resulting in smaller sampling errors of the estimates of type B genetic correlations.

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Genetic Variation of Oaks (*Quercus* spp.) in Switzerland

2. Genetic Structures in "Pure" and "Mixed" Forests of Pedunculate Oak (*Q. robur* L.) and Sessile Oak (*Q. petraea* (MATT.) LIEBL.)

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Abstract

Sessile oak (*Quercus petraea*) and pedunculate oak (*Q. robur*) are two closely related, interfertile taxa. They are the most frequent oak species in Switzerland. Allelic and genotypic structures at 17 isozyme gene loci were observed in 21 populations from Switzerland. Twelve populations of *Q. petraea,* six populations of *Q. robur*, and three "mixed" populations (*Q. petraea* and *Q. robur*) were investigated. The species status of the populations was confirmed by Principal Component Analysis (PCA) based on leaf morphological traits. All populations are highly variable at enzyme gene loci. Differentiation among the taxa is reflected at allelic structures at several enzyme gene loci (*ACP-C, GDH-A, IDH-B, NDH-A, PGM-A*). An excess of homozygotes relative to corresponding HARDY-WEINBERG structures was observed in all populations. Moderate levels of inbreeding are likely to contribute to these genotypic structures, but heterogeneity of inbreeding coefficients among loci suggests that deviations from random mating are not the only cause of the homozygote excess at particular loci (*AAP-A, PGM-A*). On average, expected heterozygosity is highest in the "mixed" populations, but observed heterozygosity of the "mixed" stands is in-between *Q. petraea* and *Q. robur*. A plausible

explanation is partial reproductive isolation of both species in "mixed" forests resulting in a "Wahlund effect". Seed procurement in "mixed" oak forests is encouraged. The species status of populations is of prime importance for the selection of oak genetic resources in Switzerland.

Key words: differentiation, diversity, isozyme, homozygote excess, *Quercus petraea, Q. robur,* seed procurement, Switzerland, Wahlund effect.

Introduction

Sessile oak (*Quercus petraea*) and pedunculate oak (*Q. robur*) are widely distributed forest tree species in Europe. The taxa are closely related and interfertile. Most pollination experiments revealed an asymmetric success of artificial pollination; interspecific crosses are usually more successful if female *Q. robur* flowers are pollinated by *Q. petraea* pollen rather than vice versa (e.g. AAS, 1998). Natural hybridization is very likely to occur, but the extent of hybridization is not exactly known (BACILIERI *et al.,* 1995, 1996). The species can neither be unambiguously distinguished at isozyme gene markers (ZANETTO *et al.,* 1994) nor at molecular markers such as RAPDs (BODÉNÈS *et al.,* 1997) or cpDNA haplotypes (DUMOLIN-LAPÈGUE *et al.,* 1997).

The ecological preferences of *Q. petraea* and *Q. robur* are different (AAS, 1998), but "mixed" forests with both species are frequent in Central Europe. The genetic structures of "pure" and "mixed" oak forests have rarely been investigated in detail. Harvesting of reproductive material has mainly been confined to "pure" forests in the past. Current regulations do not encourage the use of progenies from "mixed" populations for plantation establishment in most European countries.

Q. petraea and *Q. robur* are the most frequent oak species in Switzerland; 1.4% of all trees with a diameter at breast height above 12 cm are *Q. petraea* and 0.6% are *Q. robur* (BRASSEL und BRÄNDLI, 1999, loc. cit. p. 133). An investigation of cpDNA polymorphisms revealed that oaks migrated to Switzerland from two different glacial refugia (MÁTYÁS and SPERISEN, in press). Pedunculate and sessile oak are commercial species in Switzerland and have been exploited for centuries. Thus, anthropogenic seed transfer has an impact on today's distribution of the species and possibly also on patterns of genetic variation within species. However, the spatially structured occurrence of cpDNA haplotypes suggests a limited influence of human seed transfer over long distances on genetic structures of oaks in Switzerland (MÁTYÁS and SPERISEN, in press). The origin of most oak forests in Switzerland is uncertain. Clear evidence of autochthony, e.g. based on historical records, is usually lacking.

Results presented here are part of a comprehensive genetic inventory based on isozyme marker loci and leaf morphological traits in oak populations from Switzerland (FINKELDEY, submitted). I will specifically address the following questions: Are there different levels of genetic variation in "pure" and "mixed" oak stands? Is the species' status of populations reflected at particular isozyme gene loci? Are genotypic structures of mature oak forests influenced by inbreeding and/or a "Wahlund effect"?

Materials and Methods

Material

Material was collected in 21 oak populations from Switzerland (*Table 1*). With few exceptions (e.g. population "Schoren" and "Magden") populations have a minimum size of 10 hectares and are regarded as potentially suitable for the *in situ* conservation of oak genetic resources in Switzerland. All stands are mature, reproducing forests. Oaks are dominating species in all forests, but in most cases they occur in mixture with other, mainly broad-leaved species.

Table 1. – Investigated oak populations, their approximate altitude, and location.

No.	Population	Altitude (*m*)	Eastern longitude	Northern latitude
1	Bonfol	450	7° 10'	47° 28'
2	Lugnez	440	7° 06'	47° 29'
3	Wölfinswil	540	8° 02'	47° 28'
4	Tägerwilen	520	9° 08'	47° 38'
5	Uttwil	440	9° 20'	47° 34'
6	Magadino	200	8° 52'	46° 10'
7	Allschwil	350	7° 32'	47° 32'
8	Muttenz	270	7° 38'	47° 34'
9	Büren a.A.	470	7° 23'	47° 08'
10	Satigny	460	6° 00'	46° 13'
11	Jussy	500	6° 18'	46° 14'
12	Alaman	410	6° 24'	46° 28'
13	Corcelles	550-600	6° 42'	46° 51'
14	Galm	570	7° 11'	46° 57'
15	Bois de devant	570-670	6° 49'	46° 59'
16	Schoren	520	7° 47'	47° 26'
17	Magden	370	7° 48'	47° 32'
18	Bülach	430	8° 32'	47° 33'
19	Cavergno	540-1040	8° 37'	46° 21'
20	Gordevio	400-560	8° 45'	46° 14'
21	Castaneda	640-750	9° 09'	46° 16'

One hundred oak trees were randomly selected and temporarily marked in each stand. The minimum distance between trees was 30 m whenever possible. Twigs with at least 20 dormant buds were harvested from each tree. Small parts of twigs (approx. 1 cm) with dormant buds were frozen at –80 °C. In each population, 100 oak leaves were collected from the ground during the dormant season. One leaf was randomly sampled approximately every 30 m in the same area where buds were harvested. Leaves were dried and kept in an herbarium for the analysis of morphological traits.

