Forensic entomology and short-term PMI estimation: a case report [*]

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Abstract

During summer 2003, a forensic entomological study was undertaken in Brussels (Belgium) on the body of a man found in his apartment several days after he died. Diptera larvae were taken from and near the body of the victim and were reared in the laboratory under constant temperature in a monitored incubator. Once adult, the insects were identified as *Lucilia sericata* MEIGEN (1856).

The method used in the laboratory to determine the day of egg-laying was based on the measure of the quantity of thermal energy necessary for a given fly species to complete its life cycle from egg to imago (accumulated degree-days-method).

Using data from the literature, the day of egg-laying was estimated. The results of the calculations were consistent with what the criminal investigation later showed. This case report illustrates the importance of using insects in order to evaluate post-mortem interval.

Keywords: ADD-method, post-mortem interval, *Lucilia sericata*.

Résumé

Dans le courant du mois d'août 2003, une étude entomologique a été réalisée dans le cadre de la découverte d'un corps humain dans un appartement à Bruxelles (Belgique). Des larves de Diptère ont été capturées sur et à proximité du corps et mise en élevage sous température contrôlée dans un incubateur. Une fois adultes, les diptères ont été identifiés comme étant *Lucilia sericata* MEIGEN (1856).

La méthode utilisée pour déterminer la date à laquelle ces insectes ont été pondus

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est basée sur la mesure de la quantité de chaleur nécessaire pour accomplir un cycle biologique de l'œuf à l'adulte (méthode de l'accumulation des degrés-jour).

Sur base de données de la littérature, le jour de la ponte a été estimé. Le résultat des calculs était en accord avec ce que l'enquête policière a plus tard montré. Ceci illustre l'importance de l'étude des insectes pour l'évaluation de l'intervalle post-mortem.

Introduction

Knowing when a suspect death occurred is of primary importance to lead the criminal investigation, since it provides one of the first possibilities to rule out suspects with confirmed alibis, and keep focus on those who have not. The post–mortem interval (PMI) determination is even more critical when it comes to bodies decomposed beyond recognition, as it sometimes is the first indication to help identify a corpse. Furthermore, besides body stiffening and body temperature, forensic pathologists have few tools to determine the interval between death and a body's recovery, none of which having absolute precision, as many parameters can interfere (age, weight, health, ambient temperature, etc).

To elucidate suspect deaths when corpses are colonized by necrophagous insects, investigators can rely on forensic entomology (LECLERCQ, 1978; SMITH, 1986; CATTS & HASKELL, 1990; BYRD & CASTNER, 2001). Insect related evidence is one of the most powerful, but least known examples of modern forensic science (GREENBERG & KUNICH, 2002), and seemingly the one and only technique able to estimate minimum PMI for deaths older than three days.

Forensic entomology studies the biological cycle of corpse-related entomofauna in order to determine the minimum PMI. More precisely, it calculates the time interval between the moment of the egg-laying of the blowflies and the discovery of the corpse, since these insects are known to be the first colonizers and can be attracted within minutes following death (WYSS & CHERIX, 2002).

For short-term PMI estimations, one approach is based on the measurement of the thermal input (accumulated degree-days, or ADD) necessary for each insect species to complete its life cycle (REITER & GRASSBERGER, 2002; MARCHENKO, 1988 in MARCHENKO, 2001). "Short term PMI" here means the period of some weeks, strictly limited to the complete life-cycle of the *first flies* to have laid eggs on the body. Another useful technique relies on isomegalen and isomorphen diagrams (REITER & GRASSBERGER, 2002). This method is particularly adapted to estimate PMI when the corpse was left in a thermally stable environment.

Other methods, such as succession method, are said to allow longer estimations, but precision falls drastically. Anyway, even with this widened time estimation, the time of death provided is more precise than any other method (HAGLUND & SORG, 1997).

The basic principles of the ADD-method are:

- The *heat constant* of a species is the sum of effective temperatures a

species need to develop from egg to imago.
- Below a certain temperature threshold (*lower development threshold*), larvae do not grow.
- Above this threshold, the temperature that directly contributes to the insect development is the *effective temperature.* It is calculated as the difference between environmental temperature and the temperature corresponding to the lower development threshold.
- These thermal parameters are a constant value for each species.

