



Leszek Bednorz, Łukasz Myczko, Piotr Kosiński

An assessment of genetic diversity following natural regeneration of *Sorbus torminalis* in the Forest District of Jamy (northern Poland)

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Abstract: The subject of the study was a population of *Sorbus torminalis* resulting from spontaneous regeneration from seeds in northern Poland at the north-eastern limit of the species distribution. The population, occupying a fenced plot of 1.72 ha in the Forest District of Jamy, amounted to 579 individuals of variable age classes up to about 19 years old. Six polymorphic gene loci: MDH-B, ME-A, 6PGD-B, ADH-B, PGM-A, PGM-B were studied in 532 individuals recruited post-fencing and 21 potentially parental, adult trees. The analysis showed similarly high levels of genetic diversity within both subsets of individuals but there was also a noticeable change in genetic structure between them. Detailed genetic analysis of the post-fencing recruits revealed lack of significant differences between different age groups based on DBH within the fenced plot.

Additional key words: wild service tree, small population, *in situ* conservation

Addresses: L. Bednorz, Poznań University of Life Sciences, Department of Botany, Wojska Polskiego 71 C, 60-625 Poznań, Poland, e-mail: lbednorz@up.poznan.pl

Ł. Myczko, Poznań University of Life Sciences, Department of Zoology, Wojska Polskiego 71 C, 60-625 Poznań, Poland

P. Kosiński, Polish Academy of Sciences, Institute of Dendrology, Parkowa 5, PL-62-035 Kórnik, Poland; Poznań University of Life Sciences, Department of Botany, Wojska Polskiego 71 C, 60-625 Poznań, Poland

Introduction

The wild service tree *Sorbus torminalis* (L.) Crantz is a typical forest admixture species with a scattered distribution in western, central and southern Europe, in south-western Asia and north-western Africa (Rasmussen et al. 2004a). The species is a component of various oak, sometimes beech or pine-dominated, forests where it is usually found at low densities (Demesure-Musch and Oddou-Muratorio 2004). Wild service tree reproduces mainly

generatively from seeds, although vegetative reproduction, by root suckers is also common in natural populations, especially at the distribution border in north-eastern Europe (Rasmussen and Kollmann 2004b, 2007, 2008; Hoebee et al. 2006). The seeds of *S. torminalis* are dispersed by frugivorous birds and mammals.

In Poland, the species is rare and it has been protected by law since 1946. Currently, it occurs on 84 natural localities, with only eight populations containing more than 100 individuals (Bednorz 2010).

Until recently, only passive protection mechanisms have been applied in Poland and these were, unfortunately, unsuccessful (Bednorz 2007, 2009). At present, active protection of *S. torminalis* including both *in situ* and *ex situ* measures is more often implemented in Polish forests but until now this has only occurred at local or regional level (Bednorz 2007, 2009, 2010; Zwierzyński and Bednorz 2012). Both European and Polish conservation strategies for the species underline that for protection of genetic resources of the wild service tree *in situ* measures are the most important (Demesure-Musch and Oddou-Muratorio 2004; Bednorz 2007, 2009).

The Forest District of Jamy was the first local area which implemented active protection of *S. torminalis* in Poland. In 1995 Forest managers fenced a plot with abundant seedlings of the species to protect them from browsing. An inventory of the post-fencing establishment made in 2000 showed the occurrence of 360 individuals of 0.5–3 m height (Tarnawski 2001). In a demographic context the undertaking proved to be a success.

To promote natural regeneration as one of the basic tools in conservation of the species *in situ*, managers need to know the consequences of such activities for preservation of genetic polymorphism in natural populations. Although genetic diversity and the level of polymorphism in *S. torminalis* populations from eastern extremes of its range is well examined (Bednorz et al. 2006; Kučerová et al. 2010), there is a complete lack of data concerning changes of genetic structure in small and isolated populations during their natural regeneration. Loss of genetic diversity in such populations can affect population viability

and lower their capacity to respond to environmental changes (Ellstrand and Elam 1993; Kramer et al. 2008; Bacles and Jump 2011).

