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Genotyping of human DNA recovered from mosquitoes found on a crime scene

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Abstract. Many mosquitoes species are man parasitical biters commonly found in the south of Italy by crime scene investigators. Some authors already demonstrated the possibility of amplifying human DNA from blood meals of Diptera species using various methods for epidemiological issues. Here we describe a casework that occurred in Sicily: a person was killed in a room where no traces were found apart from a fresh mosquito blood meal stain. Hence an optimized DNA extraction was carefully carried out in order to yield deoxyribonucleic acid while removing insect-specific molecules. PCR amplification and STRs profiling at 15 human genetic loci was then performed on the extracted DNA, using AmpFLSTR Identifiler (Applied Biosystems). Results showed that it is possible to successfully amplify and to obtain a complete genetic profile even if DNA is recovered from small and biologically contaminated traces. The applied analytical strategy represented a powerful tool for the investigations and allowed to address the profile of the major suspect of the murder. © 2005 Published by Elsevier B.V.

Keywords: DNA STR typing; Forensic casework; Identifiler; Mosquitoes blood meal

1. Introduction

Mosquitoes (Culicidae) found on a crime scene, if properly treated and evaluated with respect to other elements, can heavily contribute to the investigators' efforts. This casework reports the murder of a transsexual prostitute found in the vicinity of a beach in Sicily. The victim's body was lying down on the sand, partially hidden by little bushes and showed evident signs of strangulation around the neck. Police enquiries focused on

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investigators' suspicions on a refined businessman, whose car was noticed in that area the night of the murder. During on-the-spot investigation at the suspect's home, which is located in a far inner area, no relevant biological evidences were found apart from a mosquito blood meal stain on a wall. Moreover police officers confiscated worn clothing presenting very small leaves fragments and a pair of sneakers, which seemed to be minimally spoiled with soil. Our goal was firstly to obtain a 15 loci profile from a poor and biologically contaminated DNA sample consisting of a single blood meal stain. Secondly we aimed to show that unusual kinds of traces may provide a significant assistance in a real investigation, if a meticulous approach to the crime scene and an interdisciplinary data analysis are pursued.

2. Materials and methods

2.1. Samples collection and storage

A mosquito blood meal stain was found on a wall of the crime scene in a dry status. It was firstly adsorbed on a wet filter paper and then gently scratched in order to recover the rest of the blood material. The insect's remains were also collected in a tube for species identification. Few grains of sand were detected in the sole relieves of the sneakers, removed with a light brush and stored into a plastic vial at room temperature.

2.2. DNA extraction and filtration

In order to obtain the best quality and yield, DNA extraction was performed by QIAmp Blood and Tissue Kit extraction protocol [1], adjusted for a 15 μl final volume. Extracted DNA samples were then filtered through Microcon™ (Amicon, Millipore) YM-30 and YM-100. DNA extraction from a reference *Culex pipiens* specimen was carried out by QIAmp Blood and Tissue Kit extraction system (QIAGEN).

2.3. Amplification and STR typing

A multiplex PCR was performed according to the AmpFlSTR Identifiler® (Applied Biosystem) manual [2] supplied by the manufacturer. An additional control, containing the reference insect's DNA, was included among samples amplified by AmpFlSTR Identifiler® kit. Each reaction was carried out by Gene Amp 9700 and 2400 thermal-cyclers (Applied Biosystem). PCR products were separated on a polymer substrate by capillary electrophoresis with 3100 AB Prism Genetic Analyzer; results analysis and automatic allele designation were performed by using the Genemapper v. 3.2. DNA extracted from reference *C. pipiens* specimen was separated by electrophoresis gel and stained with ethidium bromide (data not shown).

2.4. Sand analysis

The sand sampled during investigation was subjected to X-ray microanalysis by using a CS-24 CamScan™ Scanning Electron Microscope, coupled with both a backscattered electron and a Si–Li detector, equipped with a beryllium Super Ultra Thin Window (SUTW).

3. Results

We obtained an almost complete 15 loci profile either from the blood on filter paper or from the blood debris obtained by scratching the stain on the wall. Contaminant insect's DNA and molecules did not interfere with STR amplification nor were unspecific products yielded. Indeed the amplification of the reference insect's DNA, which had been previously detected by a smear on agarose gel, failed completely when performed by the AmpFlSTR Identifiler® kit. This way we confirmed that each signal was ascribable to a given human STR amplicon and not to unspecific amplification of alien DNA. The yielded profile fully matched the victim's one, ascertaining the presence of the latter at least in the vicinity of the suspect's home. Entomological examination attributed the mosquito to the *C. pipiens* complex, whose members are not supposed to cover, under normal condition, the distance between the beach and the suspect's home [3]. Electron microscopy and chemical X-ray microanalysis of the seized sand demonstrated clearly that the grains found under the suspect's sneakers were consistent with the ones sampled at the beach. Leave fragments resulted to belong to *Calendula maritima* (Asteraceae), whose bushes were hiding the victim's body. As regards to that given region, this plant is not reported to flourish in medium other than sandy soil.

4. Discussion

In our experience human blood DNA was typed from a smashed mosquito found on the crime scene. Few papers already showed the suitability of such DNA source for amplification and genotyping [4,5]. None of the above mentioned studies reported the use of high discriminating multiplex STR kit on a single dried blood meal. Authors were fully aware that DNA profile alone would have not allowed police officers to charge the suspect since a mosquito is still capable to move across very long distances if accidentally carried by means of transport and no clues excluded the presence of the victim in the suspect's home area. Therefore the sand and the leave fragments were submitted to specialized laboratories for further analysis. Once again geological, petrographical and botanical data alone were not decisive, whereas the cross-control with related surveys on the territory ascribed the coexistence soil/plant only to the beach where the body had been found. The chances of two independent accidental contaminations, outside the sources' ecosystem, could not be neglected. Indeed all of the aforesaid occurrences alone could not have served as an incontrovertible link between the suspect and the murder; nevertheless the extreme rareness of such joined events coupled with the compliance between police enquiry hypothesis and considerable scientific data has lead the jury toward a conviction for second degree murder.

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