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The importance of donor population identity and habitat type when creating new populations of small cow-wheat *Melampyrum sylvaticum* from seed in Perthshire, Scotland

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SUMMARY

Small cow-wheat *Melampyrum sylvaticum*, a nationally scarce annual identified as a priority species in the UK Biodiversity Action Plan, was the focus of a translocation attempt aiming to establish new populations within the extent of its former Scottish range. Seeds were collected (from wild Scottish populations) in three phases (in the years 2005, 2006 and 2008) and sown at six receptor sites where the species was absent but habitat seemed suitable. Each phase used increasing numbers of seed after the results of the first phase (89 to 103 seeds sown per site) suggested that many more than 100 seeds are needed to establish the species (at least in the short-term) at a site. Comparisons of the suitability of seed from three different wild populations showed that one resulted in higher germination rates. This donor population was associated with environmental conditions more similar to those at the receptor sites than those of the other donors. Receptor sites also differed in their suitability; those that were climatically and edaphically more similar to sites supporting wild populations appear to be more favourable to *M.sylvaticum* longer-term survival. Together, these can be seen to suggest that future seed translocation should be to sites that are ecologically similar to the donor population and within sites that fall into the cooler and wetter range of environmental conditions currently supporting Scottish populations of *M.sylvaticum*.

BACKGROUND

Small cow-wheat *Melampyrum sylvaticum* is an annual obligate hemiparasite of various herbs and woody species (Stewart *et al*. 1994), which within Europe is confined mainly to boreal-montane regions. It is now restricted to only 19 isolated sites in Great Britain. Of these, only five sites support more than 500 plants and seven support populations of 100 individuals or fewer. Additionally, many populations occupy a very small area. For example, the population in Glen Tilt (Scotland) estimated as being more than 8,000 plants, achieves densities of approximately 800 plants per m^2 with the vast majority of the population occurring in an area of only 3 m x 4 m. Although this population is vigorous, the risk of losing it to random events such as tree fall or extreme adverse weather is high given its limited distribution. This situation is repeated at many sites affecting large and small populations alike. Evidence from historical records indicate that the species was once much more widespread across upland areas of Britain; resurveys (undertaken by P.Gallagher, Scottish Wildlife Trust) between 2001 and 2005 show that this equates to a reduction of 84% of previously occupied sites, corresponding to British Red Data List inclusion (Cheffings & Farrell 2005). Indeed, since 2002 when detailed surveys of the species in the UK began, two populations have been lost, highlighting the precarious nature of the smaller populations.

Due to the small size and extent of most Scottish populations, environmental and demographic stochasticities may pose threats to the health of British *M. sylvaticum*. Further, reproductive isolation of populations will incur loss of genetic diversity as researchers at the Royal Botanic Gardens (Edinburgh) have demonstrated genetic divergence between populations at scales of just a few tens of metres (Sharp 2003). It is assumed that this genetic isolation will have deleterious effects on extant *M. sylvaticum* populations, which may be exacerbated in the future under predicted changes in climatic conditions.

This project aimed to investigate the feasibility of creating new populations within the former extent of the species in Great Britain in keeping with the targets of the Species Action Plan (UK Biodiversity Group 1999). We wanted to investigate the suitability of different wild populations as donors of seed and the degree to which habitat descriptions generated from previous studies (Dalrymple 2006) could be used to guide selection of suitable sites for translocation. We also hope to investigate the effect of mixing seed from different wild donor populations on the genetic health of the introduced populations; increasing genetic diversity of the populations was one of the original goals of the project (Dalrymple *et al*. 2008). This latter aspect of the project is ongoing and will be reported on after timescales have elapsed whereupon effects such as outbreeding depression might be discerned using molecular techniques.

ACTION

Donor populations: The translocations took place in three phases, the first in 2005, the second in 2006 and the third in 2008. Seeds were collected from three Scottish donor populations for phase I and II at the Birks of Aberfeldy, Coire na Eiridh (a site on Ciste Dhubh in West Affric), and by the side of Loch Ossian on the Corrour Estate. Phase III translocations used seed from a fourth population in Glen Tilt. This population was only discovered in 2004 and was therefore monitored to ensure consistent population size for three years before seed was collected. It is the largest *M. sylvaticum* population in Great Britain by far and this, in combination with its stable size, makes it the best available donor population for seed translocations. The Aberfeldy and Glen Tilt populations occupy sites which are geographically and ecologically closer to the receptor sites than the other two donor populations. However, the project was not set up with the aim of testing whether ecological similarity was necessary to promote successful translocations. Instead, the donor populations were the largest in Scotland and the ecological spread represented by them was a coincidental, if useful, additional factor to incorporate into the trial.