Hence, the aim of this study was to assess the genetic diversity following natural regeneration of a *Sorbus torminalis* population, derived from a small population of parental trees and to analyse the results in conservation context.

Materials and methods

The study plot of 1.72 ha is located in the area of Forest District of Jamy (18°51'31" E, 53°35'28" N). The undulating terrain, located at an altitude of 80–92 m above sea level and slightly (3%) inclined to the south-west, is a part of the edge of moraine upland. The type of soil is podzolized rusty soil, the type of habitat is mixed deciduous forest currently consisting of a low density oak-hornbeam forest (*Tilio-Carpinetum*) dominated by pine (*Pinus sylvestris*). The plot was fenced in 1995 to protect *Sorbus torminalis* seedlings from browsing. The only adult wild service tree growing on the plot died a few years later. In 2011, a total of 579 young wild service tree individuals (variable age, up to about 19 years old) were inventoried during surveys of the fenced experimental plot in 2011 (Bednorz et al. 2012).

To test the impact of duration of protection against large herbivores we divided the recruits into four age groups based on DBH: 1. up to 3.0 cm; 2. 3.1–6.0 cm; 3. 6.1–9.0 cm; 4. over 9.1 cm. Diameter at breast high was used as a surrogate for age of

Table 1. Genetic diversity in recruits and adult trees of *Sorbus torminalis*

Locus	N_a	N_e	I	H_o	H_e	uH_e	F
Post-fencing recruits (N=532)							
MDH B	2.000	1.928	0.674	0.507	0.481	0.482	-0.053
ME	4.000	2.867	1.190	0.667	0.651	0.652	-0.024
6PGD	2.000	1.658	0.586	0.406	0.397	0.397	-0.024
ADH	3.000	2.174	0.871	0.532	0.540	0.541	0.015
PGM A	2.000	1.011	0.035	0.008	0.011	0.011	0.330
PGM B	2.000	1.015	0.044	0.015	0.015	0.015	-0.008
Mean	2.500	1.776	0.567	0.356	0.349	0.350	0.039
SE	0.342	0.292	0.187	0.114	0.112	0.112	0.059
Adult trees (N=21)							
MDH B	2.000	1.893	0.665	0.762	0.472	0.483	-0.615
ME	4.000	2.571	1.083	0.857	0.611	0.626	-0.403
6PGD	2.000	1.265	0.365	0.238	0.210	0.215	-0.135
ADH	3.000	2.513	0.992	0.762	0.602	0.617	-0.266
PGM A	2.000	1.170	0.276	0.158	0.145	0.149	-0.086
PGM B	2.000	1.153	0.257	0.143	0.133	0.136	-0.077
Mean	2.500	1.761	0.606	0.487	0.362	0.371	-0.264
SE	0.342	0.271	0.149	0.139	0.092	0.094	0.087

N_a – number of alleles per locus, N_e – effective number of alleles, I – Shannon's information index, H_o – observed heterozygosity, H_e – expected heterozygosity, uH_e – unbiased expected heterozygosity, F – fixation index (inbreeding coefficient), SE – standard error.

Table 2. Mann–Whitney U test of mean individual heterozygosity across loci for groups of recruits and adult trees

Rank Sum recruits	Rank Sum adults	U	Z (Z adjusted)	p-value (p-value adjusted)	Valid N recruits	Valid N adults
144878.0	8303.000	2099	-3.46086 (-3.51004)	0.000539 (0.000448)	532	21

