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An Attempt to Infer on the Origin of a Beech (*Fagus sylvatica* L.) Stand in Rheinland-Pfalz (Germany)

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Summary

Information on whether a stand represents a part of an autochthonous population is important for control of the seed trade and for the declaration of gene resources.

The genetic structure of a planted beech stand of questionable origin with an outstanding high proportion of forked trees, was compared with those of stands of the same region in western Germany that were either known or supposed to be autochthonous. Genetic structures were estimated for 11 enzyme gene loci. Comparisons were performed by determining genetic distances and applying measures of genetic differentiation. The degree of differentiation of beech populations at enzyme gene loci is generally low, which causes additional difficulties. Nevertheless, it may be concluded that the plants used to establish the stand in question could hardly have been raised from seed collected from a stand in the alleged part of the natural distribution range.

Key words: Faglls sylvatica L., enzyme gene loci, regional genetic differentiation, planted and autochthonous stands, seed trade.

FDC: 165.3; 165.5; 176.1 *Fagus sylvatica*; (430).

Zusammenfassung

Die Kenntnis der Zugehörigkeit eines Bestandes zu einer autochthonen Population ist sowohl für die Kontrolle des Vertriebs von Vermehrungsgut als auch die Ausweisung von Genressourcen bedeutsam.

Die genetische Struktur eines Buchenpflanzbestandes fraglichen Ursprungs mit außergewöhnlichem hohen Anteil

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mehrfacher Zwiesel wurde mit der Struktur anderer Bestände der gleichen Gegend in Westdeutschland verglichen. Die Strukturen wurden an 11 Enzymgenloci erhoben. Vergleiche geschahen durch genetische Abstände und Maße der genetischen Differenzierung. Der Grad der genetischen Differenzierung von Buchenbeständen an Enzymgenloci ist im allgemeinen gering, was zusätzliche Schwierigkeiten bedingt. Trotzdem legten die Ergebnisse nahe, daß das für die Begründung des fraglichen Bestandes verwendete Pflanzgut schwerlich aus Saatgut angezogen worden sein dürfte, welches in dem vom Lieferanten angegebenen Teil des natürlichen Verbreitungsgebiets geerntet wurde.

1. Introduction

Autochthonous tree populations have long been given importance in forestry, since their stands are considered to be well-adapted to the environmental conditions in their habitat. The expectation of a high degree of local adaptedness is valuable in making decisions on the approval of stands for seed collection and on the declaration of genetic resources. A population may, of course, be expected to be locally adapted only if the environment has essentially remained unchanged during recent generations. Moreover, adaptedness to past conditions does not necessarily lead to adaptedness to a new factor which has never before occurred during evolution. In almost all of a number of beech populations, MÜLLER-STARCK (1993) studied genetically controlled variation for tolerance to air pollution and presented evidence for the initiation of adaptational processes that could be traced at several enzyme gene loci.

The degree of differentiation of predominantly cross-fertilising plant populations at enzyme gene loci is expected to be low due to similar effects of past environmental selection pressure in various parts of the distribution range of a species (GREGORIUS and BERGMANN, 1995) on the one hand and efficient gene flow between populations on the other hand (MÜLLER-STARCK, 1996), Therefore, it is diff1cult to classify a stand as having been derived from a given natural population and to locate the presumable origin of planted stands of unknown origin on the basis of enzyme gene markers. Only in rare cases of isolated populations occupying extreme sites or of strong differentiation between races can it be possible to assign stands of unknown origin to one of several races (BERGMANN, 1984; LEINEMANN, 1996). Statements are even more difficult in situations where data on the parental material is not available, If questionable material is actually derived from only a small number of seed parents, ADAMS (1983) calls the determination of its region of origin "a hopeless task". Besides genetic drift, the local conditions of fertility selection, mating system, and of gene flow by pollen influx from differentiated donor populations (MÜLLER-STARCK, 1996) shape the genetic structures of seed stands and their progeny stands and may give them unique characteristics. The decision whether questionable material (seed or a derived stand) can be assigned to an autochthonous population is generally made by formulating a detailed working hypothesis and then testing it according to the exclusion principle on the basis of the observations. Prior knowledge about the respective tree species helps both in formulating and testing such hypotheses.

Further aspects can be taken into account which serve the decision whether a given population is autochthonous or otherwise appropriate for seed procurement. As early as in 1935, LANGER searched in older records for information on the descent of planted larch stands in Saxony, He could derive plausible conclusions on the historical practice of larch seed procurement and was able to set up guidelines for the choice of appropriate regions of origin, thus demonstrating the efficiency of historical studies in preventing silvicultural failures.

It is nearly impossible to review all situations and procedures of "identification" (BERGMANN, 1995). In general, GREGORIUS *et al*. (1984) advocated conservative statements following the exclusion principle. These authors concentrated on the reconstruction of descent of a population by comparing it with its alleged parent populations.

