

GENETIC STRUCTURE OF A NATURALLY REGENERATING SCOTS PINE POPULATION TOLERANT FOR HIGH POLLUTION NEAR A ZINC SMELTER

W. PRUS-GŁOWACKI and R. NOWAK-BZOWY

A. Mickiewicz University, Department of Genetics, ul. Dąbrowskiego 165, 60-594 Poznań, Poland

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Abstract. A comparison of the genetic structure for a group of 10 to 15 year old Scots pine trees originating from natural regeneration in an extremely polluted area with the genetic structure of 9 reference populations from the same geographical region but only slightly polluted, have demonstrated differences in alleles and genotypes frequencies. The observed heterozygosity (H_o) was lower by 12% for the group of trees from the polluted area. Fixation index (F) exhibited significant excess of homozygotes ($F=0.15$) in the group of tolerant trees. Average numbers of alleles per locus was higher by 8% in the group of trees from the polluted site. This differs from results from areas with the novel forest decline. Based on the differences in allele and genotype frequencies one might concluded that some of the alleles have a selective disadvantage or a selective advantage in the group of trees in the studied polluted area.

1. Introduction

The 20 yr of activity of the Miasteczko Śląskie zinc smelter in Upper Silesia resulted in the decline of the Scots pine forest stand located to the south-west of the factory. The zone of the tree decline is shifted to south-west about 50 to 100 m yr⁻¹ (Rostański, personal communication). At the moment the Scots pine stand appeared about 3 km from the smelter. In 1988 we found 29, 10 to 18 yr old trees apparently originated from natural regeneration. These trees were growing 1000 to 1150 m from the zinc smelter, and do not show any traces of injuries caused by the industrial emission. Therefore it was interesting from the theoretical and practical point of view to study the genetic structure of these tolerant trees grown in the extremely polluted environment and to compare the genetic parameters of this tolerant population with the genetic structure of other pine populations originated from the same geographical region but not so polluted. We are fully aware of the fact that it is difficult to compare directly adaptive strategies of the population from Miasteczko Śląskie and the reference populations due their different origin and different gene pools. But since the parental stand from which the tolerant population originated does not exist, we assume that the reference populations represents 'average' genetic structure of scots pine characteristic for this geographical region.

Comparing several genetic parameters of these two groups of trees we believe that the observed differences between the population from MS and the reference

populations reflect the changes in genetic structure linked to selection pressure close to zinc smelter.

2. Material and Methods

Dormant buds collected in winter 1988 were taken in order to perform isoenzymatic analyses and to determine the genetic structure of the group of trees from the polluted area. As a reference (control) nine populations from south-western Poland were sampled from a provenance trial established by the Institute of Dendrology in Kórnik. Localization of the seed sources for 'control' populations and of origin of experimental material from polluted area are listed in Table I. In the material the variability of 10 enzymatic loci were analyzed: glutamate dehydrogenase (GDH) E.C.1.4.1.3., malate dehydrogenase (MDH) – two loci, E.C.1.1.1.37, shikimate dehydrogenase (SkDH) – two loci, E.C.1.1.1.14, alcohol dehydrogenase (ADH) E.C.1.1.1.1., glutamate oxale-acetate transaminase (GOT) – two loci, E.C.2.6.1.1., diaforase (Diaf), E.C.1.6.99, and fluorescent esterase (Fest) E.C.3.1.1.1. Isoenzyme patterns in gels, staining procedure and genetic interpretation of the results correspond to those described by Rudin and Ekberg (1978), Szmidt and Yazdani (1984), Yazdani and Rudin (1982) and Gullberg *et al.* (1982). Calculations of genetic parameters such as heterozygosity observed (H_o) and expected (H_e), genotype polymorphism index (P_g) acc. to Kahler *et al.* (1980), fixation index (F) and genetic similarity indices based on gene (SN) and genotype (SH) frequencies and also the genetic distances according to formula $DN = 1 - SN$, and $DH = 1 - SH$ in the studied groups of trees were conducted as described by Nei and Roychoudhury (1974), Jain and Workman (1967) and Hedrick (1974). Significance of differences in allele and genotype frequencies in the studied populations was evaluated using a chi-square test assuming their equal frequency in the population from zinc smelter