trees (Madéra et al. 2012). Genetic variation between the post-fencing recruits and those of adult trees growing up to 3 km from the experimental plot was investigated using isozyme markers. Dormant buds were taken from 532 recruits and 21 adult trees. The buds were ground in the tris-HCl buffer pH 7.5 (Wendel and Weeden 1989). The enzymes were separated in 12% starch gel, prepared in tris-citrate electrode buffer pH 7.0, and histidine-tris gel buffer pH 7.0 (Conkle et al. 1982; Wendel and Weeden 1989). The extracts were stored at -80°C until the electrophoretic analysis. Five polymorphic enzyme systems were analysed: MDH, E.C. 1.1.1.37 (malate dehydrogenase); ME, E.C. 1.1.1.40 (malic enzyme); 6PGD, E.C. 1.1.1.44 (6-phosphogluconate dehydrogenase); ADH, E.C. 1.1.1.1 (alcohol dehydrogenase); PGM, E.C. 5.4.2.2 (phosphoglucomutase). The alleles were numbered from the fastest to the slowest. Standard staining procedures with some minor modification were used (Rothe 1994). Genotypes were scored at six polymorphic gene loci: MDH-B, ME-A, 6PGD-B, ADH-B, PGM-A, PGM-B.

The GenAlEx 6.5 (Peakall and Smouse 2006, 2012) computer program was used to estimate the main genetic parameters: N_a – the average number of alleles per locus; N_e – the effective number of alleles per locus; H_o , H_e , uH_e – the observed, expected and unbiased expected heterozygosities; I – Shannon's information index; F – inbreeding coefficient, F_{st} – fixation index (via AMOVA, with permutational test for calculating probability value). The Chi-squared test was used to check for differences in allele frequencies at all examined loci between the post-fencing recruits

and adult trees, and between DBH classes of recruits. The Mann–Whitney U and Kruskal–Wallis nonparametric tests were performed in Statistica 10 software (StatSoft Inc. 2011) for comparing mean individual heterozygosities across loci for the chosen groups.

Results

Genetic diversity and structure in recruits and adult trees

Comparing the group of parental trees and the post-fencing recruits group showed no differences in the percentage of polymorphic loci (100%) as well as the number of alleles per locus. The effective number of alleles was also similar in both groups. There was modest but statistically significant difference in observed heterozygosity, but at the same time expected heterozygosities were very similar (Tab. 1–2, Fig. 1). This dissimilarity between H_o and H_e in the case of adult trees were reflected in distinctly lower inbreeding coefficient in this group (Tab. 1).

Analysis of genetic structure of post-fencing recruits and adult trees showed that all alleles were found in both groups, although in different frequencies and that these two groups of specimens differed statistically significantly in allele frequencies in all examined loci except ME and ADH (Tab. 3). Estimate of F_{st} (0.058) was statistically significant, indicating differentiation between the compared groups (Tab. 4).

Impact of age on the genetic structure of the recruits

Comparing genetic diversity among recruits from four age groups showed some differences in the number of alleles (Tab. 5, 7). We found only two significant differences in allele frequencies between DBH (age) dependent groups of recruits (Tab. 7). Never-

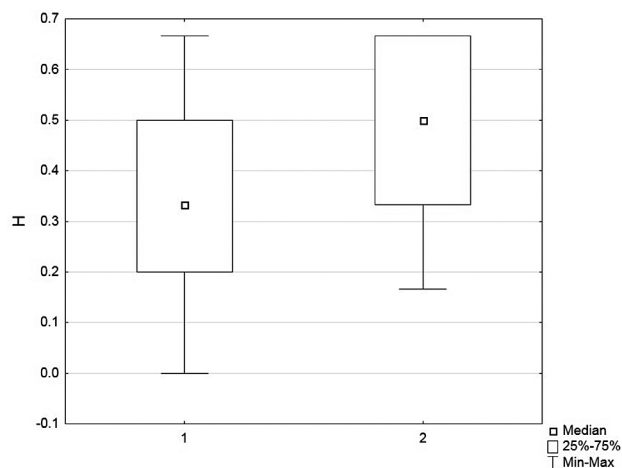


Fig. 1. Boxplot of mean individual heterozygosity across loci (H) by groups of recruits (1) and adult trees (2)

