Chloroplast DNA variation in planted and natural regenerated stands of black locust (*Robinia pseudoacacia* L.)

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Abstract

Black locust (*Robinia pseudoacacia* L.), native in mountainous regions in USA, is increasing in importance for forestry in many countries as a „multi-purpose tree" associated with breeding efforts at the family and clonal levels. A few population genetic studies exist with nuclear, codominantly inherited markers. Here we present an additional marker type, the maternally inherited chloroplast haplotypes. The studied material included samples from the natural range and from artificial stands from three European countries as well as samples from a clone collection. Eleven haplotypes belonging to two clearly separated groups of related haplotypes were found using the PCR-RFLP method. The variation pattern of chloroplast haplotypes in artificial stands is strongly influenced by the local silvicultural management.

Key words: Robinia pseudoacacia L.; cpDNA haplotypes; PCR-RFLP; artificial populations; silvicultural management.

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Introduction

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

Black locust was introduced to Europe from its natural range in the southeastern 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 (DEGOMEZ and WAGNER, 2001). Several varieties of this multipurpose tree known to increase wood production, production of biomass for animal feeding, recultivation of devastated lands or nectar production, have been selected. The species is known for its very hard and resistant wood with high natural durability and density (WELLING, 2005). Black locust delivers a construction wood which is very stable under moist conditions without chemical treatment.

At present, cultivation and breeding is undertaken in the United States (BONGARTEN et al., 1992; MEBRAHTU and HANOVER, 1989) and many countries outside the natural range of this tree species, such as in Hungary (KERESZTESI, 1983; RÉDEI et al., 2002; RÉDEI et al., 2008), Greece (DINI-PAPANASTASI and PANETSOS, 2000), Germany (EWALD et al., 1992; NAUJOKS et al., 1993), India (SWAMY et al., 2002), South Korea (KIM and ZSUFFA, 1994) and China (HONG, 1985; HUO et al., 2009).

In Germany black locust covers about 34,000 ha with the main part in the eastern regions of the country (BWI²). Many stands are of bad quality in terms of stem and crown form and branching. As a minor species, black locust was not an object of tree breeding until 1990. Only in the 1950s some individuals with straight trunks were selected for seed orchards (SCHRÖCK, 1953; SCHRÖCK, 1965). Since the beginning of the 1990s, the demand for black locust wood has increased in Germany. In this time new activities were started for selection of plus trees, provenance and progeny testing and for developing tissue culture methods for vegetative propagation (DAVIS and KEATHLEY, 1992; NAUJOKS and EWALD, 1996; NAUJOKS et al., 1999). First considerations were made to include black locust clones in forest management concepts (EWALD et al., 2001). In the last years the significance of this species for biomass production in short rotation has become more and more evident in Germany (ERTLE et al., 2008; GRÜNEWALD et al., 2009; PETERS et al., 2007).

Since 2003, the production and marketing of seeds and plant material of black locust fall under the rules of the Act on Forest Reproductive Material in Germany. Recommendations for the approval of seed stands have to include adequate knowledge on phenotypic variation and the genetic structures of populations and their progenies. As a first step, a progeny trial with black locust from different seed sources from Germany, Hungary, Slovakia and the United States was established by the task group of forest tree breeders in Germany (SCHNECK et al., 2002).

The description of population genetic structures of black locust with isozyme markers was started by SURLES et al. (1989) using 23 seed sources representing the natural range. A high genetic diversity within seed sources was assessed accompanied by a lower differentiation among populations ($G_{ST} = 0.12$). Omitting 4 populations from peripheral areas, no significant correlations between genetic and geographic distances were observed. The mean outcrossing rate was estimated at 0.87 in the same 23 seed sources (SURLES et al., 1990). Clonal structures in natural populations were detected with genetic markers (CHANG et al., 1998; MCCAIG et al., 1993). These structures occur due to the ability of black locust to propagate vegetatively with root suckers. An investigation with isozyme markers in a single stand in Germany confirmed the clonal structure for artificial stands (HERTEL and SCHNECK, 2003). A study with isozyme markers in the material of a progeny test with black locust from different seed sources demonstrated the strong influence of the managing system in adult stands on the genetic variation in progenies (LIESEBACH et al., 2005). Progenies from nearly unmanaged stands with probable clonal structures in Germany appear relatively low within-population variation and a higher portion of variation among populations compared to progenies from managed stands in Hungary, which were established with planted seedlings, showing a high within-population variation and low differentiation among populations.

