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09:20-09:50 **Physical Evidence in the Investigation of Crime" – An introduction to the work of the Physical Evidence section of the National Crime & Operations Faculty (NCOF)**

Jonathan Smith

National Crime and Operations Faculty, Bramshill, Uk

This paper describes the role of the National Crime and Operations Faculty (NCOF) in serious crime investigations. The NCOF was established in 1996 to support UK police forces in the investigation of offences of murder, rape, abduction, and series sexual offences.

In order to carry out this support function to greatest effect the NCOF often needs to call on experts from many different disciplines. This co-ordinated response is carried out through a helpdesk at Bramshill and through regional support officers around the U.K.

The Physical Evidence Section at Bramshill is comprised of experienced forensic scientists operating as specialist advisers; National Injuries Database, who have detailed records of types of injuries found on bodies; consultant pathologists; and consultant toxicologists.

In some investigations the need to incorporate a greater range of forensic capability is apparent. For instance issues such as time of death may be crucial to a murder enquiry, and such information can be assisted by reference to entomological studies at the scene.
At the same time it may be necessary to consult with other experts at the scene such as archaeologists, palynologists, and anthropologists. In these instances the way that the experts work together, and issues of primacy are of paramount importance.

The work of scientific experts such as forensic entomologists in serious crime investigation has become of increasing importance in recent years. The need for communication between experts in this field and investigators can be assisted by providing an integrated team approach to assist Senior Investigating Officers and scene examiners. The NCOF are in a position to facilitate this approach whereby all aspects of scene expertise can be drawn together.

Presenter – Jonathan Smith

A Forensic Biologist with over 20 years experience. Originally working for the Home Office Forensic Science Laboratory in Nottingham, specialising in conventional blood grouping techniques and scene examination. Subsequently working for the Forensic Science Service in the Huntingdon, Aldermaston, and London laboratories. Between 1989 – 1997, Head of DNA profiling in the Aldermaston Laboratory covering forensic casework from Southern England, Wales, and from overseas forces.
Currently a Specialist Advisor working with both the Forensic Science Service, and the National Crime & Operations Faculty, assisting in the use of physical evidence in investigations across the UK.

09:50-10:10 **Distribution of the blowflies *Phormia regina* and *Protophormia terraenovae* in the Netherlands and Western Europe**

Hans Huijbregts

National Museum of Natural History, Leiden, Netherlands

About eight different blowflies are regularly observed during forensic investigations in The Netherlands. Most species can be found everywhere in The Netherlands, but *Phormia regina* and *Protophormia terraenovae* are restricted to certain areas. Both species are widespread in both the Nearctic and the Palaearctic region. In literature they are reported to be cold adapted, but this does not explain the Dutch distribution patterns.

Literature is vague about the European distribution of *P. regina*, but it seems to be extremely rare in most European countries. From the Netherlands, Fennoscandia and the UK together only 4 old records from the last century of *Phormia regina* do exist. Much to my surprise the species turned up in a forensic sample from The Netherlands in 2001; last year it was even collected on 4 separate occasions. This may indicate it is expanding its range. Relative frequencies in forensic samples are reported as follows: Hamburg 0%, Frankfurt 1%, and Vienna <1%. More information is needed to get a precise idea about the European distribution and its possible expansion.

Within The Netherlands *Protophormia terraenovae* is only rather common in the large cities along the coast, in inland rural areas it is very rare. Nuorteva (1960, 1963) describes it as a primarily arctic species, which in more southern latitudes only occurs under conditions created by man. According to scattered data available from different parts of Europe there it seems to be a typical city species rarely occurring in rural surroundings, except in mountain areas. Its preference for cities can not be explained by a supposed preference for cooler areas, but Nuorteva gives a convincing explanation for that fact. He noted in Finland that *P. terraenovae* is able to tolerate unfavourable conditions (low humidity, high and low temperatures, food deficiency, crowding etc.) and that it is only dominating in conditions where no other blowflies occur. He concluded that the species apparently has a low competitive ability. This could explain why it is unable to survive in the natural conditions of more southern latitudes with a larger number of competing species. When attempting to explain the distribution of *P. terraenovae* it is therefore wrong to concentrate on the preferences of *P. terraenovae*, but one rather has to examine why certain areas are unfavourable for other blowflies.

