SHORT COMMUNICATION

A GENE CODING FOR A NON-SPECIFIC NAD-DEPENDENT DEHYDROGENASE SHOWS A STRONG DIFFERENTIATION BETWEEN *QUERCUS ROBUR* AND *QUERCUS PETRAEA*

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Received March 5, 2000; accepted July 15, 2000

ABSTRACT

Genetic variation in a substrate-unspecific NAD-dependent dehydrogenase was analyzed in five geographically dispersed pedunculate oak and sessile oak populations. Mendelian inheritance of this isozyme system has not been tested, but it was supposed on the basis of phenotype distribution (alternative occurrence of single-banded and triple-banded phenotypes in a single zone of activity), and the conformance of the putative genotype distribution with Hardy-Weinberg proportions. Two rare variants (R_m = 134 and 83, respectively), which appear to be species-specific, were found. Frequent variants (R_m = 117 and 100, respectively) were present in both species, but the allelic profiles were strikingly different. The interspecific component of variation accounted for 54.3% of the total gene diversity, what is much more than the values reported for this pair of oak species.

Key words: *Quercus robur* L., *Q. petraea* (Matt.) Liebl., non-specific dehydrogenase, interspecific differentiation

INTRODUCTION

The most intensively studied species among the European white oaks are *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. Generally, these two species are understood as species *sensu lato*. Several further taxa have been described and found in natural forests over Europe (mainly in Eastern and Southern Europe), like *Q. dalechampii* Ten., *Q. polycarpa* Schur., and *Q. pedunculiflora* C. Koch, which are considered separate species by some botanists. However, they are not generally recognized, because of a great intraspecific morphological variation, making them difficult to distinguish. Therefore, they are frequently included into *Q. petraea* and *Q. robur*, respectively.

Interspecific genetic differentiation among European white oak species has extensively been studied by means of isozyme as well as DNA genetic markers. In general, there are significant differences in allelic frequencies between pedunculate and sessile oaks at several isozyme loci (MÜLLER-STARCK & ZIEHE 1991, ZANETTO *et al.* 1994), but no species-specific alleles were found (the alleles, which were found in samples of one species but which were missing in samples of the other one, are generally of low frequencies, so that their absence may be due to sampling error). An analysis of total proteins using two-dimensional electrophoresis brought the same result – no species-specific spots, but significant differences in the frequency of occurrence of some protein fractions between both oak species were found (BARRENECHE *et al.* 1996). Molecular markers do not seem to differentiate the species either. Sessile and pedunculate oaks share the same cytoplasmic genes when they occur in the same stand (PETIT *et al.* 1993). There are no differences between *Q. robur* and *Q. petraea* in the distribution of rDNA length variants (PETIT *et al.* 1993). RAPD fragments do not allow to discriminate both species either (MOREAU *et al.* 1994, BODÉNÈS *et al.* 1997).

BORDÁCS & BURG (1997) found two species-specific RAPD markers differentiating *Q. robur* (*sensu lato*, including ssp. *slavonica*) from *Q. petraea* (again *sensu lato*, including *Q. dalechampii*). However, this study is based on a fairly limited material. Very recently, MUIR *et al.* (2000) presented mikrosatellite markers allowing to discriminate both species, what confirms that they actually represent different taxonomical units.

During a screening of the genetic variation in isozyme loci within the *Quercus robur* L./*Q. petraea* complex in East Europe, we tried several isozyme systems which have not been reported in previous studies. One of these systems, a substrate-unspecific NAD-dependent dehydrogenase (stained using D-glucose, D-sorbitol or L-glutamic acid as substrates) exhibited allelic differentiation between both species to an extent, which has not been found in other isozyme loci.

MATERIALS AND METHODS

Within this study, we present results from one mixed

Table 1. Location of the investigated populations.

Population name	Species	Longitude (E)	Latitude (N)
Bieň (Slovakia)	mixed	19°06'	48°40'
Boky (Slovakia)	*Q. petraea*	19°04'	48°38'
Palárikovo (Slovakia)	*Q. robur*	18°04'	48°02'
Volzhsk (Russia)	*Q. robur*	48°20´	55°55´
Assenovgrad (Bulgaria)	*Q. petraea*	24°52'	41°59'

stand near Zvolen, Slovakia, and four pure stands of either pedunculate oak or sessile oak, situated in Slovakia, Bulgaria and Russia. The exact position of the investigated populations is given in Table 1. In each stand, at least 50 trees were sampled. Dormant winter buds were homogenized in a 0.1 M Tris-HCl buffer pH 7.2 with addition of PVP 40, PVP 360, EDTA II (1 g per 100 ml each), Tween 80, PEG, 2-mercaptoethanol (1 ml per 100 ml each), DTT (25 mg per 100 ml) and 1 M Na-ascorbate (4 ml per 100 ml). For the electrophoresis in a 12 % starch gel, the system following ASHTON & BRADEN (1961) was used (slightly modified: electrode buffer 0.028 M LiOH-boric acid pH 8.1, gel buffer 0.051 M Tris-citric acid pH 8.1 + 12% electrode buffer). Gels were stained in 25 ml of a 0.2 M Tris-HCl buffer pH 8.0, using 500 mg of sorbitol (preferably) or glucose, NAD, NBT, MTT (10 mg each) and PMS (1 mg) at least 3 hours (or overnight) at 37 °C.

