STUDIES ON GENETIC DIVERSITY IN EUROPEAN OAK POPULATIONS

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Abstract. The possession of genetic variation is an indispensable precondition for the ability of forest tree populations to survive spatial and temporal variation of the environment. Therefore, genetic variation is the basis of any evolutionary development. Genetic inventories give an idea of the ammount of genetic variation in a forest tree population. In European oaks, with the exception of some recent studies in juvenile populations of pendunculate oak *(Quercus robur)* and sessile oak *(Quercus petraea),* employing biochemical genetic marker systems, such inventories do not yet exist. The results of the present study using biochemical markers on different European populations of sessile oak revealed relatively small genetic variation within individuals in terms of heterozygosity. In contrast, the variation within populations is large. Genetic differentiation among populations of each species is relatively small. In contrast to earlier results, which yielded smaller values for pendunculate oak as compared to sessile oak, the present results indicate the opposite trend. As can be expected, mixtures of population samples from different locations are less differentiated than samples from single locations. The genetic information of pendunculate oak and sessile oak is quite similar. So far, no species-specific alleles have been found. However, allele frequencies can vary between species. In particular, genetic distances are found to be greater between populations of the two different species than between populations of the same species. Oaks are carrier tree species of important forest ecosystems. Compared to other species, oaks are long-lived and thrive in a wide range of ecological site conditions. These peculiarities should require very large intrapopulational variation and in fact, such variation was observed in all previous preliminary studies. However, many alleles are rare in all populations in which they occur. As a consequence, further intensive studies on genetic structures with special consideration of appropriate measures for the conservation of genetic resources are required.

Keywords: Quercus, genetic variation, genetic resources.

INTRODUCTION

Oaks belong to the major deciduous tree species in Europe. Two species, *i.e. Quercus robur* (pedunculate oak) and *Quercus petraea* (sessile oak) are quantitatively predominating in most parts of Europe. They are carrier species of complex, economically as well as ecologically important forest ecosystems. They range from the

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fertile plains of the lowlands to the submontane or even montane regions. Oaks are relatively long-lived species with forest rotation cycles of 200 and more years. Thus, they are exposed to more heterogeneous environmental conditions than most other predominant tree species and may serve as model organisms in the study of genetic variability and its implications for the survival of tree populations, especially in complex environmental situations.

The objective of the present study is to proceed in the characterization of the genetic variation in pedunculate oak and sessile oak populations and to supplement the genetic inventory of European oak populations initiated by preliminary studies of MÜLLER-STARCK and ZIEHE (1991), KREMER *et al.* (1991), and
et *al.* (in press). Additionally, it should contribute to the knowledge of the natural variability of forest ecosystems in general. Data on patterns of genetic variation will lead to a better understanding of principles of adaptation and survival of long-lived tree species. Such data is needed to develop criteria for the choice of reproductive material, for silvicultural treatment as well as for the conservation of genetic ressources.

MATERIAL AND METHODS

Nine stands of sessile oak *(Quercus petraea* LIEBL.) from Scotland, France, Danmark and Deutschland were studied (Figure 1).

The samples represent presumably indigenous populations of sessile oak and also include marginal provenances of this species. The sample size was 100 trees per population. In addition, first results of an unpublished study of HERZOG and KRABEL on pedunculate oak *Quercus robur* are discussed in the context of the present study.

Buds or young leaves were sampled and immediately frozen in liquid nitrogen before storage at -80° Celsius. In the present study the sample size was 100 per population. This means a probability of 95 per cent to detect alleles with a frequency of at least a=5.99.

Isoenzyme analysis was modified following MÜLLER-STARCK and ZIEHE (1991). The buds were thawed and homogenized in a 0.08/0.02 mol/lTRIS/HCl buffer at pH 7.3. To inhibit phenols and tannins, 2 to 5 %[w/v] polyvinylpyrrolidone, 10 to 130 mmol/l mercaptoethanol, 3 mmol/l ethylenediaminetetraacetic acid (EDTA) as well as 3 to 6 mmol/l dithiothreitol were added. The resulting slurry was absorbed onto filter paper wicks and loaded onto gel slabs. Horizontal starch gel electrophoresis was performed using a starch concentration of 11.5%[w/v]. The bridge distance was 12 cm with a voltage distribution of 20 to 30 V/cm.

