Gene Conservation Through Seed Orchards – A Case Study Of *Prunus spinosa* L.

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Summary

In Germany plantations of indigenous shrub species with regionally harvested propagation material become more and more important to compensate for the encroachment of natural habitats. The use of such material is based on the expectation of its high adaptedness and vitality. The urban sprawl and land use make it difficult to identify populations which correspond to such expectations. For some species gene flow between populations and hybridization with cultivars blur potential patterns of adaptation. Results of systematic provenance trials are missing for indigenous shrub species. Thus the use of regional material is a strategy to safeguard against unintentional change of potential patterns of adaptation.

To contribute to this discussion we analysed 13 natural stands, two conservation seedling seed orchards and two seed lots of *Prunus spinosa* L. using isozyme gene markers.

This paper studies the following questions:

1. Are naturally occurring populations of *P. spinosa* L. genetically differentiated?
2. Is there any evidence for a link between genetics and spatial proximity?
3. Do the existing seed orchards of *P. spinosa* L. represent their source populations?
4. Are imported seed from southeast Europe (Hungary) significantly differentiated?
5. Do genetics support the hypothesis of naturally arisen source populations?

The natural stands are genetically differentiated. Evidence exists for a link between genetic differentiation and spatial proximity. The genotypes of the seed orchards represent the gene pool of the natural stands well. Minor genetic differentiation exists between the German study material and the seed lot from Hungary. Cluster analysis supports the hypothesis of naturally arisen source populations.

Introduction

According to considerations of nature conservation plantations of indigenous shrub species with regionally harvested propagation material become more and more important to compensate for the encroachment of natural habitats in Germany. Over 150 million trees and shrubs are produced annually in German nurseries for plantations in urban areas and the open landscape. On top of this amount a considerable quantity of propagation material is imported from other countries for the same purpose. There exist no legal regulations on harvest and procurement of these species. Especially widely distributed species like blackthorn (*Prunus spinosa* L.) have a considerable economic relevance. But the costs for harvesting seed especially from shrub species are much higher in Western Europe than in other countries. Thus 50 to 80% of the plant material is imported from low income countries (Spethmann 1995, 2003). During the last years a debate arose concerning this practice. Two partially conflicting objectives have to be reached: avoiding risks for nature and landscape by planting potentially maladapted material and allowing economically interesting production of woody plant species.

For indigenous shrub species results of systematic provenance trials are missing. Nevertheless first exploratory studies show some disadvantage of material which has been transferred over wide geographic distances (Liesebach et al. 2007). In terms of nature conservation the use of regional material is supposed to be a strategy to safeguard against unintentional change of potential patterns of adaptation. Thus the federal Ministry of Food, Agriculture and Consumer Protection published recommendations for provenance regions for shrub and minor tree species (BMVEL 2003). The use of local material is based on the expectation of its high adaptedness and vitality (McKay et al. 2005). Unfortunately the urban sprawl and land use make it difficult to identify populations which correspond to such expectations. The human as "migration factor" might alter naturally occurring adaptation processes. For some species gene flow between populations and hybridization with cultivars blur potential patterns of adaptation and might even have the potential to endanger species (Allendorf et al. 2001).

Seed orchards of shrub species were established during the last decade to overcome the shortage of reproductive material originating from local populations and to allow an economically interesting seed production. Within the process of conservation of forest genetic resources populations of indigenous tree and shrub species were identified, which according to historical records evolved without artificial introduction of plant material (Paul et al. 2000). These populations should be separated from artificially planted material of the same species.

A sample of those populations was used as source population to set up conservation seed orchards for different shrub species. This paper describes the example of a blackthorn (*Prunus spinosa* L.) seedling seed orchard.

Example of blackthorn (*Prunus spinosa* L.)

Black thorn (*Prunus spinosa* L.) is a character species of hedges along forest edges and tracks as well as on open farm land (Scholz and Scholz 1995). The phenotypic appearance of *P. spinosa* L. is quite variable. The species is widely planted in the open landscape. The taxonomic status and possible hybridization with *Prunus domestica* ssp. *Insititia* is discussed in Hegi 1995. The species is tetraploid (4n=32). Blackthorn propagates vegetatively through root suckers in nature. As an allotetraploide species *P. spinosa* is supposed to be self fertile (Hanelt, 1997). Experimentally Guitán et al. (1993) could not support this hypothesis. Population genetic studies have been carried out by Leinemann et al. 2002 and Schmitt (2003).

