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Impact of forest management on genetic diversity of *Quercus petraea* populations: a case study from the Křivoklátsko Protected Landscape Area (Czech Republic)

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Abstract. In the Křivoklátsko Protected Landscape Area (Czech Republic), the relation between genetic diversity and intensity of management was studied in 3 populations of *Quercus petraea* (Mattuschka) Liebl. (sessile oak). Microsatellite analysis was used to assess genetic diversity. The results indicate that differences between populations in average number of alleles per locus are so small that no genetic difference can be proved between them. The values of heterozygosity for all 3 studied populations confirm the absence of significant genetic differences among them.

Key words: *Quercus petraea*, genetic diversity, microsatellites, forest management

1. Introduction

Forest management has been used for a long time throughout most forest stands in Europe (e.g. Bradshaw 2004). To help protect the diversity of populations of forest woody species, information on how this management influenced their genetic variability should be known. We can anticipate that the narrow selection of seed-producing trees will result in a lower genetic variability of planted stands. Intensive forest management also introduces a risk of disturbing the populations' initial genetic structure as a result of planting the same species but of alien origin, i.e., seedling material from different geographic regions within the country, or from abroad. In the future, this can also result in outbreeding depression, as described e.g. by Woessner (1975).

Using DNA analysis allows for a more detailed view of genetically conditioned variability of populations, facilitating a more detailed study of genetic variability of populations based on molecular markers (Petit *et al*. 1993; Lexer *et al*. 1999; Wang & Szmidt 2001, etc.).

Quercus petraea (Mattuschka) Liebl. (sessile oak) – a widely distributed, economically important woody species in the Czech Republic and in most of Europe – was selected for this study. Several works have focused on the study of genetic structure and variability of its populations (Streiff *et al*. 1998; Finkeldey 2000; Bakker 2001; Cottrell *et al*. 2003). Till now, work in this field focused mainly on the assessment of the genetic diversity of natural populations. In our work, attention was paid to the relation between genetic diversity and management intensity, hence the level of the populations' autochthony.

Using the example of 3 populations of *Q. petraea* in the Křivoklátsko Protected Landscape Area, we examined whether any genetic differences exist between the populations, depending on management intensity.

2. Materials and methods

2.1. Localities of the populations studied

The Křivoklátsko Protected Landscape Area was selected to study the populations of *Q. petraea*. In this territory, within a relatively small area, we found various types of vegetation, including those of natural origin and planted forest stands. For this study, the following populations of *Q. petraea*, all to a certain extent influenced by human activities (forest management – see Fig. 1) were selected.

1. Brdatka Nature Reserve. The locality represents natural vegetation in the specially protected area, which

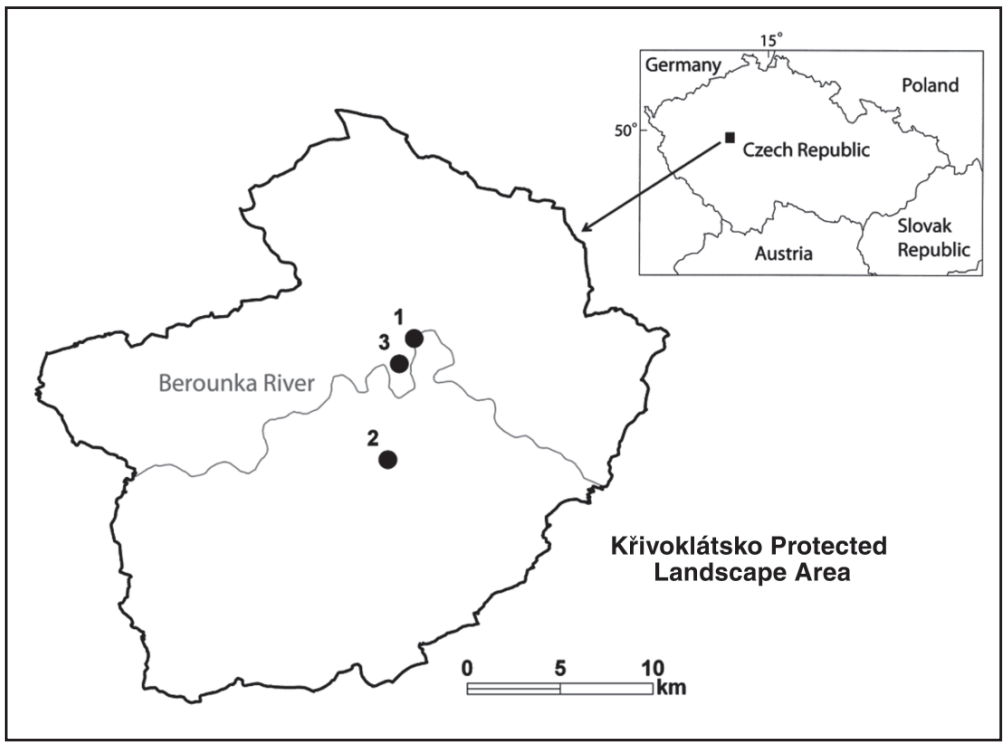

Fig. 1. Location of the studied sessile oak (*Quercus petraea*) vegetation in the Křivoklátsko Protected Landscape Area
Explanations: 1 = Brdatka National Nature Reserve, 2 = Červený kříž Nature Reserve, 3 = Křivoklát (vegetation planted near the town)

