Council of Italy (CNR). Porano-Orvieto Italy, October 11—13 (1988). —— Allard, R. W., Jain, S. K. and Workman, P. L.: The genetics of inbreeding populations. Adv. Genet. 14: 55—131 (1968). —— Barrett, J. W.: Knowles, P. and Cheliak, W. M.: The mating system in a black spruce clonal seed orchard. Can. J. For. Res. 17: 379—382 (1987). —— Brown, A. D. H.: The estimation of Wrights fixation index from genotypic frequencies. Genetica 41: 399—406 (1970). —— Brown, A. D. H.: Enzyme polymorphism in plant populations. Theor. Pop. Biol. 15: 1—42 (1979). —— Brown, A. H. D., Barrett, S. C. H. and Moran, G. F.: Mating system estimation in forest trees: models, methods and meanings. In: (Ed.) Gregorius, H.-R.: Population genetics in forestry. Springer-Verlag, Berlin. pp. 32—49 (1984). —— Brown, A. H. D., Matheson, A. C. and Eldridge, K. G.: Estimation of the mating system of *Eucalyptus obliqua* L'Herit. by using allozyme polymorphisms. Aust. J. Bot. 23: 931—949 (1975). —— Cheliak, W. M.: Mating system dynamics in a Scots pine seed orchard. In: Proc. IUFRO Working Party on Ecological Population Genetics. Göttingen, 1984. pp. 107—117 (1984). —— Cheliak, W. M., Pitel, J. A. and Murray, G.: Population structure and mating system of white spruce. Can. J. For. Res. 15: 301—308 (1985). —— Clegg, M. T.: Measuring plant mating systems. Bioscience 30: 814—818 (1980). —— Crow, J. F. and Kimura, M.: An introduction to population genetics theory. Harper and Row, New York (1970). —— Dyakowska, J.: Research on the rapidity of the failing down of pollen of some trees. Bull. Intern. Acad. Polon. Sci. Lett. Ser. Sci. Natur., Cracovie: 155—168 (1936). —— El-Kassaby, Y. A., Fashler, A. M. K. and Sziklai, O.: Reproductive phenology and its impact on genetically improved seed production in a Douglas-fir seed orchard. Silvae Genet. 33: 120—125 (1984). —— El-Kassaby, Y. A., Meagher, M. D., Parkinson, J. and Portlock, F. T.: Allozyme inheritance, heterozygosity and outcrossing rate among *Pinus monticola* near Ladysmith, British Columbia. Heredity 58: 173—181 (1987). —— Farris, M. A. and Mitton, J. B.: Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. Evolution 38: 1151—1154 (1984). —— Hamrick, J. L., Mitton, J. B. and Linhart, Y. B.: Levels of genetic variation in trees. Influence of life history characteristics. In: Isozymes of North American Forest trees and forest insects. USDA Gen. Tech. Rep. PSW-48. pp. 35—41 (1981). —— King, J. N., Dancik, B. P. and Dhir, N. K.: Genetic structure and mating system of white spruce (*Picea glauca*) in a seed production area. Can. J. For. Res. 14: 639—643 (1984). —— Knowles, P., Furnier, G. R., Aleksiuk, M. A. and Perry, D. J.: Significant levels of self-fertilization in natural populations of tamarack. Can. J. Bot. 65: 1087—1091 (1987). —— Kosinski, G.: Przyczyny powstawania pustych nasion u modrzewia europejskiego (*Larix decidua* Mill.). Arbor. Kornickie 31: 107—182 (1986). —— Koski, V.: On self-pollination, genetic load, and subsequent inbreeding in some conifers. Communic. Inst. For. Fenn. 78 (10): 1—42 (1973). Kulesza, W.: Modrzew polski na Górze Chelmowej i w Majdowie pod Skarzyskiem. Sylwan 45 (4): 221—227 (1927). —— Latocha, A. and Hawrys, Z.: Wzrost i rozwój introdukowanych gatunków drzew w rejonach przemyslowych. Sylwan 120: 29—38 (1976). —— Lewandowski, A. and Mejnartowicz, L.: Inheritance of allozymes in *Larix decidua* Mill. Silvae Genet. 39: 184—188 (1990). —— Linhart, Y. B., Mitton, J. B., Sturgeon, K. B. and Davis, M. L.: Genetic variation in space and time in a population of ponderosa pine. Heredity 46: 407—426 (1981). —— Mejnartowicz, L. and Bergmann, F.: Genetic studies on European larch (*Larix decidua* Mill.). employing isoenzyme polymorphism. Genet. Polon. 16: 29—35 (1975). —— Mitton, J. B.: Conifers. In: Isozymes in plant genetics and breeding. Ed.: S. D. Tanksley and T. J. Orton. Elsevier, Amsterdam. pp. 443—472 (1983). —— Müller-Starck, G: Sexual asymmetric fertility selection and partial self-fertilization. 2. Clonal gametic contributions to the offspring of a Scots pine seed orchard. In: Proc. Symp. Population Genetics of Forest Trees, Helsinki 1981. Silva Fennica 16 (2): 99—106 (1982). —— Müller-Starck, G. and Gregorius, H.-R.: Analysis of mating system in forest trees. In: Proc. of the Second International Conference on "Quantitative Genetics". Eds.: B. S. Weir, E. J. Eisen, M. M. Goodman, G. Namkong. Sinauer, Sunderland, Massachusetts, 573—597 (1988). —— Muona, O.: Population genetics in forest tree improvement. In: A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir: Population genetics, plant breeding and gene conservation. Sinauer, Sunderland, Massachusetts (in press). —— Muona, O. and Har'u, A.: Effective population sizes, genetic variability, and mating system in natural stands and seed orchards of *Pinus sylvestris*. Silvae Genet. 38 (5—6): 221—228 (1989). —— Neale, D. B.: Genetic implications of the Douglas-fir shelterwood regeneration system in southwest Oregon. For. Sci. 31: 995—1005 (1985). —— Neale, D. B. and Adams, W. T.: The mating system in natural and shelterwood stands of Douglas-fir. Theor. Appl. Genet. 71: 201—207 (1985a). —— Neale, D. B. and Adams, W. T.: Allozyme and mating system variation in balsam fir (*Abies balsamea*) across a continuous elevational transect. Can. J. Bot. 63: 2448—2453 (1985b). —— Nei, M.: Molecular population genetics and evolution. North-Holland Publishing Company, Amsterdam (1975). —— Olaczek, R.: Zarys ekologii i fitocenologii. In: Nasze drzewa lesne 6 — Modrzewie. Ed. S. Bialobok, PWN, Poznan — Warszawa. pp. 381—440 (1986). —— O'Malley, M. M., Allendorf, F. W. and Blake G. M.: Inheritance of isoenzyme variation and heterogeneity in *Pinus ponderosa*. Biochem. Genet. 17: 233—250 (1979). —— Park, Y. S. and Fowler, D. P.: Effects of inbreeding and genetic variances in a natural population of tamarack (*Larix laricina* (Du Roi) K. Koch) in eastern Canada. Silvae Genet. 31: 21—26 (1982). —— Rao, C. R.: Linear statistical inference and its applications. 2nd ed. John Wiley and Sons, New York (1973). —— Ritland, K. and Jain, S.: A model for the estimation of outcrossing rate and gene frequencies using independent loci. Heredity 47: 35—52 (1981). —— Sarvas, R.: Investigations on the flowering and seed crop of *Pinus* sylvestris. Communic. Inst. For. Fenn. 53 (4) 1—198 (1962) —— Schoen, D. J. and Stewart, S. C.: Variation in male reproductive investment and male reproductive success in white spruce. Evolution 40: 1109—1120 (1986). —— Shaw, D. V. and Allard, R. W.: Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theor. Appl. Genet. 62: 113—120 (1982a). —— Shaw, D. V. and Allard, R W.: Isozyme heterozygosity in adult and open-pollinated embryo samples of Douglas-fir. Silva Fennica 16: 115—121 (1982b). —— Shaw, D. V., Kahler, A. L. and Allard, R. W.: A multilocus estimator of mating system parameters in plant populations. Proc. Natl. Acad. Sci. U.S.A. 78: 1298—1302 (1981). —— Sokal, R. R. and Rohlf, R. F.: An introduction to biostatistics. Freeman, San Francisco (1973). —— Stern, K. and Roche, F.: Genetics of Forest ecosystems. Springer-Verlag, Berlin (1974). —— Yeh, F. C.: Analyses of gene diversity in some conifers. In: Isozymes of North American Forest trees and forest insects. USDA Gen. Tech. Rep. PSW-48. pp. 48—52 (1981). —— Yeh, F. C. H. and Layton, C.: The organization of genetic variability in central and marginal populations of lodgepole pine, *Pinus contorta* spp. *latifolia*. Can. J. Genet. Cytol. 21: 487—503 (1979). —— Yeh, F. C. and Morgan, K.: Mating system and multilocus associations in a natural population of *Pseudotsuga menziesii* (Mirb.) Franco. Theor. Appl. Genet. 73: 799—808 (1987).

