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Globally rare oceanic-montane liverworts with disjunct distributions:
Evidence for long-distance dispersal

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Abstract

Disjunct distributions in bryophytes are well known, but when it comes to the mechanisms that have shaped these distributions, especially for apparently sterile species, the historical processes leading to inter-continental disjunctions remain enigmatic. In this study, we developed microsatellite markers to investigate the spatial distribution and extent of genotypic diversity of 147 *Anastrophyllum alpinum* samples collected from three populations each in Nepal and Scotland (UK). For a more general insight into genetic differences, sequence divergence in *A. alpinum* and eight other globally rare and disjunct oceanic-montane liverworts was also assessed. A nested allele distribution of Scottish populations of *A. alpinum* within the allele range of the Nepalese populations, and lower levels of genetic diversity within Scottish samples indicate that the Scottish populations likely have their origins in the Sino-Himalaya. The evidence for long-distance dispersal was supported by a lack of sequence divergence in chloroplast DNA between Scottish and Nepalese populations, with only a single substitution detected from 5160 bp of plastid sequence. Low levels of sequence divergence were also detected in species with similar distributions. While Scottish populations of *A. alpinum* do not appear to produce spores, they do still harbour a considerable genetic diversity. This indicates that sexual reproduction has been important at some point in their history. However, the current absence of evidence for sexual reproduction needs to be taken into account when designing conservation actions for these montane species, to ensure population maintenance and ability to track suitable climate and habitat space.

Keywords Disjunct distributions, bryophytes, microsatellite loci, biogeography, conservation genetics, mixed northern hepatic mat

Introduction

Anthropogenic environmental change is a major threat to plants, especially rare plants with restricted ability to track climate and habitat space due to dispersal limitations (Travis 2003). This is the case for many of the large and enigmatic oceanic-montane liverworts belonging to the mixed northern hepatic mat community (Ratcliffe 1968), several of which are globally rare and disjunct between Europe and climatically similar areas in Asia (Flagmeier et al. 2013; Hodgetts et al. 2019). In Europe, the most species-rich examples of this community are found in Scotland but it also occurs elsewhere in Britain and Ireland, the Faroe Islands and Norway (Ratcliffe 1968). The community has been termed an 'internationally significant feature' of the British uplands due to its

uniqueness and the rarity of its component taxa (Ratcliffe and Thompson 1988). Conservation concerns surrounding several species are reflected in their inclusion in the UK and Norwegian Red Lists (Hodgetts 2011; Hassel et al. 2010) as well as the European Red List (Hodgetts et al. 2019). Generally, these plants are rare due to dispersal limitations (Flagmeier et al. 2016; Wangen et al. 2016) and habitat specificity (Averis 1994), requiring an oceanic climate with high rain frequency as well as a topography (altitude, slope, aspect) that accentuates humid micro-climatic conditions (Averis 1992; 1994). The community is best represented on rocky slopes and cliffs, in boulder-fields and in dark, sheltered mountain corries, often in association with dwarf shrubs (Ratcliffe 1968; Averis 1994; Flagmeier et al. 2014). The community is also termed oceanic-montane liverwort-rich heath (Flagmeier et al. 2013), subsequently referred to here as oceanic-montane heath (OMH).

Most OMH species have never been observed with sporophytes in the British Isles, and only some produce asexual propagules (Paton 1999; Table 1). Field observations suggest they rely on vegetative spread and fragmentation of the gametophyte with subsequent local dispersal to maintain local populations (Flagmeier et al. 2013). Given their disjunct global distributions, it is thus puzzling how they dispersed to the places where they are today. Vicariance associated with continental drift and changes in climate were initially proposed to explain many disjunct bryophyte distributions (e.g. Crum 1972; Pócs 1976; Frahm 2008). However, based on a growing number of studies using phylogenetic analyses and molecular dating techniques, there is now a consensus that long-distance dispersal (LDD), is a major factor shaping disjunct distributions in bryophytes (e.g. Feldberg et al. 2007), as phylogenetic splits are generally reconstructed as having occurred later than continental separation events (Patino and Vanderpoorten 2018).

Investigating the population genetic structure of species of conservation concern may provide valuable insights into population dynamics, including mode of reproduction, and thus inform conservation efforts. This is relevant to the OMH community as there is considerable uncertainty about how these liverworts reproduce and consequently their evolutionary potential and ability to colonise new sites through dispersal. Sexual reproduction as well as dispersal between populations can generate and maintain genotypic variation and increase gene flow, which is linked to a greater ability to adapt to environmental changes (e.g. Laaka-Lindberg et al. 2000). Bryophytes with frequent sexual reproduction are found to be genetically less differentiated and experience more gene flow than species where asexual reproduction predominates (Korpelainen et al. 2005; Pohjamo

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et al. 2008). Also, cryptic speciation is a common phenomenon in bryophytes and disjunct populations with similar morphologies may show significant amounts of underlying genetic variation (reviewed in Shaw 2001).

In this study, we focus on the leafy liverwort *Anastrophyllum alpinum* Steph. (Scapaniaceae, Jungermanniales) as a representative of the OMH community, and use microsatellite markers to investigate population structure and history. To provide context by revealing more general patterns in the community as a whole, we also generated sequence data from the plastid genome of eight other dioicous liverwort species that show similar disjunct distributions (*Anastrophyllum donnianum*, *Anastrophyllum joergensenii*, *Anastrepta orcadensis*, *Adelanthus lindenbergianus, Bazzania pearsonii*, *Plagiochila carringtonii*, *Pleurozia purpurea* and *Mastigophora woodsii*). The ability of these species to reproduce sexually is largely unknown or unclear, with either only one sex represented or no production of spores reported despite gametes having been observed (Table 1).

