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Genetic diversity of *Betula nana* in Sweden and conservation implications for protection of relict Polish populations

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Abstract

The natural range of the dwarf birch (*Betula nana* L.) includes the boreal, subarctic and arctic regions of Europe, Asia and North America, where it is relatively common. In Poland, it is a relict species occurring in fragmented populations. Using the random amplification of polymorphic DNA (RAPD) technique, we investigated the genetic diversity of the four Swedish populations representing a part of the continuous range of dwarf birch. With the knowledge of the level of genetic diversity of a population from a continuous distribution, we can assess the genetic status of polish populations and answer the question if habitat fragmentation and a decrease in population size lead to a loss in genetic diversity. Knowledge of genetic diversity is important for species conservation, especially to predict their ability to respond to environmental pressures. We found that the populations Abisko, Malbo, Gällivare and Storlien, which are located at the edge of the natural range of *B. nana* and occupy different habitats, are genetically diverse to varying degrees. The northern populations from Abisko and Gällivare showed a lower level of genetic polymorphism than the population from Malbo, the southernmost site of dwarf birch in Sweden. The data presented indicate higher genetic diversity existed within populations, whereas genetic differentiation between populations was lower. The high level of genetic differentiation within *B. nana* populations that were analysed in the present study may be explained by a limited capacity for dispersal among populations via both pollen and seeds.

We found that the level of genetic diversity in one of the Polish populations of *B. nana* is comparable to that in areas in Scandinavia where populations are large and continuous. Based on these studies, we conclude that the "Linje" population has sufficient genetic resources.

Key words

dwarf birch, RAPD, population, relict species, genetic diversity

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Introduction

Genetic variability determines the adaptability to changing environmental conditions (gradation of pests, diseases, climate change). Therefore, it is crucial for the protection of populations, especially of endangered species, to keep it at a high level.

Dwarf birch (*Betula nana* L.) is a common shrub in boreal, subarctic and arctic areas of North America and Europe, including Sweden, where it occurs in tundra and acidic raised (ombrotrophic) bogs (Przybylski 1960; Drzymulska 2014). In Poland, it is a glacial relict species occurring in fragmented populations in three nature reserves: in the Chełmno Lake District, in "Torfowiska Doliny Izery" and "Torfowisko pod Zieleńcem" in the Bystrzyckie Mountains. Analysis of chloroplast DNA diversity (cpDNA) showed that all Polish populations are fixed with respect to the most common birch haplotypes (Jadwiszczak et al. 2012). "Linje" and Torfowisko pod Zieleńcem reserves share the same haplotype, while the Torfowiska Doliny Izery reserve is characterised by a different haplotype. Based on this result, it was hypothesised that Poland was either colonised by distinct glacial refugia or haplotype fixation resulted from genetic drift acting strongly in relict populations (Jadwiszczak et al. 2012). To protect these sites, studies on genetic parameters and monitoring of genetic diversity have also been conducted in populations occurring in the compact and continuous range of dwarf birch in Fennoscandia (Jadwiszczak et al. 2017; Tsuda et al. 2017; Borrell et al. 2018). Such studies provide the reference material necessary to assess the status of relict populations and develop strategies to protect them. Analysis of genetic structure and variability of isolated species is critical to assess whether stochastic or human-induced factors are affecting rare species.

Low genetic diversity affects the ability of populations to evolve and reduces their chances of survival in the face of environmental change. Molecular markers are widely used in assessing the genetic diversity of many plant species (Arif et al. 2010; Dąbrowska et al. 2006). Among the various molecular tools for genetic analysis, random amplification of polymorphic DNA (RAPD) is the simplest technique for determining DNA diversity (Williams et al. 1990; Hafzari et al. 2019; Sadoon and Shobrak 2010). In previous studies, RAPD markers had been used to examine the genetic variability of other species in the genus *Betula*: *Betula alnoides* (Zeng et al. 2003), *Betula platyphylla* and *Betula pendula* (Martin et al. 2008; Jiang et al. 2011). In our studies, we used the RAPD technique to analyse genetic variation in the Polish reserve Linje, for which a high level of genetic variation was found (Dąbrowska et al. 2006). A similar, high level of variability in Linje samples was determined by Jadwiszczak et al. (2012) using the microsatellite marker system. AFLP (Amplified Fragment Length Polymorphisms) analyses showed that the Polish populations of *B. nana* have comparable levels of genetic variation to samples from the centre of the species range.