Phenotypically, the stand which is the object of this study is very different from any other beech stand in the region and thus attracted attention. Because multiple forking is to be observed in almost all individuals, it will hardly yield any timber whatsoever. The morphological homogeneity of the stand and the sharp contrast with the phenotypes of the adjacent stands make it likely that its particular phenotype is genetically conditioned. This has also been indicated by the studies of BURGER (1948), KRAHL-URBAN (1952, 1962), and DUPRÉ *et al*. (1986). The supplier of the seed used for raising the planting stock stated that it was collected from a stand in the same region. The present paper focusses on the question of whether this information may be trusted.

2. Genetic Variation in Beech

According to the recent review by PAULE (1995), beech shows little regional differentiation in comparison to conifers. Also in the present study region in southwestern Germany, beech populations are only slightly differentiated at 11 enzyme gene loci according to STARKE *et al*. (1995); some of the detected minor differences at these nuclear loci correspond to thedistribution of two morphs of cpDNA (VORNAM and HERZOG, 1996).

In beech, low differentiation is not the result of low variability. Beech populations are more variable at enzyme gene loci than many other tree species (MÜLLER-STARCK *et al.,* 1992). Most of the variation still resides within populations. Applying the measures D and o of genetic differentiation (GREGORIUS and ROBERDS, 1986 GREGORIUS, 1988) to all populations and to several groupings of populations, TUROK (1995) was unable to attribute the differentiation among 50 western German populations to regions of provenance defined by legal regulations (ANONYMUS, 1966, 1994) or to ecological conditions such as elevation or mean temperature. At least in all cases of major polymorphisms, average differentiation δ between populations was substantially less than the total differentiation δ_T between trees of the same population.

Similar findings were reported by KONNERT (1995) in a study of enzyme gene loci of beech populations in southeastern Germany.

3. Material

A beech stand in the forest district of Otterberg (No. 23 in *Fig.1*), III (Birkenköpfe) 1 d^2 (Lichtenbruch), was planted in 1971 and was 21 years of age when sampled in 1992, Buds were collected from 101 trees approximately equally distributed over various parts of that stand.

A total of 22 adult stands between 90 and 160 years of age were used for comparison; the locations of the stands are shown in *figure 1*. These stands had been chosen as potential gene resources (TABEL and MAURER, 1992) and therefore been the subject of a genetic inventory. Buds were collected from 200 trees per stand that were chosen as those nearest to the points of a regular grid system (TABEL and MAURER, 1992).

The 22 reference stands can be regarded as autochthonous with the following exceptions: Stands No. 17 (Neuerburg) and

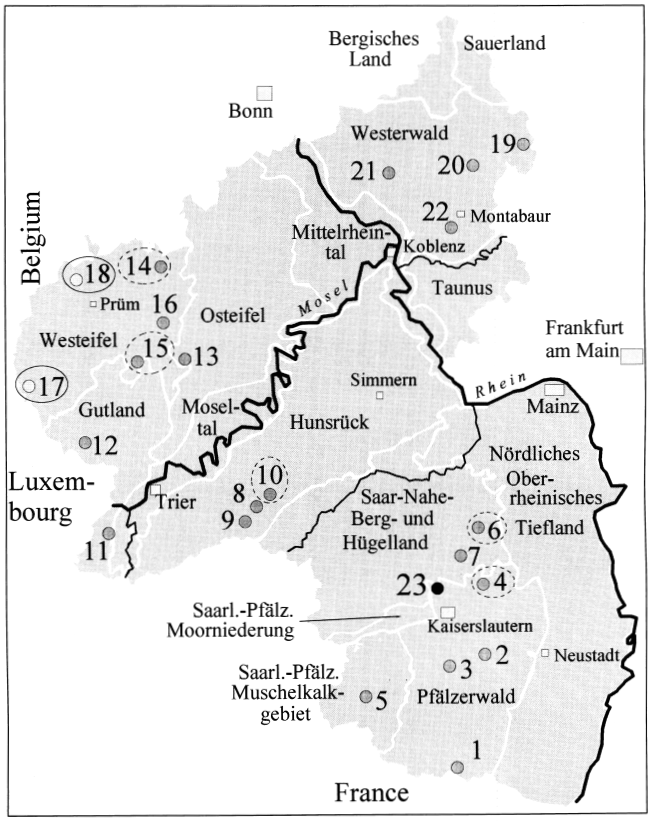

Figure 1. – Location of the 22 adult stands according to STARKE *et al.* (1995) and stand No. 23 of doubtful origin in the forest district of Otterberg. Artificial stands are encircled with solid lines; stands encircled with a broken line are not safely confirmed to be autochthonous. The forests of Rheinland-Pfalz have been subdivided into growing districts on the basis of site conditions. These districts are also indicated in the map.

18 (Schneifel) are artificial; according to information on file with the forest administration, the latter was sown. Furthermore, it could not be determined with certainty that stands No. 4 (Hochspeyer), 6 (Kirchheimbolanden), 10 (Morbach), 14 (Hillesheim), and 15 (Bitburg) are autochthonous (TUROK, 1995). The other stands not only originated from natural regeneration. Moreover it is highly likely that they represent the local populations that re-immigrated after the most recent glaciation.

