GENETIC DIVERSITY AND DIFFERENTIATION IN A BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.) PROGENY TEST

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ABSTRACT

Progenies of 18 seed lots from black locust (*Robinia pseudoacacia* L.) originating from Germany, Slovakia, Hungary and from the natural distribution area in USA were characterised by isozyme markers. A high genetic within-population variation was ascertained in six Hungarian progenies, combined with a low between-population variation. In contrast to these findings, the within-population variation was low in eight German progenies. Indeed, the genetic between-population variation was remarkable.

Black locust has been cultivated in Hungary for a long period of time in a short rotation management that was carried out by plantation of seedlings. The seed stands themselves originate from common seedling plantations. This procedure seems to be responsible for the relatively high genetic variation within the progenies and the low differentiation between them. In comparison, black locust in Germany was introduced in the past with nearly no subsequent forest management. After the first establishment of a black locust population, it is more likely that asexual reproduction has dominated for many generations. The large genetic differentiation between the German progenies combined with the relatively low level of genetic variation is the immediate consequence of these actual conditions.

Key words: *Robinia pseudoacacia*, population genetics, isozyme markers, forest management, clonal structure

INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is a deciduous tree that belongs to the *Fabaceae* (legume) family. Insects, especially bees, pollinate the flowers. The tree species is well adapted for growth in a wide variety of soils and environmental conditions. Due to its symbiosis with the nitrogen fixing bacteria *Rhizobium* sp. *Robinia* is capable of colonising very low nutrient substrates.

Black locust was introduced to Europe from its natural range in south-eastern United States more than 300 years ago. *Robinia* species are among the most widely planted tree species in the world because they are fast growing, drought tolerant, have very hard durable wood, and are adaptable to many sites and climates (reviewed by DEGOMEZ & WAGNER 2001).

Several varieties of this multipurpose tree have been selected, which increase wood production or production of biomass for animal feeding or which are suitable for recultivation of devastated lands and nectar production. The very hard and resistant wood with high natural durability and density plays the most important role in utilization of resources of this tree species. Black locust delivers a construction wood very stable under moist conditions without chemical treatment. Thus, it became an alternative for some tropical tree species.

At present, cultivation and breeding is undertaken in the United States (MEBRAHTU & HANOVER 1989, BONGARTEN *et al.* 1991, 1992, BLOESE *et al.* 1992, DAVIS & KEATHLEY 1992) and many countries outside the natural range of this tree species, such as in Hungary (KERESZTESI 1983, REDEI *et al.* 2002), Greece (DINI 1993, DINI-PAPANASTASI & PANETSOS 2000), Germany (EWALD *et al.* 1992, NAUJOKS *et al.* 1993), India (SHARMA 2000, SWAMY *et al.* 2002), South Korea (KIM & ZSUFFA 1994), China (RICHTER 1999).

In Germany black locust covers about 14000 ha. One half is located in the eastern part of the country (SEELING 1997). Many stands are of a bad quality concerning stem form and thick branches. The breeding of black locust was concentrated on the selection of individuals with straight trunks (SCHRÖCK 1953, 1965) and their vegetative propagation including tissue culture methods (NAUJOKS &

