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Somatic mutation and the Antarctic ozone hole

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Somatic mutation and the Antarctic ozone hole

Abstract

1 Previous studies of Antarctic clonal moss populations using RAPD markers have reported extraordinarily high levels of genetic variation. This has been claimed to reflect somatic mutation, possibly resulting from elevated UV-B radiation. 2 Our study used microsatellite markers to compare the genetic variation present within continental Antarctic, sub-Antarctic and temperate populations of the moss *Ceratodon purpureus*. 3 In contrast to the RAPD studies, microsatellite data revealed that *C. purpureus* populations from continental Antarctica display less intra-population genetic diversity than populations from a range of temperate and sub-Antarctic sites. 4 Analysis of Molecular Variation (AMOVA) revealed that populations within the Windmill Islands region of Antarctica were more genetically differentiated than populations spread among more widely separated temperate regions. 5 Synthesis Our data provide no evidence of elevated mutation rates in the Antarctic, and imply climate change will present ongoing challenges for continental Antarctic moss populations that appear weakly interconnected and with less potential than temperate populations to adapt to environmental change.

Keywords

Antarctica, *Ceratodon purpureus*, climate change, clonal, genetic variation, microsatellites, ozone depletion, RAPDs, somatic mutation, Windmill Islands.

Disciplines

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Somatic mutation and the Antarctic ozone hole

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Running headline: No evidence of UV-induced mutation in Antarctic moss

Summary

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2 Our study used microsatellite markers to compare the genetic variation present within continental Antarctic, sub-Antarctic and temperate populations of the moss *Ceratodon purpureus*.

3 In contrast to the RAPD studies, microsatellite data revealed that *C. purpureus* populations from continental Antarctica display less intra-population genetic diversity than populations from a range of temperate and sub-Antarctic sites.

4 Analysis of Molecular Variation (AMOVA) revealed that populations within the Windmill Islands region of Antarctica were more genetically differentiated than populations spread among more widely separated temperate regions.

5 Synthesis Our data provide no evidence of elevated mutation rates in the Antarctic, and imply climate change will present ongoing challenges for continental Antarctic moss populations that appear weakly interconnected and with less potential than temperate populations to adapt to environmental change.

Keywords: Antarctica, *Ceratodon purpureus*, climate change, clonal, genetic variation, microsatellites, ozone depletion, RAPDs, somatic mutation, Windmill Islands.

Introduction

Stratospheric ozone depletion, and the subsequent increase in UV-B radiation (UV-BR) at the Earth's surface, has occurred over Antarctica each austral spring since the late 1970s, with the 2006 ozone hole being the largest ever recorded (Cracknell & Varotsos, 2007). The mosses, lichens and algae that comprise the sparse terrestrial vegetation of continental Antarctica are potentially susceptible to UV-BR as they require sunlight for photosynthesis, are unable to behaviourally avoid excess UV-BR and generally lack the epidermal or cuticle tissues that provide important protection against UV-BR in vascular plants (e.g. Robberecht & Caldwell, 1980). Elevated UV-BR as a result of ozone depletion has been shown to increase DNA damage in the perennial herb *Gunnera magellanica* from Tierra del Fuego (Rousseaux *et al.*, 1999). DNA damage that is not repaired or replicated correctly will cause mutation, and consequent DNA sequence variation (Hutchinson, 1987).

Although no study has directly examined whether elevated UV-BR, as a result of ozone depletion, causes mutation in Antarctic plants, a series of genetic (RAPD) surveys have reported extraordinarily high levels of genetic variation in Antarctic populations of several moss species, including the widespread *Anomobryum subrotundifolium* (referred to as *Bryum argenteum*) and *Ceratodon purpureus* (Skotnicki *et al.*, 1998a; Skotnicki *et al.*, 2000; Skotnicki *et al.*, 2004). These studies suggested that many putatively clonal continental Antarctic populations possessed greater genetic variation than sexually reproducing populations of the same moss species in temperate regions (Skotnicki *et al.*, 2000; Skotnicki *et al.*, 2004). Indeed they reported surprising genetic variation within individual plants, such that separate shoots within a single compact moss clump (approximately 600 shoots/cm²) displayed

many different RAPD profiles, with occasional variation in RAPD banding patterns along the length of a single moss shoot (Skotnicki *et al.*, 2004). Skotnicki *et al.* attributed these high levels of genetic variation measured in continental Antarctic moss populations to mutation, with the likely mutagen being UV-BR (e.g. Skotnicki *et al.*, 2000; Skotnicki *et al.*, 2004).

