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GENE CONSERVATION IN EUROPEAN BEECH (*FAGUS SYLVATICA* L.)

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ABSTRACT

The European beech belongs to the most important broadleaved tree species in Europe occurring in various ecological conditions. After establishment of numerous provenance experiments (the last one containing 188 provenances and 23 trials), extensive genetic inventories in most part of Europe has been carried out. *In situ* and *ex situ* gene conservation in European beech is outlined.

Key words: *Fagus sylvatica*, genetic resources, gene conservation, genetic differentiation

INTRODUCTION

The European beech (*Fagus sylvatica* L.) is at present considered to be the most common economically important broadleaved tree species in Europe. The extent of beech forests (*Fagus sylvatica* and *F. orientalis* together) in Europe and Asia Minor varies between 17 and 20 million ha (*e.g.*, MILESCU *et al.* 1967 estimate 16.8 million ha) and represents approximately 10 % of European forests. The proportions of beech forests in individual regions represent frequently up to 30%, *e.g.*, the former Yugoslavia, Slovakia, Romania etc.

Both *Fagus sylvatica* and *F. orientalis* belong to the forest tree species with the widest natural range in the western part of Eurasia. *F. sylvatica* is distributed in western, central and southern Europe with individual occurrences in southern England and southern Scandinavia. *F. orientalis* is distributed in Asia Minor, in Caucasus, in the Amanus mts. (Syria), and in the Elburz mts. (Iran). Contact zone between the natural ranges of both species runs in northern Greece and Bulgaria (Fig. 1). Isolated occurrences of *F. orientalis* outside the natural range were recorded in eastern Serbia (GLIŠIĆ 1973), in Macedonia (ČERNAVSKI *ex* MILESCU *et al.* 1967), in Banate and Moldova (MILESCU *et al.* 1967), and in Dobrudja and Central Bulgaria (CZECZOTT 1932).

Problematic taxonomic identity of beech is in Crimea. POPLAVSKAJA (1928) described the beech in the Crimean peninsula as an independent tree species – *F. taurica*. Beech occurs in Crimea in two altitudinally separated zones. The lower zone was more frequently described as *F. orientalis* and the upper one as *F. taurica* (MOLOTKOV 1966, MILESCU *et al.* 1967), but WULFF (1932) describes it as *F. sylvatica*. It is necessary to point out, that the name *F. taurica* is used in a different way in the literature. While, POPLAVSKAJA described it as the intermediate form between *F. sylvatica* and *F. orientalis*, MILESCU *et al.* (1967) considers it an independent species and its occurrence is recorded not only in Crimea.

Further dubious taxonomic unit is *F. moesiaca* with the most frequent occurrence in Balkan. MIŠIĆ (*ex* GLIŠIĆ 1973) considers it an independent species of tertiary origin. It is most frequently considered the subspecies or variety of *F. sylvatica*.

From the given statements it is obvious that taxonomical status of the beech populations in a rather large zone is unclear. The original description of these taxa (*F. moesiaca* and *F. taurica*) was based mainly on the morphological traits of leaves, and seldom these presumed taxa occur in comparative provenance trials together with *F. sylvatica*. The aim of recent investigations carried out in Slovakia, France, and other countries is to describe both species – *F. sylvatica* and *F. orientalis* – using gene markers, to define the zone of introgressive hybridization and the limits or the direction of the gene flow exchange between both species, and to characterize the structure of diversity within the genus *Fagus* in Europe and western Asia.

NATURAL RANGE AND PRESENT DISTRIBUTION OF *FAGUS SYLVATICA*

The European beech (*F. sylvatica*) is a tree species of oceanic and suboceanic climate. Its eastern limit runs on the limit of continentality (STANESCU 1979). Except the continentality, the eastern limit is defined by the air humidity and late frost (PUKACKI 1990). Although it is resistant to fairly low winter temperatures which does not exclude it from the higher altitudes, it is sensitive to late spring frost – a limiting factor for lower altitudes with greater accumulation of cold air (BECKER 1981). This is a reason why the European beech does not occur in frost valleys or in regions with a more continental climate (*e.g.*, the valley of Ebro, Languedoc, Provence, the central Alps, the Hungarian and Vienna lowlands and the Hungarian plane as well as the inner part of Transsylvania (SVOBODA 1955). In southern Europe its occurrence is limited by the line dividing the zone with spring and autumn rains from the zone with winter rains. That is why in southern Europe it is a tree species of the higher altitudes (*e.g.*, Greece over 1,300 m, Apennines over 1,000 m, Sicily over 1,500 m). Thus European beech is a tree species of mountainous regions.

