

in showing very large fully heterochromatic satellites attached to the short arm.

P. tremula

The chromosomes are m to sm and have mostly proximally located C-bands. Some small chromosomes appear to be devoid of heterochromatin. The distinct feature of the complement is the presence of a very large pair (3.27 μm) (Fig. 3 arrows), which is 1.74 times longer than the second longest pair (1.87 μm). This pair is sm, the short arm is fully heterochromatic as is also the long arm except for two narrow intercalary regions of euchromatin. This shows that during the course of evolution this particular pair has gained C-band material disproportionate to that in rest of the chromosomes. It might be that it is concerned with sex determination! However, its exact nature and significance can only be ascertained by the detailed karyotypic studies in some more species.

Acknowledgements

D.O is grateful to Deutsche Forschungsgemeinschaft for providing financial assistance for visiting West Germany under INSA-DFG Exchange of Scientists Programme.

References

- FEDOROV, A. A.: Chromosome numbers of flowering plants. Reprint by Otto Koeltz Science Publishers, Königstein, West Germany, 1974 (original in 1969). — GOLDBLATT, P.: Index to Plant Chromosome Numbers 1975 to 1978. Missouri Botanical Garden, Braun-Brumfield, Inc., Ann. Arbor, Michigan (1981). — LEVAN, A., FREDGA, K. and SANDBERG, A. A.: Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220 (1964). — OHRI, D., and AHUJA, M. R.: Giemsa C-banded karyotype in *Quercus L.* (oak). *Silvae Genet.* 39: 216–219 (1990). — SCHLARBAUM, S. E.: Cytogenetic manipulation in the forest trees through tissue culture. In: Cell and Tissue Culture in Forestry. Vol. 1. General Principles and Biotechnology. J. M. BONGA and D. J. DURZAN (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 330–352 (1987). — STEBBINS, G. L.: Longevity, habitat and release of genetic variability in the higher plants. Cold Spring Harb. Symp. Quant. Biol. 23: 365–378 (1958).

Genetic Structure and the Mating System in an Old Stand of Polish Larch

By A. LEWANDOWSKI, J. BURCZYK and L. MEJNARTOWICZ

Institute of Dendrology, Polish Academy of Sciences,
62-035 Kornik, Poland

(Received 22nd March 1990)

Summary

The genetic structure and the mating system were investigated in a 250 year old stand of Polish larch (*Larix decidua subsp. polonica* [RACIB.] DOMIN) using six allozyme marker loci (Gdh, Mdh-1, Mdh-2, Mdh-3, Shdh, Srdh). The average expected heterozygosities, based on these loci were 0.189 and 0.187 for the parental and filial generation respectively, and the mean expected heterozygosity for the mature stand based on 18 gene loci was calculated to be 0.193. An excess of heterozygotes was found in the mature forest population. Single-locus estimates of outcrossing rate (\hat{t}_s) ranged from 0.776 to 1.132 (minimum variance mean 1.048), while the multilocus estimate (\hat{t}_m) was 0.943. Detected amount of selfing and heterogeneity of single-locus estimates were statistically significant. The calculated ratio of genetically effective male individuals to receptive female individuals was only 0.22 in this population.

Key words: Isozymes, heterozygosity, inbreeding, outcrossing, variance effective population size.

Introduction

Polish larch (*Larix decidua subsp. polonica* [RACIB.] DOMIN), is of high wood quality, fast growth and relative high resistance to air pollution (LATOCHA and HAWRYS, 1976), thus it belongs to valuable coniferous trees in Europe. However, despite the economic value of this species, little is known about its biology and especially about its genetic properties.

Larch in Poland occurs in small groups or even as individual trees within stands of pine, spruce or fir. It is suggested that such a structure of populations and heavy

pollen create significant barriers for the free passage of genes between and within populations (MEJNARTOWICZ and BERGMANN, 1975). Larch produces many empty seeds and it is supposed that self-fertilization plays an important role in this process. KOSINSKI (1986) studying the causes of empty seed formation in larch has shown, that embryo degeneration took place in 85% to 100% of the pollinated ovules, after controlled self-pollination. However, there is no information about the rates of natural self-fertilization. A significant level of self-fertilization was found for natural populations of another larch (*Larix laricina* (Du Roi) K. KOCH) (KNOWLES et al., 1987). Thus it is very interesting to study the mating system of Polish larch, which could be helpful in explaining empty seed formation in natural stands and could extend our knowledge about this species.

