FORESTRY IDEAS, 2022, vol. 28, No 1 (63): 45–54

ALLOZYME VARIATION IN *QUERCUS FRAINETTO* TEN. POPULATIONS IN BULGARIA

Evgeni Tsavkov and Petar Zhelev*

Department of Dendrology, University of Forestry, 10 Kliment Ohridski Blvd., 1797 Sofia, Bulgaria. *E-mail: petar.zhelev@ltu.bg

Received: 04 January 2022 Accepted: 02 February 2022

Abstract

The paper presents a survey on the genetic variation of *Quercus frainetto* Ten., an economically important tree species. Allozyme markers were applied to detect intra- and inter-population variation. The level of genetic diversity within populations was relatively high, like in other oak species with similar life history characteristics – mean expected and observed heterozygosities were 0.231, and 0.196, respectively. The major part of genetic diversity was attributed to within-population component of variation. Genetic differentiation measured by F-statistics was 0.027. No significant isolation by distance and small-scale geographic subdivision was detected, which indicates substantial gene flow among populations.

Key words: conservation, differentiation, genetic diversity, Hungarian oak.

Introduction

The Hungarian oak (*Quercus frainetto* Ten.) is widely distributed on the Balkan Peninsula. It is drought tolerant species, occupies mostly dryer and moderately rich habitats and has an exceptional good quality timber (Kalinkov 1960, 1961; Kalinkov and Shipchanov 1976; Yurukov and Panayotov 2015; Bordács et al. 2019). Therefore, the species is considered important from both economic and ecological points of view. It grows in pure and mixed stands, most often with *Quercus cerris* L. and significant part of *Q. frainetto* stands are of coppice origin.

Genetics of European oaks was studied extensively in western, and in part of Central Europe (Aas 1993, Kleinschmit et al. 1995, Stejnhoff 1997, but see Kremer and Hipp 2020, for a review). The methods of documenting the genetic diversity of oaks consist mostly of isozyme and DNA gene markers, but the quantitative adaptive traits are considered very important, too (Kremer and LeCorre 2012). Genetic diversity of *Q. robur* L. and *Q. petraea* (Matt.) Liebl. is relatively well documented (Zanetto et al. 1994, Zanetto and Kremer 1995, Kremer and Zanetto 1997, Bacilieri et al. 1996, Finkeldey 2001, Gömöry et al. 2001). However, while the biology and sylviculture of *Q. frainetto* have been extensively studied during the past decades (Marinov et al. 1995) its genetic diversity remained less studied, mostly because its

natural area of distribution is in southeastern Europe, and particularly, on the Balkan Peninsula (Bordács et al. 2019). Recent studies have shown that it attracts the attention of geneticists in its area of natural distribution and its genetic variation was studied in different aspects – hybridization, distribution of genetic diversity, differentiation, adaptation and growth performance of different provenances (Belletti et al. 2005; Curtu et al. 2007, 2011; Fortini et al. 2015, Apostol et al. 2020).

In Bulgaria, most studies are related to describing its phenotypic variation, which is extremely high, especially in leaf traits (Stefanoff 1943, Kalinkov 1960, Garilov and Stoykov 1978). According to the taxonomic scheme of Schwarz (1937–1939, 1964), applied to the Flora of Bulgaria (vol. 3; Ganchev and Bondev 1966) *Quercus frainetto* belongs to the section *Dascia* Kotschy, together with three other species of the Bulgarian flora – *Q. brachyphylla* Kotschy, *Q. virgiliana* Ten. and *Q. pubescens* Willd. (Ganchev and Bondev 1966). *Q. frainetto* is characterized by the presence of stipitate-fasciculate trichomes fused at the base, as well as by the presence of large amount of secretory trichomes. An important differentiating trait is the ratio between covering and secretory trichomes, which is 1:21 for *Q. frainetto*, thus differentiating it from the other species of the section (Uzunova and Palamarev 1992, Uzunova et al. 1997).

The knowledge of the genetic diversity and differentiation of *Q. frainetto* is important due to several reasons: First, it could be very useful for developing strategies for gene conservation; second, it could serve as a basis for sustainable management of its genetic resources; and third, it would be very useful for practical breeding programs.

