Isoenzymes' Structure of Norway Spruce (*Picea abies* (L.) Karst.) in Natural Populations in Estonia, Latvia and Byelorussia

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Abstract

Using isoenzymes as genetic markers in studies of Norway spruce populations situated in four natural stands in Estonia, five in Latvia and six in Byelorussia, an evaluation of the genetic resources of the populations was given.

Northwards from Byelorussia the parameters characterising genetic diversity increase: polymorphism P_{99} from 67.3% in Byelorussia to 78% in Estonia, and the number of alleles per locus from 2.13 to 2.41 respectively. Empirical heterozygosity increased from 17.8% to 19.7% respectively. Heterozygosity parameters varied least between the Latvian populations. The mean genetic diversity indices for the entire Balto-Byelorussian region were the following: polymorphism P_{99} 73.1%, number of alleles per locus 2.25 and empirical heterozygosity 18.7%. It appeared from polymorphism analysis that virtually all stands in the natural habitat of Norway spruce in Estonia, Latvia and Byelorussia have sufficient genetic resources for restoring their gene pools.

Key words: Norway spruce, genetic structure, diversity, isoenzymes, differentiation

Introduction

Norway spruce is known as a sustainable and valuable natural resource constituting an important structural component of the ecosystem and determining the diversity of coniferous forests. Norway spruce has been frequently identified as a priority species in national gene conservation strategies (Koski 1993). Many efforts have been devoted to the conservation of genetic material prior to or even without an explicit elaboration of national gene conservation programmes, mainly in tree breeding and nature protection (Turok 1997).

Integration with Europe brings more challenge to the national strategy of forest genetic resource conservation. In order to draw up effective gene pool conservation programmes one must know the current population structures and understand the factors influencing them (Savolainen *et al.* 1992). In this regard the evaluation of the gene pool situation in different natural populations of Norway spruce has proved a topical issue in modern population genetics.

For a long time only phenotypic traits of trees were analysed. However, these traits are greatly influenced by the environment and have a low coefficient of heritability. Thus, it was impossible to determine the genotypic structure and the levels of genetic variation in populations and species. The situation changed drastically once isoenzyme electrophoresis became widely used in population genetic studies. At present isoenzyme electrophoresis is considered one of the most accurate and suitable methods for the study of gene pools, which allows researchers not only to evaluate genetic resources but also, in certain respects, to make recommendations for their management (Goncharenko *et al.* 1996).

Between regions, in Slovakia, the component of the gene diversity was 1.59%, between populations within regions 0.89% and within populations 97.52% (Gömöry and Paule 1993). However, the diversity of stands in the Baltics, Byelorussia and Russia, based on the material collected from there, was found to be significantly higher than that in central Europe. While heterozygosity on average was 14% it reached

17% in some populations. On the basis of the data Lagercrantz and Ryman (1990) are of the opinion that the populations of *Picea abies* in central Europe lost their diversity after the last glaciation. Some scientists (Krutovskii and Bergmann 1993) have a different opinion as they have found a very high level of allozyme variation in the populations of *Picea abies* studied in Europe (Westerhof in Germany, Sorlieden in Sweden), Ukraine (Chernigov), Byelorussia (Vitebsk) and west-central Russia. The regular parameters of genetic diversity – 2.8 for the mean number of alleles per locus, 61.5 for the percentage of polymorphic loci (95% criteria) and 0.252 for the expected heterozygosity – equalled with the corresponding averages for *Picea abies*.

The main parameters of morphological and isoenzyme variations of natural populations of *Picea abies* in Karelia and neighbouring regions were determined. The indices of genetic diversity and differentiation were determined by means of electrophoretic analysis of 25 genes. More than 64% of the loci of Norway spruce populations were found to be polymorphic and the heterozygosity of each tree on average was under 19.2% (Ilynov 1996).

The objective of our research was to assess the level of genetic diversity and characterize the structure of the genetic resources of natural populations of *Picea abies* in Estonia, Latvia and Byelorussia, by using isoenzymes as genetic markers.

Material and methods

Baltic-Byelorussian region lies in the middle of the natural habitat of Norway spruce, thus providing very favourable growth conditions for the species. Spruce is fairly numerously represented in the forests of the regions, accounting for 20.3% of the area of forests in Estonia (Pärt 2005), 21.3% in Latvia (Forest…2003) and 10.9% in Byelorussia (Goncharenko and Padutov 1996).

Conservation of the gene pool of forest trees became a priority also on the territories of the former Soviet Union already. The governmental policy for the conservation of genetic resources was formulated for the first time in 1982 and is known as "The Guideline for the Selection and Conservation of the Gene Pool of Forest Trees in the Forests of the USSR". This document laid the basis for later work aimed at the conservation of forest genetic resources *in situ* and *ex situ,* including the designation of forest genetic reserves, stands with superior phenotypic performance (Routkowski *et al.* 1997).

10 gene pool conservation units with a total area of 3,540 ha were established in Estonia; Norway spruce was the dominant tree species on five of the units (1,136 ha). This provided the basis for the conservation of forest genetic resources in Estonia. By way of comparison, the area of forests is ten times bigger in Finland, yet, the area of gene reserve forests is just 7,500 ha (Koski 2000). In Byelorussia genetic forest reserves have been established on an area of 4,167 ha, including 1,127 ha of Norway spruce.

The genetic analysis of populations was based on the seeds collected from 530 trees in 15 natural populations of Norway spruce. Using isoenzymes as genetic markers in the studies of four natural stands in Estonia (Kabala, Purdi, Vihula and Õisu), five in Latvia (Ligatne, Ranki, Rezekne, Rozeni and Saldus) and six in Byelorussia (Begoml, Belynichi, Gorodok, Podsvilje, Turov and Vetka) an evaluation of the genetic resources of the stands was given. The experiments were done at the Laboratory of Molecular Genetics and Forest Tree Breeding in the Byelorussian Forestry Research Institute.

Electrophoretic fractionation was performed using tissues of haploid endosperms and of diploid embryos as the material for analysis. Eight to twenty endosperms were analysed to determine the individual genotype of each tree. The endosperms were selected randomly from at least 500 seeds obtained from a minimum of 20 cones taken from different sections of the crown of each tree. Electrophoresis and histochemical analysis were used to explore 25 genes controlling a system of 15 enzymes in seeds of each Norway spruce tree.

Electrophoresis of fifteen enzymes was performed in 13–14% starch gel, using three different buffer systems: tris-borate-EDTA buffer solution, pH-8.6 (A); tris-citrate buffer solution, pH-6.2/tris-HCL, pH-8.0 (B); and tris-citrate, pH-6.2 (C). All the particulars of electrophoretic fractionation as well as the methods of extraction and isoenzyme histochemical analysis, have been presented in more detail in some previous studies (Гончаренко *и др.* 1989; Гончаренко и Потенко, 1991; Гончаренко 1999).

Electrophoresis was performed by cooling down to 0–5˚C and at 280–320V (40–80 mA) for 3.5–4.5 hours. After resolution 4–6 horizontal segments of the gel were cut and painted using somewhat modified standard methods (Cheliak and Pitel 1984; Гончаренко *и др.* 1989). The enzyme names and code numbers are in compliance with the isoenzyme classification (Номенклатура ферментов 1979). The buffer systems preferred in the analysis as well as the number of loci involved are given in Table 1.

