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Assessment of the molecular cytogenetic, morphometric and biochemical parameters of *Deschampsia antarctica* from its southern range limit in maritime Antarctic

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Abstract: Different chromosomal forms of *Deschampsia antarctica* Desv. (Poaceae), including diploids (2n=26), hypotriploid (2n=36–38) and a genotype with an occasional occurrence of B chromosome (2n=26+0-1B) that originated from southern marginal populations (Argentine Islands region, maritime Antarctic) were studied using molecular cytogenetic, morphometric and biochemical methods. FISH analysis revealed variations in the number of rDNA sites between the diploid and hypotriploid plants. The genome size varied among plants with a different chromosome number and was on average 10.88 pg/2C for diploids and 16.46 pg/2C for hypotriploid. The mean values of leaf length of plants grown *in vitro* varied within a range of 5.23–9.56 cm. The total phenolic content ranged from 51.10 to 105.40 mg/g, and the total flavonoid content ranged from 1.22 to 4.67 mg/g. The amount of phenolic compounds did not differ significantly between the genotypes, while a variation in the flavonoid content was observed for L59 and DAR12. The diploids did not differ significantly among each other in terms of the number of rDNA loci, but differed slightly in their genome size. The individuals of DAR12 carrying B chromosome were similar to other diploids in terms of their genome size, but

statistically differed in leaf length. The hypotriploid had both a greater number of rDNA sites and a larger genome size. No statistical correlations were observed between the genome size and leaf length or genome size and accumulation of phenolic and flavonoid compounds. The results of this study suggest that *D. antarctica* plants from the southern edge of the range are characterised by the heterogeneity of the studied parameters.

Key words: Antarctic, *Deschampsia antarctica*, marginal populations, variation, phenotypic characteristics.

Introduction

According to the concept of a 'species range' or the 'abundant centre' model, geographically peripheral populations exhibit a lower genetic diversity and a higher genetic differentiation than populations from the central part of a range (Sagarin and Gaines 2002; Hampe and Petit 2005; Johannesson and Andre 2006; Eckert *et al*. 2008). At the same time, in marginal populations, which are often isolated and under the extreme selection pressures of anomalous ecological conditions, intensive processes of speciation may occur that contribute to the maintenance and generation of biological diversity (Grant 1981; Kirkpatrick and Barton 1997). Genetic changes combined with karyotype repatterning enable a species to survive as a new form or even as a new species under intensive environmental pressure (Belyayev and Raskina 2013).

The studies of *Deschampsia antarctica* Desv. (Poaceae), the only grass species native to Antarctica (Alberdi *et al*. 2002), indicated a low level of molecular genetic diversity in the populations from different regions of the maritime Antarctic (Holderegger 2003; Van de Wouw *et al*. 2008). The low genetic variability between the plants of this species from the vicinity of the Antarctic Peninsula (South Shetland Islands) and Falklands Islands was also revealed by AFLP analysis (Chwedorzewska and Bednarek 2008, 2011). Similarities in the ITS1 and ITS2 sequences of the rRNA genes were found in the populations from two geographically distant regions of maritime Antarctic, *i.e*. the South Shetland Islands and the Argentine archipelago (Volkov *et al*. 2010; Andreev *et al*. 2010).

On the other hand, plant forms with atypical chromosome numbers or chromosomal races have been found for the *Deschampsia* genus, specifically for *D. caespitosa*, in unfavourable environmental conditions. For *D. alpina*, forms with 2n=26, 39, 48 and 52 chromosomes have been found in Sweden and Norway. As was described by Nygren (1949), the polyploids had probably arisen as a result of the fusion of reduced and unreduced gametes. These were able to produce seeds from which plants with aberrant chromosome numbers developed (Rothera and Davy 1986). The chromosome number of *D. antarctica* has been mentioned only in a few studies (Moore 1967; Cardone *et al*. 2009; Navrotska

et al. 2014; Amosova *et al*. 2015). The phenomenon of aneusomaty was revealed in plants from King George Island, the South Shetland Islands region of the maritime Antarctic, by Cardone *et al*. (2009). For the more southern range limit of the species (Argentine Islands region, maritime Antarctic), new chromosomal forms of *D*. *antarctica*, *i.e*. a hypotriploid and a genotype that supposedly had a B chromosome were recently reported (Navrotska *et al*. 2014). The peculiarities of the karyotype structure of the *D*. *antarctica* that originated from the Darboux, Galindez, Great Yalour and Skua Islands of the maritime Antarctic were revealed through a comprehensive molecular cytogenetic analysis by Amosova *et al*. (2015). In addition, a polyploid genotype of *D*. *antarctica* (2n=52) was reported in Central Patagonia (Chubut, Argentina) close to the northern limit of its distribution range (Gonzalez *et al*. 2016).

It is known that differences in the ploidy level are manifested as variations in the number of chromosome sets, the cell volume and the nuclear DNA content per cell. Furthermore, it may also affect the phenotypic characteristics of a plant such as various morphological, physiological, cellular and biochemical parameters (Yildiz 2013). Plants that grow in polar environments have been shown to demonstrate a reduced morphogenetic plasticity (Grime 2006). By contrast, Antarctic flowering plant species have been found to have variable morphometric indices and evolve morphological forms that are specific to particular habitats (Giełwanowska 2005). The biochemical parameters of plants, especially the content of secondary metabolites such as phenolic and flavonoid compounds, may also play an important role in adaptation to extreme conditions. Flavonoids are a group of phenolics that display a remarkable array of biochemical actions. It is known that these secondary metabolites are synthesised by plants during both their normal development and in their response to stress conditions such as infection, wounding or UV radiation (Ahmed *et al*. 2017). They also play an important role as antioxidants that are involved in many physiological processes and can be used as phenotype markers at the biochemical level (Quattorocchio *et al*. 2006).

In this paper, we present molecular cytogenetic, morphological and biochemical data for *D*. *antarctica* plants from marginal populations, including some genotypes that were previously described cytogenetically. We aim to characterise the different chromosomal forms within the species and to examine the possible impact of alterations in genotypes on their phenotypic characteristics.

