

Mitochondrial DNA and STR analyses for human DNA from maggots crop contents: A forensic entomology case from central-southern China

Li, X.¹, Cai, J.F.^{1*}, Guo, Y.D.¹, Xiong, F.¹, Zhang, L.¹, Feng, H.², Meng, F.M.¹, Fu, Y.³, Li, J.B.³ and Chen, Y.Q.⁴

¹ Department of Forensic Science, School of Basic Medical Sciences, Central South University, Changsha 410013, Hunan, China

² The Beijing Public Security Bureau, Beijing 100192, China

³ The Changsha Public Security Bureau in Hunan, Changsha 410008, Hunan, China

⁴ Department of Forensic Science, Hunan Police Academy, Changsha 410013, Hunan, China

* Corresponding author email: cjf_jifeng@163.com

Received 14 January 2011; received in revised form 26 February 2011; accepted 5 March 2011

Abstract. Insect larvae and adult insects found on human corpses can provide important forensic evidence however it is useful to be able to prove evidence of association. Without this, it could be claimed that the insect evidence was a contaminant or had been planted on the body. This paper describes how mitochondrial DNA (mtDNA) and STR analysis of the crop contents of larvae of the blowfly *Aldrichina grahami* collected from separated body parts was used to provide evidence of association.

INTRODUCTION

Insect larvae and adult insects found on human corpses provide important clues for the estimation of the postmortem interval (PMI) and judgment the identity of individuals (Wells & LaMotte, 2001). Because fly eggs are usually deposited on a corpse before death, determining the age of immature insect specimens collected from a corpse can be an accurate way to analyse the forensic cases. (Linville *et al.*, 2004). Maggots are commonly used to estimate the period of insect activity based on aging the oldest insect specimen associated with the corpse (Goff, 1993; Wells & LaMotte, 2001). In certain cases, when maggots but no corpses are found, the association between the specific maggots and the particular corpse is exceptionally important (Zehner *et al.*, 2004a).

The DNA analysis of insects has the advantage of being applicable to any life

stages of insects, including the immature specimens (Zehner *et al.*, 2004b). For the identification of humans, mitochondrial DNA analysis is useful because the high copy number within cells means that it can be employed when the tissues are degraded. (Holland & Parsons, 1999; Morley *et al.*, 1999). Within the blowfly larva crop the liquefied food is temporarily stored. Primary digestion does not occur in the crop because proteolytic enzymes are not secreted into this area (Campobasso *et al.*, 2005).

Inferring close relationship between two individuals or human bodies typically relies on the same sort of loci such as microsatellites (Wells & Stevens, 2008). In previous studies, STR typing (Butler, 2001) and two particularly hyper-variable regions (HVI and HVII) within the non-coding control region or D-loop (Lutz *et al.*, 1998) were used to identify human tissue, and for insects it was the 16S rDNA (Li *et al.*, 2010). In this study, mtDNA (HVII) and STR typing were

applied for the analyzing of human tissue, and 16S rDNA gene was picked for the identification of insects. The crop contents of maggots collected from human corpses was subjected to mtDNA and STR genetic analysis.

CASE DESCRIPTION

In March 2008, a male headless corpse (Body I) dressed only in underwear was discovered in a garden in the city of Changsha (28.23°N, 112.94°E) Figure 1A. Without the relative certification, the identity of the corpse was difficult to identify. Soon after a separated skull (Body II), which was wrapped in clothes, was found 500m away from the former scene, and the identity of the separated skull was certified soon Figure 1B. On these two bodies, a large number of nearly full-grown maggots were discovered. Whether these two bodies were from the same victim was difficult to judge because of their state of decay. The daily average temperature record of the region was 14°C and the relative humidity in the recent weeks was 70%. These data were obtained at the website of the China Meteorological Administration (CMA).

MATERIAL AND METHODS

Maggot samples

Third instar maggots were collected from the Body I and Body II during autopsy, and killed in boiling water and preserved in 1.5 mL tubes separately at -70°C without any preservation fluid before the analysis (Linville *et al.*, 2004). Tissue samples (human costal cartilage) from the two bodies were also collected and stored at -70°C prior to examination.

Wash method and maggot dissection

In order to remove the potential external contaminants, each of the maggots was soaked in 20% bleach solution before dissection (Linville & Wells, 2002). Then the bleach solution was removed and each maggot was rinsed twice with 1 mL distilled water (Linville *et al.*, 2004). The dissection

process was followed by the method described by Linville & Wells (2002). The remainder of each maggot was preserved for molecular identification of blowfly species.

