039
Pgi	1	0.917(0.056)	0.944	0.958(0.041)	0.922
	2	0.083(0.056)	0.056	0.042(0.041)	0.078
Pgm	1	0.0 (0.0)	0.003	0.0 (0.0)	0.002
	2	1.0 (0.0)	0.997	1.0 (0.0)	0.997
	3	0.0 (0.0)	0.0	0.0 (0.0)	0.001
Sample size		12	960	12	960

* Significant at 0.05 level.

Table 2. Single-locus and multilocus population estimates of outcrossing rate for GLFP and CP11 populations (S.E.in parentheses)

	GLFP	CP11
Single-locus		
Aco	0.446 (0.047)[a]	0.551 (0.033)[a]
F-est3	---	---
Gdh	---	---
Mdh1	0.978 (0.033)	---
Me	---	---
6pgd1	0.946 (0.054)	0.889 (0.046)[a]
6pgd2	0.997 (0.040)	0.989 (0.035)
Pgi	0.988 (0.042)	0.884 (0.076)
Pgm	---	---
Mean		
t_s[b]	0.897 (0.019)[c]	0.795 (0.020)[c]
Multilocus		
t_m[d]	0.942 (0.018)[a]	0.824 (0.022)[a]
t_m[e]	0.952 (0.017)[a]	0.829 (0.021)[a]
Sample size	960	960

a: Significant ($P < 0.05$) departure from Ho: $t=1$
b: Minimum variance mean
c: Significant ($P < 0.05$) heterogeneity of t_s among loci
d: Multilocus estimate excluding the maternal genotype loci that have insufficient variation for single-locus estimates
e: Multilocus estimate for all nine loci
---: Insufficient maternal genotype classes for estimation of t_s

The mean estimates of t_{f1} in the two populations were close to the population estimates of t_m (Table 3). Estimates of t_{f2} ranged from 0.543 to 1.584 (Table 3) and were homogeneous ($P>0.05$) among trees in both populations (see the first two lines of Table 4), indicating that the assumption of uniformity of t_f for all maternal trees was valid for the population estimates of t. Estimates of t_{f2} were significantly less than 1 for two trees in GLFP and one tree in CP11 (Table 3). Wilcoxon signed-rank tests indicated no significant differences between t_{f1} and t_{f2} in either population (see the first two lines of Table 4), suggesting that population estimates of p may adequately describe the outcross pollen pool of individual trees, *i.e.*, the assumption of pollen pool homogeneity appeared valid for the population estimation of t.

G-tests indicated significant heterogeneity of pollen allele frequencies in viable offspring among trees in 3 of 9 loci for GLFP and in 4 of 9 loci for CP11 (Table 5). Spearman rank correlations, $r_{s(t)}$, showed two negatively significant values at *Gdh* and *Mdh1* in GLFP and one negatively significant value at *Gdh* in CP11 (Table 5). This result suggests that epistatic selection favoring combinations of genotypes (Epperson and Allard 1989) might act on these two loci. If heterogeneity of the observed pollen pool was due to varying outcrossing rates among trees, positive $r_{s(t)}$ would be expected. Significant

Table 3. Multilocus single-tree estimates of outcrossing rate for two populations using two methods: (1) $\underline{p}$ set constant and equal to estimated population frequencies, $\underline{t}_{f1}$; and (2) $\underline{p}$ allowed to vary among trees (joint estimation of $\underline{t}$ with $\underline{p}$, $\underline{t}_{f2}$) (S.E.in parentheses).

	GLFP		CP11	
Single-tree	$\underline{t}_{f1}$	$\underline{t}_{f2}$	$\underline{t}_{f1}$	$\underline{t}_{f2}$
1	1.031	1.123 (0.182)	0.891	---
2	0.784	1.584 (0.819)	0.945	1.021 (0.086)
3	1.017	0.940 (0.089)	0.499	1.051 (0.908)
4	1.148	---	0.872	0.915 (0.203)
5	0.813	0.543 (0.171)	0.839	0.840 (0.097)
6	0.946	0.894 (0.065)[a]	1.129	1.016 (0.056)
7	0.840	1.482 (0.419)[a]	1.060	0.929 (0.094)
8	0.905	1.575 (1.270)	0.654	0.855 (0.152)
9	0.940	---	0.811	0.759 (0.244)[a]
10	---	1.008 (0.034)	0.847	0.921 (0.073)
11	0.905	0.950 (0.107)	0.722	1.034 (0.256)
12	0.965	1.004 (0.064)	0.498	0.828 (0.557)
Mean				
$\underline{t}_f$[b]	0.936	1.110 (0.160)	0.814	0.924 (0.106)
$\underline{t}_f$[c]		0.977 (0.025)		0.954 (0.032)
Population				
$\underline{t}_m$[d]		0.936 (0.021)[a]		0.824 (0.023)[a]

a: Significant ($P<0.05$) departure from Ho: $\underline{t}=1$
b: Arithmetic mean was calculated because the variance for the single-tree is unobtainable using the first method
c: Minimum variance mean
d: Population mulitlocus estimate of outcrossing rate excluding non-convergent single-trees (see Ritland and El-Kassaby 1985)
---: Single-tree was not convergent (see Ritland and El-Kassaby 1985)

Table 4. The variation in $\underline{p}$ and its effect on the population estimation of outcrossing rate (degrees of freedom in parentheses).