Table 3. Differences in allele frequencies between recruits and adult trees of *Sorbus torminalis*

Locus	df	χ^2	p
MDH	1	7.4419	p<0.01
ME	3	3.8174	n.s.
6PGD	1	4.8078	p<0.05
ADH	2	3.6219	n.s.
PGM-A	1	24.7086	p<0.01
PGM-B	1	17.0689	p<0.01

Table 4. Summary of F_{st} via AMOVA for recruits and adult trees

Source	df	SS	MS	Est. Var.	%
Among groups	1	7.043	7.043	0.073	6%
Within groups	1 104	1293.979	1.172	1.172	94%
Total	1 105	1301.022	–	1.245	100%

$F_{st} = 0.058$

p-value = 0.001

df = degrees of freedom, SS = variation (sum of squares), MS = variance (mean of squares), Est. Var. = estimated variance

theless, there were very small and statistically insignificant differences in the observed heterozygosities (Tab. 5–6, Fig. 2). Lack of differentiation between the DBH groups was confirmed also by very low estimate of F_{st} (Tab. 8).

Discussion

Conservation strategies for *Sorbus torminalis* prepared for Europe and Poland underline the great importance of *in situ* measures for protection of genetic resources of the species (Demesure-Musch and Oddou-Muratorio 2004; Bednorz 2007, 2009). *In situ* conservation measures are of course also fundamental for the physical persistence of local populations of *S. torminalis*, especially the small ones. Ensuring the potential for natural regeneration, especially regeneration involving sexual recombination, should be paramount in conservation strategies for these populations. In examining Danish populations of *Sorbus torminalis* on the northern distribution limit of the species, Rasmussen and Kollmann (2004b, 2007) observed its poor sexual reproduction abilities. In this context, an abundant natural regeneration from seeds in *S. torminalis* population on north-eastern limit of the species range, such the examined population in Jamy, was rather unusual.

In this study, high and comparable levels of genetic variation, both in a small population of adult trees and from numerous recruits originating from seeds were revealed. At all six loci, the same alleles

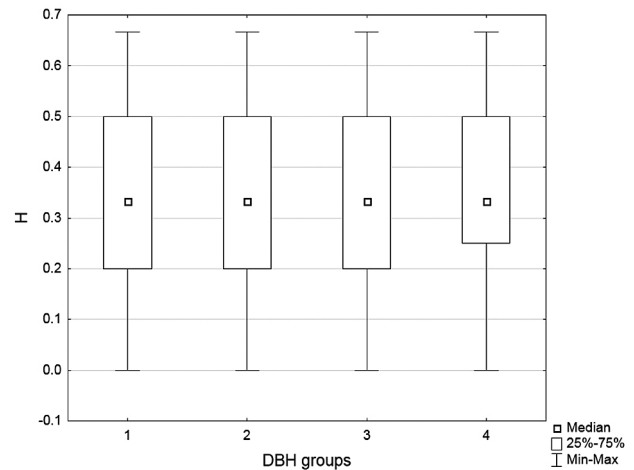


Fig. 2. Boxplot of mean individual heterozygosity across loci (H) by DBH groups

were found in both groups which indicates no loss in allelic diversity between the adult and recruit groups. However, the recruitment group was over 25 times more numerous than parental one and does not reflect a single cohort but rather the sequential recruitment of individuals through several years within a fenced plot.

Noticeable changes in genetic structure between two compared generations were found. They were manifested in relatively high value of F_{st} (0.058). Parental population displayed also distinctly higher observed heterozygosity. The estimated inbreeding coefficient for recruits was close to zero with very small deficiency of heterozygotes, indicating the genotypic frequencies in this group were close to Hardy-Weinberg expectations. This evidence leads to the conclusion that despite the small size of parental population, no effective signs of increased genetic inbreeding in the progeny occurred. The apparent modest excess of heterozygotes (negative F value) observed in the mature populations may be explained by the overdominance hypothesis (better fitness of heterozygous specimens) or selection against deleterious alleles found in homozygotes arising from kinship mating and their elimination from popula-