TABLE I
Description of *Pinus sylvestris* populations used in the study

Population		Localization	No. of trees	Origin	Age
1. Bystrzyca Kłodzka	BK	16°36'E 50°17'N	29	Provenance	22
2. Bolewice	BO	16°03'E 52°28'N	30	trial	22
3. Brody	BR	14°50'E 51°46'N	30		22
4. Janów Lubelski	JL	22°25'E 50°40'N	30	in Kórnik	22
5. Oleszyce	OL	23°00'E 50°15'N	30		22
6. Pionki	PI	21°20'E 51°30'N	29	17°10'E	22
7. Spała	SP	20°10'E 51°30'N	30		22
8. Zw. Lubelski	ZL	23°00'E 50°38'N	30	52°25'N	22
9. Radawnica	RD	16°58'E 53°30'N	30	Natural regeneration	22
10. Miasteczko Śl.	MS	18°75'E 50°50'N	29		10

TABLE II

Concentration in the air and content of major pollutants in the soil in Kórník and Miasteczko Śląskie area

Pollutants	Miasteczko Śląskie	Kórník
mg kg ⁻¹ of soil		
Zn	595.0	11.5
Pb	425.0	2.9
Cd	5.25	0.0
mg m ⁻² a ⁻¹		
SO ₂	92.0	2.0
N ₂ O ₅	81.0	5.0
F	1.25	0.0

area and in the reference populations (Greń, 1972). The data about the deposition and concentration of the major air pollutants in the zinc smelter region are from WSSE Katowice (Environmental Pollution Monitoring Laboratory). Soil samples were taken from the polluted area analyzed for the content of three main heavy metals (Zn, Pb, Cd) emitted by the smelter. Also from Kórník area the level of pollution was determined.

3. Results

3.1. POLLUTION

The concentration of SO₂, N₂O₅, F and NH₃ and the deposition of Pb, Zn and Cd in the polluted area from which the analyzed group of trees was originated is presented in Table II. The content of Zn, Pb, Cd in the soil samples were in average 595 mg Zn, 425 mg Pb and 5.25 mg Cd kg⁻¹ of soil taken from 5 to 10 cm below the ground surface in Miasteczko Śląskie region and much less from Kórník (reference populations).

3.2. ALLELES

Comparison the frequency of alleles in the 10 studied populations showed that in the case of 5 alleles i.e. GDH1, GDH2, SkDH-A2, SkDH-B1 and SkDH-B2 population from Miasteczko Śląskie (MS – polluted area) differ from 9 reference populations. Allele GDH1 is observed in MS population much more frequently and allele GDH2 is rare than in the controls. Statistical significance of these differences were proved by chi-square test. Allele SkDH-A2 which was less frequent in the population from the polluted area (0.052), is also less frequent in population from Plonki (PI – 0.086) and Spała (SP – 0.068). In contrary, allele SkDH-B1 is less frequent in the 'control' group of populations and SkDH-B2 more frequent in population from Miasteczko Śląskie (MS). Rare allele ADH3 was noted only in the population from the polluted area. Rare alleles i.e. Fest5, GOT-A3, SkDH-

TABLE III

Average frequencies of alleles in the group of unpolluted populations and in population from Miasteczko Śląskie. S – standard deviation. * – statistically significant differences (χ^2 test)

Alleles		References populations	S	Miasteczko Śl.
FEst.	1	0.735 (min.0.583 max.0.879)	0.111	0.654
	2	0.092 (min.0.050 max.0.167)	0.038	0.077
	3	0.145 (min.0.017 max.0.300)	0.096	0.269
	5	0.028 (min.0.000 max.0.067)	0.025	0.000
GOT-A	1	0.988 (min.0.948 max.1.000)	0.017	1.000
	3	0.012 (min.0.000 max.0.052)	0.017	0.000
GOT-B	1	0.574 (min.0.467 max.0.667)	0.067	0.554
	2	0.379 (min.0.250 max.0.517)	0.094	0.393
	3	0.047 (min.0.000 max.0.183)	0.043	0.054
Diaf	1	0.866 (min.0.750 max.0.948)	0.061	0.929
	2	0.096 (min.0.017 max.0.167)	0.055	0.054
	3	0.038 (min.0.000 max.0.183)	0.059	0.018
GDH	1	0.647 (min.0.500 max.0.750)	0.092	0.828*
	2	0.353 (min.0.250 max.0.500)	0.092	0.172*
ADH	1	0.636 (min.0.440 max.0.750)	0.087	0.636
	2	0.364 (min.0.250 max.0.560)	0.087	0.295
	3	0.000		0.069
SkDH-A	1	0.828 (min.0.750 max.0.917)	0.059	0.897
	2	0.144 (min.0.086 max.0.250)	0.058	0.052
	3	0.015 (min.0.000 max.0.052)	0.018	0.017
	4	0.006 (min.0.000 max.0.050)	0.017	0.000
	5	0.007 (min.0.000 max.0.017)	0.009	0.035
SkDH-B	1	0.951 (min.0.917 max.0.983)	0.021	0.828
	2	0.045 (min.0.017 max.0.083)	0.022	0.155*
	3	0.002 (min.0.000 max.0.017)	0.006	0.017
	4	0.002 (min.0.000 max.0.017)	0.006	0.000
MDH-A	1	0.965 (min.0.933 max.1.000)	0.019	0.984
	2	0.035 (min.0.000 max.0.052)	0.019	0.017
MDH-C	1	0.749 (min.0.647 max.0.833)	0.080	0.741
	2	0.251 (min.0.167 max.0.353)	0.080	0.259

