Genetic Changes in Populations of Scots Pine Growing Under Industrial Air Pollution Conditions

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Abstract

Using isozymes of 5 polymorphic loci the frequency of mutations and mutation-like events in populations of Scots pine (*Pinus sylvestris* L.) in South Urals (Russia) were studied. The frequency of rare electrophoretic variants of isozymes is shown to be significantly higher in 2 populations growing under industrial air pollution conditions ($p=5.0 \times 10^{-3}$ and $p=5.2 \times 10^{-3}$) than that of the control (1.6×10^{-4}).

Key words: Pinus sylvestris, air pollution, genetic diversity, isozymes, mutation, rare electrophoretic variants.

FDC: 165.6; 181.45; 425.1; 174.7 *Pinus sylvestris.*

Introduction

Currently the world is witnessing increasing anthropogenic influences on the environment. One of the most evident consequences of these anthropogenic influences on natural ecosystems is the degradation of genetic architecture of coniferous forests as result of air pollution. Many research scientists (MEJNARTOWICZ et al., 1978; MÜLLER-STARCK, 1985; BERGMANN and SCHOLZ, 1985; SCHOLZ and VENNE, 1987) have studied this problem. However, considerably less attention has been devoted to the study of mutation processes in populations of woody plants growing under conditions of industrial air pollution and only several works were published in this field of science (GONCHARENKO et al., 1991; KORSHIKOV et al., 1991).

According to classical genetics the influence of mutations on population and species as a whole is contradictory. On the positive side mutations are elementary units of hereditary variability and suppliers of evolutional material. Alternatively, mutations can be lethal or they can reduce vitality of individuals. It is usually considered that for each species under "normal" conditions an optimum background of spontaneous mutation processes exists and its frequency varies in the limits of $p=10^{-4}$ to 10^{-6} (DUBININ and GLEMBOTSKY, 1967). Since these data are based on morphological mutations and chromosomal abberations it is necessary to compare the available results with those generated by molecular biology, in particular isozyme analysis. In this content we may pose two additional questions: First, is there an increase in mutation rates under changed environmental conditions, in particular, under industrial air pollution; and secondly, does an increased frequency of mutations resulted in increased population adaptivity leading to wider genetic diversity?

Coniferous plants are convenient for addressing these problems because it is possible to study diploid (genotypes of maternal trees and embryos) and haploid (endosperms) tissues from the same individual plants to processes at the level of gametes and to observe mutant genotypes at later ontogenetic stages.

Mutant gene variants which are revealed using isozyme analysis include 3 main types:

A. Change of electrophoretic mobility of enzyme during substitution of DNA base and correspondingly change of aminoacid sequence in polypeptide chain.

B. Absence of enzyme activity as a result of deletion with multiple break of chromosomes and loss of separate genes or their blocks, DNA base change in gene site controlling synthesis of catalytic centre of enzyme, destruction of the activity of regulatory gene, etc.

C. Appearance in haploid tissues of two isozymes corresponding to both "normal" alleles of a locus. This phenomenon may be caused by unequal crossing-over or "mosaic" diploidy of a gamete when endosperm from 2 or more megaspores is formed.

This manuscript reports on a study of rare isozyme variants in three populations of Scots pine (*Pinus sylvestris* L.), 2 in the regions of heavy industrial air pollution, and the third population, a control one, in an ecologically benign area.

Materials and Methods

The populations under investigation are situated in the western part of Chelyabinsk region (South Urals, Russia). Population 1 borders a magnesite plant in the town of Satka, in which the main pollutants are magnesium oxide (MgO), sulphur dioxide (SO_2), nitrogen oxides (NO_X) and carbon dioxide (CO_2). Population 2 is located not far from the copper-melting plant in Karabash. The main pollutans from this plant are sulphur dioxide (SO_2), heavy metal pollutans include arsenic, hydrogen chloride (HCl), hydrogen fluoride (HF), nitrogen oxides (NO_X) and others. In these populations significant degradation of forests resulting from damage to trees (top-die back of crown, chlorosis and necrosis of needles, etc.) was observed in an 58000 hectars area. Population 3 (control) is situated approximately 35 km and 20 km from populations 1 and 2, respectively. Tree age in all 3 populations changes from 100 years to 120 years and soil-climatic conditions are similar in all 3 locations.

