Managing small populations—using genetic data and trial translocations to help inform suitable conservation measures for the alpine blue-sowthistle (*Cicerbita alpina*) in Scotland

ENVIRONMENTAL RESEARCH
ECOLOGY

PAPER

Managing small populations—using genetic data and trial translocations to help inform suitable conservation measures for the alpine blue-sowthistle (*Cicerbita alpina*) in Scotland

A Finger[1,*], M Smedley[2], D Halley[3], N Frachon[1] and A Ahrends[1]

[1] Royal Botanic Garden Edinburgh, Genetics & Conservation, Edinburgh EH3 5LREH3 5LR, United Kingdom
[2] NatureScot, Inverdee House, Baxter Street, Torry, Aberdeen AB11 9QA, United Kingdom
[3] Norsk institutt for naturforskning, PO Box 5685 Sluppen, NO-7485 Trondheim, Norway
[*] Author to whom any correspondence should be addressed.

E-mail: afinger@rbge.org.uk

Keywords: conservation genetics, translocations, gene flow, microsatellites, grazing, Scotland, Asteraceae

Supplementary material for this article is available online

OPEN ACCESS

RECEIVED
10 January 2023

REVISED
10 June 2023

ACCEPTED FOR PUBLICATION
16 June 2023

PUBLISHED
28 June 2023

Abstract

Habitat fragmentation is causing an increasing amount of species loss around the world and creates problems at the population level. Many species are left as only small and isolated populations, which are vulnerable to genetic erosion and inbreeding depression. Here we present a study on the alpine blue-sowthistle (*Cicerbita alpina*). Due to intensive grazing the species is very rare in Scotland, where it occurs at only four small, montane sites, has never been reported to reproduce and is in need of conservation interventions. As the species can grow clonally it is unknown how many individuals remain and whether populations are affected by genetic isolation. We (1) quantified genetic diversity, inbreeding and between-population differentiation in Scotland and Norway using 15 microsatellite loci, and (2) experimentally translocated plants to new sites. Genetic diversity in Scotland was low (H_E: 0.35; Allelic Richness: 1.84; 4 sites) compared to Norway (H_E: 0.52; Allelic Richness: 2.56; 5 sites). The transplants were able to grow at new sites and are therefore not restricted to steep, montane ledges. While grazing is likely to be the main factor preventing range expansion, long-term genetic isolation has possibly further lowered population viability. To avoid local extinction of this species, conservation translocations and genetic rescue might be appropriate conservation interventions, but this needs to be further tested in a controlled environment and away from wild sites to avoid potential risks of outbreeding depression.

1. Introduction

Habitat fragmentation has major negative impacts on terrestrial biodiversity around the world, particularly in combination with climate change, and is threatening two in five plant species with extinction (Haddad *et al* 2015, IPBES 2019, Lughadha *et al* 2020). The direct and immediate negative impacts of fragmentation affect species' abundances and range sizes, indirect impacts can arise in the form of genetic problems following population isolation (Aguilar *et al* 2006, 2008, Schlaepfer *et al* 2018, Püttker *et al* 2020). The management of small and fragmented populations can be challenging and costly, as many species require continuing, species-specific interventions (Scott *et al* 2010). Developing effective and decisive management action may therefore be crucial to prevent declining population sizes and could ultimately avert extinction (Meek *et al* 2015).

Anthropogenic habitat fragmentation has a strong negative effect on plant genetic diversity (Schlaepfer *et al* 2018, González *et al* 2020). Restricted gene flow between small, fragmented populations can lead to loss of genetic variation, genetic drift, inbreeding depression, and the fixation of deleterious alleles (Leimu *et al* 2010, Angeloni *et al* 2011, Hedrick and Garcia-Dorado 2016). A large body of empirical research has demonstrated causal links between reduction in genetic diversity, elevated inbreeding, and reduced fitness in

wild plant populations, characterised e.g. by lowered seed production, germination or survival rates (reviewed by Leimu *et al* 2006, Finger *et al* 2011, Pickup *et al* 2013). Furthermore, small populations are at a higher risk of being lost altogether due to stochastic events, such as landslides, outbreaks of pests, or grazing. To effectively manage and conserve small populations it is therefore crucial to include genetic information in conservation programmes (Gaitán-Espitia and Hobday 2021).

An example of a country that has experienced strong habitat fragmentation is Scotland. A long history of deforestation has left Scotland with only ∼4% cover of native woodlands, and intensive grazing by sheep and deer and burning regimes prevent woodlands from re-establishing (Anderson 1967, Oosthoek 2013). Consequently, many plant species in Scotland are of conservation concern (Stroh *et al* 2023), may require genetic restoration (Honnay and Jacquemyn 2007) and habitat conservation may simply no longer be sufficient to prevent declines due to fragmentation. Conservation translocations, the intentional movement of species for conservation purposes (IUCN/SSC 2013), might also be needed as a measure of last resort. Which conservation measures are the most appropriate and effective will depend on a good understanding of a species' genetics and ecological requirements.

We explored the genetic state of the nationally vulnerable alpine blue-sowthistle *Cicerbita alpina* (Asteraceae). In the UK, this species survives at only four small, inaccessible sites in the Cairngorms National Park, Scotland. While there is no documentation on this it is likely that prior to deforestation and grazing intensification, *C. alpina* populations were more widespread in Scotland. To guide the most appropriate conservation measures we explored whether (i) *C. alpina* is genetically depauperate in Scotland, (ii) remnant populations are genetically isolated, (iii) plants in Scotland can be translocated away from their natural sites.

To answer these questions, we conducted a near complete genetic inventory across the four known populations in Scotland and sampled a subset of larger and less isolated populations in Norway (which we expected to be genetically more diverse). Furthermore, we planted *C. alpina* into six new sites to test whether translocations can be done successfully.

2. Material and methods

2.1. Study species

Cicerbita alpina is a boreal-montane plant, which is found across Fenno-Scandinavia and in most European mountain ranges (Fries 1949, Wagenitz 1987, Meusel *et al* 1992). It is a perennial plant with up to 1.5 m tall panicles, flowering between July and September. The plants grow straight with each stem producing over 100 flower heads per season. Each composite flower head is about 2.5 cm wide and made up of about 40 individual violet blue flowers. The flowers are visited and presumably pollinated by bees, bumblebees, hoverflies, moths and butterflies (Marren *et al* 1986, Sell and Murrell 2002). Each fruit head contains up to 40 seeds (∼4 mm length × 1 mm width), which are crowned by unbranched hair and readily dispersed by wind. Seed production is between August and October.

Prior to this study, it was unknown how many individuals remain in Scottish populations. Although plants produce flowers and seeds, seedlings have rarely been recorded. It was unclear whether this was due to recruitment failure, survey errors, or other demographic reasons. The species can also reproduce asexually through clonal growth. It is therefore challenging in the field to differentiate between seedlings and new shoots of an existing plant, as it is not possible to find cotyledons in field conditions, and seedlings may consequently have been missed during surveys.

2.2. Habitat and populations

2.2.1. Scotland

In Scotland *C. alpina* is considered a montane species, exclusively found on wet, north-facing ledges and gullies at higher altitudes (700–1100 m a.s.l.). These provide protection from grazing animals, direct sunlight, and permanently moist but not waterlogged soils. They offer island refugia, surrounded by largely unsuitable areas of open heathlands and grasslands. However, landslides in and around some of these ledges and gullies happen frequently and the populations are at risk of being lost altogether. The species is a representative of the nationally rare tall herb community. It is possible that the species used to be more widespread in the UK prior to deforestation and heavy grazing by livestock and deer. According to Marren *et al* (1986), at least eight populations have been lost since 1848, with the most recent extinction occurring in Canness Glen in 1978. Historical grazing has likely led to its current restricted distribution in Scotland, current levels of grazing might hinder natural expansion, and warming temperatures could further threaten this species' existence in Scotland. Consequently, without human intervention, such as removing grazing pressure, increasing population sizes, and facilitating gene flow, long-term persistence of this species in Scotland seems unlikely.