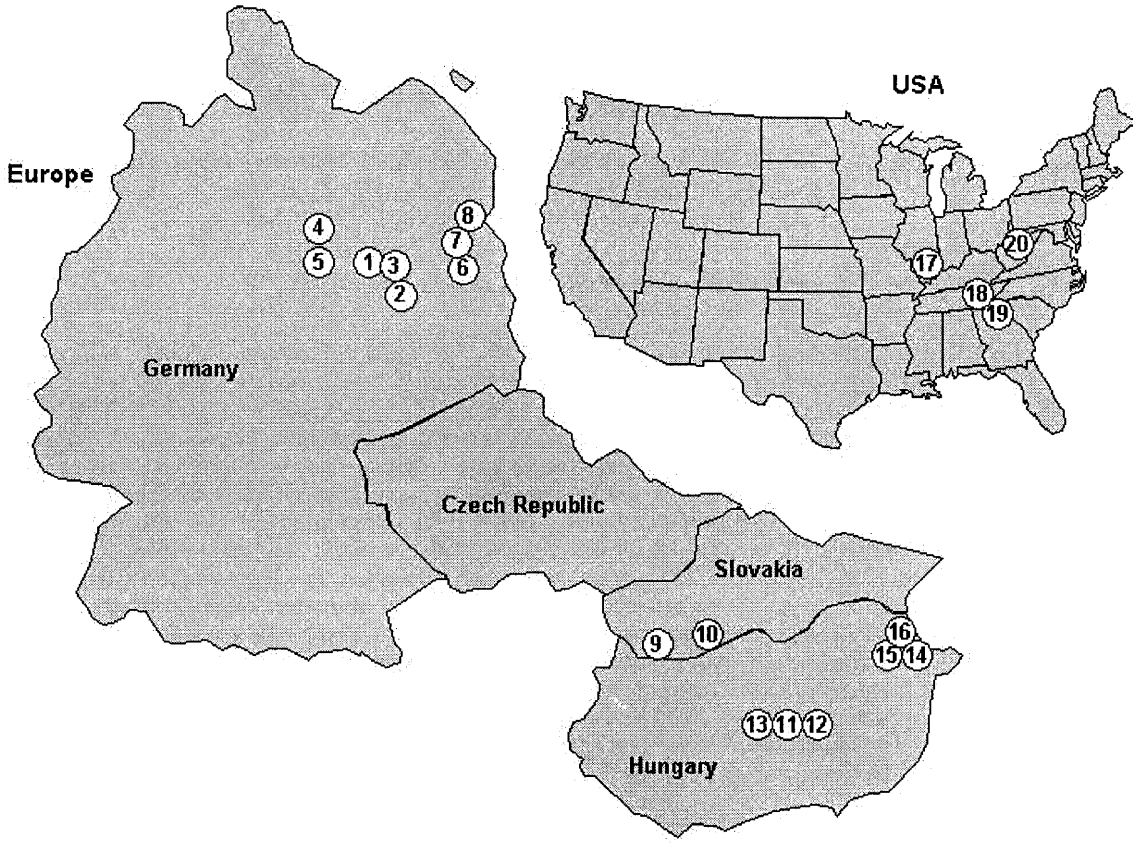

Figure 1. Locations of origin of the tested progenies.

EWALD 1996, NAUJOKS *et al.* 1999). Some field tests with several clones and populations were established between 1995 and 2004 to check the stability of stem form and for selection of suitable propagation material of black locust.

An increasing demand for black locust wood in Germany was ascertained by BÜSSOW *et al.* 1997. First considerations were made to include black locust clones in forest management concepts (EWALD *et al.* 2001). At present, there is no knowledge from field experiments about provenance differences.

The description of population genetic structures of black locust with isozyme markers was started by SURLES *et al.* in 1989 and continued by SURLES *et al.* 1990 and BONGARTEN 1992. A very high genetic diversity was assessed within seed sources with low geographic variation in the natural range. The outcrossing rate was 0.87. Clonal structures in natural populations were detected with genetic markers (MCCAIG *et al.* 1993, CHANG *et al.* 1998). These structures occur due to the ability of black locust to vegetative propagation by root suckers. An investigation with isozyme markers in a single stand in Germany confirmed the clonal structure in consequence of vegetative propagation by root suckers (HERTEL & SCHNECK 2003). Only little is known about genetic diversity and differentiation of European populations of black locust.

Clonal breeding is one of the possibilities to provide material for commercial use (e.g. REDEI *et al.* 2002). Furthermore, family selection is very promising because of high family heritabilities for growth, biomass and morphological traits (BONGARTEN *et al.* 1992, DINI-PAPANASTASI & PANETSOS 2000). As a first step a progeny trial with black locust from different seed sources from Germany, Hungary, Slovakia and the the United States was started, organized by the group of forest tree breeders in Germany (SCHNECK *et al.* 2003). The aim of this field experiment is the collection of first data on differences between several seed sources. These data should result in identification and approval of basic material for generative reproduction of this tree species that was affiliated in the German Law on Forest Propagation Materials in 2003.