4. Methods

Isoenzyme analysis

Nine enzyme systems were analysed for isoenzyme variation, The zymograms were interpreted according to MÜLLER-STARCK and STARKE (1993) and 11 gene loci (see *Table 1*) were scored for the present purpose. The same gene loci had previously been employed in the beech study by STARKE et al. (1995) which we use for comparison.

The locus MDH-A turned out to be almost fixed. Information about genotypes at gene loci 6PGDH-B and -C were excluded in this study, because the zones controlled by these 2 gene loci overlap, and 1 allozyme of 6PGDH-B and 1 of 6PGDH-C migrate to the same position in the gel (HATTEMER et al. 1994). Therefore these 2 gene loci do not seem appropriate for genetic inventories in spite of the unambiguous results on Mendelian segregation presented by MÜLLER-STARCK and STARKE (1993). The variability ofthe other enzyme systems also makes appropriate inheritance analysis indispensable.

Table 1. – Surveyed enzyme systems and gene loci scored.

	Enzyme system	E.C.Ref No.	Gene loci
1	glutamate oxalacetate transaminase	2.6.1.1	*GOT-B*
2	isocitrate dehydrogenase	1.1.1.42	*IDH-A*
3	leucine aminopeptidase	3.4.11.1	*LAP-A*
4	malate dehydrogenase	1.1.1.37	*MDH-A, -B, -C*
5	menadione reductase	1.6.2.2	*MNR-A*
6	6-phosphogluconate dehydrogenase	1.1.1.44	*6-PGHD-A (-B, -C)*
7	phosphoglucose isomerase	5.3.1.9	*PGI-B*
8	phosphoglucomutase	2.7.5.1	*PGM-A*
9	shikimate dehydrogenase	1.1.1.25	*SKDH-A*

Data analysis

Population genetic analyses were performed by using the GSED software (GILLET, 1994), Gene pool diversity was determined as described in GREGORIUS (1987), Genetic differentiation was computed according to GREGORIUS and ROBERDS (1986). For graphic representation, their "snail" diagram was applied. The radii of the 23 specific sectors are proportional to that portion D_j of the alleles not shared by population sample No. j and its complement consisting of all other 22 (equally weighted) populations. The sectors are arranged in decreasing order of D_j so that the larger radii represent the more strongly differentiated populations. The smaller radii of the innermost circles refer to less differentiated populations, the genetic information of which is more representative of the whole set. The broken circle indicates the (equally weighted) average o of the 23 values of Dj. Dendrograms were based on genetic distances according to GREGORIUS (1974) and constructed by the UPGMA method.

5. Results

Gene Pool differences

Considering the gene pool of all 11 gene loci in all 23 stands, the average genetic differentiation δ between the samples amounts to δ=0.029. The inclusion of minor polymorphisms, as for example MDH-A which is fixed in 21 of the 23 samples, lowers the gene pool differentiation and eventually explains the relatively small amount of δ.

As illustrated in *figure 2*, some stands differ from their respective complements more clearly than others. The strongest deviation is observed for the artificial stand No. 18

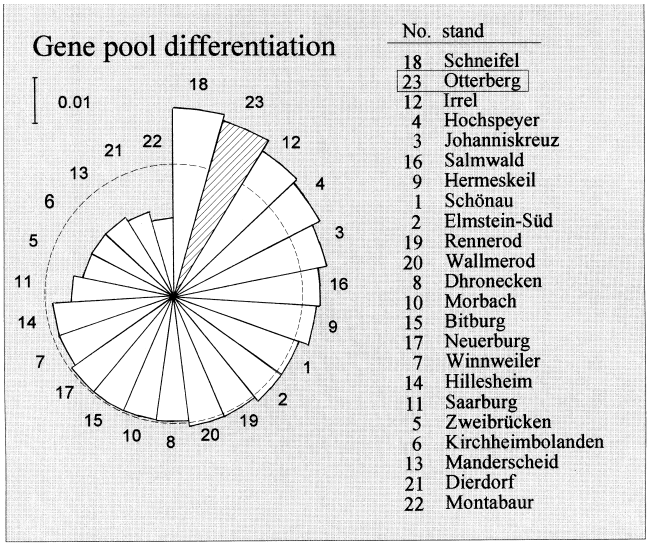

Figure 2. – Measures D_j of genic differentiation at 11 enzyme gene loci of the 22 reference stands and stand No. 23 of doubtful origin.

(Schneifel) with maximum differentiation D_{18}=0.041. This stand still shares 1- D_{18}or 95.9% of its genes with the bulked structure of all other samples. It is followed by No. 23 (Otterberg) to which main attention is given in this study. In contrast to these 2 stands, Neuerburg (No. 17), the second stand confirmed as artificial, represents a gene pool differentiation close to the average.

