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Genetic Variability of Service Tree (*Sorbus domestica* L.) in the Hungarian Middle Mountains – Based on cpDNA Analysis in Two Regions

László NYÁRI*

Chair of Ecology and Genetics, Institute of Environmental and Earth Sciences,
University of West Hungary, Sopron, Hungary

Abstract – A genetic inventory was conducted at maternally inherited chloroplast DNA (cpDNA) gene loci of 196 adult service trees (*S. domestica*). The sampled trees represent autochthonous collectives/populations originating from 2 distant regions, from contrasting habitats, a forested area (eastern part of the Dunazug Mountains) and cultured habitats (Zemplén Mountains), respectively.

Strong intrapopulation variation was observed; percentages of molecular variance were: between regions 27%, among populations/regions 6%, within populations 67%. Considering all samples, the major part of total diversity (h_t = 0.752) was contributed by intrapopulation diversity (h_s = 0.583).

Species diversity was represented differently in individual populations. E.g. the population Kácsárd contains only one haplotype: the doubtless sign of local human cultivation. The population Buda Hills has an average differentiation considering the whole sampled material but the highest when evaluating the region north from Budapest separately. That points to the dispersion after an introduction event, probably parallel to adaptive radiation under selection influence.

In the study genetically polymorphic populations containing unique haplotypes were detected, providing important information for forest management, gene conservation and nature protection activities. The described work is part of *ex situ* gene conservation projects of the species in Hungary.

cpDNA diversity / PCR-RFLP / differentiation / representativity / genetic distances

Kivonat – Középhegységi házi berkenye (*Sorbus domestica* L.) populációk genetikai variabilitása – két régió cpDNS vizsgálata alapján. 196 házi berkenye egyed vizsgálatát végeztük el cpDNS-markerekkel. Az idős fák kollektívumait két tájegységben mintáztunk: a Dunazug-hegység keleti felének erdőborította részéből, illetve a Zempléni-hegység szőlőhegyi kultúr-élőhelyeiről. Az élőhelyek ugyan különbözőek, viszont a mintázott populációk őshonosaknak tekinthetőek.

A molekuláris variancia 27%-a régiók közötti, a régiókon belüli populációk között ez 6%, míg a populációkon belüli érték 67%. A minták összességét tekintve az összdiverzitás (h_t = 0,752) meghatározó részét a populáción belüli diverzitás teszi ki (h_s = 0,583). Az elkülönített populációk eltérő reprezentativitási avagy differenciálódási értékeket mutattak a minták összességéhez viszonyítva. A kácsárdi populáció pl. csak egyetlen haplotípust őriz, mely a helyi kultiváció biztos jele. A Budai-hegyek populációja különlegesnek bizonyult: a teljes növényanyaghoz viszonyítva

* lnyari@emk.nyme.hu, H-9400 SOPRON, Cházár A. tér 1.

átlagos differenciálódást mutatott, viszont a Budapesttől északra levő populációk között a legjobban differenciált. Mindez a behurcolást követő szétáramlásra utal, amely együtt járhatott szelektív befolyás alatti adaptív radiációval is.

cpDNS-diverzitás / PCR-RFLP / differenciálódás / reprezentativitás / genetikai távolságok

1 INTRODUCTION

Forest species, particularly wind pollinated species, exhibit a very effective gene flow. Unlike the patterns of nuclear DNA encoded alleles, maternal inheritance of the DNA of cell organelles maintain local patterns of allelic structure for long time periods (Mátyás 2002). Intraspecific chloroplast DNA (cpDNA) diversity is very effective in characterisation of population structures and in phylogeographic studies (Demesure et al 1996; Fineschi et al 2000). Organelle DNA markers are suitable for tracking of postglacial species migration routes (Petit et al 2002/b), for introgression or hybridisation testing, for kinship relations or descent analysis (Heinze 1998 in: Mátyás 2002).

Service tree (*Sorbus domestica)* is an insect pollinated and animal dispersed, rare species, and is of high interest both from silvicultural as well as from nature and gene conservation aspects. The natural distribution area of the species is concentrated on Southern Europe. Core areas are the Balkans and Appenines, eastern Spain and southern France (Rotach 2003). The easternmost occurrences are found on the Crimean peninsula and in Asia Minor. The northern boundary of natural occurrence is uncertain, because since the Roman period this species was repeatedly cultivated and dispersed for its fruits (Kausch Blecken von Schmeling 2000, Gyulai 2001). A contiguous distribution is present in southern Germany (Kausch Blecken von Schmeling 2000), Lower Austria (Klumpp – Kirisits1998) and in southern Slovakia (Kárpáti 1959/60, Paganová 2008).

In Hungary, the species is distributed mostly along the Hungarian Middle Mountains range and in South and West Transdanubia *(Figure 1).* The habitats are in mountainous regions, usually dry oak or scrub forests, rocky, open woodlands, but also forest margins, abandoned vineyards and fruit orchards.