The designations of enzyme alleles are given according to the commonly used S. Prakash's (Prakash *et al.* 1969) classification, under which the most

Table 1. Enzymes, buffer systems and the number of loci analysed in Norway spruce

Enzyme	Abbreviation	Buffer system	Code number	No of involved loci
Aspartate aminotrasferase	AAT	A	2.6.1.1.	2
Alcohol dehydrogenase	ADH	A, B	1.1.1.1.	1
Diaphorase	DIA	A	1.6.4.3.	3
Glutamate dehydrogenase	GDH	A	1.4.1.2.	1
Glucose phosphate isomerase	GPI	B, C	5.3.1.9.	1
Hexocinase	HK	B	2.7.1.1.	1
Isocitrate dehydrogenase	IDH	B	1.1.1.42	2
Leucine aminopeptidase	LAP	A, B	3.4.11.1	2
Malate dehydrogenase	MDH	B, C	1.1.1.37.	3
Malic enzyme	ME	A	1.1.1.40.	1
Sorbitol dehydrogenase	SDH	A	1.1.1.14.	1
Phosphoglucomutase	PGM	A, B	2.7.5.1.	2
Fluorescent esterase	FL-EST	A	3.1.1.2.	1
6-Phosphogluconate dehydrogenase	6-PGD	B, C	1.1.1.44.	3
Shikimate dehydrogenase	SKDH	B	1.1.1.25	1

common electromorph in each locus of *Picea abies* and the respective encoding gene allele were marked with the symbol 1.00 and all the other allele variants with numerical symbols depending on their electrophoretic mobility in relation to 1.00. The leucine aminopeptidase allele variants occurring as several fractions were designated with double numerical symbols (e.g. 1.00/1.04).

To assess the level of genetic variation and characterize the structure of genetic resources of the species in a population, the following parameters were used: the percentage of polymorphic loci (P_{95} and P_{99}), the mean number of alleles per locus (A) and the expected (He) and observed (Ho) heterozygosities.

The percentage of polymorphic loci (P) is calculated by dividing the number of polymorphic loci (having two and more different alleles) by the overall number of the loci surveyed. This parameter is normally computed in accordance with two criteria of reliability: estimated at the 99% level (the frequency of the most common allele is not greater than 99% – P_{99}) and at the 95% level (P_{95}).

The mean number of alleles per locus (A) is computed by dividing the number of alleles discovered by the overall number of the loci analysed.

Expected and the observed heterozygosities permit the most accurate estimation of the level of genetic variation within populations. The observed heterozygosity (Ho) is calculated for a particular locus by dividing the number of heterozygous trees with the overall number of the individuals surveyed. The expected heterozygosity (He), which depends on the population sample to a lesser extent than on the other parameters, is calculated for a particular locus using allelic frequencies as calculated by the formula:

$$He = 1 - \sum_{i=1}^{n} x^2$$

where x is the frequency of the i^{th} allele.

Both the expected and the observed mean heterozygosities are estimated as means of the H values for all the loci:

$$\overline{H} = \frac{1}{L}\sum H_j$$

where H_j is the heterozygosity for the j^{th} locus and L is the number of the loci assayed.

Results and discussion

The material for analysis originated from four natural populations from different regions in Estonia, five in Latvia and six in Byelorussia (Figure 1).

Figure 1. The locations of the Norway spruce stands analysed in Estonia, Latvia and Byelorussia

In electrophoretic analysis 99 different electromorphs were identified in 15 enzymes from natural populations of Norway spruce. Figures 2 and 3 provide the variants as examples occurring in the electrophoretic spectra of alcohol dehydrogenase, glutamate dehydrogenase and glycose phosphate isomerase.

The nature of Norway spruce allele variants was determined by analysing the divergence between haploid endosperms of heterozygous trees. In a locus encoding a particular isoenzyme, the meiotic activity of a heterozygous tree should result in the formation of haploid endosperms carrying alternative electrophoretic allele variants in the proportion 1:1. Table 2 presents summary data on the divergence of isoenzyme allele variants in heterozygous trees.

The table clearly shows that marked divergences from the expected ratio 1:1 were only observed in occasional allele combinations in the loci Aat-1, Aat-2, Mdh-3, Sdh, Skdh, Gpi, Lap-1, Me and Fl-Est. The results of our research, presented in Table 2, reflect the genetic nature of the electrophoretic variants found in Norway spruce populations. Further analysis of diploid germs, which should demonstrate the occurrence of two different allele variants in the electrophoretic spectrum of heterozygous trees, also corroborated the genetic nature of the electrophoretic variants found.

Our findings confirm the genetic nature of the electrophoretic variants found in the Norway spruce populations. The analysis led to the conclusion that

Table 2. The divergence of allele variants of heterozygous Norway spruce trees in electrophoresis

Locus	Combination of alleles	Divergence	χ^2	Locus	Combination of alleles	Divergence	χ^2
Aat-1	1.00/1.10	206:163	5.01*	**Lap-1**	1.00/0	106:101	0.12
Aat-2	0.65/1.00	1339:1221	5.44*		0.94/1.00	190:172	0.90
	0.30/1.00	12:15	0.33		0.97/1.04	11:5	2.25
	0.30/0.65	15:15	0		1.00/1.04	474:410	4.63*
Adh	0.90/1.00	44:48	0.17		1.00/1.10	122:110	0.62
	1.00/1.10	11:12	0.04		1.00/0.97/1.00	15:15	0
Gdh	0.75/1.00	624:636	0.11		0.94/1.04	38:37	0.01
Idh-1	0.90/1.00	69:73	0.11		1.00/1.000/1.04	6:3	1.00
	1.00/1.10	151:156	0.08		1.04/1.10	21:25	0.35
Idh-2	0.70/1.00	33:28	0.41		1.04/0	4:8	1.33
	0.90/1.00	15:9	1.50		0.97/1.00	13:19	1.13
	1.00/1.20	22:17	0.64		0.94/1.10	16:10	1.38
6-Pgd-1	0.80/1.00	46:50	0.17		0.97/1.10	7:4	0.82
	0.90/1.00	89:97	0.34	**Lap-2**	0.95/1.00	116:129	0.69
	1.00/1.10	55:54	0.01		1.00/1.05	609:553	2.70
6-Pgd-2	0.65/1.00	812:883	2.97		1.00/1.10	119:105	0.88
	0.85/1.00	25:29	0.30		0.95/1.05	14:9	1.09
	0.65/0.85	8:17	3.24		1.05/1.05/1.10	15:13	0.14
	1.00/1.20	5:4	0.11		1.00/0	4:4	0
6-Pgd-3	0.50/1.00	685:755	3.40		1.05/1.10	17:19	0.11
	0.80/1.00	24:14	2.63	**Me**	0.60/1.00	104:144	6.45*
	0.90/1.00	6:10	1.00		0.60/0	16:8	2.67
	1.00/1.15	14:21	2.63		1.00/1.20	87:71	1.62
	0.50/0.80	7:9	0.25		0.10/1.00	90:97	0.26
Mdh-1	1.00/1.10	8:6	0.28		1.00/0	32:44	1.89
Mdh-2	0.80/1.00	5:9	1.14		0.10/0.60	12:5	2.88
Mdh-3	0.70/1.00	12:16	0.57	**Pgm-1**	0.90/1.00	45:65	3.64
	1.00/1.15	246:195	5.90*	**Pgm-2**	0.85/1.00	27:1	0.28
	1.00/1.15	61:53	0.56		1.00/1.15	165:138	2.41
	1.00/0	18:20	0.11	**Hk**	0.90/1.00	102:120	1.46
	0.90/1.00	388:471	8.02**		1.00/1.10	42:33	1.08
	0.90/1.15	6:3	1.00	**Dia-1**	1.00/0	15:11	0.62
	1.15/1.30	15:10	1.00		0.80/1.00	16:14	0.13
Sdh	0.95/1.00	188:186	0.01	**Dia-2**	1.00/0	27:17	2.27
	1.00/1.05	65:38	7.08**		0.70/1.00	15.15	0
	1.00/0	4:4	0		1.00/1.30	29:32	0.15
Skdh	0.80/1.00	15:30	5.00*	**Dia-4**	1.00/1.10	896:939	1.01
	1.00/1.05	58:38	4.17*		1.00/1.15	22:24	0.09
Gpi	0.80/1.00	457:519	3.94*		1.00/1.15	129:141	0.53
	0.80/1.15	28:16	3.27	**Fl-Est**	0.70/1.00	59:33	7.35**
	1.00/1.15	170:166	0.05		1.00/1.30	105:79	3.67
	1.00/1.25	79:71	0.43		0.70/1.30	19:19	0