DNA extraction and amplification

For the DNA samples produced from the human tissue samples and crops of maggots that fed on Body I and Body II, the segment of the HVII was amplified using primers L48/H408 (Wilson *et al.*, 1995). Besides the segment of 16S rDNA was produced for the insect species identification. The detailed extraction and amplification processes were based on Li *et al.* (2010).

STR Analysis

STR analysis was performed on all extractions of crop of maggots on Body I and Body II and human liver. Samples were amplified using Applied Biosystems Identifiler System according to the manufacturer's protocol. Amplified fragments were separated using an Applied Biosystems' 3130XL Genetic Analyzer (Foster City, CA). Data was analyzed using Genemapper ID-3.2 Software (Applied Biosystems).

RESULTS

Insect identification

The maggots were collected from the corpse during autopsy. The third instar larvae measured from 1.1cm to 1.6cm (Figure 1C), and the crop could be observed as a dark colored object, as seen from the exterior part (Figure 1D). All the third instar larvae and pupas were identified as *Aldrichina grahami* using morphological keys (Wang *et al.*, 2002a,b; Zhao *et al.*, 2010). The molecular method using 16S rDNA fragment confirmed the above morphological identification results.

Human and non-human mtDNA

The fragment of HVII was successfully amplified for the human tissue obtained from the Body I, Body II and crop of maggots, and the agarose-gel electrophoresis gel yield of PCR products are shown in Figure 2.

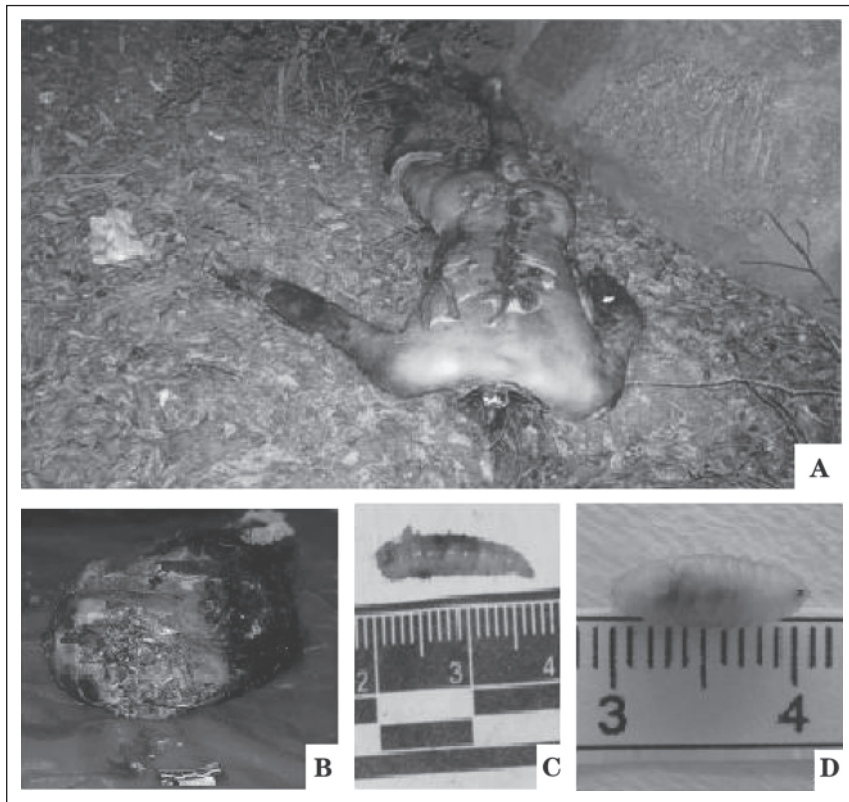


Figure 1. (A) The male headless corpse (Body I) and the discovery site; (B) The separated skull (Body II). Maggots were found on the skull; (C) The obtained third instar larvae and the length. (Length values were varied from 1.1cm-1.6cm); (D) The observed crop in a dark color at the exterior part of the maggot