A. alpinum has a highly disjunct distribution, being found in North West Scotland and the Sino-Himalaya, with a single locality in the Aleutian Islands, Alaska (Schill and Long 2003; Long et al. 2006; Fig. 1). Other associated species of the community show similar disjunctions (Table 1). In the UK and European Red Lists *A. alpinum* is considered a near threatened species (Hodgetts 2011; Hodgetts et al. 2019), although its preference for late snow-lie areas (Paton 1999) may make it particularly vulnerable to changes in climate. In the eastern Himalaya it is typically an alpine species growing in similar habitats, but extending into sub-alpine *Abies/Rhododendron* forests where it grows in moist liverwort mats. Gemmae are unknown, but it may spread via fragmentation of the gametophyte (Flagmeier et al. 2013). Sporophytes are unknown in Scottish populations (Paton 1999), but occur in the Himalaya (Schill and Long 2003).

The aims of this study were (1) to assess the patterns of genetic variation and the extent of genotypic diversity in populations of *Anastrophyllum alpinum* over its disjunct distribution, (2) to elucidate the role that LDD may have played in shaping the current disjunct distribution, and (3) to compare and place in context our results from (1) and (2) with nucleotide sequence data from eight other disjunctly distributed oceanic-montane liverworts.

Materials and Methods

Sampling and DNA extraction

Anastrophyllum alpinum was collected from three populations in Scotland and three in Nepal (Table 2). Within each population a mat of *A. alpinum* was considered as a single individual. From each mat several stems were collected and dried on the same day in silica gel for long term storage. Between 22 and 32 individual mats were sampled from each population. In total, 147 samples were collected. Voucher specimens from each population were deposited at the Royal Botanic Garden Edinburgh (E). Total genomic DNA was isolated from ca. 50 mg of dried material (2-4 stems) using a modified CTAB protocol (Squirrell et al. 2002).

Microsatellite isolation and screening

Five microsatellite loci were newly developed for this study (Table 3), using the following process: Random genomic microsatellites (two loci) were isolated from an enriched microsatellite library using a modified protocol described in Squirrell et al. (2004). Modifications included use of a PCR-Script Amp Cloning kit (Stratagene) to clone enriched DNA. A cDNA library was constructed using mRNA from Scottish *A. alpinum* material and a further three EST-derived microsatellite loci were isolated using the method detailed in Woodhead et al. (2003). Microsatellite loci were screened for polymorphism using a small subset of samples from Nepal and Scotland. PCR products were amplified as in Squirrell et al. (2004) and Woodhead et al. (2003) using the annealing temperatures in Table 3 and for the EST-derived microsatellite loci the conditions detailed in Woodhead et al. (2003). Resultant PCR products were analysed by electrophoresis through 4.2 % (w/v) acrylamide gels with an ABI 377 automated sequencer, using either Genescan Tamra 350 or Rox 500 as an internal size standard. Gels were subsequently analysed using the programs Genescan and Genotyper.

Microsatellite analysis

For both genomic and EST-derived microsatellites the mean number of alleles (A), percentage polymorphic loci (P) and mean genetic diversity (H_E) were estimated at the population, country and global levels using Microsatellite Toolkit (Park 2001) and FSTAT (Goudet 2002). Allele frequencies were estimated at the country level using Microsatellite Toolkit. Population differentiation was estimated with F_{ST} (Weir and Cockerham 1984), using FSTAT, and an analysis

of molecular variance was performed using Arlequin (Schneider et al. 2000). The total number of multilocus genotypes (hereafter genotypes), based on individuals with complete 5 locus genotypes, and the number of private (occurring only in one population) or shared (occurring in more than one population) genotypes were established in GENALEX 6.5 (Peakall and Smouse 2012).Population structure as indicated by the number and distribution of genetic clusters was assessed with the software STRUCTURE (Pritchard et al. 2000). The Bayesian cluster analysis is based on a correlated allele frequency model where the model infers a population structure with K numbers of clusters (Falush et al. 2003). An admixture model was applied, but to balance the sample size where populations did not have the same number of samples, a separate Alpha for each population was applied, as suggested by Wang (2017). The analysis was run with different values of K, ranging from 1-6, to define the K with the highest probability (log likelihood ln Pr(X/K)). For each K, 10 independent runs with a burnin length of 20000 iterations and 100000 MCMC repetitions after Burnin were conducted. Finally, Delta K values were calculated to examine which K best explained the data.

A bottleneck test, using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996), was applied to determine if any statistically significant signal could be detected to help disentangle the mechanisms that have shaped the current distribution of *A. alpinum*. Recent founding events could have led to a genetic bottleneck and a reduction in effective population size. Reduction in number of alleles is assumed to occur faster than reduction in heterozygosity (H_E) in a population that experienced a recent bottleneck, resulting in a larger H_E (heterozygosity excess), compared to the expected heterozygosity (H_{EQ}) at mutation-drift equilibrium (i.e. where the effective population size has remained constant in the past; Cornuet and Luikart 1996). Although we might expect to have a limited capacity to detect such a pattern with only five microsatellite loci (thus making a negative result dubiously conclusive), a statistically supported positive result would be informative. We thus tested the null hypothesis of no significant heterozygosity excess versus the alternative hypothesis of a significant heterozygosity excess (indicating a recent bottleneck) with 10 000 permutations, applying a two-phase model of mutation (TPM) with 5% multi-step mutations and a variance among multiple steps of 12, under a Wilcoxon's test ($p<0.05$), as recommended for microsatellite loci (Piry et al. 1999).

Sequence analysis

The chloroplast regions *trn*H-*trn*K, *trn*K-*trn*K, *psb*C-*trn*S (Demesure et al. 1995); *psb*C-*trn*T (Dumolin-Lapegue et al. 1997); *rps*4 (Nadot et al. 1994; Cox et al. 2000) and *trn*L (Taberlet et al. 1991) were amplified using primers and conditions described within these references. The *trn*H-*trn*K, trnK$_1$-trnK$_2$, *psb*C-*trn*S and *psb*C-*trn*T regions were too large to be sequenced in their entirety without the development of internal primers, so the sequences obtained result from single pass sequencing at either end of the PCR fragment. Not all regions were amplified for all OMH species (see Table SM2), and this was accounted for when making comparisons between species with regards to the differences detected in sequences (Table 5; % nucleotide differences). PCR products were purified using a Qiagen PCR purification kit. Modified dideoxy cycle sequence reactions were performed using either DTCS Quick Start mix (Beckman Coulter) run on a CEQ 800 and output files analysed using the associated Sequence analysis software (Beckman Coulter), or Thermosequenase II dye terminator kit (Amersham) run on an ABI 377 automated sequencer and output files edited using Sequence Navigator (Applied Navigator). Sequence alignments were unambiguous and performed by eye. Aligned sequences were screened for mutations, with base pair changes and indels scored as single mutational events.