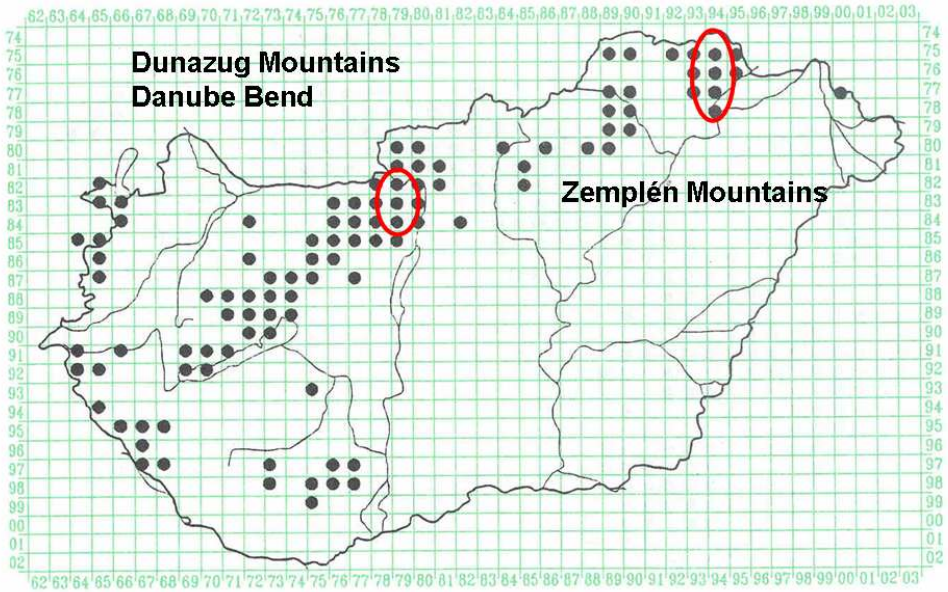

Figure 1. Natural distribution range of Sorbus domestica in Hungary (after Bartha – Mátyás 1995) and the location of the study areas

The occurrences in the last two habitat types reflect the human influence on distribution of the species. Gyulai (2001) states that archaeological findings (seeds, fruit residuals) prove that Roman Villa-farms had a highly developed fruit and winery culture in the Roman province of Pannonia (in present-day Hungary). Seed finds from the Migration Period, and finally the study of medieval wells in Buda castle confirm the historically continuous consumption of service tree fruits and the cultivation of the tree species. Rapaics (1940) considers the service tree (*S. domestica*) as indigenous in Hungary.

S. domestica has a certain economic potential if plant material of excellent genetic quality is used (Rotach 2003). However, in most regions of Central Europe, service tree is threatened, and is regarded as a valuable biological and plant genetic resource worth to be conserved.

2 MATERIAL AND METHODS

2.1 Plant material

Samples *(Table 1.)* were collected in the Zemplén Mountains (North-Eastern Hungary), and in the eastern part of the Dunazug Mountains (Danube Bend, north of Budapest). Fresh leaves were collected from trees, afterwards frozen and stored at -80°C. 196 individuals were sampled and analysed. In the Zemplén Mts. the service trees are mostly located in cultured or semi-cultured habitats: vineyards and extensively used village margins (Nyári 2002). In the Danube Bend *S. domestica* appears in some close-to-nature forest associations, while in the Buda Hills (western limits of Budapest) the species is considered as subspontaneous (Kárpáti 1959/60, Nyári 2003).

Table 1. Sampled service tree populations in the Zemplén Mountains (codes 1-5), and in the eastern part of Dunazug Mountains (codes 6-9). The coordinates of populations represent the means of individual tree coordinates

Code	Populations	Coordinates		Number of trees
		Longitude	Latitude	
1.	Hegyköz	48°31’ 6”	21°30’38”	28
2.	Kácsárd	48°23’26”	21°35’ 5”	17
3.	Hegyalja	48°15’54”	21°23’35”	43
4.	Tokaj	48° 9’51”	21°19’54”	15
5.	Meződűlő	48°27’39”	21°18’12”	9
6.	Buda Hills	47°34’12”	18°57’33”	11
7.	Szentendre	47°43’53”	19° 3’45”	30
8.	Visegrád	47°45’ 7”	18°56’19”	29
9.	Pilismarót	47°46’31”	18°51’ 5”	14

When defining the dividing borders of sampled populations in the two regions, first of all the reproduction biology of the species was considered. Therefore, the occurrences in larger groups or significant presence in contiguous landscapes (convergent valleys, coherent vine yards, basins) were defined as populations because of favourable geographical circumstances for gene flow.

2.2 DNA extraction, amplification and digestion

For extracting total DNA from frozen leaf samples the DNeasy® 96 Plant Kit (6) (QIAGEN, Hidden, Germany) was applied. The concentration and the quality of the extracted DNA was checked by 1.5% agarose gel electrophoresis with 1xTAE as running buffer (Sambrook et al. 1989).

The fragment length polymorphisms obtained by primer pairs, combined with restriction enzymes, allow the detailed study of intraspecific variability and investigation of putative relationships between sampled genotypes. The details of amplification and restriction digestion conditions are described in Dumolin – Lapégue et al. (1997) and Demesure (1999). Seven cpDNA primer pairs HK, KQ, DT, ST, VL, CS and SFM, described originally for *Sorbus torminalis* by Demesure (1999) were tested. Amplifed products of these primers were digested with the restriction enzymes Alu I, Dde I, EcoR I, Hae III, Hinf I, Mse I and ScrF I. The restriction fragments were separated by overall 4 hours of electrophoresis on 8% polyacrylamide gel (Dumolin – Lapégue et al. 1995). The analysed samples revealed at KQ digested by Mse I seven, and at KQ digested by Hinf I four polymorphic bands, enabling the description of different cpDNA haplotypes.

