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Research article

STR and SNP analysis of human DNA from *Lucilia sericata* larvae's gut contents

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ABSTRACT

Importance of forensic entomology becomes inevitable when come across some incident where corpse is unidentifiable and lot of maggots or other insects are present. The most common application of forensic entomology is to use insects for the identification of specimens or human remains. DNA analysis recovered from a larva's gut contents can be used to identify a missing body. The obtained human STR and SNP profile support the association of a maggot to a specific patients or corpse. Main aim of this research was the identification of human DNA from gut contents of third instar maggots (larvae of *Lucilia sericata*) placed on diabetic patient's wounds for treatment purpose. Maggots (8–15) were taken from each diabetic patients (no. of the patients 8) and DNA was extracted from the gut contents manually by using Qiagen tissue protocol. Agarose gel electrophoresis was performed and the total size of DNA was seen using UV transilluminator. PCR amplification, STRs and SNPs profiling was then performed using PCR 9700 and AmpFLSTR Identifiler and SnaPshot Multiplex Kit (Applied Biosystems) respectively. The results were analyzed on ABI 310. SNP profiles were good and identifiable compared to the STRs where amplification was poor and the peaks were low. This may be the fact of the enzymatic activity present in the gut of the larvae which cause tremendous reduction in DNA size and thus yield. The results of this study reveals that it is possible to obtain a complete human profile using STRs and SNPs even if DNA is recovered from gut contents of maggots.

1. Introduction

Entomological evidence is generally used to estimate the postmortem interval. The most common application of forensic entomology is to use insects for the identification of specimens or human remains by using molecular techniques like DNA typing. It is possible to extract DNA from the crop of a maggot and thereby determine what it has been feeding on [1]. Crop-content analysis may be useful in helping to identify a missing corpse that has been removed from a crime scene, or answering questions about whether a maggot has fed on multiple food sources [2]. Biochemical alterations during food digestion from the maggot may represent a factor for further degradation, leading to a failure of typing from the crop material. Degradation of DNA may occur even within the crop [3]. In cases where STR typing is not possible due to degraded DNA or too low amounts of target molecules, for example, in telogen or rootless hair, heavily putrefied tissue, bone or faeces [4] the analysis of SNP is an alternative and more sensitive method than STR typing. This strategy is applicable also in case of examination of crop content. Main aim of this research was the identification of human STR and SNP profile from gut contents of third instar maggots (larvae of *Lucilia sericata*).

2. Materials and methods

2.1. Samples collection

Feeding third instar larvae were collected from diabetic patient's wounds for treatment purpose. The maggots were killed by freezing at −20 °C. Exterior DNA contamination eliminated by 20% bleach solution for 2 h after that larvae were washed with sterile distilled water. A saliva sample was obtained from each diabetic patients and served as a DNA reference sample.

2.2. Dissection of maggot

Maggots were dissected under stereomicroscope (Olympus, SZX10). Anterior segments were cut with iris scissors. Crop was put out of the body by a little dorsal pressure and posterior connection to mid gut was cut with a scalpel. Before and after digestion of the gut of third instar maggots shown Fig. 1.

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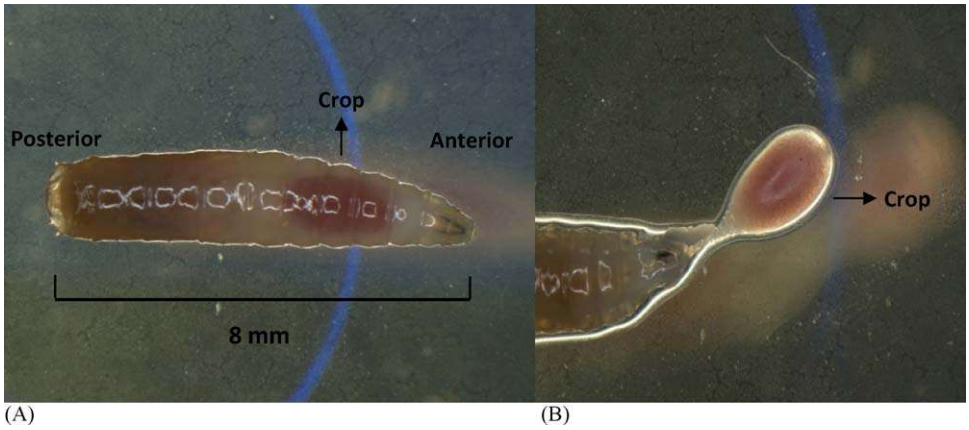

Fig. 1. (A) Before digestion, Crop with third instar *Lucilia sericata* larvae (dorsal view). Magnification: 0.8×, Stereo microscope Olympus SZX10. (B) After digestion, Crop is out of the larvae (dorsal view). Magnification: 3.2×, Stereo microscope Olympus SZX10.

2.3. Human STR and SNP analysis

Gut contents and reference samples DNA extraction was performed by using QiagenTM DNA MiniKit. Extracted DNA samples were visualized after agarose gel electrophoresis and ethidiumbromide-staining under UV light. For STR analyses, extracted DNA samples were amplified according to AmpFℓSTR Identifiler® PCR Kit (Applied Biosystem) and separated by capillary electrophoresis on ABI Prism 310 Genetic Analyzers; results were analyzed by GeneScan 3.7 (Applied Biosystem).

29 SNPs were amplified in one PCR reaction in a GeneAmp PCR System 9700 (Applied Biosystems) according to Sanchez at al. [5]. The final volume of the PCR reaction was 12.5 μl. Exo-SAP was used to remove unincorporated primers and dNTPs. Single base extension reaction was performed with SNaPshot multiplex kit (Applied Biosystems) using specific primers according to Sanchez at al. [5]. Free ddNTPs were removed with SAP. SNP detection was performed in a ABI Prism 310 Genetic Analyzer, results were analyzed by GeneScan 3.7 (Applied Biosystem).

3. Result and discussion

All samples yielded complete genotyping data. In most cases, STR typing of the crop content from third instar maggots was successful. In three samples complete STR profiles were obtained. In three cases incomplete STR profiles (amplification was poor and the peaks were low and/or allelic drop-out) were observed. In two samples STR typing failed may be due to highly degradation of DNA within the gut of the maggot. Reports show clearly though there is not a significant effect of gut enzymes on degradation of DNA yet there may be some effect. Enzymes along with food comes down in pre gut region and thus caused the degradation of DNA. Tying failure may be due to the low amount of DNA obtained after extraction.

SNP typing was performed and genotypes were obtained successfully after amplification from all third instar maggots extracts and from reference sample. STR and SNP profiles obtained from the gut content matched the profile of the corresponding volunteers in all samples. This clearly shows that the samples where the highly degraded samples are present, SNP typing may be useful and can be obtained successfully.

Enzymes are present in the saliva of the maggot for pre-oral digestion and are re-incorporated with the food into the crop [3]. Therefore, degradation of DNA may occur even within the crop, but the extent of degradation may vary and redictions about the time period during which SNP typing can be successfully performed are difficult to evaluate.

From this study, it can be concluded that STR analysis of a maggot's crop content can be used to associate particular maggots to a given corpse of forensic interest. Because crop-content DNA is likely to be degraded, SNP analysis is recommended. This suggests that in many cases SNP can be analyzed, if STR profiles are not obtained.

Conflict of interest

None.

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