Active conservation: augmenting the only British population of *Bryum schleicheri* var. *latifolium* via *in vitro* cultivation

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Background

Bryum schleicheri var. *latifolium* is only known from one site in the UK, growing in a single spring by a small burn in the Touch Hills, SW of Stirling, a site from which it was first recorded in 1880. There are five other records, all from Perthshire, but it has not been seen at these sites since the 19th century, despite targeted survey work. The loss of this moss from a number of sites and its reduction to such a small and vulnerable population resulted in *B. schleicheri* var. *latifolium* being included in the first tranche of bryophytes added to Schedule 8 of the Wildlife and Countryside Act. It is rated as Critically Endangered in the bryophyte Red Data Book (Church *et al.*, 2001). Despite its restriction to a single vulnerable site (just one dead sheep in the wrong place would finish it off), the moss was not included in the priority list of Biodiversity Action Plan species. The site has no statutory protection.

GPR first visited the site in 1988 with Peter Pitkin when the moss was seen in reasonable quantity in the main spring and a few stems were seen in the next flush downstream. GPR's next visit was in 1993, as part of survey work for the Royal Botanic Garden Edinburgh, and financed by Scottish Natural Heritage (SNH). The population in the main spring seemed much the same as in 1988 but no trace of the moss could be found in the lower spring (Rothero, 1993; Long & Rothero, 1996). It seems probable that the main agent in the loss from this second spring was the luxuriant growth of *Montia fontana*. As a result of this visit, a thorough survey of the moss in the main spring was made by Allen (1993). Allen mapped and counted all the growing shoots of *B. schleicheri* var. *latifolium* in the spring. He also visited similar sites in the surrounding area of the Touch Hills but did not find any other populations. His total count in the main spring was 854 stems, growing in two areas, the largest stand being in the centre of the spring.

GPR's next visit was in 2003 and it seems likely that no one else had visited the site to look at the moss in the interim period. There was a large suckler herd of cattle in the area and, during a dry summer, the cattle had preferentially used the wetter ground by the burn. The spring containing *B. schleicheri* var. *latifolium* was badly poached and, although stems of the moss could be found, the damage looked to be considerable. This was reported to Stephen Ward, then SNH Lower Plants Officer, and as a result of his

promptings, Stirling SNH office arranged a site visit with GPR, the SNH Area Officer and the landowner, Jim McLaren, and financed an assessment of the site. A count of stems of *B. schleicheri* var. *latifolium* was made in October 2003 and this gave a total of 372 stems, a more than 50% reduction since 1993. This situation clearly called for urgent action. It was therefore decided, after due consideration of the Joint Nature Conservation Committee guidelines on translocations (McLean, 2003) and consultation with the BBS Conservation Committee, that SNH would finance a project to attempt to reintroduce the moss to the spring downstream from which it had been lost after 1988, via the novel strategy of *in vitro* culturing of plants from the extant site. SNH and Jim McLaren also arranged to put together a management agreement for the site whilst cultures were being grown at Queen Mary from three stems of the moss collected by GPR. At the very least these cultures would ensure the survival of the germplasm of British *B. schleicheri* var. *latifolium* if it became extinct in the wild. More hopefully, they might provide a means of increasing the existing population and pioneer a *modus operandi* for the conservation of other endangered taxa.

As part of this project a full survey of the extant site was made the following spring (April 2004) when it was apparent that the poaching by cattle, far from damaging the population of *B. schleicheri* var. *latifolium*, had probably been beneficial, now that time for recovery from the initial damage had elapsed. Another 'head count' of the moss was made and this gave a total of 2004 stems. This rather exact figure gives a spurious degree of accuracy and confidence limits of 10% should certainly be attached as the stems are not easy to count without doing an unacceptable amount of damage. Even so, this figure is considerably higher than that given by Allen in 1993 (854 stems) and very different from the count in October 2003. It was apparent that the poaching had opened up the sward of *Montia fontana*, providing more potential sites for the moss, and also that stems of the moss, as well as being flattened and moved around, had sent up many adventitious shoots from leaf axils. It was also apparent from the mapping of the groups of stems that the distribution of the moss in the spring was different from that mapped in 1993. This emphasises the fact that these springs are dynamic habitats in which change must be expected to occur.

