PHENOTYPICAL AND GENETIC CHARACTERISTICS OF SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL.) SEEDLINGS AFTER STORAGE OF ACORNS

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ABSTRACT

Artificial stand establishment with sessile oak (*Quercus petraea*) needs sufficient amounts of acorns and nursery plants every year. Therefore, the storage of seeds for periods with low or without seed yield is desired. The objective of our investigation was to obtain information on the influence of storage of acorns over two winters on seedling traits and population genetic structures in comparison to sowings in next spring after harvest.

Acorns from an approved seed stand were collected in autumn 1997. The seeds were sorted according to visible or non-visible radicle and to acorn size.

An early emergence and large seeds led to a maximum height growth. Storage over two winters caused a decreased germination capacity (–46 %), delayed germination (for 6 weeks), decreased height growth of 2-year-old seedlings (–30 %), and an increased percentage of multiple shoots (+71 %) in comparison to normal storage over one winter (100 %).

Population genetic structure of acorns before storage and of seedlings after storage over one and after two winters was analysed by isozyme markers (11 polymorphic loci). An identical selection process occurred during storage and germination, as it was obvious by significant shifts of allele frequencies at the loci ACP-C and MR in all tested subpopulations.

The consequences of sorting and acorn storage over two winters are discussed.

Key words: *Quercus petraea*, acorn storage, seedling development, viability selection, population genetics, isozyme markers

INTRODUCTION

Sessile oak (*Quercus petraea* (Matt.) Liebl.) is a tree species with a broad natural range and high economic importance in forestry. It is adapted to atlantic and sub-atlantic climate in Western and Central Europe.

Besides natural regeneration, artificial stand establishment is usual and necessary in managed forests in many cases. In ancient times, the part of oaks in their habitat decreased in favour of conifers and other rapid growing tree species. Site-adequate silviculture has the aim to promote broad-leaved trees in their natural vegetation type.

Oaks are trees with irregular intervals of fruiting. Hence, there is a strong need for sufficient amounts of acorns and nursery plants every year, the storage of seeds for periods with low or without seed yield is desired.

For sustained forest management and for an effective protection and conservation of forest genetic resources we need knowledge on the level of genetic variation. Especially the genetic processes during reproduction and stand establishment, representing developmental stages under influence of human activity, should be considered. An adequate level of genetic variation is recognized as a prerequisite for adaptability of tree populations to survive different environmental conditions during their long life span.

The genus *Quercus* belongs to temperate forest species with recalcitrant seeds. Acorns lose viability progressively when dried to water content less than 40 %. This problem has generated much interest and a range of storage methods has been developed, also long-term storage. Such studies were carried out e.g. by KRAHL-URBAN (1952), B. SUSZKA & TYLKOWSKI (1980) and GUTHKE (1992). More actual experiments were focussed on optimising temperature and humidity regimes (SCHLEGEL & SPETHMANN 1999, SCHRÖDER *et al.* 1999a, J. SUSZKA 1999a, LEPICHON & GUIBERT 2001) or on phytopathological aspects (WERRES *et al.* 1992, GILLE 1999, PROCHAZKOVA & SIKOROVA 1999, SCHRÖDER *et al.*

1999b, FINCH-SAVAGE *et al.* 2003). Sometimes the germination capacity was included into the research projects. However, in almost all cases, the observation of seedling development and their growth was not regarded.

The objective of our investigation was to obtain information on the influence of long-term storage of acorns on seedling traits in comparison to sowings in next spring after the harvest. Furthermore, different classifications of acorns should be tested for their suitability for long-term storage. The reduced germination power of acorns after storage could be caused by random losses and/or by selection processes. Thus, population genetic analyses by isozyme markers were carried out in acorn samples as well as in seedlings to check genetic consequences of storage and sorting.

MATERIAL AND METHODS

Acorns from an approved seed stand (Müllrose, Germany) were collected in an early and a late batch in 1997 (Batch 1: Oct 27, mean weight per 100 acorns 297 g, Batch 2: Nov 11, mean weight per 100 acorns 445 g). The early seed lot was sorted according to visible (>0.5mm) or non-visible radicle (64 % and 36 % resp.) and the second seed lot was sorted according to acorn size (small 44 % and large 56 %). Only the late seed batch was used for storage over two winters. Seeds were stored in dagged plastic boxes covered by moistened cotton fabric under controlled conditions of cold-air drying.