The mating system, being part of the whole genetic system of the species, defines the mode of transmission of genetic information from the parental to the filial generation (STERN and ROCHE, 1974), and is an important determinant of plant population structure (CLEGG, 1980). In recent years the mating system was studied in many conifer trees in natural populations, however statistically efficient multilocus estimates of mating system parameters have been obtained only for a relatively small number of conifers (see reviews: MITTON, 1983; ADAMS and BIRKES, 1988; MUONA, 1989).

Coniferous forest tree species are wind pollinated and genetically highly variable (HAMRICK et al., 1981). Many of them display strong inbreeding depression (KOSKI, 1973; PARK and FOWLER, 1982), which following selfing results in reduced seed set, decreased survival and growth of prog-

eny. Some studies indicate that a small but significant proportion of selfed progeny exists (KNOWLES et al., 1987; YEH and MORGAN, 1987). Therefore the knowledge about the rates of selfing for particular populations would allow foresters to predict the losses caused by inbreeding depression, when seeds from these populations are used for reforestation.

In this paper we report the results of studies on the genetic structure and the mating system parameters using allozymes as genetic markers.

Materials and Methods

Material for this study came from 21 randomly chosen Polish larch (*Larix decidua subsp. polonica* [RACIB.] DOMIN) trees from Ciechostowice (Poland), growing within an area of about 5 ha. The investigated population is a remnant of one of the best documented natural populations of Polish larch (KULESZA, 1927). Sampled trees build upper story were about 200 years to 300 years old and were 30 m to 40 m tall with 2.5 m to 3.3 m girth. They were distributed relatively uniformly throughout the stand with density about 40 trees/ha. Associated species build second story include 30 years to 40 years old *Abies alba* and *Fagus sylvatica* with density of 1200 trees and 500 trees per ha respectively. Cones were collected in 1986 from the middle part of the crown during a moderate cone crop. The seeds were kept separately for the maternal parent trees and stored dry below 0 °C until laboratory analysis.

Genotypic identification of the 21 parent trees was made using the seed megagametophyte tissue for 10 enzyme systems: esterase (EST; 3.1.1.2), glutamate dehydrogenase (GDH; E.C. 1.4.1.2), glucose-6-phosphate dehydrogenase (G6PDH; E.C. 1.1.1.4), isocitrate dehydrogenase (IDH; E.C. 1.1.1.42), leucine aminopeptidase (LAP; E.C. 3.4.1.1), malate dehydrogenase (MDH; E.C. 1.1.1.37), menadione reductase (MNR), shikimate dehydrogenase (SHDH; E.C. 1.1.1.25), sorbitol dehydrogenase (SRDH; E.C. 1.1.1.14), and superoxide dismutase (SOD; E.C. 1.15.1.1).

The progeny was identified at six polymorphic loci: Gdh, Mdh-1, Mdh-2, Mdh-3, Shdh and Srdh. Other polymorphic loci could not be clearly resolved in the embryo tissue. At least 12 embryo-megagametophyte pairs were analyzed for each tree. Details of the electrophoretic procedures for detecting these allozymes, their banding

patterns and analyses of their Mendelian genetics are reported elsewhere (LEWANDOWSKI and MEJNARTOWICZ, 1990).