Methods

From each leaf (100 leaves per population) the following data were recorded: petiole length (PL), lamina length (LL), number of intercalary veins (NV), abaxial lamina pubescence (HR), basal shape of petiole (BS), and number of lobes (NL). NV and NL were directly counted, PL and LL measured in mm, and HR and BS assessed on a relative scale ranging from 0 to 6 and 0 to 9, respectively. The petiole ratio (PR) was computed as PR = PL/(LL+PL). Principal component analysis (PCA; BACKHAUS *et al.,* 2000, loc. cit. 252ff.) was performed based on five characters: PR, NV, HR, NL, BS. Previous studies proved the suitability of these traits to differentiate between *Q. petraea* and *Q. robur* (GRANDJEAN and SIGAUD, 1987; DUPOUEY and BADEAU, 1993). Factor scores of the first and second component were computed for all leaves and plotted in two-dimensional graphs. Leaves were assigned to one of three groups (*Q. petraea*, *Q. robur*, or intermediate) according to their factor scores of the first and second component.

Fourteen polymorphic enzyme systems were observed: (Alanine)-aminopeptidase (E.C.-No. 3.4.11.1; *AAP*); aconitase (E.C.-No. 4.2.1.3; *ACO*); acid phosphatase (E.C.-No. 3.1.3.2; *ACP*); alcohol-dehydrogenase (E.C.-No. 1.1.1.1; *ADH*); glutamate-dehydrogenase (E.C.-No. 1.4.1.3; *GDH*); glutamate-oxaloacetate-transaminase (E.C.-No. 2.6.1.1; *GOT*); isocitrate-dehydrogen-

ase (E.C.-No. 1.1.1.42; *IDH*); malate-dehydrogenase (E.C.-No. 1.1.1.37; *MDH*); menadione-reductase (E.C.-No. 1.6.99.2; *MNR*); NADH-dehydrogenase (E.C.-No. 1.6.99.3; *NDH*); 6-phosphogluconate-dehydrogenase (E.C.-No. 1.1.1.44; *6-PGDH*); phosphoglucose-isomerase (E.C.-No. 5.3.1.9; *PGI*); phosphoglucomutase (E.C.-No. 2.7.5.1; *PGM*); shikimic-acid-dehydrogenase (E.C.-No. 1.1.1.25; *SKDH*). Allelic and genotypic structures based on 100 trees per population were computed for 17 isozyme gene loci: *AAP-A; ACO-A; ACP-C; ADH-A; GDH-A; GOT-B; IDH-A; IDH-B; MDH-A; MNR-A; NDH-A; 6-PGDH-A; 6-PGDH-B; PGI-A; PGI-B; PGM-A; SKDH-A.* Laboratory methods and genotyping followed standard procedures (MÜLLER-STARCK *et al.,* 1996; ZANETTO *et al.,* 1996) with slight modifications as described in FINKELDEY (submitted). The genetic control of isozyme phenotypes has been studied for most gene loci (MÜLLER-STARCK *et al.,* 1996; ZANETTO *et al.,* 1996), but not for *ACO-A, ADH-A, IDH-A, MDH-A, NDH-A,* and *PGI-A.* Enzyme phenotypes were empirically interpreted at these putative loci. Genotyping was straightforward at these loci since clear and repeatable banding patterns were observed. Only the *A* and *B* zones of the *IDH* enzyme system overlapped. These zones could unambiguously be separated by their different staining intensity.

Genetic variation within populations was computed as the average number of alleles per locus (A/L), expected heterozygosity (H_e) (e.g. BERG and HAMRICK, 1997), which equals the "differentiation within populations" δ_T according to GREGORIUS (1987), and hypothetical gametic multilocus diversity v_{gam} (GREGORIUS, 1978). Allelic differentiation among populations was assessed following NEI (1973) and GREGORIUS and ROBERDS (1986), and illustrated as cluster diagram (UPGMA; SNEATH and SOKAL, 1973) based on genetic distances d_0 (GREGORIUS, 1984). Heterozygosities expected under HARDY-WEINBERG assumptions (H_e) and observed heterozygosities (H_o) were compared. Differences between H_e and H_o were tested for statistical significance by a goodness-of-fit test (G-test; SOKAL and ROHLF, 1995; loc. cit. pp. 686ff.). Inbreeding coefficients (F) were computed as $F = 1 - (H_o/H_e)$.

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

Results

Leaf morphological data

Multivariate analysis of leaf morphological data revealed that most of the leaves can be assigned to one of two main groups. For the majority of leaves it holds: factor score (1st component) > [–1.5 × factor score (2nd component)]. These leaves are of the *"Q. petraea*-type". The other main group consists of leaves with factor score (1st component) < [-1.5 × factor score (2nd component) – 1]. These leaves are of the *"Q. robur*-type". Intermediate forms are comparatively rare (*Figure 1*). The frequency of leaf types based on scores of their 1st and 2nd component are reported in *table 2* for each population. Six populations predominantly contain leaves of the *"Q. robur*-type" (*Table 2* and *Figure 2a*). However, a few trees of the *"Q. petraea*-type" and intermediate forms were observed in all of these populations. The *"Q. petraea*-type" clearly dominates in twelve populations (*Table 2* and *Figure 2b*), but intermediate forms and leaves of the *"Q. robur*-type" were observed in most of these populations in low frequencies as well. Three populations contain leaves of the *"Q. robur*-type" and the *"Q. petraea*-type" in roughly equal frequencies (*Table 2* and *Figure 2c*). Intermediate forms are comparatively rare in these populations as well.