Population or simulation scenarios	Replication	$\underline{G}$-test for p^a	Likelihood ratio test for $\underline{t}_{f2}$	Wilcoxon signed-rank test for $\underline{t}_{f1}-\underline{t}_{f2}$	% Bias $\underline{t}_m$	% Bias $\underline{t}_s$
GLFP		3	14.4(10) ns	13(9) ns		
CP11		4	12.2(11) ns	16(11) ns		
P_{ho}	S11	0	6.7(12) ns	20(12) ns	-3.0	-6.9
	S12	0	12.5(11) ns	29(11) ns	0.4	0.4
	S13	0	5.5(11) ns	29(11) ns	0.8	0.0
	mean	0			-0.6	-2.1
P_{her}	S21	7	13.7(12) ns	18(12) ns	-0.3	2.2
	S22	8	12.3(12) ns	27(12) ns	-0.4	-4.1
	S23	8	13.2(12) ns	24(12) ns	-0.4	1.9
	mean	7.7			-0.3	0.0
P_{hec}	S31	3	8.3(11) ns	1(11)***	-16.4	-30.9
	S32	1	10.2(12) ns	0(12)***	-18.9	-32.1
	S33	2	6.3(12) ns	3(12)***	-14.3	-28.8
	mean	2			-16.5	-30.5

a: The number of significant ($P<0.05$) loci (out of 9 loci).
ns: Not significant at 0.05 level.
***: Significant at 0.01 level

Table 5. The results of G-test and Spearman rank test for pollen pool heterogeneity (degrees of freedom in parentheses).

	G-test	Spearman rank test	
		r_s(t)	r_s(p)
Population		GLFP	
Locus			
Aco	81.75 (18)***	---a	---a
F-est3	15.99 (22)	0.14 (8)	0.96 (8)***
Gdh	19.48 (11)	-0.65 (8)*	0.97 (8)***
Mdh1	19.08 (9)*	-0.74 (6)*	0.97 (6)***
Me	8.35 (11)	-0.34 (8)	0.99 (8)***
6pgd1	17.36 (9)*	0.80 (2)	0.20 (2)
6pgd2	28.33 (25)	0.23 (6)	0.26 (6)
Pgi	8.70 (10)	0.29 (7)	0.74 (7)*
Pgm	8.35 (11)	0.19 (8)	0.98 (8)***
Population		CP11	
locus			
Aco	157.08 (13)***	0.60 (3)	1.00 (3)***
F-est3	10.38 (11)	-0.07 (9)	0.94 (9)***
Gdh	84.00 (11)***	-0.91 (9)***	0.98 (9)***
Mdh1	18.83 (11)	-0.35 (9)	0.98 (9)***
Me	7.19 (11)	0.40 (9)	1.00 (9)***
6pgd1	36.38 (9)***	-0.40 (2)	1.00 (2)***
6pgd2	38.83 (30)	0.36 (8)	0.89 (8)***
Pgi	19.80 (10)*	-0.34 (8)	1.00 (8)***
Pgm	12.12 (22)	0.13 (9)	0.99 (9)***

* P<0.05 *** P<0.001

a: no homozygous maternal genotypes included in sampled trees

positive $r_{s(p)}$ were obtained at all loci in CP11 and in 6 of 8 loci in GLFP (Table 5). These results indicate that the heterogeneity of the pollen pool allele frequencies in viable progeny observed among trees at most loci was likely due to actual heterogeneity of allele frequencies within the pollen cloud rather than variation in t.

The results of the simulation studies are given in Table 4 and in Table 6, and are summarized below.

(1) In all simulations, estimates of t_{f2} were homogeneous ($P>0.05$) (Table 4), reflecting the true nature of the data.

(2) The G-test was substantially less sensitive in detecting pollen pool heterogeneity when consanguineous mating existed (Table 4).

