GENETIC VARIATION OF OAKS (*QUERCUS* SPP.) IN SWITZERLAND. 1. ALLELIC DIVERSITY AND DIFFERENTIATION AT ISOZYME GENE LOCI

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ABSTRACT

Genetic variation of 28 oak populations (*Quercus cerris, Q. petraea, Q. pubescens, Q. robur*) in Switzerland has been assessed at 17 isozyme gene loci. Individual *Q. cerris* trees were identified by the morphology of their buds; the species status of the other taxa was confirmed by Principal Component Analysis (PCA) of leaf morphological data. All populations proved to be highly variable (expected heterozygosity H_e in-between 0.180 and 0.262). On average, *Q. robur* and *Q. petraea* populations show similar levels of genetic diversity ($H_e = 0.241$ and $H_e = 0.249$ respectively). Diversity is slightly lower for *Q. pubescens* ($H_e = 0.215$) and *Q. cerris* ($H_e = 0.221$). The average number of alleles per locus is highest for *Q. pubescens* populations. The two *Q. cerris* collectives are strongly differentiated from all other populations ($D_j > 0.5$) indicating wide systematic separation and complete reproductive isolation from the other species. Differentiation among *Q. petraea, Q. pubescens,* and *Q. robur* is much lower and in close correspondence to differentiation patterns of leaf morphological traits. A hierarchical division of the total genetic diversity for the three species reveals that most of the variation is within populations (93.8 %), and a comparatively high amount of the variation is between species (4.3 %). Differentiation among populations within species is low (1.9 %). Results support the close systematic relationship between *Q. petraea, Q. pubescens,* and *Q. robur*. The significance of the results for the systematics of the "white oaks" and for the conservation of genetic resources of oaks in Switzerland is discussed.

Keywords: genetic variation, diversity, isozyme marker, population differentiation, *Quercus* spp., Switzerland

INTRODUCTION

Oaks (*Quercus* spp.) are dominant species in many temperate forests, but they are comparatively rare in Switzerland; only 2.24 % of all trees with a diameter at breast height (DBH) of more than 12 cm are native oaks (BRASSEL & BRÄNDLI 1999). Four deciduous oak species are indigenous to Switzerland: sessile oak (*Q. petraea* (Matt.) Liebl.; 62.1 % of all native oak trees in Swiss forests; cf. BRASSEL & BRÄNDLI (1999)), pedunculate oak (*Q. robur* L.; 26.6 %), pubescent oak (*Q. pubescens* Willd.; 10.7 %), and Turkey oak (*Q. cerris* L.; 0.6 %). The occurrence of *Q. cerris* is confined to a few forests in the southernmost part of Ticino. The other species can be found throughout the country up to an altitude of approximately 1400 *m* (BRÄNDLI 1996). Oaks are mainly distributed in mixed deciduous forests up to the submontane vegetation belt. The occurrence of oak species in various forest types is connected to different ecological preferences of the species (AAS 1998). Less than 20 % of all forests dominated by oaks are older than 120 years in Switzerland (BRASSEL & BRÄNDLI 1999). *Q. petraea* and *Q. robur* are important commercial species in several Swiss forest districts in spite of their low overall abundance. The topography and environmental conditions suggest that the rarity of oaks in Switzerland is mainly caused by natural factors and not by the recent history of forests. Thus, losses of genetic variation due to isolation and bottlenecks cannot be ruled out at least for populations in the Alps.

Q. petraea, Q. pubescens, and *Q. robur* are closely related taxa. Pollination experiments revealed that interspecific hybridization among these species is possible. However, the success rate of artificial hybridization depends on the species involved and is often asymmetric (e.g. MÜLLER 1999). *Q. cerris* is also a "white oak" species (Subgenus: *Quercus*; Section: *Quercus* according to NIXON (1993)), but is often separated from the three other investigated species, which all belong to the section *Lepidobalanus* according to CAMUS (1938), in a different section *Cerris* (AAS 1998). There are no confirmed results of hybridization of *Q. cerris* with any of the other species.

Genetic variation of European oak species has been intensively studied at marker loci in the recent past. Variation patterns of maternally inherited cpDNA has been used to identify refugia and to investigate the postglacial recolonization history of oaks (DUMOLIN-LAPÈGUE *et al.* 1997). The postglacial history of oaks in

Switzerland has been elucidated by MÁTYÁS (1999) based on variation of cpDNA. He found clear evidence for the recolonization of Switzerland by oaks from at least two different refugia. His results suggest that long-distance seed transfer by humans had little impact on the genetic structures of oak populations in Switzerland. Genetic variation of biparentally inherited nuclear genes has been assessed using several types of molecular markers (e.g. microsatellites; STREIFF *et al.* 1998) and for biochemical markers, i.e. isozymes (e.g. ZANETTO & KREMER 1995). MÜLLER (1999) investigated mainly *Quercus pubescens* populations from Switzerland and the Mediterranean region at isozyme gene loci.

The objectives of this study are to observe patterns of genetic variation at isozyme gene loci and of variation at leaf morphological traits in order to address the following questions: Are there different levels of genetic variation within oak populations in Switzerland? If yes, does the amount of genetic variation vary among species and/or are there indications of losses of genetic variation due to bottlenecks e.g. in alpine oak forests? Are Swiss oak populations differentiated from each other? If yes, is there a correspondence between patterns of genetic variation at marker loci and patterns at leaf morphological traits? Is the close taxonomic relationship and the possibility of hybridization between *Q. petraea, Q. pubescens, Q. robur* reflected at enzyme gene loci and to what extent is *Q. cerris* differentiated from the other species?