It requires a humid and moderate climate during the summer period, the sum of precipitations has to be higher than the sum of evaporation. Optimal sites for beech require at least a 5 month vegetation period, a mean annual temperature of 10 °C, a mean July temperature about 18 °C, and precipitation over 1,000 mm (precipitation optimum 800 to 1,000 mm). It is indifferent to soil substrata, except sandy, wet, and otherwise poor soils. In optimal conditions it, however, grows best on rich soils.

Climatic conditions in beech regions differ significantly from those in coniferous ones, especially Norway spruce. In the lowland regions these two species cannot coexist, in the mountains they are partially overlapping. Where the natural occurrence of beech ceases that of Norway spruce starts. In some regions protected from the sea beech forms the upper tree limit (Apennines, Vosges, Pyrenees, Eastern Carpathians, Balkan Peninsula etc.)

The largest continuous beech region in Europe occurs in the low forested Carpathians, which provide a range as optimal as possible for beech in west and southwest Europe. The growth and yield optimum for beech is in the fir-free beech altitudinal zone and in the lower parts of the fir altitudinal zone.

Eastern Europe and Carpathians

In eastern Europe, the European beech probably originated from the principal Balkan glacial refugium, from where it started to spread 13,000 years ago. Since the late Atlantic period, *i.e.* since approx. 3,000 years, it has become dominant practically in its whole present distribution range (HUNTLEY & BIRKS *ex* HORVAT-MAROLT 1992). Postglacial migration ways can be besides refugia other factors modelling the genetic differentiation pattern. In some parts of the distribution range of the European beech the allelic structure may be affected by gene flow from adjacent populations of the other beech species (*Fagus orientalis* in European Turkey and eastern Bulgaria, *F. taurica* in Crimea).

The forests of the Carpathians cover in total approximately 8 million ha, and the beech covers along the Carpathians 3.4 million ha. The proportion of beech in the Carpathians is approximately 32 %. It covers along the Carpathians the altitudes from 250 –300 m up to the upper tree limit. In Slovakia and Poland, the upper tree limit is formed by Norway spruce and the mountain pine belt, while in the western Ukraine the upper tree limit is formed by beech in altitudes of 1,250–1,380 m. In the eastern part of the Ukrainian Carpathians and in Romania, the upper tree limit is formed by spruce. Within the Carpathians European beech is besides Norway spruce and silver fir one of the most important tree species which form in mountainous regions the single-species as well as mixed forest stands.

GEOGRAPHICAL VARIATION

The number of provenance experiments with European beech is much lower than with any conifer species of economical importance. Within the last fourty years numerous provenance trials were established in Germany, Slovenia, Czech Republic, Slovakia, France, Denmark and Poland (see for review GIERTYCH 1990). They are characterized by a small numbers of used provenances and replications under different ecological conditions.

Due to the lack of provenance experiments with higher number of provenances which were established in more replications, there has not been the possibility to make any broader statements on geographic variation of growth traits. There is general tendency in west European trials (Denmark and Germany) confirming that the Carpathian provenances were growing better than those from Western Europe. However, there are many exceptions from this general statement and some provenances (*e.g.*, Sihlwald, Forêt Soignes etc.) showed very good growth performance and high quality. In the Czech and Slovak provenance experiments, the east Slovakian provenances were in general top- ranking; however, in other regions there were also found some provenances characterized by good growth performance. In general, the European beech is rather characterized by ecotypic mode of variation rather than by clinal variation.

Apart of growth traits (height, diameter and volume) and some qualitative traits (forking), the variation of flushing is one of the adaptive traits of importance. It reflects the existing possibilities of the utilization of individual, suitable performing provenances in afforestation or reforestation programs.

In one older provenance experiment BRINAR (1962) investigated the growth of 29 provenances of beech from Slovenia occurring along the altitudinal gradient and originated from the altitudes 310 to 1,360 m. Based on this material he found that the delay in flushing by one day corresponds to an altitude difference of 122 m, and a delay by one day in autumn coloring corresponds to 113 m in altitude.

VON WUEHLISH *et al.* (1993, 1995) found significant differences and a pronounced east-west trend. Eastern and southeastern provenances started to flush in German, French and Belgian provenance trials earlier than western ones, while the high correlations among flushing ranks in individual trials showed high stability with respect to this adaptive trait.

GENETIC RESOURCES

In most parts of the natural occurrence of the European beech, such as Carpathians, Balkan, the prevailing forest management practice in beech stands was the application of natural regeneration. Due to this fact, that natural composition of mixed forests with participation of beech has been preserved in many parts of the European natural range, the genetic structure of beech populations can be expected not to deviate significantly from the 'natural' one.

