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Original article

Investigations on vitality and genetic structure in oak stands

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Summary — Six oak stands with the two indigenous species *Quercus petraea* and *Q robur* were investigated in order to establish relationships between the vitality of oak trees and their genetic structure. The stands were affected by the 'European oak decline'. The registered traits of every tree were branching habits, defoliation, discoloration of foliage, necrosis on stems, epicormic branches at stems and in the crowns. The several traits were integrated into a vitality coefficient. Isozyme analyses were carried out to characterize the genetic structure of oak stands and subpopulations distinguished by their vitality. In principle, the results indicate the same tendency for the relationship between vitality and genetic structure for *Q robur* and *Q petraea:* increase of excess of homozygotes from the tolerant group to the sensitive group, decrease of observed heterozygosity from the tolerant to the sensitive group, maximum hypothetical gametic diversity and minimum subpopulation differentiation in the intermediate group as an indication for a directed selection.

***Quercus* / vitality / isozyme marker / genetic structure / selection / decline**

Résumé — Recherches sur la vitalité et la structure génétique de peuplements de chênes. *Six peuplements comportant les deux espèces indigènes* Quercus petraea *et* Q robur *ont été analysés dans le but de relier la vitalité des arbres à leur structure génétique. Les peuplements en question souffraient de dépérissement marqué. Les caractères notés sur chaque individu étaient la branchaison, le degré de défoliation, les décolorations du feuillage, l'existence de nécroses sur les troncs, et la présence de rameaux anticipés sur les troncs et dans les branches. Tous ces caractères ont été utilisés pour la définition d'un index de vitalité. Des analyses d'isozymes ont été entreprises pour caractériser la structure génétique des peuplements, ou de sous-populations différant par leur degré de vitalité. Les résultats indiquent des tendances identiques entre les deux espèces : augmentation de l'excès d'homozygotes entre les groupes tolérants et ceux sensibles au dépérissement, baisse du degré d'hétérozygotie depuis les plus tolérants aux plus sensibles, diversité gamétique maximale et faible degré de différenciation entre subpopulations dans les groupes intermédiaires. Les deux groupes extrêmes, à fort degré de différentiation intrapopulation, peuvent constituer des sous-populations résultant d'un processus de sélection lié au dépérissement. Ils présentent un potentiel réduit de production d'une nouvelle*

génération avec une large variabilité génétique, par rapport au groupe intermédiaire à forte variabilité et à bonne capacité d'adaptation à des modifications des conditions d'environnement.

Quercus / *vitalité / isozymes / structure génétique / sélection / dépérissement*

INTRODUCTION

Oak trees are dominant forest tree species in Germany and an important ecological and economical factor. Both species *Quercus petraea* and *Q robur* covered more than 38% of the total forest area in the eastern part of Germany in the past. This area has been strongly decreased in the last centuries in favour of conifer tree species. Today, oaks are growing in a tenth of their natural range as major species (Kohlstock, 1993). Therefore, their conservation and promotion is interesting because oak stands are more and more endangered by the increasing process of 'European oak decline' and the shifting of climatic zones.

The current process of oak decline is not limited to East Germany but is found all over Europe. According to the report of forest damage survey of Germany, distinct damages were found on 45% of all oaks (Anonymus, 1993), thus, nearly every second oak shows visible symptoms. The vitality decrease appears to be stronger especially in East Germany. Here 55% of all oaks are clearly damaged.

This process of decreasing vitality is expressed in several phenotypical traits of the trees. The level of damage varies from tree to tree and includes the dying of members of the stands. A regeneration of oak stands cannot be noted until now and because of its complexity it is not foreseeable.

The capability of forest trees to survive is based on their adaptation to the existing environment and their adaptability to changing environmental conditions. Long-living forest trees need genetic variability at the level of individuals, among individuals in populations and among populations in the natural range of species in order to adapt to heterogeneous environmental conditions.

From the view of gene conservation and forest tree breeding, it is important to gain information about the genetic structure effect on the sensitivity to 'European oak decline' and the influences of decline on genetic structure of stands in the next generation. Preconditions for that are investigations concerning the state of damage of oak trees and the complete evaluation of their vitality in the stands based on phenotypic-morphological traits and the description of the genetic structure in the research area.

