Comparison of Morphological and Genetic Traits of Pedunculate Oak (*Q. robur* L.) and Sessile Oak (*Q. petraea* (MATT.) LIEBL.)

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Summary

Morphological and genetic traits are compared for pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* (MATT.) LIEBL.) by means of controlled crosses and 2 mixed stands. The existence of hybrids as morphological intermediate forms and of disjunctive characters for distinction between the 2 taxonomic species was tested.

It was shown that the leaf morphology of the juvenile and the adult trees differ from each other. On the level of single individuals a recognition of species' hybrids is not possible by means of an intermediate morphology. On the level of families it is sometimes possible using statistical tests.

Morphological, isoenzymatic and DNA-analytic methods are compared for their different abilities to detect a differentiation between pedunculate and sessile oak. 111 trees of a German stand, were divided into groups as well by means of discriminant functions as by the direct comparison of density distributions (VERGA, 1995), which derived from the measurements of the characters. None of the characters has disjunctive expressions. The 2 methods of species' discrimination, using morphological traits, differ in respect of their selectivity between *Q. robur* and *Q. petraea* and their classification of individuals with intermediate morphology.

Phenotypic and genetic parameters were calculated for the groups derived from VERGA's method. Eight RAPD fragments whose dominant Mendelian inheritance was proven were used for DNA analysis. The total phenotypic differentiation (δ_{TP}), based on the RAPD analysis, is 0.30 for the "robur type" and 0.44 for the "petraea type".

On the basis of 10 enzyme loci the genetic parameters were calculated according to Gregorius. The mean number of alleles is 2.9 for the „robur type" resp. 3.1 for the "petraea type". The observed heterozygosity is 26.7 % for the "robur type" and 20.6 % for the "petraea type" (the conditional heterozygosities are 59.9 % resp. 55.5 %), the total differentiation of the collective (δ_T) is 0.25 for the "robur type" and 0.28 for the "petraea type". The distances between the types are: morphological distance (d_m) 0.45, phenotypic distance (d_{0P}) 0.22 and genic distance (d_0) 0.14.

The values of the German stand are compared to those of a French stand. The characters which contribute to the discrimination between the types within a stand are the same for both stands. The geographically separated groups of one type are differentiated above all because of differences of their leaf length and leaf width. The distance between the same types in different locations is small compared to the distance between different types within one stand.

The "petraea type" of the German stand is more variable for all traits analysed than the "robur type".

Due to the possibility of a hybridization between *Q. robur* and *Q. petraea,* their different ecological requirements and because of the lack of characters' disjunctive expressions between the different types, it seems to be appropriate to state that sessile and pedunculate oak belong to the same biological species for which they represent different ecotypes.

Key words: Quercus robur, Quercus petraea, hybridization, species' identification, morphology, RAPDs, isozymes.

FDC: 165.71; 165.72; 165.3; 165.4; 176.1 *Quercus robur;* 176.1 *Quercus petraea.*

Zusammenfassung

Morphologische und genetische Unterscheidungsmerkmale für Stieleiche (*Quercus robur* L.) und Traubeneiche (*Quercus petraea* (MATT.) LIEBL.) werden an kontrollierten Kreuzungen und an 2 Eichenmischbeständen verglichen. Es wird überprüft, inwieweit Arthybriden eine intermediäre Stellung zwischen den reinen taxonomischen Arten einnehmen und ob sich disjunkte Merkmale zwischen beiden Arten finden.

Die Blattmorphologie juveniler Individuen unterscheidet sich von der adulter Bäume. Eine Erkennung von Arthybriden auf der Ebene von Einzelindividuen ist nicht aufgrund intermediärer Morphologie möglich. Auf der Ebene von Familien ist eine Erkennung von Arthybriden zum Teil mit Hilfe statistischer Tests möglich.

Morphologische, isoenzymatische und DNA-analytische Untersuchungsmethoden werden verglichen in bezug auf ihre Aussagen über die Differenzierung von Stiel- und Traubeneiche. 111 Bäume eines deutschen Bestandes werden Gruppen zugeordnet zum einen mit Hilfe von Diskriminanzfunktionen und zum anderen durch einen direkten Vergleich der sich aus den Messungen ergebenden Dichtefunktionen (VERGA, 1995) jedes einzelnen Merkmales.

Keines der Merkmale zeigt disjunkte Ausprägungen. Die 2 Methoden der Zuordnung unterscheiden sich in ihrer Trennschärfe zwischen *Q. robur* und *Q. petraea* und der Klassifizierung von Individuen als Intermediärformen. Für die nach der Methode von VERGA gebildeten Gruppen werden Parameter aufgrund von DNA Analysen (RAPDs) und Isoenzymuntersuchungen berechnet. Acht RAPD Fragmente mit bekanntem Vererbungsmodus wurden zur DNA Analyse benutzt. Die phänotypische Gesamtdifferenzierung (δ_{TP}) der Gruppen bezogen auf RAPD Daten beträgt 0,30 für den „robur Typ" und 0,44 für den „petraea Typ".

Aufgrund der Analyse von 10 Enzymgenloci ergab sich eine mittlere Anzahl von Allelen pro Genlocus von 2,9 für den „robur Typ" bzw. 3,1 für den „petraea Typ". Die beobachteten Heterozygotiegrade lagen bei 26,7 % für den „robur Typ" und 20,6 % für den „petraea Typ" (die bedingte Heterozygotie lag bei 59,9 % bzw. 55,5 %), die Gesamtdifferenzierung der Kollektive (δ_T) betrug 0,25 für den „robur Typ" und 0,28 für den „petraea Typ". Der errechneten Abstände zwischen den Gruppen betragen für die Morphologie $d_m = 0{,}45$, für die DNA Analyse $d_{0P} = 0{,}22$ und für die Isoenzymanalysen $d_0 = 0{,}14$.

Die errechneten Parameter der einzelnen Merkmale für die Gruppen des deutschen Bestandes werden mit den Werten eines französischen Eichenbestandes verglichen. Die morphologischen Unterscheidungsmerkmale zwischen den Typen (Arten) sind in den beiden Beständen dieselben, aber zwischen geographisch getrennten Kollektiven eines Typs (einer Art)

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treten Unterschiede in der Morphologie auf, die auf Unterschieden in Längen- und Breitenabmessung der Blätter basieren. Der genetische Abstand (d_0) zwischen gleichen Typen verschiedener Bestände ist gering im Vergleich zu den Abständen verschiedener Typen innerhalb desselben Bestandes. Der „Traubeneichentyp" im deutschen Bestand ist über alle betrachteten Merkmale hinweg (Morphologie, RAPDs und Isoenzyme) variabler, als der „Stieleichentyp".

Aufgrund der Hybridisierbarkeit von *Q. robur* und *Q. petraea,* ihren unterschiedlichen ökologischen Ansprüchen und dem Fehlen disjunkter Ausprägungen von Merkmalen zwischen den Typen scheint es angebracht zu sein, von einer biologischen Art zu sprechen und Stiel- und Traubeneiche als deren Ökotypen zu bezeichnen.

Introduction

Botanical description

The genus *Quercus* includes more than 200 species (HEGI, 1912). It is subdivided into 3 subgenera: Cyclobalanopsis, Erythrobalanus and Lepidobalanus. The subgenus Lepidobalanus contains 7 sections, of which the section Robur is one. It includes most of the important European oak species. *Quercus robur* L. and *Quercus petraea* (MATT.) LIEBL. are 2 of these species. They are – together with beech (*Fagus silvatica* L.) – the most important European deciduous tree species. These 2 species are defined botanically by means of morphological traits (OELKERS, 1913; SCHWARZ, 1937; CAMUS, 1938 to 1939; JONES, 1959; KRAHL-URBAN, 1959; TUTIN et al., 1964).

Range of species

The natural range of *Q. robur* and *Q. petraea* is described by MEUSEL (1965). The distributions of *Q. robur* and *Q. petraea* reach from the low lands up to the sub-alpine mountains (SCHÜTT et al., 1992).

Ecological requirements

Q. robur and *Q. petraea* have different ecological requirements (KRAHL-URBAN, 1959; GRANDJEAN and SIGAUD, 1987). *Q. robur* is typical for rich, humid sites in the lowlands and "Aue" forests. *Q. petraea* has its characteristic populations in a medium altitude on dryer and warmer sites, which are less rich. Both species require light but sessile oak tolerates a little bit more shade. Nevertheless the 2 species are sympatric in a lot of places (e.g. ELSNER, 1993). For northern and western Germany the morphological analysis of more than 1200 stands showed that only 25 % of the stands consisted of only one of the species. All other stands included a certain percentage of the other species (ELSNER, 1993; KLEINSCHMIT, 1993). This is especially true for sites with more humid and dry mosaics. In these places intermediate morphological forms are found more or less frequently (ELSNER, 1993; AAS, 1988; KRAHL-URBAN, 1959). The different ecological requirements do not imply that some provenances of these species do not yet grow well on sites which show the opposite extremes of their typical site characteristics (JENSEN, 1993).

Hybridization and controlled crosses

The 2 species flower at the same time (KRAHL-URBAN, 1959; KLEINSCHMIT, 1993; BACILIERI, 1994). Artificial hybridization between the 2 species is possible (DENGLER, 1941; RUSHTON, 1977; AAS, 1988; STEINHOFF, 1993). The rates of acorn production for interspecific crosses with *Q. robur* as female parent and *Q. petraea* pollen tree showed about 60 % of the success compared to the intraspecific crosses of *Q. robur.* For *Q. petraea* as a female parent the interspecific crosses had only 8 % of the success in single tree crosses and 54 % in crosses with pollen mix (KLEINSCHMIT and KLEINSCHMIT, 1994). Isozyme analysis in a mixed French oak stand with natural regeneration support these results (BACILIERI, 1994).

