Our friends in the North: On the Arctic genetic affinity of *Arenaria ciliata* L. and *A. norvegica* Gunn. in Ireland

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Since the beginning of modern natural history research in Ireland, the origins of Ireland's longest-established plant populations have been the subject of much interest. Arenaria ciliata *(Fringed Sandwort) and* A. norvegica *(Arctic Sandwort), two closely related Arctic-Alpine red-list species with isolated populations in Ireland, have long been noted for their unusual biology in this regard. Both display disjunct European distributions and characteristic ecological traits that align with possible survival since the last glacial maximum, however to date their genetic affinities with sister populations in Europe have not been analysed. Here we present an AFLP fragment analysis of genetic affinities in the wider* A. ciliata *species complex, which includes both* A. ciliata *and* A. norvegica*, as well as a number of unresolved taxonomic identities including* A. gothica*. We identify a clear association between genetic and geographic distance among populations in the group across Europe, and a clear pattern of genetic subdivision that is strongly associated with ploidy identity.* A. norvegica *is the most genetically distinct taxon in the wider group, and displays a narrow genetic signal consistent with rapid population expansion across its present range, including Ireland. The data also confirm that* A. ciliata *is a biogeographic anomaly within the native Irish flora, showing a discrete genetic identity with clear affinity to Arctic rather than continental European populations.*

Keywords: phylogeography, postglacial, cryptic refugia, arctic-alpine flora, *tabula rasa*

Introduction

The origins of the early Irish Flora

A long-standing question in Irish natural history has focused on whether the entire native flora is postglacial in origin, and in particular whether any extant species might survive here from before the last glacial maximum (LGM) *c.*20,000 years BP (Webb 1982, Rasmussen e*t al.* 2006). The quasi-Arctic flora that assembled in Ireland in the aftermath of deglaciation, and well documented in the palaeoecological record, included characteristic early postglacial colonists such as *Artemisia*-grass-sedge communities, and later Crowberry (*Empetrum nigrum* L.), Juniper (*Juniperus communis* L.), Dwarf Willow (*Salix herbacea* L.) and Mountain Avens (*Dryas octopetala* L.). These examples align with a continent-wide northward colonisation of newly deglaciated landscapes by these groups of species, with broad synchrony in patterns between Ireland, Britain and Northern Europe (Watts 1977). After the arid Younger Dryas cold phase 13,000-11,700 years BP, the fossil record describes a pattern of new immigrant arrivals from the east and south (Huntley and Birks 1983, Mitchell 2008). This is synchronous with the expansion of temperate species across Europe at that time, first to the western shores of Britain and France (then a single landmass) before crossing the Irish Sea to Ireland (Huntley and Birks 1983, Mitchell 2008, Giesecke and Brewer 2018).

Though geographically adjacent, Ireland and Britain have experienced a divergent recent biogeographical history. Ireland became isolated as an island between 20,000 and 16,000 years BP following the contraction and division of the British-Irish Ice Sheet and the breakup of sea-ice cover over the Irish Sea (Ó Cofaigh and Evans

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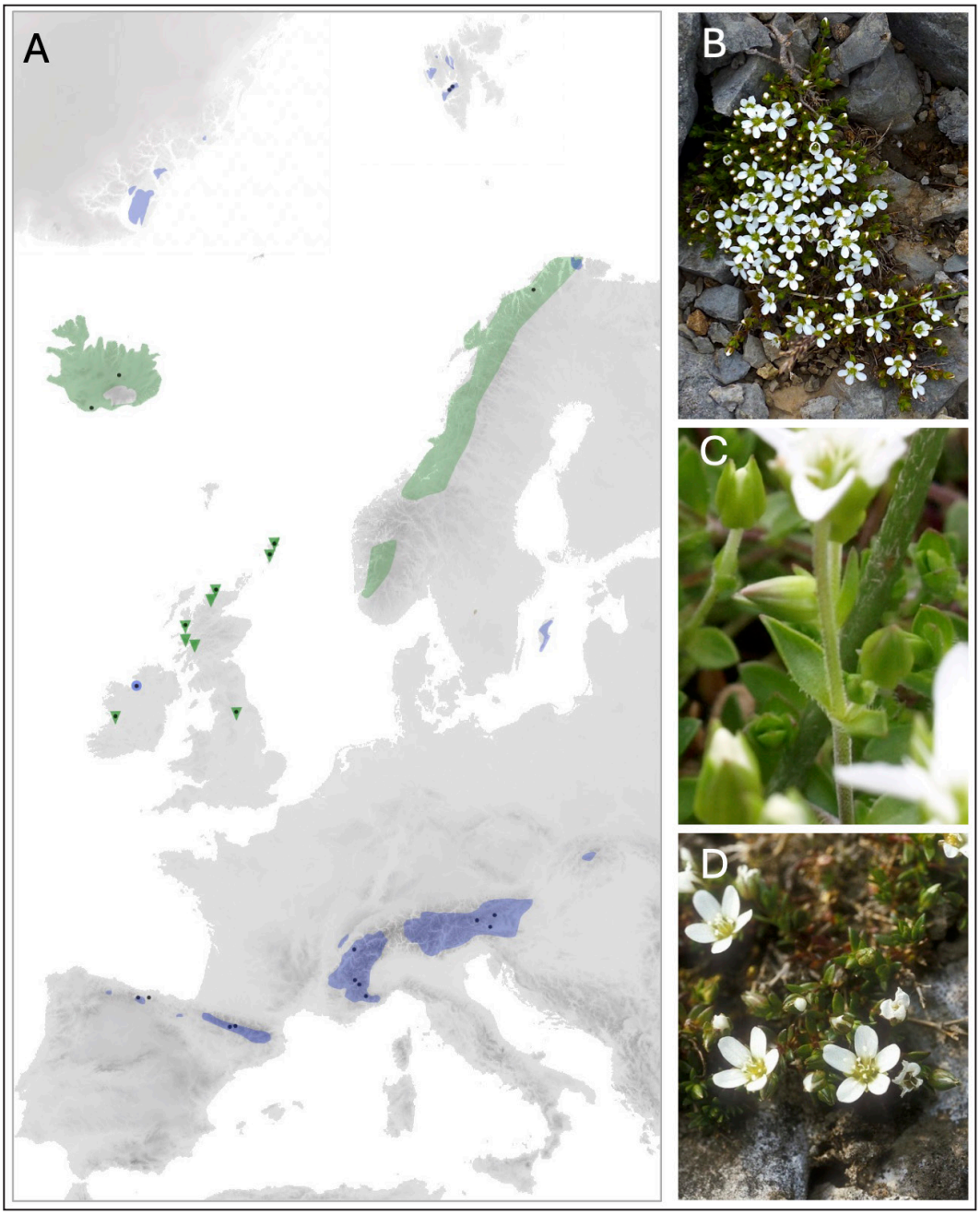