The validity of claims of elevated mutation in Antarctic moss has recently been thrown into doubt by a study that found the presence of DNA from non-target fungal and protozoan organisms prevents the accurate measurement of genetic variation in Antarctic mosses using RAPD markers (Stevens *et al.*, 2007). Moreover, a claim of elevated mutation in the continental Antarctic mosses is striking because their life-history might be predicted to result in relatively low levels of genetic variation. The arid, polar climate of Antarctica combined with the short growing season has led to the conclusion that most moss species present in continental Antarctica rely exclusively on asexual reproduction and hence exist solely as haploid gametophytes (Lewis Smith & Convey, 2002). Approximately 0.3% of the Antarctic continent is ice-free, restricting terrestrial vegetation to small isolated patches of suitable habitat. Such isolation, together with clonality, is expected to produce small effective population sizes (Orive, 1993) and reduce genetic variation through drift, so that even in the event of infrequent episodes of sexual reproduction and recombination, reduced levels of genetic diversity would be expected. It is important to note however that the genetic structure of these populations has not been established and it is conceivable that continental Antarctic populations retain diversity from past sexual episodes or receive immigrant wind blown spores.

If the life history of the continental Antarctic mosses has been correctly interpreted then the conflicting genetic scenarios have potentially contrasting implications for the evolution, and indeed survival, of Antarctic mosses. On the one hand, the level of genetic variation is expected to be a strong predictor of the capacity for adaptive change in response to environmental variation (Frankham, 2005). On the other hand, the potential benefit to continental Antarctic mosses of elevated genetic variation as a result of UV-BR induced mutation will be reduced in the absence of sexual recombination, as multiple independent mutation events would be required for more than one beneficial mutation to ever become present in a single plant (Greig & Travisano, 2003).

Clearly moss-specific markers are required to resolve whether continental Antarctic mosses possess elevated levels of genetic variation as a result of mutation. The cosmopolitan moss *Ceratodon purpureus* (Hedw.) Brid. has been used as a model organism for studies of plant genetics (Wood *et al.*, 2000), and thus has more DNA sequence data available than any other plant species in Antarctica. We developed species-specific microsatellite markers from published DNA sequence data available on GenBank and used them to examine the genetic structure and compare genetic variation in continental Antarctic, sub-Antarctic and temperate populations of *C. purpureus*. Specifically, we aim to answer four questions; 1) are microsatellites a useful marker in the genus *Ceratodon*; 2) Are continental Antarctic populations more clonal than temperate populations; 3) Are *C. purpureus* populations from continental Antarctica more or less differentiated than temperate populations; 4) Is there more genetic variation in continental Antarctic *C. purpureus* compared to sub-Antarctic and temperate populations?

Methods

The species

Ceratodon purpureus is a cosmopolitan moss species found on all continents as well as many islands (Burley & Pritchard, 1990). As in all mosses, the sexual lifecycle is dominated by the photosynthetic haploid gametophyte. *Ceratodon purpureus* is a dioecious moss, thus sperm must swim from the antheridia of the male gametophyte to the archegonia of a female. After fertilisation a diploid sporophyte grows from the archegonia, remaining attached to the maternal gametophyte for several months before releasing abundant wind-dispersed spores at maturity (Shaw, 2000). This species is also capable of asexual reproduction through the regeneration of vegetative fragments. Although temperate *C. purpureus* populations reproduce both sexually and asexually, sporophyte production is less common in Arctic and sub-Antarctic populations of *C. purpureus* (Burley and Pritchard, 1990) and has never been observed in continental Antarctica (Selkirk, 1984; Burley & Pritchard, 1990; Skotnicki *et al.*, 1998b; Lewis Smith & Convey, 2002).

Burley and Pritchard (1990) described Antarctic *Ceratodon* as a separate species (*C. antarcticus*) from temperate *C. purpureus* on the basis of morphological characteristics; however, sequence analysis of four separate DNA regions suggests that the Antarctic material is not genetically divergent from temperate *C. purpureus* (Skotnicki *et al.*, 2004; McDaniel & Shaw, 2005). We therefore refer to all material in this study as *C. purpureus*. If Antarctic *Ceratodon* was found to be a distinct species, it would nevertheless still be valuable to compare the microsatellite variation present in Antarctic and temperate *Ceratodon* populations.

