Detection of parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) by HPLC in insects of forensic importance in Medellín, Colombia

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A Public Publication Experiment*

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There are three things that we need in forensic entomology: More young researchers entering the badly paid and sometimes a little too exciting field, open discussion about possible flaws in our methods and PMI calculations, and access to information for scientists all over the world, allowing discussion and experimentation in all cultures, and independently from access to very expensive literature.

After the great success of the Forensic Entomology Special Issue in *Forensic Sci Int* 120(2001)1-160 (that even brought unexpected impact points to the editorial house), we decided that one thing would not come true fast enough if we did not do it quick, and by ourselves: A collection of cases and observations with as much raw data and photographs as possible.

Thanks to the responses of my colleagues, and thanks to Anil Aggraval who became more enthusiastic than any other journal editors yet, this special issue will go online at the same time that the European Association of Forensic Entomologists Meeting in London (March 28–30, 2004) takes place.

I am very proud of all of you who submitted articles, and wish to thank you very much. You knew that you would never receive an impact or citation point from one of the companies who sell these things. At the same time, I wish to thank Anil Aggrawal and Zeno Gerards who maintain and offer web space to put this issue out. Neither of them receives any money for their extra work. The same is, of course, true for the Guest Editor and the Main Reviewer.

It was not easy for the reviewers – most importantly Jeff Wells, who put in a lot of his time to not only read through the draft versions but also the final submissions of the articles – to decide which articles should be included. Jeff became the scientific brain of the operation whereas Anil encouraged me to include an interview, and a case report from a journalist. He also wanted photographs of us, the scientists, but we could not bring ourselves to include them in this .pdf version of the journal. Feel free to check the web site, and you may find some. (Be warned and do not expect too much, though: Forensic entomologists are considered to be yucky even by forensic pathologists.)

Finally, some technical points: This .pdf file has an actual resolution of 600 dpi (for photographs and drawings) but on the screen, you will most likely see a quick preview mode. Using a high resolution printer – ink preferred over laser – you should receive fine pictures. And hey, this special issue does indeed contain some excellent colour material. Any questions – send an E-mail to me.

Dear colleagues, students and forensic investigators in all types of countries and cultures – it was and is a serious pleasure to work and discuss with you. Stay tuned, and let´s prepare the next generation to get ready.

Mark Benecke

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A Case of *Megaselia scalaris* (Loew) (Dipt., Phoridae) breeding in a human corpse

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Abstract

The first Italian case of *Megaselia scalaris* (Loew) breeding in a human corpse is reported from an exhumed body in Southern Italy. Based on predilection of some Phoridae for older carrion and their delayed arrival at a corpse, the scuttle flies are usually relegated to a secondary forensic role. However, they may occur even in the early stages of decay as the only insect evidence especially in bodies that have somehow been at least partially sheltered from colonization by larger flies through burial.

Introduction

The most commonly reported species of Phoridae breeding in buried human corpses in Europe is the coffin fly, *Conicera tibialis* Schmitz, whereas corpses disposed of in more exposed situations (such as under floorboards or in outbuildings) tend to be colonized by *Megaselia rufipes* (Meigen) [1]. Some other Phoridae such as *Triphleba hyalinata* Meigen and *Megaselia scalaris* (Loew), have also been found associated with human remains [2].

The scuttle fly *Megaselia scalaris* is a polyphagous saprophage species that has been transported around the world by man [3]. It is essentially a warm climate species and is common in the lowlands bordering the Mediterranean. In cooler climes it tends to occur in buildings, or other situations where it escapes frost. As a result of a forensic investigation in Italy, we now detail the first Italian report of a case of *Megaselia scalari*s (Loew) breeding in a human corpse.

Case description

On 6 February 1999, at Bari in Southern Italy, the body of an adult male was exhumed. He had been buried at a depth of 30-40cm in a wooden coffin on 4 February 1998, approximately one year before the exhumation, having been killed by gunshot wounds to the head and the chest. He was 33 years old at the time of death. The fully clothed corpse was covered with numerous larvae, pupae and empty puparia. Pupae and puparia were particularly evident on the clothing, especially on the cotton-wool covering the head (Fig. 1).

Subsequent examination during autopsy, performed by two forensic pathologists, revealed that the body was quite completely mummified with high densities of larvae, especially on the hands and feet (Fig. 2-3). The larvae and adult specimens collected during the autopsy were identified as individuals of *Megaselia scalaris* (Loew). This species was the only insect evidence associated to the human remains. The examination of the coffin revealed some very small holes of the wooden axis through which only small flies like *M. scalaris* were able to get the body.

Discussion

In many exhumations a vast number of flies and puparia can be found and it appear that many

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generations had successfully developed within the coffins. As in the case reported above, it is unusual to find only one species of Diptera such as *Megaselia scalaris* (Loew).

Fig. 1. *Pupae and puparia of* Megaselia scalaris *(Loew) on the clothing and on the cotton-wool covering the head.*

They may occur as the only insect evidence in bodies that have somehow been at least partially sheltered from colonization by larger flies through burial. Due to the predilection of *Conicera tibialis* for soft tissues of older carrion and its typically delayed arrival at a corpse, approximately 4-8 months after death [4], these flies are usually relegated to a secondary forensic role. However, other Phoridae may be found on corpses even in the early stages of decay, associated with some other species of Diptera as well as alone; and thus helping forensic experts to estimate the minimum post-mortem interval.

Megaselia scalaris can arrive at a carrion within a short time of its exposure and will lay eggs straight away. This species frequently breeds in human corpses in warmer climes, but only a few cases have been published. We review some of these below.

In South Africa the scuttle fly *Megaselia scalaris* has been reared from exposed corpses that were 4-6 months old and had been treated with a bleaching agent. The corpses had begun to mummify [3]. In Argentina this species seems to replace *Conicera tibialis* in buried corpses [5]. In the Neartic region, *Megaselia scalaris* has been reared from an exposed corpse 90-98 days old, partially mummified, found in Chicago, IL [6]. It has been reported breeding in a black and tarry, partially skeletonized, corpse buried in Puerto Rico for about one year [7]. Puparia of this species were recovered from the mummified remains of a woman drug addict who had died in her home in New England (U.S.A.), 33 months previously.

Some drugs such as amitryptilyne and nortriptyline were isolated from the chitinized insect remnants of its puparial cases as well from cerebral fragments and from stomach contents and, furthermore even from cast beetle (Dermestidae) skins and beetle fecal material. Results showed that the above drugs were greater in Phoridae puparia than exuviae or frass as this most likely reflects the High densities of larvae on the hands and feet of the mummified body.propensity of Phorids for soft tissues where drug concentrations are likely to be higher [8].

Megaselia scalaris was the only insect observed breeding in a body found within a tightly sealed 7th-floor apartment in Kochi City, Shikoku, Japan [6]. In Belgium, Dewaele et al [9] describe a case of human myasis in a body of an adult female found dead in her apartment on 13. October 1999 in which very young larve of *Megaselia scalaris* were in the genital area (vagina); some specimens of these larvae were reared and eclosion of adult flies was observed after 9-10 days.

Figs. 2, 3. *High densities of larvae on the hands and feet of the mummified body.*

This time interval was considered too short for a complete life-cycle at body temperature of 22.8°C and ambient temperature of 15°C. Some experimental rearings demonstrated that *M. scalaris* develops from egg to adult in 22 days at 22°C, in 14-16 days at 27°C and in 11.1 days at 29°C, consistent with data recorded

by Greenberg and Wells in 1998 who provided growth curves for the scuttle fly at different constant temperatures [6].

The above developmental data were essential to the final estimation of the time since death in the Belgium case and supported the inference of a Phorid vaginal myasis occurred before death. In the literature there are several cases of Phorid myasis illustrated worldwide: two cases in North America among which one of vaginal myasis [10], two were of urogenital and intestinal myasis caused by *M. scalaris* in India [11-12]. Other such cases are reported from elsewhere and also a few cases of wound myasis in humans [3], thus further indicating the medical and forensic importance of this species.

The occurrence of this species of scuttle fly in forensic cases, such as reported above, relates to the shallow burial of the corpse and to the southerly lowland location. In fact, burial of cadavers usually restricts the access of many carrions insects to the body; but not of smaller flies, such as the *Megaselia scalaris* (Loew), that are particularly skilled in locating decaying tissues. By gaining access through very small holes in a closed coffin, they are able to avoid competition from larvae of larger Diptera species.

Acknowledgements

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Detection of Parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) by HPLC in insects of forensic importance in Medellín, Colombia

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Abstract

High Performance Liquid Chromatography (HPLC) was used to determine and quantify Parathion in insects collected from decomposing rabbits previously injected with 5mg/kg of weight of commercial Methyl Parathion. Its effect on the succession of medically and legally important insects was studied.

Three rabbits were given a lethal dose of Parathion by intracardiac injection and a fourth was killed by cervical dislocation. Samples of liver were taken from each to verify the presence of Parathion in the tissue. A high concentration of the product was found with values between 1.379 mg and 1.68 mg per Kg of weight.

Presence of Parathion was detected in insects collected at each stage of decomposition, from fresh to dry remains and at different stadia of development of 10 species of arthropods from the orders Diptera, Coleoptera, Hymenoptera, Isopoda and Acari.

Introduction

Insects can be used as an alternative for toxicological analysis when it is not possible to obtain samples such as blood, urine or internal organs due to the advanced state of decomposition or skeletonisation of a corpse [1-4].

Several studies illustrate the great potential of entomotoxicology for providing additional information on cause of death, postmortem interval, and geographical area where the body was found [5], [1], [3]. Psychoactive substances and antidepressants have been detected by diverse methods in different stadia of insects, such as pupae of Phoridae, exuviae and faecal material of Dermestidae [6], *Cochliomyia macellaria* larvae [7] and other diptera [8,9].

The majority of studies in entomotoxicology, either at experimental level or case studies, have dealt with barbiturates [5,7,10,11], opiates [9,12-18], diazepam [4], cocaine [8,19], MDMA (ecstasy) [20], Amitriptyline, nortriptyline [6,11,21,22] and heavy metals [23]. However, very few studies have focused on organophosphorus compounds [24].

It has been shown that toxicological analysis by liquid chromatography is more sensitive using insect larvae than body tissue [25]. This increases the importance of using insects in this type of research and investigation.

Parathion (C10H14NO5PS) is an insecticide and acaricide widely used in agriculture. It is strongly absorbed by the soil surface but is degraded by photolysis. It is estimated that it disappears from the soil within one week [26,27]. It is broken down into p-nitrophenol, diethyl thiophosphoric acid and paraoxon [28,29].

Although this type of research is at its initial stages in Latin America [4] the number of cases of intoxication by toxic substances or drugs is considerably high. This study contributes to forensic research in Colombia in the field of entomotoxicology.

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Methods

This study was carried out in urban conditions, within the campus of the Universidad de Antioquia, at an altitude of 1450 m above sea level, temperature between 17 and 26 ºC and average rainfall of 1031 mm [30].

Four rabbits were used (2.50 – 2.80 kg in weight). Three of them (S1, S2, S3) were used to evaluate the effect of Parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) and a fourth as the control (Sctrl). The four rabbits were first given 1 ml/kg of weight of Sodium Penthotal to anaesthetise them (suggested by the Universidad de Antioquia Committee on Ethics). The rabbits S1, S2 and S3 were given a lethal dose of 5 mg/kg of Parathion by intracardiac injection and 2 ml orally. The control rabbit (Sctrl) was killed by cervical dislocation. The rabbits were subsequently placed in metal cages (60 x 50 x 50 cm) with a 20 m distance between each.

Arthropod samples and data collection

Samples for the study of insect succession were collected over a period of 28 days, three times per day for the first 12 days, 2 times per day for the next three days and once per day for the last 12 days. The arthropods were collected in the following order: First, those flying over and/or landing on the carcasses; then those which were found in natural cavities (eyes, nose, mouth, anus) and in the wound; and finally those that were under the carcass and in the soil to a depth of 10 cm. The collected material was fixed in 70% alcohol in the case of immature specimens. Adults were killed with ethyl acetate and mounted on entomological pins. Environmental and body temperatures were noted at each sampling. Each carcass was weighed once a day.

Toxicological Analysis

A toxicological analysis was carried out on the liver of each rabbit and the insects collected.

As soon as the rabbits were killed, a small incision was made in the abdomen and a 20 g liver sample was taken. This was stored in a dry tube and frozen at -4ºC.

Every 48 hours an additional sample of the different species of insects was collected for toxicological analysis. The same process was followed as for the liver samples.

The method used to quantify the amount of Parathion was High Performance Liquid Chromatography (HPLC). The following HPLC condition was used in the chromatographic analysis: Mobile Phase: water/acetonitrile; Programme: 15:85 (ACN:H2O) 5 minutes at a flow rate of 1 ml per minute, then changed to 70:30 (ACN:H2O) for 15 minutes; DAD Detector: λ = 274 nm; Solvent: 70:30 ACN:H2O; Column: Waters μBondapak S18: 3.9 x 300 mm; Retention time for Parathion: 12.89 minutes.

Preparation of Samples

The samples were prepared following Thompson *et al* [31]. The sample was weighed, macerated and 0.5 ml of HPLC grade water was added. It was sonicated in order to liberate the analyte, centrifuged to separate the supernatant fraction (to obtain the greatest amount of analyte) and isolated by decantation. This extraction process was carried out in triplicate and the supernatant was collected in one container. The analyte was then extracted with solvents – three extractions of 0.5 ml each time. It was dried in the dark and reconstituted with 1.5 ml of ACN:H20 (70:30) and transferred directly to an HPLC vial for analysis.

Validation of the method

Calibration Curve

The standard solution of Parathion was prepared from pure Parathion, diluted with HPLC grade acetonitrile. Standards in the elution solvent (ACN: Water: 70:30) were prepared from the diluted Parathion. In the table 1 shows the raw data used for the calibration curve (tab. 1, fig. 1). The calibration curve was produced by plotting Area (UA) against Concentration (ppm) of the pure standard using the statistics package, Statgraphic 5.0. To establish the quantifiable limits, linearity was evaluated at concentration values at both extremes of the curve. It was found that Parathion is quantifiable within a range of 0.1 ppm to 20 ppm (tab. 1, fig. 2). Figure 2 clearly shows a linear relationship between the variables area and concentration. Correlation coefficient = 0.998765 and R2 = 99.7532%.

Conc. (ppm)	Area	Area / Conc.	log conc.
0.4	1734150	4335375	-0.397940008672
0.4	1812787.75	4531969.375	-0.397940008672
0.6	2468823.25	4114705.41667	-0.221848749616
0.6	2539573.50	4232622.5	-0.221848749616
0.8	3359754.5	4199693.125	-0.0969100130081
0.8	3405949	4257436.25	-0.0969100130081
1	4112264	4112264	0
1	4105239.75	4105239.75	0
1	4194453	4194453	0
5	20208050	4041610	0.698970004336
10	42588016	4258801.6	1
15	62593800	4172920	1.17609125906
20	92452288	4622614.35	1.30102999566
0.192	808193.5	4209341.14583	-0.716698771296
0.192	806933.19	4202777.03125	-0.716698771296
0.122	533457.56	4372602.95082	-0.913640169325
0.122	553866.44	4539888.85246	-0.913640169325

Tab. 1. *Raw data for calibration curve*

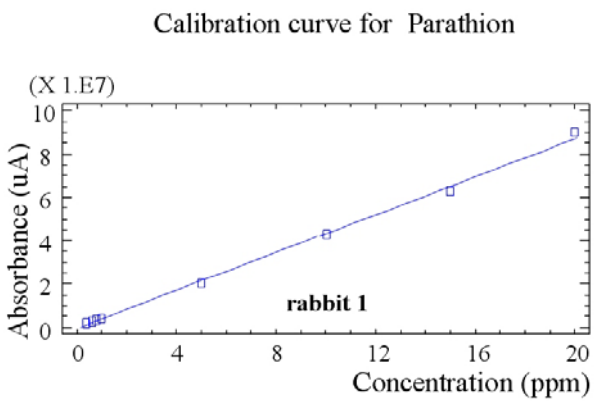

Fig. 1. *Calibration curve for parathion by HPLC*

Retrieval

An exact quantity of control larvae was weighed (6 in total), four of them were dosed with 200 µl of methyl parathion at 5 ppm. They were put through the same process as the initial sample. For the final extraction, in two of the dosed samples the analyte was extracted with 3 portions of 0.5 ml ethylic ether GR and the other two with the same quantity of reactive grade Toluene. The other two were treated as a control. A recovery of 82% was found for the dosed rabbits, showing that the extraction method was reliable.

Results

Arthropod Succession

Over a period of 28 days, a total of 987 individuals were collected, belonging to 10 orders, 34 families, 17 genera and 7 species.

Each of the rabbits went through 5 stages of decomposition: fresh (day 0 to 2); bloated (day 3 to 5); active decomposition (day 6 to 9), advanced decomposition (10 to 13) and dry remains (day 14+). However, in S1 a mummification stage was seen from day 12 to 24, not observed in the other rabbits.

Detection of Parathion in the liver

The livers showed a high concentration of parathion. Values between 1.4 mg and 3 mg of Parathion per Kg of rabbit were detected. No presence of the product was found in the control (fig. 3 and 4, tab. 2).

Sample	Weight (g)	Area (UA)	mg / Kg
Rabbit 1 Liver	0,5312	60522390 ± 200097.08	1,38
Rabbit 2 Liver	0,6825	148278472 ± 239228.37	2.97
Rabbit 3 Liver	0,9374	87589624 ± 1066225.8	1,68

Tab. 2. *Values for the detection of Parathion in the liver samples*

Detection of Parathion in arthropods

A total of 53 specimens, collected at different stages of decomposition of the rabbits were analysed for parathion: 29 diptera (24 larvae, 3 pupae, 1 pupa case, 1 adult), 13 coleoptera (adults), 6 hymenoptera (adults), 1 hemiptera (adult), 1 isopod and 3 acarids (adults).

Parathion was detected in the following diptera: *Phaenicia sericata, Musca domestica, C. Albiceps, Fannia scararis*, coleoptera: Tenebrionidae, Dermestidae and Staphylinidae, and in ants of the genus *Doymyrmex*, in 1 isopod and acarids. However, the small amount assimilated only allowed its detection, not quantification (Tab. 3 and 4).

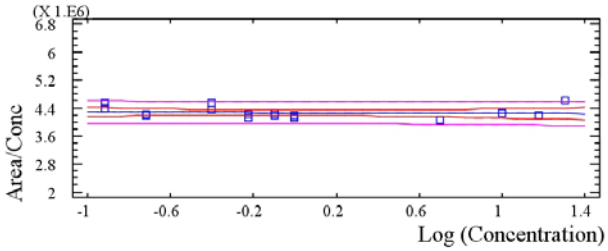

Fig. 2. *Evaluation of the linearity of the calibration curve (90-110% confidence limit)*

Conclusions

The presence of parathion only repelled arthropods or had insecticidal effects at the mouth of the treated rabbits. No relevant differences in insect succession were observed between the four rabbits. This contrasts to observations with Malathion, where oviposition was delayed and differences were seen in the number of taxa found on carcasses with or without the insecticide [24].

Phaenicia sericata was the first to arrive at the bloated stage and remained until the end of the active decomposition. The same was true of *Cochliomyia macellaria*, although it was observed in smaller quantities.

The process of mummification observed in rabbit 1 meant that the dry remains stage began later than in the other rabbits. This may have been because the carcass was located in a sunnier area causing the body to dry out, avoiding the stages of putrefaction.

It was possible to detect and quantify appreciable levels of parathion in the liver samples by means of HPLC. As was expected, parathion was not detected in the control.

