CASE REPORT

Application of Forensic Entomology to Postmortem Interval Determination of a Burned Human Corpse: A Homicide Case Report from Southern Taiwan

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Determining the postmortem interval (PMI) is strongly impacted by several variables, which consequently results in inaccuracy in the estimation of PMI used in court trials. A PMI experiment was conducted in Kaohsiung County by disposing a burned pig corpse in the woods. One month later, unexpectedly and interestingly, a homicide case, very similar to this mock study, occurred at a distance of 6 km away from the experimental site. The female victim had been killed and burned. The maggots collected from the victim were identified to be *Chrysomya megacephala* by morphologic observation and were then confirmed by mitochondrial DNA sequence. A PMI of 50 hours was concluded for the burned human body, based on the information of the maggots from the pig corpse. The murderer was eventually arrested and confessed to the crime. According to his statement, the elapsed time since death was calculated to have been 46 hours. In this case, the PMI was estimated successfully and it was almost precise. It would appear that the more similar the surrounding environment between the mock study and the actual case, the more precise can be the PMI estimation. [*J Formos Med Assoc* 2007;106(9):792–798]

Key Words: fly larvae, forensic entomology, insect species, mitochondrial DNA, postmortem interval

Knowledge of the insect species found on exposed human remains and their succession patterns has been used by forensic entomologists in postmortem interval (PMI) estimation in cases of homicide, suicide, accident, and unattended death due to natural causes. Based on the identification of specific insects present in the body and examination of the developmental stages of the fly larvae, investigators can approximate how long a body has been left exposed. Accordingly, the correct typing of insect species is a critical requirement in the estimation of PMI. However, the traditional morphology-based approach may be imprecise or even inaccurate. Recently, several studies on this issue were reported by using the mitochondrial DNA (mtDNA) sequence method to identify immature insects.[1–5] Also, the development cycle of individual fly and arthropod succession patterns are well described.[6–8] Although these studies and techniques have been successfully applied worldwide, their practical application in Taiwan has rarely been demonstrated. Such a situation might be attributed to the challenge of estimating PMI in this country, for example, due to geographical situation, ambient air temperature, humidity, climate and weather

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conditions, which would regulate or influence the developmental stage and insect succession.

A PMI experiment was performed by disposing a burned pig in the woods. Maggots and flies were collected for species identification two times each day. For confirmation, the DNA was extracted from adult flies for mtDNA sequence analysis at the cytochrome oxidase b subunit I between nucleotide position 2495 and 2800. Coincidentally, a homicide case occurred at a location 6 kilometers away from the experimental site. Maggots were collected from the victim's body that had been burned and then disposed of. In this case, PMI was successfully pinpointed by referring to the pig study that had been carried out under similar circumstances.

Case Report

A girl was murdered, burned and found on a side path close to a sugarcane field in Kaohsiung County, Taiwan, on August 29, 2003. The victim was initially identified by her parents, who found a necklace on her neck. Her identity was then confirmed by short tandem repeat DNA typing. The girl had last been seen alive on August 27 by her sister, who claimed that the victim went out at 7:00 pm after receiving a phone call, and did not come back.

Her body was discovered after she had been missing for 2 days. Fly maggots were crawling around on the head and lower part of the body. The underclothes and pants had been taken off and disposed of on the upper part of the body. An autopsy was conducted, which revealed that the corpse had been burned after gasoline had been poured on the body. Eight knife wounds were located around the chest, neck, abdomen and back. Several wounds including ecchymosis and laceration were also uncovered on her face, presumably caused by being heavily hit with a brick. A great number of fresh fly maggots were observed crawling around the body orifices, primarily on the head (Figure 1).

Representative collection of 40 larvae from the victim's face and lower part of the body was made for later identification. Fly maggots were put in KAA (fix solution: kerosene 10 mL, acetic acid 20 mL, ethyl alcohol 80–100%) for 4 hours before preservation in 75% ethanol. The insect species was identified by a stereomicroscope and morphology comparison,[9] and then confirmed using DNA sequencing. The representative larvae collected from the victim's body were identified to be *Chrysomya megacephala*, and most were at instar II, with a few at instar III (Table 1).