Recommendations for the approval of seed stands have to consider an adequate knowledge on genetic structures of populations and their progenies. This paper presents the results of a population genetic study of the progenies in nursery stage by means of isozyme markers.

MATERIALS AND METHODS

Materials

Seeds were collected in 20 populations of black locust. Eighteen of them are included in this study, representing a part of the artificial European range of the species as well as the natural range in North America (Figure 1). Seed harvesting was performed according to the local situation. Unfortunately, the number of seed trees is unknown in many cases. The seeds were sawn in the nursery in spring 2000 and seedlings were transplanted in spring 2001. Names of harvested stands, geographic data and sample sizes for isozyme analyses are given in Table 1.

Electrophoresis

Leaves of 2-year-old nursery plants (sample size 48 ... 52 from each progeny) were collected for isozyme analysis in summer 2002. A total of 895 plants were included in the investigation. Fresh leaves were ground with mortar and pestle in extraction buffer (0.1 M Tris pH 8.0 with 16 % sucrose, 1 % DIECA, 1 % soluble PVP, 1 % mercapto ethanol). Details on electrophoresis and enzyme specific staining methods are described by HERTEL & MAURER (1999). The seedlings were assayed for 11 Enzyme systems (Table 2, Table 3).

Data evaluation

The banding patterns were evaluated visually. The assessment of gene loci and alleles was carried out empirically using the known quarternary structure of enzyme proteins (Table 3). Unfortunately, suitable full sib or half sib material was not available to test segregation for the marker loci. In fact, application of the qualitative inheritance analysis program DIPLOGEN (Gillet 1998) yielded no hypothesis of inheritance for lack of complete combinations of rare variants.

All parameters and statistics were calculated with SAS (SAS Institute Inc., procedures allele, cluster, freq, nparlway, and tree). The comparison between observed genotype frequencies and frequencies expected under Hardy-Weinberg equilibrium (HWE) was carried out with the SAS permutation version of the exact test given by GUO and THOMPSON (1992).

Genetic differentiation G_{ST} was calculated to specify the partitions of within and among population differentiation (NEI 1977).

Special SAS macros were used for additional population genetic parameters (STAUBER & HERTEL 1997). The genetic distances (GREGORIUS 1978) and the subpopulation differentiation (GREGORIUS 1986) were used to quantify the between population variation. The subpopulation differentiation measures the distance of each single subpopulation to the complement of all other subpopulations.

Population genetic parameters of groups of progenies were compared using the nonparametric Wilcoxon rank-sum test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

RESULTS

Description of isozyme loci

The marker loci of the enzyme systems were described according to their electrophoretic mobility with A, B and C. Alleles within a single locus were described with 1, 2, 3, ... Twelve polymorphic loci seem to be available after an empirical evaluation of banding patterns (Table 4). The loci *Aat-A, Gdh, Pgdh-A* and *Pgi-A* are monomorphic in the tested material. The observation at activity zones *Aat-B* and *Pgi-B* give a strong indication for duplication, resulting in two overlaying loci with interlocus hybrid bands due to the dimeric protein structure of these enzymes. At least, 3 resp. 6 alleles were assumed for these loci. They had to be excluded from the data evaluation because of the impossibility to resolve the patterns into diploid loci. Furthermore the data evaluation was impossible for the enzyme system DIA and for *Fest-A* because of slurry bands.