With regard to the detection of parathion in the insects, its extraction was possible from 10 specimens of the following orders: Diptera, Coleoptera, Hymenoptera, Isopoda and Acari in different stages of

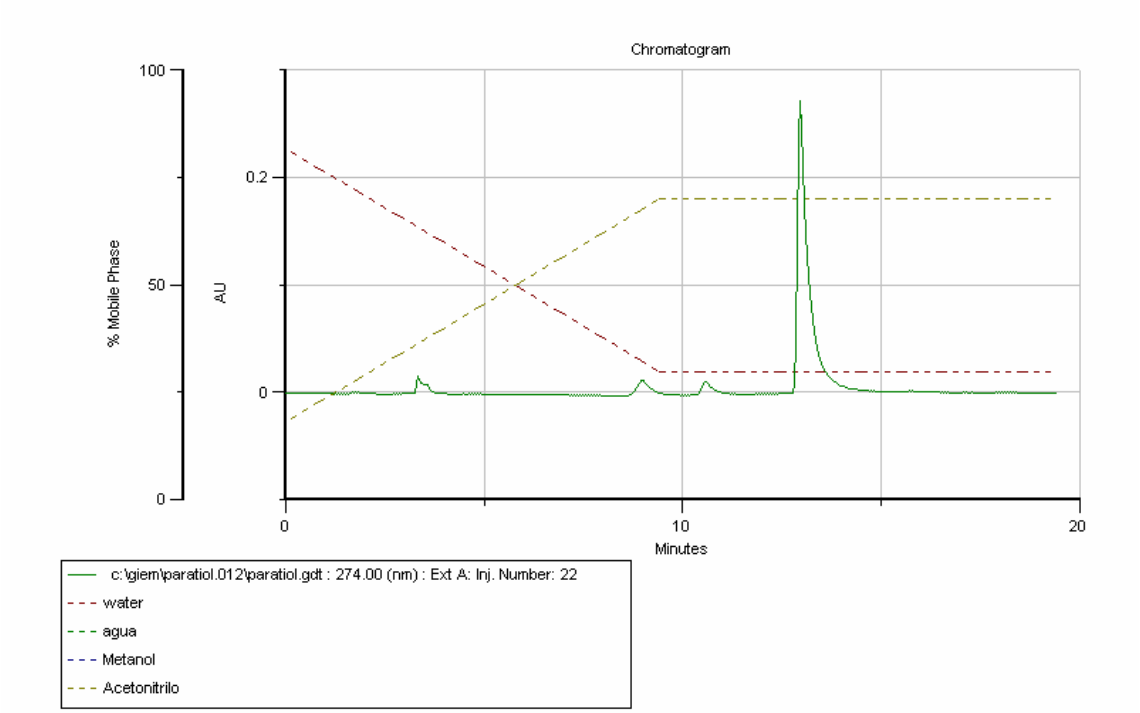

Fig. 3. *Chromatogram for Methyl Parathion extraction from the liver of rabbit 1.*

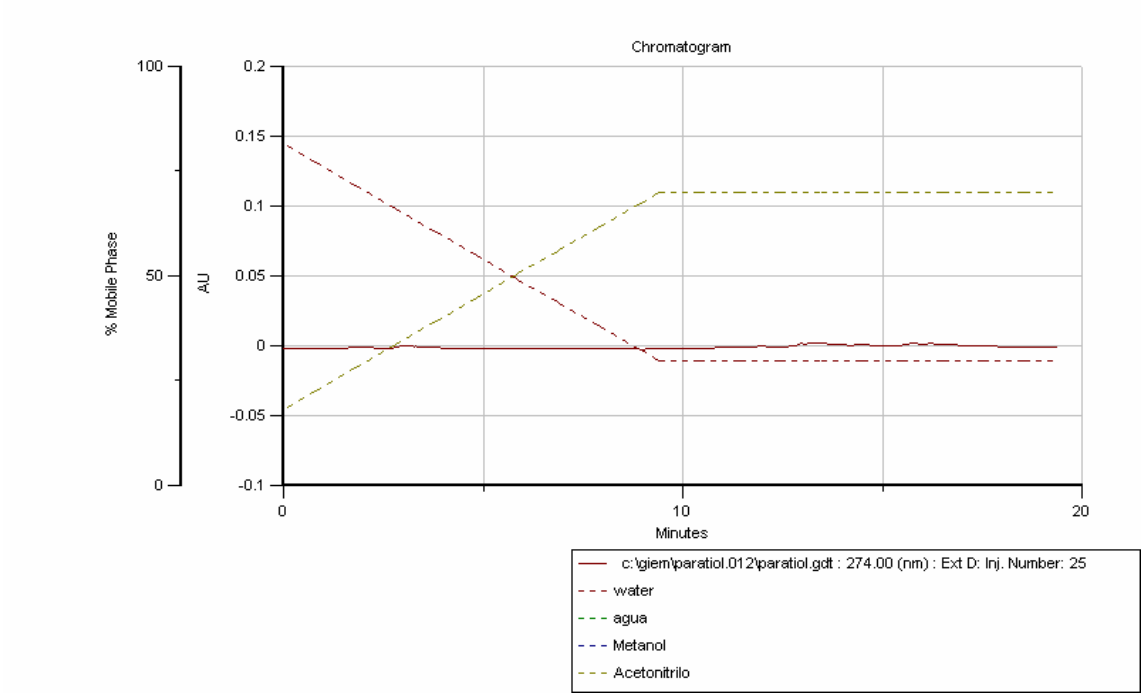

Fig. 4. *Chromatogram for Methyl Parathion extraction from the liver of the control rabbit.*

development (larva, pupae, pupae cases and adult) and at all stages of decomposition. At the bloated stage, parathion was detected in 3 larvae of *P. sericata*. At active decomposition in L3 of *C. albiceps* and in pupae and pupae cases of *M. domestica*. The highest number of species found containing parathion was at the dry remains stage; in pupae of *Musca* sp, in adults of Coleoptera (Dermestidae, Tenebrionidae and Staphylinidae), in *Dorymyrmex sp* ants, which were very abundant throughout the study. Parathion was also detected in isopods and acarids, which despite their small size accumulated high enough levels to enable its detection. This is probably due to their chitinous epidermis [3].

High Performance Liquid Chromatography (HPLC) is an efficient technique for detecting and quantifying parathion in tissues as well as in arthropods present from the initial to the final stages of decomposition, including dry remains.

Sample	Material analysed		Stage	Average area	Obs.
Rabbit 1	Diptera	*P. sericata*	L3		ND
		P. sericata	L1		ND
		M. domestica	L3	20540,43	NQ
		Muscidae	Pupa	393,50	NQ
		Stratiomyidae	L3		ND
		Stratiomyidae	L3		ND
		Stratiomyidae	L3		ND
	Coleoptera	Tenebrionidae (*Strogylium sp*)	Adult		ND
		Tenebrionidae (*Strogylium sp*)	Adult		ND
		Tenebrionidae (*Strogylium sp*)	Adult	23201,88	NQ
		Dermestes sp	Adult		ND
		Dermestes sp	Adult		ND
		Dermestes sp	Adult	194,96	NQ
		Staphylinidae	Adult	22423,72	NQ
	Hymenoptera	*Dorymyrmex sp*	Adult	437,13	NQ
		Dorymyrmex sp	Adult	335,45	NQ
	Hemiptera	Pentatomidae	Adult		ND
	Isopoda		Adult	535,85	NQ
	Acari		Adult	569,99	NQ
			Adult	1155,43	NQ
			Adult	645,94	NQ
Rabbit 2	Diptera	*P. sericata*	L1		ND
		P. sericata	L3		ND
		P. sericata	L3	6174,07	NQ
		M. domestica	L3		ND
		M. domestica	L3		ND
		M. domestica	L3		ND
		Muscidae	Pupa	102307,71	NQ
		Muscidae	Pupa case	115883,31	NQ
		Sarcophagidae	Adult		ND
		F. scalaris	L3		ND
		F. scalaris	L3		ND
		F. scalaris	L3		ND
	Coleoptera	*Dermestes sp*	Adult		ND
		Dermestes sp	Adult		ND
	Hymenoptera	*Dorymyrmex sp*	Adult		ND
		Dorymyrmex sp	Adult	133869,04	NQ
		Dorymyrmex sp	Adult		ND
Rabbit 3	Diptera	*P. sericata*	L3		ND
		P. sericata	L3	22689,37	NQ
		P. sericata	L3		ND
		C. albiceps	L3	22937,76	NQ
		M. domestica	L3		ND
		M. domestica	L3		ND
		M. domestica	L3	22852,53	NQ
		M. domestica	L3	85753,965	NQ
		M. domestica	L3		ND
		Muscidae	Pupa		ND
		F. scalaris	L3	41883,86	NQ
	Coleoptera	Tenebrionidae (*Strogylium sp*)	Adult		ND
		Tenebrionidae (*Strogylium sp*)	Adult		ND
		Tenebrionidae (*Strogylium sp*)	Adult		ND
		Tenebrionidae (*Strogylium sp*)	Adult		ND
	Hymenoptera	*Dorymyrmex sp*	Adult		ND

ND: not detectable, NQ: not quantifiable

Tab. 3. *Arthropods analysed by HPLC to detect parathion*

Order	Species	Dev. Stage	Stage of decomposition
Diptera	*P. sericata*	L3	Bloated
	C. albiceps	L3	Active D
	M. domestica	L3	Active D/advanced
	F. scalaris	L3	Dry remains
	Muscidae	Pupa and pupa case	Dry remains
Coleoptera	*Strongylium sp.*	Adult	Dry remains
	Dermestes sp.	Adult	Dry remains
	Staphylinidae	Adult	Dry remains
Isopoda		Adult	Bloated
Acari		Adult	Active D/advanced/ Dry remains
Hymenoptera	*Dorymyrmex sp.*	Adult	Fresh/Dry remains

Tab. 4. *Arthropods with positive detection of parathion, stadium of development and stage of decomposition at which it was collected.*

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Forensic evaluations on a crime case with monospecific necrophagous fly population infected by two parasitoid species

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Abstract

The entomoforensic investigation carried out on a decaying corpse found indoor has shown an unusual insect cenoses, that created some problems in estimating the actual time since death. Except for very few beetles, the body had been colonized by a monospecific population of the fly, *Hydrotaea capensis* Weidemann 1818 (Diptera, Muscidae), parasitized by two species of parasitoid wasps of the superfamily Chalcidoidae: *Nasonia vitripennis* (Walker, 1836) (Hymenoptera, Pteromalidae) and *Tachinaephagus zealandicus* Ashmead 1904 (Hymenoptera, Encyrtidae). The possible implications of the parasitoids on the fly population growth and density, the life cycle length, the maggots' and pupae survival and their hindering the PMI evaluation and other entomologico-forensic inferences are discussed.

Keywords: Forensic Entomology, P.M.I., Fly monospecific population, *Hydrotaea capensis*, Fly parasitoids, *Nasonia vitripennis*, *Tachinaephagus zealandicus*

Introduction

The entomoforensic science is nowadays used mostly for estimating the postmortem interval (P.M.I.) [1-10], even if various new applications, such as drug and chemical assumption, toxins or xenobiotics presence, abuse and rape before the death and so on [11-13], are in progress. The time since death is calculated by taking into account the largest number of variables, both intrinsic to the involved species and extrinsic to the insects, interfering one other. Among the first group of variables, we can include the kind of species feeding on the remains, the number of specimens, the time of development of the species, the composition of arthropod biocenoses, the successional patterns, the autoecological and synecological features of each species.

In the second group we can include all the external factors referred to the macro-ecosystem of the environment and to the micro-ecosystem of the body: the kind of biotope, the season, the climatic conditions, the composition of the local fauna and flora, the soil or litter composition, xenobiotic pollution, treatments with pesticides or other anthropic interferences. Regarding the corpses we have also to consider, the position and the state of the body (indoors, outdoors, burned, buried, hanging, under branches, underneath or inside plastic bags, etc.), the age and the general status (newborn, young, old, ill, whole, torn, with injuries or wounds, etc.) and the rate of decomposition of the human remains.

Nevertheless the most widespread, and less known, biotic factor affecting the growth and the survival of the insects is the presence of their parasites and parasitoids[1]. Arthropod parasitology is an immense world, only a very little part of which has been studied so far: the parasitology of useful bred insects (honey bees, silk worms, etc.), the parasitology of arthropods of medical and veterinary interest (considering the arthropods as vectors of parasites pathogenic for humans or animals) and, only recently, the parasitology applied to biological pest management. The last applications which include the control of fruit flies, in agriculture, and the elimination of flies inducing

[1] A parasitoid is a symbiotic species that always kills its host.

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myasis, in the veterinary science, employ parasitoids more than true parasites.

Many species of Hymenopteran have been introduced for such purposes, often in an uncontrolled fashion in countries and environments far away and different from their native area.

The pest flies, against which this biological control is performed, are often the same species or very closely related to the species found in corpse, and parasites and parasitoids are not strictly selective. Therefore we can reasonably suppose that many of them could also infest the flies used as PMI indicators, altering their development or killing them and, consequently, hindering entomoforensic evaluations [14, 15].

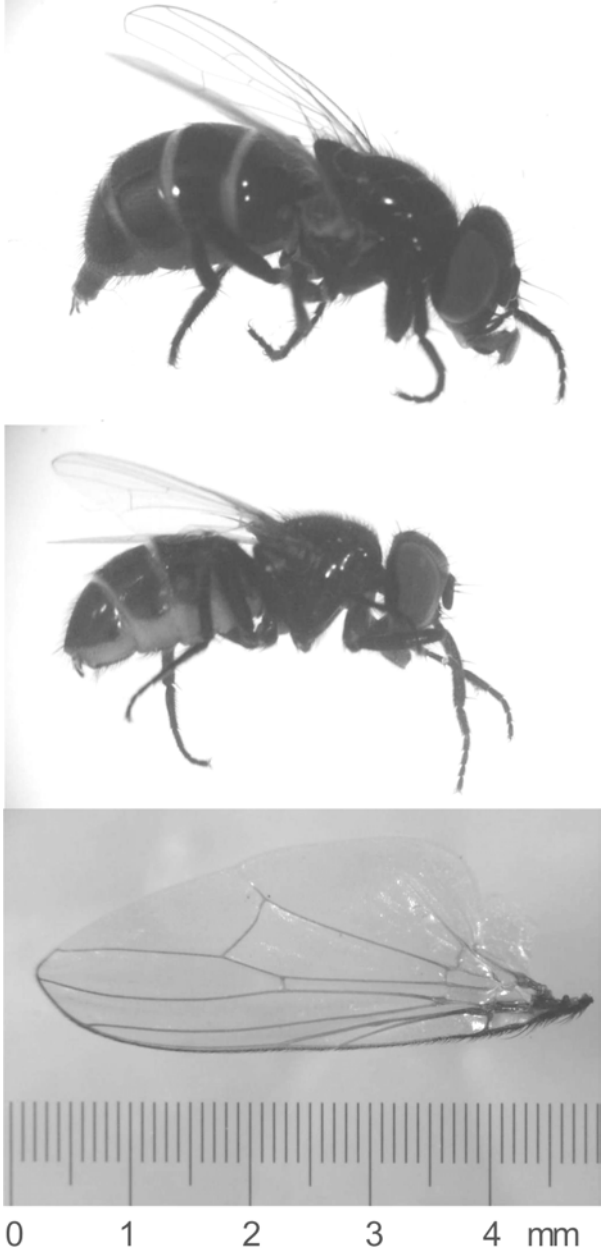
Case history

Crime scene

In mid September 2000 in a house of Eraclea, a little town in the inland of Venice (N-E Italy), the decaying remains of a young woman were found. The house, empty and with tightly shut doors and windows, Venetian blinds included, had not been inhabited for long time. The environmental temperature had been warm during the summer (25-30°C daily average) whereas indoors a temperature a few degrees lower, but more constant, had been recorded. The body, partially dressed, lying face-downward, was near the front door. The tiled floor and the walls bore trace of dried blood, as if the women had dragged herself towards the way out. The uncovered skin was drying out, except for the organic matter, in ammoniacal fermentation, protruding out from a wound in the skull (the crime weapon was later identified as the hooked support of a radiator). No papers nor other objects identifying the woman were found.

Fig. 1. *Full view on puparium*

Forensic entomology remarks



The investigators called us saying that the body was full of “a lot of worms and mouse faeces”, instead, as we arrived at the scene of the crime we found a very large number of maggots, pupae and puparia. The fly larvae of all instars were feeding on the uncovered skin parts and, among the hairs, on the matter in ammoniacal fermentation, whereas many pupae and pupal cases were found on the driest parts and especially on the clothes. Very few necrophagous beetles were present, while no other arthropod was found in the room. It must be presumed that the insects had come in through a little space under the door, attracted by the cadaver’s smell. At first sight, both the maggots and the pupae seemed to belong all to just one species. Pupae and puparia were characterized by two conspicuous prothoracic horns (fig. 1); some flies had successsfully emerged as indicated by an empty puparium missing an apical hood, while others pupae

had been killed as indicated by one or two parasitoid emergence holes.

The largest possible amount of beetles and flies was collected from the corpse at the crime site and immediately taken alive to the our laboratory, in the Biological Department, University of Padova, a few kilometers away from the death recovery site. Through an additional collection, made in the mortuary during the autopsy, the same type of specimens were collected.

Materials and methods

In the laboratory the few Coleoptera found (4 adults, 6 pupae and 2 larvae) were prepared and determined with specific keys [5,16-18].

The maggots were certainly all of the same species, but they appeared to be of a species not common on carrion and not easy to identify. A portion of the collected larvae, of each instar, were killed by dropping them into hot water and then stored in alcohol 70% for further study [5,19]. The remaining specimens were reared to obtain the adults for a sure species identification and to calculate the length of the complete life cycle.

Breeding was carried out in small cages covered with a fine-meshed net, with damp sand on the bottom and the maggots were fed on rotten minced meat [19,20]. Temperature and humidity were maintained near to those measured inside the house at the moment of the crime discovery (25°C; 70% rh., in average). Individuals beginning to pupariate were daily carefully collected and transferred into dryer cages, as naturally occurs, without food and wet sand, to avoid the bacterial and fungal attack during the quiescent phase.

The emerged adults (fig. 2) were collected and some of them were dry prepared, pinned and determined, while others were transferred alive into new clean cages with food and wet sand. These were mantained through two generations to study two full life cycles. With the intention of obtaining diapausing pupae, the offspring of the third generation were kept without food in a dry, airtight jar and left in a cooler thermostatic room (10°C) for six months.

Rearing the flies, we also obtained some small parasitoid wasps, responsible for the holes observed in the pupa cases (fig. 3). Some adults were sent to taxonomic specialists for species' determination.

All the observations of the insects or of the diagnostic parts of them were performed under a stereomicroscope (Leica MZFL III) and a microscope (Leica DMR); photographs were taken with a digital camera (Nikon D1X and Leica DC 200).

Results and Discussion

The collected beetles belonged to two families, usually associated with carrion: the skin beetle *Dermestes maculatus* Payne (Coleoptera: Dermestidae) and the bone beetles *Necrobia rufipes* De Geer and *Necrobia violacea* L. (Coleoptera: Cleridae). In Italy *D. maculatus* and *N. rufipes* are widespread beetles, while *N. violacea* is not a common species, reported only from the northern regions [16,21] and never cited, as far as we know, in the forensic literature. According to Mégnin [1] and Smith [5], these insects are typical of the 5th-7th waves, arriving at the remains after the butyric fermentation, when the carrion is drying out or is almost dry. Many other findings confirm the time of their arrival in the last stages of decay. They are gnawers on dry skin, sinews and bones and probably maggot predators [5,22]; nevertheless in our case their small amount could not affect the fly population.

Fig. 3. *Emerged adults (parasitoids)*

The identification of the collected maggots and pupae and of the adults from the rearing confirmed that the body had been colonized by a monospecific population of the genus *Hydrotaea* Robineau-Desvoidy (=*Ophyra*) (Diptera:Muscidae). The genus was determined using as diagnostic features the mouthparts and the anterior and posterior spiracles (fig. 4.1) of the larvae [23-25] and the prothoracic spiracular horns of the puparium (fig. 4.2). The species was determined as *Hydrotaea capensis* (Widemann, 1818), determination confirmed by A. Pont (personal communication, 2001)[2]. This is a

[2] A. Pont distinguishs *Hydrotaea capensis* Wiedemann from the very similar american species, *H. aenescens* Wiedemann, by the palpi, black in the first and yellow in the second.

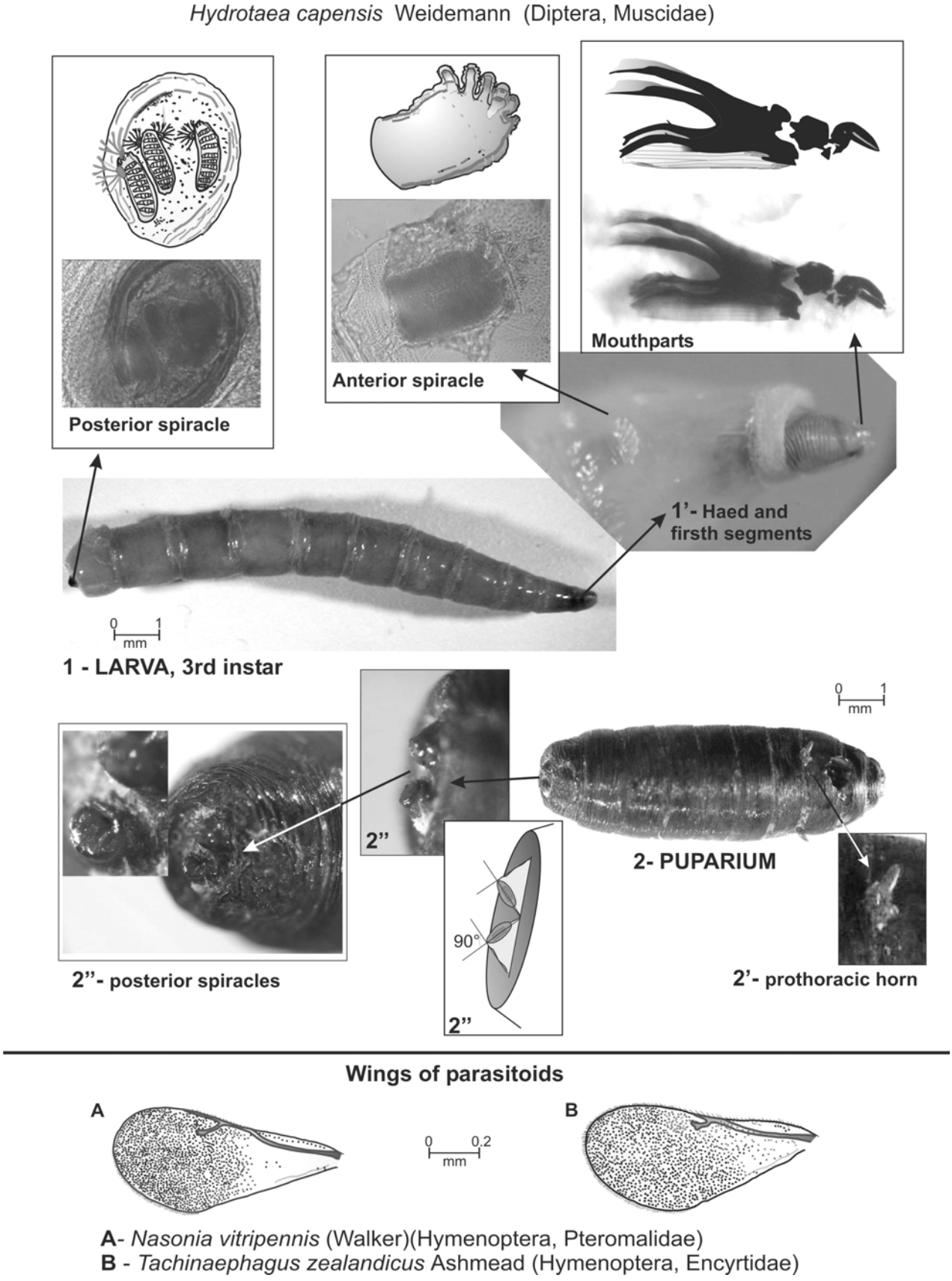


Fig. 4. *Anatomical features*

Mediterranean and Afrotropical species, known, but not widely in northern Italy [26-28].

