# Vorwort

"What are you doing here so shortly after midnight?", LANGNER used to address a doctoral student who happened to turn up at the Schmalenbeck institute before 9 o'clock in the morning. One must know that LANGNER was a hard-working man who had seen the student's lamp burning when he left the building around midnight the night before. Although he himself hardly ever arrived only second or even third at the office in the morning, he had never expected the student to be this early.

This attitude toward his coworkers was not the least among the reasons for the atmosphere of industriousness and scientific productivity which he created. LANGNER directed the Institute of Forest Genetics and Forest Tree Breeding in Schmalenbeck for the 22 years between 1949 and 1971 in a way that made those who worked there feel like members of a family.

This family and many other friends all over the world express their cordial congratulations on the occasion of LANGNER's 80th birthday. We also extend our warmest wishes for the continuation of his proverbial health and youthfulness.

LANGNER has published far more than one hundred articles on diverse aspects of forest tree breeding and the evolving field of forest genetics. In 1971, when LANGNER retired from active service, his *curriculum vitae* was presented in this — his — journal. Fifteen years later, a few words may be added concerning his presumable satisfaction to observe that the population genetics of forest trees is being widely studied with modern techniques. He not only paved the way for the establishment of this scientific field in forestry. During the fifties and sixties, when only a limited body of population genetic concepts existed, he published papers which may be considered as landmarks in the field of forest genetics. These articles both enlarged the store of experimental evidence and advanced the manner of thinking. The world of forest genetics and forest tree breeding has benefited greatly from this strong contributor.

*Ad multos annos!*

H. H. HATTEMER
Abteilung Forstgenetik
und Forstpflanzenzüchtung
der Universität Göttingen

# Genetic Studies by Isozyme Gene Loci on Tolerance and Sensitivity in an Air Polluted Pinus sylvestris Field Trial[1])

By TH. GEBUREK*, F. SCHOLZ*, W. KNABE** and A. VORNWEG*

* Federal Research Centre for Forestry and Forest Products,
Institute of Forest Genetics and Forest Tree Breeding,
Sieker Landstrasse 2, D-2070 Grosshansdorf 2
Federal Republic of Germany

** State Institute for Ecology, Landscape Development,
and Forest Planning of Northrhine-Westphalia,
Leibnizstrasse 10, D-4350 Recklinghausen
Federal Republic of Germany

(Received 26th January 1986)

**Summary**

Two sets of trees, 46 relative sensitive and 45 relative tolerant individuals, were selected from six open pollinated *Pinus sylvestris* families in a field trial stressed by air pollutants in Northrhine-Westphalia, Fed. Rep. of Germany.

The trees were genetically identified at nine electrophoretically-detectable enzyme gene loci. Comparisons of genetic parameters between the sensitive and the tolerant set showed the following results:

Allele and/or genotype frequencies at gene loci of glutamate dehydrogenase (GDH) and glutamate oxaloacetate transaminase (GOT) differed significantly between the two sets. The tolerant trees had higher multilocus heterozygosities (actual and conditional) that exceeded with $\bar{H}_a = .41$ and $\bar{H}_c = .80$ the values among the sensitive trees by about 30% and 10%, respectively. This indicates that GDH- and GOT-gene loci, as well as the other gene loci were partly involved in the sensitivity against the environmental stress. Genic and genotypic multilocus diversities were each about 2.5 times greater in the tolerant than in the sensitive set. Single locus genetic distances between the two sets did not exceed $\bar{d} = .32$.

*Key words: Pinus sylvestris*, genetic differentiation, isozymes, air pollution.

**Zusammenfassung**

Von sechs *Pinus sylvestris* Nachkommenschaften — entstanden aus freier Abblüte — wurden zwei Kollektive gebildet, die Bäume unterschiedlicher Immissionstoleranz umfaßten. Dafür wurden 46 relativ immissionsensitive und 45 relativ tolerante Kiefern in einem immissionsbelasteten Feldversuch in Nordrhein-Westfalen (Bundesrepublik Deutschland) ausgewählt.