MATERIAL AND METHODS

Material

Material was collected from 28 oak forests throughout Switzerland (Table 1). It was aimed to sample only autochthonous populations, but the inclusion of allochthonous stands cannot be ruled out due to the scarcity of historical records on seed transfer in Switzerland. The species-status of oaks in the forests was preliminarily assessed by field observations of morphological traits. The forests were pure oak stands or mixed stands with oaks being one of the dominant species. The minimum area of the forests was with only few excep

Table 1. Investigated populations; number, name, approximate altitude (*m* above sea level), location (eastern longitude; northern latitude), and species status according to the analysis of morphological data (*rob: Q. robur*; *rob/pet:* mixed *Q. robur/Q.petraea*; *pet: Q. petraea*; *pub: Q. pubescens*; *cer/pub:* mixed *Q. cerris/Q. pubescens*).

No.	Name	Altitude (*m*)	Eastern longitude	Northern latitude	Species
1	Bonfol	450	7° 10'	47° 28'	*rob*
2	Lugnez	440	7° 06'	47° 29'	*rob*
3	Wölfinswil	540	8° 02'	47° 28'	*rob*
4	Tägerwilen	520	9° 08'	47° 38'	*rob*
5	Uttwil	440	9° 20'	47° 34'	*rob*
6	Magadino	200	8° 52'	46° 10'	*rob*
7	Allschwil	350	7° 32'	47° 32'	*pet/rob*
8	Muttenz	270	7° 38'	47° 34'	*pet/rob*
9	Büren a.A.	470	7° 23'	47° 08'	*pet/rob*
10	Satigny	460	6° 00'	46° 13'	*pet*
11	Jussy	500	6° 18'	46° 14'	*pet*
12	Alaman	410	6° 24'	46° 28'	*pet*
13	Corcelles	550-600	6° 42'	46° 51'	*pet*
14	Galm	570	7° 11'	46° 57'	*pet*
15	Bois de devant	570-670	6° 49'	46° 59'	*pet*
16	Schoren	520	7° 47'	47° 26'	*pet*
17	Magden	370	7° 48'	47° 32'	*pet*
18	Bülach	430	8° 32'	47° 33'	*pet*
19	Cavergno	540-1040	8° 37'	46° 21'	*pet*
20	Gordevio	400-560	8° 45'	46° 14'	*pet*
21	Castaneda	640-750	9° 09'	46° 16'	*pet*
22	Le Landeron	700	7° 04'	47° 04'	*pub*
23	Fully	530-800	7° 06'	46° 08'	*pub*
24	St. Luc	1040-1800	7° 34'	46° 16'	*pub*
25	Gampel	900-1000	7° 44'	46° 19'	*pub*
26	Tamins	600-820	9° 26'	46° 50'	*pub*
27	Mt. Caslano	300-530	8° 52'	45° 58'	*cer/pub*
28	Novaggio	740-840	8° 51'	46° 01'	*cer/pub*

Table 2. Investigated enzyme systems, their abbreviations, E.C. numbers, and controlling gene loci. Investigated gene loci are printed in bold.

Name	Abbreviation	E.C. No.	Controlling gene loci
(Alanine)-aminopeptidase	AAP	3.4.11.1	***Aap-A***, *Aap-B*
Aconitase	ACO	4.2.1.3	***Aco-A***
Acid phosphatase	ACP	3.1.3.2	*Acp-A*, *Aco-B*, ***Acp-C***
Alcohol dehydrogenase	ADH	1.1.1.1	***Adh-A***
Glutamate dehydrogenase	GDH	1.4.1.3	***Gdh-A***
Glutamate oxaloacetate transaminase	GOT	2.6.1.1	*Got-A*, ***Got-B***, *Got-C*
Isocitrate dehydrogenase	IDH	1.1.1.42	***Idh-A***, ***Idh-B***
Malate dehydrogenase	MDH	1.1.1.37	***Mdh-A***, *Mdh-B*, *Mdh-C*
Menadione reductase	MNR	1.6.99.2	***Mnr-A***
NADH dehydrogenase	NDH	1.6.99.3	***Ndh-A***
6-Phosphogluconate dehydrogenase	6-PGDH	1.1.1.44	***6-Pgdh-A***, ***6-Pgdh-B***
Phosphoglucose isomerase	PGI	5.3.1.9	***Pgi-A***, ***Pgi-B***
Phosphoglucomutase	PGI	2.7.5.1	***Pgm-A***
Shikimic acid dehydrogenase	SKDH	1.1.1.25	***Skdh-A***, *Skdh-B*

tions (Populations Schoren, St. Luc) 10 *ha*. All stands were regarded as potentially suitable as *in situ* conservation areas for genetic resources of oaks in Switzerland.

Twigs with dormant buds were sampled from 100 randomly selected trees in each population for the extraction of isozymes. The minimum distance between sampled trees was 30 *m* whenever possible. Trees were temporarily marked in order to avoid sampling of close neighbours. Twigs with dormant buds were cut into short pieces and stored at –80 °C until investigation. Leaves were collected after leaf fall from the ground or during the dormant season from sampled trees for the analysis of morphological data. Single leaves were collected by keeping a minimum distance of 30 *m* between sampled sites. One hundred leaves were sampled in each population and preserved in a herbarium.