According to many Authors [26,29,30] different species of the genus *Hydrotaea* feed on dry faeces, manure, dung, carcasses and lavatories in the summer, the adults being most abundant in August. In human remains the species appears during the period of ammoniacal fermentation (i.e. four months after death and over); K. G. V. Smith [5] wrote that "if the body has not been exposed to open air for that period of time, these may be the only maggots or puparia present and the time of their arrival may be affected" and this seems to be the same situation we found. Mégnin [1] found flies of this genus in corpses about one year old. From the rearings we obtained two complete cycles of *H. capensis*, from eggs to adults. In both the experiments, adults emerged after 12-17 days (the whole life cycle being of 15 days, in average, at 25°C).

Together with the adult flies, in the rearing cages some adults of parasitoid wasps were also found, belonging to two different species of Hymenoptera Chalcidoidae. Specialists identified them as the Pteromalidae *Nasonia vitripennis* (Walker, 1834) and as the Encyrtidae *Tachinaephagous zealandicus* Ashmed, 190[4] [31] (fig. 4 A, B).

Hydrotaea capensis

breedings	time of eclosion	pierced puparia
1st	12-17 days	5%
2nd	24-30 days	10%
3th	6 months	36%

Tab. 1. *Time of eclosion of* Hydrotaea capensis *and percent of parasitized papae and puparia*

The former is a polyphagous species, living in various habitats, and which may also parasitize several Cyclorrhapha dipterans [32-34]; its gregarious larvae develop inside the fly pupae, attached to the host's nymphs and also into maggots paralyzed by their venom or, as idiobiont ectopararasites, on nymphs of Cyclorrhapha flies. Cosmopolitan in its distribution, this species is the only European of the genus.

The latter wasp species, *T. zealandicus*, probably native to Australia and New Zealand, is reported as a parasitoid of synanthropic flies (Calliphoridae, Muscidae, Sarcophagidae), that attacks the third instar of the maggots, the postfeeding or the prepupae [35, 36]. It has been introduced into the Southern Hemisphere and several States of U.S.A., as a biological control agent against myasis-inducing flies [37-40]. This is the first record of *T. zealandicus* for the Paleartic Region [31].

The number of holed puparia in the two experiments were 5% and 10% respectively. In the experiment with the maggots kept in the jar hermetically closed, after six months we found 66 pupae and puparial cases: 42 unholed and 24 pierced (36% of the total), 20 of which by a single hole, 4 by two or three holes (Tab I). The only wasps present were, in the last experiment, *N. vitripennis*, species reported as winner competitor, in the long run, over other parasitoids [41]. The dissections yielded some unholed still pupae, parasitized by 6-8 adult wasps, ready for eclosion (fig. 3), as observed by Nasser and Eraky [42].

Conclusions

This murder appears as a textbook case, as if it had been performed in laboratory under controlled conditions. The indoor environment was constant in temperature and humidity, the insect fauna was very simple, and there was no evidence that a vertebrate had disturbed the corpse. Crime scene conditions were reproduced in our laboratory in order to establish the length of the whole life cycle of *Hydrotaea capensis*, a datum not reported in literature, for an estimation of the exact time when this crime was committed. In fact in long-term deaths the medico-legal inferences about the time since death are very difficult inasmuch as the degradative process decelerates, getting to a final steady state. In most cases a criminal investigation and a medical evaluation may be aided by the entomological observations.

However in this case, owing to the lack of other insects, usually present in the early stages of decomposition, it was only possible to state that: the murder was committed into the house (the same room), the body had been inside for four months or more and nobody had come in before the police's discovery of the corpse. The presence of only a *Hydrotea capensis* population and the lack of the usual cadaveric fauna could be explained by the physical isolation of the remains and by the ability of *H. capensis* to find an indoor corpse [5].

Final remarks

Until today, lacking any kind of clue (neither documents nor personal objects, nor registration of the fingerprints, nor even adisappearance report), the victim has not been nameidentified. All that it is possible to say about her is that she was a young Caucasic woman, probably from Eastern Europe. The entomological inferences agree with those of the forensic-pathologists and legal-doctors: death had occurred at least four months prior to discovery. Surely the lack of the necrophagous biodiversity and the presence of the parasitoids did not help the entomologists to precisely establish the PMI.

The person guilty of this murder has not yet been found, as it often happens when the crime involves irregular immigrants, but it may be linked with prostitution or drug dealing.

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Information is Everything - A Case Report Demonstrating the Necessity of Entomological Knowledge at the Crime Scene

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Abstract

The authors present a case where a man was found shot on the edge of a forest. Lack of information produced various difficulties in entomological PMI-estimation. The calculation was first based on the information that the man had lain on his back and the larvae were collected from his face. Furthermore all of the larvae had been stored in 70% alcohol solution and no further development under laboratory conditions could be observed. The determination of the eldest organisms as 2nd instar larvae of *Calliphora vomitoria* Linné could be achieved by PCR-RFLP identification.

With data from the local weather station first a PMI of 5 days was calculated, overlapping with an interval when the deceased still had been seen alive. Further investigation revealed that the dead body was originally found with a leather jacket over his head that was removed by the emergency physician. Assumption of higher environmental temperature for the development of the maggots was leading to a PMI of 4 days which was in accordance with results of the police investigations. The necessity of comprehensive investigation of the environmental conditions influencing the growing rate of blowfly *larvae* and a more widespread education and training of persons involved in crime scene work as a possible task for forensic entomologists is discussed.

Introduction

Forensic Entomology as a valuable tool for PMI-estimation became more and more popular recently. The developmental rate of the necrophagous insects used for calculation highly depends on external factors, e. g. on the ambient temperature. For a precise PMI-estimation a appropriate collection of the colonizing organisms is required
[1-5,8].

Case Report

A 37-year old man was found shot July, 21st , 11.48 a. m., on the edge of a forest in Northern Germany.

Neither forensic pathologists nor entomologists were present at the crime scene. A post mortem was performed and as cause of death a pericard-tamponade due to a gunshot injury was diagnosed. An additional gunshot caused a fracture of the right jaw bone. Green discoloration of the abdominal wall was reported by the pathologists, no more *rigor mortis* was detected. In the course of the autopsy fly larvae and eggs were collected ”from the face” of the deceased and sent to our department for PMI-estimation.

3 samples stored in 70% ethanol arrived at our department, 2 of which contained fly eggs and one with several blow fly larvae. The ethanol solution of the last sample showed brownish discoloration, indicating that the maggots might have been feeding on blood. No living organisms were available. The larvae with a length ranging from 6 to 7 mm, were diagnosed as 2nd instar Calliphoridae on binocular investigation. As commonly known a further morphological determination in this developmental state - indispensable for PMI-estimation - has no real chance of success. Therefore PCR-RFLP identification of the larvae was applied [7,9] and the larvae could be determined as *Calliphora vomitoria* Linné, the eggs as *Lucilia sericata* Meigen and *Calliphora vicina*

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Robineau-Desvoidy. The meteorological data were available from the nearest weather station with a maximum of 20°C and a minimum of 12°C, mean: 16,8°C (tab. 1).

A PMI of 5 days was calculated based on the data of Marchenko [6], referring to a total developmental time egg to imago of 33,7 days for 17°C and an assumed developmental period of 15% for the 2nd instar *larvae* investigated. These results were also in accordance with our own data [10]: Under laboratory conditions with a constant temperature of 20°C concerning larvae of *Calliphora vomitoria* L. after 4 to 5 days a length of 6 to 7 mm was measured. The PMI calculated would have led to the conclusion that the deceased died at July, 16th. However the police investigation revealed that the man was last seen alive 9.30 in the morning of July, 17th. This was stated by his girl friend whom the police thought to be absolutely reliable.

Fig. 1. *Original position the dead body was found with a leather jacket over his head. Note the accessibility for flies.*

After discovering these irregularities a comprehensive reinvestigation of the circumstances of the case in its entirety was required. A reconstruction of the events at the crime scene revealed that the dead body was first found lying on his right side with a leather jacket over his head, which was removed by the emergency physician (fig. 1).

	17.07.	18.07.	19.07.	20.07.	21.07.
maximum	20°C	16°C	18°C	19°C	20°C
minimum	18°C	15°C	14°C	12°C	(13°C)

Tab. 1. *Meteorological data*

Discussion

The ambient temperature is one of the main factors influencing the developmental rate of necrophagous insects [1-5,8]. The specimen age first calculated in this case was based on the information that the corpse had lain on his back and the maggots were collected ”from his face”. The circumstances, i. e. that the dead body was found with a leather jacket over his head, lead to the conclusion that the eggs and larvae were exposed to a higher temperature than first assumed. On inquiry it was finally reported by the pathologists who performed the autopsy that the maggots were actually collected from the oral cavity which was filled with blood due to the fracture of the jaw bone. As shown in fig. 1, the face of the deceased was not totally inaccessible for flies. Regarding the circumstances it seems very likely that the flies of *Calliphora vomitoria* L., which is known to be chiefly a forest species in Europe [8], were attracted by the bleeding wound and deposited their eggs close to the oral cavity. At this moment the body temperature might still have been very warm and probably decreased slowly in this area, because the face was covered. It also can be assumed that after the larvae hatched and migrated into the oral cavity due to the attractive feeding conditions that they remained constantly in this protected environment where the temperature was rather close to the maximum ambient temperature or even higher. Under these conditions a PMI of 4 days seemed more likely, meaning that the man was probably shot shortly after he was last seen alive by his girl friend. This was also in accordance with the police investigations.

Conclusions

Lack of information on both sides produced apparent inaccuracy in entomological PMI-estimation. If the time of death may be of interest in cases with insects colonisation of human corpses, preferably a person with experience in the field of forensic entomology should be present at the crime scene. Due to the enormous influence of ambient factors careful and comprehensive history taking is required if the entomologist responsible for PMI-estimation cannot visit the corpse at the scene. Collecting living organisms from the dead body and observing their further development under laboratory conditions leads to much better results in PMI-estimation because data from the particular species can be obtained and referred to. Also species determination is mostly difficult and

sometimes impossible with young larvae but indispensable for calculating the time of death. The persons first involved in the presented case, policemen and pathologists, were apparently not familiar with these facts. Fortunately in the presented case the important species for PMI-estimation, *Calliphora vomitoria* L., could be identified by PCR-RFLP analysis, which would not have been possible for various other species. Regarding the possible consequences a more detailed education and special training of policemen, pathologists and other persons involved in the work at the crime scene as a task of forensic entomologists should be considered.

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A forensic entomology case from the Southeastern Iberian Peninsula

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Abstract

We present a forensic case in which the time of year when death occurred was established from the entomological evidence after medical examination had been unable to offer any precise information regarding the same, proffering, instead, a time that ran from three months to one year or more. The entomological evidence consisted mainly of Coleoptera, although remains of Diptera were also collected. The minimum postmortem interval (PMI) was established by relating the entomological evidence with the available data concerning the sarcosaprophagous fauna in the geographical area and with our knowledge concerning the development of the principal Coleoptera.

Introduction

It is well known that a cadaver constitutes a dynamic system that shelters and supports a rich community, of which arthropods form an important part, not only because they consume decomposing tissue but also because they permit those same decomposition processes to speed up. The arthropod community varies with time, specific species that feed on the tissues or exudates appearing at a given moment of the decomposition process. The community is also affected by seasonal dynamics, certain species appearing in one season but not in others, This factor is of special importance when trying to establish the time of death in cases where the PMI is quite long. For this reason, too, knowledge of the sarcosaprophagus communities associated with different environments and of their seasonal dynamic is important in forensic practice.

This contribution describes a forensic case, for which entomological evidence provided a more precise answer when medical evidence offered too wide a range for the possible time of death. The data take on an added interest because they are taken from a region where this type of evidence has not traditionally been taken into account in forensic science and for which, therefore, there is no reliable database which may be of use in future cases.

Case description

On 25 February, the corpse of an initially unidentified male was found on the floor of a room in Lorca (SE Spain), which did not receive direct sunlight (fig. 1). The cadaver was found in a prone position (fig. 2) dressed in a short-sleeved shirt and light-weight tracksuit top. The building was abandoned with access officially barred, although it must have been easy to enter because the body was found by playing children.

The cadaver was skeletonized (fig. 3) and showed bites of, presumably, rodents. Medical evidence suggested that death had been caused by craneo-cephalic traumatism. Entomological evidence was abundant and present in all parts of the body. In zones covered by clothes there were live insects. During the autopsy, which was carried out on 26 February, numerous Coleoptera moving over the joints and tendons were observed.

Fig. 1. *General view of the room where the corpse was found.*

Fig. 2. *General view of the corpse* in situ

Fig. 3. *Skeletonized cadaver: Detail of upper part of the cadaver* in situ

Fig. 4. *Adult* Necrobia rufipes *(Coleoptera: Cleridae)*

Fig. 5. *Adult* Dermestes maculatus *(Coleoptera, Dermestidae) -- note hair colour and pattern*

The entomological material collected during the autopsy was preserved live at room temperature and, after inspection in the laboratory, was preserved in 70% ethanol.

Fig. 6. *Remains of arthropod activity between clothes*

Fig. 7. *Peritrophic membranes of Dermestidae larvae*

Fig. 8. *Empty puparium of* Chrysomya albiceps

The entomological study was conducted with no additional information, such photographs, about the case.

Results

The material consisted of live adult samples of *Necrobia rufipes* (Coleoptera. Cleridae) (fig. 4), one live adult of *Dermestes maculatus* (Coleoptera, Dermestidae) (fig. 5), abundant remains of activity (faeces included in peritrophic membranes) (fig. 6,7) and more or less fragmented remains of Dermestidae larval exuviae, empty *Piophila casei* puparia (Diptera, Piophilidae), empty puparia of *Chrysomya albiceps* (Diptera, Calliphoridae) (fig. 8), one empty puparium of *Ophyra* sp. (Diptera, Muscidae) and two live caterpillars of Tineoida (Lepidoptera).

Discussion and conclusion

In the area in question, *Chrysomya albiceps* is by far the most abundant species of Diptera found in autumn, while in winter its presence is extremely rare. It is also common in summer, although in lower numbers than in autumn [1].

Ophyra sp. has been described in human cadavers from the fourth month after death and species of the genus have been described as being active between June and October [2,3,4], although no information is available regarding this genus in the area under consideration. As regards *Piophila casei*, they are known to be associated with advanced stages of decomposition. Although adults can be found in the first few days after death, oviposition and therefore preimaginal stages of development occur later, so that the evidence obtained in the cadaver pointed to a long PMI. There are no details referring to their preferred flight period, although Fuller [4] mentions the presence of adult *Piophila casei* at the end of summer. Benecke [5] considered *Piophila casei* to be typical of cadavers exposed for a period of 3-6 months.

As regards Dermestidae, adults specimens are known to appear in cadavers from a very early time, although their larvae, which are the real indicators of PMI, are only characteristic of the most advanced stages of decomposition, when the tissues are dry. According to Raspi and Antonelli [6], *Dermestes maculatus* only reaches full development when the temperature remains constant at above 18ºC, in which conditions, according to the same authors, the species takes 96 days after oviposition to reach the adult stage. However, this information must bear in mind the environmental characteristics of the region [7], where the mean temperature in autumn is 15ºC, with a large difference between the first and last days of the season (mean temperatures 20 and 10ºC, respectively). In winter the mean daily temperatures vary between 10 and 15ºC from the beginning to the end of the season. Bearing these temperatures in mind, we may conclude that Dermestidae development may well have been slower than in [6]. Whatever the case, the remains of Dermestidae feeding activity were abundant, although no larva or pupa was found, suggesting that the growth cycle had finished.

The Cleridae are predatory Coleoptera, although Payne and King [8] claim that *Necrobia rufipes* is only

a carrion feeder that is found in skeleletonized bodies. Fuller [4] observed recently emerged adults in four month old cadavers and Oliva [2] cites larvae, pupae and adults of this species alongside exuviae of *Dermestes maculatus*, puparia of *Ophyra* sp.and of Sarcophagidae in a six months old cadaver. This finding is of particular importance because the degree of similarity with our evidence as a whole.

Another pointer of a long PMI is the presence of Tineidae, which are commonly found in exposed cadavers since they complete their cycle in desiccated remains. However, because these specimens were not identified to the species level, their presence provides little additional help.

The evidence as a whole showed the synchronous presence of arthropods which are known to exist early (*Chrysomya albiceps*) and late (*Dermestes, Piophila*) in decomposing bodies. Of note is the fact that no remains of other Diptera of a primary character were found in the decomposing body**,** which may be due to different reasons: the strong competition with *Chrysomya albiceps* and *Ophyra* sp., whose larvae are predatory, the small presence of other species of Diptera or the fact that other species, such as *Phaenicia sericata*, or *Calliphora vicina*, are known to be much more likely than *Ch. albiceps* to crawl away from the corpse to pupate.

Insect samples were collected at autopsy, so any specimens that were away from the body would not have been detected. Since the cadaver was clothed, it is possible that, although most of the migratory larvae had left the body, a few specimens remained among the folds of the clothing, as occurred with *Chrysomya albiceps* and *Ophyra* sp. The sole presence of these two Diptera strongly suggests that other members of this order had been poorly represented, which, together with the predatory pressure of the other species, would have eliminated the evidence of their presence.

We considered *Chrysomya albiceps* to be the principal taxon present of the primary Diptera, which suggests colonisation of the cadaver at the end of summer or beginning of autumn, since this when *Chrysomya albiceps* is the most abundant species of Diptera [1]. This first seasonal indication pointed to this time of the year being the time of death and agreed with the other evidence found. The evidence as a whole indicated a minimum PMI of six months, which put the beginning of colonisation and therefore of death in the month of September (end of summer/beginning of autumn).

The case remains unsolved, but circumstantial evidence pointed to the man last being seen alive towards the end of the summer about 17 months prior to discovery the corpse. This fact accords with our estimate of the time of the year that death occurred according to the entomological evidence. On the other hand it also agrees with the estimation of a longer PMI made by the medical examiners who had access to the remains themselves.

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Insects as forensic indicators: methodological aspects

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Abstract

Forensic entomology is the study of insects associated with human corpses during legal investigations. Insect evidence can help to determine the time elapsed after death and other circumstances of each crime scene. Insects that first colonize a dead body usually belong to the Order Diptera and in particular to the families Calliphoridae, Sarcophagidae and Muscidae.

In the Botanical Gardens and Natural History Museum of Parma, Northern Italy, from April to June 2002 experimental trials using pig meat as bait were carried out to collect and define the insect fauna of forensic interest in an urban habitat. In these experiments Diptera and Coleoptera samples were collected, analysed and identified. Different climatic conditions such as temperature and relative humidity values were recorded to indicate the influence of biotic and abiotic factors on the insects' life cycle and on the entomological conclusions in a real forensic case.

Numerous individuals of the species *Calliphora vicina* (Calliphoridae), *Sarcophaga africa* (Sarcophagidae), *Megaselia scalaris* (Phoridae) and Coleopteran Dermestidae and Histeridae were sampled. The first two species were attracted to fresh meat and the third one was attracted to rotting meat and had a complete development cycle in indoors. Environmental parameters such as temperature and relative humidity variations can affect the presence and abundance of Diptera specimens and their life cycle as well as other arthropods.

Introduction

Forensic entomology is a discipline that can be used as a tool in crime scene investigations. Insects are usually the first visitors to a dead body and blowflies are able to oviposit on carrion within a few hours after death. Insects in criminal investigations could help an investigator estimate the post mortem interval (PMI), indicate that the body has been moved or disturbed after death, shed light on the causes and related circumstances of death (i.e. detecting the presence of drugs), match a suspect with the scene of a crime and produce information in cases of neglected children [1-3].

Insects are the most numerous group among the invertebrates in carrion, owing, in part, to their ability to quickly colonize different habitats. Recently, many studies on entomological fauna associated with criminal events have been carried out to better define insect groups as "forensic entomo-indicators". Many species belonging to the families Calliphoridae, Sarcophagidae, Fannidae, Phoridae, Drosophilidae and Piophilidae among Diptera and to the families Dermestidae, Cleridae, Histeridae, Staphilinidae and Tenebrionidae among Coleoptera associated with decomposing bodies have been identified from forensic institutes and police crime scene investigations [4-10].