The occurrence of pathogenic fungi and insects in acorns was recorded visually at each sample immediately after the harvest. Damaged acorns with exit holes from insects or blackening by fungi were rejected before storage. The acorns were re-assessed before sowing in spring 1998 and 1999. Fungal pathogens were observed by cutting of random samples of acorns at one and two thirds of its length. Discoloured cotyledons or embryonic axes were disposed on 2 % malt agar to determine pathogenic fungi. Furthermore, the proportion of living or dead embryonic axes was recorded.

Acorn storage was started with a temperature of +1°C. In the first 2 to 4 weeks the temperature was decreased slowly to – 4°C for the late and early batch respectively. During this hardening period, the water content of acorns decreased from 48 % to 45 %. In the course of storage the water content was checked and adjusted for about 40 % to 42 % by spraying water on the surface of the seeds and on the cotton fabric in intervals of several weeks. The frost period of storage was finished 10 days before planting and the seeds were adapted to air temperatures of 5 to 8°C for outdoor sowings at March 31st 1998 and for outdoor sowings after storage in April 22nd 1999. The temperatures were comparable for the periods of sowing to emergence of the plants in both years. The mean air temperature at 20 cm above soil ranged between 13 and 16°C in 1998 and between 11 and 16°C in 1999 for this period. The seedling emergence (recorded every week starting from the first week after sowings), the height growth and root collar diameter for seedlings from each seed lot were recorded individually for every vegetation period. An overview on the treatments and sample sizes for each subpopulation is given in Table 1.

Population genetic structure of acorns before

Table 1. Overview on sorting variants and sample sizes for assessing genotypes, seedling growth traits and acorn infection.

Batch	Sorting variant	Sowings	Mean weight per 100 acorns	Number of sowed acorns	Sample size Genotype	Sample size Seedling growth	Sample size Acorn infection
1	Visible radicle	No	319 g		62		total sample
1	Closed acorn	No	265 g		62		total sample
2	Small	No	323 g		60		total sample
2	Large	No	634 g		60		total sample
1	Visible radicle	1998		365	120	140	40
1	Closed acorn	1998		222	80	92	40
2	Small	1998		538	120	175	40
2	Large	1998		325	120	150	40
2	Visible radicle	1999		1000	80	318	100
2	Closed acorn	1999		1000	80	203	100
2	Small	1999		1000	80	205	100
2	Large	1999		1000	80	142	100

storage and of seedlings was analysed by isozyme markers with tissue of radicle of acorns or dormant buds of young plants. One-year-old seedlings were used from the sowings in 1998 and two-year-old plants from the sowings in 1999 because of the delayed development after storage over two winters. The electrophoretic separations of enzyme proteins in starch and polyacrylamide gels, staining solutions and the interpretation of banding patterns for 11 polymorphic loci (Aspartate aminotransferase *Aat-B*, Acid phosphatase *Acp-C*, Aminopeptidase *Ap-B*, Isocitric dehydrogenase *Idh-B*, Malate dehydrogenase *Mdh-A*, Menadione reductase *Mr, Nadh* dehydrogenase *Ndh-A*, 6-Phosphogluconic dehydroenase *Pgdh*, Phosphoglucose isomerase *Pgi-B*, Phophoglucomutase *Pgm-A*, Shikimate dehydroenase *Skdh*) were carried out according to standard methods (MÜLLER-STARCK *et al.* 1996, HERTEL & ZASPEL 1996).

All calculations and statistic tests were carried out with the SAS statistic package. Wilcoxon rank test was used for the comparison of quantitative traits between the groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Exact Fisher-Test was used for the comparison of frequency distributions (germination capacity, multiple shoots, allele frequency distribution, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Population genetic parameters were calculated with the help of special SAS macros (STAUBER & HERTEL 1997).

