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## Practical Implications of the Forest Genetic Resources Conservation Program in Germany

By J. KLEINSCHMIT

Lower Saxony Forest Research Institute, Dept. Forest Tree Breeding, Escherode

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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<sup>3</sup> 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.

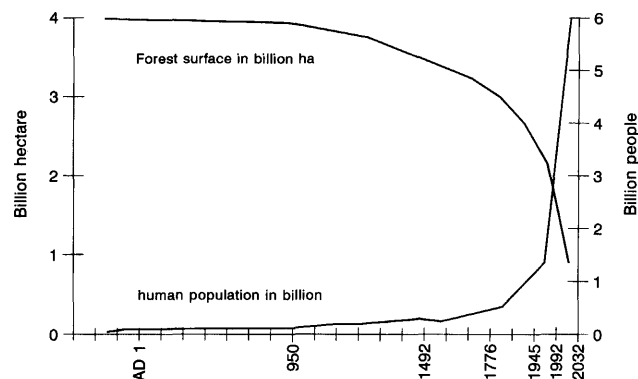
**Key words:** conservation, genetic resources, concept, in situ conservation, ex situ conservation, rare species.

**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



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

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

States signed in Helsinki guidelines for the conservation of biologic diversity of European forests.

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.

The program is under execution since 1989 and coordinated by the „Bund-Länder-Arbeitsgruppe Erhaltung Forstlicher Genressourcen“ (Federal-State-Working-Group conservation of forest genetic resources).

An increasing number of scientific meetings discuss the topic on national and international level (Arbeitsgemeinschaft für Forstgenetik und Forstpflanzenzüchtung, 1985; Forum Genetik-Wald-Forstwirtschaft, 1985; Institut für Pflanzenbau und Pflanzenzüchtung (FAL), 1985, ARBEZ, 1987; a meeting organized by MELCHIOR: STEPHAN (Ed.), 1990; HATTEMER (Ed.), 1990; MÜLLER-STARCK and ZIEHE, (Eds.) 1991; KLEINSCHMIT and WEISGERBER (Eds.), 1993; BML, 1993; MUHS and VON WÜHLISCH (Eds.), 1993; Dachverband Agrarforschung, 1994; BEGEMANN and HAMMER, 1994; TUROK et al., 1995; FRISON et al., 1995; e.g.) and the research efforts allocated to conservation of forest genetic resources are increasing. The international activities will be summarized during an FAO meeting in Leipzig 1996 where a global plan of action shall be disposed.

## 2. Present Situation of Forest Tree Species in Germany

Central Europe has, due to the losses during the glaciations, comparatively few tree species. The genepool of local populations is influenced by the size of the refugia, the ways the populations reinvaded and the size of the founder populations. After reestablishment competition between species became a major factor for population size.

In spite of the fact that existing older stands of European tree species are generally wild populations only marginally affected by artificial selection or breeding with the exception of *Populus*, human activities had severe implications on the population size, distribution and genetic structure of tree species.

This started with the clearing of two thirds of the land surface from forests, mostly on the fertile low elevation sites which affected before all hardwood forests adapted to these site conditions. The intense utilization of the remaining forest lands for wood, cattle grazing, forest litter utilization, charcoal production, and many other purposes since the early Middle Ages supportet certain species like oak and beech on expense of other hardwood species and led to an overutilization of forest lands (HASEL, 1985). During the 18th Century wood became rare regionally which gave rise to a forest management on sustained yield base. Extensive plantation forestry started partly with seed transfer over large distances and a preference of conifers. This resulted in a drastic change of species composition. Compared to 1300 where hardwood forest covered 75% of the forest land and conifers only 25%, in 1937 72% of the forests were conifers and only 28% hardwoods (HASEL, 1985). Regular forest management concentrated on few species which further reduced the population size of all other forest tree and shrub species.

Parallel to this development in forestry the changes in agriculture led to intensive utilization of the land and destroyed the ecological niches of many minor tree species with low competitive ability.

Since 1980 the consequences of air pollution on forests became obvious and tree populations in exposed areas had severe losses.

