

Monitoring the genetic variability in Norway spruce populations in Europe

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Abstract

Maintenance of biodiversity and protection of genetic resources has arisen as new target in the modern silviculture. As consequence, in the forest practices of several European countries special attention has been focused on seed sources. The variability of maternally inherited mtDNA can be associated to the amount of mother trees used for seed production.

Commercial samples of Norway spruce covering the entire natural area of this species were obtained by Seed Agencies and Forest Services. Genetic variability of mtDNA was investigated in 25 populations of Norway spruce (*Picea abies* Karst.) along 8 European countries: Switzerland, Italy, Czech Republic, Slovakia, Romania, Lithuania, Byelorussia and Sweden. The populations were characterised by a mitochondrial VNTRs primer set. This approach showed to be reliable, fast and easy to perform in order to analyse genetic biodiversity within a species and population.

The primer used gave a reliable and polymorphic pattern of amplification. The primer *nad-1* turned out to be the most informative in order to assess the range of variability within the populations. There were high differences of within-variability values of the populations ranging from 0.00 to 0.80. These values showed also a significant trend of variability among the countries that may be related to different management procedures for the seed collection. Seed collecting procedures are analysed and discussed.

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Introduction

Conservation of biological diversity and protection of genetic variability has arisen as new target in modern silviculture (Tewari, 1994). Biodiversity is important for many reasons and it has been defined and monitored at different levels: diversity between different ecosystems, between species inside the same ecosystem and between individuals inside the same species.

Monitoring biodiversity at each of these three levels is fundamental in order to give the natural environment the possibility for a future development. Research has showed that the more diverse an ecosystem the better it can withstand environmental stresses. This means that when a species is lost in an ecosystem, the ability of the ecosystem to face an environmental stress decreases. Furthermore, variation of quantitative traits is a fundamental prerequisite in establishing future heterogeneity: the processes of evolution and speciation, from which new species arise, are strictly correlated to the level of genetic variability among the individuals of the same population. While it is relatively easy and effective to monitor the variation of quantitative traits, the monitoring of distribution of genetic variability between and within populations require an higher effort.

Many conifer species are threatened by forestry activities in Europe. This has led to the development of silvicultural methods to meet the demand for maintaining biological diversity. Norway spruce is one of the most widespread distributed conifer species in Europe. There are differences in biodiversity of spruce forests between European countries depending on the history of land use: the amount of Norway spruce plantations is much higher in Central-European and Nordic countries than in Southern Europe.

The most prevalent system of forest cultivation across the world is "clear-felling with monocultures and replantation" (Faehser, 1997). This system of forest management operates under the directive of maximising production of the marketable product, and consequently it fails to acknowledge many of the biological realities facing forests. The technical measures that characterise this system include: felling all of the trees in a fairly large area; "reforestation" by artificially planting or sowing. In this context, the seed collection procedures are very important in order to maintain the biodiversity of forest trees, especially for those species, like Norway spruce, in which single mother trees can yield a luxuriant amount of seeds in the mast years. It is possible that the seed collection practices in many cases may significantly reduce the genetic diversity of this species.

In recent years molecular techniques have provided useful tools to monitor biodiversity quality of propagative materials in forestry. Mitochondrial DNA (mtDNA) has proved to be a useful source of DNA markers for the study of population genetics and biogeography. They have been used to elucidate population structure, migration routes, mating systems and reproductive behaviour (Lunt *et al.* 1998; Palmer *et al.* 2000). The development of mtDNA markers in plants has been hampered by the complex structure and changes in the gene order and organisation of the mitochondrial genome, also because of the frequent exchange of DNA material between the mitochondria and the nucleus (Palmer *et al.* 2000). These markers are maternally inherited, and thus less subject to gene flow and more informative regarding geographical population structure. In conifers mtDNA represents the sole source of DNA markers useful to track the effects of seed dispersion on population structure. These features of the plant mitochondrial

genome reduce the opportunities for wide cross-species transferability of primers designed to amplify more variable non-coding regions. However, several mtDNA markers specific for *Picea* spp. and other close conifers have been recently optimised (Grivet *et al.*, 1999; Mitton *et al.*, 2000; Sperisen, *et al.*, 2001; Jaramillo-Correa *et al.*, 2003).

This paper is a preliminary report showing that the current forest management practices in some cases may significantly altering the genetic structure and dynamics of Norway spruce in several European countries and how it is possible to effectively monitor these processes by means of molecular approaches.