RESULTS

Infection of acorns

The infection of acorns with *Sclerotinia pseudotuberosa* (anamorph: *Ciboria batschiana)*, the causal agent of black rot disease, was very low in autumn 1997 and attained less than 2 % in each sample. The infection increased during the storage period, but only the sample of large acorns showed a serious loss of 30 % by this pathogen in spring 1998. The infection rate of the small seeds amounted to 11 %. In the course of storage over the second winter, the part of mummified acorns increased and ranged between 17 and 42 % in spring 1999. Additionally, about 5 % of seeds stored over this long period were infected by *Cylindrocarpon didymum*.

The part of visible insect damages caused by the bright marble tortrix (*Cydia splendana*) and the acorn weevil (*Balaninus glandium*) was very low at all samples before seeds were stored (2 to 4 %). The losses increased to 21 % in spring 1999.

An accumulation of seeds with dead embryonic axes was observed at all samples when they were concurrently affected by several pathogens. In some cases, the acorns were infected but the germination was possible due to one unaffected cotyledon and a healthy embryonic axis.

Seedling traits

In general, the height and diameter growth in the first and second year of seedling development are positively correlated for all seed lots (batch 1 – sowings 1998, batch 2 – sowings 1998, batch 2 – sowings 1999, six correlation coefficients $r = +0.37 \ldots +0.72$).

For sowings in first spring after harvest an early emergence led to more growth in the first year (two correlation coefficients $r = -0.24, -0.50$). However, the influence of the acorn size seems to be more evident. Seedlings derived from batch 2 with a 100-acorn weight of 445 g and an earlier emergence are higher and have a more stretched habit than seedlings from batch 1 with a 100-acorn weight of 297 g. Within batch 2, seedlings developed from large acorns reached a height growth of about 50 % above the height of seedlings from small acorns. The percentage of multiple shoots differed between the early and late seed batch and was higher in the seedling sample derived from closed acorns than from acorns with visible radicle (Table 2).

Sowings in second spring after harvest delayed the seedling emergence for 6 weeks and reduced the total germination capacity to the half (Table 3). Above all, the germination capacity of the large acorn sample was reduced, associated with a late emergence. Nevertheless, the seedlings developing from the large acorn sample showed a better height and diameter growth in comparison to the other variants sorted. Storage of acorns of batch 2 over two winters led to a more compact and short habit of seedlings. The percentage of multiple shoots increased due to storage of acorns for 71 % (Table 4).

Population genetic characteristics of samples

First the total material was divided into five samples considering the acorn and seedlings state and the duration of storage (acorns-batch1, acorns-batch2, seedlings98-batch1, seedlings98-batch2, seedlings99-batch2). Mean values of genetic parameters over 11 polymorphic loci did not differ significantly between these five groups, but a trend for higher genetic variation in seedling populations in contrast to acorn samples was observed (Table 5). Comparing the single locus observed heterozygosities, the heterozygosities of seedlings was significantly higher at the loci *Mr* and *Pgi-B* for both seed lots and at the locus *Skdh* for the late seed lot. At locus *Acp-C*

Table 2. Seedling traits for sowings in first spring after harvest (mean values and standard deviations, sample sizes: all available seedlings, see Table 1).

	Batch 1			Batch 2			Statistics Comparison of rows		
	Total	Visible radicle	Closed acorns	Total	Small acorns	Large acorns			
	1	2	3	4	5	6	1–4	2–3	5–6
Emergence (weeks after sowings)	6.9±2.5	5.9±2.4	8.4±1.9	3.9±2.3	4.5±2.2	3.2±2.1	***	***	***
Germination capacity (%)	42.1	40.8	44.1	40.4	36.4	46.8	n. s.	n. s.	**
Height 1-year-old (cm)	6.7±3.2	7.2±3.2	6.0±3.0	11.2±5.7	9.2±4.1	13.5±6.4	***	**	***
Height 2-year-old (cm)	16.6±7.4	17.1±7.1	15.9±7.7	25.8±13.	20.5±9.5	31.8±13.	***	n. s.	***
Root collar diameter 1 (mm)	3.5±1.0	3.6±0.9	3.3±1.0	0	3.9±1.0	8	***	*	***
Root collar diameter 2 (mm)	4.8±1.4	5.1±1.4	4.4±1.4	4.2±1.1	5.4±1.5	4.6±1.1	***	***	***
Height-Diameter-Ratio 1	19.3±6.2	19.8±6.1	18.5±6.3	5.8±1.7	23.6±7.5	6.3±1.7	***	n. s.	***
Height-Diameter-Ratio 2	34.6±10.	33.7±9.5	35.8±11.	26.0±9.1	39.0±23.	28.8±10.	***	n. s.	***
Seedlings with multiple shoots (%)	5 20.0	14.1	7 28.6	44.3±20. 9 13.1	5 12.0	1 50.3±15. 4 14.2	*	*	n. s.