Due to these facts a discussion has arisen whether the morphological intermediate forms represent natural hybrids or if they are the result of variability within each species (for a review see GARDINER, 1970). Research under different aspects was carried out to find an answer to this question. Morphological-, DNA- and isozyme-analysis were carried out to distinguish between the 2 species.

The aim of this paper is to document on the taxonomical status of morphological intermediate forms within the complex *Quercus.* This paper also tries to compare the different traits and their ability to discriminate the 2 species and to see if differences exist in this respect between geographically separated oak stands.

The *working hypothesis* are:

1. Hybrids show intermediate morphology. Therefore they can be detected morphologically.

2. *Q. robur* and *Q. petraea* are 2 clearly distinct species. This implies, that each species has its own specific characters.

Recent literature about species' differentiation in the genus Quercus

a. Morphological analysis

A lot of research has been done on the differentiation of oak species by means of morphological traits. Most of them are based on the species' botanical description. In France DUPOUEY (1983) and DUPOUEY and BADEAU (1993) studied the species' morpholocical variation and the power of different characters to separate sessile and pedunculate oak. These authors used morphological data and multivariate statistics to discriminate the 2 species. GRANDJEAN and SIGAUD (1987) studied the morphological variation and ecological requirements in the Berry region in France. They found strong correlation between one species' occurence and the ecological characteristics of the analysed sites. KISSLING (1977 and 1980) did some studies especially on the pilosity of the leaves in the section robur in the Swiss Jura and discovered the pilosity to be a good character to separate the 2 species. He also published a key for species' differentiation for geobotanists. RUSHTON (1977) examined the hybridization between *Q. robur and Q. petraea* in Britain. He was one of the first, who analysed morphological characters of oaks by a multivariate approach (1978, 1983). In Germany SPETHMANN (1986) and ELSNER (1993) used the characters, defined by RUSHTON, to verify the purity of stands admitted for acorn collections under the rules of the German law for trade with seed and plants for forest purposes. They found a varying percentage of the other species in stands which had earlier been described as pure. AAS (1988) sampled 279 oaks throughout Germany and Poland for a morphological analysis. He found a small percentage of individuals with intermediate morphology (AAS, 1993).

It is difficult to compare the results of these papers due to the different characters which were used and due to the different statistical methods applied to handle the data. The statistical methods which have mostly been applied are factorial discriminant analysis, discriminant analysis and cluster analysis. It is not possible to divide the differences between the species, which were found as results of the different researches, into methodological and geographical effects. And also it is not possible to state whether a morphological character's variation is an expression of modification or of genetically fixed variability.

b. DNA analysis

Investigations have been done on 2 levels: the nuclear genome and the organelle genome. On the nuclear level the differentiation between the 2 species is small. MOREAU (1993, 1994) used RAPDs (Random Amplified Polymorphic DNAs) on the basis of total DNA extraction to discriminate the 2 species. She found 7 primers producing 12 fragments, which show higher frequency differences between the species than allozymes do. None of the fragments is species' specific. By means of these fragments MOREAU was able to discriminate the 2 species within 4 oak stands in France.

According to KREMER et al. (1991) no differences exist between the species within one population on the cpDNA level, but there are differences between geographically separated populations for cpDNA. PETITE (1994) reported on cpDNA polymorphisms in *Quercus petraea* and their use for analysis of recolonisation routes after the ice ages. FERRIS et al. (1993) described cpDNA polymorphism shared by the 2 oak species, indicating 2 different refugia during the last glaciation. The fact that the 2 species share the same polymorphism is taken as a sign for natural hybridization. Another group of scientists used RFLPs to detect cpDNA polymorphisms between *Q. robur* and *Q. petraea* for a small number of individuals (BURG et al., 1993).

c. Isozyme studies

Studies on the isozyme variation of *Q. robur* and *Q. petraea* investigated the differentiation within and between populations. Up to now no species' specific allele has been found for any of the enzyme systems. Allozyme surveys revealed only frequency differences, with less than 20% of the genes differing from one species to another (ZANETTO et al., 1994; BACILIERI et al., 1995). Most of the studies were done on acorns (MÜLLER-STARCK et al., 1993; ZANETTO et al., 1993) or juvenile trees, only a few used material from adult trees (e. g. HERZOG and MÜLLER-STARCK, 1993). BACILIERI found proof for unidirectional gene flow in a mixed oak stand in France using data from isozyme studies (BACILIERI, 1994).

VULICEVIC and ROTHE (1995) from the university at Mainz reported on differences between the 2 species *Q. robur* and *Q. petraea* for isozyme activity rates of the primary metabolic chain's enzymes. They found that the expression of this character is dominantly inherited.

Material and Methods

Material

Two mixed oak stands were analysed for their morphological, ecological and genetic traits. The first stand – compartment 66a – is situated in northern Germany, the second – Petite Charnie – in the north west of France. In addition to these stands controlled inter- and intraspecific crosses were morphologically and genetically analysed. *Table 1* gives a summary of the material, the kind of analysis carried out and the reference material.

a. Controlled crosses of the seed orchards at Berkel in Lower Saxony

In the year 1989 controlled crosses were done at the seed orchards at Berkel by the Department of Forest Tree Breeding of the Lower Saxony Forest Research Institute. The species' identification of the parents was proved using ELSNER's discriminate score (1993). The family numbers, the parental clones, the number of offspring and the species' group is shown in *table 2.*

Table 1. – Summary of the material, the kind of analysis realized within the scope of this work and the reference material.

Übersicht über das verwendete Material, die angewandten Methoden und Vergleichsmaterial.

	material analysed	kind of analysis	reference material
A	**controlled crosses (1989)** 9 families	**1.** leaf morphology 18 characters 1991-1994	parental clones of seed orchards at Berkel; trees of compartment 66a, Germany
	families no.1 and 2	**2.** DNA analysis (using RAPD with 7 primers counting 12 fragments)	parents, at the seed orchards at Berkel
B	**Compartment 66a, Germany** 111 trees	**1.** leaf and fruitstand morphology (26 characters)	Petite Charnie, France only leaf morphology (15 characters) 113 trees
	111 trees	**2.** DNA analysis (RAPD using 7 primers, counting 8 fragments)	families no.1 and 2 of controlled crosses; Petite Charnie, France, 38 trees
	111 trees	**3.** isozyme analysis 10 enzyme loci	Petite Charnie, France 8 enzyme loci, 407 trees

Table 2. – Families of controlled crosses 1989 from the seed orchards at Berkel, Lower Saxony, with parents, number of offspring and distribution on species' groups.

Familien der kontrollierte Kreuzungen 1989 von den Samenplantagen in Berkel, Niedersachsen, mit ihren Eltern, der Anzahl an Nachkommen und ihrer Einteilung in Arten-Gruppen.

family no	parents mother		father	number of plants	Species' group
1	Q.robur Koberg 6	x	Q.petraea Lüß 6	24	hymr
2	Q.robur Lensahn 2	x	Q.petraea Reinhausen 2	24	hymr
3	Q.robur Koberg 6	x	pollen mix Q.robur	37	robu
4	Q.robur Lensahn 2	x	pollen mix Q.robur	41	robu
5	Q.robur Koberg 6	x	Q.robur Lensahn 2	20	robu
6	Q.petraea Reinhausen 2	x	pollen mix Q.petraea	3	petr
7	Q.petraea Lüß 6	x	pollen mix Q.petraea	17	petr
8	Q.petraea Saarburg 2	x	pollen mix Q.robur	8	hymp
9	Q.petraea Ritzerau 9	x	Q.robur Lensahn 2	2	hymp

Table 3. – Measured and calculated characters on the leaf and the fruit morphology.

Gemessene und abgeleitete Blatt- und Fruchtmerkmale.

A.leaf morphology:

1. number of lobe pairs (bla) (except the terminal lobe)
2. number of sinuses (bbu)
3. number of intercalary veins (bin)(running at least 50 % into the sinus)
4. length of petiol (bls)
5. length of lamina (blb)
6. distance base of limbe to widest part (blh)
7. lamina width (bb1)
8. distance of principal veine to sinus (bb2)(adjacent to widest part)
9. auricle development (boe)
10. basal shape (bba)
11. pilosity (bha)

B.fruitstand morphology (only for compartment 66a) (Aas, 1991):

12. longitudinal stripes on acorns (est)
13. total length of acorn (el)
14. distance base to width of acorn (elb)
15. width of acorn (eb)
16. weight of acorn (eg)
17. max. length of peduncule (es1)
18. number of acorns per fruitstand (ek)
19. length of peduncule from base to first cupule (es2)

C. Combinations of the characters:

20. percentage of veniation = $\frac{\text{number of intercalary veins} \times 100}{\text{number of lamina sinuses}}$

21. Total leaf length = length of petiol + lamina length

22. Petiole ratio = $\frac{\text{length of petiole}}{\text{length of petiole + lamina length}}$

23. Obversity = $\frac{\text{lamina length}}{\text{length of lamina from base to widest part}}$

24. Leaf ratio = $\frac{\text{lamina width}}{\text{length of lamina}}$

25. Depth of sinus = lobe width - depth of sinus

26. Lobe depth ratio = $\frac{\text{lobe width}}{\text{lobe width - depth of sinus}}$

The acorns were sown in the nursery of the Tree Breeding Station at Escherode, Lower Saxony. The resulting plants are still standing in the nursery and they are exposed to the same natural conditions. In 1991, 1992, 1993 and 1994 the leaf morphology data were measured for the nine intra- and interspecific families according to RUSHTON (1983) as indicated in *table 3*. The characters were measured on 5 (1991) resp. 10 leaves (1992, 1993 and 1994) per tree.

b. Compartment 66a of the state forest district Escherode in Lower Saxony, Germany

The stand is an 145 years old mixed stand of *Quercus robur* and *Quercus petraea* originating from the plantation of "Heister" plants and from natural regeneration. The second layer of the stand is formed by *Fagus silvatica* L.. The plot is a part of the compartment 66a.

