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Original article

Genetic variation in European larch (*Larix decidua* Mill)

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Summary — Levels of electrophoretically demonstrable diversity of 7 allozyme loci were estimated in 7 populations representing the natural range of *Larix decidua* (Mill). On average the gene diversity was 0.223 and the number of alleles per locus was 2.28. Only 5.1% of the total genetic diversity resided among populations with a mean genetic distance among populations of 0.029. The populations could be assigned to two geographic groups, a large one containing populations from the eastern Alps as well as from Poland and Czechoslovakia and a rather restricted one with a single population from the Western Alps. The most homogeneous populations are those from eastern Europe (Poland, Sudetan region and Tatra mountains).

***Larix decidua* / allozymes / genetic variation**

Résumé — Variation génétique du mélèze d'Europe (*Larix decidua* Mill). *Le niveau de diversité de 7 marqueurs génétiques a été étudié en électrophorèse pour 7 populations représentatives de la variation naturelle du* Larix decidua *(Mill). En moyenne, la diversité génétique était de 0,223 et le nombre d'allèles par marqueur 2,28. Seulement 5,1% de la diversité génétique totale étaient représentés dans des populations d'une distance génétique moyenne entre populations de 0,029. Les populations purent être assignées à 2 groupes géographiques, dont l'un, le plus important, comprend les populations des Alpes orientales, ainsi que celles de Pologne et de Tchécoslovaquie, alors que l'autre, plus réduit, ne comprend qu'une seule population des Alpes occidentales. Les populations les plus homogènes sont celles d'Europe orientale (Pologne, Sudètes, chaîne du Tatra).*

Larix decidua / *diversité génétique* / *marqueurs génétiques*

INTRODUCTION

Larix decidua Mill has its natural range restricted to four distinct areas of Central and eastern Europe: the Alps, the Sudetan region, the Tatra Mountains and scattered throughout Poland (fig 1). It has been the subject of both numerous provenance experiments (Cieslar, 1899, 1914; Varma, 1949; Leibundgut, 1959; Barnes, 1977; Giertych, 1979; Schober, 1977, 1985) and other studies concerning phenotypic traits (Bouvarel and Lemoine, 1958; Gathy, 1959; Schreiber, 1960, 1961, 1963; Kral, 1966, 1967; Simak, 1967; Lang, 1976; Leibundgut, 1985). These investigations underline the fact that European larch is a highly variable species. Allozyme surveys estimating gene diversity among populations are not known for *Larix decidua*, but within the genus *Larix* for *L laricinia* (Cheliak *et al*, 1988) and for *L occidentalis* (Fins and Seeb, 1986). However, for *L decidua* from Poland segregation studies of allozyme loci are already available (Mejnartowicz and Bergmann, 1975; Kosinski and Szmidt, 1984; Lewandowski and Mejnartowicz, 1990a, b, 1991). In this study seeds from provenances covering all four parts of the range of European larch were analysed by gel electrophoresis. The genetic interpretation of six enzyme gene markers as well as the calculation of genetic differentiation parameters will be presented.

MATERIALS AND METHODS

Geographic location and background information for the seven populations of *Larix decidua* are given in figure 1 and table I. Bulked seed samples, where seeds from several trees were pooled, as well as seed lots from individual trees

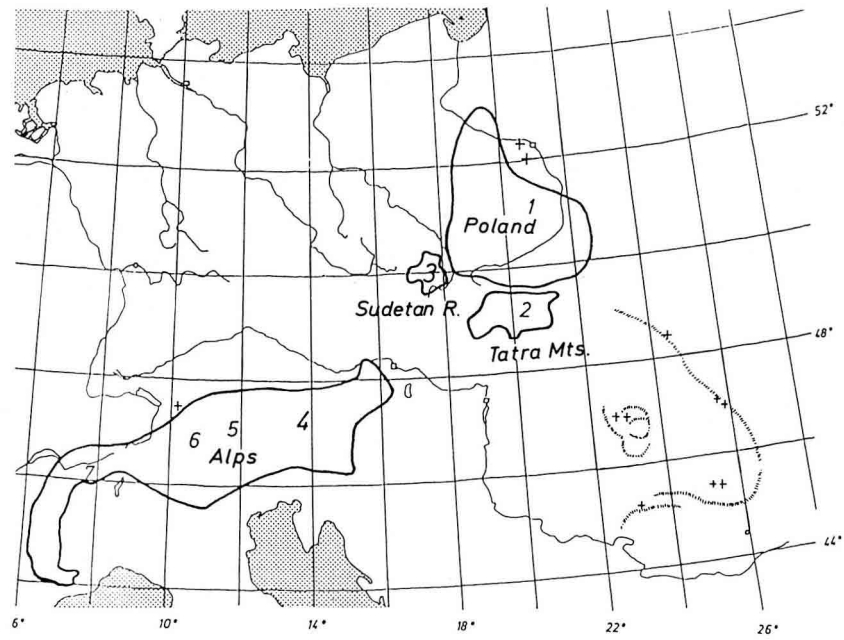

Fig 1. Natural range of *Larix decidua* (Mill). Numbers 1-7 refer to populations in table I (redrawn from Schober, 1977).