Botanical surveys of the populations have included counting the number of flowering stems, but due to vegetative growth and plants growing in proximity it is not possible to distinguish between genetically

distinct individuals. The most extensive stand at Corrie Fee (gully size ~300 m^2, 730 m a.s.l.) had a stem count of 239 in 1999 (Alexander 2000). Populations on Lochnagar and in Corrie Kander are dispersed over several ledges. In 1999 the number of stems recorded at Lochnagar (total ledge sizes ~50 m^2, 1030 m a.s.l.) was 349 stems and at Corrie Kander (total ledge sizes ~30 m^2, 850 m a.s.l.) 242 stems. The smallest population with a stem count of 31 is Caenlochan (one ledge of ~4 m^2, 850 m a.s.l.) (Alexander 2000). Recent surveys undertaken as part of this study have rediscovered a stand at Lochnagar (1120 m).

2.2.2. Scandinavia

Scandinavia is best suited for a comparative analysis with Scottish populations. It has the geographically closest populations to Scotland, growing at similar latitudes and altitudes. In Scandinavia *C. alpina* is a common and widespread plant and its National Red List status is 'Least Concern'. It is mainly found in fairly open to moderately dense birch and conifer woodlands. Shade and reliably moist but not waterlogged soils are a requirement. It can also be found in shaded places above the treeline, generally north facing. It is found from sea level to above the treeline throughout Norway, except for some Finnmark areas in the north, and throughout Sweden, except for Skåne (Solstad *et al* 2021). Where grazing pressure is high it is confined to cliff ledges (Bakkeveig 1983). It is climatically tolerant, ranging from very mild, wet, and almost snowless 'hyperoceanic' coasts to cold continental inland regions with seven months of snow; and with annual precipitation ranging from over 4000 mm in the most oceanic areas to less than 500 mm in parts of eastern Sweden. There is no evidence for recruitment issues in Scandinavian populations (Solstad *et al* 2021).

2.3. Genetic sampling

In 2015–17 we collected leaf samples for DNA extraction from all four populations in Scotland (102 samples) and five populations in Norway (52 samples), see figure 1. We attempted an exhaustive sampling in Scotland while avoiding resampling the same plant by collecting leaves across each ledge but leaving 0.5 metre between leaf samples. At Lochnagar and Corrie Kander some ledges were inaccessible, therefore we will have missed some plants there.

In Norwegian populations samples were taken across the entire population and collected at least a few metres apart to avoid sampling clones. This was to give us an indication of the levels of genetic diversity and inbreeding to expect for large, and more continuous populations. This data can then act as a baseline and indication for what we expect in a 'genetically healthy' population and be used for comparisons with Scottish populations. Populations were collected from central and southern latitudes, high and low altitudes, and large and small patches (see table 1 and figure 1). For the purpose of this study, we defined a population in Norway as a patch of plants in a certain area. The STRUCTURE analysis (figure 2) confirmed that this definition was adequate.

Due to small ledge sizes in Scotland it is more likely that we have collected clones in Scottish populations (closer proximity of collected leaf samples) compared to Norway.

2.4. *Cicerbita alpina ex-situ* conservation collection

Plant root stock and seeds were collected (under licence) from all four natural UK populations (2 genets from Corrie Fee, 1 Caenlochan, 2 Lochnagar, 4 Corrie Kander) and plants grown in pots and bulked up at the nursery/polytunnel of the Royal Botanic Garden Edinburgh (RBGE). Root stock from each plant can be divided repeatedly, allowing accumulation of a large number of (genetically identical) plants over time, which can be used for ecological and growing experiments.

2.5. DNA extraction, short sequence repeat (SSR) marker development, polymerase chain reaction (PCR) amplifications and genotyping

Leaf material was immediately dried and stored in silica gel. DNA was extracted from the leaves using the QIAGEN DNeasy 96 Plant Kit, following the manufacturer's protocol. All samples were screened at a total of seventeen nuclear microsatellite loci.

Enriched libraries were created by Ecogenics (Microsynth, Switzerland) with TrueSeq nano and sequenced on Illumina Miseq v2 2 × 250 bp. Microsatellites were enriched in silico using Tandem Repeats Finder, v 4.09 (Benson 1999) and primer design was done with Primer 3 (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). Out of 91 microsatellite containing sequences we developed primers for 68 markers to test for polymorphism. Of these, we chose the best working markers (seventeen) which had at least three alleles. These were optimized and labelled with an M13-tag at its 5′-end described by Schuelke (2000). For marker details see table 1.

PCR was carried out in 10 μl reactions with 1 μl of 1X PCR buffer (Promega colorless Flexi GoTaq PCR buffer), 2.5 mM MgCl2, 0.2 mM dNTPs, 0.05 μl of the 0.1 μM M13 forward primer, 0.1 μl of the 0.1 μM reverse primer and 0.1 μl of the 0.1 μM M13 primer, 0.03 U *Taq* polymerase (Promega), and 2 μl DNA