The investigation of chloroplast DNA variation should be a next step in the genetic characterisation of European black locust populations. Chloroplasts are maternally inherited in *Robinia pseudoacacia* (HU et al., 2005) as in nearly all Angiosperms. Thus, they are transferred from mother trees to the next generation without pollen contributions and without recombination. This type of inheritance leads to a low or missing variation within populations and rather large scale variation patterns within the natural range of species. These large scale patterns can be influenced by the location of refugia and colonisation histories after glacial periods in Europe and America.

Chloroplast DNA markers, the so-called cpDNA haplotypes, are potentially a very good marker type to describe geographic variation patterns. The analysis of the noncoding regions of chloroplast DNA have the potential to detect variation within species associated with geographic areas. Consensus primers derived from the tobacco chloroplast DNA sequence are suitable to amplify noncoding regions of chloroplast DNA of plants (GRIVET et al., 2001). The method was successfully adapted to many European tree and shrub species, i.e., *Quercus* sp. in an extensive phylogeographic study (DUMOLIN-LAPOEGUE et al., 1997; PETIT et al., 2002), *Fagus sylvatica* (DEMESURE et al., 1996), *Corylus avellana* (PALMÉ and VENDRAMIN, 2002), *Prunus spinosa* (MOHANTY et al., 2002), *Tilia cordata* (FINESCHI et al., 2003), *Betula pendula* (PALMÉ et al., 2004), *Fraxinus excelsior* (HEUERTZ et al., 2004), *Populus nigra* (COTTRELL et al., 2005) and *Cornus sanguinea* (LIESEBACH and GÖTZ, 2008). Similar studies were carried out for American tree species like, i.e., *Liriodendron tulipifera* (SEWELL et al., 1996) and *Quercus rubra* (ROMERO-SEVERSON et al., 2003). There is no published data on chloroplast DNA variation for *Robinia pseudoacacia* to date, neither in its natural range nor in other regions of cultivation.

The provision of a method to get information on a uniparental inherited marker type including first data presented here are a step to detect within-species variation of chloroplast DNA in introduced *Robinia pseudoacacia* populations, and can give an impression of the amount of within- and among-population variation. Such information is important for the assessment of different management systems and the approval and harvest of seed stands.

Materials and Methods

Material

Population samples were taken from a progeny trial with black locust from different seed sources (SCHNECK

et al., 2002; LIESEBACH et al., 2004) located near population No. 2. Seeds for this field trial were collected in 20 populations of black locust. Sixteen of them represent part of the artificial European range of the species (Germany, Hungary and Slovakia) and four originate from the natural range in North America (*Table 1*). These populations are characterised by different histories and structures. The seeds from Hungary came from approved seed stands after commercial harvests by sieving the upper layer of soil. The German populations differ in age and size. Two of them represent clonal plantations (seed orchard, clone collection). Seed harvesting was performed by collecting pods from the ground or pick them from the trees. Seeds were sown in the nursery in spring 2000 and seedlings were transplanted in spring 2001. At least 10 individuals from each population should be genotyped. Exact sample sizes are given in *Table 4*.

Additionally, 31 clones from the collection of the Thünen – Institute of Forest Genetics in Waldsieversdorf, located near population No. 6, were included into the study. The majority of clones was selected from German populations for their straight stem form. Some additional clones were included, among them the clone 'Algonquin' from the „Steiner Group black locusts" from the National Plant Materials Center in Beltsville/Maryland (USA), the clone 'Monophylla Pendula' (*R. pseudoacacia*), the clone 'Casque Rouge' (*R.* × *margarettiae* Ashe, *R. hispida* L. × *R. pseudoacacia*) and one clone from a Korean partner. The clone 'Algonquin' originates from West Virginia near provenance No. 20. The origin from the other clones is unknown. Altogether, 251 individuals were genotyped for their cpDNA haplotype.

Laboratory methods

The isolation of total DNA from frozen leaves followed a modified CTAB protocol (DUMOLIN et al., 1995).

The amplification of noncoding regions of chloroplast DNA based on a selection of four consensus primer pairs derived from the tobacco chloroplast DNA sequence (*Table 2*). Additionally, one primer pair derived from *Lotus japonicus* (Genbank Accession AP002983), the

Table 1. – Information about geographical and climatic data, stand structure and seed harvest of 20 selected populations (n.i. = no further information).