Two seedling seed orchards have been established with 15 seedlings of each source population in the year 2000. One seed orchard is supposed to represents natural populations of the lowlands in the north of Lower Saxony the other those of the hilly region in the south. Even though some degree of spatial isolation to neighboring populations of the same species was sought gene flow from outside cannot be excluded.

In the light of the above mentioned concerns the following questions have to be answered:

1. Are naturally occurring populations of *P. spinosa* L. genetically differentiated?
2. Is there any evidence for a link between genetics and spatial proximity?
3. Do the existing seed orchards of *P. spinosa* L. represent their source populations?
4. Are imported seed from southeast Europe (Hungary) significantly differentiated?
5. Do genetics support the hypothesis of naturally arisen source populations?

Material

In total we analyzed 17 different objects consisting of 13 natural stands which are the source populations of the northern *P. spinosa* L. seedling seed orchard (SPL-HSF), two seed orchards and two seed lots, one originating from the northern seed orchard, the second coming from Hungary. Buds were harvested in autumn 2006 and seeds were obtained from

one private enterprise and the tree seed center of the Lower Saxony State Forest. As a rule 70 samples per source population were analyzed (except DAN-ST: 36 and NIE-96: 26), 80 samples of each seed lot and 100 of the southern and over 300 of the northern seed orchard, respectively. Figure 1 shows the locations of the different source populations and the seed orchards.

figure 1: Map of Lower Saxony, Germany with the location of source populations (red dots) and seed orchards (green romboids)

Source populations	abbreviation
Danndorf	DAN-AK
Danndorf	DAN-ST
Göhrde	GDE-BL
Harsefeld	HSF-HE
Neuenburg	NEU-109
Neuenburg	NEU-137
Neuenburg	NEU-UP
Nienburg	NIE-67
Nienburg	NIE-96
Rotenburg	ROT-AL
Rotenburg	ROT-DI
Wolfenbüttel	WOL-HO
Wolfenbüttel	WOL-KA
Seed orchards	
Grohnde	SPL-GR
Harsefeld	SPL-HSF
Seed lots	
Harsefeld	S-HSF
Hungary	S-UNGA

Methods

Five polymorphic enzyme systems, representing seven gene loci were analyzed (table 1). The methods for isoenzyme analysis of blackthorn are described in Leinemann (2000), Leinemann and Bergman (2000) and Leinemann *et al.* (2002). Data analysis was carried out using the program Tetraploide, Version 1 (Decarli and Leinemann 2003). The dendrogram illustrating the genetic distances between populations is based on the software NTSYS, Version 2.01, Applied Biostatistics. The enzyme systems and their E.C. numbers are described in table 1.

table 1: Analyzed enzyme systems, zones and number of observed alleles
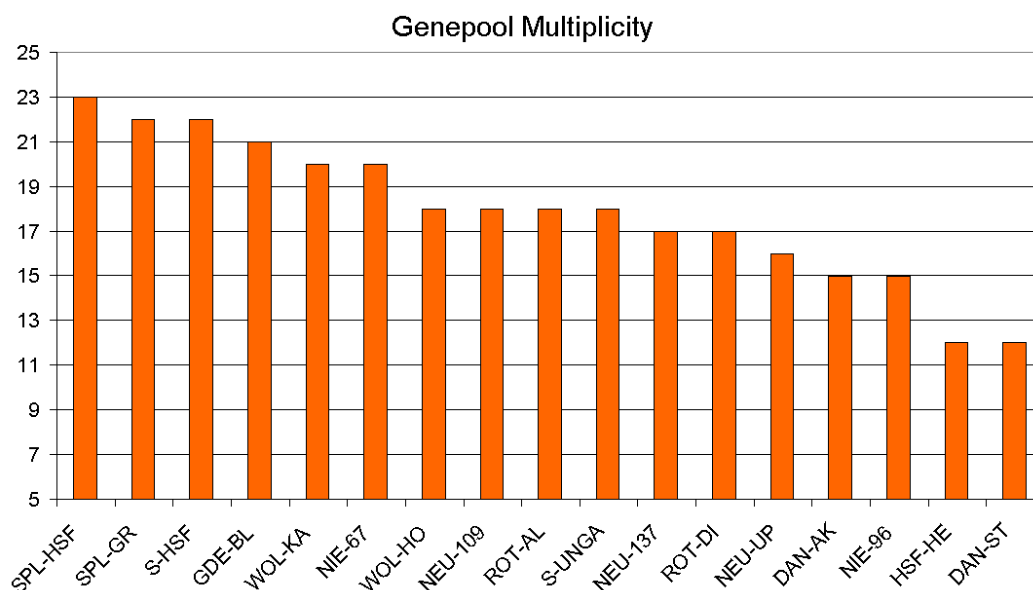
Enzyme system	gene loci	number of alleles
Alcohol-Dehydrogenase E.C. 1.1.1.1	Adh-A	4
Malat-Dehydrogenase	Mdh-A	4
E.C. 1.1.1.37	Mdh-B	3
Isocitrat-Dehydrogenase E.C. 1.1.1.42	Idh-A	3
6-Phosphogluconat-Dehydrogenase	6-Pgdh-A	5
E.C. 1.1.1.44	6-Pgdh-B	3
Phosphoglucose-Isomerase E.C. 5.3.1.9	Pgi-B	5