The aims of this study are A, to determine which insects, Diptera in particular, first colonize a corpse and B, to better define their ethology by describing the development cycles and variability of these species as they correlate to climatic conditions in the urban habitat of Parma, Northern Italy. Comparing the species obtained from these experiments with those reported in literature, we propose a suitable and quick method to estimate the presence of insects associated with a dead body in an area in which a crime occurred that is climatically comparable to the one studied. The influence of parameters such as temperature, relative humidity, season and habitat on the activities and life

cycles of the insects collected in the two environments, potentially simulating two different crime scenes, are described.

Materials and Methods

The study was carried out in the Botanical Gardens and Natural History Museum of Parma in an urban habitat. As bait we employed pork meat with the knowledge that pigs are used in human research in both medical and forensic fields.

Six pieces of meat, weighing from 56 gr. to 90 gr. (fresh wt.) have been used. Each piece was placed in a Petri dish with 20-25 gr. of sterilized sand at the bottom. To avoid rapid desiccation, 5 ml of distilled water was added daily to keep the sand moderately moist.

The baits were located in an open area (in shade on a terrace) and were exposed for 72-90 hours to allow oviposition time of Diptera attracted to fresh meat.

Two experiments were carried out:

Exp. 1 from April 24 to May 17

To simulate the post-mortem movement of the body to another location after a bit of time, six Petri dishes were left exposed for 90 hr. After the exposure period, three Petri dishes with meat were transferred to a plastic container (length 40 cm, width 20 cm, height 20 cm), closed with gauze to avoid further Diptera oviposition and were moved to the top floor of a building in a room with glass walls after the exposure time. The remaining three Petri dishes were transferred to the same kind of container and were moved to a cellar with constant temperature and relative humidity values. This was a dark room with a window partially opened.

Exp. 2 from May 28 to June 24

Again, six Petri dishes were exposed on the terrace for 72 hr, three of the Petri dishes with meat were transferred to a plastic container (length 40 cm, width 20 cm, height 20 cm) and after the exposure time were closed with gauze to avoid further Diptera oviposition, and left on the terrace. To simulate the post-mortem movement of the body to another location after a bit of time, the remaining three Petri dishes were transferred to the same kind of pot and were moved to the cellar with constant temperature and relative humidity values as in the exp. 1.

During each experiment, pig meat pieces were monitored daily and data of ambient, temperature/humidity and atmospheric conditions were recorded. All insect forms (eggs, larvae, pupae and adults) were collected from the meat and their activity was described. In the laboratory collected samples were preserved in 70% alcohol; pupae and adults were preserved both in 70% alcohol and in a freezer (-4°).

Pupae and adults of Diptera and adults of Coleoptera were observed under a stereo microscope and identified to genus and family. For each group, further analysis to recognize species specific characters were carried out; some specimens were carefully dissected and morphological features mounted on slides and analysed.

Results

Three families of Diptera of forensic interest: Calliphoridae, Sarcophagidae and Phoridae, and three families of Coleoptera: Dermestidae, Histeridae and Staphilinidae, were collected during this study.

Among Diptera, *Calliphora vicina* Robineau-Desvoidy, 1830 (=*erythrocephala* Meigen, 1826), *Sarcophaga africa* (Wiedemann, 1824) and *Megaselia scalaris* (Loew, 1866) were identified [4,11-14].

During the first experiment *Calliphora vicina* (Fig. n. 1) was the only species ovipositing; 1730 specimens from a total of the 208 gr. (fresh wt.) of meat moved to the cellar, were obtained.

Regarding meat moved to the top floor of the building, a small number of blowflies reached the stage of pupa but eclosion of the adults did not take place. Pupae appeared extremely dried, it is likely that the strong daily fluctuations of the temperature and humidity (average maximum 37° and minimum 15°, relative humidity 80% and 38%) registered could have interfered with the blowflies development. This environment was not used in the second experiment.

During the second experiment 356 specimens of *Sarcophaga africa* (fig. 2) from a total of the 253 gr. (fresh wt.) of meat were obtained in outdoor conditions but no adults emerged due to 11% parasitism by Hymenoptera as well as a strong rainfall that submerged pupae. In indoor conditions 70 specimens of *Sarcophaga africa* and only two samples of *Calliphora vicina* developed into adults.

It can be assumed that oviposition of *Calliphora vicina*, occurring in late April, was replaced by *Sarcophaga africa*, being the dominant species larvipositing in late May.

Also in indoor conditions, *Megaselia scalaris* (Phoridae) was identified, using the taxonomic keys of the Natural History Museum of London [13]. The genus *Megaselia* Rondani, 1856, includes around 1400 described species but it is thought it could be between 5,000 and 20,000 species [13]. In the experiment more than 2000 specimens of *M. scalaris* were counted and the adults showed sexual dimorphism in which female size was 4.11 cm of length with a standard error of 0.09 (N=24) and male size 2.72 cm of length with a standard error of 0.06 (N=24). The Phoridae is a family, in which larva is always enclosed in the puparium, characterised by the presence of respiratory horns.

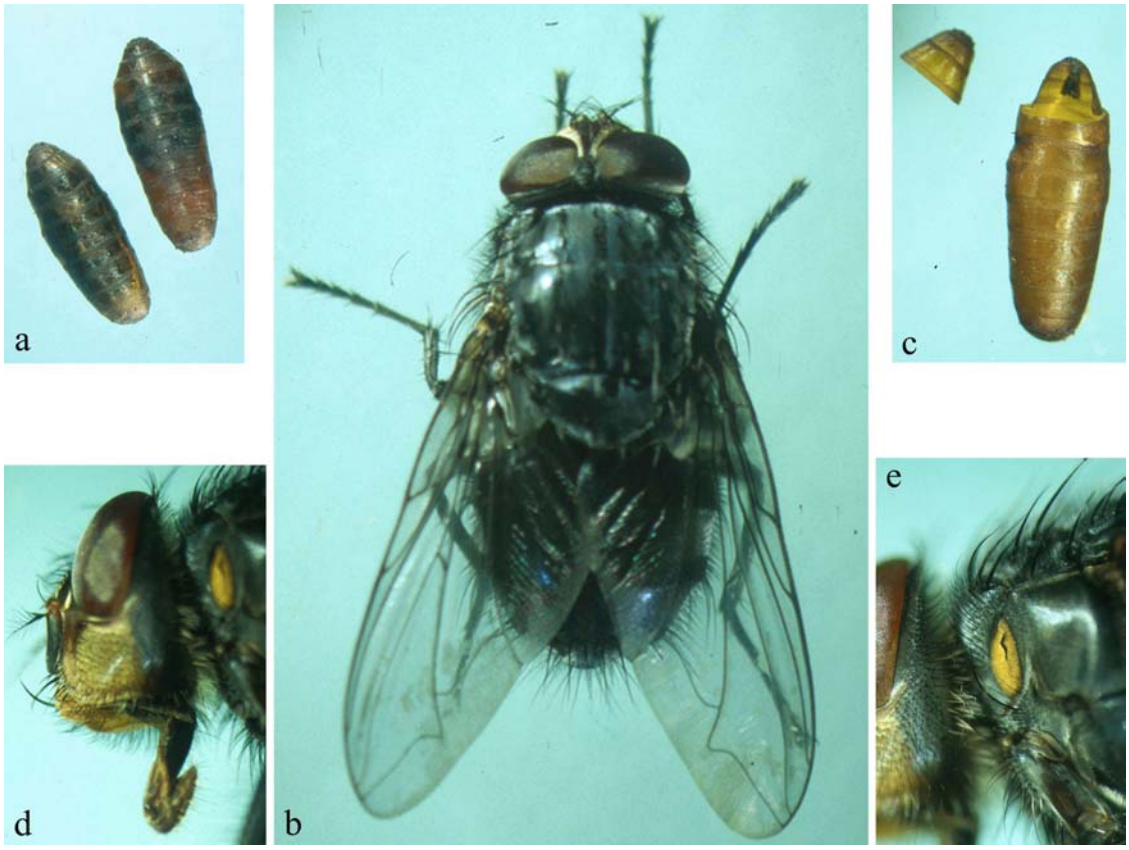

Fig. 1. Calliphora vicina *Robineau-Desvoidy (= erythrocephala Meigen), a) pupae (on the left length 7.1mm x width 3.0 mm; on the right length 7.5 mm x width 3.0 mm); b) adult (thorax length 4.1 mm); c) empty puparium; d) head in lateral view; e) anterior spiracle.*

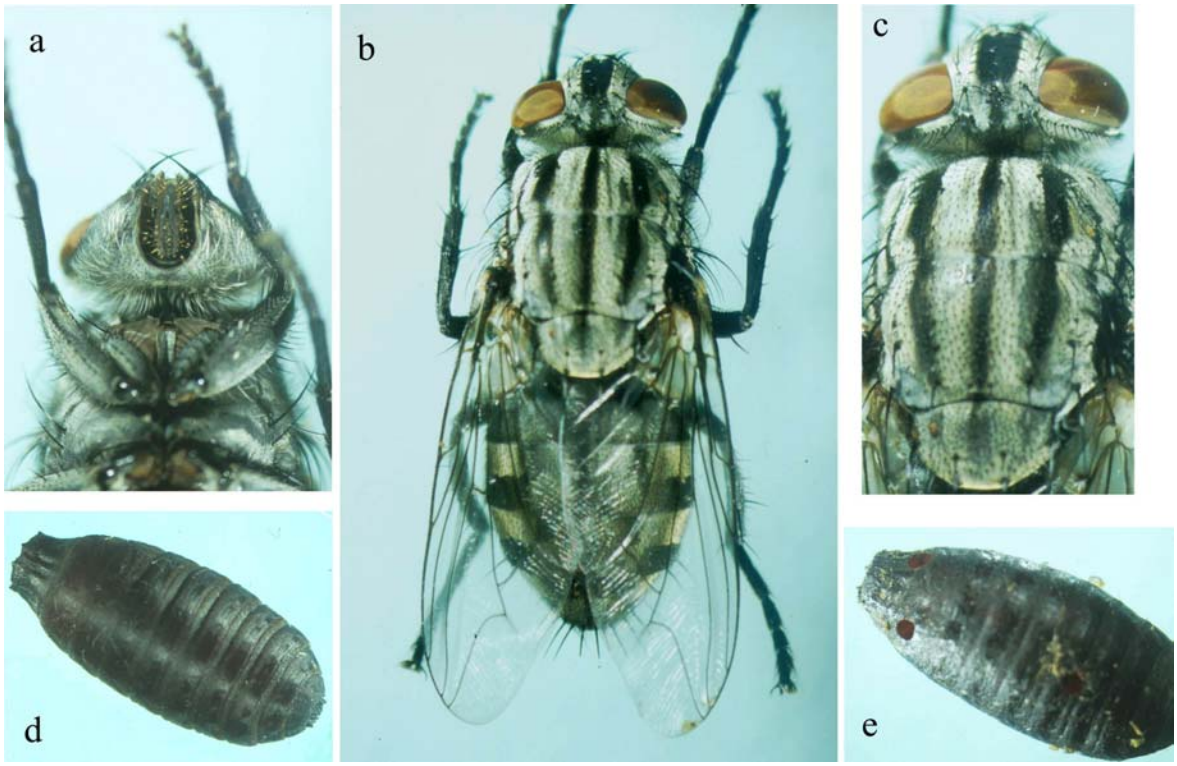

Fig. 2. Sarcophaga africa *Wiedemann, a) head in ventral view; b) adult; c) thorax (length 5.8 mm); d) pupa (length 11.0 mm x width 5.0 mm); e) emerging holes of Hymenoptera parasitoids.*

Regarding Coleoptera, species identification is quite difficult, especially for Staphilinidae, so only the genus *Dermestes* sp. (Dermestidae) and *Saprinus* sp. (Histeridae) were identified (fig. 3). Adults of *Dermestes* sp. brown coloured have elytra covered by hairs while adults of *Saprinus* sp. are shiny black coloured. They were found feeding under the meat in hidden places, moving fast when disturbed and they, generally, produce recognizable feces similar to sawdust.

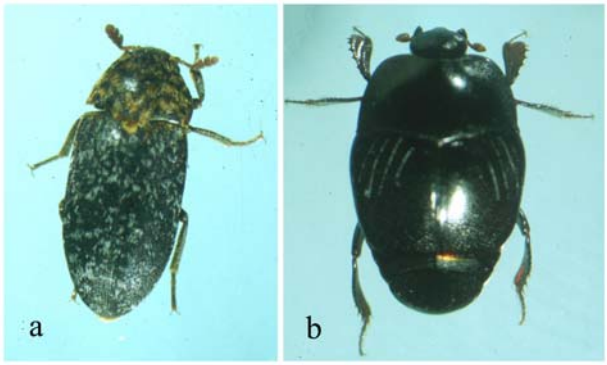

Fig. 3. *Coleoptera, a)* Dermestes sp. *(Dermestidae, length 6 mm); b)* Saprinus sp. *(length Histeridae 5 mm).*

In outdoor and indoor conditions, temperature and relative humidity data were quite different. From late April to late June their trends are reported in Fig. n. 4-7.

From April 24th to 27th during meat exposition on the terrace, the average maximum temperature was 28° and the minimum temperature was 13°; the maximum relative humidity was 91% and the minimum 31%.

Environmental temperature and relative humidity had high daily maximum and minimum variation compared to the cellar.

After June 20th, the maximum environmental temperature reached 40-42°, the highest registered in the entire experimental period.

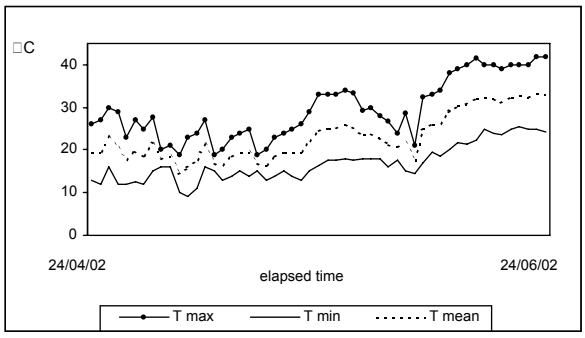

Fig. 4. *Outdoor conditions (terrace): temperatures during the period of study*

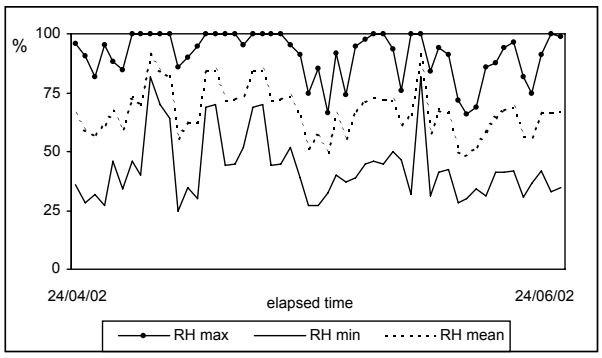

Fig. 5. *Outdoor conditions (terrace): relative humidity during the period of study*

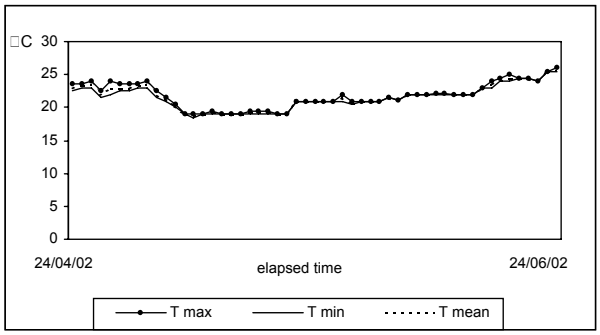

Fig. 6. *Indoor conditions (cellar): temperatures during the period of study*

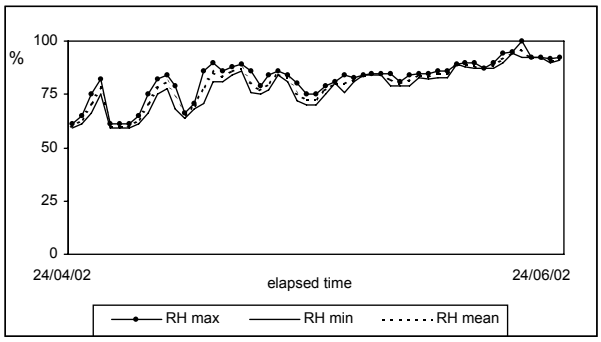

Fig. 7. *Indoor conditions (cellar): relative humidity during the period of study*

Regarding the indoor conditions in the cellar, temperature and relative humidity values had no daily variation and temperature had an average ranging from 20° to 25° during the whole period. In the literature for controlled dipteran rearing conditions, temperature values of 22-27° are reported [14-16]. So the indoor conditions were suitable for the complete life cycle of dipterans feeding on the meat.

In the first experiment with pig meat pieces, moved to the cellar on April 28th, eggs were observed after two days on those exposed on April 24th. On the 28th many maggots were feeding under the meat and two days later mature migrating larvae were observed. On May 3rd, the first three pupae appeared and on the 14th the

first adult of *Calliphora vicina* emerged. *Calliphora vicina* underwent complete development in the climatic conditions described in fig. 8. Under these relatively stable conditions, *Calliphora vicina* had a development cycle from egg to adult of 19-22 days (2 days: eggs; 5 days: maggots with the last 3 days spent as postfeeding larvae; 11 days: pupae).

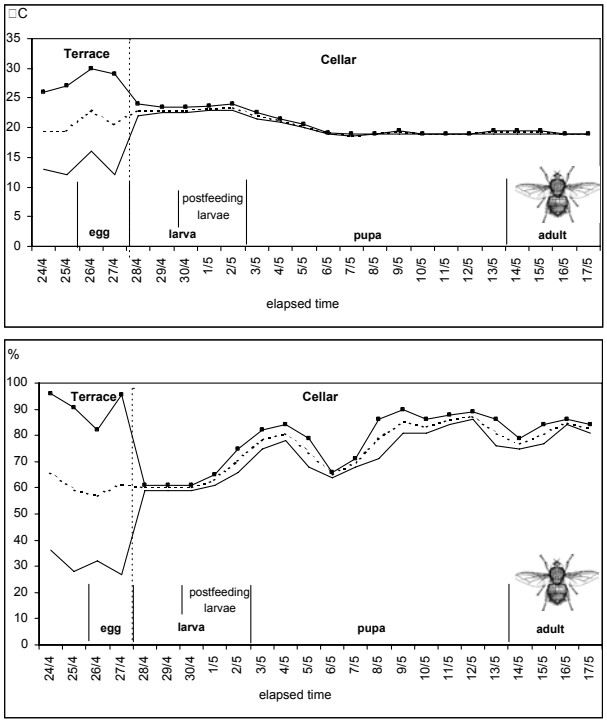

Fig. 8. *Temperature (above) and relative humidity (below) trends and the life cycle of* Calliphora vicina *in the cellar from April 24 to May 17. (Above: dotted black line: T max.; black line T min.; dashed line: T mean; below: dotted black line: RH max.; black line RH min.; dashed line: RH mean).*

During the second experiment, six meat pieces were exposed on the terrace from May 28th to the 31st and on the 30th larvae at the first developmental stage (L1) were feeding on the meat. In outdoor conditions on June 3rd postfeeding larvae belonging to the genus *Sarcophaga* spp. were identified. On the same day, Coleopteran adults of Dermestidae, Histeridae and Staphilinidae appeared and remained until the end of the experiment.

Two days later, 25 pupae, together with flesh fly postfeeding larvae, were observed. On the 6th, Hymenoptera parasitoids were observed flying around flesh fly maggots. On the 9th a strong rainfall, two days long, submerged puparia. Small parasitoid emergence holes on flesh fly puparia were observed and more than 20 parasitoid wasps inside some dissected puparia were found.

In conclusion, in outdoor conditions *Sarcophaga* sp. were in the larval stage for 6-8 days including 3 days as postfeeding larvae. Adults of Coleoptera appeared 6 days after meat was exposed and adults of Hymenoptera parasitoids appeared 9 days after, when the highest number of flesh fly mature migrating larvae was registered (fig. 9).

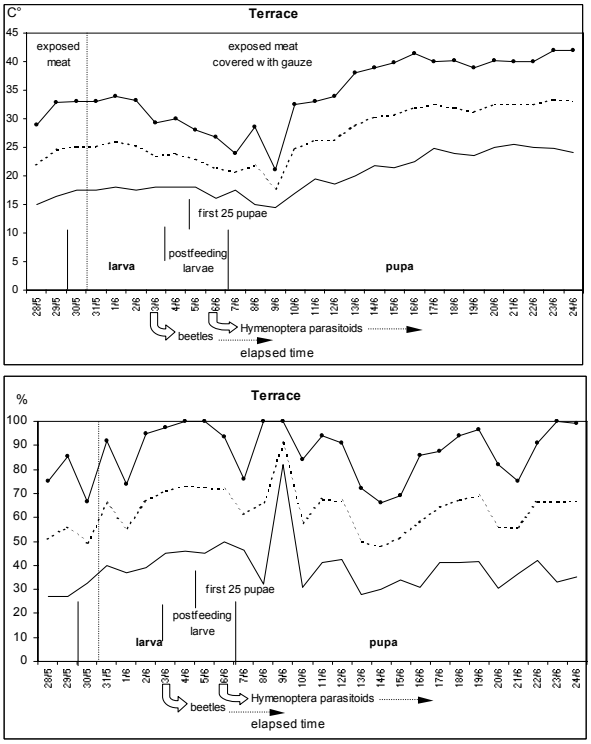

Fig. 9. *Temperature (above) and relative humidity (below) trends and the life cycle of* Sarcophaga africa *on the terrace from May 28 to June 24. (Above: dotted black line: T max.; black line T min.; dashed line: T mean; below: dotted black line: RH max.; black line RH min.; dashed line: RH mean).*

The remaining meat chunks transferred to the cellar had different results. On the 30th larvae L1 were observed and on June 2nd, together with flesh fly larvae, small Diptera were attracted to the rotting meat and many small eggs were detected. On the 6th, 20 pupae of *Sarcophaga* sp. appeared and on June 12th many small dipteran pupae belonging to the species *Megaselia scalaris* were observed. On the 21st the first adult of *Sarcophaga africa* and 10-15 adults of *Megaselia scalaris* emerged (fig. 10).