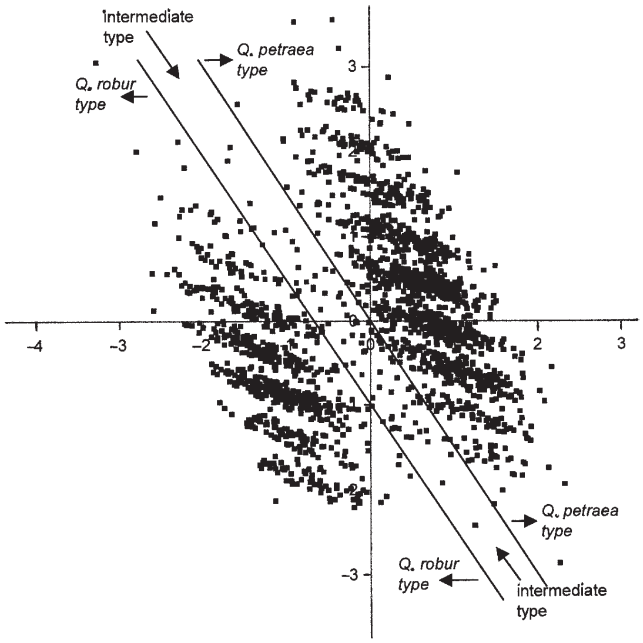

Figure 1. – Principal component analysis (PCA) of morphological data from 2100 oak leaves collected in Switzerland. Further explanations in the text.

Table 2. – Frequencies of leaf types based on PCA of morphological data in oak populations from Switzerland. Sample size: 100 leaves in each population.

No.	Population	*Q. robur* type	*Q. petraea* type	Intermediary type	Dominating species
1	Bonfol	88	8	4	*Q. robur*
2	Lugnez	88	7	5	*Q. robur*
3	Wölfinswil	87	9	4	*Q. robur*
4	Tägerwilen	95	1	4	*Q. robur*
5	Uttwil	98	1	1	*Q. robur*
6	Magadino	96	2	2	*Q. robur*
7	Allschwil	50	46	4	"mixed"
8	Muttenz	39	55	6	"mixed"
9	Büren	45	48	7	"mixed"
10	Satigny	5	91	4	*Q. petraea*
11	Jussy	7	91	2	*Q. petraea*
12	Alaman	1	97	2	*Q. petraea*
13	Corcelles	0	97	3	*Q. petraea*
14	Galm	0	99	1	*Q. petraea*
15	Bois de devant	0	92	8	*Q. petraea*
16	Schoren	1	98	1	*Q. petraea*
17	Magden	4	95	1	*Q. petraea*
18	Bülach	6	92	2	*Q. petraea*
19	Cavergno	0	99	1	*Q. petraea*
20	Gordevio	1	97	2	*Q. petraea*
21	Castaneda	1	84	15	*Q. petraea*
	all populations (in %)	712 33.9 %	1309 62.3 %	79 3.8 %	

Genetic variation within populations and differentiation among populations

Genetic variation at isozyme gene loci is high within all investigated populations (*Table 3*). A total of 70 alleles were observed at the 17 gene loci (4.12 alleles per locus). The average expected heterozygosity H_e is 0.247 for all 21 populations. Levels of genetic variation are slightly higher in *Q. petraea* populations as compared to *Q. robur*. On average, all computed measures of genetic variation within populations (A/L, H_e, and v_{gam}) are highest in the "mixed" populations (*Table 3*).

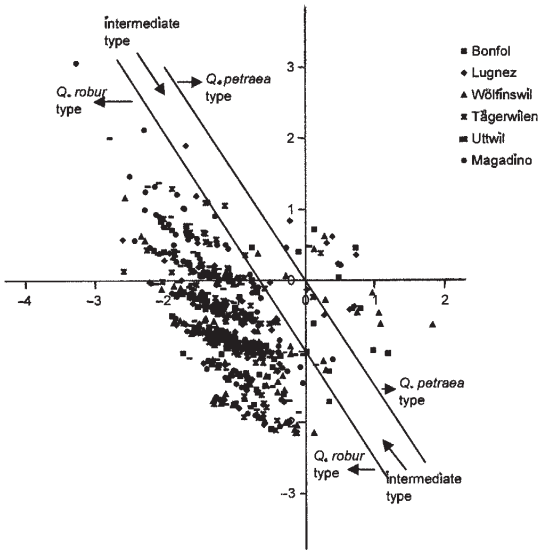

figure 2a

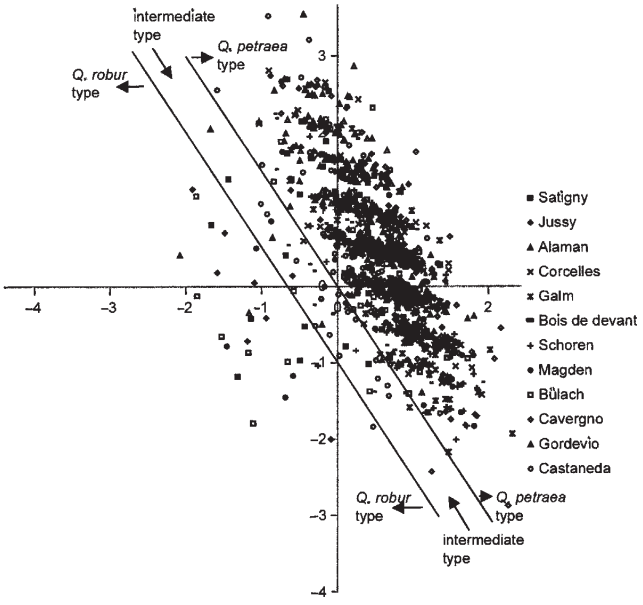

figure 2b

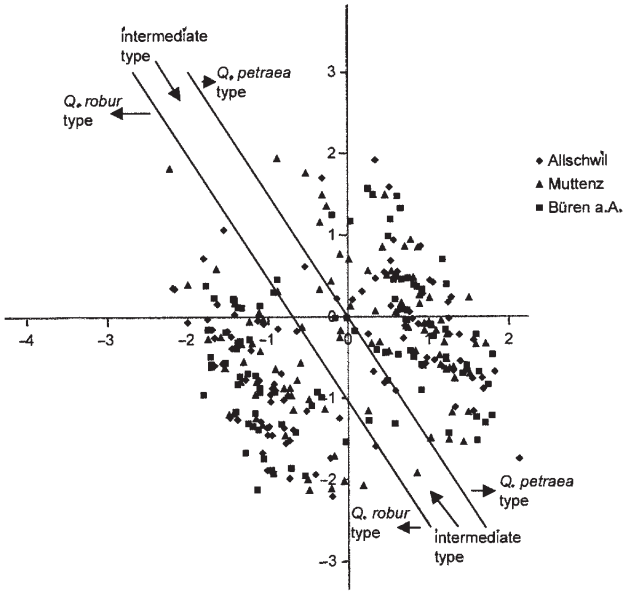

figure 2c

Figure 2a to 2c. — Principal component analysis (PCA) of leaf morphological data in Swiss populations dominated by *Quercus robur* (*Figure 2a*), *Q. petraea* (*Figure 2b*), and in "mixed" populations (*Figure 2c*). Further explanations in the text.