Die Versuchsbäume wurden an neun elektrophoretisch nachweisbaren Enzym-Genloci genetisch identifiziert. Vergleiche genetischer Parameter zwischen dem sensitiven und toleranten Kollektiv erbrachten folgende Resultate:

[1]) dedicated to Prof. Dr. W. LANGNER, on the occassion of his 80th birthday.

Allel- und/oder Genotypfrequenzen an Genloci der Glutamatdehydrogenase (GDH) und Glutamatoxalacetattransaminase (GOT) unterschieden sich signifikant zwischen den beiden Kollektiven. Die toleranten Bäume wiesen mit $\bar{H}_a$ = 0,41 und $\bar{H}_c$ = 0,80 eine um etwa 30% bzw. 10% höhere Multilocus-Heterozygotie (aktuell und konditioniert) auf, als die sensitiven. Dies läßt erwarten, daß neben Genloci der GDH und GOT auch die anderen untersuchten Genloci die Immissionstoleranz mit beeinflussen. Die genische und genotypische Multilocus-Diversität war in der toleranten Gruppe jeweils um etwa das 2,5-fache größer, als die entsprechenden Werte der sensitiven Gruppe. Der genetische Abstand zwischen den Kollektiven war an den einzelnen Genloci nicht größer als $\bar{d}$ = 0,32.

## Introduction

Man-made environmental pollution has caused a rapid increase of stress factors in forest ecosystems. Severe ecological damage has to be expected. One of the long-term effects of air pollution is selection. In extreme cases extinction, i.e. a break down of the genetic system of forest tree species by a high reduction of the population size will be the result of environmental stress.

Beginning with the sixties, clonal, family, and provenance studies have already indicated that sensitivity to different air pollutants has a genetic basis (for a literature review see: MEJNARTOWICZ 1984). However, only the application of the isozyme technique made a quantification of selection on basis of genes possible. Indications of such selection processes were published by several authors. MEJNARTOWICZ (1983), investigating a *Pinus* stand affected by $SO_2$ and fluoride, could differentiate sensitive and tolerant trees by isozyme gene markers. Fumigation experiments with $SO_2$ showed, that the genetic structures at isozyme gene loci of sensitive and tolerant Norway spruce clones differed significantly (SCHOLZ and BERGMANN 1984). Genetic differences between tolerant and sensitive beeches (*Fagus sylvatica*) as studied by isozyme gene loci were also found in an adult forest stand under environmental stress (MÜLLER-STARCK 1985).

The objective of this investigation was the question, whether the so called field resistance against air pollutants of young pines *(Pinus sylvestris* L.) was associated to a genetic differentiation based on isozyme data.

## Material and Methods

The investigated population is located in the Northrhine-Westphalia forest district Xanten, at an elevation of 45 m above sea level. The trees originated from open pollination of three relative sensitive and three relative tolerant seed parents, formerly located in Mengeder Heide. For this investigation 46 sensitive and 45 tolerant trees were selected from in total 640 individuals. The selection of the experimental trees based on scorings of the number of needles per year, colour of needles and needle necrosis, when the trees were four years old in 1972 and air pollution was mainly due to $SO_2$.

For genotyping meristem tissue of young vegetative buds was collected. As far as seeds from the experimental trees were obtainable, additional endosperms were used for the enzyme separation.

The isozymes assayed, their acronoyms, the applied gel- und electrode buffer systems, the number of gene loci scored and references refering to the genetic control are given in *Table 1.*

The enzymes were extracted by 0.05 M tris/0.04 M HCl buffer, pH 7.5, with addition of 30 mg/ml water-insoluble polyvinylpyrrolidone (PVP) and 2 mg/ml ethylenediaminetetraacetic acid (EDTA II). The raw extracts were separated by horizontal starch gel electrophoresis: Gel-concentration 12%, voltage 15—30 V/cm, bridge distance 10—14 cm.

The staining recipes for SDH (SHAW and PRASAD 1970), IDH (NICHOLS and RUDDLE 1973), 6-PGDH (BREWER 1970), GDH (SHAW and KOEN 1968), GOT BREWBAKER *et al.* 1968), PGM (BREWER 1970), and LAP (SCANDALIOUS 1969) were slightly modified.