The study aimed to assess the genetic diversity of selected Swedish dwarf birch (*B. nana* L.) populations growing on different habitats in Abisko, Gällivare, Storlien and Malbo using RAPD markers and to compare them with the Polish population Linje. These analyses can then serve as a reference material when developing conservation strategies for Polish relict dwarf birch populations.

Material and methods

Plant material

The analysed material consisted of dry dwarf birch (*B. nana* L.) shoots collected at the sites located in Sweden (Fig. 1): Abisko (68°12' N, 18°42' E), Gällivare

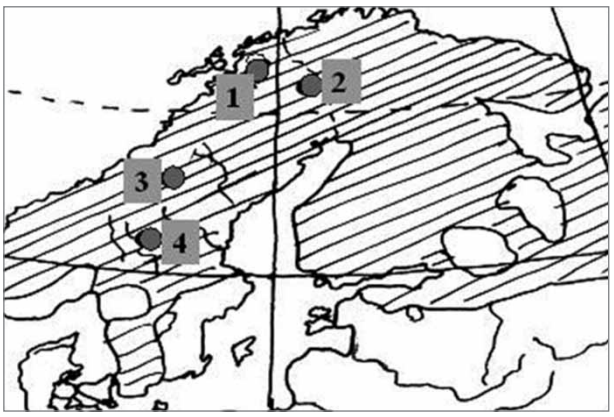

Figure 1. Geographical location of the *Betula nana* L. populations studied in this work: 1 – Abisko, 2 – Gällivare, 3 – Storlien, 4 – Malbo, 5 – The Linje Reserve. The grey area expresses the range of distribution of *B. nana* (Kruszelnicki and Fabiszewski 2001, modified)

(67°08' N, 20°42' E), Storlien (63°03' N, 12°06' E) and Malbo (60°41' N 16°83' E). At the Abisko site, *B. nana* grew in shrubby tundra, at Gällivare and Storlien sites in mountain tundra and at the Malbo site on a peat bog. The comparative material from the Polish populations of *B. nana* was collected in the Linje Reserve near Dąbrowa Chełmińska located in the northern part of Poland (53°1' N, 18°18' E). Twenty plants, growing 100 m apart, were sampled at each site.

RAPD analysis

Genomic DNA was extracted from dry dwarf birch shoots (100 mg) using a modified CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle 1990). To identify the polymorphic fragments of DNA, the following sequences of primers were used: OPA09 – 5' GGGTAACGCC 3', OPC02 – 5' GTGAGGCGTC 3', OPC14 – 5' TGCGTGCTTG 3' (Operon Technologies Inc., Alameda, CA, USA). Polymerase chain reaction (PCR) was performed in a total reaction volume of 30 µl with 20 ng of genomic DNA according to the protocol described previously (Dąbrowska et al. 2006). Each reaction was repeated three times. PCR products were separated on a 2.5% agarose gel containing ethidium bromide in 0.5× TBE (Tris-Borate-EDTA) buffer for 2 hours at 80 V. The agarose gels containing the isolated PCR products were photographed and analysed using ImageMaster 1D Elite v3.00 software (Amersham Pharmacia Biotech, Uppsala. Sweden). Following the study of Lynch and Milligan (1994), only those fragments of amplified DNA were taken into consideration which recurred within genotypes.

Data analysis

The results obtained were recorded in the 0–1 system (each PCR product represented a locus with two alternative alleles: none – 0, present – 1) and then analysed using GenAlEx 6.5 software (Peakall et al. 2012). The total number of bands, number of polymorphic loci, percentage of polymorphic loci (%), number of alleles observed on loci (Na), effective number of alleles on locus (Ne), Shannon information index (I) and gene diversity (H) according to Nei (1973) were calculated. Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed using GenAlEX 6.5 software. A dendrogram was constructed using Unweighted Pair Group Method with Arithmetic average (UPGMA) cluster analysis based on Nei's genetic distance matrix using PopGene 1.32 (Yeh 1997).

