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## Mitochondrial and nuclear DNA differentiation of *Picea abies* populations in Poland

**Abstract:** The natural stands of Norway spruce in Poland are split between the southern and the northeastern parts of the country. Two so-called “spruceless” zones separate the northern spruce locations from those in the south, one “spruceless” zone is situated in Central Poland, and the other one in the Beskid Mts. Mitochondrial (STS) and nuclear (SSR) markers were used to perform the genetic identification of Norway spruce. Four different variants of haplotypes, “a”, “b”, “c” and “d”, were found to occur in the *nad1* locus of STS markers. Populations from the northern range of *Picea abies* distribution in Poland harboured exclusively haplotypes “c” and “d”, except for the Białowieża population which had haplotypes “a” and “c”. Populations from the “spruceless” zones contained four types of haplotypes whilst those from southern Poland were mostly composed of haplotype “a”. High mean gene diversity was observed for both STS and SSR markers ( $H_T = 0.529$ , and  $H_T = 0.851$ , respectively). The total genetic differentiation of Norway spruce populations was very low ( $F_{ST} = 0.088$ ). Two main groups of populations were distinguished in the dendrogram defined by Nei’s genetic distances based on microsatellite markers. The distribution of the genotypes was scattered and did not show any connection with the spatial distribution of *P. abies* in Poland. Only the mtDNA markers were able to differentiate the northern populations of Norway spruce from the southern ones, proving the historical separation between the Baltico-Nordic and the Hercyno-Carpathian ranges of *P. abies* in Poland. By contrast, the microsatellite data suggested an overlap between the genotypes due to the human manipulation of Norway spruce stands in the past.

**Additional key words:** Norway spruce, STS markers, microsatellite markers, post-glacial migration

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### Introduction

Many approaches to the conservation of species diversity, the exploration of plant-genetic resources, and the design of plant-improvement programmes require a detailed knowledge of the amount and distribution of the genetic diversity within a species. The genetic information contained in DNA, particularly in organelle DNA and microsatellite sequences, offers a valuable input when it comes to the *in situ* and *ex situ* conservation of forest genetic resources. Notwithstanding the intensive use and management of the species, little is

still known about the genetic variability of Norway spruce (*Picea abies* (L.) Karst.) stands in Europe.

Norway spruce is one of the most widely distributed coniferous species in northern, central, and eastern parts of the continent. It enjoys major economic relevance, especially in the Alps, Carpathian Mts., and the northern and eastern European countries. Three main centres of occurrence (Baltico-Nordic, Hercyno-Carpathian, and Alpine) have been distinguished within its natural range of distribution in Europe, and have derived from three putative ice-age refuges i.e. Central Russia and the Carpathian Mts.

(Modrzyński and Prus-Głowacki 1998; Collignon and Favre 2000; Ravazzi 2002).

*Picea abies* stands in Poland account for 6% of the total forest area (Forests in Poland 2006), and are split into two main areas: the southern range including the Tatra and Sudeten Mts., and the northeastern range located in the Masuria and Podlasie regions. Spruce populations from the south of Poland are included in the Carpathian range of Norway spruce distribution, whilst those from the northeast of the country belong to the Fennoscandia and European Russia range of *P. abies* (Matras 1996). In Central Poland and a small part of the Beskid Mts., two so-called "spruceless" zones separate the northern from the southern range of spruce in Europe (Środoń 1967).

The occurrence of the "spruceless" zones is probably due to human activity. The two ranges, i.e. the Baltico-Nordic and the Hercyno-Carpathian, probably overlapped this zone in the past. In fact, the present distribution of Norway spruce stands in Poland reflects historical events and socio-economic changes, e.g. increased demand for wood, deforestation, and reforestation (Boratyński 1998). Because of the high anthropogenic pressure in recent centuries, the genetic resources of Norway spruce in Poland have been impoverished. Moreover, both the massive monocultural planting of this species during the 19<sup>th</sup> and 20<sup>th</sup> centuries in Europe, and the transfer of genetic material across many European countries have broadly modified the natural gene pools in many *P. abies* stands (Orzeł et al. 1999; Puumalainen et al. 2003).

The issue of the present genetic structure of European Norway spruce stands remains still relevant, and molecular markers may be very helpful in investigating it. Between the many DNA markers available, mitochondrial loci and nuclear microsatellite sequences seem suitable for assessing the genetic variation among coniferous trees (Neale et al. 1992; Zhang and Hewitt 2003; Nowakowska et al. 2005).

Among the mitochondrial DNA markers, RFLP (*Restriction Fragment Length Polymorphism*) and STS (*Sequence Tagged Site*) markers are commonly used (Mitton et al. 2000; Soranzo et al. 2000). These kinds of markers are uniparentally inherited via the mother tree in conifers, and are often used to study maternal lineages and the post-glacial migration of *P. sylvestris* haplotypes across Europe (Newton et al. 1999).

The nuclear microsatellite DNA sequences have so far been considered as the most informative markers, and have been used in the genetic diversity studies of many organisms (Wang et al. 1994; Pemberton et al. 1995; Idury and Cardon 1997; Nei and Kumar 2000; Delmotte et al. 2001; Li et al. 2002; Ratkiewicz and Borkowska 2002; Nowakowska 2006).