(3) For population estimations, no apparent biases of the population outcrossing estimates (t_m, t_s) were observed in p_{ho} or p_{her} simulations (Tables 4 and 6). Wilcoxon signed-rank test showed that the assumption of pollen pool homogeneity was valid for the population estimates of t in p_{ho} and p_{her} simulations (Table 4), i.e., random pollen pool heterogeneity did not appear to affect the population estimates of t. However, in p_{hec} simulations, single-locus estimates were biased down 30.5% from the true sample value, nearly twice as much as the multilocus estimates (16.5%) (Tables 4 and 6).

(4) For the t_{f2}, there are no apparent differences

Table 6. Population and single-tree estimates of outcrossing rate in the simulation studies (S.E. in parentheses).

Simulation scenario	Replication	Sample	Population[a]		Single-tree	
		$\underline{t}$	$\underline{t}_m$	$\bar{\underline{t}}_s$[b]	$\bar{\underline{t}}_{f1}$[c]	$\bar{\underline{t}}_{f2}$[b]
P_{ho}	S11	0.880	0.854 (0.018)	0.819 ns (0.022)	0.852	0.878 (0.022)
	S12	0.895	0.899 (0.018)	0.899 ns (0.021)	0.898	0.889 (0.020)
	S13	0.900	0.907 (0.016)	0.900 ns (0.022)	0.907	0.900 (0.020)
	mean	0.892	0.887NS	0.873	0.886	0.889
P_{her}	S21	0.906	0.903 (0.016)	0.926 ns (0.020)	0.911	0.910 (0.023)
	S22	0.897	0.893 (0.017)	0.860 * (0.021)	0.897	0.906 (0.020)
	S23	0.901	0.897 (0.016)	0.918 ns (0.020)	0.906	0.869 (0.020)
	mean	0.901	0.898NS	0.901	0.905	0.895
P_{hec}	S31	0.909	0.760 (0.021)	0.628 ns (0.022)	0.747	0.903 (0.035)
	S32	0.907	0.736 (0.021)	0.616 ns (0.022)	0.728	0.855 (0.033)
	S33	0.906	0.776 (0.020)	0.645 ns (0.022)	0.778	0.869 (0.032)
	mean	0.907	0.757NS	0.630	0.751	0.876

a: Estimates of $\underline{t}_m$ were based on 9 loci; $\underline{t}_s$ on 4 loci
b: Minimum variance mean
c: Arithmetic mean
NS: Not significant heterogeneity among replications ($P>0.05$).
*: Significant ($P<0.05$) heterogeneity of $\underline{t}_s$ among loci; ns, not significant ($P>0.05$)

between mean sample $\underline{t}$ and mean estimated $\underline{t}_{f2}$ in all simulations (Table 6). This result, as expected, indicates that single-tree joint estimation of $\underline{t}$ and $\underline{p}$ is unbiased regardless of pollen pool characteristics within the limits considered in this study.

DISCUSSION

The experimental studies reported above indicated that pollen pool heterogeneity did exist in two natural populations of jack pine. The G-test showed significant heterogeneity of the pollen allele frequencies in viable progeny at about 40% of the loci in both populations. Spearman rank correlations indicated that the observed pollen pool heterogeneity was due to the actual heterogeneity of allele frequencies within the pollen pool. Moreover, this conclusion is strongly supported by the fact that no tree-to-tree variation in t was observed in this study. The experimental studies aimed at assessing levels of pollen pool heterogeneity were not designed to address causal factors, and the sources of the pollen pool heterogeneity detected in the experimental portion of the present study therefore remain obscure. Theoretically, stochastic factors, microhabitat selection, and population substructure can result in pollen pool heterogeneity (Shaw et al. 1981; Bijlsma et al. 1986; Fripp et al. 1987; Merzeau et al. 1989).

According to the Wilcoxon signed-rank tests, the observed pollen pool heterogeneity did not appear to affect the population estimates of t. Comparison of the results

between the experimental and simulation studies (Tables 3, 4, and 6) indicates that the heterogeneity observed in the experimental portion most closely resembles the p_{her} scenario rather than the p_{hec} scenario of simulation. However, there was an exception. Theoretically, single-tree multilocus estimates of t (t_f as in this study) can be made and then averaged for the population t (Table 6). The simulation study confirmed this expectation, but for CP11 the differences between t_m and $\bar{t}_{f2}$ appeared large (Table 3). The simulations offered no insight regarding this deviation and its cause remains unknown. Natural pollen pools are likely more complex than the simulation models employed, and may involve factors (e.g., selection and linkage disequilibrium) and interactions which were not considered.