No.	Country	Provenance	Latitude N	Longitude E	Altitude [m]	Annual mean temperature [°C]	Annual precipitation [mm]	Stand structure and seed harvest
1	Germany	Göritz	51°58'	12°32'	80	8.6	569	Seed orchard with 21 clones, seeds were picked from the trees
2	Germany	Annaburg	51°39'	12°57'	75	8.7	573	~50-years old stand (1.2 ha), seeds were collected from the ground
3	Germany	Arensdorf	51°48'	12°59'	110	8.7	573	~15-years old stand (0.5 ha), seeds were picked from 15 trees
4	Germany	Haldensleben	52°20'	11°12'	60	9.2	543	seeds were picked from approx. 15 felled trees
5	Germany	Altbrandsleben	52°05'	11°13'	100	9.0	503	seeds were picked from approx. 15 felled trees
6	Germany	Waldsieversdorf	52°32'	14°03'	50	8.2	527	Clone collection with 39 clones, seeds were collected from the ground
7	Germany	Hasenholz	52°34'	14°03'	70	8.2	527	~80-years old stand (2.0 ha), seeds were collected from the ground
8	Germany	Gottesgabe	52°38'	14°10'	40	8.2	527	~70-years old stand (2.0 ha), seeds were collected from the ground
9	Slovakia	Luč	47°58'	17°31'	120	10.2	693	seeds were picked from trees in a progeny test
10	Slovakia	Gabčikovo	47°52'	17°32'	110	10.2	693	seeds were picked from trees in a progeny test
11	Hungary	Mikebuda 5G	47°10'	19°40'	150	10.5	542	seed stand, upper soil layer was sieved
12	Hungary	Mikebuda 27G, 28D, 30B	47°10'	19°40'	150	10.5	542	seed stand, upper soil layer was sieved
13	Hungary	Opalyi 1A,B	47°09'	19°32'	150	10.5	542	seed stand, upper soil layer was sieved
14	Hungary	Pusztavacs 60A	47°52'	22°18'	150	9.8	600	seed stand, upper soil layer was sieved
15	Hungary	Pusztavacs 56C	47°09'	19°32'	150	9.8	600	seed stand, upper soil layer was sieved
16	Hungary	Ofeherto 10B	47°56'	22°03'	150	9.8	600	seed stand, upper soil layer was sieved
17	USA	Illinois -1	37°30'	- 88°30'	200	12.3	942	n.i.
18	USA	Tennessee - 1	35°12'	- 84°54'	1000	15.2	1156	n.i.
19	USA	Georgia - 2	34°48'	- 84°00'	900	16.8	1246	n.i.
20	USA	West Virginia - 1	39°06'	- 79°36'	600	13.2	1143	n.i.

Table 2. – Primer pairs tested for black locust.

Primer pair abbreviation	Chloroplast DNA region	Reference	Annealing temperature (°C)	Elongation time (min)	Length of PCR product (bp)
B2B3	psbB - petB	Grivet et al. (2001)	53	3	2781
DT	trnD - trnT	Demesure et al. (1995)	55	2	1213
HK	trnH - trnK	Demesure et al. (1995)	62	2	1831
K1K2	trnK - trnK	Demesure et al. (1995)	53	3	2585
ED	trnE - psbD	this study	57	2	2076

next relative of *Robinia* with known chloroplast DNA sequence, was included (Forward: 5'-GTC CCG ACG TAA CCA GTC AT-3', Reverse: 5'-TGA ACC ACT AGA CGA TGG GG-3').

Amplification reactions contained 1 x PCR buffer with 2.5 mM $MgCl_2$ (AppliChem Darmstadt), 250 µM dNTP-mix, 0.5 µM of each primer, 1 U Taq Polymerase (AppliChem Darmstadt) and 20–100 ng template DNA in a volume of 25 µl. Polymerase chain reactions (PCR) were carried out in a Biometra thermal cycler using the following conditions: 1 cycle with 95°C/5 min, 30 cycles with 95°C/45 sec denaturation, annealing temperature/45 sec, and 72°C/elongation time (*Table 2*), and 7 min final elongation at 72°C. The presence of a PCR product of expected length was checked with gel electrophoresis in 1.6% agarose. Amplification products were digested with BamHI, DraI, HinfI, MboI, MspI, RsaI, SspI and TaqI in accordance with the manufacturers' instructions to find variation in restriction sites and/or fragment length. RFLP fragments were separated with agarose gel electrophoresis and stained with ethidium bromide. Gels were photographed with the Kodak EDAS 290 system.