2.3 Data evaluation

Genetic structures were characterized by population genetic parameters derived with GSED (Gillet 1998-2010) and GenAlEx (Peakal and Smouse 2005). Distances among haplotypes were calculated with NTSYS 4.0 beta (Sinauer Assotiation 1998) represented by TreeView (Page 2001). For diversity analysis the programs HAPLONSTAT and HAPERMUT were utilised (Pons – Petit 1996).

The diversity parameter v - which is usually applied for haplotype genetic interpretation and analysis - is defined as follows (Pons - Petit 1996):

$$v = \sum_{i,j} \pi_{ij} \times x_i \times y_j$$

where π_{ij} is the distance between haplotypes *i* and *j* and x_i and y_j are observed frequencies. '*h*' is calculated by ignoring the genetic distance of two haplotypes. The resulting coefficients of differentiation are defined as N_{ST} (based on v), or G_{ST} (utilising *h* diversity).

The N_{ST} and G_{ST} values are directly comparable by using permutation analysis, and their difference can be tested against 0 (Burban et al, 1999). Accordingly, after 1000 repetitions, the N_{ST} value is recalculated. Afterwards it is assessed whether the new value after certain repetitions is larger than G_{ST} (one-tailed test).

The differentiation parameter D_j of a subpopulation *j* was obtained according to Gregorius and Roberds (1986):

$$D_j = d_0(p(j), \overline{p}(j)) = \frac{1}{2}\sum_{i=1}^{n} \left| p_i(j) - \overline{p}_i(j) \right|$$

Here $\overline{p}_i(j)$ describes the relative frequency of the *i*th allele among the pooled material of all included samples except the *j*th. Thus, D_j indicates whether sample *j* represents the complete set of samples (= relatively low value) or has a special genetic setup as compared to the other samples (= relatively high value).

The δ gives the mean of the differentiation parameters D_j, weighted with the proportions c_j of the different samples (Gregorius 1984):

$$\delta = \sum_{j=1}^{n} c_j \cdot D_j$$

The total population differentiation δ_T (Gregorius, 1987, 1988) quantifies the amount of genetic variation within a single sample:

$$\delta_T = \frac{N}{N-1}\left(1 - \sum_{k=1}^{n} p_k^2\right)$$

The genetic distance d_0 between subpopulations was calculated inter alia following Gregorius (1974) and Prevosti et al. (1975). It quantifies the proportion of genetic elements which the two subpopulations do not share.

$$d_0(i,j) = \frac{1}{2}\sum_{k=1}^{n}\left|p_{ik} - p_{jk}\right|$$

Here i and j denote the subpopulations and p_{ik} the relative frequency of the k^{th} type (allele, genotype) in subpopulation i. The genetic distances following Nei (1972) were calculated as well.

The value of smallest genetic difference $\Delta(s)$ is based on the consideration that it quantifies the change to be made in the frequency of the trait states in one population in order to match the frequency distribution of the second population, under the requirement that trait states be shifted to the most similar trait state possible. The minimum change in the sense of linear programming is relevant, taking into account the characteristics of the place of traits during optimization (smallest difference) through the application of the principle (Gillet et al. 2004). Therefore:

$$\Delta(s) = \sum_{a,b} s(a,b)d(a,b)$$

$$\Delta = \min_s \Delta(s)$$

3 RESULTS

Polymorphisms were observed by means of PCR-RFLPs. The primer pair KQ was used to amplify the non-coding region between trnK and trnQ of the chloroplast genome. The amplified fragment was digested with the restriction enzymes *Mse* I and *Hinf* I and visualised on polyacrylamid (PAA) gel. Eleven polymorphic restriction fragments were observed, classifying the 196 studied trees into 16 different haplotypes (*Figure 2, 3 and Table 2*).

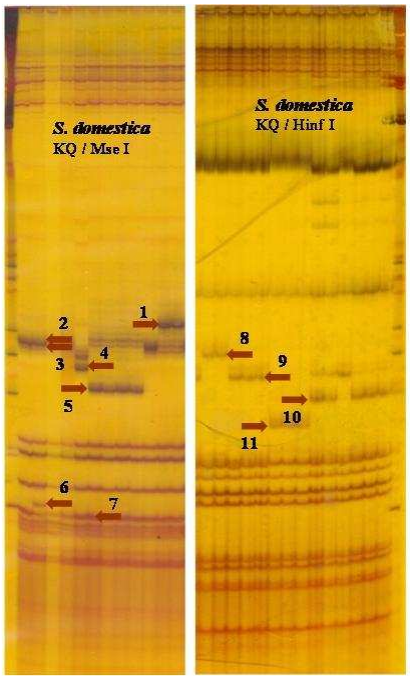

Figure 2. The RFLP restriction patterns of primer - enzyme combinations KQ - Mse I (1-7) and KQ – Hinf I (8-11) on PAA-gel. Arrows indicate the detected polymorphic bands/products.

