

Introgressive hybridization and phylogenetic relationships between Norway, *Picea abies* (L.) Karst., and Siberian, *P. obovata* Ledeb., spruce species studied by isozyme loci

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We analysed patterns of genetic variation at 26 isozyme loci across the area of two main forest-forming spruce species in Eurasia, Norway spruce (*Picea abies* (L.) Karst.) and Siberian spruce (*P. obovata* Ledeb.). Ten seed samples from distant parts of the *P. abies*–*P. obovata* area and from a supposedly wide zone of introgressive hybridization between them were investigated. A very high level of allozyme variation was found in populations of both species. As parameters of gene diversity, the mean number of alleles per locus, percentage of polymorphic loci (95 per cent criterion) and expected heterozygosity averaged 2.8, 61.5 and 0.252 for *P. abies* and 2.4, 61.5 and 0.213 for *P. obovata*, respectively. Norway and Siberian spruces turned out to be extremely similar genetically. We did not find any fixed allele differences between them, i.e. there were no diagnostic loci and only a few alleles could be characteristic of some populations. Cluster and multivariate analyses have shown that these two species should be considered as two closely related subspecies or two geographical races of one spruce species undergoing considerable gene exchange. Our genetic data agree with morphological data and confirm the existence of a wide zone of introgressive hybridization between Norway and Siberian spruces — perhaps the widest known among plants. The samples which, according to morphological and geographical data, were taken from presumably ‘hybrid’ populations showed ‘intermediate’ genetic characteristics. Clinal variation was suggested for some alleles, and the ‘rare allele phenomenon’, i.e. higher frequencies of rare and unique alleles, was observed in the ‘hybrid’ spruce populations.

Keywords: introgressive hybridization, isozyme loci, phylogeny, *Picea abies* (L.) Karst., *Picea obovata* Ledeb., population genetic structure.

Introduction

Norway spruce, *Picea abies* (L.) Karst., and Siberian spruce, *P. obovata* Ledeb., are traditional subjects of forest genetic and breeding research. However, until relatively recently, most investigations were limited to the use of morphological, physiological and other phenotypic traits with unclear modes of inheritance and unknown genetic control. Studies of isozyme genetic markers during the last two decades have resulted in additional valuable information on the genetic structure of Norway spruce (Tigerstedt, 1973,

1979; Bergmann, 1974a, 1975; Bergmann & Gregorius, 1979; Bergmann & Ruetz, 1991; Lundkvist & Rudin, 1977; Lundkvist, 1979; Brunel & Rodolphe, 1985; Altukhov *et al.*, 1986a, 1989; Paule, 1986; Paule & Gömöry, 1973; Paule *et al.*, 1990; Lagercrantz & Ryman, 1990; Muona *et al.*, 1990; Giannini *et al.*, 1991a; Goncharenko & Potenko, 1991; Morgante & Vendramin, 1991; Gömöry, 1992; see also Müller-Starck *et al.*, 1992 for review), the mating system in natural populations (Müller, 1977; Altukhov *et al.*, 1989; Muona *et al.*, 1990; Morgante *et al.*, 1991), in plantations (Xie & Knowles, 1992; Finkeldey, 1995) and in seed orchards (Cheliak *et al.*, 1987; Paule *et al.*, 1993), linkage between isozyme loci (Lundkvist, 1974a; Altukhov *et al.*, 1986a; Muona *et al.*, 1987; Geburek & von Wuehlisch, 1989), and spruce conser-

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vation strategy (Finkeldey, 1992). Such information is necessary for the development of proper breeding, reforestation and gene conservation programmes (i.e. Bergmann, 1991; Wellendorf, 1991). Clinal genetic variation (Bergmann, 1978), the effects of air pollution on the genetic structure (Scholz & Bergmann, 1984; Bergmann & Scholz, 1985, 1987, 1989), and correlation between isozyme genotypes and morphological traits (Altukhov *et al.*, 1986b; Bergmann & Ruetz, 1991; von Wuehlisch & Krusche, 1991) have also been studied in Norway spruce using isozyme loci.

However, most of the above-cited investigations have dealt with populations from the Central European part of the Norway spruce range, although this species, combined with Siberian spruce, has one of the largest parapatric areas among forest trees, covering nearly the entire area of Northern Eurasia and comparable only with Scots pine. Norway and Siberian spruces are considered to be different but very closely related species with a wide zone of introgressive hybridization along both sides of the Ural Mountains (Pravdin, 1975; Schmidt-Vogt, 1977). Both species have a great economic and ecological significance and are the subjects of intensive breeding programmes. In addition, the study of genetic processes in the zones of contact and introgression of these species may yield valuable data on spruce adaptation and evolution. Large-scale population genetic studies can also help to analyse the postglacial spruce distribution and clarify the routes of reinvasion. However, neither the eastern range of Norway spruce nor introgressive hybridization and phylogenetic relationships between Norway and Siberian spruces have been sufficiently studied using isozyme loci as genetic markers (Gömöry & Paule, 1990; Goncharenko *et al.*, 1990; Goncharenko & Potenko, 1991).

Norway and Siberian spruce species have two main distinguishing morphological taxonomic traits — the shape and size of their cones and the shape of the cone scales (Pravdin, 1975). Norway spruce has large cones (10–15 cm) and egg-shaped serrated scales (Pravdin, 1975). In this paper we refer to this spruce type as 'pure' Norway spruce. According to Rubner (1953), it is found in three more or less isolated main parts of the *P. abies* area in Central Europe: (1) the Alpine south-eastern European region (Italy, former Yugoslavia, Austria, Switzerland and southern Germany); (2) the Hercynic-Carpathian region (central and eastern Germany, western Poland, the Czech Republic, Slovakia, Rumania and Bulgaria); and (3) the North-Baltic region (Scandinavian countries, eastern Poland, Byelorussia, western and central Russia). In contrast to Norway spruce, 'pure' Siberian spruce growing in northern Asia has smaller cones (4–8 cm) with oval, smooth scales (Pravdin, 1975). In the zone of intro-

gressive hybridization, a range of 'intermediate' types of cone can be found. In fact, these traits are highly variable. However, if we ignore variation among high altitude varieties and some specific 'ecotypes', the 'pure' Norway spruce cone type is nearly dominant in Central Europe and the 'pure' Siberian spruce cone type becomes dominant east of the Ural Mountains. Whereas in trees from the extreme edges of the Norway–Siberian spruce area the two cone types look like qualitative species-diagnostic traits, in the zone of introgressive hybridization the cone traits seem to vary in a more quantitative way with clinal-like variation. It would be interesting to study how this morphological variation corresponds to other genetic variation.

Thus, the main objectives of our study are the following:

- (1) to obtain data on the large-scale geographical distribution of allelic and genotype frequencies at 26 isozyme loci, and to estimate the levels of intra- and interpopulation genetic variation by studying Norway and Siberian spruce populations sampled from different parts of their ranges;
- (2) to study the genetic structure of populations sampled from the zone of supposed introgressive hybridization between Norway and Siberian spruce;
- (3) to estimate the level of genetic differentiation between Norway and Siberian spruces, as well as to reveal phylogenetic relationships between them and to find the most diagnostic species-specific alleles and loci.