The deviation of observed genotypic structure from Hardy-Weinberg equilibrium (HWE) was tested for each progeny at 12 hypothetical polymorphic loci (Table 4). All loci with exception of *Lap-A* and *Lap-B* indicate a good accordance with the hypothesis of Mendelian inheritance with less than a half of progenies with significant deviation from HWE. This could be accepted as an indication for codominance if material for a segregation analysis is not available. At the hypothetical loci *Lap-A* and *Lap-B* many thick and faint bands with identical electrophoretic mobility were observed. This fact and the distinct excess of homozygotes strongly indicate the presence of null alleles in common

Table 1. Origin and climatic data of progenies.

No	Country	Provenance	Latitude	Longitude	Altitude (m)	Annual mean temperature (°C)	Annual precipitation (mm)	Area (ha)	Seed collection	Sample size
1	Germany	Göritz	51°58′	12°32′	80	8.6	569	0.8	Seed orchard with 21 clones, seeds were harvested from the trees	50
2	Germany	Annaburg, Abt. 1451	51°39′	12°57′	75	8.7	573	1.2	Seeds were collected from the ground (total area)	50
3	Germany	Arensdorf, Abt. 1359 a1	51°48′	12°59′	110	8.7	573	0.5	Seeds were harvested from approx. 15 trees	50
4	Germany	Haldensleben	52°20′	11°12′	60	9.2	543		Seeds were harvested from felled trees	50
5	Germany	Altbrandsleben	52°05′	11°13′	100	9.0	503		Seeds were harvested from felled trees	48
6	Germany	Waldsieversdorf	52°32′	14°03′	50	8.2	527	0.5	Seed orchard with 39 clones (includes the clones of No. 1), seeds were collected from the ground (total area)	50
7	Germany	Hasenholz	52°34′	14°03′	70	8.2	527	2.0	Seeds were collected from the ground (total area)	49
8	Germany	Gottesgabe	52°38′	14°10′	40	8.2	527	2.0	Seeds were harvested from 2 trees, additionally seed collection from the ground	50
9	Slovakia	Lúč	47°59′	17°33′	117	10.2	693		Progeny of plus tree progeny test	50
10	Slovakia	Gabčíkovo	47°53′	17°34′	114	10.2	693		Progeny of plus tree progeny test	49
11	Hungary	Mikebuda 5G	47°10′	19°40′	150	10.5	542		Upper soil was sieved	49
12	Hungary	Mikebuda 27G,28D, 30B	47°10′	19°40′	150	10.5	542		Upper soil was sieved	49
13	Hungary	Opalyi 1A, B	47°09′	19°32′	150	10.5	542		Upper soil was sieved	50
14	Hungary	Pusztavacs 60 A	47°52′	22°18′	150	9.8	600		Upper soil was sieved	50
15	Hungary	Pusztavacs 56 C	47°09′	19°32′	150	9.8	600		Upper soil was sieved	49
16	Hungary	Ofeherto 10 B	47°56′	22°03′	150	9.8	600		Upper soil was sieved	50
17	USA	Illinois – 1	34°48′	-84°00′	200	12.3	942		Unknown	52
20	USA	West Virginia – 1	39°06′	-79°36′	600	13.2	1143		Unknown	50

Table 2. Protein separation by electrophoresis.

System	Gel	Gel buffer	Electrode buffer
A	12.5 % starch	0.02 M Tris-citrate buffer pH 7.5	0.15 M Tris-citrate buffer pH 7.5
B	12.5 % starch	0.05 M Tris-citrate buffer pH 8.1 plus 20 % electrode buffer	0.2 M boric acid and 0.03 M lithium-hydroxide pH 8.1
PAGE	7.5 % polyacrylamide	0.375 M Tris-HCl buffer pH 8.9	0.005 M Tris-0.038 M glycine pH 8.3

Table 3. Enzymes and their separation systems.

