

Electrophoretic Differentiation Possibilities within the Genus *Quercus* by Means of Protein Monomers

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Summary

Taxonomic differences within the genus *Quercus* were studied with SDS-poroPAGE in the case of 207 *Q. cerris*, *Q. petraea* and *Q. robur* individuals and with LiDS-poroPAGE in 75 *Q. cerris*, *Q. petraea*, *Q. pubescens*, *Q. robur* and *Q. rubra* trees. In the present material, protein monomer patterns were suitable to differentiate among 3 oak species (*Q. cerris*, *Q. robur*, *Q. rubra*) while *Q. petraea* and *Q. pubescens* had identical polypeptide profiles. Neither of the applied method was appropriate for the identification of subspecies, ecotypes or forms. Differences and identities of banding patterns may be related with the sophisticated genetics of *Quercus*.

Key words: Genetic variation, electrophoresis, protein monomers, *Quercus cerris*, *Quercus petraea*, *Quercus pubescens*, *Quercus robur*, *Quercus rubra*.

Zusammenfassung

Es wurden 207 Einzelbäume der Eichenarten *Q. cerris*, *Q. petraea*, *Q. robur* mit SDS-poroPAGE und 75 Einzelbäume von *Q. cerris*, *Q. petraea*, *Q. pubescens*, *Q. robur*, *Q. rubra* mit LiDS-poroPAGE auf mögliche taxonomische Unterschiede geprüft. Mit der poroPAGE Technologie konnten in dem bisher untersuchten Material 3 Eichenarten (*Q. cerris*, *Q. robur*, *Q. rubra*) voneinander unterschieden werden. *Q. petraea* und *Q. pubescens* zeigten gleiche Polypeptide-Strukturen.

Unterschiede zwischen den Unterarten, Formen und Ökotypen waren mit der Methode nicht nachzuweisen. Die Differenzen und Gleichheiten unter den Bandmustern von den Eichenarten werfen verschiedene genetische Fragen auf.

Introduction

Increasing silvicultural use, shortage of seeds and the general deterioration in health conditions make the study of genetic structure and variation among and within autochthonous *Quercus* populations an important task. The genus *Quercus* has an outstanding importance in Hungary and approximately 35% of forested areas consist of its species. Identification of flushing types, ecotypes, subspecies and species is required not only for tree breeding but also for practical purposes. The local importance of Slavonian oak (*Quercus robur* L. sp. *slavonica*), which was described as a subspecies or ecotype of pedunculate oak (MÁTYÁS, 1973), is also stressed. Its stands cover about 1000 hectares in Hungary, which were planted mainly at the beginning of this century. The excellent stem form of this provenance is strongly inherited and preserved among progenies (KOLOSZÁR, 1987). In Hungary there are generally mixed stands of *Q. petraea*, *Q. pubescens* and *Q. cerris*, while the occurrence of *Q. robur* with *Q. petraea* and *Q. cerris* is less frequent. Artificial plantations of *Q. rubra* are scattered over the country.

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Electrophoretic techniques may be useful tools for the analysis of genetic variability in the case of oak species (RUDIN, 1986). In the preliminary studies with *Fagaceae* mainly starch gel methods were used (FINESCHI, 1986; FINESCHI and MALVOLTI, 1987, 1988; MÜLLER-STARCK, 1985; MÜLLER-STARCK and ZIEHE, 1992; SCHROEDER, 1989; THIEBEAULT et al, 1982; YACINE and LUMARET, 1988, 1989), while some isozyme (e.g. peroxidase, esterase) patterns were determined using polyacrylamide gels (HOUSTON, 1983; MAYBERRY and FERET, 1977; OLSSON, 1975; STICH and EBERMANN, 1984; TOBOLSKI, 1978).

MANOS and FAIRBROTHERS (1987) made taxonomic studies with 6 oak species (*Erythrobalanus*) using 8 enzymes. GUTTMAN and WEIGT (1989) examined 18 isozyme loci of 478 trees belonging to 10 *Erythrobalanus* and 8 *Quercus* species. Cluster analysis revealed a substantial polymorphism (65% and 59%) of these loci. Mean values of ROGERS' Distance were 0.27 and 0.44 in the 2 subgenera and 0.68 between them. *Q. palustris* differed significantly from other species of subgenus *Erythrobalanus* in the experiments of the above authors. Several studies have been published on gene flow and introgression between *Q. robur* and *Q. petraea* (AAS, 1990; BACILIERI and DUCOUSO, 1993; DUPOUEY and BADEAU, 1992; PETIT, 1992; RUSHTON, 1978), while interspecific differentiation of oaks by biochemical markers attracted less attention.