Receptor sites: Seed from the wild populations were sown at six sites in Perthshire (eastern Scotland) within the historic range boundary of the species. Although records show that none of these sites previously supported *M. sylvaticum*, this area is thought to be core for the species in Scotland due to the presence of two of the biggest remaining populations. Sites were selected based upon Forestry Commission records of birch *Betula* and oak *Quercus* woodland that matched extant habitat of *M. sylvaticum*. Table 1 lists the site localities, describes the key features of each and compares them to conditions found in typical extant habitat. All but one site are in contiguous woodland habitat within the Highland Perthshire Forest Network.

Seed collection and sowing: Seed was collected upon ripening at the beginning of August from the wild populations. Only the lowermost seed pods were selected as these have had longest to ripen (flowers and seed pods mature sequential starting with the lowest). Seeds were transported to the receptor sites and sown as soon as possible (within 48 h of collection). Seeds were placed into the soil surface, below the moss or leaf litter layer.

Phase I was initiated in response to the requirement in the Species Action Plan that attempts be made to establish five new populations. This translocation used small numbers of seeds (between 89 and 103) to minimise risk of sowing seed into unsuitable habitat, and hence loss of a valuable and limited seed supply in case of failure. Phase II built on this by introducing greater numbers of seed (366 and 369) into two sites (one of which was a site used in phase I). It was hoped to use more sites on this occasion but access problems precluded the inclusion of a third location. Phase III used even larger numbers of seed (500 in each of three sites that had been previously used in other phases.

Phases I and II used planting protocols involving a grid-based sowing pattern and coloured labels. This allowed us to determine the identity of the donor population of any seedlings found in the subsequent growing season. These phases of translocation also used small exclosures (wooden frames measuring 30 x 30 x 30 cm covered with horticultural shade fabric, mesh size approximately 2 mm) to protect the seed from granivorous small mammals.

Germination of seed dropped *in situ* by the plants derived from the translocated seed could not be assigned to donor populations because seed from each donor had been planted in close proximity to encourage cross-pollination resulting in mixed seed-rain from the first generation. In addition, any seedlings that germinated in the second year could have resulted from either the original sowing attempt and had undergone dormancy in the intervening growing season, or might have been offspring of the original translocated generation. It has however, been possible to assign seedlings in all monitoring years to the phase of translocation where more than one has been undertaken at a site due to the spacing of sowing plots and the small dispersal distances of the dehisced seed.

The receptor sites were monitored in April-May and July (2006-2009) to record the number of seedlings and adult (i.e. flowering) plants each year.

Climate: Information on climate was generated from the Ecological Site Classification Decision Support System (Ray 2000) and soil nitrogen availability from the Forestry Commission Bulletin (Pyatt *et al*. 2001). Geological information was taken from the British Geological Survey (1979) maps. Soil data was taken from The Macaulay Institutes' soil map (1981). Meteorological data were obtained from the Met Office website (http://www.metoffice.gov.uk/climate/uk/ accessed on 16 November 2009).

CONSEQUENCES

The germination and subsequent survival of seeds translocated to the six Perthshire sites has been disappointing especially compared to data from germination trials and demographic monitoring of populations at extant sites (see Table 2 for results of monitoring in the period 2006-2009).

Phase I translocations resulted in only three or less adult (i.e. flowering) individuals in the second generation indicating that the initial sowing of 100 or so seeds was insufficient to establish viable populations and/or that conditions were unsuitable to support the plant. The population at Carie has, however, shown an encouraging increase in numbers as recorded on the last survey (July 2009) with 25 adult plants (stemming from an original 89 seeds sown August 2005). This is an order of magnitude higher than all other attempted translocations (Table 1). Carie has also supported the highest germination 134 of 500 (275) of phase III translocated seeds (sown August 2008) and an encouraging number of adult plants (42) were recorded during the last survey (July 2009); it appears to be a good receptor site candidate for future translocations.