Enzyme	Catalogue number	Separation system
Amylase (AMY)	EC 3.2.1.1	PAGE
Aspartate aminotransferase (AAT)	EC 2.6.1.1	PAGE
Diaphorase (DIA)	EC 1.6.4.3	B
Fluorescent esterase (FEST)	EC 3.1.1.56	B
Glutamate dehydrogenase (GDH)	EC 1.4.1.2	PAGE
Isocitric dehydrogenase (IDH)	EC 1.1.1.42	A
Leucine aminopeptidase (LAP)	EC 3.4.11.1	B
Malate dehydrogenase (MDH)	EC 1.1.1.37	A
6-Phosphogluconate dehydrogenase (PGDH)	EC 1.1.1.44	A
Phosphoglucose isomerase (PGI)	EC 5.3.1.9	B
Shikimate dehydrogenase (SKDH)	EC 1.1.1.25	A

Table 4. Number of individuals and alleles in the total material and means of observed and expected heterozygosity in 18 progenies.

Locus	Number of individuals	Number of alleles	Observed heterozygosity	Expected heterozygosity	Number of progenies with significant deviation from HWE ($p<0.01$)
Amy-A	890	5	0.3202	0.3791	4
Amy-B	894	3	0.4799	0.4599	0
Fest-B	857	4	0.4317	0.6353	7
Fest-C	887	4	0.3202	0.3335	2
Idh-A	889	4	0.5894	0.5893	0
Lap-A	880	4	0.3102	0.4508	11
Lap-B	890	4	0.1876	0.3730	16
Mdh-B	895	2	0.0201	0.0221	0
Mdh-C	894	3	0.5179	0.5064	1
Pgdh-B	888	2	0.3750	0.3545	1
Pgdh-C	889	2	0.3296	0.4234	3
Skdh-A	881	3	0.5358	0.5690	0

frequencies at these loci. They were excluded from the data evaluation for genetic variation within and among progenies.

The mean allele frequencies and their range among the 18 progenies for 12 polymorphic loci were given in Table 5.

Genetic variation within progenies

The investigated material shows a large spread of within-population variation (Table 6); e.g. the mean expected heterozygosity amounts to a minimum of 0.284 in progeny No. 7 and to a maximum of 0.449

Table 5. Overall allele frequencies (additional null alleles strongly assumed for loci *Lap-A* and *Lap-B*) and minimum and maximum frequency in 18 populations.

Locus	Allele	Mean frequency	Range min	Range max	Locus	Allele	Mean frequency	Range min	Range max
Amy-A	1	0.001	0	0.010	*Lap-A*	1	0.127	0.070	0.208
Amy-A	2	0.759	0.612	1.000	*Lap-A*	2	0.713	0.600	0.847
Amy-A	3	0.030	0	0.070	*Lap-A*	3	0.159	0.073	0.255
Amy-A	4	0.210	0	0.357	*Lap-A*	4	0.001	0	0.010
Amy-A	5	0.001	0	0.010	*Lap-B*	1	0.042	0	0.160
Amy-B	1	0.354	0.120	0.573	*Lap-B*	2	0.778	0.600	0.894
Amy-B	2	0.644	0.427	0.880	*Lap-B*	3	0.133	0.030	0.240
Amy-B	3	0.002	0	0.020	*Lap-B*	4	0.048	0	0.125
Fest-B	1	0.057	0	0.344	*Mdh-B*	1	0.011	0	0.060
Fest-B	2	0.485	0.276	0.929	*Mdh-B*	2	0.989	0.940	1.000
Fest-B	3	0.333	0.061	0.630	*Mdh-C*	1	0.507	0.320	0.646
Fest-B	4	0.125	0	0.398	*Mdh-C*	2	0.487	0.354	0.680
Fest-C	1	0.044	0	0.174	*Mdh-C*	3	0.007	0	0.090
Fest-C	2	0.804	0.534	0.960	*Pgdh-B*	1	0.230	0.051	0.427
Fest-C	3	0.131	0	0.421	*Pgdh-B*	2	0.770	0.573	0.949
Fest-C	4	0.021	0	0.102	*Pgdh-C*	1	0.304	0.061	0.540
Idh-A	1	0.101	0.010	0.250	*Pgdh-C*	2	0.696	0.460	0.939
Idh-A	2	0.362	0.070	0.560	*Skdh-A*	1	0.483	0.184	0.674
Idh-A	3	0.519	0.330	0.878	*Skdh-A*	2	0.438	0.260	0.765
Idh-A	4	0.018	0	0.071	*Skdh-A*	3	0.080	0	0.200

in progeny No. 10.