Material and methods

Plant material and sampling sites

Designations and geographical origins of the populations of both spruce species investigated in the study are shown in Table 1. The samples consisted of ten bulked seed lots randomly collected from hundreds (Germany and Sweden) or even thousands of trees growing in each of the ten different, large natural populations. We suppose that these samples correctly represent the alleles of the original populations. Six samples were collected from the 'pure' Norway spruce populations (Germany, Sweden, Byelorussia, Ukraine, Russia-T and Russia-V), two samples from the supposed zone of introgressive hybridization (Ural and Komi), and two from the 'pure' Siberian spruce populations (Kazakhstan and Siberia). This sampling strategy was designed to maximize the chances of finding the most species- or population-characteristic or species-specific alleles and loci.

Table 1 List of ten bulked spruce seed samples, indicating their designations and geographical origins

Geographical origin (country and name of region and/or nearest city)	Co-ordinate	Designation
<i>'Pure' Picea abies</i>		
Germany, Westerhof	51°40'N, 10°30'E	Germany
Sweden, Sörliden	64°50'N, 19°50'E	Sweden
Byelorussia, Vitebsk	55°20'N, 31°15'E	Byelorussia
Ukraine, Chernigov	51°20'N, 31°10'E	Ukraine
West-Central Russia, Tula	54°30'N, 37°40'E	Russia-T
West-Central Russia, Vyatsk	58°40'N, 49°45'E	Russia-V
<i>'Hybrid' zone</i>		
Central Russia, Ekaterinburg (Ural Mountains)	56°50'N, 60°45'E	Ural
North-Central Russia, Komi Republic (North of Ural Mountains)	63°20'N, 73°30'E	Komi
<i>'Pure' P. obovata</i>		
Eastern Kazakhstan, Leninogorsk	50°30'N, 83°50'E	Kazakhstan
Eastern Russia, Krasnoyarsk (Eastern Siberia)	51°20'N, 92°40'E	Siberia

Electrophoretic analysis

Isozyme seed haplotypes and, consequently, allele frequencies in a sample were inferred from isozyme phenotypes visualized after starch gel electrophoretic separation of haploid endosperm extracts and subsequent enzyme-specific staining. Fourteen isozyme systems encoded by 26 loci were employed. References to the inheritance and buffer systems used for isozyme separation are listed in Table 2. Detailed descriptions of electrophoretic conditions, specimen preparation, genetic interpretation of zymograms, designations of allozymes, alleles and loci have been given elsewhere (Altukhov *et al.*, 1986a; Muona *et al.*, 1987; Bergmann & Scholz, 1989; Goncharenko & Potenko, 1991). More than 135 seeds per locus, on average, were analysed for nearly every seed sample, which allowed us to search effectively for rare and unique alleles. We refer to alleles occurring in populations with a frequency less than 0.05 as rare ones, and to those occurring in one population or only in 'hybrid' populations as unique alleles.

Data analysis

Calculation of parameters of intra- and interpopulational genetic diversity (mean number of alleles per locus, A , percentage of polymorphic loci, P , mean heterozygosity expected from Hardy-Weinberg proportions, H_e), estimation of genetic differentiation (F_{ST} by Wright, 1978, and Nei, 1977) and genetic distances, clustering and construction of dendrograms of spruce populations were carried out using the IBM PC version 1.7 of the computer program BIOSYS-1 (Swofford & Selander, 1981). Mean effective number of alleles per

locus or gene pool diversity (v , Gregorius, 1987), total population differentiation of the gene pool (δ_T , Gregorius, 1987) and subpopulation genetic differentiation of the gene pool (D_j and δ , Gregorius, 1984b; Gregorius & Roberds, 1986) were computed using the GSED program by E. Gillet (unpublished). D_j is specified as the amount of genetic differentiation of the gene pool of one (sub)population compared to the remainder of the total population for infinite population size (Gregorius, 1984b; Gregorius & Roberds, 1986). D_j is the proportion of alleles by which one spruce population differs from the remaining populations of the total Norway-Siberian spruce complex. This proportion is defined as

$$D_j = \frac{1}{L} \sum_{l=1}^L \left(\frac{1}{2} \sum_{i=1}^{n_l} |p_{il} - \bar{p}_{il}| \right),$$

where n and L are the numbers of alleles and loci studied in population j , and p_{il} and \bar{p}_{il} are the frequencies of the alleles in the population j and in the remaining populations of the total Norway-Siberian spruce complex, respectively. The subpopulation genetic differentiation is then defined by

$$\delta = \sum_j c_j D_j,$$

where the weight c_j expresses the relative size of the j th (sub)population

$$\left(\sum_j c_j = 1 \right).$$

With our data we estimated different measures of genetic distance and used several methods of cluster-

Table 2 Enzymes, loci, numbers of alleles and buffer systems

Enzyme name (abbreviation, E.C. reference)	Locus	<i>N</i>	Buffer system	Reference to the genetic control
Acid phosphatase (APH, 3.1.3.2)	<i>Aph-2</i>	5	TCLiB, TC, TVB	Tigerstedt, 1973; Bergmann, 1974b; Lundkvist, 1975
Formate dehydrogenase (FDH, 1.2.1.2)	<i>Fdh</i>	4	TC	Authors' data
Glutamate dehydrogenase (GDH, 1.4.1.3)	<i>Gdh</i>	2	TCLiB, TC, TVB	Lundkvist, 1979; Brunel & Rodolphe, 1985; Altukhov <i>et al.</i> , 1986a; Cheliak <i>et al.</i> , 1987
Glutamate-oxaloacetate transaminase (GOT, 2.6.1.1)	<i>Got-1</i>	1	TCLiB,	Lundkvist, 1979; Poulsen <i>et al.</i> , 1983;
	<i>Got-2</i>	4	TVB	Brunel & Rodolphe, 1985; Altukhov
	<i>Got-3</i>	5		<i>et al.</i> , 1986a
Glucose-6-phosphate dehydrogenase (G6PDH, 1.1.1.49)	<i>G6pdh-1</i>	4	TC	Altukhov <i>et al.</i> , 1986a
Isocitrate dehydrogenase (IDH, 1.1.1.42)	<i>Idh-1</i>	4	TC, TVB,	Altukhov <i>et al.</i> , 1986a; Muona <i>et al.</i> ,
	<i>Idh-2</i>	4	TCLiB	1987
Leucine aminopeptidase (LAP, 3.4.11.1)	<i>Lap-1</i>	10	TVB	Bergmann, 1973; Lundkvist, 1974b
	<i>Lap-2</i>	9		
Malate dehydrogenase (MDH, 1.1.1.37)	<i>Mdh-1</i>	2	TC	Lundkvist, 1979; Brunel & Rodolphe,
	<i>Mdh-2</i>	4		1985; Altukhov <i>et al.</i> , 1986a; Muona
	<i>Mdh-3</i>	4		<i>et al.</i> , 1987
	<i>Mdh-4</i>	3		
	<i>Mdh-m</i>	2		
Menadion reductase (MNR, 1.6.99.2) or diaphorase (DIA, 1.6.4.3.) or NADH-dehydrogenase (NDH, 1.6.99.1)	<i>Mnr-4</i> or	6	TCLiB, TC, TVB	Authors' data; Muona <i>et al.</i> , 1987
	<i>Dia-4</i> or			
Phosphoenolpyruvate carboxylase (PEPC, 4.1.1.31)	<i>Pepca</i>	4	TC	Authors' data
6-Phosphogluconate dehydrogenase (6-PGDH, 1.1.1.44)	<i>6-Pgd-1</i>	3	TC	Poulsen <i>et al.</i> , 1983; Altukhov <i>et al.</i> ,
	<i>6-Pgd-2</i>	6		1986a; Krutovskii & Gafarov, 1987;
	<i>6-Pgd-3</i>	5		Morgante <i>et al.</i> , 1989; Giannini <i>et al.</i> ,
				1991b
Phosphoglucose isomerase (PGI, 5.3.1.9)	<i>Pgi-2</i>	7	TCLiB	Poulsen <i>et al.</i> , 1983
Phosphoglucosmutase (PGM, 2.7.5.1)	<i>Pgm-1</i>	4	TVB	Poulsen <i>et al.</i> , 1983
	<i>Pgm-2</i>	7		
Shikimic acid dehydrogenase (SKDH, 1.1.1.25)	<i>Skdh-1</i>	3	TVB	Authors' data; Muona <i>et al.</i> , 1987;
	<i>Skdh-2</i>	6		Morgante <i>et al.</i> , 1989