The knowledge of the present range, the variability in morphology, phenology and isozymes is quite good for the major tree species like *Picea abies* (34% of forests), *Pinus sylvestris* (30%), *Fagus sylvatica* (21%), *Quercus robur* and *Quercus petraea* (8,5%), *Larix decidua* (1%), Douglas-fir (1% to 2%), however very scarce, for the rest of the 35 to 40 tree species native to Germany.

It was only recently that most of the public forest administrations in Germany changed their policy in direction of an ecological oriented unevenaged forest management with the participation of minor species, more natural regeneration and the aim to increase hardwood participation considerably. This development is supported by the fact that valuable hardwood timber from tropical countries becomes more rare and prices for indigenous noble hardwood timber increases considerably. The public critics against exploitation forestry accelerated this development.

Therefore all forest owners get progressing interest into plantation of rare tree species.

## 3. Main Aims of the German Concept for Conservation of Forest Genetic Resources

The objective of the German program (*Tab. 1*) is the exploration, conservation and evaluation of all forest tree and shrub species in their regional patterns of adaptation and their genetic diversity. Research is an important component. The concept includes as well indigenous tree and shrub species as exotic tree species which were successful in plantations. In situ and ex situ conservation are handled parallel (*Tab. 2*).

*Table 1.* – Objectives of the German Program.

- Comprehensive conservation of all woody species in the forests
- Preference of in situ conservation to ex situ conservation
- Selection of 1% to 2% of species area for conservation stands in major tree species and up to 100% in rare species
- Principle of duplicate protection
- Integration into regular forest management as far as possible
- Regional work-sharing
- European cooperation
- International cooperation especially for exotics and research

*Table 2.* –

### *In situ*

Nature preservation areas

Conservation stands

Single trees

### *Ex situ*

Plantations

Seed orchards

Clonal archives

Seed in storage

Pollen in storage

Tissue in storage

(Propagations methods)

In situ conservation has preference wherever this is possible to maintain the material under the dynamic influences of evolution. This has however limitations in minor species, if populations of sufficient size do not exist and in major species if the population under consideration is endangered by human interference (e.g. pollution, lowering of ground water level). Duplicate protection is done as far as possible to compensate for unintended losses by storm, fire, insect damages or drastic environmental changes. This is most simple with long term

seed storage if possible. There are however limitations with recalcitrant species, where ex situ plantations or seed orchards have to replace seed storage. On experimental scale storage of excised embryos in liquid nitrogen is tested.

Minor tree species, where natural populations of sufficient size are missing or exceptional, are collected, grafted and planted in regional seed orchards. This procedure ensures on the one hand the reestablishment of breeding populations of sufficient size and prevents on the other hand introgression of undesired genes e.g. into wild fruit species from horticultural varieties.

In situ conservation is integrated into regular forest management as far as possible. This is usually feasible, since the only restriction for management is, that the population has to be naturally regenerated or replanted with seed originating from a representative collection of the same stand. In situ conservation of single trees finds it limits with the life-span of the tree and has to be supplemented by ex situ measures anyhow.

Regional cooperation on the base of the federal states and coordination by the „Bund-Länder-Arbeitsgruppe Erhaltung forstlicher Genressourcen“ is another principle to make optimal use of the administrative structures and to handle the constitutional situation with the responsibility of the states for forestry.

European cooperation is intended but only beginning with EUFORGEN, located at the International Plant Genetic Resources Institute in Rome, and with coordinated research efforts of different European countries supported by the European Union. International cooperation has a long tradition in the frame of IUFRO and this will have to be further extended for conservation strategies.

#### 4. Present State of the Program

The German working group "conservation of forest genetic resources" (Tab. 3) submits every second year a report to the government. The last report was in December 1993. At this time 1915 ha of in situ hardwood stands and 239 ha of conifer stands had been selected (Fig. 2).