Therefore, our objective was to study the distribution of genetic diversity within and among the populations of *Q. frainetto* in Bulgaria using allozymes as genetic markers.

Materials and Methods

Populations and sampling

Fifteen Bulgarian populations were selected for this study. The populations cover the whole natural range of the species in Bulgaria (Fig. 1, Table 2). Fifty to sixty individuals were sampled in each population. Branches with dormant winter buds were sampled and stored at 0–2 ºC until analyses.

Enzyme extraction, electrophoresis and enzyme staining

Bud tissues (2–3 buds per tree, scales removed) were homogenized in 100 µl Tris-HCl buffer pH 7.5, containing 15 mg Dithiothreitol, 5 mg Na_2EDTA, 100 mg PVP and 300 mg sucrose per 20 ml buffer. Also, ten milligrams of PVPP-40 were added as stabilizing agent. The enzymes were absorbed by wicks 3×8 mm Whatman 3MM chromatographic paper and the wicks were inserted into the gel. Standard starch-gel electrophoresis was used in two buffer systems: Lithium-borate – Tris-citrate discontinuous buffer system pH 8.1 (Ashton and Braden 1961) and Tris-citrate continuous buffer system pH 7.0 (Shaw and Prasad 1970). The enzyme systems studied and the loci scored are listed in Table 1. Staining procedures followed Shaw and Prasad (1970) and Cheliak and Pitel (1984) with slight modifications.

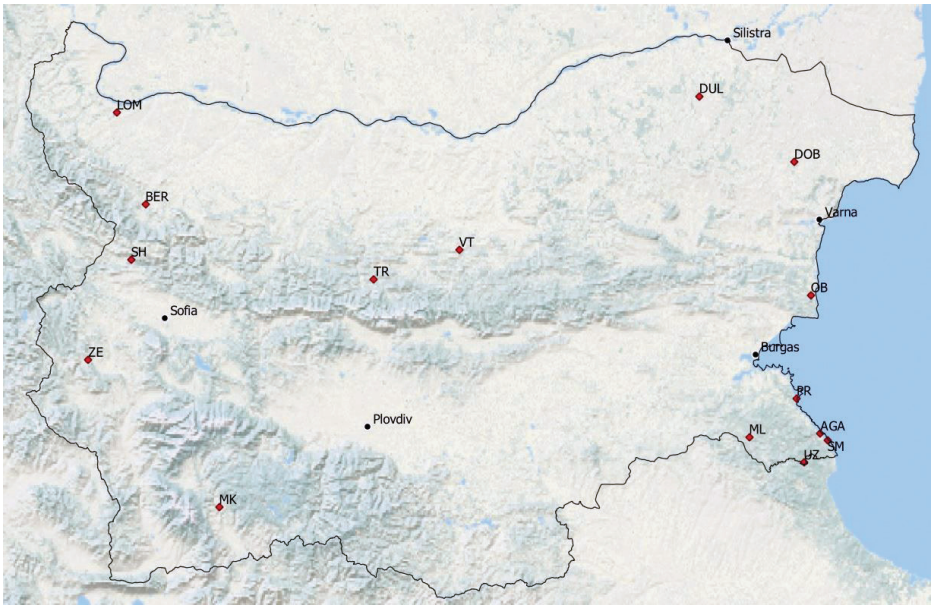

Fig. 1. Location of the populations studied.

Note: see Table 2 for the abbreviations.

Table 1. Enzyme systems studied and the loci scored.

