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Estimating the post-mortem interval (I): The use of genetic markers to aid in identification of Dipteran species and subpopulations

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Abstract. Insect evidence can be used to estimate the post-mortem interval (PMI) of a discovered corpse. They can also indicate any post-mortem movement of a body. To use insect evidence accurate identification is essential. This study examined the use of mitochondrial cytochrome oxidase I markers to differentiate *Calliphora vicina* and *Calliphora vomitoria* (Diptera:Calliphoridae). The work also explored the use of nuclear DNA markers to distinguish UK populations of these species. DNA extraction and subsequent amplification of these markers in various lifecycle stages, adult body parts and burnt/decomposing larvae ensured that the markers could be used with any insect evidence presented to the entomologist.

Keywords: *Calliphora vicina*; *Calliphora vomitoria*; Interspecific variation; Intraspecific variation; Post-mortem interval

1. Introduction

Insect evidence can be utilised in a forensic investigation in a variety of ways. Insects are most commonly used to help in the estimation of time since death of a discovered corpse (post-mortem interval, PMI). This estimation is based upon the time taken for insects developing on a corpse to reach the stage present when the body is found. The actual insect species present on a corpse will also indicate the minimum PMI as insects colonise a carcass in a distinct succession [1]. Some insects have defined geographical distributions. Their presence outside of their normal habitat could indicate post-mortem movement of a corpse or link a suspect to a scene of crime.

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All forensic entomology techniques depend upon accurate identification of insect species. Insect species have different developmental lifecycle timings and therefore accurate species identification is required to utilise the correct developmental information. At present this is mainly based upon morphological differences between species [2]. This can be difficult as the early lifecycle stages can be very hard to distinguish, especially if they are badly preserved after collection from the crime scene [3]. Rearing of immatures to the more distinguishable adult forms can be time consuming and requires insects to be collected live.

DNA techniques not only provide a suitable alternative but can also give information on populations [4]. Identification can be carried out without further rearing and on dead, preserved or live samples. DNA techniques are also relatively insensitive to the methods of preservation or age of samples [5]. Previous research work on forensically important Dipteran species has utilised the mitochondrial cytochrome oxidase (COI) gene [6–8].

The aim of this work was to use DNA molecular markers to help in identification of *Calliphora vicina* and *Calliphora vomitoria* fly species and ultimately populations within the UK. The work also considered the use of molecular markers on adult body parts, different immature stages and burnt/decomposing larvae.

2. Methodology

2.1. Samples and DNA extraction

Wild adult specimens were obtained from various locations across England and Wales. Laboratory populations of both *C. vicina* and *C. vomitoria* were used as a source for all other samples used in this work.

DNA was extracted from all *Calliphora* lifecycle stages (including empty pupal cases) along with various adult body parts including single wings, complete legs and femurs. Samples of decomposing larvae killed by incineration or drowning were also included to mimic cases of bodies burnt post-mortem and those submerged after death. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN) according the manufacturer's Tissue protocol. DNA was then quantified using PicoGreen (Molecular Probes) according to the manufacturer's protocol.

2.2. Amplification and sequencing

A region of the mitochondrial COI gene was amplified using primers described by Simon et al. [9], C1-N-2191 (5′ CCCGGTAAAATTAAAATATAAACTTC 3′) and C1-J-1718 (5′ GGAGGATTTGGAAATTGATTAGTTCC 3′). A region of the nuclear gene xanthine dehydrogenase (XDH) was amplified using forward primer (5′TAAGGGCGA ACGTGCTACTT3′) and reverse primer (5′TCAACACGGATTCATATCG3′) designed for this work.

These amplicons were then sequenced using an ABI 310 Genetic Analyser (Applied Biosystems) according to the manufacturer's protocols.

3. Results

This study found that 17 nucleotide polymorphisms were present between the COI regions amplified for *C. vicina* and *C. vomitoria*. These polymorphisms were present in all samples; there was

no intraspecific variation (sequences deposited in GenBank AY536642 and AY536643 for *C.vicina* and *C.vomitoria* respectively).

The XDH regions indicated that intraspecific variation does exist within both species. The genetic distance between *C. vomitoria* populations was correlated to the geographic distance between populations. The markers found in this work however could not distinguish specific *C. vicina* and *C. vomitoria* populations within England. XDH sequences were deposited in GenBank (accession numbers AY944470–AY944504).

DNA of varying quantities was extracted from all samples in this study. Average DNA quantities ranged from less than 30 ng (single wing) to 1520 ng (whole adult). All samples amplified the COI and XDH regions used in this study.

4. Discussion

This work concurs with previous research [6] that species identification can be conducted on any insect evidence presented to an entomologist using DNA molecular markers. This work also included burnt and decomposing larval samples and demonstrated that these can also be submitted for DNA analysis.

The COI region used in this work was sufficient to differentiate between *C. vicina* and *C. vomitoria*. The XDH sequence analysis indicates that intraspecific variation exists within *C. vicina* and *C. vomitoria*. Other markers need to be examined to aid in distinguishing between UK populations.

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