Results

Microsatellite analysis

Two genomic and three EST-SSRs identified 56 and 23 alleles respectively across both populations of *Anastrophyllum alpinum*, with expected heterozygosity vales ranging from 0.20 (2 alleles) to 0.92 (40 alleles). Their characteristics as well as the primers and conditions used to amplify them are shown in Table 3.

Large scale population screening of all microsatellite loci revealed some individuals with more than the single band expected for a haploid liverwort species. Genotyping several individual stems separately, from samples that initially produced more than one band, resulted in single bands and stems with different alleles, suggesting that mats of *A. alpinum* sometimes comprise more than one individual. The frequency of multi-individual mats was more common in Nepal (21%) compared to Scotland (5%). Extensive clonal growth was not observed in any of the populations as many different genotypes were detected, even in populations covering a small area (e.g. 20m^2). The

number of multilocus genotypes was, however, higher in the Nepalese populations (mean PD = 0.97) compared to the Scottish populations (mean PD = 0.67), based on the individuals scored for all five SSR loci (Table 4). Seven genotypes were detected that were shared between Scottish sites, while in Nepal all genotypes were private, occurring only at one site. Furthermore, sampling of the same genotype more than once was much more common in the Scottish sites than in the Nepalese sites (Fig. 1).

Mean genetic diversity estimates, mean numbers of alleles, percentages of polymorphic loci and gene diversity were consistently higher in the Nepalese populations compared to Scottish populations (Table 4). A similar level of significant population differentiation was recorded from Nepal (F_{ST} = 0.088) and Scotland (F_{ST} = 0.093). Results from a hierarchical AMOVA revealed that the majority of the variation, 71.6%, was partitioned within populations (Table SM1). Analysis of the distribution of allele frequencies at all five microsatellite loci at the country level revealed a nested distribution pattern. All, except one, of the alleles found within the Scottish populations were found within the allele range detected from Nepal (Fig. 2).

Genetic pattern analysis

STRUCTURE analyses indicated that for the six sampled populations, there were two genetic groups that best fitted the data, with one predominantly representing the three Scottish populations and the other the Nepalese populations (Fig. 3). Six individuals from the Scottish populations showed an admixture of alleles from the Scottish and Nepalese genetic groups, with four of them having close to 50% Nepalese genetic composition and another two more than 50%. Similarly, in the Nepalese populations, there were five individuals showing an admixture of alleles from the two genetic groups. However, four individuals in Scotland had genotypes which matched the genetic cluster that is associated with the Nepalese samples.

The genetic bottleneck did test not provide evidence for any of the six sampled populations having undergone a recent genetic bottleneck (Table 4).

Sequence analysis

All chloroplast regions were successfully amplified and sequenced for *Anastrophyllum alpinum*. In the 5160 bases from 10 regions aligned between *A. alpinum* from Nepal and Scotland, only a single

difference was found, comprising a single base pair change in the chloroplast *rps*4 region. For the eight other liverwort species results ranged from 0 mutations from eight regions between Scottish and Chinese individuals of *Anastrophyllum donnianum* to 19 mutations from four regions between individuals of *Adelanthus lindenbergianus* from Scotland and Tierra del Fuego. For each species the numbers of aligned bases and mutations were assessed (Table 5).

Discussion

In this study we used specially developed microsatellite markers and/or nucleotide sequences to investigate genetic diversity and divergence in several species of the oceanic-montane heath, a community of high conservation interest (Hodgetts et al. 2019). In *Anastrophyllum alpinum*, an apparently sterile species in part of its global range (Scotland), the use of microsatellites permitted the detection of unexpectedly high levels of genetic diversity and a biogeographic pattern indicative of the origin of the species' current distribution.

The Sino-Himalaya as a centre of genetic diversity

The nested allele distribution of the Scottish populations of *A. alpinum* within the allele range of the Nepalese populations and the lower levels of genetic diversity within the Scottish samples strongly suggest that the Scottish populations originated in the Sino-Himalaya. This is in accordance with the assumption that an introduced population will represent only a subset of the total genetic diversity present in the source population (Eckert et al. 2008). For *Orthodontium gracile*, a disjunctly distributed and red listed moss in the UK, it was similarly suggested that the wider Himalaya represents a centre of extant diversity based on nucleotide sequences suggestive of a greater allele diversity there than in any other area, despite more extensive sampling from other regions (Bell and Ignatov 2019). On a larger scale, the genus *Herbertus* has a Gondwanan origin, including Southeast Asia, and LDD has played an important role in its global distribution resulting in range dispersal and diversification in the Northern hemisphere (He and Sun 2017).

The Sino-Himalayan region is a hotspot for global montane biodiversity (Myers et al. 2000), home to many rare and threatened plants (Mehta et al. 2020), and the genus *Anastrophyllum* has been considered to have its centre of distribution there, this having been hypothesised to be an important area for speciation and radiation of leafy liverworts generally (Schuster 1983). The presence of both male and female gametes in the Sino-Himalayan populations of *A. alpinum*, and the lack of

observations of either in Scottish plants, further supports the hypothesis that this region represents the source from which the Scottish populations are derived. It would seem likely that the other highly disjunct population of *A. alpinum*, occurring in Alaska where it is known only from Adak Island in the central Aleutian Islands, ultimately also has its origins in the Sino-Himalaya. Such asymmetrical dispersal patterns characterise many disjunct bryophytes, with source and sink areas often consistent across multiple species (Patiño and Vanderpoorten 2018).