The consanguineous mating levels in the studied populations appeared low. The average of t_s is expected to be lower than the corresponding t_m when cross-pollination occurs among family members (Shaw et al. 1981). A comparison of t_s and t_m suggests that consanguineous mating levels were low, especially in CP11 (Table 2). A direct measure of consanguineous mating was made following Ritland and El-Kassaby's regression method. The regressions have non-significant pooled slopes for both populations (b_{glfp}=0.049 and b_{cp11}=0.019). Previous studies in jack pine (Cheliak et al. 1985; Snyder et al. 1985) also showed

low consanguineous mating levels. A study of the population substructure in these same jack pine stands using spatial autocorrelation analysis of genetic data (Xie, unpubl. data) indicated that a very weak substructure existed. Therefore, the pollen pool heterogeneity detected in the experimental portion was likely random in nature.

The simulation studies showed that random pollen pool heterogeneity did not appear to affect the population estimates of $\underline{t}$. A possible explanation for this result is that $\underline{p}$ still is an unbiased estimator for the population even if the pollen pool is randomly heterogeneous among trees. Random fluctuations in single-tree estimates of $\underline{t}_{f1}$ may result when the pollen pool is randomly heterogeneous, but the effects are eliminated when an average (population) estimate of $\underline{t}$ is obtained. Fripp et al. (1987) also reported that temporal pollen pool heterogeneity did not have a major effect on the population estimates of $\underline{t}$.

The simulation studies also showed that the consanguineous mating pollen pool is capable of biasing population estimates of $\underline{t}$ downward. When consanguineous mating exists, both $\underline{p}$ and $\underline{t}$ are biased estimators because the mixed-mating model does not accommodate consanguineous mating, and they are related to the $\underline{p}_c$ in that $\underline{p}=\underline{p}_o+\underline{p}_c$ instead of $\underline{p}=\underline{p}_o$. Since $\underline{p}$ is biased by the maternal genotype and $\underline{p}$ and $\underline{t}$ are jointly estimated, a biased population estimate of $\underline{t}$ results. This conclusion is also

supported by the previous studies of the effects of population substructure on $\underline{t}$ (Ennos and Clegg 1982; Ellstrand and Foster 1983; Shea 1987). Therefore, $\underline{p}_c$ is a potentially important conponent of the total pollen pool. Consanguineous mating influences plant mating system evolution by decreasing the "cost of meiosis" (Uyenoyama 1986; Waller and Knight 1989). Thus the study of $\underline{p}_c$ can provide important insights into species' biology. Practically, consanguineous mating may produce seeds which exhibit inbreeding depression.

In addition to quantitative estimates of population outcrossing rates in jack pine, this study provides substantial evidence for the accuracy of these estimates. First, the outcrossing rate was homogenous among all studied maternal trees. Second, the estimates of $\underline{t}$ appeared unaffected by the detected pollen pool heterogeneity. However, population estimates of $\underline{t}_m$ differed significantly between GLFP and CP11 (Table 2). This might be due to the difference in (1) stand density, (2) stand age, and (3) regeneration history between the two populations. The mean estimate of outcrossing rates obtained in this study is 89%, which is similar to those of the previous studies in jack pine using either isozyme markers (Cheliak et al. 1985; Snyder et al. 1985) or morphological markers (Fowler 1965; Rudolph 1979; Sittman and Tyson 1971). This value is also comparable to those

reported for natural populations of most other conifers (for summary see Table 1 in Muona 1990).

Interestingly, the G-tests of simulated data produced some significant results in p_{hec} but not as many as in p_{her}. This is not unexpected since (a) the pollen pool of like genotypes was similarly affected when the variation in p was correlated with the maternal genotypes as in consanguineous mating and (b) the G-test procedure tested for heterogeneity just among like genotypes. However, it is not clear why the results of the G-test should differ between p_{hec} and p_{ho} (Table 4). For this reason 11 further replications were simulated for each of these two scenarios. In these replications, significant tests obtained are 7 of the 99 tests (9 loci X 11 replications) for the p_{hec} scenario and 10 of the 99 tests for p_{ho}. These additional findings indicate that the G-test is insensitive in detecting pollen pool heterogeneity caused by consanguineous mating. The results of the G-tests presented in Table 4 were misleading but the apparent discrepancy between p_{hec} and p_{ho} may be attributed to the small numbers of replications. It is also apparent that more significant tests were obtained in p_{ho} than would be expected by chance at the 0.05 level. These spurious test results occurred mainly at loci with extremely uneven allele frequencies (e.g., Gdh and Me) and may be a result of large numbers of empty cells in the matrices tested.

