Lack of Allozyme Variation in *Larix sukaczewii* DYL. from the Southern Urals

By A. SH. TIMERJANOV

Dept. of Forest Management, Bashkir State Agrarian University, 50 let Oktyabrya Str., 34, Ufa 450001, Russia

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Summary

Populations of *Larix sukaczewii* DYL. in the Southern Urals have been studied using 20 isozyme loci controlling 12 gene-enzyme systems. The heterozygosity observed in populations averaged 0.042 ± 0.012, with an expected value of 0.043 ± 0.010. The proportion of polymorphic loci was 0.31 ± 0.08 and 0.64 ± 0.07, according to the 95% and 99% criterion of polymorphism, respectively. The average number of alleles per locus was 1.14 ± 0.03. These estimates are less than those reported for larches. Approximately 3% of the total genetic diversity occured among populations and the remainder (about 97%) resides within populations. The mean NEI's and GREGORIUS's genetic distances over all pairs of *Larix sukaczewii* populations were 0.01 and 0.08, respectively. There was no statistically significant relationship ($P < 0.05$) between genetic distance and linear geographic distance.

Key words: *Larix sukaczewii*, isozymes, electrophoresis, heterozygosity, differentiation.

FDC: 165.3; 165.53; 174.7 *Larix sukaczewii*; (470); (2348).

Introduction

Larix sukaczewii DYL. is one of the most rapid growing and widespread forest trees of Russia. The species is capable of growing under a variety of climatic and edaphic conditions. At the same time little information is available on genetic variation within the species. The most southern part of *Larix sukaczewii* is in the Southern Urals.

The objectives of the present study were to determine the level of allozyme genetic diversity of *Larix sukaczewii*, to examine the genetic variability within the species and to study the pattern of genetic differentiation among populations.

Materials and Methods

Sampling

Cones were collected from 160 trees growing in 4 natural populations of *Larix sukaczewii* in the Southern Urals (PUTENIKHIN et al., 1989). The locations of populations are presented in *table 1*.

Fourty trees were sampled in each the population and cones were collected in August. Mean tree ages ranged from 90 to 110 years. Analyses were performed using megagametophyte tissue from dry seeds. Six megagametophytes per tree were analysed.

Table 1. – Geographic location of *Larix sukaczewii* populations.

	Population name	Location (°N)	(°E)	Elevation (m)
1	Iremel	54°33'	58°57'	1200
2	Zilair	52°13'	57°25'	550
3	Uchaly	54°24'	59°23'	400
4	Karaidel	55°45'	56°58'	400

Vertical-slab polyacrylamide gel electrophoresis was used to separate the isozymes of 12 enzyme systems (DAVIS, 1964). Details of procedures, banding patterns and inheritance of the loci are discussed elsewhere (TIMERJANOV et al., 1994).

Data analysis

Allele frequency, expected heterozygosity (NEI, 1978), average expected heterozygosity, percentage of polymorphic loci per population, and the average number of alleles per locus were calculated for all 4 populations.

WRIGHT's F-statistics (WRIGHT, 1965), were used to determine deviation from HARDY-WEINBERG proportions. The homogeneity of allele frequency variation among populations was assessed using the χ^2 (chi)-procedure of WORKMAN and NISWANDER (1970). NEI's method was used to calculate the gene diversity statistics H_t, H_s, G_{st} (NEI, 1973). Genetic similarities between populations were measured by genetic distance indexes (D) of NEI (1978) and (d_o) of GREGORIUS (1974). Matrices of D and d_o values were clustered using the UPGMA method (SNEATH and SOKAL, 1973).

Results

General variability

The 12 enzymes were coded by a total of 20 electrophoretically demonstrable loci. Of these, 9 loci were polymorphic with a total of 22 alleles. The 11 monomorphic loci were Lap-1, Aph-1, Mdh-1, G-6-Pdh-1, Sod-1, Sod-2, 6-Pgd-1, Gdh-1, Dia-2, Fdh-1, G-2-dh-1.

Enzyme aspartate aminotransferase AAT (EC 2.6.1.1.): 3 loci were found; Aat-1 has 3 alleles; Aat-2 and Aat-3 each have 2 alleles.

Enzyme leucine aminopeptidase LAP (EC 3.4.11.1): 2 loci were scored; 3 alleles were found for Lap-2, Lap-1 was monomorphic.

Enzyme malate dehydrogenase MDH (EC 1.1.1.37) has 3 loci; Mdh-1 was monomorphic; Mdh-2 has 2 alleles and Mdh-3 has 3 alleles.