The present study aimed to characterise the genetic structure of Polish populations of *Picea abies* by using mitochondrial markers and nuclear DNA markers. The

genetic differences were investigated on seventeen populations belonging to the natural range of Norway spruce distribution, and on three populations originating from two "spruceless" zones in Poland.

## Material and methods

### Sample collection

Twenty natural populations of Norway spruce (*Picea abies* (L.) Karst.) were sampled across Poland, and a total of 298 samples (needles) were harvested from 12 to 19 randomly spaced adult trees, aged 68–169 years, per population (Table 1). The populations were chosen according to the register of the Forest Research Institute and the General Directorate of the State Forests in Poland (Załęski 2005).

### Isolation of genomic DNA

Following collection, 100 mg of frozen needles were ground to fine powder in liquid nitrogen, and then DNA was extracted according to QIAGEN procedure using DNeasy 250 Plant Mini Kit. The quality of the DNA was checked by electrophoresis on a 1% agarose gel containing ethidium bromide ( $0.5 \mu\text{g ml}^{-1}$ ) in 1x TBE (45 mM Tris-Borate, pH 8.3, 1 mM EDTA). The amount of DNA in each sample was quantified by Gel Doc™ 2000 Imaging System (Bio-Rad) and by spectrophotometer NanoDrop® ND-1000 v. 3.2.

### Detection of mitochondrial DNA polymorphism

PCR amplifications were based on two primer sequences designed for the second intron of the *nad1* gene: F5'-CTCTCCCTCACCCATATGATG and R5'-ACAAAGCCCCTTTGAGGG (Sperisen et al. 2001).

The PCR amplifications were carried out in 25  $\mu\text{l}$  of mix (QIAGEN) containing 50 ng of DNA template, 2 mM  $\text{MgCl}_2$ , 1  $\mu\text{M}$  of primer "F", 1  $\mu\text{M}$  of primer "R", 200  $\mu\text{M}$  of dNTPs, and 0.5 U of Taq DNA polymerase. A PTC-200™ Programmable Thermal Controller (MJ Research, Inc.) was programmed for initial 3 min at 95°C and 26 cycles of PCR reactions: 1 min at 94°C of denaturation, 1 min at 57°C of annealing, 2 min at 72°C of extension, and 8 min at 72°C of final elongation (Sperisen et al. 2001). The PCR products were diluted 1:5 with fluorescent dye YOYO-3 (Molecular Probes) before loading to 3% acrylamide/bis-acrylamide (30:1) gel in an ALFexpress II automatic sequencer (Amersham Pharmacia Biotech). An external size marker (ALFexpress™ Sizer™ 50–500) and two internal size markers (ALFexpress™ Sizer™ 200, and 300) were used according to the manufacturer protocol. All fragments were analysed using ALFwin Fragment Analyser™ 1.0 software (Amersham Pharmacia Biotech). Some alleles of unexpected size were checked via direct sequencing.

Table 1. Description of Norway spruce populations studied

| Population      | Latitude / Longitude      | Provenance region* | Number of sampled trees | Mean age (years) |
|-----------------|---------------------------|--------------------|-------------------------|------------------|
| Białowieża      | 53°16'45" N / 23°39'39" E | 208                | 15                      | 136              |
| Borki           | 54°07' N / 21°55' E       | 202                | 15                      | 117              |
| Bystrzyca Kł.   | 50°28'51" N / 16°37'9" E  | 702                | 17                      | 133              |
| Gołdap          | 54°19' N / 22°42' E       | 203                | 15                      | 123              |
| Jeleśnia        | 49°53' N / 19°33' E       | 853                | 12                      | na               |
| Kamienna G.     | 50°50' N / 16°04' E       | 701                | 15                      | 153              |
| Karnieszewice   | 54°22'3" N / 16°40'14" E  | 152                | 15                      | 108              |
| Międzylesie     | 50°21' N / 16°56' E       | 703                | 15                      | 167              |
| Myślenice       | 49°55'45" N / 19°37'28" E | 851                | 15                      | 103              |
| Nowe Ramuki     | 53°41' N / 20°34' E       | 205                | 15                      | 169              |
| Nowy Targ       | 49°53'32" N / 19°30'3" E  | 805                | 15                      | 113              |
| Piwniczna       | 49°50'56" N / 20°29'56" E | 803                | 15                      | 88               |
| Płońsk          | 52°32' N / 20°31' E       | 451                | 14                      | 98               |
| Suchedniów      | 51°26'27" N / 20°42'2" E  | 604                | 19                      | 68               |
| Suwałki         | 54°32' N / 22°54' E       | 204                | 15                      | 116              |
| Tarnawa         | 49°37'55" N / 22°34'41" E | 807                | 15                      | 106              |
| Ujsoły          | 49°43'52" N / 19°9'51" E  | 808                | 15                      | 138              |
| Węgierska Górka | 49°53'32" N / 19°2'57" E  | 801                | 15                      | 118              |
| Wisła           | 49°49'51" N / 18°51'48" E | 802                | 15                      | 113              |
| Zwierzyniec L.  | 50°28' N / 22°50' E       | 606                | 13                      | na               |