*Genetic parameters*

The allele and genotype frequencies were summed up for the sensitive and the tolerant set and the genetic structure at each gene locus was compared by the 2 × C contingency table. Actual and conditional heterozygosities (GREGORIUS *et al.* 1986), as well as genic and genotypic diversities (GREGORIUS 1978) were computed for the sensitive and tolerant set, respectively. Additionally the genetic distances between these groups were calculated according to GREGORIUS (1974), even though some criticism of this parameter was published recently (KATZ and GOUX 1986).

## Results

Comparisons of the allele and genotype frequencies resulted in significant differences between the two sets at the GDH- and GOT-gene loci (*Table 2*).

The genotype GDH-$A_2A_2$ was most frequent in the sensitive set (.74), whereas the heterozygotes GDH-$A_1A_2$ were found predominantly in the tolerant pines (.51). Corresponding to these genotypic structures the allele frequencies differed significantly. The allele GDH-$A_1$ was more

*Table 1.* — Isozymes assayed, acronyms, applied gel electrode buffer systems, number of scored gene loci and the reference(s) of the genetic control.

| Isozyme system | E.C. No. | Gel / electrode buffer system | No. of scored loci | Reference(s) |
|---|---|---|---|---|
| shikimate dehydrogenase (SDH) | 1.1.1.25 | A | 2 | SZMIDT and YAZDANI (1984) |
| isocitrate dehydrogenase (IDH) | 1.1.1.42 | A | 1 | no reference / putative genetic control |
| 6-phosphogluconate dehydrogenase (6-PGDH) | 1.1.1.43 | A | 1 | SZMIDT and YAZDANI (1984) |
| glutamate dehydrogenase (GDH) | 1.4.1.3 | B | 1 | CHUNG (1981) |
| glutamate oxaloacetate transaminase (GOT) | 2.6.1.1 | B | 3 | RUDIN (1975), CHUNG (1981), MÜLLER-STARCK (1982) |
| phosphoglucomutase (PGM) | 2.7.5.1 | B | 2 | no reference / putative genetic control |
| leucine aminopeptidase (LAP) | 3.4.11.1 | B | 2 | RUDIN (1977) |

A - Gel buffer: 0.14 M tris - 0.04 M citric acid, pH 7.0/ electrode buffer: 0.028 M tris - 0.008 M citric acid, pH 7.0 (SHAW and PRASAD (1970), modified)
B - Gel buffer: 0.08 M tris - 0.01 M citric acid, pH 8.7/ electrode buffer: 0.06 M NaOH - 0.30 M boric acid, pH 8.2 (POULIK (1957), sligthly modified)

*Table 2.* — Genotype and allele frequencies at nine isozyme gene loci in two sets of Scots pine differing in sensitivity to air pollutants.