Enzyme diaphorase DIA (EC 1.6.4.3) 2 loci were found; Dia-1 has 2 alleles; Dia-2 was monomorphic.

Enzyme shikimate dehydrogenase SKDH (EC 1.1.1.25): 1 locus (Skdh-1) with 2 alleles was analyzed.

Enzyme acid phosphatase APH (EC 3.1.3.2): Aph-1 was monomorphic; Aph-2 has 3 alleles.

Other enzymes 6-phosphogluconate dehydrogenase 6-PGD (EC 1.1.1.44), glucose-6-phosphate dehydrogenase G-6-PDH (EC 1.1.1.49), glutamate dehydrogenase GDH (EC 1.4.1.2), superoxide dismutase SOD (EC 1.15.1.1), formate dehydrogenase FDH (EC 1.2.1.2), glycerate-2-dehydrogenase G-2-DH (EC 1.1.1.29) were monomorphic.

All of these loci have been confirmed by genetic segregation tests (TIMERJANOV et al., 1994).

Parameters of genetic variability of all these 20 loci are given in *table 2*.

Table 2. – Genetic variability at 20 isoenzyme loci of *Larix sukaczewii* populations.

Population	A	$P_{95\%}$	$P_{99\%}$	H_e	H_o	F
1 Iremel	1,19	0,41	0,41	0,059	0,064	-0,08
				(0,007)	(0,006)	
2 Zilair	1,10	0,27	0,67	0,041	0,047	-0,13
				(0,006)	(0,005)	
3 Uchaly	1,15	0,27	0,81	0,039	0,030	0,30
				(0,005)	(0,003)	
4 Karaidel	1,10	0,27	0,67	0,030	0,026	0,15
				(0,003)	(0,002)	
Mean	1,14	0,31	0,64	0,043	0,042	0,02
	(0,03)	(0,08)	(0,07)	(0,010)	(0,012)	

Note: A – average number of alleles per locus; $P_{95\%}$, $P_{99\%}$ – proportion of polymorphic loci at 95% and 99% criterion; H_e, H_o – expected and observed heterozygosity; F – WRIGHT's fixation index. Standard errors are given in parentheses.

The most common alleles were predominant throughout the range of populations surveyed. Chi-frequency differences among populations were significant ($P < 0.01$) for loci Aph-2 and Mdh-3 (*Table 3*).

Table 3. – Gene diversity estimates and homogeneity tests of allele frequency variation of *Larix sukaczewii* populations.

	Gene diversity			Homogeneity tests	
Locus	H_t	H_s	G_{st}	χ^2	df
Lap-2	0,015	0,015	0,014	3,31	4
Aat-1	0,483	0,440	0,088	5,42	10
Aat-2	0,039	0,038	0,026	3,11	2
Aat-3	0,016	0,014	0,113	6,23	2
Aph-2	0,166	0,142	0,141	19,98**	10
Mdh-2	0,043	0,043	0,009	4,22	4
Mdh-3	0,279	0,262	0,062	12,06**	10
Skdh-1	0,232	0,218	0,060	3,01	4
Dia-1	0,054	0,052	0,037	2,78	4
Mean*	0,066	0,061	0,028	–	–

Note: *) calculated over 20 loci (including 11 monomorphic loci); H_t – a measure of the total gene diversity, H_s – a measure of the average diversity within populations, G_{st} – a relative measure of the proportion of interpopulational gene differentiation; **) $\chi^2 \gamma \chi^2_{01}$.

Table 2 shows that an average population of *Larix sukaczewii* is polymorphic (95% criterion) for 31% of its loci. Population levels of expected heterozygosity ranged from 0.030 in population Karaidel to 0.059 in population Iremel. The fixation index (F) is variable, the population Zilair showed a slight excess of heterozygotes.

The mean number of alleles per locus was 1.14 with a minimum of 1.10 in the Uchaly and Karaidel populations and a maximum of 1.19 in the population Iremel. Of the 25 HARDY-WEINBERG tests performed within populations, only 4 (16%) were significant ($P < 0.01$). The deviation from the expected genotypic distribution was due to an excess of homozygotes in all cases.

Degree of subdivision

An analysis of gene diversity using NEI's statistics of population subdivision indicated that about 2.8% of the total genetic variation occurred among populations (*Table 3*). Among the investigated loci, Aph-2 contributed greatly to the observed levels of interpopulation differentiation ($G_{st} = 0.141$). The pattern of allozyme variation observed in this study was lower than that reported for other larches (*Table 4*).