Microsatellites

Primers were designed for 18 *C. purpureus* microsatellites found within the *C. purpureus* DNA sequence data available on GenBank using the Primer3 program (Rozen & Skaletsky, 1998) accessed via the Australian National Genome Information Service (ANGIS, <http://www.angis.org.au>). Twelve of these primer pairs reproducibly amplified a product of the expected size. Microsatellite regions were screened for polymorphism using a worldwide sample of material (including Australia, Antarctica, Finland, sub-Antarctic Heard Island, U.S.A and Ecuador), and five of these microsatellites were found to be polymorphic and were used in subsequent genetic surveys (Table 1).

Population Sampling

We sampled 25 populations (aggregations of moss clumps separated from each other by at least 0.5 km, but typically more than 5 km) spread across five continents and the sub-Antarctic islands (Fig. 1). Continental Antarctic populations were collected from the Windmill Islands region, East Antarctica (n=5) and Australian populations (n=6) from around the cities of Adelaide (SA) and Wollongong (NSW). Because *C. purpureus* clumps are typically less than 20 cm in diameter we only sampled clumps separated by a minimum of 1-2 metres, to reduce the chance of repeatedly sampling the same clone. Samples were <1 cm² and often consisted of multiple shoots, and samples for a single population were collected within a 1-2 km radius, but typically much less. Samples were stored at -20 °C prior to molecular analysis. In addition, collaborators sampled populations from several other locations in the same fashion, including two American populations (New York, U.S.A and Otovalo, Ecuador), four

Finnish populations, and five populations from islands in the Southern Ocean (Heard Island, Île de la Possession and Île Kerguelen). Three locations within the Maritime Antarctic (consisting of the Antarctic Peninsula, South Shetland, South Orkney and South Sandwich Islands) were sampled using herbarium material and covered much larger areas (up to 20 km) than locations sampled specifically for this study. For these reasons, populations sampled using herbarium material are likely to show higher estimates for genetic variation parameters such as number of alleles per locus, expected heterozygosity and genotypic diversity relative to populations sampled specifically for the purposes of this study.

Molecular Analysis

DNA was extracted from gametophyte tissue using the DNeasy Plant Mini Kit (QIAGEN, Melbourne, Australia). To determine whether individual samples contained multiple genotypes, DNA was extracted from two single gametophyte shoots from each of 30 samples from the Wollongong-Sydney region. The selected shoots were as far apart as possible within the sample to increase the likelihood of finding multiple genotypes. Although the majority were pairs of identical genotypes, three samples contained two distinct genotypes, one of which differed at three out of five loci. Single shoots were therefore used for all subsequent DNA extractions.

Microsatellites were amplified using a PCR mix containing 1 µL of extracted DNA, either undiluted or diluted 10 to 100-fold, 0.25 units AmpliTaq Gold DNA polymerase in 1 x reaction buffer containing 2.0 mM MgCl₂ (Applied Biosystems, Melbourne, Australia), 2 nmol of each dNTP (Invitrogen, Melbourne, Australia), and 5 pmol each of the forward (FAM-labelled) and reverse PCR primer (Sigma-Genosys,

Sydney, Australia), in a total volume of 10 μ L. Reactions were PCR-amplified using a thermal cycle of 10 min at 94°C, 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 10 min on an MJR PTC100 thermal cycler (Bio-Rad, Sydney, Australia). PCR products were visualised on an ABI3130xl Genetic Analyzer (Applied Biosystems). Alleles were scored (size bp) by referencing an internal size standard in each reaction using GeneMapper (version 3.7) software.

Statistical Analysis

We carried out pairwise tests for linkage disequilibria using GENEPOP 3.4 (Raymond & Rousset, 1995; <http://genepop.curtin.edu.au>). Standard genetic parameters for each population (number of alleles, expected heterozygosity) were estimated using GenAlEx version 6.0 (Peakall & Smouse, 2006). Statistical differences between geographic regions for which multiple populations had been sampled (Australia, Finland, Southern Ocean islands, Maritime Antarctic and the Windmill Islands region of Antarctica) were then determined using one-way ANOVA performed with JMP version 5.1 (SAS Inc., Cary, NC, USA). Data were transformed where necessary to satisfy the assumptions of ANOVA. Post-hoc multiple comparisons were made using the Tukey-Kramer HSD test.

