SHORT NOTE

TWO DIMENSIONAL GEL ELECTROPHORESIS CONFIRMS THE LOW LEVEL OF GENETIC DIFFERENTIATION BETWEEN *QUERCUS ROBUR* L. AND *QUERCUS PETRAEA* (MATT.) LIEBL.

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ABSTRACT

The genetic differentiation between *Quercus petraea* and *Quercus robur* was investigated using two dimensional electrophoresis. Twenty three oaks from six European countries covering partly the natural geographic range of white oaks in Europe, were studied. Five hundred and thirty polypeptide spots were scored, among which 101 were polymorphic. We did not find any spot specific to *Quercus petraea* or *Quercus robur,* however 3 spots shown significant frequency differences between the two species. Interspecific and intraspecific dissimilarities were very close. Total proteins confirm the results obtained with other molecular markers (isozymes, RAPD and cp DNA) concerning the low level of genetic differentiation between *Quercus petraea* and *Quercus robur.*

Key words: *Quercus petraea, Quercus robur,* two dimensional electrophoresis, genetic variation, tree species

INTRODUCTION

Quercus robur L. (pedunculate oak) and *Quercus petraea* (Matt.) Liebl. (sessile oak) are two sympatric species largely distributed in Europe. Their separation in two species has been traditionally a controversial question.

Multivariate taxonomic studies of pedunculate and sessile oaks based on morphological characters showed the existence of specific morphological poles (DUPOUEY & BADEAU 1993). Moreover, the two species have different water requirements and occupy different ecological but proximal niches (BACILIERI *et al.* 1995). However, if morphological and ecological characters allowed to distinct *Quercus robur* from *Quercus petraea* on a population basis, molecular markers were not as discriminant.

In allozyme studies, the comparison of the two species showed only allelic frequency variation, but failed to reveal any specific alleles (KREMER *et al.* 1991 ; MÜLLER-STARCK *et al.* 1993; ZANETTO *et al.* 1993). In the same way, in mixed stands *Quercus robur* and *Quercus petraea* shared the same chloroplast haplotypes (PETIT *et al.* 1993a). The pattern of chloroplastic DNA variation appeared mainly geographic with no species differentiation (KREMER *et al,* 1991). Furthermore, studies concerning ribosomal DNA showed no differences in the distribution of rDNA length variants (PETIT *et al.* 1993b). Finally, in studies using random amplified polymorphic DNA, 14 fragments revealed significant frequency differences between *Quercus robur* and *Quercus petraea*, but no specific fragment was found (MOREAU *et al.* 1994).

In our study, we try to characterize proteins that possibly discriminate pedunculate and sessile oaks using two dimensional gel electrophoresis. This technique allows to identify in one single assay a large number of proteins (O'FARRELL 1975). Hence a wider prospection of the genome is accessible for localizing potential discriminant coding regions. Our objective in this contribution is to compare the level of interspecific differentiation of denatured proteins with that obtained with other markers.

MATERIAL AND METHODS

Twenty three (12 sessile and 11 pedunculate) oaks from six European countries: Romania, Poland, France, Hungary, Czech Republic and Spain, covering partly the natural geographic distribution range of white oaks in Europe were studied (Fig. 1).

Radicules of germinating acorns were crushed in the UKS buffer 20 $\mu l \cdot mg^{-1}$ of fresh weight (DAMERVAL *et al.* 1986). Extracts were analysed by two dimensional electrophoresis (BAHRMAN & PETIT 1995). Protein patterns were compared visually by superimposition