Figure 1. **A**. Current distribution of the *A. ciliata* complex in Europe and the Atlantic Arctic, including the location of sample populations included in the present analysis (Table 1). Indicated in green is the discrete distribution of *A. norvegica*. Indicated in blue are the intergrading distributions of *A. ciliata* ssp. *ciliata*, ssp. *pseudofrigida* Ostenf. and O.C. Dahl, ssp. *bernensis* Fav. and ssp. *multicaulis* (L.) Arcang.; as well as *A. gothica* ssp. *gothica* and ssp. *moerhingioides* (Murr.) Wyse-Jackson and Parnell. Outlying isolated populations are indicated, including for *A. ciliata* and *A. norvegica* in Ireland.
B. Typical growth habit of *A. ciliata* ssp. *ciliata* on Ben Bulben, Ireland at the Glebcarbury mine site. Note the thickened basal stem indicating the specimen is several decades old.
C. The characteristic diagnostic trait discriminating all other taxa in the *A. ciliata* from *A. norvegica* is the absence of basal leaf hairs, shown here on *A. gothica* ssp. *moerhingioides* at Benasque in the Pyrenees.
D. *A. norvegica* at Black Head, Co. Clare, Ireland.

2007, Patton *et al.* 2017). The Irish Sea was too deep for a direct land bridge between Ireland and Britain at this time, although low-lying island groups possibly existed in the Celtic Sea area in the period up to 20,000 years BP (Toucanne *et al*. 2009). This isolation predates the isolation of Britain from Continental Europe by some 10,000 years, and is associated with the relatively depauperate overall biota in Ireland compared to Britain, which itself experienced reduced immigration with the opening of the sea barrier to France *c*.7,000 years BP.

The contemporary Irish flora assembled between 12,000 and 7,000 years BP, by which time it included about 815 vascular plant species, all but 15 of which also occur in Britain (Huntley and Birks 1983, Webb 1983, Mitchell 2006). Irish populations in general show broad genetic affinities to contiguous populations in western Europe, including Britain, France, Spain and Portugal (*e.g*. Alder Buckthorn (*Frangula alnus* Mill.) in Hampe *et al.* 2003, Ash (*Fraxinus excelsior* L.) in Heuertz *et al*. 2004, Perennial Ryegrass (*Lolium perenne* L.) in Balfourier *et al.* 2000, and Oak (*Quercus* spp.) in Kelleher *et al*. 2004). Even among temperate species with disjunct distributions between Ireland and the Iberian Peninsula, for example, phylogeographic analysis clearly infers a postglacial migration from south to north (Beatty and Provan 2013, Eftonga 2012), suggesting the extensive ice cover of the last glacial maximum marks a *tabula rasa* in the history of the Irish flora.

Of all animal and plant taxa investigated to date, the only notable cases suggesting the existence of genetically distinct populations in Ireland that date to the late glacial period is found in respect of the Mountain Hare (*Lepus timidus* Linnaeus, 1758) and the Stoat (*Mustela erminea* Linnaeus, 1758) (Hamill *et al.* 2006, Martınkova *et al*. 2007), while the Common Frog (*Rana temporaria* Linnaeus, 1758) is the sole taxon displaying haplotype diversity consistent with possible pre-LGM origins (Teacher *et al*. 2009).

Arenaria ciliata and *A. norvegica* in Ireland

Among the group of plant species identified as potential ancient natives by Webb in his benchmark paper on the European affinities of the Irish flora, the Fringed Sandwort (*Arenaria ciliata* L.) is one of a few with an ecology suited to survival in glacial environments and a disjunct Arctic-Alpine distribution that is compatible with local *in-situ* survival since the Pleistocene (Webb 1983).