An inspection ofthe gene pool diversities (according to GREGORIUS, 1987) delivers largest values for Otterberg (No. 23), Neuerburg (No. 17), Hermeskeil (No. 9), and Schneifel (No. 18), This obviously shows that the strong differentiation of the artificial stands is not due to low genetic variation.

Similar degrees of differentiation of 2 stands do not necessarily indicate that their genetic structures are similar. For instance, the stand No. 12 (Irrel) differs almost as strongly from the rest as the stands with numbers 18 and 23 do and therefore, it assumes the adjacent position in the differentiation snail, However, it shows relatively large distances to both stand No. 18 (Schneifel) and No. 23 (Otterberg) (see *Fig. 3*). *Figure 3* also reveals relatively large differences between Schneifel and Otterberg, the 2 stands which assume the outermost position in the snail diagram (see *Fig. 2*).

According to *figure 3* the most pronounced gene pool similarity with the Otterberg stand can be observed for the artificial stand Neuerburg (No. 17). Neuerburg has in fact smaller gene pool differences with Otterberg and with Schneifel than with Montabaur (No. 22), where the latter best represents the genes of the complete material (see *Fig. 2*). Hence, the artificial stand Neuerburg shows a remarkable gene pool structure with moderate differentiation (below the average δ) on the one hand, but is also relatively similar to the substantially more strongly differentiated gene pools of Otterberg and Schneifel where the latter are additionally quite different from each other.

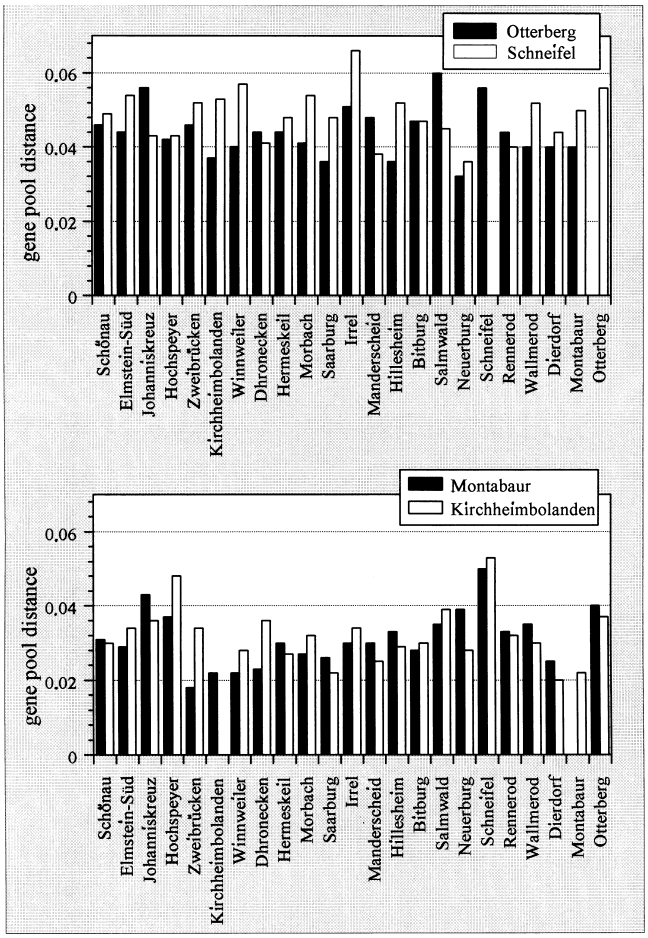

Figure 3. – Gene pool distances between strongly (Otterberg, Schneifel) and less (Montabaur, Kirchheimbolanden) differentiated populations and all other samples.

GOT-B, MDH-B, MDH-C, and relation to Fagus orientalis

When single gene loci are considered, very different effects become apparent. Gene loci GOT BIMDH-B and MDH-C are of particular interest in the present context, since according to PAULE (1995) these gene loci may serve to differentiate genetically between *Fagus sylvatica* and *Fagus orientalis* populations.

For GOT-B, *Fagus orientalis* characteristically shows a minor polymorphism. There is no tendency towards a minor polymorphism in the stands of Otterberg (No. 23), Schneifel (No. 18), or Neuerburg (No. 17). Moreover, with allele structures at GOT-B, Otterberg represents only a moderate population differentiation just below the average.

Figure 4 contains the differentiation snail and dendrogram for the allele structures at gene locus MDH-B. This gene locus is supposed to include species-specific alleles (PAULE, 1995). The allelic structures differ relatively strongly among the 23 stands. MDH-B alleles show the most deviant frequencies in the 2 autochthonous stands No. 16 (Salmwald) and No. 20 (Wallmerod) followed by No. 23 (Otterberg). However, the corresponding dendrogram illustrates that while the first 2 differ considerably from the rest, No. 23 (Otterberg) belongs to a group of more similar structures including Schönau (No. 1) and Hochspeyer (No. 4). There is no indication for a relatedness of the Otterberg material to *Fagus orientalis*.