Six isoenzyme systems (table 1) representing seven polymorphic gene loci, were identified by MÜLLER-STARCK and HATTEMER (1990) as well as HERZOG (unpublished data) as genetic markers.

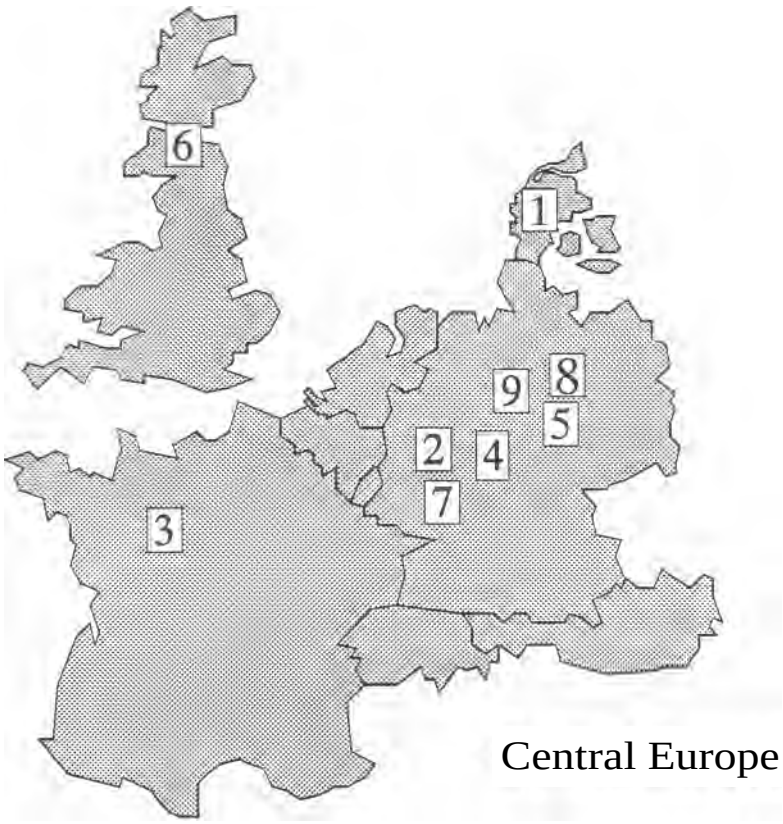

Figure 1. *Schematic map of Central Europe and the populations studied (for identification of the single populations see Table 2).*

They were studied using different electrode and gel buffer systems (table 1), modified following MÜLLER-STARCK and ZIEHE (1991). Solutions used for enzyme staining were modified following CHELIAK and PITEL (1984).

RESULTS AND DISCUSSION

Variation within populations

One commonly used measure for genetic variation of individuals and populations is the degree of heterozygosity (table 2). Its estimation relies on genotypic rather than on allelic frequencies; the ammount of heterozygosity attainable in a population ("actual amount of heterozygosity", H_A) depends on the actual allele frequencies.

The present study revealed an average (arithmetic mean) actual heterozygosity of H_A=0.229 over 9 populations of *Quercus petraea* (table 2).

The highest value was found for Scotland (Dymock), the lowest value for one population from the north of Deutschland (Lüß). The average lies near to the upper bounds

Table 1. *Enzyme systems and buffers used in* the *present study*.

Enzyme system and E.C. code	Electrode buffer and Gel buffer
Shikimate dehydrogenase E.C. 1.1.1.25	0.14/0.05 mol/l TRIS/citric acid, pH 6.5 0.04/0.014 mol/l TRIS/citric acid, pH 6.6
Isocitrate dehydrogenase E.C. 1.1.1.42	0.14/0.04 mol/l TRIS/citric acid, pH 7.8 0.04/0.011 mol/l TRIS/citric acid, pH 7.8
6-phosphogluconate dehydrogenase E.C. 1.1.1.44	0.14/0.04 mol/l TRIS/citric acid, pH 7.8 0.04/0.011 mol/l TRIS/citric acid, pH 7.8
Glutamate dehydrogenase E.C. 1.4.1.2	0.06/0.30 mol/l NaOH/boric acid, pH 8.0 0.07/0.02 mol/l TRIS/HCl, pH 8.7
Phosphoglucomutase E.C. 2.7.5.1	0.14/0.05 mol/l TRIS/citric acid, pH 6.5 0.04/0.014 mol/l TRIS/citric acid, pH 6.6
Phosphoglucose isomerase E.C. 5.3.1.9	0.05/0.19 mol/l LiOH/boric acid, pH 8.1 0.05/0.01 mol/l TRIS/citric acid, pH 8.1

of the spectrum given by previous studies for this species: in previous studies, the average heterozygosity at the species level was estimated to be H_A=0.213 for *Quercus robur* and 0.219 resp. 0.229 for *Quercus petraea* by MÜLLER-STARCK and ZIEHE (1991) and MÜLLER-STARCK *et al.* (in press). HERZOG and KRABEL (unpublished data) found an average of H_A=0.253 for *Quercus robur.*