The allelic differentiation among populations δ (GREGORIUS and ROBERDS, 1986) of the gene pool of 17 isozyme gene loci is 0.067. *Quercus robur* populations are more strongly differentiated from their respective complements as indicated by comparatively high values of Dj (*Figure 3*). The D_j-values of "mixed" populations are small, i.e. they represent the overall allelic structures well. A hierarchical partitioning of the total genetic variation according to NEI (1973) reveals that 94.0% of the genetic variation is within populations, 1.7% is among populations within species, and 4.3% is among species, if the "mixed" populations are disregarded. Inclusion of the "mixed" populations at the highest level of differentiation as a separate "type" yields corresponding values of 94.7% for the variation within populations, 1.5% for the variation among populations within "type", and 3.8% for the variation among the three different "types" (*Q. robur, Q. petraea,* and "mixed"). Clustering based on genetic distances d_0 and UPGMA reveals that the *Q. robur* populations form a separate cluster. The "mixed" populations belong to a single branch of the *Q. petraea* cluster (*Figure 4*).

The variation component due to differentiation among species is high for the gene loci *ACP-C, IDH-B,* and *PGM-A*

Table 3. – Genetic variation at 17 isozyme gene loci in oak populations (*Q. petraea* and *Q. robur*) from Switzerland. A/L: average number of alleles per locus; H_e: expected heterozygosity; v_{gam}: hypothetical gametic multilocus diversity; H_o: observed heterozygosity.

No.	Population	A/L	H_e	v_{gam}	H_o
1	Bonfol	2.76	0.229	160.9	0.208
2	Lugnez	2.82	0.251	304.8	0.232
3	Wölfinswil	2.71	0.256	388.5	0.231
4	Tägerwilen	2.59	0.235	217.1	0.221
5	Uttwil	2.65	0.233	200.6	0.229
6	Magadino	2.65	0.239	208.5	0.208
7	Allschwil	3.06	0.251	276.9	0.220
8	Muttenz	2.65	0.259	387.6	0.226
9	Büren	3.00	0.257	379.2	0.232
10	Satigny	2.76	0.246	255.0	0.227
11	Jussy	2.65	0.249	251.3	0.235
12	Alaman	2.76	0.262	375.4	0.256
13	Corcelles	2.71	0.246	240.6	0.213
14	Galm	2.88	0.247	246.5	0.237
15	Bois de devant	2.88	0.247	240.5	0.224
16	Schoren	2.76	0.260	378.8	0.253
17	Magden	2.94	0.261	324.9	0.247
18	Bülach	2.88	0.254	302.9	0.224
19	Cavergno	2.94	0.234	193.8	0.216
20	Gordevio	2.76	0.235	180.7	0.227
21	Castaneda	2.94	0.241	209.9	0.214
1 – 6	average *Q. robur*	2.70	0.241	246.7	0.222
7 – 9	average "mixed"	2.90	0.256	347.9	0.226
10 – 21	average *Q. petraea*	2.82	0.249	266.7	0.231

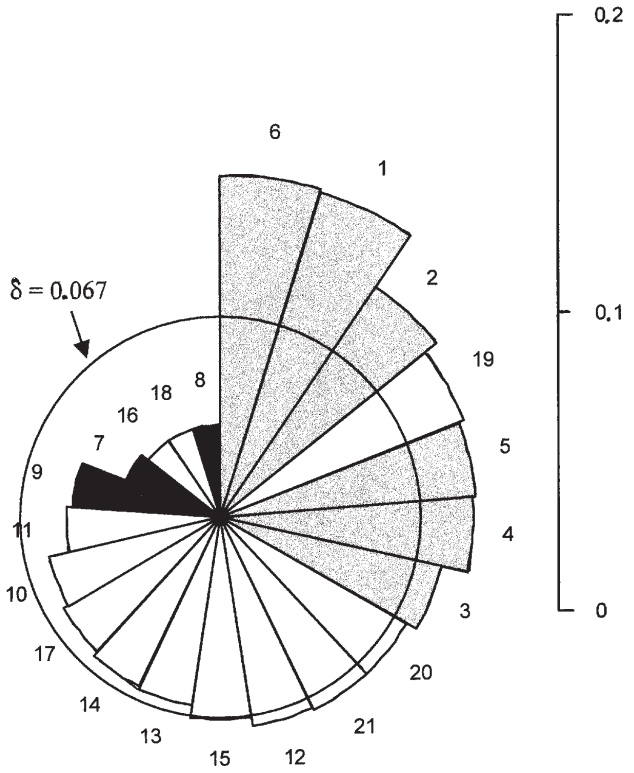

Figure 3. – Differentiation of the gene pool (17 loci) among 21 oak populations from Switzerland (D_j according to GREGORIUS and ROBERDS, 1986). Numbers as in *table 1*; grey: *Quercus robur* populations; white: *Q. petraea* populations; black: mixed populations.

(*Table 4*). Allele frequencies are reported for these loci in *table 5.* Non-overlapping ranges of allele frequencies for *Q. robur* and *Q. petraea* populations were observed at these gene loci. The "mixed" populations show intermediate frequencies of the discriminating alleles. Non-overlapping ranges of allele frequencies for *Q. robur* and *Q. petraea* populations were also observed at *GDH-A* and *NDH-A* (*Table 6*) although the differentiation among populations is much lower at these loci.

Table 4. – Hierarchical partitioning of genetic variation (NEI, 1973) at single gene loci for 18 oak populations (six *Quercus robur* and twelve *Q. petraea*) in Switzerland. G_{PS}: Variation component due to differentiation among populations within species. G_{ST}: Variation component due to differentiation among species.