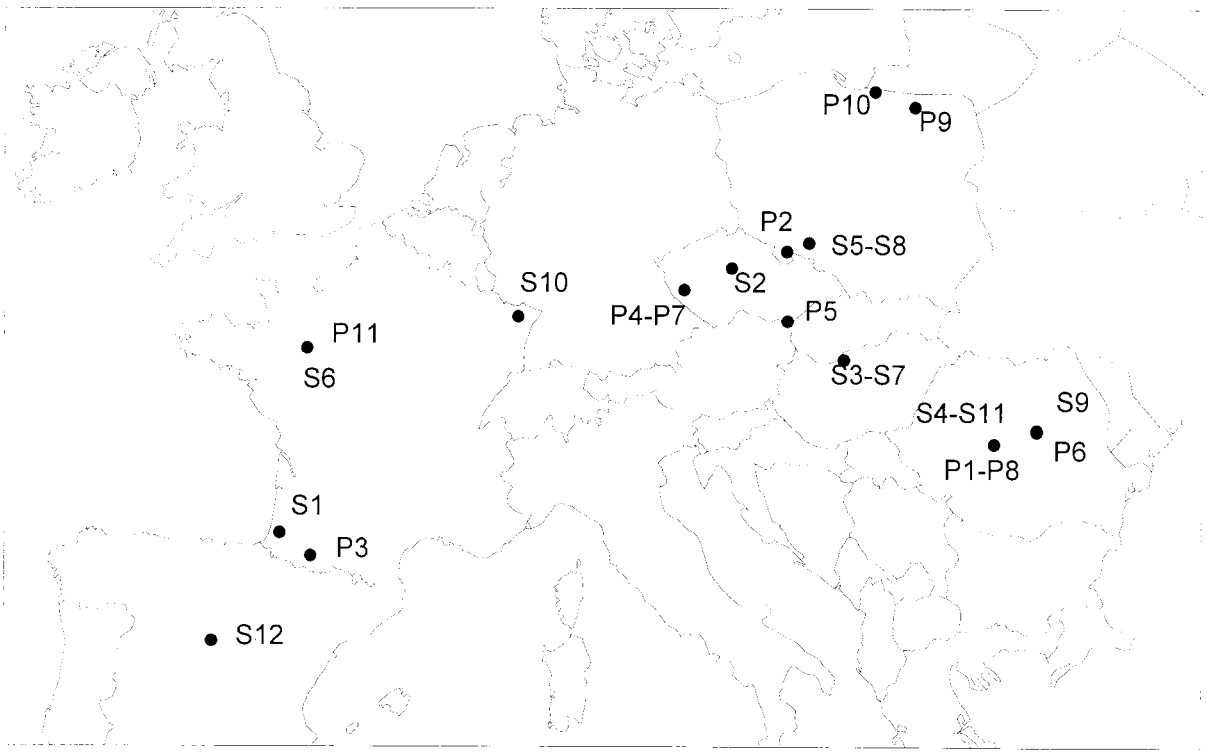

Figure 1 Geographic location of the 23 oaks studied. S = Sessile, P = Pedunculate.

of the dried gels upon a light box.

Differences between the two species were calculated on the whole of spots by two ways:

- Factorial Correspondence Analysis (FCA) was used to describe multispot patterns of variation between the two species.
- Intra and inter dissimilarity indexes were computed between all the trees to estimate the level of diversity within a species and the level of differentiation between the two species :

$$D = 1 - [N(11) + N(00)]/ N_{total}$$

where N(11) is the number of spots present in both individuals, N(00) is the number of spots absent in both individuals and N total is the total number of spots (BAHRMAN *et al.* 1994).

Although the sample size of each species is low, the analysis is conducted on more than 100 polymorphic proteins. As a result, the sampling within the genome compensates the reduced sample size.

RESULTS AND DISCUSSION

The comparison of oak protein patterns shown a total of 530 polypeptide spots, among which 101 were variable in the 23 trees (18%) (Fig. 2).

Three kinds of genetic variation were detected in two-dimensional protein patterns:

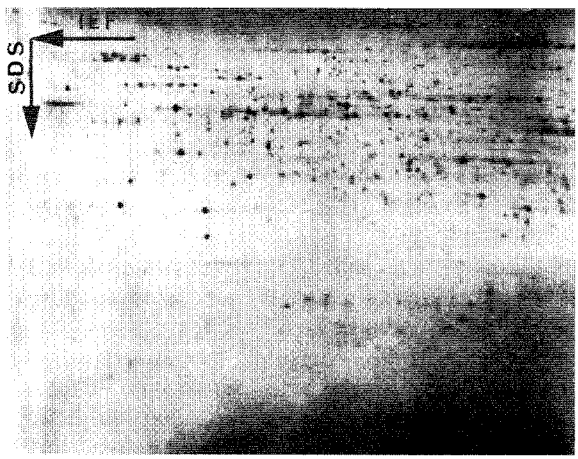

Figure 2 Two-dimensional protein pattern obtained from the radicule of germinating acorn of a sessile oak. Vertical migration separated proteins according to molecular weight whereas horizontal migration separated proteins according to isoelectric point. 530 spots were identified on the gel.

1) presence/absence variation, *i.e.* the existence of a spot in one tree and the absence of the same spot in another tree;

2) staining intensity variation of a given spot assessed by visual scoring in 2 classes;

3) position variation: the position shift of a given spot from one tree to another in one direction (same molecular weight but different isoelectric point).

Table 1 Interspecific frequency differences of spots showing presence/absence variation.

P \ S	0	1	2	3	4	5	6	7	8	9	10	11	12
0		1		1	1								
1	2	2	1	2	1		1	1 3.10^{-2}					
2		5*	1		2	1							
3	2	2	2										
4		1	1		1	3		2					
5		2	2						1		1		
6								1	2	1			
7							1		3		1	2	
8			1 1.10^{-2}				1	1				1	
9										3		2	1
10					1 9.10^{-2}		1			1	1		1
11													

Legend: The first row represents the number of occurrences in *Quercus petraea* and the first column represents the number of occurrences in *Quercus robur*. Each cell represents the number of proteins that exhibit frequency differences. For example, in cell marked with * (corresponding to column 3 and row 4): 5 proteins were present in one sessile tree and in two pedunculate trees. Cell in grey corresponds to the combination showing significant frequency differences between *Quercus robur* and *Quercus petraea* (Fisher Exact Test $p \leq 0.05$). Values of Fisher Exact Test are given for those cases.