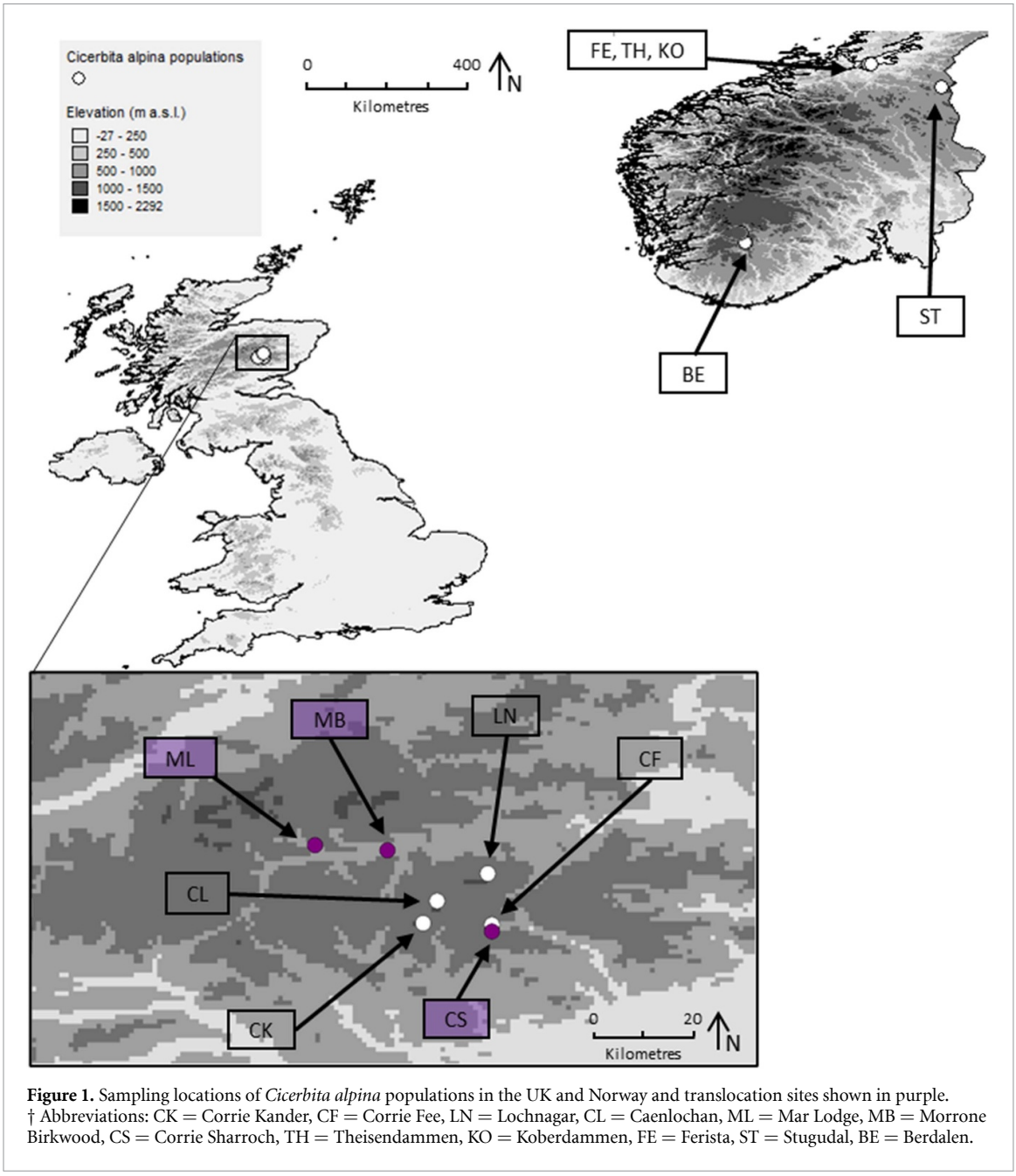

Figure 1. Sampling locations of *Cicerbita alpina* populations in the UK and Norway and translocation sites shown in purple. † Abbreviations: CK = Corrie Kander, CF = Corrie Fee, LN = Lochnagar, CL = Caenlochan, ML = Mar Lodge, MB = Morrone Birkwood, CS = Corrie Sharroch, TH = Theisendammen, KO = Koberdammen, FE = Ferista, ST = Stugudal, BE = Berdalen.

template (*c.* 10 ng). Cycling conditions were as follows: 1 × (94 °C for 5 min), 30 × (94 °C for 45 s, primer-specific temperature (57 °C) for 30 s, 72 °C for 30 s), 1 × (72 °C for 30 min).

All PCR reactions were performed on a Biorad Tetrad 2 thermal cycler and run on an ABI 3730 automated sequencer at the Edinburgh Genomics facility. LIZ-500 labelled internal size standard (Applied Biosystems, Foster City, California, USA) was added to each sample to size fragments. The data generated was analysed with the software GeneMarker (SoftGenetics, Pennsylvania, USA) scoring electropherograms manually.

2.5.1. Assessment of marker suitability

We tested the suitability of markers for population genetic analyses, i.e. their neutral and unlinked nature as well as null allele frequencies (Selkoe and Toonen 2006), using GENEPOP (Raymond and Rousset 1995) and CERVUS 3.0 (Kalinowski *et al* 2007). Significant linkage disequilibrium was observed for any pair of loci after Bonferroni correction (Hochberg 1988). All markers (except for SSR 64) had a significant excess of homozygotes. SSR 64 showed a heterozygosity of 100%, which seemed atypical and was therefore excluded from the analysis. Two markers (SSR 1 and SSR 26) had an elevated null allele frequency (0.69 and 0.75, respectively) compared to the other markers (0.11–0.55) when only analysing Norwegian samples but only SSR 26 remained higher when using the whole dataset and was therefore also excluded from further analysis,

Table 1. Characteristics of seventeen polymorphic microsatellite loci in *Cicerbita alpina*.

Locus	GenBank Accession no.	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	T_a (°C)	*A*	H_O	H_E	*PIC*	*F* (null)
1	MT995613	F CTGAGGCGGTCAGGTATGAG R ACAAAAGAAGCGCCTTGTCC	$(AG)_{17}$	103–107	57	3	0.09	0.31	0.28	0.54
3	MT995614	F TTTGAGTCCTCACCCGATCC R GTGGCTAGGATTACGTCCCC	$(TA)_{26}$	146–168	57	11	0.56	0.78	0.74	0.17
10	MT995615	F CCACTAGTTTCGCTTTTATATGGC R ACGTACGATGCCACTTTAGC	$(TA)_{25}$	238–258	57	11	0.40	0.79	0.76	0.33
15	MT995616	F AAATTGTTCCTACGACCACAC R ATGACACGTGGCTTCAAAAC	$(TA)_{27}$	146–170	57	13	0.49	0.83	0.81	0.27
16	MT995617	F ACAGACGTTACATTTTCCATGC R TAATCCCTTGACCCCAGTCG	$(TA)_{24}$	182–200	57	8	0.21	0.79	0.75	0.58
18	MT995618	F CATCGCACACCTTTAGGGAAC R ACGATTGGCTCAGAATTGTCC	$(AT)_{29}$	173–223	57	20	0.39	0.91	0.90	0.39
21	MT995619	F TACGACTGACGGCATTTTCC R GCTACACGCAATGTAGGCTC	$(AT)_{29}$	235–251	57	8	0.43	0.77	0.73	0.27
22	MT995620	F TGTGTACTCATAGCCTCGCC R TGGTACTTCCATTCAGTTCGG	$(TA)_{22}$	251–263	57	6	0.40	0.70	0.64	0.28
23	MT995621	F AAAGACGTTTCTGTCCAGCC R AATAATTTACATTCGCTAGTGTTGAG	$(ATA)_{21}$	262–277	57	5	0.24	0.67	0.60	0.48
24	MT995622	F CGTATTTACCGAAGATGGAGAGC R GCCGGGTGTTACATACATGG	$(AGTT)_{10}$	209–229	57	4	0.43	0.59	0.53	0.20
26	MT995623	F ATTACTATCCCCGCGCCATC R TTACTTTACTAACCGTGGTTTCC	$(CATA)_{11}$	125–169	57	9	0.04	0.64	0.61	0.89
42	MT995624	F AGCATGAGTTAAAAAGGTCCG R TGGTTCTACATAGCTCCGCC	$(AT)_{16}$	132–168	57	16	0.26	0.80	0.78	0.52
43	MT995625	F AGAAACAATGGATCCTAGCCC R TGCAAGGTTTGCTCCATACG	$(TA)_{16}$	157–189	57	10	0.40	0.81	0.78	0.35
54	MT995626	F TGAGTCTCGCATTCTGAACC R TGCACCCTTTTGATCTAAGCC	$(AT)_{23}$	226–246	57	10	0.20	0.83	0.81	0.62
17	MT995627	F ACGATTGGCTCAGAATTTTCC R AGGGCCGATATTTGTCCTCG	$(TA)_{27}$	193–221	57	15	0.41	0.90	0.88	0.37
64	MT995628	F ACCCACCCTAGTTGTCAGTC R CGTTTCCTGATTTTGCCAGC	$(GTAT)_7$	249–265	57	5	1.00	0.63	0.56	−0.26
68	MT995629	F CCAATGCCCCATCTAAGAGTTTG R AGTGATGCCACCATTCCTTTG	$(AC)_{13}$	246–268	57	5	0.30	0.66	0.61	0.36

[a] Abbreviations: *F*: forward primer; *R*: reverse primer; T_a: annealing temperature; *A*: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; PIC: polymorphism information content; *F* (null): null allele frequency; 106 individuals were analysed for each locus.

see table 1. SSR 26 was informative enough, though, to tell individuals apart and was used for that purpose. Further analyses were performed on multi-locus data from 15 microsatellites.

DNA isolation has been repeated for 38% of Scottish individuals. PCR and genotyping have been repeated for all samples (Scottish and Norwegian) due to the clonal nature of the species. The genotyping error rate will therefore be minimal.

2.6. Genetic diversity estimates, inbreeding, genetic structuring, kinship, and clonal growth

Number of alleles (N_A), number of effective alleles (N_E), allele frequencies, allelic richness (A_R), observed and expected heterozygosity (H_O, H_E), number of private alleles (P_A) and inbreeding coefficients (F_{IS}) using 10 000 permutations were calculated using SPAGeDi 1.5 (Hardy and Vekemans 2002) and GenAlEx 6.5 (Peakall and Smouse 2006).