\*According to Polish regionalisation of forest plant material (Załęski 2005)

na – not available

## Detection of nuclear DNA polymorphism

PCR amplifications were based on three primers designed for three microsatellite nuclear loci: SpAG-D1, SpA-G2, and SpAC1-H8, according to Pfeiffer et al. (1997) and Yazdani et al. (2003). The PCR amplifications were carried out in 25  $\mu$ l of mix (QIAGEN) containing 50 ng of DNA template, 2 mM MgCl<sub>2</sub>, 0.2  $\mu$ M of two primers labelled with Cy-5, 200  $\mu$ M of dNTPs, and 0.75 U of Taq DNA polymerase. The PCR reaction consisted of 5 min at 95°C of initial incubation, and 40 cycles of 45 sec denaturation at 94°C, 45 sec of annealing at appropriate T<sub>m</sub> (i.e. 57, 53, and 60°C for SpAG-D1, SpA-G2, and SpAC1-H8, respectively), 45 sec of extension at 72°C, followed by 10 min of final elongation at 72°C on a PTC-200™ Programmable Thermal Controller (MJ Research, Inc.). The PCR products were examined on an ALFexpress II automated sequencer (Amersham Pharmacia Biotech) using 8% ReproGel High Resolution gels in 0.5  $\times$  TBE at 1500 V. An external size marker (ALFexpress™ Sizer™ 50–500) and two internal size markers (ALFexpress™ Sizer™ 200 and 300) were used according to the manufacturer protocol.

## Data analysis

Both the mitochondrial and nuclear fragment sizes were estimated using ALFwin Fragment Analyser™

1.0 software (Amersham Pharmacia Biotech). The heterozygosity level within a single population ( $h$ ), genetic differentiation within all provenances ( $H_T$ ), mean differentiation between populations ( $G_{ST}$ ), and allele frequencies were calculated according to Nei (1987) using GenePop v. 3.2a (Raymond and Rousset 1995) and PopGene v. 1.31 (Yeh et al. 1999) software.

## Results and discussion

### Genetic structure based on mitochondrial markers

As far as STS markers were concerned, four kinds of haplotypes, i.e. "a", "b", "c" and "d", were distinguished in the *nad1* locus of Norway spruce (Fig. 1). The haplotype "a" consisted in fragment of 815 base-pair size (bp), haplotype "b" – 842 bp, haplotype "c" – 721 bp and haplotype "d" – 778 bp, previously observed by Sperisen et al. (2001).

The distribution of various haplotypes broadly followed the delimitation of the northern and the southern range of Norway spruce distribution in Poland. Precisely, the haplotype "a" predominated in populations from the southern range of spruce distribution and from the "spruceless" zones, with frequencies of 85.0% and 59.1%, respectively (Table 2, Fig. 1). Interestingly, only one population – Białowieża from

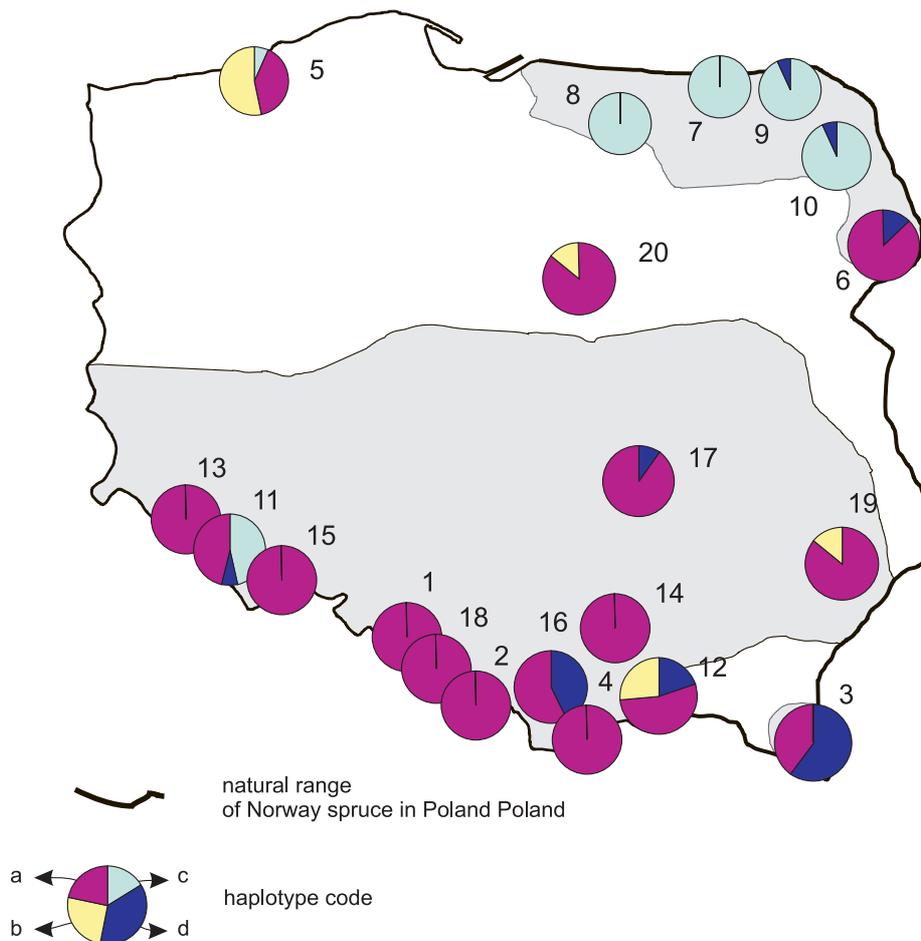


Fig. 1. Geographical distribution of four haplotypes “a”, “b”, “c” and “d” among Norway spruce populations studied

