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Application of Aldrichina grahami (Diptera, Calliphoridae) for forensic investigation in central-south China

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Abstract: Insect larvae and adult insects found on human corpses provide important clues for the estimation of the postmortem interval (PMI). We conducted postmortem interval (PMI) experiments in Changsha County by disposing animal carcasses for 3 consecutive years (2007 to 2009). In February 2010, a male corpse was found at wilderness of Changsha County. The larvae found on the corpse were identified only to be Aldrichina grahami (Aldrich, 1930) by morphologic observation and were then confirmed by mitochondrial DNA sequence. A PMI of 320±10 hours was concluded for the human body, based on the experimentally obtained entomological evidence. According to the murderer's statement, the elapsed time since death was calculated to have been 308 hours. In this case, the PMI was estimated successfully and it was almost precise. The presence of the only species is related to the climatic and micro-environmental conditions in the urban habitat of Changsha, central-south China. It would appear that the knowledge of local fauna is very useful in forensic investigations.

Key Words: Forensic science, Forensic entomology, Species identification, Aldrichina grahami, Postmortem interval

Sarcosaphagous flies that first colonize a human corpse usually belong to the families Calliphoridae [1, 2], and often play an essential role in the accurate estimation of postmortem interval (PMI) [3-5], especially when information on the postmortem phenomena is not available [2]. Investigators can approximate how long a body has been left exposed based on species identification and examination of the developmental stages of the fly larvae [6]. The age of the larva is determined by comparing its size or stage of development with published growth data from larva of the same species at a temperature the same or similar to the death scene.

Accurate identification of insects collected from the corpse is an essential requirement for forensic investigations. However, precise and rapid species identification based on the morphological keys at the immature stages is extremely difficult for almost all forensic scientists within their routine work in China [7]. DNA-based method can be used as a supplemental means of morphological method in species identification, as it can be carried out on any lifecycle stage without further rearing and on dead, preserved or live samples.

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In estimating the PMI, it is assured that all of the maggot's stage of development and feeding period occurred on the corpse [8], and the DNA analysis of maggot crop contents can solve this problem. In this study, we present details of a case in Hunan (central-south China) in February 2010. The only larvae found on the corpse were successfully identified by morphologic observation and were then confirmed by mitochondrial DNA (mtDNA) cytochrome oxidase subunits one (COI) sequence. Through STR analysis, the collected maggots were certified to feed on the body of victim. The PMI was successfully pinpointed by referring to the previous experiments that had been carried out under similar circumstances.

Case report

In the middle part of February 2010, the body of a 46-year-old male was found in Changsha County (112.87°E, 28.68°N) of Hunan province. He was lying in the weeds, supine, on a river bank. The corpse was clothed in a Jacket, sweater, shirt, trousers and leather shoes (FIG. 1A). The body was transported to the local funeral home for forensic examination and autopsy. The corpse was highly decomposed. Fatal puncture wound was observed on the chest (FIG. 1B). The cause of death was diagnosed as loss of blood from the wound of the chest. The insect larvae were found on the corpse, and particularly in wounds on the chest and on the back (FIG. 1C and D). The larvae were collected from the soft tissue of the back. The puparium was not found from the scene. The weather records of the region were at 14 oC daily average and 70 % relative humidity in the recently weeks. These data were publicly obtained at the website of the China Meteorological Administration (CMA). The estimation of PMI was puzzled the investigators as the high decay degree.

