Selection Pressure by Air Pollution as Studied by Isozyme-Gene-Systems in Norway Spruce Exposed to Sulphur Dioxide[1])

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Abstract

By analyzing isozyme-gene-systems in fumigated clones of Norway spruce a possible selection pressure by air pollutants was proven. A comparison of a clone group of high sensitivity with one of low sensitivity revealed differences in genetic structure concerning allele- and genotypic frequencies of 4 isozyme-gene loci. The results are interpreted genetically and biochemically.

Key words: Norway spruce, clones, fumigation, sulphur dioxide, genecology, selection pressure, isozyme, genetic impoverishment.

Zusammenfassung

Durch Analyse von Isoenzym-Gen-Systemen in begasten Fichtenklonen wurde ein möglicher Selektionsdruck durch Luftverunreinigungen bewiesen. Ein Vergleich einer Klongruppe hoher Empfindlichkeit mit einer Klongruppe niedriger Empfindlichkeit erbrachte Unterschiede in der genetischen Struktur bezüglich der Allel- und Genotypfrequenz an 4 Isoenzym-Genloci. Die Ergebnisse werden genetisch und biochemisch interpretiert.

Introduction

There is much evidence that air pollution is a new selective force in forest tree populations. Through adaptation to natural biotic and abiotic environmental factors during many tree generations the genetic structure has been in a dynamic equilibrium with these factors for millenia *(Figure 1)*. Since few decennia, however, the genetic structure of many forest tree populations is under the selective or even destructive influence of anthropogeneous air pollutants. It is evident that in regions with heavy air pollution, such as the Ore Mountains in Central Europe with the complete die back of populations, the genetic structure is destroyed. In regions with lower pollution, changes in the genetic structure of populations most probably take place, caused by viability and fertility selection. Viability selection is due to genotypic variation in both, sensitivity (Scholz *et al.* 1979) and tree height which causes different exposure of trees to pollutants (Scholz 1981) leading to different degree of damage. Indications for such selective processes were found by Kriebel and Leben (1981) investigating provenances from polluted areas after some decades of pollution influence.

Changes in the genetic structure of forest tree populations are very critical for several reasons. Changes in allele frequencies will affect the above-mentioned dynamic equilibrium, which may have consequences for the future existence of forest tree species. Furthermore, large changes in allele frequencies and in particular loss of alleles lead to a decrease in genetic diversity, thus reducing the adaptability of the long-lived forest tree species to temporally and spatially varying environments. Additionally, as shown in *Figure 1* the natural stress factors themselves can be influenced by air pollutants, for instance the structure of fungus or insect populations may change with adverse effects for the forest tree populations. Although there is much evidence that selection processes by air pollutants take place, there is still the lack of unequivocal experimental proof.

A strong indication of selection by air pollution was found by Mejnartowicz (1983), who studied the allele frequency variation at an acid phosphatase locus among more and less tolerant trees of a Scots pine stand in the vicinity of a factory emitting SO_2 and fluorides. Since the genotypes of individual trees were inferred from the acid phosphatase patterns found in their seedlots. Mejnartowicz's data, however, might be biassed due to selection acting directly on the seed set of these trees. Furthermore,