The distances between haplotypes were calculated utilising the PAUP 4.0 beta program (Swofford 2002). From the resulting haplotype distance matrix a tree-stucture grouping without roots was constructed (Structural grouping unrooted option, program: TreeView, Page 2001). The resulting UPGMA diagram is shown in *Figure 3.*

Table 2. Presence (1) or absence (0) of the corresponding bands 1 to 11 of S. domestica cpDNA-haplotypes (I - XVI)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
I	0	0	0	1	0	0	1	0	1	0	0
II	0	0	1	1	0	0	1	0	0	1	0
III	0	0	1	1	0	0	1	0	1	0	0
IV	0	0	1	1	0	0	1	1	1	0	0
V	0	1	0	0	1	0	1	0	0	1	0
VI	0	1	0	0	1	0	1	0	1	0	0
VII	0	1	0	0	1	0	1	1	0	0	0
VIII	0	1	1	0	0	0	0	0	1	0	0
IX	0	1	1	0	0	0	0	1	0	0	0
X	0	1	1	0	0	0	1	0	0	1	0
XI	0	1	1	0	0	0	1	1	0	0	0
XII	0	1	1	0	0	0	1	1	0	1	0
XIII	0	1	1	0	0	1	1	1	0	0	0
XIV	0	1	1	0	1	0	1	0	0	1	0
XV	0	1	1	0	1	0	1	0	0	1	1
XVI	1	1	1	0	0	0	1	0	0	0	0

3.1 Spatial distribution of cpDNA haplotypes

Haplotype “XI – Green” on the left upper arm of *Figure 3* is the generally distributed haplotype, prevalent in the Zemplén Mts. The haplotype was represented in 47% of the analysed samples. The “III – Blue” haplotype (on the upper right branch in *Figure 3*) represents 15% of the samples. The "V – Gray” haplotype on the lower right arm is present in 12% of the sampled specimens. The “III - Blue, V - Gray, XI – Green”, and “XIV – Pink” haplotypes were observed in both of the geographically distant regions.

Table 3. S. domestica cpDNA haplotypes in the studied populations (see Figure 3).

	Haplotypes	*Population code*								
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
I	Hatched light blue	0	0	0	0	0	0	0	2	0
II	Light blue	0	0	0	0	0	0	0	1	1
III	**Blue**	**2**	**0**	**0**	**0**	**0**	**1**	**15**	**9**	**2**
IV	Dark blue	0	0	0	0	0	0	2	0	0
V	**Gray**	**2**	**0**	**0**	**0**	**0**	**0**	**8**	**7**	**6**
VI	Yellow	0	0	0	0	0	0	0	0	1
VII	Orange	0	0	0	0	0	0	1	1	0
VIII	Black	0	0	0	0	0	0	0	1	0
IX	Hatched brown	0	0	0	0	0	0	0	1	0
X	Hatched pink	0	0	0	0	0	0	0	0	1
XI	**Green**	**21**	**17**	**27**	**8**	**6**	**4**	**3**	**7**	**0**
XII	Claret	0	0	1	0	0	0	0	0	0
XIII	Light green	2	0	3	3	1	0	0	0	0
XIV	Pink	0	0	3	0	0	4	1	0	3
XV	Red	1	0	9	4	2	0	0	0	0
XVI	Brown	0	0	0	0	0	2	0	0	0

Most populations include several (from 3 to 8) cpDNA haplotypes. The population of the vineyards of Kácsárd was fixed for a single haplotype which was common in other populations of the Zemplén mountains ("XI – Green"). The reason may be sought in the cultivation effect supporting the propagation of a single local haplotype (*Figure 4*). Comparing the regions, the characteristic "main" haplotypes are different (types "III – Blue" and "XI – Green" have an alternating dominance by region), or are typical for only one population. The Hegyalja, Tokaj and Meződěl populations, as well as Hegyköz contain exclusive haplotypes which occur only in the eastern, Zemplén region ("XV – Red, XIII – Light Green").

A large number of otherwise rare haplotypes ("I – Hatched light blue; II – Light blue; IV – Dark blue; VI – Yellow; VII – Orange; VIII – Black; IX – Hatched brown; X – Hatched pink") were observed in three populations from the Danube Bend (Pilismarót, Visegrád, Szentendre: *Table 3*, *Figure 4*). The population Nr. 6 of Buda Hills contains haplotypes which are typical and dominant haplotypes for both regions ("III – Blue, XI – Green, XIV – Pink") and has a private haplotype ("XVI – Brown") as well.

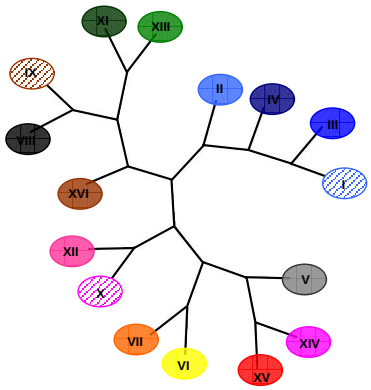

Figure 3. Unrooted UPGMA diagram of the service tree cpDNA-haplotypes (after Page 2001). The colours are corresponding to Figure 4.