the northern range of *Picea abies* in Poland, also showed a high frequency 86.7% of haplotype “a” (Table 2). The haplotype “c” was predominant (frequency of 77.3%) in the northern part of Poland. A high proportion (46.7%) of haplotype “c” was also found in one population, Bystrzyca Kł., from the southern range of spruce distribution in Poland, whilst some little proportion (2.3%) of haplotype “c” characterised the “spruceless” zones (Table 2, Fig. 1). The haplotype “b” was mainly present (frequency 31.8%) in the “spruceless” zones of Poland, and the haplotype “d” was found in stands from the northern (5.3%), the southern (10.0%) range of spruce distribution, and in one population (20%, Piwniczna) of the “spruceless” zone from the lowland of Poland (Fig. 1). Ten populations exhibited no genetic variation ( $h = 0.000$ ) because only one, “a” or “c”, haplotype variant was present.

According to Sperisen et al. in 2001, haplotypes “a” and “b” are mainly found in the southern range of *P. abies* distribution in Europe, i.e. in the Alps and the Carpathians. Haplotype “c” is present in the Baltic countries in Europe, whilst haplotype “d” occurs in the southern range of Norway spruce in Europe.

The overall level of haplotypic diversity among the Polish spruce population studied was quite high,  $G_{ST} = 0.529$  (Table 2). Similar data on the genetic differentiation in the mitochondrial *nad1* locus ( $G_{ST} = 0.676$ , and  $G_{ST} = 0.410$ ) were reported for other European populations of Norway spruce (Gugerli et al. 2001; Sperisen et al. 2001).

The greatest gene differentiation among stands,  $H_T = 0.544$ , was measured in the “spruceless” zones, compared to  $H_T = 0.369$  for populations from the northern part of Poland, and  $H_T = 0.266$  for its southern part (Table 2).

STS markers have been successfully used to study the haplotypic variation in different *Pinaceae*, e.g. *Picea mariana*, *P. glauca*, *P. sitchensis* (Perry and Bousquet 1998; Perron et al. 2000; Fournier et al. 2002; Jaramillo-Correa et al. 2003), and in *Pinus sylvestris* (Soranzo et al. 2000). These markers revealed considerable mtDNA variation in coniferous populations, and were very helpful in studying genetic similarity. Due to the maternal inheritance of mitochondrial markers in conifers, the provenance of each population can be monitored.

In the present study, the different *nad1* haplotype variants were grouped into two main lineages which

Table 2. Overall mitochondrial haplotype frequencies of *nad1* gene in Norway spruce populations in Poland

| Population         | Haplotype frequency |       |       |       | Genetic diversity ( $h$ Nei) |
|--------------------|---------------------|-------|-------|-------|------------------------------|
|                    | a                   | b     | c     | d     |                              |
| Northeast          |                     |       |       |       |                              |
| Białowieża         | 0.867               | –     | –     | 0.133 | 0.231                        |
| Borki              | –                   | –     | 1.000 | –     | 0.000                        |
| Gołdap             | –                   | –     | 0.933 | 0.067 | 0.124                        |
| Nowe Ramuki        | –                   | –     | 1.000 | –     | 0.000                        |
| Suwałki            | –                   | –     | 0.933 | 0.067 | 0.124                        |
| Total              | 0.173               | –     | 0.773 | 0.053 | $H_T = 0.369$                |
| “Spruceless” zones |                     |       |       |       |                              |
| Karnieszewice      | 0.400               | 0.533 | 0.067 | –     | 0.551                        |
| Piwniczna          | 0.533               | 0.267 | –     | 0.200 | 0.604                        |
| Płońsk             | 0.857               | 0.143 | –     | –     | 0.245                        |
| Total              | 0.591               | 0.318 | 0.023 | 0.068 | $H_T = 0.544$                |
| South              |                     |       |       |       |                              |
| Bystrzyca Kł.      | 0.467               | –     | 0.467 | 0.067 | 0.560                        |
| Jeleśnia           | 1.000               | –     | –     | –     | 0.000                        |
| Kamienna G.        | 1.000               | –     | –     | –     | 0.000                        |
| Międzylesie        | 1.000               | –     | –     | –     | 0.000                        |
| Myślenice          | 1.000               | –     | –     | –     | 0.000                        |
| Nowy Targ          | 0.571               | –     | –     | 0.428 | 0.489                        |
| Suchedniów         | 0.900               | –     | –     | 0.100 | 0.180                        |
| Tarnawa            | 0.400               | –     | –     | 0.600 | 0.480                        |
| Ujsoły             | 1.000               | –     | –     | –     | 0.000                        |
| Wiśla              | 1.000               | –     | –     | –     | 0.000                        |
| Węgierska Górka    | 1.000               | –     | –     | –     | 0.000                        |
| Zwierzyniec L.     | 0.857               | 0.143 | –     | –     | 0.245                        |
| Total              | 0.850               | 0.011 | 0.039 | 0.100 | $H_T = 0.266$                |
| All populations    | 0.642               | 0.053 | 0.221 | 0.084 | $G_{ST} = 0.529$             |

separated the Norway spruce populations of North-eastern and Central Europe from those of Southeastern Europe. The clear spatial distribution of mitochondrial haplotypes in Europe was previously suggested by Sperisen et al. (2001). Chloroplast microsatellite markers, which are paternally inherited in Norway spruce, confirmed the separation between two genetic zones: southern and northern, and two major gene pools, i.e. Alpine-Central European and Baltic, were distinguished using cpSSR markers (Bucci and Vendramin 2000; Vendramin et al. 2000).