A4 and SkDH-B4 observed in reference populations were not present in the group of trees from the polluted area. The frequencies of the other alleles did not show differences between polluted and reference populations (Table III).

3.3. GENOTYPES

Significant differences in the genotype frequencies between the population from Miasteczko Śląskie and reference populations were noted for homozygotic GDH11 genotype which was more frequent in polluted population and also for genotypes GDH12, SkDH-A12, SkDH-B11 which were less frequent in the group of trees

TABLE IV

Frequency of the most common and unique genotypes in the group of reference and polluted populations. S – standard deviation, * – statistical differences * – statistically significant differences (chi² test)

Genotypes		References populations	S	Miasteczko Śl.
FEst	11	0.554 (min.0.300 max.0.793)	0.152	0.462
	33	0.033 (min.0.000 max.0.100)	0.041	0.115
	12	0.127 (min.0.069 max.0.167)	0.043	0.077
	13	0.197 (min.0.033 max.0.400)	0.130	0.308
GOT-A	11	0.977 (min.0.897 max.1.000)	0.034	1.000
	13	0.023 (min.0.000 max.0.104)	0.034	0.000
GOT-B	11	0.354 (min.0.267 max.0.467)	0.070	0.321
	22	0.172 (min.0.033 max.0.367)	0.112	0.143
	12	0.397 (min.0.267 max.0.667)	0.120	0.429
	13	0.045 (min.0.000 max.0.167)	0.055	0.045
Diaf	11	0.747 (min.0.533 max.0.897)	0.109	0.857
	12	0.169 (min.0.104 max.0.267)	0.087	0.107
GDH	11	0.437 (min.0.233 max.0.567)	0.123	0.655
	22	0.142 (min.0.067 max.0.267)	0.087	0.000*
	12	0.421 (min.0.200 max.0.533)	0.106	0.345
ADH	11	0.365 (min.0.040 max.0.533)	0.155	0.517
	22	0.094 (min.0.000 max.0.160)	0.058	0.104
	33	0.000	0.000	0.069*
	12	0.541 (min.0.310 max.0.800)	0.157	0.310
SkDH-A	11	0.687 (min.0.533 max.0.828)	0.107	0.828
	12	0.239 (min.0.167 max.0.367)	0.107	0.069*
SkDH-B	11	0.902 (min.0.833 max.0.967)	0.043	0.724*
	12	0.090 (min.0.033 max.0.167)	0.045	0.172
	22	0.000	0.000	0.069*
MDH-A	11	0.929 (min.0.867 max.1.000)	0.039	0.897
	12	0.071 (min.0.000 max.0.135)	0.039	0.104
MDH-C	11	0.574 (min.0.414 max.0.800)	0.114	0.621
	22	0.076 (min.0.000 max.0.179)	0.058	0.138
	12	0.350 (min.0.167 max.0.448)	0.084	0.241

from Silesia. Genotype GDH22 was not found in population from Miasteczko Śląskie (MS). Genotype MDH-C12 was markedly less frequent in the population from polluted area, but also less frequent in the population from Brody (BR). The unique genotypes for MS population i.e. SkDH-A22 and ADH33 are noted for two and three trees, respectively (Table IV).