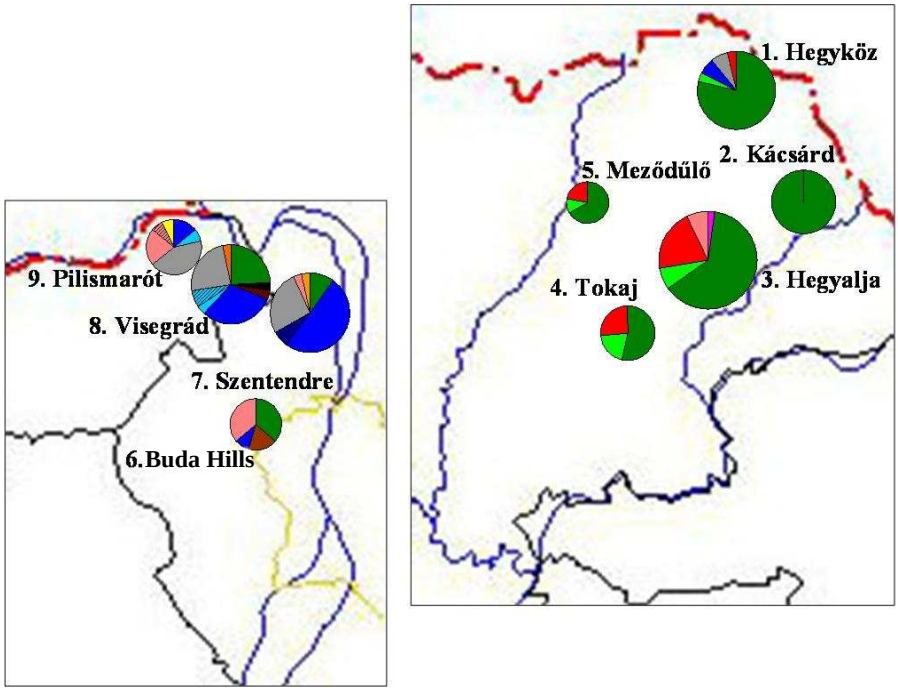

Figure 4. The spatial distribution of S. domestica cpDNA-haplotypes in the Dunazug (left) and Zemplén (right) Mountains. The circle diameters are relative to sample sizes

Considering the cpDNA-haplotype composition of the studied populations, it may be deduced that the populations dominantly indicate a mixed origin, containing numerous cpDNA haplotypes. Among these haplotypes, dominant and codominant or accompanying types are distinguishable within populations or regions. Typically, the main or dominant haplotype within a region could occupy the role of codominant haplotype in the other region.

Observed patterns of cpDNA diversity confirm the importance of endozoochorous seed dispersal and typical extinction/recolonisation dynamics. Results indicate considerable gene flow by seeds among populations within regions resulting in a high diversity within populations and sharing of haplotypes within the region, but very limited gene flow between the two regions.

An analysis of molecular variance revealed that 27% of the total variation is distributed between the two regions, 6% among populations within regions, and the remaining 67% within populations. Strong differentiation was observed between the two regions, with only 4 common haplotypes.

3.2 The cpDNA haplotype diversity and differentiation among populations

A major proportion of the total diversity (h_t = 0.752) is contributed by intrapopulation diversity (h_s = 0.583; *Table 4*). Total diversity (h_t) values are similar in the two regions (0.838 for Zemplén and 0.852 for Dunazug), however the intrapopulation differentiation is remarkably higher in the Dunazug than in the Zemplén populations (h_s = 0.761 vs. 0.678) due to other, private and rare haplotypes. The high intrapopulation differentiation indicates that a large number of mother plants participated in founding the populations.

The level of population subdivision using unordered and ordered alleles resulted G_{ST} = 0.225 and N_{ST} = 0.245, respectively, for the whole material. Analysing the two regions separately the values of population subdivision were less pronounced (*Table 4*). In the Zemplén region the G_{ST} value (0.191) was higher than N_{ST} (0.146) which is exceptional. The differences between N_{ST} and G_{ST} were not significant neither separately for the two regions, nor for all samples (U-test).

Table 4. Diversity and differentiation of S. domestica populations. Standard deviations are in parenthesis

Region	No. of populations (≥ 3 individuals)	Harmonic mean No. of individuals per populations	Number of haplotypes	h_s (standard error)	h_t (standard error)	G_{ST} (standard error)	vs (standard error)	vt (standard error)	N_{ST} (standard error)	U Nst/Gst test
Zemplén Mountains	5	21.11	9	0.678 (0.0763)	0.838 (0.0879)	0.191 (0.0135)	0.709 (0.0748)	0.830 (0.0345)	0.146 (0.1046)	-1.23 NS
Dunazug Mountains	4	17.38	13	0.761 (0.0269)	0.852 (0.0272)	0.106 (0.0303)	0.738 (0.0657)	0.858 (0.0472)	0.140 (0.0723)	0.43 NS
Overall	9	17.12	16	0.583 (0.0835)	0.752 (0.0831)	0.225 (0.0577)	0.569 (0.0932)	0.754 (0.1121)	0.245 (0.0497)	0.25 NS

NS: not significant

3.3 Genetic differentiation comparing the representativity of populations

If each service tree population is compared with the complementary material, representativity or presence of special types can be evaluated. The δ (average differentiation) value is quite high (0.51), which might be explained by the maternal inheritance, the geographical segregation, and the significant number of polymorphisms. The most representative population – including the largest number of haplotypes – is the Hegyköz population, followed by Meződűlő and by Hegyalja as well as Tokaj (*Figure 5*).

