

University of West-Hungary

PhD thesis

Genetic basis of conservation
of service tree (*Sorbus domestica* L.) and
wild service tree (*Sorbus torminalis* (L.) Crantz)
populations in the Hungarian Low Mountains

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Justification of the theme

These noble hardwood / wild fruit species *Sorbus torminalis* and *Sorbus domestica* have a forestry as well as natural and gene conservation interests.

In Hungary we have focused at first on the field work: collected a samples (incl. grafted plus trees). The main aim of this work was in and ex situ genetic conservation of these rare, scattered noble hardwood species. During this time we have established three seed orchards of Sorbus in Hungary:

1. *Sorbus torminalis* ortets from the Transdanubian Mts.;
2. *Sorbus domestica* I.; ortets from the Dunazug Mountains (north from Budapest),
3. *Sorbus domestica* II.; ortets from the Zemplén Mountains (North-East part of Hungary).

The first orchard represents a larger forest (seed) region, and is basically a synthetic population, the ramets are collected from natural populations.

The aim research was interpretation and assessment of the genetical investigation data (RAPD, PCR-RFLP, isozyme) of service tree (*Sorbus domestica*) and wild service tree (*Sorbus torminalis*) populations with the aim of utilizing of results in the practice for gene conservation of insect pollinated noble hardwood species.

Material

101 *Sorbus torminalis* - **wild service trees** were sampled and analysed. The samples represent plus trees and a control population from the forest region of Transdanubian Mountains. These samples were analysed with PCR RAPD, PCR (cpDNA) RFLP and isoenzyme methods. The genetic assessment measured variability in a synthetic population and compared with the dates of a natural control population.

The 196 **service tree** (*S. domestica*) samples represent ramets which were collected from 2 natural populations and situated mostly in a forest covered region (I. north from Budapest) or in vineyard habitats (II. Zemplén Mountains, North-East part of Hungary) too. These habitats are different but the sampled populations are presumably autochthonous. Old trees in vineyards are remaining trees or planted ones as seedlings from nearby forests.

The genetic laboratory analysis was partly realised in Budapest at the Institute of Agricultural Quality Control, and in Austria at the Austrian Research Center at Seibersdorf. The isozyme based population analysis of the *Sorbus torminalis* seed orchards was made in Sárvár at the Forest Research Institute / Dept. of Forest Tree Breeding. The data interpretation and assessment were done in Göttingen/Germany.

Method

In the case of **wild service trees** (*Sorbus torminalis*) we analysed results of 9 enzyme systems to obtain heterozygous allele pairs, their proportion and effect on the subpopulational differentiation. The 5 RAPD (random amplified polymorphic DNA) primers resulted 33 different bands that were coded analogous.

The cpDNA RFLP (restriction fragment length polymorphism) that are given on the primer pair called HK digested with enzymes named Hae III and Hinf I. We resulted 4 cpDNA-based haplotypes.

The fragment length polymorphism obtained by primer pairs combined with restriction enzymes allows detailed study of intraspecific variability and investigation of putative relationship between sampled genotypes. (We differentiated 4 haplotypes in Transdanubian-Mountains.)

At the RAPD the **service tree** (*Sorbus domestica*) analyse showed 29 different polymorphic bands. The cpDNA RFLP analyses presented polymorphism at the primer pair KQ digested with enzymes Mse I. and Hinf I. It was possible to difference 16 on cpDNA-based haplotypes. Dates were coded analogous, too. Programmes used for analyses: GSED, Popgene, GenePop, NTSYS, PAUP, PopTools and TreeView.

The formulation of our question is the measuring and calculation of genetic distances between populations, and the fixation in various populations. Results of RAPD and RFLP don't show any information for adaptation. The maternally inherited cpDNA provides information regarding colonisation routes, spatial dynamics and the likely human impact. In the total sample of 196 *S. domestica* individuals 11 restriction fragments were polymorphic, these variants grouped into 16 haplotypes.

Observed patterns of cpDNA diversity confirm the importance of seed flow in these species characterised by endozoochorous seed dispersal and typical extinction/recolonisation dynamic. G_{ST} values were used for diversity analyses of cpDNA haplotypes.

Scientific results – thesis

(1) The vital wild service tree (*S. torminalis*) populations in the Transdanubian Mountains prevalent are situated among the yield lines of the relief. Revealing are the exposition and the relations of plant associations also. The ecological optimum for the tree species can be defined as follows: yield line of the relief, south-west exposition, and dry hydrology of the sites. The *S. torminalis* specimens selected for form, vitality, fruit production, as far as possible were grouped by forest regions. We defined “subpopulations” mostly in quotation marks. Minor ecoregionally borders were used, in the case when the distribution (number of selected trees and control) showed a proper clumping. In the High-Bakony forest region it was possible to define 3 minor collectives: Bakonyszücs, South-Bakony and North-Bakony. The first collective may be regarded by its size as a true population, and the last two are real subpopulations; inside these most intense gene flow (through insect pollination) may be hypothesized.