Nuorteva, P. 1960. Förekomsten av asflugan *Phormia terraenovae* R.-D. i Finland.-- Notulae Entomologicae 40: 38-45.

Nuorteva, P. 1963. Synanthropy of blowflies (Dipt., Calliphoridae) in Finland.-- Annales Entomologici Fennici 29: 1-49.

10:10-10:30 **Post-mortem artifacts made by ants and the effects of ant activity on decompositional rates**

Carlo P. Campobasso [1], Daniela Marchetti [2] and Francesco Introna [1]

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The cadaver may be attacked by a wide variety of insects. Not only flies but a large array of arthropods, including Coleoptera (beetles) and Hymenoptera (wasps and ants) can colonize the corpse. Ants, in particular, can be present at all stages of carrion decomposition as they are opportunist feeders on fly eggs and larvae. They are typically observed shortly after death or during the early postmortem period but even after the fly maggots had left the body. The ants are capable to remove and capture adult and larvae of flies and beetles thus reducing significantly the rate of decomposition but also to feed on carrion itself. Their role in the faunal succession varies from predator on the eggs and larvae of other insects (mainly Diptera) to scavenger on the flesh or exudates from the corpse itself. They can be the numerically dominant arthropod species on the corpse and they can establish a colony. Some forensic cases dealing with ant activity and post-mortem interval (PMI) estimates will be illustrated.

However, since ants are often overlooked on the recovery site and the value of these insects as forensic indicators is still not appreciated by investigators and forensic pathologists, the goal of this brief presentation is to focuse not only on the effect of ant activity on decompositional rates and, consequently, on PMI estimation but also on interpretation of post-mortem artifacts usually associated with ant colonization. Many small punctate or scratch type lesions may be often observed on the body which are the result of post-mortem ant attack. Usually ant injuries are small and diffusely scattered over the skin surface. These injuries consist of small gnawed holes which can be easily misintepreted as ante-mortem abrasions or resulting from strong acids. In fact, ants do of course secrete formic acid causing the removal of the surface layer of the skin and no blood is usually associated with the skin lesions inflicted by the feeding action of ants.

As ants attack the uncovered areas of the body, these injuries can frequently give rise to suspicion especially if located on the neck. They are occasionally misinterpreted as patterned abrasion due to the imprinted effect of a blunt or offending object. For example, the tips of the fingers can produce contusions or erythematous marks very similar to ant bites. The features of such lesions observed in several forensic cases will be illustrated in detail. Among them the case of a 8 years-old child found dead in a rural area near Rome, during summer. At the crime scene the body was covered by ants showing diffusely post-mortem artifacts even on the neck close to ante-mortem contusions. Dissection of the throat revealed hemorrhage into the musculature just beneath the small contusions and at the anterior surface of the thyroid cartilage while no bleeding was associated with the initially suspected ant marks. The autopsy findings confirmed that the child was manually suffocated by his father during a sexual abuse.

During examination great care must be taken to determine if the artifacts made by ants have taken place in superficial ante-mortem injuries such as nail abrasions in the neck following manual strangulation. Sometimes, on closer inspection, the origin of the artifacts made by ants can be immediately apparent especially when the ants are identified upon the body but it can be only confirmed at the autopsy by gross and microscopic analysis.

10:30-10:50 **Temporal occurrence and development of Dermestid beetles (Coleoptera: *Dermestidae*) on human corpses in the domestic environment**

Heike Klotzbach, Vanessa Garbe, Lars Oesterhelweg, & Hilke Schroeder

Institute of Legal Medicine, Hamburg, Germany

The well investigated life cycle of necrophagous flies and their maggots is a useful tool in post mortem interval (PMI) estimation within the first 4-6 weeks. Cases with longer PMI are difficult to determine by entomological means. In a prospective study over 3 calendar years 32 cases of human remains, in a domestic environment, colonised by Dermestid beetles were investigated. The PMI ranged between 3 weeks and 2 ½ years and was determined mainly by police investigation. *Dermestes maculatus* was found on 21 bodies, *Dermestes lardarius* on 17 bodies, 6 corpses were colonised by both of these species. Larvae were detected in 29 cases and first occurred after a PMI of 4 weeks. Due to their feeding preferences of dry organic material a specific colonisation pattern of the Dermestid beetles on the bodies was observed concerning the process of dehydration of the remains. Adult beetles and larvae were found in each case from 3 months to 1 year PMI (n=11). Interestingly in the cases with a PMI longer than 2 years only a few adult beetles but no larvae were detected whereas empty larva exuviae indicated previous development of these organisms.