A PMI experiment had previously been carried out from July 15–20, 2003, by disposing of a burned pig in the woods. The pig weighed about 25 kg and had been killed by electric current. The pig carcass had been burned to give a level number 2 burning by pouring gasoline on it. The burned corpse was put in an iron-net cage and then dumped in the woods in an area growing luxuriantly with grass, bamboo, and several other plants. The distance between the experiment site and the crime scene was about 6 kilometers. The insect succession on the burned pig corpse was recorded two times a day after it had been placed in the woods. The adult flies were also collected by an insect net and were put into 75% alcohol for preservation. The maggots were obtained from the corpse by analytical spade and then put into KAA solution for 4–12 hours before transferring to 75% alcohol. Similar to the homicide case, the insect species was recognized by stereomicroscopic observation and then confirmed by DNA sequencing. The blow flies (*Calliphoridae*) had arrived on the pig corpse within 5 minutes; most of them were *Chrysomya megacephala*. Eggs and instar I larvae were found in 17 hours, and the size of the maggots was about 1 mm. Instar II larvae were discovered in 25 hours. The majority of the adult flies were *Chrysomya megacephala* and a minority were *Chrysomya rufifacies*. Instar III larvae were detected in 50 hours, and some of them had moved out of the corpse by 71 hours. Only two species of *Calliphoridae*, *Chrysomya megacephala* and *Chrysomya rufifacies*, were obtained in the pig experiment. Detailed data for maggots collected from the pig corpse and environmental comparison between the two cases are shown in Tables 2 and 3.

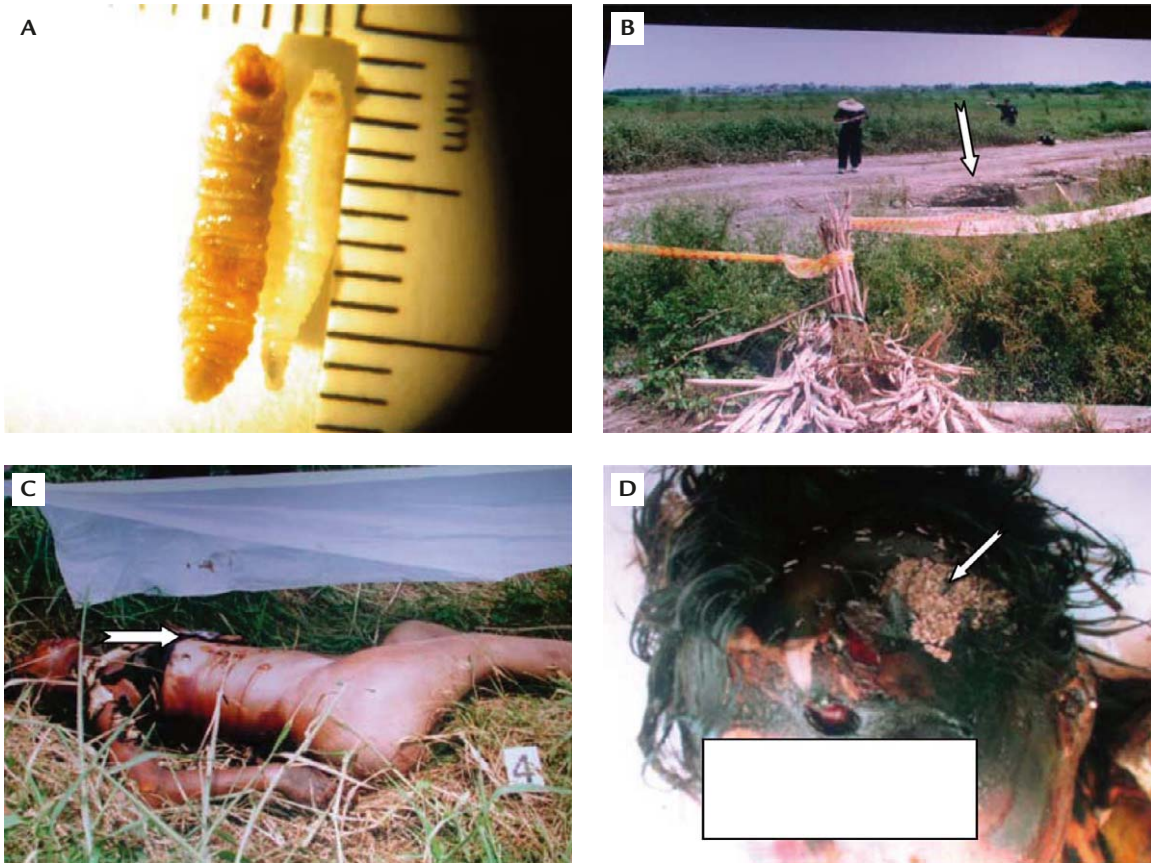

Figure 1. (A) Instar III of *Chrysomya megacephala* (left: collected from the crime scene; right: collected from the burned pig). (B) Overview of the crime scene. (C) The burned victim's corpse. (D) Maggots were found on the face and head.