Evidence for long-distance dispersal

The lack of substantial sequence divergence between samples of *A. alpinum* from Scotland and Nepal suggests that these populations do not represent cryptic species (Table 5). Although it is possible that rates of sequence evolution could be exceptionally slow, the possibility that the extant populations were separated by ancient vicariance events seems unlikely. Based on this, we propose that LDD has been the major determining factor in the current disjunct distribution of *A. alpinum*. It is unclear whether this involved direct LDD from the Sino-Himalaya to Scotland, or migration via one or more "stepping-stone" populations that are now extinct.

The spores found in Nepalese material of *A. alpinum* are small (less than 15 μm; Schill and Long 2003) and according to experiments on other liverworts should have the potential for LDD (Van Zanten and Gradstein 1988). Although these authors did not investigate the survivability of spores from any liverwort species from the Northern hemisphere, spores from the closely related *Anastrophyllum auritum* were assessed, and although susceptible to UV irradiation, 60% of spores survived 100 days of desiccation (an important trait for LDD).

In terms of establishing the likelihood of a stepping-stone dispersal pattern - this is difficult as *A. alpinum* has highly specialised habitat and climate requirements and no fossil record, and reconstructing past habitat and climatic conditions to ascertain historical distribution patterns is challenging. However, it is known that the related *Anastrophyllum donnianum* recently occurred in Poland and Slovakia (Schumacker and Váňa 2005), although it is apparently now extinct in both countries (Hodgetts 2015; Klamá and Gorski 2018). Given that this distributional change in a related species with a similar distribution to *A. alpinum* has happened over a relatively short time period, it is quite feasible that similar small populations of *A. alpinum* could have existed in a number of areas from which they have since become extinct.

In addition to the possibility that *A. alpinum* previously occurred elsewhere, it is possible that its distribution in Scotland was once larger. Very little vegetation in Scotland is completely natural, and large parts of the countryside have been modified. The preference of the OMH species is for sheltered, shaded corries and north facing slopes but they also can be found in a few remnant woodlands (Averis 1994). It is possible that the species may have previously been more common in woodlands at medium altitude, and following historical reductions in tree cover (Birks 1988), are now restricted to the remaining relictual sheltered sites such as mountain corries as suitable habitats. While there is no evidence for a recent genetic bottleneck at the population level using the Bottleneck test, our data unfortunately have little power to address the recent history of the species in Scotland, as the small number of loci gives limited statistical power for detecting genetic bottlenecks (Zachariah Peery et al. 2012).

Long-distance dispersal in other liverworts

The limited sequence divergence (% of nucleotide base pair changes; Table 5) between disjunct liverwort populations investigated within this paper strongly suggests that LDD has been dominant in shaping the current distribution patterns of several bryophyte species characteristic of the OMH, as opposed to ancient vicariance which had previously been proposed (e.g. *Mastigophora woodsii*; Schuster 1983). This is consistent with the results of our more detailed study of *Anastrophyllum alpinum* using microsatellites.

The greatest level of divergence observed was between disjunct populations of *Adelanthus lindenbergianus* with 0.96% of nucleotide base pairs different between populations (Table 5). However, this is a predominantly southern hemisphere species with highly disjunct populations in the British Isles and notably atypical of UK OMH species biogeographically. British populations of *A. lindenbergianus* have previously been considered to have originated by dispersal from South America, which is supported biologically by the presence of both sexes there while only males occur in the British Isles (Schuster 1983). Feldberg et al. (2010), using South American material of *A. lindenbergianus*, also found complex genetic structure: specimens from southern South America (Chile and Argentina) and from tropical South America occurred in two different clades, with *A. carabayensis* grouping with tropical specimens of *A. lindenbergianus*, and *A. integerrimus* with

temperate South American ones. This implies the existence of cryptic species in *A. lindenbergianus,* and/or recent speciation of the other two species from geographically isolated populations of *A. lindenbergianus.*

In contrast to *A. lindenbergianus*, all other OMH species we sampled have considerably lower levels of sequence divergence. In the case of *A. donnianum,* no sequence divergence was detected in over 4000 bp of plastid DNA. Likewise, in *Plagiochila carringtonii* only two substitutions were detected in 2500 bp of plastid DNA. This is entirely consistent with LDD, rather than ancient divergence and cryptic species. In the other five sampled OMH species we found levels of divergence falling between those detected in *Anastrophyllum donnianum* and *Adelanthus lindenbergianus,* but the general picture was of low levels of sequence divergence with all species other than *Adelanthus lindenbergianus* having less than 0.5% divergence.

Outside of the OMH community, a number of other studies have attempted to ascertain the origin of different intercontinental bryophyte disjunctions using sequence data. Shaw et al. (2003) investigated levels of sequence divergence between three moss species disjunct between western North America and southern Europe and concluded that recent LDD had shaped this distribution pattern. Similarly, the genus *Plagiochila* shows a Neotropical-African disjunct distribution, with limited ITS sequence divergence and the nesting of African populations within Neotropical clades implying that diversification occurred in the Neotropics with subsequent dispersal to Africa and Eurasia (Heinrichs et al. 2004; 2005). European populations of *P. bifaria* were morphologically more uniform than their Neotropical counterparts, suggesting either that a small number of dispersal events established the European populations or else that environmental conditions in Europe are less favourable than in the Neotropics (Heinrichs et al. 2004). Based on morphological and phylogenetic results, the moss *Lewinskya acuminata* was suggested to have originated in the Mediterranean Basin and dispersed by LDD to North America and Ethiopia (Vigalondo et al. 2016). .