Results

Genetic variation within samples

Three to five alleles were observed at each enzyme locus. The seed orchard Harsefeld comprises all the alleles, which were found in the source population, with the exception of three rare alleles: Adh-A3 was found only in GDE-BL, 6Pgdh-B1 was found only in WOL-HO and WOL-Ka and Pgi-B5 was found only in NEU-109.

The seed orchard Harsefeld has the allelic multiplicity of 23. The average over all studied objects is 17.9. The seed orchards show the highest degree of variation within the studied objects (figure 2) followed by the seedlot originating from Harsefeld. Thus the seed orchards
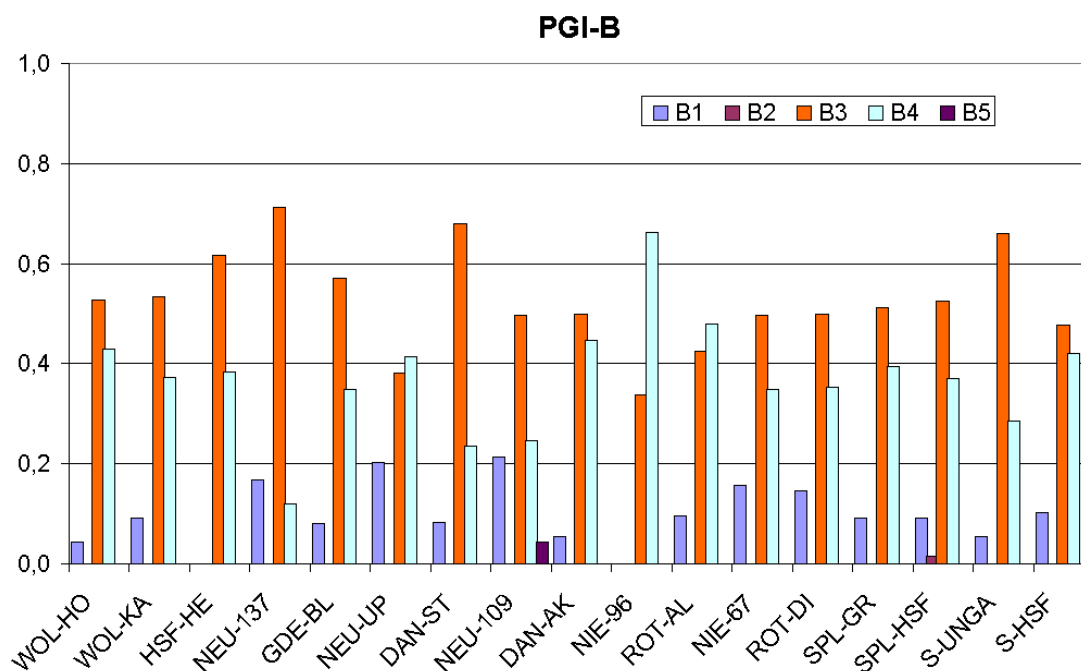

figure 1: Genepool multiplicity

represent artificial populations, which comprise more variation than the natural populations. This variation is represented in the produced seed as well. Multiplicity is influenced by sample size. In small samples there exists the chance of losing rare alleles. This might be the explanation for the values of 12 and 15 of the populations represented by small samples (DAN-ST: 36 and NIE-96). Nevertheless HSF-HE was represented by the same amount as GDE-BL for example but the first has low multiplicity of 12, the later one of 21 respectively.

The allelic diversity as measure takes the frequencies of alleles into account. The average diversity is v = 1.24. Least diverse are the sample DAN-ST with v = 1.10 and the seed lot from Hungary (S-UNGA) v = 1.16. The sample NIE-67 has the highest diversity v = 1.38.

