Habitat and leaf cytogenetic characteristics of Deschampsia antarctica Desv. in the Maritime Antarctica

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Abstract

Antarctic hairgrass (Deschampsia antarctica Desv.) was studied in the Maritime Antarctica with respect to general ecological characteristics, soil conditions, viral contamination, cell nucleus area, and relative DNA content. Material was gathered in six localities that were highly diverse in terms of the nature of soil-like substrata, presence of viral antigen determinants, and the average nucleus area and relative DNA content in leaf epidermis and parenchyma cells. Our results show that Antarctic hairgrass lives upon soils that are variable with respect to trace elements, pH, and other soil characteristics. The hairgrass is susceptible to a number of viruses, and shows substantial variation in DNA content and nucleus size. © 2007 Elsevier B.V. and NIPR. All rights reserved.

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1. Introduction

The Antarctic’s natural flora of vascular plants comprises by only two species: Antarctic hairgrass Deschampsia antarctica Desv. and Antarctic pearlwort Colobanthus quitensis (Kunth.) Bartl. (Alberdi et al., 2002; Fowbert and Lewis Smith, 1994; Lewis Smith, 1994, 1984). These species inhabit the western shore of the Antarctic Peninsula and adjacent islands (Edwards, 1972). The maritime Antarctic zone is characterized by temperatures below freezing throughout most of the year. It is only during the short Antarctic summer that the air temperature rises above 0 °C, rarely reaching 10—15 °C (Lewis Smith, 2003). It remains unclear as to why only these two species of vascular plants have successfully colonized Antarctica, when more than 100 different species of vascular plants occur in the corresponding Arctic latitudes. None of the previous studies of D. antarctica have provided an answer to this question (Lewis Smith, 2003).

The tissues of plants adapted to the extreme conditions of Arctic and mountainous regions often commonly contain polyploid cells (Hagerup, 1932;
Stebbins, 1985), and an increase in ploidy level is sometimes accompanied by increased nucleus volume (see the study by Maletskii (2005) on genotype—phenotype interaction, in particular the effect of ploidy on cell parameters such as nucleus area). A similar adaptive mechanism is also expected to exist in the Antarctic plants. The studies undertaken by Strogonov (1973)—a study on the metabolic adaptations of plants to soil salinity, particularly its influence on ploidy—and Nkongolo et al. (2001) demonstrate that polyploidy is induced by an increased level of certain cations and trace elements in the soil, as well as by viral contamination. Neither of these factors has been an-
duced by viral contamination.

In this context, we carried out detailed soil analyses in areas in which Antarctic hairgrass and Antarctic peatmoss are found, and analyzed plant samples for viral contamination. Neither of these factors has been analyzed previously in this context (Lewis Smith, 2003), which is of special interest given the increasing scale of human impact in Antarctica. We investigated the nucleus area and relative DNA content in the leaf tissues of D. antarctica tufts collected from several scattered locations within the Maritime Antarctica. The same locations were investigated with respect to vegetation conditions, including soil composition and plant viral contamination.

2. Materials and methods

2.1. Study areas

Tufts of hairgrass were collected during the 9th Ukrainian Antarctic Expedition (March, 2005) at six locations in the Maritime Antarctica (Fig. 1): Petermann Island (Site 1); Berthellot Island (Site 2); Cape Rasmussen, on the continental part of the peninsula (Site 3); Galindez Island (Site 4); and two sites on Yalour Island (Sites 5 and 6). The geographic coordinates of the sampling locations were determined using GPS. The habitats and population states of hairgrass were described in terms of the following phytocenological parameters: hill slope and exposure, total vegetation cover, and vegetation cover of hairgrass. We also determined the percentage of fruiting and dying hairgrass, where each root tuft was taken as an individual plant of D. antarctica. The phytocenological characteristics of the studied habitats were as follows.

Site 1. Petermann Island, S 65°10.453', W 64°08.452'. The study area (size, 10 m²) was located on the middle part of a hill (eastern exposure, 20–40° inclination), dominated by rock crevices approximately about 15 cm wide and 1 m long. There were no fruiting or dying specimens at this site. Total vegetation cover was 60%, made up of mosses (50%), briophytes (5%) and D. antarctica (5%). Above there are colonies of Pygoscelis papua and Pygoscelis adeliae located up-slope of the site supplying the tufts with guano.