Seventy three spots displayed presence/absence variation, 4 spots showed staining intensity variation, and position variation were observed in 24 spots (12 bi-allelic loci).

Polymorphic spot proportions corresponding to each category (23.7% position variation, 72.2% presence/absence variation and 4% of staining variation) were identical to those found in other species (BAHRMAN & PETIT 1995).

We did not find any spot specific to *Quercus petraea* or *Quercus robur*. When presence/absence variation was observed the frequency of a given spot in each species was calculated. Frequency differences between two species were tested using Fisher exact test recommended for small sample sizes. The 73 spots displaying presence/absence variation were placed in Table 1 according to their occurrence frequency in each species. Only 3 spots (numbers 1216, 2320 and 1227) expressed significant frequency differences between the two species.

The first axis of the FCA separates the 23 oaks in two groups corresponding to the two species (Fig. 3). Some individuals (s9 – s10 – s11 and p10 – p11) have an intermediate position between the two groups. Similar results were found in morphological variability of oaks studies (DUPOUEY & BADEAU 1993 ; BACILIERI *et al.* 1994).

A sessile individual from Romania (s4) was found in the pedunculate group. In a previous study, pedunculate and sessile populations from Romania were shown to be poorly differentiated (ZANETTO *et al.* 1994). Similar results were observed with RAPD data in the same populations only (C. BODÉNÈS, pers. com.). The genetically closeness of the two species in Romania is underlined again in our study.

In Fig. 3, the dispersion of the sessile cluster is larger than the pedunculate one, suggesting a greater variability of *Quercus petraea*. The dissimilarity between the two species is 0.36, whereas the within

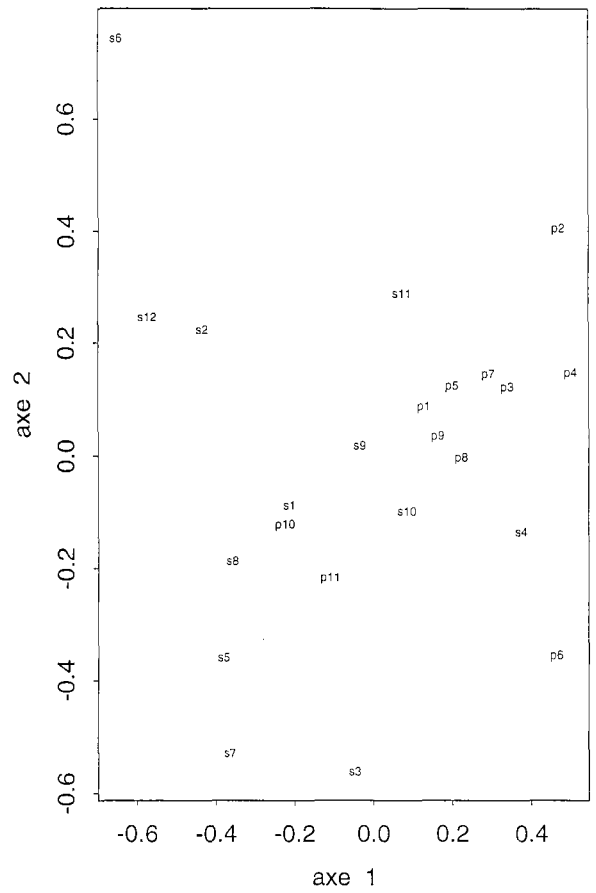

Figure 3 Distribution of the 23 trees in the 1st plane of the Factorial Correspondence Analysis. Individuals are identified as on Figure 1. The first axis explains 12.8 % of the total variation and the second axis 8.8 %.

species dissimilarities are 0.35 for *Quercus petraea* and 0.33 for *Quercus robur.*

In allozyme studies *Quercus petraea* appeared more differentiated than *Quercus robur,* despite the low level of population differentiation (ZANETTO *et al.* 1994). These results were confirmed in RAPD analysis com paring the two species, the within population diversity was higher in *Quercus petraea* than in *Quercus robur* (MOREAU *et al.* 1994). Our results showed once again the greater diversity of *Quercus petraea* and the low differentiation between the two species *Quercus robur* and *Quercus petraea.*

It is important to note on the one hand, that we did not find any characteristic spot of a species. On the other hand, interspecific and intraspecific dissimilarities were very close. In other words, total proteins failed to differentiate *Quercus robur* and *Quercus petraea.* These results confirm those obtained with other molecular markers: allozymes, cpDNA and RAPD fragments, suggesting the existence of very tenuous molecular differences between the two species.

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