* – probability <0.05; ** – <0.01; *** – <0.001.

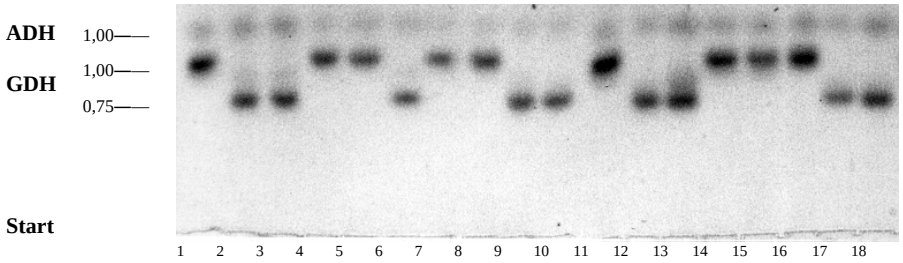

Figure 2. The Norway spruce haploid endosperm variants found in alcohol dehydrogenase and glutamate dehydrogenase zymogram: 1, 4, 5, 7, 8, 11, 14-16 – $ADH^{1,00}$. $GDH^{1,00}$; 2, 3, 6, 9, 10, 12, 13, 17, 18 – $ADH^{1,00}$ $GDH^{0,75}$

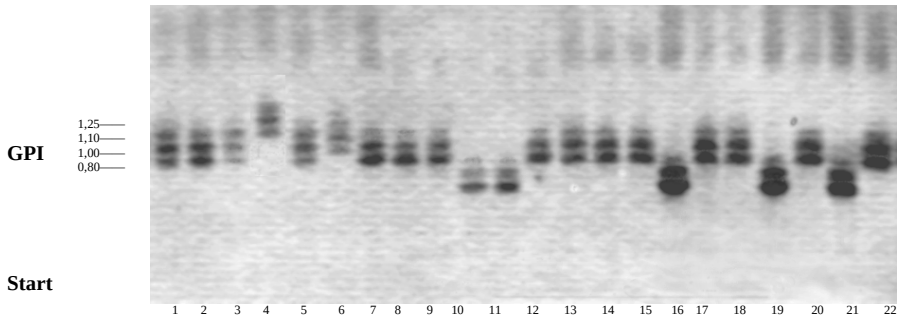

Figure 3. The Norway spruce endosperm variants found in glucose phosphate isomerase zymogram: 1–3, 5, 7–9, 12–15, 17, 18, 20, 22 – $GPI^{1,00}$, 4 – $GPI^{1,25}$, 6 – $GPI^{1,15}$, 10, 11, 16, 19, 21 – $GPI^{0,80}$

the 96 electrophoretic variants manifested in fifteen isoenzyme systems of Norway spruce are genetically controlled by 25 loci.

It must be mentioned that the issues of the genetic nature, control and variability of Norway spruce isoenzymes discussed in the present paper have been described in more detail in previously published studies (Bergmann 1973, 1974; Tigerstedt 1973; Lundkvist 1979; Lundkvist and Rudin 1977; Poulsen *et al*. 1983; Aëòóõîâ *è äð*. 1986; Cheliak *et al*. 1987; Muona *et al*. 1987; Lagercrantz *et al*. 1988; Ãîí÷àðåíêî *è äð*. 1989, 1990; Paule *et al*. 1990; Ãîí÷àðåíêî è Ï îòåíêî 1990, 1991; Giannini *et al*. 1991; Goncharenko *et al*. 1995; Ãîí÷àðåíêî 1999, 2002).

The genetic structure of natural populations of Norway spruce

The frequency of alleles in each Norway spruce population studied was taken into consideration in the genetic structure evaluation. The allele frequencies as well as the indices of theoretical and empirical heterozygosity for all the genes analysed are given in Table 3. It appeared that all the loci of Norway spruce proved to be polymorphous, with the allele marked as 1.00 dominating at almost all the loci analysed in most of the populations. This fact suggests a definite similarity between the genetic structures of the populations studied.

At the same time, differences between the populations in allelic frequency at loci Mdh-3, 6-Pgd-2, 6-Pgd-3, Me, Lap-1, Lap-2 and Dia-4 amount to 25–40% (Table 3). In the populations analysed, the most variable were 8 loci (Aat-2, Mdh-3, 6-Pgd-2, 6-Pgd-3, Gpi, Lap-1, Lap-2 and Dia-4), since their mean theoretical heterozygosity in Norway spruce as a whole exceeded 30% (Table 3). At eight loci (Aat-1, Idh-1, Gdh, Me, Fl-Est, Hk, Pgm-2 and Sdh), the value of theoretical heterozygosity varies between 7–22%, which allows them to be regarded as loci with the average level of polymorphism. The loci Adh, Dia-1, Dia-2, Idh-2, Pgm-1, Skdh, Mdh-1, Mdh-2 and 6-Pgd-1 proved to be less variable as their average heterozygosity did not exceed 6%.

In Norway spruce, three or more alleles were observed at virtually every locus. Interestingly, of the 96 alleles found in the natural populations of Norway spruce, 36 proved to be rare, with their frequency for the species as a whole being less than 1%. Of these rare alleles, eight appeared as unique "loners", occurring in just one population. It must be noted that unique alleles were found in different parts of the Norway spruce habitat studied. One of the characteristic features of Norway spruce allelic diversity was the existence of the so-called "null alleles" (alleles encoding an inactive form of an isoenzyme) at almost half of the loci.