This is, however, not true in the western part of Europe, where the large proportion of indigenous beech forests were converted during the last two centuries into coniferous stands. This is true in Germany, the Czech Republic, the western part of Poland etc., where the originally higher proportion of beech stands was converted into Scots pine and Norway spruce stands, frequently on inappropriate sites for conifers. At present, these inappropriate silvicultural practices have significant consequences since non-indigenous coniferous stands suffer in many cases from environmental stress and air pollution.

This is the basic situation which we have to take into account when evaluating the genetic resources of beech in Europe. The natural range of the European beech in central, eastern and the southeastern Europe includes a large proportion of primary and well preserved secondary genetic resources of beech (virgin and natural forests) with original genetic structure. In the Carpathians remnants of beech virgin forests from several hundreds to several thousands of hectares (*e.g.*, Uholka, Carpatho-Ukraine with 11,000 ha) occur.

GENETIC MARKERS

Unlike the variation of phenotypic traits, marker gene loci in European beech have only recently become available for research. This was made possible through experiments conducted by earlier research workers. 40 years ago, NIELSEN and SCHAFFALITZKY DE MUCKADELL (1954) crossed several beeches in the Arboretum Hørsholm and out-planted their progenies. This enabled KIM (1979) to identify the first enzyme gene locus by studying the zymograms of parent trees and their offspring. Later THIÉBAUT *et al.* (1982) also used the Hørsholm material for his investigation.

PAULE (1992) and HATTEMER *et al.* (1993) published reviews of the isozyme systems, their biochemical analyses and controlling gene loci which were applied in genetic inventories of beech. They have listed in total 17 isozyme systems controlling 27 gene loci: ACO – 2, ACP – 2, DIA – 1, GDH – 1, GOT – 2, IDH – 2, LAP – 2, MDH – 3, MNR – 1, NDH – 1, PEPCA – 1, 6PGDH – 3, PGI – 1, PGM –1, PER – 2, SKDH – 1, and SOD – 1 gene loci.

MÜLLER-STARCK and STARKE (1993) and MERZEAU *et al.* (1989) studied inheritance patterns of the enzyme gene loci in progenies from controlled crossings and single trees, respectively.

Recently, SDS-PAGE was applied to find species-specific differences in the seed protein content of *F. sylvatica* and *F. orientalis* populations from Bulgaria. The comparative analysis of the their patterns showed a greater similarity than dissimilarity between both species BUSOV (1995) .

Finally, the polymorphism in the chloroplast genome have been detected by resolutive restriction site studies of PCR-amplified fragments. Eleven haplotypes, which could be phylogenetically ordered, were detected in a large survey (399 individuals in 85 populations) encompassing most of the natural range of the species (DEMESURE *et al.* 1996).

GENETIC DIVERSITY AND DIFFERENTIATION OF BEECH POPULATIONS

Extensive studies on the geographical variation of the European beech based on isozymes started in France. The first investigations used quite a small number if isozyme loci (3–6) (COMPS *et al.* 1987, 1990. In later studies the number of polymorphic isozyme loci increased to 10–16 (MÜLLER-STARCK & ZIEHE 1991, GÖMÖRY *et al.* 1992, HATTEMER *et al.* 1993, TUROK 1993, 1996 , LEONARDI & MENOZZI 1995, TRÖBER, 1995, LARSEN 1995).

In general, the genetic differences between stands of the same geographic region are ususally described to be low. The variation is reported to be greater in southern parts of the distribution range. With the exception of gene loci coding for peroxidases, the differences in allele frequencies do not indicate correlations with the climate of the place of origin (BARRIÈRE *et al.* 1984, COMPS *et al.* 1987, 1990). Allele frequencies at other loci such as *Got-1* seem to be related to merely geographic gradients of unknown selective pressure (BARRIÈRE *et al.* 1984, COMPS *et al.* 1991). If similar correlations of the type mentioned were detected independently in various parts of the distribution range, selection would have been considered as the causal