Isozyme Variation within the Fraser Fir (Abies fraseri (Pursh) Poir.) Population on Mount Rogers, Virginia: Lack of Microgeographic Differentiation

By K. E. Diebel[1]) and P. P. Feret[2])

(Received 13th June 1990)

[1]) Postdoctoral Research Associate, Forestry Department, Kansas State University, Manhattan, Kansas 66506, USA

[2]) Professor. Forestry Department, Virginia Polytechnic and State University, Blacksburg, Virginia 24061, USA.

Summary

The objective of this study was to determine the amout of microgeographic differentiation within the Fraser fir (*Abies fraseri* (Pursh) Poir.) on Mt. Rogers, Virginia, an

isolated relic population. Three hundred and four trees from 35 plots were analyzed for 13 isozyme loci. Four loci were polymorphic but there were no significant differences in gene frequencies among three subpopulations. WRIGHT's F-statistics, and GREGORIUS' "δ" index indicated little or no substructuring of the population. Lack of barriers to gene flow between subpopulations contributed to the lack of population differentiation.

Key words: genetic diversity, subpopulation, F-statistics.

Introduction

For most tree species 80% to 90% of isozyme variation is within populations (MITTON, 1983); and because of this there has been considerable interest in quantifying genetic diversity on a microgeographic scale. Results from previous studies lead to conflicting conclusions. Some studies have detected microgeographical differentiation of gene or genotype frequencies (LINHART et al., 1981; KNOWLES, 1984; KNOWLES and GRANT, 1985; GREGORIUS et al., 1986) and it has been assumed that this genetic substructuring is primarily the result of limited seed dispersal and mating of related individuals. Other studies have not detected any microgeographic patterns (GURIES and LEDIG, 1977; ROBERDS and CONKLE, 1984; NEALE and ADAMS, 1985; EPPERSON and ALLARD, 1989; MORAN and ADAMS, 1989), suggesting that high levels of outcrossing, and extensive gene flow by pollen and seed dispersal are dominant forces in population structuring.

It is difficult to draw generalized conclusions about within population genetic structure in forest trees when the evidence is so conflicting. More studies of different species in various stand conditions are needed to provide definitive conclusions about the relative frequency and importance of within population patterns of genetic variation.

The Fraser fir (*Abies fraseri* (PURSH) POIR.) population on Mt. Rogers, Virginia (Latitude 36° 40'N, Longitude 81° 30'W) provides an excellent opportunity for studying microgeographical patterns of isozyme variation. This population occupies a great variety of sites and varying stand conditions. The disturbance history of the population is variable as well. Some stands in the population have been heavily disturbed by logging, grazing and fire. Others have not been subjected to extensive anthropogenetic disturbance (PYLE et al., 1985). We hyphothesized that these differences in site and stand conditions, and disturbance histories, could create conditions suitable for the development of microgeographic genetic variation.