Material and methods

In this study, 11 genotypes of *D*. *antarctica* plants that were grown from seeds that had been collected in the Argentine Islands (maritime Antarctic) near the Ukrainian Antarctic Station *Academician Vernadsky* were analysed. The material was collected during the 9th (2004/05), 11th (2006/07), 12th (2007/08),

14th (2009/10), 17th (2012/13) and 18th (2013/14) Ukrainian Antarctic Expeditions (Table 1). Aseptic seedlings were obtained as described in Zahrychuk *et al*. (2012) and were cultured on a Gamborg B5 medium (Gamborg *et al*. 1968). The plants were propagated *in vitro* and were grown at 18°C, 16-h photoperiod and light intensity of 30 µmol/m^{2}s^1 in a growth chamber. The rationale for this was to obtain a sufficient amount of genetically identical plant material (Spiridonova *et al*. 2016), and to examine the possible effects of genetic factors, in particular the ploidy level and nuclear DNA content, on some of the morphometric and biochemical parameters of the plants.

Cytogenetic analysis and fluorescence *in situ* hybridisation. — Plant root tips 1.5–2.0 cm long were immersed in ice-cold water for 24 hours, fixed overnight in 3:1 (v/v) freshly prepared methanol/glacial acetic acid at 4°C for 24 h and stored at -20°C until required. After a 15-minute wash in a 0.01 M citric acid-sodium citrate buffer (pH 4.8), the root tips were digested in an enzyme mixture comprising 2% (w/v) cellulase "Onozuka RS" (Serva) and 20% (v/v) pectinase from *Aspergillus niger* (Sigma) at 37°C. The meristems were dissected from the root tips and squashed in a drop of 45% acetic acid. After squashing and freezing, the preparations were dehydrated in absolute ethanol, air dried and stained with 0.5 µg/µl DAPI (Serva) as described in Jenkins and Hasterok (2007).

Table 1

Locations and years of collecting the *D. antarctica* material.

№	Genotype	Locality	Geographical coordinates	Year of collection
1	G/D4-1	Galindez Island	S65°14.919' W64°14.332'	2012/2013
2	G/D12-2a	Galindez Island	S65°14.528' W64°14.332'	2006/2007
3	G/D12-1	Galindez Island	S65°14.528' W64°14.332'	2013/2014
4	L59	Lahille Island	S65°55.220' W64°39.426'	2011
5	R35	Rasmussen Cape	S65°14.819' W64°05.156'	2004/2005
6	S22	Skua Island	S65°14.039' W64°09.761'	2009/2010
7	W1	Winter Island	S65°14.851' W64°15.482'	2013/2014
8	Y62	Great Yalour Island	S65°14.039' W64°09.761'	2004/2005
9	Y67	Great Yalour Island	S65°14.039' W64°09.761'	2004/2005
10	DAR12	Darboux Island	S65°23.424' W64°12.543'	2007
11	Y66	Great Yalour Island	S65°14.039' W64°09.761'	2004/2005

The fluorescence *in situ* hybridisation (FISH) procedure was adopted from Hasterok *et al*. (2002). The G/D12-2a, Y66, S22, R35 and DAR12 genotypes were studied with the following probes: (i) 5S rDNA from *Triticum aestivum*, clone pTa794 (Gerlach and Dyer 1980); (ii) 2.3 kb *Cla*I fragment of the 25S rDNA coding region from *Arabidopsis thaliana* (Unfried and Gruendler 1990), (iii) HT100.3 telomeric repeats $(TTTAGGG)_n$ from *A. thaliana* (Hajdera *et al*. 2003) and (iv) cereal centromeric sequence 1 (CCS1; 260 bp) from *Brachypodium sylvaticum* (Aragon-Alcaide *et al*. 1996). The preparations were examined using an Olympus Provis AX70 epifluorescence microscope with the appropriate filter sets. Images were taken with a Hamamatsu C5810 colour CCD camera and processed using Adobe Photoshop and CorelDRAW software.

Flow cytometry analysis. — The nuclear DNA content of *D. antarctica* was calculated based on the results of the flow cytometry. Young leaves of the *D. antarctica* plants and an internal standard were chopped together with a razor blade in a Petri dish in 500 µl of a nuclei extraction buffer (CyStain PI Precision P Sysmex 05-5022) that had been supplemented with 1% β-mercaptoethanol (Sigma) and 1% Triton X-100 (Sigma). The nuclei suspension was filtered through a 30 µm mesh (CellTrics, Sysmex) into a clean tube. Next, the samples were stained with 2 ml of a staining buffer containing propidium iodide and RNase (CyStain PI Sysmex Precision P 05-5022) and incubated for 45 min at room temperature in the dark. Then, the samples were analysed using a CyFlow Space flow cytometer (Sysmex) equipped with a 532 nm green laser. At least 10 000 nuclei were analysed for each sample. *Secale cereale* subsp. *cereale* (16.01 pg/2C, GeneBank Gatersleben accession number: R737) was selected as the internal standard for the diploid genotypes, whilst *Vicia faba* cv 'Tinova' (26.21 pg/2C, GeneBank Gatersleben accession number: FAB602) was used as the internal standard for the hypotriploid. For each genotype, at least three plants were used and each plant was analysed twice. The measurements were performed in three replicates on different days.

Morphometric measurements of leaf length. — Two-month-old plants that had been grown *in vitro* were used to measure the leaf length. All of the leaves of each individual were analysed. The number of plants from each genotype that were used for analysis is presented in Table 2. Standard statistical methods were used to calculate the mean value (X) and standard deviation (SD) (Pollard 2008).

Quantification of the total phenolic and flavonoid content. — The leaf compounds were extracted in diethyl ether for 24 h from the dried plant material (200 mg) of at least three individuals of each genotype. Profiling the phenolic and flavonoid content in the *D. antarctica* leaves was performed using reversed-phase high-performance liquid chromatography (HPLC) as described in detail

Table 2

Morphometric, cytogenetic and biochemical characteristics of the *D. antarctica* plants.