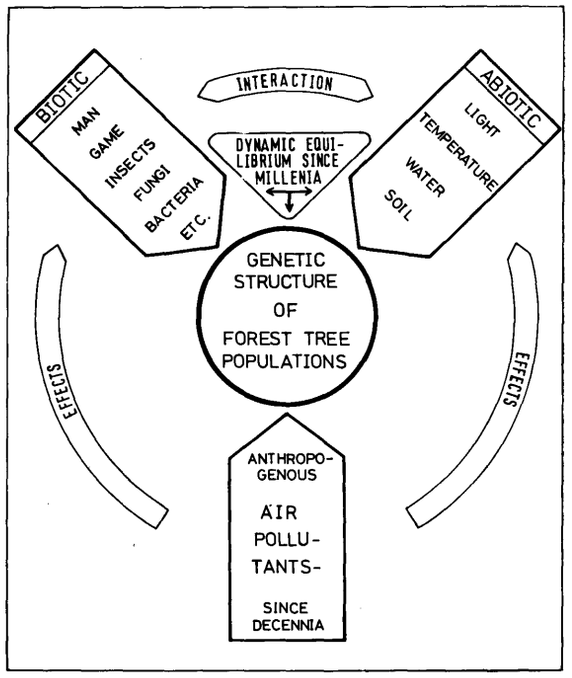

Figure 1. — Schematic illustration of effects of air pollutants on the genetic structure of forest tree populations in relation to natural stressfactors (after Scholz, 1984).

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it must be assured that the isozyme patterns observed in zymograms were not modified by biochemical action of air pollutants (Pahlich 1972, Ziegler 1974 a, b). Therefore, our investigations on selection pressure by air pollution were primarily concerned with controlled fumigation experiments using clones from defined plant material. The genetic structure of groups comprising more and less sensitive clones was determined by isozyme-gene-systems electrophoretically identified in the ortets. Norway spruce *(Picea abies)* was chosen as forest tree species, because it is severely damaged in Central Europe.

Material and Methods

The fumigation experiments were carried out with clones from material consisting of several provenances from CSSR of which seed from five mother trees was harvested. Twenty plants of each progeny were repeatedly propagated by cuttings and fumigated with SO_2. The phenotypic variation of damage ranged from "total necrosis of current year needles" to "not affected" after a fumigation period of about 14 days. In such fumigation experiments, it was shown that the phenotypic variance is predominantly due to genetic factors (Scholz *et al.* 1979 for HF and unpublished data for SO_2). From 123 clones, fumigated with SO_2 and scored for their damage for characterizing their sensitivity, the 31 most sensitive (S^+) and the 30 least sensitive ones (S^-) were chosen for a comparison of their genetic structure determined by isozyme systems the genetic control of which was known. The clone means ranged from 2.3 to 59.1.

For isozyme analysis, meristem tissue of young vegetative buds collected from the nonfumigated ortets was extracted, and the extracts subjected to horizontal starch gel zone-electrophoresis. The following isozyme systems (and their corresponding gene loci) were used: Glutamate oxaloacetate transaminase (GOT-B), glutamate dehydrogenase (GDH-A), malate dehydrogenase (MDH-C), isocitrate dehydrogenase (IDH-A, IDH-B), phosphoglucomutase (PGM-A, PGM-B), and glucose-6-phosphate dehydrogenase (G6PDH-A). Details of the electrophoretic procedures, staining recipes and identification of the genetic control were described elsewere (Lundkvist 1979, Poulsen *et al.* 1983, Bergmann in preparation).

Results and Discussion

As the phenotypic variation of the response of Norway spruce to SO_2 fumigation is governed by genetic factors to a great extent, differences in genotype and allele frequencies are expected to exist between the clone group with high (S^+) and that with low (S^-) sensitivity.

When considering isozyme-gene-systems to be investigated, especially such gene loci were selected which code for enzymes involved in metabolic processes particularly affected by SO_2 as stated by Jäger and Klein (1980), Rabe and Kreeb (1980), and Rabe (1981). Thus, the data obtained in this study may not only reveal the possible occurence of selection pressure and gene loss but may also indicate the mode of selection involved in resistance mechanisms at the biochemical level.

The allele and genotype frequencies of the two sensitivity groups listed in *Table 1* show that genetic differences occur at four out of the eight enzyme loci so far investigated. At the locus GOT-B, for instance, the genotype B_1B_2 is absent in the S^- clone group but showing a frequency of 0.1 in the S^+ group, which results in the allele frequencies of 0.0 in S^- and 0.05 in S^+, respectively. For the MDH-C locus the allele frequencies in S^- and S^+ are 0.05/0.00 for C_1, 0.02/0.08 for C_3, and 0.02/0.00 for C_0, respectively.