Materials and Methods

Populations and sampling

Commercial samples of Norway spruce seeds were obtained by the National Forest Genetic Resources Centre of Canadian Forest Service, the Lattecaldo Swiss Forest Nursery and the Forest Service of Dogana di Peri, Italy. Twenty-five populations were sampled, coming from 8 European countries: Switzerland, Italy, Czech Republic, Slovakia, Romania, Lithuania, Byelorussia and Sweden and distributed over the whole natural range of the species. In Table 1 and Figure 1 the sites of sampling are shown.

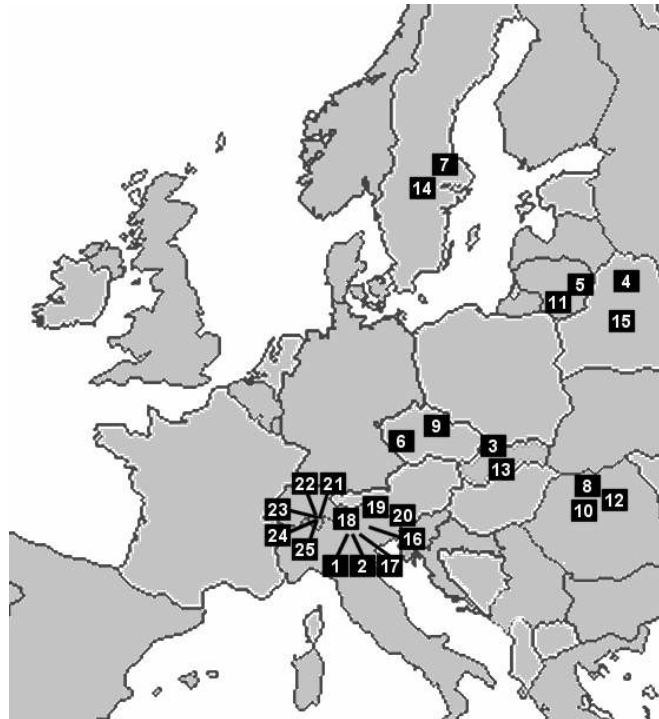


Figure 1. Geographical map of the 25 Norway spruce populations in Europe (site codes are defined in table 1).

Table 1. Seed sources and site details of 25 populations of Norway spruce.

Lot	Provenance	Country Code	Latitude	Longitude
1	Val di Fiemme	IT	46.18	11.44
2	Val di Fiemme	IT	46.17	11.27
3	Novot	SVK	49.26	19.18
4	Polotsk	BLR	55.30	28.45
5	Vilniaus	LTU	54.48	25.18
6	Boubin	CZE	48.58	13.47
7	Ivantjarn	SWE	60.48	16.45
8	Bistrita Nasaud	ROM	47.17	24.56
9	Nachod Opocno	CZE	50.23	16.16
10	Moldovita	ROM	47.45	25.44
11	Prienai	LTU	54.45	23.47
12	Suceava	ROM	45.45	26.00
13	Brezno	SVK	48.48	19.38
14	Sor Amsberg	SWE	60.31	14.09
15	Minsk	BLR	53.52	27.35
16	Latemar	IT	46.24	11.32
17	Val di Fiemme	IT	46.15	11.32
18	Valdidentro	IT	46.29	10.17
19	Brajes	IT	46.43	12.70
20	Comelico	IT	46.34	12.33
21	Motto Bartola	CH	46.26	08.59
22	Bosco Isola	CH	46.23	09.13
23	Fontana	CH	46.30	08.33
24	Pian Taioii	CH	46.16	08.29
25	Faura d'Isra	CH	46.30	08.42

Fifteen seeds for each of populations were analysed; therefore molecular analyses were carried out on a total of 375 individuals originating from the three macroareas usually distinguished within the natural distribution area of *Picea abies* (Schmidt-Vogt, 1978; Huntley and Birks, 1983): Baltic (Fennoscandia and European Russia), Carpathian (Central Europe and Carpathian Mountains) and Alpine (Alpine and Dinaric massifs) areas.