The results of this study have the following implications. First, the mixed-mating model has practical limitations. A complex model that really reflects the genetic transmission process would be valuable. Second, when the mixed-mating model is used, it is necessary to consider the possibility of consanguineous mating. A simple, indirect method is to compare $\underline{t}_m$ with $\underline{t}_s$ (Shaw *et al.* 1981). The simulation portion of this study confirmed the reliability of this method. The direct methods are (1) to detect the effect using Wilcoxon signed-rank test, and (2) to assess the level of consanguineous mating using the regression method developed by Ritland and El-Kassaby (1985). It is also important to note that conventional tests for pollen pool heterogeneity (Brown *et al.* 1975) may be ineffective when existing heterogeneity is caused by consanguineous matings. Third, this study presents an approach to investigate pollen pool heterogeneity. The advantage of this approach can provide information as to whether pollen pool heterogeneity exists and whether it affects a population estimate of $\underline{t}$. And fourth, this study has provided an unbiased estimate of $\underline{t}$ with which tree breeders may accurately predict potential losses due to inbreeding depression in tree improvement programs of jack pine.

LITERATURE CITED

Barrett, J.W., P. Knowles, and W.M. Cheliak. 1987. The mating system in a black spruce clonal seed orchard. Can. J. For. Res. 17:379-382.

Bijlsma, R., R.W. Allard, and A.L. Kahler. 1986. Nonrandom mating in an open-pollinated maize population. Genetics 112:669-680.

Boyle, T.J.B. and E.K. Morgenstern. 1986. Estimates of outcrossing rates in six populations of black spruce in central New Brunswick. Silv. Genet. 35(2-3):102-106.

Bradshaw, A.D. 1972. Some of the evolutionary consequences of being a plant. Evol. Biol. 5:25-47.

Brown, A.H.D. 1990. Genetic characterization of plant mating systems. Pp. 145-162. In: Plant Population Genetics, Breeding and Genetic Resources. Edited by A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir. Sinauer, Sunderland, Massachusetts.

Brown, A.H.D. and R.W. Allard. 1970. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. Genetics 66:133-145.

Brown, A.H.D., S.C.H. Barrett, and G.F. Moran. 1985. Mating system estimation in forest trees: Models, methods and meaning. Pp. 32-49. In:Population Genetics in Forestry, H. Gregorius (ed). Goettingen: Springer-Verlag.

Brown, A.H.D., A.C. Matheson, and K.G. Eldridge. 1975. Estimation of mating system of Eucalyptus obliqua L, Herit. by using allozyme polymorphisms. Aust. J. Bot. 23:931-949.

Cheliak, W.M. 1985. Mating system dynamics in a Scots pine seed orchard. Pp. 107-117. In:Population Genetics in Forestry, H.Gregorius (ed). Goettingen: Springer-Verlag.

Cheliak, W.M., B.P. Dancik, K. Morgan, F.C.H. Yeh, and C. Strobeck. 1985. Temporal variation of the mating system in a natural population of jack pine. Genetics, 109: 569-584.

Cheliak, W.M., K. Morgan, B.P. Dancik, C. Strobeck, and F.C.H. Yeh. 1984. Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, Pinus banksiana Lamb. Theor. Appl. Genet. 69:145-151.

Cheliak, W.M., K. Morgan, C. Strobeck, F.C.H. Yeh, and B.P. Dancik. 1983. Estimation of mating system parameters in plant populations using the EM algorithm. Theor Appl. Genet. 65:157-161.

Cheliak, W.M.and J.A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Inf. Rep. PI-X-42. Can. For. Serv., Environment Canada, Ottawa.

Clegg, M.T. 1980. Measuring plant mating systems. Bioscience. 30:814-818.

Clegg, M.T., A.L. Kahler, and R.W. Allard. 1978. Estimation of life-cycle components of selection in an experimental plant population. Genetics 89: 765-792.

Conkle, M.T. 1972. Analyzing genetic diversity in conifers: Isozyme resolution by starch gel electrophoresis. USDA, P. NW. For. Rang. Exp. Sta., Res. Note PSW-264. 5pp.

Dancik, B.P. and F.C. Yeh. 1983. ALlozyme variability and evolution of lodgepole (Pinus contorta var. latifolia) and jack pine (Pinus banksiana) in Alberta. Can. J. Genet. Cytol. 25:57-64.

Denti, D. and D.J. Schoen. 1988. Self-fertilization rates in white spruce: Effect of pollen and seed production. J. Heredity 79: 284-288.

El-Kassaby, Y.A., M.D. Meagher, J. Parkinson, and F.T. Portlock. 1987. Allozyme inheritance, heterozygosity and outcrossing rate among Pinus monticola near Ladysmith, British Columbia. Heredity 58:173-181.

El-Kassaby, Y.A., J. Parkinson, and W.J.B. Devitt. 1985. The effect of crown segment on the mating system in a Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seed orchard. Silv. Genet. 35,4:149-155.

Ellstrand, N.C. 1984. Multiple paternity within the fruits of the wild radish, Raphanus sativus. Amer. Natur. 123:819-828.