Table I. List of provenances under study.

No	*Population*	*Country*	*Altitude (m)*	*Seed type**	*No of trees/ seeds analysed*
1	Lysa Gora	Poland	300	b	144
2	Smokovec	CFSR	1200	s	30
3	Sudetan	CFSR	300 – 650	b	136
4	Eastern Alps	Austria	900 – 1700	b	50
5	Pfitsch	Italy	1100 – 1900	s	56
6	Wintschgau	Italy	900 – 1300	b	144
7	Simplon	Switzerland	1400 – 1500	s	23

* b = bulked seed sample, s = single tree seed lots.

were sampled. Both of them originate from indigenous populations; the former were provided by forest research stations in Poland, Czechoslovakia, and Austria, and the latter were collected by us. Seeds from many small stands from the Sudetan region as well as from a limited area in the eastern Alps were grouped, respectively.

Electrophoretic analysis was carried out on the endosperm. Between 50 and 144 have been examined for the bulk provenance collections. For the provenances with single tree seeds, six endosperms per tree were analysed. Horizontal starch gel electrophoresis was carried out to separate isozymes for six enzyme systems:

GDH	(EC 1.4.1.3)
IDH	(EC 1.1.1.42)
G6PDH	(EC 1.1.1.49)
6PGDH	(EC 1.1.1.43)
SKDH	(EC 1.1.1.25)
MDH	(EC 1.1.1.37)

Details of gel and electrophoresis buffer and staining mixtures were taken from Conkle *et al* (1982) and from Müller-Starck (personal communication). Enzyme band phenotypes, evidence from gametic segregation ratios and close analogy to results from other isozyme investigations in larch (Cheliak and Pitel, 1985; Fins and Seeb, 1986; Lewandowski and Mejnartowicz, 1990a, b; Ying and Morgenstern, 1990) were the basis for genetic interpretation of the zymograms. Capital letters refer to gene loci, number 1 being assigned to the most mobile band at any locus. A locus was considered polymorphic if more than one allele was observed, regardless of allelic frequencies.

Nei's (1972) genetic distance (D) was used to quantify the degree of differentiation among populations. Cluster analysis, using the UPGMA-method, was performed with SPSS (Norusis, 1986) on the matrix of Nei's genetic distances. Gene diversity analysis was calculated according to Nei (1973). A measure of total gene diversity is $H_T = 1 - \Sigma p_i^2$, where p^2 is the mean frequency of the i th of k alleles. H_T is partitioned in $H_T = H_S + D_{ST}$, where H_S and D_{ST} are average gene diversities within and among populations, respectively. G_{ST} is the proportion of interpopulation gene diversity H_T.

RESULTS

IDH and GDH were found to be monomorphic. No variation was observed for the allozyme encoded by these loci (fig 2a, b). Two zones of activity were observed on gels stained for G6PDH. The lower zone stained inconsistently and was therefore not scored. The fastest migration zone exhibits 3 bands differing in mobility (fig 2c). Thus, 1 locus with 3 alleles was postulated. A heavily stained zone with 2 single-

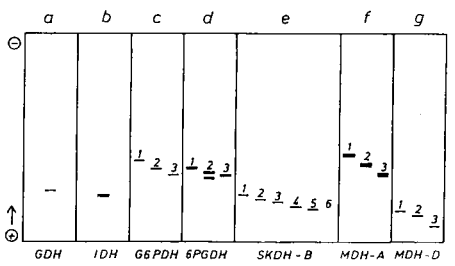

Fig 2. Zymograms with locus designations and allozyme numbers for seven loci.

banded and 1 double-banded phenotype was found for 6PGDH, suggesting a 3-allele locus (fig 2d). Gametophytes scored for SKDH showed 2 zones of activity. The faster zone was unreliably stained and could not be considered. The more cathodal zone exhibits 5 bands differing in mobility and staining intensity. In addition, a null allele was observed. Thus, this zone was interpreted as 1 locus with 6 alleles (fig 2e). A 5 zone banding pattern, inferred as four loci and an interlocus heterodimer, was recorded for MDH. On account of poor band resolution, MDH-B and MDH-C were not further analysed. Both MDH-A and MDH-D performed 3 bands suggesting 2 loci with 3 alleles each (fig 2f and 2g).

