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Populations of Antarctic Hairgrass (*Deschampsia antarctica*) Show Low Genetic Diversity

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Abstract

Populations of the only two flowering plants native to the Antarctic have recently increased in number and size possibly due to climate warming. We have undertaken a preliminary study of the population genetics of one of these species by surveying variation in amplified fragment length polymorphisms (AFLP) within the Antarctic Hairgrass, *Deschampsia antarctica*. Populations of *D. antarctica* from two widely separated regions of the maritime Antarctic, namely Signy Island in the north and Léonie Islands 1350 km farther south, were characterized by low genetic diversity (only 15.95% of total genetic variation found within populations). Populations from the northern and southern maritime Antarctic were genetically distinct from each other ($F_{CT} = 37.10\%$), and low levels of historical gene flow occurred among them ($N_{m} = 0.05$). This genetic structure suggests that new populations of *D. antarctica* are founded by one or few individuals, which mainly reproduce by self-fertilization and/or vegetative propagation. Vegetative reproduction and selfing are, therefore, likely to have been key factors in the establishment of *D. antarctica* at new sites in the Antarctic during recent years.

Introduction

Only two species of flowering plants, the Antarctic Hairgrass, *Deschampsia antarctica* Desv. (Poaceae) and the Antarctic Pearlwort, *Colobanthus quitensis* (Kunth.) Barl. (Caryophyllaceae) have succeeded in colonizing the Antarctic during the Holocene (Lewis Smith, 1984; Chapman, 1996). These two species occur on many of the southern oceanic islands, but, within the antarctic biome, are only found on the Antarctic Peninsula and offshore islands, usually defined as the maritime Antarctic (Lewis Smith, 1984). Ecophysiological studies have shown that both species are sensitive to the rapid climate warming trend ($2.5^\circ C$ in summer air temperatures during the last 50 yr; Oppenheimer, 1998) and to the biologically damaging high UV-B radiation in spring (two fold increase) observed in the Antarctic (Day et al., 1999).

Throughout its antarctic distribution, the freeze-tolerant grass *D. antarctica* (Bravo et al., 2001) exhibits, at least in some years, successful sexual reproduction (Lewis Smith, 1994; Fowbert and Lewis Smith, 1994), and this appears to have been enhanced by regional climate warming (Lewis Smith, in press). *Deschampsia antarctica* is known to form a substantial buried seed population (McGraw and Day, 1997), while dispersal of detached tillers is important at the local scale (Convey, 1996). Experimental studies on the effects of temperature and UV-B radiation on growth of *D. antarctica* have produced conflicting results. Increased temperature has either reduced (Day et al., 1999) or stimulated (Xiong et al., 2000) vegetative growth of *D. antarctica*, while exposure to UV-B radiation reduced growth in one study (Ruhland and Day, 2000) and improved it in another (Lu et al., 2001). However, both seed production and seedling establishment increased during the current trend of climate warming (Fowbert and Lewis Smith, 1994; Day et al., 1999). *Deschampsia antarctica* is self-compatible, and its flowers often remain closed, such that self-pollination is enforced through cleistogamy (Moore, 1983). In climatically favorable years, production of viable seed through selfing or outcrossing occurs even at the species’ southernmost localities ($68^\circ S$; Fowbert and Lewis Smith, 1994; Convey, 1996).

Antarctic populations of *D. antarctica* have recently increased in number and size, which has been related to climate change (Lewis Smith, 1994; Walther et al., 2002). To estimate the relative importance of sexual reproduction and/or vegetative propagation in the observed increased colonization rate and enhanced population growth of *D. antarctica*, we carried out a preliminary population molecular genetic study by surveying variation in amplified fragment length polymorphisms (AFLP) within and among several antarctic populations of this grass species. AFLPs sample predominantly the nuclear genome and usually provide high levels of neutral polymorphisms (Mueller and Wolfenbarger, 1999). Thus, this technique of genetic fingerprinting is well suited for investigating demographic and historical processes at the level of populations. The present study is the first to report on genetic diversity in antarctic flowering plants.