The present results confirmed the distinctiveness of the two ranges of Norway spruce distribution in Poland as far as the mitochondrial haplotypes of the intron 2 of *nad1* locus are concerned. However, the location of the suture zone between the two ranges of spruce distribution remains unclear, and probably more populations need to be examined from the lowland of Poland. A high degree of mtDNA variation in all populations from the “spruceless” zones in Poland may confirm that this region represents a contact zone of re-colonisation pathways after glaciation.

### Genetic structure based on nuclear microsatellite markers

Among 298 individuals, 123 different variants of microsatellite alleles were detected, with the fragment size being from 57 to 253 base-pairs. For all the tested loci, i.e. SpAG-D1, SpA-G2 and SpAC1-H8, high levels of expected heterozygosity ( $H_e = 0.934$ ), observed heterozygosity ( $H_o = 0.729$ ), and intra-population diversity ( $H_T = 0.852$ ) were noted (Table 3). For all loci, the departure from the Hardy-Weinberg equilibrium was observed with the probability lower than 1% (Table 3).

Regardless of the provenance region, the populations studied exhibited a similar level of intra-population gene diversity ( $H_S = 0.925$ ,  $H_S = 0.928$ , and  $H_S = 0.922$ ), and exactly the same inter-population gene diversity,  $H_T = 0.851$  (Table 4).

Among the spruce populations from northeastern Poland, Gołdap was the most differentiated ( $h = 0.901$ ), and Suwałki was the least differentiated ( $h = 0.791$ ; Table 4). In the southern “spruceless”

Table 3. Level of genetic diversity and deviation from Hardy-Weinberg equilibrium (HWE) of observed genotypes assessed with microsatellite loci

| Population      | N   | SpAG-D1        |                | SpA-G2         |                | SpAC1-H8       |                | Mean           |                |                |
|-----------------|-----|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                 |     | A <sub>0</sub> | H <sub>e</sub> | A <sub>0</sub> | H <sub>e</sub> | A <sub>0</sub> | H <sub>e</sub> | H <sub>o</sub> | H <sub>e</sub> | H <sub>o</sub> |
| Białowieża      | 15  | 30             | 0.947**        | 30             | 0.825ns        | 30             | 0.942ns        | 0.866ns        | 0.905          | 0.822          |
| Borki           | 15  | 30             | 0.926**        | 30             | 0.885*         | 30             | 0.763ns        | 0.866ns        | 0.858          | 0.666          |
| Bystrzyca Kł.   | 15  | 30             | 0.947ns        | 30             | 0.917**        | 28             | 0.952*         | 0.714*         | 0.939          | 0.660          |
| Goldap          | 15  | 30             | 0.956**        | 30             | 0.899**        | 30             | 0.942**        | 0.600**        | 0.932          | 0.666          |
| Jeleśnia        | 12  | 24             | 0.927ns        | 24             | 0.840ns        | 24             | 0.913*         | 0.916*         | 0.893          | 0.833          |
| Kamienna G.     | 14  | 28             | 0.977**        | 28             | 0.928ns        | 28             | 0.871ns        | 0.933ns        | 0.925          | 0.889          |
| Karnieszewice   | 15  | 30             | 0.924ns        | 30             | 0.853**        | 28             | 0.944**        | 0.571**        | 0.907          | 0.546          |
| Międzylesie     | 15  | 30             | 0.846ns        | 30             | 0.882ns        | 30             | 0.945ns        | 0.800ns        | 0.891          | 0.866          |
| Mysłenice       | 15  | 30             | 0.903**        | 30             | 0.857ns        | 30             | 0.931**        | 0.600**        | 0.897          | 0.689          |
| Nowe Ramuki     | 15  | 30             | 0.848ns        | 30             | 0.804**        | 30             | 0.908ns        | 0.933ns        | 0.853          | 0.755          |
| Nowy Targ       | 15  | 28             | 0.949*         | 30             | 0.915ns        | 30             | 0.942ns        | 0.866ns        | 0.935          | 0.885          |
| Piwniczna       | 15  | 30             | 0.961**        | 30             | 0.919ns        | 28             | 0.965**        | 0.643**        | 0.948          | 0.747          |
| Płońsk          | 14  | 28             | 0.915**        | 28             | 0.910ns        | 24             | 0.927ns        | 0.666ns        | 0.917          | 0.674          |
| Suchedniów      | 20  | 40             | 0.957**        | 40             | 0.929*         | 40             | 0.731**        | 0.700**        | 0.872          | 0.700          |
| Suwałki         | 15  | 30             | 0.970**        | 30             | 0.924ns        | 30             | 0.561ns        | 0.733ns        | 0.818          | 0.689          |
| Tarnawa         | 15  | 30             | 0.825**        | 30             | 0.878ns        | 30             | 0.791**        | 0.533**        | 0.831          | 0.822          |
| Ujsoly          | 15  | 30             | 0.947**        | 30             | 0.912ns        | 30             | 0.687*         | 0.533*         | 0.849          | 0.600          |
| Węgierska G.    | 15  | 28             | 0.934ns        | 30             | 0.905ns        | 30             | 0.581ns        | 0.600ns        | 0.807          | 0.728          |
| Wisła           | 15  | 30             | 0.869ns        | 30             | 0.931ns        | 30             | 0.503ns        | 0.533ns        | 0.767          | 0.755          |
| Zwierzyniec L.  | 13  | 24             | 0.909**        | 26             | 0.904ns        | 24             | 0.840**        | 0.500**        | 0.885          | 0.585          |
| All populations | 298 | 29.6           | 0.958**        | 29.9           | 0.920**        | 29.3           | 0.924**        | 0.706**        | 0.934          | 0.729          |