Our main goal was to find simple and fast electrophoretic methods which can be used safely for the identification of taxonomic units within the genus *Quercus*. Porosity gradient polyacrylamide gel electrophoresis (poroPAGE) techniques were adapted and improved for the separation of solubilized bud protein monomers using different detergents (e.g. SDS, LiDS). The analysis of *Q. cerris*, *Q. petraea*, *Q. pubescens*, *Q. robur* and *Q. rubra* populations, located in 4 Hungarian geographical areas, was performed in 2 series of experiments. Regarding morphological characters, which were used for identification of species, the descriptions of AAS (1990), DUPOUEY (1983) and MÁTYÁS (1973) were followed.

Materials and Methods

Samples of 207 pedunculate, sessile and Turkey oak trees originating from 10 natural stands and from the Botanical Garden of Sopron University were examined in the first series of experiments utilizing SDS-poroPAGE (Table 1). Samples were collected between February 1989 and March 1990 from 80 to 100 years old trees. For the second series of experiments with LiDS-poroPAGE samples were collected from 75 20 to 120 years old trees at 4 sites (Table 2) between November 1990 and March 1991. (samples collected from 10 trees were used in both sets of experiments.) There parallel samples of 20 cm to 40 cm long shoots were collected from each individual and transported in cooled polyethylene bags.

Table 1. — Numbers of sampled trees in the oak populations examined by the SDS-poroPAGE. The trees marked with * were examined also by LiDS-poroPAGE.

SPECIES	Q. robur	Q. robur f.	Q. robur ssp.	Q. petraea	Q. cerris	TOTAL
	robur	"Fastigiata"	slavonica			
LOCATION OF POPULATIONS	NUMBER OF TREES					
Sopron I.	1	—	4	—	—	5
Sopron II.	7	—	—	21	17	45
Sopron Bot. G.	10	5	4	10	10	39
Zákány I.	2	—	6	3	—	11
Zákány II.	3	—	4	2	2	11
Zákány III.	—	—	1	1	4	6
Zákány IV.	15	—	22	1	2	40
Bejczyertyános	—	—	10	—	—	10
Nagylózs	—	—	5	—	—	5
Sopronkövesd	—	—	5	—	—	5
Budapest	6+4*	—	—	7+3*	7+3*	30
TOTAL	48	5	61	48	45	207

Table 2. — Numbers of sampled trees in the oak populations examined by the LiDS-poroPAGE. The numbers marked with * refer to trees sampled also in the SDS-poroPAGE examinations.

SPECIES	Q. petraea	Q. robur	Q. pubescens	Q. rubra	Q. cerris	TOTAL
	NUMBER OF TREES					
LOCATION OF POPULATIONS	NUMBER OF TREES					
Sopron - Bot. Garden	2	3	1	5	2	13
Budapest - Mt. Széché.	6+2*	2*	12	—	7+2*	31
Budapest - Normafa	4+1*	4+2*	2	2	3+1*	19
Budapest - Cinkota	—	4	—	8	—	12
TOTAL	15	15	15	15	15	75

Solubilized protein monomers were separated with SDS- or LiDS-poroPAGE. 50 mg bud material, using at least 10 replicates per each sample, was ground thoroughly with a mortar and pestle in 0.5 ml extract solution (20.0% w/v sucrose, 5.0% v/v 2-mercaptoethanol, 2.0% w/v SDS or LiDS in 50 mM Tris-HCl, pH 6.5). For complete solubilisation these total protein extracts were incubated at 100 °C for 10 minutes and centrifuged at 2,500 rpm for 20 minutes.

Sample volumes of 20 µl were loaded into the wells of 10% to 15% linear acrylamide gradient gels. Gels and stock solutions were made according to LAEMMLI (1970).

Lithium dodecyl sulfate was used in the same quantity as SDS and N,N,N',N'-tetramethylethylenediamine was replaced by dimethylaminopropionitrile. Electrophoresis was carried out as described earlier (KORÁNYI, 1989). 8x8x0.2 cm slabs were used in a 2-gel vertical system. To improve the separation of close bands, runs were continued for an additional 30 minutes after the bromophenol blue tracking dye had left the gels. The electrode buffer was Tris-glycine, pH 8.3 supplemented with 0.1% SDS or LiDS.

Coomassie Brilliant Blue R-250 staining was performed according to FAIRBANKS et al. (1971), however, times were

changed in the 3 staining steps to 5 hours, 12 hours and 5 hours, respectively.

The samples were generally analyzed by electrophoresis directly after their preparation, however polypeptide extracts can be stored at -24°C for at least 1 year without any detectable degradation. After staining, the patterns were evaluated visually from magnified gel photos.