Arenaria ciliata itself is not a discrete taxon, and forms the core of a species complex that ranges across Europe and the Atlantic Arctic. All taxa in the *A. ciliata* group are slow-growing cold-tolerant mostly perennial herbs found on shallow calcareous sediment and in bedrock fissures. Morphometric analysis implies three intergrading taxa: *A. ciliata s.s.*, Swedish Sandwort (*A. gothica* Fr.) and the Arctic Sandwort (*A. norvegica* Gunn.) comprising multiple subspecific identities, however the morphological traits that distinguish these taxa are continuous rather than discrete, and no taxon displays floral, fruit or ploidy traits that uniquely set it apart (Tutin *et al*. 1993, Wyse Jackson and Parnell 1987). This lack of taxonomic differentiation is not especially unusual: many Arctic-Alpine species show patterns of weak morphological differentiation among biogeographic types, a characteristic often associated with repeated refugial isolation and migrant mixing during Quaternary glacial cycles (Bennett, 2004).

Among the red-listed Arctic-Alpine element of the Irish flora, *A. cilata* is restricted to limestone bedrock outcrops atop the Ben Bulben massif, particularly on the north-facing slopes. Despite a much more abundant potential habitat of a similar kind in Britain, it is absent from the larger island. The nearest *A. ciliata* populations are *c*.1,000 km distant from Ireland in the Cordillera Cantabrica in Northern Spain, and 1,300 km distant in the Jura Mountains on the Swiss French border, respectively (Fig. 1).

The disjunct distribution pattern of *A. ciliata* is shared by a small number of other native Arctic-Alpine species for example Snow Saxifrage (*Saxifraga nivalis* L.) and Dwarf Willow (*Salix herbacea* L.) (Webb 1983), but it contrasts markedly with that of its closely related sister species, also a red-list Irish species, *Arenaria norvegica* Gunnerus. The only Irish site for *A. norvegica* is located at Black Head, in the Burren in Co. Clare (Walker *et al.* 2013). In contrast to *A. ciliata*, the nearest populations are relatively nearby, in the karst beds of the Yorkshire Dales, and base-rich bedrock localities in the Highlands and the island of Rum, and beyond Britain the species is widespread in the Arctic and sub-Arctic lands of the northeast Atlantic.

Unlike the lowland species Strawberry Tree (*Arbutus unedo* L.) and St Dabeoc's Heath (*Daboecia cantabrica* (Huds.) K. Koch) that share a disjunct Irish-Iberian affinity with *A. ciliata*, the latter is distinctly an Alpine species in Iberia, and at Ben Bulben is surrounded by a quasi Arctic-Alpine vegetation habitat unique in Ireland (Barrington and Vowell 1888). *Arenaria norvegica*, by contrast, occurs at sea level in Ireland and Britain, and is accompanied by a diverse Arctic-Alpine, warm-temperate and cold-temperate plant community (Walker *et al*. 2013).

The Burren and Ben Bulben comprise the two most significant refuges for Arctic-Alpine

Table 1. Distribution and taxonomic and cytology diversity of the *A. ciliata* species complex across Europe, with a list of population samples and sampling localities for AFLP analysis, according to recorded taxonomic identity. At the time of publication, taxon status is unresolved for many subspecies, with the indicated distribution of taxa provided for countries and regions.

*Taxonomic Identity^*	*2N**	*Region*	*Elevation Range*	*AFLP Sample Population Locality*	*Latitude*	*Longitude*	*#AFLP Samples*	*AFLP Population Group (Based on PCoA)*
Arenaria ciliata subsp. ciliata								
Ireland	40	Ireland	<500m	Ben Bulben, Co. Sligo	54.350	8.467	5	*A. ciliata* Ireland-Svalbard
Switzerland	40, 80	Western Alps	<2300m	-				
Austria	120, 160, 200	Eastern Alps	<2500m	Nieder Tauren, Steiermark	47.267	-14.483	3	*A. ciliata* E Alps
Italy	40, 80	South Western Alps	<2300m	Rifugio Mongoie, Piemonte	44.167	-7.783	2	*A. ciliata* SW Alps
France	40, 80	South Western Alps	<2500m	Col Agnel	44.783	-6.683	1	*A. ciliata* SW Alps
Slovakia	N/A	Tatras Mountains	<2500m	-				
A. ciliata subsp. multicaulis								
Switzerland	40	Western Alps	<2500m	Leukerbad	46.400	-7.583	1	*A. gothica* ssp *moerhingioides* Iberia Alps
Italy	40, 80	South Western Alps	<2500m	-				
France	40, 80	South Western Alps	<2500m	-				
A. ciliata subsp. pseudofrigida								
Greenland	40	Sermersook, Tunu	<200m	-				
Norway	40	Finnmark	<500m	-				
Norway	40	Svalbard	<500m	Bohemanflya	78.383	-14.733	2	*A. ciliata* Ireland-Svalbard
A ciliata subsp. bernensis								
Switzerland	200	Bernese Alps	2-3000m	-				
A. gothica subsp. moeringioides								
Spain	40, 80	Iberia	<2500m	Corarrobres, Picos de Europa	43.167	4.817	3	*A. gothica* ssp *moerhingioides* Iberia Alps
	40, 80	Iberia	<2500m	Benasque, Pyrenees	42.683	-0.600	2	*A. gothica* ssp *moerhingioides* Iberia Alps
A. gothica subsp. gothica								
Switzerland	100	Jura Mountains	<2000m	-				
Sweden	100	Gotland island	<100m	-				
A. norvegica								
Ireland	80	Ireland	<200m	Black Head, Co. Clare	53.133	9.267	1	*A. norvegica*
Britain	80	Britain	<500m	Yorkshire	54.283	-2.550	1	*A. norvegica*
Britain	80	Britain	<1000m	Isle of Rum	56.983	6.317	1	*A. norvegica*
Britain	80	Britain	<1000m	Inchnadamph, Highlands	58.117	4.917	2	*A. norvegica*
Britain	80	Shetland	<200m	Shetland	60.517	1.367	1	*A. norvegica*
Iceland	60, 80	Iceland	<500m	Hofn	64.000	16.367	2	*A. norvegica*
Norway	80	Norway	<1000m	Tromso	69.73	-18.80	1	*A. norvegica*