Enzyme system (abbreviation and EC code)	**No of loci scored**	**Buffer system**
Glutamate-oxaloacetate transaminase (GOT, 2.6.1.1)	2	A
Isocitrate dehydrogenase (IDH, 1.1.1.42)	1	TC
Leucine aminopeptidase (LAP, 3.4.11.1)	1	A
Malate dehydrogenase (MDH, 1.1.1.37)	4	TC
Menadione reductase (MNR, 1.6.99.2)	1	A
Phosphoglucomutase (PGM, 5.4.2.2)	1	TC
Phosphpoglucose isomerase (PGI, 5.3.1.9)	2	A
6-phosphogluconate dehydrogenase (6PG, 1.1.1.44)	2	TC
Shikimate dehydrogenase (SkDH, 1.1.1.25)	2	TC

Legend: A – Lithium borate (pH 8.1) + Tris Citrate (pH 8.1) discontinuous buffer system according to Ashton and Braden (1961); TC – Tris Citrate (pH 7.0) continuous buffer system according to Shaw and Prasad (1970).

Data analysis

The allelic frequencies were calculated based on diploid genotypes. They are available from the authors upon request. The expected and observed heterozygosities, mean number of alleles per locus and effective allele numbers were calculated by means of software Genepop v. 3.1c (Raymond and Rousset 1995) and Popgene v. 1.31. (Yeh and Boyle 1997).

Deviations from Hardy-Weinberg expectations due to heterozygote deficiency or excess, and to linkage disequilibrium,

were tested by Genepop (Raymond and Rousset 1995).

Genetic diversity among populations was evaluated by Nei's genetic distance (Nei 1978) and the differentiation measure was Wright's F-statistics (Wright 1965), calculated by the method of Weir and Cockerham (1984) using F-STAT software (Goudet 1995). Genetic distances (Nei 1978) were obtained by using the software Popgene, and cluster analysis was applied by means of the software DendroUPGMA (http://genomes.urv.cat/UPGMA, Garcia-Vallve et al. 1999).

Results

All loci showed minor polymorphism (*sensu* Lewontin 1985), which means that the frequency of the most common allele was always higher than 0.5.

Statistically significant linkage disequilibrium was detected in 12 of 344 possible two locus combinations in all populations (2.6 %) and concerned the populations Obzor (3), Ahtopol (2), Sinemorets (3) and Dulovo, Troyan, Momina kula and Veliko Tarnovo (1 each). The loci involved in the linkage disequilibrium were: PGI-2 (9 combinations), GOT-2 (5), LAP1 (3), PGM (3), MNR (2), and SKD2 (2). At overall level, when the data of all populations were pooled, significant linkage disequilibrium was detected in only one of 28 (3.6 %) two-locus combinations (PGI-2-LAP1). As a whole, the amount of linkage disequilibrium was negligible and therefore, we retained all loci for further analyses.

Nine significant cases of deviation from Hardy-Weinberg equilibrium were detected (one in each of nine populations), and all deviations were due to heterozygote deficiency. The most polymorphic loci were PGI-2 and MNR – each with 5 alleles, while 2 to 3 alleles were detected in the other loci.

Mean allele number per locus (Table 2) varied from 1.9 (Lom) to 3 (Sinemorec). Effective allele number varied from 1.15 (Veliko Tarnovo) to 1.35 (Berkovitza). Percent of polymorphic loci ranged from 25 to 75. Expected heterozygosity was relatively high, ranging from 0.147 (Veliko Tarnovo), to 0.253 (Berkovitza). Observed heterozygosities had values close to the expected ones, which resulted in low values of the inbreeding coefficient (-0.043 to 0.190).

Shannon index of diversity was the highest in locus Pgi-2, and also in the loci Got-2, Mnr and Lap1 (Table 3). The average value of all population was 0.36.

Nei (1978) genetic distances are presented in Table 4. The distance values are low indicating little genetic differences among the populations. The cluster dendrogram based on genetic distances (Fig. 2) revealed some grouping without clear trend – Obzor is the most distant population, while in the pairs and groups formed by the other populations, the closely situated Sinemoretz and Uzunbodzhak form a mini-cluster, like Shuma and Zemen. The other populations are placed without correlation among the genetic differences and geographic distance.

F-statistics showed that the overall differentiation is low – only about 3 % of the total variation was due to genetic differences among populations; the remaining variation could be attributed to intra-population genetic diversity (Table 5). Number of migrants was 27 using the private allele method), and 9, calculated by using F_{ST} method, The isolation-by-distance test calculated by plotting pairwise differentiation $F_{ST} \cdot (1-F_{ST})^{-1}$ against the log-transformed distance in km was not significant ($p = 0.089$).