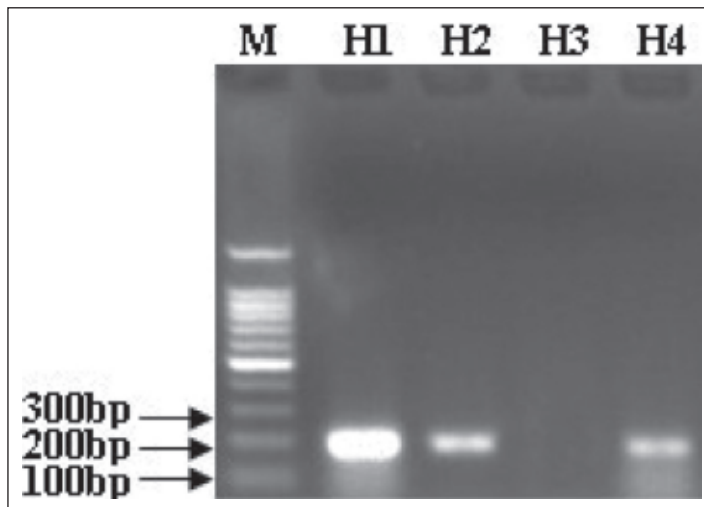


Figure 2. The yield agarose-gel electrophoresis gel of PCR products. (M: marker, H1: Body I, H2: maggot crop, H3: maggot remains, H4: Body II.)

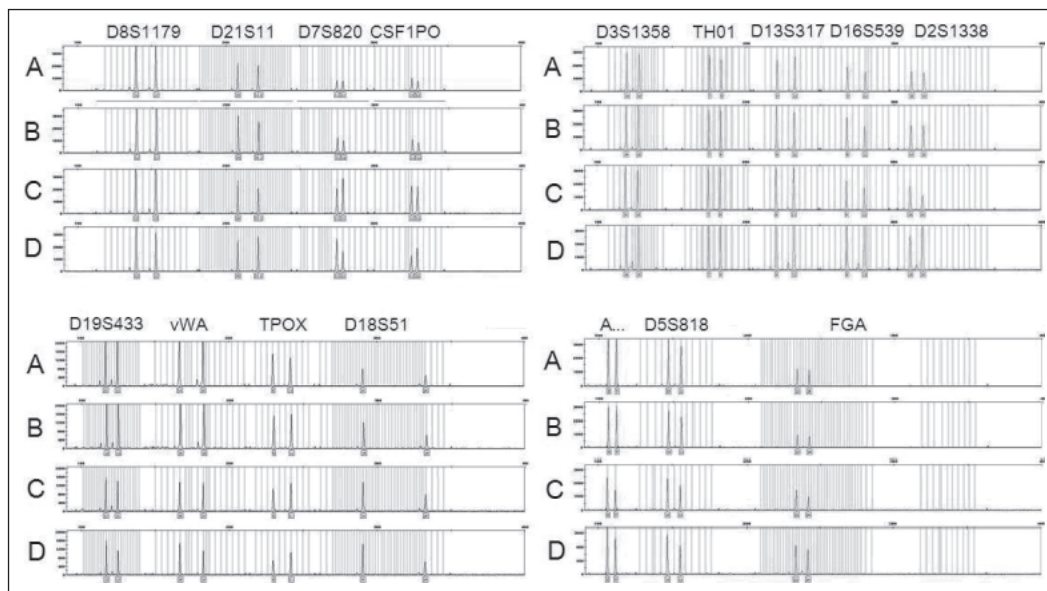


Figure 3. The complete STR profiles (16 loci). A: maggot obtained from Body I; B: maggot obtained from Body II; C: Body I; D: Body II.

STR analysis

STR typing of the human tissue and the crop content from maggots was successful, and the complete STR profiles (16 loci) were obtained for all the specimens. The STR profiles obtained from the human tissue matched each other, and the profiles obtained from the crop of maggots were identical and matched the ones from the human tissues completely (Figure 3).

DISCUSSION

A considerable amount of research effort has been focused on the forensic value of the molecular genotyping methods for identifying specimen, including the identification of the characterization of the population genetic structure of forensically important insect species and insect gut contents (Wells & Stevens, 2008). Previous studies had demonstrated the potential use of the genetic analysis of insect gut contents, including those of the mosquito (Mukabana *et al.*, 2002), lice (Mumcuoglu *et al.*, 2004) and carrion ies (Zehner *et al.*, 2004a). The carrion y maggots gut contents proved to be suitable for all of the typical human identity

genetic procedures (Wells *et al.*, 2001; Linville *et al.*, 2004; Zehner *et al.*, 2004a; Wells & Stevens, 2008), as the gut contents present similar material for associating its last food (Wells *et al.*, 2001).