A preliminary screening of a subset of 24 samples representing several populations was carried out to find combinations of primers and restriction enzymes revealing polymorphisms. The informative combinations were then used to screen all black locust samples.

Data evaluation

All combinations of primers and restriction enzymes with variation in RFLP patterns were scored for their polymorphic sites, which are the presence or absence of a restriction site (coded by 1 and 0) or length variations of DNA fragments (largest fragment coded by 1). Haplotypes were defined as certain combinations of polymorphic sites.

The parameter of the total genetic diversity hT of populations is defined as $h_T = 1 - \Sigma p_i^2$, whereas p_i is the frequency of the i-th haplotype. It measures the genetic variation within the range between 0 and 1. The total variation can be divided into a portion of within-population and among-population variation. G_{ST} is the parameter of differentiation among populations between 0 in the case of total identity of all populations and 1 in the

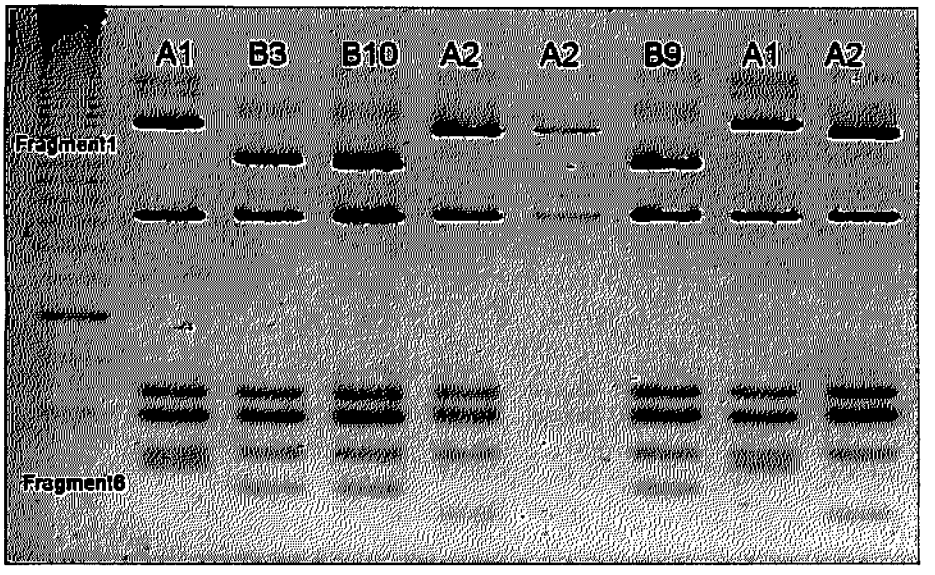

Figure 1. – RFLP patterns of the HK fragment after digestion with HinfI of *Robinia pseudoacacia* chloroplast DNA, from left to right: Lane 1: 50 bp-ladder (increased intensity at 250 bp), Lane 2 to Lane 9: Examples for several cpDNA haplotypes.

Table 3. – Coding of 11 cpDNA haplotypes in *Robinia pseudoacacia* by 19 polymorphic sites.

	HK-HinfI	HK-HinfI	HK-SspI	HK-SspI	HK-TaqI	HK-MboI	HK-MboI	HK-DraI	HK-DraI	KK-RsaI	KK-BamHI	KK-DraI	BB-HinfI	BB-MspI	BB-MboI	DT-HinfI	DT-MspI	ED-HinfI	ED-HinfI
	F1	F6	F2	F5	F2	F1	F4	F2	F4	F1	F2	F1	F2	F1	F1	F1	F1	F2	F4
A1	2	1	1	3	0	2	1	1	2	1	1	3	1	1	1	2	0	2	1
A2	3	3	1	3	1	2	1	1	2	1	1	3	1	1	1	2	0	2	1
A5	2	2	1	3	0	2	2	1	2	1	1	3	1	1	1	2	0	2	1
A6	1	2	1	1	0	1	1	1	0	1	1	3	1	1	1	2	1	2	2
A7	3	2	1	3	0	2	1	1	2	1	1	3	1	1	1	2	0	2	1
A11	3	3	1	3	0	2	1	1	2	1	1	3	1	1	1	2	0	2	1
B3	4	2	2	2	0	3	1	2	1	1	1	1	2	2	0	2	0	2	1
B4	4	2	2	3	0	3	1	2	2	1	1	1	2	2	0	2	1	2	1
B8	4	2	2	3	0	3	1	2	2	0	2	2	2	2	0	1	0	1	1
B9	4	2	2	3	0	3	1	2	2	0	1	1	2	2	0	2	0	2	1
B10	4	2	2	3	0	3	1	2	2	0	2	2	2	2	0	1	0	2	1