Thre have been several studies of range-wide morphological, monoterpene, and isozyme variation of *Abies* species in eastern North America which have included samples from Mt. Rogers (ROBINSON and THOR, 1969; CLARKSON and FAIRBROTHERS, 1970; ZAVARIN and SNAJBERK, 1972; THOR and BARNETT, 1974; JACOBS et al., 1984). None of these studies sampled more than 20 to 30 trees from the Fraser fir population on Mt. Rogers. While such limited sample sizes may be adequate to characterize average allele frequencies over the entire population, they are insufficient for investigating within population genetic structure. The primary objective of this study was to quantify microgeographic patterns of isozyme variation in the Fraser fir population on Mr. Rogers.

As an aid in determining the role that gene flow may play in influencing population substructuring, the second objective was to determine the potential for gene flow via pollen exchange by observing variation in dates of pollen relase and female receptivity. Extensive gene flow may prevent subpopulation differentiation. Few studies have quantified the potential for gene flow via pollen and at the same time determined the genetic structure of the sampled populations. SCHUSTER et al. (1989) documented that some populations of limber pine (*Pinus flexilis* JAMES) on an elevational transect do not have overlapping pollination periods which may be a significant factor contributing to the observed differences in gene frequencies between the two most widely separated populations.

Materials and Methods

Seven transects were established to traverse nearly all elevations, aspects and stand characteristics of the Mt. Rogers area (*Figure 1*). Plots were located along each transect at 30.5 m intervals of elevation with the aid of an altimeter and topographic map. A total of 35 plots were established. This design ensured hat trees from all the various site and stand conditions were sampled.

Up to 13 trees per plot were chosen randomly for cone collection (mean number collected per plot = 8.69; range 5 to 13). Cones were taken from the top third of the crown, brought to the lab, air dried, and seed separated from cone scales by hand. After 45 to 60 days of cold stratification, seeds were germinated on moist filter paper in a growth chamber at 25 °C, under continuous light (9.5 μE m^{-2} sec^{-1}). Germinated seeds (radicle extending at least 2 mm but less than 5 mm from the seed coat) were then used for isozyme analysis.

Electrophoretic Procedures

Megagametophytes from at least five seeds were sampled from each parent tree to infer its genotype. Sampling this number of megagametophytes ensured at least a 93.75% confidence that the genotype was correctly identified (TIGERSTEDT, 1973). While the stratification and

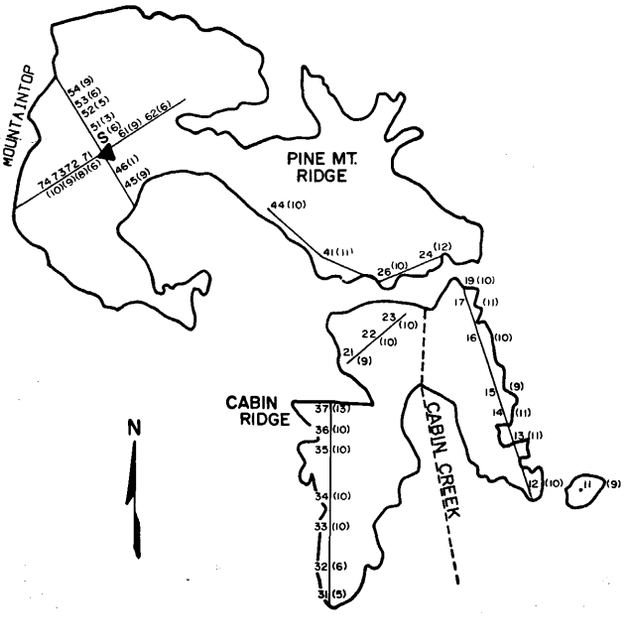

Figure 1. — Distribution of Fraser fir stands on Mt. Rogers, Va. Lines indicate transects and numbers plot locations. Numbers in parentheses are the number of parent trees per plot for which genotypes were determined. The first number of each site is the transect number, the second is the plot number within the transect; thus 37 is transect 3, plot 7 in transect 3.

germination procedures previously described worked well for most single tree seedlots, in some the minimum number of seeds failed to germinate. Thus for a few plots the number of trees sampled were low. The total number of trees sampled was 304. Sample sizes per plot are given in *Figure 1*.

Megagamethophytes were homogenized in three drops of a soultion of 1% phlyvinlypyroledone (PVP-40) and distilled water. The extract was absorbed onto filter paper wicks and the wicks inserted into an 11% starch gel (Sigma Chemical Co.). Two electrode and gel buffer systems were utilized, a Tris-Citric acid buffer (pH 6.2, NEALE and ADAMS, 1981), and a Tris-Citric acid/Lithium hydroxide-boric acid buffer (pH 8.5, CHELIAK and PITEL, 1984). Seven enzyme systems were assayed for each tree. These enzyme systems were:
phosphoglucomutase (PGM, EC 2.7.5.1),
glutamine oxaloacetate transaminase (GOT EC 2.6.1.1),
6-phosphogluconate dehydrogenase (6-PGP, EC 1.1.1.44),
leucine aminopeptidase (LAP, EC 3.4.11.1),
malate dehydrogenase (MDH, EC 1.1.1.37),
glutamate dehydrogenase (GDH, EC 1.4.1.3),
and isocitrate dehydrogenase (IDH, EC 1.1.1.42).

Gel slices from the Tris-Citric acid/Lithium hydroxide-Boric acid buffer system were stained for GOT, LAP, and GDH. Gel slices from the Tris-Citric acid buffer system were stained for 6-PGD, IDH, MDH, and PGM. Enzyme staining solutions were as described by CHELIAK and PITEL (1984).