Deviations from Hardy-Weinberg proportions were observed at several loci, but in most cases expected genotype frequencies are less than 5, which may erroneously suggest rejection of the hypothesis using the x^2-test. However, provided the deviations were truly significant, they may suggest an influence of selection or the mating system, but the present data base does not allow further conclusions in this context.

Diversity is measured using the gene pool diversity (v, GREGORIUS 1978, 1987) and the total population differentiation (S_T*, GREGORIUS 1987; table 2). Especially the calculation of v makes the data comparable to other studies. Whereas the present study revealed a total population differentiation S_T* ranging between 0.210 and 0.322, the gene pool diversity v was found to range between v=1.26 and v=1.47. MÜLLER-STARCK and ZIEHE (1991) as well as MÜLLER-STARCK *et al.* (in press) calculated gene pool diversities between v=1.29 and v=1.49 for *Quercus petraea.* For *Quercus robur* the respective values were found to range between v=1.33 and v=1.41 (MÜLLER-STARCK and ZIEHE 1991, MULLER-STARCK *et al.* in press) resp. between v=1.35 and v=1.47 (HERZOG and KRABEL unpublished data).

Table 2. *Genetic parameters for 9 sessile* oak *(Quercus petraea) populations: heterozygosity (H_A),* gene *pool diversity* (v, GREGORIUS *1978, 1987), population differentiation (S_T *,* GREGORIUS *1987), and subpopulation differentiation (D_o,* GREGORIUS *and* ROBERDS *1986).*

Origin	*HA*	v	(5T*	
(1) Danmark (Horbylunde)	0,227	1,47	0,322	0,103
(2) Deutschland (Bad Münstereifel)	0,211	1,34	0,257	0,059
(3) France (Youille Saint Hilaire)	0,217	1,33	0,252	0,052
(4) Deutschland (Wolfgang)	0,253	1,36	0,266	0,048
(5) Deutschland (Lüi3)	0,191	1,26	0,210	0,049
(6) Scotland (Dymock)	0,261	1,30	0,230	0,044
(7) Deutschland (Johanniskreuz)	0,233	1,34	0,254	0,045
(8) Deutschland (Göhrde)	0,224	1,33	0,250	0,074
(9) Deutschland (Seelzerthurm)	0,246	1,35	0,262	0,078

Thus, the present data correspond well to previous studies on oak populations. However, the values are high compared to the results of studies on other plant species. HAMRICK and GODT (1990) reanalyzed more than 600 studies and found an average "effective number of alleles" of 1.24. This measure is comparable to the diversity v what means a relatively high diversity of oaks. This may result from the above mentioned spatial and temporal heterogeneity of the environments.

Differentiation between populations

In the present study, the genetic distances do (GREGORIUS 1974, 1984) as well as the gene pool subpopulation differentiation D_j (see Table 2) and S (GREGORIUS and ROBERDS 1986, GREGORIUS 1987; Table 3 and 4) were applied.

Table 3. *Pairwise genetic (gene pool) distances do* between populations *of sessile* oak *(Quercus petraea)*

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1)	–								
(2)	0,088	–							
(3)	0,088	0,071	–						
(4)	0,114	0,079	0,039	–					
(5)	0,114	0,075	0,042	0,053	–				
(6)	0,098	0,054	0,053	0,045	0,041	–			
(7)	0,108	0,081	0,061	0,056	0,054	0,061	–		
(8)	0,150	0,106	0,077	0,064	0,078	0,087	0,070	–	
(9)	0,157	0,106	0,090	0,073	0,071	0,071	0,077	0,061	–

Genetic distances d_os of, say, 0.1 and more provide evidence for a substantial genetic differentiation. However, the genetic distances are poorly correlated to the geographical distances. This means that we also should not expect a good correlation between genetic differentiation and geographic distance of the sites.