Methods

The following data were recorded from each sampled leaf: lamina length (LL), petiole length (PL), lobe width (LW), sinus width (SW), number of intercalary veins (NV), basal shape of the lamina (BS), and abaxial lamina pubescence (HR) (AAS 1993; DUPOUEY & BADEAU 1993). LL, PL, LW, and SW were measured in mm, NV was directly counted, and BS as well as HR were scored on a scale ranging from 1 to 9 and 1 to 6 respectively. The following secondary characters were computed: petiole ratio (PR = PL/(PL+LL)) and width ratio (WR = LW/SW). *Q. cerris* trees were excluded from the leaf morphological analysis due to the high variability of their leaves. However, they could be easily and unambiguously recognised by the long, pointed ends of their bud scales.

Arithmetic mean values were computed for each population for NV, BS, HR, PR, SW, and WR. These traits proved to be of significance for species identification in previous studies (e.g. DUPOUEY & BADEAU 1993). Principal component analysis (PCA; CURETON & D'AGOSTINO 1983) was performed based on the mean values, and populations were plotted in a two-dimensional graph according to their factor scores. The final assessment of the taxonomic status of the populations was based on the results of the PCA of leaf morphological traits.

Enzyme electrophoresis followed standard procedures with slight modifications (MÜLLER-STARCK *et al.* 1996; ZANETTO *et al.* 1996). Fourteen enzyme systems were investigated (Table 2). Only zones with clear, repeatable phenotypes were interpreted. Isozyme phenotypes were scored following biochemical staining of horizontally sliced starch gels. Staining followed with slight modifications standard procedures (CONKLE 1982; CHELIAK & PITEL 1984). All doubtful or previously unobserved zymograms were scanned and electronically conserved. They were repeated at least once. Phenotypes which could not be reliably scored after one or two repetitions were not interpreted. The corresponding genotypes were regarded as missing data at the respective gene loci (total frequency approximately 1.2 %). A total of 17 gene loci code for the observed variation at the 14 enzyme systems.

Inheritance of most isozyme systems has been studied by MÜLLER-STARCK *et al.* (1996) and ZANETTO *et al.* (1996). A single putative gene locus is assumed to control the polymorphic zones *Aco-A*, *Adh-A*, *Idh-A*, and *Ndh-A*. No inheritance study has been performed for these zones, but the observed simple and clear isozyme phenotypes strongly suggest the control of each

zone by one gene locus. Genotyping was based on the confirmed or, in the case of the four zones mentioned above, presumed genetic control of the isozyme phenotypes. Alleles were numbered according to the relative mobilities of corresponding enzymes in the gels. Number 1 was assigned to the allele coding for the fastest migrating band.

Genetic variation within populations was quantified as the average number of alleles per locus (*A/L*) and as "expected heterozygosity" (H_e) (BERG & HAMRICK 1997), which computationally equals the differentiation within populations (δ_T) according to GREGORIUS (1987) if a correction for finite sample sizes is applied. Hypothetical gametic diversity v_{gam} was computed by multiplication of the single locus diversities v of all gene loci (GREGORIUS 1978). Average genetic distances d_0 (GREGORIUS 1984) between population pairs from the same and from two different taxonomic units were computed. In addition, genetic differentiation among populations was assessed by the partitioning of total gene diversity following NEI (1973; G_{ST}) and by the computation of genetic distances between populations and their respective complements (D_j and δ; GREGORIUS & ROBERDS 1986). Cluster analysis (UPGMA; SNEATH & SOKAL 1973) based on a matrix of genetic distances d_0 (GREGORIUS 1984) was used to illustrate patterns of genetic differentiation among populations.

The computer programmes BIOSYS 1.7 (SWOFFORD & SELANDER 1981), GSED 1.1d (GILLET 1994), and WinSTAT 3.1 (BRAEUNIG & FITCH 1998) were used for the computations.

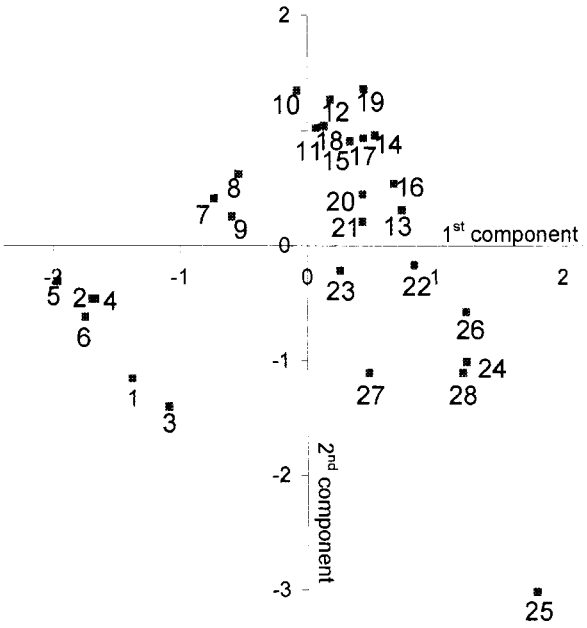

Figure 1. Principal Component Analysis (PCA) of leaf morphological data from 28 oak (*Quercus* spp.) populations in Switzerland; population numbers as in Table 1.

RESULTS

Analysis of leaf morphology

Trees with long, pointed ends of bud scales typical of *Q. cerris* were found in only two populations in Ticino (Mt. Caslano and Novaggio). Leaves from these trees were excluded from further morphological studies, i.e. only leaves from trees without pointed bud scales were taken from these two populations. The result of the PCA is shown in Figure 1. The first component (x-axis) explains 86.6 % of the total variation, the second component (y-axis) explains 9.2 %.