Materials and Methods

We collected widely scattered living individuals of *D. antarctica* from seven populations on Signy Island in the northern maritime Antarctic ($60^\circ 43' S, 45^\circ 38' W$; Fig. 1). These populations were separated from each other by 0.5 to 4 km. We also sampled one population of *D. antarctica* from each of Anchorage Island, Lagoon Island, and Léonie Island situated in the southern maritime Antarctic ($67^\circ 36' S, 68^\circ 17'$), more than 1350 km distant from Signy Island (Fig. 1). Each of the latter three islands, called the Léonie Islands, was 1 to 3 km apart. Plants were taken to the University of St. Andrews, U.K., potted in domestic compost M2 and cultivated in a growth chamber (12 h light at 9°C; 12 h night at 5°C; light intensity: 60 $\mu$M m$^{-2}$ s$^{-1}$). Fresh plant material was sampled from these individuals and sent to the University of Zurich, Switzerland, for AFLP analysis.
A total of 41 individuals from 10 populations (two to six individuals per population on Signy island and six individuals from each of the three southern maritime islands) was investigated. Genomic DNA was extracted with a DNeasy kit (Qiagen) and cleaned with a Wizard DNA clean up kit (Promega). The AFLP protocol followed Stehlik et al. (2001). Three selective AFLP primer combinations were used: E⁻¹ACC/M⁻¹CTT, E⁻¹AGC/M⁻¹CAG, and E⁻¹AGC/M⁻¹CTT (preselective primer E: 5'-GACTGCGTACCAATTCA-3'; preselective primer M: 5'-GATGAGTCTGAGTAAG-3'). The PCR products were run together with an internal size standard (ROX 500; PE Biosystems) on an ABI 377 automated sequencer (PE Biosystems) in 6% polyacrylamide gels using GENESCAN 3.1 (PE Biosystems). Reproducibility was tested by repeating AFLP procedures in case of uncertain individual electropherograms and by running PCR products on different gels. Fragment lengths were determined and transcribed into a binary matrix of band presence and absence with GENOTYPER 2.1 (PE Biosystems).

Genetic variance components either within or among populations of *D. antarctica* in the northern and/or the southern maritime Antarctic were determined with a set of analyses of molecular variance (AMOVA; Excoffier et al., 1992) using WINAMOVA 1.55 (Excoffier, 1993). Significance testing relied on 999 permutations. To assess evolutionary distances among genotypes, a minimum spanning tree was constructed with NTSYS-PC 2.02i (Rohlf, 1998). Historical gene flow was determined as the number of migrants per generation ($N_{m} = [1/F_{ST} - 1]/4$; Slatkin, 1987) using the respective $F_{ST}$-values from the AMOVA analyses (Excoffier, 1993; Table 1).

### Results

Antarctic populations of *D. antarctica* were characterized by overall low genetic diversities. Only 23 of the 182 scored AFLP fragments were polymorphic (13%). The northern and southern Antarctic populations harbored, on average, only 1.20 and 1.67 genotypes, respectively. Thus, levels of genetic variation within populations were low (15.95% in the whole data set, 85.05% in the northern region with regard to genetic variation among local populations. On the northern Signy Island, the level of genetic variation among local populations was relatively high (88.86%, $P < 0.001$; Table 1), whereas genetic variation among the local populations on the more southern Anchorage Island, Léonie Island, and Lagoon Island was much lower (45.00%, $P = 0.017$; Table 1). Estimates of historical gene flow among populations were low both within the northern ($N_{m} = 0.03$) and the southern ($N_{m} = 0.31$) Antarctic regions.

A large proportion of the total genetic variation was due to differences found between the southern and northern Antarctic region (37.10%, $P < 0.01$; Table 1). No genotype occurred in both regions (Fig. 2). The minimum spanning tree, showing the evolutionary relatedness of the seven multilocus genotypes detected, mainly separated the genotypes according to their geographic origin. Nevertheless, not all northern Antarctic genotypes clustered together in the minimum spanning tree (Fig. 2). One genotype was found on all three southern Antarctic islands, whereas no ubiquitous genotype was present in the northern Antarctic populations on Signy Island (Fig. 2). Gene flow in the whole data set was estimated to be low ($N_{m} = 0.05$).