Table 3.Seedling traits for sowing in second spring after harvest (mean values and standard deviations, sample sizes: all available seedlings, see Table 1).

	Batch 2					Statistics Comparison of rows	
	Total	Visible radicle	Closed acorns	Small acorns	Large acorns		
	1	2	3	4	5	2–3	4–5

Table 4. Consequences of acorn storage over two winters on seedling development (batch 2, compared with sowings 1998 = 100 %).

	Total		Small acorns		Large acorns	
Emergence (weeks after sowings)	+6.1 weeks	***	+5.9 weeks	***	+8.2 weeks	***
Germination capacity (%)	–46 %	***	–43 %	***	–69 %	***
Height 1-year-old (cm)	–22 %	***	–12 %	*	–38 %	***
Height 2-year-old (cm)	–30 %	***	–15 %	***	–26 %	***
Root collar diameter 1 (mm)	–21 %	***	–19 %	***	–30 %	***
Root collar diameter 2 (mm)	+37 %	***	+46 %	***	+41 %	***
Height-Diameter-Ratio 1	+4 %	n. s.	+13 %	*	–8 %	*
Height-Diameter-Ratio 2	–48 %	***	–43 %	***	–48 %	***
Seedlings with multiple shoots (%)	+71 %	***	+112 %	**	+108 %	**

Table 5. Mean values of population genetic parameters.

	Acorns-batch1	Acorns-batch2	Seedlings98-batch1	Seedlings98-batch2	Seedlings99-batch2
Sample size	124	160	200	240	320
Alleles/Locus	3.818	3.909	3.909	4.000	4.091
Genpool-Diversity	1.327	1.313	1.350	1.364	1.347
Multilocus-Diversity	35.4	33.3	40.3	44.7	37.3
Observed heterozygosity H_o	0.208	0.216	0.230	0.237	0.233
Expected heterozygosity H_e	0.247	0.238	0.259	0.267	0.258

Table 6. Single locus observed heterozygosities (Stat.: Comparison between acorns and seedlings).

Marker locus	Stat.	Acorns-batch1	Acorns-batch2	Seedlings98-batch1	Seedlings98-batch2	Seedlings99-batch2
Aat-B	n. s.	0.124	0.102	0.108	0.105	0.101
Acp-C	**	0.496	0.516	0.412	0.318	0.362
Ap-B	n. s.	0.541	0.579	0.569	0.555	0.555
Idh-B	n. s.	0.268	0.310	0.246	0.253	0.262
Mdh-A	n. s.	0.016	0.000	0.032	0.042	0.018
Mr	***	0.090	0.149	0.275	0.285	0.263
Ndh-A	n. s.	0.128	0.053	0.171	0.083	0.095
Pgm-A	n. s.	0.240	0.250	0.181	0.281	0.190
PgdH	n. s.	0.063	0.143	0.096	0.142	0.159
Pgi-B	*	0.218	0.231	0.362	0.317	0.288
Skdh	***	0.107	0.044	0.080	0.223	0.276

the heterozygosities were higher in acorns than in seedlings (Table 6).

The dendrogram in Figure 1 clearly shows two separated groups: The acorn batches on the one hand and seedlings developing from these acorn batches on the other hand. This is obvious despite the significantly different allele frequency distribution at the loci *Mr* and *Ndh-A* between the early and the late acorn batch (Fisher's exact test, *Mr**** and *Ndh-A**).

The sorting within each acorn batch led to further differences in allele frequency distributions (batch 1 – visible radicle resp. closed acorns: *Ap-B* **, batch 2 – small resp. large acorns: *Mr* * and *Pgdh* *). Nevertheless, the separation into a group of acorn samples and a group of seedling samples is nearly the same (Figure 2).