The group of progenies from Hungary (n = 6) tend to have higher genetic variation than the group of progenies from Germany (n = 8). This is significant for the number of polymorphic loci (**), for the number of alleles per locus (*), for the expected heterozygosity (*) and for gene pool diversity (*).

The two Slovakian progenies are similar to the Hungarian and the two progenies from USA tend to be similar to the German ones, with exception of a higher number of alleles per locus in progenies from the natural range in USA.

Genetic variation among progenies

Only a small part of 5.8 % resp. 10.7 % of the total genetic variation was found to be among the progenies by the calculations according to NEI 1977 and GREGORIUS 1986 (mean G_{ST} = 0.058 and mean δ = 0.107 over 10 polymorphic loci). The parameters G_{ST} and δ are strongly correlated for the set of gene loci used (r = 0.958). The most contributing loci for variation among progenies are *Fest-B* and *Idh-A* for both methods (Table 7). Furthermore, the subpopulation differentiation is suitable to characterise the genetic differentiation of single subpopulations among all other populations. The higher the differentiation value of a subpopulation, the more different is it from the total variation (Table 8).

In comparison to the German progenies the Hungarian progenies exhibit lower values for the subpopulation differentiation (**). With their higher genetic variation they are better representing the total genetic variation within this data set. The German progenies with lower genetic variation more differ from the total genetic variation (Table 8). This general relationship persists for several subsets of progenies. Each single Hungarian progeny only slightly differs from the total genetic variation of Hungarian material (subset 1). Despite their relatively low genetic variation within progenies, distinct differences exist between the German progenies (subsets 2 and 3, with or without the most deviant progeny No. 7).

An UPGMA cluster analysis was carried out with the pair wise genetic distances between 18 progenies based on allele frequencies at 10 polymorphic loci. The dendrogram (Figure 2) clearly confirms the results of subpopulation differentiation. Progenies with lower subpopulation differentiation values are clustered together in one group; among them all Hungarian progenies occur. Another group is formed by two offspring populations. The seed orchard No. 6 with 39 clones completely includes the 21 clones of the seed orchard No 1. Thus, their progenies are genetically more similar one another than to other progenies. No. 7, the most deviant progeny from subpopulation differentiation, is

Table 6. Population genetic parameters of 18 progenies (means of 10 polymorphic Loci).

No.	Country	Number of polymorphic loci	Number of alleles per locus	Observed heterozygosity	Expected heterozygosity	Gene pool diversity
1	Germany	9	2.3	0.426	0.395	1.6524
2	Germany	9	2.4	0.370	0.377	1.6054
3	Germany	10	2.8	0.405	0.435	1.7694
4	Germany	10	2.7	0.368	0.387	1.6317
5	Germany	9	2.6	0.402	0.392	1.6453
6	Germany	9	2.6	0.405	0.376	1.6037
7	Germany	9	2.5	0.299	0.284	1.3975
8	Germany	9	2.6	0.408	0.401	1.6682
9	Slovakia	10	2.5	0.366	0.417	1.7167
10	Slovakia	9	2.7	0.454	0.449	1.8165
11	Hungary	10	2.8	0.429	0.432	1.7616
12	Hungary	10	2.6	0.425	0.421	1.7273
13	Hungary	10	2.8	0.420	0.424	1.7367
14	Hungary	10	2.8	0.389	0.421	1.7285
15	Hungary	10	2.7	0.409	0.443	1.7962
16	Hungary	10	2.7	0.316	0.382	1.6183
17	USA	10	2.8	0.434	0.423	1.7346
20	USA	9	2.7	0.333	0.335	1.5042
1... 8	Germany	9.25	2.56	0.385	0.381	1.6217
9...10	Slovakia	9.50	2.60	0.410	0.433	1.7666
11...16	Hungary	10.00	2.73	0.398	0.421	1.7281
17...20	USA	9.50	2.75	0.384	0.379	1.6194

Table 7. Genetic variation among progenies.