The estimates of genetic distances for all combinations of populations over all loci are presented in *table 5*.

Table 5 shows the matrix of the 2 measures of genetic distance. The mean NEI's standard genetic distance was 0.013. The maximum value was between populations Iremel and Karaidel ($D = 0.041$). The GREGORIUS's genetic distance (mean value: 0.078, maximum value between populations Iremel and Uchaly: $d_o = 0.138$) confirmed the results obtained with NEI's formula on the whole.

There was no statistically significant relationship between genetic and geographic distances.

Discussion

General variability

The parameters of general variability observed in this study (*Table 2*) were lower than those reported for this species by other investigators. For example, in a range-wide investigation of *Larix sukaczewii*, MILYUTIN et al. (1993) observed an average of 2.0 alleles per locus, with about 75% of the loci polymorphic over all populations. The lower level of variation observed in this study could simply be due to the more restricted geographic range of the sample as compared to the range-wide sampling of MILYUTIN et al. (1993). Of their 12 loci, the study of MILYUTIN et al. (1993) includes 3 monomorphic ones. In the present study, where 20 loci are included which examined 11 monomorphic loci, the larger number of loci examined could cause the differences in the levels of variation.

Degree of subdivision

The G_{st} analysis confirms there is relatively little genetic differentiation in *Larix sukaczewii* as regards allozymes. Approximately 97% of the genetic variation in *Larix sukaczewii* resides within stands, as compared to 91% to 95% for this species as reported by other investigators (see *Table 4*). The level of interpopulational gene differentiation was similar to measures ($G_{st} = 97\%$) for other conifers – *Pseudotsuga menziesii* (YEH and O'MALLEY, 1980) and *Pinus rigida* (GURIES and LEDIG, 1982).

The values of NEI's genetic distance between populations vary from 0.001 (between population Uchaly and Karaidel) to 0.041 (between population Iremel and Karaidel). The population Iremel is markedly divergent from all other populations with NEI's genetic distances 0.013, 0.015, 0.041 and GREGORIUS's genetic distances 0.096, 0.125, 0.138 (*Table 5*). The average NEI's genetic distance among all populations was relatively small ($D = 0.013$), about 3 to 4 times smaller than the average genetic distance among populations *Larix sukaczewii* found by other investigators (LARIONOVA, 1988; ALTUKHOV et al., 1980; MILYUTIN et al., 1993). Because this study differs from

Table 4. – Levels of genetic diversity and differentiation of some species of the genus *Larix*.

Species	Range	P_{99}	F_{st}	G_{st}	D	Reference
L.decidua	d	–	–	0,051	0,029	Maier,1992
L.decidua	d	0,36*	–	0,041	0,008	Lewandowski, Mejnartowicz,1991
L.laricina	c	0,62	–	0,055	0,032	Cheliak et al., 1988
L.laricina	c	0,47	0,038	–	0,008	Ying, Morgenstern,1991
L.laricina	c	0,32	0,022	–	0,003	Liu, Knowles, 1991
L.occidentalis	d	0,30	–	0,086	0,017	Fins, Seeb,1986
L.sibirica	c	0,77	–	0,070	0,052	Altukhov et al., 1989
L.sibirica	c	0,47	0,082	–	0,024	Semerikov, Matweev, 1995
L.sukaczewii	d	0,75	–	0,069	0,048	Milyutin et al., 1993
L.sukaczewii	d	0,69	–	0,045	0,043	Altukhov et al., 1989
L.sukaczewii	d	0,69	–	0,085	0,041	Larionova,1988
L.sukaczewii	d	0,64	–	0,028	0,013	This study

Note: c – continuous range, d – distinct range, P_{99} – proportion of polymorphic loci at 99% criterion (*at 95% criterion), $G_{st}(F_{st})$ – proportion of interpopulational gene differentiation, D – NEI's genetic distance.

Table 5. – Genetic distances between populations of *Larix sukaczewii* (above the diagonal calculated following NEI (1978) and below the diagonal calculated following GREGORIUS (1974)).