The sampling in the source populations was done along a line. The changes of multilocus genotypes along these lines were analyzed. The maximum estimated extent of a clone is 72 meters (average 20 meters). This is an indication for naturally arisen populations as compared to plantations, where we expect different multi locus genotypes next to each other. The later situation is given in the two seed orchards.

No association exists between the number of multi locus genotypes per source population and its size. Thus small populations potentially harbor high amounts of genetic variation as well as big populations.
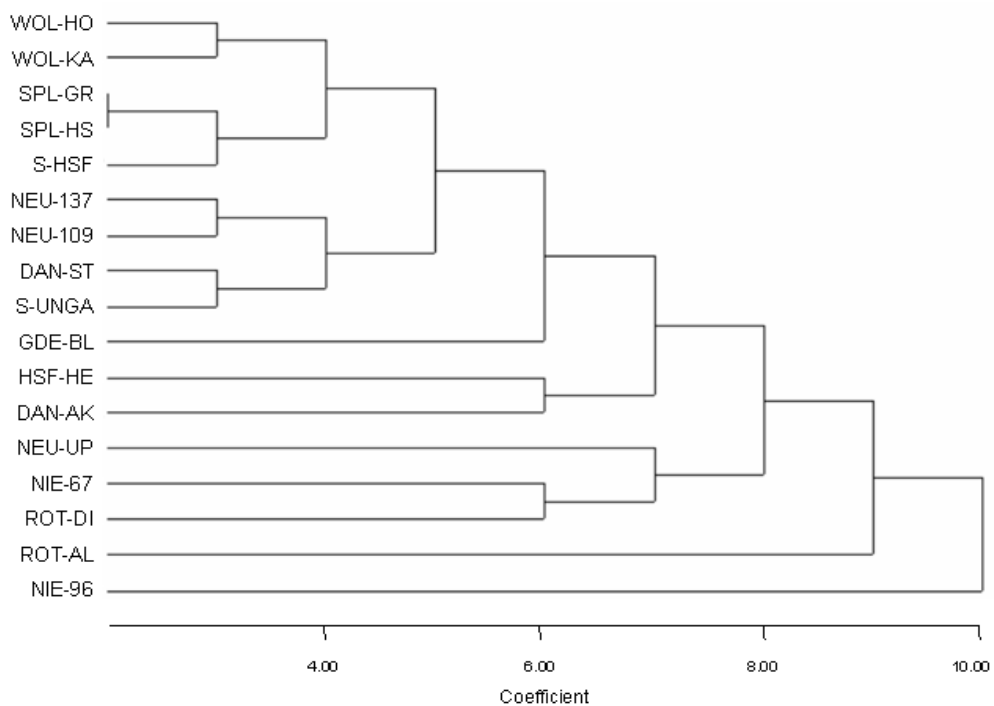

figure 2: Allelic frequencies at enzyme gene locus PGI-B
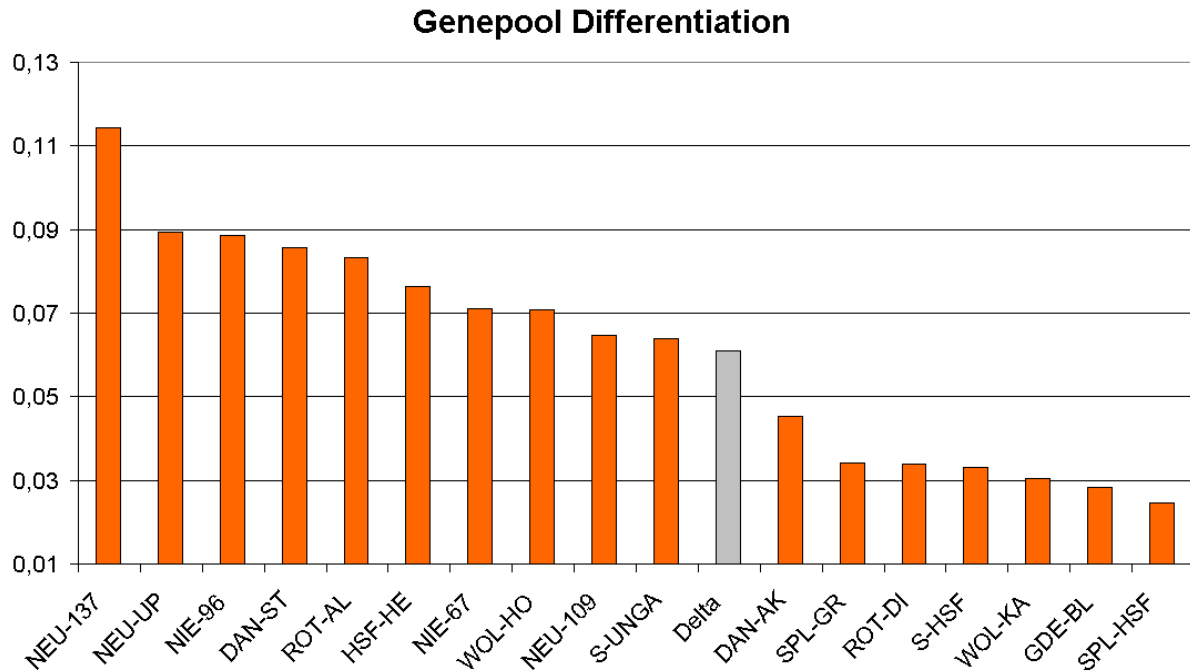
Genetic differentiation between samples