Gene locus	G_{PS}	G_{ST}
AAP-A	0.024	0.017
ACO-A	0.010	0.000
ACP-C	0.013	0.085
ADH-A	0.032	0.002
GDH-A	0.012	0.022
GOT-B	0.004	0.004
IDH-A	0.018	0.016
IDH-B	0.007	0.096
MDH-A	0.000	0.000
MNR-A	0.010	0.015
NDH-A	0.006	0.018
6-PGDH-A	0.007	0.000
6-PGDH-B	0.012	0.000
PGI-A	0.010	0.001
PGI-B	0.008	0.013
PGM-A	0.016	0.180
SKDH-A	0.029	0.001
gene pool	0.017	0.043

Genotypic structures and heterozygosity

Genotypic structures were analysed by testing deviations between observed heterozygosities (H_o; *Table 3*) and corresponding heterozygosities based on HARDY-WEINBERG structures (expected heterozygosities H_e) for statistical significance. Goodness-of-fit tests (G-Tests) were only performed if the absolute number of expected heterozygotes was at least 5 (SOKAL and ROHLF, 1995, loc. cit. 698ff.). Thirty-one tests out of 259 tests which could be performed resulted in deviations between observed and expected heterozygosities at a significance level of at least 5%. A significant excess of observed heterozygotes relatively to the expectation was observed in a single case only (Population "Schoren" at locus *GDH-A*). All other significant deviations resulted in positive inbreeding coefficients F, i.e. an excess of observed homozygotes relatively to HARDY-WEINBERG proportions.

Significant deviations between observed and expected heterozygosities were observed in 14 out of the 21 investigated populations at *AAP-A* and in nine populations at *PGM-A*. Deviations at all other loci (218 tests) were observed at the 5% level of significance in five cases only and at the 1% level in three further cases, i.e. roughly in a frequency as expected by chance alone.

The high frequency of heterozygote deficiencies at the *AAP-A* and *PGM-A* gene loci may not be caused by inbreeding or other peculiarities of the mating system but may have different causes (see below). Particular attention was paid to gene loci showing unambiguous differentiation between *Q. robur* and *Q. petraea*. Thus, inbreeding coefficients [$F = 1 - (H_o/H_e)$] were calculated for different sets of gene loci (*Table 7*): All 17 investi-

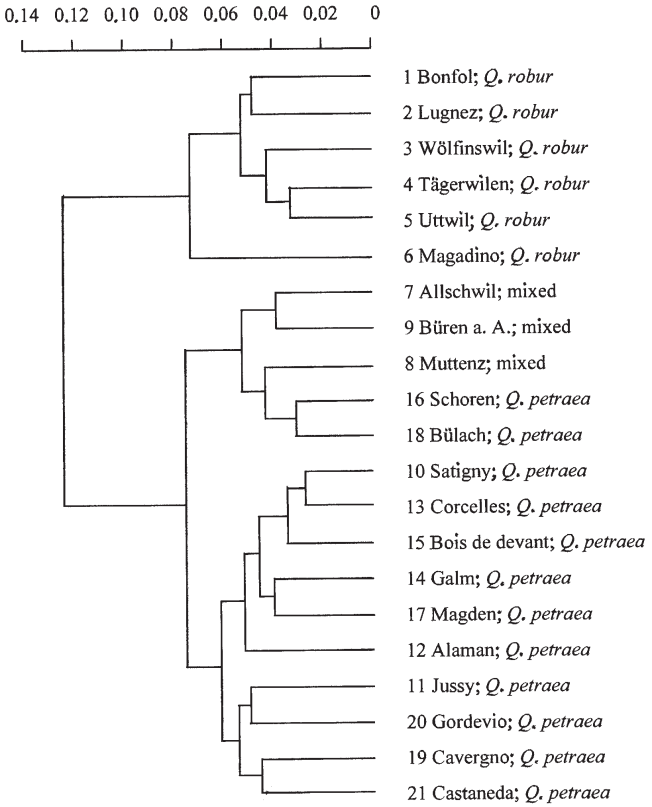

Figure 4. – Cluster diagram (UPGMA based on genetic distances d_0 of the gene pool (17 loci)) illustrating allelic differentiation among 21 oak populations from Switzerland.

Table 5. – Allele frequencies at the isozyme gene loci *ACP-C, IDH-B,* and *PGM-A* in 21 oak populations from Switzerland. Frequencies may not add to unity since frequencies of rare alleles are not reported.

Gene locus	ACP-C		IDH-B			PGM-A	
Allele	1	2	2	3	4	2	3
Bonfol	0.820	0.180	0.000	0.355	0.645	0.540	0.450
Lugnez	0.795	0.205	0.005	0.405	0.590	0.615	0.380
Wölfinswil	0.785	0.215	0.005	0.320	0.665	0.505	0.465
Tägerwilen	0.795	0.205	0.000	0.350	0.645	0.505	0.475
Uttwil	0.810	0.190	0.000	0.305	0.685	0.580	0.415
Magadino	0.720	0.280	0.005	0.380	0.615	0.610	0.355
range *Q. robur*	0.720 – 0.820	0.180 – 0.280	0.000 – 0.005	0.305 – 0.405	0.590 – 0.685	0.505 – 0.615	0.355 – 0.475
Allschwil	0.640	0.360	0.035	0.150	0.800	0.345	0.645
Muttenz	0.635	0.365	0.040	0.165	0.790	0.325	0.675
Büren	0.714	0.286	0.020	0.235	0.735	0.394	0.535
range "mixed"	0.635 – 0.714	0.286 – 0.365	0.020 – 0.040	0.150 – 0.235	0.735 – 0.800	0.325 – 0.394	0.535 – 0.675
Satigny	0.475	0.525	0.045	0.040	0.905	0.155	0.835
Jussy	0.510	0.490	0.080	0.065	0.830	0.167	0.818
Alaman	0.465	0.535	0.056	0.010	0.919	0.207	0.783
Corcelles	0.490	0.510	0.035	0.010	0.950	0.160	0.830
Galm	0.455	0.545	0.085	0.020	0.875	0.145	0.845
Bois de devant	0.430	0.565	0.065	0.045	0.875	0.125	0.855
Schoren	0.625	0.375	0.080	0.070	0.850	0.230	0.740
Magden	0.410	0.590	0.125	0.040	0.815	0.136	0.833
Bülach	0.585	0.415	0.085	0.110	0.795	0.210	0.775
Cavergno	0.410	0.590	0.085	0.020	0.860	0.015	0.975
Gordevio	0.520	0.480	0.160	0.020	0.805	0.075	0.925
Castaneda	0.380	0.620	0.130	0.000	0.860	0.075	0.885
range *Q. petraea*	0.380 – 0.625	0.375 – 0.620	0.035 – 0.160	0.000 – 0.110	0.795 – 0.950	0.015 – 0.230	0.740 – 0.975

Table 6. – Allele frequencies at the isozyme gene loci *GDH-A* and *NDH-A* in 21 oak populations from Switzerland. Frequencies may not add to unity since frequencies of rare alleles are not reported.