Observed and expected genotypic diversity was estimated for populations and regions using the program Divers11.c (Milgroom *et al.*, 1992). As the program accepts a maximum of five alleles at each locus, where there were more than five alleles at a locus in a population or region, the rarest alleles were pooled. Observed genotypic diversity was estimated as $G_O = 1/(\sum[(f_x)(x/n)^2])$, where n is the sample size, and f_x is the number of genotypes observed x times (Stoddart & Taylor, 1988). G_O was

compared with the genotypic diversity expected under conditions of sexual reproduction and random mating (G_E). G_E was generated using a resampling procedure based on observed allele frequencies to generate a null distribution based on 1000 randomizations. The P -value is the proportion of the null distribution less than or equal to G_O . This procedure was repeated excluding allele frequencies <0.10 and >0.90 for populations and <0.05 and >0.95 for regions as recommended by the program's author, but this had no significant effect on G_E and the associated P -value. Values reported are those based on all alleles.

The partitioning of genetic variance within and among populations and regions with multiple sampled populations was estimated using Φ , an analogue of F_{ST} (Weir & Cockerham, 1984), calculated within the Analysis of Molecular Variance (AMOVA; Excoffier *et al.*, 1992) framework using GenAlEx version 6.0 (Peakall & Smouse, 2006). The significance of the variance components was tested using a permutational method.

Results

The microsatellites we developed for this study proved useful in quantifying population differentiation and genotypic diversity in all populations and all regions. The number of alleles per locus ranged from five to 43 (Table 1) and the percentage of distinct multilocus genotypes detected per site ranged from 20 to 100%. We detected no evidence of significant linkage disequilibrium between any of the loci at any location, nor did we find evidence of null alleles in *C. purpureus* from continental Antarctica or any other location. All alleles present in the continental Antarctic populations also occurred in populations from other regions.

In contrast to predictions that mutation is producing greatly elevated levels of genetic variation (Skotnicki *et al.*, 2000; Skotnicki *et al.*, 2004), our microsatellite data revealed lower levels of variation in continental Antarctic *C. purpureus* populations compared with populations from sub-Antarctic and temperate regions. These findings instead support our prediction that the life-history of continental Antarctic *C. purpureus* may favour reduced genetic variation. Continental Antarctic *C. purpureus* populations contain an average of only 1.48 ± 0.08 alleles per locus (mean \pm SE), compared to 3.4 ± 0.4 and 3.7 ± 0.3 alleles per locus in the temperate Finnish and Australian populations, respectively ($F_{4,18}=16.3918$, $P<0.0001$, Fig. 2). Continental Antarctic populations also tended to possess fewer alleles per locus than those from the more temperate Southern Ocean islands (2.4 ± 0.2) and Maritime Antarctic (2.4 ± 0.1), but the difference was only significant for the Maritime Antarctic. Continental Antarctic populations also displayed lower levels of expected heterozygosity (0.13 ± 0.03) than temperate (Finland, 0.41 ± 0.02 , Australia 0.45 ± 0.01) and sub-Antarctic populations (Southern Ocean islands, 0.30 ± 0.05 , Maritime Antarctic, 0.37 ± 0.05 , $F_{4,18}=15.8339$, $P<0.0001$). We also detected a reduced number of polymorphic loci in the continental Antarctic populations compared to other regions ($F_{4,18}=16.3918$, $P<0.0001$). We did not include the single populations from New York and Ecuador in any formal analysis; however, the population from New York displayed levels of genetic variation comparable to other temperate populations from Finland and Australia, whereas the population from Ecuador displayed lower variation with allelic diversity and expected heterozygosity similar to the sub-Antarctic populations.

Unsurprisingly we found that *C. purpureus* populations from continental Antarctica and Southern Ocean islands displayed less multilocus genotypic diversity than populations from a range of temperate sites. Continental Antarctic and Southern Ocean islands populations had significantly lower G_O values (continental Antarctica - 2.2 ± 0.5 , Southern Ocean islands - 3.1 ± 0.7) than Australian and Finnish populations (9.3 ± 0.6 and 6.9 ± 0.9 , respectively, $F_{4,18}=20.2852$, $P<0.0001$).

In contrast to published expectations however we found little evidence of clonality in any of our samples. The ratio of G_O to G_E was close to unity for most populations except those from the Southern Ocean islands, which had significantly lower estimates of $G_O:G_E$ than all other regions ($F_{4,18}=5.1048$, $P=0.0063$, Fig. 3). Multilocus genotype frequencies were never significantly different to values expected under conditions of sexual reproduction and random mating, except for a single population from the Southern Ocean islands (Île Guillou, $P<0.01$). We also estimated multilocus genotypic diversity for all samples within each region to increase the sample size and power of the analysis to detect deviations from random mating. This increased both the average observed and expected genotypic diversity. However, no significant deviation from expected genotypic diversity under sexual reproduction was found in any region, except the Southern Ocean islands ($P<0.001$). This suggests that the reduced genotypic diversity in the continental Antarctic is due to low allelic diversity, and not increased clonality.