N – number of sampled trees, A<sub>0</sub> – observed number of alleles, H<sub>e</sub> and H<sub>o</sub> – expected and observed heterozygosities, H<sub>T</sub> – genetic variation in all populations  
For each population the departure from HWE is shown; ns – nonsignificant, \*p < 0.05, \*\*p < 0.01

**H<sub>T</sub> = 0.852**

Table 4. Genetic parameters of Norway Spruce populations according to microsatellite markers

| Region             | Population    | Provenance region | $A_0$ | $n_e$  | $I$           | $h$              |
|--------------------|---------------|-------------------|-------|--------|---------------|------------------|
| Northeast          | Białowieża    | 208               | 30    | 9.345  | 2.326         | 0.874            |
|                    | Borki         | 202               | 30    | 6.770  | 2.118         | 0.829            |
|                    | Goldap        | 203               | 30    | 10.704 | 2.497         | 0.901            |
|                    | Nowe Ramuki   | 205               | 30    | 6.079  | 2.015         | 0.825            |
|                    | Suwałki       | 204               | 30    | 9.210  | 2.063         | 0.791            |
|                    | Total         |                   | 30    | 14.546 | 2.900         | $H_T = 0.851$    |
|                    |               |                   |       |        | $H_S = 0.925$ | $F_{ST} = 0.087$ |
| "Spruceless" zones | Karnieszewice | 152               | 29    | 8.757  | 2.311         | 0.876            |
|                    | Piwniczna     | 803               | 29    | 12.527 | 2.626         | 0.916            |
|                    | Płońsk        | 451               | 27    | 8.562  | 2.333         | 0.883            |
|                    | Total         |                   | 28    | 1.466  | 16.175        | $H_T = 0.851$    |
|                    |               |                   |       |        | $H_S = 0.928$ | $F_{ST} = 0.039$ |
| South              | Bystrzyca Kł. | 702               | 29    | 10.972 | 2.512         | 0.907            |
|                    | Jeleśnia      | 853               | 24    | 7.381  | 2.226         | 0.856            |
|                    | Kamienna Góra | 701               | 30    | 11.373 | 2.556         | 0.895            |
|                    | Międzyzylesie | 703               | 30    | 7.948  | 2.276         | 0.861            |
|                    | Myślenice     | 851               | 30    | 7.913  | 2.284         | 0.867            |
|                    | Nowy Targ     | 805               | 29    | 10.594 | 2.496         | 0.904            |
|                    | Suchedniów    | 604               | 40    | 9.746  | 2.242         | 0.851            |
|                    | Tarnawa       | 807               | 30    | 5.269  | 1.832         | 0.803            |
|                    | Ujsoły        | 808               | 30    | 7.771  | 1.990         | 0.820            |
|                    | Węgierska G.  | 801               | 29    | 6.790  | 1.964         | 0.779            |
|                    | Wisła         | 802               | 30    | 6.066  | 1.891         | 0.742            |
| Zwierzyniec L.     | 605           | 25                | 6.869 | 2.067  | 0.849         |                  |
|                    | Total         |                   | 94.9  | 14.262 | 2.955         | $H_T = 0.851$    |
|                    |               |                   |       |        | $H_S = 0.922$ | $F_{ST} = 0.085$ |
| All populations    |               |                   | 97.0  | 16.165 | 3.074         | $H_T = 0.851$    |
|                    |               |                   |       |        | $H_S = 0.933$ | $F_{ST} = 0.088$ |

$A_0$  – observed number of alleles,  $n_e$  – effective number of alleles,  $I$  – Shannon index (Lewontin 1972)  
 $h$  – mean heterozygosity level,  $H_S$  – mean gene diversity within populations

zone, the Piwniczna population was the richest in heterozygotes ( $h = 0.916$ ), and the Karnieszewice population was the least rich ( $h = 0.876$ ). Among the spruce stands from southern Poland, Bystrzyca Kł. and Nowy Targ were the most differentiated ( $h = 0.907$  and  $h = 0.904$ , respectively), whilst Węgierska G. and Wisła were the least differentiated ( $h = 0.779$  and  $h = 0.742$ , respectively; Table 4).

The highest Shannon index of gene variation ( $I = 16.175$ ) was found in three populations of the "spruceless" zones in Poland, whereas the lowest values of this index were calculated for the populations from the northeast ( $I = 2.900$ ) and the south ( $I = 2.955$ ) of Poland (Table 4).