In general our data point out that there are regularities in the development and colonisation pattern of Dermestid beetles on human corpses in regard to the stages of decay and dehydration of the remains. The role of these insects as a valuable tool in PMI estimation shall be confirmed by further evaluation particularly in cases with extended PMI.

11:20-11:40 **Use of time-lapse photography in forensic science**

Matthew Wright

Image Impact, The Old Rectory, Woodlane, Clapton in Gordano, Bristol, BS20 7RQ

This talk will give an overview of time-lapse photography techniques. There will be a brief demonstration of the equipment commonly used by professional film makers and recommendations of apparatus suitable for use in scientific studies.

A few clips will be shown from the BBC forensic film, The Witness Was a Fly, before a concluding discussion on the benefits of time-lapse photography to forensic science.

The company Image Impact (an independent production company) is currently making a documentary on forensic science, in which forensic entomology will be a major part. I am very keen to make contact with potential contributors with whom I can discuss research and actual case studies. There will be an opportunity for this at the end of the session.

11:40-12:00 **Decomposition in cars: A Forensic Entomology Perspective**

Ian Dadour

Centre for Forensic Science, University of Western Australia, Nedlands, 6009 Western Australia

To determine the entomological succession on a decomposing pig inside a vehicle following its sacrifice by CO poisoning (suicide pig), to calculate the time of decomposition of a suicide pig and determine temperature fluctuations inside vehicles

Decomposing animal tissues provide an opportunity for flies to utilise the fauna and flora of microorganisms. Cadavers undergo a series of predictable changes during decomposition, and are "visited" by a succession of flies and other insects. This ordered process has handed us an invaluable tool for estimating the age of cadavers (Post mortem interval) and the biology of this process has resulted in the science of Forensic Entomology. One current program at UWA is determining the post mortem intervals of pigs decomposing in vehicles (simulated suicides).

The significance of this research is the:

- requirement by police, coronial and justice system for accurate information on cadavers, particularly in regard to homicide and suicide and the
- accuracy of the Post Mortem Interval (PMI) depends on the available knowledge base, which in most cases is dependent on a number of facts and assumptions concerning insect biology, behaviour and ecology. It is the assumptions that require investigation especially in cases of suicide and suicide/homicide. The Police Service and the Justice system are constantly expressing concern about the accuracy of the PMI.

Most suicides conducted in vehicles happen in isolated areas where the vehicle is parked in wooded areas for the purposes of concealment. Generally this is a shaded area or an area with minimal exposure to the sun. The methodology includes sacrificing 45kg pigs using CO gas. Following death the pigs are quickly dressed and placed inside a vehicle. CO gas is pumped into the vehicle for another 5 hours. Decomposition is recorded using infrared video cameras as well as daily observations of insect activity. Decomposition rates are compared with two other pigs (one pig sacrificed by CO poisoning and the other by head bolt) decomposing under normal conditions. Each pig has a datalogger placed inside its throat, abdomen and colon. The temperature inside the car is also measured and compared with the recorded ambient temperature.

12:00-12:20 **Effect of Arson on Entomological Evidence**

Gail S. Anderson

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Arson is a common method used by a killer in an attempt to destroy evidence. An experiment was conducted to determine the impact of arson on entomological evidence on pig carrion. Three freshly killed pigs (*Sus scrofa* L.) were placed in a small detached house, in Edmonton Alberta, in summer 2003. The house was approximately 50 years old and had a single storey plus basement. One carcass was placed in a bedroom, one in the living room and one in the bathtub. A small cut was made in the screen of an open window in a room unoccupied by a carcass.

The remains were allowed to be naturally colonised by insects. Extensive blow fly (Diptera: Calliphoridae) colonisation occurred, and the remains were allowed to decompose to the remains stage. At the time of the fires, the entomological evidence consisted of very large numbers of larval Calliphoridae, pupae and empty puparia.

Four successive simulated arson fires were set by the Edmonton Fire Department, and extinguished. The first fire involved pouring gasoline on a blanket over the carcass, then covering the carcass with a mattress, also liberally coated in gasoline. The second fire was begun in the furniture, using common inflammatory materials such as newspapers and the third fire was set using gasoline in the basement. Finally, the entire house was torched and allowed to burn itself out.