Fifty trees were randomly selected in each stand and their seeds were collected for these experiments. Preparation of seed for electrophoresis consisted of the removal of the seed cover and of the separation of endosperm and embryo. For homogenization of endosperm 100 µl of extraction buffer (0.1M Tris-HCl, pH 8.0 containing 17% sucrose, 0.1% bovine serum albumin and 0.1% cysteine) was used. 50 µl of the same buffer was used for homogenization of embryos. For moving away cellular fragments and substances of lipid nature the homogenate was centrifugated during 15 minutes at 13000 g.

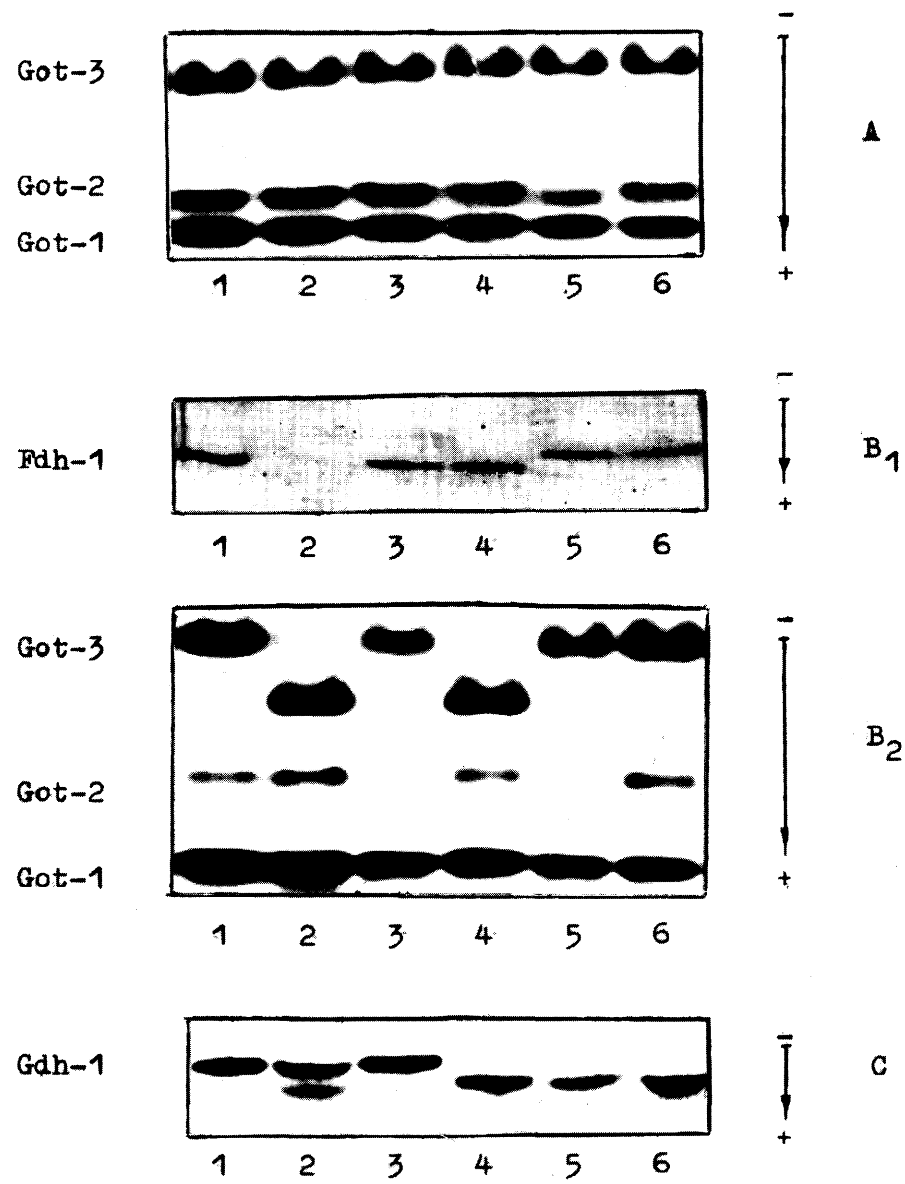
Polyacrylamide vertical slab gel electrophoresis was prepared according to DAVIS (DAVIS, 1964).