Results

To identify polymorphic DNA fragments, seven primers were tested, three of which (OPA09, OPC14, OPC02) produced distinct electrophoretic profiles. The number of polymorphic loci varied from 12 to 16, and the size of the diagnostic DNA fragments ranged from 90 to 1200 bp (Tab. 1).

Table 1. Range of length and number of DNA fragments of *Betula nana* determined by RAPD

Primer name	Number of polymorphic DNA fragments	Size range of polymorphic DNA fragments (bp)
OPA09	14	90–900
OPC14	12	90–1030
OPC02	16	120–1200

At the population level, indices of genetic diversity varied among populations of *B. nana*, as shown in Table 2. On average, the percentage of polymorphic loci, P (%), across all populations ranged from 30% for Gällivare to 72.5% for Malbo. The number of observed alleles (Na) per population varied from 0.900 (Gällivare) to 1.675 (Malbo), with a mean of 1.300. The number of effective alleles (Ne) across all populations was 1.245

Table 2. Genetic diversity within populations and genetic differentiation parameters of analysed populations of *Betula nana*

Population	P (%)	Na	Ne	I	He
Abisko	52.5	1.325	1.265	0.259	0.167
Malbo	72.5	1.675	1.458	0.396	0.265
Storlien	52.5	1.300	1.177	0.200	0.121
Gällivare	30.0	0.900	1.090	0.111	0.066
Mean	51.9	1.300	1.245	0.242	0.155
Linje	66.7	1.333	1.256	0.290	0.179

P (%) – percentage of polymorphic loci; Na – no. of alleles; Ne – no. of effective alleles; I – Shannon's information index; He – expected heterozygosity.

and varied from 1.090 (Gällivare) to 1.458 (Malbo). The expected heterozygosity (He) across all populations ranged from 0.066 (Gällivare) to 0.265 (Malbo) with a mean of 0.154. The mean Shannon's information index (I) was 0.242 over a range of 0.111–0.396.

AMOVA revealed that the majority (68%) of the observed variation was explained by differences within populations and a small but significant amount of variation (32%) occurred among populations (Tab. 3).

Table 3. Results of an AMOVA showing the distribution of genetic variation among *Betula nana* populations and within populations based on RAPD

Source of variations	Degree of freedom	Sum of squares	Variance components	% of total variance	*P* value
Among populations	3	126.738	1.912	32	<0.001
Within populations	76	304.350	4.005	68	<0.001
Total	79	431.088	5.917	100	

AMOVA – analysis of molecular variance; RAPD – random amplification of polymorphic DNA.

Within-population genetic diversity (Hs) and total species genetic diversity (Ht) were recorded as 0.224 and 0.1548, respectively. The observed genetic differentiation among populations (Gst) was 0.3171, which shows the presence of 32% genetic variation among populations. This result is consistent with the result of the AMOVA, which revealed 32% of genetic variation existing among populations and 68% within populations. The differences between populations were found to be highly significant ($P = 0.001$; Tab. 3). The result was further supported by the presence of gene flow between populations, as indicated by the high estimate of Nm (1.1). We also calculated genetic differentiation and gene flow for the two groups separately (northern populations Abisko/Gällivare and southern populations Storlien/Malbo) and found that Gst between the two southern populations was 0.2460 and Nm was 1.532, while Gst between the northern populations was 0.180 and Nm was 2.220. These results indicate weak genetic differentiation between the northern populations. Gene flow was slightly stronger between northern populations than between southern populations (Tab. 4).