DNA extraction and microsatellite assay

DNA was extracted from seeds using a DNeasy Plant Mini Kit (Qiagen, Mississauga, Ontario) according to the manufacturer's instructions. DNA samples were subjected to Polymerase Chain Reaction (PCR) using a primer set for a mitochondrial locus *nad-1*, sequence:

F: CTCTCCCTCACCCATATGATG

R: ACAAAGCCCCTTTGAGGG

Ta=58°C, [Mg²⁺]=1.5 mM, polymorphism detection on SSCP (Sperisen, *et al.*, 2001). Reaction mixtures containing 20 ng of DNA, 0.2 µM of each primer, 0.4 mM of each dNTP, 0.1 units/µl of Taq DNA polymerase, 1X of supplied buffer reaction, and MgCl₂ adjusted at 1.5 mM were prepared. Amplification was carried out in a DNA thermal

cycler Biometra T-Gradient Thermoblock with the following program: an initial denaturation at 95 °C for 5 min, then 40 cycles of 1 min at 94 °C, 1 min 30 s at 57.9 °C, 2 min at 72 °C and a final elongation at 72 °C for 10 min. Eight microliters of the PCR products were electrophoresed on 8% SSCP polyacrylamide gel (in TBE). Gels were photographed under UV light.

Statistical analysis

Variability indexes (Nei & Li, 1979) and distance matrixes were calculated using Arlequin 2.0 and PHYLIP 3.5 packages. Cluster analysis (UPGMA) was carried out using Web-PHYLIP (<http://biocore.unl.edu/webphylip/>) software.

Results and Discussion

DNA amplification with the *nad-1* primers was successful for all of the 325 samples tested. Some examples of the denaturing polyacrilamide gel after electrophoresis of PCR products derived from *nad-1* locus are shown in Figure 2.

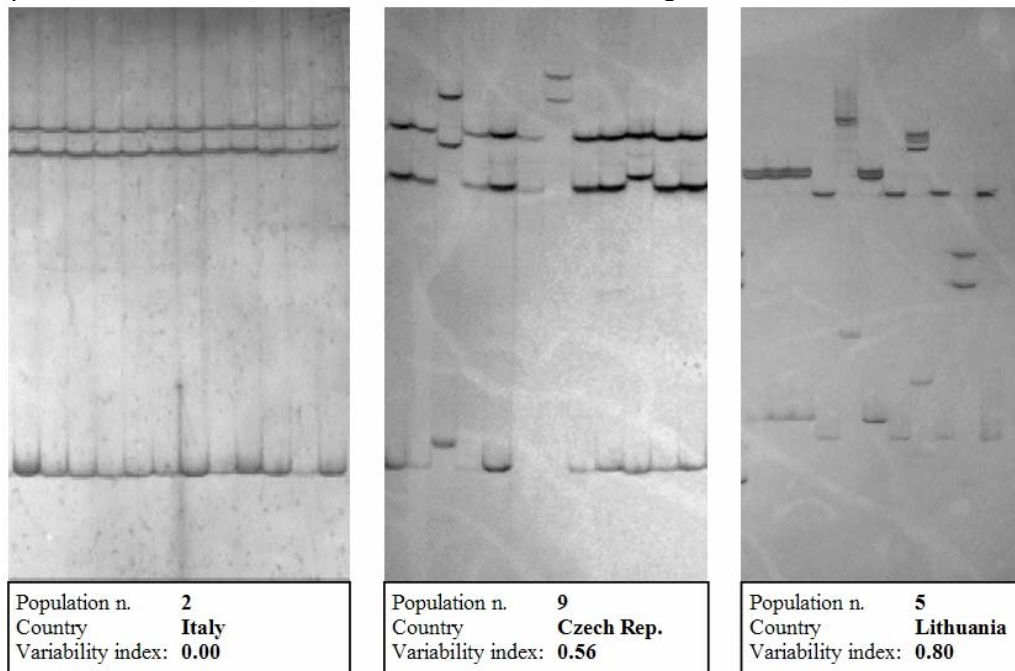


Figure 2. Example of different polymorphisms detected in three populations of *Picea abies*. Denaturing polyacrilamide gel after electrophoresis of PCR products derived from *nad-1* locus.

This figure clearly represents different patterns of polymorphism detected in three populations of Norway spruce: no. 2 (Italy, low variability), no. 9 (Czech Republic, medium variability) and no.5 (Lithuania, high variability). Easily scorable on polyacrilamide gels, this maternally inherited locus have proved to be useful for biodiversity monitoring studies in this tree species.