Isozymes of glutamate oxalate transaminase (GOT, EC 2.6.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.2) and formiate dehydrogenase (FDH, EC 1.2.1.2) were used as molecular-genetic markers. Loci were named using abbreviated names of enzymes with small second and third letters. If an enzyme was controlled by 2 or more loci, they were numbered with arab figures, the most rapid isozymes were numbered 1. Enzyme systems were chosen because genetic control and population polymorphism of GOT, GDH, FDH was studied to a great extent in previous experiments (YANBAEV, 1989; YANBAEV et al., 1991). The substrates of these enzymes show affinity for metabolic products of coniferous plant cells. The last fact is taken into consideration because a number of research scientists suggest the relation between the facts of discovery of null-alleles and the use of synthetic substrates in histochemical staining of enzymes (GURIES and LEDIG, 1978).

Results

Analysis of allele segregation in haploid material (endosperm) indicated that glutamate oxalate transaminase in Scots pine was under control of 3 loci: 7 alleles were assimilated with locus Got-3, 6 alleles with Got-2, and 4 alleles with Got-1. Glutamate dehydrogenase and formiate dehydrogenase were each controlled by 1 gene. Three alleles were detected in glutamate dehydrogenase, though according to the data of our previous experiments and those of other scientists Scots pine is characterized by diallelic genetic control of glutamate dehydrogenase. Six alleles were detected in formiate dehydrogenase. Taking into account null-alleles, the polymorphism of these enzymes turns out to be greater than previously indicated. Each locus had 2 to 3 alleles.

Rare electrophoretic variants of isozymes which can be considered mutations and mutation-like events were observed in the populations. Rare isozymes found in gametes that are absent in maternal trees were regarded as new-formed mutations by us. For example, in *figure 1* (A) sample N5 is represented by isozyme with altered mobility (genotype



A – Isozyme with the changed electrophoretic mobility (sample N 5).
B_1 – Mutant variant of isozyme with the absence of activity (sample N 2) – null-mutation.
B_2 – Null-alleles (samples N 3 and 5).
C – Appearance in haploid (endosperm) tissue of products of two alleles (sample N 2).

Fig. 1. – Electrophoregrammes of isozymes demonstrating mutations (mutation-like events) in 4 trees.

Table 1. – Frequency of mutations and mutation-like events in populations of Scots pine.

Stand	Volume of sample (number of endosperms)	Method of calculation, by loci	Frequency (x 10^{-3})				In all	Per one locus
			A	B_1	B_2	C		
1	1632	Heterozygous	0,61	1,20	46,00	0	48,00	9,60
	1721	Homozygous	2,90	0	0	2,30	5,20	1,00
	3353	All	1,80	0,60	22,00	1,20	26,00	5,20
2	3165	Heterozygous	0,63	0,95	6,30	10,00	18,00	3,70
	6798	Homozygous	2,60	3,40	0	22,00	28,00	5,70
	9963	All	2,00	2,60	2,00	19,00	25,00	5,00
3	1550	Heterozygous	0	0	0	0,65	0,65	0,13
	3581	Homozygous	0,84	0	0	0	0,84	0,17
	5131	All	0,19	0	0	0,58	0,78	0,16

of maternal tree is Got–$2^{c/c}$). In *figure 1* (B_1) sample N2 shows null-mutation-absence of isozyme activity (genotype of maternal tree is Fdh–$1^{c/d}$).

Null-alleles producing isozymes with the absence of activity when segregated in heterozygous trees are inherited mutations (*Figure 1* (B_2), genotype of maternal tree is Got–$2^{f\text{-null}}$).

