The Population Structure of Larix laricina in New Brunswick, Canada

By L. YING and E. K. MORGENSTERN

Dept. of Forest Resources, University of Brunswick,
Fredericton, NB E3B 6C2, Canada

(Received 10th April 1990)

Summary

Genetic diversity within and among eight natural populations of tamarack (*Larix laricina* (DU ROI) K. KOCH) was investigated using allozyme frequencies at 24 independently segregating loci. For these eight populations the average number of alleles per locus ranged from 1.6 to 1.8, the proportion of polymorphic loci from 0.38 to 0.54, and the mean observed heterozygosity from 0.135 to 0.162. These values are somewhat higher than those found by two investigators in northern Ontario.

F-statistics based on the 13 polymorphic loci indicated that 3.8% of total variation resides among populations. The correlation between NEI's genetic distance and elevational distance was stronger than between genetic distance and linear geographic distance. The results suggest that in spite of inefficient pollen distribution in this species, inbreeding has played only a very limited role in subdivision of New Brunswick populations. Large genetic variability within populations implies that tree improvement should be concentrated within populations.

Key words: tamarack, eastern larch, isozymes, electrophoresis, heterozygosity, subdivision.

Zusammenfassung

Die genetische Diversität zwischen und innerhalb von 8 Populationen von *Larix laricina* wurde durch elektrophoretische Methoden untersucht. Es wurden die Genhäufigkeiten an 24 unabhängig segregierenden Loci ermittelt. Für diese 8 Populationen lagen Mittelwerte für einzelne Parameter in folgenden Bereichen. Allele pro Locus: 1,6 bis 1,8; Anteil polymorpher Loci: 0,38 bis 0,54; mittlerer beobachteter Heterozygotiegrad: 0,135 bis 0.162. Diese Werte sind etwas höher als die, welche von zwei Forschern in Nordontario gefunden worden sind.

Parameter von F, welche auf 13 polymorphen Loci basieren, zeigten an, daß Populationen 3,8% zur Gesamtvariation beitragen. Korrelationen zwischen NEI's genetischem Abstand (D) und Unterschied in der Höhenstufe der Herkunft der Elternpopulation waren stärker als zwischen genetischem Abstand und linearer geographischer Entfernung. Die Ergebnisse deuten an, daß Inzucht infolge beschränkten Pollenfluges nur eine geringe Rolle in der Unterteilung von Populationen in New Brunswick gespielt hat. Die große Variabilität innerhalb der Populationen bedeutet, daß die Auslese an dieser Stufe konzentriert werden sollte.

Introduction

Larix laricina (DU ROI) K. KOCH, tamarack or eastern larch ,is the most widely distributed North American larch species, ranging from the Atlantic Coast and Pennsylvania to Alaska. Tamarack is well adapted to a variety of sites and grows rapidly, making it a promising reforestation species particularly in areas where wood will be scarce early in the next century. Considerable information on its growth, yield, management and protection has accumulated in recent years (GRAHAM *et al.*, 1983; New Brunswick Forest Research Advisory Committee, 1986; STIELL, 1986).

Studies of genetic variation have also been initiated and these must take the mating system into account. Like other species of the genus *Larix* Mill., tamarack produces spherical pollen grains without air bladders (ADAMS and MORTON, 1972), and studies have shown that most pollen grains drop within a distance of about 15 m from a source tree (EAVY *et al.*, 1985). The cones open readily in late August or early September and all seeds are shed within a few days. Only about 2% of the seed is distributed as far as 60 m away from parent trees (DUNCAN, 1954). As a result of inefficient pollen distribution in this genus, several authors have hypothesized that *Larix* species are more prone to inbreeding than other conifers (MEJNARTOWICZ and BERGMANN, 1975; SIMAK, 1979; EAVY *et al.*, 1985). This hypothesis is supported to some extent by low germination which in tamaracs usually ranges from about 20% to 50% (U. S. Forest Service, 1974). PARK and FOWLER (1982) have shown by means of controlled pollination that in natural stands of tamarack inbreeding declines with distance between parent trees. On the other hand, REHFELDT (1970) demonstrated that the gene pool of many small and isolated tamarack populations in Wisconsin has remained variable and unsegmented. In northern Ontario, KNOWLES *et al.* (1987) found significant levels of inbreeding, but in spite of this, variation within populations remained very high and only about 4% of total variation could be attributed to differences among populations (DICKINSON *et al.*, 1988; LIU, 1988). Even in a range-wide study of 36 populations sampled from Newfoundland to Alberta, no more than 5.5% of total variation was contributed by populations (CHELIAK *et al.*, 1988).