N, number of alleles observed. TC, Tris-citrate, pH 6.5–7.4 (Siciliano & Shaw, 1976, with modifications); TCLiB, Tris-citrate/Li-borate, pH 8.1 (Ashton & Braden, 1961); TVB, Tris-EDTA-borate, pH 8.0 (Siciliano & Shaw, 1976, with modifications).

ing, but almost all dendrograms resulted in nearly the same topology. Thus, we will present the most commonly used measures: Nei's (1972, 1978) genetic distance *D*, Cavalli-Sforza & Edwards's (1967) chord distance, Gregorius's (1974, 1978, 1984a) distance d_o , and the UPGMA-dendrogram based on the *D* matrix (Sneath & Sokal, 1973). The last two distances are metric ones and can be used in other clustering methods, such as the Wagner tree method (Farris, 1972), which unlike UPGMA does not require the assumption of equality of evolutionary rates. Thus, we will also present the phylogenetic tree obtained by the Wagner tree method of clustering based on the d_o matrix. This method minimizes the total branch length

at each stage of clustering of OTUs. Nei's (1972) genetic distances matrix was also used for principal coordinate analysis of spruce populations and for analysis of correlation between geographical and distance matrices (normalized Mantel statistic *Z*) with the aid of the computer program NTSYS-PC (Rohlf, 1988). Pearson's linear and Spearman's rank correlations of allozyme allele frequencies with latitude and longitude of *P. abies* and *P. obovata* populations were studied using the SYSTAT statistical computer package (Wilkinson, 1987).

Differences in rare and unique allele frequencies between *P. abies*, *P. obovata* and 'hybrid' populations were estimated by Fisher's criterion statistics,

$$F = (\varphi_1 - \varphi_2)^2 \frac{N_1 \cdot N_2}{N_1 + N_2},$$

where $\varphi_1 = 2 \arcsin \sqrt{p_1}$ and $\varphi_2 = 2 \arcsin \sqrt{p_2}$, p_1 and p_2 are the frequencies of rare or unique alleles, and N_1 and N_2 are the total number of alleles studied in populations 1 and 2, respectively.

Results and discussion

Levels of genetic diversity and intraspecific genetic differentiation among populations

The genetic parameters estimated from allele frequencies of 26 isozyme loci are presented in Table 3 (the allele frequencies are available on request). Expected heterozygosity (H_e), calculated from corresponding Hardy-Weinberg proportions, averaged 0.252, ranging from 0.192 in the German population to 0.284 in the Swedish population of Norway spruce (Table 3). The mean number of alleles per locus (A) was 2.8, again

with a minimum of 2.5 in the German population and a maximum of 2.9 in several other populations (Byelorussia, Ukraine and Russia-T). The average percentage of polymorphic loci (P) was 61.5 and ranged from 53.8 per cent (Germany) to 65.4 per cent (Byelorussia, Ukraine and Sweden). The significantly lower level of genetic diversity in the German population compared to other Norway spruce populations agrees with previous data indicating that central European populations have consistently less genetic variability than north-eastern and Scandinavian populations (Lagercrantz & Ryman, 1990; Bergmann, 1991). This may have resulted from a relatively recent decrease in effective population size ('bottleneck' effect) during the last glaciation, which is assumed for many Central European populations. An artificial origin of the German population cannot be completely excluded, although specialists consider this Westerhof population in the foothills of the Harz Mountains to be indigenous (Schmidt-Vogt, 1977).

Table 3 Genetic variation in ten populations of *Picea abies* and *P. obovata* determined at 26 isozyme loci

Population name	$N \pm SE^1$	$A \pm SE^2$	$P, \%^3$	$H_e \pm SE^4$	ν^5	D_j^6
'Pure' <i>Picea abies</i>						
Germany	132.8 ± 6.7	2.5 ± 0.2	53.8	0.192 ± 0.038	1.236	0.132
Sweden	125.2 ± 7.9	2.8 ± 0.3	65.4	0.284 ± 0.047	1.393	0.084
Byelorussia	137.5 ± 7.2	2.9 ± 0.3	65.4	0.255 ± 0.042	1.340	0.081
Ukraine	144.2 ± 10.2	2.9 ± 0.2	65.4	0.259 ± 0.042	1.349	0.077
Russia-T	147.0 ± 15.0	2.9 ± 0.4	57.7	0.250 ± 0.045	1.331	0.054
Russia-V	142.7 ± 10.9	2.8 ± 0.3	61.5	0.272 ± 0.046	1.371	0.073
Mean	138.2 ± 9.7	2.8 ± 0.3	61.5	0.252 ± 0.043	1.337	0.084
'Hybrid' zone						
Ural	141.4 ± 7.9	2.9 ± 0.3	65.4	0.278 ± 0.045	1.382	0.072
Komi	136.8 ± 7.2	2.9 ± 0.4	57.7	0.274 ± 0.050	1.375	0.106
'Pure' <i>P. obovata</i>						
Kazakhstan	137.8 ± 9.3	2.5 ± 0.3	61.5	0.209 ± 0.040	1.264	0.148
Siberia	143.4 ± 14.5	2.3 ± 0.2	61.5	0.216 ± 0.040	1.274	0.134
Mean	140.6 ± 11.9	2.4 ± 0.2	61.5	0.213 ± 0.040	1.269	0.141

¹ Mean seed sample size per locus studied and standard error (SE).

² Mean number of alleles per locus and standard error (SE).

³ Percentage of polymorphic loci (a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95).

⁴ Mean heterozygosity expected from Hardy-Weinberg proportions (unbiased estimate according to Nei, 1978) and standard error (SE). It equals total population differentiation of the gene pool, δ_T , for large sample size (Gregorius, 1987).

⁵ Mean effective number of alleles per locus or gene pool diversity (Gregorius, 1987).

⁶ The amount of genetic differentiation of the gene pool of one subpopulation to the remainder of the total population for infinite population size (Gregorius, 1984b; Gregorius & Roberds, 1986).

Siberian spruce populations have less genetic diversity than Norway spruce. However, our preliminary data do not allow us to conclude whether it is a species-specific phenomenon and we need to confirm this by further studies.

Because of the differences in allele frequencies at several loci between Norway and Siberian spruces the 'hybrid' populations have the highest levels of genetic diversity among all populations studied.