MATERIALS AND METHODS

Trials

Six experimental sites were established as permanent observation plots. They are situated on Pleistocene Forest soils formed by the last two stages of the Weichselian Glaciation Period. The sites are located in the eastern part of Germany, in the area of Brandenburg (fig 1).

The mean annual temperature ranges from 8.2 to 8.4 °C, the average annual rainfall amounts to 520 and 570 mm. The vegetation types of the sites are pine(linden)-sessile oak forests or beech-pedunculate oak forests.

The site type of all six experimental stands is characterized by sufficient supply with nutrients and average but varying water supply (K2). All stands are established artificially. The planted material of five stands came from surrounding forests, the origin of the trees of the stand 'Blumenthal' 1 is unknown.

Three of the six stands are mixed stands with *Q petraea* and *Q robur*. Every tree was assigned to the species belonging to leaf traits (leaf shape,

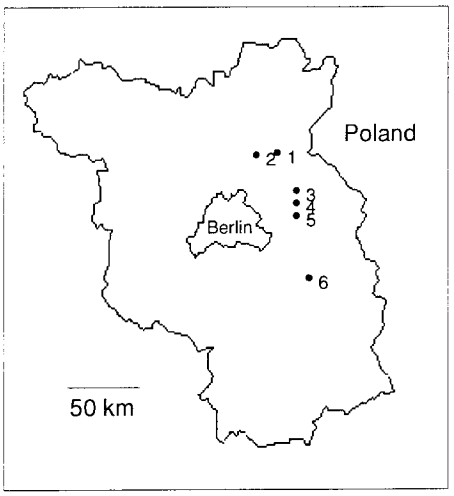

Fig 1. Map of Brandenburg with the location of six experimental plots.

nervature) and acorn traits according to Aas (1988). There was no individual with indifferent traits in our study which possibly could be a hybrid. An overview on the six trials is given in table I.

Assessment of damage

The estimation of vitality includes several traits which are described by their classification in table II. The stands were evaluated for the traits branching structure, water sprouts of stem, water sprouts of crown, and bark necrosis in the time between December and March. The traits discoloration of leaves and defoliation were examined in late spring or early summer. The trait defoliation contains the assessment of feeding activity of insect pathogens and abscission of twigs after dry periods especially.

The branching structure of the crowns were classified after the estimation key of Roloff (1989).

Table I. Survey of experimental sites.

Site no	*Designation of trial*	*Size (ha)*	*No of trees/ Ratio of* Q robur: Q petraea	*Age (years)*	*Other tree species*
1	Rosinsee	0.4	100 70:30	100	*Carpinus betulus, Fagus sylvatica*
2	Plagefenn	0.4	138 83:17	90	*Fagus sylvatica*
3	Tiefensee	0.3	49 22:78	130	*Fagus sylvatica, Carpinus betulus, Betula pubescens*
4	Blumenthal 1	0.13	68 100:0	85	*Pinus sylvestris*
5	Blumenthal 2	0.3	34 100:0	120	*Carpinus betulus, Acer pseudoplatanus, Tilia cordata*
6	Rehhagen	2	38 0:100	130	*Pinus sylvestris, Carpinus betulus*

Table II. Quantification of phenotypical traits which characterized harmful effects.

Traits	*Score*	*Estimation key*	*Weighing factor*
Branching structure	1	Exploration	
	2	Degeneration	0.5
	3	Stagnation	
	4	Resignation	
	5	Dead	
Defoliation	1	< 10%	
	2	< 30%	0.1
	3	< 60%	
	4	> 60%	
Discoloration of foliage	1	< 10% of foliage	
		< 30%	0.1
	2	< 60%	
	3	> 60%	
	4		
Epicormic branches of crown	1	No water sprouts	0.1
	2	Isolated	
	3	Many	
Epicormic branches of trunk	1	No water sprouts	0.1
	2	Isolated	
	3	Many	
Bark necrosis	1	No	0.1
	2	Isolated	
	3	Many	

The scores of the other visible symptoms were fixed with regard to the situation of oak decline of all six stands and the possibility of their actual estimation.