In New Brunswick, tamarack is one of the species receiving priority in selection programs (FOWLER, 1986). Studies of population structure are thus important to guide plus-tree selection, breeding methodology, and gene conservation. A study of New Brunswick populations was therefore initiated in 1986 and inheritance and linkage have already been described (YING and MORGENSTERN, 1990). The objective of the present paper is to report on population structure in the same populations.

Materials and Methods

Sampling

The area sampled is part of the Acadian Forest Region (ROWE, 1972) which was subdivided into seed zones by FOWLER and MACGILLVRAY (1967). *Figure 1* delineates these and the location of the eight natural populations sampled. Latitudes, longitudes and elevations of the parent populations have been given earlier (YING and MORGENSTERN, 1990). All populations were fast growing stands on upland sites with an average age of 14 years (*Table 1*). In each population, 50 trees were selected on the basis of flowering in May and cones collected in late August of 1986. There were 388 trees with sufficient viable seed.

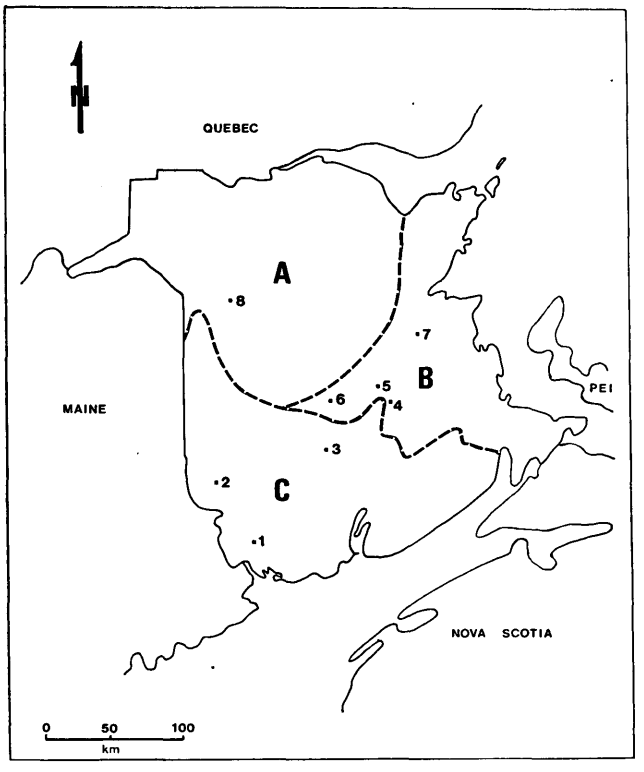

Figure 1. — The Province of New Brunswick with three major seed zones (simplified from FOWLER and MACGILLIVRAY, 1967) and location of eight populations sampled in this study.

Horizontal starch gel electrophoresis was used to separate the isozymes of 15 enzyme systems. Details of the procedures, banding patterns, and inheritance and linkage relationships of the loci have been given elsewhere (YING and MORGENSTERN, 1990).

Data Analysis

Allele frequency, expected heterozygosity (NEI, 1975), average expected heterozygosity (YEH and EL-KASSABY, 1980), percentage of polymorphic loci per population, average number of alleles per locus, and the effective number of alleles per locus (CROW and KIMURA, 1970) were calculated for all populations.