From this study, it is 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.

In the analysis, the 16 loci in STR analysis were identical for Body I and Body II. Moreover it showed that the maggots were feeding on certain body and the possibility that the maggots were from other places was excluded, which was ensured by the accordance among the HVII sequences extracted from the crop and human tissues. Therefore the accuracy and possibility of estimation PMI relying on these maggots were ensured. The previous study has also demonstrated the usefulness of mtDNA (Wells & LaMotte, 2001; Guo *et al.*, 2010a; Guo *et al.*, 2010b) and STR (Zehner *et al.*, 2004a) technology in the maggot crop analysis. Besides, some researchers (Hopwood *et al.*, 1995; Lutz *et al.*, 1996; Hellmann *et al.*, 2001;

Zehner *et al.*, 2004a) discussed the reason for failure of STR analysis, to degraded DNA or too low amounts of target molecules including in telogen or rootless hair, faeces or bones, and they advised the analysis of human mtDNA as the alternative method. In this study, both the analysis of mtDNA and STR were included to ensure the accuracy of the results.

In this study, only *A. grahami* were observed and collected. *Aldrichina grahami* is a necrophagous insect since the larvae usually feed on putrid animal corpse (Shinonaga, 1965). It is widely distributed in China (except Xinjiang and some alpine areas), and is one of the most dominant species in spring and summer (Ma *et al.*, 2000; Chen *et al.*, 2003).

In conclusion, this study demonstrated that it is possible to prove the association of maggots with specific human remains.

Acknowledgements. This study was funded by National Natural Science Foundation of China (NSFC, No. 30672354), the Graduate degree thesis Innovation Foundation of Central South University and National university student innovation test plan (NMOE:YC09139, YC10107, YC10117).

REFERENCES

- Butler, J.M. (2001). Biology and technology behind STR markers. In: *Forensic DNA Typing*. San Diego: Elsevier Academic Press. pp.335.
- Campobasso, C.P., Linville, J.G., Wells, J.D. & Introna, F. (2005). Forensic Genetic Analysis of Insect Gut Contents. *The American Journal of Forensic Medicine and Pathology* **26**: 161-165.
- Chen, L.S., He, Y.X., Chen, J.G., Wang, P., Liu, J.Y., Qi, X., Li, J.P., Chen, L., Tian, J.B., Wu, Z.K. & Wang, L.P. (2003). Investigation of the sarcophagous fly in Guizhou Province. *Chinese Journal of Forensic Medicine* **18**: 269-272.
- Guo, Y.D., Cai, J.F., Wang, X.H., Lan, L.M., Liu, Q.L., Li, X., Chang, Y.F., Ming, Z., Wang, X. & Wen, J.F. (2010a). Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) based on COI gene in China. *Romanian Journal of Legal Medicine* **18**: 217-224.
- Guo, Y.D., Cai, J.F., Li, X., Xiong, F., Su, R.N., Chen, F.L., Liu, Q.L., Wang, X.H., Chang, Y.F., Zhong, M., Wang, X. & Wen, J.F. (2010b). Identification of the forensically important sarcophagid flies *Boerttcherisca peregrina*, *Parasarcophaga albiceps* and *Parasarcophaga dux* (Diptera: Sarcophagidae) based on COII gene in China. *Tropical Biomedicine* **27**: 451-460.
- Goff, M.L. (1993). Estimation of postmortem interval using arthropod development and successional patterns. *Forensic Science Review* **5**: 81-94.
- Hellmann, A., Rohleder, U., Schmitter, H. & Wittig, M. (2001). STR typing of human telogen hairs-a new approach. *International Journal of Legal Medicine* **114**: 269-273.
- Holland, M.M. & Parsons, T.J. (1999). Mitochondrial DNA sequence analysis: validation and use for forensic casework. *Forensic Science Review* **11**: 21-50.
- Hopwood, A.J., Mannucci, A. & Sullivan, K.M. (1995). DNA typing from human faeces. *International Journal of Legal Medicine* **108**: 237-243.
- Linville, J.G. & Wells, J.D. (2002). Surface Sterilization of a Maggot Using Bleach Does Not Interfere with Mitochondrial DNA Analysis of Crop Contents. *Journal of Forensic Sciences* **47**: 1055-1059.
- Linville, J.G., Hayes, J. & Wells, J.D. (2004). Mitochondrial DNA and STR analyses of maggot crop contents: Effect of specimen preservation technique. *Journal of Forensic Sciences* **49**: 341-344.
- Li, X., Cai, J.F., Guo, Y.D., Wu, K.L., Wang, J.F., Liu, Q.L., Wang, X.H., Chang, Y.F., Yang, L., Lan, L.M., Zhong, M., Wang, X., Song, C., Liu, Y., Li, J.B. & Dai, Z.H. (2010). The availability of 16S rRNA for the identification of forensically important flies (Diptera: Muscidae) in China. *Tropical Biomedicine* **27**: 155-166.
- Lutz, S., Weisser, H.J., Heizmann, J. & Pollak, S. (1996). mtDNA as a tool for identification of human remains.