### Discussion

Although *D. antarctica* has recently undergone substantial and rapid population increase in size and number, probably in response to
regional climate warming in the Antarctic (Fowbert and Smith, 1994; Lewis Smith, 1994; Day et al., 1999), there has apparently been no population genetic study of this species. AFLP markers have generally high-resolution power allowing the detection of different genetic fingerprints or genotypes (Mueller and Wolfenbarger, 1999), even if only few individuals per population are investigated. Nevertheless, the populations studied were all characterized by low levels of genetic variation in AFLPs (Table 1), although one of the southern populations harbored three different AFLP genotypes and was the most diverse population in the analysis (Fig. 2). Because *D. antarctica* is self-compatible and often cleistogamous (Moore, 1983), seed production might often result from self-fertilization. In addition, vegetative propagation is believed to be important, and local dispersal of tillers of *D. antarctica* is enhanced by birds sampling nest material (Komárková et al., 1985). Indeed, the species' distribution in the maritime Antarctic may be largely due to progressive short-distance dispersal by birds, especially skuas and gulls, introducing the grass to new sites (Lewis Smith, in press). Our finding of low genetic diversity within populations support the hypothesis that the establishment of new populations and increases in population size result predominantly from vegetative propagation and/or, in warmer summers, from recruitment of seeds produced by self-fertilization (Edwards, 1974). In contrast to what we have found for *D. antarctica*, the majority of studies on arctic and/or alpine species have been conducted on mainly outcrossing species showing that almost all individuals possess unique nuclear genotypes (see references in Stehlik et al, 2001; Abbott and Brochmann, 2003).

All estimates of historical gene flow in the present study were low. Gene flow values of $N_m < 1$ indicate that migration among populations is so small that it will not counteract the effects of random genetic drift (Slatkin, 1987). Our results thus suggest that *D. antarctica* is normally characterized by low capacity for long-distance dispersal, which might explain why the species is reported to be slow to colonize distant, but seemingly suitable habitat sites such as recently deglaciated areas (Fowbert and Lewis Smith, 1994; Convey, 1996). Low gene flow among populations after colonization will also restrict the accumulation of genetic diversity within populations as found in the present study. Hence, populations of *D. antarctica* from Signy Island in the northern maritime Antarctic were genetically highly isolated from each other ($F_{ST} = 88.86\%$), despite the small spatial distance of only several hundred meters to a few kilometers separating them. In fact, one genotype from Signy Island was evolutionary more distant from the other genotypes found on the island than it was from genotypes sampled from southern antarctic islands (Fig. 2).

One common genotype (Fig. 2) was found in all three populations investigated in the southern maritime Antarctic. The Léonie Island population contained two additional genotypes and might, therefore, have served as a source for the colonization of the two nearby Lagoon and Anchorage Islands. Each island has a large breeding population of skuas, which are probably responsible for the local or even regional dispersal of the grass. Such long-distance dispersal is nevertheless rare in *D. antarctica* as only the common genotype occurred in more than one population (Fig. 2). Long-distance dispersal has often been invoked to explain the amphiatlantic distributions of several arctic plant species, and molecular-genetic studies have recently contributed unequivocal proof of this (Abbott and Brochmann, 2003). However, local populations of arctic flowering plants are usually characterized by rather low among population genetic variation at a regional scale, which is in marked contrast to the pattern found in *D. antarctica*.

In conclusion, this preliminary assessment suggests that new populations of *D. antarctica* are founded by one or few individuals and that vegetative reproduction and selfing are likely to have been key factors in the species’ establishment at new sites in the Antarctic during recent years. The AFLP method, which we have applied here, could also be used to elucidate past events such as the colonization of the subarctic islands and the Antarctic Peninsula. A comparison of genetic variation in antarctic populations with that existing within and among populations from the subantarctic islands would be most worthwhile in this respect.

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