The analysis of the population genetic structures and the level of genetic variation made it possible to integrally assess the structure of genetic resources of coniferous species and provide the basis for the development of specific technologies of conservation and reproduction of gene pools of the populations. The results obtained suggest that the strategy of the management of genetic resources should be worked out with regard to individual peculiarities of each

Table 3. Isoenzyme allele frequency in 25 loci of natural populations of Norway spruce in Estonia, Latvia and Byelorussia

Locus	Populations															
Alleles	Ka	Pr	Vi	Õs	Li	Ra	Re	Ro	Sa	Be	Bl	Gr	Pd	Tr	Vt	Mean
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Aat-1																
N	30	28	30	30	33	30	50	30	49	29	29	28	77	31	26	530
0.95	0.000	0.000	0.017	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
1.00	0.983	0.964	0.950	0.967	0.955	0.883	0.980	0.933	1.000	0.948	1.000	0.946	0.974	0.968	0.962	0.964
1.10	0.017	0.036	0.033	0.033	0.045	0.100	0.020	0.067	0.000	0.052	0.000	0.054	0.026	0.032	0.038	0.034
h-o	0.033	0.036	0.100	0.067	0.091	0.233	0.040	0.133	0.000	0.034	0.000	0.036	0.052	0.065	0.077	0.062
h-e	0.033	0.069	0.096	0.064	0.086	0.210	0.039	0.125	0.000	0.099	0.000	0.102	0.051	0.062	0.073	0.068
Aat-2																
N	24	28	30	30	33	30	50	30	49	29	29	28	77	31	25	523
0.30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.017	0.000	0.000	0.000	0.000	0.000	0.002
0.65	0.500	0.585	0.583	0.500	0.470	0.450	0.550	0.450	0.531	0.586	0.586	0.554	0.513	0.581	0.520	0.529
1.00	0.500	0.415	0.417	0.500	0.530	0.550	0.450	0.550	0.459	0.397	0.414	0.446	0.487	0.419	0.480	0.469
h-o	0.792	0.464	0.500	0.667	0.455	0.567	0.380	0.633	0.510	0.379	0.483	0.536	0.610	0.645	0.400	0.533
h-e	0.500	0.486	0.486	0.500	0.498	0.495	0.495	0.495	0.507	0.499	0.485	0.494	0.500	0.487	0.499	0.496
Adh																
N	30	28	30	30	33	30	50	30	47	29	29	28	77	31	20	522
0.00	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
0.90	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.017	0.000	0.000	0.017	0.000	0.006	0.000	0.025	0.005
1.00	1.000	1.000	1.000	0.983	0.970	1.000	1.000	0.983	1.000	1.000	0.983	1.000	0.994	1.000	0.975	0.993
1.10	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
h-o	0.000	0.000	0.000	0.033	0.061	0.000	0.000	0.033	0.000	0.000	0.034	0.000	0.013	0.000	0.050	0.013
h-e	0.000	0.000	0.000	0.033	0.059	0.000	0.000	0.033	0.000	0.000	0.033	0.000	0.012	0.000	0.049	0.013
Idh-1																
N	30	28	30	30	33	30	50	29	49	29	29	28	76	21	26	518
0.00	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.003
0.70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.001
0.90	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.002
1.00	0.917	0.966	0.917	0.950	0.970	0.917	0.980	0.862	0.949	0.966	1.000	0.982	1.000	0.976	0.962	0.959
1.10	0.083	0.034	0.083	0.017	0.030	0.083	0.020	0.138	0.031	0.017	0.000	0.018	0.000	0.000	0.038	0.036
h-o	0.167	0.036	0.033	0.100	0.061	0.167	0.040	0.276	0.102	0.069	0.000	0.036	0.000	0.048	0.077	0.073
h-e	0.152	0.066	0.152	0.097	0.058	0.152	0.039	0.238	0.098	0.066	0.000	0.035	0.000	0.047	0.073	0.077
Idh-2																
N	30	28	30	30	33	30	50	30	49	29	29	28	76	31	26	529
0.70	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.003
0.90	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
1.00	0.983	1.000	1.000	1.000	1.000	1.000	0.980	0.983	1.000	1.000	1.000	1.000	0.987	1.000	1.000	0.994
h-o	0.033	0.000	0.000	0.000	0.000	0.000	0.040	0.033	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.011
h-e	0.033	0.000	0.000	0.000	0.000	0.000	0.039	0.033	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.011
Mdh-1																
N	30	28	30	30	33	30	50	30	49	29	29	28	77	31	26	530
0.95	0.017	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.003
1.00	0.983	1.000	0.983	1.000	1.000	1.000	1.000	1.000	0.908	1.000	1.000	1.000	0.994	1.000	1.000	0.989
1.10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.092	0.000	0.000	0.000	0.000	0.000	0.000	0.009
h-o	0.033	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.102	0.000	0.000	0.000	0.013	0.000	0.000	0.015
h-e	0.033	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.012	0.000	0.000	0.021
Mdh-2																
N	30	28	30	30	33	30	50	30	49	29	29	28	77	31	26	530
0.80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.001
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000	1.000	1.000	0.999
h-o	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.002
h-e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.002
Mdh-3																
K	30	28	30	30	33	30	50	30	49	29	29	28	67	31	26	520
0.00	0.033	0.027	0.017	0.000	0.000	0.033	0.000	0.017	0.031	0.017	0.000	0.000	0.007	0.000	0.019	0.013
0.70	0.000	0.000	0.000	0.017	0.000	0.017	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
0.90	0.067	0.027	0.017	0.017	0.045	0.117	0.070	0.017	0.143	0.207	0.034	0.107	0.201	0.226	0.135	0.103
1.00	0.817	0.757	0.883	0.850	0.894	0.717	0.810	0.933	0.786	0.707	0.948	0.804	0.746	0.726	0.827	0.808
1.15	0.083	0.162	0.083	0.083	0.045	0.117	0.060	0.033	0.020	0.052	0.017	0.054	0.045	0.032	0.019	0.058
1.30	0.000	0.027	0.000	0.033	0.015	0.000	0.020	0.000	0.020	0.017	0.000	0.036	0.000	0.016	0.000	0.012
h-o	0.367	0.286	0.233	0.300	0.212	0.433	0.380	0.133	0.367	0.483	0.103	0.393	0.507	0.419	0.346	0.346
h-e	0.320	0.399	0.213	0.269	0.196	0.457	0.330	0.128	0.360	0.454	0.100	0.338	0.401	0.421	0.297	0.320
6-Pgd-1																
N	26	28	30	30	33	30	50	30	49	29	29	28	77	31	26	526
0.90	0.000	0.000	0.017	0.017	0.045	0.050	0.000	0.000	0.031	0.052	0.000	0.000	0.019	0.000	0.038	0.018
1.00	0.923	1.000	0.983	0.983	0.955	0.950	1.000	1.000	0.969	0.948	1.000	0.946	0.981	1.000	0.962	0.975