Genotype	Cytogenetic characteristics			Genome size			Leaf length			Biochemical characteristics	
	No. of metaphases	No. of chromosomes (2n)	Ploidy	2C DNA content, X ± SD (pg)	CV ± SD (%)	C_x (pg)	No. of investigated		X ± SD (cm)	Phenolic content, X ± SD (mg/g)	Flavonoid content, X ± SD (mg/g)
							plants	leaves			
G/D4-1	21	26	2x	11.01 ± 0.03	4.00±0.10	5.50	55	417	6.22±2.01	96.33±4.54	2.92±0.80
G/D12-2a	61	26	2x	10.84±0.09	3.50±0.10	5.42	36	230	6.50±1.83	88.42±2.36	2.80±0.85
G/D12-1	24	26	2x	11.02±0.03	3.90±0.13	5.51	59	372	8.01±3.84	105.40±6.25	3.86±2.47
L59	32	26	2x	11.01±0.01	3.92±0.04	5.50	38	317	8.08±3.15	51.10±3.65	4.67±0.35
R35	37	26	2x	10.77±0.02	3.70±0.09	5.38	97	599	7.96±2.36	92.00±4.38	2.72±0.56
S22	45	26	2x	10.94±0.04	3.90±0.12	5.48	50	410	6.37±1.73	89.12±2.41	3.74±0.74
W1	17	26	2x	10.91±0.05	4.10±0.20	5.45	28	182	6.73±1.72	91.72±1.28	2.23±0.35
Y62	67	26	2x	10.85±0.13	4.33±0.20	5.42	48	298	5.53±1.51	92.82±2.42	1.62±0.93
Y67	29	26	2x	10.79±0.07	4.26±0.26	5.39	40	290	5.23±1.43	101.25±5.33	2.64±0.70
DAR12	56/12	26+0B/26+1B	2x	10.86±0.05	4.25±0.16	5.43	22	168	9.56±2.87	66.22±3.12	1.22±0.02
Y66	52/40	36/37, 38	~3x	16.46±0.24	4.28±0.20	5.49	65	408	7.97±2.30	80.22±3.21	2.23±0.35

X – mean value, SD – standard deviation, CV – coefficient of variation, C_x – DNA content of the monoploid genome.

by Belemets *et al*. (2014). The total phenolic content was determined as the gallic acid equivalent, and the total flavonoids were determined as the rutin equivalent according to Sytar *et al*. (2014). The processing and visualisation of the chromatogram and absorption spectra were performed using Agilent ChemStation software.

Statistical analysis. — Based on our previous observations, a normal distribution of genome size and leaf length was assumed. The parametric ANOVA analysis followed by the *post hoc* LSD (Least Significant Difference) test were applied to detect a significantly different genome size and leaf length in particular genotypes. To compare the phenolic and flavonoid content in the genotypes, the non-parametric Kruskal-Wallis ANOVA and *post hoc* multiple comparison tests (whenever necessary) were used. Correlations between the genome size and three other variables, *i.e*. leaf length, phenolic content and flavonoid content were tested separately using Pearson r statistics. In the correlation tests, the mean values of the variables of particular genotypes were used. The statistical analysis was performed using Statistica 13.1 software (Dell Inc., Tulsa, OK, USA).

Results

Molecular cytogenetic studies of *D. antarctica*. — Plants from all of the genotypes (Table 1 and Table 2) were analysed. It was revealed that G/D12-2a, R35, S22, Y62 and Y67 have 26 chromosomes in their karyotypes. In addition to the plants with 2n=26, which is the chromosome number that is typical for this species, a genotype with the occasional presence of one B chromosome in some of its cells (DAR12, 2n=26+0–1B) and with a hypotriploid chromosome set (Y66, 2n=36–38) were also found (Navrotska *et al*. 2014). In order to expand the sample size, we conducted an analysis of additional *D. antarctica* plants from other populations of the Argentine Islands region. It was found that the karyotypes of the G/D4-1, G/D12-1, L59 and W1 plants also consisted of 26 chromosomes (Table 2).

Dual-colour fluorescence *in situ* hybridisation revealed variations in the numbers of rDNA loci between the diploid and hypotriploid genotypes. The 5S rDNA and 35S rDNA signals were located on different chromosomes of the complement. Ten sites of 5S rDNA and four sites of 35S rDNA were observed in the karyotypes of the diploids (G/D12-2a, R35, S22 and DAR12). 5S rDNA sites were allocated to the proximal regions of six chromosomes and the terminal regions of four chromosomes. Signals of the 35S rDNA were observed in close proximity to the centromere of two chromosomes and in the terminal regions of the other two chromosomes (Fig. 1A–D). For the plants of the Y66 hypotriploid genotype in which individuals with 36 chromosomes

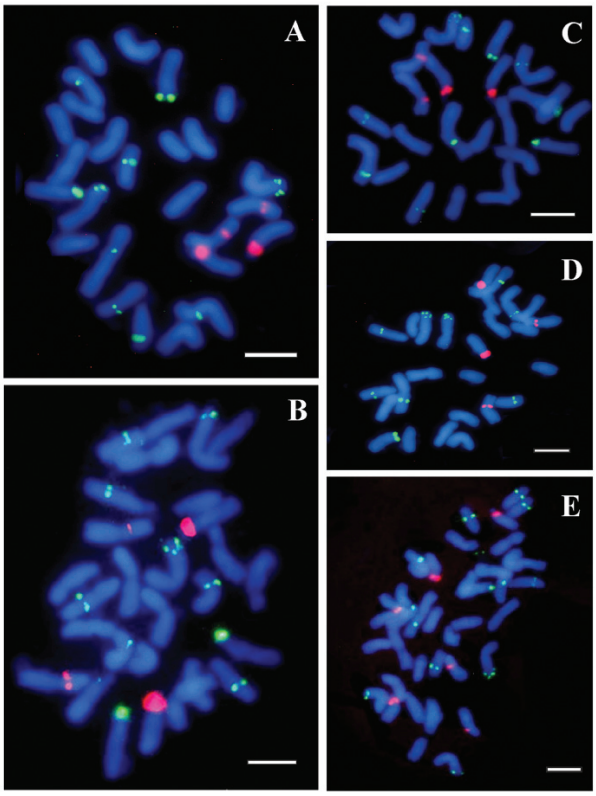

Fig. 1. Fluorescence *in situ* hybridisation of 5S rDNA (green) and 25S rDNA (red) probes with somatic metaphase chromosomes of different genotypes of *D. antarctica*. **A.** S22, 2n=26. **B.** DAR12, 2n=26+0B. **C.** G/D12-2a, 2n=26. **D.** R35, 2n=26. **E.** Y66, 2n=36. Chromosomes were stained with DAPI (blue). Scale bar is 5 µm.

