

PROTECTION OF GENETIC VARIABILITY IN POLLUTED STANDS: A CASE STUDY WITH SILVER FIR (*ABIES ALBA* MILL.)

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ABSTRACT

Stability of forest ecosystems is a common goal of forest management and nature protection. Stability is given when the populations of the involved forest tree species are persistent. Persistence of a population results from its current state of adaptedness and from its adaptive potential, both determined by the genetic structure and regulated by the genetic system. The adaptive potential of long-lived forest tree populations is dependent on the extent of their genetic variability. The management of populations under threat, e.g., by air pollution has to integrate strategies for the protection or for the increase of their genetic variability.

A case study on severely threatened silver fir (*Abies alba* Mill.) in the Ore Mountains demonstrates the steps which are necessary for a genetic management of the residual populations. Within a joint project, a comprehensive investigation on abundance and vitality as well as on the genetic constitution of residual silver fir provided basic results for decision-making in the fields of forest management and nature protection. The results of the genetic investigations are the basis for *in situ* and *ex situ* conservation strategies.

Key words: forest ecosystems, genetic system, genetic variation, genetic management, *Abies alba*, air pollution, Ore Mountains

INTRODUCTION

Air pollution means a severe threat to the stability of forest ecosystems. In face of forest decline, forest management is challenged to elaborate strategies of preservation, regeneration or even reforestation.

Investigations on the dynamics of matter and energy fluxes have been a common approach to judge on the stability of forest ecosystems (ULRICH 1992). Recently, the genetic information governing these fluxes is in the focus of interest for obtaining an increased knowledge of the functioning of forest ecosystems (SCHOLZ 1993; DEGEN 1995). The results of genetic investigations on endangered species should be integrated into preservation strategies. There is need for **genetic management** (GREGORIUS *et al.* 1979; GREGORIUS *et al.* 1985) to support silviculture and nature protection in endangered forest ecosystems.

Two lines will be followed to trace the genetic approach towards preservation strategies.

1. Main working hypotheses for understanding the genetic aspects of ecosystem stability will be given. From these, population genetic questions will be

deduced and methodological approaches will be presented.

2. A case study on endangered silver fir populations presents the results of an inventory of the residual populations (abundance, number of individuals, regeneration, and vitality), and the results of a comprehensive analysis of their genetic structure and conclusions for a genetic management.

Genetic aspects of ecosystem stability

Working hypotheses

Stability of an ecosystem is given when the populations of the “carrier“ species are persistent. Persistence results from their current state of adaptedness and from their capacity to adapt to environments changing in time and space (adaptive potential). The adaptive potential of a population is determined by the genetic structure and is regulated by the **genetic system** (DARLINGTON 1939, JAIN 1979, DEGEN 1995). According to a modification of the original definition by DARLINGTON (1939), the **genetic system** comprises all processes which generate, modify and preserve genetic variation and

transfer it to the following generations (STERN & ROCHE 1974). Investigations on the genetic implications of adaptation, adaptability and adaptedness therefore have to focus on investigations of the genetic structure and on aspects of the genetic system, *e.g.*, mating system, processes of genetic drift and selection (GRANT 1963; SOLBRIG *et al.* 1979).

Forest tree populations with their long-lived individuals are particularly challenged to adapt to nowadays rapidly changing environments. A basic working hypothesis says that the adaptive potential of a forest tree population depends on the extent of its genetic variation (GREGORIUS 1989; HATTEMER *et al.* 1993). There is much evidence for this hypothesis from the results of population genetic investigations on various forest tree species (for review, see MITTON 1995). Genetic studies of polluted forest tree stands using isozyme gene markers revealed that genetic diversity was low in subsets of individuals with visible damages. Also, heterozygous genotypes were found to show higher rates of survival compared with more homozygous genotypes. Due to selection processes and decreased population size, rare or low frequency alleles might be lost causing an impoverishment of the genetic multiplicity necessary for adaptation (SCHOLZ & BERGMANN 1984; BERGMANN & SCHOLZ 1985; GEBUREK *et al.* 1986; GREGORIUS 1989; MÜLLER-STARCK 1989; SCHOLZ *et al.* 1989; KONNERT 1992; SCHOLZ & BERGMANN 1994).

Hence, a genetic management of endangered forest tree populations has to aim at the protection or even increase of genetic variability of the relevant species. Besides a comprehensive inventory on a species' abundance, regeneration and vitality, the following population genetic items have to be worked on as a basis for any preservation activity.