A group of six populations (nos. 1–6) with scores below -1 for the first component and negative scores for the second component is clearly separated from all other populations. The great majority of leaves of these populations are of the *Q. robur* type. These populations are regarded "pure" *Q. robur* stands (Table 1), although a few leaves from some stands showed different morphological characters (FINKELDEY 2001). A second group (nos. 7–9) consists of three populations with scores of the first component between – 0.5 and – 0.75, and positive scores for the second component. These stands contain leaves of the *Q. robur* and the *Q. petraea* type in roughly equal frequencies with only few intermediate leaves (FINKELDEY 2001). They are regarded as "mixed" (*Q. robur/Q. petraea*) populations (Table 1). The remaining populations are less clearly differentiated. This group contains populations with leaves of the *Q. petraea* and the *Q. pubescens* type. An unambiguous distinction at the level of single leaves between these two botanical species is impossible if it is based on the observed leaf morphological traits. However, it is still possible to group populations according to their dominating type: populations with a positive score for the second principal component are regarded as *Q. petraea* (nos. 10–21) and those with negative scores are regarded as *Q. pubescens* (nos. 22–28). The resulting assessment of the species-status of the populations (Table 1) was in most cases in accordance to initially made observations in the forest.

The analysis of leaf morphology showed that it was possible to unambiguously identify six *Q. robur* (nos. 1–6) populations and three mixed *Q. petraea / Q. robur* populations (nos. 7–9). Twelve "pure" *Q. petraea* populations (nos. 10–21) are poorly differentiated from the seven morphologically similar *Q. pubescens* populations (nos. 22–28). In two of these populations *Q. pubescens* occurs in mixture with *Q. cerris*.

Allelic structure at isozyme gene loci

The strong differentiation (see below) between *Q. cerris*

Table 3. Mean allele frequencies at 17 isozyme gene loci in Q. robur, mixed Q. robur/Q. petraea, Q. petraea, Q. pubescens, and Q. cerris populations from Switzerland. Allele frequencies may not add to unity for single gene loci since very rare alleles (mean frequency below 0.02 for all five taxonomic units) are not listed.

Locus	Allele	*Q. rob.*	mixed	*Q. pet.*	*Q. pub.*	*Q. cer.*
Aap-A	1	0.000	0.002	0.002	0.006	0.087
	2	0.360	0.465	0.549	0.643	0.203
	3	0.000	0.000	0.000	0.000	0.472
	4	0.196	0.113	0.084	0.088	0.122
	5	0.406	0.371	0.321	0.241	0.041
	6	0.037	0.050	0.044	0.021	0.040
	7	0.000	0.000	0.000	0.000	0.035
Aco-A	2	0.958	0.957	0.957	0.949	0.949
	3	0.023	0.023	0.003	0.014	0.029
	4	0.018	0.013	0.035	0.021	0.015
Acp-C	1	0.788	0.663	0.480	0.778	0.012
	2	0.213	0.337	0.520	0.222	0.988
Adh-A	1	0.003	0.030	0.040	0.029	0.000
	2	0.576	0.635	0.633	0.821	0.273
	3	0.421	0.333	0.324	0.148	0.707
	4	0.000	0.002	0.003	0.002	0.020
Gdh-A	1	0.009	0.002	0.003	0.019	0.093
	2	0.276	0.373	0.433	0.438	0.000
	4	0.713	0.623	0.548	0.529	0.000
Got-B	1	0.011	0.032	0.048	0.062	0.322
	2	0.957	0.950	0.944	0.918	0.614
	3	0.032	0.018	0.008	0.018	0.000
	4	0.001	0.000	0.000	0.001	0.064
Idh-A	1	0.149	0.125	0.026	0.029	0.013
	2	0143	0.213	0.316	0.323	0.042
	3	0.639	0.587	0.592	0.620	0.830
	4	0.069	0.075	0.066	0.028	0.108
Idh-B	1	0.000	0.002	0.000	0.000	0.000
	2	0.003	0.032	0.086	0.033	0.000
	3	0.353	0.183	0.038	0.009	0.000
	4	0.641	0.775	0.862	0.951	0.977
	5	0.000	0.000	0.000	0.000	0.023
Mdh-A	2	1.000	0.998	0.999	0.991	1.000
Mnr-A	1	0.011	0.000	0.020	0.017	0.000
	3	0.902	0.867	0.790	0.800	0.000
	4	0.008	0.035	0.040	0.062	0.000
	5	0.033	0.005	0.006	0.006	0.000
	6	0.000	0.000	0.000	0.000	1.000
	7	0.047	0.092	0.137	0.107	0.000
Ndh-A	1	0.001	0.027	0.058	0.035	0.000
	2	0.999	0.973	0.942	0.965	1.000
6-Pgdh-A	1	0.003	0.005	0.003	0.003	0.063
	2	0.973	0.983	0.985	0.976	0.808
	3	0.024	0.012	0.013	0.020	0.000
	4	0.000	0.000	0.000	0.000	0.124
6-Pgdh-B	3	0.993	0.990	0.993	0.907	0.011
	4	0.000	0.000	0.001	0.007	0.000
	5	0.001	0.000	0.005	0.083	0.000
	6	0.000	0.000	0.000	0.003	0.989
Pgi-A	1	0.004	0.003	0.000	0.000	0.000
	2	0.996	0.997	1.000	0.996	0.017
	3	0.000	0.000	0.000	0.004	0.955
	4	0.000	0.000	0.000	0.000	0.028
Pgi-B	2	0.028	0.032	0.049	0.050	0.000
	3	0.045	0.060	0.051	0.034	0.000
	5	0.917	0.848	0.813	0.847	0.017
	6	0.000	0.000	0.000	0.000	0.241
	7	0.009	0.058	0.087	0.067	0.006
	8	0.000	0.000	0.000	0.001	0.736
Pgm-A	2	0.559	0.355	0.142	0.094	0.000
	3	0.423	0.618	0.842	0.894	0.145
	4	0.017	0.024	0.015	0.012	0.855
Skdh-A	2	0.913	0.962	0.951	0.960	0.448
	3	0.086	0.037	0.049	0.039	0.552

