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Genetic diversity of postglacial relict shrub *Betula nana* revealed by RAPD analysis

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Abstract: A sample of *Betula nana* from the Linie reserve near Dąbrowa Chełmińska, have been fingerprinted using random amplified polymorphic DNA (RAPD). The high level of genetic variation was detected. All individuals had unique genotypes, supporting the generally high resolving power of RAPD's. For the conservation strategy, information about the distribution of the genetic variation within and among populations plays very important role. Thus, extensive study in other populations of dwarf birch is needed.

Additional key words: genetic resources, conservation, population genetics.

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Introduction

Genetic variation exists within every species and forms the basis for selection and evolution (Järvinen et al. 2003). The analysis of the distribution of genetic diversity in a species provides useful information for conservation programs and management at the species level (Petit et al. 1998). In widespread, diploid outcrossers, most of the total genetic variation is found within populations, and only small proportion of diversity occurring among them. Large effective population sizes, a predominantly outcrossing mating system, long generation intervals, high fecundity and potential for long-distance gene flow have been cited as determinants of their genetic structure (Loveless and Hamrick 1984). Surveys of enzyme polymorphisms in plant populations have revealed that forest tree species usually have higher genetic diversity and little population differentiation than other groups of plants (Hamrick and Godt 1989; Hamrick et al. 1992). However, small and isolated populations may experience genetic drift and loss of genetic diversity. Disjunct distribution, environmental gradients (Xie et al. 1992), multilocus Wahlund and founder effects (Yang and Yeh 1993) may reduce the genetic diversity. Population or even species extinction may be the ultimate fate of this process (Hilfiker et al. 2004).

The genus *Betula* comprises 35–60 species of deciduous trees and shrubs, widely occurring at temperate, boreal, and arctic zones of northern hemisphere (Kulju et al. 2004, Pekkinen et al 2005). *Betula nana* is a hardy pioneer, monoecious and wind pollinated species. The nutlets are winged and wind dispersed (de Groot et al. 1997), but lack of germinable seeds or seed banks in arctic regions has been noted, even where the species is locally dominant (Alsos et al. 2003). Common vegetative layering compensates rare sexual reproduction.

Dwarf birch has a circumpolar distribution, it occurs in the western coastal regions of Greenland, across Iceland, British Isles, Northern Europe and southward to N. Alps, and across northern Asia to western Siberia and northern latitudes of North America (de Groot et al. 1997). In Poland, it is thought to be a climatic late-glacial survivor, occurring at three isolated populations: in the Linie Reserve near Dąbrowa Chełmińska, in the Torfowisko Izerskie peat-bog in the Izera Mountains (Sudeten Mountains) and in the Torfowisko pod Zieleńcem peat-bog in the Bystrzyca Mountains (Sudeten Mountains). Because present populations of *Betula nana* are probably fragmented relicts of larger populations in postglacial warm period, they may be more sensitive to genetic drift and depauperation. To provide a population genetic background for conservation efforts of dwarf birch we used random amplified polymorphic DNA (RAPD) analysis.

Material and methods

Plant material was collected in the Linie Reserve near Dąbrowa Chełmińska, located in the northern part of Poland. *Betula nana* occupies area of 1.37 ha, therefore to avoid sampling clones or close relatives, we collected shoots from 20 individuals separated by at least 100 m. DNA was extracted from 1.0 g of bark and secondary cambium tissue, following Doyle and Doyle (1990) protocol. The quantity and quality of the DNA was tested spectrophotometrically (Gene Quant, Pharmacia Biotech) and by electrophoresis in 1% agarose gel, stained with ethidium bromide.

Preliminary extractions of DNA revealed high RNA contents. Thus, we used incubation with 2 μl RNase (10 mg/ml), then with phenol-chloroform-isoamylic alcohol (25:24:1) mixture and dilution with two volumes of TE. To remove DNA contamination we added sodium chloride until obtaining 2.5 M concentration. The mixture was centrifuged and to the upper fraction, three volumes of 96% ethanol was added and incubated at –20°C for 1 hour. DNA was centrifuged for 15 min at 4°C by 10 000 × g, supernatant was discarded and pellet was washed with 1 ml of 70% ethanol and centrifuged again. The DNA was resuspended in 50 μl of TE.

