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

Heike Liesebach¹, Min Sheng Yang² & Volker Schneck¹

¹) Institute for Forest Genetics and Forest Tree Breeding, Federal Research Centre for Forestry and Forest Products
Eberswalder Chaussee 3A, D-15377 Waldsieversdorf, Germany
²) Agricultural University of Hebei Baoding 071000, China

Received December 14, 2004; accepted February 20, 2005

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 BUSSOW 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 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 (Gillett 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 ($^\ast p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

**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.

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

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

Table 3. Enzymes and their separation systems.

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

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

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

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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Mean frequency</th>
<th>Range</th>
<th>Locus</th>
<th>Mean frequency</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amy-A</td>
<td>0.001</td>
<td>0</td>
<td>0.010</td>
<td>Lap-A</td>
<td>0.127</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.759</td>
<td>0.612</td>
<td>1.000</td>
<td>Lap-A</td>
<td>0.713</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.030</td>
<td>0</td>
<td>0.070</td>
<td>Lap-A</td>
<td>0.159</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.210</td>
<td>0</td>
<td>0.357</td>
<td>Lap-A</td>
<td>0.001</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.001</td>
<td>0</td>
<td>0.010</td>
<td>Lap-A</td>
<td>0.042</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.354</td>
<td>0.120</td>
<td>0.573</td>
<td>Lap-A</td>
<td>0.778</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.644</td>
<td>0.427</td>
<td>0.880</td>
<td>Lap-A</td>
<td>0.133</td>
</tr>
<tr>
<td>Amy-A</td>
<td>0.002</td>
<td>0</td>
<td>0.020</td>
<td>Lap-A</td>
<td>0.048</td>
</tr>
<tr>
<td>Fest-B</td>
<td>0.057</td>
<td>0</td>
<td>0.344</td>
<td>Mdh-B</td>
<td>0.011</td>
</tr>
<tr>
<td>Fest-B</td>
<td>0.485</td>
<td>0.276</td>
<td>0.929</td>
<td>Mdh-B</td>
<td>0.989</td>
</tr>
<tr>
<td>Fest-B</td>
<td>0.333</td>
<td>0.061</td>
<td>0.630</td>
<td>Mdh-C</td>
<td>0.507</td>
</tr>
<tr>
<td>Fest-B</td>
<td>0.125</td>
<td>0</td>
<td>0.398</td>
<td>Mdh-C</td>
<td>0.487</td>
</tr>
<tr>
<td>Fest-C</td>
<td>0.044</td>
<td>0</td>
<td>0.174</td>
<td>Mdh-C</td>
<td>0.007</td>
</tr>
<tr>
<td>Fest-C</td>
<td>0.804</td>
<td>0.534</td>
<td>0.960</td>
<td>Pgdh-B</td>
<td>0.230</td>
</tr>
<tr>
<td>Fest-C</td>
<td>0.131</td>
<td>0</td>
<td>0.421</td>
<td>Pgdh-B</td>
<td>0.770</td>
</tr>
<tr>
<td>Idh-A</td>
<td>0.012</td>
<td>0</td>
<td>0.102</td>
<td>Pgdh-C</td>
<td>0.304</td>
</tr>
<tr>
<td>Idh-A</td>
<td>0.101</td>
<td>0.010</td>
<td>0.250</td>
<td>Pgdh-C</td>
<td>0.696</td>
</tr>
<tr>
<td>Idh-A</td>
<td>0.362</td>
<td>0.070</td>
<td>0.560</td>
<td>Skdh-A</td>
<td>0.483</td>
</tr>
<tr>
<td>Idh-A</td>
<td>0.519</td>
<td>0.330</td>
<td>0.878</td>
<td>Skdh-A</td>
<td>0.438</td>
</tr>
<tr>
<td>Idh-A</td>
<td>0.018</td>
<td>0</td>
<td>0.071</td>
<td>Skdh-A</td>
<td>0.080</td>
</tr>
</tbody>
</table>

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 $\delta = 0.107$ over 10 polymorphic loci). The parameters $G_{ST}$ and $\delta$ 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. 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).