The appearance of products of 2 alleles simultaneously in a haploid endosperm (*Figure 1* (C)) cannot be called mutations in the exact meaning of this term. That is why this phenomenon should be conditionally considered as a mutation-like phenomenon. These isozymes coincide with that of the alleles present in population in their electrophoretic mobility. In enzymes with dimeric structure (GOT, FDH) there is no hybridization of isozymes in gametes possessing both alleles.

Rare variants of isozymes (null-mutations and isozymes with altered electrophoretic mobility) revealed in these populations may also appear as a result of intracystronic recombinations due to unequal crossing-over. The frequency of intracystronic recombinations must be much lower in homozygous populations (ALTUKHOV et al., 1983) approaching the frequency of true mutations. For verifying of this supposition rare electrophoretic variants were united separately in homo- and heterozygous groups of trees (*Table 1*).

Analysis of *table 1* shows that there are no great differences ($p=5.2 \times 10^{-3}$ and 5.0×10^{-3}, correspondingly) between stands 1 and 2 per locus if all types of mutations and mutation-like events are united. However, stands 1 and 2 differ in comparison of the analysed characteristics – the stand in the region of copper-melting plant (population 2) is characterized by a fewer number of trees with null-allele heterozygotes and by mutation-like events related with the destruction of the processes of gametogenesis and/or crossing-over.

The frequency of mutations and mutation-like events turned out to be more than one order higher under the conditions of air pollution.

The main reason for such large differences was because in the control (population 3) no heterozygous null-alleles trees and null-mutations were observed, and the frequency of isozymes with altered electrophoretic mobility turned out to be one order lower.

The primary ecologic and site characteristics of the analysed populations don't differ significantly from each other. Therefore, we assume air pollution to be the main factor responsible for the increase of frequency of mutations and mutation-like events and greater survival of heterozygous null-alleles trees. Under optimum growing conditions the frequency of such events is considerably lower in Scots pine.

The rate of mutation processes varies depending on the loci chosen for analysis. The maximum frequency of rare electrophoretic variants of isozymes was determined for locus Got–3 ($p=6.0 \times 10^{-3}$), and the minimum – for locus Fdh–1 ($p=7.6 \times 10^{-4}$). Mutations and mutation-like events are observed at intermediate frequency in all other loci. The comparison of 3 stands by loci also shows lower frequency of rare electrophoretic variants of isozymes in the control populations.

In scientific literature there are reports of studies of the mutation rates in Scots pine populations using isozymes as molecular-genetic markers. Spontaneous mutations in gametes are shown to occur with frequency $p=2.9-5.8 \times 10^{-4}$ (DUKHAREV and PRAVDIN, 1983), $p=7.7 \times 10^{-4}$ (ALTUKHOV et al., 1983). The data of control population 3 ($p=1.6 \times 10^{-4}$) confirm these results. The rate of spontaneous mutations in Scots pine populations is likely to be on the order of 10^{-4}. Correlations between the frequency of rare allozyme variants and the level of environmental extremes independent of the fact whether they are caused by natural-climatic conditions (KRUTOVSKY et al., 1986) or anthropogenic influence have been reported in the literature. The rate of mutations in swamp populations of Scots pine under severe ecological conditions turned out to be twice as great as in Scots pine under optimum environmental conditions (DUKHAREV and PRAVDIN, 1983). In irradiated Scots pine the frequency of mutations increases up to $p=6.3 \times 10^{-3}$, while in the control the frequency is $p=7.8 \times 10^{-4}$ (KALCHENKO and SPIRIN, 1989). These results and also discoveries of greater genetic diversity in populations under extreme conditions (mainly due to rare alleles with the frequency less than 0.05) imply that survival under extreme conditions is dependent upon the existence of variability in populations. In this case mutations as elementary hereditary units of variability extend the scope of potentialities for natural selection.