has not been markedly influenced by human activities. The habitat is located above the Berounka River, on an extremely jagged slope with a southern to southeastern exposure and occasional rock outcrops. In the area, the oak population forms a layered dwarf thermophilous oak forest that is classified as a typical wild service-oak forest (*Sorbo torminalis-Quercetum* Svoboda ex Blažková 1962). The dominant *Q. petraea* accounts for 25%-50% of the tree layer, and the admixture of *Pinus sylvestris* L. accounts for 5%-25%. The shrub layer is mostly formed of young *Q. petraea* individuals. In the herb layer, reaching the coverage of approximately 30%, especially *Vincetoxicum hirundinaria* Med., *Anthericum liliago* L., *Galium glaucum* L., *Q. petraea*, *Hieracium pilosella* L., and *Festuca ovina* L. are frequently present. The moss layer covers approximately 30% of the area. With regard to a complicated morphology of the terrain, the material for analysis was collected on two separate plots (plot 1 defined by the following geographic coordinates: 13°53’56.0” E, 50°03´07.6” N; 13°53’55.9” E, 50°03’06.9” N; 13°53’39.4” E, 50°03’07.2” N; 13°53’39.0” E, 50°03’07.1” N; and plot 2: 13°53’36.4” E, 50°03’05.8” N; 13°53’33.2” E, 50°03’05.9” N; 13°53’28.7” E, 50°02’57.8” N; and 13°53’28.6” E, 50°02’58.4” N).

2. Červený Kříž Nature Reserve. The locality represents natural vegetation managed for a long time, in the so-called „coppice system” that is considered an environmentally friendly management system. The habitat is located in a plain terrain. The oak population in the area is a part of a thermophilous oak forest (*Potentillo albae-Quercetum* Libbert 1933).

The tree layer is formed almost exclusively of the oak population, with a cover of 75-100%. Individuals of *Carpinus betulus* L., *Tilia cordata* Mill, and *Sorbus torminalis* (L.) Crantz can be found there only sporadically. The shrub layer is missing in this community, mainly because of wild animals. The species-rich herb layer covers approximately 80% and is dominated by *Potentilla alba* L., *Hepatica nobilis* Schreber, *Brachypodium sylvaticum* (Huds.) P. B., *Calamagrostis arundinacea* (L.) Roth, and *Anemone nemorosa* L.

The material for analysis was collected in a plot defined by the following geographic coordinates: 13°55’49.5” E, 49°59’31.8” N; 13°56’03.9” E, 49°59’31.6” N; 13°56’04.1” E, 49°59’28.5” N; 13°55’45.7” E, 49°59’32.4” N; 13°55’49.7” E, 49°59’34.5” N; and 13°55’49.5” E, 49°59’34.4” N.

3. Křivoklát. The locality represents a stand of *Q. petraea* planted approximately 80 years ago on municipal land at the town Křivoklát. The tree layer is formed predominantly of the planted *Q. petraea*, with an admixture of *Carpinus betulus* L., *Fagus sylvatica* L., *Larix decidua* Mill., and *Pinus sylvestris* L. The herb layer with a cover of 30-50% is relatively poor in species, and is dominated particularly by *Poa nemoralis* L.

The material for analysis was collected on the plot defined by the following geographic coordinates: 13°53'00.7" E, 50°02'01.0" N; 13°53'04.1" E, 50°01'58.5" N; 13°52'43.8" E, 50°01'59.2" N; and 13°52'42.6" E, 50°01'52.2" N.

2.2. Sampling design and laboratory techniques

The plant material was collected on 19-20 June 2007. The geographic coordinates were surveyed by using the GPS equipment eTrex Summit in the WGS 84 coordinate system. The corners of polygons, from which the material was collected, were surveyed with an error range of 5-11 m. The material was collected in a grid system, where the distance of individual tree samples was approximately 50 m. Leaf samples from 30 trees were taken at all 3 localities.