In indoor conditions *Sarcophaga* sp., under the climatic conditions described in fig. 10, had a development cycle of 23-26 days (7 days: maggots with 2 days of postfeeding larvae; 15 days: pupae), larviposition occurred two days after meat was exposed. *Megaselia scalaris*, at the same temperature and relative humidity conditions, had a life cycle of 19-22 days (7-8 days: larvae; 10 days: pupae), oviposition occurred five days later.

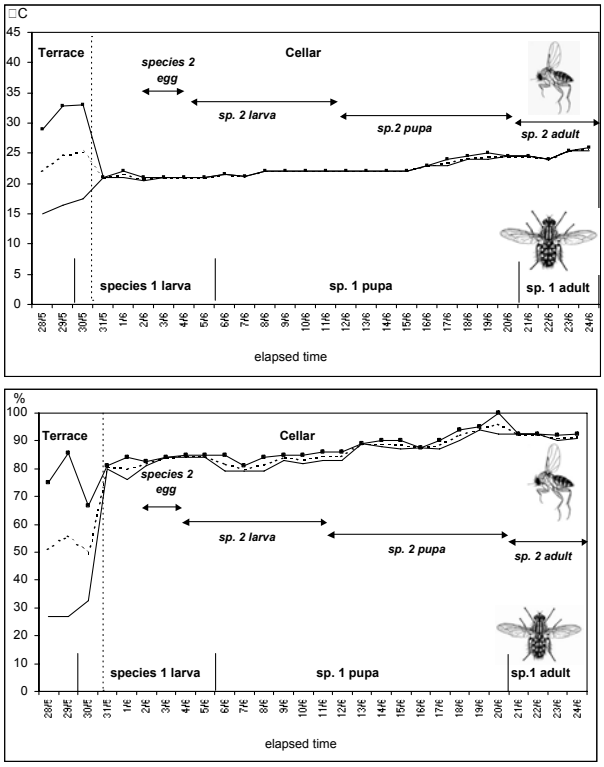

Fig. 10. *Temperature (above) and relative humidity (below) trends and the life cycle of* Sarcophaga africa *(species 1, sp.1) and of Megaselia scalaris (species 2, sp. 2) in the cellar from May 28 to June 24. (Above: dotted black line: T max.; black line T min.; dashed line: T mean; below: dotted black line: RH max.; black line RH min.; dashed line: RH mean).*

Discussion

The use of pig meat pieces both in outdoor and indoor conditions, represents a methodological procedure to get an estimation of insects' presence as "forensic indicators". Pig meat is a cheap food substrate without the social implications associated with the use of human material and is suitable for the development cycles of necrophagous Diptera like Calliphoridae, Sarcophagidae and Phoridae. By attracting insects of forensic interest this kind of meat can be considered to estimate the forensic fauna in different Italian geoclimatic areas (different climatic areas, for example north vs. south, can have different insects attracted to a dead body so it is possible to give an experimental estimation in this way). Moreover this methodology can be used to check Diptera species collected at the crime scene and their life cycle; in this work the results obtained on the precence of Calliphoridae and Sarcophagidae and their development cycles have been reported in real cases from cadavers in fields and/or during autopsy [17-19] and from experiments with carrion [15, 20].

In the urban area of Parma, Northern Italy, from late April to late June, Diptera belonging to the species *Calliphora vicina*, *Sarcophaga africa* and *Megaselia scalaris* were collected and identified. In this period the species that first appeared, were *Calliphora vicina* and *Sarcophaga africa* confirming the primary role of sarcophagids in carrion colonization [16]. *Calliphora vicina* were the dominant species in the colder months while *Sarcophaga* sp. were dominant in the warmer months [21]. Development rates of *Calliphora vicina* obtained in this experimental work coincides with conclusions reported by Professor Pekka Nuorteva in two real cases (case 806/65 and case 757/66) [17-19].

From this study the use of *Megaselia scalaris* in real forensic cases has been pointed out; the abundance of each life cycle stage of this species, together with *Sarcophaga* sp., could contribute to an estimation of post mortem interval (PMI). *Megaselia scalaris*, associated with dark indoor conditions and attracted to rotting meat has a secondary forensic role [22-23].

The appearance of Coleoptera in outdoor conditions confirm their role in carrion colonization after Diptera when the suitable stage of desiccation of the flesh is reached [23-24].

In indoor conditions in the cellar, for temperature and relative humidity values registered, the period of dipteran development was consistent, suggesting that the entomological conclusions based on PMI are precise and useful. In outdoor conditions, some biotic and abiotic factors could interfere with the insects' life cycle. For example, rainfalls flooding flesh fly puparia caused them to die of anoxia. Parasitism by Hymenoptera [9], even if in low percentage, or predation by Coleoptera and other insects, could contribute to a limitation of the number of flies emerging from the carrion [25]. In this work, the influence of abiotic factors on frequency and abundance of Diptera was highlighted. Environmental temperature, relative humidity, presence or absence of wounds on a corpse and access of insects to the body can affect the presence and abundance of Diptera specimens as well as other arthropods. The influence of all of these factors is specific to aspects of each crime scene.

Acknoledgments

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Observations on the Succession Patterns of Necrophagous Insects on a Pig Carcass in an Urban Area of Southeastern Brazil

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Abstract

Carcasses of the domestic pig (*Sus scrofa* L.) were exposed in an urban area in the vicinity of Campinas SP, Brazil, to determine stages of decomposition and insects of forensic significance exploiting the carcasses. Four species of Calliphoridae (Diptera) were collected and considered to be of potential forensic significance for urban situations in the regions: *Chrysomya albiceps*, *Chrysomya megacephala*, *Chrysomya putoria*, and *Lucilia eximia*. Unlike many other studies, Sarcophagidae were relatively late arrivals at the carcass with activity beginning on day 5, while Muscidae species arrived early. Ant activity, which began on day 1 of the study, was observed to retard the rate of biomass removal.

Key Words: Succession, Decomposition, Calliphoridae, PMI, Forensic Entomology, Brazil

Introduction

Over the past several years, applications of entomological evidence have contributed significantly to legal investigations [1,2,3,4]. This field, referred to as medicolegal forensic entomology, combines aspects of insect development, behavior and ecology with other investigative techniques. The most common application is to the estimation of the period of time since death or postmortem interval by determining a minimum period of insect activity on a decomposing body. Additionally, entomological evidence can provide significant data concerning postmortem movement of the body, assessment of wounds on a body, and circumstances of the death [1-6]. Another developing area is in the use of insects to detect drugs and toxins in a decomposing body, termed entomotoxicology [7].

In estimating the postmortem interval, an assessment of the physical condition of the body must be combined with an assessment of the fauna associated with the body [2,4]. Basic to the faunal analysis is the correct identification of the species encountered, their biologies and ecological relations with the fauna surrounding the body [4,8,9]. In this assessment, attention must be given, not only to those taxa present, but also to those absent from the body, frequency, specificity to a given geographic region, seasonality, and biotic or abiotic factors that may serve to alter the developmental periods for the taxa and thus the potential errors in the postmortem interval estimate [8]. Studies on the insect fauna associated with decomposing carcasses have previously been conducted in the area of Campinas in both an urban setting [10] and in wooded areas [11]. These studies have been general in nature and more detailed studies of decomposition and arthropod species succession patterns are needed, particularly with respect to daily variations in carcass visitation by species. The present

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study concentrates on species of forensic significance present in the urban situation.

Materials and Methods

Carcasses of 2 domestic pigs (*Sus scrofa* L.), 17 kg in weight, were exposed in an open urban area on the campus of UNICAMP (State University of Campinas) in the city of Campinas – SP, Brazil, for 40 days during the months of Aug. and Sept. 2000. The carcasses were in an open area, exposed directly to sunlight and 2 m apart. One carcass was placed directly on the soil and was not manipulated or otherwise disturbed during the study. The second carcass was placed on a wire mesh platform to allow for weighing and collection of specimens. Both carcasses were photographed daily. Carcasses were sampled twice daily, once in morning and again during the afternoon. At each visit, weight of the experimental carcass was recorded to give an estimate of the rate of biomass removal. Internal temperature was recorded as well at temperatures in the mouth and anus. Ambient temperature was recorded, along with daily maximum/minimum temperatures, relative humidity, precipitation, and wind conditions from the university weather station adjacent to the study site. Representative specimens were collected at each visit. Adult Diptera were collected using a hand net. Adult Coleoptera and other adult insects were collected by hand as well as immature stages of both Coleoptera and Diptera. Adult specimens were preserved in 70% ETOH. Immature specimens collected were divided into 2 lots: One was fixed in hot water and preserved in 70% ETOH. The other lot was placed on beef liver and reared to the adult stage for positive identification. All specimens collected were deposited in the Laboratory of Parasitology, Department of Parasitology, UNICAMP.

Results and Discussion

As shown in Fig. 1, daily minimum temperatures during the study period showed little variation, remaining around 15ºC for most of the study and rainfall was considerable. As shown in Figs. 2-4, ambient temperatures and wind speeds were generally higher during the afternoons than mornings, and correspondingly, relative humidity was generally lower during the afternoon periods. Internal temperatures of the carcass also varied from morning to afternoon measurements (figs. 7,8). The mouth and anus temperatures were taken only while was feasible due to carcass condition. During the morning period, internal carcass temperature approximated the ambient air temperatures while afternoon measurements were generally above ambient air temperatures.

There were adults of 5 families of Diptera collected from the carcasses. Adults in the families Calliphoridae and Muscidae were the initial colonizers of the carcasses, arriving shortly after exposure of the carcasses (tab. 1). Beginning on days 4 and 5, adults in the families Sarcophagidae and Phoridae were attracted to the carcass, followed by the Piophilidae beginning on day 11. This pattern is somewhat different from observations in other decomposition studies [12,13], where Sarcophagiodae were early arrivals, sometimes arriving ahead of the Calliphoridae. In these same studies, Muscidae species often delayed several days prior to their colonization of a carcass. Coleoptera were also relatively late arrivals during this study (tab. 1), with adult Histeridae first appearing on day 12 and Staphylinidae on day 13. Other studies have indicated an earlier arrival of predatory Coleoptera taxa, although not a precisely predictable event. Arrivals of adults of other families (Dermestidae, Cleridae, and Scarabaeidae) were more closely similar to observations in other studies. Ants (Formicidae) arrived on day 1 and their activities continued throughout the study. These were omnivorous, feeding on both the carcass and exerting a significant predation pressure on other taxa colonizing the carcasses.

There were 4 species of Calliphoridae recorded from the carcasses in this study as immatures: *Chrysomya albiceps, Chrysomya megacephala, Chrysomya putoria,* and *Lucilia eximia.* A total of 9 species of Diptera of forensic significance have been recorded from the region of Campinas, 8 Calliphoridae and 1 Sarcophagidae (*Patonella intermutans)*. In addition to those Calliphoridae recovered during the study are: *Cochliomyia macellaria, Hemilucilia segmentaria, Hemilucilia semidiaphana* and the Muscidae, *Ophyra chalcogaster*. Absence of *H. segmentaria* and *H. semidiaphana* is consistent with results of previous studies conducted in an urban habitat by Souza [10]. While *L. eximia* was among the first to arrive at the carcasses, it was the last to complete development, with *C. megacephala* and *C. putoria* producing the first emerging adults, followed by *C. albiceps* (tab. 2). Among the Muscidae, only *Musca domestica* was observed to complete its development on the carcasses.

Five stages of decomposition were recognized during this study as defined by Goff [14]: Fresh (figs. 1,2), Bloated (fig. 10), Decay (fig. 11), Postdecay (fig. 12) and Skeletal (fig. 13). Rate of biomass removal is shown in Fig. 14. In this study, the rapid initial loss of biomass reported by several other workers was not observed. For example, Early & Goff [13] reported a reduction to only 20% of the original weight by day 9 working in Manoa Valley on the island of Oahu, whereas Richards & Goff [15] showed similar results by day 11 on the island of Hawaii. Payne [16], working in a continental situation in South Carolina, reported a reduction to approximately 10% of the original biomass by day 5. In the present

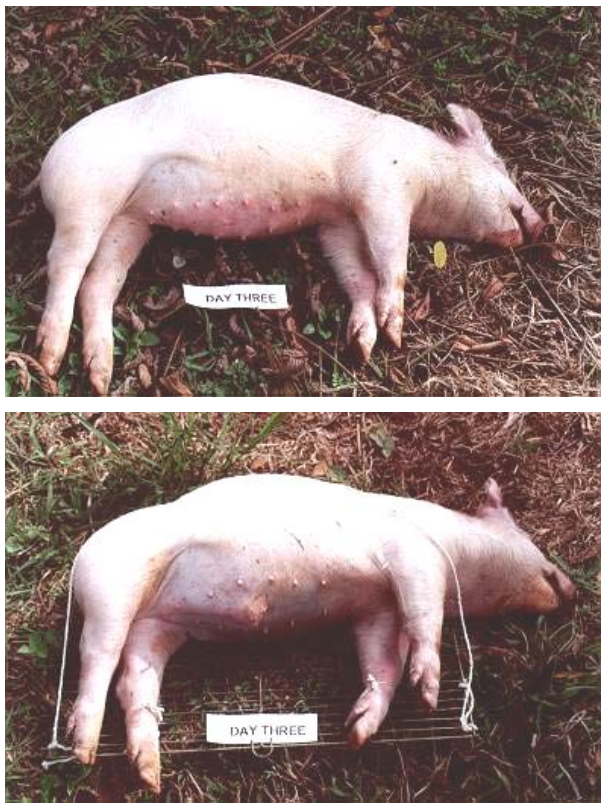
Day	Stage	Family	Species
1	Fresh	Formicidae	spp
2	Bloated	Formicidae	spp
		Calliphoridae	Chrysomya albiceps
		Muscidae	Musca domestica, spp
		Tephritidae	spp
3	Bloated	Formicidae	spp
		Muscidae	M. domestica, spp
4	Bloated	Formicidae	spp
		Muscidae	Ophyra chalcogaster, Musca domestica, spp
		Phoridae	Megaselia scalaris
5	Bloated	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O chalcogaster, M. domestica, spp
		Sarcophagidae	Patonella intermutans, spp
6	Bloated	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	M. domestica, spp
		Sarcophagidae	P. intermutans, spp
7	Bloated	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O chalcogaster, M. domestica, spp
		Sarcophagidae	spp
8	Bloated	Formicidae	spp
		Calliphoridae	C. albiceps, L. eximia
		Muscidae	spp
		Sarcophagidae	spp
9	Bloated	Formicidae	spp
		Muscidae	spp
10	Decay	Formicidae	spp
		Calliphoridae	C. albiceps, Cochlyomia macellaria, C.
		Muscidae	megacephala
		Sarcophagidae	O. chalcogaster, spp
			P. intermutans, spp
11	Decay	Formicidae	spp
		Calliphoridae	C. albiceps, C. megacephala
		Muscidae	M. domestica
		Piophilidae	P. casei
		Sarcophagidae	P. intermutans, spp
		Histeridae	spp
		Staphylinidae	spp
12	Decay	Formicidae	spp
		Calliphoridae	C. albiceps, spp
		Muscidae	M. domestica, O. chalcogaster, spp
		Phoridae	M. scalaris
		Sarcophagidae	spp
		Histeridae	spp
13	Decay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	M. domestica, O. chalcogaster, spp
		Piophilidae	P. casei
		Sarcophagidae	spp
		Histeridae	spp
		Staphylinidae	spp
14	Decay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	M. domestica, O. chalcogaster, spp
		Piophilidae	P. casei
		Sarcophagidae	spp
		Histeridae	spp
		Scarabaeidae	spp
15	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O. chalcogaster, spp
		Sarcophagidae	spp
		Histeridae	spp
		Cleridae	N. rufipes
16	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O. chalcogaster
		Sarcophagidae	spp
		Histeridae	spp
		Cleridae	N. rufipes
17	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	M. domestica, O. chalcogaster, spp
		Piophilidae	P. casei
		Sarcophagidae	spp
		Histeridae	spp
		Cleridae	N. rufipes
18	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	M. domestica, O. chalcogaster, spp
		Histeridae	spp
		Cleridae	N. rufipes
		Dermestidae	D. maculatus
19	Postdecay	Formicidae	spp
		Calliphoridae	C. megacephala
		Muscidae	M. domestica, O. chalcogaster, spp
		Piophilidae	P. casei
		Sarcophagidae	P. intermutans, spp
		Histeridae	spp
		Cleridae	N. rufipes
		Dermestidae	D. maculatus
20	Postdecay	Muscidae	O. chalcogaster, spp
21	Postdecay	Formicidae	spp
		Muscidae	O. chalcogaster, spp
		Phoridae	M. scalaris
		Neriidae	spp
		Histeridae	spp
		Cleridae	N. rufipes
		Dermestidae	D. maculatus
22	Postdecay	Formicidae	spp
		Muscidae	O. chalcogaster
		Calliphoridae	C. albiceps
		Phoridae	M. scalaris
		Vespidae	ssp
23	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O. chalcogaster, spp
		Phoridae	M. scalaris
		Sarcophagidae	spp
		Cleridae	N. rufipes
24	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O. chalcogaster
		Piophilidae	P. casei
		Cleridae	N. rufipes
		Dermestidae	D. maculatus
25	Postdecay	Formicidae	spp
		Muscidae	O. chalcogaster
		Calliphoridae	C. albiceps
26	Postdecay	Formicidae	spp
27	Postdecay	Formicidae	spp
		Muscidae	O. chalcogaster
		Cleridae	N. rufipes
		Dermestidae	D. maculatus
28	Postdecay	Formicidae	spp
		Calliphoridae	C. albiceps
		Muscidae	O. chalcogaster
		Vespidae	spp
29	Postdecay	Formicidae	spp

		Muscidae	O. chalcogaster
		Vespidae	*spp*
		Dermestidae	D. maculatus
30	*Posdecay*	*Formicidae*	*spp*
		Calliphoridae	C. albiceps, C.
		Muscidae	megacephala
		Cleridae	O. chalcogaster
		Dermestidae	N. rufipes
			D. maculatus
31	*Postdecay*	*Cleridae*	N. rufipes
		Dermestidae	D. maculatus
32	*Postdecay*	*Dermestidae*	D. maculatus
33	*Postdecay*	*Dermestidae*	D. maculatus
34	*Postdecay*	*Dermestidae*	D. maculatus
35	*Postdecay*	*Formicidae*	*spp*
		Dermestidae	D. maculatus
36	*Skelettal*	*Formicidae*	*spp*
37	*Skeletal*	*Formicidae*	*spp*

Tab. 1. *Adult specimens collected on a daily basis in the carcass in the decomposition different stages.*

Day	Stage	Family	Species
8	Bloated	Calliphoridae	*C. megacephala; C. albiceps; L. eximia*
9	Fermentation	Calliphoridae	*C. albiceps*
		Muscidae	*M. domestica*
10	Fermentation	Calliphoridae	*C. megacephala; C. albiceps; L. eximia*
11	Fermentation	Calliphoridae	*C. megacephala; C. albiceps; L. eximia*
12	Fermentation	Calliphoridae	*C. megacephala; C. albiceps; C. putoria*
13	Fermentation	Calliphoridae	*C. megacephala*
14	Fermentation	Calliphoridae	*C. albiceps*
15	Putrefaction	Calliphoridae	*C. megacephala; C. albiceps; L. eximia*
16	Putrefaction	Calliphoridae	*C. albiceps; C. putoria*
17	Putrefaction	Calliphoridae	*C. albiceps*
18	Putrefaction	Calliphoridae	*C. albiceps*
19	Putrefaction	Calliphoridae	*C. albiceps*
20	Putrefaction	Calliphoridae	*C. albiceps*
25	Putrefaction	Calliphoridae	*C. albiceps*
28	Putrefaction	Calliphoridae	*C. albiceps; C. megacephala*
29	Putrefaction	Calliphoridae	*C. albiceps; C. megacephala*
30	Putrefaction	Calliphoridae	*C. albiceps; C. megacephala*
31	Putrefaction	Calliphoridae	*C. albiceps; C. megacephala*
32	Putrefaction	Calliphoridae	*C. albiceps*
33	Putrefaction	Calliphoridae	*C. albiceps*

Tab. 2. *Third instar larvae specimens collected on a daily basis in the carcass in the decomposition different stages.*



Figs. 1,2. *Carcass in "fresh" stage placed directly on the soil (top); in "fresh" stage placed on a wire mesh platform (bottom).*

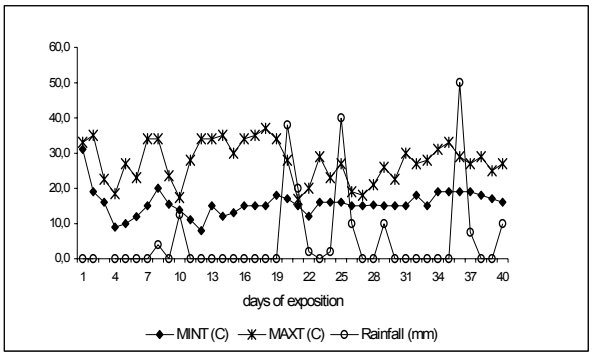


Figure 3: Daily minimum and maximum temperatures and precipitation during the carcass exposure time in the urban region.