The results for gene locus *MDH-C* are illustrated in *figure 5*. Here, No. 23 (Otterberg) has the highest differentiation and forms a relatively homogeneous group together with the autochthonous stand No. 11 (Saarburg), which is next in line with only slightly less differentiation. Both stands show the highest frequencies of the less frequent allele at this locus, i.e their polymorphism is most pronounced. Only a minor polymorphism would be expected for *Fagus orientalis* populations.

The structures of clusters based on the 2 gene loci MDH-B and MDH-C (see dendrograms in *Fig. 4* and *5*) differ profoundly, Geographically close populations do not necessarily turn up in the same cluster. The same holds with dendrograms based on several other gene loci. This may partly be explained by the generally low level of differentiation, since minor deviations of allele frequencies may have a marked effect on clustering.

Differences at the enzvme gene locus PGM-A

High levels of differentiation with particular effects for the artificial stands were observed for the genetic structures at gene locus PGM-A, Figure 6 illustrates the situation for the allele frequencies. Here, Otterberg (No. 23) and the artificial stands Schneifel (No. 18) and Neuerburg (No. 17) represent the most differentiated populations and form a cluster characterized by the lowest frequencies of the most frequent allele A3. The largest frequencies of A3 can be found in the group consisting of the stands No. 2, 12, 10, 20, 14, and 7, representing material which is mostly confirmed as autochthonous.

A further outstanding example is the extreme position of No. 23 when genotype frequencies at the gene locus PGM-A are considered (*Fig. 7*). Since D_{23}=0.176, the Otterberg stand (No. 23) shares only 83% of its genotypes at this locus with the

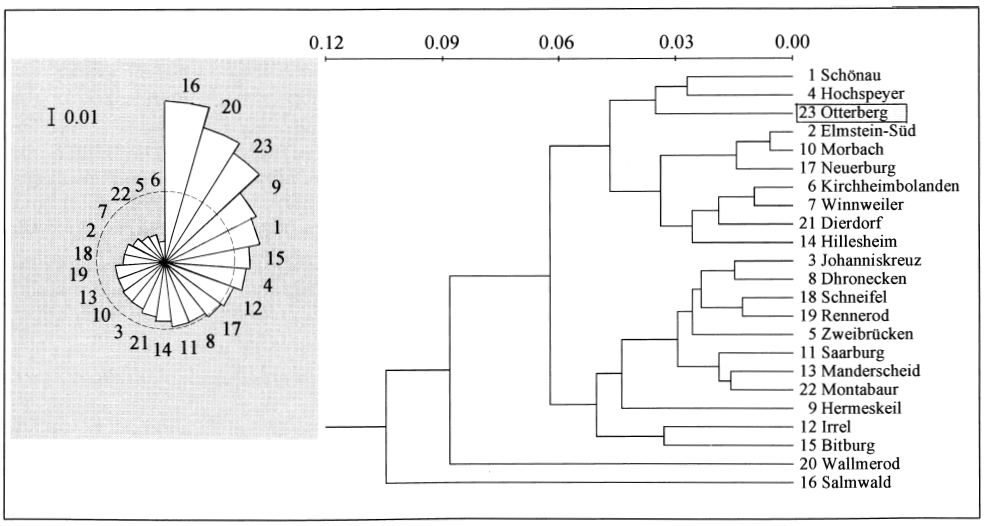

Figure 4. – Population differentiation of stand No. 23 and the 22 reference stands and dendrogram (UPGMA) based on allele frequencies at the gene locus *MDH-B*.

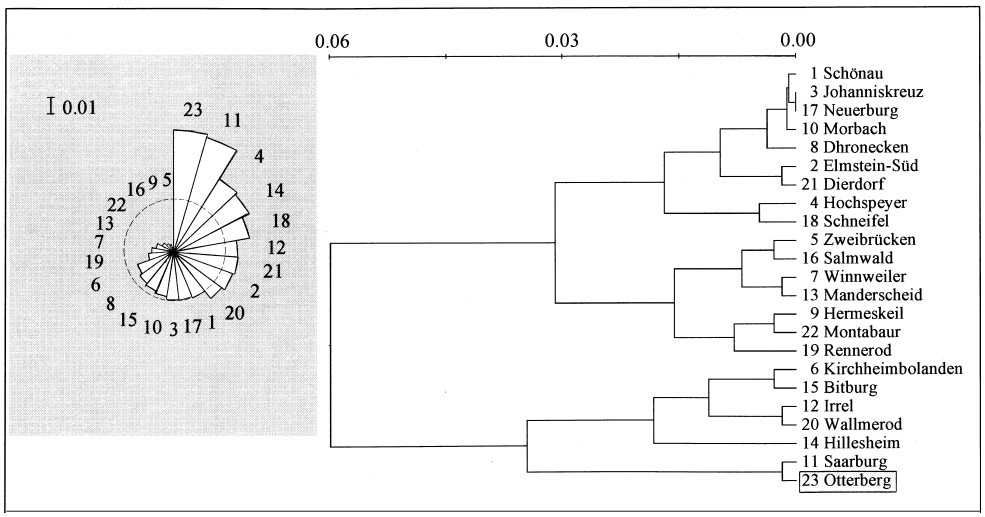

Figure 5. – Population differentiation of stand No. 23 and the 22 reference stands and dendrogram (UPGMA) based on allele frequencies at the gene locus *MDH-C*.