The data obtained confirm the high level of intrapopulation variation found in previous studies on spruce species, including Norway and Siberian spruce (Table 4), as well as in conifers in general (Müller-Starck *et al.*, 1992). According to the published data on Norway and Siberian spruce, our values of genetic diversity exceed all previous estimates based on a similar number of loci. However, such comparisons should be made with caution because the sets of isozyme loci used are not the same in every case. On the whole, our data correspond quite well with previously published data.

Some rare alleles of the loci *Fdh* and *Mnr-4* were specific to one or a few populations of Siberian spruce, and some rare and unique alleles of loci *Aph-2*, *Fdh*, *Got-2*, *Got-3*, *G6pdh-1*, *Idh-2*, *Lap-2*, *Mnr-4*, *Pepca* and *6-Pgd-2* were specific to one or a few populations of Norway spruce.

Allele frequencies of some loci vary clinally across the whole Norway–Siberian spruce area (Table 5). To answer the question of whether this is the result of gradient selection on these alleles or the result of gradual gene flow between Norway and Siberian spruce through incomplete isolation-by-distance, additional investigations are necessary.

Genetic differentiation among populations was analysed using F -statistics (Nei, 1977; Wright, 1978) and the δ -measure (Gregorius, 1984b; Gregorius & Roberds, 1986). In spite of the very wide distribution of the populations studied, levels of intraspecific genetic differentiation among populations were comparatively low — only about 1–4 per cent of the total intraspecific isozyme gene variation resulted from interpopulation variation ($F_{ST}=0.044$ for Norway spruce and $F_{ST}=0.011$ for Siberian spruce) and the overwhelming proportion of the total variation, over 95 per cent, belonged to intrapopulation variation (Table 6). The loci *Gdh*, *G6pdh-1* and *Pgm-2* of Norway spruce, and *Pgm-2* of Siberian spruce make the most significant contributions to the intraspecific differentiation of these species (Table 6). Since these same loci also show clinal variation (Table 5), geographical variation in these loci should be studied in detail in future searches for possible adaptive loci.

Genetic distances between populations were also small (e.g. for Nei's (1972) genetic distance between

populations of Norway spruce, D averaged 0.020 and ranged between 0.007 and 0.051 (Table 7)). These values agree with the calculations carried out on three other spruce species. Nei's genetic distance averaged 0.012 among six populations of *P. glauca* (Yeh & Arnott, 1986; Alden & Loopstra, 1987), 0.013 among 32 populations of *P. mariana* (O'Reilly *et al.*, 1985; Yeh *et al.*, 1986; Boyle & Morgenstern, 1987), and 0.014 among 13 populations of *P. sitchensis* (Yeh & El-Kassaby, 1980; Yeh, 1981; Yeh & Arnott, 1986). We have also summarized data on interpopulation genetic differentiation of these three spruce species using F_{ST} or analogous G_{ST} parameters of differentiation (Table 4). These values ranged from 0.010 to 0.079 (average 0.048) and are very similar to our data (Table 6).

The parameter D_j gives a good estimation of the contribution of each population to the total differentiation of the Norway–Siberian spruce complex (Table 3). The populations of Germany (Norway spruce) and Kazakhstan (Siberian spruce), from the remote parts of the Norway–Siberian spruce area, made the greatest contribution to the total genetic differentiation and turned out to be the most divergent. On the other hand, Russian populations exhibited the smallest differentiation and their gene pools were the most 'representative' of the total gene composition, which, hypothetically, can be explained by their geographical proximity to the ancient refuge in the Central Russian area. From this point of view, they can be considered as the most original populations keeping most of the 'ancient' spruce variation. Gene flow from the extreme populations could be another explanation of the weak differentiation of the Russian populations, taking into account their central position. However, both considerations are rather speculative because of the generally low F_{ST} levels.

The low levels of interpopulation differentiation obtained for allozyme loci of Norway–Siberian spruce are usual for conifers. Such factors as outcrossing, wind-pollination, seed dispersal by wind (most conifers) or by birds (stone pines, some white pines and pinyons), wide continuous ranges, high population density and large effective size are expected to reduce the influence of genetic drift and, therefore, decrease heterogeneity of allele frequencies and interpopulation genetic differentiation for allozyme alleles that are largely equivalent selectively (Hamrick *et al.*, 1981; Hamrick, 1983; Loveless & Hamrick, 1984; Hamrick & Loveless, 1986; Hamrick & Godt, 1989). On the other hand, recent analysis of isozyme alleles in forest tree populations suggests that isozymes of the primary metabolism have been optimized during evolutionary epochs and, therefore, are present in all populations, and that major allozyme polymorphism (where there is

Table 4 Isozyme loci variation in the populations of spruce (*Picea*) species compiled from previously published data

Pop.	Loci	P, % ¹		A ²	H _o ³	H _e ⁴	F _{ST} ⁵	G _{ST} ⁶	D ⁷	Reference
		95%	99%							
<i>Picea abies</i> (L.) Karst.										
9	4			2.7				0.04		Bergmann, 1974a
2	4			2.5	0.430					Tigerstedt, 1973
15	6			2.6						Bergmann, 1975
10	6							0.05		Tigerstedt, 1979
21	7	71.0-100		2.14-3.14		0.360-0.450				Bergmann & Gregorius, 1979
2	8	88.0-100		2.37-2.50	0.230-0.320	0.247-0.397				Bergmann & Ruetz, 1991
14	8	77.0-85.0			0.225-0.376				0.001-0.087	Gömöry, 1992
4	12	91.7		3.54	0.341				0.011-0.042 ⁸	Lundkvist, 1979
4	13	53.8	76.9	1.86	0.232	0.237	0.050	0.049	0.052	Altukhov <i>et al.</i> , 1989
2	9-14					0.198-0.230		0.12		Muona <i>et al.</i> , 1990
10	10					0.322-0.367				Bergmann, 1991
9	15				0.217-0.267			0.042	0.0005-0.0322	Paule, 1986; Paule <i>et al.</i> , 1990
9	21	45.5		1.83		0.165		0.042	0.019	Giannini <i>et al.</i> , 1991a
19	19	43.2		1.78		0.162				Morgante & Vendramin, 1991
70	22	41.0		1.58		0.115		0.052	0.007	Lagercrantz & Ryman, 1990
5	27	50.6	75.0	2.17	0.215	0.194	0.025	0.032	0.005-0.030	Goncharenko & Potenko, 1991
9	34	65.0	82.0			0.220				Cited by Ledig, 1986
6	26	61.5		2.8		0.252	0.044		0.005-0.049	This paper
<i>P. obovata</i> Ledeb.										
1	8					0.282				Gömöry & Paule, 1990
2	27	57.1	66.2	2.02	0.199	0.186	0.011	0.017	0.009	Goncharenko & Potenko, 1991
2	26	61.5		2.4		0.213	0.011		0.007	This paper
<i>P. jezoensis</i> (Sieb. et Zucc.) Carr.										
1	8					0.174				Gömöry & Paule, 1990