The traits were weighed differently and added up into a vitality coefficient. All trees were arranged into the vitality groups 'tolerant', 'intermediate', and 'sensitive'. The limits between the vitality groups were determined according to the accumulation of the individual values of the tree's vitality. The values of the vitality coefficient of the tolerant group ranged from 1.1 to 2.0; the values of the vitality coefficient of the intermediate group ranged from 2.1 to 2.6; the values of the sensitive group enclosed values of more than 2.6. Dead trees were recorded in favour of a description of the structures of the stands and the oak decline course inside the stands.

Description of the genetic structure

Isozyme analyses were carried out for ten enzyme systems encoded by 11 loci listed in table III. Dormant buds of the trees were homogenized in extraction buffer (modified from Lundkvist, 1974) containing 1% (v/v) 2-mercaptoethanol and 5% (w/v) Polyclar AT. The proteins were separated by horizontal starch gel electrophoresis with the following buffer systems: (A) 12.5% starch in 0.02 M Tris citrate buffer pH 7.5, electrode buffer: 0.15 M Tris citrate buffer pH 7.5; (B) 12.5% starch in 0.05 M Tris citrate buffer pH 8.1, electrode buffer: 0.2 M lithium borate buffer pH 8.1; (C) 12.5% starch in 0.075 M Tris citrate buffer pH 8.7, electrode buffer: 0.3 M sodium borate buffer pH 8.3. For the enzyme systems AAT and GDH, the proteins

Table III. Enzyme systems used for genetic analysis of oak trees.

Enzyme	*Separation method* [a]	*Protein structure*	*Isozyme loci scored*
Acid phosphatase E.C.3.1.3.2.	C	Monomeric	ACP-C
Aminopeptidase E.C.3.4.11.1./2.	B	Monomeric	AP-B
Aspartataminotransferase E.C.2.6.1.1.	PAGE	Dimeric	AAT-B
Glutamate-dehydrogenase E.C.1.4.1.2.	PAGE	Tetrameric	GDH
Isocitrate-dehydrogenase E.C.1.1.1.42	A	Dimeric	IDH-B
Menadione-reductase E.C.1.6.99.2.	C	Tetrameric	MR
NADH-dehydrogenase E.C.1.6.99.3.	A	Polymeric Monomeric	NDH-A NDH-B
Phosphoglucomutase E.C.2.7.5.1.	A	Monomeric	PGM-A
6-Phosphogluconate-dehydrogenase E.C.1.1.1.44.	A	Dimeric	PGDH
Phosphoglucose-isomerase E.C.5.3.1.9.	B	Dimeric	PGI-B

For a further explanation, see *Materials and Methods*.

were separated by electrophoresis in polyacrylamide slab gels (PAGE, 7.5% polyacrylamide in 0.375 M Tris-HCl buffer pH 8.9, and 0.19 M Tris glycine electrode buffer pH 8.3; Maurer, 1968).

Specific staining solutions for the enzymes 6PGDH, GDH, AAT and PGI were modified from Yeh and O'Malley (1980), and stains for the enzymes ACP, IDH, PGM and AP followed Vallejos (1983). The staining solutions for MR and NDH were modified from Cheliak and Pitel (1984).

The observed heterozygosity H_o at a locus is equal to the proportion of heterozygous trees among all tested trees. The expected heterozygosity H_e is the proportion of heterozygotes at the Hardy-Weinberg equilibrium. The total population differentiation δ_T was used to even out the different sample sizes (Gregorius, 1987). The fixation index F was calculated as $F = 1 - H_o / H_e$. The gene pool diversity was calculated as the harmonic mean of the allelic diversities $v_l = 1 / \sum p_l^2$ (Gregorius, 1987). The genetic distances and the hypothetical gametic diversity ($V = \prod v_l$) were calculated according to Gregorius (1978). The calculation of the subpopulation differentiation D_j and the differentiation δ of subdivided gene pools followed Gregorius and Roberds (1986).

Statistics

The comparison of samples based on ordinal scale was realized by the test of Kruskal-Wallis.

The test of homogeneity of the population's sampling distribution was realized by the Fisher test or by the Maximum-Likelihood test when the sample size was sufficient. The cluster analysis (average linkage method) was carried out with pairwise genetic distances based on allele frequencies. All data were computed by the Statistical Analysis System (SAS Institute, Inc, USA).

