



EXTRACTING ANCIENT DNA FROM THE NORTH SLOPE



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Abstract

- Ancient spruce DNA found frozen on the North Slope
 - 8 Samples
- Carbon dated 50,000 years, so the samples are significantly older
- 50,000 years ago, the North Slope was glaciated
 - No areas capable of inhabiting live spruce trees
 - This places the samples to be about 80,000-130,000 years old
- The goal is to identify the species of spruce that was found
- Knowing the species will give us more insight into how the landscape once looked on the North Slope
- This research is significant because no records have been found extracting DNA from a tree specimen this old
- Currently, it appears we have successfully extracted DNA from 1 sample

Background

We have access to spruce wood that was found frozen on the North Slope, North of the Brooks Range. It was melting out of permafrost and peat, so the wood itself has remained frozen since it has remained submerged this entire time. It has been carbon-dated 50,000 years, which means that it is significantly older than that. We know that 50,000 years ago, the North Slope was completely glaciated at that time; meaning that there were no areas capable of inhabiting live spruce trees. This spruce wood is most likely from the last interglacial period when it was warm with no glaciers. That is placing the spruce wood to be about 80,000-130,000 years old.

The last interglacial period, the Eeiman, is thought to be the last time the global climate was significantly warmer than that of the present (Muhs et al. 2001). Pollen, macrofossils, and fossils have migrated by route of glaciers and can tell us a lot about past ecosystems. We do not know exactly what the landscape looked like on the North Slope ~100,000 years ago. Identifying the species of spruce that was found there will tell us give us more insight into how the landscape of the North Slope looked during the last interglacial period when it was warm with no glaciers. This study is significant because no records have been found extracting DNA from a tree specimen this old.

The plan is to use the labs that are available on the UAF campus to extract DNA from the spruce wood, use PCR to amplify many copies of a particular gene, sequence it, and compare the sequences to a large public database to determine its species.

Objectives

- Identify a way to grind rock-hard frozen spruce wood
- Extract DNA from spruce wood
- PCR amplification of ITS1, mitochondrial and chloroplast DNA from the aDNA
- Sequence the DNA
- Accurately identify extracted DNA sequences to the family, genus, and species
- Create a picture of Northern Alaska's plant communities from 80-130,000 years ago

Methods & Materials

Sample Collection and Storage in aDNA lab:

- 8 samples, kept frozen at -17.8 °C
- Scalpel used to scrape contaminants off sample surface; Ground sample with SPEX SamplePrep Freezer/Mill

DNA Extraction in aDNA lab:

- Latorre 2020 Protocol w/o the Dabney modification
- DNeasy Plant Mini Kit
 - PVP added for wood, polyvinylpyrrolidone (PVP=PVP40000)

Identifying Levels of DNA Concentration in IAB Genomics Core lab:

- Qubit Fluorometer - dsDNA
 - Successfully shown to extract DNA from one sample at this time.

Quality Assessment of DNA in IAB Genomics Core lab:

- Agilent 4200 TapeStation System
- NanoDrop Microvolume Spectrophotometer

Extraction ID	Weight (g)	Qubit Reading (ng/mL)	DNA Conc. (ng/ul)
Blank	0	<0.50	0
5.13	0.011	1.39	0.278
5.14	0.0108	1.37	0.274
5.17	0.0152	1.9	0.38
5.18	0.0162	1.67	0.334
5.20	0.014	1.77	0.354



Images: 1) Dr. Go Iwahana using a band saw to subsample one of the 8 samples of ancient Spruce wood. 2) Sample 5 out of 8; the freshest sample of Spruce wood and was used in this extraction. 3) Specimen ground up, ready for extraction. 4) Bree using the DNeasy Plant Mini Kit to extract DNA from the ancient Spruce in the ancient DNA lab at the UA museum. Protective gear is required to avoid contaminating samples with modern DNA.

Anticipated Results

The preservation of biological remains depends on an array of processes and conditions that involve physical, chemical, and biological agents. Such processes can affect the DNA of waterlogged tissues at different steps of taphonomy: during the transport to and within the environment, at the sediment-water interface, and after burial in the sediment. An increasing number of studies indicate that waterlogged plant remains represent a rich source of aDNA sequences (Shworer et al. 2021). Because of this data, we anticipate finding well-preserved DNA in the frozen spruce that has remained submerged in permafrost. Data also shows that the oldest wood specimen to have DNA extracted from it is ~10,000 years old, and was not frozen. Our specimens have remained frozen so we anticipate DNA should be better preserved. I plan to sequence plant DNA sequences and use the results to identify what species of spruce was living in Northern Alaska from 80,000 to 130,000 years ago. This will give us an idea of what the landscape looked like North of the Brooks Range ~100,000 years ago.

Future Research

- Extract DNA from the other 7 samples
 - Identify species
- PCR amplification of ITS1, mitochondrial and chloroplast DNA from the aDNA
- Sequence the DNA
- Compare the samples of aDNA to modern Spruce DNA
 - Compare bp lengths

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