Allele frequencies are given in table II. When comparing gene frequencies of the 7 populations, qualitative differences occur only in rare variants. The same allele pre-

Table II. Allele frequencies of seven provenances.

	Populations						
	Lysa Gora	*Smoko-vec*	*Sude-tan*	*E-Alps*	*Pfitsch*	*Vint-gau*	*Simplon*
Allele							
G6PDH-1	0.319	0.256	0.320	0.429	0.385	0.445	0.123
–2	0.500	0.611	0.524	0.469	0.375	0.444	0.833
–3	0.181	0.133	0.156	0.102	0.240	0.111	0.044
6PGDH–1	0.069	0.067	0.072	0.182	0.027	0.201	0.349
–2	0.171	0.139	0.231	0.091	0.080	0.167	0.007
–3	0.760	0.794	0.697	0.727	0.893	0.632	0.644
SKDH –1	0.049	–	0.007	–	–	–	–
–2	0.090	0.183	0.171	0.279	0.289	0.270	0.058
–3	0.153	0.222	0.173	0.186	0.169	0.042	0.008
–4	0.652	0.545	0.642	0.512	0.535	0.681	0.934
–5	0.021	0.033	–	0.023	–	–	–
–6	0.035	0.017	0.007	–	0.007	0.007	–
MDH –A1	0.069	0.100	0.014	–	–	–	–
–A2	0.931	0.883	0.986	1.000	1.000	1.000	1.000
–A3	–	0.017	–	–	–	–	–
MDH –D1	–	–	0.007	0.040	–	0.014	–
–D2	1.000	1.000	0.971	0.900	1.000	0.972	0.942
–D3	–	–	0.022	0.060	–	0.014	0.058

vails at every locus in all populations with the exception of the G6PDH. The frequency of the G6PDH-1 allele exceeds that of G6PDH-2 at the provenances Pfitsch and Vintschgau, while for the rest the opposite is true.

The estimates of genetic distances for all combinations of provenances averaged over the 7 loci are presented in table III. The distances (average 0.029) are lowest among the eastern European samples from Poland, the Sudetan region and the Tatra Mts (0.004–0.009). The Simplon population appeared to be the most divergent from all other populations with genetic distances rising up to 0.099. All alpine samples have relatively large average distances between each other.

Figure 3 shows the dendrogram resulting from UPGMA clustering based on NEI's genetic distance. The general analysis showed that two large groups were delineated. The Simplon material from the western Alps is clearly distinct from the other 6 populations. In this cluster containing 6 populations the 3 provenances from eastern Europe form a relatively homogeneous sub-cluster which is slightly different from the Alpine provenances.

Gene diversity per locus varies widely from 0.027 at MDH-A to 0.653 at G6PDH (table IV). The mean heterozygosity per population ranges from 0.140 in the Simplon population to 0.260 in the Ostalpen population with a mean of 0.223 over all (E-Alps) populations. The mean number of alleles per locus was 2.28 with a minimum of 2.0 in the Pfitsch and Simplon populations and a maximum of 2.6 in the Sudetan region. On the average, 94.9% (Hs/Hr x 100%) of the gene diversity resided within stands and 5.1% among stands (G_{ST} x 100%, table V).

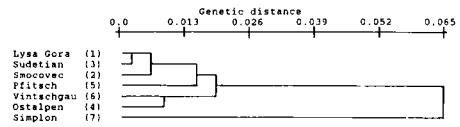

Fig 3. UPGMA cluster analysis of NEI's genetic distance.

DISCUSSION

Monomorphic loci at GDH are reported for *L laricina* (Cheliak and Pitel, 1985; Ying and Morgenstern, 1990) and at IDH for *L laricina* (Cheliak and Pitel, 1985) as well as for *L occidentalis* (Fins and Seeb, 1986). Lewandowski and Mejnartowicz (1990a) found these 2 enzyme systems controlled by 1 locus with 1 dominating allele and 2 rare alleles each. Corresponding

Table III. Estimates of genetic distances based on data from seven loci.

	Lysa Gora	*Smokovec*	*Sudetan*	*E-Alps*	*Pfitsch*	*Vintschgau*
Smokovec	0.008					
Sudetan	0.004	0.009				
E-Alps	0.018	0.018	0.013			
Pfitsch	0.016	0.019	0.017	0.013		
Vintschgau	0.017	0.029	0.011	0.010	0.024	
Simplon	0.052	0.056	0.052	0.099	0.054	0.070

Table IV. Population gene diversity.