Table 1. List of used primers and their sequences

Primer name	Sequence
OPA-05	5' AGGGGTCTTG 3'
OPA-09	5' GGGTAACGCC 3'
OPB-07	5' GGTGACGCAG 3'
OPC-02	5' GTGAGGCGTC 3'
OPC-14	5' TGCGTGCTTG 3'

The 30 μl of PCR reaction mixture contained 1× polymerase buffer (Polgen), 2.5 mM $MgCl_2$, 200 mM of each of dNTP, 250 nM of primer, 1U of Taq polymerase (Polgen) and 10 ng of DNA. In a preliminary primer screening we tested five 10-mer primers (Operon Technologies, Table 1). Negative controls containing TE buffer instead of DNA extract in the reaction mixture confirmed that no artifacts such as primer bands occurred. An overlay of 30 ml of mineral oil prevented evaporation of the reaction mix during heating. The following thermal cycles were run on PTC-100 thermocycler (MJ Research): first denaturation step at 94°C for 5 min, then 40 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C and 10 min of the final extension at 72°C. For each DNA sample the amplification was conducted at least twice in order to eliminate possible mistakes. The PCR products were separated on 2.5% agarose gel in 0.5 TBE buffer at 80–120 V for 5 h. The mix in ratio 1:1 of pUC19/MspI (Fermentas) and Lambda DNA/EcoRI + HindIII (Fermentas) molecular weight markers were used for sizing PCR products. The ethidium bromide was added to the gels for band visualization, photographs were made in the UV-light for further analyses. Amplification products were analyzed using Image Master 1D Ellite v3.00 computer software (Pharmacia Biotech). For each band two states were described: 1 – if the band was present or 0 – if band was absent.

Genetic variation of population was assessed by (A_e) effective number of alleles per locus (Kimura and Crow 1964), (h) Nei's gene diversity (Nei 1973) and (I) Shannon's information index (Lewontin 1972). Statistically analyses were performed using PopGene ver. 1.32 (Yeh et al. 1997) and TFPGA ver 1.3 (Miller 1997) software's.

Results

From a set of 5 primers, only three showed clear, polymorphic and reproducible RAPD banding patterns and therefore were used in further analyses. With these primers, we scored a total of 56 interpretable marker bands, but 17 of which were monomorphic (69.6% of polymorphic loci, 100% criterion, see Table 2). Based on 39 polymorphic products we found that all individuals were separated genotypes, which supports the generally high resolving power of RAPD's. The genetic diversity is high, because: the ef-

Table 2. The approximate lengths of the interpreted DNA fragments of *Betula nana* revealed after RAPD amplification

Primer	Marker bands (bp)
OPA-09	1400, 1100, 1010, 950, 830, 700, 620, 550, 500*, 410*, 374*, 350*, 320*, 300*, 250*, 242*, 220*, 200*, 180*, 160*, 147, 120, 111*, 90
OPC-02	730, 620, 564, 500, 410*, 350, 300, 242, 220, 200, 160*, 143, 120, 100, 90
OPC-14	1400, 1070, 950, 750, 650*, 560, 520, 450*, 350, 330, 250, 220, 190, 170, 150, 120, 100

*monomorphic RAPD product

fective number of alleles is 1.30, Nei's gene diversity is 0.217 and Shannon's information index is 0.367. Measures of genetic diversity are presented in Table 3.

Discussion

RAPD markers are well suited for population genetic analysis because they are primarily neutral, polymorphic and predominantly nuclear loci. Each random primer typically yields several variable RAPD's, and since many random primers are available, a large number of RAPD's may be analysed. RAPD's have been shown to inherit in a biparental dominant Mendelian manner. The drawback of restricted reproducibility can be limited by stringent and consistent handling procedures during DNA isolation and PCR amplification, and by controlling for artifacts with repetition and control samples (Hilfiker et al. 2004). Although RAPD analyses have recently been applied to study many of the forest tree species (Newton et al. 1999; Nybom and Bartish 2000), patterns of genetic variation in the birch species have been relatively little studied. The aim of the study was to assess the gene pool diversity of *Betula nana* from the Linie reserve. To our knowledge, this is the first report on genetic diversity in dwarf birch using RAPD markers. Although, only three primers were used in our study, we were able to achieve a high degree of individual resolution. It confirms, that sampling individuals separated by at least 100 m is enough to avoid sampling clones or close relatives. Few small and disjunct present-day populations of *Betula nana* in Poland may be fragmented relicts of larger and more continuous populations from warmer climatic period. Despite rare species and small populations often have reduced allelic variation due to genetic drift, bottlenecks, and strong natural selection in limited range of environments (Karron 1987; Ellstrand and Ellam 1993), the population studied, preserved a high level of genetic diversity. The variation was a little bit higher than the average for outcrossing plants based on allozyme loci study, which in general possess lower levels of variation (Hamrick and Godt 1989). On the other hand, Alsos and Engelskjøn (2002) found some signs of genetic depauperation within northern populations of *Betula nana* in Svalbard.