Ellstrand, N.C. and K.W. Foster. 1983. Impact of population structure on the apparent outcrossing rate of grain sorghum (Sorghum bicolor). Theor. Appl. Genet. 66:323-327.

Ennos, R.A. and M.T. Clegg. 1982. Effect of population substructuring on estimates of outcrossing rate in plant populations. Heredity 48:283-292.

Epperson, B.K. and R.W. Allard. 1989. Spatial autocorrelation analysis of genotypes within populations of lodgepole pine. Genetics 121: 369-377.

Epperson, B.K. and R.W. Allard. 1984. Allozyme analysis of the mating system in lodgepole pine populations, J. Heredity 75: 212-214.

Farmer, R.E., P. Knowles, and W.H. Parker. 1983. Genetic resources of the North American Boreal Forest. Pp. 40-51. In: Resources and dynamics of the Boreal Zone. Proc. of a conference held at Thunder Bay, Ontario, August.

Farris, M. A. and J.B. Mitton. 1984. Population density, outcorossing rate, and heterozygote superiority in ponderosa pine. Evolution 38:1151-1154.

Fowler, D. P. 1965. Natural self-fertilization in three jack pines and its implications in seed orchard management. For. Sci. 11(1):56-58.

Franklin, E.C. 1970. A survey of mutant forms and inbreeding depression in species of the family Pinaceae. USDA For. Ser. Res. Pap. SE-61.

Fripp, Y.J., A.R. Griffin, and G.F. Moran. 1987. Variation in allele frequencies in the outcross pollen pool of Eucalyptus regnans F. Muell. throughout a flowering season. Heredity 59:161-171.

Furnier, G.R. and W.T. Adams. 1986. Mating system in natural populations of Jeffrey pine. Am. J. Bot. 73:1002-1008.

Fyfe, J.L. and N.T.J. Bailey. 1951. Plant breeding studies in leguminous forage crops. I. Natural crossbreeding in winter beans. J. Agric. Sci. 41:371-378

Govindaraju, D.R. and B.P. Dancik. 1987. Allozyme

heterozygosity and homeostasis in germinating seeds of jack pine. Heredity 59:279-283.

Green, A.G., A.H.D. Brown, and R.N. Oram. 1980. Determination of outcrossing rate in a breeding population of Lupinus albus l. (white lupin). Z. Pflanzenzucht 84:181-191.

Greenwood, M.S. 1986. Gene exchange in loblolly pine: the relation between pollination mechanism, female receptivity and pollen availability. Amer. J. Bot. 73(10):1443-1451.

Guries, R.P. and F.T. Ledig. 1981. Genetic structure of populations and differentiation in forest trees. Pp. 42-47 in: Proc. Symp. on Isozymes of N. Amer. For. Trees and For. Insects. USDA. Rep. PSW-48, 64pp.

Hamrick, J.L. 1982. Plant population genetics and evolution. Amer. J. Bot. 69(10):1685-1693.

Hamrick, J.L. and A. Schnabel. 1985. Understanding the genetic structure of plant populations: some old problems and a new approach. Pp. 50-70, In: Population Genetics in Forestry, H.Gregorius (ed). Goettingen: Springer-Verlag.

Jain, S.K. 1979. Estimation of outcrossing rates: some alternative procedures. Crop Sci. Vol.9:23-26.

Jones, D.F. 1916. Natural cross-pollination in the tomato. Science 43: 509-510.

Kahler, A.L. M.T. Clegg, and R.W. Allard. 1975. Evolutionary changes in the mating system of an experimental population of Barley (Hordeum vulgare L.) Proc. Natl. Acad. Sci. USA 72:943-946.

King, J.N., B.P. Dancik, and N.K. Dhir. 1984. Genetic structure and mating system of white spruce (Pinus glauca) in a seed production area. Can. J. For. Res. 14:639-643.

Knowles, P. 1985. Comparison of isozyme variation among natural stands and populations: jack pine and black spruce. Can. J. For. Res. 15:902-908.

Knowles, P., G.R. Furnier, M.A. Aleksiuk, and D.J. Perry. 1987. Significant levels of self-fertilization in natural populations of tamarack. Can. J. Bot. 65(6):1087-1091.

Levin, D.A. 1988. The paternity pools of plants. Amer. Natur. 123(3):309-317

Levin, D.A. and H.W. Kerster. 1974. Gene flow in seed plants. Evol. Biol. 7:139-220.

Loveless, M.D. and J.L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15:65-95.

Magnussen, S. and C.W. Yeatman. 1988. Height growth and survival of within- and between-provenance crosses in jack pine. Can. J. For. Res. 18:1145-1151.