Table 1. Maggot data from the victim's corpse

Species	Instar stage	Size (mm)	Maggot number
C. megacephala	II	0.45	2
C. megacephala	II	0.5	1
C. megacephala	II	0.6	3
C. megacephala	II	0.7	30
C. megacephala	III	0.9	1
C. megacephala	III	1.2	3

For further confirmation of insect species, genomic DNA was extracted from the maggots collected from both the pig and victim's corpses using QIAamp DNA kit (QIAGEN Inc., Valencia, CA, USA). Each maggot was ground into powder using a disposable plastic pestle inside a 1.5 mL microfuge tube immersed in liquid nitrogen. The DNA extracted was used as template DNA for PCR reactions. The region of mitochondrial cytochrome oxidase b subunit I (COI) gene was amplified using primers 2495 (5′-CAGCTACTTTATGAGCTTTAG-3′) and 2800 (5′-CATTTCAAGT/CTGTGTAAGCAT-3′).[10] An aliquot of PCR reaction solution comprised 5 μL of 10X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 8 μL of 1.25 mM dNTP mixture, 5 μL of 1.5 μM forward and reverse primers, 2.5 units of *Taq* DNA polymerase, 10 ng of DNA, and distilled water to a final volume of 50 μL. The PCR conditions were as follows: 35 cycles of 94°C for 1 minute, 45°C for 1 minute, and 72°C for 1.5 minutes. A PCR fragment of 305 bp was amplified. Prior to the sequencing reaction, the PCR products were purified using a QIAquick® PCR purification kit (QIAGEN Inc.). Cycle sequencing was performed using 3.0 μL of ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction solution with AmpliTaq FS DNA polymerase (PE Applied Biosystems, Foster City, CA, USA),

Table 2. Maggot data obtained from the burned pig corpse

Sampling no.	Time interval (hr)	Development			
		Stage	Species	Size (mm)	Number
1	17	Egg	Unknown	0.12	20
		I	*C. megacephala*	0.3	82
		I	*C. megacephala*	0.2	8
		I	*C. megacephala*	0.15	12
2	25	Egg	Unknown	0.1	90
		II	*C. rufifacies*	0.4	1
		II	*C. megacephala*	0.38	20
		II	*C. megacephala*	0.42	3
		II	*C. megacephala*	0.48	30
3	40	I, II	*C. rufifacies*	0.45	2
		I	*C. megacephala*	0.2	3
4	50	III	*C. megacephala*	1.1	7
		III	*C. megacephala*	0.65	5
		II	*C. rufifacies*	0.48	2
		III	*C. rufifacies*	0.6	3
5	61	III	*C. rufifacies*	1.1	2
		III	*C. rufifacies*	0.6	1
		III	*C. rufifacies*	0.7	1
		III	*C. rufifacies*	0.8	1
		III	*C. rufifacies*	0.9	1
		II	*C. rufifacies*	0.6	8
		III	*C. rufifacies*	1.1	4
6	71	III	*C. rufifacies*	1.1	2
		III	*C. rufifacies*	1	1
		III	*C. rufifacies*	0.8	1
		III	*C. rufifacies*	0.65	1

Table 3. Environmental comparison between the homicide case and the mimic experiment

	Occurrence	Scene	Surroundings	Temperature* (°C)	Humidity (%)	PMI
Homicide	Body found on August 29, 2003	Kaohsiung County in Southern Taiwan	Side path in a sugarcane field	28.6	78.4	46 hr, based on murderer's confession
Pig experiment	During July 15–20, 2003	A distance of 6 km away from the homicide	In wooded area with luxuriant plant growth	30.3	69.3	Estimated to be 50 hr

**The average temperature data during analysis was provided by the Central Weather Bureau, Taiwan. PMI = postmortem interval.*

10 pmol of forward or reverse primer, 2.3 μL of purified PCR product, and sterile distilled water to a final volume of 10 μL for each sample. For cycle sequencing, a Perkin Elmer 9600 thermal cycler was used under the following conditions: 25 cycles at 96°C for 30 seconds, 50°C for 15 seconds, and 60°C for 4 minutes. After sequencing, each sample was added to a sephadex-G-50 column (spin 50-mini-column; BioMax Inc., Odenton, MD, USA) and centrifuged at 1500*g* for 3 minutes.