| Gene locus | Genotype | Allele | Frequency (Sensitive Set) | Frequency (Tolerant Set) | $\chi^2$ |
|---|---|---|---|---|---|
| SDH-A | $A_1A_2$ | | .13 | .08 | 2.59 n.s. |
| | $A_2A_2$ | | .75 | .76 | |
| | $A_2A_3$ | | .05 | .12 | |
| | $A_2A_4$ | | .05 | .02 | |
| | $A_3A_3$ | | .02 | .02 | |
| | | $A_1$ | .07 | .04 | 2.12 n.s. |
| | | $A_2$ | .86 | .87 | |
| | | $A_3$ | .05 | .08 | |
| | | $A_4$ | .02 | .01 | |
| SDH-B | $B_1B_2$ | | .09 | .12 | .21 n.s. |
| | $B_2B_2$ | | .91 | .88 | |
| | | $B_1$ | .04 | .06 | .20 n.s. |
| | | $B_2$ | .96 | .94 | |
| 6-PGDH-A | $A_1A_1$ | | - | - | 1.96 n.s. |
| | $A_1A_2$ | | .38 | .54 | |
| | $A_2A_2$ | | .62 | .46 | |
| | | $A_1$ | .19 | .27 | 1.31 n.s. |
| | | $A_2$ | .81 | .73 | |
| GDH-A | $A_1A_1$ | | .02 | .07 | 9.47 ($P \leq .01$) |
| | $A_1A_2$ | | .24 | .51 | |
| | $A_2A_2$ | | .74 | .42 | |
| | | $A_1$ | .14 | .32 | 8.39 ($P \leq .01$) |
| | | $A_2$ | .86 | .68 | |
| GOT-A | $A_1A_1$ | | - | .02 | .02 n.s. |
| | $A_1A_2$ | | 1.0 | .98 | |
| | $A_2A_2$ | | - | - | |
| | | $A_1$ | .50 | .51 | .02 n.s. |
| | | $A_2$ | .50 | .49 | |
| GOT-B | $B_1B_1$ | | .09 | .09 | 14.94 ($P \leq .01$) |
| | $B_1B_2$ | | - | - | |
| | $B_1B_3$ | | .38 | .43 | |
| | $B_2B_2$ | | .04 | .02 | |
| | $B_2B_3$ | | - | .23 | |
| | $B_3B_3$ | | .49 | .23 | |
| | | $B_1$ | .28 | .30 | 5.36 n.s. |
| | | $B_2$ | .04 | .14 | |
| | | $B_3$ | .68 | .56 | |
| GOT-C | $C_1C_1$ | | .18 | .25 | 4.52 n.s. |
| | $C_1C_2$ | | .47 | .59 | |
| | $C_2C_2$ | | .35 | .16 | |
| | | $C_1$ | .41 | .55 | 3.22 n.s. |
| | | $C_2$ | .59 | .45 | |
| PGM-A | $A_1A_1$ | | .02 | .02 | 5.50 n.s. |
| | $A_1A_2$ | | - | .11 | |
| | $A_2A_2$ | | .93 | .80 | |
| | $A_1A_3$ | | - | - | |
| | $A_2A_3$ | | .05 | .07 | |
| | $A_3A_3$ | | - | - | |
| | | $A_1$ | .02 | .08 | 3.05 n.s. |
| | | $A_2$ | .96 | .89 | |
| | | $A_3$ | .02 | .03 | |
| LAP-B | $B_1B_1$ | | - | - | .88 n.s. |
| | $B_1B_2$ | | - | .02 | |
| | $B_1B_3$ | | - | - | |
| | $B_2B_2$ | | .97 | .96 | |
| | $B_2B_3$ | | - | - | |
| | $B_3B_3$ | | .03 | .02 | |
| | | $B_1$ | - | .02 | .89 n.s. |
| | | $B_2$ | .97 | .97 | |
| | | $B_3$ | .03 | .01 | |

frequent in the tolerant set (.32) than in the sensitive one (.14). The allele GDH-$A_2$ predominated with a frequency of .86 and .68 in the sensitive and tolerant set, respectively.

The frequencies of the GOT-B genotypes were very similar in both sets, except GOT-$B_2B_2$ which was only observable in the sensitive set, at frequencies of .23. The allele GOT-$B_2$ was about four times as frequent in the sensitive set as in the tolerant one. However these differences were not significant as the other gene loci did not differ at a significant level.

Actual and conditional multilocus heterozygosities among the sensitive trees were $\bar{H}_a = .31$ and $H_c = .72$, whereas $\bar{H}_a = .41$ and $\bar{H}_c = .80$ were calculated among the tolerant trees. Genotypic multilocus diversities were $\bar{V}_{SEN} = 51.7$ for the sensitive and $\bar{V}_{TOL} = 124.2$ for the tolerant trees. The genetic multiplicity was not different between the two groups of trees. But the genic multilocus diversity differed to a great extent ($\bar{V}_{SEN} = 21.7$, $\bar{V}_{TOL} = 52.5$).