RESULTS

Comparison between oak species

The six experimental sites possess different numbers of trees in pure stands of *Q petraea* and *Q robur,* respectively, or mixed stands with both species (for sample sizes, see table I). Their distribution is irregular and is a consequence of the use of mixed acorns in the time of stand establishment.

The vitality coefficients of both oak species differ significantly at the level of $P = 0.001$. They amount to 2.15 for the species *Q petraea* and to 2.41 for *Q robur.* The comparison of the vitality coefficients of both species growing on the three mixed stands also shows a significant difference at the same significance level. In this case, they amount to 2.27 for *Q petraea* and to 2.44 for *Q robur.*

Q petraea shows a better branching structure in the crown than *Q robur.* Pedunculate oak trees tend more to discoloration of leaves and to the formation of epicormic branches of the crown. Furthermore, the trait 'defoliation' is expressed more intensively in *Q robur,* as this species shows lower disposition to form epicormic branches of stems. The total number of necroses of stems is higher in the species *Q robur,* but *Q petraea* shows larger necroses. Thus, *Q petraea* has a stronger reaction in the case of development of necrotic bark tissue (fig 2).

The genetic structure of 262 individuals of *Q robur* trees and 118 individuals of *Q petraea* trees was described by isozyme gene markers. A total number of 44 alleles at all 11 loci tested was detected, 41 alleles in case of pedunculate oak and 37 in case of sessile oak. The allelic frequencies of all loci are presented in table IV. The enzyme gene loci PGM-A, ACP-C, GDH, IDH-B and AP-B exhibit the most substantial differences in the allelic frequencies between the indigenous oak species in the region of eastern Germany. Their genetic distances range from 0.446 to 0.192, but it is impossible to identify the species of an individual by its isozyme genotype because there are no alleles specific for species.

The dendrogram demonstrates clearly that the genetic distances between the

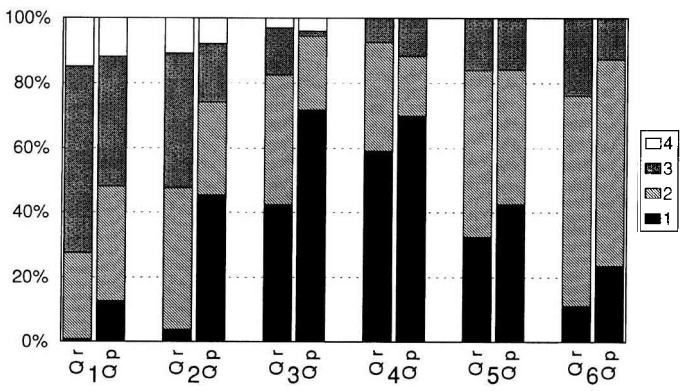

Fig 2. Distribution of damage classes for *Q robur* (Qr) and *Q petraea* (Qp) (score numbers on the right). 1 = branching structure, 2 = defoliation, 3 = discoloration, 4 = necrosis, 5 = epicormic branches of stem, 6 = epicormic branches of crown.

Table IV. Allelic frequencies in experimental plots of oak stands in Bradenburg.

