RAPD analysis of genetic variation and dispersal of the moss
*Bryum argenteum* in Ross Island and Victoria Land, Antarctica

**Abstract** The Random Amplified Polymorphic DNA technique was used to assess the level of genetic diversity in *Bryum argenteum* from Ross Island and southern Victoria Land, Antarctica. Samples were collected from three separate transects, and from other geographically distinct populations within 150 km of Ross Island. Moss growth in two transects, sampled down small exposed meltstream channels at Cape Royds and Cape Chocolate, was very sparse with no other moss colonies found within 0.4 or 4 km, respectively. However, samples from these channels showed similar levels of genetic variation to those from a transect at Granite Harbour, where moss colonies were large, luxuriant and turf-like between boulders. In all transects, high levels of genetic diversity were apparent both within and between colonies, and some spatial relationships were observed down the length of the channels, with more extensive variation at the top than the bottom of two transects. Samples from other sites in the region showed varying but high levels of genetic diversity; overall, the majority showed some clustering according to site of collection, with short-distance dispersal of propagules by water and transmission between sites presumably by wind. The extensive genetic diversity observed appears mainly due to somatic mutation within colonies, with some contribution by immigration of propagules from elsewhere into established colonies.

**Introduction**

*Bryum argenteum* Hedw. is widespread in both the northern and southern hemispheres, including Antarctica. There is currently some uncertainty as to the precise identity of the Antarctic material, with the name *Bryum argenteum* sensu lato being the most appropriate until further taxonomic research is completed (Skotnicki et al. 1998a; J. Spence, personal communication).

A study of genetic variation amongst populations of two Antarctic moss species, *Hennediella heimii* and *Bryum argenteum* in the Garwood Valley (78°00′S, 164°05′E) showed definite clustering of *B. argenteum* samples (but not of *H. heimii*) within drainage channels in a small area of the valley, indicating dispersal of *B. argenteum* over short distances by water (Selkirk et al. 1998). We now report an extended Random Amplified Polymorphic DNA (RAPD) investigation of genetic diversity in *B. argenteum* in the Ross Sea region. This study examines the extent of variation at a small scale within three meltstream channel populations, one at Cape Royds on Ross Island, and one each at Cape Chocolate and Granite Harbour in southern Victoria Land. At a larger scale, the diversity in these populations is compared with that found in 13 other geographically separate populations on Ross Island, small offshore islands and the mainland of southern Victoria Land.

The RAPD technique (Rafalski and Tingey 1993) has proven very useful for analysis of the extent of genetic diversity in Antarctic mosses (Adam et al. 1997; Selkirk et al. 1997; Skotnicki et al. 1997, 1998a, 1998b, 1998c) with several distinct advantages over other genetic techniques: no other genetic information about the species is required, many samples can be quickly and simply compared, and minute samples of material are sufficient for analysis. The method has been adapted to give reproducible comparisons of genetic diversity in several Antarctic moss species (Skotnicki et al. 1998a, 1998b, 1998c), using single shoots for DNA extraction, and is the most appropriate method for an initial, overall
insemination of genetic variation in sparse populations where minimum impact sampling is essential.

**Materials and methods**

Samples of *B. argenteum* were collected from three small melt-steam channels. One was near Discovery Bluff at Granite Harbour in southern Victoria Land (77°00′S, 162°30′E; GH isolates), where the colonies were found growing in an almost continuous 4 m x 1 m turf between large protective boulders, and with shoots up to 15 mm in length. No other *B. argenteum* plants were seen for at least 100 m. Sixteen samples were collected, in pairs 5 cm apart, with each pair separated by 50 cm, and numbered GH1 to GH16 from top to bottom down the channel.

Another 10-m channel was at Cape Royds on Ross Island (77°35′S, 166°10′E; CR samples), at an isolated exposed site at least 300 m from the next *B. argenteum* colonies, on gravel below a small snowpatch; moss colonies were much smaller (generally 2-4 cm diameter) with short stems 2-3 mm in length. Fifteen samples were collected, each separated by 0.5-1 m from its next neighbours, and numbered CR1 to CR16 from top to bottom of the channel.

A third transect was collected at Cape Chocolate in southern Victoria Land (77°57′S, 164°30′E), where 1- to 3-cm diameter colonies were found growing in an isolated, exposed wide depression in dry gravelly ground beneath a very large snowpatch. This transect was at least 4 km from the next moss colonies found at Cape Chocolate. Very small samples were collected from each of the eight clumps (CC1–CC8) found within 25 m down this shallow transect was at least 4 km from the next moss colonies found at Cape Chocolate. Very small samples were collected from each of the eight clumps (CC1–CC8) found within 25 m down this shallow channel, but unlike the other two transects, these samples were scattered in an area 3 m wide.