Table 2. Information about the populations studied, and results of polymorphism and diversity.

Population (abbreviation)	Geographic coordinates	Altitude, m	S_m	A_m	A_e	P	H_o (s.e.)	H_e (s.e.)	F
Akhtopol (AGA)	42° 06' N 27°55' E	20	41.6	2.5	1.266	75	0.210 (0.038)	0.233 (0.043)	0.102
Berkovitza (BER)	43°17 'N 23°11' E	250	49.4	2.0	1.346	50	0.257 (0.062)	0.253 (0.059)	- 0.015
Dobrich (DOB)	43°30' N 27°44' E	200	46.6	2.25	1.304	62.5	0.233 (0.060)	0.237 (0.055)	0.016
Dulovo (DUL)	43°50' N 27° 04' E	200	49.9	2.5	1.244	37.5	0.196 (0.050)	0.208 (0.056)	0.057
Lom (LOM)	43°45' N 22°59' E	50	21.9	1.9	1.198	50	0.165 (0.066)	0.167 (0.062)	0.012
Mladezhka River (ML)	42° 05' N 27°25' E	250	55.8	2.63	1.256	75	0.204 (0.059)	0.213 (0.057)	0.043
Momina kula (MK)	41°43' N 23°42' E	650	53.4	2.25	1.176	37.5	0.150 (0.064)	0.147 (0.062)	0.020
Obzor (OB)	42°49' N 27°51' E	200	55.3	2.38	1.276	62.5	0.216 (0.047)	0.207 (0.051)	0.043
Perla (PR)	42°17' N 27°45' E	20	47.2	2.5	1.222	50	0.182 (0.035)	0.224 (0.045)	0.190
Shuma (SH)	43°00' N 23° 05' E	800	47.5	2.5	1.183	62.5	0.155 (0.061)	0.159 (0.051)	0.025
Sinemorec (SM)	42° 04' N 27°58' E	30	59.1	3	1.319	62.5	0.242 (0.046)	0.264 (0.053)	0.085
Troyan (TR)	42°54' N 24°47' E	500	55.7	2.25	1.248	50	0.199 (0.054)	0.210 (0.059)	0.053
Uzunbodzhak (UZ)	41°57' N 27°48' E	180	48.6	2.62	1.235	75	0.190 (0.055)	0.210 (0.057)	0.037
Veliko Tarnovo (VT)	43° 03' N 25°23' E	400	48.1	2.13	1.147	25	0.128 (0.062)	0.147 (0.067)	0.129
Zemen (ZE)	42°29' N 22°47' E	600	43.7	2.37	1.282	62.5	0.220 (0.059)	0.225 (0.059)	0.023
Mean			48.3	2.38	1.247	55.8	0.196 (0.059)	0.231 (0.056)	0.046

Note: A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95. Legend: S_m – mean sample size; A_m – mean number of alleles per locus; A_e – effective allele number (Crow and Kimura 1970); P – percent of polymorphic loci (0.05 criterion); H_o – observed heterozygosity; H_e – expected heterozygosity under Hardy-Weinberg equilibrium; s.e. – standard errors; F – inbreeding coefficient ($F = 1-H_o \cdot H_e^{-1}$).

Discussion

The polymorphism and diversity established in the studied populations showed that *Q. frainetto* exhibits levels of genetic diversity well within the range of these reported for other oak species (Hamrick et al. 1992). The levels of genetic diversity

Table 3. Shannon information diversity index (*I*).