The heat constant of some of the usual necrophagous Diptera species are known as well as their corresponding lower development threshold (KAMAL, 1958; MARCHENKO, 2001; WYSS, 2000). The insects collected on the body (larvae or pupae) will already have accumulated a part of this thermal energy. The rest of the necessary thermal input will be provided in the laboratory, under the controlled thermal conditions of an incubator. The thermal input received at the scene of death before the body is found (and thus the date of the egg-laying) results from the difference between the theoretical total thermal requirement and the thermal input provided in the laboratory.

$$ADD_{total} = ADD_{in\ incubator} + ADD_{at\ the\ scene\ of\ death}$$

Thus, it is of primary importance to know the temperature conditions at the scene of death before the body was found (HASKELL, 2002; REITER & GRASSBERGER, 2002). It is the main parameter used to monitor the insects' development. Temperature data-loggers are left at the scene of death, measuring the thermal conditions for one or two weeks. These data will be compared to data from an official meteorological station over the same time period, and the temperature conditions of the scene of death *before* the body's recovery will then be calculated backwards.

It is important to note that in certain conditions (e.g. too low or too high temperatures, heavy rain, body concealment) the time of egg-laying and the time of death can be significantly different (WYSS & CHERIX, 2005).

Scene of death

On august 11^{th} 2003, the corpse of a man was discovered in the dry bathtub of his apartment in Brussels. The apartment (Fig. 1) was clean, except for numerous dried bloodstains covering the floor of the bathroom. One window of the apartment was slightly open (slit opening about 1 cm wide). This slit was situated at more than 10 meters from the body. The bathroom itself had absolutely no direct communication with the outside, except a ventilation pipe emerging on the roof several floors higher, but the bathroom door was half-open. The man, almost naked, presented injuries on the wrists and marked signs of degradation. The conclusions of the medical examiner were: "suicide, supposedly not more than 8 days ago".

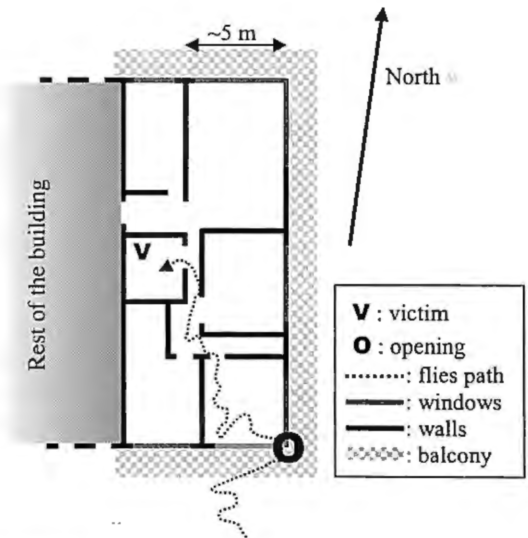

Fig. 1. Map of the apartment.

Larvae of calliphorids were sampled on and near the body (wrists injuries, eyes, mouth, bath tub). No larvae did (or could) crawl outside the bathtub, no pupae were found, and no larval masses were seen. Dead adult flies were lying on the ground near the windows of the room, north of the apartment. No other insect orders or families were found. A temperature data-logger was left in the bathroom for two weeks. No larvae coming from the death scene were killed. Therefore, a PMI estimation based on isomegalen and isomorphen diagrams was not possible.

Materials and methods

Insect rearing and identification of specimens

The larvae collected on the body were brought to the laboratory, and reared on minced heart beef substrate. The rearing boxes were made of clear polystyrene (~10 × 18 × 24 cm), with uetted ventilation holes in the top cover. The bottom of these boxes was covered with a layer of moistened sand (2 cm-thick) as a pupation medium and to avoid escape of the post feeding larvae. Rearing took place in controlled incubators (Sanyo MIR553) until the emergence of adult flies. The temperature conditions were strictly controlled (23°C), with a photoperiodism of 16L:8D. Observation of emergences occurred daily between 10 and 12 am (Table 1).