- Identification using mtDNA. *International Journal of Legal Medicine* **109**: 205-209.
- Lutz, S., Weisser, H.J., Heizmann, J. & Pollak, S. (1998). Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany. *International Journal of Legal Medicine* **111**: 67-77.
- Ma, Y.K., Hu, C. & Min, J.X. (2000). A preliminary study on the constitution and succession of insect community on pig carcass in Hangzhou District. *Acta Entomologica Sinica* **43**: 388-393.
- Morley, J.M., Bark, J.E., Evans, C.E., Perry, J.G., Hewitt, C.A. & Tully, G. (1999). Validation of mitochondrial DNA minisequencing for forensic casework. *International Journal of Legal Medicine* **112**: 241-248.
- Mukabana, W.R., Takken, W., Seda, P., Killeen, G.F., Hawley, W.A. & Knols, B.G. (2002). Extent of digestion affects the success of amplifying human DNA from blood meal of *Anopheles gambiae* (Diptera: Culicidae). *Bulletin Entomological Research* **92**: 233-239.
- Mumcuoglu, K.Y., Gallili, N., Reshef, A., Brauner, P. & Grant, H. (2004). Use of human lice in forensic entomology. *Journal of Medical Entomology* **41**: 803-806.
- Shinonaga, S. (1965). The altitudinal distribution of flies on Mt. Fuji in summer season. *Japanese Journal of Sanitary Zoology* **16**: 263-269.
- Wang, J.F., Hu, C., Hen, Y.C., Min, J.X. & Li, J.T. (2002a). Chronometrical morphology of *Aldrichina grahamae* and its application in the determination of postmortem interval. *Acta Entomologica Sinica* **45**: 265-270.
- Wang, J.F., Hu, C., Chen, Y.C., Min, J.X., Li, J.T. (2002b). Application of the pupal morphogenesis of *Aldrichina grahamae* (Aldrich) to the deduction of postmortem interval. *Acta Entomologica Sinica* **45**: 696-699.
- Wells, J.D. & LaMotte, L.R. (2001). Estimating the postmortem interval. In: *Forensic entomology – the utility of arthropods in legal investigations*. Byrd, J.H. & Castner, J.L. (editors). Boca Raton: CRC Press, pp.263-285.
- Wells, J.D., Introna, F.Jr., Di Vella, G., Campobasso, C.P., Hayes, J. & Sperling, F.A. (2001). Human and insect mitochondrial DNA analysis from maggots. *Journal of Forensic Sciences* **46**: 685-687.
- Wells, J.D. & Stevens, J.R. (2008). Application of DNA-based methods in forensic entomology. *Annual Review of Entomology* **53**:103-120.
- Wilson, M.R., DiZinno, J.A., Polanskey, D., Replegle, J. & Budowle, B. (1995). Validation of mitochondrial DNA sequencing for forensic casework analysis. *International Journal of Legal Medicine* **108**: 68-74.
- Zehner, R., Amendt, J. & Krettek, R. (2004a). STR Typing of Human DNA from Fly Larvae Fed on Decomposing Bodies. *Journal of Forensic Sciences* **49**: 337-340.
- Zehner, R., Amendt, J., Schütt, S., Sauer, J., Krettek, R. & Povolný, D. (2004b). Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). *International Journal of Legal Medicine* **118**: 245-247.
- Zhao, B., Wang, H., Wang, L., Wang, W.J. & Li, Z.M. (2010). Chronometrically morphological change of larvae of two species of necrophagous flies and its implication in forensic medicine. *Chinese Bulletin of Entomology* **47**: 360-367.