Table 5. Genetic diversity in DBH (age) depending groups of recruits; means and SE (in brackets)

DBH group	N_a	N_e	I	H_o	H_e	uH_e	F
1	2.167	1.805	0.557	0.353	0.351	0.352	0.005
	(0.477)	(0.314)	(0.196)	(0.121)	(0.116)	(0.117)	(0.033)
2	2.500	1.771	0.565	0.349	0.347	0.348	-0.011
	(0.342)	(0.293)	(0.187)	(0.110)	(0.111)	(0.112)	(0.024)
3	2.500	1.738	0.560	0.363	0.344	0.345	-0.042
	(0.342)	(0.269)	(0.181)	(0.113)	(0.108)	(0.109)	(0.017)
4	2.333	1.812	0.598	0.378	0.365	0.371	0.078
	(0.422)	(0.291)	(0.183)	(0.122)	(0.108)	(0.110)	(0.148)
Total	2.375	1.782	0.570	0.361	0.352	0.354	0.004
	(0.189)	(0.136)	(0.087)	(0.055)	(0.052)	(0.052)	(0.036)

Table 6. Kruskal-Wallis non-parametric ANOVA of mean individual heterozygosity across loci for DBH groups: H (3, N= 532) = 0.6595459, p =0.8827

DBH group	Valid N	Rank Sum	Mean Rank
1	137	36757.50	268.3029
2	185	48059.00	259.7784
3	176	47524.00	270.0227
4	34	9437.50	277.5735

tion by the time the trees reached the reproductive age. Stoeckel et al. (2006) found similar results in small stands of the self-incompatible *Prunus avium*, although they attributed it mainly to clonal propagation (asexuality effect). The frequencies of alleles in most cases significantly differed between the group of adult trees and recruit one. This may be explained by random changes caused by very limited number of individuals of parental generation (21 individuals) and the possibility of large differences in reproductive success between specimens of unique genotypes. This finding is similar to the results obtained in the studies of spatial genetic structure generated by random events (eg.: Epperson et al. 2001; Vekemans and Hardy 2004; Piotti et al. 2013).

We did not find clear differences between the DBH (age) groups: F_{st} was close to zero and they had very similar observed heterozygosities (Tab. 6, 8; Fig. 2). They differed only in allele frequencies of two loci (Tab. 7). These data might imply lack of distinct influence of the duration of reproduction time for genetic structure. However we should remember that different DBH classes included individuals belonging to cohorts from a few successive reproductive seasons.

The research of Biedenkopf et al. (2007) showed that sampling *S. torminalis* population for commercial harvest can result in significant reduction in the genetic diversity of the sampled group relative to the overall population. The example of the population at Jamy showed that natural regeneration even in a small population of wild service tree may be effective both in demographic and genetic aspects. It should encourage foresters to promote and protect natural regeneration of this rare species as one of the basic tools of conservation of the species *in situ*. The persistence of small populations of *S. torminalis* relies on effective protection of its offspring against browsing

Table 7. Differences in allele frequencies between DBH (age) depending groups of recruits

Locus	df	χ^2	p
MDH	3	1.922	n.s.
ME	9	8.758	n.s.
6PGD	3	1.052	n.s.
ADH	6	14.181	p<0.05
PGM-A	3	19.996	p<0.01
PGM-B	3	4.723	n.s.

Table 8. Summary of F_{st} via AMOVA for DBH groups

Source	df	SS	MS	Est. Var.	%
Among groups	3	3.279	1.093	0.000	0%
Within groups	1 062	1244.576	1.172	1.172	100%
Total	1 065	1247.856	-	1.172	100%

$F_{st} = 0.000$
p-value = 0.486

and eliminating an excessive competition from other tree species. Our results also show that even a few successive cohorts of recruits within fenced plot of considerable area seem to be sufficient to maintain genetic polymorphism of parental population.

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