predominated, 14 sites of 5S rDNA and six sites of 35S rDNA were observed. The 5S rDNA sites were located in the proximal regions of eight chromosomes and in the terminal regions of six chromosomes. The signals of the 35S rDNA were found in the proximal regions of three chromosomes and in the terminal regions of three other chromosomes (Fig. 1E).

FISH using telomeric and centromere-specific probes revealed a typical distribution of these repeats in the chromosomes of the genotypes of *D. antarctica* that were investigated. The telomeric repeats formed distinct blocks at the termini of all of the chromosomes. The centromeric probe mainly hybridised to the primary constrictions of all of the chromosomes in both the diploid and hypotriploid genotypes (Fig. 2). Furthermore, the presence of a B chromosome in the karyotype of the DAR12 plants confirmed our previous findings (Navrotska

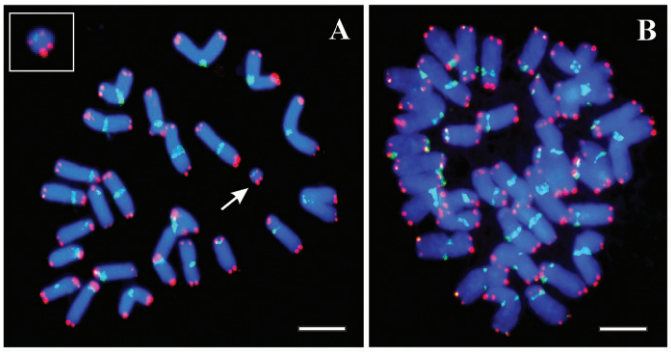

Fig. 2. Distribution of telomeric (red) and centromeric (green) repeats in the metaphase chromosomes of *D. antarctica*. **A.** DAR12, 2n=26+1B. **B.** Y66, 2n=36. The B chromosome is indicated by the arrow and is shown in the inset. Chromosomes were stained with DAPI (blue). Scale bar is 5 μm.

et al. 2014). This additional chromosome was significantly smaller than all of the other chromosomes, but had clear hybridisation signals at both the centromeric and terminal telomeric sites (Fig. 2A).

Genome size of plants with different chromosome numbers. — The flow cytometry analysis revealed variations in the nuclear DNA content between plants with different chromosome numbers. The 2C-value of the diploid plants varied from 10.77 pg for R35 to 11.02 pg for G/D12-1 with an average of 10.88 pg. The nuclear DNA content for the DAR12 that contained a B chromosome was 10.86 pg/2C DNA and fell within the range of the values that were obtained for the diploid genotypes. Thus, the presence of this B chromosome did not significantly affect the genome size. The DNA content of the hypotriploid plants (Y66) was 16.46 pg/2C (Table 2, Fig. 3). The calculated C_x value, the size of the monoploid genome (Greilhuber *et al*. 2005), fluctuated between 5.38 pg to 5.51 pg with a mean of 5.45 pg. The C_x value for the individuals with a B chromosome (DAR12; 5.43 pg) and the hypotriploid (Y66; 5.49 pg) fell within this range. The coefficient of variation for all of the genotypes varied within a range of 3.5% to 4.33% (Table 2). The diploid plants were found to differ statistically in their nuclear DNA content (ANOVA test, $p = 0.0$ and LSD *post hoc* test; see Table 3), for example between the L59 and R35 genotypes (0.000057). The differences in genome size between the genotypes with the highest DNA content (G/D12-1, G/D4-1 and L59) and with the smallest DNA content (Y67 and R35) were larger than the standard deviation that was calculated for genotypes. Statistically, the largest differences were observed for the hypotriploid Y66 compared to the diploid genotypes, which is associated with the much larger genome of Y66.