Locus	*Allele*	Quercus robur					Quercus petraea			
		1	*2*	*3*	*4*	*5*	*1*	*2*	*3*	*6*
ACP-C	1	0.892	0.776	0.864	0.781	0.767	0.483	0.583	0.515	0.471
	2	0	0	0	0.016	0	0	0	0	0
	3	0.108	0.224	0.136	0.203	0.233	0.483	0.417	0.485	0.529
	4	0	0	0	0	0	0.034	0	0	0
AP-B	1	0	0.005	0	0	0	0	0	0.029	0
	2	0.314	0.385	0.273	0.391	0.379	0.400	0.553	0.485	0.457
	3	0	0.016	0	0	0.009	0.033	0	0.029	0.029
	4	0.279	0.154	0.273	0.250	0.164	0.217	0.132	0.029	0.029
	5	0.007	0.016	0.045	0	0	0	0	0.044	0.157
	6	0.307	0.269	0.364	0.359	0.388	0.300	0.158	0.265	0.214
	7	0.093	0.154	0.045	0	0.060	0.050	0.158	0.118	0.114
AAT-B	1	–	–	0	0.052	0.019	–	–	0.083	0.077
	2	–	–	1.000	0.897	0.971	–	–	0.917	0.923
	3	–	–	0	0.052	0.010	–	–	0	0
GDH	1	0.152	0.137	0.136	0.125	0.138	0.464	0.211	0.309	0.286
	2	0.167	0.082	0.091	0.016	0.095	0.196	0.158	0.221	0.143
	3	0.682	0.780	0.773	0.859	0.767	0.339	0.632	0.471	0.571
IDH-B	2	0	0.011	0	0	0	0.100	0.132	0.103	0.029
	3	0	0	0	0	0.009	0	0	0	0
	4	0.314	0.214	0.455	0.313	0.207	0.067	0.026	0.044	0.014
	5	0.014	0.011	0.045	0	0	0	0	0	0
	6	0.671	0.764	0.500	0.688	0.784	0.833	0.842	0.853	0.957
MR	1	0	0.017	0	0	0.034	0.033	0.029	0	0.029
	2	0.934	0.943	0.955	0.906	0.879	0.900	0.853	0.897	0.800
	3	0.044	0.034	0	0.047	0.052	0.033	0.088	0.074	0.014
	4	0.022	0.006	0.045	0.047	0.034	0.033	0.029	0.029	0.157
NDH-A	2	0.174	0.042	0.045	0	0	0.093	0.029	0.074	0.114
	3	0.826	0.958	0.955	1.000	1.000	0.907	0.971	0.926	0.886
NDH-B	2	0.051	0.022	0.045	0	0.009	0.017	0	0	0.014
	3	0.014	0	0	0	0	0	0	0	0
	4	0.935	0.978	0.955	1.000	0.991	0.967	1.000	1.000	0.971
	5	0	0	0	0	0	0.017	0	0	0.014
PGM-A	1	0	0	0	0	0	0	0	0.015	0
	2	0.534	0.525	0.500	0.484	0.621	0.182	0.132	0.088	0.043
	3	0.466	0.456	0.500	0.422	0.362	0.795	0.868	0.868	0.914
	4	0	0.019	0	0.078	0.017	0.023	0	0.029	0.043
	5	0	0	0	0.016	0	0	0	0	0
PGDH	1	0.077	0.093	0.045	0.031	0.138	0	0.088	0.015	0.029
	2	0.923	0.907	0.955	0.969	0.862	1.000	0.912	0.985	0.971
PGI-B	1	0	0.005	0	0	0	0	0	0	0
	2	0.086	0.060	0.045	0	0.079	0.083	0.026	0.059	0.086
	3	0	0	0	0.016	0	0	0	0.044	0
	6	0.900	0.929	0.955	0.984	0.921	0.883	0.921	0.868	0.886
	8	0.014	0.005	0	0	0	0.033	0.053	0.029	0.029

stands, regarding the species separately, are small in comparison with the distance between the two species (fig 3).

Comparison between sites

The comparison of the vitality coefficients of the three experimental sites with mixed stands show lower values in sessile oaks. Thus, *Q petraea* possesses a better vitality compared with *Q robur* at the stands (table V).

Generally, the variability of vitality coefficients among single trees in the stands was greater than the differences between the stands. The single morphological traits tend to differ more between the stands and show partly significant differences. In some cases, significance appears between very small differences and, in contrast, greater differences are not significant. This depends on the sample size of the single stands and the standard deviation.

In order to compare the genetic structure of the experimental sites, genetic parameters were surveyed separately for *Q robur* and *Q petraea* (table VI).

The stand "Rosinsee" is remarkable for its maximum gene pool and hypothetical gametic diversity for *Q robur* as well as *Q petraea*. Although there is a rank correlation between the average observed heterozygosity and the stand's average value of the 'branching structure' as well as the average vitality's coefficient for *Q robur*, we can state that the most variation is among the individuals in a stand and not among the stands. Therefore, an attempt was made to pool all trees of each species for the following comparison of vitality classes.

Comparison between the vitality classes

The division of all investigated trees into three vitality groups based on their calculated vitality coefficients allowed the coherent consideration of results of damage assessment and of the genetic parameters for both oak species.