Overall G'_{ST} and Josts'D (Jost 2008) values and pairwise comparisons between populations were calculated using GenAlEx 6.5. To test for the presence of geographical groupings of related samples, we applied a Bayesian cluster analysis to all individuals using the software STRUCTURE (Pritchard *et al* 2000). We carried out a total of 150 runs, 1–15 clusters (K1 to K15) each repeated ten times, with the 'admixture', 'Separate α for each Population', and 'Correlated allele frequencies' options selected. For each run the burn-in and simulation length was 50 000 and 500 000 MCMC iterations, respectively. To identify the most likely number of distinct genetic groups K we used the ΔK statistic (Evanno *et al* 2005), as well as $Pr[X|K]$ of Pritchard *et al* (2000). They were calculated using the online version of the software CLUMPAK (Kopelman *et al* 2015), http://clumpak.tau.acil). STRUCTURE output files were processed using the online version of 'pophelper' (Francis 2016), http://pophelper.com/.

To gain a better insight into the amount of selfing in populations, multilocus kinship coefficients F were calculated for all possible pairwise combinations for all plants and mean kinship coefficients for each population estimated using SPAGeDi 1.5.

Clonal growth has not been analysed systematically but when samples scored the same alleles at all 15 microsatellite markers, we assumed that these samples originated from the same genetic individual.

2.7. Trial translocations to assess plant establishment away from natural sites

To ensure that we have a good knowledge on growing and habitat requirements for this species in Scotland we conducted experimental translocations in 2017. While short term results (five year trial) will not provide evidence that the species can complete its entire life cycle at that site it helped to understand whether (i) *C. alpina* can grow away from its natural sites, (ii) altitude affects establishment of pregrown transplants, and (iii) plants need protection from grazing when large herbivore control is in place. We used clones originating from two plants, one from Lochnagar and one from Corrie Kander. These have been repeatedly divided resulting in large numbers of separate clones. As such we were able to repeatedly plant the same two genetic individuals into different environments. This approach allowed us to control for genetic effects impacting survival. Using only two genets (genetic individuals) has limitations and is not sufficient to provide a true representation of population level fitness. Though it needs to be stressed that due to the low number of remaining genets in populations, and the close relateness within populations (see results), one genet per population can still give an indication of the survival potential of these populations in different habitats.

Plants were controlled for pest and tested for occurrence of *Phytophthora ramorum* before being planted out with a small amount of soil remaining between roots. Rootball size was not measured for each plant prior to planting but is estimated to have been around 25 cm in circumference. Initially 240 rootballs were planted into three new sites: 80 at Mar Lodge, 80 at Morrone Birkwood and 80 at Corrie Sharroch, all within the Cairngorms National Park. Plants had a ratio of 80% Lochnagar to 20% Corrie Kander clones, which represents the plant material available at the time. Planting at each site was done in eight groups of five, with each group about 1–3 m apart. After grazing by voles and slugs became apparent at Mar Lodge in the first year of planting a further 75 plants were planted at this site in 2018 with additional protection by vole caging. This was done to test the effectiveness of vole cages for potential further application for larger scale conservation translocations.

All three translocation sites lie within the species' 'natural range' in Scotland, and are protected from livestock and deer either by fencing or rigorous deer stalking. The Corrie Sharroch site is situated on steep mountain ledges (750 m a.s.l.) and similar to the habitat currently occupied within Scotland. Locations at Mar Lodge (415 m a.s.l.) and in Morrone Birkwood (390 m a.s.l.) are at lower altitudes and more similar to where the species is found in Norway. Survival, rough estimate of plant size (height x width at widest point, measure for each plant), flowering, grazing pressure (estimate % of damaged leaves and stalk per plant), and plant competition (% encroaching vegetation within a 30 cm $\times$ 30 cm plot measured for each plant) were recorded over a period of four years.

3. Results

After removing all clones (individuals with the same genotype at 15 SSR loci), from our genetic dataset as well as samples where DNA extraction had failed, a total of 104 sampled individuals remained for further analysis, 56 from Scotland and 48 from Norway (table 2).

3.1. Genetic diversity and inbreeding

At the species level each of the 15 loci yielded between three and 19 alleles, with a total of 144.
A comparison of genetic diversity over all loci and populations is given in table 2. In Scottish populations significant inbreeding (F_{IS}) was detected for Lochnagar and Corrie Fee, and while not significant ($p < 0.1$) Corrie Kander also had a lower H_O than H_E. In contrast, Caenlochan exhibited a homozygote deficit, possibly suggesting a recent population bottleneck, lack of selfing or an advantage for heterozygotes at this site. Values for H_E ranged from 0.20 (±0.05 SE) in Corrie Fee to 0.42 (±0.06 SE) in Caenlochan. Allelic richness (A_R), based on five diploid individuals used for rarefactions, was similar across three sites but lowest in Corrie Fee 1.56 (±0.12 SE).

Comparisons with larger Norwegian populations showed that significant inbreeding is also present with similar values to Scottish populations. Genetic diversity indices (H_E, A_R) though were consistently higher in Norwegian populations, on average H_E was 0.56 (± 0.03 SE) and A_R 3.29 (± 0.16 SE) (table 2).

3.2. Genetic differentiation

Mean pairwise genetic distances were significantly different and very high among all populations (table 3). In Scotland genetic distances were lowest between the geographically closest Corrie Kander and Caenlochan (JostD 0.35, $p = 0.01$; 4.6 km between populations), both located on the west side of a mountain range (The Mounth). Populations are also differentiated in Norway; even populations Theisendammen and Ferista, which are only 150 m apart, have a JostD value of 0.28 ($p = 0.01$). The STRUCTURE analysis identified five and nine distinct genetic clusters (K5 and K10) as the most likely solution. In both solutions this results in clustering Corrie Kander and Caenlochan into one genetic group and completely isolating Corrie Fee and Lochnagar, both of which are at least 7 km away from any other population (see figure 2). Equally, all analysed Norwegian populations form separate genetic clusters in the $K = 9$ solution with indication of a degree of gene flow as some individuals are not assigned to their respective population (see figure 2). The solution of nine genetic clusters remained when analysing Scottish and Norwegian population separately (data not shown).

3.3. Within population kinship coefficient

The average individual kinship coefficient within all analysed *C. alpina* populations is high. For Scotland Corrie Kander had the lowest kinship values, 0.42 ± 0.02 SE, and Corrie Fee had the highest average value, 0.62 ± 0.01 SE (see figure 3). Such high values can only be obtained through repeated selfing and inbreeding.

Kinship values in Norway were also high though slightly lower than Scotland, ranging from 0.37 ± 0.07 SE in Ferista to 0.23 ± 0.01 SE in Stugudal (see figure 3).

3.4. Clonal growth

Each Scottish population exhibited some clonal growth. In Corrie Kander, of 39 collected leaves 12 (31%) were different genets, Corrie Fee: of 32 sampled leaves 19 different genets (59%), Lochnagar: of 18 sampled leaves 14 different genets (78%), and Caenlochan: of 13 collected leaves 11 different genets (85%). In Norwegian populations detected clonal growth was lower, most likely due to a different sampling strategy, leaving more space between samples. For Ferista of 12 collected leaves 9 (75%) were different genets, and Berdalen of 9 collected leaves 8 (89%) were different genets.

3.5. Trial translocations

3.5.1. Survival and plant size

Of 315 planted C. alpina clones, 31% survived four years after planting, (44% in Mar Lodge, 30% in Corrie Sharroch, and 6% in Morrone Birkwood, table 4). Poor performance at Morrone Birkwood is likely due to poor micro-siting (e.g. planting in waterlogged soils). Plant survival stayed similar between years 3 and 4 at

Table 2. Genetic variability of 15 microsatellite loci calculated for all populations of *Cicerbita alpina*, 104 individuals.

