A137 Genotyping Diptera Using Amplified Fragment Length Polymorphism (AFLP): Development of a Genetic Marker System for Species in the Families Calliphoridae and Sarcophagidae

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After attending this presentation, attendees will gain an understanding of the Amplified Fragment Length Polymorphism (AFLP) technique and its applicability to genyotyping non-human organisms.

This presentation will impact the forensic community by presenting a technique that can be used to genotype DNA of any origin and complexity. The AFLP method is rapid, robust, and many steps can be automated.

In forensics carrion-breeding insects are used primarily to estimate postmortem interval (PMI). Particular species are attracted to specific states of decay and colonize a body for a limited period of time. Forensic entomologists must correctly identify carrion breeding species in order to associate a particular developmental pattern with succession. This is problematic since the morphology of larvae, particularly of closely related species, is very similar if not identical. Rearing the larvae to adulthood delays the determination of PMI and may compromise the specimens by exposing the larvae to contamination, parasitism, and predation. Amplified Fragment Length Polymorphism (AFLP) is a powerful method that combines techniques from classical hybridization- based and PCR-based genotyping strategies. AFLP can be used to genotype DNAs of any origin and complexity. The AFLP technique has several advantages for forensics. The method is rapid, robust and many steps can be automated. Therefore, an identification system based on genetic markers would be a useful tool for forensic entomologists. AFLP profiles were obtained using larval samples from *Cochliomyia macellaria, Phormia regina* and *Sarcophaga bullata.* Genomic DNA was isolated using the DNeasy Tissue Kit (Qiagen, Valencia, CA), double- digested by two restriction endonucleases (*Eco*RI and *Mse*I) and ligated to oligonucleotide adapters. Two consecutive PCR reactions (preamplification and selective amplification) were performed using a modification of the AFLP protocol described by Gibco (Invitrogen, Rockville, MD). The DNA fragments were separated by capillary electrophoresis using the CEQ 8000 DNA Fragment Analyzer. Successive selective amplifications using the *Mse*I M-CAT and *Mse*I M- CA primers produced a set of markers that, taken as whole, comprise a species specific profile. Peaks at 103, 107, 119, 127, 135, 151, 274 nt were found in the *C. macellaria* samples. The species specific profile for *S. bullata* contained peaks at 100, 102, 109, 113, 126, 128, 133, 137, 143, 165, 171, 183, 188, 242 nt. The electropherograms of the *P. regina* samples exhibited species specific peaks at 113, 131, 138, 148, 178 nt. The results indicate that the AFLP technique is a viable and valuable technique for identification of entomological material. AFLP analysis can provide answers in certain situations where traditional forensic entomology can offer no (dead larvae), or only limited (fragmented larvae), information.

Forensic Entomology, AFLP, Genotyping

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