Table 5. Genetic differentiation among the S. domestica populations (Program: GSED, author: Gillet 1998-2010)

	1. Hegyköz	2. Kácsárd	3. Hegyalja	4. Tokaj	5. Meződűlő	6. Buda Hills	7. Szentendre	8. Visegrád	9. Pilismarót
C_j	0.143	0.087	0.219	0.077	0.046	0.056	0.153	0.148	0.071
D_j	0.351	0.575	0.431	0.431	0.417	0.508	0.686	0.531	0.714

C_j: differentiaton proportionally to sample sizes, D_j: differentiation versus the complementary material

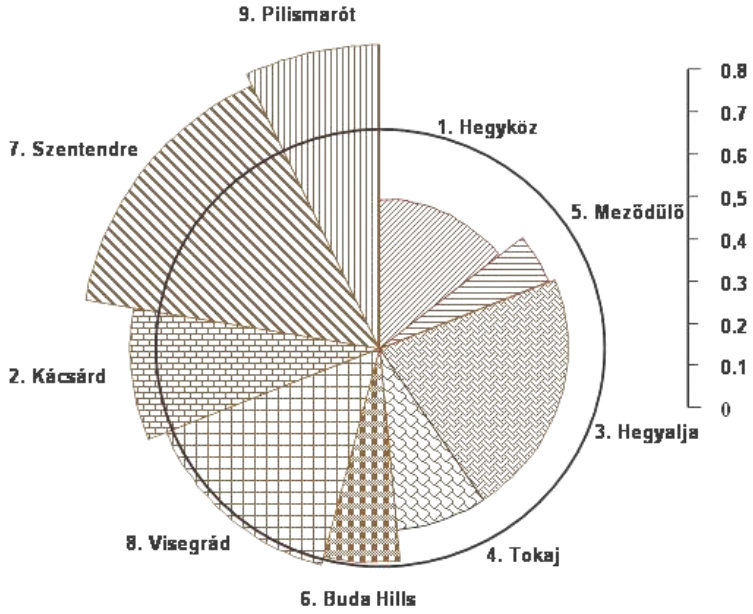

Figure 5. Genetic differentiation among populations based on cpDNA haplotypes after Gregorius and Roberds (1986). The circle displays the mean differentiation among populations (δ = 0.51)

According to the differentiation values, populations in Zemplén are more representative. Kácsárd, containing just one haplotype, is the exception. This locally cultivated cpDNA type is an autochthonous and well distributed haplotype in the Zemplén Mountains.

The differentiation of the Buda Hills population (D_j = 0.508) corresponds to the mean value among populations. It contains haplotypes commonly found in both regions and has a private haplotype: this is also reflected by the 'intermediate' position in the dendrogram (*Figure 8*).

The other populations in the Danube Bend (Visegrád, Szentendre, Pilismarót) north of Budapest, show only a lower representation (D_j > mean δ) caused by the appearance of 'new' and rare haplotypes (*Figure 5*).

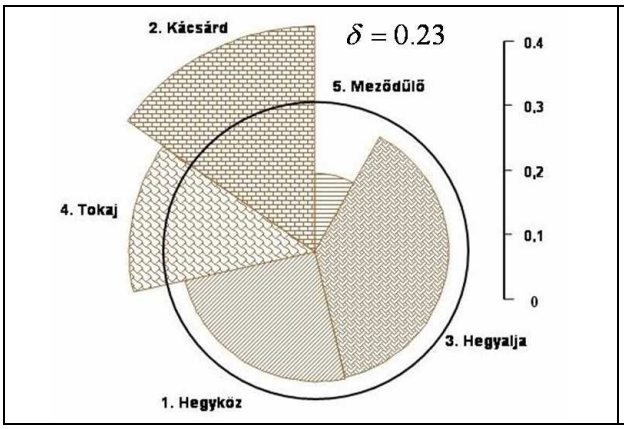

Figure 6. Genetic differentiation among the Zemplén Mts. populations (see Figure 5 for explanations)

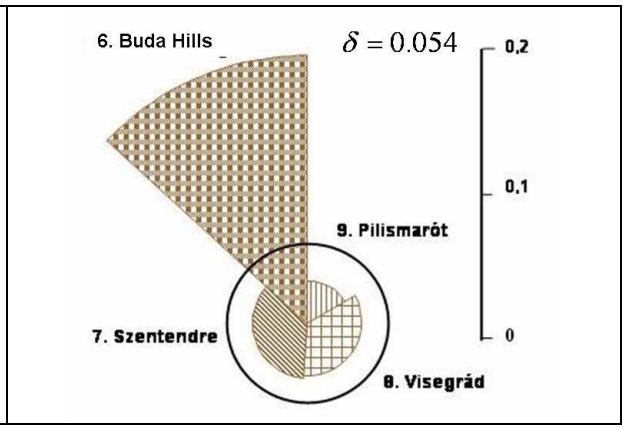

Figure 7. Genetic differentiation among the Dunazug Mts. populations.