Genotypes of open pollinated embryos from four heterozygous by null-alleles trees are presented (*Table 2*). Genotypes f/f are formed by allele f of maternal gamete and allele f of paternal gamete or may be a result of self-pollination. Genotypes f/c, f/d, f/e are formed by pollination of the analysed trees with the pollen carrying alleles c, d, e, correspondingly. The following 3 embryo genotypes do not posess maternal alleles (f) and are likely to be genotypes with maternal alleles which have mutated at the stage of formation and development of zygote. Embryos homozygous by null allele (null/null) are likely to be formed as the result of self-pollination or mergence of maternal gamete carrying null allele with parental gamete carrying null allele. The last genotype f^1/f^1 was found as a result of fertilization of individuals with mutant allele ($f-f^1$) of maternal gamete (isozymes of allele f^1 coincide with those of

Table 2. – Frequency of embryos from open pollination of trees heterozygous by null-allele in locus Got-2.

Number of tree	Volume of sample	Alleles of maternal gamete								
		f	f	f	f	f	f	f	null	f^1
		Genotypes of embryos								
		f/f	f/c	f/d	f/e	null/c	null/e	null/null	null/null	f^1/f^1
46	45	35	2	0	0	2	0	2	4	0
48	45	33	3	4	0	0	0	0	4	1
61	70	48	5	5	3	1	0	4	4	0
69	59	30	3	11	2	0	2	2	9	0
In all	219	146	13	20	5	3	2	8	21	1
%	100	66,7	5,9	9,1	2,3	1,4	0,9	3,6	9,6	0,5

allele e in electrophoretic mobility) and parental gamete carrying allele f^1 or as a result of self-pollination.

We observed a low frequency of null allele genotypes in endosperms in 1:1 ratio. We believe this was caused by deviations of allelic segregation in 4 trees under investigation (*Table 3*).

Table 3. – Segregation of alleles in endosperms of trees geterozygous by null-allele in locus Got-2.

Number of tree	Volume of sample	Alleles				x^2
		null	f	null, %	f, %	
46	85	15	70	17,6	82,4	36,03*
48	84	17	67	20,2	79,8	29,76*
61	110	10	100	9,1	90,9	73,64*
69	99	19	80	19,2	80,8	37,98*
In all	378	61	317	16,1	83,9	173,38*

*) reliable at the level of significance 0.01%

In other loci under conditions of air pollution in heterozygous by null alleles trees reliable or close to reliable deviations in segregation of alleles were observed. This phenomenon may be explained by the selective elimination of gametes or meiotic destructions.

Discussion

The results of the present study should be compared with the reports of genetic diversity in populations of Scots pine not suffering from anthropogenic influences. As result of the observed regularities (STAROVA et al., 1990) it was established that under extreme environmental conditions the frequency of heterozygosity increased, promoting increased genetic diversity. As compared to populations growing under relatively optimum conditions there is observed an increased heterozygosity and a greater number of alleles per locus in spite of isolation of these small populations in high mountains growing under extreme and more heterogenous conditions.

The results of the present work testify to the fact that changes in genetic structure of populations in response to the influence of natural and anthropogenic stress factors have similar regularities. We observed an increase in genetic diversity under air pollution conditions as compared to controls. These changes include:

- an increase in rates of de novo mutations and mutation-like events (null-formations and rare isozymes with altered electrophoretic mobility);
- a greater number of trees with rare genotypes with inherited mutations (heterozygous by null-alleles).

Mutation pricesses may be responsible for the increase in genetic diversity, broadening the scope of possibilities for natural selection under the conditions of increasing anthropogenic influence on forest ecosystems.