The plot contains 115 oak trees of the complex *Q. robur/petraea*. In October 1992 branches of 113 trees with leaves and fruits were collected from the upper external part of the crown. Ten leaves and 10 fruitstands with acorns were sampled for each individual to carry out morphological analysis. In addition twigs with buds were taken for isozyme and DNA analysis. The twigs were kept fresh and sent to France where the buds were separated and stored at –80 °C for further manipulation.

Morphological data were measured according to RUSHTON (1978) and AAS (1991) at the Forest Tree Breeding Station of the Lower Saxony Forest Research Institute (*Table 3*).

c. Compartment no.26 of the state forest of Petite Charnie, France

The plot, located in the north-west of France, is an approximatly 120 years old mixed oak stand with 426 trees. The forest "Petite Charnie" (Sarthe) is situated 35 km west of the city of Le Mans. A detailed description of the site is given in the report from MAILAIT (1993). The plot is situated on a slope from the northeast to the southwest. Using a digitaizer table the leaf morphology data were measured for 5 leaves per tree according to DUPOUEY (1983) (BACILIERI, 1994; BACILIERI et al., 1995). The data on RAPDs of this stand was supplied by FABIENNE MOREAU (1993).

Methods

a. Morphological analysis

The leaf morphology was measured according to RUSHTON (1983). Acorn morphology was measured according to AAS (1991).

Additionally to the original characters combinations were calculated as new variables. Two different ways of data analysis were used to evaluate the morphological data and to compare the results of these methods. The first method was the "classical" way of discriminating the 2 oak species.

Classical analysis of morphological data

For the controlled crosses' data and the trees' data of compartment 66a means of every character were calculated for each tree. With these means discriminant scores were calculated (ELSNER, 1993). For further analysis the 9 families were combined into 4 species' groups, one group per intraspecific cross and one group for each interspecific cross, by means of their female parent's species. The discriminant scores' frequency distribution of every group was plotted. Thus the evolution of the leaf morphology over 4 years could be compared statistically within each species' group and also for each year between the species' groups. In 1993 and 1994 the pilosity of the leaves' lower side was measured for the controlled crosses and illustrated as a frequency distribution for every species' group and for each year. The variation of the frequency distributions was statistically tested between the years within one species' group and between the species' groups within each year.

A cluster analysis with the characters' means of the trees was performed using the average linkage method of "proc cluster" of the Statistical-Analysing-System's (SAS) (1989) statistic package to see, if there is any structure in the data which represents the biological relationship of the individuals.

To compare the 2 data sets from Escherode and Petite Charnie, it was necessary to transform the characters "number of lobes, angle at the base of the lamina, pilosity on the lower side of the lamina" into classes. The character no.8 "distance from the base of the lamina to the sinus adjacent to the widest point of the lamina (bb2)" was not measured for the trees of Petite Charnie. Therefore it was not possible to calculate ELSNER's discriminant score (1993) for these trees.

To group the trees for further calculations according to their leaf morphology the discriminant score from DUPOUEY and BADEAU (1993) was used where the number of intercalary veins and the length of the petiole are included.

This simple discriminant function was used to get a comparable grouping for the French and the German stands and to see within the compartment 66a how the species distinction with the different discriminant functions differs from each other concerning the classification of trees.

Comparison of frequency distributions of morphological data

The second method of analysing the morphological data is a method developed by VERGA (1995) on the basis of the genetic distance (GREGORIUS, 1974).

The genetic distance (d_0) is used to calculate the distance between collectives of organisms on the basis of a genetic inventory (isozyme or DNA data). With the morphological distance (d_m) from VERGA (1995) it is possible to calculate the distances between individuals based on morphological data, because one can obtain several repetitions of measurements (e. g. leaf lengths) for a single individual.

For the controlled crosses the matrix of morphological distances was calculated between the families within one year (1993) and between the species' groups within one year and also over the years using all leaf morphological characters except pilosity. On the basis of the matrix a cluster analysis was done with the UPGEMA method (unweighted pair-group method, arithmetic average) by the SAHN Clustering procedure of the NTSYS program. For compartment 66a the morphological distance (d_m) was calculated between the single trees using the 19 measured and 7 calculated characters. A phenogram was constructed using the same method as described beforehand for the controlled crosses to see if any differences exist in the species' distinction between the "classical method" represented by DUPOUEY and BADEAU's discriminant score and this new method. On the basis of this phenogram the collective of the trees was classified into 2 new groups which were named "type robur" and "type petraea". The morphological distance (d_m) between these types was calculated for every character to see which were the most powerful for the species discrimination. The variablity of the morphological characters within each type was measured as the mean of the coefficient of variance (CV) over all analysed characters.

The resulting morphological distance (d_m) was compared with the distances derived from the DNA analysis and the isozyme studies using the same material and the same classification of the trees.

With a reduced number of morphological characters (leaf morphology, 15 characters) the values for every type of compartment 66a were then compared with the values of a systematical sample (every fourth tree) of the french stand "Petite Charnie".

b. DNA analysis

The DNA analysis was done using RAPDs (*R*andom *A*mplified *P*olymorphic *D*NA) and the PCR technique. The RAPD assay is based on the use of short, random-sequenced oligonucleotides as primers for the amplification of segments of the target genome (RAFALSKI et al., 1991). The technique was first described 1990 independently by 2 groups (WILLIAMS et al., 1990; WELSH and MCCLELLAND, 1990). 15 ng to 25 ng DNA are necessary per assay. The primers, which are generally decamers, should have the following characteristics: at least 50% GC content and no palindromique sequences to avoid a hybridization with itself. To enable-the Taq-Polymerase to synthesise the DNA between 2 fixation sites the distance between the sites has to be smaller than 2000 to 3000 nucleotides.

In 1993 2 interspecific families (no.1 and 2 of the controlled crosses) were used for DNA-analysis. The total DNA was extracted out of the buds (1.0 g) following a modified CTAB method of SAGHAI-MAROOF et al. (1984). The DNA quality and concentration of each sample was estimated firstly by comparing the intensity of bands with known standards of lambda DNA on an agarose gel at 8% and secondly by measuring the concentration of DNA with a fluorimeter. The DNA analysis was done using the polymerase chain reaction (PCR) technique and the 7 random primers OPF1, OPF4, OPF12, OPF14, OPF15, 174 and $[GACA]_4$ (MOREAU, 1993; KLEINSCHMIT, 1995). These 7 primers were chosen by MOREAU (1993) out of 45, to discriminate *Q. robur* and *Q. petraea* (MOREAU et al., 1994). The DNA fragments were separated by means of electrophoresis on polyacrylamid gels, stained with ethidium bromide and revealed on an UV-table (320 nm). Black and white photos of the gels were taken to conserve the results. The molecular sizes of the DNA fragments were estimated by utilizing a 1 Kb DNA ladder. For the used primers, segregation proportions of absence/presence of 12 fragments were analysed and compared with the phenotypes of the parents. The same fragments were used to analyse the 111 trees of compartment 66a. The fragments were counted as present or absent. Using the program GSED (Genetic Structure from Electrophoresis Data) (GILLET, 1994) the "phenotypic distance" (d_{0P}) and the "total phenotypic differentiation" (δ_{TP}) of the collective were computed for the two morphological types by means of the RAPD data. For the calculation of the "phenotypic" parameters the equations of the genetic distance (d_0) (GREGORIUS, 1974) and the total population differentiation (δ_T) (GREGORIUS, 1987 and 1988) were used.

For the French stand there were only 38 individuals analysed for eight fragments with the technique of RAPD (MOREAU, 1993). The data of these trees were used for the comparison of the two stands.

c. The isozyme analysis

The extraction, migration and staining of the gels was done as described in FOUGERE (1988) and ZANETTO (1989).

Migration was performed on starch gels at 12% with electrolyte buffers differing in the pH according to the enzyme systems. The following pH was used for the enzyme systems:

pH = 7 for: IDH, MDH, PGI, PGM, 6PGDH
pH = 8.3 for: AAP, ACP, GOT, LAP, MNR

Ten enzymesystems of the primary and secondary metabolism were analysed (*Table 4*). These systems had beforehand been chosen and analysed genetically by ZANETTO. The enzymatic systems showed a simple Mendelian inheritance in controlled crosses (ZANETTO, unpublished).