Table 3. (Continuation)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0.50	0.500	0.342	0.233	0.417	0.379	0.278	0.280	0.260	0.389	0.259	0.362	0.321	0.312	0.238	0.295	0.321
0.80	0.024	0.024	0.017	0.000	0.015	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
1.00	0.452	0.610	0.717	0.550	0.576	0.704	0.710	0.740	0.611	0.741	0.638	0.679	0.688	0.762	0.705	0.664
1.15	0.024	0.000	0.033	0.000	0.030	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
h-o	0.524	0.464	0.400	0.433	0.636	0.481	0.500	0.520	0.407	0.379	0.310	0.429	0.494	0.381	0.500	0.463
h-e	0.545	0.510	0.430	0.523	0.523	0.427	0.417	0.385	0.475	0.384	0.462	0.436	0.429	0.363	0.416	0.446
Gdh																
N	30	28	30	30	33	30	50	30	49	29	29	28	77	31	25	529
0.75	0.117	0.305	0.150	0.117	0.121	0.117	0.150	0.253	0.082	0.103	0.138	0.179	0.130	0.081	0.200	0.144
1.00	0.883	0.695	0.850	0.883	0.879	0.883	0.850	0.767	0.918	0.897	0.862	0.821	0.870	0.919	0.800	0.857
h-o	0.100	0.286	0.233	0.167	0.242	0.233	0.260	0.400	0.122	0.207	0.276	0.286	0.234	0.161	0.400	0.234
h-e	0.207	0.424	0.255	0.207	0.213	0.207	0.255	0.357	0.151	0.185	0.238	0.294	0.226	0.149	0.320	0.239
Skdh																
N	30	18	30	30	33	30	50	30	20	29	29	28	77	31	4	469
0.60	0.117	0.050	0.083	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
0.80	0.000	0.000	0.033	0.017	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.005
1.00	0.850	0.900	0.900	0.967	1.000	1.000	0.990	0.983	1.000	0.948	1.000	1.000	1.000	1.000	1.000	0.973
1.05	0.000	0.050	0.000	0.000	0.000	0.000	0.010	0.017	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.005
h-o	0.300	0.111	0.133	0.067	0.000	0.000	0.020	0.033	0.000	0.103	0.000	0.000	0.000	0.000	0.000	0.047
h-e	0.263	0.185	0.183	0.064	0.000	0.000	0.020	0.033	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.050
Gpi																
N	30	28	30	30	26	30	50	30	49	29	29	28	77	31	26	523
0.80	0.117	0.162	0.100	0.217	0.058	0.067	0.080	0.150	0.102	0.052	0.190	0.125	0.084	0.129	0.038	0.108
1.00	0.817	0.757	0.883	0.733	0.769	0.900	0.900	0.850	0.837	0.914	0.759	0.857	0.818	0.677	0.808	0.823
1.15	0.050	0.081	0.017	0.050	0.173	0.033	0.020	0.000	0.051	0.017	0.017	0.000	0.078	0.194	0.154	0.061
1.25	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.017	0.034	0.018	0.019	0.000	0.000	0.009
h-o	0.300	0.321	0.167	0.400	0.385	0.200	0.200	0.233	0.224	0.172	0.414	0.286	0.299	0.613	0.269	0.293
h-e	0.316	0.394	0.210	0.413	0.375	0.184	0.183	0.255	0.286	0.161	0.386	0.250	0.317	0.487	0.322	0.298
Sdh																
N	30	28	30	30	33	30	50	27	24	29	29	28	77	31	24	500
0.00	0.017	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
0.95	0.033	0.033	0.017	0.167	0.061	0.017	0.030	0.074	0.063	0.034	0.000	0.000	0.013	0.032	0.021	0.037
1.00	0.867	0.934	0.933	0.833	0.909	0.983	0.960	0.926	0.917	0.966	1.000	1.000	0.968	0.968	0.979	0.945
1.05	0.083	0.000	0.050	0.000	0.030	0.000	0.010	0.000	0.021	0.000	0.000	0.000	0.019	0.000	0.000	0.015
h-o	0.267	0.071	0.133	0.200	0.182	0.033	0.080	0.148	0.167	0.069	0.000	0.000	0.065	0.065	0.042	0.098
h-e	0.240	0.125	0.127	0.278	0.169	0.033	0.077	0.137	0.155	0.066	0.000	0.000	0.062	0.062	0.041	0.101
Me																
N	8	9	10	10	32	30	50	28	48	19	10	28	64	21	25	392
0.00	0.188	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.039	0.000	0.020	0.017
0.10	0.000	0.000	0.000	0.100	0.031	0.067	0.030	0.107	0.052	0.026	0.000	0.018	0.023	0.000	0.040	0.037
0.60	0.000	0.200	0.000	0.200	0.031	0.033	0.100	0.018	0.052	0.026	0.100	0.018	0.070	0.000	0.020	0.053
1.00	0.688	0.800	1.000	0.700	0.875	0.900	0.860	0.875	0.896	0.947	0.800	0.964	0.859	1.000	0.920	0.886
1.20	0.125	0.000	0.000	0.000	0.031	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.008
h-o	0.250	0.111	0.000	0.600	0.250	0.200	0.240	0.250	0.188	0.105	0.400	0.036	0.250	0.000	0.160	0.199
h-e	0.476	0.320	0.000	0.460	0.231	0.184	0.249	0.223	0.192	0.102	0.340	0.070	0.255	0.000	0.151	0.203
Lap-1																
N	30	18	30	30	33	30	50	28	49	29	29	28	77	31	26	518
0.00	0.017	0.045	0.017	0.000	0.030	0.000	0.020	0.018	0.010	0.086	0.052	0.000	0.045	0.000	0.000	0.024
0.94	0.050	0.045	0.033	0.083	0.076	0.017	0.100	0.036	0.051	0.121	0.034	0.036	0.097	0.113	0.058	0.068
0.97	0.000	0.045	0.000	0.050	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.071	0.000	0.000	0.058	0.012
1.00	0.750	0.774	0.783	0.533	0.773	0.700	0.460	0.607	0.675	0.690	0.793	0.786	0.669	0.726	0.827	0.687
1.04	0.133	0.091	0.167	0.267	0.106	0.267	0.340	0.286	0.235	0.052	0.034	0.107	0.156	0.129	0.058	0.173
1.10	0.050	0.000	0.000	0.067	0.015	0.017	0.080	0.054	0.020	0.052	0.086	0.000	0.032	0.032	0.000	0.036
h-o	0.433	0.222	0.367	0.800	0.424	0.500	0.640	0.464	0.490	0.586	0.379	0.321	0.558	0.452	0.269	0.484
h-e	0.415	0.387	0.358	0.631	0.384	0.438	0.656	0.545	0.486	0.496	0.359	0.364	0.516	0.442	0.306	0.469
Lap-2																
N	30	18	30	30	32	30	50	30	49	29	29	28	77	31	26	519
0.00	0.017	0.042	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
0.95	0.000	0.083	0.150	0.000	0.016	0.000	0.010	0.067	0.010	0.069	0.017	0.036	0.019	0.016	0.019	0.030
1.00	0.733	0.750	0.667	0.783	0.797	0.750	0.720	0.833	0.683	0.569	0.776	0.768	0.786	0.613	0.827	0.738
1.05	0.183	0.125	0.117	0.200	0.188	0.217	0.230	0.083	0.276	0.293	0.207	0.143	0.149	0.290	0.135	0.192
1.10	0.067	0.000	0.067	0.017	0.000	0.017	0.020	0.017	0.031	0.069	0.000	0.054	0.032	0.081	0.019	0.033
05/10	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.004
h-o	0.300	0.333	0.500	0.267	0.344	0.367	0.480	0.267	0.449	0.586	0.276	0.429	0.390	0.516	0.346	0.397
h-e	0.424	0.413	0.514	0.347	0.329	0.390	0.428	0.294	0.456	0.581	0.355	0.386	0.358	0.533	0.297	0.406
Hk																
N	18	2	30	28	32	30	50	30	44	19	19	17	77	21	24	441
0.90	0.056	0.000	0.017	0.018	0.016	0.017	0.090	0.017	0.045	0.026	0.000	0.000	0.032	0.048	0.042	0.034
1.00	0.944	1.000	0.983	0.964	0.969	0.983	0.910	0.983	0.920	0.974	1.000	0.971	0.968	0.952	0.938	0.958