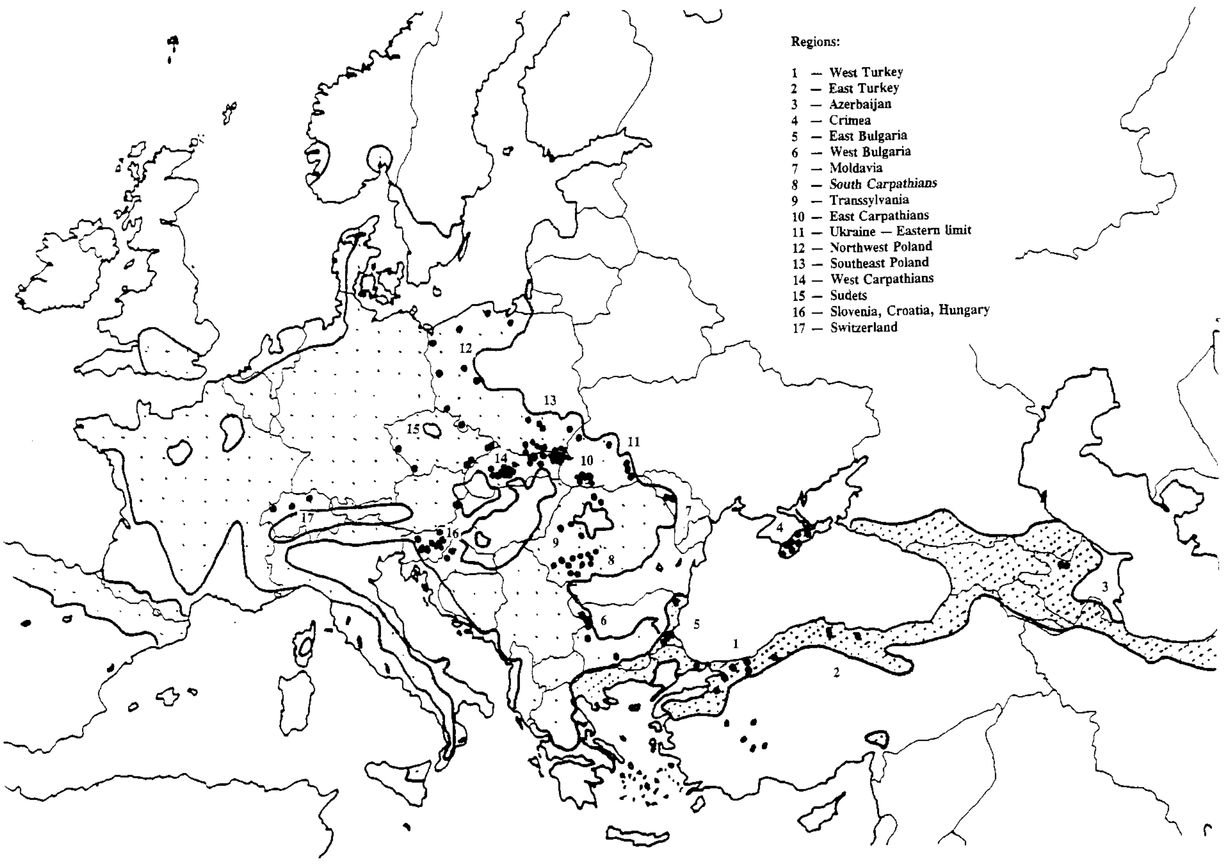

Figure 1 Natural range of *Fagus sylvatica* (free dotted) and *Fagus orientalis* (densily dotted). Bold dots represent investigated populations within the genetic inventory carried out in Zvolen laboratory (PAULE *et al.* 1995)

agent for differentiation between populations at these loci (PAULE 1992).

Western Europe

In Europe there have been several groups investigating the genetic diversity and differentiation of beech populations. The first one was the French group (THIÉBAUT, COMPS etc.) which started to investigate the genetic variation of west European populations. They have applied from the beginning only few isozyme systems (PX, GOT, IDH, MDH, PGI). Later the number of isozyme systems was increased (ACP, GOT, MDH, MNR, 6-PGD, PGI, PGM, PX, SOD). The German group (MÜLLER-STARCK) has independently developed procedures for staining and interpretation of numerous isozyme systems on starch gels which could be utilized for *Fagus sylvatica* as well as for *F. orientalis* MÜLLER-STARCK 1985, MÜLLER-STARCK & STARKE 1993)

Beech occurs in the larger part of western Europe except the Meridional part with a drier climate. In the southeastern part of France beech is growing in higher altitudes with more humid climate, but it can occur also in the lower altitudes with drier climate (THIÉBAUT 1984). In one of the first papers this group investigated populations from the two types of plant associations *Fagetum* and *Quercion pubescenti* with the help of isozyme markers. For the mutual comparison of peroxidases (isozyme systems sensitive to ecological conditions) were applied. The distribution of allelic frequencies showed correlation with the environmental factors. The allelic frequencies are not randomly distributed but they reveal geographic clines. They correspond to geographic variation, or they also correspond with the genetic migration, stochastic processes and the history of beech stands.

In another paper, COMPS *et al.* (1987) investigated 104 stands from the Atlantic region using four polymorphic loci (*Px–1*, *Px–2*, *Pgi–1* and *Got–1*). They found, that populations with the highest diversity occurred in southern regions – Spain, the Pyrenees, and Aquitaine. Those originating from the Pyrenees were characterized by the highest diversity. In contrast to the previous paper the allelic frequencies in the Mediterranean populations are independent from the ecological parameters. The selection effect seems to be revealed only at one single locus (*Px–1*). The total diversity of the

Pyrenees population could reflect great variation of microhabitats.