Population level genetic diversity in Anastrophyllum alpinum
In Nepal the presence of a large number of private genotypes for *A. alpinum* suggests ongoing sexual reproduction and very little evidence for clonal growth. In contrast, the populations in Scotland show a much greater tendency for genotypes to be shared between individuals and populations (Fig. 1), and this may indicate clonal growth. The observed aggregation of identical

genotypes at individual Scottish sites at a small spatial scale may mean that asexual growth is occurring at that scale (Fig. 1). Formation of clonal clusters has been demonstrated in other mainly asexually reproducing bryophytes, e.g. the moss *Rhytidiadelphus squarrosus* (Korpelainen et al. 2012) and the liverworts *Barbilophozia attenuata* (Korpelainen et al. 2011) and *Crossocalyx hellerianus* (Hola et al. 2015). However, sampling of more loci is required to gain further insights into the extent of vegetative spread in *A. alpinum*. In the absence of observed gemmae or other specialized asexual propagules, vegetative fragments (less common in liverworts that in mosses; Longton and Schuster 1983) would appear to be the only mode of asexual spread available to this species, although such fragments may not travel very far. In another leafy liverwort, even though it produced gemmae, aggregation of the same genotype only occurred up to a distance of 80 m (Hola et al. 2015). However, vegetative fragments could be transported in the plumage or on the feet of birds (Chmielewski and Eppley 2019) or endozoochorously (Russo et al. 2020), even over long distances (Lewis et al. 2014). Nonetheless, the lack of aggregation of the same genotype in Nepalese populations suggests that vegetative spread is not a common strategy for this species and that LDD is much more likely to have occurred by spores than by vegetative fragments.

Surprisingly however, we detected multiple genotypes not only within Scotland, but within all of our sampled Scottish sites. In the absence of field observations of sexual reproduction in Scottish *A. alpinum*, this genotypic diversity presents something of a paradox. Several other studies have reported similar patterns in bryophyte species with absent or rare sexual reproduction (e.g. Pohjamo et al. 2008; Korpelainen et al. 2012), as well as, for example, in other Scottish rare plants such as sub-arctic willow species (e.g. Stamati et al. 2007). The most likely explanation in the case of *A. alpinum* is that multiple introductions of founders from the Sino-Himalaya have combined with very sporadic sexual reproduction (currently not occurring at all), resulting in the observed genotypic diversity, which has been able to persist due to the longevity of individual plants. Low mortality rates of shoots (consistent with the 'perennial stayer' strategy; During 1979), coupled with relatively frequent new branch growth (Flagmeier et al. 2013) indicating effective ramet production, may ensure population persistence. This ability to persist may explain the low population differentiation even in the apparently sterile Scottish populations (Table 4).

Although sexual reproduction does not appear to be taking place currently, the observed genetic diversity suggests that it has occurred in the past. The production of gametes, especially male, has

been shown to be affected by unfavourable climatic conditions, this having been cited as a possible cause of the highly skewed sex ratios seen in many bryophyte populations (Hugonnot et al. 2014). In the Sino-Himalaya, where conditions are potentially at an optimum, both expressed sexes of *A. alpinum* are known. This suggests that the lack of gamete production in Scotland may be a consequence of recent climatic and/or habitat changes.

Conservation application

These liverworts are sensitive to changes that alter their microclimate (Flagmeier et al. 2014), and in the absence of spores and specialized asexual propagules their ability to disperse to otherwise climatically suitable sites may be limited. Species distribution modelling for some species of the OMH indicates that suitable sites in Scotland (Flagmeier et al. 2016) and Norway (Wangen et al. 2016) are not occupied, while under future climate change scenarios in Ireland, suitable habitat is expected to shift northwards (Hodd et al. 2014). Although the Sino-Himalayan region is an important area of genetic diversity for *Anastrophyllum alpinum* and likely also for other liverworts of the OMH, the populations in Scotland have apparently lost the ability to reproduce sexually and likely present populations at their distributional climatic limit.

The oceanic-montane heath in Scotland has become less distinct as a community over the last 50 years and is threatened to eventually become homogenised with other upland plant communities through conversion to grassland (Flagmeier et al. 2014). To avoid this, the community should be safeguarded from threats such as over-grazing (Moore and Crawley 2015) and burning (Averis 1992). Although the specific assemblage of these disjunct liverworts is globally rare, at the moment only one species, *Adelanthus lindenbergianius,* is red listed globally. The extant occurrence of liverwort heath species in Britain (except for *Adelanthus lindenbergianus*, *Herbertus borealis* and *Herbertus norenus*) does not currently warrant assignments to threatened IUCN red list categories (see Hodgetts el al. 2019). However, it has been shown that at several sites in Scotland declines in abundances have taken place at the population level, even when numbers of occupied hectads have remained stable (Flagmeier et al. 2014). In Ireland, rapid and dramatic losses in cover of liverwort heath along with heathland more generally due to sheep grazing have been reported (Holyoak 2006). *Plagiochila carringtonii* is thought to have been lost from one of only two known sites in Ireland despite having been fairly abundant there in the recent past, while *Adelanthus lindenbergianus* has been severely restricted (Long 2010). Unfortunately it is plausible, therefore, that anticipated

changes in climate and land use could have rapid and dramatic impacts on Scottish OMH communities generally, despite few individual species currently having IUCN threatened status.

Within Europe, *Anastrophyllum alpinum* is more restricted in its range than other species of the community, not occurring in Ireland or Norway (Long et al. 2006). It also tends to occur in or at the lower boundaries of areas of late snow-lie, potentially making it more vulnerable as temperatures are predicted to rise by up to 4°C over parts of Scotland, particularly in summer and autumn (Barnett et al. 2006), leading to snow disappearing much earlier in the year. Furthermore, the Scottish populations at least of *A. alpinum* are likely to be at their distributional climatic limit. Considering all of this, *A. alpinum* may deserve a higher conservation and protection status than it currently has.