Country	Population	Patch size (m^2)	Pop alt (m)	n	N_A	N_E	H_O	H_E	$A_{R(5)}$	P_A	F_{IS}
UK	Corrie Kander	25	850	12	2.33 (±0.25)	1.84 (±0.23)	0.33 (±0.07)	0.38 (±0.06)	2.16 (±0.21)	1	0.11
	Corrie Fee	100	730	19	1.80 (±0.19)	1.32 (±0.10)	0.11 (±0.07)	0.20 (±0.05)	1.56 (±0.12)	2	0.49***
	Lochnagar	50	1030	14	2.80 (±0.24)	1.75 (±0.16)	0.31 (±0.08)	0.38 (±0.06)	2.22 (±0.18)	4	0.19**
	Caenlochan	4	850	11	2.13 (±0.33)	1.86 (±0.18)	0.62 (±0.10)	0.42 (±0.06)	2.03 (±0.24)	1	−0.50
	Average			**14**	**2.27 (±0.12)**	**1.74 (±0.09)**	**0.34 (±0.05)**	**0.35 (±0.03)**	**1.99 (±0.18)**	**2.0 (±0.58)**	
NO	Theisendammen	130	160	12	3.60 (±0.45)	2.62 (±0.31)	0.40 (±0.06)	0.51 (±0.07)	2.97 (±0.32)	5	0.22***
	Ferista	195	173	9	3.53 (±0.31)	2.63 (±0.23)	0.39 (±0.06)	0.58 (±0.04)	3.06 (±0.24)	2	0.34***
	Kobberdammen	10	300	10	3.93 (±0.45)	3.26 (±0.46)	0.34 (±0.06)	0.61 (±0.05)	3.38 (±0.32)	11	0.46***
	Stugudal	40	620	9	5.47 (±0.56)	4.56 (±0.67)	0.56 (±0.07)	0.68 (±0.06)	4.33 (±0.40)	10	0.19***
	Berdalen	10	1107	8	4.53 (±0.51)	4.17 (±0.76)	0.43 (±0.08)	0.65 (±0.06)	4.01 (±0.41)	8	0.36***
	Average			**10.3**	**3.89 (±0.22)**	**3.16 (±0.25)**	**0.41 (±0.03)**	**0.56 (±0.03)**	**3.29 (±0.16)**	**7.2 (±1.66)**	

[a] Abbreviations: n, number of genotyped individuals; N_A, Mean number of alleles; N_E, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; A_R, Allelic richness, based on five diploid individuals; P_A, total number of private alleles (mean frequency); F_{IS}, Inbreeding coefficient, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Table 3. Pairwise G''_{ST} values (below diagonal) and pairwise Jost's D values (above diagonal) between *Cicerbita alpina* populations, all samples $p < 0.01$. Shaded in grey are Norwegian pairwise values, shaded in teal Norwegian/Scotland pairwise values.

	CK	CF	LN	CL	TH	KO	FE	ST	BE
CK	0	0.65	0.54	0.35	0.68	0.57	0.70	0.66	0.50
CF	0.86	0	0.72	0.66	0.76	0.70	0.83	0.71	0.68
LN	0.76	0.90	0	0.70	0.57	0.64	0.60	0.59	0.54
CL	0.58	0.86	0.85	0	0.67	0.70	0.74	0.79	0.66
TH	0.83	0.90	0.75	0.82	0	0.48	0.28	0.27	0.52
KO	0.72	0.85	0.78	0.82	0.62	0	0.43	0.34	0.62
FE	0.83	0.92	0.76	0.85	0.41	0.55	0	0.31	0.50
ST	0.78	0.85	0.73	0.87	0.38	0.44	0.41	0	0.40
BE	0.66	0.83	0.69	0.78	0.65	0.71	0.61	0.49	0

[a] Abbreviations: CK = Corrie Kander, CF = Corrie Fee, LN = Lochnagar, CL = Caenlochan, TH = Theisendammen, KO = Kobberdammen, FE = Ferista, ST = Stugudal, BE = Berdalen.

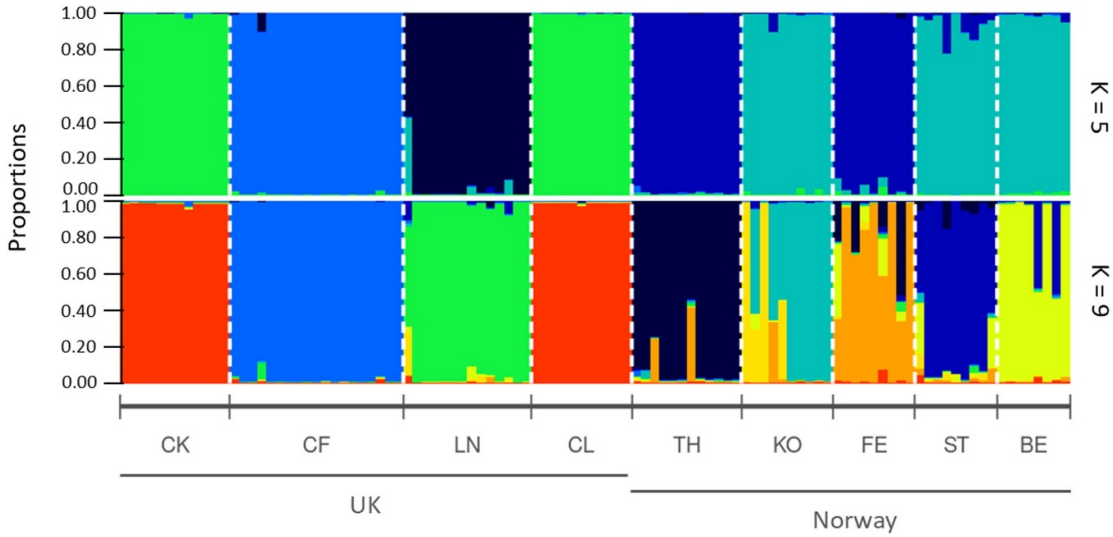

Figure 2. Bayesian structure analysis of *Cicerbita alpina* populations (*x* axis) using STRUCTURE (Pritchard *et al* 2000). Bars represent individual plants with their assignment proportions (*y* axis) to the different genetic clusters. Performing the analysis for $K = 10$genetic clusters. † Abbreviations: CK = Corrie Kander, CF = Corrie Fee, LN = Lochnagar, CL = Caenlochan, TH = Theisendammen, KO = Kobberdammen, FE = Ferista, ST = Stugudal, BE = Berdalen.

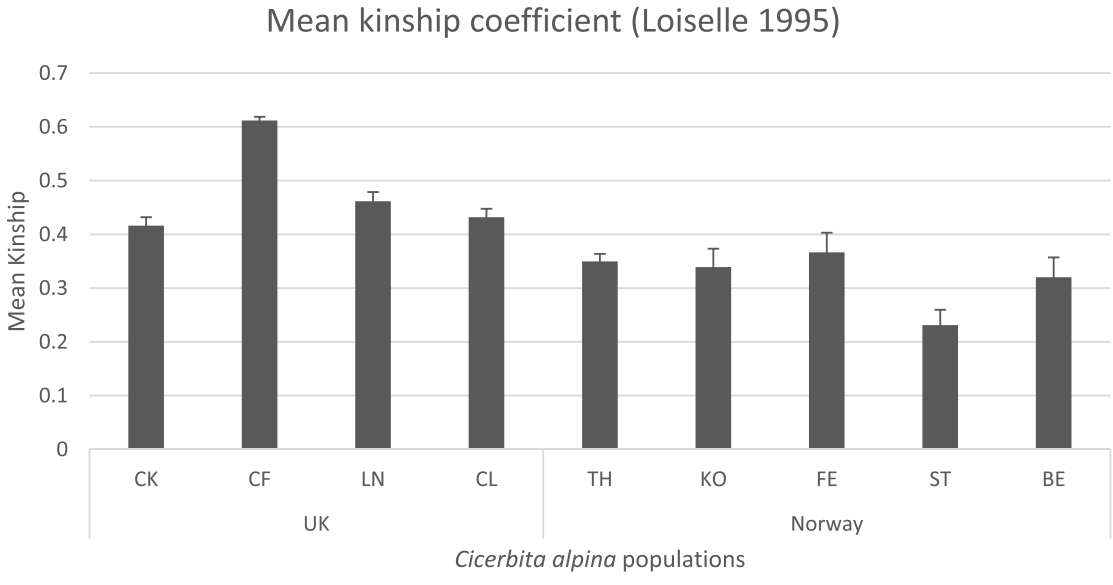

Figure 3. Average kinship coefficient (Loiselle *et al* 1995) of *Cicerbita alpina* populations, including standard error bars. † Abbreviations: CK = Corrie Kander, CF = Corrie Fee, LN = Lochnagar, CL = Caenlochan, TH = Theisendammen, KO = Koberdammen, FE = Ferista, ST = Stugudal, BE = Berdalen.