Table 3. (Continuation)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.10	0.000	0.000	0.000	0.018	0.016	0.000	0.000	0.000	0.034	0.000	0.000	0.029	0.000	0.000	0.021	0.008
h-o	0.111	0.000	0.033	0.071	0.063	0.033	0.140	0.033	0.114	0.053	0.000	0.059	0.039	0.000	0.125	0.066
h-e	0.106	0.000	0.033	0.070	0.061	0.033	0.164	0.033	0.150	0.051	0.000	0.056	0.062	0.091	0.118	0.079
Pgm-1																
N	23	28	30	30	32	30	50	30	44	29	29	28	77	31	22	513
0.90	0.043	0.034	0.000	0.033	0.000	0.033	0.050	0.000	0.011	0.000	0.000	0.036	0.013	0.000	0.000	0.017
1.00	0.957	0.966	1.000	0.967	1.000	0.967	0.950	1.000	0.989	1.000	1.000	0.964	0.987	1.000	1.000	0.983
h-o	0.087	0.036	0.000	0.067	0.000	0.067	0.100	0.000	0.023	0.000	0.000	0.071	0.026	0.000	0.000	0.033
h-e	0.082	0.066	0.000	0.064	0.000	0.064	0.095	0.000	0.022	0.000	0.000	0.069	0.026	0.000	0.000	0.034
Pgm-2																
K	23	28	30	30	32	28	18	30	43	29	29	28	77	31	22	478
0.85	0.022	0.063	0.083	0.050	0.000	0.037	0.111	0.033	0.000	0.017	0.052	0.000	0.013	0.032	0.000	0.030
1.00	0.913	0.875	0.867	0.783	0.891	0.963	0.806	0.833	0.942	0.948	0.897	0.929	0.968	0.903	0.886	0.904
1.15	0.065	0.062	0.050	0.167	0.109	0.000	0.083	0.133	0.058	0.034	0.052	0.071	0.019	0.065	0.114	0.066
h-o	0.174	0.143	0.233	0.433	0.219	0.071	0.278	0.333	0.070	0.103	0.207	0.071	0.065	0.194	0.227	0.172
h-e	0.162	0.227	0.239	0.357	0.194	0.071	0.331	0.287	0.109	0.100	0.190	0.132	0.062	0.179	0.202	0.172
Fl-Est																
N	30	28	30	30	24	29	50	30	47	29	29	28	58	31	26	499
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.001
0.70	0.017	0.032	0.017	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.081	0.038	0.014
1.00	0.950	0.903	0.950	0.983	0.937	0.948	0.970	1.000	0.989	0.948	1.000	0.982	0.983	0.806	0.923	0.955
1.30	0.033	0.065	0.033	0.017	0.063	0.017	0.030	0.000	0.011	0.052	0.000	0.018	0.000	0.113	0.038	0.030
h-o	0.100	0.107	0.100	0.033	0.125	0.103	0.060	0.000	0.021	0.103	0.000	0.036	0.034	0.387	0.115	0.082
h-e	0.096	0.179	0.096	0.033	0.118	0.100	0.058	0.000	0.022	0.099	0.000	0.035	0.034	0.331	0.145	0.083
Dia-1																
N	25	28	30	30	32	30	50	30	49	29	29	28	76	31	25	522
0.80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.001
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.984	1.000	0.999
h-o	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.002
h-e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.002
Dia-2																
N	14	28	30	30	32	30	50	30	37	29	29	28	76	31	25	499
0.00	0.000	0.000	0.000	0.000	0.000	0.017	0.040	0.000	0.014	0.000	0.000	0.000	0.007	0.000	0.000	0.007
0.70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.001
1.00	1.000	0.964	1.000	0.950	0.969	0.983	0.940	1.000	0.973	1.000	1.000	1.000	0.987	1.000	1.000	0.982
1.30	0.000	0.036	0.000	0.050	0.031	0.000	0.020	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.010
h-o	0.000	0.036	0.000	0.100	0.063	0.033	0.120	0.000	0.054	0.000	0.000	0.000	0.026	0.000	0.000	0.034
h-e	0.000	0.069	0.000	0.095	0.060	0.033	0.114	0.000	0.053	0.000	0.000	0.000	0.026	0.000	0.000	0.035
Dia-4																
N	28	28	24	29	32	29	50	30	38	29	20	28	76	31	26	498
0.80	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
1.00	0.393	0.469	0.521	0.431	0.563	0.517	0.420	0.433	0.605	0.534	0.750	0.393	0.546	0.484	0.481	0.501
1.10	0.589	0.531	0.479	0.569	0.437	0.466	0.570	0.567	0.395	0.466	0.200	0.607	0.454	0.516	0.519	0.494
1.15	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.003
1.5	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
h-o	0.393	0.750	0.625	0.448	0.375	0.483	0.420	0.533	0.526	0.517	0.350	0.500	0.645	0.516	0.577	0.520
h-e	0.498	0.498	0.499	0.490	0.492	0.515	0.499	0.491	0.478	0.498	0.395	0.477	0.496	0.499	0.499	0.491

(Ka – Kabala, Pr – Purdi, Vi – Vihula, Õs – Õisu, Li – Ligatne, Ra – Ranki Re – Rezekne, Ro – Rozeni, Sa-Saldus, Bg – Begoml, Bl – Belynichi, Gr – Gorodok, Pd – Podsvilje, Tr – Turov, Vt – Vetka)

population, or blocks of populations if the structures of their gene pools are similar (Goncharenko *et al*. 2000).

The level of genetic diversity in natural populations of Norway spruce

The key genetic polymorphism parameters of the 25 genes investigated were considered (Table 4) in the 15 populations studied for the purpose of determining the level of genetic variation,. It appears from the table that the level of polymorphic loci (P_{99}) in Norway spruce populations in Estonia, Latvia and Byelorussia ranges from 0.520 to 0.840. The mean number of alleles per locus varies from 1.96 in the Belynichi population, Byelorussia, to 2.56 in the Kabala population, Estonia.

Based on the genetic polymorphism parameters for the species as a whole, it can be claimed that 78% of the isoenzyme loci in Estonia and 67% in Byelorussia are polymorphous (the average number of alleles per locus is 2.41 in Estonia and 2.13 in Byelorussia), and that more than 20% of the genes studied in each tree are heterozygous in Estonia and 18% in Byelorussia. On the basis of the data obtained from Latvia we can say that more than 76% of the genes analysed in

the stands are polymorphic and each tree on average is heterozygous 18.9% of its genes.

Isozyme polymorphism studies provided direct evidence of extensive genetic variation within natural Norway spruce populations of open-pollinated forest tree species in Lithuania. 74% of the loci studied were polymorphic, 21% of the alleles in each tree studied were heterozygous and the mean number of alleles per locus was 2.20 (Gabrilavičius and Danusevičius 2003). Similar results were obtained in Lithuania from a study of Norway spruce populations in which the RAPD (random amplified polymorphic DNA) method was used. The average percentage of polymorphic loci was 75.5% and the average number of alleles per locus was 1.755 (Areškeviciene *et al*. 2005).

Considering the above and based on Table 4 we may claim that genetic variability in Norway spruce increases from central Europe northwards. Based on the findings obtained from our research, the same tendency can be observed in the Baltic-Byelorussian region.