To demonstrate the amount of the genetic differentiation, we decomposed gene diversity (as measured by expected heterozygosity) within the *Q. robur/Q. petraea* complex by subdividing the total gene diversity (H_T) into three components: H_S - the within-population diversity, D_{SQ} - diversity among populations within species (*Q* stands for *Quercus*), and D_{QT} - diversity among species within the complex, following NEI (1973).

Differences of allelic frequencies between populations and sets of individuals were tested using the Fisher exact test following RAYMOND & ROUSSET (1995). Deviations from the Hardy-Weinberg equilibrium were tested using the probability test (WEIR 1996).

RESULTS AND DISCUSSION

We do not dispose of material (progenies from artificial crosses or single-tree progenies), which would allow a genetic analysis of the variation of the mentioned isozymes. However, a Mendelian inheritance can be supposed based on the following facts:

(1) There is only a single zone of activity, where only two phenotypes occur alternatively - single bands or triple bands (Fig. 1, see also YAKOVLEV *et al.* 1999). This pattern corresponds to a dimeric enzyme, where homozygotes are single-banded, since they produce one

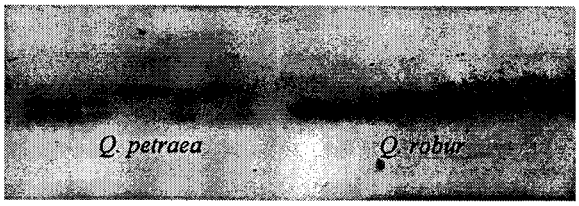

Figure 1. Zymograms of the non-specific dehydro-genase in the mixed population of *Q. robur* and *Q. peatraea* Bieň.

proteine fraction, and heterozygotes are triple-banded, since they produce an additional hybrid protein fraction.

(2) Populations of widespread wind-pollinated forest tree species (like both pedunculate and sessile oaks) are generally nearly-panmictic. We tested the deviations from the Hardy-Weinberg genotype properties in all five investigated populations (whereby the mixed Bieň population was divided into separate individual sets of the respective oak species). No significant deviations from the equilibrium were found. The phenotype distributions corresponded to the expected distributions of putative genotypes in all populations.

Adopting this interpretation, we found 4 putatively allelic variants with relative mobilities *134, 117, 100,* and *83*. However, only two of them (*117* and *100*) were found in homozygous state.

Frequencies of the variants are presented in Table 2. Two variants appear to be species-specific: the variant *134* for *Q. petraea* and *83* for *Q. robur*. However, a very limited material does not allow statements about specificity of variants. In addition, there is a regional component of variant occurrence, since the variant *134* is has not been found in the Bulgarian sessile oak population.

Allelic profiles of the respective oak species are clearly different, although both most frequent variants occur in both species. The variant *100* predominates in pedunculate oak with frequencies over 90 %, whereas in sessile oak, its frequency does not exceed 25 %. On the other hand, the frequency of the most common variant in sessile oak, *117*, is more variable, it ranges from over 60 % in Slovakia to almost 90 % in Bulgaria. The differences of frequencies of variants within the *Q. robur* population set are insignificant (Table 3). Within

Table 2. Allelic frequencies of the *Gludh-A* locus in the investigated populations.

Species	Population	Variant (*Rm*)			
		134	*117*	*100*	*83*
Q. robur	Bieň	-	.062	.938	-
	Palárikovo	-	.049	.941	.010
	Volzhsk	-	.028	.934	.038
Q. petraea	Bieň	.062	.688	.250	-
	Boky	.155	.643	.202	-
	Assenovgrad	-	.893	.107	-

Table 3. Fisher's exact tests of genic differentiation among populations.

Species	Population	BI*r*	PA	VO	BI*p*	BO
Q. robur	Bieň (BI*r*)	-				
	Palárikovo (PA)	ns	-			
	Volzhsk (VO)	ns	ns	-		
Q. petraea	Bieň (BI*p*)	***	***	***	-	
	Boky (BO)	***	***	***	ns	-
	Assenovgrad (AS)	***	***	***	**	***

ns - non-significant, *** - $P > 0.001$, ** - $P > 0.01$

Q. petraea, Bulgarian population differs significantly from the Slovak ones.

To quantify the amount of the genetic differentiation, we decomposed the total gene diversity into within-population, within-species, and between-species component. The within-population component is low, it represents only 43.5 %. There is also an inter-population (maybe inter-regional) variation, but it accounts for only 2.2 % of the total heterozygosity. The major component is the interspecific one, representing 54.3 % of variation. The values of this component given by ZANETTO *et al.* (1994) for isozyme loci range from 0.4 % to 11.1 %, with an average of 3.3 %.

In spite of the introgression occurring in mixed stands and a subsequent occurrence of intermediate forms, both species can be distinguished on the basis of morphological traits of leaves, fruits, buds and bark (AAS 1993), and they also differ partially by ecological requirements. However, isozyme genes (especially the genes controlling the enzymes of primary metabolism - group I enzymes) are unlikely to participate in the control of such traits. The enzyme investigated within this study seems to belong to the group II due to its substrate-unspecificity, so that it is probably more sensitive to selection (ZANETTO *et al.* 1993). Anyway, the presented results must be considered preliminary, since before this isozyme can be used in a routine screening of the differentiation patterns, its Mendelian inheritance must be properly proved.

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