Table 4. *Cicerbita alpina* translocation experiment results for survival, flowering, plant size, grazing damage (as an estimated percentage of damage per plant), and encroaching vegetation (as an estimated percentage of encroachment within a 30 cm × 30 cm plot per plant) over four years of monitoring. Monitoring was done once a year in the summer at the peak of the growing season. At each site 80 plants were planted in 2017.

	Survival				Plants flowering				Size height*width			Grazing damage			Encroaching vegetation	
Site	Year 1	Year 2	Year 3	Year 4	Year 1	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4	Year 3	Year 4
Corrie Sharroch (750 m a.s.l.)	60%	51%	43%	30%	14%	26%	na	8%	905 cm (±150 SE)	Na	1256 cm (±195 SE)	10%	74%	29%	5%	14%
Mar Lodge (415 m a.s.l.), fenced	48%	63%*+	43%	44%	0%	0.6%	3%	15%	233 cm (±31 SE)	589 cm (±99 SE)	1108 cm (±197 SE)	15%	21%	11%	25%	23%
Morrone Birkwood (390 m a.s.l.), fenced	35%	21%*	6%	6%	0%	0%	0%	0%	92 cm (±36 SE)	29 cm (±8 SE)	208 cm (±71 SE)	10%	10%	13%	80%	14%

* Vole cages added to protect plants from grazing damage. +75 plants supplemented; Na = no data analysed due to heavy grazing.

Mar Lodge, these plants have extra protection through vole cages. Lochnagar and Corrie Kander clones showed similar survival rates.

Corrie Sharroch and Mar Lodge developed the largest plants (height multiplied by width, 1256 cm ± SE 195 and 1198 cm ± SE 197, respectively) compared to Morrone Birkwood (208 cm ± SE 71). At Corrie Sharroch this was particularly true for plants located in a gully with steep walls, which may have sheltered the plants from wind, direct sunshine and dehydration. Third year size data for Corrie Sharroch was not available as most plants were heavily grazed down to the stem. Plant size at all sites increased over the years which may indicate that those plants surviving are starting to establish.

3.5.2. Flowering

Overall in year 4, 13% of surviving plants flowered. No plants reached the flowering stage at Morrone Birkwood. At Mar Lodge flowering increased each year. One plant (0.6%) flowered in year 2, four (3%) in year 3 and 10 (15%) in year 4. Flowering plants were only found within vole cages which provided protection from grazing but possibly also wind shelter. In Corrie Sharroch eleven plants (14%) flowered in year 2 and two plants (8%) in year 4, see table 4. Flowering plants consisted of both Lochnagar and Corrie Kander clones. Variations in flowering between years could also be linked to temperatures and other environmental factors.

3.5.3. Grazing and encroaching vegetation

Mar Lodge was heavily grazed by voles and slugs and needed additional protection through fencing and vole cages in year 1, whereas plants at Corrie Sharroch did not show much grazing damage until year 3 when grazers, likely hare, found the site. In the third year grazing damage was highest at Corrie Sharroch with an average of 74% damage per plant, while Mar Lodge had 21% damage, 16% within vole cages and 34% without vole cages. Only low grazing damage was detected at Morrone Birkwood (10%).

Encroaching vegetation, mainly grasses (e.g. *Molinia caerulea*), measured for each plant was highest at Morrone Birkwood in Year 3. In Year 4 this figure reduced as plants survived in places where encroachment was low (table 4).

4. Discussion

4.1. Are small populations genetically depauperate and isolated?

We have identified 56 genets in Scotland, although we are likely to have missed some, it is evident that fewer than 100 *C. alpina* genets remain in Scotland, which is a very low number. Moreover, genetic diversity is relatively low and kinship high within populations compared to larger Norwegian populations. Another study on *C. alpina* shows that Scandinavia has the highest AFLP diversity, and that other European regions are less diverse in comparison (though excluding Scotland) (Michl *et al* 2010). Extended periods of selfing, very low numbers and resulting genetic drift could explain the observed high kinship and low genetic diversity within populations in Scotland. High inbreeding and kinship values are also characteristic for Norwegian populations, suggesting that selfing is not unusual for the species. Self-compatible clonal plants will have increased levels of geitonogamy (pollination between different flowers of the same genetic individual) as even high visitation rates by pollinators will result in high selfing rates if clones are large or where clonal diversity of (small) populations is low.

Strong genetic differentiation between populations indicates that gene dispersal rates in *C. alpina* may be lower than expected for a wind-dispersed and insect-pollinated species (Dick *et al* 2003, Lander *et al* 2010, Finger *et al* 2014). Population Corrie Fee has a slightly different leaf shape compared to other populations, see figure 4. Though leaf shape has not been systematically analysed it may be a further indication that this populations has been isolated and restricted to within population reproduction since many generations. Limited gene flow was also detected between populations in Norway, even when only 150 m apart. Occasional, long-distance gene flow events there may though be enough to prevent genetic isolation in more continuous habitats.

Cicerbita alpina can reproduce clonally which further affects its ability to persist and expand in Scotland, both positively and negatively. While clonality increases population survival, predominant clonal reproduction can affect long-term phenotypic evolution which could impact on the species' abilities to adapt to changing environments (Orive *et al* 2017). It has been suggested in previous research that increased clonal growth might be a response to small population size (Duwe *et al* 2017). The smallest population Caenlochan though had the lowest amount of clonal growth (eleven of the 13 collected leaf samples belonged to different genets). Though one reason may simply be the lack of space on a 4 m^2 ledge. Where ledges are larger (Corrie Kander and Corrie Fee), clonal growth is more frequent. Therefore a mechanistic link between reduced

Figure 4. Lower leaves collected in the nursery of plants representing five *Cicerbita alpina* populations, CF = Corrie Fee, CA = Caenlochan, LN = Lochnagar, CK = Corrie Kander, NW = Norway. Leaf shape of plants from population CF differ from all other populations which tend to have winged petriole with longer lateral lobes.

population size and increased clonality is unlikely and for *C. alpina* this rather reflects that conditions for successful germination and seedling establishment are rare.

The observed high inbreeding values, strong between population structure in both Scotland and Norway suggest that the species is naturally selfing. It is therefore possible that through many generations of selfing it has purged its genetic load and that inbreeding does not necessarily lead to inbreeding depression (Crnokrak and Barrett 2003, Hedrick and Garcia-Dorado 2016, Glémin *et al* 2019). Whether inbreeding depression might be an issue for this species needs to be further investigated.

4.2. Can plants establish away from wild sites?

Our trial translocations demonstrated that transplant survived at high (750 m a.s.l.) and low altitudes (400 m a.s.l.), but that grazing can be an issue for plant establishment and survival, even at sites where grazing by large herbivores has been almost completely eliminated. A study analysing 249 plant species translocations worldwide showed that on average survival for translocated plants was low with 52%, flowering 19% and fruiting 16% (Godefroid *et al* 2011). In this study, success rates for *C. alpina* were even lower, with only 31% survival and 13% of surviving plants flowering. This may in part be due to the low genetic diversity of plants (only two genetic clones used). Grazing at lower altitudes had a strong negative impact on plants from the first year of planting. While the higher altitude site (Corrie Sharroch) was only mildly affected by grazing in the first two years and year four, it did get heavily damaged in its third year. Thus, our study only partially confirms the notion that montane sites provide refuges from herbivory (Scheidel and Bruelheide 2001). In the absence of changing grazing levels, future translocations will likely rely on additional protection through (expensive and often inefficient) fencing to limit grazing pressure (Scheidel and Bruelheide 2001, Fenu *et al* 2016).