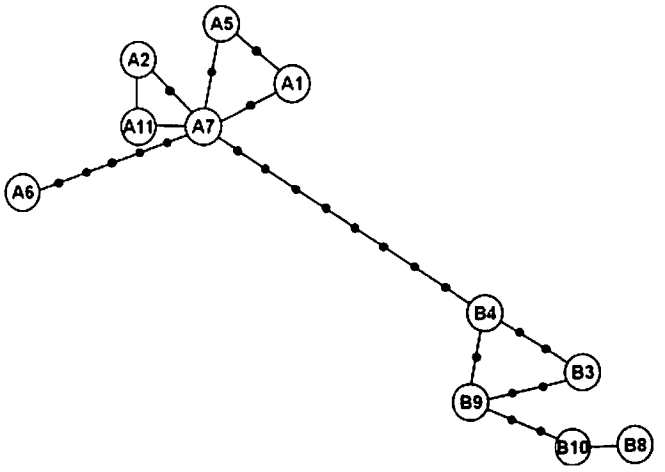

Figure 2. – Minimum spanning network, representing the genetic relationships among 11 cpDNA haplotypes detected in progenies of 20 selected populations of *Robinia pseudoacacia* and in a collection of 31 clones.

case of a lack of consistencies. The variation parameters of cpDNA haplotypes and their standard errors were calculated with the help of the TURBO PASCAL program Haplodiv (PONS and PETIT, 1995).

To visualise the genetic relationships between cpDNA haplotypes, a minimum spanning network was created with data from all polymorphic sites with the help of the software Arlequin (EXCOFFIER et al., 2005). Arlequin was also applied to calculate the percentage of variation among populations (F_{ST}) for diploid allozyme data from the same material (recalculated from LIESEBACH et al., 2005).

Results

Variations of banding patterns of PCR-RFLPs were observed for 14 different primer restriction arrangements. An example is given in *Figure 1*. The patterns resulted in 19 polymorphic sites (*Table 3*, fragments

Table 4. – Frequency of cpDNA haplotypes in 20 selected progenies of *Robinia pseudoacacia*.

Population	Number of samples	A1	A2	A7	A11	B3	B4	B8	B9	B10
1	11	8	1			2				
2	12								12	
3	11	1	2		2			3	3	
4	12	3	4			5				
5	12	4	3			1				4
6	12	1	11							
7	11	10								1
8	11	6	5							
9	11	3	3			4			1	
10	11		1			7			3	
11	11		8			3				
12	11	1	2		1	4			1	2
13	12	4	3			1			2	2
14	7	3	3							1
15	10	2	3			2	1	1		1
16	12	3	4			4			1	
17	11		6			1		2	1	1
18	10						7			3
19	11			11						
20	11							8	3	
Total	220	49	59	11	3	34	8	14	27	15
%		22.3	26.8	5.0	1.4	15.5	3.6	6.4	12.3	6.8