Table 4 Continued

Number		P, % ¹		A ²	H _o ³	H _e ⁴	F _{ST} ⁵	G _{ST} ⁶	D ⁷	Reference
Pop.	Loci	95%	99%							
<i>P. glauca</i> (Moench) Voss										
1	20					0.140				King <i>et al.</i> , 1984
1	14	64.3	85.7	1.86	0.183	0.195				Chełiak <i>et al.</i> , 1985
2	18		83.4	1.97		0.174			0.005	Yeh & Arnott, 1986
4	13		92.0	2.31-2.69	0.262	0.270		0.015	0.015	Alden & Loopstra, 1987
<i>P. mariana</i> (Mill.) B.S.P.										
5	15	47.5-57.3			0.210-0.230		0.048-0.069		0.020	O'Reilly <i>et al.</i> , 1985
21	23		38.1	1.44	0.120	0.107	0.059		0.014	Yeh <i>et al.</i> , 1986
6	12	63.9	88.9	2.37	0.223	0.229	0.010		0.003	Boyle & Morgenstern, 1987
<i>P. schrenkiana</i> Fisch. et Mey.										
6	24	45.8	45.8	1.88	0.140	0.114	0.113	0.118	0.049	Goncharenko <i>et al.</i> , 1992
<i>P. sitchensis</i> (Bong.) Carr.										
10	24	46.0	51.3	1.90	0.150			0.079	0.014	Yeh & El-Kassaby, 1980; Yeh, 1981
3	18		75.9	1.89	0.199		0.059		0.014	Yeh & Arnott, 1986

¹ Percentage of polymorphic loci (a locus is considered polymorphic if the frequency of the most common allele does not exceed 95% or 99%).

² Mean number of alleles per locus.

³ Mean observed heterozygosity.

⁴ Mean heterozygosity expected from Hardy-Weinberg proportions.

⁵ Wright's (1978) or Nei's (1977) measure of genetic differentiation.

⁶ Nei's (1973) measure of genetic differentiation of the gene pool.

⁷ Nei's (1972 or 1978) genetic distance.

⁸ Estimated only from polymorphic loci.

Table 5 Correlations (*r*) of allozyme allele frequencies with latitude and longitude of ten *Picea abies* and *P. obovata* populations

Allele	Latitude				Allele	Longitude			
	Pearson ¹		Spearman ²			Pearson		Spearman	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<i>Fdh</i> ⁴³	0.699	0.025	0.815	<0.01	<i>Aph</i> -2 ⁹⁰	0.672	0.033	0.503	NS
<i>Fdh</i> ¹⁰⁰	-0.659	0.038	-0.614	NS	<i>Aph</i> -2 ⁹⁶	0.858	0.002	0.796	<0.01
<i>Gdh</i> ⁸⁰	0.647	0.043	0.644	<0.05	<i>Aph</i> -2 ¹⁰⁰	-0.802	0.005	-0.758	<0.05
<i>Gdh</i> ¹⁰⁰	-0.647	0.043	-0.644	<0.05	<i>G6pdh</i> -1 ⁸⁹	0.919	0.000	0.939	<0.01
<i>Got</i> -2 ¹⁰⁰	-0.697	0.025	-0.636	<0.05	<i>G6pdh</i> -1 ¹⁰⁰	-0.921	0.000	-0.939	<0.01
<i>Lap</i> -1 ¹⁰⁰	-0.752	0.012	-0.772	<0.05	<i>Got</i> -3 ⁷⁰	-0.738	0.015	-0.600	NS
<i>Lap</i> -1 ¹¹²	0.922	0.000	0.863	<0.01	<i>Got</i> -3 ¹⁰⁰	0.751	0.012	0.699	<0.05
<i>Lap</i> -2 ⁹⁷	0.632	0.050	0.720	<0.05	<i>Lap</i> -1 ¹⁰³	-0.670	0.034	-0.673	<0.05
<i>Lap</i> -2 ¹⁰⁰	-0.907	0.000	-0.857	<0.01	<i>Lap</i> -1 ¹⁰⁸	0.452	NS	0.902	<0.01
<i>Lap</i> -2 ¹⁰⁵	0.635	0.048	0.564	NS	<i>Mdh</i> -3 ⁸¹	0.681	0.030	0.464	NS
<i>Mdh</i> -3 ¹⁰⁰	0.611	0.060	0.778	<0.05	<i>Mnr</i> -4 ¹⁰⁰	-0.833	0.003	-0.709	<0.05
<i>6-Pgd</i> -2 ⁷⁶	0.645	0.044	0.736	<0.05	<i>Mnr</i> -4 ¹¹²	-0.626	0.053	-0.491	NS
<i>6-Pgd</i> -2 ¹⁰⁰	-0.627	0.053	-0.815	<0.01	<i>Mnr</i> -4 ¹²¹	0.848	0.002	0.930	<0.01
<i>Pgm</i> -1 ¹⁰⁰	-0.767	0.010	-0.677	<0.05	<i>Pepca</i> ¹⁰⁰	0.645	0.044	0.661	<0.05
<i>Pgm</i> -1 ¹⁰⁵	0.639	0.047	0.490	NS	<i>Pepca</i> ¹¹⁶	-0.658	0.039	-0.661	<0.05
<i>Pgm</i> -2 ⁹⁴	0.802	0.005	0.438	NS	<i>6-Pgd</i> -1 ⁹³	0.746	0.013	0.784	<0.05
<i>Pgm</i> -2 ¹⁰⁰	-0.923	0.000	-0.863	<0.01	<i>6-Pgd</i> -1 ¹⁰⁰	-0.659	0.038	-0.288	NS
<i>Skdh</i> -1 ⁸⁸	-0.691	0.027	-0.829	<0.01	<i>6-Pgd</i> -2 ⁷⁶	-0.607	0.063	-0.333	NS
<i>Skdh</i> -1 ¹⁰⁰	0.722	0.018	0.829	<0.01	<i>6-Pgd</i> -2 ¹⁰⁰	0.627	0.052	0.273	NS
<i>Skdh</i> -2 ⁸⁷	0.710	0.021	0.732	<0.05	<i>6-Pgd</i> -3 ⁷²	-0.820	0.004	-0.842	<0.01
					<i>6-Pgd</i> -3 ¹⁰⁰	0.825	0.003	0.855	<0.01
					<i>Pgi</i> -2 ¹⁰⁰	0.651	0.042	0.430	NS
					<i>Pgi</i> -2 ¹²³	-0.762	0.010	-0.809	<0.01
					<i>Skdh</i> -2 ⁵³	0.900	0.000	0.952	<0.01
					<i>Skdh</i> -2 ¹⁰⁰	-0.851	0.002	-0.842	<0.01

¹ Pearson's linear correlation.² Spearman's rank correlation.

more than one prevalent allele in the population) is the result of heterozygote advantage arising from ontogenetic differentiation in enzyme function (Bergmann & Gregorius, 1993; Gregorius & Bergmann, 1994).

In spite of a generally low differentiation among spruce populations within species and the low values of genetic distances (Table 7), dendrograms based on these distances basically reflect the geographical origin of the spruce samples (Fig. 1). Moreover, the genetic similarity found between the Swedish population and some Central Russian populations of Norway spruce supports the hypothesis that this species immigrated from the Central Russian area into Scandinavia during the postglacial expansion (Schmidt-Vogt, 1977; Lagercrantz & Ryman, 1990).

Principal coordinates analysis based on the matrix of Nei's genetic distances between spruce samples has also been performed (Fig. 2). This multidimensional analysis produced almost the same result as the cluster analysis. Genetic relationships between spruce popula-

tions, graphically presented in the three-dimensional space of the first three principal coordinates, clearly reflected the actual geographical distribution of these populations (Fig. 2).