Particularly striking are the results found at the G6PDH-A locus, where the heterozygote A_1A_2 is relatively rare and the homozygote A_1A_1 completely absent in group S^-. This results in a great difference in occurrence of allele A_1 which is 7.5 times more frequent in the more sensitive clone group than in the less sensitive one.

In *Figure 2* the frequency of particular alleles in both groups is related to the sensitivity of the respective plant material. It demonstrates that, assuming a predominant elimination of the more sensitive genotypes, a selection pressure by air pollutants should lead to a decrease in frequency of certain alleles or even to a loss of alleles (GOT-B_1) in the surviving plants of the population. Such gene loss may occur due to viability selection through direct influence of gaseous air pollutants on the crown (Scholz 1984) or through indirect influence acting via the soil (Scholz and Geburek 1983).

Table 1. — Genotype and allele frequencies at eight enzyme loci in two groups of Norway spruce clones of different sensitivity to SO_2 fumigation.

enzyme locus	genotype	allele	clone group S^- (n=30)	clone group S^+ (n=31)	
GOT-B	B_1B_2		-	0.10	
	B_2B_2		0.43	0.42	
	B_2B_3		0.47	0.26	$\chi^2 = 6.24$
	B_3B_3		0.10	0.22	n.s.
		B_1	-	0.05	
		B_2	0.67	0.60	
		B_3	0.33	0.35	
GDH-A	A_1A_2		0.03	0.06	n.s.
	A_2A_2		0.97	0.94	
		A_1	0.02	0.03	
		A_2	0.98	0.97	
MDH-C	C_1C_2		0.10	-	
	C_2C_2		0.83	0.87	
	C_2C_3		0.03	0.10	n.s.
	C_2C_0		0.03	-	
	C_3C_3		-	0.03	
		C_1	0.05	-	
		C_2	0.91	0.92	
		C_3	0.02	0.08	
		C_0	0.02	-	
IDH-A	A_3A_3		0.83	0.81	
	A_3A_4		0.17	0.19	
		A_3	0.92	0.90	n.s.
		A_4	0.08	0.10	
IDH-B	B_2B_2		0.97	1.00	
	B_2B_3		0.03	-	
		B_2	0.98	1.00	n.s.
		B_3	0.02	-	
PGM-A	A_1A_2		0.03	0.03	
	A_2A_2		0.97	0.97	
		A_1	0.02	0.03	n.s.
		A_2	0.98	0.97	
PGM-B	B_1B_2		0.03	0.13	
	B_2B_2		0.97	0.87	
		B_1	0.02	0.07	n.s.
		B_2	0.98	0.93	
G6PDH-A	A_1A_1		-	0.03	
	A_1A_2		0.03	0.23	$\chi^2 = 6.20^*$
	A_2A_2		0.97	0.74	
		A_1	0.02	0.15	
		A_2	0.98	0.85	$\chi^2 = 6.69^{**}$