Populations	*Gene diversity*						*No of alleles per locus*
	G6PDH	*6PGDH*	*SKDH*	*MDH-A*	*MDH-D*	*mean**	
Lysa Gora	0.615	0.388	0.538	0.128	0.000	0.238	2.4
Smokovec	0.543	0.345	0.618	0.210	0.000	0.245	2.4
Sudetan	0.598	0.455	0.528	0.027	0.056	0.237	2.6
E-Alps	0.585	0.430	0.624	0.000	0.184	0.260	2.3
Pfitsch	0.653	0.195	0.594	0.000	0.000	0.206	2.0
Vintschgau	0.592	0.532	0.461	0.000	0.054	0.234	2.3
Simplon	0.289	0.463	0.124	0.000	0.109	0.140	2.0

* = calculated over 7 loci (including 2 monomorphic loci).

Table V. Gene diversity analysis.

Locus	*Total* (H_T)	*Within populations* (H_S)	*Among populations* (D_{ST})	*Proportion of among-population differentiation* (G_{ST})
G6PDH	0.588	0.553	0.035	0.060
6PGDH	0.425	0.401	0.024	0.056
SKDH	0.521	0.498	0.023	0.044
MDH-A	0.054	0.052	0.002	0.037
MDH-D	0.060	0.057	0.003	0.050
mean*	0.235	0.223	0.012	0.051

* = calculated over 7 loci (including 2 monomorphic loci).

to the present results a 3-allele locus at G6PDH was found for *L decidua* (Lewandowski and Mejnartowicz, 1990a) and for *L laricina* (Cheliak and Pitel, 1985). At SKDH Lewandowski and Mejnartowicz (1990a) detected 1 locus with four alleles. Further studies of SKDH in *Larix* have been done on one single clone of both European and Japanese larch (Bergmann and Ruetz, 1987). The finding of four MDH loci in many other conifers (Wheeler *et al,* 1983; Cheliak and Pitel, 1985; Yeh *et al,* 1985; Fins and Seeb, 1986; Ernst *et al,* 1987; Merkle and Adams, 1987; Bergmann, 1988; El-Kassaby, 1989; Lewandowski and Mejnartowicz, 1990a) are in agreement with the present results. For *L decidua* Lewandowski and Mejnartowicz (1990a) observed at MDH1 (MDH-A) and MDH4 (MDH-D) a deviating number of alleles. For Polish larch however, the postulation of a monomorphic locus at MDH-D

(table II: Population Lysa Gora) is consistent with observations by Lewandowski and Mejnartowicz (1990b). This indicates that deviation in number of alleles per locus at GDH, IDH, MDH and SKDH in present results compared to those by Lewandowski and Mejnartowicz (1990a) may be due to different populations investigated. At 6PGDH one (Cheliak and Pitel, 1985) or two (Fins and Seeb, 1986) polymorphic loci had been reported for *L laricina* and *L occidentalis,* respectively.

Levels of genetic distances and gene diversity among provenances of *L decidua* reveal very similar average values for *L laricina* (Cheliak *et al,* 1988); however, the average number of alleles per locus is higher in *L decidua.* On the other hand, *L occidentalis* (Fins and Seeb, 1986) differs considerably from *L laricina* and *L decidua* by relatively low genetic variability as well as genetic distances among populations. This is surprising considering the extent of the species ranges. *L laricina* expands continent-wide, while *L decidua* and *L occidentalis* are localized in restricted regions. Fins and Seeb (1986) suggest that low genic differentiation and diversity among stands of western larch may be the result of isolated refugia during Pleistocene glaciation and founder effects after fires. On the other hand, with regard to the relatively low number of enzyme systems asseyed, the results for *L decidua* should be interpreted carefully. Nevertheless, a substantial difference in gene diversity between *L decidua* and *L occidentalis* remains.

Genetic variability of *L decidua* evaluated for two Polish stands and for a seed orchard in Poland was considered to be low (Mejnartowicz and Bergmann, 1975; Kosinski and Szmidt, 1984). In this study no obvious low gene diversity in the Polish population (Lysa Gora) was found.

Nei's (1972) genetic distance indicated relatively large genetic differences among larch populations. The isolated position of the Simplon stand in genetic distance matrix and in cluster analysis may be due to the relatively small sample size (23 trees). However, cluster analysis derived from monoterpene data of larch seedlings (unpublished data) exhibits full correspondence to the isozyme results. In addition, provenance experiments support the results from both the isoenzyme and resin oil analyses. Substantial differences in growth rate between western and eastern Alpine provenances have been pointed out (Schober, 1977, 1985). Considerable amounts of differentiation in several traits among provenances resulted in several authors speaking of alpine larch races and/or ecotypes (Wettstein, 1946; Rubner, 1954; Mayer, 1961; Kral, 1967; Leibundgut, 1985). In contrast, the eastern European provenances form a quite uniform group in respect to larch canker susceptibility and growth rate (Schober, 1977, 1985). According to these and present results, the larches from Poland, the Tatra Mts and the Sudetan region may be regarded as one single race.

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