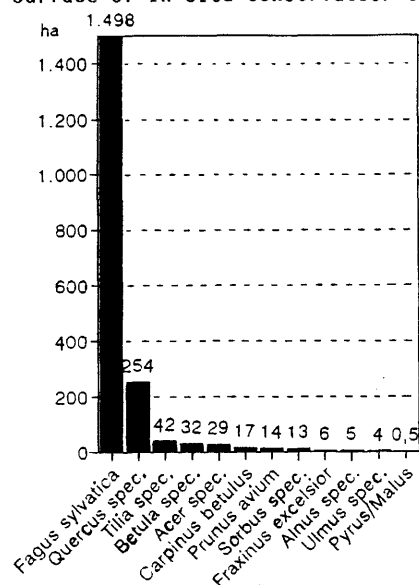
In the hardwood stands *Fagus sylvatica* has the highest representation with 1498 ha due to the fact, that this species was very little planted and thus a selection of conservation populations can follow ecological gradients. The indigenous *Quercus* species (*Q. robur* and *Q. petraea*) are situated on second position. All the other hardwoods have up to now only minor areas contributed which also reflects the fact, that these species are comparatively rare today.

In conifers *Picea abies* and *Pinus sylvestris* are prevailing. These stands however represent only a minor part of the

Table 3. - Members of the German Federal-State-Working Group "Conservation of forest genetic resources".

A. BEHM	Bavarian Institute for Seeding and Planting, Teisendorf
H. DÖRFLINGER	Federal Ministry for Food, Agriculture and Forestry, Bonn
A. FRANKE	Forestry Research Institute of Baden-Württemberg, Freiburg
Dr. J. KLEINSCHMIT	Lower Saxony Forest Research Institute, Escherode
Prof. Dr. N. KOHLSTOCK	Federal Research Institute for Forest Tree Breeding, Waldsiedersdorf
Prof. Dr. H.-J. MUHS	Federal Research Institute for Forest Genetics, Großhansdorf
H. P. SCHMITT	State-Institute for Forestry Northrhine-Westphalia, Arnsberg
Prof. Dr. B. R. STEPHAN	Federal Research Institute for Forest Genetics, Großhansdorf
U. TABEL	Forestry Research Institute of Rhineland-Palatinate, Trippstadt
Dr. F. WEISER	Forestry Research Institute Eberswalde, Waldsiedersdorf
Dr. Dr. habil. WEISGERBER	Hessian Forest Research Institute, Hann. Münden
Dr. H. WOLF	Saxonian Institute for Forestry, Graupa

Surface of in-situ-conservation stands (hardwoods)



Surface of in-situ-conservation stands (conifers)

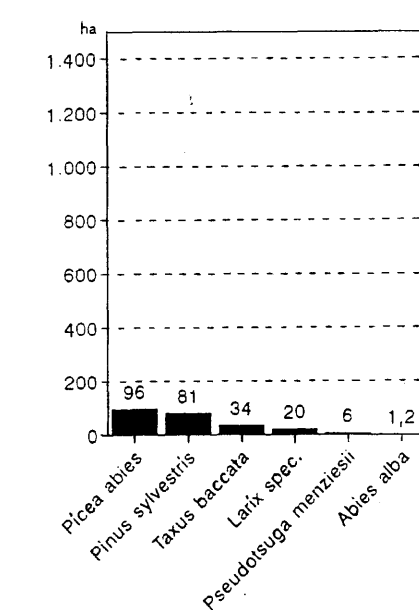


Figure 2.

interesting populations. Efforts were directed towards hardwoods during the recent years since these had been largely neglected in breeding and selection programs in the past but at the same time have increasing importance in plantation programs today. Only in *Taxus baccata* the conservation stands represent a considerable part of the existing populations.

The in situ stands are supplemented by ex situ plantations, which include for example more than 800 ha for Douglas-fir.

Figure 3 presents the situation for in situ single tree conservation. Here again hardwoods are prevailing with altogether 6375 trees. The species with the highest numbers are the most rare species where no or only few in situ populations were found and where the main efforts were concentrated to get an as complete exploration as possible.

For the main hardwood species in situ single trees mostly represent special forms or outstanding individuals.