After each fire was extinguished, the carcasses were examined for entomological evidence. In all, cases, enough entomological evidence was found after every fire, including the final destruction of the house, to allow an entomologist to analyse the evidence and to determine elapsed time since death. In several cases, live insects were recovered.

12:20-12:40 **Effect of various substances on the delay of colonisation by necrophagous insects**

Benoit Bourel [1,2] , Valéry Hedouin [1], Didier Gosset [1]

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The aim of this work is the study of the effects of chemical substances (petrol, patchouli, HCl, NaOH, insecticide, citronella and lime) on the delay of colonisation by necrophagous insects. It is a synthesis of five experiments carried out in September 1999, July 2001, July 2002, July 2003 and August 2003. For each experiment, there were two controls and the other rats were splashed with one of each of the substances. Animals were placed in plastic boxes whose sides were replaced by wire netting. The boxes were disposed on the flat roof of the Forensic Institute. The distance between each rat was about five meters. Adult insects, eggs and larvae were noted and sampled regularly, every hour at the beginning and less frequent at the end of experiments. Meteorological data also were noted.

The time of colonisation for all rats varied from three days (2001, 2002, 2003) to eleven days (1999). It can be interpreted by meteorological data less favourable to insect activity in September 1999.

Three intensities of effect can be described after the synthesis of all results : NaOH, citronella and lime had no or few effect on the delay of colonisation and were similar to the controls, the first flies (Calliphoridae, Sarcophagidae) were attracted rapidly (less than one day). Petrol, HCl and insecticide had an average retarding effect: one or two days in 2001, 2002, 2003; seven days in 1999. Finally, patchouli presents an important retarding effect: two to three days in 2001, 2002, 2003; eleven days in 1999. Note that eggs or larvae were seen rapidly after the first adults. The number of taxa was also greater in control than in other rats. The minimal number of species was seen in the patchouli rats.

This study highlights that it is possible to delay colonisation of a corpse by necrophagous insects simply by splashing substances on to the corpse. Even if the precise estimation of the delay is impossible because it is also dependent on meteorological data, it is important to take it into account in forensic expertises.

14:00-14:20 **Autoerotic Accident, Shooting or Stabbing? Staphylinid Beetles Tell the Story**

Mark Benecke

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Scene of Crime

A corpse was found during a very hot period of the extremely hot summer of 2003 in a shaded forest area on the border of Cologne (with one million inhabitants the fourth largest city of Germany). The area is intensively used by persons of both genders cruising for sexual encounters; however, no decomposing organic material in the sense of scattered trash was found in the area.

On the scene of crime, the most prominent findings at the corpse were (a) that the eyes were intact with relatively few maggots present, (b) the presence of several holes in the skin of ca. 7 mm in diameter, and (c) a blood-like substance that was found on the insides of the thighs of the corpse including the jeans trousers that partially looked as if they were soaked in blood.

Two prominent holes were seen on the inner part of the partially exposed left thigh and under the chin. The holes were investigated by the author, and seemed to be missing tissue up to a depth of several mm. Also, thin stripes of body tissue were visible bridging the empty spaces.

The length of the maggots did not exceed 5 mm. Due to the early developmental stage, anatomical determination was not performed. Some green shiny adult flies were seen at the forest site on the corpse but could not be captured. In experiments performed at the same time in a location similar to the forest site (where the corpse was found), we caught mostly the heat-adapted *Lucilia species.* At the forest site, a single adult Silphid beetle *Nicrophorus species* was observed approaching the corpse by flight but could not be caught either. Several adult wasps approached the corpse on the scene.

Whilst undressing of the body before autopsy three days later, dozens of adult beetles *Necrophorus (Nicrophorus) humator* (Goeze) and few *Necrophorus (Nicrophorus) vespilloides* (Herbst) tried to escape and were collected.

Since the body was found hanging in a belt, it was unclear where the blood came from, if this was an autoerotic accident, and – due to the skin lesions – if shooting or stabbing was involved.

Material and Methods

One live beetle was collected at the scene of crime in the morning of July 26, 2003; many more were collected before autopsy in the morning of July 29, 2003. Collected animals were stored in 94 % EtOH (methylated spirits) [1,2].