Table 4. – Enzyme systems analysed after electrophoresis and their interpretation.
Auflistung der Enzymsysteme und ihre Interpretation nach der Elektrophorese.

abbreviation	name of system	E.C. ref.	loci code	identification numbers of alleles	quartaire structure
AAP	Alanine aminopeptidase	3.4.11.1	A	4, 5, 6, 7	monomere
ACP	Acide phosphatase	3.1.3.2	B	1, 2, 4	monomere
GOT	Glutamate oxaloacetate transaminase	2.6.1.1	B	2, 3	dimere
IDH	Isocitrate dehydrogenase	1.1.1.42	A	1, 2, 3, 4, 5	dimere
LAP	Leucine aminopeptidase	3.4.11.1	B	2, 4, 5	monomere
MDH	Malate dehydrogenase	1.1.1.37	A	2	dimere
MNR	Menandione reductase	1.6.99.2	A	1, 2, 3, 4, 6	tetramere
6PGDH	6-phosphogluconate dehydrogenase	1.1.1.44	B	1, 6, 7	dimere
PGI	Phosphoglucose isomerase	5.3.1.9	B	1, 2, 3, 4	dimere
PGM	Phosphogluco mutase	2.7.5.1	A	1, 3, 4	monomere
total			*10*	*33*	

After reading the gels, genetic parameters were computed for the morphological "types" using GSED (GILLET, 1994). The mean number of alleles per locus (A/L), the gene pool diversity (v) (GREGORIUS, 1978, 1987), the observed and conditional heterozygosities (H_a and H_c) (GREGORIUS et al., 1986) and total differentiation of a collective (δ_T) (GREGORIUS, 1987, 1988) were used to characterize the variation within the morphological types. The variation between the different "types" was quantified by the genetic distance (d_0) (GREGORIUS, 1974).

A reduced number of eight enzyme loci (PGM, PGI, IDH, ACP, LAP, AAP, MR, GOT) was chosen to compare the genetic parameters of the French and the German stand by means of the genic distance (d_0). The species' discrimination for the French data was done using a factorial discriminant analysis with all morphological data (leaf morphology). Therefore the direct comparison of the genetic parameters for the French stand Petite Charnie with compartment 66a is not exactly possible.

Results and Discussion

Controlled crosses

Leaf morphology: classical analysis

Using ELSNER's definition for intermedinate forms [(-1) to 1 of the discriminant score], the 2 species are clearly separated in regard of the parental trees' mean scores. The discriminant scores' means of the offspring are situated between the parents' means. From this point of view the offspring show an intermediate morphology concerning the discriminant scores. The offspring of the intraspecific crosses is not separated into two distinct groups. Eight out of the 9 families show individuals with discriminant scores between (-1) and 1. There is just one interspecific cross (fam. 9) with *Q. petraea* as female parent's species, which has scores greater than one for all of its members. But this family has only 2 individuals (see *Table 2*). One obtains 2 clusters, running a cluster analysis using the average linkage method of "proc cluster" of

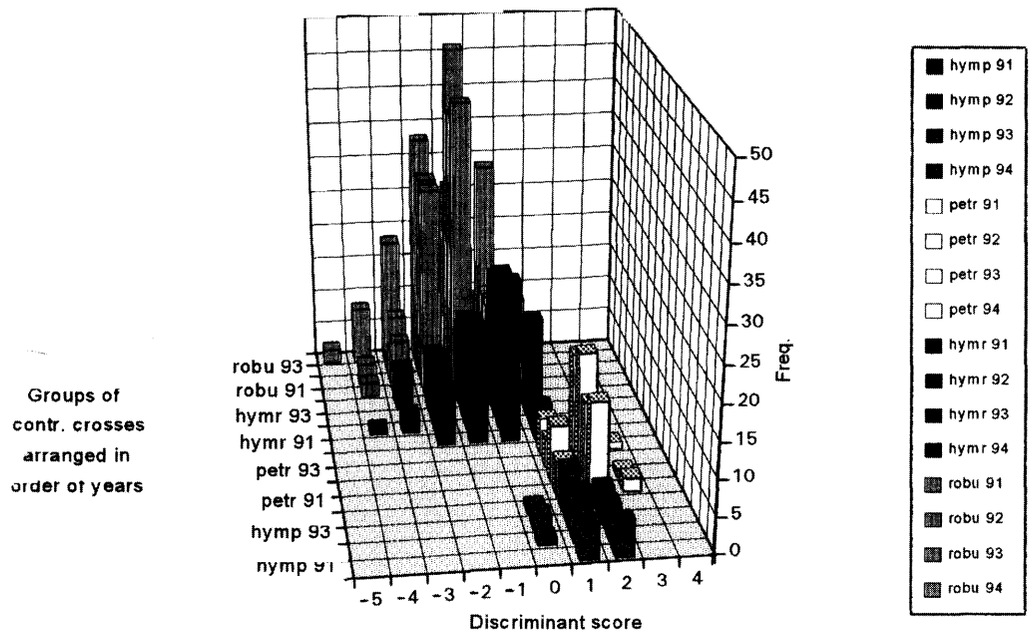

Figure 1. – Controlled crosses: Distribution of discriminant scores according to ELSNER for species' groups over years.

Kontrollierte Kreuzungen: Verteilung der Diskriminanzwerte nach ELSNER für die Artengruppen über die Jahre.

SAS/STAT with the progeny data from 1993 including abaxial pubescence as additional variable. One cluster includes all the plants with *Q. robur* as female parent, the other all the trees with *Q. petraea* as female parent. Just 2 plants, one of family 9 the other of family 6, are situated in the "opposite" cluster.

Running a cluster analysis with the leaf morphology data of both the parents and the offspring together it becomes obvious that a direct comparison of juvenile leaves with the leaves of the adult parental trees is not possible. The analysis separates the adult and the juvenile trees, but does not differentiate between the two species. Therefore the leaves of the pure species' families (intraspecific crosses) are compared with the hybrid families (interspecific crosses) in *figure 1.*

The hybrids were situated with their discriminant scores close to the group of the pure female parent's species and had some tendency towards intermediate scores. The intraspecific *Q. petraea* crosses and the crosses with *Q. petraea* as female parent show over the years no statistically significant

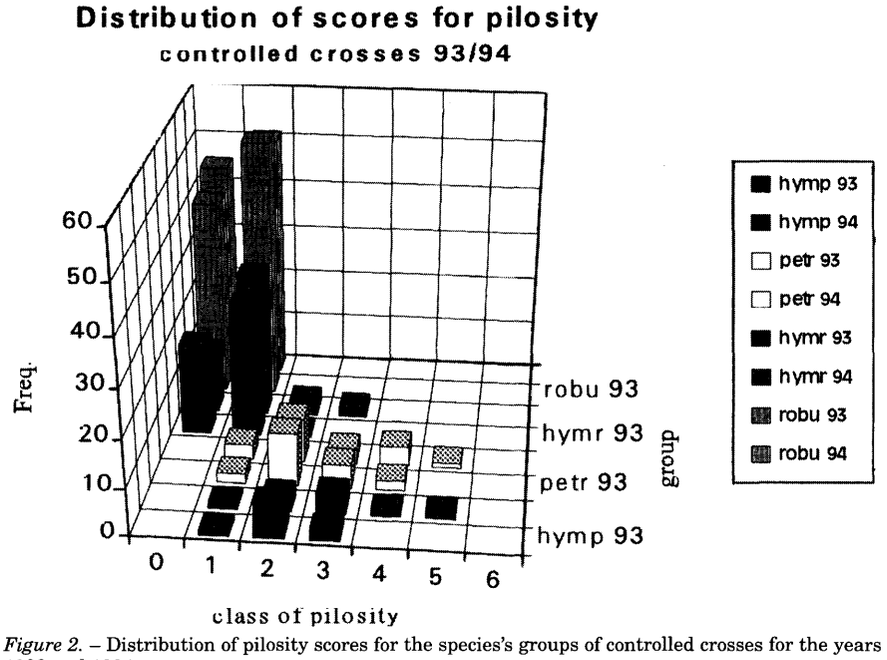

Figure 2. – Distribution of pilosity scores for the species's groups of controlled crosses for the years 1993 and 1994.

Verteilung der Behaarungswerte der Artengruppen der kontrollierten Kreuzungen für die Jahre 1993 und 1994.

difference in their distributions of the discriminant scores. The variation of the discriminant score's distributions over the 4 years is significantly different within the pure *Q. robur* crosses (***) and is also significantly different within the interspecific crosses with *Q. robur* as female parent (**). In the years 1991 and 1992 the difference of their discriminant score's distributions within one year is not significant for the groups "petr – hymp". In the year 1993 it is significant for the same groups at the 1% level and in 1994 at the 5% level. In the years 1991, 1993 and 1994 there are significant differences in the discriminant score's distributions for the groups "robu – hymr" at the 0.1% level. In 1992 there is no significant difference between these 2 groups.

The distributions of the pilosity classes shown in *figure 2* stresses once more, that the groups of the species' hybrids are allocated closer to the group of the female parent's species than towards the species' group of the male parent. Not only within the species' groups over the 2 years but also between the species' groups with the same species as female parent within 1 year no statistical significant difference of the distributions exist, with the exception of the groups "robu 94 – hymr 94" which have a significant difference of the distributions at the 1% level.

Leaf morphology: comparison of frequency distributions

Calculating morphological distances (d_m) for the controlled crosses' data of 1993 including information about abaxial pubescense (altogether 18 leaf morphological characters) one obtains the phenogram given in *figure 3*. The method of comparing morphological data's frequency distributions is able to differentiate between the different families of the controlled crosses. This was not possible with the discriminant score from ELSNER. The families are separated into 2 groups. The common characteristic of each group is the species of the female parent. This result corresponds to the outcome in the previous chapter. The range and location parameters of a character within one individual seem to be an important information for the discrimination between the species.