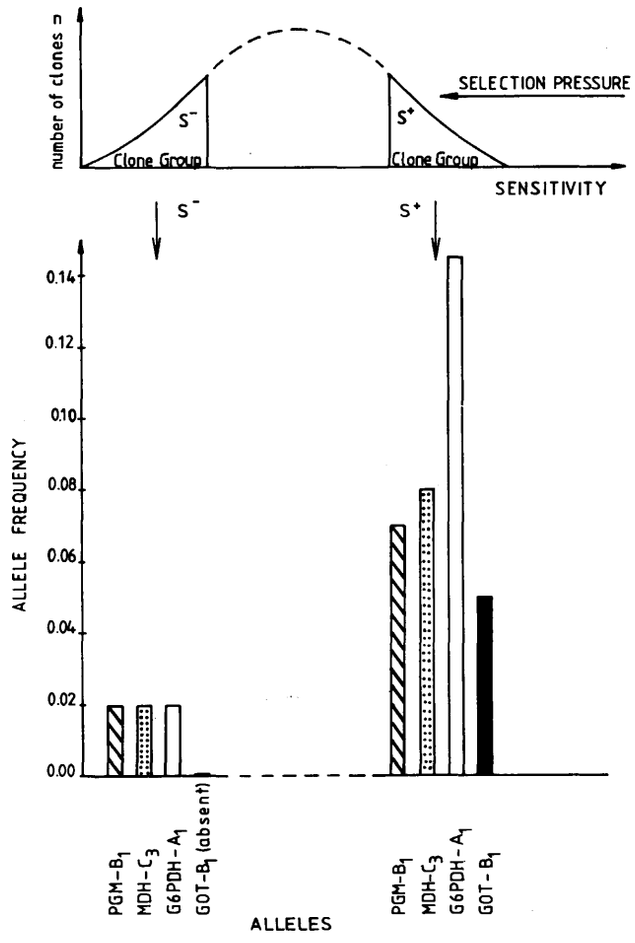

Figure 2. — Schematic illustration of the relationship between sensitivity and the frequencies of individual alleles at four enzyme loci.

For the results presented here, it is important to point out that the isozyme investigations were carried out with unfumigated clone material, while the sensitivity score was obtained by fumigation of other ramets of the same clones. Thus, by investigating nonfumigated ramets the different isozyme patterns in the low sensitive and the high sensitive clone group were not biassed by air pollution, influencing the gene expression or the gene products. That such influence can occur, has been demonstrated e.g. by PAHLICH and ZIEGLER (1974 a, b). The data compiled in *Table 1*, although showing clear genetic differences between the two sensitivity groups, do not verify in each case that the selection acts on the enzyme locus itself, rather it may affect other loci closely linked to the respective enzyme locus (hitch-hiking effect). The allele and genotype differences at the G6PDH-A locus *(Table 1)*, however, provide great evidence that his enzyme- genesystem is directly involved in the selection process caused by SO_2 fumigation. In particular, the presence of the allele A_1 in a tree gives rise to higher sensitivity in most of the clones fumigated.

Table 2. — Genotypes at the G6PDH-A locus in a progeny of a heterozygous mother tree (A_1A_2) in relation to the scored sensitivity to SO_2 fumigation.

clone	genotype	sensitivity scoring
C7/4/3	A_2A_2	14.6
C7/4/1	A_1A_2	50.1
C7/4/16	A_1A_2	54.8
C7/4/2	A_1A_1	58.1

This relationship between the allele A_1 and the high sensitivity score of its carriers becomes even more prominent in one half-sib family where the mother tree is obviously heterozygous A_1A_2. The relationship between the genotype at G6PDH-A and the sensitivity score in the progeny of this tree is shown in *Table 2.* With increasing number of alleles A_1 from 0 to 2 the sensitivity score shifts to the highest value of 59,5.

A biochemical interpretation of these results may contribute to a better understanding of phytotoxic effects of SO_2 on one hand and of resistance mechanisms on the other hand. It is well known, that stress by air pollutants such as SO_2, Ozone, and HF (ref. in RABE and KREEB, 1980) leads to an increased activity of the pentose phosphate cycle which is a shunt to glycolysis or gluconeo genesis, respectively. To what extent the increased activity of the pentose phosphate cycle is due to an increased demand for pentoses or $NADPH+H^+$ as assumed by RABE and KREEB (1980) or to the inhibition of the glycolysis may depend on the respective plant species or type of pollutant. Ross *et al.* (1968) found that *Gladiolus* varieties which are more resistant to HF can use the pentose phosphate cycle more efficiently. SCHOLZ (1975) showed that Pine species with a more fluoride resistant glycolysis enzyme (Enolase) are more resistant to HF.

G6PDH is the key enzyme for entering the pentose phosphate cycle. As isozymes differing in their electrophoretic mobility may also possess different catalytic activity and/or different sensitivity to detrimental chemicals such as air pollutants, the possession of two alleles A_2 and their corresponding isozyme product can be responsible for a higher degree of resistance. Biochemical experiments in-vitro with preparations of these isozymes or with extracts of G6PDH from homozygous trees A_1A_1 and A_2A_2 may provide information on the function of these isozymes in resistance mechanisms.