A contingency chi-square test (WORKMAN and NISWANDER, 1970) was used to test the homogeneity of allele frequency variation among populations. WRIGHT's F-statistics (WRIGHT, 1965) were used to determine deviation from HARDY-WEINBERG proportions and to measure the correlation between uniting gametes within populations (F_{IS}), among populations (F_{ST}), and for the population as a whole ($F_{IT} = F_{IS} + (1 - F_{IS}) F_{ST}$. Genetic distance among populations, D (NEI, 1972), was estimated, and the correlation calculated between genetic distance and geographic distance, between genetic distance and elevational distance, and between parent tree age and WRIGHT's fixation index. No general method has been devised to determine degrees of freedom for pairwise comparisons of matrices of intercorrelated populations and therefore significance tests of these correlations were not made (JORDE, 1980).

Table 1. — Genetic variability at 24 loci, expressed by effective number of alleles (N_e); average number of alleles (A) per locus; proportion of polymorphic loci (P); mean heterozygosity observed (H_o) aid expected (H_e). Standard errors are given in parentheses. F is the fixation index calculated from the 13 polymorphic loci.

Population	Mean age	N_e	A	P	H_o	H_e	F
1	21	1.49	1.7 (0.2)	0.46	0.148 (0.042)	0.143 (0.041)	-0.051
2	7	1.42	1.6 (0.1)	0.46	0.150 (0.043)	0.159 (0.044)	0.138
3	12	1.45	1.7 (0.2)	0.50	0.151 (0.043)	0.151 (0.042)	0.005
4	12	1.46	1.8 (0.2)	0.54	0.150 (0.042)	0.156 (0.041)	0.061
5	17	1.47	1.7 (0.2)	0.42	0.137 (0.043)	0.142 (0.042)	0.081
6	14	1.61	1.7 (0.2)	0.38	0.137 (0.045)	0.145 (0.046)	0.063
7	12	1.38	1.8 (0.2)	0.54	0.135 (0.036)	0.150 (0.038)	0.136
8	13	1.49	1.8 (0.2)	0.50	0.162 (0.046)	0.160 (0.044)	0.001
Mean	14	1.47	1.7	0.47	0.146	0.151	

Results

General variability

For the 15 enzyme systems, 27 loci were identified. When one member of a pair of linked loci is excluded, 24 loci are available for further analysis (YING and MORGENSTERN, 1990).

Five parameters of genetic variability at these 24 loci are given in *Table 1*. Ranges for the following three parameters are: effective number of alleles per locus (N_e): 1.38 to 1.61; average number of alleles per locus (A): 1.6 to 1.8; and proportion of polymorphic loci (P): 0.38 to 0.54. The average observed heterozygosities of populations (H_o) were not significantly different from those expected under HARDY-WEINBERG conditions (H_e) as indicated by standard errors.

Eleven of the 24 loci were monomorphic in all eight populations (YING, 1988): *Aat-1, Aco-2, Gdh, Lap-2, Mdh-1, Mdh-2, Mdh-4, Me-1, Pgi-1, Sod, Sdh-1.* This left 13 polymorphic loci for further analyses. The fixation index

Table 2. — The expected heterozygosity (H_e) at 13 polymorphic loci.

	Population								
Locus	1	2	3	4	5	6	7	8	Mean(h)
Aco-1	0.314	0.389	0.227	0.364	0.314	0.036	0.323	0.475	0.305
Acp-2	0.453	0.407	0.437	0.419	0.431	0.500	0.287	0.473	0.426
Fum	0.020	0.000	0.041	0.038	0.000	0.000	0.059	0.000	0.020
G6pd	0.352	0.462	0.342	0.355	0.392	0.375	0.425	0.378	0.393
Lap-1	0.475	0.449	0.500	0.493	0.473	0.493	0.437	0.476	0.474
Mdh-3	0.381	0.527	0.226	0.466	0.288	0.473	0.289	0.445	0.387
Mdr-2	0.380	0.459	0.497	0.402	0.422	0.494	0.430	0.435	0.440
Pgi-2	0.643	0.556	0.613	0.592	0.671	0.707	0.557	0.582	0.615
Pgm-1	0.059	0.019	0.000	0.074	0.089	0.000	0.133	0.038	0.051
Pgm-2	0.133	0.038	0.041	0.019	0.000	0.000	0.059	0.019	0.039
6Pgd-1	0.222	0.284	0.359	0.225	0.249	0.165	0.312	0.300	0.264
6Pgd-2	0.000	0.000	0.021	0.056	0.000	0.000	0.020	0.057	0.019
Sdh-2	0.000	0.225	0.324	0.250	0.080	0.236	0.278	0.092	0.186