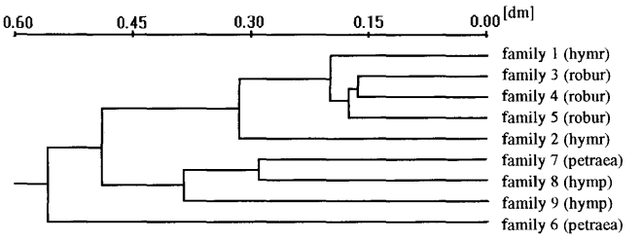

Figure 3. – Phenogram of the controlled crosses based on the leaf morphology data (1993) including abaxial pubescence as additional character.

Dendrogramm der kontrollierten Kreuzungen der Blattmorphologie (1993) einschließlich der Behaarung.

Figure 4 shows the phenogram resulting from the distance matrix calculated for the leaf morphology over 4 years. The controlled crosses are separated into 2 groups. The common characteristic in each group is the species of the female parent. This is indicative of maternally inherited leaf morphology. If the leaf morphology is maternally inherited the variation of an offspring's character of a single tree cross should not be less than that of trees resulting from pollination with a pollenmix. This hypothesis can be tested with the families no.1, no.3 and no.5 and with the families no.2 and no.4. The KRUSKAL-WALLIS-Test indicates significant differences between the distributions of every single character of the families no.1, 3 and 5 in at least one year, except for the character "number of lobe pairs (bla)". For the families no.2 and no.4 the KRUSKAL-WALLIS-Test also shows significant differences between every single character's distribution in at least 1 of the 4 years. These results show that there are other effects beneath those of maternal inheritance which influence the expression of leaf morphology characters.

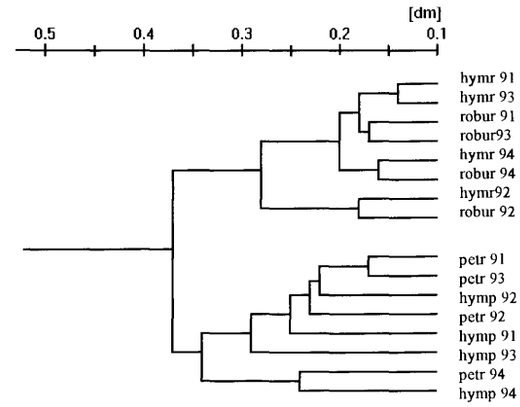

Figure 4. – Phenogram of species' groups of the controlled crosses based on morphological distances (d_m).

Dendrogramm der Artengruppen der kontrollierten Kreuzungen aufgrund der errechneten morphologischen Abstände (d_m).

Table 5. – Morphological distance (d_m) of each character and level of significane for difference between 2 main groups according to KRUSKAL-WALLIS-test.

Morphologischer Abstand (d_m) der einzelnen Unterscheidungsmerkmale und Signifikanzschranke der Unterscheidungsmerkmale zwischen den beiden Hauptgruppen nach dem KRUSKAL-WALLIS-Test.

character according to table no.4	**morphological distance (d_m)**
length of peduncule to first cupule (es2)	0.9096***
length of petiole (bls)	0.8691***
maximum length of peduncule (es1)	0.8373***
petiole ratio (petratio)	0.7856***
percentage of veniation (perven)	0.7260***
pilosity (bha)	0.7018***
number of intercalary veins (bin)	0.6058***
auricle developement (boe)	0.5919***
longitudinal stripes on acorns (est)	0.5629***
number of sinuses (bbu)	0.5224***
basal shape of lamina (bba)	0.5083***
distance of sinus adjacent to widest part of lamina to principal vein (bb2)	0.5064***
total leaf length (totleaf)	0.4839***
lobe depth ratio (lobdepra)	0.4531***
distance from base to widest part of acorn (elb)	0.4493***
number of lobe pairs (bla)	0.4379***
length of acorn (el)	0.3999***
weight of acorn (eg)	0.3360***
lamina length (blb)	0.2758***
depth of sinus (sindepth)	0.2318***
width of acorn (eb)	0.1951***
maximum width of lamina (bb1)	0.1828***
number of cupules per fruitstand (ek)	0.1664***
distance from base of lamina to widest part (blh)	0.1538**
obversity (obversi)	0.1302***
ratio of lamina width to lamina length (leafrati)	0.0916**
mean distance over all characters	*0.4487*

The results of the different methods show similar tendencies for the morphological status of the species' hybrids. The families of the hybrids seem to have intermediate morphology if one compares the discriminant scores' means. The problem with this method is that one uses 2 fold means. So the information get lost about every character's distributions within each individual and about the discriminant scores' distribution within each group. The uses of discriminant scores also implies the

comparability of characters between the "standard" and the analysed material. It does not take into account the problems associated with the juvenile leaf morphology. The cluster analysis carried out with the character's means of every single tree made doubtful whether the comparison of juvenile and adult trees is correct. VERGA's method (1995) using the morphological distance avoids some of the disadvantages of the "classical" methods. Here the density distributions of single characters within individuals are directly compared, which keeps an important potential information. As a result the separation of the different families with VERGA's method is much clearer. By running a cluster analysis with morphological distances between individuals of the controlled crosses, their parents and the trees of compartment 66a one obtains a phenogram separating the 2 species on a level of about 70 %. Within every species the adult and the juvenile trees are separated, but none of the clusters contains individuals of just one family. This indicates that the variability within the families of the controlled crosses is considerably high. Another advantage of VERGA's method is, that parameters which are compatible with those arising from genetic inventories can be produced, because they have the same theoretical basis.

The trend of the different methods is the same. The leaf morphology of interspecific families is more similar to the leaf morphology of their female parent's species than to that of the male parent's species. At this age it is not possible to state on an individual level whether a tree is a hybrid or if it is an offspring of an intraspecific cross. Differences between inter- and intraspecific crosses can be detected in some cases on the level of families.

DNA analysis

The 7 primers produce 12 fragments which show significantly higher frequency differences between *Q. robur* and *Q. petraea* than alleles of isozyme analysis. No primer was found with a species' specific fragment. A DNA analysis of the hybrids was done to prove a Mendelian inheritance of the fragments (MOREAU et al., 1994). Eight fragments showed Mendelian inheritance assuming dominance relationships. They were used in the further analysis. These fragments do not give an exact information about the allelic frequencies. One can detect the homozygotes which have the recessive allele. But it is not possible to distinguish between the homozygotes and the heterozygotes which both have the dominant allele. In a strict sense the description of individuals by these RAPD fragments therefore is phenotypical because it is not possible to detect the heterozygotes. Another problem of this technique is that one does not know where the binding sites of the primers are located in the genome. That implies that the produced fragments do not have strictly defined characteristics. There is a good chance to end up in a non coding region, but nevertheless correlations can occur between interesting traits – morphological characteristics – and the occurence of an analysed fragment. Another problem of the RAPD technique is its great sensibility for changing reaction conditions. This problem can be handled if one regards very strictly the developed protocols of manipulations in the laboratories. The advantage of the RAPD technique is that one can test a potential infinitive number of primers. A knowledge of specific base sequences in the genome, as it is necessary for the RFLP technique, is not necessary for the RAPDs. The RAPD technique can also be used as a first step for the analysis of an unknown genome. The fragments can be

Table 6. – Summarizing parameters for the analysed RAPD fragments: OPF1b, OPF4a and b, OPF12a, OPF14c, OPF15a, 174h and $[GACA]_4g$.

Zusammenfassende Maße für die betrachteten RAPD Fragmente Abteilung 66a, 7 Primer mit 8 gelesenen Banden (OPF1b, OPF4a und b, OPF12a, OPF14c, OPF15a, 174h und $[GACA]_4g$).

group	**robur type**	**petraea type**
δ_{TP}	0,30	0,44
Phenotypic distance d_{op}	0,217	

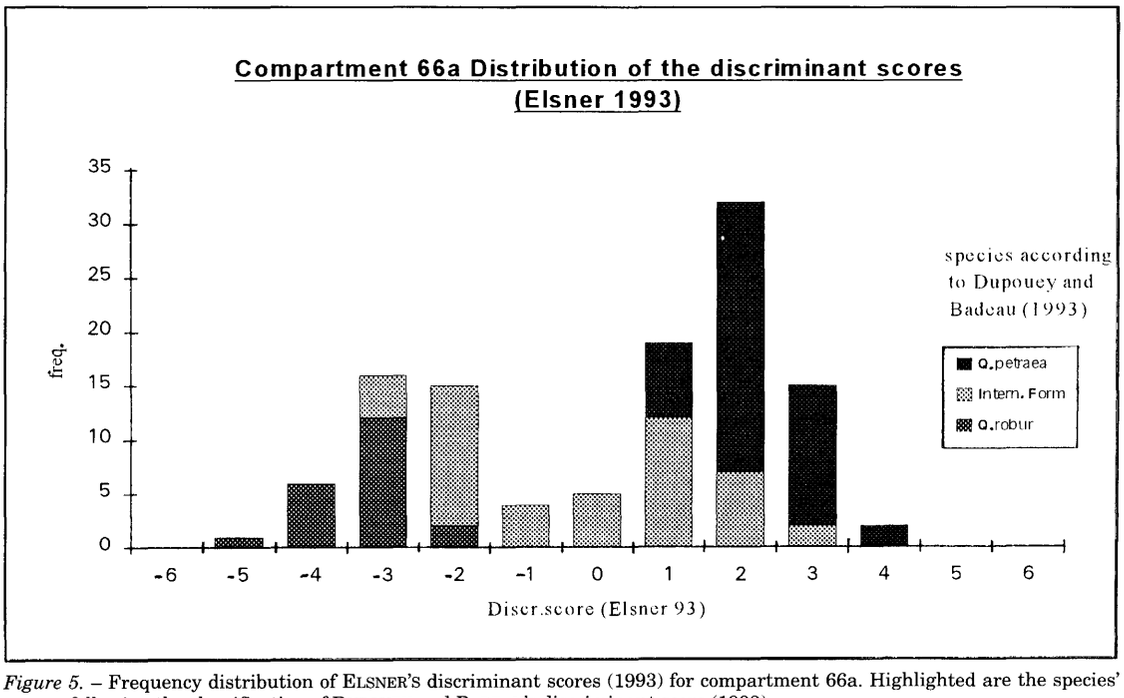

Figure 5. – Frequency distribution of ELSNER'S discriminant scores (1993) for compartment 66a. Highlighted are the species' groups following the classification of DUPOUEY and BADEAU's discriminant score (1993).