Microsatellite analysis (STR) was used to assess genetic diversity, as these markers are efficient for DNA fingerprinting of individuals and determining their genetic relationships. The markers are also suitable to study pollen dispersal, genetic structure, diversity, and differentiation of populations, or to assess effective population size or genetic drift. Total DNA was extracted from single leaves by using a DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. The primers for amplification of polymorphic microsatellite regions of the genus *Quercus* were adopted from the literature (Steinkellner *et al*. 1997; Dow *et al*. 1995; Kampfer *et al*. 1998). The first 15 primer pairs were tested in 54 variants of PCR reaction conditions (PCR solution and cycling protocol). The 9 most polymorphic loci with high-quality banding patterns were chosen, and optimal PCR conditions for these loci were found. These are loci MSQ4 and MSQ13 (Dow *et al*. 1995), ZAG110, ZAG16, ZAG104 and ZAG9 (Steinkellner *et al*. 1997) and ZAG11, ZAG30 and ZAG90 (Kampfer *et al*. 1998). Amplified fragments were resolved on sequencing polyacrylamide gels and visualized by using silver staining according to the Promega Silver Sequence DNA Sequencing System. Allele size was determined by comparing with the DNA size ladder.

2.3. Data processing

Genetic diversity was determined as the mean number of alleles per locus (A) and observed (H_o) and expected (H_e) heterozygosity (Nei 1978). For each locus, deviation from Hardy-Weinberg equilibrium was examined by calculating Wright's fixation index as $F_{is} = 1 - (H_o/H_e)$. The population F_{is} values were estimated as the mean of F_{is} values over all loci. The genetic distances between populations were calculated from allele frequencies according to Nei (1978). POPGENE 1.31 program (Yeh *et al*. 1999) was used for analysis of the results. The differences in genetic diversity of the studied populations were also compared by correspondence analysis, using CANOCO program (Ter Braak & Šmilauer 1998).

3. Results and discussion

The differences between average values for individual populations are so small that no genetic difference can be proved between them (Table 1). This is confirmed by the result of correspondence analysis (Fig. 2). Also small differences in the values of heterozygosity for all 3 studied populations (Table 1) confirm the absence of significant genetic differences. This is documented by the result of correspondence analysis in Fig. 2, too.

The smallest genetic distance (0.1044) was found between the natural population 1 (Brdatka) and the planted population 3 (Křivoklát). In contrast, the largest distance was between population 2 (Červený Kříž) and population 1 (0.1199). The genetic distance between populations 2 and 3 reached 0.1087.

Among the 3 studied populations of *Q. petraea,* differing in forest management, no significant differences have been found in genetic structure. Similar results are reported also from the territory of Denmark by Siegismund & Jensen (2001). In their work, no inter-population genetic differences were shown with the use of biochemical markers for each of the studied taxa: *Q. robur, Q. petraea* and *Q. robur* of Danish origin, that has specific phenological and growth characteristics (see Jensen 1993). Siegismund & Jensen (2001) have not proved any difference in frequencies of alleles with regard to geographic distribution of populations. Similar results are also listed by Bakker (2001). Those authors state that biochemical markers indicate only high gene flow and small genetic drift, but do not reflect adaptations to habitats. A low genetic differentiation among populations of *Q. petraea* is also reported from Ireland by Muir *et al*. (2004). Those authors attribute small differences in the genetic structure of populations to high gene flow among populations, related to extensive pollen flow in *Q. petraea*, documented e.g. by Streiff *et al*. (1999).

Using microsatellites, Cottrell *et al*. (2003) compared genetic structure at 2 localities, where both *Q. robur* and *Q. petraea* occur. One of the stands was natural, with no human-mediated planting, but with coppice management, while the second stand was planted and managed intensively. Genetic diversity of both plantations was high for both species, only slightly higher in the case of *Q. petraea*. According to those authors, the possible reasons include differences in seed transfer and dynamics of regeneration between those species, one-way hybridization, and a higher degree of outcrossing in *Q. petraea*. Although in the natural vegetation expected heterozygosity was higher (and at the same time, there was a higher number of alleles per locus), observed heterozy-

Table 1. Genetic diversity in 3 populations of *Q. petraea*, expressed by the number of alleles per locus (*A*), and observed and expected heterozygosity (H_o and H_e) for the monitored loci. In brackets are given standard errors. Fixation index (F_{is}) shows variation from the Hardy-Weinberg equilibrium