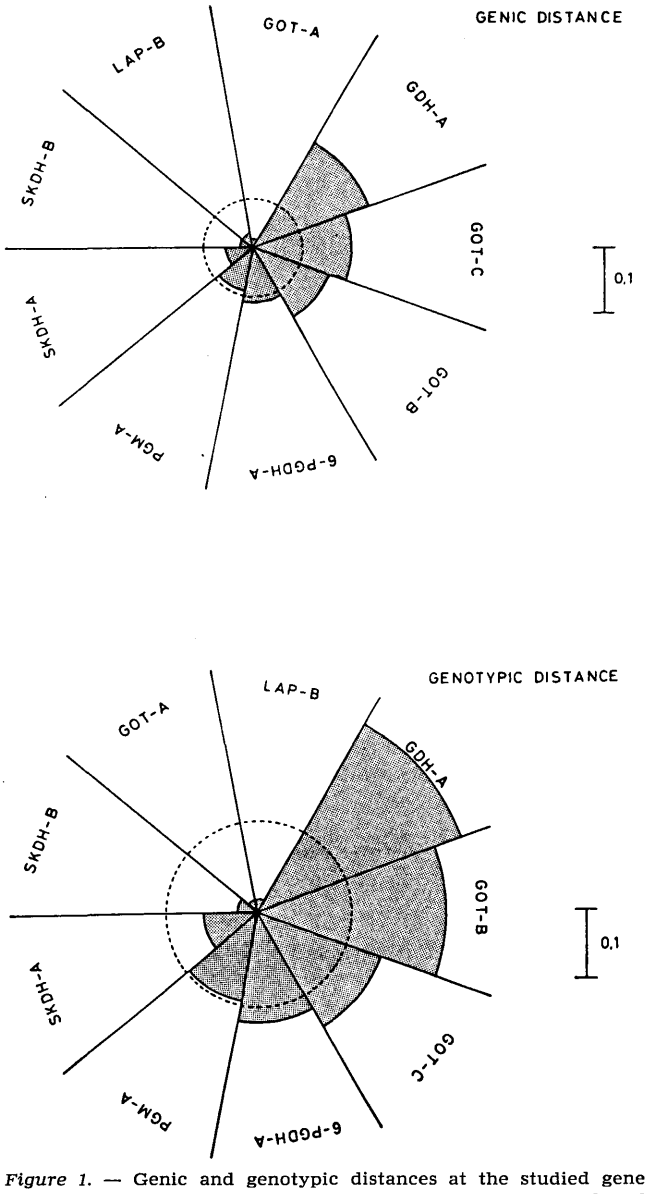


*Figure 1.* — Genic and genotypic distances at the studied gene loci. The radii of the circle sectors represent the distance based on the respective gene loci. Radii of the dotted circles represent the mean distance.

The genetic distance, which will result in 0.0 if the compared sets are genetical identical, reached maximum values at the GDH gene locus. Distances of .18 and .32 were computed as allelic and genotypic distances, respectively, whereas the mean distances were $\bar{d}_{genic} = .08$ and $\bar{d}_{genotypic} = .14$. *Figure 1* demonstrates the distance values for each gene locus as well as the mean distance values.

## Discussion

The recent forest decline is caused by complex stress, based on interactions between several pollutant components and natural stress factors. Therefore, it would be difficult to relate certain effects, genetic ones or others, to a certain pollution component. However, in this study the separation of the experimental trees into two sets is based on characters, which were measured and scored in the beginning seventies, when sulphur dioxide was very probably one of the main stress factors in Northrhine-Westphalia.

For monitoring genetic differentiation between the sensitive and the tolerant set of trees by isozyme systems, isozymes itself or products controlled by gene loci closely linked to the isozyme gene loci should be involved in metabolic processes affected by toxic components.

The enzymes GDH and GOT are essential for the aminoacid metabolism, whereas IDH is involved in the tricarbonacid cycle. The activity of these enzymes is altered by $SO_2$ and other toxic gases. Low concentrations of $SO_2$ increased the activity of these enzymes (Rabe and Kreeb 1980). Hence considering the study mentioned above, there was reason to expect a certain relationship between the gene loci coding for these isozymes and field resistance of the respective trees. Indeed, genetic differences could be found at GDH- and GOT-gene loci comparing two sets differing in the field resistance. Since the IDH-gene locus was monomorphic in all trees, an important role of this isozyme in the variation of tolerance mechanism is not very probable in the Scots pine trees of this investigation.