The average genetic distance (Gregorius 1974) of the gene pool is d_0=0.16. This is an extraordinarily high value for isoenzymes. The biggest allelic distance between the source populations was found for PGI-B ($d_{0\_Nie-96\_Neu-137}$=0.54) (see as well figure 3).

The neighbor joining dendrogram of average pair wise genetic distances (figure 4) shows an association between genetic and spatial proximity for some of the source populations (Wolfenbüttel and Neuenburg). The two seed orchards are grouped together joined on the next level by the seed lot from the seed orchard Harsefeld.

figure 3: phylogenetic (neighbourjoining) tree based on pair wise genetic distances between source populations

The subpopulation differentiation D_j (figure 5) extends the concept of genetic distances between two to multiple samples. According to Gregorius (1984) and Gregorius and Roberds (1986) D_j measures the genetic distance between a sample and its complement (the average of all other samples). The sample with the lowest value of D_j represents best all the rest of the samples. Samples with high values of D_j might represent special genetic information linked to genetic processes like adaptive differentiation.

figure 4: subpopulaition differentiation of the genepool; the gray column represents the average differentitation

The mean differentiation $D_j = 0.065$ (gray bar). The population NEU-137 shows the biggest differentiation from all other populations as compared to the seed orchard Harsefeld (SPL-HSF) with the lowest value of differentiation. The later is especially representative for the rest of all samples. The seed lot of Hungary (S-UNGA) does not show a remarkable differentiation.

Discussion

Prunus spinosa L. shows a high degree of genetic variation based on isoenzyme gene markers. Seeds from 13 natural stands are represented in the seed orchard Harsefeld (SPL-HSF). The natural stands are genetically differentiated. There is now evidence for strong gene flow into the natural stands. Otherwise we would have found new allelic variants in the seed orchard.

Setting up a seedling seed orchard (SPL-HSF) to represent the variation within the natural populations was successful. The comparison between the genetic structures of the seed orchard Harsefeld (SPL-HSF) and the seed orchard Grohnde (SPL-GR) gives no evidence for the assumption of genetic differentiation between lowland's and hilly region's populations at the isoenzyme level. But physiological studies point out the possibility of such adaptive

differentiation (see Liesebach et al. 2007). Therefore it can be seen as a strategy to safeguard against unintentional change of potential patterns of adaptation to keep two separate seed orchards for the lowlands and the hilly region as long as no results of studies of adaptive traits are available.

Evidence exists for a link between genetic differentiation and spatial proximity. Thus an adaptive significance of the observed genetic differentiation cannot be excluded. The use of different types of markers is discussed in Holderegger et al. (2006). The authors advocate for a combination of adaptive and neutral marker types.

The genotypes of the seed orchards represent the gene pool of the natural stands well. Minor genetic differentiation exists between the German study material and the seed lot from Hungary. The seed lot of Hungary (S-UNGA) is less genetically diverse than the seed lot (S-HSF) originating from the seed orchard Harsefeld.

The size of clonal structures within the stands supports the historical records that these populations were in place for already long time. The source populations probably existed before the period when intensive planting of shrub species began in the open landscape. The cluster analysis of the natural stands according to their genetic distances supports the hypothesis of naturally arisen source populations. Genetic markers represent a useful tool to analyze the different types of reproductive strategies (vegetative vs. generative propagation) of blackthorn.

For future seed procurement the seed orchards represent the chance to harvest seeds economically while safeguarding genetic variability. Genetic analysis of potential source populations prior to the establishment of seed orchards would allow to optimize the genetic variability and representativeness of the seed orchard material (e.g. Hosius et al. 2000).

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