The genetic variation of beech stands depends on the ecological conditions. In the central part of the natural range it is continuous. In the marginal populations the variation is discontinuous being influenced by small effective population sizes and, besides that, a small gene flow occurs with regard to the distances and phenological differences.

Based on the French investigations, it could be concluded that there are no greater differences among individual regions based on allelic frequencies. The exception represent two gene loci (*Px–1* and *Got–1*). In the gene locus *Px–1*, the frequency of the most common allele increases from the 54 % in Scandinavia and in the Mediterranean region to over 72 % in Mediterranean region and the Pyrenees, 83 % in the Atlantic populations and up to 94 % in central Europe.

The frequency of the most common allele at *Got–1* increases from 49 % in the south European populations to over 75 % in the Mediterranean region and in Atlantic stands and up to 86 – 96 % in the remaining stands. The genetic diversity in peroxidases is smaller in populations growing under optimal ecological conditions.

VYŠNÝ (1991) and GÖMÖRY *et al.* (1992) investigated the genetic structures of 48 French beech populations which were selected within the gene pool conservation project. They used multilocus records based on 12 gene loci. The results have shown that the allelic frequencies of several loci correspond with the geographical longitude, or with the mean temperature or precipitations. The biggest difference were found between individual populations and regions in the following loci: *Px–2, Acp–1, Got–1* and *Idh–1*. The results have shown that the populations from northwest and northeast France are best separated from those originating from the Pyrenees, and the remaining populations have been characterized by the intermediate position. This fact can be considered to prove that these two population groups originated from two different refugia and were distributed by two different migration ways.

The German investigations were aimed on three different aspects of genetic studies:

- comparison of sensitive and tolerant sets of beech individuals within populations and of initial population and survivors (MÜLLER-STARCK 1993)
- genetic inventories of beech populations for gene conservation purposes (TUROK 1996)
- population genetic studies and reproduction processes (comparison of different ontogenetic stages) (MÜLLER-STARCK, STARKE).

Within the first studies, MÜLLER-STARCK (1985, 1989, 1993) investigated the impact of environmental stress on demes of "tolerant" and "sensitive" beeches. He found that genetic structures deviate significantly between tolerant and sensitive demes in adult stands and between initial populations and juvenile survivors. Tolerant subsets were characterized by greater heterozygosity and greater gene pool diversity. Losses of subsets were evident in surviving subsets.

TUROK (1993, 1996) found in Nordrhein-Westfalia and Rheinland-Pflaz high values of diversity as well as genetic differentiation within the populations. Genetic differentiation among populations revealed only modest geographic and/or ecological patterns. A group of indigenous populations from altitudes ranging between 300 and 600 m showed a higher degree of genetic similarity. Non-indigenous populations have rather heterogeneous genetic structures. Significant contribution to gene conservation are genetic investigations of reproductive processes in beech stands (HATTEMER *et al.* 1993, GREGORIUS *et al.* 1986, GREGORIUS & DEGEN 1994).

Within last years several other studies were published which aimed at genetic diversity and differentiation of indigenous beech stands included in the gene conservation programs. KONNERT (1996) investigated differentiation of beech populations from Bavaria and based on 16 isozyme loci found that very high proportion of variation is represented within individual stands (98 %). In a similar study, TRÖBER (1996) analyzed the remnants of indigenous Saxon beech populations (five stands). Based on 10 isozyme loci, she compared the diversity of parent stands with progenies grown in different nursery conditions (greenhouse and open nursery beds). LÖCHELT (1993) initiated genetic inventory in Baden-Württenbergian beech populations. She found that the differences among the individual stands were smaller that the variation within the stands.

LEONARDI & MENOZZI (1995) compared Italian populations and found higher levels of genetic variation in southern parts of peninsular Italy which provides evidence for a southern origin of beech and its northward recolonization.

Central and Eastern Europe

The first larger investigation of genetic diversity and differentiation was done with the collaboration of the French groups of Bordeaux and Montpellier and a Slovakian group (COMPS *et al.* 1990) and was aimed at populations originating from the transect leading from Poland through Slovakia and Croatia to Sicily and Corsica. This study comprised of 140 beech stands and was based on 6 common isozyme loci. It confirmed a strong differentiation of Corsican and Italian populations from those of Balkans and Central Europe. Surprisingly, Polish populations seemed to be also quite different, whereas those from Czecho-Slovakia, Bulgaria, Romania and Serbia formed a relatively homoge-