Before going ahead with the reintroduction of the moss into the lower spring, an exhaustive search of the site was made to make sure that no stems of *B. schleicheri* var. *latifolium* were lurking unseen. Other springs in the area were surveyed in the hope of finding further populations but none were found, although a number of flushes had broadly similar bryophyte communities. The best-localised of the old Perthshire sites is on Ben Chonzie, and this area was also re-visited. Here, wonderful springs occur with many square metres of *Pseudobryum cinclidioides* and stands of *Oncophorus virens* and the Red Data Book species *Tayloria lingulata*, but no *B. schleicheri* var. *latifolium* could be found.

Meanwhile, back in the laboratory *B. schleicheri* var. *latifolium* proved to be extremely vigorous in Phytagel cultures (see Duckett *et al.* (2004) for full details of media and growth conditions) as anticipated from previous work on *Bryum* species, including several rare coastal dune taxa. Within 3-4 weeks of initiating the cultures from stem fragments, the surface of the medium in 5 cm Petri-dishes was completely covered with protonemata bearing young shoots (Figure 1). We then entered completely uncharted territory: how might the cultured specimens be reintroduced into the wild? The only protocol we had previously tried (Pressel & Duckett, 2004) involved 'rock cultures' whereby saxicolous mosses, grown in culture onto pieces of their native substrates, were simply stuck back in the wild with superglue, and was clearly unsuitable for *B. schleicheri* var. *latifolium*. Cultures placed in the wild would be washed away during the first rain before the moss could become established. We therefore adopted the approach

of placing the cultures in muslin bags and pegging these down in small areas from which vegetation had been cleared. Twelve such muslin-encased cultures were planted in September 2004 (Figure 2) and their development monitored.

After just one month, stems of the moss had grown through the muslin in all of the sachets (Figure 3) but it was clear that some cultures were performing better than others and by the time of the next visit in November 2004, one culture was moribund. At the same time a further nine cultures were pegged out in the spring using the same procedure.

The next visit was in March 2005, when it was clear that the second tranche of nine cultures had failed. This failure was probably due to a combination of mistakes. The muslin sachets used on this occasion were too flimsy and all had come apart, the cultures had been less 'advanced', with less protonemal bulk and fewer stems, and they were planted out at the beginning of winter, giving them little chance to grow on before the weather deteriorated. However, only one further culture of the first tranche had failed, leaving ten with at least some growth and some with several robust stems, all associated with the original muslin bags (Figure 4).

No further visits were made until September 2005, which is unfortunate and simply due to lack of both time and funding. The cattle had been on the hill over the summer and there were signs of trampling in both the original and the transplant sites. The main population in the upper spring looked to be in reasonable condition but the cultures in the lower spring had fared much less well and it was possible to verify continued growth in only four of the spots in which cultures had been pegged out. Nevertheless, stem counts for these four sites were >21, 15, 34 and >120, and there were clear signs that new stems, that had been shifted from the original sachet by trampling, were regenerating (Figures 5 and 6).

The end of the summer is not the best time to assess the growth of the moss as the biomass of vascular plants is at its greatest at this time; it is much better to check growth in the spring. One other problem was locating some of the culture sites, despite marking and photographing them. The sachets had been pegged out with tent pegs marked with white tape and the sites marked with white plastic markers and photographed. All of the plastic markers had gone, some being found close by, much mangled, presumably through chewing by cattle or sheep. Some of the taped pegs had also disappeared, presumably either overwhelmed by the growth of vascular plants or trodden into the spring.

So, out of a total of 21 cultures reintroduced into the lower spring, only four survived a full year. However, this is only the first attempt at a completely new approach to moss conservation. Given our ignorance about conditions at the spring and hence the optimal season for introducing the cultures, not to mention using the wrong kind of bag for the second tranche of cultures, the results can be viewed as most promising. It is a pity that no funding was available to continue monitoring of the site and that visits by GPR in 2005 had to be fitted in on an *ad hoc* basis. However, the hope is that SNH will fund a further transplant experiment in 2006, and that lessons learnt in this initial trial will lead to the long-term re-establishment of *B. schleicheri* var. *latifolium* in the lower spring and, in the future, the formulation of similar conservation strategies for other endangered mosses.