Adult flies were slightly anaesthetized with CO_2 to enable their capture once a day, and then killed in a freezer. The species identification was performed under a Leica MZ8 stereomicroscope, using standard identification keys (SMITH, 1986; ROGNES, 1991).

Collection of ambient temperature data and statistical treatment

A temperature data logger (Testo 175-T1) was left in the bathroom from August 11th (5 pm) to August 27th (8 am), measuring and storing room temperature hourly (precision: 0.5°C, resolution: 0.1°C).

The reference weather station data used in the calculations were obtained from the Royal Meteorological Institute whose nearest station is located 6.5 km away, at Uccle, Belgium. Average 24 hours effective temperature was used (MARCHENKO, 2001). The correlation between temperature data sets was calculated and verified by a t-test.

Results

Correlation with weather data and temperature adjustments

A linear regression curve was calculated between temperature data coming from the scene itself (bathroom) and the reference weather station for the period from August 11th (5 pm) to August 20th (12 pm). We selected this time interval for the calculation of the regression curve because the reference temperature profile changed drastically from August 21st when the daily mean air temperature dropped from about 28°C to about 22°C. There was no rainfall period during the considered period of time. Two hundred fourty seven hourly data points were taken into account for calculations (Fig. 2).

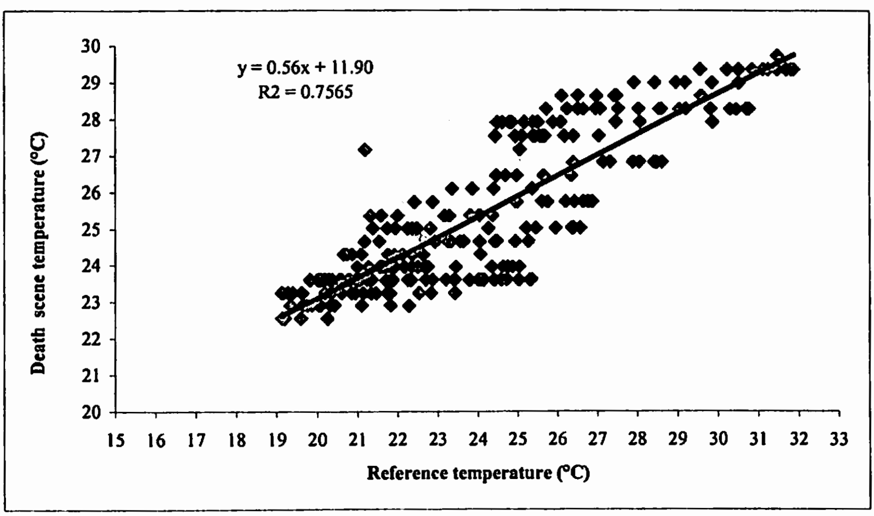

Fig. 2: Statistical regression between temperature data from the official weather station used as reference (Uccle) and the data from the bathroom (death scene) for the period going from August 11th to August 20th.

The linear regression showed a multiple R^2 value of 0.7565 and adjusted R^2 value of 0.7555 (F-statistic: 761.2 on 1 and 245 DF, $p \leq 2.2e-16$). The linear equation was:

$$y = 0.56x + 11.90$$

x = hourly temperature (°C) from the reference weather station (Uccle)
y = hourly temperature (°C) in the bathroom.

The t-test showed that the values of a and b were highly significant (t-values 21.26 and 47.12).

Fly emergences and identification of the collected material

Emergence of the first adults began on August 22nd (Fig. 3). The emerging flies were identified as *Lucilia sericata* MEIGEN (1856) (Fig. 4) that has a heat constant of 207°C days and a lower development threshold of 9°C (MARCHENKO, 2001; WYSS, 2002).