A statistically significant positive correlation between geographical and genetic distances has been found for Norway spruce populations ($r = 0.62-0.78$; Table 8) indicating that long-distance migration with gene flow and gradually changing selection, at least at several allozyme loci, could play a significant role in genetic differentiation. Analogous results have also been obtained for many other conifers (see for review Krutovskii *et al.*, 1994), including spruce (Lagercrantz & Ryman, 1990; Giannini *et al.*, 1991a).

We also analysed the correlation between geographical and Nei's distances for individual loci. Of the 26 loci studied only *Aph*-2, *Fdh*, *Gdh*, *G6pdh*-1, *Lap*-1, *Lap*-2, *Mnr*-4, *6-Pgd*-2, *6-Pgd*-3, *Pgi*-2 and *Skdh*-2 exhibit significant correlation (Table 8). This may suggest a selective influence on certain alleles. Patterns

Table 6 Hierarchical F_{ST} -analysis of allozyme allele variation among ten populations of *Picea abies* and *P. obovata*

Locus	Without two populations from 'hybrid' zone				Including two populations from 'hybrid' zone						
	Population/Species			Species/ Total	Population/Total		Population/Total				
	<i>Picea abies</i>	<i>Picea obovata</i>	Both		Wright, 1978	Nei, 1977	Population/ Species	Species/ Total	F_{ST} , Wright, 1978	F_{ST} , Nei, 1977	δ , Gregorius, 1987
<i>Aph-2</i>	0.023	0.013	0.027	0.034	0.060	0.064	0.026	0.025	0.051	0.055	0.124
<i>Fdh</i>	0.027	0.019	0.036	0.010	0.026	0.036	0.032	0.093	0.121	0.130	0.130
<i>Gdh</i>	0.119	0.011	0.117	0.029	0.091	0.094	0.099	0.001	0.099	0.102	0.133
<i>Got-2</i>	0.016	0.000	0.023	0.004	0.019	0.022	0.015	0.001	0.014	0.017	0.017
<i>Got-3</i>	0.020	0.017	0.028	0.178*	0.201	0.204	0.027	0.141	0.164	0.167	0.180
<i>G6pdh-1</i>	0.162	0.011	0.203	0.198*	0.361	0.363	0.179	0.188	0.333	0.335	0.267
<i>Idh-1</i>	0.041	0.008	0.044	0.005	0.039	0.042	0.069	0.010	0.060	0.063	0.076
<i>Idh-2</i>	0.006	0.003	0.008	0.003	0.005	0.009	0.008	0.003	0.005	0.008	0.009
<i>Lap-1</i>	0.056	0.003	0.060	0.012	0.071	0.075	0.055	0.012	0.066	0.070	0.240
<i>Lap-2</i>	0.048	0.000	0.052	0.008	0.045	0.051	0.071	0.001	0.071	0.077	0.181
<i>Mdh-1</i>	0.007	0.000	0.008	0.003	0.006	0.009	0.009	0.003	0.006	0.010	0.005
<i>Mdh-2</i>	0.007	0.000	0.011	0.003	0.008	0.012	0.005	0.006	0.011	0.015	0.013
<i>Mdh-3</i>	0.007	0.007	0.010	0.020	0.030	0.034	0.011	0.023	0.033	0.037	0.043
<i>Mdh-m</i>	0.025	0.019	0.035	0.005	0.030	0.042	0.034	0.008	0.026	0.036	0.094
<i>Mdh-4</i>	0.017	0.009	0.023	0.004	0.018	0.022	0.023	0.005	0.018	0.022	0.024
<i>Mnr-4</i>	0.009	0.010	0.013	0.128*	0.140	0.142	0.011	0.104	0.114	0.116	0.197
<i>Pepca</i>	0.029	0.000	0.039	0.005	0.043	0.049	0.035	0.002	0.037	0.044	0.047
<i>6-Pgd-1</i>	0.012	0.005	0.012	0.071*	0.082	0.087	0.012	0.074	0.085	0.090	0.061
<i>6-Pgd-2</i>	0.011	0.013	0.017	0.092*	0.107	0.110	0.014	0.077	0.090	0.093	0.139
<i>6-Pgd-3</i>	0.054	0.005	0.068	0.038	0.104	0.107	0.063	0.038	0.099	0.102	0.123
<i>Pgi-2</i>	0.006	0.022	0.013	0.014	0.026	0.029	0.021	0.011	0.032	0.035	0.098
<i>Pgm-1</i>	0.010	0.005	0.014	0.003	0.011	0.015	0.009	0.003	0.012	0.015	0.023
<i>Pgm-2</i>	0.074	0.034	0.089	0.015	0.076	0.079	0.087	0.010	0.078	0.082	0.146
<i>Skdh-1</i>	0.005	0.000	0.002	0.030	0.032	0.035	0.003	0.035	0.038	0.041	0.036
<i>Skdh-2</i>	0.004	0.000	0.003	0.048*	0.051	0.054	0.004	0.037	0.041	0.044	0.086
Mean	0.044	0.011	0.050	0.051	0.099	0.103	0.048	0.048	0.095	0.099	0.096

*Most diagnostic loci (variation at these loci is mainly determined by allele frequency differences between *P. abies* and *P. obovata*).

of spatial differentiation seem to be the result of complex interaction of gene flow and selection. Distribution of genetic variability may be also affected by 'evolutionary footprints', such as, for example, spruce populations descendant from different refuges.

Genetic differentiation between Norway and Siberian spruces and their phylogenetic relationships

Genetic distances estimated between Norway and Siberian spruce were relatively small (Table 7). Nei's distance (1972) equalled 0.046–0.103 (average 0.072). Such levels of differentiation correspond to that between subspecies or geographical races. For example, the genetic distance between two closely related North American spruce species *P. glauca* and *P. sitchensis* averaged 0.121 (Yeh & Arnott, 1986).

Good crossability, high variation of morphological traits used as diagnostic features, existence of a wide zone of introgressive hybridization and the lack of species-specific diagnostic loci between Norway and Siberian spruce also suggest that they be considered as two subspecies or races (see also Lindquist, 1948; Schmidt-Vogt, 1974). The loci *Got-3*, *G6pdh-1*, *Mnr-4*, *6-Pgd-1*, *6-Pgd-2* and *Skdh-2* make the most significant contribution to interspecific differentiation of these spruces, but only 5 per cent of the whole Norway–Siberian spruce genetic variation results from interspecific differences (Table 6). According to δ -values, *Got-3*, *G6pdh-1*, *Lap-1*, *Lap-2*, *Mnr-4*, and *Pgm-2* are, on the whole, the most diverged loci in the Norway–Siberian spruce complex (Table 6).