Locus	H_T	H_S	$D_{ST} = H_T - H_S$	$G_{ST} = D_{ST}/H_T$	δ (Subpopulation differentiation)
Amy-A	0.3791	0.3609	0.0182	0.0481	0.0950
Amy-B	0.4599	0.4317	0.0282	0.0613	0.1010
Fest-B	0.6353	0.5610	0.0743	0.1169	0.2264
Fest-C	0.3335	0.3124	0.0211	0.0634	0.1139
Idh-A	0.5893	0.5479	0.0415	0.0703	0.1463
Mdh-B	0.0221	0.0216	0.0005	0.0229	0.0114
Mdh-C	0.5064	0.4877	0.0187	0.0370	0.0878
Pgdh-B	0.3545	0.3384	0.0161	0.0454	0.0744
Pgdh-C	0.4234	0.3979	0.0256	0.0603	0.0998
Skdh-A	0.5690	0.5394	0.0296	0.0521	0.1158
Mean	0.4273	0.3999	0.0274	0.0578	0.1072

clearly separated from all other progenies in the dendrogram. A single linkage cluster analysis resulted in a similar dendrogram concerning the above-mentioned structures.

DISCUSSION

Robinia pseudoacacia is a diploid species with 2n = 22 chromosomes (KUMARI & BIR 1990). Within the *Fabaceae* family, a large variation in chromosome numbers exists including many polyploid species. The basic chromosome number seems to be x = 6 (KUMARI & BIR 1990).

Therefore, it is not surprising that the loci *Pgi-B* and *Aat-B* appear to be duplicated. An "unusually high number of loci per enzyme" for black locust

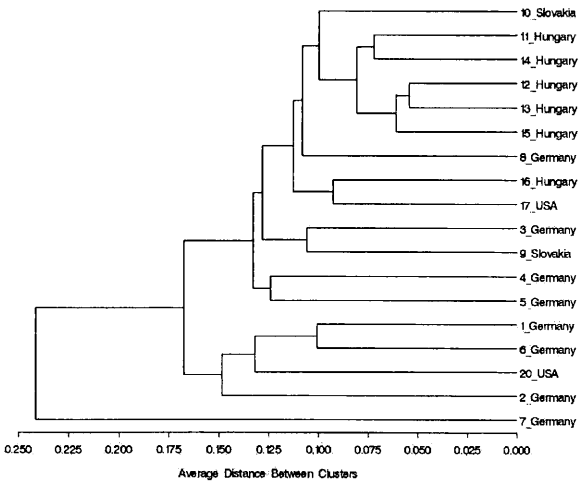

Figure 2. UPGMA dendrogram based on pair wise genetic distances between 18 progenies (GREGORIUS 1978).

was ascertained by SURLES *et al.* 1989 as well. The investigation on single tree progenies indicated the accordance to diploidy for a restricted number of isozyme loci (SURLES *et al.* 1990). Their loci *Idh-1, Lap-2, Mdh-1, Mdh-3* and *6-Ppdh-5* probably correspond to our *Idh-A, Lap-B, Mdh-A, Mdh-B* and *Pgdh-C*. A diploid status with codominant inheritance of alleles can be accepted if there is no substantial deviation from Hardy-Weinberg equilibrium.

An extreme deviation from the HWE for the loci *Lap-A* and *Lap-B* could be explained by the existence of null alleles. At least at the locus *Lap-A* strong and faint bands at the same position were observed, resp. hypothetical genotypes A_2A_2 and A_0A_2. These differences in staining intensity were reproducible for many individuals. Another possible explanation for the deviation from HWE could be the overlapping of several Leucine aminopeptidase loci with partially identical positions of alleles. Both loci are not suitable for calculation of parameters based on allele frequencies.