trees and other trees (mainly of the *Q. pubescens*-type; cf. Figure 1) from Mt. Caslano and Novaggio made it meaningful to separate two populations at both sites based on the bud morphology of trees. Thus, thirty different populations from 28 different sites were distinguished. Sample size was 100 for each population apart from those containing *Q. cerris* types. At Mt. Caslano, 17 trees of the *Q. cerris* type and 83 of other types (mainly *Q. pubescens*) were investigated. The respective sample sizes were 44 *Q. cerris* and 56 others for the two populations from Novaggio.

Average allele frequencies at the 17 observed enzyme gene loci were computed for the groups *Q. robur*, mixed *Q. robur* / *Q. petraea*, *Q. petraea*, *Q. pubescens*, and *Q. cerris*. Only alleles with a frequency of at least 2 % in any of these groups are reported in Table 3.

Genetic variation within populations

Measures of genetic variation within populations are summarized in Table 4 for the gene pool of 17 observed isozyme gene loci. A total of 83 different alleles were observed at 17 isozyme gene loci (on average 4.88 alleles per locus). Seven alleles were observed only in *Q. cerris*, i.e. 76 different alleles (on average 4.47

Table 4. Genetic variation within populations of oaks (*Quercus* spp.) in Switzerland; gene pool values for 17 enzyme gene loci. *A/L* (no. of alleles/locus), expected heterozygosity H_e (= differentiation within populations δ_T), and hypothetical gametic diversity v_{gam}.

	A/L	$H_e = \delta_T$	v_{gam}
Bonfol	2.76	0.229	160.9
Lugnez	2.82	0.251	304.8
Wölfinswil	2.71	0.256	388.5
Tägerwilen	2.59	0.235	217.1
Uttwil	2.65	0.233	200.6
Magadino	2.65	0.239	208.5
Allschwil	3.06	0.251	276.9
Muttenz	2.65	0.259	387.6
Büren	3.00	0.257	379.2
Satigny	2.76	0.246	255.0
Jussy	2.65	0.249	251.3
Alaman	2.76	0.262	375.4
Corcelles	2.71	0.246	240.6
Galm	2.88	0.247	246.5
Bois de devant	2.88	0.247	240.5
Schoren	2.76	0.260	378.8
Magden	2.94	0.261	324.9
Bülach	2.88	0.254	302.9
Cavergno	2.94	0.234	193.8
Gordevio	2.76	0.235	180.7
Castaneda	2.94	0.241	209.9
Le Landeron	2.88	0.239	175.2
Fully	2.94	0.194	60.0
St. Luc	2.94	0.213	104.9
Gampel	2.94	0.180	46.6
Tamins	2.76	0.240	161.3
Mt. Caslano *pub.*	2.94	0.203	76.4
Novaggio *pub.*	2.94	0.236	150.1
Mt. Caslano *cer.*	2.00	0.199	88.1
Novaggio *cer.*	2.71	0.244	238.5

alleles per locus) were identified in *Q. robur, Q. petraea,* or *Q. pubescens*. The average number of alleles per locus (*A/L*) in single populations is much lower (cf. Table 4) since many alleles were identified at low frequencies in only one or a few populations. The particular low allelic richness (*A/L* = 2.00) at Mt. Caslano is likely to be related to the small sample size since only 17 *Q. cerris* trees were sampled at this location. Expected heterozygosity (H_e) among populations varies from 0.180 (Gampel) to 0.262 (Alaman). As expected, differences between populations are more pronounced for the hypothetical gametic diversity (v_{gam}).

Average values of genetic variation within populations are shown for the four investigated *Quercus* species and the mixed *Q. petraea/Q. robur* populations in Table 5. *Q. petraea, Q. robur*, and the mixed populations harbour similar levels of genetic variation. The expected heterozygosity of *Q. pubescens* and *Q. cerris* is slightly reduced. However, a large number of rare alleles in *Q. pubescens* populations is responsible for the average highest number of alleles per locus.

Genetic differentiation among populations

Both *Q. cerris* populations are highly differentiated from the remaining populations (Figure 2 and Table 6). These populations share less than half of their alleles with their respective complements (D_j = 0.521 for Novaggio *cer.* and D_j = 0.508 for Mt. Caslano *cer.*). Complete or nearly complete differentiation between *Q. cerris* and the other species is observed at four gene loci (*Mnr-A, 6-Pgdh-B, Pgi-A, Pgi-B*; Table 3). Strong differentiation is also observed at the gene loci *Acp-C, Gdh-A, Pgm-A*, and *Skdh-A* (Table 3 and Table 7). Hierarchical partitioning of genetic variation reveals that 17.8 % of the total variation of the gene pool is due to differences among species, if the *Q. cerris* populations are included. The total population differentiation δ according to GREGORIUS & ROBERDS (1986) among 30 populations, i.e. including the two *Q. cerris* populations, is 11.8 % for the gene pool.