Conclusions

Anastrophyllym alpinum is part of a community of large liverworts occurring in the high rainfall areas of the northern hemisphere that show highly disjunct distributions. Here we provide the first evidence from molecular population genetics for long-distance dispersal as an explanation for the distribution of a member of this community and offer further insights into these species of conservation concern. The centre of genetic diversity for *A. alpinum* lies in the Sino-Himalayan region. The European populations may be at their climatic range limit as suggested by lack of sexual reproduction, and whereas reliance on clonal growth may be sufficient for relictual population persistence, it remains to be determined how far vegetative fragments can spread. Investigating the population genetic structure of species of conservation concern can inform conservation practice, and the results presented here contribute to the growing evidence that bryophytes are able to disperse over long distances. However, as dispersal events may be rather rare, knowledge of smaller-scale population dynamics is equally important for assessing the prospects of species to maintain populations and establish new ones, tracking suitable climate and habitat space. This needs to be taken into account when measuring the biodiversity response and planning the management of other montane species threatened by climate change.

Acknowledgements

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Table 1 Details of the sexual condition and disjunctions of liverworts of the oceanic-montane liverwort heath in the present study. Information compiled from Hill et al. (1991), Paton (1999) and Long et al. (2006).

Species	Gemmae	Male	Female	Sporophytes	Regeneration from fragments (Flagmeier et al. 2013)	World occurrence	Disjunction (Schofield and Crum 1972)
Adelanthus lindenbergianus (Lehm.)Mitt.	Unknown in Europe	Yes	Unknown in Europe	Unknown in Europe	Untested	Scotland (Islay, Jura), Ireland, Africa, C and S America, Antarctica	Widely disjunct
Anastrepta orcadensis (Hook.)Schiffn.	Yes (frequent)	Occasional	Yes (rare)	Yes, but unknown in the British Isles	Untested	Europe, WN America, Hawaii, Sino-Himalaya	Widely disjunct in Northern hemisphere
Anastrophyllum alpinum Steph.	Unknown	Yes, but unknown in the British Isles	Yes, but unknown in the British Isles	Yes, but unknown in the British Isles	Yes	Scotland, Aleutians, Sino-Himalaya	Widely disjunct in Northern hemisphere
Anastrophyllum donnianum (Hook.) Steph**.**	Unknown	Yes	Yes	Yes (very rare)	Yes	British Isles, Faeroes, Norway, WN America, Sino-Himalaya	Widely disjunct in Northern hemisphere
Anastrophyllum joergensenii Schiffn.	Unknown	Unknown	Yes	Unknown	Untested	Scotland, Norway, Sino-Himalaya	Widely disjunct in Northern hemisphere
Bazzania pearsonii Steph.	Unknown	Yes, but unknown in the British Isles	Yes	Yes (very rare), but unknown in the British Isles	Yes	British Isles, Sicily, Macaronesia, North America, Sino-Himalaya	Widely disjunct in Northern hemisphere
Mastigophora woodsii (**Hook.**) Nee**s**	Unknown	Unknown	Unknown	Unknown	Untested	British Isles, Faeroes, WN America, Sino-Himalaya, Taiwan	Widely disjunct in Northern hemisphere
Plagiochila carringtonii (Balf.)Grolle	Unknown	Yes	Unknown	Unknown	Yes	British Isles, Faeroes	Endemic to western Europe
Pleurozia purpurea Lindb.	Unknown	Yes, but unknown in the British Isles	Very rare	Unknown	Untested	British Isles, Faeroes, Norway, Guadeloupe, WN America, Sino-Himalaya, Taiwan, Japan	Widely disjunct in Northern hemisphere

Table 2 *A. alpinum* population details

Population	latitude	longitude
Scotland		
Beinn Dearg, Wester Ross	57.794349	-4.940856
Carn na Criche, Wester Ross	57.705166	-5.025959
Sgurr Mor, Wester Ross	57.699167	-5.013708
Nepal		
Gosainkund	28.080833	85.415555
Bhairabkund	28.079166	85.406666
Nyengang Kharka	28.198333	85.567222

Table 3 Characteristics of the five microsatellite loci for six populations of *A. alpinum* from Scotland and Nepal; this includes the repeat motif, annealing temperature, primers, the origin of the locus, number of alleles, total expected heterozygosity (H_T), and estimate of F_{ST}

Locus	Origin	Repeat motif	Allele size range	T_{an} (^{0}C)	Primer sequences (5'-3')	No. of alleles	H_T	F_{ST}
Aj5	Genomic	(GA)$_{25}$	159-199	55	F: TATAAGGTGCCTAGTGGAACACC R: TGATTCTCCATAACTCTACACTCG[1]	16	0.88	0.128**
Aj8[+]	Genomic	(TC)$_{36}$	142-210	55	F: TTAAGGCCGTAGACAGTCAGTGC R: ACCGGATGATCTTACACCGAA[2]	40	0.92	0.069**
1J11[+]	EST	(AT)$_8$	235-275	#	F: GCCCTGTTTTCGATACCTGA[1] R: CCTCTCACAGGGGAAAAAGA	16	0.70	0.368**
M22[+]	EST	(AT)$_5$	280-281	#	F: GCTGATGCGGCTTTATC[1]	2	0.20	0.138**
C03	EST	(GT)$_{15}$	109-119	#	R: CAGCAGTCGTTAAGGTGTA F: GATTTCGGTCCAGCAAGAGT[1] R: TTACGTCTGGGTTAGGTCCG	5	0.28	0.302**

** *p*<0.001
# touch-down PCR, see Woodhead et al. (2003) for details

Table 4 Genetic variability parameters of three populations of *A. alpinum* from Scotland and three from Nepal as well as the result (Wilcoxon test *p*-value) of a Bottleneck test, based on 5 microsatellite loci at the population, regional and global level. The AMOVA analysis from data in this table is shown in Table SM1.