An AMOVA including all regions with multiple populations sampled showed most variation to be present within populations (68%, $\Phi_{PT}=0.319$, $P=0.001$), but 13% is present among populations within regions ($\Phi_{PR}=0.164$, $P=0.001$), and 19% among

regions ($\Phi_{RT}=0.186$, $P=0.001$). However separate AMOVA analyses within each region revealed contrasting patterns. Within the Windmill Islands, 20% of variation was among populations ($\Phi_{PT}=0.205$, $P=0.001$), compared to only 1% in Finland ($\Phi_{PT}=0.006$, $P=0.339$, Table 2). For Australia, our sampling arguably included two regions (Adelaide and Wollongong-Sydney, separated by more than 1000 km), each containing three sampled populations. Here AMOVA revealed no variation among regions ($\Phi_{RT}=-0.006$, $P=0.684$), although there was again 5% of variation among populations within regions ($\Phi_{PR}=0.054$, $P=0.005$). This suggests much lower connectivity of *C. purpureus* populations within the Windmill Islands compared to temperate regions. In the Maritime Antarctic, 12% of variation was among populations ($\Phi_{PT}=0.117$, $P=0.006$), whereas in the Southern Ocean islands this increased to 39% ($\Phi_{PT}=0.388$, $P=0.001$). The among population variation component is likely to be elevated in the Southern Ocean islands due to the greater distances between populations (up to 1700 km, compared to 10-270 km in other regions) and the lack of suitable habitat between populations. There were similar distances between populations in the Maritime Antarctic (up to 2000 km), but this region is likely to have elevated estimates of within population variation due to sampling larger areas for each population.

Discussion

Stratospheric ozone depletion and the subsequent increase in UV-BR has been most severe at high latitudes, especially over the Antarctic (Madronich *et al.*, 1998). There is some evidence that plants from historically low UV-BR environments at higher latitudes are more UV-BR sensitive (Robberecht & Caldwell, 1980; Barnes *et al.*, 1987), and that bryophytes, which are more dominant in terrestrial ecosystems at

higher latitudes, may be especially sensitive to UV-BR (Gehrke, 1999; Gwynn-Jones *et al.*, 1999). Since the onset of ozone depletion, ambient UV radiation has been shown to negatively affect growth of the only vascular plants native to the Antarctic (Day *et al.*, 1999) and cause chlorophyll bleaching in an endemic Antarctic moss species (Robinson *et al.*, 2005). Furthermore, elevated UV-BR as a result of ozone depletion has been shown to cause DNA damage in a perennial herb and a terrestrial alga from Tierra del Fuego and the Antarctic Peninsula, respectively (Rousseaux *et al.*, 1999; Lud *et al.*, 2001). In contrast to the negative effects of ozone depletion observed in these studies, others have found that UV-screening pigments provide effective protection against elevated UV-BR for some Antarctic plant species (Newsham *et al.*, 2002; Green *et al.*, 2005). Similarly, our data refute the suggestion that UV-B induced DNA damage is causing a general increase in levels of mutation in Antarctic moss (Skotnicki *et al.*, 2000; Skotnicki *et al.*, 2004). Indeed our comparison of genetic diversity and population structure revealed that, as predicted, continental Antarctic *C. purpureus* populations are apparently isolated and display lower levels of genetic and genotypic diversity than the temperate populations of Australia and Europe. Although reduced genotypic diversity was expected in continental Antarctica as a consequence of increased clonality, our sampling was designed to minimise the contribution of clonality and we found no evidence of deviation from the expected genotypic frequencies for randomly mating, sexual populations in continental Antarctica. Rather our data suggest the reduced allelic variation in continental Antarctica may reflect the effects of genetic drift acting on populations with limited dispersal and small effective population sizes (Frankham *et al.*, 2002).