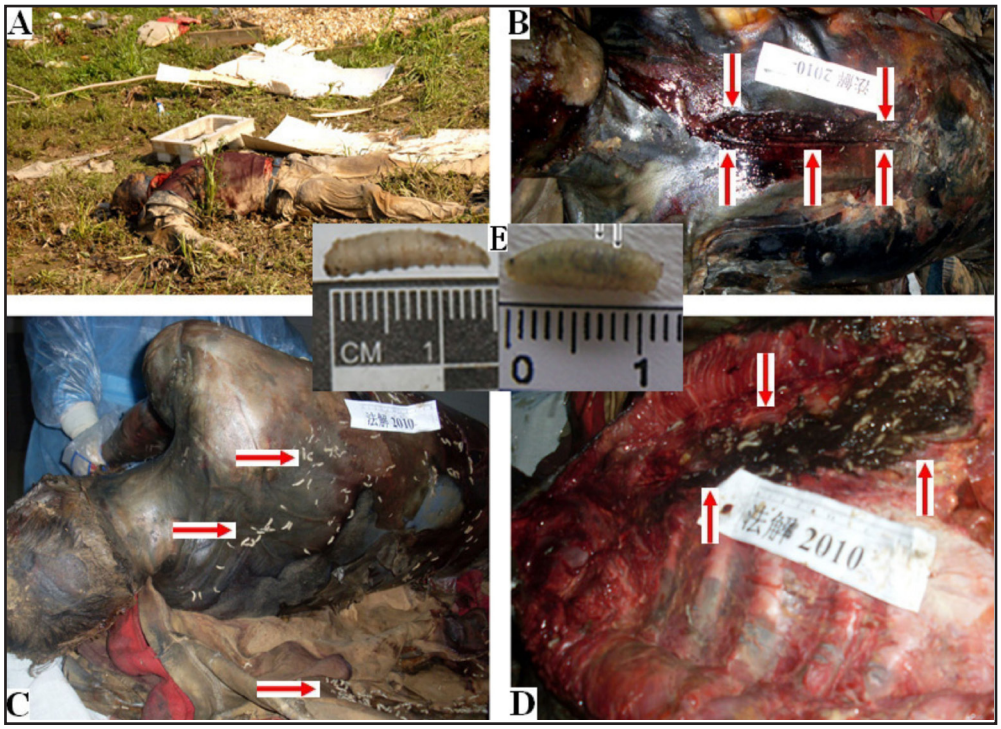

Figure 1. (A) The cadaver was found in the morning in middle February 2010 at wilderness of Changsha County (Hunan Province, central-south China). (B) Fatal puncture wound was observed on the chest. (C) The insect larvae were found on the back (D) Maggots were found under the wound. (E) Instar III of A. grahami (left: The longest larvae; right: the crop could be observed in a dark color from the exterior part).

The maggots were collected from the corpse during autopsy. The obtained third instar larvae were varied from 1.1cm to 1.5cm, and the crop could be observed in a dark color from the exterior part (FIG. 1E). The cuticle of the maggot could be folded back and the crop can be easily removed with forceps [9]. All the third larvae were identified to be Aldrichina grahami using morphological keys [10,11]. DNA of the specimens (including crop, maggots' remains and human costal cartilage sample) were extracted using QIAamp tissue columns (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. STR analysis was performed on all extractions of crop of maggots and human tissue. 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). The STR profiles obtained from the human tissue and the crop of maggots matched each other (FIG. 2). Then the segment of 272-bp COI was amplified and sequenced for the DNA samples from all of the maggots remain.

The PCR amplification primers and conditions used are described in [7]. The resulting sequences were

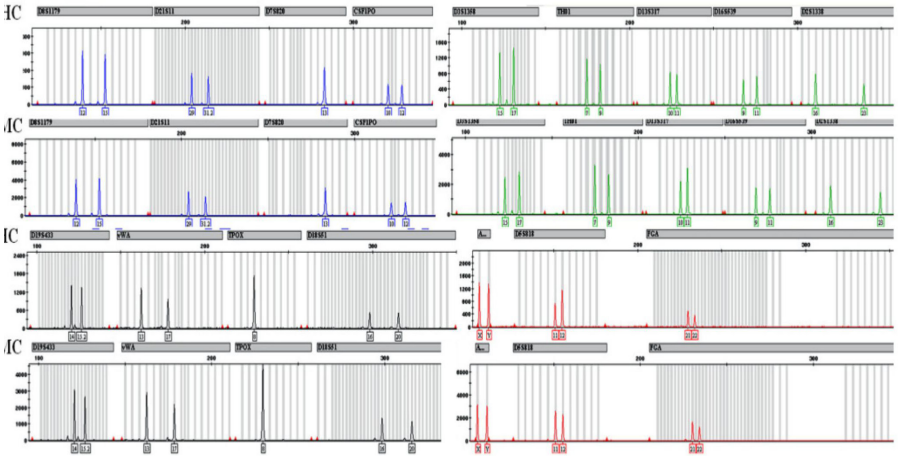

Figure 2. The STR profiles (16 loci) obtained from the human tissue and the crop of maggots matched each other. (HC: Human costal cartilage; MC:maggots crop)

