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Scientific note

Preliminary genetic study on species from genus *Deschampsia* from Antarctic (King George I.) and Arctic (Spitsbergen)

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Abstract: The aim of this study was to analyze the variation among three polar species *Deschampsia antarctica*, *Deschampsia alpina* and *Deschampsia brevifolia*. Genomic DNA samples were extracted from individual plants and analyzed by AFLP technique. More than 322 polymorphic AFLP bands that allowed determination of genetic relationships among three *Deschampsia* species were obtained. The present investigation reveals that *D. antarctica* was more similar to a species from the Arctic (*D. brevifolia*) than was *D. alpina*. Also, the genetic diversity within population of *D. antarctica* was greater than those within the analyzed populations of *D. brevifolia* and *D. alpina*.

key words: *Deschampsia* spp., AFLP, Antarctica, Arctic

Introduction

Many of species from the genus *Deschampsia* are highly tolerant to variable and stressful environmental conditions this tolerance allow them to colonize and dominate plots of land that are uninhabited by other plant species (Nkongolo *et al*., 2001). Many of species from the genus *Deschampsia*, such as for example, *D. alba*, *D. arctica*, *D. nubigena*, *D. alpina*, *D. brevifolia* and *D. antarctica*, inhabit extreme polar environments (Index Kewensis, 1997).

In the Antarctic and Arctic, the sites of *Deschampsia* species occurrence are disjunct, situated mainly on islands and coastal areas, often separated from one another by natural barriers like open sea, glaciers or mountains. So, contact between populations might not be easy. We hypothesize that gene exchange between the populations from those harsh environments might be hard and some isolated populations might have very low genetic variability, when a limited number of individuals establish a new population. To verified those hypothesis we apply the AFLP (Amplified Fragment Length Polymorphism) (Vos *et al*., 1995) technique that generates highly reproducible results with many polymorphisms visualized on a single gel. AFLP is considered to be more reproducible than other PCR based techniques (Bednarek and Chwedorzewska, 2001). This makes this method useful for studies of closely related species, inbred lines (Bednarek *et al*.,

1999) or inter-population diversity in the same species (Chwedorzewska *et al*., 2004). In this paper we analyze the variation among three species of genus *Deschampsia* collected from opposite parts of the world: one from Antarctica, *Deschampsia antarctica* (King George I., South Shetlands Is.), and two from the Arctic, *Deschampsia alpina* and *Deschampsia brevifolia* (Spitsbergen, Horsundu) and try to take first step to compare population genetic structure of species of genus *Deschampsia.*

Materials and methods

Plant sampling

Species from genus *Deschampsia* were collected from King George Island (South Shetlands), Antarctica—*Deschampsia antarctica* (Fig. 1), and from the Horsundu area on Spitsbergen, *Deschampsia alpina* and *Deschampsia brevifolia.* Shoots were dried between two pieces of paper at room temperature.

DNA extraction

DNA was extracted from about 100 mg dry tissue following the manufacturer's recommendation (Qiagen). Purity and quantity of the samples were determined spectrophotometrically. DNA integrity and lack of RNA impurities were tested in 1.4% agarose gels (1^{x} TBE buffer and ethidium bromide (0.5 μg/m*l*) at 20 V/cm). For routine purposes, standard dilutions 10 μg/m*l* were prepared.

AFLP analysis

The AFLP technique was performed according to the procedure described by Vos *et al.* (1995) with minor modification (Bednarek *et al*., 1999). Briefly, 250 ng of genomic DNA was digested with *Eco*RI and *Mse*I, and ligated to the appropriate adaptors. A pre-(weakly selective) amplification step was performed in the presence of primers with

Fig. 1. *Deschampsia antarctica* DESV (H. Galera).

Table 1. Adapter and primer sequences.

Adapter/Primer	Sequence (5'->3')
Adapter *Eco*RI	CTCGTAGACTGCGTACC
	CATCTGACGCATGGTTAA
Adapter *Mse*I	TACTCAGGACTCATA
	GAGTCCTGAGTAGCAG
*Eco*RI preselective primer	GACTGCGTACCAATTCA
*Mse*I preselective primer	GATGAGTCCTGAGTAAC
E-Azz - selective primer	GACTGCGTACCAATTCAzz
M-Cyy - selective primer	GATGAGTCCTGAGTAACyy

E and M are for any selective primer complementary to *Eco*RI and *Mse*I adaptors respectively; -zz, -yy-any combination of the nucleotides at the primers 3'ends.

one selective nucleotide ("A" for *Eco*RI and "C" for *Mse*I). For selective amplification, primer combinations (E-AAA/M-CAA, E-AAA/M-CAC, E-AAG/M-CAG, E-AGG/M-CAG) with two additional nucleotides at the 3′-ends were used (Table 1). The *Eco*RI compatible primers were labeled at their 5′-ends with gamma-^{32}P ATP. PCR products were separated on 5% PAGE and exposed to X-ray film at -70°C overnight.

Data analysis

Visible, reproducible and polymorphic bands were scored as presence (1) and absence (0) and arranged in a matrix for further evaluation. The frequencies of all the markers were calculated.

Clustering analysis

The estimates of similarity were based on Euclidean distance. Clustering was performed using unweighted pairgroups with the arithmetic averages (UPGMA) clustering method (Sokal and Michener, 1958) using the software *STATISTICA*® version 5. The results were presented in the form of dendrogram (Fig. 2).