Population	n	*A*	*P*	H_E	F_{ST}	PD	*Wilcoxon test*
Scotland							
Beinn Dearg	19	3.2 ± 2.17	80	0.404 ± 0.165		0.78	0.84
Carn na Criche	31	3.0 ± 2.12	60	0.322 ± 0.163		0.61	0.81
Sgurr Mor	25	2.2 ± 1.79	40	0.262 ± 0.164		0.62	0.13
mean	**25**	**2.8**	**60**	**0.329**		**0.67**	
Nepal							
Gosainkund	23	7.0 ± 5.70	100	0.609 ± 0.178		0.92	0.84
Bhairab Kund	19	6.4 ± 4.04	100	0.676 ± 0.128		1	0.69
Nyengang Kharka	30	7.4 ± 4.10	100	0.730 ± 0.125		1	0.41
mean	**24**	**6.9**	**100**	**0.672**		**0.97**	
Regions							
Scotland	75	4.6 ± 3.58	80	0.345 ± 0.173	0.093**	46.3	
Nepal	72	13.2 ± 10.94	100	0.704 ± 0.129	0.088**	96.6	
Global overall	147	14.0 ± 11.77	100	0.587 ± 0.150	0.199**		

A, mean number of alleles per locus ± standard deviation; *P*, percentage polymorphic loci; H_E, expected gene diversity ± standard deviation; F_{ST}, population differentiation; PD, proportion of multilocus genotypes distinguished, based on individuals with complete 5 loci genotypes.
** *p*<0.001.

Table 5 Levels of sequence divergence detected for nine liverwort species showing major geographical disjunct distributions.