Site 2. Berthellot Island, S 65°19.731', W 64°08.613'. The study area (size, 8 m²) was located on a west-facing slope inclined at 20–40°. Total vegetation cover was 50%, made up of briophytes (30%) and D. antarctica (20%). Of the D. antarctica specimens, 60% were fruiting and 30% were dying. The island is inhabited by Phalacrocorax atriceps and P. adeliae.

Site 3. Cape Rasmussen, S 65°14.819', W 64°05.156'. The study area (size, 10 m²) was located on a west-facing slope inclined at 10°. Total vegetation cover was 40%, made up of briophytes (30%) and D. antarctica (10%). Of the D. antarctica specimens, 20% were fruiting and 40% were dying. The study area is a nesting site for Catharacta maccormicki and Larus dominicanus.

Site 4. Galindez Island, S 65°14.783', W 64°14.799'. The study area (size, 10 m²) was located on upper part of an east-facing slope inclined at 10°. Total vegetation cover was 25%, made up of briophytes (10%) and D. antarctica (15%). Of the D. antarctica specimens, 10% were fruiting and 50% were dying. The island is a nesting place for C. maccormicki and Oceanites oceanicus.

Site 5. Yalour Island, S 65°14.139', W 64°09.330'. The first study area on this island (size, 6 m²) was located on the upper part of a west-facing slope inclined at 20°. Total vegetation cover was 45%, made up of briophytes (25%) and D. antarctica (20%). Of the D. antarctica specimens, 40% were fruiting and 30% were dying. The island is inhabited by P. atriceps and P. adeliae.

Site 6. Yalour Island, S 65°14.039', W 64°09.761'. The second study area on this island (size, 12 m²) was located on the upper part of a north-facing slope inclined at 30°. Total vegetation cover was 10%, made up of briophytes (1%) and D. antarctica (9%); we found no dying plants of D. antarctica. The island is a nesting site for P. papua and P. atriceps.

From every location listed, we collected more than one visibly undamaged generative tuft. The plants were delivered to Kyiv within a 1 week of sampling, transported in impenetrable paper bags. Leaves, floriferous shoots and roots were fixed for cytogenetic analysis, while the remaining biomass was used to determine viral contamination. The soil under each tuft was also analyzed for trace elements and content of biogens.
2.2. Soil analysis

Analyses of exchange acidity and the total amount of carbon, nitrogen, and phosphorus were carried out using common techniques of soil analysis (see Bulygin (2000) for a review of methods commonly employed in the post-Soviet era). Because the analyzed soil substrate was essentially composed of partially decomposed plant remains, analyses of potassium, microelements, and trace elements were performed using analytical methods developed for organic materials derived from plants. The soil acidity was evaluated by ionometry of the extract after treating the substrate with potassium chloride solution. The carbon content was determined according to Bulygin (2000), the procedure involving the oxidation of organic carbon with potassium dichromate in an acidic environment and subsequent titration of residual potassium dichromate by Mohr’s salt solution.

The amounts of nitrogen, phosphorus, and potassium were determined after wet ashing of the substrate in the presence of concentrated sulfuric and chloric acids. Nitrogen and phosphorus were determined by photocolorimetry, with nitrogen determined by iodide mercury complex stained with Nessler reagent, and phosphorus by the stained complex of phosphoric acid and ammonium molybate in the presence of sodium chloride. Potassium concentrations were determined by flame photometry. Total contents of trace elements and microelements were determined by atomic absorption spectrometry after dry ashing of soil substrate in a muffle furnace (Bulygin, 2000).

Fig. 1. Locations of sample sites for D. antarctica tufts in the Maritime Antarctica.
We compared the content of biogenic elements in the substrata from the six study sites with those of peat, bird guano and Ukrainian soils (the data for bird guano and Ukrainian soils were sourced from Dmytrenko’s manual on soil fertilization in Ukraine (Dmytrenko, 1987). The concentrations of trace elements and other microelements in the sampled Antarctic soils were also compared to those of penguin guano reported by Andreev et al. (2004) and the Clark values (mg/kg) of microelements and trace elements reported in Vinogradov’s classic work on soil science (Vinogradov, 1957).

2.3. Analysis of viral contamination

Five sites were selected in terms of investigating viral contamination: Petermann Island, Berthellot Island, Cape Rasmussen, and Yalour Island (Sites 5 and 6). These sites were chosen because human impact was less than in Galindez Island, where Vernadsky Station (Ukraine) is located.