3.4. GENETIC MULTIPLICITY

The average numbers of alleles per locus are different in particular populations and fluctuate from 2.3 to 2.5. For Miasteczko Śląskie population the value is 2.6

TABLE V

Average number of alleles and genotypes per locus in the group of unpolluted populations and in the population from Miasteczko Śląskie

Locus	Unpolluted populations		Miasteczko Śląskie	
	Alleles	Genotypes	Alleles	Genotypes
FEst	3.7	5.2	3.0	5.0
GOT-A	1.4	1.4	1.0	1.0
GOT-B	2.9	4.2	3.0	5.0
Diaf	2.5	3.0	3.0	3.0
GDH	2.0	2.9	2.0	2.0
SkDH-A	3.1	3.8	4.0	5.0
SkDH-B	2.2	2.2	3.0	4.0
MDH-A	1.9	1.9	2.0	2.0
MDH-C	2.0	2.9	2.0	3.8
ADH	2.0	2.9	3.0	4.0
Mean	2.4	3.0	2.6	3.4
Range	2.3-2.5	2.7-3.7		

alleles per locus. An average of 2.38 alleles per locus was observed for the 9 populations from 'unpolluted' region. (Table V). Average numbers of genotypes per locus in particular reference populations varied from 2.7 to 3.7 (an average 3.0) and for population from Miasteczko Śląskie the value is 3.4. genotypes per locus. Therefore the average number of alleles per locus is higher for population from polluted area than for the reference.

3.5. HETEROZYGOSITY AND GENETIC DIVERSITY

Comparison of the levels of multilocus heterozygosity (H_o) for the group of reference populations to the level of heterozygosity of population from polluted area showed that the heterozygosity of this population is slightly lower ($H_{oMS} = 0.244$, $H_{ocontr.} = 0.288$). When individual loci are taken into consideration, the most extensive differences between population from Miasteczko Śląskie and 'control' group for GDH, ADH, SkDH-B and MDH-C loci are noted. Average heterozygosity (H_o) for these four loci is about 12% higher for reference populations than for population from Miasteczko Śląskie. For locus SkDH-B however, the heterozygosity is higher for the MS population. One should stress, however, that the values of the heterozygosity showed marked fluctuations in particular loci and populations (Table VI). Average genotype polymorphic index (P_g) for polluted population and group of reference populations does not differ significantly. However it is distinctly lower for GDH and higher for SkDH-B loci in the MS population as compared to the reference group. For the four most differing loci (GDH, ADH, MDHC and SkDH-B) the average P_g index is about 15% higher for polluted population (Table VI). The average fixation index (F) values for 10 loci in the studied populations are shown in Table VI. The excess of homozygotes in populations from Oleszyce (OL) and

TABLE VI

Average heterozygosity (H_o and H_e), fixation index (F) and genotype polymorphic indices (P_g) for 10 enzymatic loci in tolerant population (Miasteczko Śląskie) and 'control' populations. S – standard deviation

Population	H_e	H_o	F	P_g
BK	0.266	0.258	0.031	0.368
BO	0.283	0.291	-0.026	0.422
BR	0.312	0.316	-0.013	0.428
JL	0.295	0.310	-0.050	0.412
OL	0.293	0.266	0.092	0.413
PI	0.289	0.289	0.000	0.412
SP	0.286	0.301	-0.052	0.376
ZL	0.281	0.282	-0.002	0.412
RD	0.277	0.286	-0.034	0.393
Average	0.287	0.288	0.000	0.405
S	0.013	0.019	0.045	0.021
MS	0.289	0.244	0.155	0.415

Miasteczko Śląskie (MS) is noted. In the remaining populations, the index (F) is close to zero showing slight excess homo or heterozygotes as expected from Hardy-Weinberg equilibrium. The most extensive differences between populations are noted for GDH, ADH, MDH-C and SkDH-B loci. For the SkDH-B locus the most pronounced excess of homozygotes in population from polluted area is observed (Figure 1).