The level of genetic variation within populations in this investigation is higher than in many other conifer and broad-leaved trees. Also SURLES *et al.* 1989 assessed a relatively high degree of genetic variation and high levels of polymorphism in 23 seed sources from the natural range of black locust. This material might be influenced by planted populations and did not show a geographic pattern.

In our study, clear differences exist between the group of German progenies with lower genetic variation and Hungarian progenies with higher genetic variation within populations. In contrast to this fact, the differentiation between progenies is low in Hungary and very high in Germany. Except for

Table 8. Subpopulation differentiation of 18 progenies (mean values of 10 polymorphic loci).

No.	Country	All progenies	Subset 1	Subset 2	Subset 3
			Hungarian progenies	German progenies	German progenies without No. 7
1	Germany	0.1201		0.1107	0.1333
2	Germany	0.1669		0.1549	0.1503
3	Germany	0.1045		0.1183	0.1231
4	Germany	0.1115		0.1385	0.1196
5	Germany	0.1274		0.1465	0.1316
6	Germany	0.0961		0.0818	0.0719
7	Germany	0.2317		0.2300	
8	Germany	0.0771		0.0843	0.0944
9	Slovakia	0.1208			
10	Slovakia	0.0789			
11	Hungary	0.0807	0.0600		
12	Hungary	0.0728	0.0571		
13	Hungary	0.0780	0.0556		
14	Hungary	0.0472	0.0549		
15	Hungary	0.0884	0.0675		
16	Hungary	0.0849	0.0908		
17	USA	0.0964			
20	USA	0.1459			
Mean		0.1072	0.0643	0.1331	0.1177

the very similar Hungarian progenies, no geographic pattern is visible in the dendrogram. Population 7 (Hasenholz) with the maximum deviation from all others is concurrently the seedling population with the lowest genetic variation. It is originated from a stand with very straight stems. The clustering of the progenies 1 and 6 was expected because of the partially identical clone composition of the seed orchards. The two Slovakian progenies and the two progenies from the natural range are located more or less at random within the dendrogram.

Black locust has been cultivated in Hungary for a long period of time. Traditionally a short rotation management was carried out by plantation of seedlings. In Hungary the common method of seed collection is sieving the top approximately 20 cm of soil of the selected seed stand (RÉDEI 2002). So seeds of relatively large areas and many different trees and probably different years are collected. The seed stands themselves originate from common seedling plantations. This procedure seems to be responsible for the relatively high genetic variation within the progenies and the low differentiation between them.

In contrast, black locust in Germany was introduced in the past with nearly no subsequent forest management. After the first establishment of a black locust population, it is more likely that asexual reproduction has dominated for many generations. This seems to be typical for many local occurrences in Germany. As a special case, the parent population in Hasenholz (No. 7 in this study) was investigated with isozyme markers. At an area of app. 50m × 100m, 5 clones could be detected by their multilocus isozyme genotypes at a sample size of 140 trees. Two dominating clones covered more than 80 % of this area (HERTEL & SCHNECK 2003). Other populations in Germany, from which seeds were collected, were not tested for their possible clonal structure so far. Similar results were obtained from natural populations in North America, were the largest clone with the most ramets covered more than 100m × 100m (CHANG *et al.* 1998). The seed collection in Germany was carried out in relatively small areas, which probably included clonal structures, by harvesting or collection of the pods. Seed orchards (No. 1 and No. 6) are rather an exception. The eight German progenies could be characterised by a substantial maternal bias because of the assumed low number of mothers. The clonal structure of seed stands and the method of seed collection strongly differ from the Hungarian situation. The large genetic differentiation between the German progenies combined with the relatively low level of genetic variation is the immediate consequence of these actual conditions.

Further observations of the field trials with the progeny tests will result in some more knowledge on similarities and differences among the tested material.

An additional field of further investigation will be a detailed analysis of population genetic structure, as well as mating system and pollen and seed dispersal. Suitable nuclear microsatellites markers are now available from LIAN & HOGETSU (2002).

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