Viral antigens were detected using indirect and sandwich immunoferment analyses (Crowther, 1995; Catty, 1991; Hill, 1984). We used polyclonal rabbit antibodies to Tobacco mosaic virus (TMV) and Cucumber green mottle mosaic virus (CGMMV), generated at the Department of Virology of Taras Shevchenko Kyiv National University (Ukraine). Potato virus X (PVX), Cucumber mosaic virus (CMV), Alfalfa mosaic virus (AMV), and Tomato spotted wilt virus (TSWV) were detected using antisera from Aschersleben, Germany. The results were detected using an automatic ELISA-reader (Dynametech) at 405 nm wavelength ($E_{405}$). Signals that were at least double that of the negative control (sap of a healthy plant) were considered to be positive. To test for statistical significance, analyses were repeated three times and the standard deviation calculated (Crowther, 1995).

2.4. Cytogenetics

The basal third of a leaf and the rootlets of a visibly undamaged mature generative plant were used for the DNA cytophotometry assays. After fixation in 100% alcohol-acetic acid (3:1 v/v), the material was stored in 70% alcohol. Leaf parenchyma and epidermis cells (Fig. 2) were used to calculate average nucleus area and the relative DNA content (RDC). Cells were stained using the Feulgen technique (Kiernon, 1990).

We employed the green light filter of an optical microscope and the red PAL-N filter of a video adapter (Konus Asus V 3000) in analyzing four samples from each site, with each sample comprising 25 nuclei. The analysis was undertaken using a digital camera (Samsung CCD SAC-410 PA) with an Asus V 3000 video adaptor. The nucleus area within the images was measured in pixel units and then converted into SI units using an experimentally derived formula. RDC was estimated by comparing the staining intensity of the nuclei to that of the anaphase nuclei of *D. antarctica* rootlet cells (where the quantity of DNA was assumed to be 4C). Distribution curves were plotted for each parameter over all sites. We applied the median test to compare the curves and to determine the confidence intervals for correlation values (Pollard, 1982).

3. Results

In the study area, the Antarctic hairgrass, a turfy herbaceous plant, occupies considerable territory and grows in dense clonal tufts without breaking gaps between shoots. The nature of the habitats in this study varied widely in terms of slope orientation and the presence of bryophytes and lichens in the cenoses.

Fig. 2. Leaf cells of *D. antarctica* collected from Galindez Island: (a) epidermis, (b) parenchyma.
The soil samples showed considerable variations in pH and biogen content. pH varied from 6.85 (Yalour Island, Site 5) to 3.80 (Cape Rasmussen) (Table 1). Notably, all tufts were found near guano sources, suggesting that guano may play a role in determining the distribution of the plants. The spatial heterogeneity in pH might also arise from the irregular dispersal of decomposing bryophytes. The investigated substrata, however, can be classified as more or less acidic: Petermann Island, Berthellot Island, and Cape Rasmussen all contained relatively acidic substrata, while less acidic substrata were found on Galindez Island and both sites on Yalour Island.

The measured concentrations of biogenic elements varied among the different sites by a factor of between 2 and 3. Phosphorus concentrations varied among the different sites by a factor of approximately 11 (Table 1). Antarctic soils are rich in carbon, nitrogen, and phosphorus, but poor in potassium, meaning that the amounts of these elements in the analyzed soils were substantially different to those in common soils (e.g. Ukrainian soils); therefore, the substrata can be referred to as soils only conditionally.

Trace elements and microelements also showed substantial variations in their concentrations (Table 2), exceeding the range of variation recorded for biogens. Among the different sites, zinc concentrations varied by a factor of 40, while iron concentrations varied by a factor of 60. The values obtained for other elements did not exceed the Clark values for Ukrainian soils, except for copper (Petermann Island, Galindez Island, and Yalour Island Site 6) and cadmium (most substantial value exceeded the Clark value by a factor of 77 at Yalour Island Site 5; Galindez Island, and Yalour Island Site 6). The concentrations of some of the analyzed elements were higher in Antarctic soils than in penguin guano (nickel at all locations, and iron at all locations excluding Yalour Island Site 5), suggesting that these elements are largely derived from rocks (Juchnowicz-Bierbasz and Rakusa-Suszczewski, 2002; Milne and Millar, 1989). The concentration of zinc, however, was higher in guano than in soils. Copper amounts were comparable in the guano and the soils of Petermann Island, Galindez Island, and Yalour Island Site 6; however, conservations recorded at the other sites were lower than concentrations in the guano.