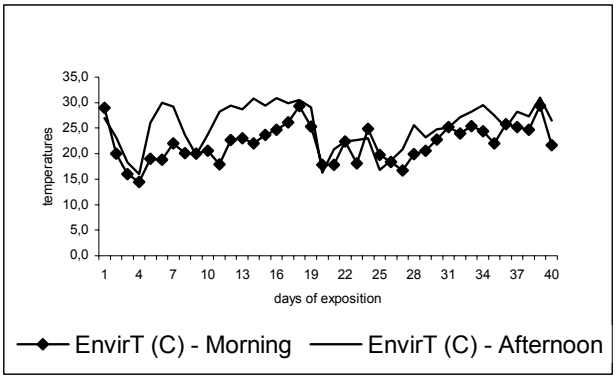


Fig. 4. *Fluctuation of ambient temperature in two distinct periods of the day during the time of carcass exposure in the urban region.*

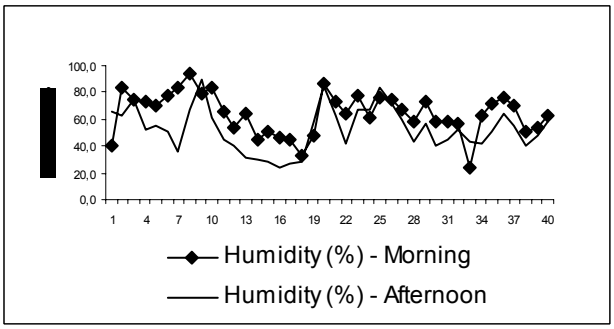


Fig. 5. *Fluctuation of relative humidity of ambient air in two distinct periods of the day during the time of carcass exposure in the urban region.*

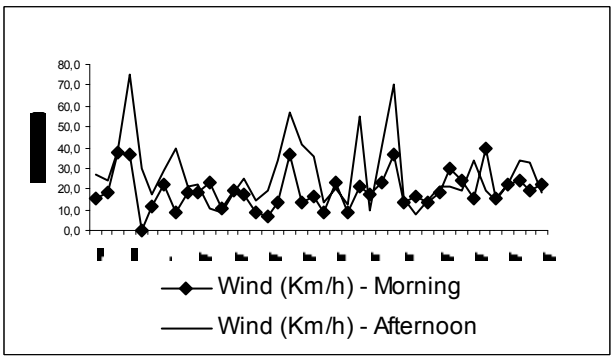


Fig. 6. *Daily variation of the speed of the surrounding wind in two distinct periods of the day during the time of carcass exposure in the urban region.*

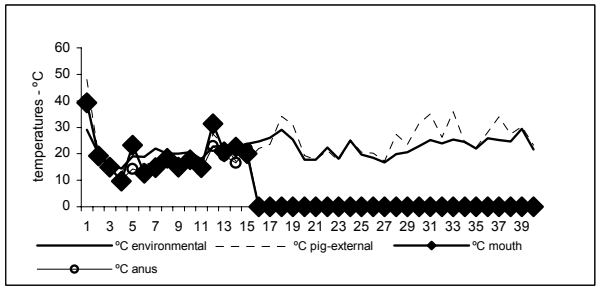


Fig. 7. *Daily variation of the of the external and environment temperatures, mouth and anus of the carcass in the period of the morning during the carcass exposure time in the urban region.*

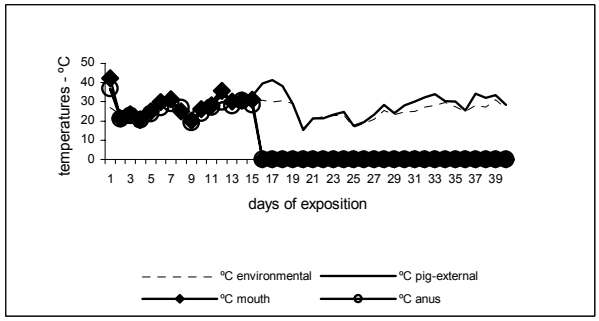


Fig. 8. *Daily variation of the of the external and environment temperatures, mouth and anus of the carcass in the period of the afternoon during the carcass exposure time in the urban region.*

Fig. 9. *Artifacts produced by lizards*

Figs. 10-14. *Carcass in bloated, decay, postdecay, and skeletonized state.*

study, this level was not reached until 23 days following exposure of the carcass. This pattern is similar to that observed by Early & Goff [13] for a carcass inside Diamond Head Crater, Oahu, Hawaii, where there was significant predation by ants. Similar effects have been noted by Stoker et al. [17] in Texas and Houston [18] in Brazil. As ant activity began on day 1 and continued throughout the study, it is probable that ant predation could be a factor in slowing the rate of biomass removal by necrophagous taxa, particularly Diptera larvae.

These results are important to development of Forensic Entomology in Southeastern region once they can be add in brazilian database.

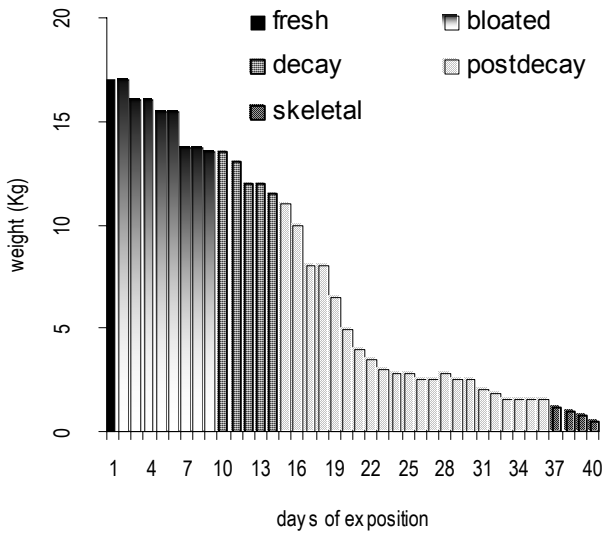


Fig. 14. *Biomass removal during the time of carcass exposure in the urban region.*

Conclusions

As in other studies, adult Calliphoridae were the first to arrive at carcasses. However, the early arrival of Muscidae and delayed arrival of Sarcophagidae species were departures from previous observations. Of the 9 species of Calliphoridae reported from carrion in the area around Campinas, only 4 species were of significance in the urban habitat in which the study was conducted: *C. albiceps*, *C. megacephala*, *C. putoria*, and *L. eximia*.

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Application of Forensic Entomology to estimate of the postmortem interval (PMI) in homicide investigations by the Rio de Janeiro Police Department in Brazil

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Abstract

This work presents three case studies, in which estimates of the *postmortem* interval (PMI) were based on the concept of accumulated degree-days (ADD). In two cases, the PMI estimates based on the biology of *Chrysomya megacephala* (Fabr.) and *Cochliomyia macellaria* (Wied.) were close to that obtained by other investigation means. In the third case, based on *C. megacephala,* the PMI estimate greatly differed from the real interval, which is considered to be caused by restricted access of sarcophagous insects to the body.

Keywords: Forensic Entomology, Postmortem Interval, Diptera, Brazil

Introduction

In the last decades, Forensic Entomology has begun to play an important role as an investigative procedure for cases of homicide throughout the world, mainly for the determination of death chronology (*postmortem* interval – PMI). International scientific institutions already have specific laboratories to assist law officers at the death scene. Unfortunately, there are no such resources in Brazil and the application of this technique is unusual. In a pioneering action, the Department of Technical and Scientific Police of Rio de Janeiro is trying to bring the association of law officers with entomologists of the Museu Nacional – UFRJ into a conventional practice.

Decomposition studies have been conducted around the world. However, these succession patterns cannot be accurately applied to studies of PMI in Brazil [1,2]. This is only possible if the habitats are similar with regard to especially temperature and humidity, because such environmental conditions have a direct influence upon the rate of postmortem decay. Recently, some studies have been carried out in Rio de Janeiro in order to establish some baseline data [3, 4, 5]. This work presents the application of Forensic Dipterology in three homicide cases, the PMI estimates of which were based on larval development rate.

Methods

It is desirable that in a crime report most evidence be analyzed as well as the factors that may influence the occurrence of carrion insects, including conditions which may accelerate or delay the arrival of insects attracted by odors given off by the corpse.

The local meteorological data from the five days prior to that on which the corpse was found was obtained from the meteorological station of Nova Iguaçu county, the nearest weather station to the crime scene. In addition, the ambient temperature was

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Source	Stage	Thresold Min. (ºC)	Environ. Temp. (ºC)	Develop. Time (h)	Exp.ADH	Exp. ADD
Wells & Kurahashi. (1994)	Egg hatch	10	27	18	306	12.75
	1st moult			30	510	21.25
	2nd moult			72	1224	51
	Pupariation			144	2448	102
	Emergence			234	3978	165.75

Tab. 1. *The time of development obtained from literature and expected accumulated degree-hours (ADH) and degree-days (ADD) to* Chrysomya megacephala.

measured in loco, including temperature from the mass of larvae and corpses as well as soil temperature in 10-20 cm depth, in order to relate the meteorological data from the nearest weather station to the microclimate of the place of the crime.

The methods used in this work were based on the adaptation of methodologies for collection, transportation and rearing of the Diptera specimens described by various authors [6,7,8].

The stage of decomposition of the corpse was specified according to the morphological characteristics observed when it was found (fresh, bloated, early decomposition, advanced decomposition and remains) and the accuracy of PMI estimate was evaluated by reference to the statements from witnesses.

Adult insects were collected with the aid of a modified entomological net [9] and transported in the same plastic bag that was coupled to the net. This avoids contact of the collector with the insects, safeguarding his/her health. Eggs and larvae were collected from the corpses with the aid of forceps and brushes, and the living specimens were put in jars with moistened filter paper for the eggs and ground bovine meat for the larvae. The jars were closed with gauze to allow proper ventilation. Wandering larvae and pupae were also sought in the area around the corpse and taken to the lab in pots with a little sample of soil from the place. All the jars were labeled, indicating hour, date and environmental data.

In the laboratory, the immature insects were sorted by instar and morphological similarity and transferred to separate rearing jars. Adult insects collected at the crime site as well as those reared in the lab were killed by freezing. Later, they were identified to species.

Temperature is the most important factor affecting the growth and development rate of insects. The metabolic heat generated by maggot-mass can be sufficient to raise their micro-environmental temperature by several degrees above ambient [10] so it is essential to take into consideration the maggot mass temperature in determining insect development, specifically for last instar maggots. Consequently, ambient temperature was used to determine development time for earlier stages, maggot-mass temperature for later instars and soil temperature for pupae. A common approach is to use maggot-mass temperature as a constant which would provide the fastest growth of maggots (around 90 to 95º F or 32 to 35º C) [11].

As hourly temperatures were not available, the data were limited to daily maximum and minimum temperatures used to calculate the daily mean temperature. So, PMI estimates had to be based on accumulated degree-day (ADD) units. The calculation used for ADD was based upon a standard technique called the rectangle method [12]. The heat accumulation is the difference between the average temperature and the lower threshold times the days taken to develop [13]. Calculations without an appropriate threshold will overestimate the heat accumulation or degree-days. The equation used to calculate degree-days per day is:

$$\text{Degree-days} = \frac{(\text{Max. temp.} + \text{min. temp.}) - (\text{lower threshold}) \times 1\ \text{day}}{2}$$

This method ignores the upper threshold temperature because the highest temperatures rarely reaches levels where they may have any lethal effect. Determining the lower developmental threshold is an important prerequisite to use the degree-day concept. For the two species encountered in the present paper, a lower threshold of 10º C was applied [11].

The development data (time and temperature) were obtained from literature for calculation of the ADD required for the involved species [14, 15]. The equation used to calculate degree-days per day from literature (ADD expected) is:

$$\text{ADD expected} = \frac{(\text{temp.} - \text{lower threshold.}) \times \text{hours of development}}{24}$$

For an estimate of the time elapsed since oviposition and, consequently, the minimum PMI, it is important to use the insect development in backtracking from the oldest instar collected to the time of oviposition [11]. Expected ADD was obtained from the literature for each instar (egg, larvae or pupae) and subtracted from each computed degree-day until the beginning (oviposition).

Case studies

Case study 1

By the morning of July 6, 1999, the remains of a woman were discovered in a residential property. The doors and windows were shut and the corpse was found covered with varied blankets (fig. 1). Local environmental temperature was measured at 26º C, which was coherent with daily maximum and minimum temperature (27º C and 15º C respectively) obtained from the weather station. The body was in a bloated, partially decomposed state. The abdomen was bloated and its skin greenish-brown colored. The rigor mortis was generalized and lividity was fixed. The report of the investigation indicated that the woman had been seen alive three days before finding the body. In spite of these observations, just some eggs were located behind the ear and they hatched out in laboratory. These maggots were reared to adults. On July 16, after 240 hours, some specimens of *Chrysomya megacephala* emerged. The expected accumulated degree-day (Exp. ADD) was figured out as 12.75 basing on the time of development determined for this species under laboratory conditions [14], as 18 hours at 27º C (tab. 1). Considering that the expected ADD was calculated for egg hatch, but only eggs had been collected and that oviposition frequently occurs more than 12 hours after death, as Lothe [16] observed to *C. albiceps* and *C. chloropyga* and Oliveira-Costa [3] to species collected in this search, the PMI came to slightly more than 1 day (tab. 2). This result has not corroborated the estimate obtained by the usual Forensic Medicine methods (physical appearance of the body, lividity, rigor mortis and mainly by investigation report or witness' statement). Probably, the delay in flies locating and ovipositing on the corpse derives from the fact that the doors and windows were shut and the corpse was found covered with blankets, creating a barrier and hindering the access of insects to the remains as well as the release of attractive odours.

DAY	Soil temp.	Environ. Temp.		maggot mass temp.	DD-B10	ADD-B10
		Max. Temp.	Min. Temp.			
July 6		27º C	15º C		11	12.75

Tab. 2. *Case 1: Calculation of the degree-day (DD) and accumulated degree-days (ADD) with lower threshold temperature of 10º C.*

Day	soil temp.	Environ. Temp.		maggot mass temp.	DD-B10	ADD-B10
		Max. Temp.	Min. Temp.			
August 9	25º C				15	77.5
August 8				32º C	22	62.5
August 7		27º C	19º C		13	40.5
August 6		26º C	18º C		12	27.5
August 5		28º C	20º C		14	15.5
August 4		28º C	19º C		13.5	1.5
Day	soil temp.	Environ. Temp.		maggot mass temp.	DD-B10	ADD-B10
		Max. Temp.	Min. Temp.			
July 6				31º C	21	51
July 5		25º C	16º C		10.5	30
July 4		26º C	19º C		12.5	19.5
July 3		25º C	13º C		9	7

Tab. 3. *Case 2: Calculation of the degree-day (DD) and accumulated degree-days (ADD) with lower threshold temperature of 10º C.*

Source	Stage	Thresold Min. (ºC)	Environ. Temp. (ºC)	Develop. Time (h)	Exp.ADH	Exp. ADD
Byrd & Butler (1996)	Egg	10	25	12	180	7.5
	1st instar			18	270	11.25
	2nd instar			24	360	15
	3rd instar			62	930	38.75
	Pupation			116	1740	72.5
	Pupa			124	1860	77.5
	Adult			240	3600	150

Tab. 4. *The time of development obtained from literature and expected accumulated degree-hours (ADH) and degree-days (ADD) to* Cochliomyia macellaria.

Day	Soil temp.	Environ. Temp.		maggot mass temp.	DD-B10	ADD-B10
		Max. Temp.	Min. Temp.			
August 9	25º C				15	77.5
August 8	25º C				15	62.5
August 7				32º C	22	47.5
August 6		26º C	18º C		12	25.5
August 5		28º C	20º C		14	13.5

Tab. 5. *Case 3: Calculation of the degree-day (DD) and accumulated degree-days (ADD) with lower threshold temperature of 10º C.*

Case study 2

On July 6, 1999, in the morning, the corpse of a man in a bloated stage of decomposition was discovered in a vacant lot with an earthen floor covered by low vegetation (fig. 2). The abdomen's skin was colored greenish-brown and it was bloated. The rigor mortis was generalized and lividity was fixed.

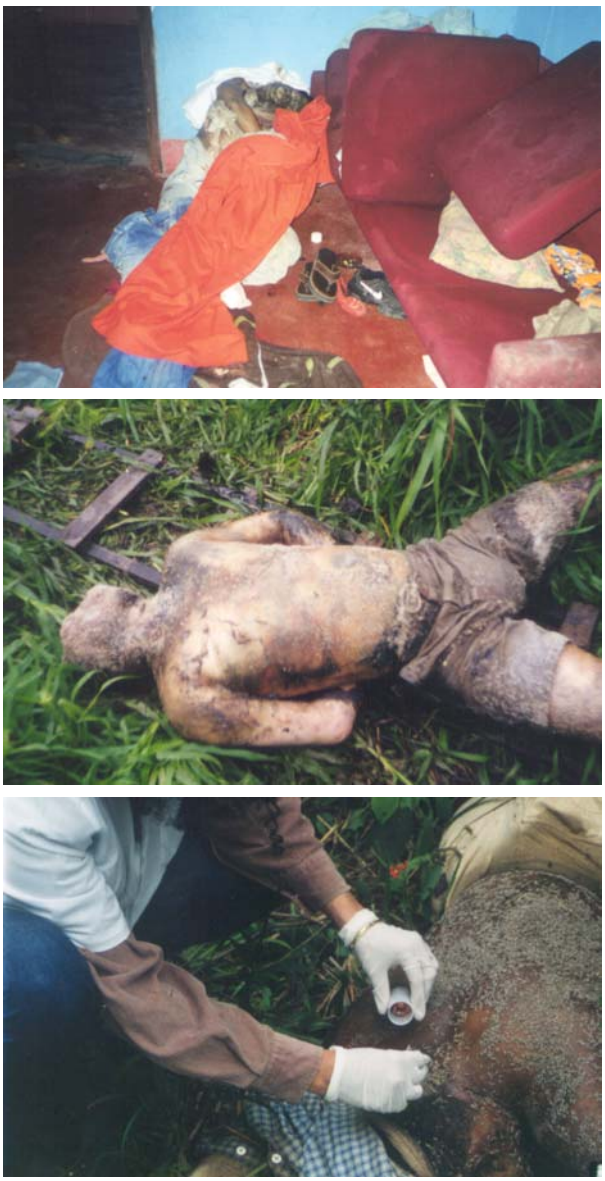

Fig. 1-3. *Bodies of victims at the scene where they were discovered: Case 1 (top), Case 2 (middle), Case 3 (bottom).*

Environmental temperature was measured to 27º C, which was coherent with daily minimum and maximum temperature (27º C and 15º C respectively) obtained from the weather station. The investigation report indicated that the man had been seen alive four days before finding the body. The investigation indicated the authorship ratifying the date of death. Adult specimens of *Chrysomya megacephala*, *Cochliomyia macellaria* and *Sarcophaga (Liopygia) ruficornis* (Fabr.) were present. As for immature stages, a lot of third instar larvae were found, especially in the cranial area. Maggot-mass temperature was measured at 31º C. On July 14, after 141 hours, some specimens of *Chrysomya megacephala* emerged. Considering that oviposition frequently occurs more than 12 hours after death [3, 16], the accumulated degree-days (ADD) were calculated and the estimate of minimum postmortem interval was 4,5 days based on the time of development determined for the 2nd moult of this species under laboratory conditions as 72 hours at 27º C [14] (tab.1), corroborating the interval obtained by the traditional method (tab. 3).

Case study 3

In the morning of August 9, 1999, the corpse of a man was discovered in a mixed forest (fig. 3), the local environmental temperature was 27º C and soil temperature was 25º C. The investigation report pointed out that the man had been seen alive six days before finding the body. The body was in an early decomposition stage and adult specimens of Calliphoridae and Sarcophagidae were present. As for immature stages, a lot of third instar larvae were found on the corpse and dark puparia were found in the soil near the body.

On August 13, after 92 hours, some specimens of *Cochliomyia macellaria* emerged. The expected ADD was based on the time of development determined for pupation of this species under laboratory conditions as 116 hours at 25º C [15] (tab. 4). As puparia were already dark, they had developed for at least 24 hours subject to the soil heat (tab. 5). Considering that oviposition frequently occurs more than 12 hours after death [3, 16], the estimate of postmortem interval for this case was 5.5 days, thereby confirming the interval indicated by the investigation report.

Discussion

The estimate of postmortem interval based on entomological evidence was in agreement with the PMI obtained by standard means, provided that all evidence from the death scene is taken into consideration, such as the delayed arrival of flies to a corpse when in enclosed environments. The estimate of PMI can be different from the real interval due to physical circumstances in the surroundings of the remains [17]. The application of the entomological method requires extensive knowledge of the mechanical and environmental factors that can interfere with the processes of colonization, the development time and the decomposition of the corpses by insects.

The forensic entomologist has to do careful measurements of the temperature which the immature insect was submitted to, in order to determine which temperature should be used for obtaining a more precise calculation.