	GDH-A		*NDH-A*	
Allele	1	2	1	2
Bonfol	0.215	0.775	0.005	0.995
Lugnez	0.285	0.700	0.000	1.000
Wölfinswil	0.350	0.645	0.000	1.000
Tägerwilen	0.268	0.722	0.000	1.000
Uttwil	0.365	0.630	0.000	1.000
Magadino	0.175	0.805	0.000	1.000
range *Q. robur*	0.175 – 0.365	0.630 – 0.805	0.000 – 0.005	0.995 – 1.000
Allschwil	0.360	0.630	0.017	0.983
Muttenz	0.359	0.641	0.045	0.955
Büren	0.401	0.599	0.020	0.980
range "mixed"	0.360 – 0.401	0.599 – 0.641	0.017 – 0.045	0.955 – 0.983
Satigny	0.460	0.500	0.045	0.955
Jussy	0.403	0.566	0.045	0.955
Alaman	0.440	0.510	0.066	0.934
Corcelles	0.470	0.525	0.051	0.949
Galm	0.364	0.626	0.080	0.920
Bois de devant	0.505	0.495	0.045	0.955
Schoren	0.398	0.602	0.035	0.965
Magden	0.375	0.573	0.111	0.884
Bülach	0.375	0.620	0.035	0.965
Cavergno	0.446	0.554	0.085	0.915
Gordevio	0.555	0.430	0.055	0.945
Castaneda	0.410	0.580	0.041	0.959
range *Q. petraea*	0.364 – 0.555	0.430 – 0.626	0.035 – 0.111	0.884 – 0.965

Table 7. – Inbreeding coefficients F in 21 oak populations from Switzerland based on different sets of gene loci: Values of F for all 17 investigated isozyme gene loci, for 15 loci (i.e. without *AAP-A* and *PGM-A*), for 5 loci (*ACP-C, GDH-A, IDH-B, NDH-A, PGM-A*), 4 loci (*ACP-C, GDH-A, IDH-B, NDH-A*), 3 loci (*ACP-C, IDH-B, PGM-A*), and 12 loci (all but not *ACP-C, GDH-A, IDH-B, NDH-A, PGM-A*).

No.	Population	17 loci	15 loci	5 loci	4 loci	3 loci	12 loci
1	Bonfol	0.092	0.038	0.141	0.082	0.146	0.056
2	Lugnez	0.075	0.016	0.126	0.039	0.121	0.040
3	Wölfinswil	0.095	0.031	0.118	0.056	0.149	0.079
4	Tägerwilen	0.060	–0.008	0.016	–0.087	0.040	0.092
5	Uttwil	0.014	–0.051	0.021	–0.081	0.051	0.009
6	Magadino	0.129	0.069	0.088	0.056	0.143	0.159
7	Allschwil	0.124	0.082	0.165	0.104	0.196	0.094
8	Muttenz	0.129	0.069	0.178	0.139	0.220	0.095
9	Büren	0.097	0.040	0.119	0.001	0.184	0.080
10	Satigny	0.079	0.054	0.104	0.112	0.156	0.064
11	Jussy	0.059	0.013	0.070	0.020	0.095	0.052
12	Alaman	0.025	0.019	0.048	0.035	0.161	0.011
13	Corcelles	0.137	0.114	0.146	0.167	0.198	0.131
14	Galm	0.040	0.020	0.008	0.012	0.073	0.059
15	Bois de devant	0.091	0.051	0.043	0.056	0.042	0.120
16	Schoren	0.025	–0.011	–0.002	–0.065	0.096	0.042
17	Magden	0.051	0.005	0.032	0.015	0.028	0.065
18	Bülach	0.117	0.075	0.178	0.133	0.230	0.075
19	Cavergno	0.076	0.081	0.086	0.089	0.125	0.071
20	Gordevio	0.035	0.010	0.002	–0.033	0.060	0.057
21	Castaneda	0.113	0.126	0.128	0.141	0.096	0.104
1 – 6	average *Q. robur*	0.077	0.016	0.085	0.011	0.108	0.073
7 – 9	average "mixed"	0.116	0.064	0.154	0.081	0.200	0.090
10 – 21	average *Q. petraea*	0.071	0.046	0.070	0.057	0.113	0.071

gated gene loci, 15 investigated loci (i.e. all loci excluding *AAP-A* and *PGM-A*), the five loci with non-overlapping allele frequencies for *Q. robur* and *Q. petraea* (i.e. *ACP-A, GDH-A, IDH-B, NDH-A, PGM-A*), four of these gene loci (i.e. without *PGM-A*), the three loci showing strongest differentiation (*ACP-A, IDH-B, PGM-A*), and twelve loci showing poor differentiation between *Q. robur* and *Q. petraea* (i.e. all loci but not *ACP-A, GDH-A, IDH-B, NDH-A, PGM-A*).

Inbreeding coefficients are moderately positive in all populations if all 17 loci are considered (*Table 7*). The coefficients decrease in most populations if *AAP-A* and *PGM-A* are excluded, but are still positive for 18 of the 21 populations. Thus, there is some evidence for weak inbreeding in most of the populations even though at single gene loci the observed heterozygosities are only rarely significantly different from expected heterozygosities. The average inbreeding coefficients are higher for the "mixed" populations than for *Q. petraea* and *Q. robur* populations irrespective of the chosen set of gene loci. However, differences between "pure" and "mixed" populations are small if the set of twelve loci showing low differentiation between the species is taken. On the other hand, the average inbreeding coefficient of "mixed" populations is considerably higher than the average F of both types of "pure" populations, if only loci are considered showing unambiguous differentiation between *Q. petraea* and *Q. robur* populations (three, four, and five loci).

Discussion

Leaf morphology and species status

A principal component analysis based on the observation of six leaf morphological traits allowed to assign the majority of the sampled leaves to one of the two "types" *Q. petraea* or *Q. robur*. Only few intermediate types (average frequency: 3.8%) were observed. These intermediate types may be the leaves of species hybrids, but they may also be within the natural variation range of one or both species. Similiar results, i.e. the possibility to separate *Q. petraea* and *Q. robur* based on simple morphological traits with few intermediate forms, were also obtained in previous studies (e.g. DUPOUEY and BADEAU, 1993). Thus, the observation of leaf morphological traits is suitable to confirm the species status of populations. The overall frequency of sampled *Q. petraea* and *Q. robur* leaves (62.3% and 33.9%, respectively) is comparable to the relative abundance of both species in Switzerland (70% and 30%, respectively; cf. BRASSEL und BRÄNDLI, 1999, loc. cit. p. 133).