The isozyme data elaborated in this study suggest that certain isozyme genotypes may have a viability advantage. However monogenic effects are not very probable, since in trees tolerance and resistance are predominantly controlled by polygenes. Therefore a close association of biochemical markers and tolerance/resistance genes, as for example found in *Aegilops ventricosa* against the fungal desease eyespot (McMillin *et al.* 1986), cannot be expected in long-lived forest tree species.

In the sensitive set the frequency of the allele GDH-$A_2$ is higher than in the tolerant set. If the influence of the respective pollutants persists, selection against this allele is to be expected, resulting in changes of the genetic structure. In the extreme case this biallelic gene locus can become monomorphic.

The classical hypothesis of heterozygotes superiority formulated by Lerner (1954) supports reason to expect that in a heterogeneous environment as found in field trials plants with a higher degree of individual genetic multiplicity have higher viability. This is supported by the present study, where the mean heterozygosity was greater in the tolerant pines than in the sensitive ones. Similar results were found by Mejnartowicz (1983) in an environmental stressed *Pinus sylvestris* population and by Müller-Starck (1985) in a *Fagus sylvatica* forest stand that suffered from air pollution.

Since heterozygosity is limited by the genic structure, the actual values were weighted by the maximum values, which theoretically can be obtained. This so called conditional heterozygosity is bounded by the most frequent allele at the gene locus. At three gene loci (6-PGDH-A, GOT-A, SKDH-B) $H_a$ coincide with the maximum attainable. This gives expectation that $H_a$ is an inappropriate parameter describing the variation of this set of data. At the other gene loci the tolerant trees had higher H-values also after conditioning than the sensitive ones, but the relative difference became smaller.

Likewise, recent genetic studies in other plant species support the classical hypothesis of heterozygote superiority. An association between heterozygosity of isozyme gene loci and quantitative traits (especially concerning growth characters) was found for example in *Pinus rigida* (Ledig *et al.* 1983), in *Populus tremuloides* (Mitton and Grant 1980). and in *Zea mays* (Kahler and Wehrhahn 1986). Nevertheless, it should be mentioned that not always an association of heterozygosity at studied gene loci and quantitative traits is observable in forest trees (El-Kassaby 1982).

Under homogeneous environmental conditions as in controlled fumigation experiments there is also no reason to expect inferiority of individuals with low heterozygosity. Indeed, in such experiments with controlled application of single stress factors, heterozygosity was not higher in the tolerant group, as found in two sets of Norway spruce clones differing in $SO_2$ sensitivity in fumigation experiments (Scholz and Bergmann 1984).

The genetic multiplicity of the tolerant and sensitive pines did not differ in this investigation. Since the experimental trees originated from only six open-pollinated seed parents, different genic multiplicity between the two sets was not very probable. However, in a natural stand of beech Müller-Starck (1985) found that the tolerant trees contained one-third more different alleles and genotypes than the sensitive ones.

Since high genetic multiplicity of a gene pool is a basic requirement of high heterozygosity and since high heterozygosity renders a high adaptive potential of a plant to varying stress factors (Gregorius *et al.* 1985), the genetic multiplicity of forest tree populations is to be preserved to a great extent.


## Acknowledgements

The technical assistance of Renate Reck is greatly appreciated. We thank Sabine Neumann for preparing the drawings. This study was partly funded by the State Institute of Ecology, Landscape Development and Forest Planning of Northrhine-Westphalia, Fed. Rep. of Germany.