plants in Ireland, and many species share their distribution between the two localities; however *A. ciliata* and *A. norvegica* are mutually exclusive at the two sites. In a wider context, the regional distribution of *A. norvegica* clearly follows that of the Irish flora in general in also being present in Britain, however *A. ciliata* remains a genuine botanical anomaly. Their discrete distribution in Ireland may be an artefact of recent habitat loss, population contraction, immigration or a combination of all of these, as well as possibly reflective of a longer distinct population history, however for two species so closely related to share such similar ecological niches but different distributions in Ireland is noteworthy.

European affinities of Irish *A. ciliata* and *A. norvegica* populations

Resolving the European genetic affinities of these two native species can help elucidate their migratory histories to the island, and in so doing, provide new evidence about some of the potentially earliest plant populations of early Holocene Ireland.

We have previously presented analysis of ploidy and chloroplast haplotype diversity across the *A. ciliata* complex in Ireland and Europe, establishing a baseline genetic survey of the species group (Abukrees *et al.* 2018, Dang *et al.* 2012, Kozlowski *et al.* 2024, Kozlowski *et al.* 2022). As a first step in presenting our exploration of the biogeographic history of *A. ciliata* in Ireland and Europe, we examine here genetic affinities among a subset of European populations of *A. ciliata*, *A. gothica* and *A. norvegica* using AFLP (Amplified Fragment Length Polymorphism) markers, set in the context of known chromosome ploidy counts.

The AFLP fingerprinting technique (Vos *et al.* 1995) is a PCR-based assay of genetic similarity, revealing similarities and differences in DNA identity from across the combined nuclear, mitochondrial and (in the case of plants) chloroplast genomes, and is most useful for studying accessions of closely related populations and species (*e.g.* Reisch and Rosbakh 2021, Sharbel *et al.* 2000). The AFLP technique involves the digestion of genomic DNA using restriction enzymes followed by two rounds of

PCR amplification of a subset of these fragments using selective primers.

Our objective is to provide a preliminary overview of genetic identity across the sampled populations based on whole genome similarity between individuals. These data will in turn act as a compliment for other genetic investigations focused on DNA sequence-based methodologies.

MATERIALS AND METHODS

Sampling

A total of 31 individuals from 11 populations were included in this analysis (Table 1), comprising a representative sampling of the biogeographical and taxonomic range of the A. ciliata species complex. Tissue samples were collected and desiccated in silica gel. DNA extraction was completed using a modified 2 x CTAB protocol, after Doyle and Doyle (1987), with extracted DNA at a target concentration of 50 ng/µl stored in TE Buffer at -20 °C until AFLP analysis.

Molecular Analysis

For initial sample digestion, 5.5µl of genomic DNA (10-200 ng/µl) was digested 40 units of EcoRI and 100 units of Mse1. Digestions also included accompanying reaction buffers at 1X concentration and 32.5µl of Bovine Serum Albumen (1mg/ml) for 1 hour at 37 °C. Forward and reverse adaptor pairs were prepared by adding 25µl of EcoR1forward adaptor at 1000µM to 25 µl of Mse1 reverse adaptor at 100µM and mixed with 450 µl of TE buffer. The restriction ligation enzyme master mix was prepared comprising 0.325µl H_2O, 1µl T4 DNA ligase buffer, 1.1µl NaCl (0.5M), 0.55µl BSA (1 mg/ml), 1µl EcoRI Adaptor (5 uM), 1µl Mse1 Adaptor (50µM), 0.125µl EcoRI (40 U/µl), 0.1µl Mse1 (10 U/µl), and 0.2µl T4 DNA Ligase (340 U/µl). 5.5µl of this mix was added to 5.5µl of digested template