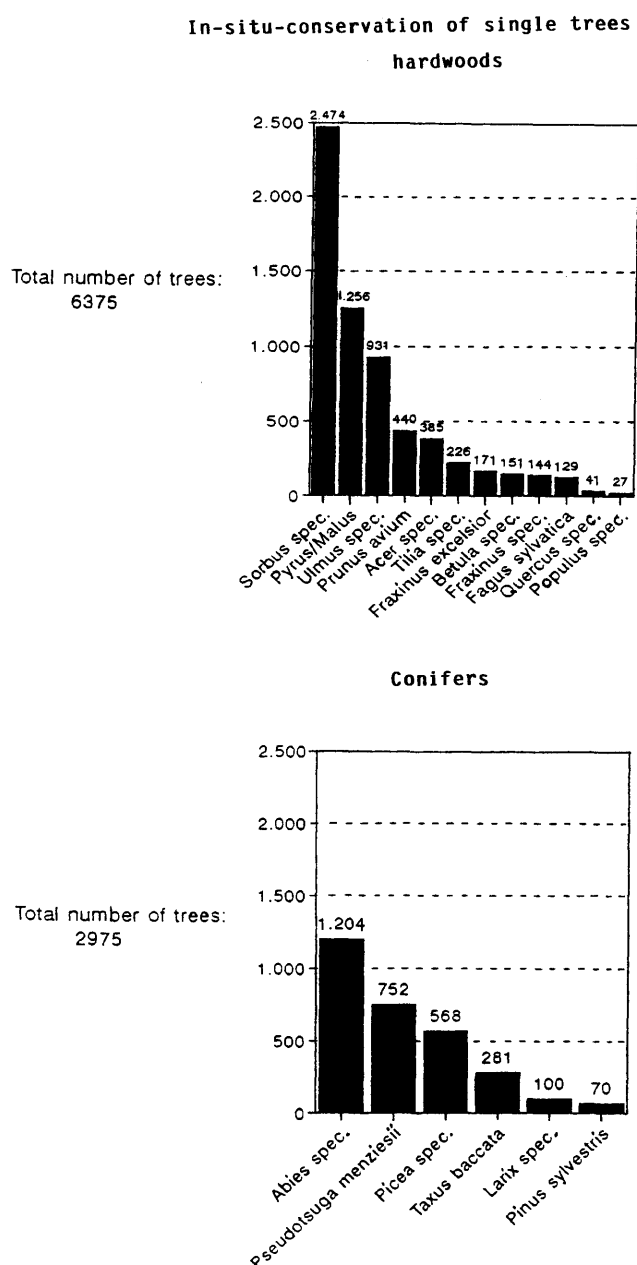


Figure 3.

For conifers less than 50% of the single trees in hardwoods were selected for conservation. Here *Abies alba*, which is most affected by immission damages, is prevailing with 1204 trees followed by *Pseudotsuga menziesii*. These trees originate from the outstanding old plantations of the first Douglas-fir introductions, probably Columbia river origin, which gave rise to include Douglas-fir into the plantation programs.

The seed orchards (Tab. 4) include as well the already existing seed orchards from breeding programs – before all conifers (550 ha) – as seed orchards of hardwood species (368 ha) which mostly have been established within the frame of the conservation program. The considerable number of seed orchards with minor species reflects the efforts into conservation of these species during the recent years. At the same time every seed orchard can be used as a separate breeding population to procure valuable material to forest management.

Table 4. – Seed orchards establishment for conservation and breeding (species or genera).

Conifers	Number	ha	Hardwoods	Number	ha
Abies	7	8.3	Acer	14	27.8
Larix	42	80.0	Alnus glutinosa	15	25.6
Picea abies	39	86.7	Betula	14	8.2
Pinus sylvestris	78	285.2	Carpinus betulus	1	1.0
Pseudotsuga menziesii	31	87.8	Fagus sylvatica	9	9.9
Sequoiadendron giganteum	2	1.3	Fraxinus excelsior	11	18.4
Taxus baccata	1	0.3	Pyrus/Malus	58	65.3
			Populus	23	17.0
			Quercus	16	27.2
			Sorbus	12	10.6
			Tilia	22	41.0
			Ulmus	14	11.4
			others	3	4.1
	200	549.6		202	267.5
Sa. 402 Seed orchards with 817.1 ha					

20 000 kg of seed originating from 2200 stands and 8600 single trees are stored in longterm cool storage. 25 000 cm<sup>3</sup> pollen originating from 6400 single trees is in storage too.