Meagher, T.R. 1986. Analysis of paternity within a natural population of Chamaelirium luteum. I. Identification of most-likely male parents. Amer. Nat. 128:199-215.

Merzeau, D.,F. Di Giusto, B. Comps, B. Thiebaut, J. Letouzey, and J. Cuguen. 1989. Genetic control of isozyme systems and heterogeneity of pollen contribution in beech (Fagus sylvatica L.). Silv. Genet. 38, (5-6):195-201.

Moran, G.F., J.C. Bell, and A.C. Matheson. 1980. The genetic structure and levels of inbreeding in a Pinus radiata D. Don seed orchard. Silv. Genet. 29:190-193.

Morris, R.W. and P.T. Speith. 1978. Sampling strategies for using female gametophytes to estimate heterozygosity in conifers. Theor. Appl. Genet. 51:217-222.

Muller, G. 1977. Cross-fertilization in a conifer stand inferred from enzyme gene-markers in seeds. Silv. Genet. 26:223-226.

Muller-Starck, G. 1982. Reproductive systems in conifer seed orchards: I. Mating probabilities in a seed orchard of Pinus sylvestris L. Silvae Genet. 31: 188-197.

Muller-Starck, G. 1979. Estimates of self- and cross-fertilization in a Scots pine seed orchard. Pp. 170-179, Proc. Conf. Biochem. Genet. of For. Trees. Umea, Sweden 1978.

Muona, O. 1990. Population genetics in forest tree improvement. Pp. 282-298. In: Plant Population Genetics, Breeding and Genetic Resources. Edited by A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S.

Weir. Sinauer, Sunderland, Massachusetts.

Neale, D.B. 1983. Population genetic structure of the Douglas-fir shelterwood regeneration system in southwest Oregon. Ph.D. Thesis, Oregon State Uni. Corvallis, 179 pp.

O'Reilly, C., W.H. Parker, and J.E. Barker. 1982. Effect of pollination period and strobili number on random mating in a clonal seed orchard of Picea mariana. Silv. Genet. 31(2/3):90-94.

Perry, D.J. and B.P. Dancik. 1986. Mating system dynamics of lodgepole pine in Alberta, Canada. Silv. Genet. 35:190-195.

Perry, D.J. and P. Knowles. 1990. Evidence of high self-fertilization in natural populations of eastern white cedar (Thuja occidentalis L.). Can. J.Bot. vol. :663-668.

Perry, D.J., P.Knowles and F.C. Yeh. 1990. Allozyme varaition of Thuja occidentalis L. in northwestern Ontario. Biochemical Systematics and Ecology (in press).

Rao, C.R. 1973. Linear statistical inference and its applications. Wiley, N.Y. USA

Ritland, K. 1984. The effective proportion of self-fertilization with consanguineous matings in inbred populations. Genetics 106:139-152

Ritland, K. 1989. Correlated matings in the partial selfer Mimulus guttatus. Evolution, 43(4): 848-859.

Ritland, K. and Y.A. El-Kassaby. 1985. The nature of inbreeding in a seed orchard of Douglas-fir as shown by an efficient multilocus model. Theor. Appl. Genet. 71:375-384.

Ritland, K. and F.R. Ganders. 1985. Variation in the mating system of Bidens menziesii (Asteraceae) in relation to population substructure. Heredity 55:235-244.

Ritland, K. and S.K. Jain. 1981. A model for the estimation of outcrossing rate and gene frequencies using an independent loci. Heredity 47:35-52.

Ross, H.A. and J.L. Hawkins. 1986. Genetic variation among local populations of jack pine (Pinus banksiana).

Can. J. Genet. Cytol. 28:453-458.

Rudolf, P.O. 1958. Silvical characteristics of jack pine (Pinus banksiana) USDA For. Serv. Pap. 61. Lake States For. Exp. Stn. 31p.

Rudolph, T.D. 1979. Seed production in the first eight years and frequency of natural selfing in a simulated jack pine seedling seed orchard. Pp.33-47, In:Proc., Thirteen Lake States For. For. Tree Improv. Conf.

Rudolph, T.D. 1981. Four year height growth variation among and within S_o , S_1 x S_1, S_1 open-pollinated, and S_2 inbred jack pine families. Can. J. For. Res. 11:654-661.

Rudolph, T.D. and C.W. Yeatman. 1982. Genetics of jack pine. Res. Pap. WO-38. USDA For. Serv. 60 p.

Sarvas, R. 1967. Pollen dispersal within and between subpopulations: role of isolation and migration in microevolution of forest tree species. Pp. 332-345 in: Proc. XIV IUFRO Congr., Vol. III, Munich, 1967.

