Protection of genetic variability in polluted stands. A case study with silver fir (*Abies alba* Mill.)

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Abstract

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Adaptability of forest ecosystems is a common goal of forest management and nature protection. The adaptive potential of long-lived forest tree populations depends on the amount of their genetic variability. The management of populations under threat (e.g. by air pollution) has to integrate strategies for the protection or if necessary, for the increase of their genetic variability.
A case study on severely threatened silver fir (*Abies alba* Mill.) in the Ore Mountains illustrates the research activities 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.

Introduction

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 1996).

The results of genetic investigations on endangered species should be integrated into preservation strategies. There is need for genetic management (Gregorius & al. 1979, Gregorius & 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. Conclusions for a genetic management are drawn and addressed to forest management and nature protection.

Genetic aspects of ecosystem stability

Working hypotheses

Forest tree populations with their long-lived individuals are particularly challenged to adapt to today'ss 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 & al. 1993). A considerable amount of evidence siupporting this hypothesis is already available from population genetic studies carried out 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 & al. 1986, Gregorius 1989, Müller-Starck 1989, Scholz & al. 1989, Konnert 1992, Scholz & Bergmann 1994).

Hence, a genetic management of endangered forest tree populations has to aim at the protection or even at an 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 out as a basis for any preservation activity.

Population genetic investigations

These studies 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 of such investigations reflect the genetic variation of a species throughout time scale: They are for assessment of 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 for future tree generations, estimations on *future* potential genetic structures can be made, e.g. in terms of hypothetical gametic multilocus diversity.

Methodological approaches

Genetic markers

Isozyme polymorphism has been the most common - instrument for quantification of genetic variation anddetermination of genetic structures during the past 25 years. Due to their codominance in gene action, isozyme alleles classify as useful gene markers and thus as a principal tool in population genetics (Bergmann 1991).

DNA polymorphism: The recently developed DNA molecular methods provide additional information on the variation found in the genome.

Variation in DNA coding regions, as well as non-coding nuclear and organelle DNA can now be effectively analyzed. 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 (cpDNA), provided they are uniparentally inherited, are expected to differentiate between maternal and paternal gene flow. A lot of investigations confirmed cpDNA polymorphism to be predominantly paternally inherited in conifers (see references in Wagner 1992). Many angiosperm tree species show predominantly maternal inheritance of chloroplast DNA, e.g. poplar (Mejnartowicz 1991) and pedunculate oak (Dumolin & al. 1995).

For two European oak species (*Q. robur* and *Q. petraea*), cpDNA polymorphisms revealed a promising potential for population differentiation and for phylogeographic studies (Ferris & 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 & al. 1996).

Systems analysis approach

A computer simulation programme (ECO-GENE) has most recently been developed (Degen 1996). It simultaneously simulates the dynamics of several population genetic processes in time and space such as reproduction effective population size, gene flow within 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 and adjacent Saxonian Elbsandstein Mountains (Scholz & al. 1994). In this area silver fir is at the northern border of its natural range (see Fig. 1). It 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 & al. 1994). In general, this species' decline has already been discussed as related to different levels of genetic variation (Bergmann & al. 1990).