Population genetic investigations aim at:

- assessment of the genetic structure of all populations in a study area
- assessment of their genetic multiplicity and genetic diversity,
- assessment of genetic differentiation within and among the populations in question, and
- comparisons of the genetic structure of parental and filial generations.

Furthermore the results have to be integrated into and compared with data of the genetic structure of neighbouring populations or even of broad-ranged populations within the natural range of the involved species.

The results reflect the genetic variation of a species throughout time scale: They are expected to judge on the *present* "genetic constitution" of the investigated populations. *Retrospectively*, assumptions can be made

on preceding events of selection and/or drift and on the mating system. And estimations on *future* potential genetic structures can be made, *e.g.*, in terms of hypothetical gametic multi-locus diversity.

Methodological approaches

Genetic markers

Isozyme gene systems have been the most common and traditional instrument for the determination of genetic structures and the quantification of genetic variation during the past 25 years. Due to their codominance in gene action they classify as gene markers and thus are a useful tool in population genetics (BERGMANN 1991).

The recently developed molecular methods are supposed to provide additional information on the genome. By means of **DNA markers** coding regions, but also non-coding DNA, and organelle DNA have become accessible. This implies several advantages in population genetic investigations. The detection of hypervariable DNA polymorphisms with capacity to differentiate between individuals promises to be well suited for parentage analyses and by this for investigations of the mating system. Also, selectively neutral DNA markers might be helpful in separating adaptive sub-population differentiation from stochastic differentiation by genetic drift. Polymorphisms detected in chloroplast DNA, provided they are uniparentally inherited, are expected to differentiate between maternal and paternal gene flow. A lot of investigations confirmed chloroplast DNA polymorphism to be predominantly paternally inherited in conifers (see references in WAGNER 1992). Apart from the occurrence of biparental inheritance, many angiosperm tree species show predominantly maternal inheritance of chloroplast DNA, *e.g.*, poplar (MEJNARTOWICZ 1991) and pedunculate oak (DUMOLIN *et al.* 1995). For two European oak species (*Q. robur* and *Q. petraea*), cpDNA polymorphisms revealed a promising potential for population differentiation and for biogeographic studies (FERRIS *et al.* 1993; KREMER & PETIT 1993).

With special regard to recombinant nuclear DNA, DNA polymorphisms as a tool in population genetics have to undergo inheritance analysis for proving them to be codominant, respectively gene markers (DEGEN *et al.* in press).

System analysis approach

A computer simulation programme (ECO-GEN) has most recently been developed (DEGEN, in prep.). It simultaneously simulates the dynamics of several population genetic processes in time and space such as reproduction effective population size, gene flow within

Table 1 Genetic multiplicity (A/L, P%), genetic diversity (v_{pool} , v_{gam}), heterozygosity (H_o , H_e) and genetic differentiation (δ_T) of silver fir in the Ore Mountains, assessed in 21 populations of each 14-53 individuals per population at 6 isozyme loci - data are given as means (according to LLAMAS GÓMEZ & BRAUN, in SCHOLZ *et al.* 1994)

	A/L	P%	v_{pool}	v_{gam}	δ_T	H_o	H_e
Mean	1.94	87.4	1.423	18.79	0.251	19.40	24.02

and between populations, fertility, and viability selection, competition and genetic drift. This instrument allows to simulate genetic effects of air pollution and forest management in forest tree populations and has been validated by isozyme gene data sets from a model population.

CASE STUDY ON ENDANGERED SILVER FIR POPULATIONS (*ABIES ALBA* MILL.)

Introduction

The case study to be presented reflects the results of a joint project on endangered silver fir populations in the Ore Mountains, Saxony (SCHOLZ *et al.* 1994). Silver fir is known to be one of the most important tree species in Saxonian mixed forest stands. Nowadays, it is suffering from severe dieback, showing symptoms of novel forest decline (LLAMAS GÓMEZ & BRAUN, in SCHOLZ *et al.* 1994). In general, this species' decline has already been discussed as related to different levels of genetic variation (BERGMANN *et al.* 1990).

Within the joint project three institutions have been working on three main tasks to answer the question: "Are vitality and genetic structure of the silver fir populations in the Ore Mountains still sufficient to guarantee a successful preservation of the residual populations and, furthermore a successful re-expansion of indigenous silver fir?"