When evaluating the Zemplén and Dunazug regions separately, the calculated mean differentiation values are lower (δ = 0.23 and δ = 0.054 respectively, *Figures 6 and 7*) compared to the pooled analysis. The reason is the regional separation regarding the main haplotypes (see *Figure 4*), which determines a relatively higher homogeneity within both regions. In the Zemplén Mountains the population of the vineyards of Kácsárd is the least representative due of its single haplotype. The populations of Hegyköz and Hegyalja with a large number of sampled trees and haplotypes have close to average differentiation (*Figure 6*). The Tokaj and Meződűlő display the same haplotypes but their proportion varies. This fact has a remarkable influence on the differentation calculation.

In the Dunazug region, the Buda Hills population is deviant and considerably increases the value of the average differentiation. The other populations have high representativity.
The mean differentiation values (δ) are analogous to the N_{ST} and G_{ST} population differentiation *(Table 4).*

3.4 Genetic distances among populations

Genetic distances among the 9 subpopulations were calculated according to Nei (1972), Gillet et al. (2004, smallest genetic difference), and Gregorius (1974) (software: GeneAlex /Peakall – Smouse 2006/ and GSED /Gillet 1998-2010/). When comparing the geographic and genetic distances, the Buda Hills population shows deviant values (underlined in *Table 6*).

The distances (after Gregorius 1974) are less in the eastern Zemplén region than in the Danube Bend region (*Table 6*).

The intermediate situation of the Buda Hills population was previously described: it includes main or typical haplotypes from both regions and is remarkably differentiated from the other, Danube Bend populations within the region (*Figure 7*). Based on the distances d_0 after Gregorius (1974), a cluster analysis of the analysed populations was executed (*Figure 8*). Within the SAHN (Sequential, Agglomerative, Hierarchical, Non-overlapping) classification the 'single linkage' method separates, based on the principle of minimum differences. The clustering using the single linkage method is clearly taking into consideration the geographic differentiation as well.

Table 6. Matrix of genetic distances (after Gregorius 1974) (left lower triangle) and geographic distances (right upper triangle) of the sampled populations

Geographic distance (km)

Hegyköz	Kácsárd	Hegyalja	Tokaj	Meződűlő	Buda Hills	Szentendre	Visegrád	Pilismarót	
—	15.227	29.488	41.572	16.573	*217.011*	*201.681*	*209.026*	*213.904*	Hegyköz
0.250	—	19.893	31.398	22.184	*215.666*	*201.259*	*209.035*	*214.263*	Kácsárd
0.267	0.372	—	12.119	22.761	*197.153*	*183.283*	*191.298*	*196.733*	Hegyalja
0.360	0.467	0.188	—	33.063	*188.885*	*175.710*	*183.983*	*189.638*	Tokaj
0.226	0.333	0.093	0.133	—	*200.520*	*185.121*	*192.454*	*197.334*	Meződűlő
0.565	*0.636*	*0.567*	*0.636*	*0.636*	—	19.551	20.291	24.208	Buda Hills
0.757	*0.900*	*0.867*	*0.900*	*0.900*	0.776	—	9.534	16.516	Szentendre
0.616	*0.759*	*0.759*	*0.759*	*0.759*	0.668	0.315	—	7.020	Visegrád
0.857	*1.000*	*0.930*	*1.000*	*1.000*	0.695	0.557	0.581	—	Pilismarót

Genetic distance

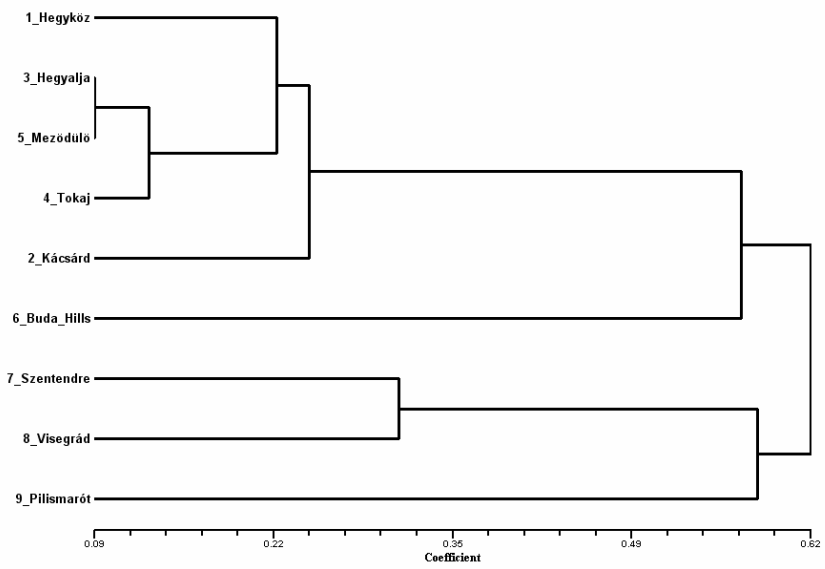

Figure 8. Single linkage dendrogram of nine S. domestica populations, based on the genetic distance d_0 after Gregorius (1974)

In our clustering, using the distances in *Table 6*, the Buda Hills was always grouped to the eastern region. It seems, that the Danube Bend populations constitute a distinctly different region regarding their haplotype composition.

4 DISCUSSION

The cpDNA analysis of this insect-pollinated, scattered species is well suited for gene flow analysis based on seed dispersal. The fruit has numerous consumers (especially thrush species) which transport the seed over greater distances and through the digestion process neutralise the effects of germination inhibitors which are in the fruit flesh (Yagihashi et al. 1998).