Table 4. Summary of genetic diversity statistics for all loci in relation to the geographic location of *Betula nana* populations in the north and south of Sweden

	Ht	Hs	Gst	Nm
All populations	0.2240	0.1548	0.3171	1.100
Abisko/Gällivare	0.1428	0.1166	0.1800	2.220
Malbo/Storlien	0.2559	0.1930	0.2460	1.532

Gst = (Ht – Hs)/Ht – heterozygosity level, Nei (1973); Ht – total genetic diversity in the pooled populations; Hs – mean diversity within each population; Nm = 0.5(1 – Gst)/Gst – migration rate.

PCoA was performed to determine the spatial representation of genetic distances observed between individuals of different populations and to verify the consistency of genetic differentiation among populations. The first three principal coordinates for the sampled individuals of the four populations described 30.85%, 17.52% and 12.08% of the total variance, respectively. The plots of the first two coordinates are shown in Fig. 2. The individuals of the four populations of *B. nana* were distributed in the plot in accordance with the cluster analysis. Individuals belonging to Storlien were scattered separately from the remaining populations (Fig. 2). The populations Abisko, Malbo and Gällivare were clustered as one group, with individuals more or less genetically diverse as shown in the cluster

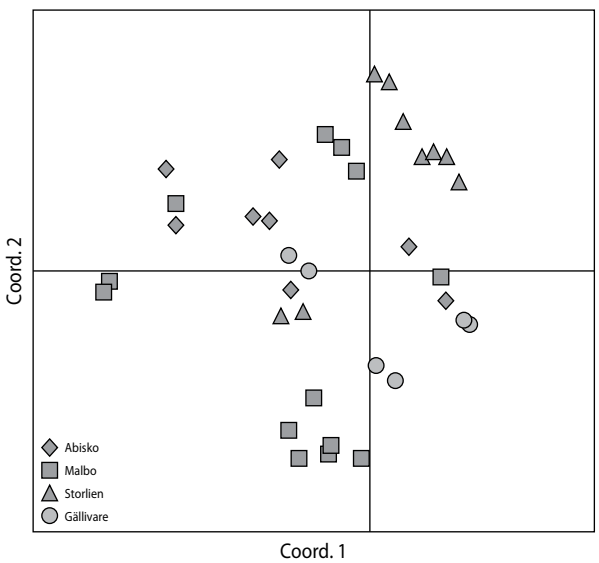

Figure 2. Principal coordinates analysis (PCoA) using random amplification of polymorphic DNA (RAPD) data of the studied populations of *Betula nana*

analysis. The results of PCoA and neighbour-joining dendrogram showed the same pattern, that is, that the three populations (Abisko, Malbo and Gällivare) were genetically close and distinct from the Storlien population (Fig. 3).

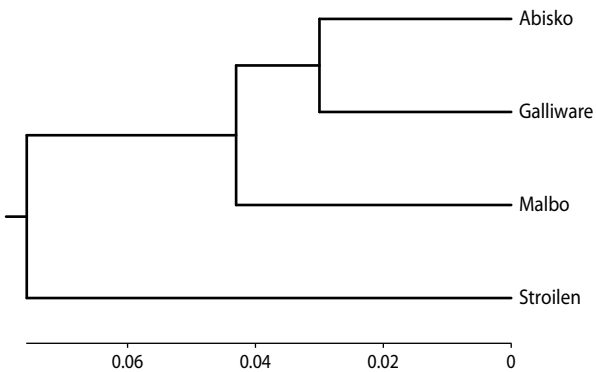

Figure 3. Dendrogram based on Unweighted Pair Group Method with Arithmetic average (UPGMA) of random amplification of polymorphic DNA (RAPD) profiles, showing the relationships among the four Swedish populations of *Betula nana*

Discussion

High level of genetic variation increases the potential of the species to respond to selective pressure. Previous studies on *B. nana* showed that endangered central European populations are not genetically extinct compared to widespread localities from Finland and Russia, which may result from rare outcrossing events in long-lived clonal populations (Jadwiszczak et al. 2017). Based on RAPD markers, the results reported here show that the populations Abisko, Malbo, Gällivare and Storlien, which are located at the edge of the natural range of *B. nana* and occupy different habitats, are genetically diverse to varying degrees. The highest variability was observed in the Malbo population, in which the percentage of polymorphic loci had the highest value of 72.5%, while the effective number of alleles and the mean diversity according to Nei were 1.458 and 0.265, respectively. It is the southernmost population among the studied populations. Two populations, Abisko and Gällivare, are geographically close (213 km) and have similar habitat structure, but the level of genetic diversity for Malbo and Storlien was higher (Ht – 0.2559) than between Abisko and Gällivare (Ht – 0.1428), which could be due to a reduction in heterozygosity. The Gällivare population – one of the northern populations – proved to be the most genetically homogeneous population. The Abisko and Storlien populations showed a similar degree of variability.