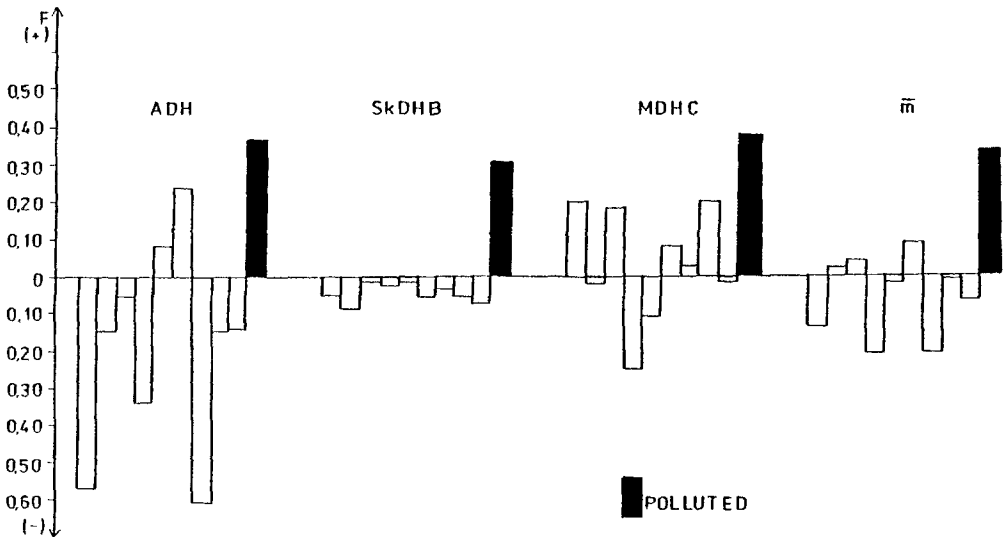


Fig. 1. Fixation index (F) for ADH, SkDH-B and MDH-C loci for reference populations (open bars) and population from Miasteczko Śląskie \bar{m} – mean value for these loci.

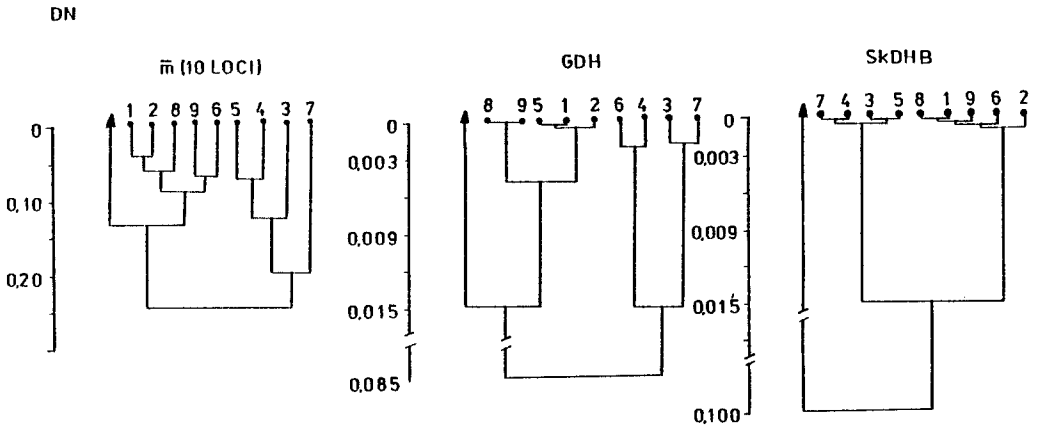


Fig. 2. Dendrograms showing the genetic similarities of the studied populations based on Nei genetic distances (DN) for GDH and SkDH-B loci and also for the all 10 investigated loci (\bar{m}). Description of population as in Table I.

3.6. GENETIC SIMILARITIES

The genetic similarities of the studied populations based on allele (Nei) and genotype (Hedrick) frequencies in form of dendrograms are presented in Figures 2 and 3. The MS population exhibited the most pronounced specificity as compare to reference

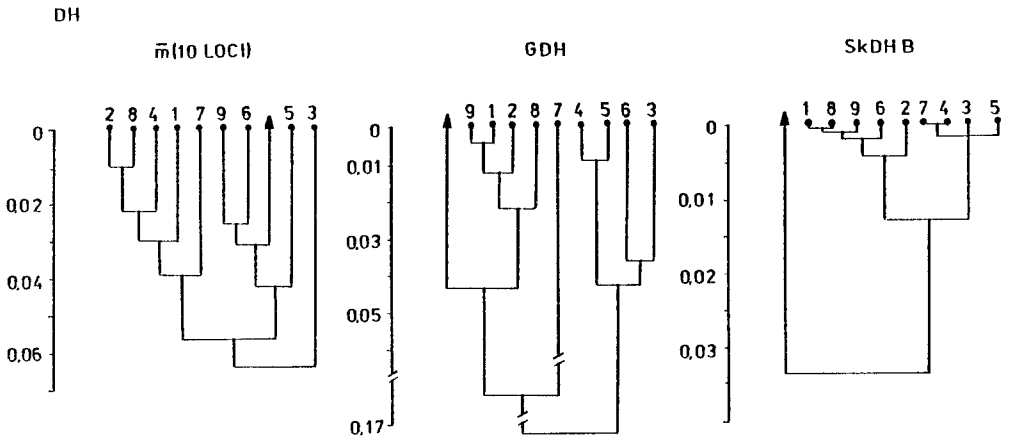


Fig. 3. Dendrograms showing the genetic similarities of the studied populations based on Hedrick genetic distances (DH) for GDH and SkDH-B and also for the all 10 investigated loci (\bar{m}). Description of populations as in Table I.