Literature

ALTUKHOV, Yu. P., DUKHAREV, V. A. and ZHIVOTOVSKY, L. A.: Otbor protiv redkih elektroforeticheskih variantov belka i tempi spontannogo mutatsionnogo protsessa v populyatsiyah. (Spontaneous mutation rate in populations under selection pressure against rare protein variants). Genetica **19**, 264–275 (1983). — BERGMANN, F. and SCHOLZ, F.: Effects of selection pressure by SO_2 pollution on genetic structures of Norway spruce (*Picea abies*). In: Population Genetics in Forestry. Ed.: H.-R. GREGORIUS. Lecture Notes in Biomathematics. Springer Verlag, Berlin. 267–275 (1985). — DAVIS, B. J.: Disc electrophoresis. II. Methods and application to human serum proteins. Ann. New York Acad. Sci. **121**, 404–427 (1964). — DUBININ, N. P. and GLEMBOTSKY, Ya. L.: Genetika populyatsy i selektsiya. Moskva, Nauka. 591s. (1967). — DUKHAREV, V. A. and PRAVDIN, L. F.: Estestvenii mutatsionnii protsess v populyatsiyah sosni obiknovennoi. (Natural mutation process in Pinus sylvestris populations). Lesovedenie **4**, 10–14 (1983). — GONCHARENKO, G. G., KRIVKO, V. G., POTENKO, V. V. and KHOTYLEVA, L. V.: Endospermi kak test-sistema dlya opredeleniya tempov mutirovaniya v raionah s radioaktivnim zagryazneniem. (*Picea abies* endosperms as a test system for determining mutation rates in the regions with radioactive pollution). Doklady of the Academy of Sciences of the BSSR **35**, 365–369 (1991). — GURIES, R. P. and LEDIG, F. T.: Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* MILL.). Heredity **40** (1), 27–32 (1978). — KALCHENKO, V. A. and SPIRIN, D. A.: Geneticheskie effekti v populyatsiyah sosni obiknovennoi, proizrastayutchih v usloviyah hronicheskogo oblucheniya malimi dozami. (Genetic effects revealed in populations of *Pinus sylvestris* L. growing under exposure to small doses of chronic irradiation). Genetica **25**, 1059–1064 (1989). — KORSHIKOV, I. I., DUKHAREV, V. A., KOTOVA, A. A., RYABOKON, S. M. and MIKHEENKO, I. P.: Allozyme polymorphism of GOT, GDH and SOD loci in Scots pine under conditions of technogenously polluted environment. Tsitologia i genetica **25** (6), 60–64 (1991). — KRUTOVSKY, K. V., GAFAROV, N. I., ALTUKHOV, YU. P. and DUKHAREV, V. A.: Allozyme polymorphism in a natural population of Norway spruce *Picea abies* (L.) KARST. II. Frequency of rare alleles and de novo mutations. Genetica **22**, 2310–2316 (1986). — MEJNARTOWICZ, L., BIALOBOK, ST. and KAROLEWSKI, P.: Genetic characteristics of Scots pine specimens resistant and susceptible to SO_2 action. Arboretum Kornickie **23**, 233–238 (1978). — MÜLLER-STARCK, G.: Genetic Differences between "Tolerant" and "Sensitive" Beeches (*Fagus sylvatica* L.) in an Environmentally Stressed Adult Forest Stand. Silvae Genetica **34**, 241–247 (1985). — SCHOLZ, F. and VENNE, H.: Untersuchung von Genotyp-Umwelt-Interaktionen mit Fichtenklonen auf unterschiedliche Standorten sowie unter Begasung. J. BAUCH und W. MICHAELIS (Herausgeber). Das Forschungsprogramm Waldschaden am Standort „Postturm", Forstamt Farschau, Ratzeburg. GKSS 88/E/55. S. 371–382. (1987). — STAROVA, N. V., ISHBIRDIN, A. R., PUTENIKHIN, V. P. and YANBAEV, YU. A.: Coniferous forests of the South Ural, their population structure and ways of preservation and reproduction of gene pool. Boreal Forests Arkhangelsk, 14–28 (1990). — YANBAEV, Yu. A.: Analiz geneticheskoi structury prirodnykh populyatsii sosny obyknovennoi Yuzhnogo Urala. Avtoref. kand. dis. Minsk, In-t genetiki i tsitologii. 16s. (1989). — YANBAEV, Yu. A., BAKHTIYAROVA, R. M. and SHIGAPOV, S. H.: Polymorphism fermenta formiatdegidrogenasi v populyatsii sosny obyknovennoi v usloviyakh promyshlennogo zagryazneniya. Ekologocheskaya genetica rastenii, zhivotnykh, cheloveka. Mat. IV Vsesoyuznoi nauchnoi konf. Kishinev, Shtiints, 491–492 (1991).