An in-depth test of the inheritance of all the enzymes we assayed in Fraser fir has been reported previously for the closely related balsam fir (*Abies balsamea* (L.) MILL.; NEALE and ADAMS, 1981). However, inheritance patterns were verified for Fraser fir by sampling 20 megagametophytes from 15 presumed heterozygotes for each of the variable loci. A chisquare "goodness-of-fit" test was used to determine whether segregation patterns fit the expected 1:1 ratio for each individual tree.

Isozyme Data Analysis

The isozyme allele and genotype frequency data from all plots was arbitrarily grouped into three subpopulations (*Figure 1*) based on topography, disturbance history, and stand characteristics. The subpopulation here after called Mountaintop encompasses stands growing on the mountain itself. These stands grow on all possible aspects from 1600 m to 1746 m in elevation. In the Mountaintop subpopulation the average tree age is 101 years (Personal communication Dr. S. M. ZEDAKER, Virginia Polytechnic Institute and State University) and according to PYLE et al. (1985) it probaply has never been logged.

Stands of the Cabin Ridge and Pine Mt. Ridge subpopulations are considerably younger than those on Mountaintop (average ages 50.4 and 36.7 years respectively, Personal communication Dr. S. M. ZEDAKER), probably because they were extensively logged in the 1900's, 1930's, and 1950's (PYLE et al., 1985). The stands on Cabin Ridge grow at elevations ranging from 1515 m to 1600 m. The terrain is relatively flat and the Fraser fir primarily grow on south facing slopes. The fir on Pine Mt. Ridge grow in a narrow band between 1600 m and 1636m and only on a north facing slope.

A chi-square "goodness-of-fit' test was used to determine if observed genotype frequencies were in accordance with expectations under HARDY-WEINBERG equilibrium. This was done for the entire population and for each of the three hypothesized subpopulations separately.

For each locus, CROW and KIMURA's (1970) "effective number of alleles" (v) was calculated for the total population, and for each subpopulation. Gene pool allelic diversities were estimated by calculating the harmonic mean of the effective number of alleles of all sampled loci including the monomorphic loci (GREGORIUS, 1978).

The distribution of genetic variation within and between subpopulations was evaluated and described using a variety of statistical tests (contingency table chi square, WORKMAN and NISWANDER, 1970; F statistics, WRIGHT, 1978; GREGORIUS' "δ" index, GREGORIUS and ROBERDS, 1986). The results of these tests all led to the same conclusions. Therefore, only Wright's F statistics (WRIGHT, 1978), as modified by WEIR and COCKERHAM (1984), are presented here because they are most commonly used in the literature. These F-statistics were used to partition the total (T) genetic variation of the Mt. Rogers population into components attributable to variation among individuals (I) within plots, among plots (P) within subpopulations, and among subpopulations (S). The results are interpreted in terms of correlations of uniting gametes (F). FIT, FIS ,and FIP are correlations of uniting gametes relative to gametes drawn at random from the total population, from within a subpopulation, and within a plot, respectively. FST, FPT, and FPS are correlations of uniting gametes within subpopulations, within plots, and within individuals from the same plot respectvely, relative to gametes drawn at random from the entire population. FST, FPT, and FPS can be interpreted as the percentages of the total variation explained by variation among subpopulations among plots, and among plots within subpopulations, respectively. WEIR and COCKERHAM's (1984) modifications incorporate corrections for small and/or unequal sample sizes. They also devised a method for calculating the variances of each of the components. The variance estimate is obtained by jackknifing across loci (i. e. by recalculating statistics after loci are sequentially removed).

Phenological Observations

To determine the potential for gene flow among subpopulations, dates of female receptivity and pollen shed were recorded in the spring of 1987. The intent was to sample flowering in more than one year, but little or no flowering occurred in 1988 or 1989 so additional data could not be collected. Weekly observations began before bud break and continued until no further anthesis was observed. Male and female strobili on three branches on each of four sides of up to 10 trees per plot were scored using the following scale:
0 = no activity,
1 = bud swell,
2 = bud break, but no pollen shed or female strobilus receptivity,
3 = pollen shed, females receptive.
Female strobili were considered receptive when the cone scales were open at 90^0 angles to the strobilus axis. Male strobili were considered to be shedding pollen when manual movement of branches caused pollen release. Scores were averaged to obtain a single tree value. Observations were made for a five week period, between April 23 and May 30, 1987.

Results and Discussion

Enzyme staining patterns were similar to those NEALE and ADAMS (1981) observed for balsam fir. Therefore, the enzyme nomenclature in this paper follows that used by

NEALE and ADAMS. The seven enzyme systems were coded by 13 loci, four of which were polymorphic (*Table 1*). The polymorphic loci were PGM (1), GOT (3), 6-PGD (1), and LAP (1) (the number in parentheses is the locus designation). None of the chi-square tests for segregating allelic pairs were significantly different from the 1:1 expectation, verifying simple Mendelian inheritance, as was observed in balsam fir for the same polymorphic enzymes (NEALE and ADAMS, 1981; p values ranged from 0.1 to 0.9 with 34 of the 60 tests having p values 0.5 or greater).

The proportion of polymorphic loci observed in the Mt. Rogers population in this study (30.8%) is similar to that observed by JACOBS et al. (1984) for the same population based on 20 loci (40%), and for two other Fraser fir populations (35% and 45%). Balsam fir populations, however, may have somewhat higher levels of polymorphism (50% to 64%; NEALE and ADAMS, 1981; JACOBS et al., 1984). This may be an indication that Fraser fir populations are less genetically diverse than balsam fir.

Heterozygosity and Diversity

Observed heterozygosities at three of the four polymorphic loci were not significantly different from expected heterozygosities under HARDY-WEINBERG equilibrium (*Table 2*). The exception was GOT(3), where significantly (p = 0.05) fewer heterozygotes than expected were observed in two (Mountaintop and Cabin Ridge) of the three subpopulations. There are many possible explanations for the lack of heterozygotes including sampling error ,the Whalund effect, selection, and mating between related individuals. The exact cause of the deficiency of heterozygotes for the GOT(3) locus cannot be determined on the basis of the results of this study alone. Although observed heterozygosities were not reported by JACOBS et al. (1984), expected heterozygosities in the Mt. Rogers population were calculated from their allele frequency estimates for the same loci we sampled. At three of the four loci estimates of expected heterozygosity differed substantially between the two sets of data (*Table 2*). For two of the loci, 6-PGD(1) and LAP(1), our estimates were lower than JACOBS et al. and for GOT(3) our estimate was higher. Perhaps the great difference in sample size (304 trees in our study versus 20 to 30 in JACOBS et al.) and/or differences in electrophoretic technique are responsible for the discrepancies.