A considerable number of studies including morphology, phenology, isozymes and DNA characters has been carried out along with practical conservation work to get better knowledge of the variability and genetic structure of the species under consideration. Minimum standards for common data bases were developed.

There have been many discussions how far it is justified to include material from the breeding programs into the conservation concept. The following arguments are in favour of a general view to conservation:

1. Conservation has to consider utilization of genetic resources too, otherwise it is a dead end.
2. All breeding material is a genetic resource at the same time.
3. Breeding concepts have to reflect long term conservation aspects too (e.g. ERIKSSON et al., 1993).
4. Increasingly the forests will be used intensively to cover human needs. Therefore conservation aspects have to enter into regular forest management.
5. Integrated approaches of breeding and conservation are economically and ecologically in a long term view the only

sound solutions. Of course local and temporal variations are necessary.

6. Conservation programs and breeding programs have to be monitored with the same methods.

### 5. Experiences and Problems (Tab. 5)

A main constraint is the lack of knowledge about the present range, the genetic structure and the hybridization especially in the rare species. But even in major species like oak the knowledge is by far not complete (see e.g. KLEINSCHMIT, BACILIERI, KREMER and ROLOFF, in this volume).

Table 5. – Experiences and problems.

1. Knowledge of present range of most tree species-except major ones – is insufficient.
2. Species delineation is often difficult due to hybridization. This causes especially problems with fruit tree species (Malus, Pyrus, Prunus).
3. Information about genetic structure and differentiation of species is lacking or scarce.
4. Especially integrated approaches, considering characters of adaptive relevance (e.g. phenology, physiology, morphology) and biochemical and genetic characters of the same material are lacking.
5. In a long term view in situ conservation is only possible with tree species which still have sufficiently large populations.
6. In Germany most of the rare tree species only occur as single trees or in small groups, very seldom in small stands. These have to be joined in regionally structured seed orchards.
7. Completely protected nature reservation areas usually can be used as in situ conservation stands only for major tree species of strong competitive ability. Only under extreme ecological conditions minor species can survive without support.
8. Identification problems occur in many species due to hybridization and to insufficient knowledge of the different developmental stages.
9. Knowledge of long term storage conditions for many species is lacking.
10. Propagation methods have to be developed for many species to efficiently transfer material to application.
11. The economic environment supports the plantation of rare tree species under normal forestry conditions.
12. The time horizon for complete exploration exceeds 25 years with the present funding of the activities.

For *Ulmus campestris* and *Ulmus glabra* conservation is especially difficult due to the Dutch elm disease. The collection of material in seed orchards attracts the beetles and has therefore problems. A good strategy is to plant few trees scattered in the forests.

Hybridization is more frequent in many species than anticipated. This causes problems for species delineation and for conservation strategies. In wild fruit trees all transitions between horticultural varieties and the original species occur. This is a major problem in Malus, where the horticultural varieties are species hybrids with strong participation of non indigenous species, less a problem in Pyrus, where horti-

cultural varieties are more or less the extreme forms of the indigenous species.

Since most minor species usually grow under severe competition of other tree species, identification is often even more difficult due to the lack of a fully developed crown and sunleaves.

Practical difficulties occur with the complete protection in nature reserves since often the species of interest cannot be maintained in a long view if other species, like Fagus, have higher competitive potential.

The long time needed for a complete survey makes it necessary to start immediate actions for endangered species and noble hardwoods. These species are requested by foresters but reliable seed sources are often not existing.

Therefore the practical actions work on 2 lines:

1. Complete exploration of all species on all forest lands by mapping (time horizon more than 25 years), decision on in situ stands and additional ex situ measures.
2. Specific inquiries for endangered and rare species and collection of material for seed orchard establishment.

With specific inquiries less than 50% of the material existing is detected, but it can be used immediately.

### 6. Discussion

The concept for the conservation of forest genetic resources in Germany forms a good base for practical actions. One has however to accept, that many practical decisions are not based on sound scientific knowledge at the moment. This knowledge will increase parallel to the actions. Therefore the program includes security strategies which probably conserve more than absolutely necessary.

