D2 DNA Analysis of Submerged Pine Logs

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After attending this presentation, attendees will understand methods of extracting and analyzing DNA from woody tissue for the purposes of historic or genetic reconstruction of forests.

This presentation will impact the forensic community and/or humanity by demonstrating more reliable protocols for wood DNA extraction are needed for timber certification, forensics and criminal prosecution, nautical archaeology, paleobotany and especially for reconstructing colonial history. We demonstrate here the use of DNA extraction and analysis from submerged logs as a reliable tool in reconstructing North American forest genetic composition and diversity. Submerged logs constitute an "accidental museum" for reconstructing pre-settlement forests along the Atlantic seaboard and the Gulf of Mexico. This method also holds promise in archaeology for DNA analysis of early wooden buildings, wooden ships, wooden forts and wooden tools of early European colonists.

DNA analysis of submerged logs is a novel approach to historic and genetic reconstruction of North American forests. Along the Atlantic seaboard, Gulf of Mexico, Great Lakes and Puget Sound, logs were rafted along flat rivers and estuaries in the 19th century as a means of transport. During rafting, many logs sunk and were preserved from degradation in deep anaerobic silt 7 to 15 m below the water's surface. The value of these submerged logs for historic purposes depends on reliable methods for extracting high molecular weight DNA. DNA analysis of submerged logs has value for identifying species and for measuring polymorphism for population genetics studies. Criteria for a reliable protocol include 1) high-molecular weight DNA extraction, 2) amplification of rbcL, a chloroplast gene indicating DNA origin from a photosynthetic plant, 3) a close homology between ribosomal DNA sequences and those of putative forest tree species and 4) assaying for DNA polymorphism. A DNA protocol was developed for submerged pine logs dredged in the Cape Fear River near Wilmington, NC. DNA extraction was based on a CTAB protocol modified with proteinase K, RNAase and polyvinyl pyrrolidone (PVP) steps. Crude extraction of DNA from five submerged log samples was followed by stringent DNA purification. Chloroplast gene rbcL (ribulose-1,5bisphosphate carboxylase) could be amplified in all samples. Intergenic transcribed spacer (ITS) sequences from ribosomal DNA were 98 to 94% homologous to sequences from two indigenous pine species, Pinus taeda and P. palustris. Assaying nuclear polymorphism required a variant of the DNA protocol purification step to improve amplification of singleand low-copy DNA sequences. A nuclear microsatellite was assayed; its polymorphism matched 3 of the 14 known P. taeda alleles. This is a robust DNA protocol for wood, which will have broad research applications for reconstructing genetic patterns in pre-settlement forests as well as wood certification, forensics, paleobotany and nautical archaeology.

Gymnosperms, Forest History, Forensics

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