Although they have received less attention than the reports of high levels of variation detected using RAPD markers, our data add to several other lines of evidence suggesting that UV-B induced mutation is indeed of little significance for Antarctic moss. It could be argued that our microsatellite data simply fails to detect the point mutations produced by UV-B damage since microsatellite variation normally arises through strand-slippage (Jackson *et al.*, 1998). If this is true, our results would nevertheless represent a baseline estimate of genetic variation expected in continental Antarctic *C. purpureus* populations. However, our finding that continental Antarctic populations lack variation is supported by several other studies, albeit with much smaller sample sizes (n=2-16) than in this study. No intraspecific allozyme variation was found in *C. purpureus* and the co-occurring *Schistidium antarctici* from the Windmill Islands (Melick *et al.*, 1994), and a similar lack of ITS DNA sequence variation was found in three moss species, including *C. purpureus*, from the Victoria Land region in continental Antarctica (Skotnicki *et al.*, 2004; Skotnicki *et al.*, 2005), suggesting the low levels of variation detected in our larger scale study is representative of the genetic diversity in continental Antarctic mosses.

Genotypic diversity in the sub-Antarctic and continental Antarctica

Surprisingly, we found no deviation from genotypic frequencies expected for sexual reproduction with random mating in any population or region, including continental Antarctic populations that are presumed to reproduce solely by asexual means, except in the Southern Ocean islands region. Our sampling regime was chosen to reduce the chance of sampling multiple ramets of the same clone, however, the fact that the observed genotypic diversity in the Windmill Islands populations did not deviate from that expected for random mating suggests that sexually produced spores are

continuing to arrive in the Antarctic (Lewis Smith, 1991), or that populations have retained substantial diversity from past episodes of sexual reproduction (Hsiao & Rieseberg, 1994; Sherman *et al.*, 2006). We have conducted other more fine scale sampling that confirms the presence of localised asexual reproduction in both temperate and continental Antarctic populations.

Lower values of $G_O:G_E$ in the Southern Ocean islands as compared with both temperate and continental Antarctic sites may reflect a lower input of sexually produced spores relative to asexual propagation in this region. Populations from both the Southern Ocean islands and continental Antarctic are separated from temperate continental populations by more than 2500 kilometres, reducing the possibility of genotypically diverse input from sexually produced windblown spores. In addition sexual reproduction is less frequent in sub-Antarctic *C. purpureus* than in temperate regions (Burley & Pritchard, 1990) although greater than on the continental Antarctic. Moreover, growth and thus potential for asexual propagation is likely to be more vigorous in the wet, cool but relatively constant conditions of these ocean islands than in the frozen desert conditions of continental Antarctica where growth is extremely slow (0.5-2 mm per year; Clarke, Hua, Fink and Robinson, unpublished work).

Evolutionary implications and climate change

Importantly, given the stresses now facing the Antarctic terrestrial vegetation, the limited genetic variation present within individual *C. purpureus* populations in the Windmill Islands, combined with their apparent genetic isolation from sexual populations in temperate regions, may limit their ability to adapt to environmental change (Frankham, 2005). The apparently reduced connectivity among populations in

continental Antarctica should also limit the potential for recolonisation in the case of local extinction. Moreover, in the Antarctic, recovery from any damage is likely to be extremely slow due to slow growth rates, with existing moss turfs dated to 50 years old or more (Clarke, Hua, Fink and Robinson, unpublished work). Increased UV-BR as a result of stratospheric ozone depletion, increased human activity (Frenot *et al.*, 2005) and climate change, especially in the region of the Antarctic Peninsula (Hansen *et al.*, 1999; Vaughan *et al.*, 2003), will present ongoing challenges for Antarctic moss populations with less potential to adapt to change than their temperate counterparts.

Not all of the effects of regional climate change in the Antarctic may be detrimental to its moss populations. Indeed regional warming has the potential to increase the incidence of sexual reproduction and thus significantly alter the population genetics of Antarctic *C. purpureus*. The southernmost record of *C. purpureus* with sporophytes is from James Ross Island (63°56' S, 57°49' W) in the northern region of the Antarctic Peninsula. Mean temperature for the summer and winter months is similar for Casey station (Windmill Islands region, December-February -1.0 ± 0.8 °C, June-August -14 ± 2 °C) and Marambio station (Seymour Island, adjacent to James Ross Island, December-February -2.2 ± 0.6 °C, June-August -15 ± 3 °C), although mean temperatures are higher at Marambio than Casey throughout the spring (Jones & Reid, 2001). Presumably photosynthesis can resume earlier after the austral winter in the northern Antarctic Peninsula, extending the growing season compared to the Windmill Islands and allowing sexual reproduction to occur in particularly favourable years. If continental Antarctica experiences regional climate amelioration as the Antarctic Peninsula has, constraints to sexual reproduction may be removed. Climate warming would also increase the likelihood of propagules becoming established,

allowing more gene flow within and between regions, but also the likelihood of exotic species becoming established, which could drastically alter species interactions in what is currently one of the simplest ecosystems in the world (Robinson *et al.*, 2003). However, recent evidence suggests that parts of East Antarctica are not warming but becoming cooler and drier (Doran *et al.*, 2002; Hodgson *et al.*, 2006), conditions which are likely to reduce moss growth rates still further (Robinson *et al.*, 2000; Wasley *et al.*, 2006). It is thus difficult to predict the impact of future climate change on these populations.