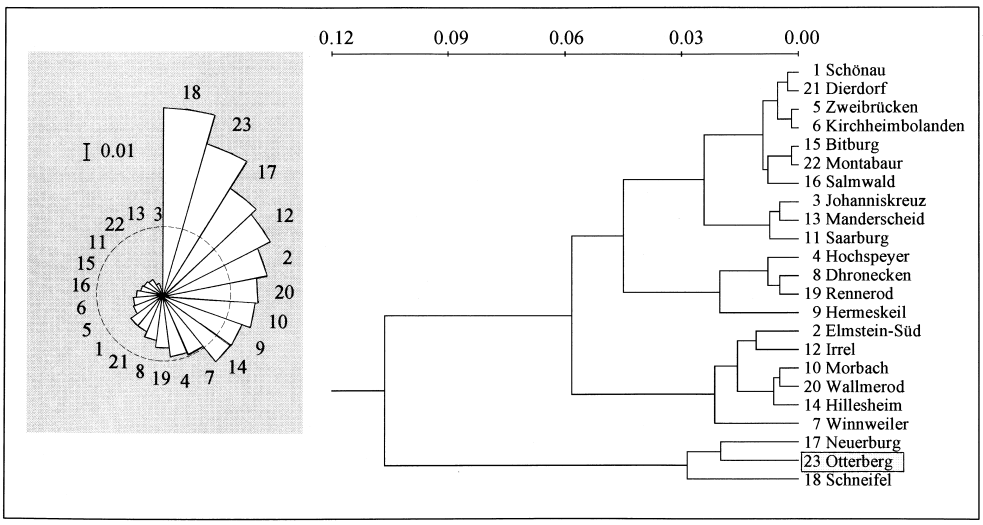

Figure 6. – Population differentiation of the 23 stands including No. 23 and dendrogram (UPGMA) based on allele frequencies at the gene locus *PGM-A*.

bulked material of all other stands. An extraordinarily large frequency of 57% for the heterozygous genotypes A2A3 is mainlyresponsible for this result. According to the respective dendrogram, No. 4 (Hochspeyer) which is not confirmed to be autochthonous, and the autochthonous stand No. 8 (Dhronecken) join the group of Otterberg (No. 23), Schneifel (No. 18), and Neuerburg (No. 17). All members of this group show frequencies of the heterozygote A_2A_3 of more than 45%.

Figure 8 illustrates the genotypic distances at gene locus PGM-A between each one of the stands Otterberg (No. 23), Schneifel (No. 18), Neuerburg (No. 17), Montabaur (No. 22) and Kirchheimbolanden (No. 6) on the one hand, and all remaining mostly autochthonous populations on the other hand. The consistently large genotypic distances of Otterberg from the other stands except from Hochspeyer are obvious. The artificial stands Schneifel and Neuerburg provide consistently lower distances but these are in general substantially larger than distances of the less differentiated stands Montabaur and Kirchheimbolanden.

The extraordinarily large frequency of 57% for the heterozygous genotypes A_2A_3 in the Otterberg stand can hardly be due to peculiarities of the reproductive system in the parental stand alone. One further possibility is that heterozygotes may have been favored by the conditions during production of the planting stock. This would be in accordance with observations of MÜLLER-STARCK (1993), who found under almost all ofthe considered environmental conditions a substantial viability advantage for the heterozygote A_2A_3 during the juvenile stage of beech populations.

Based only on the genetic observations at this gene locus, the conclusion about the origin of the Otterberg material would unambiguously lead to populations outside the alleged region of Rheinland-Pfalz.

Other enzyme gene loci

LAP-A represents the enzyme gene locus with the largest average differentiation of the stands, followed by *PGM-A*. However, previous authors observed not only strong viability