Acknowledgements

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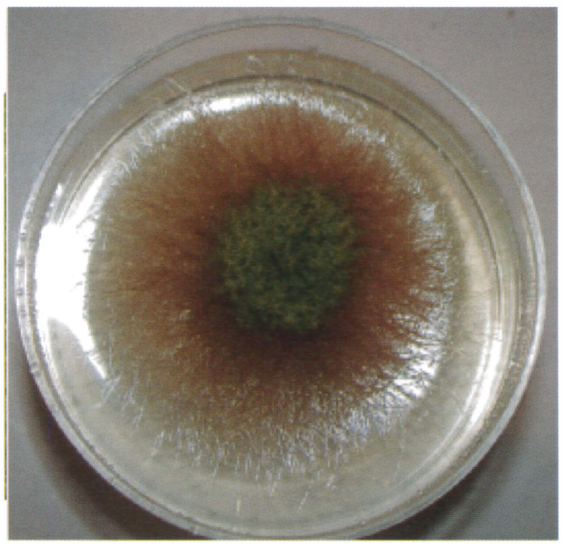

Figure 1. Phytagel culture of *B. schleicheri* var. *latifolium* used in the reintroduction trials. Photo: J.G. Duckett.

Figure 2. Newly pegged-out muslin bag containing a culture of *B. schleicheri* var. *latifolium.* Photo: G.P. Rothero.

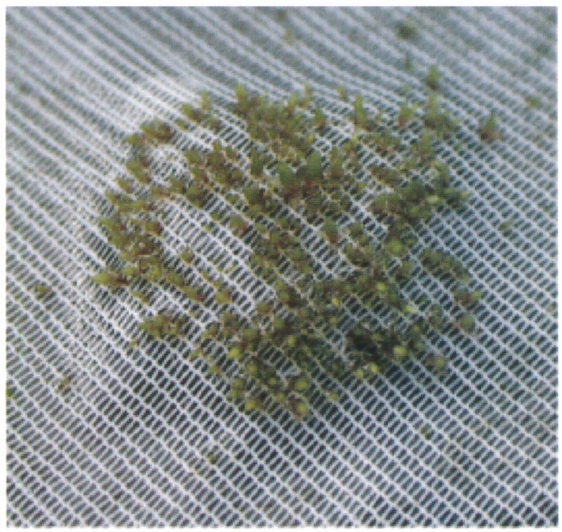

Figure 3. Shoots of *B. schleicheri* var. *latifolium* growing through a muslin bag one month after planting. Photo: G.P. Rothero.

Figure 4. Colony of *B. schleicheri* var. *latifolium* on a muslin bag six months after planting. Photo: G.P. Rothero.

Figure 5. A well-grown colony of *B. schleicheri* var. *latifolium* one year after planting. Photo: G.P. Rothero.

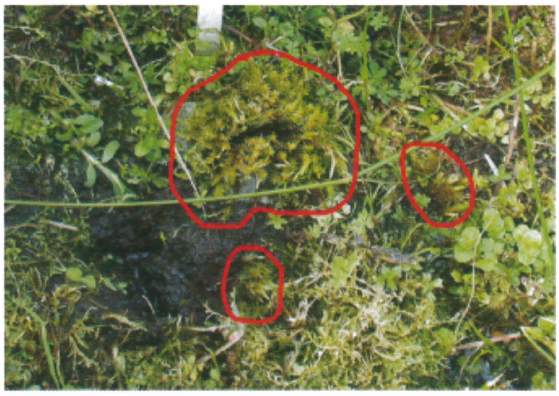

Figure 6. Two groups of new shoots of *B. schleicheri* var. *latifolium* (small circles) becoming established away from the original colony illustrated in Figure 5. Photo: G.P. Rothero.

Bazzania trilobata on sandstone outcrops in Sussex

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Methodology

As part of an undergraduate Certificate in Field Biology course at the University of Sussex I undertook a field-based project looking at bryophytes on the Sussex sandrocks. The Wealden sandrock bryophyte flora has been well studied for nearly 100 years, most recently by Paton (1953) and Rose (1992). However, comparatively little is known about the ecology of species, and the aim of this study was to investigate the relative importance of a range of environmental variables on the occurrence of *Bazzania trilobata*.

The following environmental variables were investigated:

- moisture of substrate (on a scale where 1 = dry, 2 = damp, 3 = wet)
- height above ground (cm)
- shade (percentage cover)
- aspect
- incline of substrate
- competition from other bryophytes

Principal Components Analysis (PCA) was used to analyse the results.