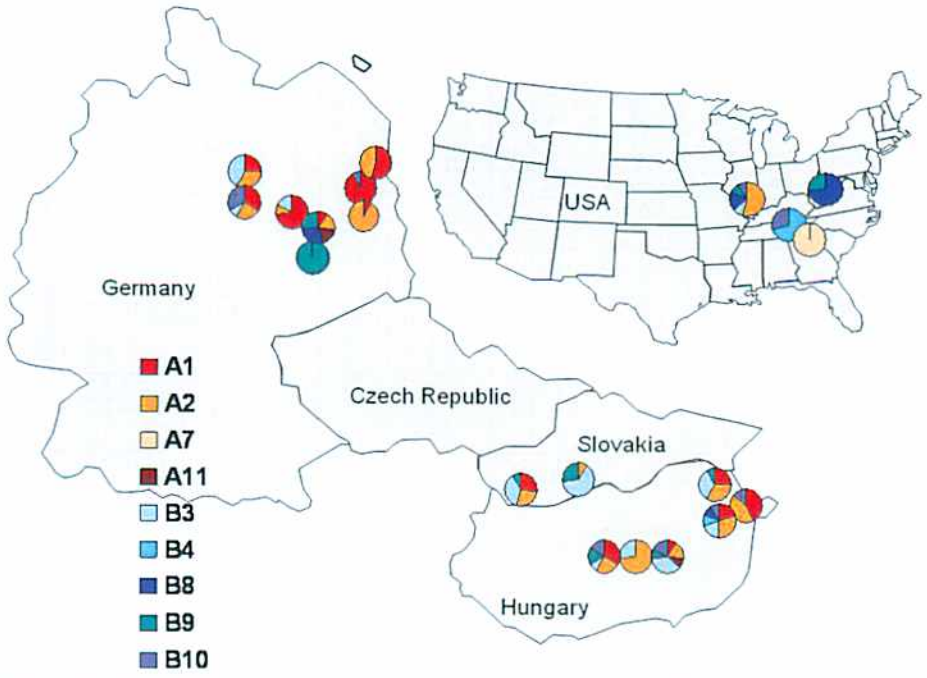

Figure 3. – Map of 20 seed stands and relative frequencies of cpDNA haplotypes in their progenies, also pointed the location of progeny trial and clone collection.

were described as F1, F2, ... with descending length). Thus, 11 different haplotypes were recognized by combinations of all variable PCR-RFLP patterns. The pedigree of 11 haplotypes inferred from 19 polymorphic sites was visualised with the minimum spanning network (*Figure 2*). Two lineages of closely related cpDNA haplotypes A and B could be identified.

The 31 samples of the clone collection exhibit 5 haplotypes A1, A5, A6, B4 and B9. Haplotypes A5 (clone from Korea) and A6 ('Algonquin') were found only in the clone collection. The clone 'Monophylla Pendula' exhibits haplotype B4 and the clone 'Casque Rouge' exhibits haplotype B9. All clones selected for their straight stem form from German populations possesses haplotype A1. Nine different haplotypes were detected in the 20 sampled offspring populations in the progeny trail. Their frequencies are given in *Table 4* and visualised in a map (*Figure 3*).

Several variation parameters were calculated for groups of populations established by assignment to source countries Germany, Hungary and USA (*Table 5*). With only 2 Slovakian populations, the sample is too small to create a group for calculations.

The four populations from the natural range reveal a maximum total genetic variation despite their lower number of haplotypes. The total genetic variation in the German and in the Hungarian group of populations is somewhat smaller. Populations from the natural range and German populations are comparable for the percentage of variation among their populations amounting to approximately half of the total variation of haplotype composition. In contrast, the Hungarian group has no significant variation among populations. This means that all variation is within populations associated with a maximum number of haplotypes per population.

Table 5. – Mean parameters of genetic variation in subgroups of tested progenies of *Robinia pseudoacacia* (variation ± standard error) in according to PONS and PETIT (1995).

	Mean number of haplotypes per population	Mean effective number of haplotypes per population	Total variation (H_T)	Variation among populations % (G_{ST})
Populations 1-8 (Germany)	2.75	2.24	0.797 ± 0.054	41.9 ± 15.5
Populations 11-16 (Hungary)	4.33	3.56	0.775 ± 0.042	3.2 ± 6.4
Populations 17-20 (USA)	2.50	1.80	0.969 ± 0.051	58.4 ± 16.8
All populations 1-20	3.25	2.60	0.844 ± 0.037	33.9 ± 8.8

Formerly published allozyme data for the same material (LIESEBACH et al., 2005) were used to compare percentages of among population variation with cpDNA-variation. The percentage of variation among eight German populations amounts to 42% for cpDNA haplotypes and to 9% for allozymes, whereas the variation among six Hungarian populations amounts to non-significant 3% and 1% respective.

Discussion

A total of eleven haplotypes was found belonging to two clearly separated groups of related haplotypes, the lineages labelled as A and B. From the minimum spanning network, the haplotypes A7 and B4 could be recognised as putative ancestors of the two lineages A and B, respectively. They differ in 9 mutation steps out of 19 detected polymorphic sites. Nine different haplotypes were detected in 20 progenies of selected populations in the natural range and in artificial stands in Europe, and two other haplotypes were only present in the clone collection.