Population		I	2	3	4
I	Iremel	–	0,013	0,015	0,041
2	Zilair	0,096	–	0,004	0,005
3	Uchaly	0,I38	0,040	–	0,001
4	Karaidel	0,125	0,034	0,036	–

the other investigations in the systems used and the number of loci, the results must be carefully compared. The population Iremel may have been isolated long enough during the last glacial period to develop a strong differentiation. Spatial isolation (1200 m altitude) may also be a reason for this divergency. Thus, NEI's and GREGORIUS's genetic distances indicated a relatively small amount of accumulated genetic differences among populations *Larix sukaczewii*.

The relatively small genetic differentiation among populations *Larix sukaczewii* is in agreement with our earlier observations based on morphological variation (PUTENIKHIN et al., 1989). Cluster analysis derived from monoterpene data of branches of this species also are in full agreement with the

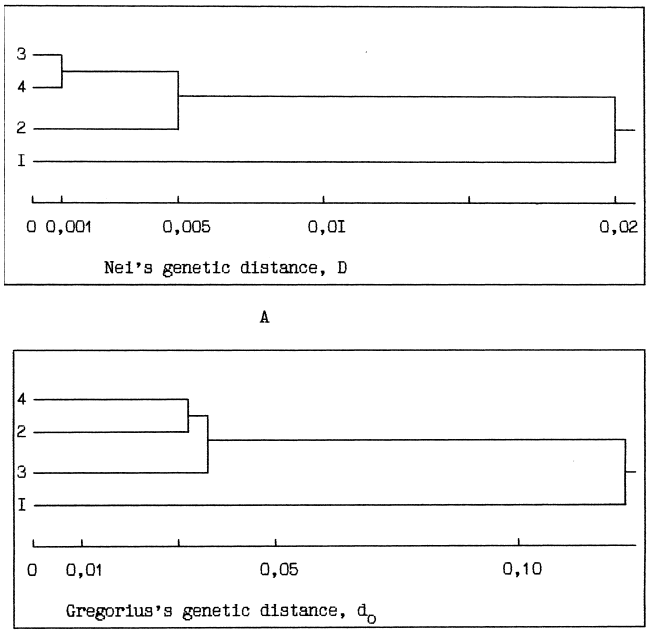

Figure 1. – Dendrograms from clustering based on NEI's (A) and GREGORIUS's (B) genetic distances between populations of *Larix sukaczewii* (number of populations as in the *table 5*).

allozyme results (TIMERJANOV, 1994). The similarity between patterns of morphological, monoterpene and allozyme differentiation may indicate that they reflect the effect of events related to glaciation. Paleobotanical evidence indicates that refugia probably existed in the Ural mountains. *Larix sukaczewii* may have been restricted to small refugia during Pleistocene glaciation, as *L. occidentalis* (FINS and SEEB, 1986). These small refugia could have acted as "genetic bottlenecks" and resulted in low genetic variation.

The results reported in this paper suggest that historical events have had a marked effect on the pattern of distribution of genetic variation of *Larix sukaczewii* in the Southern Urals. It appears that the genetic characteristics of *Larix sukaczewii* largely reflect the effects of evolutionary factors imposed by relatively recent historical events related to the last glaciation.

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Effect of Age on Selected Wood Quality Traits of Poplar Clones

By C. MÁTYÁS and I. PESZLEN[1])

University of Sopron, Hungary

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Summary

Anatomical properties of 3 Euramerican hybrid poplar (*Populus* x *euramericana* (DODE) GUINIER) clones, the Italian 'I-214' and the Hungarian 'Kopecky' and 'Koltay', were investigated. Six trees from each clone were sampled at 2 sites in Hungary. Plantations on the 2 sites were 15 and 10 years old, respectively. Disks were removed at breast height from each tree to study the effect of age on variation of selected wood properties.

Age had significant effect on wood quality traits. Differences in ring widths between clones were significant in the first few years and in the favorable years only.

For anatomical properties, most of the variation was detected within tree. From pith to bark anatomical properties showed a rapid change first, followed by a decreasing rate of change, finally a constant value for each clone on both sites; this has been interpreted as a sign of maturation. The maturation process was affected by site. The better site accelerated maturation but at lower values. The ages of levelling off were not the same for all properties; however, the sequence of maturation was the same on both sites. Fibre length and vessel lumen area were the last to become constant.

For specific gravity, there were significant differences between clonal means. Within-tree specific gravity was generally high near the pith, but each clone exhibited different radial patterns. Regarding strength properties, clonal effect was found non-significant; means were higher near the bark than close to pith. Specific gravity was not the most important

[1]) The authors are, respectively, Professor, Department of Plant Sciences and Associate Professor, Department of Wood Science, 9401 Sopron, POB 132, Hungary.