Population Genetics of Sub-Arctic Willow
(S. lanata, S. lapponum and *S. herbacea).*

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S. lanata

S. lapponum

S. herbacea

Introduction

Sub-arctic willow scrub is largely confined to the Scottish highlands[1], existing as isolated, fragmented populations that are considered to be remnants of a formerly more widespread vegetation type of which many are now in danger of terminal decline. In order to effectively conserve these habitats, an understanding of the diversity and inter-relationships of ecosystem components is essential.

To investigate patterns of clonal diversity in sub-arctic willow populations, 2 isolated locations (Figure 5); C. Sharroch (*S. lapponum* and *S. lanata*) and the M. Ghaordie (*S. herbacea*) were chosen and a detailed sampling was undertaken. All individuals were screened for polymorphism using a series of microsatellite markers (SSRs), developed for this project[2] to establish the importance of clonal diversity and composition. One primer (PMGC 223) developed for poplar studies[3] was also used for this study.

Materials & Methods

Total genomic DNA was extracted from 50mg of silica-dried *S. lanata*, *S. lapponum* and *S. herbacea* leaves using the protocol for 'isolation of DNA from plant tissue', from the DNeasy plant Mini Extraction Kit (Qiagen).

Coire Sharroch
All sampled bushes were labelled with individually numbered aluminium foil tags. Leaf samples were taken every 30-50cm intervals. In total 100 *S. lanata* and 310 *S. lapponum* individuals were collected from 6 sub-sites (Figure 1)

Meall Ghaordie
In contrast to the other two species, *S. herbacea* is abundant and widespread throughout Scotland, being found in more open, higher altitude sites (Figure 2).

The different habitat of this species allows a more methodical sampling strategy. At each of the 6 sub sites on the mountain a transect was taken with 5 quadrats. Three samples were taken from each quadrat at 20cm intervals, with the distance between quadrat from 1 to 2m and then to 4 m. (Figure 3).

Figure 1: Spatial representation of the six sub-sites used on C. Sharroch mountain.

Figure 2: Spatial representation of the six sub-sites used on M. Ghaordie mountain.

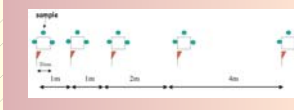

Figure 3: Representation of a transect, the samples collected and the distances between them.

Results

All SSR loci were polymorphic across all sub-sites for all three species. With 19 alleles for *S. lapponum*, 18 for *S. lanata*, and 16 for *S. herbacea* at their most variable and 8 for *S. lapponum*, 4 for *S. lanata*, and 2 for *S. herbacea* at their least variable locus. From the six microsatellite loci surveyed, 62 alleles were identified for *S. lapponum*, 44 for *S. lanata* and 29 for *S. herbacea*, accounting for a total of 78 different alleles across the three species. Additionally, genetic diversity measures obtained for each species are summarised in Table 1.

	N	A	P_D	H_E	H_O	F_{IS}
S. lanata	82	8.4	0.99	0.706	0.5634	0.174
S. lapponum	224	12.4	0.95	0.7027	0.4152	0.378
S. herbacea	90	5.8	0.54	0.5275	0.4996	-0.228

Table 1: Number of samples analysed for each species, their mean allele frequencies, proportion of distinguishable alleles, expected, observed heterozygosity and inbreeding coefficient.

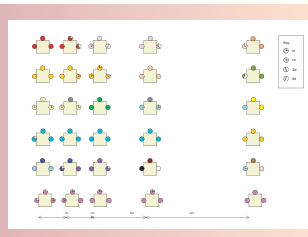

Figure 4: Map showing the spatial distribution of S. herbacea clones at M. Ghaordie. Each colour corresponds to a singe (different) genotype.

The proportion of distinguishable genotypes, Pd, was high for both *S. lanata* and *S. lapponum* (0.95 and 0.99 respectively) suggesting that in both species almost all of the individuals are unique. In the very rare cases of the detection of shared genotypes, individuals were geographically very close and appear to be examples of extreme local clonal growth. In contrast, the proportion of distinguishable genotypes, Pd, for *S. herbacea* was 0.54, making it much less diverse than the other two species.

These distinguishable genotypes are shown in Figure 4, which is a representation of all six subsites (Figure 3) of M. Ghaordie. In this figure, every different colour represents a different genotype. It is obvious that the same genotype is found in multiple samples. Some of these patches are large; with the same genotype being found at distances up to 8m within transects suggesting extensive clonal growth. These identical multi-locus genotypes show strong spatial aggregation, which fits well with a hypothesis of identical genotypes relating to clonal growth and local patch formation.

Ongoing work

Population genetic structure
Comparative assessments of population differentiation in *S. lanata, S. lapponum* and *S. herbacea.* (Figure 5).

Gene flow
Assessment of parentage of seed families from a single population of *S. lapponum* at B. Lawers (Figure 5).

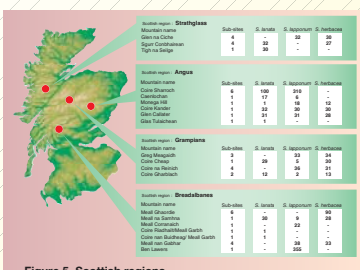

Figure 5. Scottish regions, mountain sites and numbers of plants collected

Conclusions

S. lanata and *S. lapponum*, intensive sampling of sub-sites within a single large population detected little evidence of clonal growth. Instead, the vast majority of individuals possessed distinct multi-locus genotypes. Thus, sexual rather asexual reproduction is the predominant means of perpetuation and dispersal despite the extensive suckering abilities of willows and the limited amount of observed sexual recruitment in these populations. However, for *S. herbacea*, the genetic data provides evidence for local patch formation with identical genotypes being detected from multiple samples within transects up to 8m apart.

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References
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2. Stamati , K *et al*., (2003). A set of polymorphic SSR loci for subarctic willow (*Salix lanata*, *S. lapponum* and *S. herbacea*) Molecular Ecology Notes (2003) 3, 280–282
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