Germination of translocated seed varied greatly according to site (Fig. 1). Rumbling Bridge supported very poor germination rates in phase I and the lowest germination in phase III. This is possibly due to environmental factors; this site has warmer and drier conditions than those at extant populations (Table 1). This is supported by germination in April 2007 of 16 seedlings despite no surviving adults from the previous generation. These seedlings must have emerged from dormant translocated seeds from the phase I plantings in 2005.

Dormancy is thought to be induced by deviation from normal weather conditions, particularly drought (Dalrymple 2007), and can allow the progeny to survive unfavourable conditions. However, the remaining ungerminated seeds translocated to Rumbling Bridge succumbed to some other cause of mortality before reaching maturity and we therefore conclude that where environmental conditions induce dormancy this carries the risk of deleteriously impacting on seed viability. It is not known to what extent dormancy of seed incurs higher mortality in *M. sylvaticum*, but it is assumed that there must be some loss if dormancy is induced in the majority of individuals. The phase III translocation to Rumbling Bridge may help elucidate whether site conditions are the cause for low survival but unless this population shows much better recruitment, this site will not be used in future translocations.

Table 2. Seed translocation site characteristics over three phases of the project. The proportional survival at most recent survey is expressed as a proportion of seed input and therefore reflects population size as a factor of the original translocation. With the exception of phase III translocations which were undertaken in 2008 and first surveyed in 2009, the proportional survival will be dependent on intervening years and therefore should be interpreted accounting for the difference in time since each introduction was initiated.

Receptor site	**Project phase**	**Seed input**	**Date sown**	**Number of seedlings (April or May) or adults (July) counted during surveys**								**Proportional survival at most recent survey**
				May 2006	July 2006	April 2007	July 2007	April 2008	July 2008	April 2009	July 2009	
Lower Deil's Cauldron	I	91	August 2005	15	0	10	2			1		0.011
Upper Deil's Cauldron	I	103	August 2005	22	8	7	3			0		0.000
Rumbling Bridge	I	92	August 2005	7	0	16	0			4		0.043
	III	500	August 2008							64	10	0.020
Carie	I	89	August 2005	22	9	3	2			24	25	0.281
	III	500	August 2008							134	42	0.084
Kynachan	I	99	August 2005	28	12	0	1			19	3	0.030
	II	366	August 2006			60	36	16	12	15	14	0.038
	III	500	August 2008							75	25	0.050
Glen Artney	II	369	August 2006			46	24	9	3	5		0.014

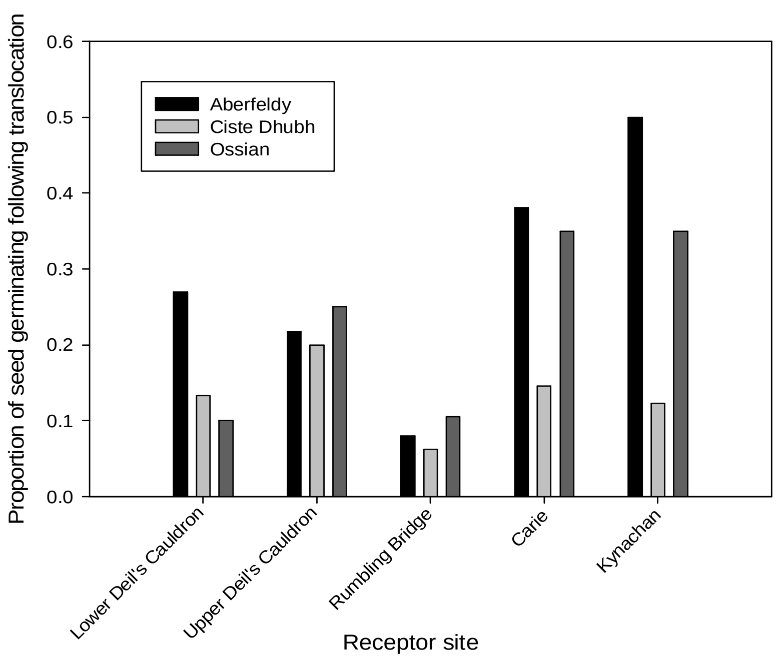

Figure 1. Proportion of germinated seed at each of the five sites used in phase I of the *Melampyrum sylvaticum* Recovery Project initiated in 2005. The differently shaded bars denote the identity of the donor population. Two way ANOVA showed that both receptor site and identity of donor population were almost significant in terms of their effect on germination (site: F = 3.737, p = 0.053; donor population: F = 4.145, p = 0.058).