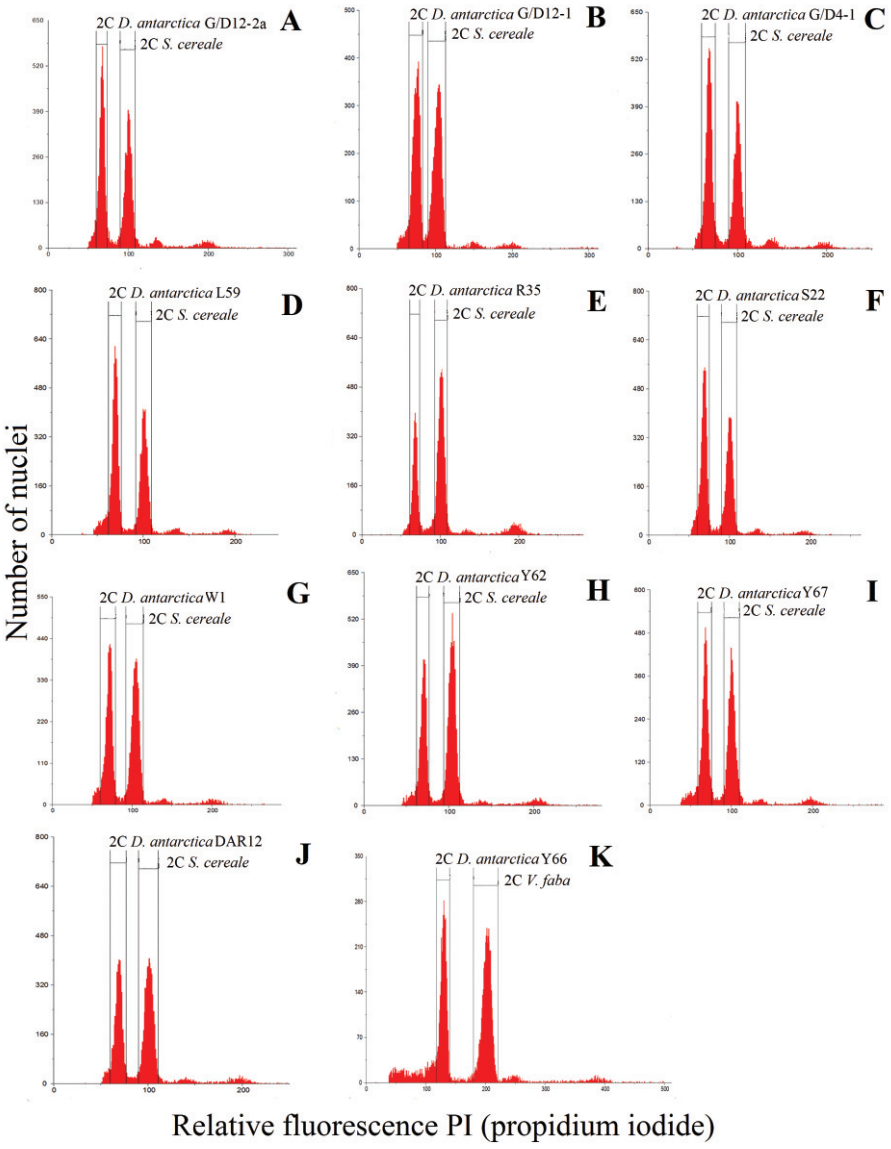

Fig. 3. Estimation of the 2C DNA content in *D. antarctica* genotypes using flow cytometry. **A.** G/D12-2a, 2n=26. **B.** G/D12-1, 2n=26. **C.** G/D4-1, 2n=26. **D.** L59, 2n=26. **E.** R35, 2n=26. **F.** S22, 2n=26. **G.** W1, 2n=26. **H.** Y62, 2n=26. **I.** Y67, 2n=26. **J.** DAR12, 2n=26+0–1B. **K.** Y66, 2n=36–38.

Table 3

p-values in the *post hoc* LSD test comparing the means (M) of the 2C DNA content in the genotypes, pairs of means are significantly different for $p < 0.05$ (bold).

Genotype	G/D4-1 M=11.014	G/D12-2a M=10.840	G/D12-1 M=11.001	L59 M=11.012	R35 M=10.773	S22 M=10.942	W1 M=10.913	Y62 M=10.884	Y67 M=10.791	DAR12 M=10.873	Y66 M=16.482
G/D4-1		**0.006670**	0.829640	0.973067	**0.000251**	0.244287	0.108351	**0.003524**	**0.000420**	**0.021709**	**0.00**
G/D12-2a	**0.006670**		**0.008507**	**0.002814**	0.261667	0.091808	0.220866	0.941532	0.387669	0.565596	**0.00**
G/D12-1	0.829640	**0.008507**		0.837089	**0.000283**	0.318180	0.143369	**0.004294**	**0.000477**	**0.028452**	**0.00**
L59	0.973067	**0.002814**	0.837089		**0.000057**	0.204545	0.078206	0.000977	**0.000093**	**0.010849**	**0.00**
R35	**0.000251**	0.261667	0.000283	0.000057		**0.006024**	**0.020974**	0.185110	0.760916	0.084554	**0.00**
S22	0.244287	0.091808	0.318180	0.204545	0.006024		0.635767	0.070420	**0.010302**	0.234828	**0.00**
W1	0.108351	0.220866	0.143369	0.078206	0.020974	0.635767		0.195361	**0.035227**	0.482941	**0.00**
Y62	**0.003524**	0.941532	0.004294	0.000977	0.185110	0.070420	0.195361		0.2935765	0.567398	**0.00**
Y67	**0.000420**	0.387669	0.000477	0.000093	0.760916	0.010302	0.035227	0.293576		0.137000	**0.00**
DAR12	**0.021709**	0.565596	0.028452	0.010849	0.084554	0.234828	0.482941	0.567398	0.137000		**0.00**
Y66	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00**

Leaf length of *D. antarctica* plants. — The morphometric measurements revealed that the mean values of the leaf length for most of the genotypes (G/D12-2a, G/D4-1, G/D12-1, L59, R35, S22, W1, Y62 and Y67) varied within a range of 5.23–9.56 cm (Table 2). Statistically significant differences in leaf length (ANOVA test, p = 0.0) were found between the genotypes. Most pairs of the mean values of the leaf length varied between each other (LSD *post hoc*, see Table 4). The longest leaves (9.56 cm) were observed in DAR12, which differed from all of the others. The leaf length in G/D4-1, G/D12-2a and S22 did not vary significantly and was 6.22 cm, 6.50 cm and 6.37 cm, respectively. In the group of genotypes G/D12-1, L59, R35 and Y66, the values were also very close (8.01 cm, 8.08 cm, 7.96 cm and 7.97 cm, respectively) and had high p-values (Table 4).

Determination of the total phenolic and flavonoid content in *D. antarctica*. — The total content of phenolics in the plants ranged from 51.10 mg/g for L59 to 105.40 mg/g for G/D12-1. The genotype with a B chromosome (DAR12) had a lower phenolic content of 66.22 mg/g, whereas the hypotriploid (Y66) had a total phenolic content of 80.22 mg/g (Table 2, Fig. 4A).

The total flavonoid content in the plants ranged from 1.22 mg/g for DAR12 to 4.67 mg/g for L59. The content of flavonoids for the diploid genotypes was within the range of 1.62 mg/g (Y62) to 4.67 mg/g (L59). The amount of these compounds for the B chromosome-containing plants was the lowest and reached only 1.22 mg/g. The hypotriploid contained 2.23 mg/g of total flavonoids, which falls within the range of this parameter for the diploid genotypes (Table 2, Fig. 4B). Furthermore, the chromatographic profiles of the flavonoids were almost similar for the leaf extracts of the different genotypes and only slight quantitative differences were observed for individual peaks of the hypotriploid genotype compared with the diploid ones (Fig. 5).

