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# Human DNA extraction from larvae: a brief review of the literature

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ABSTRACT

The analysis of the insects present on the corpses is a new frontier of Forensic Entomology Sciences useful for medico-legal evaluation, in order to extract human DNA and facilitate the estimation of the post-mortem interval.

Starting from a case of an unidentified and mummified body, colonized by insects at different developmental stages, we searched in the Literature the procedures of extraction and analysis of human DNA from the larvae. Our analysis found no trace of human genetic material in larvae's puparia and crops.

This case report adds to the scarce literature available on the human DNA extraction from insects and highlights the analytical challenge of genetic analysis related to post-mortem tissue degradation.

**Keywords:** *Forensic entomology, larvae extraction, mummified corpse, genetic analysis*

## 1. Introduction

It is reported in literature that the genetic analysis of human DNA extracted from larvae, puparia and adult insects, found on human corpses, could provide important medico-legal information about the estimation of the post-mortem interval (PMI), in particular when the time of death is beyond 72 h [1-2].

Holometabolous insects, such as Diptera, perform a complete metamorphosis based on three stages: larvae, pupas and adult flies. During the first phase, after the hatching of the eggs, the maggots eat decomposing organic material and mature through a series of changes (feeding stage), but, before starting the formation of the pupal-cage, the maggots reduce their metabolism, stop eat and, in some cases, move away from the corpse (post-feeding stage). For this reason, it is common to find insects on the corpse, at any stage of development, always considering the many factors that influence the rate of colonization and the composition of the insects, such as for example temperature, environment, clothes and cause of death [3].

In many cases, morphological and environmental analysis is the first approach of entomologic evaluation [4].

Recent studies have shown that the analysis of intestinal contents of insects and flies that feed carrion has a genetic potential to be used in forensic sciences [1-2, 5]. In particular, they demonstrated that, after ingestion of human tissue, during the digestion process, the hydrolysed host tissues are normally stored in the maggot's crop. Therefore, it is possible to sample the host tissue residues from the crop, subject it to STR analysis and generate a genetic profile for the identification of an unknown body.

After a forensic investigation performed on a "mummified" human body and the subsequent genetic analysis performed on larvae puparia and crop, without success [6], we decided to perform a review of the literature about the forensic genetic entomology in order to highlight the conclusions of other researches.

## 2. Material and Methods

We have searched articles in Pubmed and Scopus databases, with the keywords "Human DNA extraction" matched at "Larvae", "Puparia", "Crop", "Human DNA" matched at "Larvae", "Puparia", "Crop" and "Human DNA" AND "Extraction" matched at "Larvae", "Puparia", "Crop".

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We included those studies that evaluated the use of the intestinal contents of insects and flies at different stages as carrier of human genetic material. We excluded studies regarding other use of insects for genetic purposes different from human DNA identification or non-English language articles.

Our research was not limited by chronological parameters. The articles were selected based on the review of their titles and abstracts. In addition to a critical review of each abstract, an evaluation of the full text was made in the case of articles whose summary was not conclusive.

We identified 25 articles, of which 12 were finally included in the review and summarized in Table 1 distinguishing them by: author, year of publication, type of study (case-work vs experimental study), utilized sources, remarks.

## 3. Discussion

Entomology has become a promising part of forensic sciences able to provide essential informations and, in future, through a more in-depth study of insects, it will be a useful tool to obtain new crucial elements in the global forensic evaluation of real caseworks.

In particular, the study of the insects is important for toxicological and genetic researches.

Indeed, the material contained in the digestive system of larvae and flies can be a source from which to derive genetic profiles and or the presence of drugs [1-2, 7].

After ingestion of human tissue, the digestion process causes the hydrolysis of the host tissues stored in maggots' crop. Therefore, it is possible to sample the host tissue residues from the crop and perform a short tandem repeats (STR) analysis to generate a genetic profile, comparable with the profile of the corpse or hypothetic relatives.

In our knowledge, there are just few studies about human DNA analysis sampled from larvae's puparia and crop.