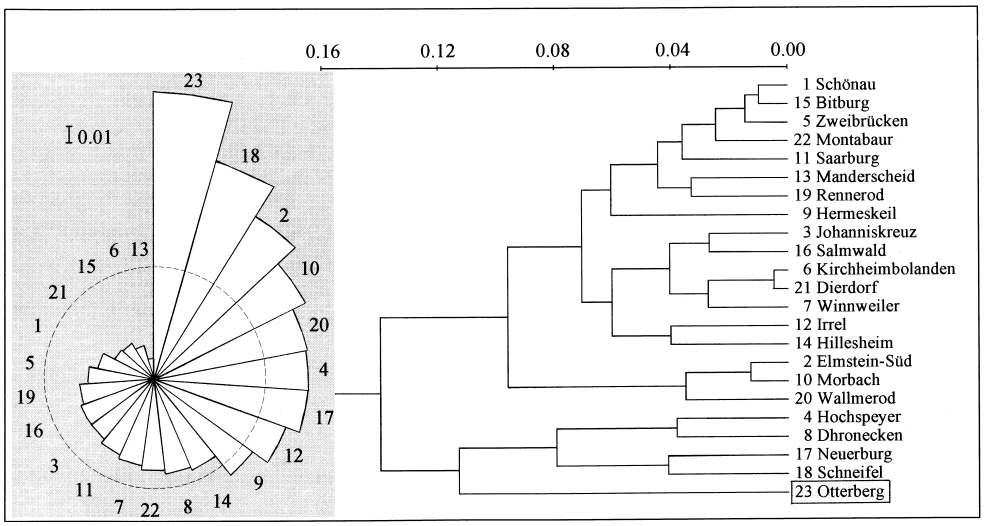

Figure 7. – Population differentiation of the 23 stands including No. 23 and dendrogram (UPGMA) based on genotype frequencies at the gene locus *PGM-A*.

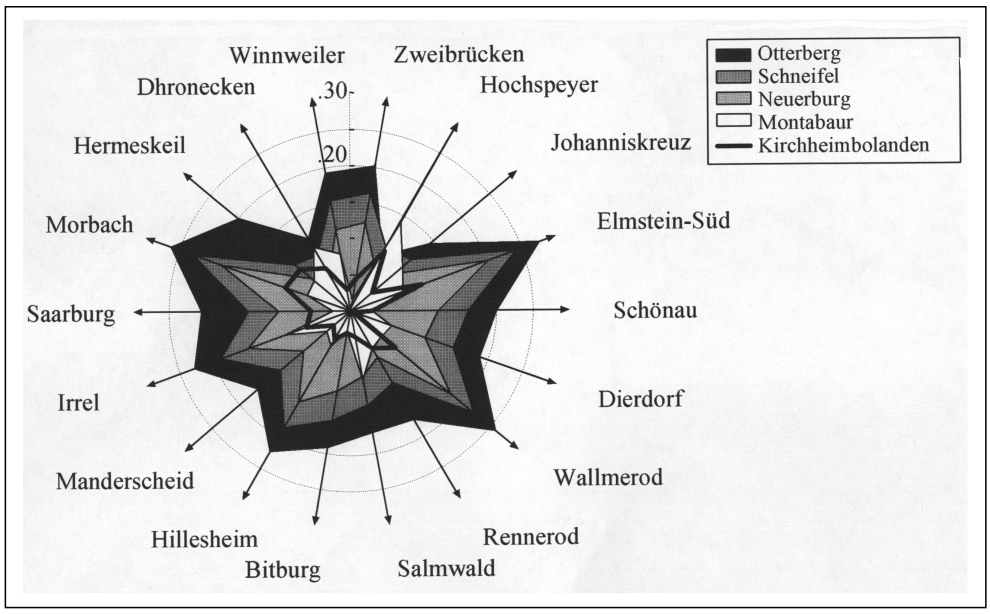

Figure 8. – Radial diagram with genotypic distances between stands of particular interest (Nos. 23, 18, 17, 6, and 22) and all other reference stands at gene locus PGM-A. The genotypic distance is represented by the total radius.

selection (KIM, 1985; MÜLLER-STARCK, 1993) but also inferred fertility selection and found differentiation between seeds collected in different years with different procedures (ZIEHE *et al*., 1997). Additionally, in contrast to *PGM-A,* no relatively consistent ranking effects of allelic and genotypic distances can be observed for *LAP-A.* This makes it difficult to reconstruct an ancestral population of the Otterberg material from the genetic distances at gene locus *LAP-A.*

At the gene locus *IDH-A,* Otterberg (No. 23) is similar to Neuerburg (No. 17) but it differs from Schneifel (No. 18). This gene locus is of particular interest, since Gora *et al*. (1994) have detected significant differences among genotypes in the intensity of attack by beech scale. The latter results have been confirmed with a more recently studied population (unpublished results). GORA et al. (1994) also reported results of the gene locus *GOT-B* similar to those for *IDH-A. GOT-B* agrees with *IDH-A* in showing a small distance between No. 23 (Otterberg) and No. 17 (Neuerburg). However, the distance to No. 18 (Schneifel) is not extreme but rather moderate and consequently does not allow further speculations in this context.