group of populations in the case of GDH and SkDHB loci. In the case of genetic similarities based on alleles frequencies, for all studied loci the populations clustered in two groups. To one of this group, population from Miasteczko Śląskie (MS) is loosely connected. When the genetic similarities based on genotype frequencies are considered, the studied populations formed also two groups, but this time population from Miasteczko Śląskie is incorporated to one of them.

4. Discussion and Conclusions

Comparison of the frequencies of alleles and genotypes in the group of populations from only slightly polluted regions with those located in extremely polluted area has shown distinct differences in the genetic structure of the two groups of trees. The specificity of the population from Miasteczko Śląskie could be caused by two reasons. One of them is genetic drift resulting from not random set of the seeds from which the natural regeneration of *Pinus sylvestris* in the polluted area was created, or most probably the specificity of this group of trees is connected with selection pressure caused by various pollutants, especially heavy metals emitted by zinc smelter. It should be also mentioned that atmospheric pollutants could have influence on reproductive processes and the viability of the parental trees. These phenomenon additionally differentiated the genetic pool of the seeds of naturally regenerating populations of trees in clean and polluted areas (Venne *et al.*, 1989). Differences in the genetic structure of the tolerant and sensitive to pollution groups of trees were reported by several authors (Müller-Starck, 1985, 1989; Bergmann and Scholz, 1984, 1987, 1988; Scholz and Bergmann, 1984; Mejnartowicz, 1983; Mejnartowicz and Palowski, 1989; Geburek *et al.*, 1987; Prus-Głowacki and Nowak-Bzowy, 1989; Prus-Głowacki and Godzik, 1990). However the results of the works are not convergent in some points. Hitherto studies indicate that for some of loci, heterozygotic genotypes are more frequent in tolerant groups of trees in natural populations as well as in the controlled conditions, indicating the fitness superiority of heterozygotes (Bergman and Scholz, 1989; Geburek *et al.*, 1987; Müller-Starck, 1989). On the other hand, our studies (Prus-Głowacki and Godzik, 1991) on *Pinus sylvestris* seedling populations grown in extremely polluted environment have shown significant increase of the homozygosity in the groups of survivors for some of the loci. It is generally accepted that the advantage of high genetic multiplicity and heterozygosity is connected with the spatially and temporally heterogenous environment. Any low level stress coinnected with antropogenic factors will increase the heterogenity of environment and the genetic variability of population. But with high concentration of stress a decrease of environmental heterogenity will occur and becomes the prevailing environmental factor. Under such conditions, genetic diversity may be no advantage or becomes even a load, because only special genotypes can survive in such conditions (Scholz, 1990). This kind of situation seems to exist in the investigated polluted population. In comparison with 9 slightly polluted populations, the population from extremely polluted area has shown lower

heterozygosity (H_o) for several loci. Also Wright fixation index (F) has revealed an excess of homozygotic genotypes most extensive for ADH, SkDH-B and MDH loci (Figure I). Comparison of the frequency of alleles and genotypes in population from Miasteczko Śląskie and in 'control' populations has shown that the frequency of alleles GDH2 and SkDH-A2 is significantly lower in Miasteczko Śląskie population and that the alleles exist in the population only in heterozygotes. It is in accordance to results of Geburek *et al.* (1987), where the frequency of allele GDH2 was much lower in the group of *Pinus sylvestris* trees tolerant to SO. On the other hand, allele SkDH-B2 is more frequent in the population from the polluted area. There is a growing body of evidence that the two enzymatic loci e.g. GDH and SkDH are directly connected to detoxication mechanisms in environment polluted by SO, NO, as well as heavy metal ions (for review see Srivastava and Singh, 1987; Tomset and Thruman, 1988). From this point of view, one might conclude that these alleles have a selective disadvantage or advantage in the studied polluted area. Our investigations have shown that the group of trees from the polluted area is decisively different in genetic structure and also in the level of genetic variability from the group of trees from unpolluted region. Now, when the industrial pollution in Europe is no longer a local problem, the studies on adaptive strategy and long term consequences of changes in genetic pool of forest tree populations are fundamentally important.

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