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2496 ATTTGTATTT TTATTTACTG TAGGAGGATT AACTGGAGTT GTTTTAGCTA ATTCATCAAT
2496 ---------- ---------- ---------- ---------- ---------- ----------
2496 ---------- ---------- ---------- ---------- ---------- ----------

2556 TGACATTATT TTACATGATA CATATTATGT AGTAGCTCAC TTCCATTATG TTCTATCAAT
2556 ---------- ---------- ---------- ---------- ---------- ----------
2556 ---------- ---------- ---------- ---------- ---------- ----------

2616 GGGAGCTGTA TTTGCTATTA TAGCAGGATT TGTTCATTGA TTCCCTCTAT TTACTGGATT
2616 ---------- ---------- ---------- ---------- ---------- ----------
2616 ---------- -C-------- ---------- ---------- ---------- ----------

2676 AACTTTAAAT AGCAAGTTAT TAAAGAGTCA ATTTGCTATT ATATTTATCG GAGTAAATTT
2676 ---------- ---------- ---------- ---------- --------T- ----------
2676 ---------- ---------- ---------- ---------- ---------- ----------

2736 AACATTCTTC CCTCAACATT TCTTAGGATT AGCAGGTATA CCTCGACGAT ACTCAGACTA
2736 ---------- ---------- ---------- ---------- ---------- ----------
2736 ---------- ---------- ---------- ---------- ---------- ----------

2796 TCCA (Type 1)
2796 ---- (Type 2)
2796 ---- (Type 3)
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Figure 2. Mitochondrial DNA sequence between np 2495 and 2800 in the cytochrome oxidase b subunit I region of *Chrysomya megacephala*. The variation (defined as type 1 and 2) was detected at np 2724 from three maggots (the human corpse) and six maggots (the pig). In addition, sequence variation of type 3 was found at np 2627 in one maggot from the pig.

Each purified sample was recovered from the bottom of the collection tube and dried in a vacuum centrifuge. Automated DNA sequencing was performed on an ABI 310 sequencer.

Results of mtDNA sequences of the maggots from both the pig and human corpse revealed that they were the same species, *Chrysomya megacephala*, by comparing them with published sequence data.[11] However, sequence variations were observed between the two. Type 1 and type 2 variations (Figure 2) were found at np 2724 among the three maggots collected from the human corpse and among six maggots from the burnt pig. Sequence variation at np 2627 was observed in one of the six maggots collected from the pig corpse (type 3 in Figure 2). Nevertheless, according to the literature,[10] these nine maggots should be identified as the same species in that the nucleotide difference is less than 1% in the PCR fragment of 305 bp.

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. An accumulated degree hours (ADH) method[12] was used to estimate PMI. The assumption of ADH is that the relation between the development rate of insects and temperature is linear in the middle range of the correlation curve. It took 50 hours for *Chrysomya megacephala* to develop into identical instar III measuring 11 mm in length in the pig experiment. Based on this data, it was estimated that the PMI of the victim was 50 hours.

Discussion

Compared to one prominent study,[13] it took 32 hours for *Chrysomya megacephala* to develop from eggs to the end of instar II at a constant breeding temperature of 30°C, and another 72 hours to stay in instar III. Our result was similar to those of that report. Nevertheless, it should be noted that the oviposition of the *Calliphoridae* family could occur earlier on a burned carcass than a control one,[14] indicating that if the estimated PMI of a burned carcass was based on the arthropod succession pattern on an unburned carcass, the estimate would be longer than the actual time.

With the help of PMI data and other investigative clues, one suspect was finally arrested. As expected, he admitted that the girl was killed at 10 pm on August 27. The time period between death and discovery of the body was calculated to be 46 hours. The estimated PMI almost pinpointed the exact time. In practice, matching the time of death in the closest PMI is currently imprecise, such that only a wide window of estimation, i.e. 8 hours, is the best that can be obtained.[15] Therefore, the estimated PMI of 50 hours in this homicide case can be regarded as quite accurate and precise, though there was a time difference of 4 hours.

It is reasonable to assume that the oviposition of *Chrysomya megacephala* occurred shortly after the disposal of the burned pig corpse, because the blow flies arrived on the pig corpse within 5 minutes and their instar larvae were found in 17 hours. As for the homicide case, it was difficult to determine the time of arrival of the blow flies. However, it was inferred that the victim was disposed of at the crime scene after 10 pm. A well known phenomenon is that there is no oviposition activity during darkness. But, judging from the larvae data, the eggs should have been oviposited before the midnight of August 27, otherwise the instar III larvae (12 mm) could not have been obtained within 46 hours of disposal.

In conclusion, the species of collected maggots from the victim's corpse was simple and identified to be *Chrysomya megacephala* of *Calliphoridae*. In this homicide case, a PMI of 50 hours was accurately determined from the maggots collected from the burned human body when compared with the information of maggots growing on the remains of the pig experiment. 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. Luckily, the difference between the pig experiment and the real case in space and time was only 6 kilometers and 1 month, respectively. This precise PMI determination can be attributed to the similarity of environmental factors influencing the maggots' developmental stages. Thus, we conclude and emphasize that the more similar the ambient conditions between the experiment and the real case, the more accurate can be the PMI obtained.

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