A relatively low level of divergence between Norway and Siberian spruce can be explained by the existence

Table 7 Genetic distance between *Picea abies* and *P. obovata* populations and species

Measure of genetic distance	Species	Number of populations	<i>P. abies</i>	'Hybrid'	<i>P. obovata</i>
Nei (1978) unbiased	<i>Picea abies</i>	6	0.019 (0.005–0.049)		
	'Hybrid'	2	0.034 (0.011–0.084)	0.017 (0.017–0.017)	
	<i>P. obovata</i>	2	0.071 (0.044–0.102)	0.048 (0.033–0.061)	0.007 (0.007–0.007)
Nei (1972)	<i>P. abies</i>	6	0.020 (0.007–0.051)		
	'Hybrid'	2	0.035 (0.012–0.085)	0.019 (0.019–0.019)	
	<i>P. obovata</i>	2	0.072 (0.046–0.103)	0.049 (0.035–0.063)	0.008 (0.008–0.008)
Gregorius (1974)	<i>P. abies</i>	6	0.092 (0.061–0.151)		
	'Hybrid'	2	0.116 (0.077–0.181)	0.095 (0.095–0.095)	
	<i>P. obovata</i>	2	0.173 (0.140–0.198)	0.159 (0.134–0.180)	0.068 (0.068–0.068)
Cavalli-Sforza & Edwards (1967) chord distance	<i>P. abies</i>	6	0.140 (0.096–0.221)		
	'Hybrid'	2	0.162 (0.113–0.263)	0.136 (0.136–0.136)	
	<i>P. obovata</i>	2	0.235 (0.203–0.276)	0.207 (0.185–0.225)	0.114 (0.114–0.114)

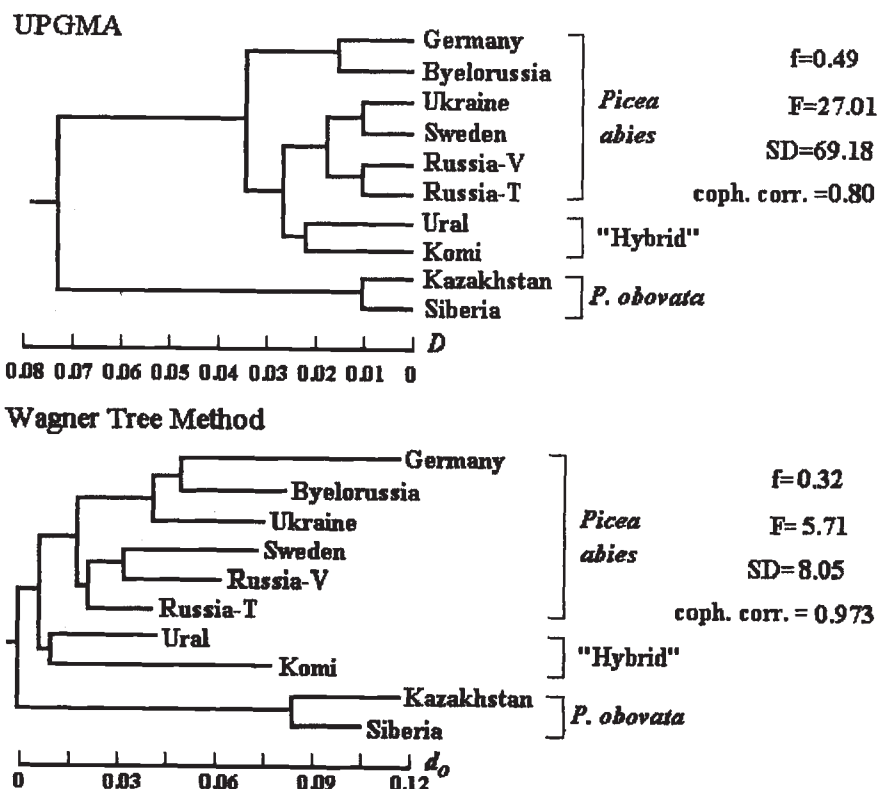
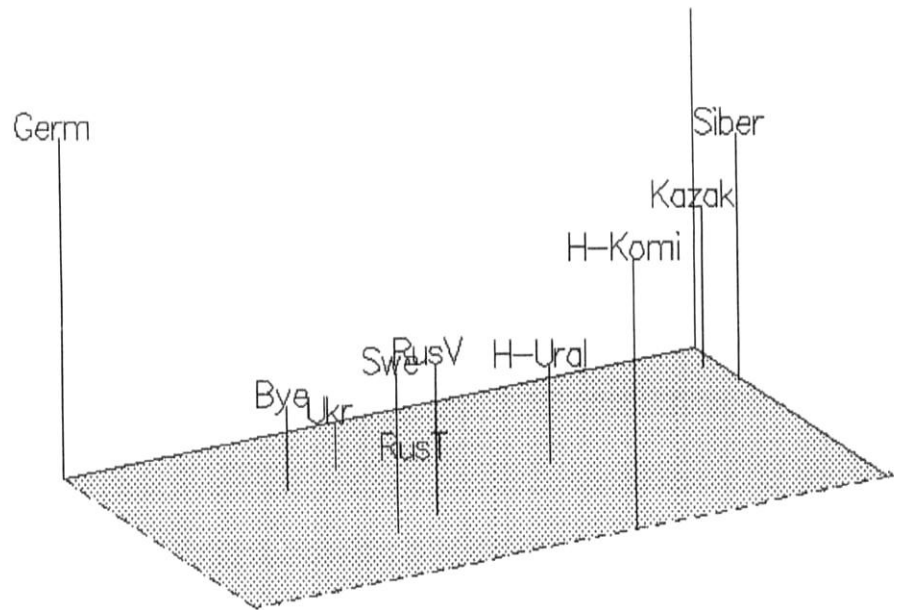


Fig. 1 UPGMA and Wagner Tree dendrograms of ten *Picea abies* and *P. obovata* populations based on 26 isozyme loci and D (Nei, 1972) and d_0 (Gregorius, 1974, 1978, 1984a) genetic distance matrices, respectively. Goodness of fit statistics: f , summarized absolute difference between patristic and genetic distances (Farris, 1972); F , congruence measure by Prager & Wilson (1976); SD , percent standard deviation (Prager & Wilson, 1976); $coph. corr.$, cophenetic correlation (Farris, 1972).

Fig. 2 Principal Coordinate Analysis using D (Nei, 1972) genetic distance matrix based on 26 isozyme loci allele frequencies of ten *P. abies* and *P. obovata* populations. 'Pure' *Picea abies*: **Germ**, Germany; **Bye**, Byelorussia; **Ukr**, Ukraine; **Swe**, Sweden; **RusV** and **RusT**, West-Central Russia. 'Hybrid' zone: **H-Ural**, Central Russia (Ural Mountains); **H-Komi**, North-Central Russia (North of Ural Mountains). 'Pure' *Picea obovata*: **Kazak**, Eastern Kazakhstan; **Siber**, Eastern Siberia (for more details see Table 1).



of several or many large common refuges of ancient spruce species which occupied a large area in Eurasia in the past. During glaciation, these large refuges could have conserved essential genetic variation and have thereby decelerated divergence and speciation processes. Subsequent reinvasion along with secondary contacts and hybridization between different populations could have prevented the establishment of new species if the duration of isolation was not sufficient to develop reproductive isolation mechanisms and genetic 'incompatibility'. Thus, the low level of divergence between Norway and Siberian spruce can be explained by the incomplete isolation within large refuges and the short duration of their existence. Nevertheless, a considerable amount of genetic difference accumulated during that time, because genetic distances between Norway and Siberian spruce are 3–4 times larger, on the average, than those between populations of the same species (Table 7) and are comparable with distances reported by other authors for subspecies or very closely related conifer species with introgressive hybridization (Dancik & Yeh, 1983; Wheeler *et al.*, 1983; Jacobs *et al.*, 1984; Conkle *et al.*, 1988; Millar *et al.*, 1988).