Differentiation among the 28 populations consisting only of *Q. petraea, Q. pubescens,* and *Q. robur* is much lower (Table 7). This tendency is also revealed by the pairwise genetic distances between populations (Table 6). Variation mainly occurs within populations (93.8 %), if the *Q. cerris* populations are excluded. However, the remaining diversity is still mainly found amon species (4.3 %). Only a small fraction of the overall diversity is due to differentiation among populations within species (1.9 %). Differentiation among populations within species is particularly low for *Q. robur* and *Q. petraea*. It is slightly higher for *Q. pubescens* (Table 8).

Cluster analysis based on a matrix of genetic distances d_0 reveals that the overall pattern of genetic differentiation among populations is primarily influenced by the species-status of the populations even if the two *Q. cerris* populations are not considered (Figure 3). Populations belonging to the same botanical species cluster with genetically similar groups. The three mixed *Q. petraea/Q. robur* populations are assigned to a branch of the *Q. petraea* cluster. *Q. petraea* and *Q. pubescens* populations belong to separate clusters, but are genetically more similar to each other than to *Q. robur* populations (see also Table 6). Thus, there is a close correspondence between the grouping of populations according to their leaf morphology and the grouping based on variation patterns at isozyme gene loci.

Table 5. Genetic variation within populations of oaks (*Quercus* spp.) in Switzerland at 17 enzyme gene loci; average for four species and comparison to other studies. Abbreviations as in Table 4.

Species	Region	*A/L*	$H_e = dT$	Source
Q. robur	Switzerland	2.70	0.241	this study
Q. robur / Q. petraea.	Switzerland	2.90	0.256	this study
Q. petraea	Switzerland	2.82	0.249	this study
Q. pubescens	Switzerland	2.91	0.215	this study
Q. cerris	Switzerland	2.35	0.221	this study
Q. robur	Europe	2.70	0.252	ZANETTO *et al.*, 1994
Q. patraea	Europe	2.72	0.245	ZANETTO *et al.*, 1994
Q. robur	mainly Germany	-*	0.252	HERZOG, 1996
Q. petraea	mainly Germany	-*	0.253	HERZOG, 1996
Q. pubescens	Switzerland, Italy	2.97	0.252	MÜLLER, 1999
Picea abies	Switzerland	2.52	0.265	MÜLLER STARCK, 1995
Abies alba	Switzerland	2.16	0.206	HUSSENDÖRFER, 1999
196 woody species	worldwide	1.76	0.148	HAMRICK *et al.*, 1992

*: not reported

Table 6. Average pairwise genetic distances do of the gene pool (17 isozyme gene loci) between populations belonging to the same (main diagonal) or different (below main diagonal) taxonomic units.

	Q. robur	Mixed *Q.robur - Q.petrea*	*Q. petraea*	*Q. pubescens*	*Q. cerris*
Q. robur	0.057				
Mixed *Q.robur - Q.petrea*	0.081	0.047			
Q. petraea	0.135	0.079	0.056		
Q. pubesens	0.148	0.103	0.089	0.069	
Q. cerris	0.541	0.536	0.523	0.544	0.124

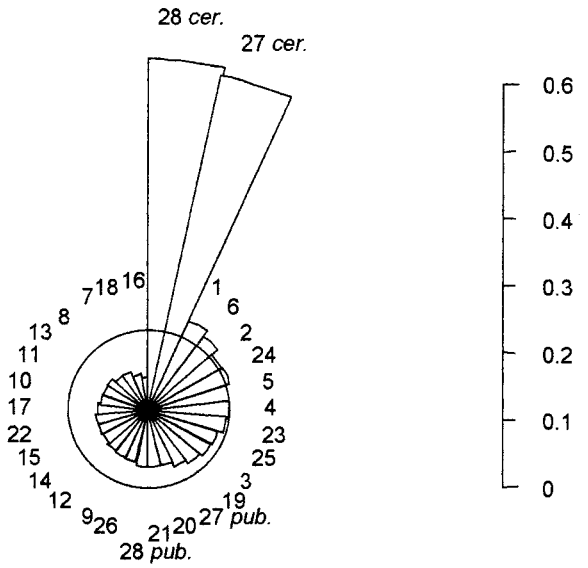

Figure 2. Differentiation D_j (GREGORIUS & ROBERDS 1986) of the gene pool (17 gene loci) for 30 oak (*Quercus* spp.) populations from Switzerland; populations nos. as in Table 1.

DISCUSSION

Leaf morphological data

A clear distinction is easily possible between *Q. robur* and the other investigated species based on leaf morphological traits. Multivariate statistics has been successfully used to differentiate between *Q. robur* and *Q. petraea* also in previous studies (e.g. GRANDJEAN & SIGAUD 1987; AAS 1993). However, differences between *Q. pubescens* and *Q. petraea* are much less pronounced at the recorded leaf morphological traits. In north-eastern France both species form a continuum and cannot be clearly distinguished based on their leaf morphology (DUPOUEY & BADEAU, 1993). A large proportion of "intermediate forms" between these two taxa has been identified by MÁTYÁS (1999) and MÜLLER (1999) in Switzerland based on observations of trichomes on leaves and twigs. *Q. pubescens sensu stricto* dominates in the Mediterranean region (MÜLLER 1999). His observations suggest that *Q. pubescens*

Table 7. Hierarchical partitioning of genetic variation according to Nei (1973) for 30 (including *Q. cerris*) and 28 (excluding *Q. cerris*) oak (*Quercus* spp.) populations from Switzerand at 17 isozyme gene loci and the gene pool.