Most of the investigated populations are dominated by a single species, but leaves with characteristics of the alternate species were sampled at low frequencies in all populations dominated by *Q. robur* and most populations dominated by *Q. petraea*. Leaves were sampled from the ground after leaf fall. Thus, it cannot be ruled out that leaves from surrounding stands were transported by the wind into the sampled populations. However, sampling was mainly within the closed forest and is unlikely to be strongly disturbed by long-distance wind transport of leaves. Direct observations on trees during the growing season confirmed the occurrence of both species in the majority of Swiss oak forests, although the frequency ratio seems to be highly unbalanced in most stands.

Three "mixed" forests with both species occurring in roughly equal frequencies were sampled. The average frequency of intermediate types is only slightly higher in theses stands as compared to stands dominated by a single species (*Table 2*). Thus, species hybrids which can be identified by the chosen method seem to be rare both in "mixed" stands and stands dominated by a single species. The observation of a comparatively high number of intermediate leaf types in population "Castaneda" possibly reflects the occurrence of *Q. pubescens* s.l. trees in this population rather than a high frequency of hybrids.

Genetic variation within and differentiation among populations

Genetic variation is high within all investigated populations. The average number of alleles per locus (A/L) varies from 2.59 to 3.06, and the average genetic diversity ($H_e = \delta_T$) lies between 0.229 and 0.261. The average levels of genetic variation are very similiar for *Q. robur* and *Q. petraea*. Comparable levels of variation at enzyme gene loci for both species were reported in previous studies on a regional or range-wide scale in spite of different sets of investigated loci (e.g. ZANETTO and KREMER, 1995; HERZOG, 1996). All computed measures characterising the allelic variation in "mixed" populations are slightly higher than the corresponding average values in stands dominated by a single species (*Table 3*). However, this does not hold for the average level of observed heterozygosity, which is highest for populations dominated by *Q. petraea* (*Table 3*). "Mixed" populations do not only show high levels of genetic variation; they also represent the overall genetic structures well as indicated by small genetic distances between the populations and their respective complements (D_j; *Figure 3*).

Allelic differentiation is mainly among species (4.3%) and is very weak among populations within species (1.7%) in Switzerland. ZANETTO and KREMER (1995) investigated seven pairs of populations of *Q. petraea* and *Q. robur*. They found the among populations within species component and the among species component of the total gene diversity in the same order of magnitude (3.1% and 3.3%, respectively). The contrasting results from the two studies may be explained by the locations of sampled populations: ZANETTO and KREMER (1995) investigated populations from a wide geographic range from Scandinavia to Southwest France and Romania. Differentiation among populations within species is likely to increase if populations are sampled from such a wide range.

The comparatively strong differentiation of *Q. robur* populations (*Figure 3*) is an effect of an unbalanced representation of populations from different species in this study and in Swiss forests. Most populations are of the *Q. petraea* type, and the respective complements of all populations are thus also dominated by *Q. petraea*.

Differentiation patterns among populations are not uniform for all loci. Strong differentiation is evident for the loci *ACP-C, IDH-B,* and *PGM-A* (*Tables 4* and *5*). Similiar allelic structures differentiating *Q. robur* and *Q. petraea* were observed at these three loci by HERTEL und DEGEN (1998, 2000) in German oak populations and by ZANETTO and KREMER (1995) in populations from a wide geographic range. Non-overlapping ranges of allele frequencies for *Q. petraea* and *Q. robur* populations at least for a single allele were also observed at the gene loci *GDH-A* and *NDH-A* (*Table 6*). Differentiation among species is low for all other loci (*Table 4*). These contrasting differentiation patterns suggest that evolutionary forces and in particular selection act differently on the investigated enzyme gene loci.

Differentiation among populations within species is very low, but reflects the geographical location of populations to a certain extent. For example, population "Magadino" (pop. no. 6) is most strongly differentiated from all other populations (*Figure 3*) and forms a separate branch of the „*Q. robur* cluster" in *figure 4*. "Magadino" is located in Ticino and the only *Q. robur* population sampled south of the Alps. Similar, "Gordevio" (pop. no. 20), „Cavergno" (pop. no. 19), and "Castaneda" (pop. no. 21) are the three *Q. petraea* populations sampled in Ticino. The allelic differentiation from their respective complements is also above average (*Figure 3*), and they belong to a common main branch of the „*Q. petraea* cluster" in *figure 4*.

Genotypic structures and heterozygosity

Expected heterozygosities are higher than observed heterozygosities in all populations (*Table 3*). Thus, inbreeding coefficients are positive if all gene loci are considered (*Table 7*). However, significant deviations between observed and expected heterozygosities are mainly confined to two gene loci: *AAP-A* and *PGM-A*. Misinterpretation of enzyme phenotypes at these loci is an unlikely cause of the observed heterozygote deficit since both enzymes are monomeric. Thus, homozygotes and heterozygotes are easily identified by the occurrence of one and two bands, respectively. BACILIERI *et al.* (1994) also found positive inbreeding coefficients F for both loci in the *Q. petraea* and the *Q. robur* demes of a "mixed" population. They suspect the occurrence of null-alleles at both loci to be responsible for the positive F-values. Their hypothesis is supported for *AAP-A* by the observation of a single tree of population "Castaneda" showing no activity in the *AAP-A* zone. Strong staining of the sample was observed for all other investigated enzyme systems and for the *AAP-B* zone, which was not interpreted in this study. Electrophoresis of this sample was repeated twice and the duration of the staining was extended, but still no enzyme activity was detected in zone *AAP-A*. Thus, the tree is apparently homozygous for a null-allele at *AAP-A*.

As expected, inbreeding coefficients decrease if *AAP-A* and *PGM-A* are excluded from the computation, but remain slightly positive for most populations (column „15 loci" in *Table 7*). STREIFF *et al.* (1998) observed similiar inbreeding coefficients

for both species at isozyme and microsatellite gene loci. F-values are rather homogeneous across loci if *AAP-A* and *PGM-A* are not considered. Weak inbreeding, possibly mainly by preferential mating of neighbouring, related trees, is a plausible explanation for the slightly positive F-values in most populations (BACILIERI *et al.,* 1994).