Brdatka	A	H_o	H_e	F_{is}
MSQ4	13	0.81	0.82	0.014
MSQ13	10	0.63	0.74	0.159
ZAG104	16	0.94	0.91	-0.032
ZAG16	20	0.91	0.91	0.009
ZAG9	12	0.91	0.87	-0.044
ZAG110	14	0.88	0.88	0.002
ZAG90	17	0.55	0.91	0.398
ZAG11	13	0.75	0.75	-0.004
ZAG30	12	0.66	0.61	-0.073
Average	14.1 (3.1)	0.78 (0.14)	0.82 (0.10)	0.048
Červený kříž	A	H_o	H_e	F_{is}
MSQ4	11	0.91	0.83	-0.090
MSQ13	10	0.84	0.80	-0.051
ZAG104	18	0.96	0.91	-0.060
ZAG16	16	0.97	0.91	-0.069
ZAG9	12	0.88	0.86	-0.022
ZAG110	15	0.91	0.86	-0.057
ZAG90	23	0.79	0.94	0.153
ZAG11	12	0.77	0.76	-0.014
ZAG30	13	0.75	0.73	-0.024
Average	14.4 (4.1)	0.86 (0.07)	0.84 (0.07)	-0.026
Křivoklát	A	H_o	H_e	F_{is}
MSQ4	16	0.84	0.87	0.032
MSQ13	10	0.81	0.75	-0.080
ZAG104	17	0.90	0.91	0.013
ZAG16	17	0.91	0.91	0.000
ZAG9	15	0.90	0.85	-0.068
ZAG110	16	0.91	0.88	-0.036
ZAG90	25	0.62	0.94	0.333
ZAG11	11	0.72	0.78	0.081
ZAG30	13	0.59	0.55	-0.072
Average	15.6 (4.4)	0.80 (0.13)	0.83 (0.12)	0.072

gosity was lower than in the controlled, managed stand, and the whole system in the coppiced stand significantly diverged from the Hardy-Weinberg equilibrium, probably due to frequent vegetative propagation.

Among the individual populations of *Q. petraea*, Bruschi *et al.* (2003) found a slight, but statistically significant correlation between nuclear genetic distance and geographic distance. When analyzing the genetic

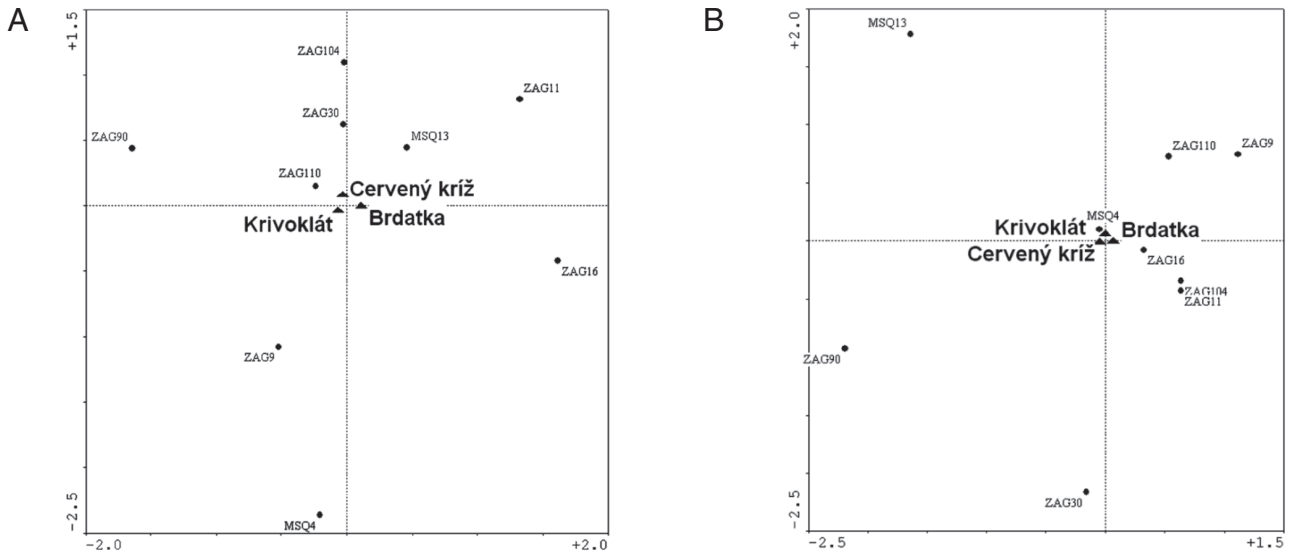

Fig. 2. Results of correspondence analysis of the number of alleles per locus (A) and heterozygosity – number of heterozygotes in respective locus (B)

structure of populations by using enzymes, Zanetto & Kremer (1995) observed significant differences in frequencies of alleles in populations of *Q. petraea* on the European scale. However, from the methodological point of view, these results cannot be compared with the analysis of microsatellites.

The results of our monitoring do not significantly differ from the general data on genetic diversity of populations listed by the above authors. Our work thus has not proved that forest management had a significant adverse impact on genetic diversity of the populations of *Q. petraea*.

In addition, a more detailed testing of genetic distance of the populations studied by us showed that genetic difference between the natural population and the planted stand in its vicinity is smaller than the genetic difference between more distant but more natural populations. Hence, we can assume that planting material from the closest vicinity was still used 80-100 years ago.

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