Zehner et al. first tried to perform a STR typing and HVR amplifications using the crop contents of maggots collected from 13 corpses after various postmortem intervals. In seven cases, a complete STR profile was established, in two cases, an incomplete set of alleles was obtained, and in four cases, STR typing was not successful. The obtained human STR profiles supported the association of a maggot to a specific corpse. The time of storage of the maggots and the length of the post-mortem interval up to 16 weeks appeared to have no particular influence on the quality of the results. [8].

Similarly, Linville et al. dissected maggots after 2 weeks, 8 weeks and 6 months of preservation. They were able to amplify mtDNA (mitochondrial DNA) and STRs from maggots stored in ethanol or without any preservation fluid. Each control maggot produced a complete HVII haplotype and STR profile. Both the mtDNA haplotype and STR genotype matched those of the maggot's food source (human spleen). [9].

Differently, Carvalho et al. considered the potential to detect the ingested human DNA from immature stages of Calliphora dubia which had fed on sheep liver. They detected the host DNA by day 2, even if the crop was visually empty, while from day 3 the material was no longer detectable as it was eliminated, reduced to pieces below 87 bp, or was perhaps present in such a low number of copies that it couldn't be detected by PCR [10].

An Italian group headed by Di Luise made a comparison between different specimen preservation and DNA extraction strategies from the crop of third instar maggots (larvae of Calliphoridae) recovered from a cadaver in decay stage of decomposition with the aim of obtaining autosomal and Y-STR profiles. They observed that ethanol-based preservation dramatically decreased the quantity of typeable human DNA whereas preservation by simple refrigeration produced the best results. None of the batches conserve at room temperature, both in ethanol and dry condition, yielded useful results. Furthermore, extraction methods based on the use of silica columns (i.e. QiagenTM DNA MicroKit) showed the higher DNA yield and purity. DNA IQTM system resulted in useful profiles although a great degree of variation between samples. ChelexTM system followed by filter purification resulted in useful profile only for specimen stored in dry condition [11].

Similarly, Gulden Onur Kondakci et al. tried to identify human DNA from gut contents of third instar maggot (larvae of Lucilia sericata) placed on diabetic patient's wound for treatment purpose. In three samples complete STR profiles were obtained. In three cases incomplete STR profiles were observed. In two samples STR typing failed may be due to highly degradation of DNA within the gut of the maggot. SNP typing was performed and genotypes were obtained successfully after amplification from all third instar maggots extracts and from reference sample, so they concluded that if STR profiles are not obtained, because of crop-content DNA degradation, SNP analysis should be recommended [12].

Also Xi Li et al. showed that the mtDNA and STR analysis of maggot crop contents may potentially be used to associate the maggots with human corpse, even if physical contact between the maggots and corpse is not observed [13].

Afterwards, De Lourdes Chávez-Briones et al. obtained complete STR profiles from groups of 20 third-instar larvae of Calliphoridae albiceps, left in bovine ground meat and human blood for a period of 48 h, even after 2 months of storage in 70% ethanol. They concluded confirming that ethanol is a useful preservative for tissue that has to be analysed for DNA [14].

Oliveira at al. left a group of 20 third-instar larvae of Calliphoridae albiceps in bovine ground meat and human blood for a period of 48 h to ensure higher levels of larval activity with the same diet. Their results showed complete profiles of human STRs for a short period during degradation of the material, concluding that within the first 48 h of death, full-DNA profiles can be obtained from larvae [15].

Similarly, Njau et al. studied the period in which it is

possible to obtain successfully human DNA, using STR analysis, from third instar maggots of Protophormia terraenovae present on decomposing human corpses. In particular, they investigated the degradation and disappearance times of human DNA in the larvae's crop after their removal from the corpse and/or a feeding phase with different food source (for example beef meat). Results showed that the amount of human DNA recovered from maggots decreased with time in all cases. For maggots fed on beef, the human DNA could only be recovered up to day two and up to day four for the starved maggots [16].

Powers et al. observed that human DNA profile could be obtained from second and third instar life stages, as well as pupal and casing samples, of the forensically relevant blowfly species Calliphora augur and Calliphora stygia that have consumed human semen. In particular, the results of this study indicate that the second and third instar, as well as the pupal life stages, would be most pertinent samples to collect at a crime scene where a sexual assault is suspected, and conventional sources of genetic material are not suitable [17].

