B189 Using DNA Barcoding to Assess DNA Viability in Plant and Insect Fragments Isolated From Forensic Soil Samples

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After attending this presentation, attendees will understand the fundamentals of DNA barcoding and whether the DNA of biological fragments collected in forensic soil samples is viable toward molecular analysis.

This presentation will impact the forensic science community by providing an assessment as to whether the individual biological fragments commonly present in soil samples could provide additional information for provenance and thus be implemented in casework.

Every year, debris samples are submitted to forensic laboratories as evidence, but the insect and plant materials commonly found in such samples are rarely analyzed. This is despite the fact that individual insect and plant materials could add to the characterization of the sample that is provided by inorganic analysis.[1] In addition, biological material could provide information for soil geo-attribution, since insects and plants inhabit specific ecosystems. Seasonal distribution of plant and insect material will provide additional information for the sample. One reason such biological material is not analyzed is because microscopic identification is not straightforward, especially for examiners who not appropriately trained. Different individual specimens may present similar morphologies that are impossible to distinguish and there may be fragments instead of the whole insect or plant as well as specimens at different developmental stages. In these circumstances, using DNA for identification is an attractive alternative approach as DNA is present in all biological tissues.

Species-level identification is possible by using sequence data. DNA barcoding, which utilizes a standardized short sequence of DNA, typically 400–800 base pairs in length, is now a commonly accepted forensic approach for molecular identification.[2,3] The barcode sequence of the DNA from the item of interest is compared with sequences in public databases for classification of the sample. The mitochondrial Cytochrome Oxidase subunit 1 (CO1) gene has been adopted as the standard barcoding region for insect identification as it has a fast mutation rate and is found in high copies within tissues.[4] Two plastid regions (an organelle found exclusively in the cells of plants and algae), rbcL and matK, have been adopted as the core barcode regions for land plants as CO1 evolves too slowly to facilitate species-level discrimination of plant species.[4]

Using surface soil samples collected from various locations in Virginia with varied geology and ecohabitats to represent soil evidence associated with shoes and digging tools, the following will be discussed: (1) what types of plant and insect fragments are commonly recovered with surface soil samples; (2) whether such fragments contain viable DNA; and, (3) whether the appropriate DNA barcode regions could be amplified and sequenced using traditional Sanger methods. Ten insect and ten plant fragments were collected from each site (n=200). Various commercial kits for DNA extraction from the insect and plant material were compared. The results of these kits as well as the use of degenerate primers for COI, rbcL, and matK will be presented along with the success rate of the published barcoding protocols. Issues encountered when searching the data against two public databases, the National Center for Biotechnology Information (NCBI) and the Barcode of Life Database (BOLD) will be described in order to assess congruence and taxonomic identification.

Reference(s):

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2. Dalton D.L., Kotze A. (2011) DNA barcoding as a tool for species identification in three forensic wildlife cases in South Africa. *Forensic Sci. Int.* 2011 207(1-3) 51-4doi: 10.1016/j.forsciint.2010.12.017.
3. Yan D., Luo J.Y., Han Y.M., Peng C., Dong X.P., Chen S.L., Sun L.G., Xiao X.H. (2013) Forensic DNA barcoding and bio-response studies of animal horn products used in traditional medicine. *PLoS ONE* 8(2):e55854.
4. Kress W.J., Erickson D.L. Editors: DNA Barcodes: Methods and Protocols. Humana Press, New York, 2014.

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