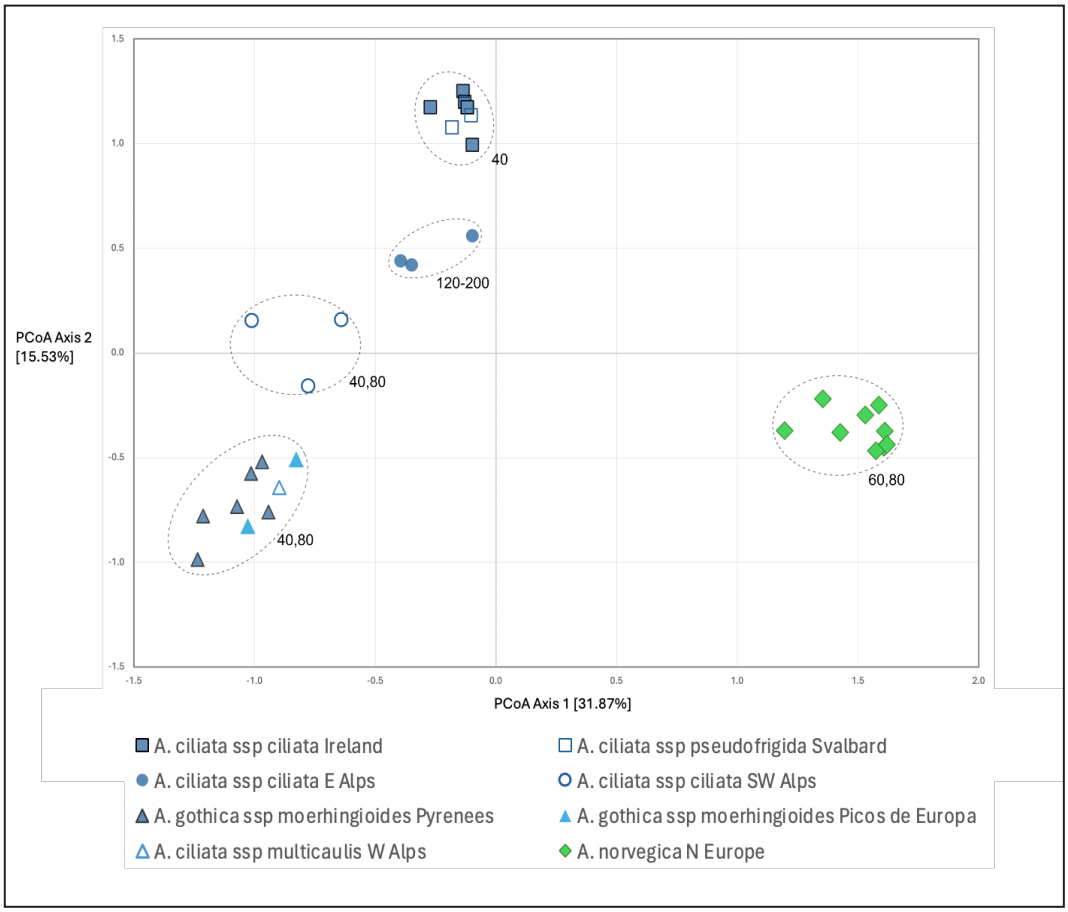

Figure 2. Principal Co-ordinates Analysis (PCoA) of genetic identity among sampled individuals ordinated using derived Principal Axes 1 and 2. Individual samples are coded according to their recorded taxonomic identity. The observed range of ploidy identities for individuals are indicated for each discrete sample cluster.

Table 2. Population Genetic Structure of the sampled population group based on AFLP frequency data, with sample allelic diversity for the ploidy-based grouping of species and populations. Except where indicated, all calculations have been completed using GenAlEx 6.5.

Analysis of Population Genetic Structure

		Dataset Structure		AMOVA			Mantel
Sample Group	N Samples	Populations	AFLP loci	Φ'_{PT}	Among Populations	Within Populations	rXY
All samples	31	17	146	0.551***	55.06	44.94	0.454**
A. ciliata	14	8	146	0.427***	42.66	57.33	0.615**
A. norvegica	9	6	146	0.072 ns	7.17	92.82	-0.439 ns
A. gothica	8	3	146	0.112**	11.15	88.84	0.232 ns
2N Ploidy Groups	31	5	146	0.565***	56.53	43.46	0.454**

Allelic Diversity 2N Ploidy Population Groups

2N Ploidy Group	N Samples	Ploidy[1]	No. AFLP Bands	No. Private AFLP Bands	Polymorphic loci	uh (Unbiased Diversity)
A. ciliata Ireland -Svalbard	7	40	60	9	32.19%	0.132
A. ciliata E Alps	3	120, 160, 200	56	6	19.18%	0.128
A. ciliata Ligurian Alps	3	40, 80	58	10	31.51%	0.210
A. gothica ssp moehringioides Iberia W Alps	9	40, 80	75	20	44.52%	0.169
A. norvegica	9	60, 80	63	12	19.86%	0.057
Mean					29.45%	0.139
S. E.					4.67%	0.009

genomic DNA, and samples were incubated for 2 hours at 37 °C prior to dilution with 90µlsterilized ddH_20.