## Literature Cited


Brewbaker, J. L., Upadhya, M. D., Makinen, Y. and McDonald, T.: Isoenzyme polymorphism in flowering plants III: gel electrophoretic methods and applications. Physiologia Plantarum **21**, 930—940 (1968). — Brewer, G. J.: An introduction to isozyme techniques. Academic Press, New York (1970). — Chung, M.-S.: Biochemical methods for determining population structure in *Pinus sylvestris* L.. Acta Forestalia Fennica **173**, 4—28 (1981). — El-Kassaby, Y.: Associations between allozyme genotypes and quantitative traits in Douglas-Fir (*Pseudotsuga menziesii* (Mirb.) Franco). Genetics **101**, 105—115 (1982). — Gregorius, H.-R.: Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. Silvae Genetica **23**, 22—27 (1974). — Gregorius, H.-R.: The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Math Biosci. **41**, 253—271 (1978). — Gregorius, H.-R., Hattemer, H. H., Bergmann, F. und Müller-Starck, G.: Umweltbelastung und Anpassungsfähigkeit von Baumpopulationen. Silvae Genetica **34**, 230—241 (1985). — Gregorius, H.-R., Krauhausen, J. and Müller-Starck, G.: Spatial and temporal genetic differentiation among the seed in a stand of *Fagus sylvatica* L..

Heredity, in press (1986). —— KAHLER, A. L. and WEHRHAHN, C. F.: Associations between quantitative traits and enzyme loci in the F2 population of a maize hybrid. Theor. Appl. Genet. **72**, 15—26 (1986). —— KATZ, M. and GOUX, J.-M.: The statistical properties of genetic absolute distance. Biom. J. **28**, 729—739 (1986). —— LEDIG, F. T., GURIES, R. P. and BONNEFELD, B. A.: The relation of growth to heterozygosity in pitch pine. Evolution **37**, 1227—1238 (1983). —— LERNER, I. M.: Genetic homeostasis. Oliver and Boyd, Edinburgh (1954). —— MCMILLIN, D. E., ALLAN, R. E. and ROBERTS, D. E.: Association of an isozyme locus and strawbreaker foot resistance derived from *Aegilops ventricosa* in wheat. Theor. Appl. Genet. **72**: 743—747 (1986). —— MEJNARTOWICZ, L. E.: Changes in genetic structure of Scots pine (*Pinus sylvestris* L.) population affected by industrial emission of fluoride and sulfur dioxide. Genetica Polonica **24**, 41—50 (1983). —— MEJNARTOWICZ, L. E.: Enzymatic investigations on tolerance in forest trees. In: KOZIOL, M. J. and WHATELY, F. R. (eds.): Gaseous air pollutants and plant metabolism. Butterworths, London, 381—398 (1984). —— MITTON, J. B. and GRANT, M. C.: Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. Am. J. Bot. **67**, 202—209 (1980). —— MÜLLER-STARCK, G.: Sexually asymmetric fertility selection and partial self-fertilisation 2. Clonal gametic contributions to the offspring of a Scots pine seed orchard. Silvae Fennica **16**, 99—106 (1982). —— MÜLLER-STARCK, G.: Genetic differences between "tolerant" and "sensitive" beeches (*Fagus sylvatica* L.) in an environmental stressed adult forest stand. Silvae Genetica **34**, 241—247 (1985). —— NICHOLS, E. A. and RUDDLE, F. H.: A review of enzyme polymorphism, linkage electrophoretic conditions for mouse and somatic cell hybrids in starch gels. Histochem. and Cytochem. **21**: 1066—1081 (1973). —— POULIK, M. D.: Starch gel electrophoresis in a discontinuous system of buffers. Nature **180**, 1477—1478 (1957). —— RABE, R. and KREEB, K, H.: Wirkungen von $SO_2$ auf die Enzymaktivität in Pflanzenblättern. Z. Pflanzenphysiol. **97**, 215—226 (1980). —— RUDIN, D.: Inheritance of glutamate-oxalate-transaminases (GOT) from needles and endosperms of *Pinus sylvestris*. Hereditas **80**, 296—300 (1975). —— RUDIN, D.: Leucine-amino-peptidases (LAP) from needles and macrogametophytes of *Pinus sylvestris* L. Inheritance of allozymes. Hereditas **85**, 219—226 (1977). —— SCANDALIOUS, J. G.: Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Gen. **3**, 37—79 (1969). —— SCHOLZ, F. and BERGMANN, F.: Selection pressure by air pollution as studied by isozyme-gene-systems in Norway spruce exposed to sulphur-dioxide. Silvae Genetica **33**, 238—241 (1984). —— SHAW, C. R. and KOEN, A. L.: Starch gel electrophoresis of enzymes. In: SMITH, I. (ed.): Chromatographic and electrophoretic techniques. Vol. II., Pittman Press, England, 325—364 (1968). —— SHAW, C. R. and PRASAD, R.: Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Gen. **4**, 297—320 (1970). —— SZMIDT, A. E. and YAZDANI, R.: Electrophoretic studies of genetic polymorphism of shikimate and 6-phosphogluconate dehydrogenases in Scots pine (*Pinus sylvestris* L.). Arboretum Kornickie **29**, 63—71 (1984).

