EXTRACTING ANCIENT DNA FROM THE NORTH SLOPE

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Background

- Spruce wood discovered from a permafrost bluff on the North Slope.
- The wood has remained frozen in the permafrost, preserving it for thousands of years.
- All samples radiocarbon-dated to 50,000 years, indicating it is significantly older.
- At 50,000 years ago, the North Slope was fully glaciated—live spruce trees could not have grown there.
- The wood likely originates from the last interglacial period (Eemian, ~80,000–130,000 years ago) when the region was ice-free and warm.
- The Eemian interglacial period was the last time global climate was significantly warmer than today (Muhs et al. 2001).
- Fossil pollen, macrofossils, and plant remains can provide insight into past ecosystems.
- The North Slope's landscape ~100,000 years ago is unknown—identifying the spruce species will improve our understanding.
- This study is pioneering DNA extraction from a tree specimen of this age, with no prior records of success.

Objectives

- Identify a way to grind frozen ancient wood without thawing
- Extract DNA from ancient wood
- PCR amply ITS1, mitochondrial and chloroplast DNA from the ancient DNA
- Sequence the DNA
- Identify wood to family, genus, and species
- Reconstruct Northern Alaska's plant communities 80,000–130,000 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

PCR Amplification:

- ITS1, nad5, rbcL, trnL, and trnT/R

Anticipated Results

- Because the spruce wood had been preserved in the permafrost, we anticipate finding well-preserved DNA.
- Sequence the extracted DNA and compare it to known plant DNA databases to identify the species of spruce that lived in Northern Alaska.

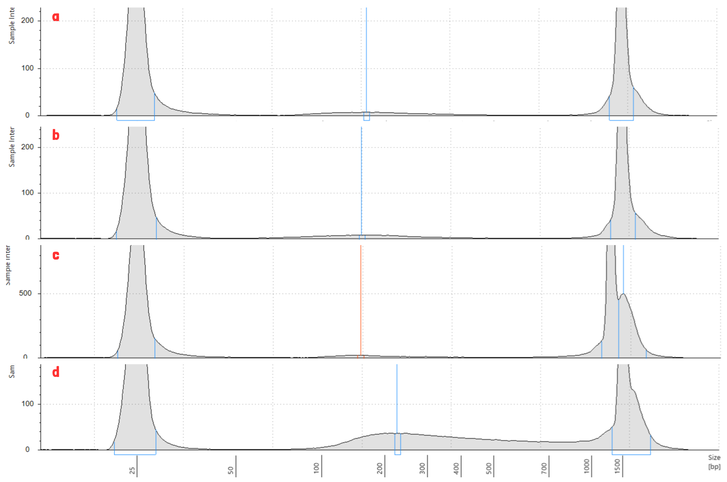

Figure 1: The electropherogram from the Agilent TapeStation shows the fragment size distribution of the ancient DNA samples 1A.1(a), 0.1(b), 5.17(c), and 6.4(d). The two prominent peaks at 25 bp and 1500 bp correspond to the internal lower and upper markers. Each sample exhibits a characteristic degraded DNA profile, with small peaks observed at 169 bp (1A.1), 161 bp (0.1), 157 bp (5.17), and 228 bp (6.4).

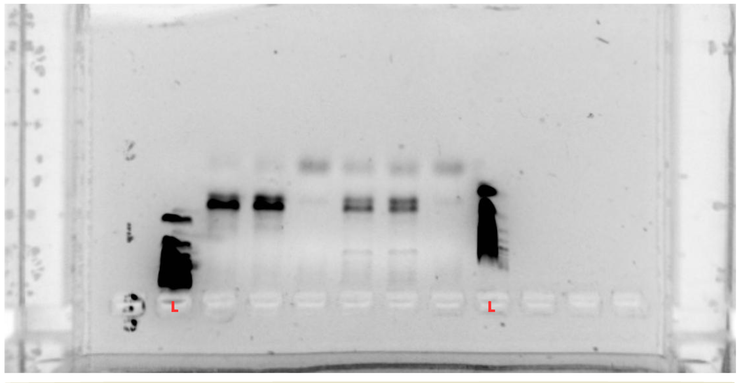

Image: This agarose gel shows PCR amplification of ancient DNA using trnL primers. Ladders (L) on both sides provide size references (100–200 bp). Amplified fragments appear within this range, indicating successful target amplification. Variations in band intensity reflect DNA preservation differences. A negative control confirms no contamination. These results support the use of trnL for plant identification in degraded samples.

Images: 1) Sample 5 out of 8; the freshest sample of Spruce wood and was used in this extraction. 2) 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. 3) The location where these samples were found thawing out of permafrost (N68.99, W150.28).

Future Research

- PCR amplification of ITS1, mitochondrial and chloroplast DNA from the aDNA
- Sequence the DNA
- Identify Species

References

- D.R. Muhs, T.A. Ager, J. E. Beget. (2001). Vegetation and paleoclimate of the last interglacial period, central Alaska. *Quaternary Science Reviews*. 10.1016/S0277-3791(00)00132-3
- Latorre, S. M., M. Lang, P. L., Burbano, H. A., & Gutaker, R. M. (2020). Isolation, Library Preparation, and Bioinformatic Analysis of Historical and Ancient Plant DNA. Current Protocols in Plant Biology, 5(4), e20121. https://doi.org/10.1002/cppb.20121
- Li, X., Yang, Y., Henry, R.J., Rossetto, M., Wang, Y. & Chen, S. (2014). Plant DNA barcoding: from gene to genome. *Biological Reviews* 90, 157–166.
- Schwörer, C., Leunda, M., Alvarez, N., Gugerli, F., & Sperisen, C. (2022). The untapped potential of macrofossils in ancient plant DNA research. New Phytologist, 235(2), 391–401. https://doi.org/10.1111/nph.18108
- Wagner, S., Lagane, F., Seguin-Orlando, A., Schubert, M., Leroy, T., Guichoux, E., Chancerel, E., Bech-Hebelstrup, I., Bernard, V., Billard, C., Billaud, Y., Bolliger, M., Croutsch, C., Čufar, K., Eynaud, F., Heussner, K. U., Köninger, J., Langenegger, F., Leroy, F., . . . Orlando, L. (2018). High-Throughput DNA sequencing of ancient wood. Molecular Ecology, 27(5), 1138–1154. https://doi.org/10.1111/mec.14514
- Wang, X., Liu, C., Huang, L., Bengtsson-Palme, J., Chen, H., Zhang, J., Cai, D., & Li, J. (2015). ITS1: a DNA barcode better than ITS2 in eukaryotes? Molecular Ecology Resources 15, 573-586.

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