Table 4. The main parameters of the genetic diversity of Norway spruce populations in Estonia, Latvia and Byelorussia

Population	Polymorphism		The average number of alleles		Heterozygosity	
	P_{95}	P_{99}	A	A_1	The average expected He	The average observed Ho
Kabala	0.680	0.840	2.56	2.56	0.222	0.204
Purdi	0.560	0.720	2.36	2.36	0.213	0.168
Vihula	0.600	0.720	2.32	2.32	0.180	0.182
Õisu	0.560	0.840	2.40	2.40	0.221	0.234
Mean	**0.600**	**0.780**	**2.41**	**2.41**	**0.209**	**0.197**
Ligatne	0.520	0.760	2.36	2.36	0.185	0.192
Ranki	0.560	0.760	2.28	2.28	0.183	0.191
Rezekne	0.560	0.800	2.52	2.24	0.199	0.190
Rozeni	0.560	0.720	2.08	2.08	0.180	0.194
Saldus	0.600	0.760	2.48	2.28	0.188	0.178
Mean	**0.560**	**0.760**	**2.34**	**2.25**	**0.187**	**0.189**
Begoml	0.600	0.720	2.32	2.32	0.181	0.176
Belynichi	0.440	0.520	1.96	1.96	0.156	0.153
Gorodok	0.480	0.680	2.08	2.08	0.167	0.161
Podsvilje	0.400	0.760	2.56	2.20	0.177	0.194
Turov	0.440	0.640	2.00	2.00	0.187	0.201
Vetka	0.520	0.720	2.24	2.24	0.177	0.180
Mean	**0.480**	**0.673**	**2.19**	**2.13**	**0.174**	**0.178**
Mean	**0.539**	**0.731**	**2.30**	**2.25**	**0.188**	**0.187**

The theoretical and empirical values for heterozygosity vary over a fairly broad range in most of the populations. Theoretical, or expected, heterozygosity (He) increases from 0.156 in the Belynichi population, east Byelorussia, to 0.222 in the Kabala population, Estonia. Empirical, or observed, heterozygosity (Ho) was at its lowest in the Belynichi population (0.153) and at its highest in the Õisu population (0.234). The averages for the theoretical and the empirical heterozygosities were at their highest in the Estonian populations, which also evidenced the greatest difference in heterozygous indices between the populations (4.2%). By way of comparison, in four of the five Latvian populations studied the variation in empirical heterozygosity was 0.4% and it was 0.8% in theoretical heterozygosity.

The data obtained show that the studied stands of *P. abies* in different regions of Estonia have a high level of variability and a common gene pool of isoenzymes. In Estonia, the maximal values for genetic polymorphism (P_{99}) and expected heterozygosity (He) were evidenced in the Kabala and Õisu populations. The Kabala population in Estonia was characterised by the highest values for allelic diversity. Based on the findings from polymorphism analysis it must be mentioned that virtually all the stands studied in Norway spruce natural populations in Estonia have sufficient population genetic resources to restore their gene pools.

Conclusions

In conclusion, it should be noted that the population genetic approach, which takes the results of the analysis of microevolutionary processes and genetic diversity into account, allows to realise the strategy of the conservation of forest genetic resources. Using isoenzymes as genetic markers in the studies, an evaluation was given of the status of population genetic resources of Norway spruce in 15 natural stands. It was determined that in the Baltic and Byelorussian populations of the natural habitat of Norway spruce approximately 73% of the loci studied are in a polymorphic state. However, an average of 19% of all the genes analysed per tree are heterozygous, and the allelic diversity is at least 2.25 alleles frequently occurring per locus. It appeared that the key parameters of genetic variability fluctuated around a relatively broad range.

Based on the literature and the data obtained it can be claimed that in the Baltic-Byelorussian region it is practical to collect seed of Norway spruce from the stands where the genetic variability parameters determined using the isoenzyme test system described herein do not fall below the average indices for the country in question. It is impractical to collect seed from a population having indices for its genetic diversity, which are below the average.

Considering the literature we may claim that the genetic variability of Norway spruce increases from central Europe northwards. Based on the findings obtained from our research, the same tendency can be observed in the Baltic-Byelorussian region.

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References

Areškeviciene, R., Žvingila, D., Gabrilavičius, R. and Kuusiene, S. 2005. The estimation of genetic diversity within and between Lithuanian populations of Norway spruce (*Picea abies* (L.) karst.) using RAPD. *Baltic Forestry,* 11 (2): 2–8.

Bergmann, F. 1973. Genetische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. Genetische Kontrolle von Esterase- und Leucinaminopeptidase Isoenzymen im haploiden Endosperm ruhenden Samen. *Theoretical and Applied Genetics*, 43, 222–225.

Bergmann, F. 1974. The genetics of some isoenzyme systems in spruce endosperm (*Picea abies*). *Genetika*, 6: 353–360.

Cheliak, W. M. and Pitel, J. A. 1984. Genetic control of allozyme variants in mature tissues of white spruce trees. *Heredity*, vol. 75: 34–40.

Cheliak, W. M., Skroppa, T. and Pitel, J. A. 1987. Genetics of the polycross. 1. Experimental results from Norway spruce. *Theoretical and Applied Genetics*, 73: 321–329.

Forest sector in Latvia. 2003. 34p.

Gabrilavičius, R. and Danusevičius, D. 2003. Genetics and breeding of Norway spruce in Lithuania. Vilnius, 360p.

Giannini, R. M., Morgante, M. and Vendramin G. G. 1991. Allozyme variation in Italian populations of *Picea abies* (L.) Karst. *Silvae Genetica*, 40: 160–166.

Goncharenko, G. G., Zadeika, I. V., and Birgelis, J. J. 1995. Genetic structure, diversity and differentiation of Norway spruce (*Picea abies* (L.) Karst.) in natural populations of Latvia. *Forest Ecol. Manag*., 72: 31–38.

Goncharenko, G. G. and Padutov, A. E. 1996. Forest genetic resources of the Republic of Belarus. Sustainable forest genetic resources programmes in the Newly Independent States of the former USSR. Proceedings of a workshop, 23-26 september 1996, Belvezha, Belarus. Rome, Italy, p. 20–24.

Goncharenko, G. G., Silin, A. E., Padutov, A. E. and Padutov, V. E. 1996. Genetic resources pine, spruce and fir species in the former Soviet Union: analysis of their gene pools, phylogenetic relationships and genome organization. Sustainable forest genetic resources programmes in the Newly Independent States of the former USSR. Proceedings of a workshop, 23–26 september 1996, Belvezha, Belarus. Rome, Italy, 84–101.

Goncharenko, G. G., Padutov, V. E., Vorobyev V. N., Vorobyeva N. A., Tamm, Y., Korczyk, A. F., Baranov, O. Y. and Petrova, E., A. 2000. Microevolutionary process and genetic diversity in populations of main structural components of forest ecosystems of Northern Eurasia. Biodiversity and dynamics of ecosystems in north Eurasia. Boene, p. 44–46.

Gцmцry, D. and Paule, L. 1993. Isozyme polymorfism in Norway spruce (*Picea abies* Karst.) from Slovak Carpathias. – Norway spruce provenances and breeding. Proc. of the IUFRO S 2.2. 11 Symposium Latvia 1993. Riga, p. 60–67.

Ilyinov, 1996. Intraspecific variability and population structure of Norway spruce in Karelia. Genetics and Breeding in Forest Service Papers. Theses of International Scientific Practical Conference (June 28–29, 1996, Voronezh). Voronezh, p. 100–101.

Koski, V. 1993. Norway spruce gene conservation network. 2nd Interim Reports on the Follow-up Work with the Strasbourg Resolutions. Ministry of Agriculture and Forestry, Helsinki, p. 41–45.