Master mixes for the preselective amplification were prepared as follows: 4µl diluted DNA prepared by restriction-ligation was added to 0.5µl AFLP preselective primer pairs (10µM, Applied Biosystems), 1.25µl Ampli Taq Buffer (Applied Biosystems), 0.75µl $MgCL_2$ (25mM), 0.25µl DNTPs (10mM), and 0.1µl Amplitaq (5U/µl, Applied Biosystems). Preselective PCR cycles comprised an initial hold at 72 °C for 2 minutes, followed by 30 cycles of denaturing at 95 °C for 30 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 2 minutes; followed by 10 minutes at 60 °C, and a final hold at 4 °C. 5µl of each reaction was then run on a 1.5 % agarose gel containing 5µl/100ml SYBR-green (10,000x solution) to confirm the appropriate PCR product smear in the 100-1500 bp range. 10µl of the preselective amplification product was diluted with 190µl of TE0.1M and stored at 4 °C for immediate use and at 20 °C for long term storage.

For the selective amplification step, trial evaluation of 9 primer pairs identified the pairs ACT-CAG and AGC-CAA as generating the

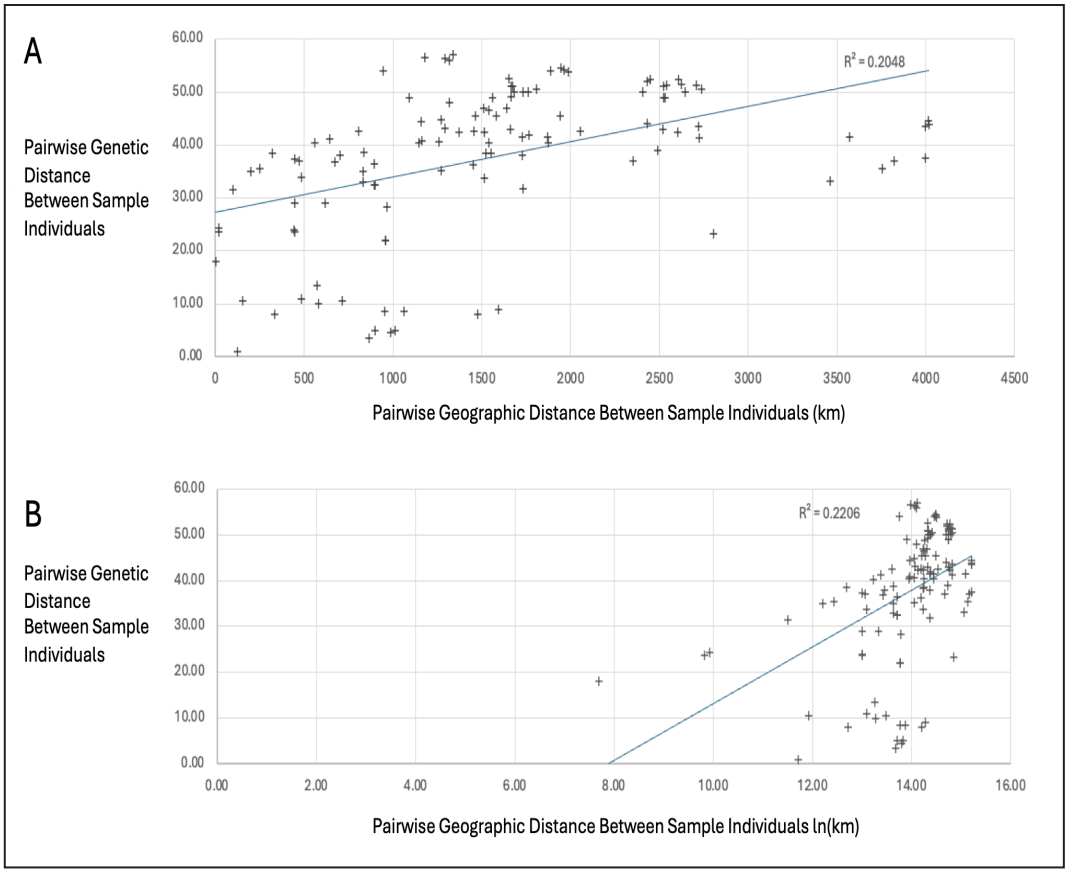

Figure 3. Pairwise genetic compared to geographic distance among all sample pairs in the dataset, with linear regression summary line and R^2 value. A – Genetic compared to raw geographic distance. B – genetic compared to log-transformed geographic distance. Genetic distance calculated after the method of Huff *et al*. (1993), implemented in GenAlEx 6.50.

highest density of variable loci in the target species populations, and were used here. Master mixes for the selective amplification step were prepared in 1.5 ml tubes comprising 6.55µl ddH_2O, 1.25µl of 10x Amplitaq Buffer (Applied Biosystems), 0.75µl of 25mM $MgCl_2$, 1µl of 10mM DNTPs, 0.1µl of EcoRI-NNN+ Dye Primer, 0.25µl Mse1-NNN Primer and 0.1µl 5 U/µl Ampli Taq Gold (Applied Biosystems). 2.5µl of template DNA prepared by preselective amplification was added to10µl of selective master mix and run on the selective amplification cycle of an initial hold at 95 °C for 2 minutes, followed by 13 cycles of denaturing at 94 °C for 30 seconds, annealing at 65 °C for 1 minute, reducing by 0.7 °C with each cycle, and extension at 72 °C for 2 minutes; followed by 24 cycles of denaturing at 94 °C for 30 seconds, annealing at 56 °C for 1 minute, and extension at 72 °C for 2 minutes; with a final extension step of 72 °C 10 minutes, followed by indefinite hold at 4 °C. Selective amplification samples were analyzed on an Applied Biosystems 3730 capillary sequencer, using the Rox500 internal size standard, with run data scored using the GeneMapper 4.0 application.