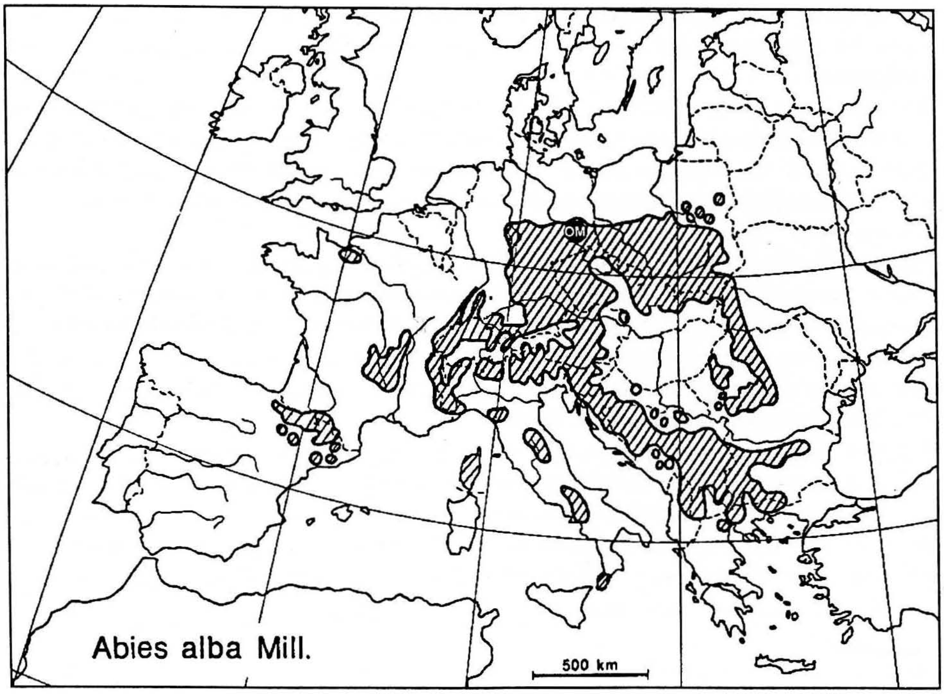

Fig. 1. Natural range of silver fir (*Abies alba* Mill.) according to Meusel (1965). The study area (Ore Mountains including Elbsandstein Mountains) is characterised by the circled initials **OM**.

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 / Elbsandstein 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:

(1) Inventory of all residual populations (Braun & Llamas Gómez): Monitoring of abundance, regeneration and vitality, and comprehensive genetic investigations of 21 populations by means of isozyme gene markers.

(2) Investigations on the genetic basis of adaptation and adaptedness (Bergmann): Comparison of the genetic structure of silver fir populations in the Ore / Elbsandstein Mountains with neighbouring populations in Poland and Czech Republic, as well as with populations of Fichtel Mountains and Black Forest. The investigations were performed by means of 6-7isozyme gene markers.

(3) Adaptation and establishing of molecular screening technics to the forest tree species (*Abies alba* Mill.), and to the questions of the project (Scholz & Ziegenhagen).

Summarised results

(1) 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 / Elbsandstein Mountains in Saxony. Their 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 to be found in a population was 118. From the inventory, 21 populations were chosen for genetic investigations.

Genetic monitoring: Table 1 gives a summary on the results of isozyme gene analyses in the 21 silver fir populations under study.

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 gene loci - data are given as means (according to Llamas Gómez & Braun, in Scholz & 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

A/L = average number of alleles per locus, P% = average percentage of polymorphic loci (loci with at least one allele $P \geq 95\%$, excluded), v_{Pool} = gene pool diversity, v_{gam} = hypothetic gametic multilocus diversity, δ_T = population differentiation, H_o = observed heterozygosity, H_e = expected heterozygosity

On the basis of 6 analysed isozyme gene systems, 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 cluster analysis of the populations based on data of the genetic distance (according to Gregorius 1974) resulted in their sub-division into 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 (data not shown, for detailed results see Llamas Gómez & Braun, in Scholz & al. 1994).

(2) 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, 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 (= Dxy) 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; it never exceeds 0.1. Also there is genetic similarity to the populations from Thuringian Forest and Fichtel Mountains. In contrast, genetic distances between the Saxonian Ore/Elbsandstein mountains populations and the populations from the Black Forest are relatively high (for detailed results see Bergmann, in Scholz & al. 1994) Ore Mountains populations are assumed to originate from the re-expansion along two post-glacial immigration routes: The East Alpine route from an Italian refuge and the East Carpathian route from refuges in the Balkan Peninsula (Konnert & Bergmann 1995).

Table 2. Gene pool distances (= Dxy, see also Gregorius 1974) between the populations of Saxonian Ore/Elbsandstein Mts. and other silver fir populations (according to Bergmann in Scholz & al. 1994).

Ore/Elbsandstein Mts.	Northern Czech Republic		South-west Poland		Thuringia Forest	Fichtel Mts.	Black Forest	
	Klasterec	Frydland	Miedzygorze	Ladek	Vessertal	Weissen-stadt	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

(3) Genetic investigations at the DNA level

DNA extraction from needles could be adapted to this species, high in phenols and polysaccharide substances, and could also be scaled down to minipreparation procedure (Ziegenhagen & al. 1993). Thus also in *Abies* population genetic studies based on large sample sizes become possible.

DNA polymorphisms were detected on different levels.

Within-population-level: Individuals of a selected Elbsandstein 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 & 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.

Between-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 *psb*C and adjacent non-coding sequences which were confirmed to be paternally inherited in an intra- as well as in an interspecific cross. These two variants can be observed throughout the range of silver fir (Ziegenhagen & 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 & 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 / Elbsandstein Mountains is a most desirable goal of forestry as well as of nature protection in Saxony. Investigations on vitality and genetic structure of the residual silver fir populations clearly revealed that their persistence is endangered for genetic reasons. The project's results suggest a genetic management as follows:

In situ *preservation*

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 storage 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 Mountains or Thuringian Forests is recommended. 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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