Current distribution patterns in Scandinavia demonstrate that (under lower grazing pressure) *C. alpina* grows at lower altitudes and warmer temperatures. We therefore expected that grazing was the main driver for the species' retreat onto high elevation ledges (Scheidel and Bruelheide 2001, Oosthoek 2013). It is likely though that changing plant species compositions (through changed land management) has further contributed to this retreat (Alexander *et al* 2015). While low plant competition appeared to be linked to higher survival in our translocation experiment, this contrasts partly with Norway, where *C. alpina* creates

patches able to compete and persist within luxuriant, species-rich assemblages. Whether this increased ability to compete is due to higher genetic diversity, more suitable environments, or due to other reasons is currently unclear.

It should be noted that our observations are based on four years of monitoring which is too short to indicate whether plants are likely to persist long-term in these sites and able to produce seedlings (Godefroid *et al* 2011, Albrecht *et al* 2019, Bubac *et al* 2019). Varying plant performance between years and sites as shown in our data, further emphasise the need for long-term datasets. Furthermore, using two single genetic individuals for these trial translocation might not be sufficient to provide a true representation of population level fitness. The trials have demonstrated that plants can grow at new sites, in habitats more typical of where Norwegian *C. alpina* are found which will significantly increase site options for future conservation translocations.

4.3. Conservation recommendations

Increasing population sizes and creating or maintaining gene flow between populations can halt fitness declines and the extinction of small populations (Ellstrand 2014, Ellstrand and Rieseberg 2016). Limiting grazing around wild populations might help plants to expand but as the surrounding vegetation has become largely unsuitable (except possibly on other small ledges) it is questionable whether deer control by itself will allow establishment and/or expansion into surrounding areas. Given that in some cases habitat conservation may simply no longer be sufficient to prevent declines, as is the case for *C. alpina*, there is an increasing interest in the potential of conservation translocations, the intentional movement of species for conservation purposes (IUCN/SSC 2013). Such translocations should only be considered if suitable habitat is available. Our results have shown that a small proportion of pre-grown transplants can grow at a range of new sites when micro-siting is correct and conservation translocations are therefore likely to succeed. This needs to be further tested at a larger scale though. Such experiment would ideally also have to determine whether transplants can complete the full life cycle, produce seeds which can then germinate, establish and grow up to form new plants and form a viable population, though this would likely take decades. Other trials could also include growing new populations from seed (if enough can be generated), mimicking the natural selection process during germination, establishment and first growth as much as possible. This approach would allow to immediately determine whether the new habitat is suitable for the completion of the entire life cycle, and not only for the survival of planted adults.

To increase genetic diversity and lower inbreeding it would also be beneficial to mix plants from different populations and plant them into new sites where they have enough space to expand. We recommend doing controlled crossing experiments (using Scottish population as well as Norwegian and European provenance) to determine whether mixing between populations is likely effective for this species before planting them out. Such a conservation intervention would resemble genetic rescue, the supplementation of genetically impoverished populations with new individuals (or genotypes) with the purpose of reducing genetic load (Thrall *et al* 1998, Bell *et al* 2019) and enhancing population viability (Tallmon *et al* 2004). Planting into new sites rather than introducing genes into existing populations would further avoid any potential risks of outbreeding depression by undermining evolved local adaptation among recipient populations (Edmands 1999, Tallmon *et al* 2004, Barmentlo *et al* 2018) and genetic swamping (Hufford and Mazer 2003). These sites will need to have significant deer control in place and be part of wider habitat restoration programmes to create suitable habitat for the species long term and a more balanced herbivore to plant balance.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary information files).

Acknowledgments

We thank NTS (Mar Lodge), Mar estate, and NatureScot (Glen Doll) for providing planting sites, and more specifically S Rao for ongoing support and valuable advice throughout the project. This work has been supported by advice and information provided by D Hetherington of the Cairngorms National Park Authority. Valuable help in the field and the nursery by G Powell is gratefully acknowledged. We thank E and E Sandnes for collecting leaf material from Stugudal and M de Vitis and Y Knockaert for their help with data processing. We would like to thank the landowners (NTS, Mar Estate, Balmoral, Tulchan-Glenisla Estate, and Invercauld Estate) for their continuous support. The Royal Botanic Garden Edinburgh is supported by the Scottish Government's Rural and Environment Science and Analytical Services Division. This work was supported by the players of the People's Postcode Lottery.

Author contributions

A F designed the research and analysed the data. A F and N F performed the research. M S and D H contributed samples. N F provided horticulture support. A F, M S, D H and A A wrote the paper.

Conflict of interest

The authors have no competing interests to declare.

ORCID iD

A Finger https://orcid.org/0000-0003-1859-7741

References

Aguilar R, Ashworth L, Galetto L and Aizen M A 2006 Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis *Ecol. Lett.* **9** 968–80
Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y and Lobo J 2008 Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches *Mol. Ecol.* **17** 5177–88
Albrecht M A *et al* 2019 Effects of life history and reproduction on recruitment time lags in reintroductions of rare plants *Biol. Conserv.* **33** 601–11
Alexander J M, Diez J M and Levine J M 2015 Novel competitors shape species' responses to climate change *Nature* **525** 515
Alexander L 2000 *Action Plan Research on Alpine blue-sowthistle, C. alpina* (Unpublished report to Scottish Natural Heritage)
Anderson M L 1967 *A History of Scottish Forestry* (London: Thomas Nelson & Sons, Ltd)
Angeloni F, Ouborg N J and Leimu R 2011 Meta-analysis on the association of population size and life history with inbreeding depression in plants *Biol. Conserv.* **144** 35–43
Bakkeveig S 1983 Botanikk for fjellelskere (Botany for mountain lovers) Stavanger Turistforening (Hikers Association), Stavanger (in Norwegian)
Barmentlo S H, Meirmans P G, Luijten S H, Triest L and Oostermeijer J G B 2018 Outbreeding depression and breeding system evolution in small, remnant populations of Primula vulgaris: consequences for genetic rescue *Conserv. Genet.* **19** 545–54
Bell D A, Robinson Z L, Funk W C, Fitzpatrick S W, Allendorf F W, Tallmon D A and Whiteley A R 2019 The exciting potential and remaining uncertainties of genetic rescue *Trends Ecol. Evol.* **34** 1070–9
Benson G 1999 Tandem repeats finder: a program to analyze DNA sequences *Nucleic Acids Res.* **27** 573–80
Bubac C M, Johnson A C, Fox J A and Cullingham C I 2019 Conservation translocations and post-release monitoring: identifying trends in failures, biases, and challenges from around the world *Biol. Conserv.* **238** 108239
Crnokrak P and Barrett S 2003 Purging the genetic load: a review of the experimental evidence *Evolution* **56** 2347–58
Dick C W, Etchelecu G and Austerlitz F 2003 Pollen dispersal of tropical trees (*Dinizia excelsa*: fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest *Mol. Ecol.* **12** 753–64
Duwe V K, Muller L A H, Borsch T and Ismail S A 2017 Pervasive genetic differentiation among Central European populations of the threatened Arnica montana L. and genetic erosion at lower elevations *Perspect. Plant Ecol. Evol. Syst.* **27** 45–56
Edmands S 1999 Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence *Evolution* **53** 1757–68
Ellstrand N C 2014 Is gene flow the most important evolutionary force in plants? *Am. J. Bot.* **101** 737–53
Ellstrand N C and Rieseberg L H 2016 When gene flow really matters: gene flow in applied evolutionary biology *Evol. Appl.* **9** 833–6
Evanno G, Regnaut S and Goudet J 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study *Mol. Ecol.* **14** 2611–20
Fenu G, Cogoni D and Bacchetta G 2016 The role of fencing in the success of threatened plant species translocation *Plant Ecol.* **217** 207–17
Finger A, Kaiser-Bunbury C N, Kettle C J, Valentin T and Ghazoul J 2014 Genetic connectivity of the moth pollinated tree Glionnetia sericea in a highly fragmented habitat *PLoS One* **9** e111111
Finger A, Kettle C J, Kaiser-Bunbury C N, Valentin T, Doudee D, Matatiken D and Ghazoul J 2011 Back from the brink: potential for genetic rescue in a critically endangered tree *Mol. Ecol.* **20** 3773–84
Francis R M 2016 POPHELPER: An R package and web app to analyse and visualise population structure *Mol. Ecol. Resour.* (https://doi.org/10.1111/1755-0998.12509)
Fries M 1949 Den nordiska utbredningen av Lactuca alpina, Aconitum septentrionale, Ranunculus platanifolius och Polygonatum verticillatum *Acta Phytogeogr. Suec.* **24** 1–80
Gaitán-Espitia J D and Hobday A J 2021 Evolutionary principles and genetic considerations for guiding conservation interventions under climate change *Glob. Change Biol.* **27** 475–88
Glémin S, François C M and Galtier N 2019 Genome evolution in outcrossing vs. selfing vs. asexual species *Evolutionary Genomics: Statistical and Computational Methods* ed M Anisimova (New York: Springer) pp 331–69
Godefroid S *et al* 2011 How successful are plant species reintroductions? *Biol. Conserv.* **144** 672–82
González A V, Gómez-Silva V, Ramírez M J and Fontúrbel F E 2020 Meta-analysis of the differential effects of habitat fragmentation and degradation on plant genetic diversity *Biol. Conserv.* **34** 711–20
Haddad N M *et al* 2015 Habitat fragmentation and its lasting impact on Earth's ecosystems *Sci. Adv.* **1** e1500052
Hardy O J and Vekemans X 2002 SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels *Mol. Ecol. Notes* **2** 618–20
Hedrick P W and Garcia-Dorado A 2016 Understanding inbreeding depression, purging, and genetic rescue *Trends Ecol. Evol.* **31** 940–52
Hochberg Y 1988 A sharper bonferroni procedure for multiple tests of significance *Biometrika* **75** 800–2
Honnay O and Jacquemyn H 2007 Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation *Biol. Conserv.* **21** 823–31