Photographic evidence for forensic entomology purposes consists of 69 high resolution (4 MPixel) close-up photographs, mostly taken without flashlight to avoid whitening out of important areas [2].

Determination [3,4,7] was performed using a Leica Mz 12.5 binocular dissecting microscope (up to 10x10 fold magnification) with a Leica KL 1500 LCD circular light source.

Discussion

Exact estimation of the post mortem-interval was not performed because the macroclimatic conditions in the forest were unclear. Also, weather conditions during the actual summer were highly unusual; daytime air temperatures between June 15th and 20th, 2003 exceeded 30°C on four days. In experiments performed during our training classes all through the summer of 2003, we found a highly unusual number of blowfly (maggot) predators like Silphid beetles, ants, and wasps. Also, in experiments carried out with portions of 250 g of liver that were deposited in a shady grass and shrubbery area close to an old brick wall in August 2003 in Cologne, all (!) maggots that left the liver for pupation were caught by ants.

We focused instead on the numerous holes found in the skin of the corpse. The holes did not stretch into deeper layers of tissue and could all be explained by the activity of adult Silphid beetles, especially the large *Necrophorus humator* but also the smaller *Necrophorus vespilloides*. Both Silphids are attracted by the smell of small and large vertebrate corpses. *N. vespilloides* is typical for forest areas and is less frequently found outside of forests. *Necrophorus spec.* is among the very few insects that not only build male-female couples but also take active care of their larvae. Usually, *N. vespilloides* tries to bury the corpse which is however not possible with human corpses [4,6].

The holes that we observed were caused by two *Necrophorus* species gnawing off parts of the body tissue to build breeding balls for their larvae. The food balls are stored in crypts around 10 cm below the surface of the soil. Up to 24 eggs are deposited inside of the food balls. The larvae grow and live inside of the food balls for around 5 to 7 days, then pupate close to the crypt. *N. vespilloides* hatches in the same year and can hibernate as a young adult. The actual lifetime of the adults is around 3 months.

The finding of *Necrophorus* is interesting not only because of the morphology of the holes in the skin of the corpse but also because in another corpse we found exclusively *Necrodes littoralis* (L.) larvae. This body was found in an area in Cologne that was covered with only few small trees (like birches) and consisted mostly of grass and shrubbery. The body (female, age 15, slim) was found earlier in the year on June 3, 2002. A girl had been killed on April 7, 2002 at the place where the corpse was found. Larvae had been collected by the author at the scene of crime from the corpse. In contrast to *Necrophorus*, *N. littoralis* is known to feed and breed only on large vertebrate corpses. In this case, the larvae were found inside of the arms, legs and the chest. It is very likely that all these locations were entered through stab wounds. However, the freely exposed anal region was severely damaged by the activity of the beetles, too.

It is also interesting to note that *Necrophorus* adults did hide and remain on the corpse inside of the clothing after the body was transferred to the Institute of Legal Medicine. Since the animals prefer to be active during night time, it must be stressed that an intensive search for *Necrophorus* is strongly recommended in cases where one or several holes are found on any corpse. Furthermore, *Necrophorus* beetles follow hissing chirping sounds that are produced both by male and female adults. They can fly very well even if they frequently avoid this under the influence of light.

The presence of Histerid and Silphid beetles in forensic cases is well documented for over 100 years [5]. Holes in the skin produced by *Necrophorus* and *Necrodes* can be restricted to one area of the corpse, or be distributed over softer parts of the skin all over the corpse, depending on the storage conditions. They start with diameters of a few millimetres but over time extend

in size. Another group of beetles that produces circular lesions, even in dried out skin, are Histerid beetles like *Hister cadaverinus* (Hoffm.) [1].

These cases very clearly show that *high resolution close-up* photographs of the actual state of the corpse at the crime scene are necessary to determine the entomological state of the corpse.

Acknowledgement
The author thanks Det. Sgt. Markus Weber of the Homicide Bureau KK 11 of the Cologne Police. His and his colleague's very quick reactions make useful forensic entomological investigation possible.