Table 3. — Results of the X^2 contingency tests, and F-statistics within populations (F_{IS}), among populations (F_{ST}), and the total population (F_{IT}).

Locus	X^2	d.f.	F_{IS}	F_{IT}	F_{ST}
Aco-1	32.6**	7	0.076	0.132	0.060
Acp-2	23.6**	7	0.144	0.172	0.032
Fum	9.8	7	-0.023	-0.010	0.013
G6pd	9.5	7	-0.064	-0.051	0.012
Lap-1	29.2**	7	-0.125	-0.083	0.037
Mdh-3	121.6**	28	0.133	0.191	0.067
Mdr-2	16.1*	7	-0.001	0.021	0.021
Pgi-2	118.9**	28	-0.024	0.026	0.050
Pgm-1	15.7*	7	0.256	0.271	0.020
Pgm-2	17.9*	7	0.213	0.232	0.024
6Pgd-1	42.2**	14	0.089	0.107	0.020
6Pgd-2	21.5	14	-0.022	-0.008	0.014
Sdh-2	21.3**	7	0.150	0.188	0.044
Mean			0.030	0.067	0.038

Significance levels: *) 5%; **) 1%.

(F) calculated for the eight populations (*Table 1*) is variable; the oldest population (population 1) shows a slight excess of heterozygotes while other populations have values near zero or a slight excess of homozygotes. Based on this parameter, Populations 2 and 7 are clearly inbred (F = 0.138; 0.136). However correlation with age was not strong (r = —0.340).

Expected heterozygosity at these 13 loci is given in *Table 2.*

Degree of subdivision

Contingency chi-square tests of homogeneity *(Table 3)* showed that only three loci (*Fum, G6pd* and *6Pgd-2*) were homogeneous in their allele frequency distribution. The other 10 loci exhibited variation in allele frequency among the eight populations, which was significant at least at the 5% probability level.

Table 4. — Pair-wise genetic distance (D) (below diagonal) and geographic distance (km) (above diagonal between the eight populations.

Population	1	2	3	4	5	6	7	8
1		72	70	127	130	109	183	185
2	0.015		73	138	137	89	181	128
3	0.007	0.019		66	67	41	116	132
4	0.002	0.011	0.006		8	60	58	148
5	0.001	0.013	0.006	0.002		55	53	141
6	0.007	0.014	0.008	0.008	0.006		91	97
7	0.005	0.017	0.007	0.003	0.004	0.013		147
8	0.004	0.010	0.011	0.003	0.004	0.011	0.007	

The F-statistics (*Table 3*) show that, within populations (F_{IS}), there is an excess of heterozygotes at four loci shown by negative values; but the mean value, 0.030, is positive, indicating a 3% heterozygote deficiency relative to HARDY-WEINBERG expectations. This demonstrates a low level of inbreeding. The exent of genetic differentiation among populations (F_{ST}) ranges from 0.012 to 0.067 among loci and averages 0.038. Thus about 96% of genetic variation resided within population and 4% among populations.

Genetic distance values (D) ranged from 0.001 to 0.019 and averaged 0.008 (*Table 4*). The correlation between genetic distance and geographic distance was 0.056, and the correlation between genetic distance and elevational distance 0.625.