Mean observed heterozygosity for the four polymorphic loci was 25.8%. By way of comparison, NEALE and ADAMS (1985) estimated the mean observed heterozygosity of eight polymorphic loci to be 26.6%. JACOBS et al. (1984) did not report an estimate of mean observed heterozygosity.

The estimated effective number of alleles were low in this fir population (*Table 3*). Pine Mt. Ridge was the least diverse subpopulation ($v = 1.08$) although the differences among subpopulations were small (range = 1.08 to 1.10). JACOBS et al. (1984) estimated that the mean number of alleles per locus was 1.4 for the Mt. Rogers population. Their estimates of mean number of alleles for other Fraser fir populations were identical. Some examples of estimates of the effective number of alleles in other conifer species are: black spruce (*Picea marianna* MILL.) 1.24 to 1.38 effective alleles per locus (BOYLE and MORGENSTERN, 1987), Norway spruce (*Picea abies* (L.) KARST.) 1.65 to 1.75 (LUNDVIST, 1979), and bristlecone pine (*Pinus aristata* ENGELM.) with an average of 1.49 (HEIBERT and HAMRICK, 1983). An example of the effective number of alleles for an anginosperm species is Europeam beech

Table 1. — Observed allelic frequencies in subpopulations of Fraser fir on Mt. Rogers.

Locus[1]	Mountaintop	Cabin Ridge	Pine Mt. Ridge	Overall
N[2] =	87	74	43	304
PGM(1)				
1	.69	.74	.65	.72
2	.31	.26	.35	.28
GOT(3)				
2	.88	.87	.92	.88
1	.12	.13	.08	.12
6-PGD(1)				
2	.89	.88	.87	.89
1	.11	.12	.13	.11
LAP(1)				
1	.79	.79	.87	.80
4	.21	.21	.13	.20
6-PGD(2)				
1	1.00	1.00	1.00	1.00
GOT(1)				
1	1.00	1.00	1.00	1.00
GOT(2)				
2	1.00	1.00	1.00	1.00
PGM(2)				
2	1.00	1.00	1.00	1.00
LAP(2)				
2	1.00	1.00	1.00	1.00
GDH				
1	1.00	1.00	1.00	1.00
MDH(1)				
1	1.00	1.00	1.00	1.00
MDH(2)				
1	1.00	1.00	1.00	1.00
IDH				
2	1.00	1.00	1.00	1.00

[1]) Allelic designations follow NEALE and ADAMS (1981).
[2]) N = number of adult trees sampled.

Table 2. — Observed (O) and expected (E) heterozygote frequencies for each of the variable loci sampled in the Fraser fir population on Mt. Rogers[1]) Numbers in parentheses are the expected heterozygote frequencies for the Mt. Rogers population calculated from JACOBS et al. (1984).

	Subpopulation			
Locus	Mountain-top	Cabin Ridge	Pine Mt. Ridge	Overall
PGM(1)				
O	.414	.351	.419	.378
E	.428	.386	.454	.410
				(.403)
GOT(3)				
O	.126*	.167*	.163	.155*
E	.212	.233	.149	.216
				(.164)
6-PGD(1)				
O	.218	.213	.116	.201
E	.194	.215	.149	.201
				(.354)
LAP(1)				
O	.299	.322	.209	.299
E	.328	.334	.223	.318
				(.461)

[1]) Expected when population is in HARDY-WEINBERG equilibrium.
*) Indicates significant deviation from expected frequency of heterozygotes, p = 0.05.

Table 3. — Estimated numbers of effective alleles (Crow and Kimura, 1970) in the Mt. Rogers Fraser fir population.

	Subpopulation			
	Mountaintop	Cabin Ridge	Pine Mt. Ridge	Overall
PGM(1)	1.75	1.63	1.83	1.69
GOT(3)	1.27	1.30	1.18	1.27
6-PGD(1)	1.24	1.27	1.18	1.25
LAP(1)	1.49	1.50	1.29	1.47
Gene Pool[1]	1.10	1.10	1.08	1.10

[1]) Harmonic mean of all loci sampled, which includes 9 monomorphic loci not shown (see *Table 1*).

(*Fagus sylvatica* L.) with an average of 1.55 (Gregorius et al., 1986).

Organization of Genetic Variation

All indices and statistical tests indicated very little genetic differentiation among the population subdivisions (i.e. among subpopulations, transects, and plots). FST, FPT and FPS values (*Table 4*) are all less than 1%. These F's can be thought of as the percentage of the total genetic variation explained by variation among subpopulations, among plots, and among plots within subpopulations, respectively. Summing these three values indicated that the total percentage of genetic variation present in the population explained by interplot or among subpopulation differences is 0.35%. Thus more than 99% of the genetic variation, as measured by this index, is due to within plot (i.e. tree-to-tree) variation.

Theoretically FST, FPT, and FPS cannot be negative (Weir and Cockerham, 1984). However, because of the corrections for unequal/small sample size it is sometimes possible to have values less than zero. This is most likely the cause for the negative estimates at individual loci (*Table 4*).

Table 4. — F statistics describing the hierarchical organization of genetic variation within the population of Fraser fir on Mt. Rogers[1]). Numbers in brackets are plus or minus one standard error.