Data analysis

Single-locus (t_s) and multilocus (t_m) mating system parameters were estimated based on a mixed mating model, using the maximum likelihood procedures and programs described in NEALE and ADAMS (1985a). The mixed mating model divides the mating process into two components: random mating and self-fertilization (CLEGG, 1980). This model is based on the following assumptions: (1) each viable progeny is the result of a random outcross (with probability t) or a self-fertilization (with probability $s=1-t$), (2) the probability of an outcross is independent of the genotype of the maternal plant, (3) allele frequencies of outcross pollen pools are homogeneous over the array of mature plants sampled (SHAW et al., 1981). Multilocus data contain more information about outcrossing. When more loci are assayed at once, more outcrosses are detected directly, and then multilocus estimation is statistically more efficient than single-locus estimation, however independence among studied loci should be assumed (SHAW et al., 1981; RITLAND and JAIN, 1981). Estimates of t are occasionally greater than 1.0 (SHAW and ALLARD, 1982a; NEALE and ADAMS, 1985a; NEALE and ADAMS, 1985b; BARRETT et al., 1987; EL-KASSABY et al., 1987). This may be due to violations of the mixed mating model (eg. disassortative mating), but sampling error is also a reasonable explanation (BROWN et al., 1984; RITLAND and EL-KASSABY, 1985). Sampling error is especially likely, when t is near 1.0 and sample sizes are small (ADAMS and BIRKES, 1988).

Spatial heterogeneity of pollen alleles was tested across maternal homozygous trees in a $2 \times m$ contingency table, where m is the number of homozygous females in the sample (BROWN et al., 1975). The detectable outcrosses were compared with homozygotes for each maternal tree by way of a chi-square analysis.

Observed (H_o) and expected (H_e) heterozygosity were calculated for each polymorphic locus in parental and filial population after NEI (1975). The observed genotypic distribution was compared with that expected under HARDY-WEINBERG equilibrium by means of a G-test (SOKAL and ROHLF, 1973).

WRIGHT's fixation index (F) was estimated to measure the proportional extent of inbreeding using the formula: $F = 1 - H_o/H_e$. The variances of fixation indices were cal-

Table 1. — Summary of genetic characteristics for a mature stand of Polish larch near Ciechostowice, Poland. H_o — observed heterozygosity, H_e — expected heterozygosity, F — WRIGHT's fixation index, G — G-test

	Gene locus																	Mean	
	Est-1	Est-3	Gdh	G6pdh	Idh	Lap-1	Lap-2	Mdb-1	Mdb-2	Mdb-3	Mdb-4	Mnr-2	Mnr-3	Mnr-4	Shdh	Sod-1	Sod-2		Srdh
Allele frequencies																			
Allele 1	0.826	1.000	0.913	0.413	1.000	1.000	0.957	0.956	0.044	0.956	1.000	0.978	0.565	0.413	0.522	1.000	1.000	0.956	
Allele 2	0.044	-	0.087	0.326	-	-	0.021	0.044	0.956	0.044	-	0.022	0.435	0.587	0.174	-	-	0.044	
Allele 3	0.130	-	-	0.217	-	-	0.022	-	-	-	-	-	-	-	0.217	-	-	-	
Allele 4	-	-	-	0.044	-	-	-	-	-	-	-	-	-	-	0.087	-	-	-	
H_o	0.348	-	0.174	0.696	-	-	0.087	0.087	0.087	0.087	-	0.044	0.609	0.565	0.652	-	-	0.087	
SB(H_o)	(0.099)	-	(0.079)	(0.096)	-	-	(0.059)	(0.059)	(0.059)	(0.059)	-	(0.043)	(0.102)	(0.103)	(0.099)	-	-	(0.059)	
H_e	0.299	-	0.159	0.674	-	-	0.084	0.083	0.083	0.083	-	0.043	0.492	0.485	0.643	-	-	0.083	
F	-0.165	-	-0.096	-0.033	-	-	-0.034	-0.046	-0.046	-0.046	-	-0.022	-0.239	-0.166	-0.015	-	-	-0.046	
SB(F)	(0.321)	-	(0.046)	(0.151)	-	-	(0.247)	(0.028)	(0.028)	(0.028)	-	(0.002)	(0.200)	(0.203)	(0.184)	-	-	(0.028)	
G	1.697	-	0.382	7.601	-	-	0.091	0.091	0.091	0.091	-	0.022	1.330	0.640	9.766	-	-	0.091	
df	3	-	1	6	-	-	3	1	1	1	-	1	1	1	6	-	-	1	

Table 2. — Summary of genetic characteristics of the filial population from a stand of Polish larch near Ciechostowice, Poland.