Locus	Sample size	*I*
PGI-2	1520	0.8222
LAP-1	1538	0.3987
GOT-2	1134	0.5710
MNR	1508	0.3462
6PGD	1534	0.2626
PGM	1548	0.0990
SKD-1	1372	0.1751
SKD-2	1434	0.2152
Mean	1448	0.3612 ±0.24 (SD)

were more or less typical for species with similar life-history characteristics like *Q. frainetto*. Shannon's index of diversity confirmed the trend detected by the heterozygosity levels in separate loci. Analogously to heterozygosity, this index has the highest values when the alleles are evenly represented. Originally developed for information theory (Crow 2001), it has been successfully applied in ecology, but seems of little use in population genetics. Similar levels of genetic diversity were recorded in other studies. Curtu et al. (2011) found a bit higher differentiation (F_{ST} = 0.067); however, *Q. pubescens* populations were included in their dataset. Most studies report relatively high genetic diversity within populations and lower differentiation among populations, which was detected in the present study. There was no spatial trend in the distribution of genetic diversity. The cluster dendrogram revealed some grouping of closely situat-

Table 4. Genetic distances among populations (Nei 1978).

Populations*	SH	OB	UZ	PER	AGA	MLAD	SIN	DUL	ZEM	TROY	DOB	BER	MOM	VT
OB	0.0079													
UZ	0.0034	0.0124												
PER	0.0077	0.0155	0.0058											
AGA	0.0064	0.0136	0.0033	0.0009										
MLAD	0.0095	0.0168	0.0026	0.0069	0.0047									
SIN	0.0041	0.0051	0.0039	0.0070	0.0062	0.0076								
DUL	0.0059	0.0137	0.0116	0.0160	0.0112	0.0209	0.0152							
ZEM	0.0004	0.0092	0.0029	0.0069	0.0066	0.0066	0.0045	0.0093						
TROY	0.0026	0.0108	0.0069	0.0192	0.0144	0.0161	0.0084	0.0072	0.0050					
DOB	0.0028	0.0105	0.0027	0.0018	0.0006	0.0063	0.0046	0.0084	0.0020	0.0097				
BER	0.0076	0.0146	0.0065	0.0089	0.0074	0.0023	0.0071	0.0174	0.0053	0.0133	0.0071			
MOM	0.0015	0.0077	0.0042	0.0040	0.0032	0.0066	0.0040	0.0084	0.0017	0.0082	0.0012	0.0043		
VT	0.0016	0.0090	0.0053	0.0071	0.0060	0.0075	0.0034	0.0107	0.0021	0.0069	0.0032	0.0035	0.0001	
LOM	0.0027	0.0133	0.0072	0.0034	0.0041	0.0085	0.0079	0.0074	0.0020	0.0108	0.0017	0.0040	0.0010	0.0021

Note: * For full names of populations, see Table 2.

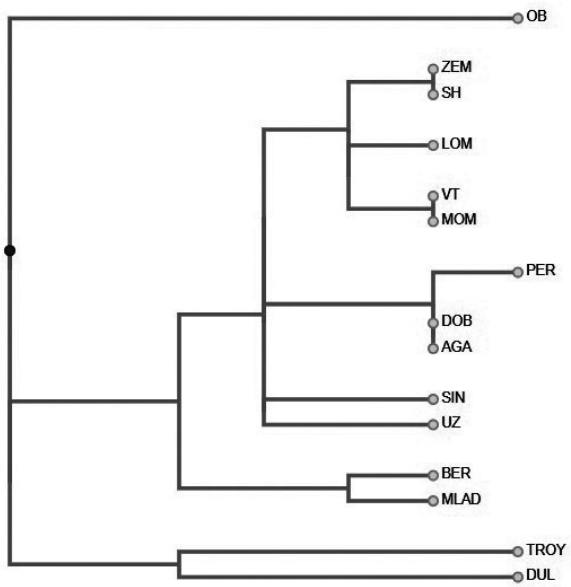

Fig. 2. Cluster dendrogram expressing the affinity among the populations based on the genetic distances.

Table 5. F-statistics and genetic differentiation.