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14:20-14:40 **Death, elderly neglect, and Forensic Entomology**

Mirella Gherardi & Gabriella Costantini

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The body of an 89-year-old women was found in her bed at 6.35 am on June 25 in the Northern Italy. The deceased's home was a very dirty and untidy flat shared with her daughter. The body was removed without any medical observation and, due to the poor condition of the body, the initial police assessment was that the lady died many days before.

Legal proceedings for "concealment of a corpse" were opened against the daughter, who however claimed that she given dinner to her mother only the evening before the body was discovered.

Therefore, the investigators requested that the body be examined by a forensic pathologist. Third instar larvae of *Lucilia sericata* (Diptera Calliphoridae) and second instar larvae of *Sarcophaga haemorroidalis* (Diptera Sarcophagidae) were collected both from the body surface and from the diapers. Despite this kind of evidence, the body temperature was still high (34,7 °C) at 11.15.am. On external examination no post-mortem changes were observed, but extensive decubitus ulcers and wide dystrophic areas, which had been misinterpreted as putrefactive patterns by the investigators, were found on the back and legs.

These findings taken together indicate the well-grown 3-4 day old larvae found on the body were the result of wound myiasis occurring before death. The development times required for the larvae to reach the recorded size suggest the old lady was not receiving proper care: therefore the legal proceeding was changed from "concealment of a corpse" to "elderly neglect".

14:40-15:00 **Forensic Entomology introduced in Basque Country: A case study**

M. I. Saloña*, M. Carles-Tolrá, P. Bahillo, V. Iraola & R. Alcaraz

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A case study from a rural area in Bizkaia (Basque Country, Spain) has allowed a first contact between the magistrate and the forensic doctor involved in the research, promoting interest in typifying the entomofauna associated with a cadaver for the clarification of death investigations in the region.

The cadaver was found in summer 2003 close to a local road, in a shaded area hidden by a dense shrub layer. As reported by the police, it was covered by a dense maggot mass of white jumping larvae and had been removed 10 days before the first author visited the scene. Nevertheless, a selection of soil samples was extracted at head, trunk and legs level as well as at several meters distance from the place. Soil fauna was manually sifted out and soil remains placed in Berlese-Tullgren funnels for a selective extraction of inhabitants. A visit to the morgue one day later allowed the collection of live insects from the corpse. It was largely skeletonized with some dark saponified fat and soft tissue covering the pelvic region and extremities. A PMI of about 3 months was reported.

The dominant species on the corpse was III^{d} instar fly larva with the peculiar jumping capability described for the family Piophilidae. Cultured to adult they were identified as *Stearibia nigriceps*. The same fly was emerging from the soil sample extracted at trunk level and abundant puparia were found in the soil, the flies emerging during the week. This species has been reported from a similar case in Venice (Turchetto et al., 2001) and adults collected on pig succession models by Anderson & VanLaerhoven, 1996 and Castillo Miralbés, 2002. Also important was the presence of *Necrobia rufipes* maggots on the corpse, cultured to adults in 1 month, abundant and cohabiting with *Necrobia violacea*, Histeridae, Staphylinidae and Nitidulidae in the soil. A new record for the Iberian Peninsula (*Omosita depressa*, Nitidulidae) has been reported from this study (Bahillo de la Puebla et al., 2004). Finally the mite *Hypoaspis aculeifer*, a predator of dipteran larvae, was also abundant both on the corpse and in soil samples.

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15:00-15:20 **Murder followed by suicide in a forest: what could be learned from a comparative field experiment**

Claude Wyss[1] & Daniel Cherix[2]

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Two corpses were discovered on 26 May 2000 in a forest situated on the property of Charlie Chaplin near Vevey (canton de Vaud, Switzerland). The first was the body of a woman, on the ground, with the head separated. As the forest is in the form of a series of banks, the body probably slipped down to the next bank. The remains were in a state of advanced decomposition and the head was partly mummified. The area next to the head was scarred, showing that the body spent some time there before slipping down. A few meters away the body of a hanging man was found. The corpse was fully clothed and in a late stage of decomposition. Eggs, larvae and pupae of flies were observed and collected, as were adult flies and beetles.

During autopsy of the female body, the medical examiners found no evidence that the head had received any ante mortem traumatic injury. Cervical vertebrae (atlas and axis) showed no impact of any sort. As to the male body, the medical examiners concluded that hanging was the cause of death. Curiously this body was less damaged than the female one. Numerous flies' larvae were collected from the abdominal cavity and chest.