The detection of the amplified products and their analysis allowed to calculate and to compare the within-variability indexes (WVI) among the 25 populations and among the 8 European countries. The results are shown in Table 2. The WVIs were ranging from 0.00 to 0.80 (provenance no. 5). All five populations from Switzerland showed 0.00 values while some country, like Lithuania, showed 2 populations with very high values (0.80 and 0.67). The WVI averages calculated by country seems to indicate, to some extent, some homogeneity among the populations belonging to the same country: it is the case of Switzerland, Italy, Slovakia, Czech Republic, Byelorussia and Lithuania. The populations tested in Sweden (0.13 and 0.49) and in Romania (0.13, 0.53 and 0.55) showed higher differences within the country. These results may be related to different management procedures for seed collection in the different countries.

Table 2. Indexes of genetic variability within each population and each country calculated using Nei and Li (1987) method for all of the 25 populations of Norway spruce. The italic numbers in the "Country average" column represent the standard error of the averages.

Country	Lot Number	Within variability*	Country aver.
ITALY	1	0.13	0,09 ± 0,03
	2	0.00	
	16	0.00	
	17	0.13	
	18	0.00	
	19	0.25	
	20	0.13	
SLOVAK (SVK)	3	0.00	0,04 ± 0,04
	13	0.07	
BJELORUSSIA (BLR)	4	0.13	0,19 ± 0,06
	15	0.25	
LITHUANIA (LTU)	5	0.80	0,74 ± 0,07
	11	0.67	
CZECH REP. (CZE)	6	0.46	0,51 ± 0,05
	9	0.56	
SWEDEN (SWE)	7	0.13	0,31 ± 0,18
	14	0.49	
ROMANIA (ROM)	8	0.55	0,40 ± 0,14
	10	0.13	
	12	0.53	
SWITZERLAND (CH)	21	0.00	0,00 ± --
	22	0.00	
	23	0.00	
	24	0.00	
	25	0.00	

Based on the calculation of the variability coefficients matrix generated by UPGMA among the 25 populations of Norway spruce, using individual samples as operational taxonomic units (Figure 3), a cluster analysis was performed (Figure 4).

The structure of the phylogenetic trees showed a good association of groups with the same geographical origin and post-glacial recolonization pathway. One lineage corresponding to the Baltic populations and the other lineage to the Alpine and the majority of the Carpathian populations. Only one Carpathian population, the no. 6, is not joined to this second group because of its peculiar pattern.

This good consistency between phylogeny and geography is congruent with the variability analyses performed by using maternally inherited genetic markers in forest trees (Dumolin Lapègue *et al.*, 1997; Newton *et al.*, 1999) . The genetic variation at the locus *nad-1* can be considered to be phylogenetically informative.

Comparison of the genetic structure derived from mtDNA markers and the colonisation pathways previously deduced from the fossil and pollen records allow to infer at least two southern and one eastern glacial populations for Norway spruce. Since the last glacial period, the re-colonisation of Norway spruce in the European continent took origin from the two distinct more important refuges. This assumption is also supported by the clinal variation observed in the collected material mentioned above.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
1																											
2	0,07																										
3	0,07	0,00																									
4	0,88	0,93	0,93																								
5	0,89	0,90	0,90	0,81																							
6	0,94	0,93	0,93	1,00	0,80																						
7	0,91	0,97	0,97	0,13	0,80	0,99																					
8	0,40	0,37	0,37	0,82	0,89	0,93	0,84																				
9	0,38	0,33	0,33	0,96	0,89	0,84	0,88	0,57																			
10	0,13	0,07	0,07	0,91	0,90	0,94	0,94	0,39	0,38																		
11	0,84	0,87	0,87	0,49	0,80	0,89	0,48	0,83	0,88	0,86																	
12	0,55	0,53	0,53	0,72	0,90	0,97	0,74	0,56	0,69	0,53	0,60																
13	0,10	0,03	0,03	0,94	0,89	0,93	0,96	0,39	0,36	0,10	0,86	0,56															
14	0,73	0,76	0,76	0,40	0,80	0,95	0,39	0,75	0,83	0,75	0,56	0,72	0,75														
15	0,88	0,93	0,93	0,19	0,80	0,98	0,18	0,83	0,96	0,91	0,50	0,74	0,93	0,41													
16	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93												
17	0,13	0,07	0,07	0,94	0,89	0,89	0,97	0,41	0,37	0,13	0,87	0,56	0,10	0,77	0,94	0,07											
18	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93	0,00	0,07										
19	0,19	0,13	0,13	0,94	0,91	0,94	0,97	0,45	0,41	0,19	0,88	0,60	0,16	0,79	0,94	0,13	0,19	0,13									
20	0,13	0,07	0,07	0,94	0,91	0,94	0,97	0,41	0,38	0,13	0,88	0,56	0,10	0,77	0,94	0,07	0,13	0,07	0,19								
21	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93	0,00	0,07	0,00	0,13	0,07							
22	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93	0,00	0,07	0,00	0,13	0,07	0,00						
23	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93	0,00	0,07	0,00	0,13	0,07	0,00	0,00					
24	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93	0,00	0,07	0,00	0,13	0,07	0,00	0,00	0,00				
25	0,07	0,00	0,00	0,93	0,90	0,93	0,97	0,37	0,33	0,07	0,87	0,53	0,03	0,76	0,93	0,00	0,07	0,00	0,13	0,07	0,00	0,00	0,00	0,00			