Effects of weather patterns on germination and survival: Based on previous experience and observations of the species at extant sites, we believe that seed introduced during phase I experienced perhaps the least ideal weather conditions for *M. sylvaticum* during the trial, being much drier than seasonal average during the seed dormancy period, cooler and wetter during germination and warmer and drier during the first growing season. The cooler germination period may explain relatively high germination results across five sites (21.8% ± 4.3), whilst a warmer and drier growing season might account for the mortality of all seedlings at the two sites that have the highest moisture deficit scores. Weather conditions experienced during phase II were drier than the seasonal average at time of sowing, much warmer and wetter during dormancy but the spring was warmer and the summer wetter during the first growing season. The two sites used for this phase attained some of the lowest germination (16.5% and 12.5%) but better survival of seedlings to maturity, possibly due to the wetter summer. Seed introduced during phase III experienced more favourable weather conditions, with weather being warmer and wetter than the seasonal norm when sown, average during dormancy and germination, and warmer and very much wetter than average during the first growing season. Average germination across the three sites was 18.2% ± 4.3, but at best, only a third of seedlings survived to maturity despite our hope that wetter conditions would be beneficial.

Effects of donor population identity: The suitability of seed from different populations was determined by analysing the mean germination of Aberfeldy, Ciste Dhubh and Ossian seed on the seven attempted translocations included in phases I and II. Aberfeldy seed consistently germinated in higher proportions than Ciste Dhubh seed although the statistical significance was only marginal (Fig. 2; 1 way ANOVA, F = 3.416, p = 0.055). This might be explained by better seed viability at Aberfeldy but also might reflect the greater ecological similarity of Aberfeldy and the translocation sites. The Birks of Aberfeldy is lower in altitude than the other donors (280 m above sea level compared to 400 m at Ossian and 460 m at Ciste Dhubh) and has upland brown earths and humic iron podzols derived from Dalradian lithology, giving rise to soils of moderate nitrogen availability. Ossian, also occurring on Dalradian lithology, has soils composed of peaty gleys and peaty podzols. Ciste Dhubh has sub-alpine soils and peats derived from the poorer Moine lithology. The translocation sites occur on the Moine, Dalradian and Devonian lithologies, but all have either upland brown earths or humic iron and peaty podzols. Overall, Aberfeldy has a much more similar edaphic conditions, climate and therefore community type to the translocation sites. Interestingly, Carie is the only receptor site that does not occur on brown forest soils, instead being associated with peaty podzols and peaty gleys. This adds further evidence to suggest that Carie may be more firmly situated within the core habitat ‘envelope’ of *M. sylvaticum* in Scotland and might also explain the promising germination of seeds from all three donor populations.

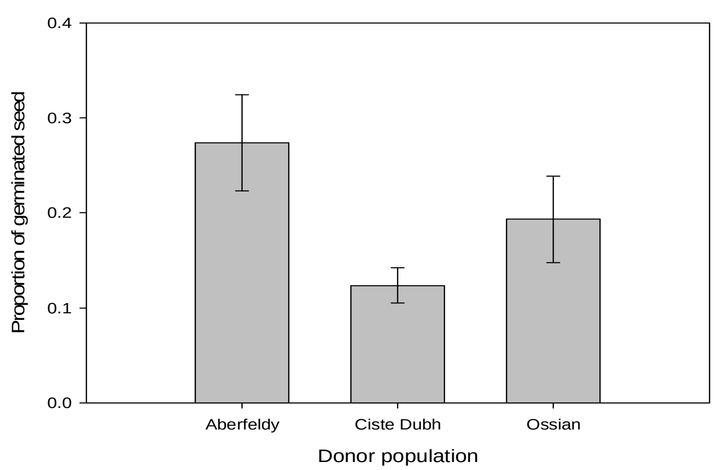

Figure 2. Means and standard error of *M. sylvaticum* seed germination of each donor population at six sites (n = 7, i.e. one received two separate seed inputs). Germination of Aberfeldy-sourced seed was greater than Ciste Dhubh-sourced seed (but not quite statistically significant; pairwise comparisons within a 1 way ANOVA (p = 0.053).