<table>
<thead>
<tr>
<th>No.</th>
<th>Country</th>
<th>Number of polymorphic loci</th>
<th>Number of alleles per locus</th>
<th>Observed heterozygosity</th>
<th>Expected heterozygosity</th>
<th>Gene pool diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Germany</td>
<td>9</td>
<td>2.3</td>
<td>0.426</td>
<td>0.395</td>
<td>1.6524</td>
</tr>
<tr>
<td>2</td>
<td>Germany</td>
<td>9</td>
<td>2.4</td>
<td>0.370</td>
<td>0.377</td>
<td>1.6054</td>
</tr>
<tr>
<td>3</td>
<td>Germany</td>
<td>10</td>
<td>2.8</td>
<td>0.405</td>
<td>0.435</td>
<td>1.7694</td>
</tr>
<tr>
<td>4</td>
<td>Germany</td>
<td>10</td>
<td>2.7</td>
<td>0.368</td>
<td>0.387</td>
<td>1.6317</td>
</tr>
<tr>
<td>5</td>
<td>Germany</td>
<td>9</td>
<td>2.6</td>
<td>0.402</td>
<td>0.392</td>
<td>1.6453</td>
</tr>
<tr>
<td>6</td>
<td>Germany</td>
<td>9</td>
<td>2.6</td>
<td>0.405</td>
<td>0.376</td>
<td>1.6037</td>
</tr>
<tr>
<td>7</td>
<td>Germany</td>
<td>9</td>
<td>2.5</td>
<td>0.299</td>
<td>0.284</td>
<td>1.3975</td>
</tr>
<tr>
<td>8</td>
<td>Germany</td>
<td>9</td>
<td>2.6</td>
<td>0.408</td>
<td>0.401</td>
<td>1.6682</td>
</tr>
<tr>
<td>9</td>
<td>Slovakia</td>
<td>10</td>
<td>2.5</td>
<td>0.366</td>
<td>0.417</td>
<td>1.7167</td>
</tr>
<tr>
<td>10</td>
<td>Slovakia</td>
<td>9</td>
<td>2.7</td>
<td>0.454</td>
<td>0.449</td>
<td>1.8165</td>
</tr>
<tr>
<td>11</td>
<td>Hungary</td>
<td>10</td>
<td>2.8</td>
<td>0.429</td>
<td>0.432</td>
<td>1.7616</td>
</tr>
<tr>
<td>12</td>
<td>Hungary</td>
<td>10</td>
<td>2.6</td>
<td>0.425</td>
<td>0.421</td>
<td>1.7273</td>
</tr>
<tr>
<td>13</td>
<td>Hungary</td>
<td>10</td>
<td>2.8</td>
<td>0.420</td>
<td>0.424</td>
<td>1.7367</td>
</tr>
<tr>
<td>14</td>
<td>Hungary</td>
<td>10</td>
<td>2.8</td>
<td>0.389</td>
<td>0.421</td>
<td>1.7285</td>
</tr>
<tr>
<td>15</td>
<td>Hungary</td>
<td>10</td>
<td>2.7</td>
<td>0.409</td>
<td>0.443</td>
<td>1.7962</td>
</tr>
<tr>
<td>16</td>
<td>Hungary</td>
<td>10</td>
<td>2.7</td>
<td>0.316</td>
<td>0.382</td>
<td>1.6183</td>
</tr>
<tr>
<td>17</td>
<td>USA</td>
<td>10</td>
<td>2.8</td>
<td>0.434</td>
<td>0.423</td>
<td>1.7346</td>
</tr>
<tr>
<td>20</td>
<td>USA</td>
<td>9</td>
<td>2.7</td>
<td>0.333</td>
<td>0.335</td>
<td>1.5042</td>
</tr>
</tbody>
</table>

Table 7. Genetic variation among progenies.

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

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

© ARBORA PUBLISHERS
Figure 2. UPGMA dendrogram based on pairwise 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-Pgdh-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 A2A2 and A1A2. 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).

<table>
<thead>
<tr>
<th>No.</th>
<th>Country</th>
<th>All progenies</th>
<th>Subset 1</th>
<th>Subset 2</th>
<th>Subset 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hungarian progenies</td>
<td>German progenies</td>
<td>German progenies without No. 7</td>
</tr>
<tr>
<td>1</td>
<td>Germany</td>
<td>0.1201</td>
<td>0.1107</td>
<td>0.1333</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Germany</td>
<td>0.1669</td>
<td>0.1549</td>
<td>0.1503</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Germany</td>
<td>0.1045</td>
<td>0.1133</td>
<td>0.1231</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Germany</td>
<td>0.1115</td>
<td>0.1385</td>
<td>0.1196</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Germany</td>
<td>0.1274</td>
<td>0.1465</td>
<td>0.1316</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Germany</td>
<td>0.0961</td>
<td>0.0818</td>
<td>0.0719</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Germany</td>
<td>0.2317</td>
<td>0.2300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Germany</td>
<td>0.0771</td>
<td>0.0843</td>
<td>0.0944</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Slovakia</td>
<td>0.1208</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Slovakia</td>
<td>0.0789</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Hungary</td>
<td>0.0807</td>
<td>0.0600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Hungary</td>
<td>0.0728</td>
<td>0.0571</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Hungary</td>
<td>0.0780</td>
<td>0.0556</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Hungary</td>
<td>0.0472</td>
<td>0.0549</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Hungary</td>
<td>0.0884</td>
<td>0.0675</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Hungary</td>
<td>0.0849</td>
<td>0.0908</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>USA</td>
<td>0.0964</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>USA</td>
<td>0.1459</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.1072</td>
<td>0.0643</td>
<td>0.1331</td>
<td>0.1177</td>
</tr>
</tbody>
</table>
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).

ACKNOWLEDGMENT

The authors would like to thank Ms Elke Ewald for their excellent technical assistance.

REFERENCES


DEGOMEZ, T., WAGNER, M.R. 2001: Culture and use of


SHARMA, K.R. 2000: Variation in wood characteristics of Robinia pseudoacacia L. managed under high density short rotation system. IUFRO World Congress held in Malaysia.

STAUBER, T. & HERTEL, H. 1997: Populationsgenetik mit

SAS. URL:
http://www.mol.shuttle.de/wspe/genetik1.htm