The three tasks had been allocated to three institutions represented by the authors as follows:

A Inventory of all residual populations (BRAUN & LLAMAS GÓMEZ): Monitoring of abundance, regeneration and vitality, and comprehensive genetic investigations of 21 populations by means of 6 isozyme gene systems.

B Investigations on the genetic basis of adaptation and adaptedness (BERGMANN): Comparison of the genetic structure of silver fir populations in the Ore Mountains with neighbouring populations in Poland and Czech Republic, as well as with populations of Fichtel Mts. and Black Forest. Also, within a few selected populations comparisons of the genetic structure of parental and filial generations were aimed at. The investigations were performed by means of 6 to 7 isozyme gene systems.

C Adaptation and establishing of molecular screening techniques to the forest tree species (*Abies alba* Mill.) and the questions of the project (SCHOLZ & ZIEGENHAGEN).

Summarized results

A: Inventory of residual populations and genetic monitoring

Inventory of residual populations

Two thousand individuals (> 50 years old) were monitored as residual silver fir trees, scattered throughout the Ore Mountains in Saxony. Vegetative and generative potential can be considered low, as 94% of the individuals were found to be visibly damaged. Mean proportion of filled seeds in cones was only 6%, suggesting high rates of inbreeding or other adverse effects.

The abundance of silver fir ranges from the occurrence of single trees to the occurrence of small-sized populations (maximum number of individuals: 118). From the inventory, 21 populations were chosen for genetic investigations.

Genetic monitoring

Table 1 gives a summary on the results of the genetic investigations of the 21 silver fir populations. On the basis of 6 analysed isozyme gene systems (IDH, 6PGDH, MNR, GOT, LAP, AAP) silver fir populations in the Ore Mountains show relatively low genetic variability, in terms of genetic multiplicity, genetic diversity, heterozygosity, and genetic differentiation (table 1). UPGMA clusteranalysis based on data of the genetic distance (according to GREGORIUS 1974) resulted in sub-division of two main groups, populations of the western and eastern Ore Mountains on the one hand and of the Elbsandstein Mountains on the other hand. Also, probably non-indigenous populations could be identified. Comparison of the genetic structure of heavily damaged silver fir sub-populations with less damaged sub-populations suggested that selection pressure by air pollution as a long lasting factor in this area, led to a balanced situation with respect to these gene markers. Both sub-populations did not differ significantly in gene pool diversity (v_{pool} = 1.411 dam-

Table 2 Gene pool distances ($=D_{xy}$, see also GREGORIUS 1974) between the populations of Saxonian Ore/Elbsandstein Mts. and other silver fir populations (according to BERGMANN in SCHOLZ *et al.* 1994)

	Northern Czech Republic		South-west Poland		Thuringian Forest	Fichtel Mountains	Black Forest	
	Klášterec	Frydlant	Międzygorze	Łądek	Vessertal	Weissenstadt	Ottenhöfen	Nagold
Bärenfels	0.078	0.095	0.076	0.058	0.092	0.102	0.173	0.168
Lauter	0.080	0.076	0.082	0.052	0.079	0.088	0.157	0.139
Bad Schandau	0.055	0.084	0.061	0.061	0.094	0.073	0.163	0.147

aged, $v_{Pool} = 1.424$ less damaged), and population differentiation ($\delta_T = 0.252$ damaged, $\delta_T = 0.251$ less damaged). (for detailed results see LLAMAS GÓMEZ & BRAUN, in SCHOLZ *et al.* 1994).

B: Investigations on the genetic basis of adaptation and adaptedness

Comparison of Ore Mountains populations with other silver fir populations

On the basis of 6 isozyme gene systems (AP, GOT, MNR, MDH, 6PGDH, IDH), 3 populations from the Saxonian Ore/Elbsandstein Mountains were compared to populations from other regions: 2 populations from north-west Czech Republic, 2 populations from south-west Poland, one population from Thuringian Forest, one from Fichtel Mountains, and 2 populations from the Black Forest. Table 2 gives the results of the genetic investigations in terms of gene pool distances ($=D_{xy}$) as a measure of average genetic distances according to GREGORIUS (1974). Table 2 shows that the average genetic distance between the Ore Mountains populations and the neighbouring populations of south-west Poland and north-west Czech Republic is low as it is never exceeding 0.1. Also there is genetic similarity to the populations from Thuringian Forest and Fichtel Mountains. In contrast, genetic distance to populations from the Black Forest is relatively high. Ore Mountains populations are assumed to originate from the re-expansion along two post-glacial immigration routes: The East Alpine route from an Italian refugium and the East Carpathian route from refuges in the Balkan Peninsula (KONNERT & BERGMANN 1995).