# Some Aspects of the Population Structure of Black Spruce in Central New Brunswick

By T. J. B. BOYLE*) and E. K. MORGENSTERN

Dept. of Forest Resources, University of New Brunswick, Fredericton, New Brunswick, E3B 6C2, Canada

(Received 9th December 1985)

## Summary

Genetic diversity within and genetic differentiation among six populations of black spruce (*Picea mariana* [MILL.] B.S.P.) in central New Brunswick were investigated using allozyme frequencies at 12 loci. Average heterozygosity ranged from 0.192 to 0.253 in the different populations. On the average, the percent heterozygous loci/individual ranged from 17.08 to 22.29, the average number of alleles/locus from 2.17 to 2.50 and the effective number of alleles/locus from 1.24 to 1.38. These values are comparable with those from other conifer species though they might be biased upwards somewhat due to the limited number of polymorphic, independently segregating loci sampled. Other measures of genetic diversity produced similar results.

Partitioning the observed variation into within- and among-population components by using F-statistics led to an estimate of within-population variation amounting to 99 per cent of total variation. These results suggest that emphasis be placed on intra-population sampling in black spruce for tree improvement. The relationship between geographic distance and Nei's genetic distance appeared to be weak, suggesting that isolation by distance may not be responsible for the observed differentiation.

*Key words:* *Picea mariana*, isozymes, population structure, gene diversity, genetic distance, heterozygosity.

## Zusammenfassung

Innerhalb und zwischen 6 Populationen von *Picea mariana* im mittleren New Brunswick wurde die genetische Diversität durch elektrophoretische Methoden untersucht. Es wurden die Genhäufigkeiten an 12 Loci ermittelt. Der durchschnittliche Heterozygotiegrad lag in den verschiedenen Populationen zwischen 0,192 und 0,253. Die Durchschnittswerte lagen in folgenden Bereichen: % der heterozygoten Loci pro Individuum, 17,08—22,29; Zahl der Allele pro Locus, 2,17—2,50; und Zahl der effektiven Allele pro Locus, 1,24—1,38. Diese Werte sind mit solchen anderer Koniferen vergleichbar, können allerdings etwas hoch eingeschätzt sein, da sie auf einer geringen Zahl von polymorphen, unabhängig aufspaltenden Loci beruhen. Andere Messungen der genetischen Diversität ergaben ähnliche Resultate.

Die Aufteilung der beobachteten Variation in Komponenten „innerhalb" und „zwischen" Populationen durch F-Statistiken ergab einen Beitrag von 99% der Komponente „innerhalb" zur Gesamtvariation. Die Plusbaumauswahl sollte deshalb hauptsächlich auf Auslese innerhalb der Populationen beruhen. Die Korrelation zwischen geographischer Entfernung der Populationen und Nei's genetischer Distanz schien schwach zu sein, sodaß Distanzisolation wenig Einfluß auf die beobachtete Differenzierung haben mag.

## Introduction

The population structure of a species has been defined by RIEGER *et al.* (1976) as: "The sum of all factors governing the pattern by which gametes from various individuals unite with each other during fertilization". BROWN (1978) recognizes two groups of evolutionary forces that affect the values which various measures of population genetic structure may assume. These are firstly, the interaction with other aspects of the population structure affecting, for

*) Present adress: Canadian Forestry Service, Petawawa Nat. for. Inst., Chalk River, K0J 1J0 Ontario, Canada.