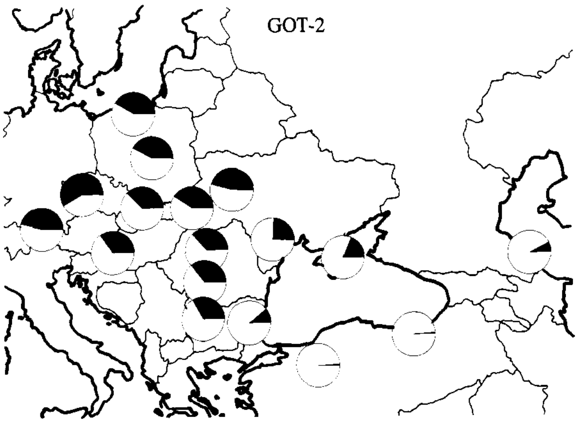

Figure 2 Allelic frequencies of *Got–2* obtained in the genetic inventory of East European beech populations

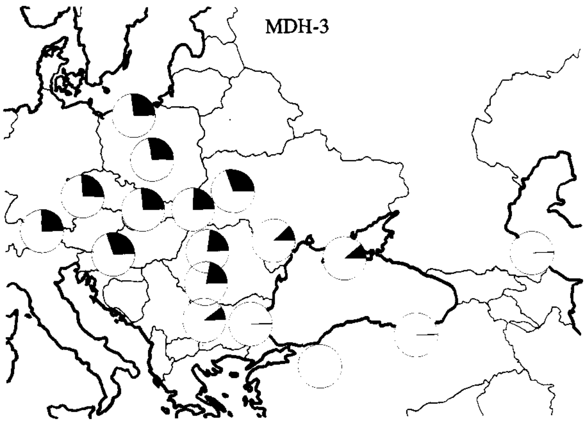

Figure 3 Allelic frequencies of *Mdh–3* obtained in the genetic inventory of East European beech populations

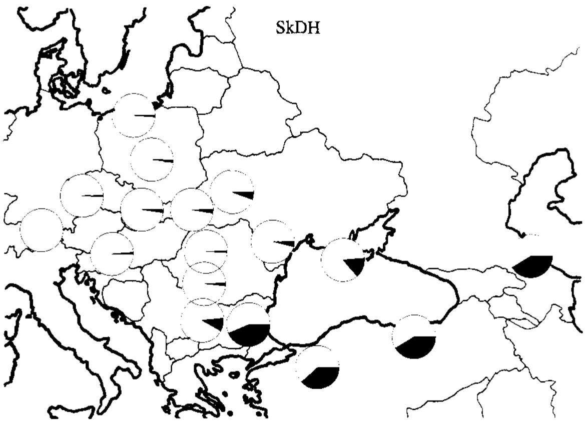

Figure 4 Allelic frequencies of *Skdh–1* obtained in the genetic inventory of East European beech populations

neous group. The distribution of genetic diversity was similar as found in the western Europe: higher diversity values were found in the southern populations.

Further studies by COMPS *et al.* (1991) were based on the same set of loci and focused on Croatia. Thirty-five populations originated from different climatic regions, soils and plant associations. They found quite few significant differences in allelic frequencies and gene diversities between the Mediterranean and continental regions, but many more differences were found between highland and lowland populations within the Mediterranean region as well as between populations belonging to the association *Seslerio-Fagetum* (on carbonate soils) and other associations.

Studies of GÖMÖRY *et al.* (1992) and VYŠNÝ *et al.* (1994) were based on the material from Czecho-Slovakia. In 10 of 12 investigated loci, a significant heterogeneity of allelic frequencies was found among populations. However, the variation among pooled Czech and Slovak populations was significant only at 4 loci. Clinal variation along the latitudinal, longitudinal, and altitudinal gradients was revealed for some alleles among the Czech populations. At the same time, significant correlations of frequencies of several alleles with indicators of air pollution were found.

In 1993, larger investigation of gene diversity and genetic differentiation of beech populations in eastern Europe and in the transition zone between *Fagus sylvatica* and *F. orientalis*, started in Zvolen (Slovakia). The results discussed here originated from the genetic inventory of 110 European beech populations from and 30 populations of *Fagus orientalis* and from the transition zone between these both species. Among the twelve isozyme loci studied only *Mdh–1* proved to be completely monomorphic in most populations. Five other loci (*Mnr–1*, *Mdh–2*, *Pgi–2*, *Pgm–1* and *Skdh–1*) exhibited generally a low degree of polymorphism as well. In total 42 allelic variants were identified, however, some of them with an extremely low frequency, thus contributed little to the allelic diversity. The overall mean number of alleles was 2.21, with slight differences among individual geographic regions. A slight marginal effect can be observed in the mean number of alleles. The Polish and Ukrainian populations situated on the range limit are poorer in alleles than the central ones. For most loci, there are significant differences in allelic frequencies among investigated regions at least at 95 % probability level. However, the differences among populations proved to be significant only in six loci, probably due to the reduced sample size. Some alleles (*Got–2(A)*, *Mdh–1(D, E, F)*, *Mdh–2(E)*, *Mdh–3(C)*, *Skdh–1(D)*) were specific for one or several adjacent regions, however, always on a very small frequency (PAULE *et al.* 1995).