6. Discussion

An attempt was made to assign an artificial beech stand of unknown origin to one or more out of 22 reference stands, regarded as pool of potential parental or ancestral stands of the region of Rheinland-Pfalz. Among the 22 stands are stands known to be either artificial or autochthonous. No strong indication was found for descent from any of the reference stands. Moreover, it turned out that the questionable material from Otterberg differs from the local autochthonous populations in the following ways:

- Similarities of genetic structures measured by genetic distances and the position of the stand Otterberg in the derived dendrograms, vary significantly with gene locus (compare for example *Fig. 3* and *Fig. 4*).
- The genetic structures of Otterberg and 2 other artificial stands, Schneifel and Neuerburg, share peculiarities. These peculiarities concern gene pool distances, gene pool diversities, or genetic structures at single gene loci such as PGM-A. However, it is not quite clear whether also the method of gaining the seed material or producing the planting stock may have had substantial influence via reproduction of the parental stand, seed collection or viability selection, eventually leading to convergence of genetic structures and parameters.

Possible effect of a small number of seed parents

The elsewhere unknown homogeneity of abnormal phenotype suggests that the seed could have been collected from a smaller number of trees. Such a situation can also contribute to a heterozygote excess as compared to HARDY-WEINBERG proportions and help to explain the peculiar genotypic frequencies observed at gene locus *PGM-A.* A simple consideration may illustrate this. If the parental stand shows allele frequencies of 20% for A_2 and of 80% for A_3, then under random mating and without any selection, the genotypes A_2A_2, A_2A_3, and A_3A_3 are expected to appear with relative frequencies of 4%, 32%, and 64%. If seed descends only from a single tree of homozygote genotype A_2A_2, the frequencies of A_2A_2, A_2A_3, and A_3A_3 among the progeny read 20%, 80%, and 0%. A mixture of both structures with equal proportions leads to genotypic frequencies of 12%, 56%, and 32%, which is quite similar to the observed genotypic structure of 10%, 57%, and 32% of the Otterberg material at *PGM-A.*

MDH-C also shows a slight excess of heterozygotes for the Otterberg stand (No. 23) as compared with HARDY-WEINBERG proportions. On the other hand, at gene locus *LAP-A,* a substantial excess of homozygotes was found, which is in accordance with observations made on different material (ZIEHE *et al*., 1997). These deviations as well as the corresponding information for the remaining loci are not sufficiently pronounced to strongly support any particular hypotheses about reproduction components of the parental stand or seed collection of the Otterberg material. Thus the above hypothesis of a mixture remains a speculation. The absence of certain rare alleles is also not conspicuous in view of the moderate sample size of 101 individuals.

There is no sufficiently strong indication that the beech stand in question was derived from a local autochthonous population, *i.e.* the seed may have been collected elsewhere. This conclusion is only heuristic in the sense of GREGORIUS *et al*. (1984), because there exists no binding evidence against its alleged descent from other seed collection stands in the same region.

It must still be stated that the morphological peculiarity of Otterberg (No. 23) is not necessarily due to the employment of seed of inappropriate geographic origin, Indications of this type can, unfortunately, not be quantified in this instance.

Introgression from Fagus orientalis

During the sixties and seventies, quite some beech seed has been imported from southeastern Europe. There is no unambiguous indication that the stand in question represents a provenance from that part of the distribution range. Furthermore, *Fagus orientalis*, the only European congener of *F. sylvatica*, has been reported to possess an introgression zone with *F. sylvatica* in southeastern Europe (PAULE, 1995). Some findings at several gene loci are similar to those published by PAULE (1995) on the introgression zone with *Fagus orientalis*. However, some autochthonous populations show greater similarities to southeastern populations than Otterberg.

Problems involved in deriving statements

The hypothesis that several lots of planting stock were derived from one and the same seed lot would have been much easier to test. In that case, the common seed population is assumed to be homogeneous and the portions of seed used for raising the different lots of plant material can be assumed to be representative of the common seed population. HÖPPNER DE RIZO (1991) and KONNERT (1994) tried to test hypotheses of this type. They could establish the genetic heterogeneity of the various plant lots. However, sampling the common seed population was no longer possible, so that no alleles could be identified that occurred only in the planting stock but not in the seed.

In the present case, the alleged basic material consists of slightly differentiated adult populations of which only a small fraction has been sampled. Furthermore, the reference stands are not a representative sample of all local populations. Therefore, it is not possible to rule out the local origin. Although no action could be brought against the vendor of the reproductive material for stand No. 23 (Otterberg), there is some evidence, that it is not of local origin.

The question of the markers

It may be asked whether the employment of enzyme gene loci in the present situation is appropriate due to the small number of allelic variants and the small regional differentiation of their frequencies. The list of problems that cannot successfully be attacked with the aid of enzyme gene loci is long. However, for the time being, this list is longer with any other type of markers. The existence of more variants of DNA

markers could not remedy the fact that the alleged local populations have not been sampled appropriately for handling problems in identification. The present approach was motivated by the fact that a larger study of genetic resources of beech was made in the same part of the country. In the absence of qualitative or very pronounced quantitative differentiation and in view of the lack of information on the geographic variation pattern, a clear decision on the basis of the exclusion principle is not possible. This is unfortunately true regardless of the encountered variation of the markers applied. The results are nevertheless considered worthwhile to demonstrate and to discuss because similar decisions have to be made rather often.

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8. Literature

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