Locus	FIT	FPT	FST	FIS	FPS	FIP
PGM1	0.081	0.003	0.004	0.015	0.002	0.078
GOT3	0.272	-0.002	-0.003	-0.007	-0.001	0.274
PGD1	0.022	-0.002	-0.005	<0.001	-0.003	0.024
LAP1	0.065	0.003	0.005	0.01	0.002	0.062
OVER-ALL	0.102 (±.04)	0.001 (±.002)	0.002 (±.003)	0.007 (±.013)	0.0005 (±.001)	0.101 (±.04)

[1]) FST, FPT, and FPS tre the F statistics which partition variation among plots and subpopulations. They are interpreted as the percentage of the total genetic variation explained by variation among subpopulations (S), among plots (P), and among plots within subpopulations, respectively. FIT, FIS, and FIP can be interpreted as the correlation of uniting gametes in individuals (I) relative to gametes drawn at random from the total (T) population, from within subpopulations, and individuals within plots, respectively.

The lack of population substructuring could be the results of high levels of gene flow. The most likely form of gene flow is through pollen dispersal. Studies of pollen flight have shown that majority of pollen falls within a short distance of the parent tree; but also, some pollen can be blown by the wind for considerable distances (Wright, 1953; Silen, 1962; Koski, 1970). Thus, although most of the pollen produced by a tree is likely to fertilize near neighbors, the small amount of pollen that travels great distances can be responsible for gene flow between widely separated subpopulations. Direct gene transport has been documented over distances exceeding 100 m (Friedman and Adams, 1985).

Phenological observations demonstrated there is the potential for mating throughout the Fraser fir population on Mt. Rogers. Since, on any one tree, timing of pollen dispersal and female receptivity were coincident, relative differences in floral phenology among plots is presented for female receptivity only. Most trees throughout the population were receptive at approximately the same time (during the fourth week of observation) with the exception of trees on south-west facing slopes on Cabin Ridge (i.e. those in plots 11 to 17, 19, 22, and 23; *Figure 2*). The trees on this part of Cabin Ridge were receptive during the third week of observation and showed no activity by the next observation. These trees may have initiated flowering relatively early because they are growing on southwest aspects and at low elevations. In contrast, the Mountaintop and Pine Mt. Ridge areas were not receptive until the fourth observation. These areas are at higher elevations and mostly on northern aspects. Elevation and

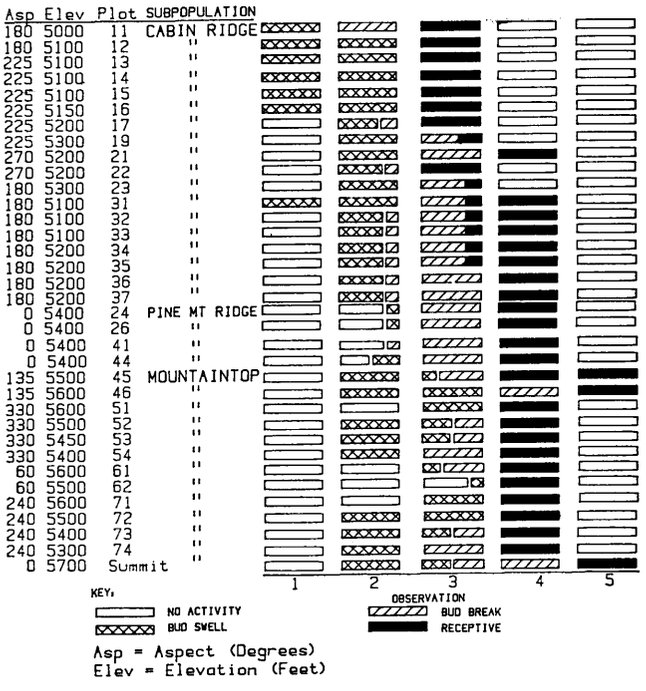

Figure 2. — Observations on female receptivity of Fraser fir on Mt. Rogers. Each plot was visited once a week for five weeks. Obeservation 1 was during the week of April 23, 1987. Hatching pattern indicates stage of floral development. Length of bars within an observation designates the proportion of trees at each stage of development.

aspect are known to be important variables affecting initiation of flowering (WRIGHT, 1976). Trees in the Cabin Ridge subpopulation which did not flower early (plots 31 to 37; Figure 2) grow in extremely dense stands relative to the early flowering trees. Perhaps high stand density slightly delayed flowering.

Once swollen, female strobili developed to mature strobili rapidly. It is likely that the trees on Cabin Ridge, in plots 31 to 37, and Pine Mt. Ridge became receptive in a very few days after the third observation as strobilus development was rapid (pers. observation). Thus, there probably is more overlap in female receptivity than indicated by *figure 2.*

Wind speed and wind direction data collected in 1987 on Whitetop mountain (directly adjacent to Mt. Rogers) indicated that the frequency of wind direction is equally distributed between winds from the northwest and from the southeast; maximum hourly wind speed was 8m/sec (MOHEN, 1988). The variability of wind direction and the relatively high wind speeds should spread pollen throughout the population. The overlap in female receptivity combined with wind patterns should allow for substantial gene flow among the subpopulations.

Gene flow is not the only possible cause of the lack of microgeographic diversity. Especially since studies of other plant species have shown that genetic differentiation among subpopulations can occur despite great amounts of gene flow (BRADSHAW, 1972). Another cause of the lack of differentiation may be population history. It is likely that a severe reduction in population size occurred approximately 4,000 years ago during the xerothermic period which followed the Wisconsinian glaciation (DELCOURT and DELCOURT, 1984; RHEINHARDT, 1984). The bottleneck may have caused in increase in the uniformity of the fir population. There may not have been sufficient time (only 4.000 years) for subpopulations to become differentiated.

The lack of subpopulation differentiation may also be a result of uniform selection pressures or the lack of intense selection pressures altogether. Uniformity of selection pressures could be the result of a uniform environment. This seems unlikely for the Mt. Rogers fir population. Despite the fact that the fir populaion covers only a small area, there is a 221 m change in elevation, stands grow on all aspects of the mountain, and there are considerable differences in the disturbance histories of each of the hypothesized subpopulations.