Gene locus	30 populations			28 populations		
	within populations	among populations within species	among species	within populations	among populations within species	among species
Aap-A	0.921	0.020	0.059	0.959	0.019	0.022
Aco-A	0.989	0.011	0.000	0.989	0.011	0.000
Acp-C	0.788	0.036	0.176	0.881	0.035	0.084
Adh-A	0.895	0.035	0.070	0.938	0.028	0.034
Gdh-A	0.828	0.011	0.161	0.974	0.010	0.016
Got-B	0.911	0.010	0.079	0.990	0.006	0.004
Idh-A	0.949	0.029	0.022	0.959	0.029	0.012
Idh-B	0.883	0.009	0.108	0.890	0.008	0.102
Mdh-A	0.973	0.027	0.000	0.973	0.026	0.001
Mnr-A	0.713	0.011	0.276	0.980	0.010	0.010
Ndh-A	0.977	0.009	0.014	0.979	0.009	0.012
6-Pgdh-A	0.944	0.009	0.047	0.990	0.010	0.000
6-Pgdh-B	0.298	0.004	0.698	0.952	0.004	0.044
Pgi-A	0.055	0.024	0.921	0.988	0.012	0.000
Pgi-B	0.764	0.009	0.227	0.984	0.009	0.007
Pgm-A	0.691	0.022	0.287	0.818	0.016	0.166
Skdh-A	0.771	0.037	0.192	0.964	0.036	0.000
Gene pool	0.801	0.021	0.178	0.938	0.019	0.043

Table 8. Differentiation among populations (G_{ST} and δ) within three oak (*Quercus* spp.) species from Switzerland and comparison to widely distributed conifers in Switzerland.

Species	G_{ST}	δ	Reference
Q. robur	0.019	0.045	this study
Q. petraea	0.012	0.042	this study
Q. pubescens	0.027	0.055	this study
Picea abies	0.023	0.043	MÜLLER-STARCK 1995
Abies alba	0.034	0.044	HUSSENDÖRFER 1999

populations in Switzerland are morphologically distinct from Mediterranean populations. Their correct taxonomic status may be *Q. pubescens sensu lato*; most trees in Swiss *Q. pubescens* populations show morphologically intermediate forms between *Q. pubescens sensu stricto* and *Q. petraea*. A likely explanation is intense hybridization between *Q. pubescens* and *Q. petraea* and introgression of *Q. petraea* genes into the gene pool of *Q. pubescens* in the northern part of the distribution of the latter species where *Q. petraea* dominates most oak forests (cf. AAS 1998; see below).

Genetic variation within populations

Genetic variation within populations of oaks in Switzerland is high if compared to the average of woody taxa (Tables 4 and 5). Results for *Q. petraea* and *Q. robur* are similar to previous findings in different regions (Table 5) although the set of investigated isozyme gene loci differs among the studies.

There are fewer reports on the level of genetic variation within *Q. pubescens*. MÜLLER (1999) found a similar trend concerning levels of genetic variation within populations of *Q. pubescens* as compared to *Q. petraea* and *Q. robur*: The average number of alleles

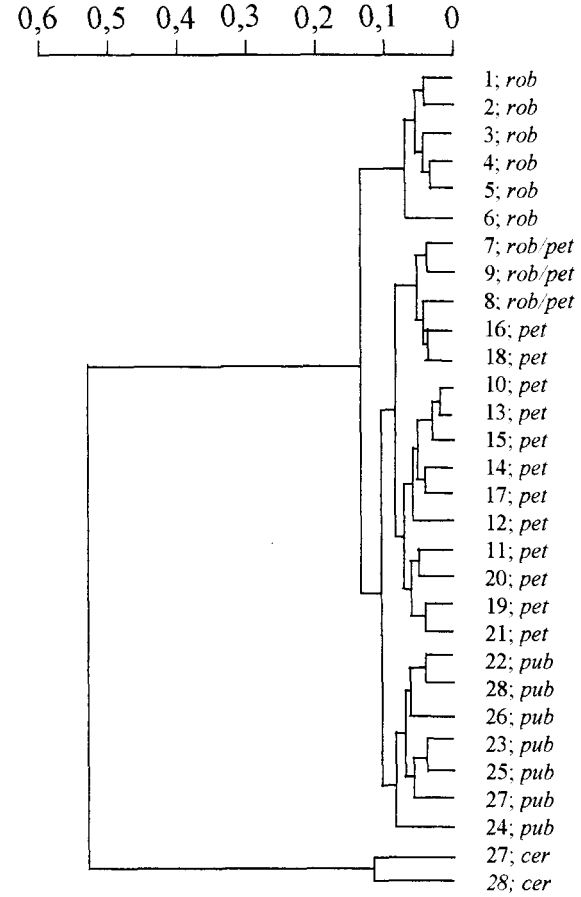

Figure 3. Cluster diagram (UPGMA) based on genetic distances do of the gene pool (17 gene loci) illustrating differentiation among 30 oak (*Quercus* spp.) populations from Switzerland; population nos. and abbreviations as in Table 1.

per locus is higher for *Q. pubescens*, but the "expected heterozygosity" is lower. His results and the results shown here suggest the occurrence of a large number of rare alleles in *Q. pubescens* populations. A large number of alleles was observed also in three highly elevated populations of *Q. pubescens* in the Valais Alps (St. Luc, Gampel and Fully). There are no hints on losses of allelic richness due to bottleneck effects in the alpine region. Thus, the low "expected heterozygosity" in the *Q. pubescens* populations from Valais is unlikely to be the result of genetic drift. It may be related to comparatively homogeneous environmental conditions within the investigated forests.