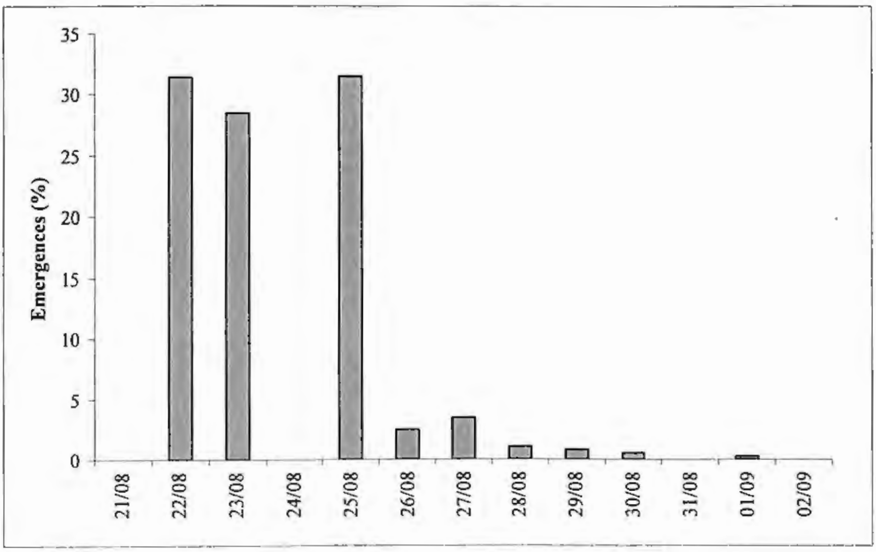

Fig. 3. Proportional repartition of the blowflies emergences.

The day of emergence selected in the calculations was August 22nd because the heat constant was measured in function of the first emergences (MARCHENKO, 2001). The subsequent days of emergence could partially correspond to later egg-layings.

Estimation of Post-Mortem Interval

Using data from the literature (MARCHENKO, 2001) we could provide the following assessment: the first egg-laying on the body occurred on August 8th.

Fig. 4. *Lucilia sericata*: lateral view of adult female.

Table 1. Estimation of the PMI with the ADD-model.

Date	Reference mean temperature (official weather station)	Extrapolated mean temperature	Incubator daily mean temperature	Lower development threshold	Daily effective temperature on death scene	Daily effective temperature in the incubator	ADD
08/08/03	27.1	27.1		9	18.1		211.6
09/08/03	26.8	26.9		9	17.9		193.5
10/08/03	27.4	27.2		9	18.2		175.6
11/08/03 - The body is found		25.8		9	16.8		157.3
12/08/03			23	9		14	140.6
13/08/03			23	9		14	126.6
14/08/03			23	9		14	112.6
15/08/03			23	9		14	98.6
16/08/03			23	9		14	84.6
17/08/03			23	9		14	70.6
18/08/03			23	9		14	56.6
19/08/03			23	9		14	42.6
20/08/03			23	9		14	28.6
21/08/03			23	9		14	14.6
22/08/03 - First emergences			23	9		0.6*	0.6

* Emergences observed at 10 am

Hourly calculation (not shown) situates the oviposition in the middle of the night, very early on August 8th, which is quite unlikely as most calliphorids usually have diurnal activity. Oviposition then probably occurred on the 7th in the afternoon, or the next morning (Tab. 1). Due to the geometrical particularities of the apartment (bathroom without direct communication with outside), it is likely that the flies needed some delay to detect and locate the body, and to find a way to go into the apartment and reach the bathroom (Fig. 1).

Epilogue

The police investigations later showed that the victim was seen last by a neighbour on the morning of August 7^{th}.

Conclusion

The results of our ADD-calculations were consistent with the results of the police investigation, and were more precise than the medical examiner's statements, although his statement was correct.

Blowflies are usually the first insects to discover and colonize dead bodies. This can happen within hours, and egg-laying can occur as soon as the body is reached. This case study illustrates the importance of using insects to help estimate minimum post-mortem interval, and to reconstruct a possible scenario of the events.

It is very important to think « forensic entomology » each time insects are encountered at a scene of death; maybe even when they are not, because their absence also can be instructive. Furthermore, entomological evidence should routinely be collected and studied, as it is the best way to increase our knowledge on insects of forensic importance. Even when it is not immediately useful for Justice, it remains important for Science.

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