The total level of genetic differentiation between the stands was very low ( $F_{ST} = 0.088$ ). The values for the northeast ( $F_{ST} = 0.087$ ) and the south ( $F_{ST} = 0.085$ ) of Poland were comparable (Table 4). The much lower genetic differentiation among

stands from the "spruceless" zones ( $F_{ST} = 0.039$ ) may be attributed to the small number of the populations studied.

The  $G_{ST}$  value reported from an analysis of European spruce populations made using nuclear isozyme loci was also low, and  $F_{ST} = 0.053$  (Lagercrantz and Ryman 1990). Isozyme studies performed on Polish populations of *P. abies* revealed their low genetic differentiation, and yielded  $F_{ST} = 0.028$  (Lewandowski and Burczyk 2002) and  $G_{ST} = 0.063$  (Modrzyński and Prus-Głowacki 1998).

Two main groups of populations were distinguished in the dendrogram drawn according to the genetic distances of Nei (1978) based on nuclear microsatellite markers data (Fig. 2). The distribution of these genotypes was scattered and did not show any affiliation to either range of *P. abies* distribution in Poland (Fig. 3). However, two main gene pools of Norway spruce (Alpine-Central European and Baltic)

deriving from different glacial refugia in Europe were detectable by means of chloroplast microsatellite markers (Vendramin et al. 2000).

The dispersed distribution of different nuclear SSR genotypes across Poland, resulting in the low degree of nuclear variation among Norway spruce populations, suggests that historical events had an impact on the species distribution, causing its fragmentation in the past. During the 18<sup>th</sup> and 19<sup>th</sup> centuries, the extensive exploitation of natural forest stands in lowland Central Europe significantly reduced the stands

of Norway spruce. Additionally, the artificial reforestation with Norway spruce in the past strongly influenced the present genetic structure of the species on the continent (Bradshaw 2004; Maghuly et al. 2006; Nowakowska et al. 2006).

In contrast to the results obtained by Gugerli et al. (2001) for Alpine populations of *P. abies*, no correlations between the genetic differentiation determined using both STS and SSR markers and the phenotypic data (mean diameter at breast height, and mean height of trees in the stand) or environmental fea-

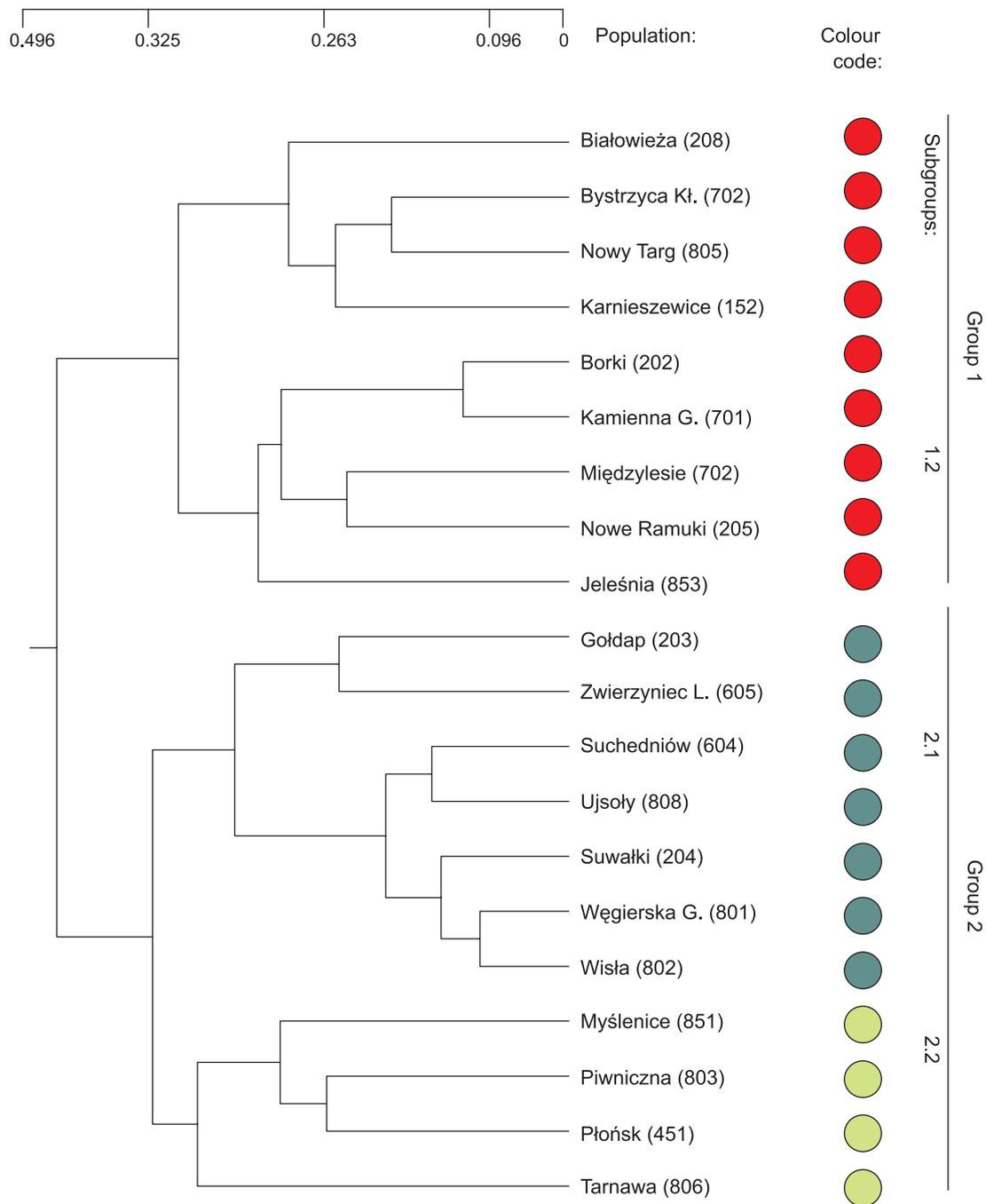


Fig. 2. Dendrogram of genetic distances between twenty Norway spruce populations of Poland. See Table 1 for provenance region numbers (in brackets)