The comparatively low abundance of oaks in Swiss forests (2.24 %; see above) did not result in losses of genetic variation with respect to the studied markers as compared to other regions (Table 5). Genetic variation within oak forests in Switzerland is comparable to variation within populations of the most frequent tree species in Switzerland, Norway spruce (*Picea abies*; 39.2 % of all trees growing in Switzerland according to BRASSEL & BRÄNDLI (1999); cf. Table 5), and higher than variation within European silver fir (*Abies alba*; 10.9 % of all trees growing in Switzerland according to BRASSEL & BRÄNDLI (1999)).

Genetic differentiation among populations

Q. cerris is strongly differentiated from the other species. A meaningful interpretation of the results required the separation of two populations (*Q. cerris vs. Q. pubescens*) at both investigated locations where *Q. cerris* occurs. Hybridization between *Q. cerris* and other species is unlikely even in mixed forests, although a few intermediate leaf forms (*Q. cerris* – *Q. pubescens*) were noticed in Novaggio (*pers. obs.*). Introgression of *Q. cerris* genes into the gene pool of the other species and *vice versa* was not observed. The complete qualitative differentiation at several isozyme gene loci and the strong differentiation of the gene pool support the common taxonomic separation between *Q. cerris* and the other species at least at the level of a section.

Differentiation among populations of *Q. petraea*, *Q. pubescens*, and *Q. robur* is rather low confirming previous results in other regions (KLEINSCHMIT *et al.* 1995; HERZOG 1996). The populations shared the most frequent alleles at all investigated loci. No gene locus showed complete qualitative differentiation, and only rare alleles were species-specific. However, the absence of rare alleles in a species may be due to limited sample sizes. The most likely explanation for the low levels of differentiation is extensive gene flow among the species. Results of pollination experiments (summarized e.g. by AAS (1998) and MÜLLER (1999)), observations at marker gene loci in mixed stands (e.g. BACILIERI *et al.* 1993), and geographical variation patterns of cpDNA (PETIT *et al.* 1997) point towards significant gene exchange among these oak species.

However, most of the genetic differentiation was observed among populations belonging to different species, and overall differentiation patterns of the gene pool reflected the taxonomic status of the populations. Thus, introgression and gene exchange did not completely wipe out any differentiation among species. Differentiation between *Q. robur* and the other species was strongest at the gene loci *Acp-C*, *Idh-B*, and *Pgm-B*, which proved to be useful for species identification of *Q. robur* and *Q. petraea* also in north-eastern Germany (HERTEL & DEGEN 1998). *Q. pubescens* is clearly differentiated from both other species at the gene locus *6-Pgdh-B*.

The easily observable differentiation at morphologi-

cal traits, which lead to the recognition of different botanical species, has a genetic basis, which can be monitored at the population level at the mentioned enzyme gene loci even in the absence of qualitative differentiation at any trait. There is no doubt that *Q. petraea, Q. pubescens,* and *Q. robur* belong to a single biological species in the sense of MAYR (1970) due to their interfertility and the importance of gene flow among the taxa for their genetic structures (KLEINSCHMIT *et al.* 1995). However, qualitative differentiation at marker loci or other traits is not a necessary requirement for the distinction of different botanical species. The observation of genetic variation patterns corresponding to the taxonomic distinction of three different botanical species supports the view of AAS *et al.* (1997) to maintain the separation of *Q. petraea, Q. pubescens,* and *Q. robur* at the species level.

The low differentiation among populations within species for *Q. petraea* and *Q. robur* is likely to be a consequence of massive gene flow among populations of both species. The comparatively low abundance of oaks in Swiss forests did not result in a stronger differentiation among populations as compared to widespread conifers (Table 8). I refrained from computing estimates of gene flow such as *Nm* based on differentiation measures (e.g. ENNOS 1994) because many of the assumptions, on which the computations rest, cannot be tested based on the data shown here. However, it seems reasonable to assume reduced levels of gene flow among populations of *Q. pubescens* as a cause of the slightly increased differentiation among populations as compared to *Q. petraea* and *Q. robur* since *Q. pubescens* is the rarest of the three species and populations are most widely separated accordingly.

The data presented here provide clear evidence for a genetic basis of the morphological differentiation on which the distinction of different *Quercus* species in Switzerland rests. This easily observable differentiation must not be overlooked if *in situ* areas for the conservation of oak genetic resources in Switzerland are selected. All botanical species should be represented in the areas. Important genetic differentiation among populations within species cannot be ruled out in view of limitations of the observed isozyme gene markers to detect patterns of adaptive differentiation (e.g. FINKELDEY & MÁTYÁS 1999).

It will be neither feasible nor desirable to prevent gene flow from outside of *in situ* conservation areas and gene flow among species. Gene flow through pollen has been a strong evolutionary force shaping the genetic structures of oaks in Switzerland in the past. Long-distance pollen movement is likely to alter the genetic structures of populations conserved *in situ* during natural regeneration. However, limited gene flow among populations is likely to promote adaptive processes in *in situ* conservation areas and may be regarded as an inherent component of dynamic conservation strategies.

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