Mukherjee et al. identified two different preservation techniques (preservation by freezing at -20 °C and preservation in Ethanol (98%)) as optimal to extract non-insect DNA from the gut contents of III instars Megaselia scalaris larvae as they not only aid the process of dissection but do not interfere with the molecular analysis. Despite these fixing methods have been proven to be better in terms of ease of dissection and in the amount of DNA yield per crop, the preservation of some morphological features useful for PMI estimation (e.g. length) is not guaranteed, so the authors strongly recommend collecting enough specimens in order to avoid the risk to lack of sufficient material to perform both the analyses as above mentioned if requested by the Court [18].

Finally, our group has recently published a study on DNA extraction from corps and puparia of Diptera and Hymenoptera's larvae recovered on a mummified unidentified human body in order to obtain a valid genetic profile. We used two different methods: the first one was the procedure reported in Marchetti et al.[19] and in Skowronek et al.[5], the second one is the one suggested by Campos et al. [20]. None of the two techniques used gave a genetic profile, not even a pattern attributable to a degraded DNA. The hypothesis of those negative results is that the process of digestion and degradation of ingested host tissues, already very compromised by the processes of putrefaction-mummification, occurs more quickly within the digestive path of the larva, reducing the time in which it is possible to derive human DNA from the larvae's crops [6].

## 4. Concluding Remarks

Forensic entomology could have a key-role in pursuing justice. It could provide a huge amount of information that can be helpful for the investigators to place someone at the scene of a crime by a more accurately determination of the time of death, the location, how long a body has been in a specific area, if it has been moved, and other important factors.

In homicides with entomological evidence, it may be important to prove the presumed association of fly larvae to a corpse, especially if it is in doubt whether all maggots used for entomological expertise developed and fed on it.

Most recently, casework and simulated studies based on short tandem repeat (STR) analysis of DNA extracted from the gut contents of larval blow flies have demonstrated that blow flies can provide molecular evidence for the identification of both victims and criminals.

The most significant limitation of the majority of the studies we presented is that most of them are conducted under experimental conditions, placing in contact specific insects with specific biological tissues in standardized experiments and only few of them focused on the evaluation of larvae directly taken from a human corpse in a forensic context. The consequence is that these protocols are often difficult to apply to real cases.

Furthermore, few studies are systematic in evaluating results over time: it seems that the effect of time is a very critical parameter in the validity of the results obtained. In the medical-legal field we often confront with unknown body badly decomposed exposed for a long time (more than the 6 weeks studied in the experimental studies presented) and to different environmental agents. In these cases, any further information that can be obtained from the body, the scene or the fauna that colonizes the body may be crucial.

Therefore in future, for the reasons explained above, further studies should necessary focus on the times of the digestive phase of the larvae in order to: characterize a ratio of time vs quantity of ingested tissue and identify the time necessary for complete DNA degradation within the larval digestive pathway, related to different special post mortal conditions in order to improve our knowledge in this context.