Genetic differentiation pattern based on genetic distances revealed that Slovenia and Croatia are rather deviating regions, probably due to a different post-glacial origin of *F. sylvatica* from the Apennine glacial refugium (PAULE *et al.* 1995). This is also indicated by a continuous area with a high beech pollen occurrence from northern Italy to Slovenia and northern Croatia in

Preboreal and Boreal periods (HUNTLEY & BIRKS *ex* HORVAT-MAROLT 1992).

The remaining regions exhibit a more or less continuous trend from the Sudets through to the Carpathian arc. This trend may be again the result of postglacial migration paths. However, the effect of selection along the longitudinal gradient (*i.e.*, the gradient of continentality) can be expected.

Transition zone of *Fagus sylvatica* and *F. orientalis*

The last mentioned study revealed a clear differentiation of populations from Bulgaria and from the former Yugoslavia from the rest. These populations belong to the putative taxon *Fagus moesiaca*, which, following the description, should be a hybrid between *F. sylvatica* and *F. orientalis*. Another quite deviating region is Moldova on the eastern limit of the distribution range.

In order to distinguish the allelic frequency patterns of both main *Fagus* species, several *F. orientalis* populations originating from Turkey and Caucasus were analyzed. Isozyme loci *Got–2* and *Mdh–3* are almost or completely monomorphic in *F. orientalis*, while in *F. sylvatica* there occur two alleles with almost equal proportions. In opposite, major polymorphism were found in *Pgi–1*, *Mdh–1*, *Skdh–1* in *F. orientalis*, in contrast to *F. sylvatica*.

The species specific alleles (*e.g.*, *Mdh–1(A)*, *Mdh–2(D)*, *Mdh–2(E)*) have generally rather low frequencies, and the differences in the proportions of common alleles are not big enough to distinguish both species unambiguously. However, in Bulgaria, where the contact of natural ranges of both species occur, the difference between them is quite clear – the genetic structure of the population from the Strandja, which could be based on the morphology classified as *F. orientalis*, are by their genetic structure most similar to the west Turkish populations, while the beech in western Bulgaria (Rodopi, Rila) is genetically more similar to *F. sylvatica*. The alleles typical for *F. orientalis* (*Skdh–1 (D)*, *Pgi–2(A*,C), *Mdh–1(B, D)* etc.) can be found in western Bulgaria in higher frequencies. Also the allele representation in some loci (*Lap–1*, *Idh–1*, *Mdh–3*) is slightly shifted towards the structure of *F. orientalis* in Strandja and western Turkey (cf. PAULE *et al.*, in prep.). Introgression of both species in southern Europe seems to be limited for the territory of western Bulgaria and probably FYR Macedonia, northern Greece, and a part of southern Serbia. Admixture of genes of *F. orientalis* is also characteristic for populations from Moldova, while the migration is from the eastern direction. However, alleles characteristic for Eastern beech are sporadically occurring also in Romanian populations from the Southern Carpathians. It is difficult to define the limits of the gene flow, and we have not analyzed the populations from eastern Romania. Based on the allelic frequencies, it is possible to conclude that the direction of the gene flow prevails from the *F. orientalis* to *F. sylvatica*, rather than the opposite.

FOREST MANAGEMENT PRACTICES AND GENE POOL CONSERVATION

In situ gene conservation

The longest proved method of genetic diversity conservation in beech stands is natural regeneration. The large scale application of natural regeneration was possible due to shade tolerance of this species. Prolongation of the regeneration period by means of phytotechnical measures might probably compensate for temporal differences in the seed production structure (HATTEMER *et al.* 1995).

In general it has been found that it is more worth while to designate for conservation larger units than many smaller units within gene conservation practices *in situ*. Within a program of gene conservation *in situ* in Poland 13 selected seed stands were established with size 10–150 ha (JANSON & SULKOWSKA 1993). The same attempt has been applied in Slovakia and in the Czech Republic where size of the gene bases as the principal tool of *in situ* conservation covers 100–500 ha (PAULE *et al.* 1993, HYNEK *et al.* 1993). Within the French gene conservation program of the European beech there 20 regions of provenances were defined and within them selected stands of different size (in total 185 stands belonging to 20 regions of provenances and representing more than 11,400 ha) (TEISSIER DU CROS & BILGER 1995). In Germany both representativeness of stands and their overall performance are being used as criteria for the selection for gene conservation purposes.