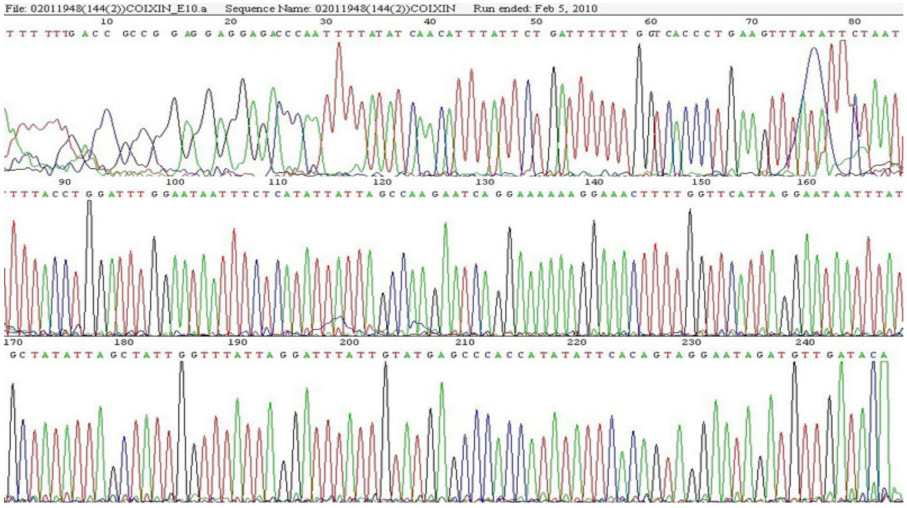

Figure 3. The 247 bp COI gene (without primers) sequence of A. grahami. One of the samples' Forward sequencing.

compared with the Diptera sequences in the NCBI web site by Blastn function (FIG. 3). And the results are consistent with the morphological identification. For the purposes of PMI estimation, it is a prerequisite to know how long it would take for a maggot to reach the same development stage in a very similar circumstance to the real case [6].

We had conducted postmortem interval (PMI) experiments in the same region by disposing animal carcasses for 3 consecutive years. According to our animal experiments, during February in Changsha county (when average temperature was 14 oC), it took 320±10 hours for Aldrichina grahami to develop into identical instar III measuring 15mm in length. According to the previous studies [12-14], it took no more than 350 hours for Aldrichina grahami to develop from eggs to the end of instar III at a constant breedingtemperature of 14°C. A least square method [15] was used to check our estimate PMI. Based on this method, it was estimated that the PMI of the victim was 331 hours. At last the assumption was confirmed by the confession of arrested perpetrator during the procedure of legal interrogation, which disclosed that the actual time of death of the victim was 308 hours when his body was recovered from the scene.

Discussion

The major mission of forensic entomology is to provide useful information for investigation of murders and suspicious deaths by helping to determine time, manner and place of death [16]. Postmortem interval has been the focus for forensic investigators in their routine work, especially when information on the postmortem phenomena is not available. Necrophagous flies collected from criminal scenes can reduce the impact of corruption on the investigation. According to the literature, entomological evidence could be found on or near the remains of the body even up to several years after death [17]. However, the utility of forensic entomology for crime scene investigation has been severely restricted, as morphological identification is difficult and the local fauna is missing [18].

In order to apply entomological evidence to the estimation of PMI or other related issues, it is essential to precisely identify the species of insects attracted to the remains. In this case, although the complex morphological keys poses a great challenge, the 272 bp COI fragment can been used as a supplemental means of morphological method in species identification [7]. Many factors affect fly larvae's growth rate on a corpse, such as climate, ambient air conditions, surrounding environment, and the wound situation on the body [6]. And luckily, we had conducted postmortem interval (PMI) experiments in Changsha County by disposing animal carcasses for 3 consecutive years. One of Our experimental sites is even only at a distance of 8 km

away from the scene. A large number of succession and development information of sarcosaphagous flies had been accumulated in this region. A. grahami can finish its cycle at a development threshold temperature above 12 oC [19], and its larvae are the only insect evidence found on the the body, which were consistent with our previous experiment findings during February. All these factors facilitate the estimation of PMI in this case.

To the best of our knowledge, this is the first record of the use of DNA analysis recovered from crop content in a real case in China. The forensic value of human DNA analysis from maggot crop content has repeatedly demonstrated [9]. A considerable amount of effort has established the validation of the analytical method and refined the involved steps, including different specimen collection, preservation, the time of storage and DNA extraction strategies. And in cases of corpse dismemberment, this approach can be used to identify the dismembered body when conventional methods cannot yield the ideal result of DNA analysis.This study contributes to the knowledge of sarcosaphagous flies associated with cadavers in central-south China. Only Aldrichina grahami of Calliphoridae were collected and indentified from the victim's corpse. It represents a case in which a species was the first and only colonizer of a human corpse and it confirms the data on the dissimilar phenology of A. grahami in different geographical areas. In this case, a PMI of 320±10 hours was accurately determined from the maggots collected from the human body when compared with the information of maggots growing on the remains of the previous experiment. Thus, we conclude and emphasize that the accumulation of local succession and development information is necessary, as the more similar climatic and micro-environmental conditions between the experiment and the real case, the more accurate can be the PMI obtained.

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