Selection processes

The genetic structure of both acorn batches was analysed immediately after harvest in autumn 1997 to characterise the initial state. The average germination capacity amounted to 41 % in spring 1998.

Table 7. Pairwise comparisons of allele frequency distributions of acorns vs. seedlings from each acorn batch, sorting variant and sowing year (Fisher' s Exact Test for 10 loci, Monte Carlo Estimation of p-values for *Ap-B*).

Batch	Sorting variant	Sowing year	*Aat-B*	*Acp-C*	*Ap-B*	*Idh-B*	*Mdh-A*	*Mr*	*Ndh-A*	*Pgm-A*	*Pgdh*	*Pgi-B*	*Skdh*
1	Visible radicle	1998	n.s.	***	*	n. s.	n. s.	***	n. s.	n. s.	n. s.	n. s.	n. s.
1	Closed acorn	1998	n.s.	***	*	n. s.	n. s.	**	n. s.	n. s.	n. s.	**	n. s.
2	Small	1998	n.s.	***	n. s.	n. s.	(n. s.)	*	n.s.	n. s.	**	n. s.	***
2	Small	1999	n.s.	***	n. s.	n. s.	n. s.	*	n. s.	n. s.	***	n. s.	***
2	Small	§	n.s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	*	n. s.	n. s.	n. s.
2	Large	1998	n.s.	***	n. s.	*	n. s.	***	n. s.	*	n. s.	n. s.	(n. s.)
2	Large	1999	n.s.	***	*	n. s.	n. s.	**	n. s.	n. s.	n. s.	n. s.	(n. s.)
2	Large	§	n.s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	(n. s.)	n. s.	n. s.	n. s.

§ seedlings 1998 vs. seedlings 1999

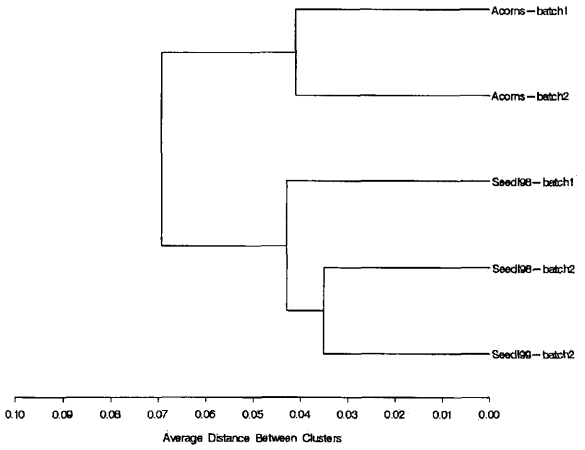

Figure 1. Dendrogram based on genetic distances and UPGMA cluster analysis (11 loci).

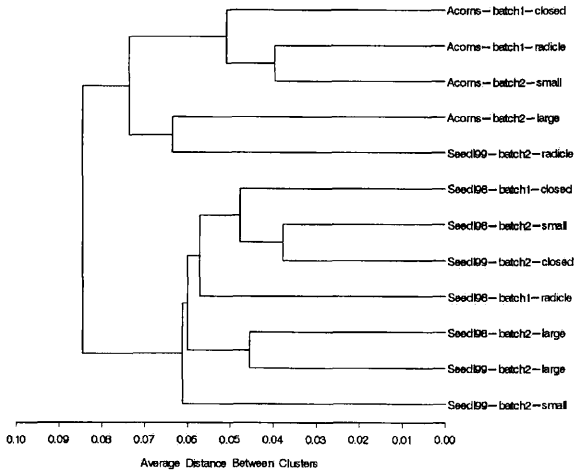

Figure 2. Dendrogram based on genetic distances and UPGMA cluster analysis (11 loci) after sorting according to morphological traits of acorns.

After one additional year of acorn storage it was reduced to 22 %. That means a strong reduction of population size during the acorns storage and emergence. Starting from all emerged seedlings, approximately 90 % survived to two-year-old plants for both sowing years. Thus, the comparison between genetic structures of acorns and seedlings more reflects the processes during storage and emergence, and it is little influenced by seedling survival.