| Species	Geographic comparison	No. regions sequenced	Total no. base pairs compared	No. of fixed differences	% difference
Anastrophyllum donnianum	Scotland vs China	8	4, 124	0	0.00
Anastrophyllum alpinum	Scotland vs Nepal	10	5,160	1	0.02
Plagiochila carringtonii	Scotland vs Bhutan	6	2, 537	2	0.07
Pleurozia purpurea	Scotland vs Bhutan	4	1, 882	4	0.21
Anastrophyllum joergensenii	Scotland vs China	6	3, 243	10	0.31
Bazzania pearsonii	Scotland vs China	7	3, 359	14	0.42
Anastrepta orcadensis	Scotland vs Bhutan	7	3, 717	17	0.43
Mastigophora woodsii	Scotland vs China	7	3, 221	16	0.50
Adelanthus lindenbergianus	Scotland vs Tierra del Fuego	4	1, 988	19	0.96
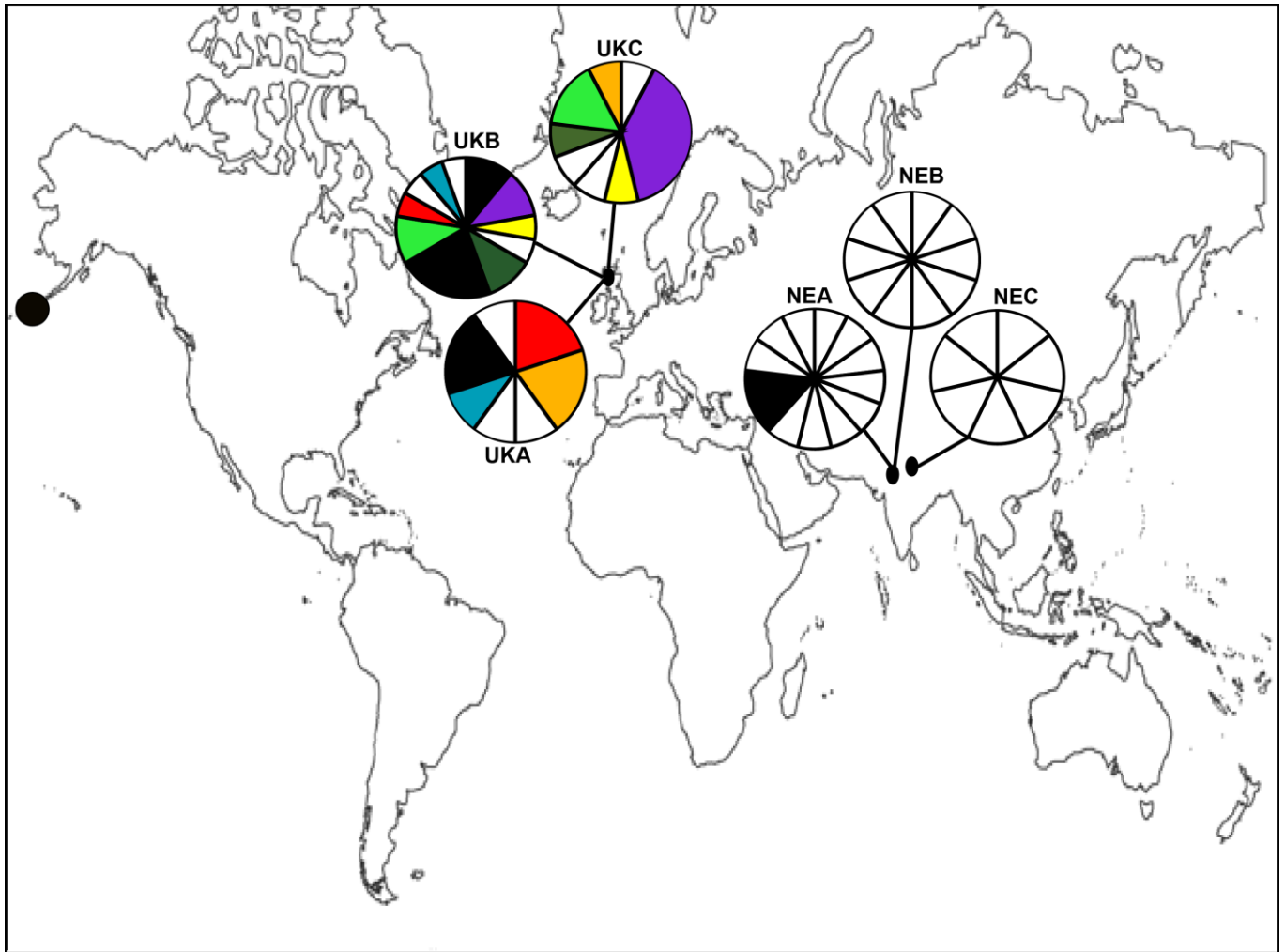
Fig. 1 Global distribution of *Anastrophyllum alpinum* with locations in Scotland (UK), Nepal and Aleutian Islands, Alaska (USA). The six populations sampled in this study are indicated by pie charts showing numbers of genotypes and their relative frequences (sizes of slices) in each population, based on samples with complete data for five SSR markers. Private genotypes, only occuring in one population, are white, and genotypes shared between populations are the same colour. Black pie slices indicate accumulation of the same private genotype. UKA (Beinn Dearg; n=10), UKB (Carn na Criche; n=18), UKC (Sgurr Mor; n=13), NEA (Gosainkund; n=13), NEB (Bairab Kund; n=10), NEC (Nyengang Kharka; n=7)
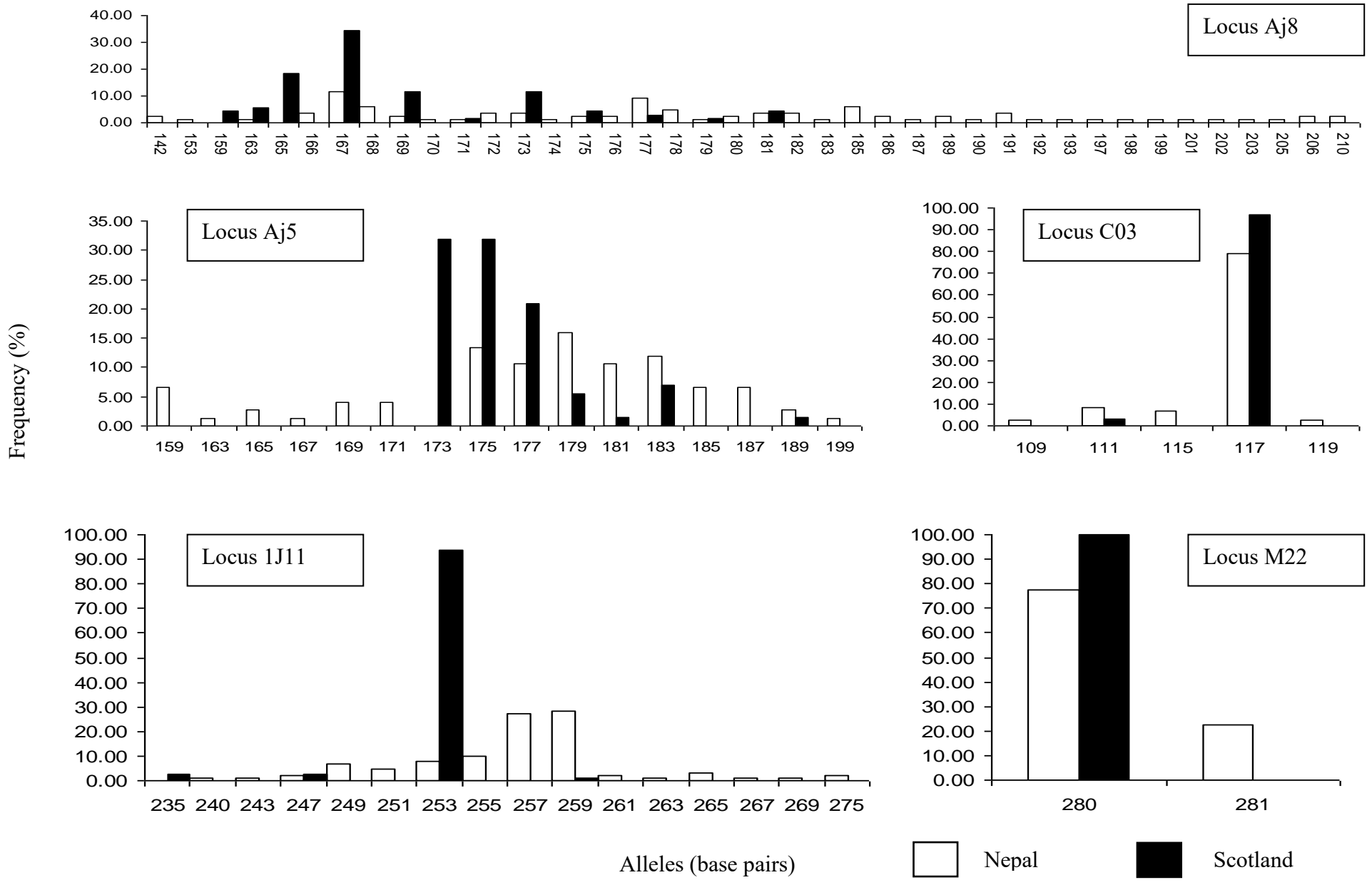
Fig. 2 Frequency and distribution of alleles in *A. alpinum* from Nepal and Scotland for 5 microsatellite loci, showing Scottish alleles nested within the allele distribution from Nepal.
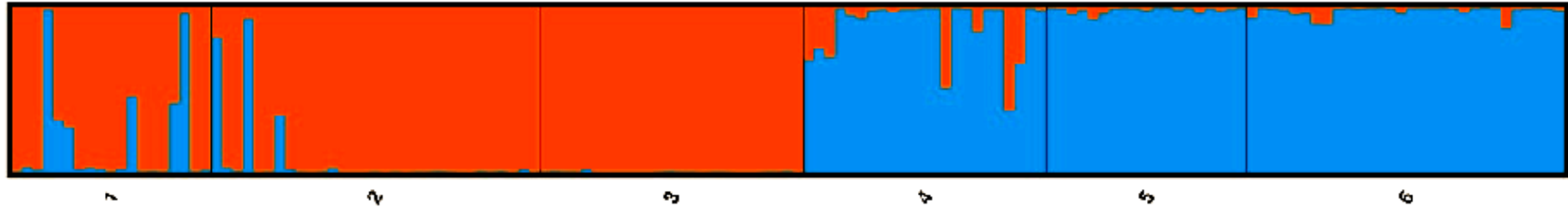
Fig. 3 Results from STRUCTURE software: proportions of alleles of each individual to the two inferred genetic clusters represented by two colours. Black lines separate the individuals from different populations (populations 1-3 from Scotland and 4-6 from Nepal) and each individual is represented by a vertical bar.