Locus	F_{IS}	F_{IT}	F_{ST}
PGI-2	-0.0353	-0.0189	0.0159
LAP-1	0.1230	0.1475	0.0279
GOT-2	-0.0223	0.0137	0.0352
MNR	0.1924	0.2395	0.0583
6PGD	0.1224	0.1216	-0.0009
PGM	0.0566	0.0624	0.0061
SKD1	0.1924	0.2098	0.0216
SKD2	-0.0058	0.0513	0.0568
All	0.0421	0.0682	0.0272
95% CI*	-0.11-0.138	0.013-0.164	0.017-0.042

Note: *CI is confidence interval.

ed populations, for example Zemen and Shuma, Perla and Ahtopol, Sinemoretz and Uzunbodzhak, but some geographically distant populations were grouped together, too, like Berkovitza and Mladezhka River. These results can be explained by the neutral character of the allozyme markers, but also, with the extensive gene flow among the populations. The number of migrants was high, calculated both by private allele method and F_{ST} method. Whitlock and McCauley (1999) questioned the utility of F_{ST} method for reliable estimate of migrants and stated that one migrant per generation can reduce the population differences, which could arise from the genetic drift.

Low differentiation makes difficult the task of selecting populations of *Q. frainetto* for gene conservation. However, in similar cases it is always recommendable to select genetic entries representing all regions of the species distribution and the whole range of environmental conditions. The low among population diversity points out the necessity of application of more polymorphic and informative genetic markers (Bordács et al. 2002, Fineschi et al. 2002). Genetic integrity of the populations is not threated by hybridization with other oak species (Curtu et al. 2011) – in Bulgaria the issue has not been studied in detail, but if existing at all, it is of minor importance.

The populations studied had different size and included also some small and isolated stands. The results indicate that even small and isolated populations from the fragmented landscape still contain significant amount of genetic variation and, therefore, they must be considered in the sustainable management activities (Zhelev et al. 2002).

Conclusions

The study on the genetic diversity of *Q. frainetto* by using allozyme gene markers allows the following main conclusions:

- The levels of genetic diversity and differentiation of Bulgarian populations correspond to these reported for species with similar life history characteristics (e.g. Hamrick et al. 1992).

- No clear spatial trend was observed, which confirms the neutrality of allozyme markers and points out the significance of gene flow as a factor minimizing the genetic differences among populations.

- The major part of genetic diversity was attributed to within-population component of variation. No small-scale geographic subdivision was detected, which indicates the presence of substantial gene flow among populations.

- Selecting populations for gene conservation should be based on territorial principle with the aim to cover the diversity in the environmental conditions.

Acknowledgements

Part of the study was supported by the former Sylvica Foundation (Bulgarian-Swiss Forestry Programme) and by the National Science Fund, project SS-1408.

References

Aas G. 1993. Taxonomical impact of morphological variation in *Quercus robur* and *Quercus petraea*: a contribution to the hybrid controversy. Annales des Sciences Forestieres 50: 107–113. https://doi.org/10.1051/forest:19930709

Apostol E.N., Stuparu E., Scărlătescu V., Budeanu M. 2020. Testing Hungarian oak (*Quercus frainetto* Ten.) provenances in Romania. iForest 13: 9–15. DOI: 10.3832/ifor3108-012

Ashton G.C., Braden A.W.H. 1961. Serum β-globulin polymorphism in mice. Australian Journal of Biological Sciences 14: 248–253. https://doi.org/10.1071/BI9610248

Bacilieri R., Ducousso A., Petit R.J., Kremer A. 1996. Mating system and asymmetric hybridization in a mixed stand of European oaks. Evolution 50(2): 900–908. https://doi.org/10.1111/j.1558-5646.1996.tb03898.x

Belletti P., Leonardi S., Monteleone I., Piovani P. 2005. Allozyme variation in different species of deciduous oaks from northwestern Italy. Silvae Genetica 54: 9–16. DOI: 10.1515/sg-2005-0002

Bordács S., Popescu F., Slade D., Csaikl U.M., Lesur I., Borovics A., Kézdy P., König A.O., Gömöry D., Brewer S., Burg K., Petit R.J. 2002: Chloroplast DNA variation of white oaks in northern Balkans and in the Carpathian Basin. Forest Ecology and Management 156(1–3): 197–209. DOI: 10.1016/S0378-1127(01)00643-0

Bordács S., Zhelev P., Schirone B. 2019. EUFORGEN Technical Guidelines for genetic conservation and use for Hungarian oak (*Quercus frainetto*). European Forest Genetic Resources Programme (EUFORGEN), European Forest Institute. 6 p.