The evaluation of the relationship between single phenotypical traits and genetic structure requires further investigation and will be presented in a separate study. The relationship between the vitality groups and the genetic parameters without regard to single phenotypical traits will be considered here.

One of the most obvious differences between the vitality classes is the increase of an excess of homozygotes (fixation index *F* in table VII) from the group of tolerant to the group of sensitive trees (significant at

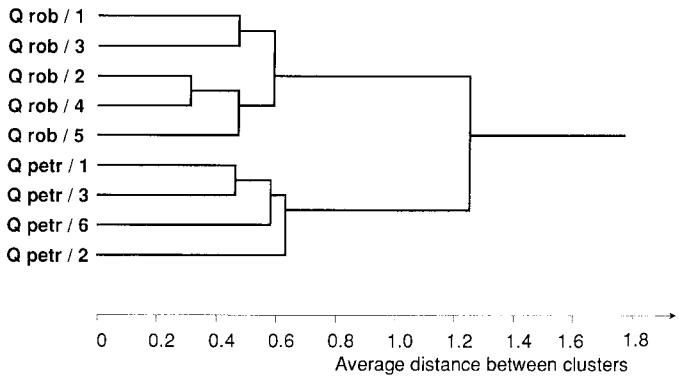

Fig 3. Dendrogram based on the genetic distances of allele frequencies of *Q robur* (Q rob) and *Q petraea* (Q petr) trees at six experimental sites.

the 0.05 level for *Q robur)*, whereas the heterozygosity H_0 tends to show a decrease (table VII). These two parameters have been influenced by the genotypical structure.

The parameters which are derived from the allelic frequencies only (expected heterozygosity, gene pool and hypothetical gametic diversities) exhibit the maximum values in the intermediate vitality class for *Q robur* and *Q petraea* (table VII). The high levels of hypothetical gametic diversities of the intermediate class demonstrate the potential of these groups to produce gametes with a high genetic variation. In addition, these intermediate vitality groups of both oak species show the lowest level of subpopulation differentiation for a majority of scored gene loci and for the average value (table VIII).

Table V. Means of vitality and morphological traits of the six experimental sites.

	Quercus robur					Quercus petraea			
Stand	*1*	*2*	*3*	*4*	*5*	*1*	*2*	*3*	*6*
No of trees	*70*	*92*	*11*	*62*	*34*	*30*	*20*	*38*	*39*
Branching structure	2.74^{b}	3.05^{b}	2.82^{abc}	2.95^{c}	2.44^{a}	2.83^{bc}	3.15^{c}	2.50^{b}	1.95^{a}
Defoliation	2.36^{a}	2.78^{b}	3.45^{c}	2.36^{a}	2.73^{b}	1.80^{a}	2.25^{b}	3.00^{c}	2.69^{c}
Discoloration	1.30^{a}	2.13^{b}	2.27^{b}	1.87^{b}	1.50^{a}	1.07^{a}	1.55^{b}	1.74^{b}	1.18^{a}
Epicormic branches of trunk	1.90^{bc}	1.92^{b}	2.27^{cd}	1.10^{a}	2.67^{d}	1.53^{a}	1.90^{b}	1.92^{b}	1.62^{ab}
Epicormic branches of crown	2.14^{c}	2.03^{b}	1.91^{abc}	2.43^{d}	1.82^{a}	2.10^{b}	2.05^{b}	1.66^{a}	1.87^{ab}
Bark necrosis	1.10^{a}	1.62^{c}	1.64^{bcd}	1.81^{d}	1.29^{b}	1.10^{a}	1.35^{a}	1.47^{ab}	1.74^{b}
Vitality coefficient	2.25^{a}	2.58^{c}	2.56^{bc}	2.44^{b}	2.22^{a}	2.18^{b}	2.49^{c}	2.23^{b}	1.88^{a}

^{abcd} Comparison among the stands for each species: means with the same letter are not significantly different; significance level $P = 0.05$.

Table VI. Genetic parameters of *Q robur* and *Q petraea* at six experimental sites.