The subpopulation differentiation was found to range between 0.044 (Dymock, Scotland) and 0.103 (Horbylunde, Danmark). Thus, the subpopulation differentiation *S* (GREGORIUS 1987; Table 4) is equalling 0.061; the corresponding value was found to be 0.055 in *Quercus robur* and 0.085 in *Quercus petraea* (MÜLLER-STARCK and ZIEHE 1991). Another study on *Quercus robur* revealed 8=0.091 (HERZOG and KRABEL unpublished data).

Table 4. Subpopulation differentiation S (GREGORIUS and ROBERDS 1986): comparison of the studies to date.

Species (author)	δ
Quercus petraea (this study)	δ=0,061
Quercus petraea (MÜLLER-STARCK and ZIEHE 1991)	δ=0,085
Quercus robur (MÜLLER-STARCK and ZIEHE 1991)	δ=0,055
Quercus robur (HERZOG and KRABEL unpublished data)	δ=0,091

These results provide evidence that the adult stands of pedunculate oak are

more differentiated than the juvenile populations of pedunculate and sessile oak. Moreover, the present data show a reduced S for sessile oak compared to the studies of MÜLLER-STARCK and ZIEHE (1991) as well as MULLER-STRACK *et al.* (in press). However, this may be at least partially caused by differences in the genetic stuctures dependent on age or by the restricted number of populations studied by HERZOG and KRABEL (unpublished data) (2 populations) or the latter authors (5 populations). The study of KREMER (1991) found juvenile populations of sessile oak especially in France to be significantly less differentiated (G_{ST}=0.017). This may be partially caused by methodological differences (S values normally exceed the corresponding *GST* values), but nevertheless it can be concluded that sessile oak in France may be less differentiated than in other parts of Europe studied to date. In general, the genetic differentiation of oak populations is of the same magnitude as obeserved for other decidous tree species (see also MÜLLER-STARCK 1991).

Another problem to be discussed is that of rare alleles. The common differentiation measures normally underestimate the influence of alleles occuring in low frequencies, say, less than 10 per cent. For example, we can consider the *PGM-A* gene locus (Table 5, Figure 2).

One allele *($PGM\text{-}A_4$)* is predominating and shows a high frequency of more than 80 per cent with one exception (Horbylunde). The other rare alleles are heterogeneously distributed what provides evidence for a locally acting selection against different rare alleles, whereas the common allele may be optimized under the present environmental conditions in general. We have to keep in mind that this differentiation pattern caused by rare alleles does not cogently correspond to that revealed by application of the common differentiation measures.

Consequences for conservation of genetic resources

Genetic variation is an important prerequisite for the ability of forest tree populations to survive spatial and temporal variation of environmental conditions. Therefore, genetic variation is the basis of any evolutionary development. Consequently, provisions on gene conservation require an inventory of genetic variation in as many populations of a species as possible. The present study should contribute to this inventory.

The present results reveal a relatively small genetic variation within individuals in terms of small actual heterozygosities. In contrast to that, intrapopulational variation is extraordinarily large.

In general, genetic differentiation among populations of each species is relatively small. In contrast to earlier results which suggest smaller values for pendunculate oak as compared to sessile oak, the present results indicate the opposite trend. As can be expected, mixtures of population samples from different locations are less differentiated than samples from single locations.

The gene pools of pendunculate oak and sessile oak are very similar: There are no species specific alleles so far although allele frequencies can vary between species.

Table 5. *Differentiation between sesile* oak *populations (Quercus petraea) due to the occurence of rare alleles using the gene locus P GM-A as* an *example (see also Figure 2)*

		PGM-A2	PGM-A_3	PGM-A_4	PGM-A_5	PGM-A,
Horbylunde	0.000	0.365	0.020	0.565	0.050	0.000
Bad Münst.	0.000	0.070	0.025	0.885	0.020	0.000
Y.-St.-Hilaire	0.000	0.005	0.005	0.935	0.035	0.020
Wolfgang	0.005	0.000	0.015	0.925	0.030	0.025
Lüß	0.000	0.000	0.000	0.985	0.015	0.000
Dymock	0.000	0.035	0.000	0.945	0.020	0.000
Johanniskreuz	0.000	0.105	0.025	0.840	0.025	0.005
Göhrde	0.000	0.030	0.000	0.910	0.055	0.005
Seelzerthurm	0.000	0.020	0.000	0.975	0.005	0.000

