G102 The Genetic Identification of United Kingdom Calliphoridae – A Multi-Gene SNaPshot® Approach to Species ID

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After attending this presentation, attendees will understand some of the problems associated with current identification methods and the advantages of molecular identifications. The sequencing results of a multi-gene approach and the development of a SNaPshot® multiplex for species identification will be presented.

This presentation will impact the forensic science community by providing a novel approach to Calliphoridae species ID. The developed SNaPshot® multiplex will provide a more time and cost effective way of analysing samples compared to the traditional Sanger sequencing. Multiple regions are analysed simultaneously, whilst the short amplicons make it suitable for degraded and comprised samples frequently found in forensic cases.

A dead body is an attractive habitat for many insect species but it is the members of the Blowfly family (*Calliphoridae*) that are usually the first to arrive, using the body as an oviposition site. The stage of larvae found on a body can be a useful indicator of time since death, but in order for species specific life cycle data to be applied, accurate species identification is critical. Damaged, unviable or immature specimens can be difficult to identify morphologically and recent work has focused on the genetic identification of Blowfly species.

The aim of this study is to assess the potential of various genetic regions to differentiate between United Kingdom Blowfly species of forensic importance. Nine genetic regions, including both nuclear (ITS2, 28s rRNA, CAD, Bicoid and Elongation factor 1 alpha) and mitochondrial DNA (Cytochrome oxidase I and II, cytochrome b and 16s rRNA), have been sequenced for six UK species commonly used in forensic investigations (*Calliphora vicina, Calliphora vomitoria, Lucilia sericata, Lucilia illustris, Lucilia caesar,* and *Photophormia terranovae*).

Existing sequences were downloaded from the sequence database and aligned. Primers were either previously published primers or manually designed based on the sequence alignments. Genomic DNA was extracted using a mini kit, from wild-caught specimens collected from nine locations throughout the United Kingdom.[1-3] PCR amplifications were performed as single plexes for each gene and purified before DNA sequencing. Sanger sequencing was conducted in-house using a cycle sequencing kit. Sequence reactions were ran on ABI 310 and 3500 genetic analysers and analysed using Sequence Analysis software v5.4 (Applied Biosystems).

Results show that while most regions are suitable for distinguishing between species, problems still exist when identifying closely related species such as *Lucilia illustris* and *Lucilia caesar*. Analysis revealed that the mitochondrial regions Cytochrome Oxidase I and II (COI and COII) and Cytochrome b are capable of distinguishing between all species examined. These regions exhibit higher levels of inter versus intra species variation making them ideal for species ID.

The nuclear markers appear more conserved, having levels of inter species variation. Each of the nuclear markers sequenced can only differentiate samples to the genus level, failing to distinguish between *Lucilia illustris* and *Lucilia caesar* at each marker (ITS2 – 400 bp, 28s rRNA – 2.2 Kb, CAD – 700 bp, Bicoid – 350 bp and Ef1α – 1 Kb).

As the rapid identification of species is advantageous in forensic investigations, a SNaPshot® approach to species ID was investigated. SNPs from the following regions were chosen to differentiate between the six UK species: 28s rRNA (4 SNPs), Ef1α (4 SNPs), COI (2 SNPs), COII (2 SNPs), Cytochrome b (2 SNPs) and 16s (1 SNP). Nuclear markers were included so that any possible hybridisation between species (with the exception of *Lucilia illustris* and *Lucilia caesar*) could be detected. This SNaPshot® multiplex gives a unique haplotype for each species. Each species can be differentiated based on between 4-12 SNPs.

References:

1. Z. Song, X. Wang, G. Liang, Species identification of some common necrophagous flies in Guangdong province, southern China based on the rDNA internal transcribed spacer 2 (ITS2), Forensic Sci. Int. 175 (2008) 17-22.
2. J. Stevens, R. Wall, Genetic relationships between blowflies (Calliphoridae) of forensic importance, Forensic Sci. Int. 120 (2001) 116-123.
3. F.A Sperling, G.S. Anderson, D.A. Hickey, A DNA-Based Approach to the Identification of Insect Species Used for Postmortem Interval Estimation. J. Forensic Sci. 39(2) (1994) 418-427.

Forensic Entomology, DNA, Calliphoridae

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