Häufigkeitsverteilung der Diskriminanzwerte nach Elsner (1993) für die Abteilung 66a, hervorgehoben sind die Artengruppen nach der Einteilung von DUPOUEY und BADEAU (1993).

isolated and then sequenced. So one gets the ability to construct new primers for the directed PCR technique, which avoids most of the problems of the RAPD assay.

Compartment 66a

Morphological analysis

Classical analysis of morphological data

Figure 5 shows the frequency distribution of the discriminant scores according to ELSNER. The interval of –1 to 1 represents intermediate forms. The distribution of the scores forms a continuum. The 2 species are by ELSNER's discriminant score not separated into 2 distinct groups. The region between the 2 maxima of the distribution represents – according to ELSNER – the intermediate forms. The groups indicated within the frequency distribution represent the species' groups according to DUPOUEY and BADEAU. This second classification is more conservative in discriminating the species than ELSNER's classification. Only the individuals with a "typical" morphology are classified as pure species, the rest is considered to be intermediate forms. The 2 discriminant scores give a different weight to the characters used for the species' discrimination.

Comparison of frequency distributions of morphological data

The results of the cluster analysis with morphological distances (d_m) between each of the trees of the compartment 66a are given in *figure 6*. All measured and estimated 26 characters were used for this analysis. Behind every number of the tree there is the species' classification from DUPOUEY and BADEAU's discriminant score indicated, with "qr" = *Q. robur,* "qp" = *Q. petraea* and "if" = intermediate form. The phenogram shows 2 main groups, which have 55% of their morphology in common. *Q. robur* and *Q. petraea,* classified by the discriminant scores, are clearly separated, whereas the trees which are classified as intermediate forms do not form a separate subgroup, but they are spread all over the 2 main groups. This fact in combination with the differences in classifying trees according to the different discriminant scores (*Figure 5*) points out clearly, no objective criterion exists for drawing limits between "pure species" and intermediate forms. Therefore the further analysis is done for the 2 main groups, which shall be called "petraea type" and "robur type". The morphological distances (d_m) of each character between these main groups are given in *table 5*. The characters are arranged in the sequence of their power to discriminate the main groups. The level of significance according to the KRUSKAL-WALLIS-Test for the differences of their expression of morphology is given for every d_m value (with * = 5%, ** = 1% and *** = 0.1% level of significance).

The characters which are the most powerful in distinguishing between the 2 main groups are those, which are given in the literature for the botanical description of the species *Q. robur* L. and *Q. petraea* (MATT.) LIEBL.. The fruitstand morphology is even more useful to distinguish between the 2 main groups than the leaf morphology. The value of the mean coefficient of variance (CV) for the "robur type" is 33%, the corresponding value for the "petraea type" is 36%. This indicates that the "petraea type" is a little bit more variable over all characters than the "robur type".

DNA analysis

The DNA analysis was carried out with the 7 primers (OPF1, OPF4, OPF12, OPF114, OPF15, 174, $[GACA]_4$). Only those fragments have been used for the analysis, which showed Mendelian inheritance assuming dominance relationships. The aim of this analysis is to show if it would be possible to distinguish between the two species by means of RAPDs. No species' specific fragment was found for the individuals of

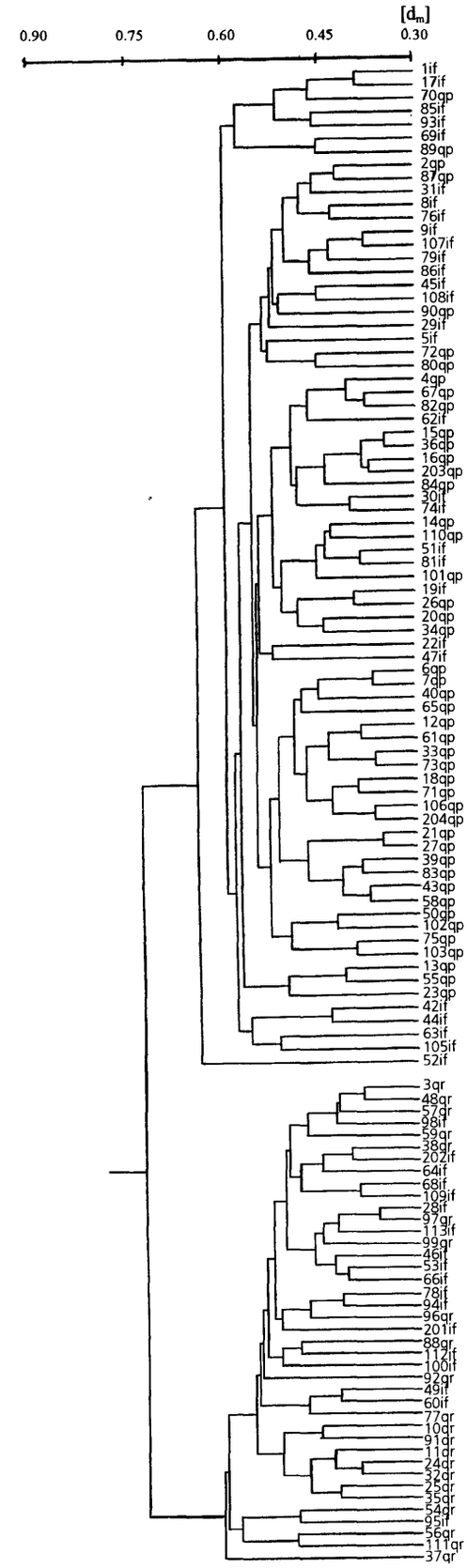

Figure 6. – Phenogram resulting of morphological distance matrix of compartment 66 a's trees.

Dendogramm aufgrund der Matrize der morphologischen Distanzen der Bäume der Abteilung 66a.

Table 7. – Allelic frequencies in sessile oak types and pedunculate oak types, and tests of differences between types.

Allelhäufigkeiten der „robur" und „petraea" Tapen und Test auf Homogenität zwischen den Typen.

		Alleles							Test of differences			
type	loci	1	2	3	4	5	6	7	N	X2	d. f.	P level
robur	PGI	0.038	0.038	0.913	0.013				80	2.4	3	n.s.
petraea		0.013	0.060	0.900	0.027				150			
robur	PGM	0.211		0.776	0.013				76	9.1	2	*
petraea		0.087		0.913					150			
robur	IDH	0.013	0.137	0.788		0.062			80	16.3	4	**
petraea		0.068	0.041	0.851	0.027	0.014			148			
robur	MDH		1.00						80		0	
petraea			1.00						150			
robur	AAP				0.405	0.149	0.466		74	10.0	3	*
petraea					0.610	0.089	0.288	0.014	146			
robur	LAP		0.538		0.423	0.038			78	15.1	2	***
petraea			0.277		0.676	0.047			148			
robur	GOT		0.013	0.987					80	5	1	*
petraea			0.087	0.913					150			
robur	MR		0.95		0.05				80	17.3	4	**
petraea		0.067	0.733	0.047	0.127		0.027		150			
robur	6PGD	0.975					0.013	0.013	80	0.2	2	n.s.
petraea		0.980					0.007	0.013	150			
robur	ACP	0.795	0.141		0.064				78	26.9	2	***
petraea		0.567	0.433						150			

N = number of individuals, X2 = Chi-square test of differences, d.f. = degree of freedom, P. levels: n.s. = non significant, *) = 0.05, **) = 0.01, ***) = 0.001

compartment 66a. The problems of RAPDs have already been mentioned in chapter "Leaf morphology: comparison of frequency distribution". As we see in *table 6* the total phenotypic differentiation of the collective (δ_{TP}) is greater for the main group "petraea type" than for the "robur type". The phenotypic distance (d_{0P}) between the 2 types is 0.206. This means that the 2 main groups which arose from morphological analysis share 79 % of their RAPD phenotypes. This distance is smaller than the morphological distance (d_m) which was discussed earlier.

The species' groups, which had been build up after the leaf morphology, were separated with a factorial correspondence analysis (FCA) using the DNA data of compartment 66a. This separation is less clear than the separation after factorial discriminance analysis or after the calculation of morphological distances (d_m) using the morphological data. If one projects the RAPD data of the controlled crosses' first 2 families into the first 2 axes of the FCA, one can see 2 phenomena. 1.) The species' hybrids of the controlled crosses are located in the middle of the 2 species' groups of compartment 66a. 2.) The adult individuals with intermediate morphology – as classified by ELSNER's discriminant score – are spread over the whole range of individuals of the 2 "pure species". The morphological intermediate forms are not only allocated to the region of the species' hybrids between the pure species. Consequently the intermediate forms are not conclusively F_1 hybrids. They may also be all levels of backcrosses and the result of genetically fixed variation within the "pure species".