Discussion

General variability

The parameters of general variability found in this study (*Table 1*) indicate a level of variability that is similar to levels found by other investigators who have investigated this species. Comparisons can be most easily made with the range-wide survey of CHELIAK *et al.* (1988) which included the largest number of loci. Of the 15 loci common to both studies, three (*Gdh, Lap-2, Aat-1*) were monomorphic and three (*Pgi, Lap-1, Mdh-3*) polymorphic, in all populations; three more (*Aco-1, G6pd, 6Pgd-1*), which were polymorphic in all populations of this study, were polymorphic in 78% to 97% of the populations in their study (CHELIAK *et al.*, 1988; YING and MORGENSTERN, 1990).

The parameters of genetic variability (*Table 1*) indicate moderate variability of New Brunswick tamarack populations. Our values of the average number of alleles per locus (1.7), the proportion of polymorphic loci (0.47), and the average observed and expected heterozygosity (0.146 and 0.151) were only slightly lower than those given by CHELIAK *et al.* (1988) from range-wide samples but heigher than those of DICKINSON *et al.* (1988) and LIU (1988) found in northern Ontario, where means of observed and expected heterozygosity ranged from 0.09 to 0.12. Although there is again only limited overlap of the loci included in northern Ontario as compared to New Brunswick, differences in heterozygosity as a result of population size are a possibility. In comparison to most areas of northern Ontario, tamarack is more common in southern and central New Brunswick and large populations are found on upland sites. Gene exchange through migration may be more frequent here than in boreal populations.

Comparisons with other species are also instructive. In western larch (*Larix occidentalis* NUTT.) mean expected heterozygosity was even lower (average 0.08) and genetic drift was considered important (FINS and SEEB, 1986). Tamarack is more variable than western larch but much less variable than many other species: the average proportion of polymorphic loci for 29 species is 0.66 and average heterozygosity 0.20 (HAMRICK *et al.*, 1981; BOYLE and YEH, 1988).

Degree of subdivision

The degree of subdivision in this species is of special interest because of limited pollen and seed distribution and the possible effect on inbreeding. The fixation index (F) given in *Table 1* indicates some differences among populations. Two of the youngest populations with ages 7 and 12 had the highest values of F (0.138, 0.136) and therefore are the most strongly inbred. Our records show

that the two parent populations, Canterbury and Rogersville, were divided into several small, somewhat isolated groups of trees which probably arose from older trees in adjacent swamps (YING and MORGENSTERN, 1990). Pollination within these groups may account for the large values of F. The other six populations have much lower values.

If inbreeding had been very significant in all populations this would have become manifest in other parameters but was not the case. As indicated by the X^2 contingency tests, significant differences among populations exist for 10 of the 13 polymorphic loci (*Table 3*). However, as is evident from the F-statistics, the mean value of F_{ST} is 0.038 indicating that only 3.8% of total variation resides among populations. Thus a very large percentage of variability is maintained within populations. Comparable figures from the range-wide study of tamarack and from northern Ontario vary from 4.2% to 5.5% (CHELIAK *et al.*, 1988; DICKINSON *et al.*, LIU, 1988). In contrast, population differences in western larch are much more pronounced and account for 8.6%, presumably because of more isolation, greater environmental diversity, and the presence not only of inbreeding but also random genetic drift at various stages in the evolution of this species (FINS and SEEB, 1986). In tamarack, a high variability within populations has also been determined for adaptation to nutrients (WANYANCHA and MORGENSTERN, 1987).

Another measure of the degree of subdivision is genetic distance, D (*Table 4*), which was compared with geographic distance. Isolation by distance appears to be less important than natural selection. The correlation between genetic distance and linear geographic distance was small ($r = 0.056$) while the correlation between genetic distance and elevational distance was much larger ($r = 0.625$). This second relationship may be based on ecological factors such as temperature which have controlled natural selection, but are difficult to assess. The area sampled is too small and climatic stations not close enough to the sampled populations. The climatic regions established by VAN GROENEWOUD (1983) in New Brunswick are not in close agreement with the seed zones of FOWLER and MACGILLIVRAY (1967). Several authors have reported relationships with geographic and climatic variables in this species (REHFELDT, 1980; JOYCE, 1987; LIU 1988).

