**Project Report:** Genetic Diversity in Ash Reproductive Material Related to Restoration of Declining Ash Forest

**Aim:**

The main objective of the project was to assess the genetic diversity of ash in the forests designated for ash restoration trials in Fjugstad Nature Reserve and compare it with seedlings grown from resistant mothers in Denmark to ascertain genetic compatibility.

**Major Activities:**

1. Sampling in August 2022 involved collecting leaf samples randomly from plants under infection pressure in Fjugstad Nature Reserve, one of the largest ash stands in Scandinavia, and scoring for the presence of disease. Leaf samples from nursery-grown seedlings for the forest restoration trial, referred to hereafter as the forest reproduction materials were also collected and immediately preserved with silica gel until DNA extraction.
2. DNA extraction was performed using QIAGEN’s DNeasy Plant Mini Kit with some modifications to optimize DNA yield.
3. DNA was amplified using 12 neutral microsatellite loci, detailed in the attached thesis.
4. Genotyping was conducted using gel electrophoresis and a standard bioinformatics pipeline for microsatellite data.
5. Genetic diversity parameters, including expected and observed heterozygosity, allelic and private allelic richness, were calculated and compared between the two study populations.
6. Inbreeding within the populations was assessed.

**Major Findings:**

1. Plants under natural selection pressure in Fjugstad Nature Reserve and forest reproduction materials exhibited similar expected and observed heterozygosity.
2. Allelic richness was similar in samples from both populations, but private allelic richness in forest reproduction materials was significantly higher. The private alleles are specific to particular population and often are developed in response to the local environment. As we used neutral marker, we however could not relate the private alleles to any specific traits.
3. Both populations showed no signature of a population bottleneck or inbreeding, suggesting Danish forest reproduction materials as suitable propagation materials.
4. Significant overlap in allele frequency was observed between the forest reproduction materials and ash plants from Fjugstad.
5. Based on measures of heterozygosity, allele frequency, and overlapping allele frequency, Danish forest reproduction materials were found genetically compatible with the ash forest's genetic diversity in the Fjugstad Nature Reserve, making them suitable for reforestation trials.

**Knowledge Dissemination:**

Besides presenting the work at the University, results were shared at the annual ForBio meeting in Bergen, attended by researchers from various countries working on biodiversity conservation. Presentation slides are attached.

**Deviation:**

Originally planning to use SNP markers for assessing genetic diversity, the project opted for microsatellite markers due to existing baseline data and cost constraints. Despite the marker change, all anticipated outcomes were achieved.

**Conclusion:**

This pioneering effort in Europe to analyze genetic diversity before introducing materials into natural forests for restoration trials confirmed the genetic compatibility of Danish reproductive materials with the natural ash forest in Fjugstad Nature Reserve. The study establishes best practices for restoration efforts involving the introduction of genetic stock of external origin.

Attached is the financial report and a copy of my thesis. Thank you so much for supporting me in this important project financially and also following up on the progress via a Zoom call.

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