Hufford K M and Mazer S J 2003 Plant ecotypes: genetic differentiation in the age of ecological restoration *Trends Ecol. Evol.* **18** 147–55
IPBES 2019 *Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services* (Bonn: IPBES secretariat)
IUCN/SSC 2013 *Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0* (Gland: IUCN Species Survival Commission)
IUCN 2010 IUCN red list of threatened species. Page Version 2010.2014 available at: www.iucnredlist.org Downloaded on March 2011
Jost L 2008 G(ST) and its relatives do not measure differentiation *Mol. Ecol.* **17** 4015–26
Kalinowski S T, Taper M L and Marshall T C 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment *Mol. Ecol.* **16** 1099–106
Kopelman N M, Mayzel J, Jakobsson M, Rosenberg N A and Mayrose I 2015 Clumpak: a program for identifying clustering modes and packaging population structure inferences across K *Mol. Ecol. Resour.* **15** 1179–91
Lander T A, Boshier D H and Harris S A 2010 Fragmented but not isolated: contribution of single trees, small patches and long-distance pollen flow to genetic connectivity for *Gomortega keule*, an endangered Chilean tree *Biol. Conserv.* **143** 2583–90
Leimu R, Mutikainen P I A, Koricheva J and Fischer M 2006 How general are positive relationships between plant population size, fitness and genetic variation? *J. Ecol.* **94** 942–52
Leimu R, Vergeer P, Angeloni F and Ouborg N J 2010 Habitat fragmentation, climate change, and inbreeding in plants *Ann. N. Y. Acad. Sci.* **1195** 84–98
Loiselle B A, Sork V L, Nason J and Graham C 1995 Spatial genetic-structure of a tropical understory shrub, *Psychotria officinalis* (rubiaceae) *Am. J. Bot.* **82** 1420–5
Lughadha N E *et al* 2020 Extinction risk and threats to plants and fungi *Plants, People, Planet* **2** 389–408
Marren P R, Payne A G and Randall R E 1986 The past and present status of Cicerbita alpina (L.) Wallr. in Britain *Watsonia* **16** 131–42
Meek M H *et al* 2015 Fear of failure in conservation: the problem and potential solutions to aid conservation of extremely small populations *Biol. Conserv.* **184** 209–17
Meusel H, Jäger E J, Weinert E and Rauschert S 1992 Vergleichende Chorologie der zentraleuropäischen Flora *Band 3* (Jena: Gustav Fischer Verlag)
Michl T, Huck S, Schmitt T, Liebrich A, Haase P and Budel B 2010 The molecular population structure of the tall forb Cicerbita alpina (Asteraceae) supports the idea of cryptic glacial refugia in central Europe *Bot. J. Linn. Soc.* **164** 142–54
Oosthoek K J 2013 *Conquering the Highlands: A history of the Afforestation of the Scottish Uplands* (Canberra: ANU Press)
Orive M E, Barfield M, Fernandez C and Holt R D 2017 Effects of clonal reproduction on evolutionary lag and evolutionary rescue *Am. Nat.* **190** 469–90
Peakall R and Smouse P E 2006 GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research *Mol. Ecol. Notes* **6** 288–95
Pickup M, Field D L, Rowell D M and Young A G 2013 Source population characteristics affect heterosis following genetic rescue of fragmented plant populations *Proc. R. Soc.* B **280** 20122058
Pritchard J K, Stephens M and Donnelly P 2000 Inference of population structure using multilocus genotype data *Genetics* **155** 945–59
Püttker T *et al* 2020 Indirect effects of habitat loss via habitat fragmentation: a cross-taxa analysis of forest-dependent species *Biol. Conserv.* **241** 108368
Raymond M and Rousset F 1995 Genepop (Version-1.2) population genetics software for exact tests and ecumenicism *J. Hered.* **86** 248–9
Scheidel U and Bruelheide H 2001 Altitudinal differences in herbivory on montane Compositae species *Oecologia* **129** 75–86
Schlaepfer D R, Braschler B, Rusterholz H-P and Baur B 2018 Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis *Ecosphere* **9** e02488
Schuelke M 2000 An economic method for the fluorescent labeling of PCR fragments *Nat. Biotechnol.* **18** 233–4
Scott J M, Goble D D, Haines A M, Wiens J A and Neel M C 2010 Conservation-reliant species and the future of conservation *Conserv. Lett.* **3** 91–97
Selkoe T and Toonen R J 2006 Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers *Ecol. Lett.* **9** 615–29
Sell P and Murrell G 2002 *Flora of Great Britain and Ireland* (Cambridge: Cambridge University Press)
Solstad H, Elven R, Arnesen G, Eidesen P B, Gaarder G, Hegre H, Høitomt T, Mjelde M and Pedersen O 2021 Vascular plants: assessment of turd Cicerbita alpina for Norway. Red list for species *Species Data Bank* (available at: www.artsdatabanken.no/lister/rodlisteforarter/2021/14940) (Accessed 24 November 2021)
Stroh P A, Walker K J, Humphrey T A, Pescott O L and Burkmar R J 2023 *Plant Atlas 2020: Mapping Changes in the Distribution of the British and Irish Flora* (Princeton, NJ: Princeton University Press)
Tallmon D A, Luikart G and Waples R S 2004 The alluring simplicity and complex reality of genetic rescue *Trends Ecol. Evol.* **19** 489–96
Thrall P H, Richards C M, McCauley D E and Antonovics J 1998 Metapopulation collapse: the consequences of limited gene flow in spacially structured populations *Modeling Spatiotemporal Dynamics in Ecology* ed J Bascompte and R V Sole (Berlin: Springer) pp 83–104
Wagenitz G 1987 Gustav Hegi. Illustrierte Flora von Mitteleuropa. Spermatophyta Band VI. Angiospermae. Dicotyledones 4 *Compositae II: Matricaria—Hieracium* 2nd edn (Berlin: Parey)