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Short Note: In Vitro Induction of Organogenesis in Juvenile and Mature Beech

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Abstract

Bud explants from three mature trees (more than 70 years old) and embryonal explants derived from the seeds of the same trees, belonging to three cultivars of European beech, *Fagus sylvatica* L., namely, 'Zlatia', 'Pendula', and var. *purpurea*, were cultured on modified Woody Plant Medium. Only bud explants from var. *purpurea* showed limited differentiation of shoots; bud explants from 'Zlatia' and 'Pendula' remained largely unresponsive under the experimental conditions. The embryonal explants from all three cultivars exhibited shoot formation, although the number of shoots was mostly limited to a few. Ooccasional plantlet regeneration from embryonal explants were observed.

Key words: European beech *(Fagus sylvatica)*, embryonal explants, bud explants from mature trees, tissue culture, shoot differentiation, plantlets.

Zusammenfassung

Knospenexplantate von drei über 70jährigen Bäumen und Embryoexplantate von Samen dieser Bäume wurden auf einem modifizierten "Woody Plant Medium" kultiviert. Die Bäume gehören zu drei Kultivaren der europäischen Buche, *Fagus sylvatica* L., und zwar 'Zlatia', 'Pendula' und var. *purpurea*. Nur Knospenexplantate von var.*purpurea* zeigten eine begrenzte Differenzierung der Sprosse, die Knospenexplantate von 'Zlatia' und 'Pendula zeigten im wesentlichen kein Wachstum. Dagegen zeigten die Embryoexplantate aller drei Kultivare Sproßdifferenzierung, obwohl jeweils nur wenige Sprosse gebildet wurden. In einzelnen Fällen konnten sich aus den Embryoexplantaten Pflänzchen bilden.

Introduction

Tissue explants from mature forest trees are generally difficult to grow and differentiate *in vitro* (Bonga 1982), unless they are obtained from juvenile material. Vegetative propagation of most mature trees is also difficult, unless the cuttings are taken from young trees. Obviously, some change occurs in the physiology/biochemistry of the mature tree that seems to interfere in the rejuvenation process. European beech (*Fagus sylvatica* L.) is extremely difficult to propagate vegetatively by cuttings from mature trees. The present investigation was aimed at *in vitro* induction of organogenesis in explants derived from juvenile material and mature trees of beech, for eventual application in clonal propagation of superior genotypes.

Materials and Methods

The present study was based on three mature trees (more than 70 years of age) belonging to three cultivars of European beech, *Fagus sylvatica* L., 'Zlatia', 'Pendula', and var. *purpurea*. Bud explants from the mature trees, taken in late autumn and early spring, and embryonal explants derived from seeds collected from the same trees were cultured on a modified Woody Plant Medium (WPM; Lloyd and McCown 1981). The WPM was variously supplemented with growth substances: 0.3—1.0 mg/l 6-benzylaminopurine (BA), 0.2—0.5 mg/l kinetin (KIN), 0.01—0.04 mg/l naphtaleneacetic acid (NAA), 0.01—0.02 mg/l indole-3-acetic acid (IAA), 0.01—0.02 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), and 10—20 mg/l adenine sulphate. In addition, 100 mg/l casein hydrolysate and 50—100 mg/l lysine were also tested in combination with phytohormones. Altogether 9 different modifications of WPM involving different combinations of growth substances were employed for the present study. Cultures were maintained at 25° C, 70% relative humidity, and 16 hours photoperiod (2000—3000 lux).

Results and Discussion

Preliminary results from the present study have indicated that bud explants from mature beech trees are, in general, difficult to grow and differentiate *in vitro*. Of the three beech cultivars investigated, bud explants from 'Zlatia' and 'Pendula' showed practically no growth and differentiation on the media tested. However, about 50% of the 206 bud explants from var. *purpurea* showed slight growth, and only a small proportion (about 10%) of the explants exhibited a limited differentiation of buds (mostly one or two) on media containing low levels of phytohor-