Comparison of the genetic structure between parental and filial generation

For assessing inbreeding/outcrossing rates within a selected population with natural regeneration, one representative isolated old parental tree and its progeny were investigated. The parental tree was proved to be

homozygous at several isozyme gene loci. Numerous young firs were found to be heterozygous at the respective loci, the proportion of outcrossed progeny was estimated 80%. It might be assumed that viability selection may have caused an early elimination of the inbred progeny (for detailed results see BERGMANN, in SCHOLZ *et al.* 1994).

C: Genetic investigations at the DNA level

DNA extraction from needles could be adapted to this species, high in phenol and polysaccharide substances, and could also be scaled down to minipreparation procedure (ZIEGENHAGEN *et al.* 1993). Thus a prerequisite is given for population genetic investigations based on large sample sizes. DNA polymorphisms were detected on different levels.

Within-population-level

Individuals of a selected Ore Mountains population were screened for DNA polymorphism. For this purpose, PCR fingerprinting was performed by means of a primer pair designed from an M13 bacteriophage sequence. Hypervariable DNA banding patterns occurred which were proved to be highly reproducible and the basis for individual identification. In order to make these patterns applicable to population genetic investigations, a test-system has been established for inheritance analysis of complex DNA banding patterns in silver fir. An *Abies alba* individual and megagametophytes (haploid primary endosperm) of a sample of its seeds provide the material for searching loci with codominant and/or dominant gene action in complex banding patterns. Two computer programmes have been developed, the programme MATRIX for digitising the patterns, and the programme CoDo for segregation analysis (DEGEN *et al.* 1996). So far, several DNA fragments generated by M13 PCR fingerprinting could be identified as candidates for bands with dominant mode of gene action.

Among-population-level

Silver fir populations from a broad range of European provenances including an Ore Mountains population were investigated for chloroplast DNA (cpDNA) variation. Restriction analyses were performed in two PCR amplified cpDNA regions. An intraspecific restriction site polymorphism could be detected within a cpDNA fragment comprising sequences of *psbC* and adjacent non-coding sequences which were confirmed to be paternally inherited in intra- and interspecific crosses. These two variants can be observed throughout the range of silver fir (ZIEGENHAGEN *et al.* 1995). One type is predominantly occurring in the very east, the other one in the uppermost south-west of the range. Also, the Ore Mountains provenance did not significantly differ from provenances in the eastern parts and provenances in the southern parts of natural range (ZIEGENHAGEN *et al.* 1995). This might support the hypothesis that the Ore Mountains populations participate in both of the above mentioned main post-glacial immigration routes.

CONCLUSIONS

The persistence of silver fir in the Ore Mountains is a most desirable goal of forestry as well as of nature protection in Saxony. Investigations on vitality and genetic structure of silver fir populations in the Ore Mountains clearly revealed that the persistence of the residual populations is endangered for genetic reasons. The project's results suggest a genetic management as follows:

In situ preservation strategies solely based on the existing genetic structure are supposed to fail. Even nature protection sites should be enriched by plant material from other parts of the Ore Mountains in order to avoid inbreeding.

Ex situ preservation. Seed orchards should be established. A great number of clones will guarantee a great size of reproduction effective population, thus allowing a high recombination rate and persistence of rare alleles. *Ex situ* preservation in gene banks is not advisable. Many of the seeds are supposed to be inbred. Furthermore, long-term conservation techniques of seeds in *Abies alba* are not yet available.

Ex situ preservation of only Ore Mountain populations, however, will not provide a sufficient basis for re-expansion activities. The results on comparisons of genetic structure in the northern distribution range clearly define where to take additional material from. For western parts of the Ore Mountains, silver fir from the Fichtel Mts. and from populations of the Bavarian and Bohemian Forest (genetic data of silver fir from Bavarian and Bohemian Forest not shown) is recom-

mended. Eastern parts should be enriched by material from Poland and Czech Republic. Plants or seeds from the Black Forest should be excluded.

The presented case study may encourage forest management and nature protection to integrate genetically based strategies into preservation activities.

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