According to the statistical analysis, no significant differences (Kruskal-Wallis test, p = 0.0813) were detected in the phenolic content between the genotypes (Table 5), while a comparison of the flavonoid content (Kruskal-Wallis test, p = 0.0237) revealed a single difference between the L59 and DAR12 plants (*post hoc* multiple comparison test, Table 6). In general, based on the Pearson r statistics analysis, we did not find any relationship between the following variables: genome size and leaf length (r = 0.23013; p = 0.496), genome size and phenolic content (r= -0.1493; p = 0.661) and genome size and flavonoid content (r = -0.1533; p = 0.653).

Table 4

p-values in the *post hoc* LSD test comparing the means (M) of the leaf length in the genotypes, pairs of means are significantly different for $p < 0.05$ (bold).

Genotype	G/D4-1 M=6.2242	G/D12-2a M=6.5013	G/D12-1 M=8.0108	L59 M=8.0789	R35 M=7.9624	S22 M=6.3707	W1 M=6.7253	Y62 M=5.5336	Y67 M=5.2310	DAR12 M=9.5625	Y66 M=7.9681
G/D4-1		0.157474	**0.000000**	**0.000000**	**0.000000**	0.377353	**0.018142**	**0.000138**	**0.000000**	**0.00000**	**0.000000**
G/D12-2a	0.157474		**0.000000**	**0.000000**	**0.000000**	0.506556	0.344122	**0.000004**	**0.000000**	**0.000000**	**0.000000**
G/D12-1	**0.00000**	**0.000000**		0.708829	0.759049	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.028452**	0.803263
L59	**0.00000**	**0.000000**	0.708829		0.482380	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.010849**	0.535407
R35	**0.00000**	**0.000000**	0.759049	0.482380		**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	0.970315
S22	0.377353	0.506556	**0.000000**	**0.000000**	**0.000000**		0.095348	**0.000000**	**0.000000**	**0.000000**	**0.000000**
W1	**0.018142**	0.344122	**0.000000**	**0.000000**	**0.000000**	0.095348		**0.000000**	**0.000000**	**0.000000**	**0.000000**
Y62	**0.000138**	**0.000004**	**0.000000**	**0.000000**	**0.000000**	**0.000004**	**0.000000**		0.124352	**0.000000**	**0.000000**
Y67	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	0.124352		**0.000000**	**0.000000**
DAR12	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**		**0.000000**
Y66	**0.000000**	**0.000000**	0.803263	0.535407	0.970315	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**	**0.000000**

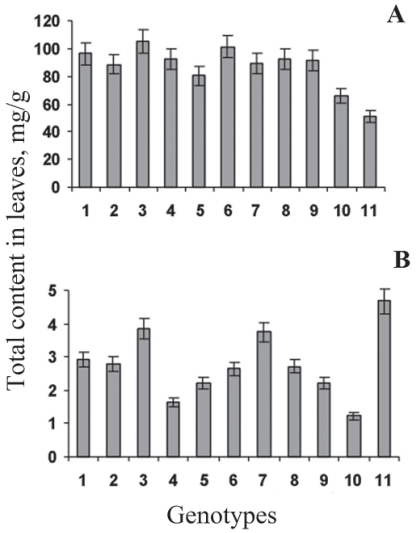

Fig. 4. Total content of the phenolic (**A**) and flavonoid (**B**) compounds in the leaves of the different genotypes of *D. antarctica*. (M±SD) **1.** G/D12-2a, 2n=26. **2.** G/D4-1, 2n=26. **3.** G/D12-1, 2n=26. **4.** Y62, 2n=26. **5.** Y66, 2n=36–38. **6.** Y67, 2n=26. **7.** S22, 2n=26. **8.** R35, 2n=26. **9.** W1, 2n=26. **10.** DAR12, 2n=26+0–1B. **11.** L59, 2n=26.

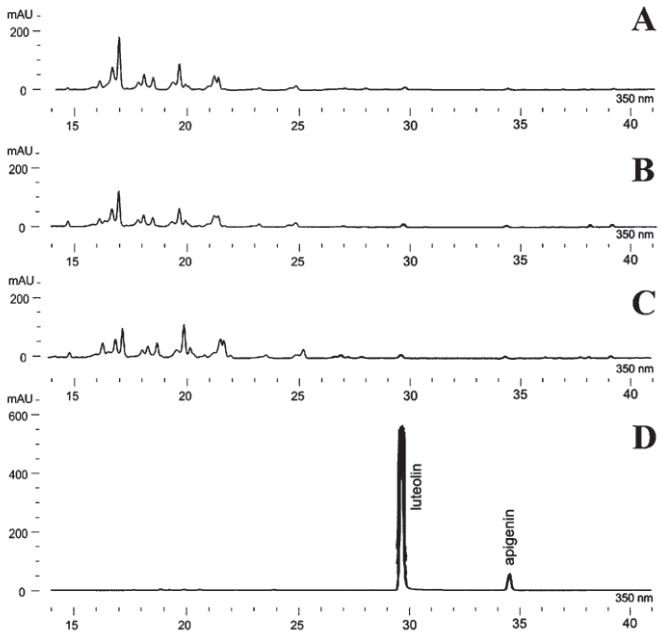

Fig. 5. Chromatographic profiles of the flavonoids in the leaf extracts of the different genotypes of *D. antarctica*. **A.** G/D12-2a, 2n=26. **B.** DAR12, 2n=26+0–1B. **C.** Y66, 2n=36–38. **D.** Standards of luteolin and apigenin flavonoids.

Table 5

p-values in the *post hoc* multiple comparison test comparing the ranges (R) of the phenolic content in the genotypes, no significant differences were detected between the samples.