Figure 3. Variability coefficient matrix generated by UPGMA among the 25 populations of *Picea abies* in Europe (site codes are defined in table 1).

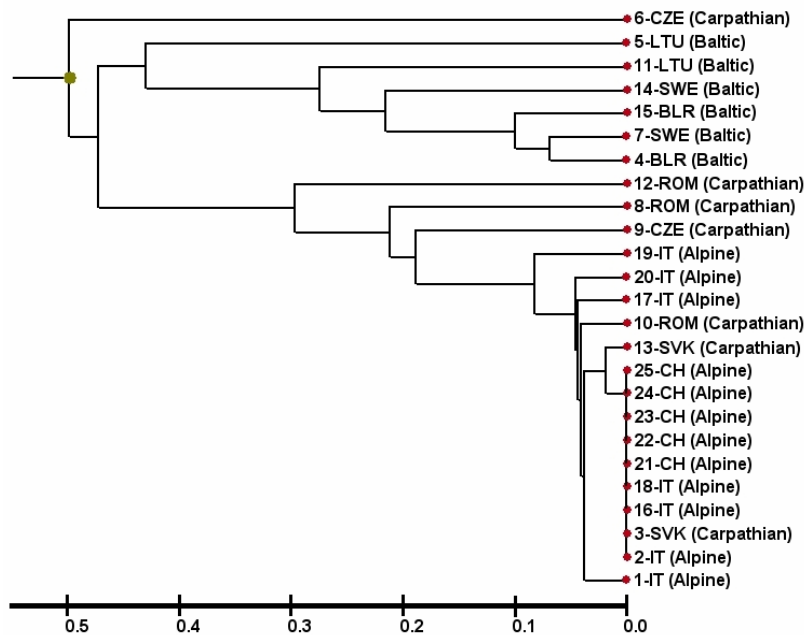


Figure 4. Cluster analysis of genetic relationships among the 25 Norway spruce natural populations studied in Europe. The phenogram is obtained from the UPGMA analysis of size variants detected at the minisatellite locus *nad-1*. Each singular population is distinguished by lot number and country code. In brackets the macroareas: Baltic, Carpathian, Alpine, represent different post-glacial recolonization pathways.

It is well known that enlargement and management of Norway spruce by humans took place during the last two centuries mainly by artificial plantation. This anthropogenic influence, still active, is going to create a genetic bottleneck effect in this species if appropriate practices are not going to be implemented and monitored by the forest managers. Especially the seed collection procedures should follow a severe criteria to protect and secure the genetic resources in all those countries where this species is cultivated and a particular monitoring control should be fulfilled on the seed quality to avoid the seed to be collected from one or only few mother trees (Figure 5).

Genetic diversity is of great significance to the survival of boreal forest tree species. Thus, the conservation of their genetic resources is of major importance, not only for national sustainable development strategies, but also for the conservation of the biodiversity that depends on these forests. The goals and methods to protect biodiversity are different in areas where forests are still undisturbed, in naturally regenerated managed forests, in forests influenced by other activities than forestry and in plantations established on agricultural land.

Key and indicator species and key habitats are commonly used in defining the protection value of forest. In conclusion, it clearly appears that the mitochondrial locus analysed in



Figure 5. Example of cone production of an adult tree of *Picea abies* in a mast year.

this study displays multiple minisatellite length variants well suited to analyse population genetics. In addition, the low level of variability detected in several European populations, despite the high population differentiation observed, confirm its utility for the monitoring the effectiveness of biodiversity conservation.

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