Introgressive hybridization between Norway and Siberian spruces

The genetic structure of 'hybrid' populations (Komi and Ural) is quite different from that of other populations. The 'hybrid' populations have significantly higher levels of genetic variation than populations from the most remote parts of the Norway and Siberian spruce area. It is interesting to note that 'hybrid' popu-

lations have 2–4 times more rare alleles and 4–10 times more unique alleles, than 'pure' *P. abies* and *P. obovata* populations (Table 9). This observation is well-known as the 'rare allele phenomenon' characteristic of interspecific hybrid zones (i.e. Barton *et al.*, 1983; Barton & Hewitt, 1985; Harrison, 1986; Cesaroni *et al.*, 1992). Woodruff (1989) even introduced the term 'hybrizyme' for these unexpected allozymes occurring within hybrid zones.

One possible explanation of their origin may be intragenic recombination which can create a 'new' genetic variant among the progeny from heterozygous parents. Thus, new variants are more likely to arise in the progeny of highly heterozygous populations. Hybridization between populations with different allele frequencies will obviously increase individual heterozygosity.

Another explanation is connected with the phenomenon of 'hybrid dysgenesis'. One of its characteristics is an increase in mutability among hybrid progeny obtained from mating between distantly isolated populations. Nuclear-cytoplasmic control of different mobile genetic elements in the genome of such hybrids can be disrupted, favouring insertion mutagenesis (Woodruff, 1989).

Certainly, both of the above-mentioned mechanisms could be responsible for the high frequency of unique alleles in the hybrid populations. More investigations are needed to clarify this phenomenon.

The genetic relationships between populations from the zone of introgressive hybridization and other spruce populations also prove the existence of wide hybridization. According to dendrograms and multivariate analysis based on genetic distances, 'hybrid'

Table 8 Correlation (r) between geographical and genetic distances among populations of *Picea abies* and *P. obovata*

Locus	'Pure' <i>Picea abies</i>						<i>Picea abies</i> , including two populations from 'hybrid' zone						All ten populations of <i>Picea abies</i> and <i>P. obovata</i>					
	D		d_0		P		D		d_0		P		D		d_0		P	
	r	t	r	t	r	t	r	t	r	t	r	t	r	t	r	t	r	t
<i>Aph-2</i>	0.46	1.47	0.929	0.43	1.32	0.907	0.52	2.30	0.989	0.43	1.79	0.963	0.57	3.00	0.999	0.63	3.34	0.999
<i>Fdh</i>	0.55	1.74	0.959	0.51	1.61	0.947	0.75	3.13	0.999	0.78	3.27	0.999	0.07	NS	NS	0.08	NS	NS
<i>G6pdh-1</i>	0.52	1.70	0.956	0.43	1.36	0.910	0.65	2.95	0.998	0.62	2.71	0.997	0.68	3.98	1.000	0.77	4.50	1.000
<i>Gdh</i>	0.62	2.05	0.980	0.57	1.93	0.973	0.67	3.16	0.999	0.59	2.75	0.997	0.17	NS	NS	0.15	NS	NS
<i>Got-3</i>	0.17	NS	NS	0.33	NS	NS	-0.18	NS	NS	-0.16	NS	NS	0.55	3.09	0.999	0.59	3.18	0.999
<i>Lap-1</i>	0.35	NS	NS	0.41	1.40	0.920	0.24	NS	NS	0.38	1.74	0.959	0.26	1.71	0.956	0.34	2.32	0.990
<i>Lap-2</i>	0.48	NS	NS	0.30	NS	NS	0.48	1.83	0.967	0.47	1.87	0.970	-0.01	NS	NS	-0.01	NS	NS
<i>Mdh-2</i>	in	in	in	in	in	in	in	in	in	0.63	2.67	0.996	0.00	NS	NS	-0.12	NS	NS
<i>Mdh-3</i>	-0.50	NS	NS	-0.34	NS	NS	-0.28	NS	NS	-0.01	NS	NS	0.45	2.25	0.988	0.44	2.26	0.988
<i>Mdr-4</i>	0.01	NS	NS	-0.05	NS	NS	0.43	2.03	0.979	0.48	2.28	0.989	0.29	1.82	0.965	0.71	3.83	1.000
<i>Pepca</i>	0.13	NS	NS	0.16	NS	NS	0.18	NS	NS	0.18	NS	NS	0.29	1.61	0.946	0.28	1.56	0.940
<i>6-Pgd-1</i>	-0.09	NS	NS	-0.08	NS	NS	-0.14	NS	NS	-0.03	NS	NS	0.54	2.77	0.997	0.58	2.98	0.999
<i>6-Pgd-2</i>	0.64	1.96	0.975	0.67	1.96	0.975	0.16	NS	NS	0.13	NS	NS	0.47	2.60	0.995	0.55	2.99	0.999
<i>6-Pgd-3</i>	0.36	1.29	0.902	0.24	NS	NS	0.47	2.13	0.983	0.41	1.93	0.973	0.52	2.93	0.998	0.56	3.21	0.999
<i>Pgt-2</i>	0.54	1.59	0.944	0.67	2.00	0.977	0.55	2.18	0.985	0.53	2.05	0.980	0.40	2.28	0.989	0.39	2.29	0.989
<i>Skdh-1</i>	-0.12	NS	NS	-0.04	NS	NS	0.08	NS	NS	0.06	NS	NS	0.50	2.74	0.997	0.53	2.92	0.998
<i>Skdh-2</i>	0.70	1.99	0.977	0.69	2.01	0.978	0.66	2.83	0.998	0.61	2.56	0.995	0.77	4.22	1.000	0.80	4.47	1.000
All 26 loci	0.62	2.00	0.977	0.62	1.92	0.973	0.78	3.38	1.000	0.75	3.23	1.000	0.86	4.88	1.000	0.81	4.55	1.000
r_p	0.62 ($P=0.013$)			0.62 ($P=0.014$)			0.78 ($P=0.000$)			0.75 ($P=0.000$)			0.86 ($P=0.000$)			0.81 ($P=0.000$)		
r_s	0.40 (NS)			0.43 (NS)			0.70 ($P<0.001$)			0.72 ($P<0.001$)			0.84 ($P<0.001$)			0.82 ($P<0.001$)		

D , comparison based on genetical distance by Nei (1972).

d_0 , comparison based on genetic distance by Gregorius (1974).

r , correlation between geographical and distance matrix (equalled normalized Mantel statistic Z).

t , approximate Mantel t -test for association between geographical and distance matrices.

r_p , Pearson's linear correlation.

r_s , Spearman's rank correlation.

P , probability that a random Z is less than the observed Z .

NS, nonsignificant value.

in, indeterminate value (genetic matrix insufficient for comparison).

Table 9 Frequencies and statistics of rare and private alleles among *Picea abies*, *P. obovata* and 'hybrid' populations

Population	Total number of studied alleles	Number of rare alleles	Frequency of rare alleles	Number of unique alleles	Frequency of unique alleles
<i>Picea abies</i>	18 964	225	0.01187	8	0.00042
<i>P. obovata</i>	6197	32	0.00516	1	0.00016
'Hybrid'	6095	131	0.02149	11	0.00181

Fisher's criterion statistics, <i>F</i>				
Compared populations	Rare alleles		Private or unique alleles	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
'Hybrid' vs. <i>Picea abies</i>	25.95	<0.001	9.34	<0.01
'Hybrid' vs. <i>P. obovata</i>	70.06	<0.001	10.70	<0.01
<i>Picea abies</i> vs. <i>P. obovata</i>	26.98	<0.001	0.92	Nonsignificant

populations always occupy intermediate positions between 'pure' Norway and Siberian spruce (Fig. 1 and 2).

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