Cheliak W.M., Pitel J.A. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Report PI-X-42 Ontario, Canada. Petawawa National Forest Institute. 49 p.

Crow J.F. 2001. Shannon's brief foray into genetics. Genetics 159(3): 915–917. DOI: 10.1093/genetics/159.3.915

Crow J.F., Kimura M. 1970. An Introduction to Population Genetics Theory. Harper & Row publ., New York. 608 p.

Curtu A.L., Gailing O., Finkeldey R. 2007. Evidence for hybridization and introgression within a species-rich oak (*Quercus* spp.) community. BMC Evolutionary Biology 7, 218. https://doi.org/10.1186/1471-2148-7-218

Curtu A.L., Moldovan I.C., Enescu C.M., Cracunesc I., Sofletea N. 2011. Genetic differentiation between *Quercus frainetto*

Ten. and *Q. pubescens* Willd. in Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 39(1): 275–282. DOI: https://doi.org/10.15835/nbha3915633

Fineschi S., Taurchini D., Grossoni P., Petit R.J., Vendramin G.G. 2002. Chloroplast DNA variation of white oaks in Italy. Forest Ecology and Management 156(1–3): 103–114. DOI: 10.1016/S0378-1127(01)00637-5

Finkeldey R. 2001. Genetic variation of oaks (*Quercus* spp.) in Switzerland. 1. Allelic diversity and differentiation at isozyme gene loci. Forest Genetics 8(3): 185–195.

Fortini P., Di Marzio P., Di Pietro R. 2015. Differentiation and hybridization of *Quercus frainetto, Q. petraea*, and *Q. pubescens* (Fagaceae): insights from macro-morphological leaf traits and molecular data. Plant Systematics and Evolution 301: 375–385. DOI: 10.1007/s00606-014-1080-2

Ganchev I., Bondev I. 1966. Genus 220 (3) Oak – *Quercus* L. In: Jordanov D. (Ed.). Flora of P.R. Bulgaria, vol. 3: 109–147 (in Bulgarian).

Garilov C., Stoykov H. 1978. Morphological and ecological forms of the Hungarian Oak (*Quercus conferta* Kit.) in Strandzha Mts. Gorskostopanska nauka 11(5): 12–28. (in Bulgarian).

Garcia-Vallve S., Palau J., Romeu A. 1999. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. Molecular Biology and Evolution 16(9): 1125–1134. DOI: 10.1093/oxfordjournals.molbev.a026203

Gömöry D., Yakovlev I., Zhelev P., Jedinakova J., Paule L. 2001. Genetic differentiation of oak populations within the *Quercus robur/Quercus petraea* complex in Central and Eastern Europe. Heredity 86: 557–563. https://doi.org/10.1046/j.1365-2540.2001.00874.x

Goudet J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics, Journal of Heredity 86: 485–486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Hamrick J.L., Godt M.J.W., Sherman-Broyles S.L. 1992. Factors influencing the level of diversity in woody plant species. New Forests 6: 95–124. https://doi.org/10.1007/BF00120641

Kalinkov V. 1960. On the distribution and silvicultural properties of the Hungarian oak (*Quercus conferta* Kit.) in Bulgaria. PhD thesis. Sofia. 177 p. (in Bulgarian).

Kalinkov V. 1961. Anatomical structure of the wood of Hungarian oak (*Quercus conferta* Kit.). Higher Institute of Forestry, Research Papers, IX: 99–113 (in Bulgarian with German summary).

Kalinkov V., Shipchanov I. 1976. Anatomical structure of the wood of some oak species. Gorskostopanska nauka 13(5): 21–34 (in Bulgarian with English summary).

Kleinschmit J.R.G., Kremer A., Roloff A. 1995. Sind Stieleiche und Traubeneiche zwei getrennte Arten? Algemeine Forstzeitung/Der Wald 26: 1453–1456.