Average inbreeding coefficients are higher in "mixed" populations as compared to populations dominated by a single species irrespective of the chosen set of gene loci. These differences are particularly pronounced if only those loci are considered which show differentiation among species. A „Wahlund effect" is the likely cause of the inflated F-values in "mixed" populations. Genotypic structures of the offspring generation will be affected by a „Wahlund effect" only if genetically differentiated populations, which are at least partially isolated from each other, contribute to the next generation (HATTEMER, 1982). It seems reasonable to assume that differentiation patterns, which were repeatedly observed between "pure" populations of *Q. petraea* and *Q. robur*, also exist in "mixed" populations and differentiate a *Q. petraea* deme from a *Q. robur* deme in these populations. This assumption is corroborated by the observation of intermediate frequencies of discriminating alleles in "mixed" populations (*Table 5*). The observed genotypic structures provide strong evidence for partial reproductive isolation of *Q. robur* and *Q. petraea* demes in "mixed" populations based on this premise.

Consequences for seed procurement and the conservation of genetic resources

This study confirms previous findings that sessile oak and pedunculate oak maintain high levels of genetic variation within species. Neither comparatively small population sizes (e.g. populations "Schoren" and "Magden") nor apparent isolation of populations (e.g. populations "Magadino", "Cavergno", "Gordevio", and "Castaneda" in Ticino) resulted in losses of genetic variation. Variation levels are similiar for both species. No recommendations are given concerning the selection of genetic resources or populations for seed procurement based on the moderate differences of genetic variation within populations for the populations dominated by a single species. Inbreeding is low at least at the age stage of adult, reproducing trees, and has only little impact on genotypic structures.

Genetic differentiation is mainly among species. Allelic structures at several gene loci allowed to unambiguously group the populations to either one of the two "pure" species or to "mixed" populations. This observation confirms the genetic basis of the morphological differentiation between sessile and pedunculate oaks although no qualitative differentiation was detected at any locus. The selection of populations for seed procurement and for the conservation of genetic resources should primarily be based on the distinction of two different taxa, which show well-studied morphological and ecological differentiation (e.g. AAS, 1998). Selection of populations should also take into account the weak, but recognisable differentiation among geographical regions within species as exemplified by the differentiation between populations from Ticino and regions north of the Alps for both species.

Several observations point towards "mixed" populations as suitable targets for seed procurement and conservation measures: "Mixed" populations contain high levels of genetic variation within species and they represent the overall genetic constitution of the investigated area well. This may be expected from genetically differentiated species mixtures; results presented here confirm the expectation at the level of genetic structures at single gene loci. An important reason for the exclusion of "mixed" populations from seed procurement and conservation measures has been the putative formation of species hybrids with unknown morphological, ecological, and adaptational properties. However, the genetic structures observed in "mixed" forests clearly indicate at least partial reproductive isolation of *Q. petraea* and *Q. robur* even if they grow in a balanced mixture. The occasional formation of species hybrids may even add to the adaptive potential of progenies from "mixed" populations. Seeds or seedlings from "mixed" populations should be preferably used on sites with spatially alternating microhabitats suitable for either pedunculate or sessile oak e.g. due to small-scale heterogeneity in water supply.

The apparent partial reproductive isolation between *Q. petraea* and *Q. robur* also implies that gene exchange and hybridization among species may be limited in forests dominated by a single species but with trees of the other species occurring in low density. The majority of the investigated populations and probably the majority of oak forests in Switzerland fall into this category. Seeds collected from trees of the dominating species may contain species hybrids in negligible frequencies. However, more detailed studies on pollen flow and the mating system in "pure" and "mixed" oak forests (e.g. BACILIERI *et al.,* 1996) will be needed in order to assess the frequency and importance of possibly asymmetric hybridization between sessile and pedunculate oak.

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Age Trends of Heritabilities and Genotype-by-Environment Interactions for Growth Traits and Wood Density from Clonal Trials of *Eucalyptus grandis* HILL ex MAIDEN

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Abstract

Obtaining accurate and precise genetic parameter estimates is fundamental to determining breeding strategies, and for choosing genotypes for commercial propagation. Results for survival and growth from seven clonal genetic tests of *Eucalyptus grandis* in Colombia supported the a priori contention of sub-dividing them into three different environments for deployment and possibly breeding purposes. The genotype-by-environment interactions (GxE) for growth traits were moderate at six years of age in the target environment (5 sites representative of 95% of the *E. grandis* planting area for the clonal program). Therefore, it is recommended to breed and select for clones that perform well across the range of sites within the target environment. The clonal rankings for growth traits at the two extreme sites differed markedly between these two distinct environments, and between each extreme environment and the five sites in the target environment. Thus, the extreme environments require separate clonal test locations and deployment populations. Broad sense heritabilities for survival, individual tree volume and mean annual increment (MAI) tended to increase over time for the three environments, but the trends for height were quite different among environments. The broad sense heritabilities for mean wood density declined with age, but GxE interaction for wood density was low indicating that clonal rankings were stable among the five sites within the target environment. The estimation of genetic gains by two methods, predicted clonal values and the classical formula, gave similar results and showed great potential for increasing productivity in the target environment through selection of the top clones.

Key words: Eucalyptus grandis, genetic parameters, clonal forestry, genetic gain.

Introduction

In the last twenty years, there has been increasing interest on the part of many *Eucalyptus* breeding programs around the world in developing clonal forestry to enhance both plantation productivity and product uniformity (LAMBETH *et al.,* 1989; DENISON and KIETZKA, 1993; BERTOLUCCI *et al.,* 1995; ARAUJO *et al.,* 1997). In this context, the estimation of basic genetic parameters is crucial in determining the best strategies for clonal breeding and testing and in predicting genetic gains from deploying the best clones (BURDON, 1992; WHITE, 1996).

In the case of *Eucalyptus grandis*, there are few reports in the literature regarding genetic parameters for growth and wood quality traits of clonal material (KAGEYAMA and KIKUTI, 1989; IKEMORI, 1990; LAMBETH *et al.,* 1994). In general, broad sense heritabilities have shown moderate genetic control for growth traits (H^2=0.22 to 0.41) and wood density (H^2=0.30). However, some of these heritability estimates are based on a single genetic test and therefore may be upwardly biased by the presence of genotype-by-environment interaction if the GxE interaction variance is larger than zero (COMSTOCK and

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