Results and Discussion

SDS-poroPAGE combined with Coomassie staining revealed highly distinctive monomer patterns regarding the 3 species (Figure 1) however, 8 out of the 114 examined individuals of *Q. robur* had the same banding pattern as *Q. petraea*. Repeated examinations of their morphological traits (e.g. number of leaf lobes, length of petiole, length of peduncle, hairiness of leaves beneath) could not reveal any sign of hybridisation. It was not possible to identify subspecies (e.g. *Q. robur ssp. slavonica*, *Q. robur* 'Fastigiata') using this method. However, there are clear and reproducible differences between certain species. Turkey oak has a clearly distinctive pattern, while pedunculate and sessile oaks show slight but reproducible differences with the exception of the above mentioned 8 trees. This may refer to the relatively short evolutionary history of these species. Their progressive speciation is an explanation to the substantial interspecific hybridisation along with the effects of human activities. According to early records significant acorn and sapling transfer took place even before the period of regular forestry (KOLLOSSVÁRY, 1975).

The described LiDS-poroPAGE method provided very clear pictures with sharp bands and enhanced the resolution compared with SDS-poroPAGE. Further studies are

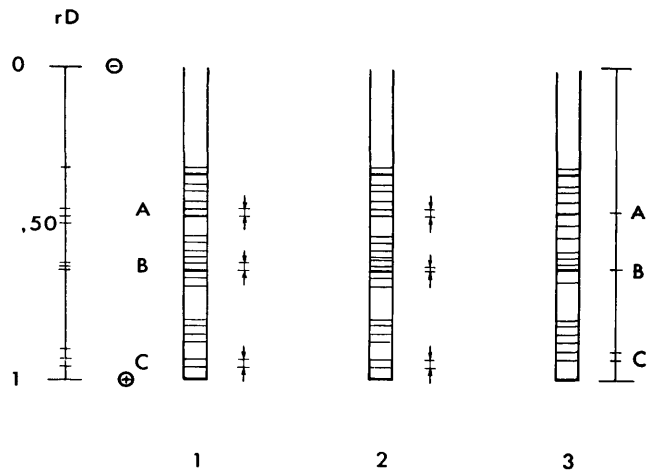


Figure 1. — Polypeptide monomer patterns of *Quercus petraea* (1), *Quercus robur* (2) and *Quercus cerris* (3) detected by SDS-poroPAGE. Alterations characteristic for the 3 species are seen at regions A, B and C. (In the case of *Q. robur* 8 individuals had the same banding patterns as *Q. petraea*.) The rD scale shows the relative distance values along the gels. The extension of specific intervals (Δxy ; x refers to regions A to C and y to species 1 to 3) shows slight differences (double arrowheads) and is characterized by the following rD values in the different species:

$\Delta a_1 = \Delta a_2 = 0.46$ to 0.48 , $\Delta a_3 = 0.46$;
 $\Delta b_1 = 0.63$ to 0.65 , $\Delta b_2 = 0.64$ to 0.65 , $\Delta b_3 = 0.62$ to 0.64 ;
 $\Delta c_1 = \Delta c_2 = 0.92$ to 0.95 , $\Delta c_3 = 0.90$ to 0.93 .

required in order to verify the genetic control and mode of inheritance of the employed protein markers. All the examined source materials showed uniform and distinctive profiles with single bud extracts which proved their homogeneity and distinctness, except in the case of *Q. petraea* and *Q. pubescens*. Figure 2 presents the monomer patterns of the 5 oak species. Monomer profile of *Q. robur* differs slightly but characteristically from the identical

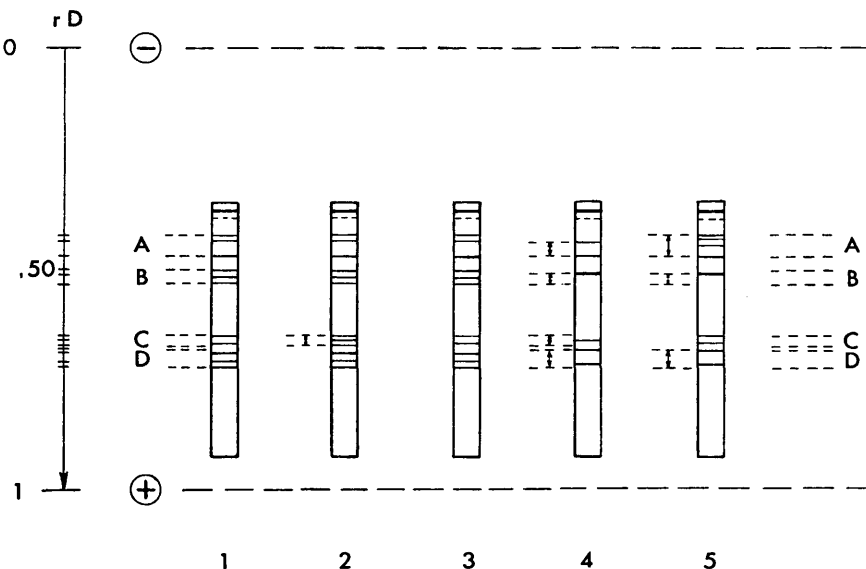


Figure 2. — Diagram of monomer patterns of *Quercus petraea* (1), *Quercus robur* (2), *Quercus pubescens* (3), *Quercus rubra* (4) and *Quercus cerris* (5). Characteristic differences are seen at regions A to D. (In the case of *Q. robur* 8 individuals had the same banding patterns as *Q. petraea*.) Compared with figure 1, A is a new region, B is identical with A, while C and D are parts of C. The extension of intervals (Δxy ; x refers to regions A to D and y to species 1 to 5) shows slight differences (double arrowheads) and is characterized by the following rD values in the different species:

$\Delta a_1 = \Delta a_2 = \Delta a_3 = 0.42$ to 0.47 , $\Delta a_4 = 0.44$ to 0.47 , $\Delta a_5 = 0.42$ to 0.47 ;
 $\Delta b_1 = \Delta b_2 = \Delta b_3 = 0.50$ to 0.54 , $\Delta b_4 = \Delta b_5 = 0.51$;
 $\Delta c_1 = \Delta c_3 = \Delta c_5 = 0.65$ to 0.66 , $\Delta c_2 = 0.65$ to 0.67 , $\Delta c_4 = 0.66$;
 $\Delta d_1 = \Delta d_2 = \Delta d_3 = 0.69$ to 0.72 , $\Delta d_4 = \Delta d_5 = 0.68$ to 0.71 .

ones of the 2 closely related species, *Q. petraea* and *Q. pubescens*. One *Q. robur* tree (Budapest-Mt. Széchenyi) had an identical pattern with *Q. petraea*, which was also observed in SDS-poroPAGE studies. It is interesting at this point that in Hungary, stands of *Q. pubescens* nearly always contain *Q. petraea* ssp. *petraea*, *Q. petraea* ssp. *DALECHAMPII* and *Q. petraea* ssp. *polycarpa* individuals showing substantial morphological variability (MAJER, 1989). Monomer bands of region B indicate that *Q. rubra* and *Q. cerris* differ considerably from the other 3 species, while the American *Q. rubra* has several significant differences in the variable regions when compared with the 4 European oaks. In this case the revealed banding patterns may relate to the evolutionary events and the closely similar monomer profiles of the 3 species may reflect their genetic relatedness. At the same time, *Q. rubra* and *Q. cerris* are very similar in Region B.

Conclusions

In the present material, SDS-poroPAGE could detect taxonomic and genetic differences within the genus *Quercus*. Individuals showed monomer patterns characteristic to 1 of the 3 analyzed species with the exception of 8 *Q. robur* trees. LiDS-poroPAGE gave the same result but the morphology of these individuals did not show any sign of hybridisation. It is supposed that they carry introgressive genes of *Q. petraea*. Some experimental findings support this hypothesis revealing asymmetric gene flow from *Q. petraea* to *Q. robur* (BACILIERT and DUCOUSSO, 1993). LiDS-poroPAGE provided clear pictures and high resolution. It may be more suitable for comparative taxonomic and genetic studies however, it is necessary to increase the number of samples.

Identity (e.g. *Q. petraea*-*Q. pubescens*, *Q. r. robur*-*Q. r. slavonica*) or close similarity (e.g. *Q. petraea*-*Q. robur*, *Q. rubra*-*Q. cerris*) of monomer patterns may be related with sophisticated genetic problems (e.g. correlations of phenotype and genotype, gene introgression, hybridisation, speciation, genetic distance and distinctness). These complicated questions were also addressed by other authors. According to MANOS and FAIRBROTHERS (1987) the patterns of genetic differentiation do not correlate with the five classical taxonomic series suggesting that they do not represent genetically differences groups of species excluding the Palustres taxon. GUTTMAN and WEIGT (1989) made isozyme studies with *Q. palustris* and *Q. virginiana* and their results contradict the traditional taxonomy of these species. Results of morphological and isozyme studies led to the conclusion that there is a unidirectional introgression from *Q. petraea* to *Q. robur* (AAS, 1990; BACILIERT and DUCOUSSO, 1993). The examination of different DNA species (KREMER et al., 1991; PETIT, 1992) revealed very little differentiation between *Q. petraea* and *Q. robur* with a strong geographical component.

The use of electrophoresis may provide new possibilities in the taxonomy of oak genotypes. For example, it was possible to detect significant biochemical (e.g. isozyme) differences between *Q. palustris* (MANOS and FAIRBROTHERS, 1987) or *Q. virginiana* (GUTTMAN and WEIGT, 1989) and their closely related species. The extension of poroPAGE studies to other oak species e.g. *Q. palustris*, *Q. macrolepis*, *Q. virginiana*) may provide further criteria in the discrimination of these species.

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