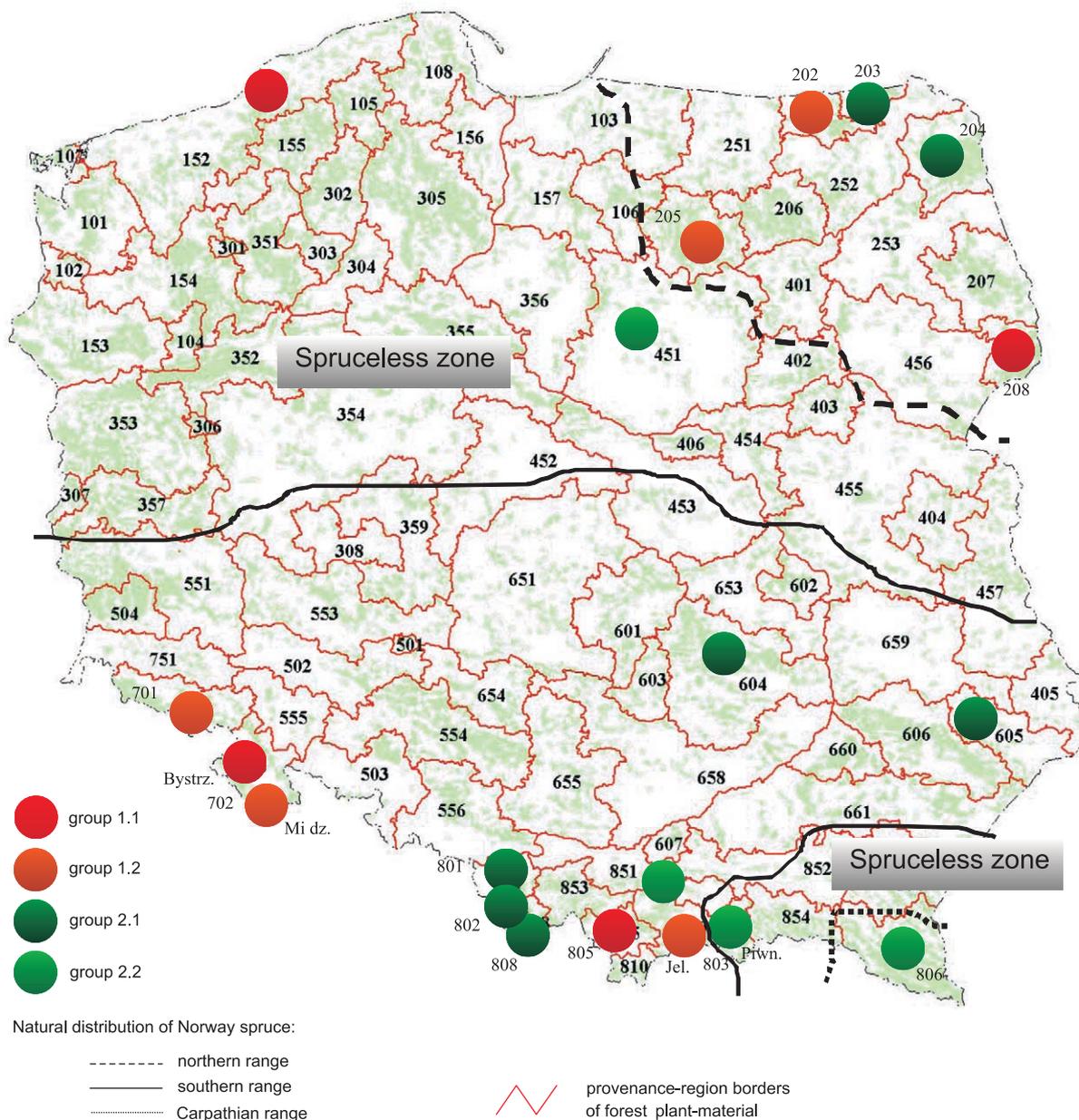


Fig. 3. Geographical distribution of genetically-related populations of Norway spruce according to dendrogram of genetic distances by Nei (1978). See Fig. 2 for colour codes

tures (mean altitude, mean annual precipitation, and mean length of the vegetation period) were found in Polish populations (data not shown).

To sum up, the mtDNA markers could only differentiate the northern populations of Norway spruce from the southern ones, indicating some historical separation between the Baltico-Nordic and the Hercyno-Carpathian ranges of *P. abies* occurring in Poland. By contrast, the microsatellite data suggest that there is an overlap between the genotypes as a consequence of the human impact made on the Norway spruce stands in the past.

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