Phenolic content (mg/g)	G/D4-1 R:22.667	G/D12-2a R:17.333	G/D12-1 R:23.667	L59 R:2.0000	R35 R:18.000	S22 R:17.000	W1 R:20.667	Y62 R:20.667	Y67 R:23.333	DAR12 R:4.5000	Y66 R:11.333
G/D4-1		1.000000	1.000000	0.383431	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
G/D12-2a	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
G/D12-1	1.000000	1.000000			1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
L59	0.383431	1.000000	0.257006		1.000000	1.000000	1.000000	0.814336	0.294182	1.000000	1.000000
R35	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
S22	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
W1	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
Y62	1.000000	1.000000	1.000000	0.814336	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
Y67	1.000000	1.000000	1.000000	0.294182	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DAR12	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
Y66	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table 6

p-values in the *post hoc* multiple comparison test comparing the ranges (R) of the flavonoid content in the genotypes, the only difference was detected between the L59 and DAR12 genotypes (p = 0.025183, bold).

Flavonoid content (mg/g)	G/D4-1 R:19.833	G/D12-2a R:19.333	G/D12-1 R:21.000	L59 R:30.667	R35 R:17.833	S22 R:26.333	W1 R:12.333	Y62 R:7.6667	Y67 R:16.667	DAR12 R:3.0000	Y66 R:12.333
G/D4-1		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
G/D12-2a	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
G/D12-1	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
L59	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	0.196771	1.000000	**0.025183**	1.000000
R35	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
S22	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	0.993471	1.000000	0.171744	1.000000
W1	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
Y62	1.000000	1.000000	1.000000	0.196771	1.000000	0.993471	1.000000		1.000000	1.000000	1.000000
Y67	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
DAR12	1.000000	1.000000	1.000000	**0.025183**	1.000000	0.171744	1.000000	1.000000	1.000000		1.000000
Y66	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Discussion

Karyological variability in the southern marginal populations. — Ecologically marginal environments (*e.g*. mountain tops, brackish-water estuaries, polar and other extreme habitats) are important sources of biodiversity, especially in the face of climate change (Hampe and Petit 2005; Eckert *et al*. 2008). The populations that inhabit such environments are typically more dynamic compared to those that inhabit the areas, which are located near the centre of species distribution, and are growing in more stable environmental conditions. At the same time, the intensive processes of raciation and speciation may occur in plant populations that are under the influence of an unfavourable environment. The genomic changes that occur during these processes can be the basis for understanding genome evolution and for predicting the response of a genome to environmental changes (Belyayev and Raskina 2013). Genomic and phenotypic-orientated studies of the representatives of *D*. *antarctica* from the marginal southern populations from the maritime Antarctic provide a unique opportunity to examine the concept of a 'species range'.

We found that most of the *D*. *antarctica* plants from the southern edge of the range that were studied are diploids and have a typical chromosome number, *i.e*. 2n=26. This confirms the chromosome number of this species that has been reported by other authors (Moore 1967; Cardone *et al*. 2009; Amosova *et al*. 2015; Gonzalez *et al*. 2016). FISH analysis allowed us to localise the 5S rRNA and 35S rRNA gene loci in the karyotypes of plants with different chromosome numbers. In the hypotriploid, six sites of 35S rDNA were observed, which is about 1.5 times greater than that of the diploid genotypes. These findings support the hypotriploid nature of the Y66 plants that had been inferred from their chromosome number. Surprisingly, an increase in the number of 5S rDNA sites was not proportional to an increase in the number of chromosome sets in the hypotriploid genotype, since only 14 sites were observed rather than the expected 15 (Fig. 1). This might indicate the presence of chromosomal rearrangements or the loss of one chromosome in the complement. In fact, a centric fusion translocation (Robertsonian rearrangement) between two homologous chromosomes of 12 pair was reported for the hypotriploid genotype of *D*. *antarctica* (Amosova *et al*. 2015).

The distribution of telomeric and centromeric FISH signals was typical on all of the chromosomes of the plants that were investigated (Fig. 2). However, in a study of *D*. *antarctica* from the maritime Antarctic, Amosova *et al*. (2015) not only revealed the presence of telomeric signals at the chromosome termini, but also at numerous interstitial sites in the proximal region of the long arm on the largest chromosome in the complement. This indicates that the hypotriploid could have arisen due to an interruption of meiosis or as a result of chromosome rearrangements, which are common in plants growing

in adverse environmental conditions (Madlung and Comai 2004; Houben *et al*. 2013). Furthermore, triploid (*D. alpina*, 2n=39) and tetraploid (*D. brevifolia, D. mackenzieana, D. mildbraedii, D. orientalis*, 2n=52) forms have been reported for some species of the *Deschampsia* genus (Chiapella 2007). The tetraploid cytotype of *D. antarctica* (2n=52) was reported for a population that is located at the northern boundary of the species range in Patagonia (Chubut, Argentina) (Gonzalez *et al*. 2016). Thus, the occurrence of different cytotypes within the species may be a common phenomenon for this genus.

FISH analysis revealed the presence of centromeric and telomeric repeats in the B chromosome of the DAR12 genotype (Fig. 2A). These results proved the structural integrity of the additional chromosome in this genotype, which otherwise could be considered to be a mitotically unstable chromosome fragment (Dhar *et al*. 2002; Jones *et al*. 2008; Houben *et al*. 2013). The occurrence of B chromosomes are also common for other species in the *Deschampsia* genus, in particular for *D. caespitosa* (2n=26+0–2B) and *D. wibeliana* (2n=26+0–5B) (Goldblatt and Johnson 2000). It is known that additional chromosomes are dispensable and can be present or absent among individuals of the same population (Dhar *et al*. 2002). Usually, such chromosomes are found in organisms that dwell in suboptimal and stress conditions (Camacho *et al*. 2000; Jones *et al*. 2008; Kunakh, 2010; Datta *et al*. 2016), and may increase the adaptive capacity of plants to survive in harsh environments (Plowman and Bougourd 1994; Rosato *et al*. 1998).