	Gene locus						Mean
	Gdh	Mdh-1	Mdh-2	Mdh-3	Shdh	Srdh	
Allele frequencies							
Allele 1	0.937	0.950	0.966	0.948	0.486	0.950	
Allele 2	0.064	0.050	0.034	0.052	0.155	0.050	
Allele 3	-	-	-	-	0.282	-	
Allele 4	-	-	-	-	0.077	-	
Ho	0.127	0.099	0.060	0.095	0.556	0.083	0.170
SE(Ho)	(0.021)	(0.019)	(0.015)	(0.019)	(0.031)	(0.017)	
He	0.119	0.094	0.065	0.098	0.654	0.094	0.188
F	-0.068	-0.052	0.087	0.027	0.151	0.116	0.043
SE(F)	(0.011)	(0.009)	(0.109)	(0.075)	(0.103)	(0.103)	
G	2.171	1.305	1.202	0.159	18.322*	2.267	
df	1	1	1	1	6	1	

* — Indicates a significant departure from the null hypothesis at $P < 0.05$.
 Ho — observed heterozygosity, He — expected heterozygosity,
 F — WRIGHT'S fixation index, G — G-test

culated according to BROWN (1970). If self-fertilization is the only factor causing deviations from random mating, an expected equilibrium coefficient F_e can be calculated: $F_e = (1-t_m)/(1+t_m)$, where t_m is the multilocus outcrossing rate (ALLARD et al., 1968).

The variance effective population size, N_e , was calculated using allelic frequencies estimated in the two generations (YASUDA, 1969, after CHELIAK et al., 1985). It defines the theoretical number of individuals contributing to the next generation. Estimates of allelic frequencies in the filial population make it possible to calculate the number of males and females in a theoretical population (CROW and KIMURA, 1970; BARRETT et al., 1987).

Results and Discussion

Mature population

The 18 loci studied in this sample of 21 trees included six monomorphic loci, eight polymorphic loci each with two alleles, two loci with three alleles, and two loci with four alleles each (Table 1). Average number of alleles per locus was 2.0 for all loci or 2.5 for polymorphic loci only.

Individual tree heterozygosity ranged from 0.05 to 0.32 with a mean of 0.177 and it is in line with values for other conifers (YEH, 1981). Expected heterozygosity values ranged from 0.0 for monomorphic loci to 0.674 for the four-allelic G6pdh locus. The average expected heterozygosity of 0.193 is similar to the results reported for many other coniferous species (HAMRICK et al., 1981). Analysis of 11 populations of *Larix decidua* revealed mean heterozygosity of 0.157 and the average number of alleles per locus as 1.90 (LEWANDOWSKI, unpublished). These values are similar, though somewhat lower than presented in this study. When observed proportions of heterozygotes were compared with heterozygosities expected for a population in HARDY-WEINBERG equilibrium a nonsignificant, but constant excess of heterozygotes was detected at all variable loci, with an average WRIGHT'S F index of -0.057. This appears to be typical for most mature populations of conifers (O'MALLEY et al., 1979; YEH and LAYTON, 1979; LINHART et al., 1981; CHELIAK et al., 1985). Mean expected heterozygosity and F values for the subset of six polymorphic loci studied in the progeny were 0.189 and -0.049 respectively.

Filial population

Analysis of the embryos in seeds did not reveal additional alleles at the six loci studied by electrophoresis (Table 2). Values of expected heterozygosity in the filial population were similar to those obtained for the same loci in the adult population, and ranged from 0.065 for Mdh-2 to 0.654 for Shdh. The negative F values for Gdh and Mdh-1 indicate an excess of heterozygotes. The remaining four loci showed a deficiency of heterozygotes. The F value appeared to be statistically significant for the Shdh locus only, which occurs occasionally for different loci (KING et al., 1984; CHELIAK et al., 1985).