An important component is the reintroduction of rare species into forestry, which in a long term assures their "pseudo" in situ conservation too. Due to the experiences it is strongly advocated to start conservation work with minor and endangered species and not with major ones like Norway spruce. These species need intensive support and are increasingly requested by forest owners.

Their genepool is partly extremely restricted (e.g. *Pyrus pyraeaster* has up to now only 572 individuals known in Germany). Most hardwoods have been severely neglected in breeding programs in the past and nearly all efforts were directed towards conifers. This improved their economical position further in comparison to hardwoods. Along with the conservation work some selection and breeding activities have to be started to be able to procure forest owners with valuable material.

The general trend in Europe towards an ecologically oriented unevenaged, multispecies forestry underlines that this is not only a necessity for Germany but for other countries too.

A constraint is the fact, that regulations for trade with forestry reproductive material do not include regulations for the conservation of genetic resources. They can however be handled in a way, which fulfills this aim too (selection of a sufficient number of seed stands, collection of seed in different populations and seed orchards e.g.).

Cooperation on European level has to be improved, since tree species usually do not respect political boundaries.

Regarding these problems in a broader frame, it has to be stressed that an intensive forest tree breeding to cover human needs will be a necessity in the future as well (see Figure 1) as the conservation of genetic resources on the broadest base possible.

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# Computer-Aided Search for Codominant Markers in Complex Haploid DNA Banding Patterns – A Case Study in *Abies alba* MILL.

By B. DEGEN<sup>1)</sup>, B. ZIEGENHAGEN<sup>1)</sup>, E. GILLET<sup>2)</sup> and F. SCHOLZ<sup>1)</sup>

Dedicated to GEORG HEINRICH MELCHIOR on the occasion of his 70th birthday

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## Abstract

A genetic trait defines a gene marker in a collection of diplophase individuals only if each phenotype is produced by only a single genotype. This requires codominance as the mode of gene action at each of the involved loci (as opposed to dominance) and the absence of epistasis. Two computer programs are presented that aid in inheritance analysis of complex DNA banding patterns of single diploid individuals by searching the banding patterns of their haploid gametophytes for pairs of segregating bands representing codominant alleles of a locus as well as for single bands showing dominance over a "null allele" defined as absence of the band. The program MATRIX<sup>3)</sup> encodes densitometrical data obtained from DNA banding patterns as a 1/0 matrix. The program CoDo<sup>3)</sup> searches this matrix for pairs of bands showing 1:1 segregation and for single bands showing 1:1 segregation with a null allele. This system is

demonstrated on both a fictitious data set and on banding patterns obtained by PCR using a primer pair designed from an M13 bacteriophage sequence in an individual of *Abies alba* (MILL.) and the haploid primary endosperm of a sample of its seeds. For the latter, no band pair showed codominance of gene action, but several bands were suggested for dominance. Reasons for the small number of bands showing 1:1 segregation are discussed.

**Key words:** DNA marker, haploid primary endosperm, inheritance analysis, gene marker, codominance, computer program, *Abies alba*, M13 fingerprint, population genetics.

**FDC:** 165.3; 165.42; 174.7 *Abies alba*.

## Introduction

In many population genetic investigations, differences between the genetic structures of different populations of a species or of subpopulations of a population are analysed. The genetic structure of a collection of individuals is given as frequency distributions of alleles and genotypes at 1 or more loci. Often, the purpose of such investigations is to find associations between genetic structures and population genetic processes (e.g., to separate adaptive subpopulation differen-

<sup>1)</sup> Bundesforschungsanstalt für Forst- und Holzwirtschaft, Institut für Forstgenetik, Sieker Landstraße 2, D-22927 Grosshansdorf, Germany

<sup>2)</sup> Abteilung für Forstgenetik und Forstpflanzenzüchtung der Universität Göttingen, Büsgenweg 2, D-37077 Göttingen, Germany

<sup>3)</sup> Microsoft Windows version of the program package CoDo and MATRIX is currently being developed and will soon be available.