Data analysis

Outputted GeneMapper files were compiled and converted to a format readable by the GenAlEx 6.5 application, implemented in Microsoft Excel (Peakall and Smouse 2012), where the presence/absence of bands at a locus was recorded as 1/0 respectively. Summarisation and visualisation of individual and population genetic affinities was completed using Principal Co-ordinates Analysis (PCoA). Evaluation of genetic diversity within and among populations was completed using Analysis of molecular variance (AMOVA); and a Mantel test was deployed to evaluate correlation between genetic and geographic distance among populations. All analyses were implemented in GenAlEx 6.5.

Results

AFLP analysis with the primers ECorI-ACT + Mse1-CAG and ECorI-AGC + Mse1-CAA generated a total of 146 variable bands across the 31 sampled individuals for which a full AFLP cycle was successfully completed. PCoA multivariate analysis of variation patterns across this dataset summarise 31.87 % and 15.53 % of variation in principal axes 1 and 2, respectively (Fig. 2). For AMOVA analysis, in addition to population-based analysis, populations were also grouped according to taxonomic identity, and according to the five identified ploidy groupings in PCoA (Table 2). Analysis of the distribution of genetic variation across the entire sample group generated a PhiT value of 0.551 ($P<0.005$). Analysis of variation based on indicated taxonomic identity of samples generated a PhiT of 0.427 ($P<0.005$) for *A. ciliata* (incl. ssp *ciliata* and ssp *pseudofrigida)*; 0.112 for *A. gothica* (ssp. *moehringioides*) ($P<0.05$) and 0.072 ($P>0.05$) for *A. norvegica*; while analysis of the ploidy-based groupings inferred from PCoA generated a PhiT of 0.565 ($P<0.005$).

Overall there was a significant positive correlation between genetic and geographic distance among all populations across the dataset, and among the 5 population groups inferred from PCoA (Mantel rXY 0.454; $P<0.05$, Table 2; Log-transformed $R^2 = 0.221$, Fig. 3). The greater proportion of this correlation is accounted for by patterns among *A. ciliata* populations, with a Mantel rXY correlation of 0.615 ($P<0.05$), compared to a non-significant correlation for both the sampled *A. gothica* ssp. *moehringioides* populations and *A. norvegica*. In terms of the grouping with highest indicated PhiT value (the 5 Ploidy Groups indicated from PCoA), the unbiased diversity values (Table 2) are highest for *A. ciliata* from the SW Alps (0.210) and lowest for *A. norvegica* (0.057), with a mean score across the dataset of 0.139 ± 0.009; while the highest number of private AFLP bands is recorded for *A. gothica* ssp. *moehringioides* (20; N=9) and lowest for *A. ciliata* ssp. *ciliata* from the Eastern Alps (6, N=3). The proportion of polymorphic sites (sites that varied between samples within a population) varied between 19 and 44 % (*A. ciliata* ssp. *ciliata* from the Eastern Alps and *A. ciliata* from SW Alps, respectively; Table 2).

Discussion

These data present a preliminary analysis of genetic affinity across the *A. ciliata* species complex in Europe, based on whole genome similarity inferred from AFLP markers. The sampled individuals and populations represent a subset of known diversity within the group (Table 1), and the AFLP markers comprise two sets of selective primers, covering 146 variable loci. Unlike datasets comprising discrete DNA sequence-based data, only limited inference is possible regarding the timing of dispersal and vicariance events among the sampled groups here, and a fuller evaluation of sequence-based data will place these findings in context. The authors are currently working on this expanded analysis. Noting these limitations, the data nevertheless present a very clear pattern of genetic structuring across the sampled populations that is consistent with patterns in sequence-based analyses (data not shown). In the PCoA ordination (Fig. 2), principal axis 1 (accounting for almost 1/3 of total variation in the dataset) primarily distinguishes *A. norvegica* from all the remaining taxa in the *A. ciliata* complex, while axis 2 captured variation distinguishing between remaining biogeographic and taxonomic groups in the core *A. ciliata* complex. The clear separation of the indicated groups in Fig. 2 is supported by an overall significant positive Mantel correlation for genetic and geographic distance across the dataset (Table 2, Fig. 3).

Populations of *A. norvegica* evidently form a quite distinct genetic group, notable also for its relatively reduced number of both private bands and genetic diversity, compared to the other population groups surveyed, as well as a non-significant correlation between genetic and geographic distance among populations sampled in this group (Table 2). For *A. ciliata* in Ireland, the data presents very strong evidence that the natural sister populations are not in Iberia or Central Europe, but in the much more distant *A. ciliata* ssp. *pseudofrigida* populations of Svalbard and the Arctic. Unlike the nearest relative population group to these two – *A. ciliata* ssp. *ciliata* from the Eastern Alps, which displays multiple polyploid types with 2N values ranging from 120–200 – both *A. ciliata* ssp *ciliata* from Ireland and *A. ciliata* ssp. *pseudofrigida* share the same fixed ploidy level of 2N = 40 (Table 2, Fig. 2).