Samples were also collected at other sites at Cape Brodo (77°35′S, 166°45′E), Cape Crozier (77°30′S, 169°25′E) and Scott Base (77°50′S, 166°45′E); on the mainland of southern Victoria Land at Cape Chocolate (77°57′S, 164°30′E), three different sites in Garwood Valley (78°00′S, 164°05′E), Miers Valley (78°06′S, 163°45′E), Canada Glacier and the adjacent Lake Fryxell (77°35′S, 163°15′E), Marble Point (77°25′S, 163°30′E) and Granite Harbour (77°20′S, 163°30′E) and on the nearby Bratina Island (78°00′S, 165°35′E) and Beaufort Island (77°00′S, 167°00′E). These sites are all within a radius of 150 km from Scott Base, and up to 12 samples were collected within an area 300 m in diameter at each location. A few samples were also collected for comparison at Edmonson Point, some 500 km further north in northern Victoria Land (74°20′S, 164°30′E).

Small moss samples, approximately 1 cm in diameter, were collected from many of the colonies; samples of 3 mm2 or less were taken from sparse, small colonies to minimise damage. Samples were collected strictly in accordance with permit conditions. The samples were air-dried in paper envelopes, and stored frozen until use. Voucher specimens are held at the University of Waikato Herbarium, Hamilton, New Zealand.

**DNA extraction and amplification**

DNA was extracted from single moss shoots by a method developed previously for *B. argenteum* and other moss species (Skotnicki et al. 1998a, 1998b). Moss DNA was amplified by PCR (polymerase chain reaction, Williams et al. 1993) in a microtitre plate (Skotnicki et al. 1998b). RAPD primers OP-A13, OP-A17, OP-C4 and OP-C13 (Operon Technologies) were used for amplification of *B. argenteum* DNA. PCR reactions were then electrophoresed through 1.5% agarose gels, stained in ethidium bromide, and photographed under UV light (Sambrook et al. 1989).

Seventy-five RAPD bands were scored as present or absent on gels, with some PCR reactions being done in duplicate or triplicate, and in different experiments, to confirm that banding patterns were always reproducible. Control reactions without added DNA gave no bands; control reactions were also done to ensure contamination of moss samples by other organisms did not present a problem or give spurious bands. The RAPDistance computer program (Armstrong et al. 1995) was used to compare pairwise the patterns of DNA fragments obtained, to produce a distance matrix and then a neighbour-joining tree using the NITREE and TDRAW programs in the package (Saitou and Nei 1987; Jin 1988; Studier and Keppler 1988; Ferguson 1990; Armstrong et al. 1995). The GRP- ANAL program of the RAPDistance package was also used, to compare populations (grouped according to collection site) rather than individuals.

A Permutation Tail Probability (PTP) test (Faith and Cranston 1991) was used to determine whether the total branch length of the tree was significantly shorter than the average of 20 randomly generated trees.

The Phylogenetic Analysis Using Parsimony program (PAUP *4.0d61* beta test version; Swofford 1998) was also used as an alternative method of analysis for the *B. argenteum* samples.

**Results**

**Genetic variation within colonies**

We previously reported that the RAPD technique can reliably detect genetic variation in single shoots of *B. argenteum*, and that it has been used to assess genetic variation within and among colonies of *B. argenteum* in Garwood Valley, southern Victoria Land (Selkirk et al. 1998) and in Australia and New Zealand (Skotnicki et al. 1998a). We now report results obtained for *B. argenteum* from a variety of locations in the Ross Sea region of Antarctica.

Substantial levels of within-colony variation were observed in samples from all three transects tested from Cape Chocolate (CC), Cape Royds (CR) and Granite Harbour (GH), with shoots usually being most closely related to others from the same clump. Figure 1 shows the results obtained for six shoots, separated by about 0.2 cm (CC transect) or 0.5 cm (CR and GH transects), from three colonies collected in the middle of each of the three transects. Considerable within-colony variation occurred in these and other clumps tested, but shoots were usually most closely related to others from the same colony.

Six shoots from at least four colonies at each site (CC, CR, GH) were analysed, from the top, middle and bottom of the three transects, but spatially related differences in the extent of variation within clumps were apparent only in the transects from Cape Royds and Granite Harbour. Both of these transects showed higher levels of within-clump variation at the top of the channel than at the bottom. In the CC transect, considerable within-clump variation occurred in all colonies tested, but levels were similar at the top and bottom of the channel.

**Genetic variation within *B. argenteum* transects**

In each of the three transects, extensive variation among colonies was observed, although some clustering of