Within the disjunctive natural distribution area of black locust we analysed three populations from the large eastern region (Appalachian Mountains: 2 southern, 1 northern) and one sample from an isolated area in the south of Illinois. There is no sample from the western part of the natural range (Central Highlands). These four sampled populations from the natural range represent different types of variation patterns. The Georgia population (No. 19) is completely composed of haplotype A7, the putative ancestor of this lineage. This haplotype was not found in the European samples tested. Two populations from Tennessee (No. 18) and West Virginia (No. 20) exhibit two haplotypes, each belonging to lineage B. The clone 'Algonquin' with its known origin south of the West Virginian provenance (No. 20) belongs to lineage A. In contrast, the Illinois population (No. 17) is an admixture, which shows 5 different haplotypes of two lineages. There is no association between the geographic location of the 4 population samples of the natural range of black locust and their composition of haplotypes.

Despite of the low number of haplotypes within these four populations compared to European artificial populations, the total variation is high in the samples from the natural range due to a high variation among populations.

Several former investigations studied geographic variation patterns of phenotypical traits in black locust in its natural range. In a common garden experiment with 145 half-sib families, height and diameter of seedlings decreased as origin was increasingly northern, with latitude explaining 5–7% of the variation in family means (BONGARTEN and MERKLE, 1996). An older experiment could not locate such patterns with numerous morphometric traits in 434 families from the natural and naturalised range (MEBRAHTU and HANOVER, 1989). SURLES et al. (1989) also could not find geographically associated variation patterns in a comprehensive study with allozyme markers in 23 seed sources representing the natural range of black locust. However, they mentioned extensive reforestations of black locust within the natural range before 1950 with seed material originating from European plantations of unknown native sources. The low number of only four populations from the natural range in the present study, and their unknown autochthony, does not allow the prediction of a geographical structure, even though maternally inherited chloroplast markers are more suitable to detect regional patterns than biparentally inherited allozyme markers. The distribution area of black locust was not covered by ice during the last glacial maximum. LOEHLE and ILTIS (1998) listed black locust as a tree species whose northern ranges stop at the ice margin. Refugees and a migration history as were detected for many tree species in Central Europe are lacking.

Outside of the natural range of black locust, the pattern of genetic variation is influenced by arbitrarily introduced founder populations and by subsequent generative and vegetative reproduction. Additionally, forest management and the transfer of seeds and planting material produce changes in genetic structures. However, the presence or absence of chloroplast haplotypes and lineages in artificial populations cannot give information on the source region within the natural range because of the unknown geographic variation pattern. Thus, the common haplotype A1 in European samples (22.3%) was not present in the four samples from the natural range. Conspicuously, all clones selected for their straight stem form possess haplotype A1. In the material analysed in this study, we can compare two groups of artificial populations, on the one hand eight German populations, and on the other hand six Hungarian populations. The number of only two Slovakian populations do not allow generalisations. The different pattern of genetic variation among and within populations between these two groups may be caused by differences in management of black locust between Germany and Hungary in the past. German populations are similar to populations from the natural range in their variation among populations of about half of total variation of chloroplast haplotype composition. The same direction of differentiation was observed for allozyme markers, but to a lower extent because of the biparental mode of inheritance. The similarity of natural populations and many German stands seems to be caused by their similar life cycles developing after longer periods without forest management. A remarkable part of vegetative propagation occurs by root suckers forming large clusters with genetically identical individuals, partially completed by generative reproduction. Thus, clonal structures of black locust were observed in the natural range (CHANG et al., 1998) and in artificial stands without human influence after initial planting (HERTEL and SCHNECK, 2003; JUNG et al., 2009).

Cultivation of black locust in Hungary is carried out by a broad planting of seedlings from nurseries for rotation periods of 30–50 years. Seeds come from two regions approved for seed production (RÉDEI et al., 2008). Obviously, Hungarian samples in this study were harvested from populations developed from planted seedlings. This form of management in Hungary over

decades generated homogeneity of black locust populations with a high level of within-population diversity and a lack of detectable differentiation among them, at least with chloroplast DNA and allozyme markers.

Seed harvesting in black locust populations should take their clonal structure into consideration. Seeds should be collected from trees belonging to different clones to minimise the risk of transferring only closely-related offspring into the next generation. Furthermore, there is a need for more studies to compare growth performance from offsprings originating from smaller or larger clonal structured populations and from seed orchards to get information on their suitability for wood production.

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