(2) The service tree (*S. domestica*) lab samples were collected in the Zemplén Mountains, and in the east part of the Dunazug Mountains: all of the sampled specimens were analysed. On the first location (Zemplén) the service trees are mostly situated in the anthropogenic culture or half culture habitats (vine yards, extensive used village margins). In the second case (Dunazug) *S. domestica* is the accompanying tree species in some close to nature forest associations, or can be defined as subsponaneous (in the Buda Hills). When determining limits of subpopulations in the regions we searched the most adequate partition of the occurrences regarding the reproduction biology of the insect pollinated rare species. Therefore the service tree occurrences in groups or in a region with higher density (convergent valleys, coherent vine yards, basin landscapes) are defined as subpopulations because the conditions of the gene flow are more favourable in these cases.

(3) The isoenzymatic analyses confirmed in numerous cases, that a species with fragmented habitat (mixed, ill-proportioned appearance) has a higher value of differentiation between populations (F_{ST}). Based on the results of the isoenzymatic analysis of the wild service tree (*S. torminalis*) the values of genetic variance (or differentiation - F_{ST}) among the subpopulations and between the synthetic populations (seed orchard) and the control population (Bakonyszücs) can be defined as particularly low. The genetical differentiation among populations and their mean (δ) is similar to the international data where low differentiation among the analysed collectives has been referred as well.

(4) The *S. torminalis* isoenzymatic results point out at some loci high heterozygote excess in analysed probes. After the permutation tests heterozygote excess at the given locus and subpopulation level has been observed as well. The heterozygote excess could be explained by the way of selection of the genotypes (plus trees) aimed at the gene conservation, breeding – a positive individual selection. The isoenzymatic values as proportion of polymorphic locus (P), mean number of alleles (n) and the expected heterozygosity are similar to the international data and showed the more favourable values for the genetical quality of the seed orchard (n and P values). Besides it should be noted that more favourable conditions of pollination exist in the synthetic population (seed orchard), which represents plus trees in the from ecologically similar forest regions.

(5) The cpDNA analysis of the wild service tree from the theoretically possible 16 cpDNA haplotypes only four types have been observed. The collectives of the Bakony Mountains include all of the 4 described cpDNA types. The Balaton Highland, Vértes, Gerecse, and the east part of the Dunazug Mountains represent just two haplotypes, namely the two dominant types. Inside the Bakony is an interesting case of the

population Bakonyszücs, where 3 haplotypes were detected. All of these types are represented in other parts of the Bakony also. This location may be described as a variable presumably cultivated regional occurrence.

(6) The genetic distances among the wild service tree populations calculated from the isoenzymatic and the cpDNA results showed mostly similar UPGMA dendrograms (grouping): the collectives in the Bakony differentiated from the others. The genetic diversity analysis of the nucleus (based on the RAPD method) showed regarding the genetic distances grouped by the UPGMA method differences between the isoenzymatic and cpDNA grouping. The grouping of the RAPD didn't show any spatial order. The supposed reason for this deviation is the failure of representation (the probes were mostly plus trees).

(7) The service tree (*S. domestica*) subpopulations include mostly more cpDNA haplotypes (from 3 to 8). The *S. domestica* population of vine yard Kácsárd comprehends just one haplotype: autochthonous, in Zemplén generally distributed and a locally cultivated one. Between the localities (Zemplén, Dunazug) the generally distributed "main" haplotypes are others (change proportionally), or are typically of only one subpopulation. The results showed nearly in all cases high cpDNA based intrapopulation differentiation. This points to the fact that the more mother plants participated in the founding of the subpopulations. The differentiation between subpopulations (G_{ST}) are lower than the widely distributed social broadleaves species.

(8) The results of cpDNA analysis of the service tree subpopulations have a different representation (Dj) by comparison of the totality of the probes and the mean value of the subpopulation differentiation (δ). After the cpDNA RFLP procedure we found 11 polymorphic bands, which made

possible to differentiate 16 cpDNA haplotypes. Based on these results according to the differentiation the types in the subpopulations, Zemplén-subpopulations are the more representatively. Exception is the population of Kácsárd, which contains just one haplotype. This cpDNA type is a locally cultivated, autochthonous and mostly distributed haplotype in the Zemplén Mountains. The subpopulations in the Danube Bend over Budapest showed just a minor (higher values than the mean δ) representation. Cause of this is the appearance of the “new” and rare haplotypes, so that this group differentiated in the single clustering methods. The subpopulation of Buda Hills has average representation ($D_j = \delta$), so that was grouped together with the east-Hungarian (Zemplén) subpopulations after single clustering.

(9) The service tree probes are representative: in the relatively small regions all genotypes were sampled. Thanks to this, the regionally typical differentiation regarding to the nuclear DNA (RAPD) and the cpDNA showed analogies. The collectives (subpopulations) are divided according to geographically distribution and distances; and calculated genetic distances between these subpopulations indicated the same grouping character due to the representative sampling.

(10) The grouping of the samples (define as subpopulations) in any cases indicated questions regarding to the reproduction equilibrium in the groups. The RAPD and cpDNA results were used for the genetic characterization and comparison of the 3 collectives: wild service tree – Transdanubian Mountains; service tree – east part of the Dunazug Mountains and the Zemplén Mountains. The genetic distances were calculated between the individuals from the RAPD and cpDNA dates in the 3 collectives. These enabled the Mantel tests between the RAPD and the cpDNA based distance matrices in the collectives. The Mantel tests showed only at the service tree

individuals in the Dunazug Mountains very low 5 % correlation.