Table 7 gives the results of pairwise comparisons of allele frequency distributions of acorns vs. seedlings from each acorn batch, sorting variant and sowing year and of seedlings 1998 vs. seedlings 1999. Significant differences for single comparisons were detected at many marker loci. However, at the loci *Acp-C* and *Mr* differences occur for all sorting variants and sowing years. Moreover, analogue shifts in favour of allele A_2 at the *Mr* locus and in favour of allele C_1 at the *Acp-C* locus were ascertained (Table 8). Additional directed shifts were observed for batch 2. The relative frequency of the rare allele A_2 at locus *Skdh* was increased at the expense of the common allele A_3 for both sowing years (significantly for small acorns, as a trend for large acorns). The relative frequency allele of $_{A2}$ at locus *Pgdh* was increased at the expense of allele A_3 for both sowing years as well, but only for the sample of small acorns. All other significant differences should rather be regarded as random effects because there is no replication on the direction of allele frequency shift.

Table 8. Shift of relative allele frequencies at marker loci Acp-C and Mr from acorns to seedlings.

Batch	Sorting variant	Sample	Marker locus *Acp-C*				Marker locus *Mr*					
			C_1	C_2	C_3	C_4	A_1	A_2	A_3	A_4	A_5	A_6
1	Visible radicle	Acorns	0.364	0	0.593	0.042	0	0.795	0.148	0.008	0.049	0
1	Visible radicle	Seedlings 98	0.632	0.011	0.352	0.006	0.013	0.788	0.058	0	0.142	0
1	Closed acorn	Acorns	0.395	0	0.565	0.040	0	0.730	0.172	0	0.098	0
1	Closed acorn	Seedlings 98	0.725	0	0.275	0	0.006	0.819	0.056	0	0.119	0
2	Small	Acorns	0.383	0.017	0.558	0.042	0	0.781	0.097	0.009	0.097	0.018
2	Small	Seedlings 98	0.719	0	0.274	0.007	0	0.832	0.050	0	0.118	0
2	Small	Seedlings 98	0.675	0.024	0.302	0	0	0.869	0.025	0	0.106	0
2	Large	Acorns	0.525	0	0.425	0.050	0	0.912	0.044	0.018	0.026	0
2	Large	Seedlings 98	0.797	0.025	0.170	0.009	0.008	0.771	0.058	0	0.163	0
2	Large	Seedlings 99	0.786	0	0.214	0	0	0.788	0.088	0.006	0.119	0

DISCUSSION

Long-term storage of acorns is possible, as it was described e.g. by SCHMALEN & HERGET (1999). GILLE (1999), SCHLEGEL & SPETHMANN (1999), B. SUSZKA (1999), J. SUSZKA (1999b) and GUTHKE (1992) observed a decreased germination capacity of stored acorns due to pathogens and frost damage in more detailed studies, but they did not include seedling growth parameters in their experiments.

The observed infections with fungal diseases and damages by insects after the long-term storage of acorns in our experiments conformed to the expectations. In addition, storage over two winters caused a decreased germination capacity (–46 %), delayed germination period (for 6 weeks), decreased height growth of two-year-old seedlings (–30 %), but an increased root collar diameter (+37 %) and an increased percentage of multiple shoots (+71 %) in comparison to normal storage over one winter (100 %). These results demonstrated a remarkable loss, not only in plant quantity, but also in quality by long-term storage of acorns. After sorting according to acorn size, the viability of large acorns was more depressed by long-term storage than the storage life of other sorting variants.

The positive correlation between acorn size and seedling height growth has been well known for a long time as reviewed by AAS (1998). Similar effects were found for *Q. ilex* (GOMEZ 2004) and *for Q. leucotrichophora* and *Q. semecarpifolia* (PUROHIT *et al.* 2003). This relationship was confirmed in our studies for the sowing in first spring after harvest, but not for the sowing after storage over two winters. Thus, the decrease in seedling height growth after storage over two winters amounted to 38 % in the sample of large acorns in contrast to a decrease of 12 % in the sample of small acorns. This might be caused by pathogens as well as physiological reasons, because seed maturation is enforced at a low level. The high water content of recalcitrant seeds like acorns promote the fungal proliferation, even in cold storage (KEHR & SCHRÖDER 1997). JUNGE *et al.* (1999) did not find significant differences in frost tolerance between size classes of acorns measured by Differential-Thermal-Analysis. However, a germination test and observation of seedling development was not carried out. GUTHKE & SPETHMANN (1993) showed a gradual reduction of germination capacity and of shoot and root dry matter after prolonged acorn storage.