When the matrix of D is examined (*Table 4*) it becomes evident that the range of D values within a seed zone is not smaller than among seed zones. For example, the greatest difference among populations in the total sample was 0.019, but even within seed zones values as large as 0.019, 0.017 and 0.011 are found. A similar trend had been discovered earlier when contingency tests showed that although the east-central zone (Zone B) was more homogeneous than the southern zone (Zone C) (*Figure 1*), there were differences significant at the 1% level both within as well as among seed zones (YING, 1988).

Overall, the degree of subdivision of New Brunswick tamarack populations reflects their distribution, ecology, population sizes, and mating system. Southern and central New Brunswick have been subjected to logging, land clearing, and farming for 200 years, and such disturbances favour the continued existence and reproduction of a pioneer species. Although many populations are confined to bogs and swamps, expansion to adjacent uplands including abandoned farm land is common. Many populations are large and not isolated so that genetic drift will only play a minor role. Short-distance pollen distribution influences only the male gamete; seed distribution is as effective as in other conifers (DUNCAN, 1954) such as white spruce (*Picea glauca* (MOENCH) Voss) but not as effective as in black spruce (*P. mariana* (MILL.) B. S. P.) which has a longer seed distribution period. Phenological differences in male anthesis and female receptivity (dichogamy) may limit self-pollination (TOSH, 1986). Embryos resulting from inbreeding will be subjected to postzygotic selection and mortality. Consequently an outbreeding system with heterozygosity is maintained. The high variability found within populations suggests that selection should be concentrated within populations.

Acknowledgements

We would like to thank Dr. F. C. YEH for making computer programs available. Mr. J. PITEL and Drs. P. KNOWLES, W. M. CHELIAK and T. J. B. BOYLE provided helpful reviews.