Schoen, D.J. 1988. Mating system estimation via the one pollen parent model with the progeny array as the unit of observation. Heredity 60(3) 439-446.

Schoen, D.J. and M.T. Clegg. 1984 Estimation of mating system parameters when outcrossing events are correlated. Proc. Natl. Acad. Sci. USA 81: 5258-5262

Schoenike, R.E. 1962. Natural variation in jack pine. Ph.D. thesis. School of For., Univ. Minn., 233p.

Schoenike, R.E. 1976. Geographic variation in jack pine. Univ. Minn. Agric. Exp. Stn. Tech. Bull. 304, 49 p.

Shaw, D.V. and R.W. Allard. 1982. Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theor. Appl. Genet. 62:113-120

Shaw, D.V., A.L. Kahler, and R.W. Allard. 1981. A multilocus estimator of mating system parameters in plant populations. Proc. Natl. Acad. Sci. USA 78:1298-1302

Shea, K.L. 1987. Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. Evolution 41:124-136.

Shen, H.H., D. Rudin, and D. Lindgren. 1981. Study of the pollination pattern in a Scots pine seed orchard by means of isozyme analysis. Silv. Genet. 30:7-15.

Sittmann, K. and H. Tyson. 1971. Estimates of inbreeding in Pinus banksiana. Can. J. Bot. 49:1241-1245.

Slatkin, M. 1985a. Rare alleles as indicator of gene flow. Evolution 39:53-65.

Slatkin, M. 1985b. Gene flow in natural populations. Ann. Rev. Eco. Syst. 16: 393-430.

Slatkin, M. and N.H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43(7):1349-1368.

Sluder, E.R. 1969. Gene flow patterns in forest tree species and implications for tree breeding. Pp. 1140-1150. In: Proc. Second World Consult. on For. Tree Breed., IUFRO, 1969 Washington.

Snyder, T.P., D.A. Stewart, and A.F. Strickler. 1985. Temporal analysis of breeding structure in jack pine (Pinus banksiana). Can. J. For. Res. 15:1159-1166.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. Freeman and Co., New York, 859 pp.

Squillace, A. E. 1974. Average genetic correlations among offspring from open-pollinated forest trees. Silvae Genet. 23:149-156.

Squillace, A.E. and R.E. Goddard. 1982. Selfing in clonal seed orchards of slash pine. For. Sci. 28:71-78.

Stern, K. and L. Roche. 1974. Genetics of forest ecosysytems, Chapman and Hall., London.

Tobolski, J.J. 1979. Inheritance of polymorphic isozymes in jack pine (Pinus banksiana Lamb) Pp. 147-154, In:Proc., First North Cent. Tree Improv. Conf.

Uyenoyama, M.K. 1986. Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. Evolution 40: 388-404.

Waller, D.M. and S.E. Knight. 1989. Genetic consequences of outcrossing in the cleistogamous annual, Impatiens capensis. II. outcrossing rates and

genotypic correlations. Evolution, 43(4): 860-869.

Wang, C.W., T.O. Perry, and A.G. Johnson. 1960. Pollen dispersion of slash pine (Pinus elliottii Engelm.) with special reference to seed orchard management. Silv. Genet. 9:78-86.

Wehrhahn, C.F. and R. Powell. 1987. Electrophoretic variation, regional differences, and gene flow in the coho salmon (Oncorhynchus kisutch) of southern British Columbia. Can. J. Fish. Aquat. Sci. 44:822-831.

Wheeler, N.C. and R.R. Guries. 1987. A quantitative measure of introgression between lodgepole and jack pine. Can. J. Bot. 65:1876-1885.

Wright, S. 1951. The genetical structure of population. Ann. Eugen. 15:323-354.

Xie, C.Y. 1989. Mating systems and genetic structure of Thuja orientalis Linn.. Ph.D dissertation, University of Alberta. Canada.

Yeh, F.C. 1988. Isozyme variation of Thuja plicata (Cupressaceae) in British Columbia. Biochemical Systematics and Ecology Vol. 16(4):373-377.

Yeh, F.C.H. and K. Morgan, 1987. Mating system and multilocus associations in a natural population of Pseudotsuga menziesii (Mirb.) Franco. Theor. Appl. Genet. 73:799-808.

Yeh, F.C.H., A. Brune, W.M. Cheliak, and D.C. Chipman. 1983. Mating system of Eucalyptus citriodora in a seed-production area. Can. J. For. Res. 13:1051-1055

Yeh, F.C. and D. O'Malley. 1980. Enzyme variations in natural populations of Douglas-fir, Pseudotsuga menziessi (Mirb.) Franco., from British Columbia. I. Genetic variation patterns in coastal populations. Silv. Genet. 29:83-92

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs. NJ. USA.