Genotypes at enzyme loci

MDH showed to be monomorphic for the compartment 66a. Seven loci produced differences in allelic frequencies which are statistically significant (*Table 7*).

Table 8 gives the genetic parameters, which were calculated on the basis of the allele frequencies within every "type" that derived from the morphological analysis. The mean number of alleles per locus (A/L) is 2.9 for the "robur type" and 3.1 for the "petraea type". MÜLLER-STARCK (1991) found values of 3.2 A/L for both species. LÖCHELT (1994) found values of 2.3 A/L for *Q. petraea* and 2.0 A/L for *Q. robur*. The differences may arise because of the different enzyme loci which were analysed and because of different sample sizes. In compartment 66a the values of the gene pool diversity (v) for the 2 types are within the range of values given in the literature for the 2 oak species (e. g. HERZOG and MÜLLER-STARCK, 1993). The values of actual heterozygosity (H_a) are 26.7 % for the "robur type" and 20.6 % for the "petraea type". ZANETTO et al. (1994) found values of H_a=18.4% for *Q. robur* and H_a=22.2% for *Q. petraea*. The conditional heterozygosity (H_c) for the "robur type" (59.9 %) is higher than the value given in literature for *Q. robur* (56.6 %, HERZOG and MÜLLER-STARCK, 1993). The conditional heterozygosity for the "petraea type" is 55.4 %, which is a little bit less than the value calculated by HERZOG and MÜLLER-STARCK (1993). The total differentiation of the genepool (δ_T) is 0.25 for the "robur type" and 0.28 for the "petraea type". These values are less than those calculated for the RAPD data, but they show the same tendency. The differentiation within the "robur

Table 8. – Summary of genetic parameters of the gene pool of compartment 66a for 10 enzyme loci.

Genetische Parameter der Abteilung 66a für 10 Enzymsysteme.

group	robur type	petraea type
A/L	2.9	3.1
gene pool diversity (v)	1.34	1.38
H_a	26.7%	20.6%
H_c	59.9%	55.5%
δ_T of the genpool	0.25	0.28
genic distance (d_0)	**0.139**	

A/L = mean number of alleles per locus, H_a = mean actual heterozygosity, H_c = mean conditional heterozygosity, δ_T = total differentiation of the collective.

type" is smaller than within the "petraea type". The genic distance (d_0) equals 0.139 which means that the 2 types have 86% of their allelic types in common. This value is smaller than the phenotypic distance (d_{0P}) and smaller than the morphological distance (d_m) between the main groups.

The study compared parameters calculated on the basis of 3 different traits: leaf and fruit-stand morphology, RAPD fragments and genotypes at enzyme loci. The analysis of the trees' collective in compartment 66a by means of the morphological distance gave evidence for the existence of the 2 taxonomic species *Q. robur* and *Q. petraea*. But non of the analysed morphological characters has 2 disjunctive species' specific expressions. The 2 "types" which were separated by means of morphological traits also show differences for the 2 other traits, for which they were analysed. These traits vary in the extent of the differentiation between the taxonomical species *Q. robur* L. and *Q. petraea* (MATT.) LIEBL.. The differentiation between the types decreases from morphology over RAPD to isozymes. The status of a morphological intermediacy depends upon the subjectively chosen limits of "pure species'" variation.

No objective criterion exists for the exact morphological description of the 2 taxonomical species. The RAPDs were thought to supply a tool of species' identification in the juvenile stage. They showed strong correlation with morphological characteristics only if one includes all 12 RAPD fragments into the analysis. The problems which arose from this technique questioned the ability of the technique to identify the species in practice. Nevertheless the technique of RAPD provides a tool for the analysis of an unknown genome.

In this study the isozymes were the only traits, which showed to be a genetic marker in a strict sense. The problem with the isozymes is that only a restricted number of enzyme systems can be analysed. Therefore only part of the differentiation of the whole genome can be detected with this method. Thus the differentiation between collectives found by isozymes is usually less than the differentiation found with other methods.

Comparison of stands

The French and the German stands were compared to find out if any geographical factor of the species' variation exists. The comparison of the French stand "Petite Charnie" and the German stand "compartment 66a" was carried out on the basis of morphological, RAPD and isozyme analysis. The morphological distances (d_m) were calculated for a dataset comprising data of the sampled trees of Petite Charnie and of all the trees of compartment 66a. Fifteen leaf morphological characters have been used. The phenogram resulting from the distance matrix showed four main groups: 2 groups consisting of the trees of compartment 66a and 2 groups consisting of the trees of Petite Charnie. The groups of compartment 66a were the same as those shown in *figure 6*. Only 2 individuals (tree no54 and tree no95) which are situated in *figure 6* in the group of the "robur type" were now found in the group of the "petraea type". This may be due to the reduced number of morphological characters, which were included in the analysis. The groups which include the trees of Petite Charnie separate the species as they are indicated by DUPOUEY and BADEAU's discriminant score (1993). The intermediate forms are located in both groups. The morphological distances between the 4 groups were calculated.

The distances between the stands within one species type are smaller than those between the different types within one stand. The phenogram resulting from the distance matrix is shown in *figure 7*. The collectives of the same species from the different stands are more similar than the collectives of different species which are situated in the same stand. Each character's morphological distance (d_m) between the different collectives was calculated.

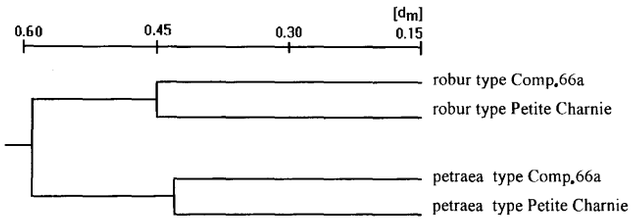

Figure 7. – Phenogram or morphological types of the oak stand Petite Charnie and the oak stand compartment 66a based on 15 leaf morphological characters (bla, bbu, bin, bls, blb, blh, bb1, boe, bba, bha, perven, totleaf, petratio, obversi, leafrati) (for abbreviations see *Tab. 3*).

Dendogramm der morphologischen Typen der Eichenbestände Petite Charnie und Abteilung 66a aufgrund der Analyse von 15 Blattmerkmalen (bla, bbu, bin, bls, blb, blh, bb1, boe, bba, bha, perven, totleaf, petratio, obversi, leafrati) (für Abkürzungen siehe *Tab. 3*).

The characters which distinguish most powerfully between the species' "types" are the same for the 2 stands (bls, petratio, perven, bha; for abbreviation see *Table 3*). The "robur type" and "petraea type" are clearly separated within one stand by the characters which are mentioned in the botanical descriptions of the species *Q. robur* L. and *Q. petraea* (MATT.) LIEBL.. The characters which discriminate the same "types" of different stands are others: The combined characters of leaf size measurements – as there are the length of the lamina, the width of the lamina and the lenght of the petiole – and the original characters discriminate mainly the geographically separated groups. These differences can have as well genetic (provenance variation) as environmental reasons (site characteristics). For oaks significant provenance differences are described for other than the leaf morphological traits (KRAHL-URBAN, 1959; JENSEN, 1993; KLEINSCHMIT, 1993). CIESLAR (1923) reports on smaller leaf sizes of oceanic provenances for *Q. robur*. Genetic differences between the stand of Petite Charnie and compartment 66a are also found in this study (see following paragraphs).

Table 9 shows the phenotypic distances (d_{0P}) based on RAPD analysis of the morphological types. The phenotypic distance between the types of compartment 66a is smaller than the morphological distance (d_m) between these types whereas the phenotypic distance (d_{0P}) between the types of Petite Charnie is greater than their morphological distance (d_m). This may be because in the French stand 38 individuals had been chosen for DNA analysis which represent with their leaf morphology the typical species. The phenotypic distances between the same types of the 2 stands is small compared to the distances between the types within one stand.

Table 9. – Phenotypic distance (d_{0P}) after analysis of 8 RAPD fragments (OPF1b, OPF4a and b, OPF12a, OPF14c, OPF15a, 174h and $[GACA]_4g$).

Phänotypischer Abstand nach der Analyse von 8 RAPD Fragmenten (OPF1b, OPF4a und b, OPF12a, OPF14c, OPF15a, 174h und $[GACA]_4g$).

group	robur type Comp.66a	petraea type Comp.66a	robur type P.C.	petraea type P.C.
robur type Comp.66a	0.000			
petraea type Comp.66a	**0.217**	0.000		
robur type P.C.	0.087	0.250	0.000	
petraea type P.C.	0.552	0.354	**0.599**	0.000

Regarding the genic distances (d_0) after isozyme analysis (*Table 10*) one finds the same tendencies. The absolute values of the genic distances are smaller than the phenotypic distances. The distance between the types in Petite Charnie (classified with a different method as mentioned above) is about the same as the corresponding distance of compartment 66a.

Table 10. – Genic distance (d_0) according to allelic frequencies of 8 loci (PGM, PGI, IDH, ACP, LAP, AAP, MR, GOT).