Lamentably, in Brazil, there is little collaboration between entomologists and law officers. Only few officers have a basic training in entomology. Therefore, this study is pioneering, and the usefulness of this method depends on how the corpse is processed before the entomological analytical methods can be

applied. As other authors have already stated [18], ideally, the team of professionals who are first called to the death scene or the autopsy should comprise a forensic entomologist.

Acknowledgments

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Conscript Carrasco: A Peacetime Casualty

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Abstract

A young man under compulsory conscription was killed in Zapala, in the southwest of Argentina. An attempt to cover up his death was prevented by medical evidence of brutal battering and by entomological evidence of a 20-30 day post-mortem interval, which implied concealement of the body. Insect samples showed a single well-developed "wave" of the greenbottle *Phaenicia sericata*, consisting in mature larvae, a dead pupa and two empty puparia; this placed the death no later than 16 days before the finding of the body, but most probably 25-30 days before it.

Since neither younger larvae of the same species nor larvae of other species of corpse-frequenting fly were found, it was thought that the body had been hidden in a dark place, after a few hours in the light (allowing oviposition by greenbottles). Coleoptera associated with butyric fermentation (*Dermestes* sp., *Necrobia rufipes*) confirmed the dating. A yellowjacket wasp (*Vespula germanica*) suggested that the remains had been taken outdoors a little before being found. A single Histerid beetle *Saprinus patagonicus* provided a first record of this species in a forensic case.

Keywords: Forensic entomology, Calliphoridae, Dermestidae, Necrophilous Coleoptera, Post-mortem Interval, Patagonian fauna

Introduction

The use of insects associated with corpses to determine the post-mortem interval has been attempted by some legal medicine experts in Argentina. However, because these professionals were not themselves entomologists, and because they never published their results, forensic entomology was not advanced in any way from such information as might be found in European books [1, 2] until the year 1993. It was then that the authors of this paper began an interdisciplinary collaboration. The entomological treatment was based at first on bibliography [2, 3], until direct information was afforded through experiments and a gradual accumulation of autopsy data.

In April 1994, a case came to public notice through the media. Compulsory military service was still active at the time, and on the 6th April a young conscript had been found dead on the barracks grounds near the town of Zapala, province of Neuquen, in the southwest of Argentina (photo 1, 2, 3). The authorities in charge claimed he had deserted just one month before, and suggested murder by person or persons unknown, who had thrown the body inside the grounds. A first post-mortem was performed at Zapala, not at the judicial Morgue but within the barracks premises. However, enough questions were raised about such a procedure, and a second autopsy was demanded.

The body was sent to the Morgue (Morgue judicial) at Buenos Aires, where insect material was collected from the body and clothes. The X-ray studies performed during the autopsy revealed grievous pre-mortem battering and traumatic luxation of the sixth, seventh and eight costochondral joints. Furthermore, the first autopsy indicated an hemothorax of 1,500 ml. The entomological evidence showed a considerable post-mortem interval, not consistent at all with the

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story given out by the local Army authorities, which had been accepted by the Provincial prosecutor.

One very obvious problem was the lack of information about development times for Argentina at the time. As a matter of fact, since the town of Zapala is sited very little to the North of parallel 39°S, extrapolation of development times in Europe would have been quite adequate, making allowance for the very dry climate of the Patagonian steppe, which causes a large circadial temperature fluctuation. As it was, times had to be estimated with great caution.

Materials and methods

Medical aspects

The corpse belonged to a 19-20 year old male, of mixed Amerindian and Caucasian descent, with a height of 163 cm and a weight of about 53 kg. (This agreed with the personal data of 19-year-old Omar Octavio Carrasco. His drafting health file listed a weight of 57 kg and a height of 163 cm).

The body was in a state of partial putrefaction and partial mummification (Photo 4, 5). Both eyeballs were putrefact, the left one dislodged from the orbit due to the action of maggots. The corpse showed traces of the previous autopsy. The left side of the face, neck, shoulder and upper thorax exhibited an increase in volume with relation to the right sides. This was not due to bleeding or bruising, as it was demonstrated by making many incisions into the swollen left-side parts. Therefore, the swelling was interpreted as the result of positional post-mortem discoloration. The location of this discoloration contradicted the position in which the corpse was found (Photograph 3).

In view of the dislodgement of the left eyeball, the facial soft tissues were removed entirely and X-rays performed to verify the presence of possible trauma; however, no such trauma was found. The X-rays of the ribs revealed luxation of the sixth, seventh and eighth chondrocostal joints.

Live maggots were observed both on the corpse and the clothes, as well as within the coffin (photo 5, 6); also a live wasp was recovered from the clothes. Routine toxicological, biochemical and histopathological tests were performed.

Entomological aspects

Label “larvae [from] clothes”: 26 Larvae III of the greenbottle *Phaenicia sericata* (Diptera: Calliphoridae).

Label “clothes 2”: 6 adults of the skin beetle *Dermestes* sp. One adult of the ham beetle *Necrobia rufipes* (Coleoptera: Cleridae) One adult *Saprinus patagonicus* (Coleoptera: Histeridae). One pupa of a Calliphorid fly (presumably *P. sericata*), puparium well preserved but contents decayed. One adult yellowjacket wasp *Vespula germanica* (Hymenoptera: Vespidae). The beetles were macerated and had a strong odour of decay, showing they had been placed in the tightly closed container when still alive. The wasp was contorted showing it had died of asphixia, also inside the container.

- Label “Bugs [from] shoes”: One adult of the skin beetle *Dermestes* sp.
- Label: “Maggots external”: 7 mature larvae of the greenbottle *P. sericata*.
- Label: “Maggots internal”: a mass of macerated larvae; the best preserved of these are identical with the precedent.
- Label: “A-2”: empty puparia of *P. sericata*. A 3 mm larva of *Dermestes* sp. had begun gnawing them, but this insect probably had hatched from the egg in the lapse between autopsies.

Results

Medical

Aetiology and mechanisms of death: the finding of luxation chondrocostal joints on the right side, together with an hemothorax of 1,500 ml suggested very strongly, in the absence of any other findings, that Carrasco had suffered severe battering with un unspecified blunt instrument, causing internal bleeding which determined death.

For a person weighting 56 kg (estimated blood volume 5,000 ml), an hemothorax of 1,500 ml represents a loss of blood volume dangerous to life, and which certainly would cause death unless immediate expert medical assistance was supplied.

Entomological

The greenbottle oviposits on recent corpses. It is true that the eggs are laid as a rule in the eyes and nose, but when the larvae are ready to pupate they spread over the body before abandoning it to bury themselves in the earth (under natural conditions). We had no reason to believe that the sample came from an abnormal oviposition, since the larvae were all of similar size and fully grown.

The finding of a pupa and empty puparia show that some individuals had completed their development. Also, the autopsy photographs show the work of maggots in the left eye socket (photo 4), which would be the prefered oviposition site for the earliest period after death. Neither *P. sericata* larvae younger than those described, nor larvae of other species were found.

Fig. 1. *The lying of the land. Barracks buildings in the middle distance. Typical Patagonian steppe in the foreground; mountains in the background.*

Fig. 2. *The dumped body.*

Fig. 3. *A closer view of the body, showing post-mortem lividities not connsistent with the declivity of the terrain.*

The presence of *Dermestes* sp. and *Necrobia rufipes* indicates a beginning of butyric fermentation at least in the limbs. Unfortunately, there is no clear-cut medical evidence as to the shortest post-mortem interval at which this stage begins. The presence of *S. patagonicus* is less conclusive, since this species preys on maggots of any size, and therefore cannot be associated with one given stage of decay.

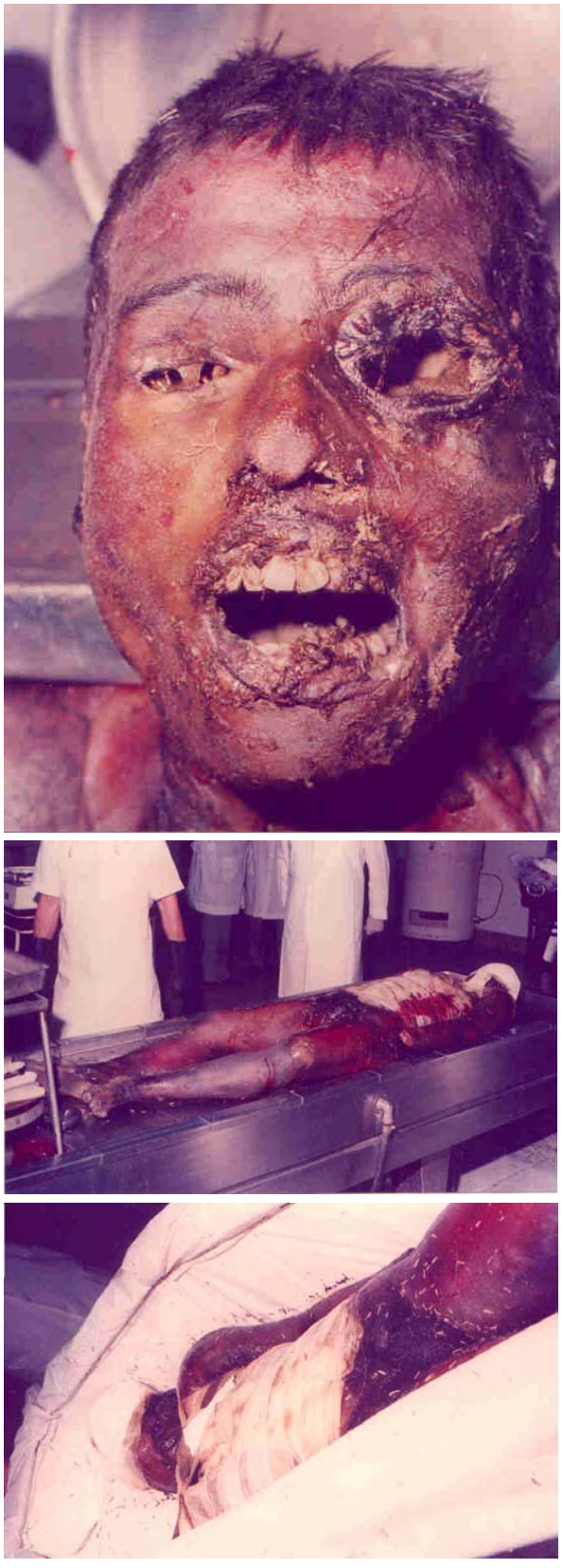

Fig. 4-6. *Autopsy: view of the face, showing the work of maggots in the left eye socket (above); body partly mummified, partly putrified, note migrating maggots (middle and bottom)*

Discussion

One or more normal ovipositions by *P. sericata* show that the corpse was exposed to daylight for a few hours at least, although it is just possible that the victim was not dead at the time of oviposition. This does not affect the case, as he would have had to be very close to passing away, and therefore the victim, if not of murder, of what Argentinian law describes as extremely grievous bodily injury. The fact that adults had begun to emerge from the puparia indicates the lapse of, at the very least, ten days, although more probably 25-30 days.

On the base of data which gave fourteen and a half days for total preimaginal development at a constant temperature of 22 °C [3], a first estimation was made of 16 days minimum. This was because the medium temperatures for March at 39S were estimated to have been a few degrees below 22 °C. On the other hand, the presence of *Dermestes* and *Necrobia* beetles indicated the beginning of butyric fermentation and pointed to a longer interval.

Fig. 7: *Map of Argentina. Province of Neuquen in yellow. The cities of Buenos Aires (capital of the country), Neuquen (capital of the province) and Zapala are marked.*

One factor with which we were not conversant at the time of the trial was the effect of wide daily temperature variation on the development rate of dipteran larvae. When the first author was giving evidence, a temperature sheet was presented in court, showing that the maximal temperatures had been very high indeed for the month of March, above 30 °C, while the minimal had descended below 5 °C, cold enough to stop insect activities.

Since at the time the author had no reliable information on the effect of such wide circadial fluctuations on the development rate of larva, it was considered more prudent to broaden the range and consider ten days as a minimum. Later on, some evidence came at hand [4] that this environmental factor produces longer development times, which supports the longer PMI estimate.

At the moment, literature in common use [2, 3] mantained that *P. sericata* oviposits on corpses only when they are in direct sunlight. It is widely known that this concept has been challenged [5]. In any case, the corpse must have been exposed to daylight, either in open air or through a window. This means that there was a period of time in which the corpse was left in a place where it was not concealed, and that later it was moved (as said) to a dark place. This time, however, may have been as brief as half an hour; even a few minutes may be enough for oviposition by *P. sericata*, and a single female, if left undisturbed, may have laid hundreds of eggs. The high number of maggots suggests rather collective oviposition by several females, but there is no conclusive evidence either way.

Some experimenting made in the city of Neuquen with domestic pig as a model produced a sample of *P. sericata* adults and pupae (that is, a sample slightly more advanced than the one in question) after 31 days of decomposition in a laboratory [6].

An interesting case of a body six weeks indoors has been described from Cologne, Germany [7]. Here, a lighted lamp had been left near the body. The left eye, further from the light, had been consumed by larvae of *P. sericata*, but at the moment of the finding there was a mass of larvae in the right eye-socket. This is explained on the grounds that, through general drying-out of the remains, the eye closest to the lamp had become the only feeding material for maggots, so that the youngest of these had to go there in spite of their aversion to light.

The body of Omar Carrasco, after (probably) four weeks, had larvae of *P. sericata* in the left eye-socket, which must have been nearer the ground judging by the post-mortem discoloration. The lower limbs had entered butyric fermentation and showed no feeding by insects. Had the corpse remained hidden for a couple of weeks more, there can be little doubt that the limbs would have begun to dry out.

In the case of Carrasco, however, no younger larvae were remarked, and it appears likely that there were none, because the body was hidden in a dark place and flies no longer had access to it. The younger larvae which switched to the right eye in the case from

Germany must have come from later ovipositions, which can be explained by the presence of the light, moderately strong, but very close to the body. Oviposition of *P. sericata* in artificial light has been recorded before [5].

Conclusion

Both medical and entomological findings showed that the death of Omar Octavio Carrasco had occurred more than a fortnight prior to the "official" finding of his body; that the said body had been concealed in conditions pointing to the participation of several persons, and therefore to collusion.

The pressure of public opinion, fed in part by indignation at this case, brought about the supression of compulsory military service in Argentina.

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Practical Note

Forensic Entomology – Past, Present and Future*

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Abstract

Entomology is derived from the Greek word *entomon* (insect) +*logos* (word, reason) meaning the study of insects. Forensic entomology is probably one of the oldest branches of forensic sciences. It has developed over the years from being used only for finding post mortem interval to being used for season of death, geographical location of death, movement or storage of remains after death etc. The development of entomotoxicology has further diversified and enhanced the role of entomology in forensic sciences. The use of DNA for identification of new species has opened a new chapter in the field. These are some of the aspects that we have to work on for future research.

Introduction

Entomology is derived from the Greek word *entomon* (insect) +*logos* (word, reason) meaning the study of insects. When we were asked to write an article on forensic entomology the first question that came to our mind was not how it is done or what are its uses and utilities but when it was started, how it has progressed over the years and what is its scope for future. This made us to search for the history of forensic entomology. In our search we found that the best work done about the history of forensic entomology was by Mark Benecke [1]. With full respect to him, we have not dealt with the history of entomology in great detail and recommend the readers to refer to his articles for any further details on the history of forensic entomology.

In this article we have tried to keep the use of medical and technical terms to the bare minimum. This is a deliberate attempt on our part as we wanted to keep the language of this article as simple as possible. We have done this so that someone who is not well versed in medical terminology is not disheartened by the complex and new-fangled expressions. Forensic entomology is a branch that has developed due to the dedicated work of a relatively less number of scientists as compared to other medical and paramedical fields. This field has developed as a continuum and there have been very few, if any, radical discoveries. As a result it was very difficult for us to differentiate the past from the present. Still we have arbitrarily labeled the period of the last couple of decades as present. Anything before that has been taken as past and the period taken as future is quite obvious.

The Past

The first instance of forensic entomology can be seen in the Chinese literature. In his book "Hsi yuan chi lu", the Chinese lawyer and death investigator Sung Tzu, in the 13th century has mentioned possibly the first case in which insects led to the murder culprit. In that case there was a stabbing near a rice field. A day after the incidence, the investigator made all the laborers to lay down their sickles. The presence of invisible bloodstains led insects (possibly blow flies) to one of the sickles. Thus the culprit was apprehended and he confessed to the crime [1].

Other instances of the presence of insects on corpses can be seen in the writings of the medieval times. There are documents dating as far back as the 15th and 16th century that clearly indicate the importance of

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insects in the decomposition of human bodies. They tell about the pattern of the destruction and skeletonisation of the bodies by the insects that have been reproduced by the modern research. The biologist Carl von Linné in 1767 said that three flies would destroy a horse as fast as a lion would [1].

Having seen the role of entomology in the medieval times let us now move on to the modern times. The credit for the first modern forensic entomology case goes to French doctor Bergeret [1]. He used forensic entomology to detect the post-mortem interval (PMI) in 1855. In that case the corpse of a child was found in a house. Bergeret was called to detect the PMI. In finding the PMI he assumed that metamorphosis involves one year and also that females lay eggs in summer so that the larvae would transform to pupae the next spring and hatch in summer. He found the eggs of *Musca carnaria L.* on the corpse that lays eggs before the body dries out. Using these findings he calculated that the body must have been left there at least a couple of years back. Thus we can see that in spite of limited knowledge and resources entomology could be of great use even in those times.

Pierre Mégnin can be regarded as the first person who undertook a scientific research on forensic entomology[1]. He worked on the subject for almost a couple of decades and compiled his findings in the form of a book titled *La faune des Cadavres* in 1894. In this book he gave the theory of eight successional waves of insects on bodies left in the open. He also mentioned that on buried bodies insects came in two waves. He also described the morphological features of various classes of insects that helped in their identification. His contribution in popularizing the subject remains unparalleled. As the reports started pouring in that Mégnin's work involved a lot of guesswork, people began modifying his findings to go with the flora and fauna prevelant at their places. This process started at the end of nineteenth century and has been continuing since.

The importance of ants and cockroaches in causing post-mortem artifacts was shown by German doctors Klingelhöffer and Maschka and the forensic pathologist from Poland (then Austria) Stefan von Horoskiewicz [1]. Both Horoskiewicz and Maschka have reported cases in which there were bites by ants or cockroaches that resembled ante-mortem abrasions or bruises. In all those cases, but for the findings and testimony of these renowned scientists, innocent people would have been punished [1]. During this time (the beginning of twentieth century) France and Germany were the main centers for the work on entomology. This is evident from the following two books of that time *Thierleben (Life of The Animals)* by Alfred Brehm and *Souvenirs entomologiques (Souvenirs of Insect Life)* by Jean Henri Fabre [1]. These books specifically dealt with carrion beetles and blowflies and went a long way in popularizing entomology among the people.

During the next few decades a lot of scientists worked on the subject and the database on the properties of insects increased. Although the amount of research increased in the field, there was no great increase in the popularity of the subject. Only a few scientists across the globe worked on insects. The main aim of this research was to prepare a database for their own geographic area and environmental conditions. All this changed in the mid 1960s. When Watson and Crick discovered DNA in 1953, even they would not have thought about its potential in forensic sciences especially forensic entomology. The use of DNA brought in a new era in the identification of the invertebrates. Soon DNA was being used to identify the insects at the scene of crime. This method was billed as more advanced and scientific than morphological features. Here we would not go into the details of the relative advantages and disadvantages of the use of DNA for identification, but would just state that because of the enormous number of species and diversity present in the invertebrates, the use of DNA is definitely a step in the right direction.

The late 1970s saw the emergence of entomotoxicology as a new branch of forensic entomology. In this the presence of toxins in the invertebrate decomposers was detected and was used as a method of finding the cause of death. So now the use of forensic entomology was graduating from finding only PMI to finding the cause of death.

The Present

In this section we would discuss the things that are currently being done in forensic entomology and their shortcomings. That is going to the basis of our next section viz. the future as the future is determined by the present. For the sake of continuity, we would start this section by discussing entomotoxicology. From detecting metals, in late 1970s it has graduated to detecting various drugs and their metabolites. Beyer et al[2]have reported a case in 1980 about a woman who was found in the stage of early skeletonisation, about 14 days after her death. They analyzed the larvae of *Cochliomyia macellaria* (Fabricius) (Calliphoridae) using Gas Chromatography (GC) and Thin Layer Chromatography (TLC); the results revealed the presence of Phenobarbital. Since then various scientists have detected the presence of quite a large number of drugs like benzodiapines, barbiturates, tricyclic antidepressants, various narcotics etc. etc. (the list is endless). The method of extraction has also improved from GC and TLC to the more advanced techniques like RIA, MS, HPLC etc. All this indicates that today virtually any drug can be detected in even minute quantities in the insect adults and larvae alike.

Newton's third law of motion states "every action has an equal and opposite reaction". This is also applicable for every new discovery. Although the use of entomotoxicology has increased and has brought in new avenues, it has also got some inherent problems that have to be solved before it can be used as an effective forensic tool. At present we don't know about the drugs that are accumulated by the insects. We are also unaware of the level upto which the various drugs are concentrated. It has been seen that various drugs alter the rate of growth of the insects. Some drugs increase the rate of growth while some others reduce it. Inability to recognize this fact can lead to an error in estimating the PMI by upto about 18 hours and sometimes even more [3-7].