Kremer A., Hipp A.L. 2020. Oaks: an evolutionary success story. New Phytologist 226: 987–1011. DOI: 10.1111/nph.16274

Kremer A., LeCore V. 2012. Decoupling of differentiation between traits and their underlying genes in response to divergent selection. Heredity 108: 375–385. https://doi.org/10.1038/hdy.2011.81

Kremer A., Zanetto A. 1997. Geographical structure of gene diversity in Quercus petraea (Matt.) Liebl. II. Multilocus patterns of variation. Heredity 78: 476–489. https://doi.org/10.1038/hdy.1997.76

Lewontin R.C. 1985. Population genetics. Annual Review of Genetics 19: 81–102. https://doi.org/10.1146/annurev.ge.01.120167.000345

Marinov M., Kostadinov K., Popov G., Stiptzov V., Bozhinov H., Dinev D., Denev D., Horozov S. 1995. The Oak Forests of Bulgaria. Sofia, Zemizdat. 255 p. (in Bulgarian).

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590. DOI: 10.1093/genetics/89.3.583

Raymond M., Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249. https://doi.org/10.1093/oxfordjournals.jhered.

a111573

Schwarz O. 1937–1939. Monographie der Eichen Europas und des Mittelmeergebietes. Repertorium specierum novarum regni vegetasbilis: 1–5.

Schwarz O. 1964. *Quercus* L. In: Tutin T.G., Heywood V.H., Burges N.A., Valentine D.H., Walters S.M., Webb D.A. (Eds) Flora Europaea, vol. 1: Lycopodiaceae to Platanaceae, Cambridge University Press, Cambridge: 61–64.

Shaw C.R., Prasad R. 1970. Starch gel electrophoresis of enzymes – a compilation of recipes. Biochemical Genetics 4: 297–320. DOI: 10.1007/BF00485780

Stefanoff B. 1943. A contribution to the study and classification of Oak forests in Bulgaria. Ann. Sofia University, Faculty of Agriculture and Forestry, vol. XXI, book 2: 125–180; 305–363; vol. XXII, book 2: 65–108.

Stejnhoff S. 1997. Results of *Quercus* hybridization work from 1989 to 1996 at Escherode. In: Steiner K.C. (Ed.), Diversity and Adaptation of Oak Species. Pennsylvania State University: 151–164.

Uzunova K., Palamarev E. 1992. Study of the leaf epidermis of the Balkan representatives of the family *Fagaceae* Durmort. III. *Quercus* L. (subgenus *Quercus*, sections *Roburoides* (Schwarz) Schwarz and *Dascia* (Kotschy) Schwarz. Fitologija 42: 22–48.

Uzunova K., Palamarev E., Ehrendorfer F. 1997. Anatomical changes and evolutionary trends in the foliar epidermis of extant and fossil Euro-Mediterranean oaks (Fagaceae). Plant Systematics and Evolution 204: 141–159. https://doi.org/10.1007/bf00989202

Weir B.S., Cockerham C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370. DOI: 10.1111/j.1558-5646.1984.tb05657.x

Whitlock M.C., McCauley D.E. 1999. Indirect measures of gene flow and migration: F_{ST}≠1/(4Nm+1). Heredity 82: 117–125. https://doi.org/10.1038/sj.hdy.6884960

Wright S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395–420. https://doi.org/10.1111/j.1558-5646.1965.tb01731.x

Yeh F.C., Boyle T.J.B. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129: 157–163.

Yurukov S., Panayotov M. 2015. Dendrology. Sofia, Ruta HB Publ. House. 211 p. (in Bulgarian).

Zanetto A., Kremer A. 1995. Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. I. Monolocus patterns of variation. Heredity 75: 506–517.

Zanetto A., Roussel G., Kremer A. 1994. Geographic variation of interspecific differentiation between *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. Forest Genetics 1: 111–123.

Zhelev P., Tsavkov E., Yurukov S. 2002. Genetic diversity in large old-growth and in small fragmented oak stands: implications for management and conservation practice. In: Dygen 'Dynamics and Conservation of Genetic Diversity in Forest Ecosystems' 2002, Strasbourg, France, Book of Abstracts: 90.