The differences between mature and filial generations in inbreeding values seemed to be typical of many coniferous trees (SHAW and ALLARD, 1982b; NEALE, 1985; NEALE and ADAMS, 1985b; EL-KASSABY et al., 1987), and is probably caused by natural selection increasing the frequency of heterozygotes in adult populations (STERN and ROCHE, 1974; BROWN, 1979).

Mating system estimation

Single- and multilocus estimates of outcrossing are presented in table 3. The single-locus rates (t_s) ranged from 0.776 for Mdh-2 to 1.132 for Mdh-1, and were significantly heterogeneous over loci studied ($\chi^2_{\text{het}} = 14.605$), based on chi-square test for heterogeneity (RAO, 1973). Because of great differences among variances of single-locus estimates, the arithmetic mean does not reflect the theoretical real mean for a population and thus the minimum variance mean over loci was used (EL-KASSABY et al., 1987). It was 1.048. The single-locus estimates for Gdh and Mdh-1 are greater than 1.0. This biologically unrealistic event could be explained by negative assortative mating following phenological differences within and among trees (SARVAS, 1962; EL-KASSABY et al., 1984), or may be due to sampling error (BROWN et al., 1984). Observed heterogeneity among single-locus outcrossing rates is probably due to the restricted number of maternal sampled trees, when each marker locus tests overlapping sets of mating events with unequal precision (BROWN et al., 1984). MÜLLER-STARCK and GREGORIUS (1988) claim that every investigated isozyme locus in a population can be related to a distinct mating system.

Table 3. — Single-locus (\hat{t}_s) and multilocus (\hat{t}_m) estimates of outcrossing rates and allelic frequencies in the outcrossed pollen pool for a stand of Polish larch near Ciechostowice, Poland (standard deviations in parentheses).

Locus	\hat{t}_s	Pollen pool		
		A1	A2	A3
Gdh	1.048(0.049)	0.958	0.042	-
Mdh-1	1.132(0.033)	0.942	0.058	-
Mdh-2	0.776(0.136) ^a	0.983	0.017	-
Mdh-3	1.000(0.163)	0.917	0.083	-
Shdh	0.803(0.138) ^a	0.458	0.170 ^b	0.372
Srdh	0.851(0.066) ^a	0.967	0.033	-
\hat{t}_s	1.048 ^c			
\hat{t}_m	0.943(0.055) ^a	F_e	0.029 ^d	

^a) significant departure from the null hypothesis $H_0: t=1$, at $p < 0.05$, based on chi-square likelihood ratio test.

^b) synthetic allele (A2+A4)

^c) minimum variance mean over loci

^d) WRIGHT'S fixation index under mating system equilibrium.

The multilocus estimate (\hat{t}_m) was calculated to be 0.943 and was unexpectedly lower than the minimum variance mean of the single-locus estimates. Spatial heterogeneity in pollen pool allele frequencies was not detected at all loci.

Multilocus and some of single-locus estimates were significantly different from 1.0 (see Table 3), indicating the existence of significant amounts of selfing compared to complete outcrossing. The value of \hat{t}_m suggests that the proportion of selfed viable embryos is likely about 5.7% ($s = 1 - \hat{t}_m$). The proportion of selfed progeny in this study is similar to values reported for natural populations of other conifers (see reviews: ADAMS and BIRKES, 1988; MUONA, 1989).

It should be noted that the \hat{t}_m estimator is much less biased by outcrosses among relatives, thus if the mean \hat{t}_s for population is substantially less than \hat{t}_m , inbreeding other than selfing is indicated (SHAW and ALLARD, 1982a). The situation, when \hat{t}_m is less than mean \hat{t}_s is unexpected in natural populations, and was found mainly for plantations such as seed orchards (SHAW and ALLARD, 1982a; BARRETT et al., 1987; MUONA and HARJU, 1989). This event suggests lack of mating among relatives, which can be explained by the absence of family structure. Larch is distinctly a light demanding species and it is a pioneer tree. Most populations of Polish larch are formed by discontinuous generations and there are problems with natural regeneration of a stand, which was also observed in the studied population. Larch regenerates and grows only where the structure of the shade forest (with beech and/or fir) was destroyed (OLACZEK, 1986), and hence the familiar structure can not be maintained.