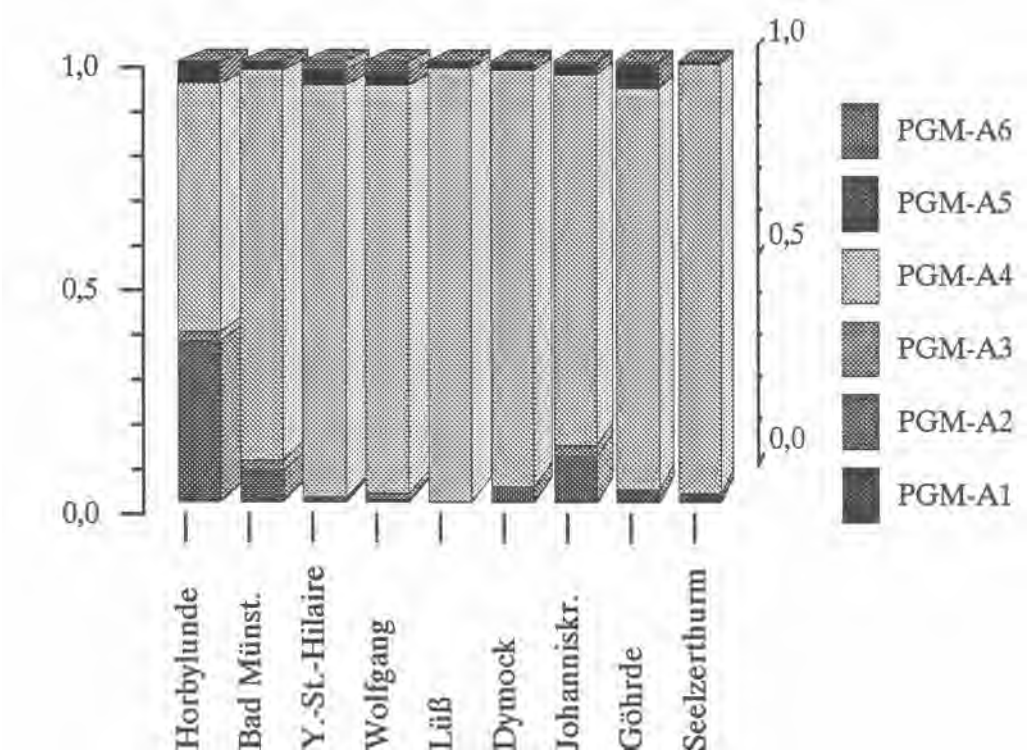

Figure 2. *Differentiation* between *sesile oak populations (Quercus petraea) due to the occurence of* rare *alleles using* the gene *locus P GM-A as an example (see also* Table *5)*

Consequently, genetic distances are greater between populations of the two different species as compared to populations within each species.

As above mentioned, oaks are carrier tree species of significant forest ecosystems. Compared to other tree species, oaks are extremely long-lived and cover a wide range of ecologically different forest sites. Genetic resources of oaks are endangered not only by the loss of living spaces such as the natural fertile plains but also by the impact of air pollution for several decades and maybe even by long-term climatic changes. Moreover, silvicultural customs, especially the limitation of seed sources may also contribute to the loss of genetic ressources. In addition, since several years we can observe an increasing impact of air pollutants even on broad-leaved trees such as oaks and beech. The results presented herein suggest that forest tree breeding and silviculture of sessile oak and pedunculate oak need to take into account large genetic multiplicities. Genetic heterogeneity should correspond to the tremendous environmental heterogeneity to which long-lived oak populations are exposed (see also MÜLLER-STARCK *et al.* in press). It appears that large genetic variation has to be incorporated in productive populations in order to maintain the potential of these populations to adapt to and to survive in complex environmental situations.

It seems not probable that we can find single stands representing the whole or nearly the whole genetic variation of the species. This would call for a management which is focussed on the *in situ* maintainance of numerous and sufficiently large, locally adapted stands. First rough estimations recommend minimum sizes of oak stands serving for gene conservation purposes of 30 to 50 hectars (HERZOG and MÜLLER-STARCK 1993). However, the present results provide good evidence that genetic conservation of oaks should be possible by means of a regular silvicultural management.

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