	Quercus robur					Quercus petraea			
Stand	*1*	*2*	*3*	*4*	*5*	*1*	*2*	*3*	*6*
H_o	0.258^{ab}	0.217^{b}	0.256^{ab}	0.235^{ab}	0.259^{a}	0.253^{a}	0.228^{a}	0.248^{a}	0.250^{a}
H_e	0.321^{a}	0.287^{a}	0.255^{a}	0.247^{a}	0.265^{a}	0.312^{a}	0.278^{a}	0.278^{a}	0.274^{a}
F	0.138^{a}	0.163^{a}	-0.034^{b}	0.030^{ab}	0.005^{b}	0.200^{a}	0.077^{a}	0.074^{a}	0.080^{a}
δ_T	0.323^{a}	0.288^{a}	0.267^{a}	0.251^{a}	0.268^{a}	0.317^{a}	0.286^{a}	0.282^{a}	0.278^{a}
Gene pool diversity	1.47^{a}	1.40^{a}	1.34^{a}	1.33^{a}	1.36^{a}	1.45^{a}	1.39^{a}	1.39^{a}	1.38^{a}
Hypoth gametic diversity	81.2	52.3	50.5	40.7	48.6	82.1	40.8	68.4	63.8
Subpopul different	0.063	0.046	0.065	0.064	0.068	0.084	0.084	0.039	0.076

^{ab} Comparison among the stands for each species: means with the same letter are not significantly different.

DISCUSSION

In this study, the attempt was made to find relationships between the vitality of oak trees described by several morphological traits and their genetic structure. The process of oak decline is marked by the temporal and spatial interrelation of biotic and abiotic factors causing a decrease of vitality of the trees. It is known that the development of the recent forest damages are also weather-induced. The variation in temperature and the amount of rainfall of former years plays a particularly important role.

The annual averages of temperature of the research area were higher in the period from 1980 to 1990 than the long-term average, especially in winter months. In addition, the annual amount of rainfall decreased in this time, especially in the summer and autumn periods (Smukalski et al, 1992).

Table VII. Genetic parameters of different vitality classes of *Q robur* and *Q petraea*.

Vitality class	Quercus robur			Quercus petraea		
	Tolerant	*Intermediate*	*Sensitive*	*Tolerant*	*Intermediate*	*Sensitive*
No of trees	55	147	60	48	54	16
H_o	0.242	0.236	0.218	0.247	0.234	0.206
H_e	0.265	0.283	0.271	0.276	0.287	0.266
F	0.022	0.114	0.166	0.096	0.119	0.198
δ_T	0.267	0.284	0.273	0.279	0.289	0.275
Gene pool diversity	1.36	1.39	1.37	1.38	1.40	1.36
Hypoth gametic diversity	51.9	68.1	55.7	63.8	83.8	54.8

Table VIII. Subpopulation differentiation (D_j) and differentiation among subpopulations (δ) at single gene loci for three vitality classes.

	Quercus robur				Quercus petraea			
	D_j			δ	D_j			δ
Locus	*Tolerant*	*Intermediate*	*Sensitive*		*Tolerant*	*Intermediate*	*Sensitive*	
ACP-C	0.022	0.008	0.028	0.019	0.162	0.019	0.163	0.114
AP-B	0.116	0.074	0.152	0.114	0.100	0.064	0.089	0.084
AAT-B	0.081	0.016	0.064	0.054	0.047	0.035	0.083	0.055
GDH	0.032	0.026	0.032	0.030	0.165	0.094	0.098	0.119
IDH-B	0.052	0.043	0.088	0.061	0.121	0.008	0.113	0.081
MR	0.051	0.017	0.048	0.039	0.071	0.018	0.067	0.052
NDH-A	0.021	0.031	0.010	0.020	0.038	0.050	0.012	0.034
NDH-B	0.015	0.019	0.005	0.013	0.012	0.008	0.020	0.013
PGM-A	0.010	0.078	0.087	0.058	0.036	0.033	0.044	0.038
PGDH	0.026	0.022	0.004	0.017	0.006	0.012	0.006	0.008
PGI-B	0.017	0.009	0.019	0.015	0.016	0.063	0.053	0.044
Gene pool	0.040	0.031	0.049	0.040	0.070	0.037	0.068	0.058

The traits which had been found and regarded as a vitality decrease of oaks corresponded with the incidence of damages described by Balder and Lakenberg (1987) and Oosterbaan (1987).