Oddou-Muratorio et al. (2001/c) distinguished 19 wild service tree (*S. torminalis*) haplotypes through Europe based on 7 cpDNS and 3 cpSSR primer pairs. Two main haplotypes were commonly occurring. In every population also minor or rare haplotypes were found.

In our study, regionally different major haplotypes and minor types occur as well in every analysed *S. domestica* population.

The G_{ST} values of *S. domestica* are extremely low: 0.106 (for the Dunazug region) < G_{ST} < 0.225 (for the two regions). Results for *S. torminalis* are similar: 0.13 < G_{ST} < 0.35 (Oddou-Muratorio et al. 2001/b, Oddou-Muratorio et al. 2001/c).

Wild service tree (*S. torminalis*) populations sampled over the eastern and south-eastern part of its range were studied with seven, presumably neutral nuclear microsatellite markers (Kučerová et al 2010). The differentiation level was relatively high (F_{ST} = 0.228), which comes close to our cpDNA results for a species with a fragmented occurrence at its limits.
Mohanty et al. (2002) found G_{ST} = 0.29 for the similarly insect pollinated and scattered *Prunus avium* among 23 European populations based on 16 described haplotypes.

These values appear to be lower than those for widely distributed social broadleaved species such as *Q. petraea*: G_{ST} = 0,82 (Dumolin-Lapegue et al. 1997); or G_{ST} = 0.835 (Petit et al. 2002); and *F. sylvatica*: G_{ST} = 0,83 (Demesure et al. 1996). The cpDNA markers show strong differentiation among social broadleaved populations and low levels of within population diversity. Conventional cpDNA primers shows for insect pollinated wild fruit species, that these populations are regularly fixed for numerous haplotypes. The presence of more – main and minor – haplotypes suggests numerous founders also through long distance seed dispersal.

The spatial distance between the two regions exceeds 200 km, therefore the structural differences between the Danube Bend area and Zemplén are remarkably high. The lack of additional or 'bridge' data between the two regions influences the evaluation and the interpretation as well. The reasons for the extraordinary structure of Buda Hill population could not be clarified undoubtedly, whether it is of natural origin or a product of human impact or cultivation. The influence of cultivation on the long distance dispersal is so far also not clear.

S. torminalis cpDNA results reveal, that a significant but slight geographical haplotype pattern was observable approximately up to a distance of 100 km (Oddou-Muratorio et al. 2001/a). The described cpDNA pattern was compared to isozyme-based (biparentally inherited and codominant) results, analysing the same plant material. Neither pollen nor seed dominated gene flow was clearly observable in the population structure of that species.

There are, however, countless examples for anthropogenous dispersal or cultivation effects on *Sorbus* species (Kárpáti 1959/60, Gräter 1996, 1997, Gyulai 2001). Kárpáti (1959/60) refers to Holuby's (1888) vascular flora description of Trencsén County. Trencsén[1] County is situated over the northern limit of service tree distribution. Holuby reported on centuries-old service trees. He explained: in case when accidentally detected seedlings were found in forests, which originated from seeds spread by birds, these were removed and planted at the edges of arable land. At the sites of abandoned orchards or vineyards, often established on forest clearings, these trees can still be found, putative remnants of transplanted specimens.

A very convincing example for local anthropogenous dispersal is the occurrence in the vineyards of Kácsárd, which belongs to the historic Tokaj-Hegyalja vine region. Here only the major and generally distributed ("XI – Green") haplotype was found.

Based on the communications of Boros (1944 in: Kárpáti 1959/60) the service tree in the Buda Hills is subspontaneous and distributed by birds from nearby forests. Our sampled trees in the Buda Hills are situated in private gardens, in former vineyards and in forested areas as well. Cultured habitats are in any case more frequent in the Buda Hills, than at other locations of the Danube Bend. On the other hand, occurrences in Zemplén are predominantly cultured habitats, but no exceptional or unique cpDNA haplotypes were found there.

[1] Presently Trenčin, N-W Slovakia

5 CONCLUSIONS FOR CONSERVATION AND FORESTRY PRACTICE

Among the main species of the genus *Sorbus*, *S. domestica* requires more ex situ gene preservation, while for wild service tree (*S. torminalis*) in situ conservation methods seem sufficient for gene conservation, because this species is less endangered (Demesure 1997). In the present study genetically polymorphic populations containing unique haplotypes were detected, providing important information for forest management, gene conservation and nature protection activities of *S. domestica*.

The service tree remains rare in closed forests, and should be planted in forest edges, hedges or clearings for game food (Vancsura 1992). From the aspect of nature protection, it is important to monitor the seedling recruitment and development in regenerated stands, and where necessary, to plant artificially.

The described genetic inventory was the preparation for gene conservation breeding of *S. domestica* in both regions. Ex situ conservation measures, i.e. the planting of two grafted seed orchards for gene conservation is in preparation. The gene conservation units will represent all identified genotypes of the respective regions. Minimum 3 grafted ramets per genotype will be outplanted. The frequency of genotypes will vary according to phenotypic trait differences, following the principles of gene conservation breeding for different traits such as fruit, seed or trunk quality. The varying representation of different genotypes in the seed orchards will be based on the rating of reproductive contribution which was first applied in Hungary, in Scots pine breeding (Bánó et al. 1978).

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