Table 3. Frequencies of 39 polymorphic RAPDs, (A_e) effective number of alleles and gene diversity value, estimated by (h) Nei's gene diversity and (I) Shannon's information index

RAPD marker	Frequency	A_e	h	I
OPA09-1	0.106	1.23	0.189	0.337
OPA09-2	0.078	1.17	0.144	0.274
OPA09-3	0.078	1.17	0.144	0.274
OPA09-4	0.106	1.23	0.189	0.337
OPA09-5	0.194	1.45	0.313	0.492
OPA09-6	0.163	1.38	0.273	0.445
OPA09-7	0.051	1.11	0.097	0.202
OPA09-8	0.106	1.23	0.189	0.337
OPA09-21	0.134	1.30	0.232	0.394
OPA09-22	0.106	1.23	0.189	0.337
OPA09-24	0.225	1.54	0.349	0.534
OPC02-1	0.106	1.23	0.189	0.337
OPC02-2	0.134	1.30	0.232	0.394
OPC02-3	0.078	1.17	0.144	0.274
OPC02-4	0.106	1.23	0.189	0.337
OPC02-6	0.194	1.45	0.313	0.492
OPC02-7	0.078	1.17	0.144	0.274
OPC02-8	0.225	1.54	0.349	0.534
OPC02-9	0.258	1.62	0.383	0.571
OPC02-10	0.194	1.45	0.313	0.492
OPC02-12	0.134	1.30	0.232	0.394
OPC02-13	0.293	1.71	0.414	0.605
OPC02-14	0.051	1.11	0.097	0.202
OPC02-15	0.106	1.23	0.189	0.337
OPC14-1	0.051	1.11	0.097	0.202
OPC14-2	0.078	1.17	0.144	0.274
OPC14-3	0.134	1.30	0.232	0.394
OPC14-4	0.051	1.11	0.097	0.202
OPC14-6	0.134	1.30	0.232	0.394
OPC14-7	0.051	1.11	0.097	0.202
OPC14-9	0.051	1.11	0.097	0.202
OPC14-10	0.078	1.17	0.144	0.274
OPC14-11	0.194	1.45	0.313	0.492
OPC14-12	0.163	1.38	0.273	0.445
OPC14-13	0.051	1.11	0.097	0.202
OPC14-14	0.163	1.38	0.273	0.445
OPC14-15	0.293	1.71	0.414	0.605
OPC14-16	0.194	1.45	0.313	0.492
OPC14-17	0.078	1.17	0.144	0.274
Mean	–	1.30	0.217	0.367

The number and sizes of populations required to preserve a minimum gene pool necessary for survival of a species depend on its vitality, reproductive strategy, and level and hierarchic distribution of the ge-

netic variation (Frankham 1995; Meffe and Carroll 1997). The distribution of the genetic variation within and among populations may have important implications for present and future conservation strategies of the species. If most of the variation is distributed within populations, preservation of just one large population might be sufficient to conserve the species. However, if a substantial amount of variation is present among populations, the conservation may require a different strategy (Molina-Freaner et al. 2001). The study in scandinavian populations of *Betula nana* revealed, that most of the variation could be attributed to differentiation between populations (F_{st} = 0.58; Palme et al. 2004). Similar, Alsos and Engelskjøn (2002) found, that the genetic differentiation among Svalbard populations of dwarf birch (F_{st} = 0.193) was higher than the average for long-lived woody perennials (7.6%), outcrossing wind-pollinated species (9.9%) and wind-dispersed species (14.3%; Hamrick and Godt 1989). Lower genetic variation within populations and strong differentiation among populations could be expected in northern populations due to infrequent sexual reproduction combined with potential bottlenecs, inbreeding, and fragmentation of previously larger populations (Alsos and Engelskjøn 2002). The high level of genetic diversity observed in this study may result from an initially large and genetically variable gene pool and relatively frequent sexual reproduction. Additional, reproduction by vegetative layering and long spans of clones may sustain considerable genetic diversity within populations (Alsos et al. 2003). *Betula* has long been known for its high levels of hybridization, and relatively frequent introgression, at least at high latitudes. Due to shorter growing seasons in the north, that reduce differences in flowering times among species (Kallio et al 1983), the higher hybridization frequencies are detected in subarctic zone (Scandinavia, Iceland, the Scottish Highlands, and Alaska), then in other regions (Palme et al. 2004). Thus, it seems that, there is a strong need to study the genetic diversity in other populations of dwarf birch, especially in Central Europe, where the species is able to reproduce sexually and hybridization may be less common.

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