Ex situ gene conservation

Due to environmental pollution in many parts of Europe it appears to be insufficient to rely on conservation *in situ* alone. Two principal possibilities for saving population resources *ex situ* are available. Static preservation in gene bank and dynamic conservation in plantations. For the first purpose the long-term term storage of beech nuts has been developed (SUSZKA 1996) which used to be in the past serious limiting factor of *ex situ* conservation.

In both cases, conservation *in situ* and *ex situ*, the limiting factor is design of gene sampling. For conservation *ex situ*, the seed collection should appear only after heavy seed crop in a large continuous stand of unreduced density. *Ex situ* conservation plantations have to be established in one year and thus representing large sample size of parent trees.

In general, the minimum sample size for preservation of gene resources for conservation *in situ* is not a significant problem for entire stands are usually a subject of natural rengeneration. In the application of conservation practices *ex situ* the sample size should be based on estimation of frequencies of rare alleles.

Natural Regeneration

The European beech is in a favorable situation in contrast to other tree species of economical importance. Due to its ecological peculiarities the most common silvicultural practice has always been natural regeneration. Sampling for the previous investigations was easy because in all cases we were dealing with naturally regenerated indigenous beech populations. There are, however, also in the eastern Europe traces of improper forest management of beech stands. Coppice stands, mainly in mixtures with oak, are characteristic for contact zones with agricultural land use in lower altitudes or in regions with lack of wood, *e.g.,* in Balkan. In western Europe, however, there were significantly more cases than in eastern Europe of previous deforestation and replacement of beech stands by more productive coniferous ones. Also known are many cases of artificially regenerated beech stands which originate in the last two centuries. One of the best examples of the introduction of non-indigenous provenances is in Danish forestry. Since the beginning of the 20th century the import of beech seed of foreign provenances was recorded and it is continuing up to the present time (LARSEN 1995).

The purpose of gene conservation is straight forward and its significance is supported by the prognostic changes of the climate. If, in the case of climatic changes the necessity of the replacement of the local populations by the southern ones occurs, it is very important to secure genetic resources will be available.

A European network for the Evaluation of Genetic Resources of Beech has been established and its main objectives are the following (MUHS & VON WUEHLISCH 1996):

1) Tree improvement:
   - testing suitability of provenances for different sites,
   - selection of basic material,
   - setting up recommendations for trade and use of provenances at national and international level;
2) Gene conservation
   - assessment of genetic and phenotypic variation,
   - development of conservation strategies,
   - evaluation methods and ecodistances;
3) Evolution biology
   - adaptedness, adaptability,
   - natural selection forces and their significance;
4) Research on impacts of global climate change;
5) Stimulation of European co-operation in forest research.

Within this network 15 provenance experiments were established in 1986, 1987, and 1988 containing a total of 188 provenances. In 1995, 23 field trials followed which include usually 49, some 100 or more provenances, and the nursery trial with 161 provenances. The field trials were established in spring of 1995 and are located in altogether 17 European countries throughout the range of distribution of beech and in one case even outside its range. The collected data will be managed centrally at a data base at Grosshansdorf (MUHS *et al.* 1996). Another provenance trial is prepared for sowing in 1996 which will include regions less represented in the previous experiments.

The main aim of these provenance experiments is to test the adaptation potential of individual provenances to changing environmental condition in reply to partially predictable climatic changes.

Artificial Regeneration

The investigations of genetic diversity and differentiation of beech populations all over Europe has been a good basis to better understand the structure of beech populations and the possibilities of seed transfer. Naturally, parallel establishment of provenance experiments is the best way to answer the questions of whether an intentional seed transfer could be a safe enough for the establishment of beech forest stands in conditions under environmental stress, or on abandoned agricultural lands outside or even inside the beech natural range.

Artificial regeneration will be a more common method of regeneration of beech stands mainly in the future. There are vast areas in western Europe which were converted from mixed or broadleaved stands to coniferous monocultures. It is a common case that these stands were established at non-appropriate sites and that their ecological stability is uncertain. Mainly in the air polluted areas of Germany, Czech Republic and Poland the consequences are visible. It is expected to reconvert these stands for mixed or broadleaved with higher resistance potential.

The second potential application of artificial reforestation is abandoned agricultural land where the selected material could be used, to establish mixed stands or broadleaved stands and to increase the potential of these newly established forest lands to adapt to environmental changes.

The act of sowing or planting stands outside the region of origin of a population is expected to induce a process of adaptation to the new local environment through viability selection. Adaptive processes will be facilitated in case of genetically highly diverse reproductive material.

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