Most empirical studies of forest trees have demonstrated that isozymes are not traits subjected to intense selection pressures (FALKENHAGEN, 1985). This study also provides little evidence of selection. Allele and genotype frequencies were not correlated with any environmental characteristic and there was no spatial pattern to observed gene frequencies. Perhaps the enzymes studied are not sensitive to environmental variation.

Literature Cited

BOYLE, T. J. B. and MORGENSTERN, E. K.: Some aspects of the population structure of black spruce in central New Brunswick. Silvae Genetica **36**: 53—60 (1987) —— BRADSHAW, A. D.: Some evolutionary consequences of being a plant. Evol. Biol. **5**: 25—47 (1972). —— CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42. Pettwawa National Forestry Institute, Canadian Forestry Service (1984). —— CLARKSON, R. B. and FAIRBROTHERS, D. E.: A serological and electrophoretic study of eastern North American *Abies* (Pinaceae). Taxon. **19**: 721—727 (1970). —— CROW, J. F. and KIMORA, M.: Introduction to Population Genetics Theory. Harper and Row, New York (1970). —— DELCOURT, H. R. and DELCOURT, P. A.: Late quaternary history of the spruce-fir ecosystem in the southern Appalachitn mountains. *In*: P. S. WHITE (ed.) The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/ Resource Management Rpt. No. 71 pp. 22—35 (1984). —— EPPERSON, B. K. and ALLARD, R. W.: Spatial autocorrelation analysis of the dstribution of genotypes within populations of lodgepole pine. Genetics **121**: 369—377 (1989). —— FALKENHAGEN, E. R.: Isozyme studies in provenance research of forest trees. Theor. Appl. Genet. **69**: 335—347 (1985) —— FRIEDMAN, S. T. and ADAMS, W. T.: Estimation of gene flow into seed orchards of loblolly pine (*Pinus taeda* L.). Theor. Appl. Genet. **69**: 609—615 (1984). —— GREGORIUS, H. R.: The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math. Biosci. **41**: 253—271 (1978). —— GREGORIUS, H. R., KRAUHAUSEN, J. and MÜLLER-STARCK, G.: Spatial and temporal genetic differentiation among the seed in a stand of *Fagus sylvatica* L. Heredity **57**: 255—262 (1986). —— GREGORIUS, H. R. and ROBERDS, J. H.: Measurement of genetical differentiation among subpopulations. Theor. Appl. Genet. **71**: 826—834 (1986). —— GURIES, R. P. and LEDIG, F. T.: Analysis of population structure from allozyme frequencies. *In*: Proc. 14th Southern Forest Tree Improvement Conf. Gainsville, Fla. pp. 246—253 (1977). —— HEIBERT, R. D. and HAMRICK, J. L.: Patterns and levels of genetic variation in Great Basin bristlecone pine. Evolution **37**: 302—310 (1983). —— JACOBS, B. F., WERTH, C. R. and GUTTMAN, S. J.: Genetic relationships in *abies* (fir) of eastern United States: An electrophoretic study. Can. J. Bot. **62**: 609—619 (1984). —— KNOWLES, P.: Genetic variability among and within closely spaced populations of lodgepole pine. Can. J. Genet. and Cytol. **26**: 177—184 (1984). —— KNOWLES, P. and GRANT, M. C.: Genetic variation of lodgepole pine over time and microgeographical space. Can. J. Bot. **63**: 722—727 (1985) —— KOSKI, V.: A study of pollen dispersal as a mechanism of gene flow in conifers. Commun. Inst. Forest. Fenn. **70**: 1—78 (1970). —— LINHART, Y. B., MITTON, J. B., STURGEON, K. B. and DAVIS, M. L.: Genetic variation in space and time in a population of ponderosa pine. Heredity **46**: 407—426 (1981). —— LUNDVIST, K.: Allozyme frequency distribution in four Swedish populations of Norway spruce (*Picea abies*). I. Estimations of genetic variation within and among populations, genetic linkage and a mating system parameter. Hereditas **90**: 127—143 (1979). —— MITTON, J. B.: Conifers. *In:* Isozymes in Plant Genetics and Breeding. Part B. S. D. TANKSLEY and T. J. ORTON (eds.). Elsevier, Amsterdam pp 443—472 (1983). —— MOHEN, V. A.: Exposure of forests to pair pollutions, clouds, precipitation, and climatic variables. U. S. Environmental Protection Agency, Areal Research Triangle Park, NC. —— MORAN, G. F. and ADAMS, W. T.: Microgeographical patterns of allozyme differentiation in Douglas-fir from southwest Oregon. For. Sci. **35** 3—15 (1989). —— NEALE, D. B. and ADAMS, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Can. J. Bot. **59**: 1285—1291 (1981). —— NEALE, D. B. and ADAMS, W. T.: Allozyme and mating system variation in balsam fir (*Abies balsamea*) across a continuous-elevational transect. Can. J. Bot. **63**: 2448—2453 (1985). —— PYLE, C., SCHAFALE, M. P. and WENTWORTH, T. R.: History of disturbance in spruce-fir forests of the SARRMC intensive study sites — Mt. Rogers National Recreation Area, Black Mountains and Great Smoky Mountains. SARRMC — southern Appalachian spruce-fir ecosystem assessment project (1985) —— REINHARDT, R. D.: Comparative study of composition and distribution patterns of subalpine forests in the Balsam Mountains of southwest Virginia and the Great Smoky Mountains. *In*: P. S. WHITE ed. The southern Appalachian spruce-fir ecosystem, its biology and threats. NPS, Southeast Regional Office, Research/Resource Management Rpt. No. 71 pp. 87—99 (1984). —— ROBERDS, J. H. and CONKLE, M. T.: Genetic structure in loblolly pine stands: Allozyme variation in parents and progeny. For. Sci.**30**: 319—329 (1984). —— ROBINSON, J. F. and THOR, E.: Natural variation in Abies of the southern Appalachians. For. Sci. **15**: 238—245 (1969). —— SCHUSTER, W. S., ALLES, D. L. and MITTON, J. B.: Gene flow in limber pine: evidence from pollination phenology and genetic differentiation along an elevational transect. Amer. J. Bot. **67**: 1395—1403 (1989). —— SILEN, R. R.: Pollen dispersal considerations for Douglas-fir. J. Forestry **60**: 790—795 (1962). —— THOR, E. and BARNETT, P. E.: Taxonomy of *Abies* in the southern Appalachians: variation in marginal and central populations of *Picea abies.* **20**: 32—40 (1974). —— TIGERSTEDT, P. M. A.: Studies on isozyme variation in marginal and central populations of *Picea abies.* Hereditas **75**: 47—60 (1973). —— WEIR, B. S. and COCKERHAM, C. C.: Estimating F statistics for the analysis of population substructure.