Recommendations: In conclusion, the *M. sylvaticum* sowing trials to the six sites within the boundaries of the former range have indicated the following:

a) at receptor sites showing good potential for supporting the species, introductions should use many more than 100 seeds; a minimum of 500 is presently recommended but this will be revised in the light of findings derived from further monitoring of phase III of the project;

b) the species is capable of forming a short-term seed bank but it is not clear how induced dormancy affects subsequent seed viability (this may be an area of future investigation);

c) receptor site selection should be informed by recent habitat descriptions particularly when considering sites at the warmer and drier end of the range of environmental conditions represented by *M. sylvaticum* habitat tolerances;

d) selecting seed from donor populations that are ecologically, and perhaps geographically, proximal to receptor sites is recommended to increase the probability of germination and establishment.

Further monitoring of all sites and molecular analysis of samples of extant individuals is hoped to strengthen some of the above recommendations and inform future attempts to create new *M. sylvaticum* populations. To test the recommendation that translocations should occur between ecologically similar sites, it will be necessary to move seed from low altitude, warmer and drier donor sites to higher altitude receptor sites (and vice versa). Without this research it is impossible to rule out the possibility that Aberfeldy seed is generally more 'fit' and better survival in ecologically similar sites was just coincidental. Proposals for new projects to address this question are now being developed for implementation in 2010.

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Table 1. Site description summaries of typical *Melampyrum sylvaticum* habitat in Britain (adapted from Dalrymple 2006) and at receptor sites, National Vegetation Classification (NVC) codes (Rodwell 1991), vegetation community descriptions (provided by F.McBirnie, Forest Research) and environmental data (recorded for site selection criteria). AT5 and moisture deficit are calculated using the Ecological Site Classification Decision Support System (Ray 2000); AT5 refers to accumulated day degrees above 5 ºC and is an indicator of site warmth (British maximum AT5 = 2000), moisture deficit is rainfall minus potential transpiration and is an indicator of site dryness (British maximum moisture deficit = 240).

Site	Community type	NVC code	Altitude	Aspect	AT5	Moisture deficit	Distance to water body	Probability of site disturbance	Other comments
Typical British habitat conditions	Broadleaf deciduous woodland often with a high proportion of birch; herb-rich ground flora.	W11/W17	110-640 m	N to W	441-1207	0-106	< 20 m	Low to medium: sites often at edge of steep gullies or in marginal woodland	Data generated from habitat surveys of 8 Scottish populations.
Receptor sites:									
Lower Diel's Cauldron, Comrie	Oak-birch woodland, the former dominating; sparse ground flora of grasses and a little *Vaccinium myrtillus*.	W17	110 m	NE	1272	113	~ 50 m	Medium: site visible from public path.	Selected only after other options rejected; lowest altitude and furthest from water body of all sites.
Upper Deil's Cauldron, Comrie	Canopy of oak, ash, birch and hazel; ground flora of forbs, grasses and *V. myrtillus*.	W11, with W7 patches	140 m	E	1232	105	~ 20 m	Medium: although near to picnic area, site is hidden amongst trees	
Rumbling Bridge, near Dunkeld	Canopy of birch (most frequent), alder, ash and elm; understorey of sparse grass and *V. myrtillus* cover.	W11	140 m	NE	1213	109	~ 10 m	Medium: close to popular beauty spot but amongst trees, not obvious from path.	
Carie, Rannoch Forest	Birch canopy, with aspen adjacent to stream; grass and *V. myrtillus* dominated ground flora but cover relatively low.	W17	260 m	NW	1052	70	~ 15 m	Low: site some distance from paths.	Bracken patches nearby should be monitored to track any expansion.
Kynachan, Tummel Bridge	Open birch canopy adjacent to conifer plantation; lush grass- and forb-dominated understorey.	W11	170 m	NW	1163	96	3-5 m	Low: close to forestry plantation but FCS notified of location. No paths nearby.	
Glen Artney, near Comrie	Open birch canopy in isolated woodland surrounded by pasture; ground flora predominantly grasses but also some forbs.	W11	195 m	N	1163	91	~ 5 m	Low: sheep grazing in area but translocated seed in 1 m high exclosure.	