The branching structure documents well the abiotic and biotic long-term effects. Therefore, the branching structure was weighed as the most essential trait. The other traits (ie, defoliation, discoloration, bark necroses and epicormic branches of crown and stem) partly represent long-term as well as short-term effects. They were weighed lower.

Strong impacts of leave-feeding insects occurred in most of the tested stands from 1991 to 1993. Mainly *Tortrix viridiana* and *Operophthera brumata* were observed. Both oak species were equally effected by them. In the year 1992, *Haltica quercetorum* occurred in the sessile oak stands "Tiefensee" and "Rehhagen" additionally and severly damaged the lower parts of crowns and epicormic branches.

The influence of repeated loss of leaves during the vegetation period by feeding insects, along with drought and high temperature, plays an important role in the process of decreasing vitality (Krapfenbauer, 1988). It is also possible that other phenotypical characters, such as flowering and fruit production, have an influence on the production of leaves during the vegetation period (Roloff, 1989). These examples may show that the loss of leaves per se is not a sign for the decline process in every case.

Löchelt and Franke (1993) did not observe any relation between the loss of leaves of investigated oak trees and one of the calculated genetic measures. Our data on the genetic characterization of oak species and oak stands are in good agreement with the published data of Müller-Starck and Ziehe (1991) and Kremer and Petit (1993).

The relative broad basis of the vitality classification of trees into tolerant, intermediate and sensitive groups might facilitate us to find out whether there is an influence of the genetic structure on the sensitivity to the European oak decline.

This is the first report about a detectable relationship between the genetic structure and phenotypical traits which characterizes the vitality for the tree species *Q robur* and *Q petraea*. The results are principally the same for both species.

We have found similar differences for the observed heterozygosity between the tolerant and sensitive vitality classes as have Bergmann and Scholz (1987) and Raddi et al (1994) for *Picea abies*, Geburek et al (1987) for *Pinus sylvestris* and Müller-Starck (1985, 1989) and Hertel and Zander (1991) for *Fagus sylvatica*. Most of the authors cited here used the pairwise sampling method (Gregorius, 1989) to compare the genetic structure of the population's subsets.

Our inclusion of all trees in small experimental plots in the investigation meant nearly the same random association between genotype and environment as the method of pairwise sampling (similar to Konnert, 1993). Additionally, we had the possibility to consider an intermediate group. The different sample sizes of the tolerant, intermediate and sensitive vitality classes for both species represent the conditions in the locations we studied.

The fixation index measures the deviation of the proportion of the observed heterozygotes to the proportion of heterozygotes at the Hardy–Weinberg equilibrium. Values below zero indicate an excess of heterozygotes and above zero an excess of homozygotes. A decreasing fixation index was used to describe the natural selection against inbreeds during the ontogenesis, which was found, for example, for pine species during the seedling development (Muona et al, 1987; Morgante et al, 1993) and for age classes from 10 to 40 years (Starova et al, 1990). In the present study, tree groups of the same

age class show a decreasing fixation index, ie, decreasing excess of homozygotes with increasing vitality. A directed selection is also derived from the level of subpopulation differentiation of the vitality classes. The subpopulation differentiation is used to describe the genetic distance of one group to the remainder of the population. Groups with low values of subpopulation differentiation better represent the gene pool of the whole population than groups with high levels of subpopulation differentiation.

The two groups, ie, tolerant and sensitive trees with higher values of subpopulation differentiation than the intermediate group, could be understood as special subpopulations after a differentiation process by the European oak decline. They have a reduced potency to form the next generation with a high genetic variation in comparison with the intermediate group with a maximum hypothetical gametic diversity and thereby a favourable general ability for adaptation at changing environmental conditions.

Until now, we did not have knowledge about the reproductive success of oak trees of different vitality. The variability in acorn production was reviewed by Ducusso et al (1993) without indication of the influence of the tree's vitality. Nevertheless, we could assume that the offspring quantities of damaged trees decrease over a long period, since, for instance, Köhler and Stratmann (1986) and Cufar et al (1994) observed this appearance for conifer species. Under this condition, the genetic structure of the next generations would approach the structure of the tolerant group, thus meaning an adaptation process at the level of populations, if the selection pressure is the same as in the previous generation.

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