Internal population diversity is probably influenced by the habitat type. The most diverse of the studied populations, that is, the Malbo population, grows on the peat bog. The other populations, further north, originate from the tundra areas. It seems that under unfavourable environmental conditions, which prevail in the tundra zone, vegetative propagation is preferred, as it has been suggested for *B. nana* populations from the Svalbard archipelago (Alsos et al. 2002). Clonal reproduction thus allows survival under unfavourable conditions, as the efficiency of sexual reproduction in plants may vary according to habitat conditions. As shown for *Betula humilis* seeds, germination efficiency was lower in dry or shaded sites than in moist or sun-exposed sites (Chrzanowska et al. 2016).

AMOVA revealed that high genetic differentiation occurred within populations. 32% of the total variation occurred among populations, while 68% occurred within populations. When all populations were considered, the coefficient of genetic differentiation among populations (Gst) was 0.3171. Furthermore, the population Nm was 1.100 at the population level. In population genetics, a gene flow lower than 1 ($Nm < 1$) is generally regarded as the threshold quantity, below which significant population differentiation may occur (Slatkin 1987), and a value of Gst above 0.25 indicates great genetic differentiation (Wright 1978; Hartl and Clark 1997; Balloux and Lugon-Moulin 2002). The high level of genetic differentiation in *B. nana* populations that were analysed in the present study may be explained by the limited capacity for dispersal via both pollen and seeds.

The results of UPGMA and PCoA showed no clear geographic trends among the studied natural populations of *B. nana* in Sweden. The pattern that the three populations (Abisko, Gällivare and Malbo) are genetically close to each other and the population from Storlien is separated was the same for PCoA and the neighbour-joining dendrogram. Research by Palme (2003) and Palme et al. (2004), using chloroplast DNA, shows the variability of *Betula* haplotypes in Sweden. Some of them are unique, typical only for northern

Scandinavia, while haplotypes A and C are widespread throughout Europe. The presence of unique genetic varieties indicates that *B. nana* was probably able to survive the glaciation in small refugia in northern Europe. After the glacier retreat, other haplotypes could "migrate" to the north from refugia located in the south or east of Europe. Such a distribution of haplotypes, and at the same time the diversity of *B. nana* populations demonstrated by our study, may reflect postglacial migration of this species, where individuals colonising the northern and southern regions may have had different migration routes. According to Taberlet et al. (1998), a hybridisation zone has been established in Scandinavia through intersection of colonisation routes, which was colonised by genetically different populations from the north and south, coming from different refugia. As a consequence, the genetic diversity in this area is higher than one could expect. Postglacial colonisation may, therefore, explain the difference between the populations of *B. nana* from different regions in Sweden. The similarity of the populations from Malbo, Gällivare and Abisko, on the other hand, may result from colonisation by individuals coming from the same refugia.

All Polish localities of *B. nana* feature small populations and are geographically isolated. In most localities, the number of individuals per population has been declining steadily in recent years due to an unstable level of ground waters. However, a high level of genetic diversity was observed in the Polish dwarf birch population from the Linje reserve, which was studied using the same RAPD markers set in previous research (Dąbrowska et al. 2006). The effective number of alleles was 1.256 and the average diversity according to Nei was 0.179. It is worth noting that both estimates are higher than across an area in Sweden where populations are large and continuous.

Conclusion

We conclude that the Linje population retains sufficient genetic resources. A future goal will be to use markers with different mutation rates to gain a better understanding of the future adaptive potential of the *B. nana* populations.

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