Results

AFLP

In total, four combinations of specific primer pairs amplified 322 bands that were generated for DNA templates isolated from individuals from three species of genus *Deschampsia* and processed according to the AFLP. The number of the DNA fragments generated by individual primer pairs varied from 27 to 106 with an average of 81.5. All primer pairs generated polymorphic signals; their number ranged between 18 and 79 with average 58.5. Nearly 73% (234) of all identified DNA fragments exhibited polymorphisms (Table 2).

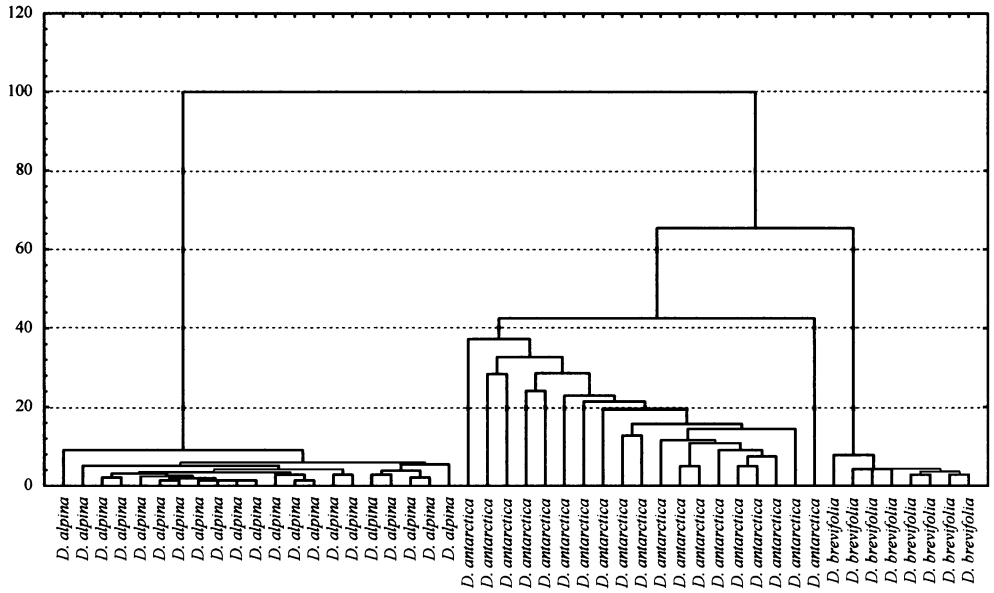

Fig. 2. Dendrogram based on all AFLPs from three *Deschampsia* species.

Table 2. Arrangement of data generated with the selected primer pairs.

Primer pair code	Detected bands (in total)	Polymorphic bands	% of Polymorphisms
E-AAA/M-CAA	27	18	66.7
E-AAA/M-CAC	106	79	74.5
E-AAG/M-CAG	83	60	72.3
E-AGG/M-CAG	106	77	72.6
Total	**322**	**234**	**72.7**

Cluster analyses

The similarity matrix was analyzed using unweighted pairgroups with arithmetic averages (UPGMA). Dendrogram was based on Euclidian distance and clustering was also performed using UPGMA clustering method for all DNA fragments obtained with four selective primer pairs for all species (Fig. 2). Cluster analyzes make it possible to clearly distinguish among taxa and also among individuals and revealed three groups. *D. alpina* formed the first cluster with similarity among individuals 97.5%, *D. antarctica* formed the second cluster with similarity 79%, and *D. brevifolia* individuals formed the third cluster with similarity 98.5%. Clusters of *D. brevifolia* and *D. antarctica* were more similar, 70%, to each other than to the *D. alpina* cluster. *D. alpina* were 57% similar to *D. brevifolia* and 52% to *D. antarctica* (similarity matrix, data not shown).

Discussion

This preliminary study verified the genetic distances among three species from the genus *Deschampsia.* The present investigation has revealed that *D. antarctica* was more similar to *D. brevifolia* than *D. brevifolia* was to *D. alpina.* Also, the genetic diversity within the population of *D. antarctica* was greater than those within populations of *D. brevifolia* and *D. alpina.* Our previous study of *Deschampsia antarctica* showed (Chwedorzewska *et al*., 2004) that the genetic structure of two populations which exhibited different morphology, divided by a natural barrier (glacier), differed although they were situated only six kilometers far from one another and a short-distance linkage by birds couldnot be excluded.

Holderegger *et al.* (2003) analyzed *D. antarctica* populations from widely separated regions of the maritime Antarctic (1350 km farther one another) and observed differences in the populations genetic structure. They observed greater polymorphisms in populations inhabiting northern and less harsh localities (Signy Island, South Orkney Islands) than in populations inhabiting at their south range limits on Anchorage Island, Lagoon Island and Léonie Island. The number of individuals from each population was too small to permit final conclusions, but the intra-population diversity seems to be much smaller in species from the Arctic than from the Antarctic. So, probably we monitored the geographic limits of those two species. Interesting results obtained for *D. antarctica* presented by many authors describe remarkably wide ecological amplitude and competitive tolerance in colonizing habitats of that species (Alberdi *et al*., 2002; Krywult *et al*., 2003; Lewis Smith, 2003; Zuniga *et al*., 1996). This result should be widened to other species from genus *Deschampsia* which inhabit similar environments. Detailed molecular analysis might reaveal common genetic factors that are responsible for survival of those species in polar conditions.

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