References

ADAMS, R J. and MORTON, J. K.: An atlas of pollen of the trees and shrubs of Eastern Canada and the United States. Part I. Gymnospermae to Fagaceae. Univ. Waterloo Biol. Ser. 8. Waterloo, Ont. (1972). — BOYLE, T. J. B. and YEH, F. C.: Within-population genetic variation — implications for selection and breeding. Proc. Twenty-first Meetg. Can. Improv. Assoc., Pt. 2, pp. 20—42 (1988). — CHELIAK, W. M., WANG, J. and PITEL, J. A.: Population structure and genic diversity in tamarack, *Larix laricina*. Can. J. For. Res. **18**: 1318—1324 (1988). — CROW, J. and KIMURA, M.: An introduction to population genetics theory. Harper and Rowe, New York. (1970). — DICKINSON, T., KNOWLES, P. and PARKER, W. H.: Data set congruence in northern Ontario tamarack (*Larix laricina*, Pinaceae). System. Bot. **13**: 442—455 (1988). — DUNCAN, D. P.: A study of some of the factors affecting the natural regeneration of tamarack (*Larix laricina*) in Minnesota. Ecol. **35**: 498—521 (1954). — EAVY, A. L., CARTER, K. K. and HOUSEWEART, M. W.: Tamarack pollen dispersal. Proc. 29th Northeast. For. Tree Improv. Conf., Morgantown, W. Va. 1984. p. 157—167 (1985). — FINS, L. and SEEB, L. W.: Genetic variation in allozymes of western larch. Can. J. For. Res. **16**: 1013—1018 (1986). — FOWLER, D. P.: Strategies for the genetic improvement of important tree species in the Maritimes. Can. For. Serv.. Inf. Rep. M-X-156. (1986). — FOWLER, D. P. and MACGILLIVRAY, G. H.: Seed zones for the Maritime Provinces. Can. For. Serv. Inf. Rep. M-X-12 (1967). — GRAHAM, C. M., FARINTOSH, H. L. and GRAHAM, B. J. (Editors): Larch symposium. Sponsored by Fac. Forestry, Univ. Toronto and Ont. Min. Nat. Res., Toronto, Ont. 1982 (1983) — HAMRICK, J L., MITTON, J. B. and LINHART, Y. B.: Levels of genetic variation in trees: influence of life history characteristics. *In*: Proc. Symp. on Isozymes of North Am. For. Trees and For. Insects, Berkeley, Calif., 1979. U. S. For Serv. Gen. Tech. Rep. PSW-48. p. 35—41 (1981) — JORDE, L. B.: The gennetic structure of subdivided human populations. A review. *In*: Current developments in anthropological genetics. Vol 1. Ed. by J. H. MIELKE and M. H. CRAWFORD. Plenum Press, New York. pp. 135—208 (1980). — JOYCE, D. G.: Adaptive variation in cold hardiness of eastern larch, *Larix laricina*, in northern Ontario. Can. J. For. Res. **18**: 85—89 (1987). — 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). — LIU, Z.: Patterns of allozyme variation in tamarack (*Larix laricina* (DU ROI) K. KOCH from northern Ontario. M. Sc. Thesis, Lakehead University, Thunder Bay, Ont. (1988). — MEJNARTOWICZ, L. and BERGMANN, F.: Genetic studies on European larch (*Larix decidua* MILL.) employing isoenzyme polymorphisms. Genet. Pol. **16**: 30—35 (1975). — NEI, M.: Genetic distance between populations. Amer. Natur **106**: 282—293 (1972). — NEI, M.: Genetic variability in natural populations. *In*: Molecular population genetics and evolution. North Holland Publ., Amsterdam. Ch. 6. p. 127—174 (1975). — New Brunswick Forest Research Advisory Committee: 1986 Larch Workshop. Fredericton, N. B. (1986). — 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). — REHFELDT, G. E.: Genecology of *Larix laricina* (DU ROI) K. KOCH in Wisconsin. Silvae Genet. **19**: 9—16 (1970). — ROWE, J. S.: Forest regions of Canada. Can. For. Serv. Pub. No. 1300 (1972). — SIMAK, M.: *Larix sukaczewii:* naturlig utbredning, biologi, ekologi och

fröanskaffnings problem. Sveriges Lantbruksuniv. Inst. Skogsköt. Rapp. Nr. 1. (1979). —— STIELL, W. M.: Fifteen-year growth of tamarack planted at six spacings on an upland site. Can. For. Serv. Inf. Rep. PI-X-62 (1986). —— TOSH, K. J.: Development and distribution of cones, pollination, and yield of seeds in young plantations of tamarack. M. Sc. F. Thesis, Univ. of New Brunswick, Fredericton, N. B. (1986). —— U. S. Forest Service: Seeds of woody plants in the United States. Agric. Handbook No. 450 (1974). —— VAN GROENEWOUD, H.: Summary of climatic data pertaining to the climatic regions of New Brunswick. Can. For. Serv. Inf. Rep. M-X-146 (1983). —— WANYANCHA, J. M. and MORGENSTERN, E. K: Genetic variation in response to soil types and phosphorus fertilizer levels in tamarack families Can. J. For. Res. 17: 1251—1256 (1987). —— WORKMAN, P. L. and NISWANDER, J. D. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Amer. J. Human Genet. 22: 24—49 (1970). —— WRIGHT, S.: The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 358—420 (1965). —— YEH, F. C. and EL-KASSABY, Y. A.: Enzyme variation in natural populations of Sitka spruce (*Picea sitchensis*). Variation patterns among trees from 10 IUFRO provenances. Can. J. For. Res. 10: 415—422 (1980). —— YING, L.: Population structure of *Larix laricina* (DU ROI) K. KOCH in New Brunswick. M. Sc. F. Thesis, University of New Brunswick, Fredericton, N. B. (1988). —— YING, L. and MORGENSTERN, E. K.: Inheritance and linkage relationships of some isozymes of *Larix laricina* in New Bruiswick, Canada. Silvae Genet. 39: 245—251 (1990).