Similarity between WRIGHT'S fixation indices, calculated from the progeny $F = 0.043$ and expected under mating system equilibrium $F_e = 0.029$, and the lack of mating among relatives suggest that self-fertilization is the most important factor influencing the level of inbreeding in the studied population.

The variance effective population size estimation indicate, that viable zygotes are equivalent to an idealized population of 9 females and 2 males, all with an equal opportunity to mate. The calculated size of 11 effective individuals is half less (52 %) than the 21 individuals studied in the population. The variance effective population size, assuming the studied population approximates a HARDY-WEINBERG equilibrium, is based on gene frequency and should be regarded with reference to the adult population (CROW and KIMURA, 1970; CHELIAK et al., 1985).

In species, where flowering is less regular, such as *Picea abies*, the ratio of effective to actual size may be very low, 0.15 to 0.35 in years with poor cone crops (LINDGREN after MUONA, 1989). The larch is regarded to be strongly irregularly flowering species, hence the small effective population number is not unexpected, the more so since cones were collected during a moderate cone crop.

Two effective males represent only 9.5 % of the actual number of males and nine effective females form 41% of the total females. The ratio of genetically effective males to receptive females observed in this sample was calculated to be 0.22, and this suggests that receptive females do not have equal opportunities to mate with a random sample of males. Asynchronous phenology, confounded with asymmetrical fertility can have a serious impact on the effective population size, which was observed in seed orchards (MÜLLER-STARCK, 1982; CHELIAK, 1984). However previous information about asymmetrical fertility in larch is not known to the authors. Additionally, direct genetic estimates of female and especially male reproductive success are very difficult to make in natural populations, because current methods are developed for isolated populations as seed orchards (SCHOEN and STEWART, 1986) or small island populations (MUONA, 1989).

Transport of larch pollen, which has no air sacs is considered restricted, compared to other conifers, however early studies have shown, that the average transport distance of male gametes can be as large as 6.7 km (DYAKOWSKA, 1936). The small ratio of genetically effective males to receptive females and spatial homogeneity of pollen allele frequencies indicate, that mating between individuals relatively distant from one another, can occur. In the situation, when almost all selfed progeny dies (KOSINSKI, 1986), only the outcross pollen pool is effective in the fertilization process.

The studied population showed a significant proportion of selfed viable embryos and other patterns of nonrandom mating. Differences of mating systems among different populations of the species were reported for many conifers (FARRIS and MITTON, 1984; NEALE and ADAMS, 1985b; KNOWLES et al., 1987), which were mainly due to different spatial structure of the populations or specific environmental conditions. Thus to fully describe the mating system of European larch further studies are needed.

Acknowledgements

Financial support for this research was provided by Polish Academy of Sciences, Grant No C.P.B.P. 04.04.

We thank Prof. W. T. ADAMS for the computer program used in mating system estimation. The technical assistance of JANINA KOZŁOWSKA and MARIA RATAJCZAK is greatly appreciated.

References

ADAMS, W. T. and BIRKES, D. S.: Estimating mating patterns in forest tree populations. In: Proc. International workshop on plant biology. Biochemical markers in the population genetics of forest trees. Institute for Agroforestry of the National Research

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 falling down of pollen of some trees. *Bull. Intern. Acad. Polon. Sci. Lett. Ser. Sci. Natur., Cracovie*: 155–168 (1936). — EL-KASSABY, Y. A., FASLER, 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., DANCİK, 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 Skarżyskiem. *Sylwan* 45 (4): 221–227 (1927). — LATOCHA, A. and HAWRYS, Z.: Wzrost i rozwój introdukowanych gatunków drzew w rejonach przemysłowych. *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, Amster-

dam. 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). — OLACZYK, 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* (DuRoi) 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, F. J.: *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* ssp. *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. DIEBEL¹) and P. P. FERET²)

(Received 13th June 1990)

Summary

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

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.