Genischer Abstand aufgrund der Analyse von 8 Enzymgenloci (PGM, PGI, IDH, ACP, LAP, AAP, MR, GOT).

group	robur type Comp.66a	petraea type Comp.66a	robur type P.C.	petraea type P.C.
robur type Comp.66a	0.000			
petraea type Comp.66a	**0.173**	0.000		
robur type P.C.	0.078	0.211	0.000	
petraea type P.C.	0.144	0.053	**0.183**	0.000

Summarizing one can say that the relatively smallest distances for every trait occur between the same morphological types of the different stands except for the phenotypical distance (d_{0P}) between the "petraea type" of Compartment 66a and the "robur type" of Petite Charnie. This exception does not occur if one includes the 4 fragments OPF12c, OPF14a, 174 g and $[GACA]_4f$ in the analysis. The morphological distances show the absolute greatest values compared with the other traits (exception: "robur type"- "petraea type" in Petite Charnie with their phenotypic distance of the RAPDs).

There is a good correlation between the distance matrixes of the morphology and isozymes ($r = 0.71$) whereas the correlation is poor between the distance matrixes of the morphology and RAPDs ($r = 0.37$) on one side and isozymes and RAPDs ($r = 0.26$) on the other sides. None of the values is significant according to MANTEL's Z-test of matrix correspondence. The values of the product-moment correlation are higher (0.71 for the morphology and RAPDs and 0.84 for isozymes and RAPDs) if one includes the four RAPD fragments into the analysis which did not show a Mendelian inheritance. The higher correlation might be important if one uses RAPDs for distinguishing between the 2 species.

Discussion

It is obvious when analysing the morphology of the controlled crosses over the first 5 years, that a juvenile leaf morphology exists, which differs clearly from the morphology of adult individuals. During the first 5 years the "species' hybrids" have a leaf morphology which resembles the morphology of the female parent very much. Only in some cases significant differences could be detected on the level of families. Difficulties for the detection of hybrids arise due to this similarity of leaf morphology of the female parent's species on one side and that of the offspring on the other side. If the leaf morphology of the controlled crosses remains stable, in the forests it is not possible to detect hybrids as intermediate morphological forms on the individual level. On the family level it may be possible, when the frequency distribution of discriminant scores for the hybrids tends towards the center between the 2 species, as it was shown for the "hymr" group of the controlled crosses. The morphological intermediate forms can be either an expression of the variability of the pure species or F_1 hybrids or all levels of backcrosses. This statement is supported by the comparison of RAPDs of the two interspecific crosses and of the individuals of compartment 66a. Due to the results derived from the leaves' morphological characters of the controlled crosses the first working hypothesis has to be rejected: On an individual level hybrids can not be detected morphologically.

The DNA analysis of controlled crosses allows an identification of hybrids and the control of crossing techniques. The analysis of the controlled crosses also revealed that the technique of PCR, using random primers to find DNA polymorphisms (RAPD), has to be used very carefully because of problems of the DNA amplification and because of the problems of band intensities.

Summarising the results of the analysis of compartment 66a with the 3 different traits it has to be stressed that up to now no species' specific marker exists for the taxonomical species *Q. robur* and *Q. petraea*. Therefore the second working hypothesis of 2 clearly distinct species *Q. robur* and *Q. petraea* has to be rejected, too. The different species' types which can be separated for morphological and ecological traits may represent different ecotypes (MAYR, 1967) of the same species "*Q. robur* L." and they should be treated as subspecies (*Q. robur*, ssp. *robur* and *Q. robur*, ssp. *petraea*) if one accepts MAYR's definition of the biological species (MAYR, 1967): "Species are defined as groups of actually or potentially intermating natural populations, which are reproductively isolated from other such groups." (authors translation).

The controlled crosses proved that hybridization between the 2 ecotypes is possible. The analysis of natural regeneration in a mixed oak stand (BACILIERI, 1994; BACILIERI et al., 1996) gave an evidence for hybridization in nature. The hybridization with *Q. petraea* as pollen parent had higher fertility rates than the reciprocal cross (STEINHOFF, 1993; KLEINSCHMIT and KLEINSCHMIT, 1994). This means that the geneflow is asymmetric. Geneflow between collectives results in the exchange of genetic information. Nevertheless the two types show differences in all traits which have been analysed. If these differences are stable there must be a selection as a second mechanism beside that of the geneflow to stabilize the system of two ecotypes within one biological species. The traits which show a great differentiation between the ecotypes should have an adaptive potential for the types. The pilosity of leaves lowers the transpiration. The pilosity of leaves of *Quercus petraea* may therefore be an adaptation to dryer site conditions. The canopy of *Q. petraea* is described to be denser than that of *Q. robur*. The length of the petiole is a criterion for the discrimination between the 2 ecotypes. Maybe this character – in combination with the different branching type – enables *Q. petraea* to be more effective in photosynthesis and as a consequence to be more competitive than *Q. robur*. The weight of the acorns, their form and their size are important characteristics for the transportation of acorns by jays (DUCOUSSO and PETIT, 1994; STIMM and BÖSWALD, 1994). This supports especially the migration of *Q. robur* ($\approx$500 m/yr) which has bigger acorns. *Q. robur* is described as pioneer species whereas *Q. petraea* follows during the succession. The size of the acorns and the juvenile growth of the plants are positively correlated. Thus *Q. robur* seedlings are able to evade herb and shrub competition faster than *Q. petraea* seedlings do. This characteristic should be more important for a pioneer species than for a species which follows primary colonisation of a site.

Morphological and RAPD data are siutable to discriminate the 2 ecotypes on the individual level using multivariate statistics. Nevertheless the exact line between the pure types has to be drawn subjectivly. Using isozymes one may find differences on the population level. The isozyme analysis detects differences in the allele frequencies of the different types. The morphological types cannot be separated on the individual level using isozyme analysis.

Comparing the results of the different traits one can see similarities in the trends. The further away a trait is from the genetic control the bigger is the differentiation between the ecotype groups. This may be due to the greater adaptive importance of these traits. The absolute amount of differentiation depends very much on the analysed characters for morphological traits. Summarizing all traits there is a trend for the variation within *Q. petraea*, measured as differentiation of the collective (δ_T), is a bit greater than within *Q. robur* (*Table 6* and *8*). Taking the results about the morphology of *Q. robur* and *Q. petraea* one has to interpret earlier studies about the genetic variability of these species carefully, if they do not give an exact description of the characters which have been used for species' discrimination.

Important for further scientific analysis of the complex *Quercus* will be the observation of leaf morphology of the controlled crosses and the comparison of the above described traits in mixed stands with natural regeneration.

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Practical Implications of the Forest Genetic Resources Conservation Program in Germany

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Abstract

Conservation of forests genetic resources has political support in Germany. A concept for conservation has been developed by a working group with the aim to include all forest tree and shrub species. In situ conservation has priority and is supplemented by ex situ activities for duplicate protection in major species. In situ conservation activities are integrated into forest management. Ex situ conservation is a main activity in rare species.

The results of the activities up to the end of 1993 are presented. 2154 ha of in situ stands and 9350 single trees were selected and 817 ha seed orchards established. In addition 20 000 kg of seed from 2200 stands and 8600 single trees was stored. More than 25000 cm^3 pollen originating from 6400 single trees was sealed and conserved.

The experiences and problems with the execution of the program are described. A major constraint is the lack of knowledge for many of the minor species. More research and international cooperation is necessary.

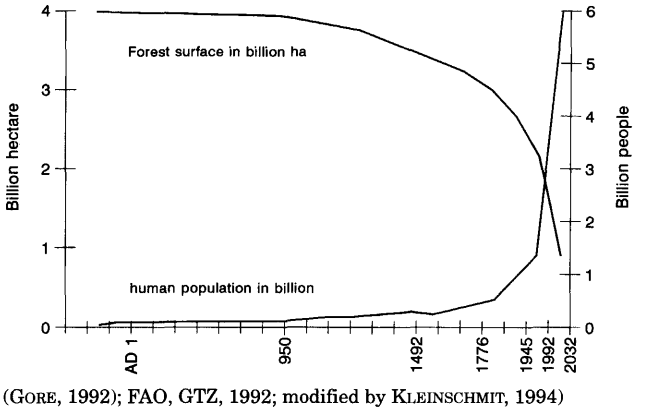
Key words: conservation, genetic resources, concept, in situ conservation, ex situ conservation, rare species.

FDC: 165.3; 165.4; (430).

1. Introduction

The intensive and increasing use of natural resources by mankind leads to a depletion in many fields. The reduction of forest surface is closely linked to the increase of human population (*Fig. 1*). Many species disappear in the tropical countries even before they are described.

Conservation of forest genetic resources became a major concern during the last 10 years. A comprehensive review of the global situation was published in 1991 (Board on Agriculture, 1991). As well during the United Nations Conference on Environment and Development, held in Rio de Janeiro, Brazil, in June 1992 as in the Conferences of European Ministers for the protection of forests in Straßburg and Helsinki conservation of biodiversity was a topic of central interest. 37 European States signed in Helsinki guidelines for the conservation of biologic diversity of European forests.



(GORE, 1992); FAO, GTZ, 1992; modified by KLEINSCHMIT, 1994)

Figure 1. – Global development of forest surface and human population.

In Germany the increasing forest damages due to immissions and the prospects of global warming gave rise to intensive discussions about the need of conservation of forest genetic resources between forest geneticists and forest tree breeders. This stimulated an unanimous resolution of the Federal Assembly in 1985 which gave the conservation of forest genetic resources high political priority. A working party was established to develop a forest genetic resources conservation program for the Federal Republic of Germany. HEINRICH MELCHIOR was the chairman of this working group. Thanks to his balanced view it was possible to finish the draft for this program in 1987, which was published in 1989 (Bund-Länder-Arbeitsgruppe, 1989). This program became part of the Federal Program for plant genetic resources (BOMMER and BEESE, 1990) and was accepted as a base for further activities by the Federal and State ministers.