Conclusion

This is, to the best of our knowledge, the first study to use species-specific microsatellite markers to investigate the population genetics of any Antarctic plant species. In contrast to previous RAPD studies, our microsatellite data reveals that continental Antarctic populations of *C. purpureus* show less genetic diversity than populations from temperate regions. Our data therefore provide little evidence of a general effect of the ozone hole on mutation rates in the Antarctic, and imply climate change will present ongoing challenges for continental Antarctic moss populations that appear weakly interconnected and with little potential to adapt to environmental change.

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Table 2. Results of analyses of molecular variance of the observed *C. purpureus* microsatellite variation within and among populations and regions.

Region	Source	df	Est. Var.	%	F	P value
Overall	Among Regions	5	0.248	19%	0.186	0.001
	Among Pops	17	0.178	13%	0.164	0.001
	Within Pops	247	0.909	68%	0.319	0.001
	Total	269	1.335	100%		
Finland	Among Pops	3	0.007	1%	0.006	0.339
	Within Pops	37	1.147	99%		
	Total	40	1.153	100%		
Australia	Among Pops	5	0.064	5%	0.051	0.002
	Within Pops	77	1.203	95%		
	Total	82	1.267	100%		
Southern Ocean islands	Among Pops	4	0.519	39%	0.388	0.001
	Within Pops	54	0.819	61%		
	Total	58	1.338	100%		
Maritime Antarctic	Among Pops	2	0.136	12%	0.117	0.006
	Within Pops	26	1.024	88%		
	Total	28	1.160	100%		
Antarctica	Among Pops	4	0.093	20%	0.205	0.001
	Within Pops	53	0.361	80%		
	Total	57	0.454	100%		