Changes Induced by Zinic Smelter Pollution in the Genetic Structure of Pine (Pinus sylvestris L.) Seedling Populations

By W. PRUS-GLOWACKI*) and ST. GODZIK**)

(Received 22nd May 1990)

Summary

Comparison of the genetic structure of a seedling population grown in an unpolluted area and in an area adjacent to a zinc smelter has demonstrated differences. When seedlings of *Pinus sylvestris* were grown in the polluted area both heterozygosity and degree of genetic polymorphism underwent a significant decrease (by 30% and 20%, respectively) compared to from the control in the unpolluted area. For studied enzymatic loci the seedling population grown on the polluted site failed to maintain HARDY-WEINBERG equilibrium, exhibiting an excess of homozygotes compared to expected levels. The changes in genetic parameters point to directional selective processes in the studied seedlings groups.

Key words: zinc pollution, genetic structure, seedlings, isozymes, Scots pine.

Introduction

Studies on the genetic structure of naturally regenerating populations in polluted regions have demonstrated more extensive changes than in populations free from pollution (PRUS-GLOWACKI and NOWAK-BZOWY, 1989). Studies on the adaptive strategy in naturally regenerating populations suffer the disadvantage that precise determination of the genetic structure of seed pools, serving as starting material for regeneration is difficult and involves some approximation. This is because in different years different individuals in the population variously participate in seed set and in the composition of the pollen cloud, and thus the gene pool of the embryo population changes each year. Also only general conclusions can be drawn from studies performed on changes in the genetic structure in two distinct populations, originating in either heavily or only slightly polluted regions and therefore representing different gene pools.

This situation induced us to undertake studies on changes in the genetic structure of pine seedling populations, using a single pool of seeds of known genetic structure for the establishment of a field experiment. Comparison of allelic and genotypic frequencies at defined time periods in the embryo population and in populations of surviving seedlings may demonstrate whether the increased mortality, observed in the polluted areas compared to controls, represents the result of a stochastic process or a directional change in the genetic structure of the population associated with selection. The need for these kind of studies is strongly recommended to reduce our gaps in knowledge, of the quantifying of genetic losses caused by anthropopressure (KARNOVSKY et al., 1989).

Material and Methods

Seeds collected from 100 trees of a pine population in Zielonka Forest, near Poznan, an area only slightly polluted by industry, served as the starting material to set up experimental plots. The choice of the seeds sown for experiments characterized as of "average" genetic structure (frequencies of genotypes) for a Scots pine population were not already changed by industrial pollution in the Upper Silesia region. From each of the 100 trees, 100 seeds were collected, pooled and distributed in portions of 3000 seeds each to be sown in the study areas. Before setting up experimental plots, the percentage of germinating seeds was established in the loboratory. Genetic structure of the seed pool was determinated by isoenzyme analysis of germinating embryos.

A total of three experimental sites were set up. Two of them were located at distances of approximately 1000 m and 1500 m, respectively from a zinc smelter in Miasteczko Slaskie (Upper Silesia) while the third, a control, was located in Zielonka Forest near Poznan the site from which the seeds were collected. At the time of sowing, soil samples were taken from each area and analyzed for content of heavy metal ions.

The plots were set up in 1987, mid-April, the recommended time for sowing pine in this part of Poland. The seeds, 3000 per plot, were spot-sown at a depth of approximately 1 cm, spaced 5 cm in rows. The distance between seedlings is designed to restrict density selection. Care

*) Adam Mickiewicz University, Genetics Department, ul. Dabrowskiego 165, PL-60-594 Poznan, Poland
**) Institute of Environmental Engineering, Polish Academy of Sciences, ul. Marii Curie-Sklodowskiej 34, PL-41-800 Zabrze, Poland