Spontaneous aneuploidy, triploidy and additional chromosomes have also been reported for marginal populations of *Aegilops speltoides* (Belyayev and Raskina 2013). Those authors implied that although aneuploidy for individual chromosomes may occur as a result of gene mutations and/or meiotic disorders, a spontaneous non-disjunction of the entire chromosome complement can also occur. They suggested that the increased ratio of self-pollination and inbreeding in stressful environments can lead to chromosomal rearrangements.

Genome size of *D. antarctica*. — Data about the nuclear DNA content of *D. antarctica* from the marginal populations at the southern range limit were very limited and were confined only to the report of Bennett *et al*. (1982). Using Feulgen staining, those authors revealed that the amount of DNA of the diploid plants from Galindez Island was 9.95 pg/2C. However, the genome size of other representatives of the genus, such as *D. caespitosa* from the British Islands (18.0 pg/2C; Bennett *et al*. 1982), *D. caespitosa* 2n=26 (10.43 pg/2C), *D. chapmanii* 2n=26 (11.05 pg/2C) and *D. tenella* 2n=26 from New Zealand (10.07 pg/2C) was determined (Murray *et al*. 2005).

Using flow cytometry technique, we found that the values of the 2C DNA content were on average 10.88 pg for the diploids and 16.46 pg for the hypotriploid (Table 2, Fig. 3). The slight differences in genome size between the diploid genotypes (10.77 pg/2C–11.02 pg/2C) can be explained

by chromosome polymorphisms, but can also be due to an observation error that is caused, for example, by the analysis of a very small plant population. The possible measurement errors were reduced using the same flow cytometer, a standardised procedure for the sample preparation and the relevant number of biological replicates for each individual. However, the observed variation between the *D. antarctica* genotypes can also be attributed to their genetic diversity, which is connected with different geographical origin, different amount of heterochromatin and/or various spontaneous chromosome aberrations, for example. Such a variation in the genome size has been observed, for instance, in different accessions of *A. thaliana* (Schmuths *et al*. 2004) or *Claytonia perfoliata* (McIntyre 2012). Moreover, the size of the monoploid genome (C_x values) was very similar for the genotypes, and no significant differences were observed between the diploids compared to the hypotriploid. That seems to indicate the absence of large genomic rearrangements (*e.g*. the loss or duplication of chromosome fragments) during the formation of the hypotriploid genotype, and that the larger genome size of this genotype is primarily the result of an increase in the chromosome number (Table 2).

Leaf length variation. — In order to determine the possible correlation between the genetic and selected phenotypic characteristics, the leaf length was measured in different chromosomal forms of *D. antarctica*. The highest value was observed for the plants of the DAR12 genotype (9.56 cm). An ANOVA statistical analysis confirmed that DAR12 was the most different from the others in its leaf length (Table 3). Long leaves were also observed in the diploids (L59, G/D12-1, R35) and the hypotriploid (Y66), which had leaf lengths of 8.08 cm, 8.01 cm, 7.96 cm and 7.97 cm, respectively (Table 2 and Table 3). It is known that leaf length is a genetically-determined trait, which can depend on the ploidy level, as has been shown for both eudicotyledon and monocotyledon species (Stebbins 1971; Madlung 2013). For example, an analysis of the closely related grasses *Lolium multiflorum* and *L. perenne* showed that their tetraploid cultivars had significantly longer leaves than the diploid ones. As was mentioned by Sugiyama (2005), the increase in leaf length in the *Lolium* polyploids primarily resulted from an increase in the cell elongation rate, and not from the duration of the period of leaf elongation. Although leaf length is a relatively stable characteristic, many species exhibit variations in leaf size in response to unfavourable environmental conditions (Tsukaya 2005). Considerable dissimilarities in the size, arrangement, shape and colour of leaves of genetically similar *D. antarctica* plants from the Admiralty Bay (King George Island, maritime Antarctic) were observed by Chwedorzewska *et al*. (2008). Those authors revealed that the anatomical variation can be due to an environmentally driven phenotypic plasticity. Additionally, morphological differences that were connected with the places of origin were observed for *Saxifraga oppositifolia* (Brysting *et al*. 1996) and the arctic species *Saxifraga caespitosa* (Chwedorzewska *et al*. 2005).

Phenolic and flavonoid content. — In this study, we could not find any statistically significant differences in the phenolic content between the different chromosomal forms of *D. antarctica* (Tables 2 and 5, Fig. 4A). However, the DAR12 and L59 plants differed from the others in the flavonoid content (Tables 2 and 6, Fig. 4B). The DAR12 plants had the lowest amount of flavonoid compounds (1.22 mg/g), while L59 had the highest amount (4.67 mg/g). We assume that such differences have an accidental character and are not genetically driven. The phenolic content may depend on environmental conditions such as the photoperiod, altitude, level of UV radiation, humidity and temperature (Day *et al*. 2001; Ruhland *et al*. 2005; Jaakola and Hohtola 2010). It is known that the concentrations of flavonoids such as orientin and luteolin increased in *D. antarctica* plants that were under the influence of UV radiation (Day *et al*. 2001; Sequeida *et al*. 2012). It is worth noting that all of the plants in our research were cultivated under the same conditions and were not subjected to any external stresses that could stimulate the accumulation of variable amounts of secondary metabolites. It is also possible that differences in the accumulation of these metabolites between plants occurred under the impact of *in vitro* conditions, which can be associated with stressful and mutagenic effects (Madlung and Comai 2004). Therefore, our findings may suggest that the total phenolic and flavonoid content in *D. antarctica* depends on growth conditions rather than on their genotype characteristics.

Conclusions

The results of this study suggest that the *D. antarctica* plants, which originated from the marginal southern populations are characterised by heterogeneity in the molecular cytogenetic, morphometric and biochemical parameters. Most probably, the variation in the leaf length and the amount of secondary metabolites depends on the impact of growth conditions rather than genotype characteristics, because no statistical correlations between the genome size and leaf length as well as the genome size and accumulation of phenolic and flavonoid compounds were observed. The ecological and adaptive significance of the different *D. antarctica* chromosomal forms is still uncertain. It seems likely that the period of existence of such plants is not sufficient for the formation of new races or cytotypes, but this requires further investigations.

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