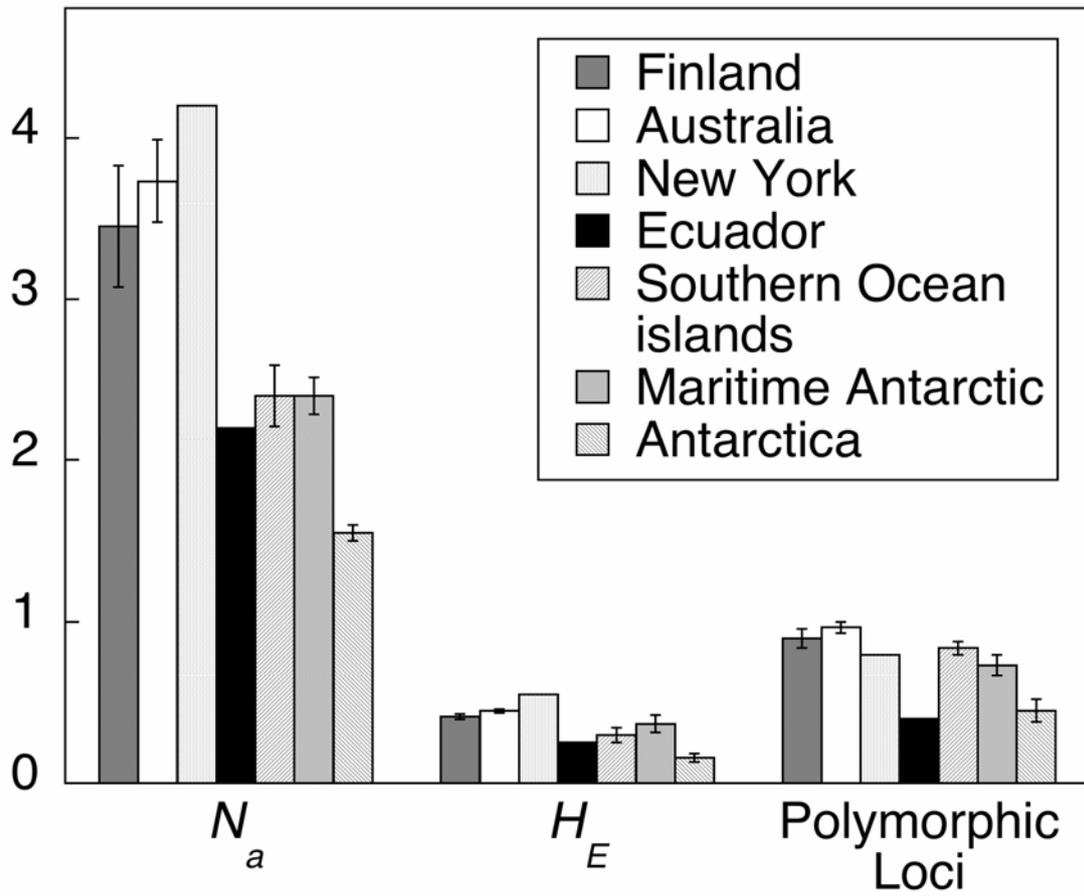


Figure 2. Number of alleles (N_a), expected heterozygosity (H_E) and proportion of polymorphic loci in *Ceratodon purpureus* populations by region. Values are means \pm SE where shown.

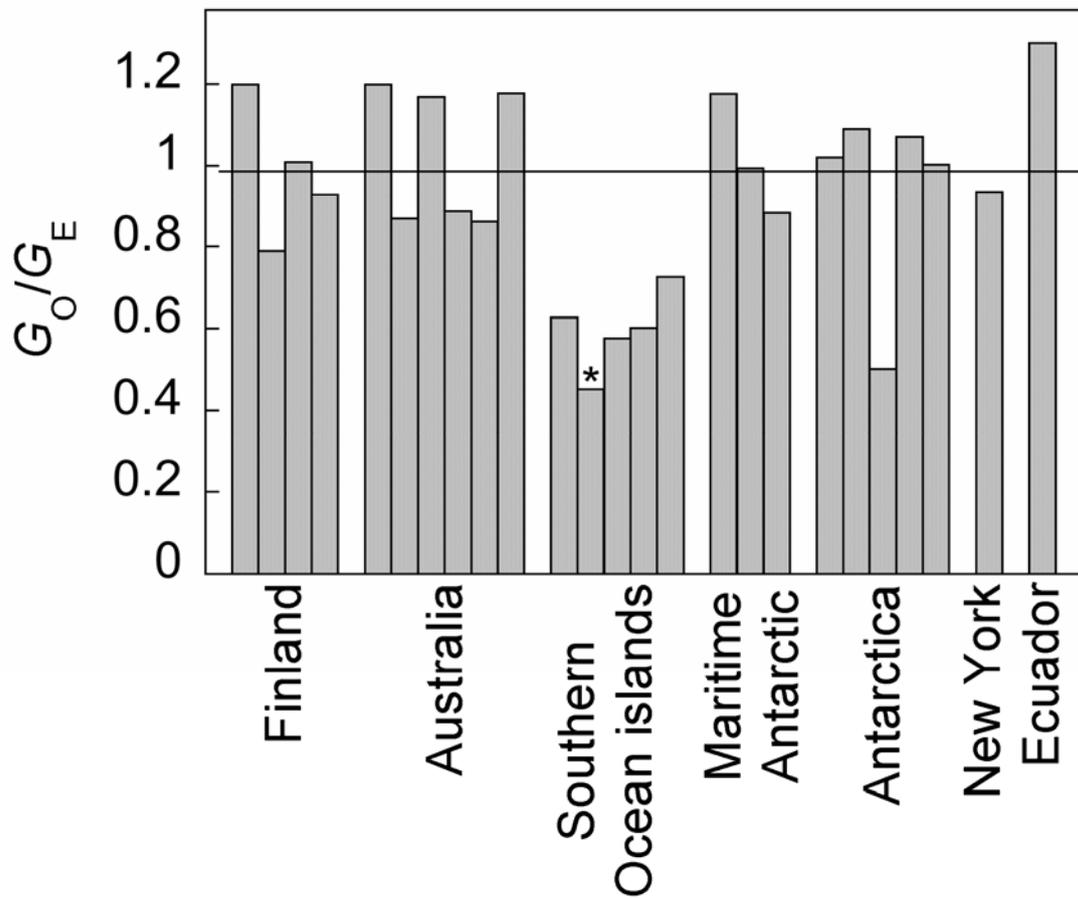


Figure 3. Ratio of observed to expected genotypic diversity in *Ceratodon purpureus* populations by region. The asterisk indicates the population that showed a significant deviation from genotypic frequencies expected in a sexual, random mating population.