The increased appearance of more than one shoot after long-term storage indicates a sensitive response of the embryo axis. Minor shifts of water content and changes in carbohydrate concentrations could damage the apical meristem irreversibly (GUTHKE 1992). Regeneration, which was probably caused by lateral meristems, is possible although the shoots were smaller.

For practical purposes, it is necessary to balance between the advantages of continuous supply of nursery plants respective the continuous demands and the obvious disadvantages of lower quantity and quality of seedlings and higher costs for storage. Long-term storage of acorns would be just even justifiable (SCHMALEN & HERGET 1999) or not to be recommended at present (D. SCHNECK 1999). The disadvantages seem to be predominating under actual conditions, even though no genetic differences were found between the seedlings of both sowing years.

The results strongly indicate that both acorn batches are not representative random samples of seeds from the respective seed stand. The different mean weights per 100 acorns of the early and the late seed batch (297 g vs. 445 g) and the significant

different allele frequency distribution at two of eleven marker loci could be explained by the contribution of different mother trees with early and later ripening acorns to the seed batches.

A former experiment with single tree seed collections of sessile oaks showed that acorns of a single mother tree are very uniform in shape, colour and size, whereas acorns from different trees may be very different. The mean weight per 100 acorns from 135 mother trees ranged between 116 g and 632 g and varied by the factor 5.5 (KEßLER 1994). The acorn size strongly influenced the seedling height growth in the first year. The correlation coefficient amounted to $r = +0.63$ ($N = 135$, ZASPEL & KEßLER 1997). The sorting of acorns according to phenotypic characteristics (visible radicle vs. closed acorns, small and large acorns) probably led to a further demixing within both seed batches. The contributions of genetic different mother trees resulted in significant differences for the allele frequency distributions at several loci. The remarkable differences between the sorting variants in emergency and seedling growth within the first two years could be explained by this cause.

Similar to our experiments, the selection for growth rate in an early stage of development (Engelmann spruce: MITTON & JEFFERS 1989) or the sizing of nursery plants (Norway spruce and silver fir: KONNERT & SCHMIDT 1996, beech: KONNERT & RUETZ 2003) was accompanied by significant differences in allele frequencies at several isozyme loci. The variation in growth does not have to be an effect of certain isozyme structures, allelic variants or their linkage groups. It could be a joint dependence from a third factor, namely the mother tree with its special genotype. In general, we conclude that genetic studies with nuclear markers from material from provenance trials, progeny tests or nursery plants should be interpreted very carefully if the number of harvested mothers is low or unknown.

Reduction of population sizes and viability selection processes are known to be accompanied by shifts in genetic structures. Often different genotypes were in advantage under different environmental conditions as described e.g. for beech (K.S. KIM 1985, MÜLLER-STARCK 1993, STARKE *et al.* 1996), for Norway spruce (BERGMANN & HOSIUS 1996), and for Scots pine (HERTEL & V. SCHNECK 1999).

In the present study, identical shifts in allele frequency distributions at the loci *Acp-C* and *Mr* occurred in all sorting variants during the reduction of population size in this early state of development. This happens despite the differences between the sorting variants, which are probably caused by genetic different mother trees. Therefore, it is rather a matter of rectified selection processes, not essentially at the locus itself, but within the linkage group.

The experiments of T.S. KIM (1999) with seed lots from three sessile oak populations resulted in similar changes in allele frequencies for the locus *Mr* from acorns to 1-year-old seedlings in nursery and greenhouse cultivation. The locus *Acp-C* was not analysed. The mean value of heterozygosity and genetic diversity as average of 7 loci decreased in his study, whereas the mean heterozygosity and gene pool diversity based on 11 loci increased in our investigation during the reduction of population size. The very low number of marker loci in both experiments implies rather random effects than reasonable estimations of mean values.

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