The chemistry of the soil-like substrata beneath D. antarctica tufts shows marked variation among the analyzed habitats, possibly related to variations in the migration of elements derived from rocks, guano and turf input.

Tests for viral contamination revealed the presence of viral antigens of different taxa (Fig. 3). CGMMV or a serologically related virus was found in the samples from Cape Rasmussen and Berthellot Island (the ratio of the signal to negative control was higher than 3). We detected neither TMV nor PVX. We did not detect any virus serologically related to these two viruses. Patterns of other investigated viruses (CMV, AMV, and TSWV) are shown in Fig. 4.
Notably, TSWV or a serologically related virus was found almost in every analyzed sample, and CMV was detected in samples from Yalour Island Site 6, Berthellot Island and Galindez Island. These findings demonstrate that the six locations show marked differences in terms of viral antigens.

Assessments of the nuclei area and the RDC in the epidermis and parenchyma cells of leaves also revealed spatial heterogeneity among the tufts. Fig. 5 shows class distribution curves for these parameters determined at all localities; some of the curves are visibly asymmetrical.

Pairwise comparison of the frequency curves for nucleus area (Fig. 5) revealed significant differences on the basis of the median test. Ten of the $\chi^2$-test values for individual pairs of class distributions for epidermis, eight for parenchyma exceeded 3.84 ($\alpha = 5\%$), indicating significant differences (Fig. 5a,c).

The distributions are notably heterogeneous. Further analysis revealed that tufts at every studied site differed significantly in terms of the nucleus area in both epidermis and parenchyma cells. It is interesting that plants from both Site 5 and Site 6 on Yalour Island were heterogeneous; this trait appeared to be relatively variable.

As expected, there were significant differences in the RDC distributions in both tissues from plants from most locations: 10 of the $\chi^2$-test values for individual pairs of class distributions for epidermis cells and 11 values for parenchyma cells exceeded 3.84 ($\alpha = 5\%$) (Fig. 5b,d).

4. Discussion

Recently molecular-biology studies have demonstrated the low genotype heterogeneity of *D. antarctica* from different regions of the Maritime Antarctica (Chwedorzewska et al., 2004; Holderegger et al., 2003; van der Wouw et al., 2006). However, in the present study we found a relatively high degree of heterogeneity among *D. antarctica* collected from six locations at a higher level of organization, namely heterogeneity of the cytogenetic parameters of relative DNA content and nucleus area. The present data are consistent with those obtained for the Arctic and mountainous regions.
Wulf's (1937) classic paper discusses the link between polyploidy and geographic distribution of the plants, while Krogulevitch and Rostovceva (1984) studied the distribution of polyploids in Stanove Nagirya, a highland region in Russia, and showed a common tendency towards increased percentage of polyploids in mountainous regions. It is commonly accepted that a change in DNA quantity can arise from a number of different impacts (Nkongolo et al., 2001; Strogonov, 1973; Topchiy and Dulevitch, 1977), which can be classified both internal and external. The substratum specifics could be considered as an external impact; the substrata showed considerable diversity in the hairgrass locations. In contrast, the observed difference in viral contamination can be considered an internal factor of heterogeneity; therefore, the obtained cytogenetic variability is not unexpected, and can result from both intended and external factors. There is probably an additional factor in explaining the ecological plasticity of the species, arising from the fact that hairgrass must adapt to available habitat rather than selecting favorable habitats. Heterogeneity is found among tufts collected from several sites within the Maritime Antarctica, but given that the plant is dispersed mostly by vegetative propagules, heterogeneity may well be found for different populations. Unfortunately, only morphological and molecular biological data are available in terms of assessing intrapopulation heterogeneity (Chwedorzewska et al., 2004; Holderegger et al., 2003; Romer et al., 1999); further research is required to cytogenetic parameters.

We demonstrated a high degree of heterogeneity in the relative DNA content of leaf tissues of the Antarctic hairgrass, similar to data obtained for Arctic and mountainous plants. The lability of the polyploidy adaptive mechanism of *D. antarctica* probably develops in...
response to the characteristics of the habitat and/or viral contamination.

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