There is some discontinuity in the literature regarding the taxonomic status of the *A. ciliata* populations of the Western and Southwestern Alps and those of Iberia, comprising taxa identified as *A. ciliata* ssp. *ciliata,* ssp. *multicaulis* or ssp. *moerhingioides*, and this appears to be captured in the ordination of the remaining populations in Fig. 2. As Wyse-Jackson and Parnell (1987) demonstrated, there are no discriminating traits that reliably identify any one type from the other, while Abukrees *et al*. (2018) and Kozlowski *et al*. (2022) confirmed that the many populations across these two regions broadly share the same

ploidy status of 2N=40 or 80. Among this group, the SW Alps types demonstrate the clearest AFLP identity, with an elevated number of private bands and unbiased genetic diversity compared to all other groups (based on a count of N=3, Table 2), while those of Switzerland, the Pyrenees and the Picos de Europa (*A. gothica* ssp. *moehringioides*) are grouped closely together in the ordination, displaying slightly lower levels of diversity compared to the SW Alps. Notably, the high level of allelic diversity in *A. gothica* ssp. *moehringioides* does not have a significant geographic correlation, whereas in *A. ciliata* ssp. *ciliata* and ssp. *pseudofrigida*, the correlation is higher than for the group as a whole (Table 2).

While these data represent a preliminary evaluation of genetic affinities across the *A. ciliata* species complex, some patterns are very clear, and suggest a number of relevant biogeographic scenarios. Firstly, *A. norvegica* appears to comprise a genetically distinct identity, with notably lower levels of diversity compared to other identities in the complex. Within this shared genetic identity for the species, the populations in Ireland and Britain are not distinct from one another or from the wider species pool. This taxon has probably the largest standing population of any species in the complex, with a near contiguous range across much of Iceland and Northern Norway, however the constricted AFLP genetic profile, combined with weak genetic-geographic signal in the dataset suggests a relatively recent range expansion. It is unclear from the current data when this expansion may have taken place, however the species distribution overlaps almost exclusively with areas formerly covered by the North European and Icelandic Ice Sheets at the end of the LGM.

The remaining *A. ciliata* complex identities display very different patterns of genetic diversity, characterised by strong genetic-geographic subdivision and diversified ploidy identities. In some cases, genetic similarity is shared over very large geographic disjunction (*A. ciliata* populations in Ireland and Svalbard), whereas in other regions quite distinct genetic identities exist in relatively close proximity (populations in the Western and SW Alps). The PCoA ordination indicates that ploidy has a clear association with broader genetic identity in the genomes of samples and populations, and combined with indicated diversity levels, and the significant positive correlation between genetic and geographic distance, suggests much longer history of population migration and localised differentiation than is evident for *A. norvegica*. While many high elevation and high latitude populations occur in once glaciated regions, they are often adjacent to Arctic-Alpine plant refugia that likely persisted through the height of the late Pleistocene glaciations (Abbott and Brochmann 2003, Eidesen *et al*. 2013, Provan and Bennett 2008). A notable exception to this pattern are the populations of *A. ciliata* in Ireland, which rest well within the implied boundary of the British-Irish Ice sheet at the end of the LGM.

Not least among the possibilities that explain these data is that the Ben Bulben populations are in fact refugial in origin. It is possible these populations derive from range contraction, either due to the loss of now-submerged LGM habitats on the coastal shelf, or as relict postglacial immigrants that shadowed the retreat of LGM ice-cover across northern Europe. Alternately they may predate the LGM, having survived *in-situ* on nunatak or coastal refugia, with local origins in the Mid-Late Pleistocene. The possibility that they may be result of a long-distance migration event, for example by migrating wintering birds, can likely be discounted, as Dang *et al*. (2013) identified diverse chloroplast haplotypes on Ben Bulben that are inconsistent with long-distance migration from a single source.

Riverine sediments at Derryvree in Northwest Ireland show that *A. ciliata* was a resident species in the late Pleistocene tundra biota of Ireland, *c*.33,000 years BP (Colhoun *et al.* 1972), while late Weichselian fossils are also recorded from central Ireland prior to the Younger Dryas *c*.10,000 years BP (Godwin 1975). The Ben Bulben massif is 30 km from Derryvree and also one of a few upland locations in Ireland where ice-scarring is not evident from the LGM (Synge 1969). Nunatak and refugial survival through the LGM are inferred from population phylogeographic data for many Arctic and Alpine plant species outside Ireland and Britain, even in cases where additional lowland areas have provided ample habitat space during glacial periods (Bettin *et al.* 2007, Schonswetter *et al.* 2005).

Further analysis of DNA sequence-based data will allow confirmation of more precise timelines for key migration and vicariance events in the *A. ciliata* complex across Europe. However the data presented here confirm, as implied by Webb (1983), that *A. ciliata* on Ben Bulben likely represents a distinctive biogeographic history compared to the remainder of the Irish flora, with a key affiliation to populations to the North.

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