Koski, V. 2000. Genetic diversity of European conifers. IPGRI Conifers Network. Report of the first Meeting. 22-24 March 2000. Brdo/Kranj, Slovenia, p. 22–24.

Krutovskii, K. V. and Bergmann, F. 1993. Genetic variation of Norway and Siberian spruce species and their zone of introgressive hybridization studied by isozyme loci. Norway spruce provenances and breeding. Proceedings of the IUFRO S 2.2 – 11 Symposium Latvia 1993. Riga, p. 93–99.

Lagercrantz, U., Ryman, N. and Stahl, G. 1988. Protein loci in diploid tissue of Norway spruce (*Picea abies* K.): description and interpretation of electrophoretic variability patterns. *Hereditas*, 108: 149–158.

Lagercrantz, U. and Ryman, N. 1990. Genetic structure of Norway spruce (*Picea abies*): Concordance of morphological and allozymic variation. *Evolution,* 44: 38–53.

Lundkvist, K. 1979. Allozyme frequency distribution in four Swedish populations of Norway spruce (*Picea abies* K.). 1. Estimations of genetic variation within and among populations, genetic linkage and a mating system parameter. *Hereditas*, 90: 127–143.

Lundkvist, K. and Rudin, D. 1977. Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. *Hereditas*, 85: 67-74.

Muona, O., Yazdani R. and Lindqvist G. 1987. Analysis of linkage in *Picea abies. Hereditas*, 106: 31–36.

Paule, L., Szmidt, A. E. and Yazdani, R. 1990. Isozyme polymorphism of Norway spruce (*Picea abies* Karst.) in Slovakia. I. Genetic structure of adjacent populations. *Acta Facultatis Forestalis Zvolen*, 32: 57–70.

Poulsen, H. D., Simonsen, V. and Wellendorf H. 1983. The inheritance of six isoenzymes in Norway spruce *Picea abies* (L.) Karst. *Forest Tree Improvement*, 16: 12–33.

Prakash, S., Lewontin, R. C. and Hubby, J. L. 1969. A molecular approach to the study of genetics heterozygosity in natural populations. IV. Patterns of genetic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. *Genetics*, 61: 841–858.

Pärt, E. 2005. Metsavarud [Forest resources]. Aastaraamat Mets 2004. [Yearbook Forest 2004] Tartu, p. 1–46.

Routkowsky, I. W., Prokazin, A. and Popivshchy, I. 1997. The main results of previous work on the conservation of Norway spruce genetic resources in Russia. In: Turok, J., Koski, V. (compilers) *Picea abies* Network. Report of the second meeting, 5-7 September 1996, Hyytiдlд, Finland. International Plant Genetic Resources Institute, Rome, Italy. 67 p.

Savolainen, O., Karvonen, P. and Kдrkinen, K. 1992. Perinnцlinen kuuntelu takaa metsien sopentumiskyvn. (Profound inquiry guarantees the ability of forests to adapt). *Metsд ja puu*, 2: 19–21.

Tigerstedt, P. M. A. 1973. Studies in isozyme in marginal and central populations of *Picea abies. Silva Fennica*, 75: 47–60.

Turok, J. 1997. Introduction. In: Koski, V., Skr?ppa, Paule, L., Wolf, H., Turok, J. (compilers). Technical guidelines for genetic conservation of Norway spruce (*Picea abies* (L.) Karst.). International Plant Genetic Resources Institute, Rome, Italy, 42 p.

Алтухов Ю. П., Крутовский К. В., Гафаров Н. И., Духарев В. А. 1986. Аллозимный полиморфизм в природной популяции ели европейской *Picea abies* (L.) Karst. Сообщение 1. Сис-

темы полиморфизма и механизмы их генного контроля [Allozymic polymorphism in natural populations of Norway Spruce (*Picea abies* (L.) Karst.). Report 1. Systems of polymorphism and mechanisms of their genetic control*]. Генетика* [Genetics], 22: 2135–2151.

Гончаренко Г. Г. 1999. Геносистематика и эволюционная филогенетика лесо-образующих хвойных Палеартики [Genosystematics and evolutional philogenesis of forest-forming conifers in the Paleoarctic region]. Минск: Тэхнология, 188 с.

Гончаренко Г. Г. 2002. Геносистематика и эволюционная филогенетика хвойных Палеартики. Сообщение III. Геносистематика и филогенетика елей [Genosystematics and evolutional philogenesis in conifers in the Paleoarctic region. Report III. Genosystematics an philogenesis in spruce]. Известия Гомельского государственного университета им. Скорины, 3(12), 81–115.

Гончаренко Г. Г., Падутов В. Е. и Потенко В. В. 1989. Руководство по исследованию хвойных видов методом электрофоретического анализа изоферментов [A guide to the study of conifer species using the method of electrophoretic analysis of isoenzymes]. – Гос. ком. СССР по лесу. Гомель, Полеспечать, 164 с.

Гончаренко Г. Г., Потенко В. В. 1990. Изменчивость и дифференциация у ели европейской *Picea abies* (L.) Karst. в популяциях Украины, Беларуссии и Латвии [Variability and differentiation in Norway spruce *Picea abies* (L.) Karst. in populations in Ukraine, Byelorussia and Latvia]. *Докл. АН СССР*, 314, 2: 492–496.

Гончаренко Г. Г., Потенко В. В. 1991. Параметры генетической изменчивости и дифференциации в популяциях ели европейской (*Picea abies* (L.) Karst.) и ели сибирской (*Picea obovata* Ledeb.) [Parameters of genetic variability and differentiation in populations of Norway spruce (*Picea abies* (L.) Karst.) and Siberian spruce (*Picea obovata* Ledeb.)]. *Генетика,* 27, 10: 1759–1772.

Гончаренко Г. Г., Потенко В. В., Слободян Я. Н., Сидор А. И. 1990. Генетикотаксо-номические взаимоотношение между *Picea abies* (L.) Karst., *P.montana* Schur. и *P. obovata* Ledeb [Genetic-taxonomic interrelations between *Picea abies* (L.) Karst., *P. montana* Schur. and *P. obovata* Ledeb.]. *Докл. АН СССР*, 34, 4: 361–364.

Номенклатура ферментов [A classification of isoenzymes]. 1979. – М., ВИНИТИ, 320 с.

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СТРУКТУРА ФЕРМЕНТОВ В ПРИРОДНЫХ ПОПУЛЯЦИЯХ ЕЛИ *P. ABIES* KARST. В ЭСТОНИИ, В ЛАТВИИ И В БЕЛОРУСИИ

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Резюме

Материал для популяционно-генетического анализа был собран из пятьнадцати природных популяциях *P. abies* Белорусско-Балтийского региона: 6 популяций из Беларуссии, 5 популяций из Латвии и 4 популяций из Эстонии. Подводя итог анализа полиморфизма в природных популяциях *P. abies*. Белорусско-Балтийского региона необходимо отметить, что практически все насаждения обладают достаточными популяцонно-генетическими ресурсами для воспроизведения своих генофондов. Установлено, что в популяциях Белорусско-Балтийской части ареала ели европейской около 73% локусов находятся в полиморфном состоянии, а каждое дерево является гетерозиготным в среднем по 19% своих генов, а количество аллелей на локус достигает 2,25. Выявлен относительно большой размах значений между исследованными популяциями по основным параметрам генетической изменчивости.

Ключевые слова: *Picea abies,* генетическая структура, генетическая изменчивость, изоферменты, дифференциация