Evolution 38: 1358—1370 (1984). —— WORKMAN, P. L. and NISWANDER, J. D.: Populations studies on southwestern Indian tribes. II. Local differentiation in the papago. Am. J. Hum. Genet. 22: 24—49 (1970). —— WRIGHT, J. W.: Pollen dispersal studies: Some practical applications. J. For. 31: 114—118 (1953). —— WRIGHT, J. W.: Introduction to Forest Genetics. Academic Press Inc., New York (1976). —— WRIGHT, S: Evolution and the Genetics of Populations. Vol. 4. Univ. Chicago Press (1978). —— ZAVARIN, E. and SNAJBERK, K.: Geographical variability of monoterpenes from *Abies balsamea* and *A. fraseri*. Phytochemistry 11: 1407—1421 (1972).

Age Trends in the Genetic Control of Stem Diameter of Eucalyptus Tereticornis and the Implication for Selection

By G. O. OTEGBEYE*)

(Received 17th July 1990)

Abstract

The trend in the genetic control of diameter at breast height of *Eucalyptus tereticornis* SM. was investigated using growth data at ages three, five and six years. The analysis showed that variance component estimates changed with age. Both absolute values of genetic variance and their relative sizes compared with environmental source of error variance increased with age. Error variance also increased with age. However the ratio of the sum of the environmental components ($\sigma^2_e + \sigma^2_r$) to the total variance decreased with age.

Stem diameter of *Eucalyptus tereticornis* was found to be under genetic control, the degree of which increased with age. Selection for diameter growth should therefore be more efficient when older trees are used in the selection process. Adaptive growth differences existed among the provenances examined, the intensity of which increased with age. The Laura, Queensland provenance was the best adapted while the Mysore landrace and the Port Moresby provenance were least adapted.

Key words: *Eucalyptus tereticornis*, provenances, diameter at breast height, ages, variance components, heritability.

Introduction

The provenance trial involving 11 provenances of *E. tereticornis* SM. established at Afaka in 1969 revealed that provenance differences exist in the growth rate of the species (OTEGBEYE, 1990). It has also been shown in another paper (OTEGBEYE, 1988a) that the pattern of genetic control on height growth of the species varies with age. The aims of the current paper are to show (i) the degree of genetic control on diameter at breast height (dbh); (ii) the pattern of the genetic control over ages three, five and six years. Stem diameter is a very important tree characteristic since it is a very important component of yield of forest tree species. In fact, in pure mathematical terms, a 10% increase in diameter will give approximately a 20% increase in basal area, hence in volume (LAURIDSEN *et al.*, 1987). Like tree height, stem diameter is therefore a very important component of commercial growth as well as fitness.

Materials and Methods

Eleven provenances of *Eucalyptus tereticornis* were used for a provenance study involving the species in 1969. The descriptions of these provenances and the establishment conditions for the trial have been given in an earlier paper (OTEGBEYE, 1990). Each provenance was composed of seed from a small number of trees, in some cases only two trees.

The seedlings were planted at Afaka (lat. 10°37'N, long. 7°17'E) in July, 1969. The experimental design used was randomized blocks with four replications.

Although, 11 provenances were involved in the original provenance study, only nine were considered for the purpose of the present paper since the other two provenances (Kennedy River, Northern Queensland and an unidentified source from Papua New Guinea) were not replicated and therefore could not be involved in the analysis of variance. The nine provenances considered include seven from Queensland Australia namely North Laura, Mt. Garnet, Conjuboy, Laura and three different seedlots from Cooktown, one from Papua New Guinea — Port Moresby and a landrace from India — Mysore.

Total heights and dbh of 16 inner trees per plot were measured in 1972, 1974 and 1975, that is when trees were three, five and six years old respectively. However, only dbh data were analysed for the purpose of this paper, the data on total height having earlier been similarly treated (OTEGBEYE, 1988a). Analysis of variance was carried out on plot means for each of the three ages examined.

Variance components and broad sense heritability (h^2) at each age were estimated. Heritability was estimated using the relationship;

$$h^2 = \frac{\sigma^2_p}{\sigma^2_p + \sigma^2_{e/r}}$$ (BURLEY and WOOD, 1976; WELLENDORF *et al.*, 1986).

where:

$\sigma^2_p = V_G$ = Genetic variance of provenance means

$\sigma^2_{e/r} = V_E$ = Environmental variance of provenance means

r = number of replications

$\sigma^2_p + \sigma^2_{e/r} = V_P = V_G + V_E$ = Phenotypic variance among provenance means.

Broad sense heritability can be used to predict gain in first generation from provenance selection (NANSON, 1968).

Results and Discussion

The form of the analysis of variance used is presented in *table 1*. The individual analysis showed that variance component estimates changed with age (*Table 2*). Similar results have been reported for height growth of the species (OTEGBEYE, 1988a), Douglas-fir (NAMKOONG et al., 1972)

*) Assistant Chief Research Officer, Forestry Research Institute of Nigeria, Savanna Forestry Research Station, P. M. B. 1039, Samaru — Zaria, Nigeria.