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| AUTHOR | YEAR | TYPE OF STUDY | SOURCES OF STUDIES | REMARKS |
|---|---|---|---|---|
| Zehner et al. [8] | 2004 | Case-work | Maggots of Calliphoridae were collected from 13 corpses after various post-mortem intervals. | In seven cases, a complete STR profile was established, in two cases, an incomplete set of alleles was obtained, and in four cases, STR typing was not successful. HVR analysis was successful in all cases except one. The time of storage of the maggots and the length of the post-mortem interval up to 16 weeks appeared to have no influence on the quality of the results. |
| Linville et al. [9] | 2004 | Experimental study | Maggots of Calliphora vicina collected from a human spleen at room temperature under a 24 h light source were dissected following 2 weeks, 8 weeks and 6 months of preservation. | Each control maggot produced a complete HVII haplotype and STR profile. Both the mtDNA haplotype and STR genotype matched those of the maggot's food source (human spleen). |
| Carvalho et al [10] | 2005 | Experimental study | Immature stages of the bluebodied blowfly Calliphora dubia (Macquart) that had fed on sheep liver. | This study suggests that the crop, although visually empty, still contains food materials. However, by day 3, the material has either been eliminated, degraded to pieces smaller than 87 bp, or is perhaps present in such low copy number by this stage that the PCR is not sensitive enough to detect it. This study shows that ingested DNA can be detected in the immature stages of C. dubia until the second day of pupal development |
| Di Luise et al. [11] | 2008 | Case-work | Comparison between different specimen preservation and DNA extraction strategies from the crop of third instar maggots (larvae of Calliphoridae) recovered from a cadaver in decay stage of decomposition. | Ethanol based preservation dramatically decreased the quantity of typeable human DNA whereas preservation by simple refrigeration produced the best results. Boiling did not seem to affect DNA recovery while the presence of thawed water into the tube increased spoiling and degradation of the crops. None of the batches conserve at room temperature, both in ethanol and dry condition, yielded useful results. |
| Kondakci et al. [12] | 2009 | Experimental study | Identification of human DNA from gut contents of third instar maggot of Lucilia sericata placed on diabetic patient's wound for treatment purpose. | 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. 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. |
| Xi Li et al. [13] | 2011 | Case-work | Third instar maggots of Aldrichina grahami were collected from a male headless corpse and a skull. | This study showed that the mtDNA and STR analysis of maggot crop contents may potentially be used to associate the maggots with human corpse, even if physical contact between the maggots and corpse (or even two different parts of corpse) is not observed. |
| de Lourdes Chávez -Briones et al. [14] | 2013 | Case-work | Three maggots of Calliphoridae and Sarcophagidae were collected from a badly burned body Several attempts to obtain a genetic profile from the fragment of liver recovered at autopsy were unsuccessful. | STR profiles obtained from the maggots were incomplete. However, the number of loci successfully amplified was sufficient to perform a comparative DNA test against the alleged father, which was adequate for conclusive identification of their mains. However, complete STR profiles could be obtained from maggots Even after 2 months of storage in 70% ethanol confirming the fact that ethanol is a useful preservative for tissue that has to be analysed for DNA. Thus, it is possible that the quality of DNA extracted from maggots was in function of the state of decomposition of their mains. |
| Oliveira et al. [15] | 2016 | Experimental study | Groups of 20 third-instar larvae of Chrysomya albicenps left in bovine ground meat and human blood for a period of 48 h. | Extraction techniques were successful in obtaining human autosomal DNA from the larvae that was compatible with a reference sample, generating full profiles that matched the reference buccal swab mouth sample. The results show complete profiles of human STRs and this only occurs for a short period during degradation of the material, typically within 48 h. This means that, within the first 48 h of death, full-DNA profiles can be obtained from larvae. |
| Njau et al. [16] | 2016 | Case-work | Third instar maggots of Protophormia terraenovae obtained from three different decomposing human corpses | Results showed that the amount of human DNA recovered from maggots decreased with time in all cases. For maggots fed on beef, the human DNA could only be recovered up to day two and up to day four for the starved maggots. |
| Powers et al. [17] | 2019 | Experimental study | Samples were taken from adult and juvenile blowflies of Calliphora augur and Calliphora stygia that had consumed human semen. | Samples taken from adult and juvenile blowflies that had consumed semen were able to generate functional profiles from second and third instar life stages, as well as pupal and casing samples. The results of this study indicate that the second and third instar, as well as the pupal life stages, would be most pertinent to collect at a crime scene where a sexual assault is suspected, and conventional sources of genetic material are not available. |
| Mukherjee et al. [18] | 2019 | Experimental study | Gut contents of III instars M. scalaris larvae fed on Sus scrofa Linneaus 1758 and Bos Taurus Linneaus 1758 tissues. | This study identifies 2 preservation techniques (preservation by freezing at -20 °C and preservation in EtOH (98%)) as optimal for this kind of analysis as they not only aid the process of dissection but do not interfere with the molecular analysis. The preservation of some morphological features useful for PMI estimation (e.g. length) is not guaranteed. |
| Sanavio et at. [6] | 2019 | Case-work | DNA extraction from corps and puparia of Diptera and Hymenoptera's larvae recovered on a mummified human body in order to obtain a genetic profile and identify the unknown body. | None of the two techniques gave a genetic profile, not even a pattern attributable to a degraded DNA. The hypothesis of those negative results is that the process of digestion and degradation of ingested host tissues, already very compromised by the processes of putrefaction-mummification, occurs more quickly within the digestive path of the larva, reducing the time in which it is possible to derive human DNA from the larvae's crops. |