Now let us come to the more orthodox use of forensic entomology i.e. for finding PMI. It has been noted that with changing times, the flora and fauna of various ecological regions of the world keeps changing. This includes the insect population also. As a result the current research is going on to note these changing patterns and update our knowledge accordingly. As has been already mentioned, the invertebrates constitute the maximum number of species on this planet. The number of species that we are aware of is still far away from being complete. This is more so in the tropical regions of the world like India. As a result the requirement for renewed research in finding as many species as possible is being felt all over the world. This is one of the most important activities going on in all parts of the world. The use of DNA for the identification and classification of species is going on in a big way. The advantages of this method are quite obvious and don't require any reiteration here.

One aspect of the use of forensic entomology that can be hard to believe and even harder to prove is in child abuse and sexual abuse cases. Its use in child abuse can be seen from the case described by Mark Benecke [8]. He has described a case in which a child's body was found. The use of forensic entomology put the time since death at about 6-8 days. But the examination of insects present in the ano-genital area of the child suggested that it had not been cleaned for about 14 days prior to the child's death. This case marked a landmark in the use of entomology in child abuse. Studies have shown that post mortem insect activity, particularly maggot masses, combined with natural decompositional changes can produce changes to clothing which mirror those seen in cases of sexual assault. This is again relevant in cases in which advanced decomposition has set in [9].

Today forensic entomology is not limited to finding PMI only. A forensic entomologist has acquired an important role in death investigation. He is required to do all sorts of works like finding time since death, season of death, geographical location of death, movement or storage of remains after death, time of decapitation and/or dismemberment, submersion interval, specific sites of injury on the body, postmortem artifacts on the body and the crime scene, use of drugs, linking a suspect to scene of crime, in child neglect, sexual molestation, identification of suspects etc [10].

The Future

Finally we come to the question: what does the future has in store for us? In which direction should the future research be directed? These are the questions one has to answer today. Before taking up these questions we should first discuss the shortcomings of the present methods.

First and foremost is the identification of correct species. Even today we are not aware of all the species of the insects. So to complete our list should be our utmost priority. The method of species identification can change e.g. from morphological features to DNA analysis, but the priority should not. Along with species identification the next thing that is to be done is to identify species in various ecosystems. For this the research has to be carried out in that particular geographical area for which we are finding the species. Not only the geographical area, the species also change with the time of the day, the season etc. So one has to take into account all these factors when conducting research on this topic.

Here we would like to mention a couple of special circumstances that can make our point clear. The first is the situation in which a corpse is found in a car. Now a car can be regarded as a special ecosystem as the points of entry in a car are bare minimum and sometimes are not present at all. As a result we are not aware what all species gain entry to this closed ecosystem and after how much time. Another case of point is the type of insects present in the water bodies viz. the lakes, rivers, oceans etc. This is important in cases where the bodies are found in water. In those cases it becomes very difficult to know the time since death as the body is usually decomposed beyond recognition. So the entomologist can help in such cases when everyone else has failed.

The next thing that we are going to talk about is entomotoxicology. Although it has made great progress in recent years, a great deal of work still has to be done in it. As we have already mentioned in the preceding section, this science has not yet developed to the level that it can be used in routine practice. The use of entomotoxicology can also be used for identification. This can be done by extracting the deceased's DNA from the invertebrates. When the insects feed on the human remains, they ingest human cells. These cells can provide the necessary sample for the sampling. Although one may say that if the DNA is present it will be present in the available sample of the human remains. But here we can say that we had come across

a case in which we got a totally charred body. The sample that we sent for DNA came negative for any DNA sample present in the remains. There were maggots present on the corpse. Although we did not send any maggots for DNA analysis, we feel that we may have been successful in getting a positive response especially because the maggots are present only on the live tissue. So we feel that this is one area on which the future research can be directed. It can be seen that DiZinno et al [11] have already done some work on this subject. They have used mitochondrial DNA to match the human DNA found in the blood in the insect's gut to that of the deceased's bone. In this case the deceased's DNA was available to match. This can be taken as a guide that in badly decomposed bodies forensic entomology can be used for the identification of the deceased.

An important thing that we must remember is that the future is in the hands of the youth. And the youth will take up the field only if we make it good enough for them to take it as a career. This is our responsibility i.e. those who are already in the field. We are saying this because forensic entomology has not progressed at the rate at which it should have. So the popularisation of the field should be one of the topmost priority of all forensic entomologists all around the globe. With more people joining the field will lead to more development paving the way for further research. This will lead to a cycle that can have only one effect, that of popularising the branch. So this should be one of the most important aspect of future development that we should strive for.

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Pratical Note

A Fly for Justice*

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* not peer reviewed

After dusk on May 13th 1998 agricultural student Russell Crookes went out with his friends Neil Sayers and Graham Wallis to the north woods near Hadlow College in Kent as they often did. Sayers claims that they returned to the college around 4.00 a.m. He saw the light go out in Crookes' room. It was the last time that he saw Russell Crookes alive. Until the discovery of Mr. Crookes' body on May 26th Wallis told the same story.

After his arrest, Wallis gave Kent Police a confession – it was more of an accusation against Sayers than a confession. According to Wallis, all three went to different woods referred to as Scene 2. At about 12.30 a.m. on May 14th Crookes was stabbed at least 23 times. After wood was collected and the fire stoked up, the body of Russell Crookes was flipped onto the pyre and burned from 1.05 to approximately 4.15. Once it had cooled sufficiently it was hidden in stinging nettles in Scene 2. Depending on which version is relied upon, Crookes' partially burned body was transferred to a grave in a depression in a wood about 200 yards away either at 9.00 p.m. or a few hours later.

The body was placed in a water-logged grave. According to the Crown's case it had lain in that grave undiscovered from the early hours of May 15th until May 26th. Apart from a missing pelvis, there was no scientific evidence of animal activity – at least none was reported by the Crown's pathologist, Dr. Michael Heath. There are many issues that arise in this case. However, for the purposes of this article I intend to concentrate on one of them – forensic entomology.

When discovered, the body of Russell Crookes was heavily maggot infested. This was the most important clue that Kent Police had at their disposal. Rigour mortis was long gone and the stomach area had sustained most fire damage. The entomological evidence was the best chance of timing the post-mortem event – in this case the partial burning of the body. It ought to have been the crux of the case for or against Neil Sayers – Wallis pleaded guilty and blamed Sayers for everything. In a nutshell, Wallis' confession was that he was there, but Sayers did everything, including mutilating the body. Both arms, a leg and a portion of the face were severed through bone after the body had been burned.

If Wallis' confession is true, oviposition – the laying of eggs by flies – would have occurred in the morning of May 14th. The maggots would therefore have been about 12.5 days old when the body was discovered.

Dr. Heath, failed to take any of the relevant temperatures at the scene as recommended by the renowned blow fly specialist, the late Dr. Zakaria Erzinçlioğlu in 1985. Forensic entomologists require data including relevant temperatures, barometric pressure, light intensity and relative humidity, among other data in order to extract all the evidence the maggots could give. Dr. Heath and Kent Police failed to obtain any of this evidence. The maggots were not collected in the manner recommended by forensic entomologists either. Half of the sample from the right rib-cage was fixed in formalin as was proper, although some entomologists prefer a solution of 80% ethanol; the remainder were for rearing.

The failure to collect the data suggested by entomologists meant that these maggots could not be reared in the same manner that they had been developing in the grave. This made it difficult to accurately time the post-mortem event. Far worse was to follow.

After placing this sample of maggots in a container with some liver they were handed to a Scene of Crime Officer (SOCO). They were transported to Tonbridge Police Station and put in the refrigerator, where they would die before reaching adulthood. The best chance to obtain the evidence that these maggots could have given had just been lost. Had Kent Police set out to kill

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these maggots deliberately they could not have done so more efficiently than this. These maggots were a precious evidential resource and should have been treated as such. Kent Police and their SOCOs were not trained forensic entomologists. Rearing maggots was a job best done by qualified experts. What made Kent Police think they were competent to do so?

Kent Police's failure to contact an experienced forensic entomologist is puzzling. Their treatment of the maggots establishes that their knowledge of forensic entomology fell far below the level required to obtain the evidence that these maggots could have given. Obtaining and interpreting the evidence that these maggots could have given was obviously a job that could only be done by someone with the requisite expertise to do so. Even in 1998 it was not difficult to obtain contact details for an experienced forensic entomologist. Kent Police could have requested such details from the National Crime and Operations Faculty (NCOF). In 1995 NCOF was established, partly to prevent investigative opportunities from being lost. Sadly Kent Police did not utilise this resource. Had they done so the entomological evidence would have been able to resolve many of the complex issues in this case.

Although the police had made a serious error of judgment in their handling of the maggots this need not have been disastrous. They could still have instructed an entomologist who could have examined the maggot carcasses and established what species they were. Their size could have been established both from examination of those maggots and comparison to the fixed sample. This would have established what stage of development they had reached and enabled the relevant post-mortem event to be timed. Wallis' account could then have been tested against scientific evidence. A data-log of the grave-site would have enabled more accurate temperature data to have been obtained that would have assisted the entomologist to calculate the time of the post-mortem event more accurately.

Sadly this did not happen. Kent Police never instructed a forensic entomologist. The prosecution lawyers never presented any entomological evidence. None of the prosecution lawyers asked the question of what had happened to the maggots. Nor did Sayers' defence. Despite instructing a pathologist, Sayers' defence never pursued the entomological evidence. Consequently, the jury which convicted Sayers did so in ignorance of the only scientific evidence that could determine when the body of Russell Crookes was partially burned.

Sayers was convicted in April 1999. I heard of his case in 2001. I noticed that maggots had been taken by Dr. Heath, but nothing seemed to have been done with them. One solicitor for Sayers would not even ask if the maggots still existed – apparently convinced that they would not, so there was no point even asking. He would be proved wrong.

In January 2003, the remaining maggots which were in a very poor condition were made available for examination by experts. Sayers' current solicitor, Jane Hickman instructed the esteemed forensic entomologists Dr. Martin Hall and Dr. Mark Benecke to examine them. Most of them were fragmented and resembled rust flakes. They were cleared up. The species was established as the bluebottle *Calliphora vomitoria.* It emerged that some time before this examination the sample of fixed maggots had been thrown away. The best opportunity to determine the precise size of the maggots upon discovery of the body had been thrown away – literally. Hall and Benecke examined several photographs. They had to work with extremely poor quality data. The most important factor in larval development is temperature. Hall and Benecke had to work with the assumption that the average of average temperatures from a nearby weather station accurately reflected the temperature within the grave with no evidence that it did. They had no choice but to rely on the data available to them. It was not their fault that this data was of such poor quality.

Hall and Benecke could not be sure that they had examined the largest maggot that was present in the body. The largest one that they examined was 12.1 mm. It was in third instar, the stage before turning into a pupa. This maggot was between young to middle third instar. Based on the poor quality data that they had available to them Hall and Benecke could not contradict Wallis' account. If better quality data had been obtained originally, their conclusions may have been different. That graphically illustrates the need to ensure that maggots are collected properly and that all the necessary data is gathered efficiently and accurately. It also demonstrates the need to fully utilise available resources such as the NCOF. In cases such as this it should be compulsory for police forces to obtain the services of relevant experts through NCOF if necessary.

Their examination of the maggots could have been the end of the matter. Had this occurred an appalling precedent would have been established. The certainty that the maggots could have offered if properly handled had been lost. This was due to entirely incomprehensible decisions by Kent Police. They failed to treat these maggots as the vital evidence that they were.

Imagine the following scenario. A brutal murder occurs. The major clue is a bloodstained handkerchief. The police split the sample in two. Instead of instructing a qualified expert they conduct their own DNA test. The results are inconclusive. Some time later they throw away the other sample. What is the difference between this scenario and what Kent Police did to the maggots in the case of Neil Sayers?

Police officers, civilian SOCOs and lawyers need to be far better informed of the possibilities of obtaining important evidence that forensic entomology offers. Had this case occurred in Brazil or the USA the treatment of those maggots by the police would have been sufficient to quash the conviction of Neil Sayers. In effect Kent Police destroyed important evidence through their incompetent handling of the maggots. But rearing maggots and interpreting entomological evidence was a job for a qualified and experienced forensic entomologist such as Dr. Hall or Dr. Benecke. There is no excuse for treating evidence in such a shoddy fashion, especially evidence that was potentially as vital as these maggots.

Dr. Heath collected the maggots without establishing all the evidence that was available from the scene. Heath took a second sample of maggots from the body at the mortuary. As things can be missed at the grave it is advisable to take a further sample in the mortuary. Dr. Heath did so. However, it is essential to know the temperatures that the body has experienced. In this case none of the temperatures that the body experienced from discovery of the body to examination in the mortuary were reported in Dr. Heath's statement. This is contrary to best practice.

Maggots taken from different areas of the body should be treated as separate samples. This occurred, but the second sample was given to a SOCO and stored in the mortuary. It was never reared or examined. It was completely wasted. This was absurd and defeats the point of taking this sample. The treatment of the second sample of maggots was a catalogue of errors from start to finish. Evidence – let alone vital evidence such as this – must never be treated in this fashion again.

The first sample could not have been reared more incompetently. Despite not being qualified for the task, the police attempted to rear – if that is the right word – maggots in the fridge without even taking the temperature in the fridge. The maggots were left there to deteriorate for years until they were in a terrible condition. The police never sought the advice of a forensic entomologist. Not surprisingly all the original sample died. Compounding the error the sample of fixed maggots was thrown away, making it impossible to obtain the evidence that the maggots could have given.

The treatment of the maggots in this case by Kent Police should serve as a model – a blueprint of how entomological samples must never be treated again. Forensic entomology is an established discipline that has been utilised for a century. Arguably the first example occurred in the Middle Ages in China. There really is no excuse for treating maggots in the fashion that they were treated by Kent Police in Sayers' case. There is also no excuse for failing to obtain the services of a forensic entomologist as contact details could easily have been obtained from the NCOF.

Kent Police treated this evidence woefully. Their conduct prevented experts of the calibre of Martin Hall and Mark Benecke from establishing the evidence that the maggots could have given in this case. The police should face severe sanction for their conduct in this case, as the entire blame for this fiasco rests fairly and squarely with Dr. Heath and them. Their incompetence deprived Sayers of the best opportunity to prove his innocence. It has also deprived the public of the answer to the pressing question of who is the real Graham Wallis. Is he a courageous young man who sent a brutal murderer to jail at the cost of his own liberty? Or is he a manipulative and cowardly murderer who not only savagely killed a 'friend,' but also framed another 'friend' for that murder? The negligent treatment of the maggots by Kent Police has deprived us of the definitive answer to these questions, so far. We can only wonder how many other cases have been tainted by such methods. Forensic entomology must be treated with the respect it is due from every tier of the criminal justice system throughout the world. Nothing less will suffice.

Despite having no control over these unfortunate events Sayers alone pays the price. However, Kent Police are rewarded for shoddy practices not only by depriving Sayers of the best chance to obtain scientific evidence to challenge Wallis' version of events, but also by making it extremely difficult to use entomological evidence on appeal. This case sets a potentially appalling precedent: if entomological evidence may damage the case one wishes to bring, sabotage it before it can wreck that case. As with all other disciplines of forensic science entomology must be allowed to lead investigators to whatever conclusions are justified by the evidence – inconvenient though that may be. Properly constituted forensic entomology can convict the guilty and clear the innocent as can DNA or other techniques. It must be allowed to do so.

This could have been the end of the story for Neil Sayers and the maggots. Sayers is fortunate that his plight attracted the interest of the Spanish entomologist Dr. Alfredo Piera Pellicer, of the Centro de Entomología Forense y Radiología Experimental in Valencia, who has developed a very interesting technique that can retrieve the entomological evidence of this case from the mess caused by Kent Police.

Dr. Piera uses a reconstruction chamber to artificially recreate the conditions in actual cases. I discussed Sayers' case with him and he very kindly offered to conduct such an experiment. The precise parameters for this experiment are being considered as I write. The technique depends on the quality of data provided. In Sayers' case that data is of a woeful quality. However, further experiments and research can be conducted even now to obtain better quality data in order to enable Dr. Piera's technique to perform to the

best of its ability. It will also provide data that will increase our understanding of forensic entomology.

Piera uses his technique to teach his students best practice. Sayers' case is ideal for that purpose. Hopefully it can also provide a definitive answer to the question of who is the real Graham Wallis. Dr. Piera believes that it can tell to a margin of error of 2-3% when oviposition occurred on the body of Russell Crookes, despite the negligent and incompetent treatment of the original maggots in this case. Piera's technique offers the chance of obtaining useful entomological evidence even in cases such as this. Forensic entomology has well and truly come of age.

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Interview

"I don´t know" can be the best answer

Madison Lee Goff[1] & Mark Benecke[2]

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M.B.: Lee, is forensic entomology a science? Or what else is it?

L.G.: I regard forensic entomology as applied science. The estimates we generate are based on results of carefully conducted experiments and field observations. These should be documented and published in peer-reviewed journals, subject to review and comment. They must not be "unpublished personal observations" as a general course. The results must be repeatable and documented.

As for all applied sciences, there is a measure of skill and "art" in the application of the data to a particular situation. This is the same for crop pest control or putting a satellite into orbit.

M.B.: How did you as a biologist manage to start communication and co-operation about FE with medical people, the police, the coroner/forensic pathologist/chief medical examiner? Any hints for the next generation about bridging that cultural gap?

L.G.: When I first began to interact with law enforcement and other forensic disciplines some 25 years ago, no one knew what forensic entomology was and I was regarded as being a "little strange." To a certain extent, I am still regarded as being a little strange, but the discipline is now accepted.

Acceptance was a long slow process. I was determined and kept contacting the agencies until they began to realize that I was not going to go away and, more significantly, the results were useful to their investigations.

For those beginning to work in the field it is important to have a realistic grasp of the role of the forensic entomologist. We are not law enforcement, with rare exceptions.

Stay within your area of expertise and do not attempt to inflate your own importance. Stay open to change. It will continue to happen and you need to be able to adapt.

M.B.: You are now head of your own forensic program at Chaminade University in Hawai´i Which topics do you focus on there for the students?

L.G.: My program here at Chaminade University leads to a BS degree in Forensic Sciences. We provide a strong background in the basic sciences and math combined with course work in criminal law.

Our forensic sciences courses include a core of general forensic sciences, physical forensic sciences and forensic biology, crime scene investigation, seminar and an internship served with an agency directly involved in forensic analyses. Electives include forensic photography, entomology, anthropology, computer crime and several criminal justice courses.

Our aim is to provide a general education in a wide spectrum of forensic science disciplines that will allow the student to specialize during graduate training.

M.B.: What do you do if you calculate PMI but it does not match to what the police would prefer to hear, or found out yet?

L.G.: My calculations are based on available data and are not dependent on the desires of the police. There are instances where I do not receive all the relevant information initially and an estimate may change based on those data. Never on the desires of an investigative agency. The estimates are based on an impartial analysis of evidence submitted. Not always popular but respected.

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M.B.: From your experience, which type of person will enjoy forensic entomology as a profession(al career)?

L.G.: Based on my experiences, an individual wanting to work in forensic sciences in general has to be a little strange. We go to situations that most people avoid.

You must be able to distance yourself from the situation and view the evidence as evidence, not becoming emotionally involved.

You need to remain objective. You are looking for the truth of the situation and that is not as variable as lawyers would lead you to believe. You need to be someone who enjoys puzzles and does not want life to be routine. If you want a 9 to 5 job, this is not for you.

Life can be much like having Christmas every morning. You never know what will be waiting for you. You need to be a little nosey and not accept the easy answers all of the time.

You also need to remember that sometimes things are exactly what they appear to be. Develop a sense of humor. You'll need it.

M.B.: Which of your own papers do you like best?

L.G.: Actually my favorite paper would be the book I wrote: *A Fly for the Prosecution*. Primarily because I got to insert a little of myself into the situations. More that the publishers might have wanted but less than I started with.

Past that my first paper *A short note on distributions of chigger mites on islands in the North Central Pacific*. Not earth shattering, but I'm still amazed that I actually had nerve enough to write it, given my position as a technician in the Entomology Department at the Bishop Museum at the time.

M.B.: Was there any scientific paper or observation that made your mind blow?

L.G.: In retrospect, I was fascinated by S. J. Gould's work on the ideas on evolution. Can't say they changed my existence, but they stared me thinking in different ways. Past that, I had some wonderful conversations with him.

M.B.: How did you manage to get your family, especially your daugther, enthusiastic about forensics?

L.G.: My family is amazingly tolerant of my activities. Before I became interested in forensics, I worked on ectoparasitic mites. This meant trips to catch and skin rodents and other small mammals, collection of amphibians and reptiles. My wife went with me and helped in these projects. Forensics may have actually been a relief.

I don't keep those specimens in the freezer at home. My daughters grew up with my research. They went with me to visit the decomposition studies and based school projects on some of these.

I never pushed what I do on their futures. One has decided she enjoys it and is now working on her graduate degrees in forensic anthropology. The other has gone into communications and education, working with a pre-headstart program aimed at low income/recent immigrant families here in Hawaii. I'm very proud of both.

M.B.: In one sentence, what is your basic guideline for forensic entomologists?

L.G.: Follow the evidence and be objective in your analyses.

M.B.: Anything else?

L.G.: One of the major problems I have seen recently is in entomologists inflating their roles in the investigation. Too often I see individuals forgetting they are scientists and not law enforcement.

One should provide testimony only with one's own area of expertise and be certain not to extend opinions past the limits of the available evidence. Deviations from this do not serve the legal system or justice. Sometimes, in fact quite often, "I don't know" is the best answer in the world.

M.B.: Thanks, Lee.

L.G.: Aloha.