In order to estimate post mortem interval (PMI) a temperature recorder was left at the crime scene for a month, and comparisons with the two nearest recording weather stations allowed us to make an accurate estimation of temperatures during the month preceding the discovery of the two corpses. We were able to provide the following assessment as to the day of the presence of the first egg laying flies on the two bodies: 25 April (+/- 24 hours). We could assume that the two victims had died between April 24 and April 26. Other evidence from the investigation showed that the couple was last seen on April 25 at 11 a.m.

Because of the great discrepancy observed between the two bodies (decomposition stage, weight, fly colonisation) we embarked on an experiment on May 1 2002 with two pigs (about 50 kg each), one hanging, the second one laying on the ground. Nearly each day during two months we checked both pigs[1]: visual observations, collection of flying adult insects, location of major insect infestations etc. We found no difference between the colonising fly species, but significantly less egg masses on the hanging pig. Moreover large amounts of larvae fell to the ground when moving over the body surface in order to reach natural orifices (i.e. mouth, ears), or they were washed down by rainwater. We concluded that on a hanged body fly behaviour (egg laying activity and competition for suitable egg laying places) and mechanical factors (dropping of larvae) affect body decomposition, but this does not distort an accurate estimation of PMI.

[1] see also www.entomologieforensique.ch

09:30-10:00 **So just how good is Forensic Entomology?**

Bryan Turner

Department of Life Sciences and the Forensic Science Unit, King's College London

The purpose of this presentation is to sound a warning to forensic entomologists about the need to ensure that the statements we make are drawn from pertinent, correct and verified datasets with known variation limits.

There is growing evidence that expert witnesses are coming under greater challenge in the courts. The evidence from several established methodologies and experts has been cast into doubt in recent months in the UK. We must be careful in this increasingly sceptical world not to fall into a similar situation.

The author identifies some of the areas where our science is often weak:

- An understanding of the relationship between the population characteristics of blowflies in the locality and those for whom we have published data.
- A detailed knowledge of the species that are of key interest in a locality at different times of year
- The impact of temperature is still poorly understood in the development processes of many blowfly species
- The characteristics of time, season, population density, location etc. that affect the time it takes for a blowfly female to find a corpse and begin to oviposit

A number of areas of focused study are suggested to begin to address these difficulties.
These include

- A focus on understanding basic blowfly biology in specific geographic areas
- Understanding the behaviour and predilections of species
- Population dynamics, dispersal and range studies of forensically important species

It is easy to be side-tracked, both in interest and in finding funding sources, to more esoteric topics, for example the roles of DNA in forensic entomology. There is nothing wrong with this, but without solid foundational information we are building a house of cards and it will not be long before the wind blows.

10:00-10:20 **Towards Guidelines in Estimating PMI by Forensic Entomology**

Christian Reiter

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Having its historical beginnings in the 19th century, extensive research in the last decades has developed forensic entomology into a scientific method in medico legal death investigation and post mortem interval estimation. Research on ecological aspects of succession on dead bodies, taxonomic investigations, studies about the developmental behaviour of forensically important insects and mathematical calculations on larval development, depending on environmental conditions, provide the possibility to answer questions that occur in crime cases in court. The rapid scientific development of forensic entomology and its potential to answer questions of forensic relevance was not well recognised by forensic pathologists (and relevant persons in justice) in the past.

In the last decade different working groups all over the world have developed methods and strategies in the field of PMI estimation by forensic entomology, resulting in many different methods and techniques in practice today. This leads to divergent results or to conclusions that cannot be compared easily. This heterogeneity within our discipline should be prevented in future, to prevent loss of scientific reputation.

One aim of the new founded European Association for Forensic Entomology should be to recommend standardised working methods in forensic entomology leading to quality assurance in the work of forensic experts.
This contribution should give assistance toward guidelines in forensic entomological working techniques to estimate the PMI.

First of all it must be conceded, that entomologists need not do the collecting of forensic entomological evidence in every case, if the forensic pathologist or crime scene investigator is well trained in this field and there is a co-operation with the entomologist, who can input their expertise later on.

Onsite micro-climatic temperatures prevailing in the maggot's immediate environment have to be recorded for at least three to five days and correlated retrospectively with the temperature records from the nearest weather station. After regression analysis the temperature curve of crime scene and its daily maximum and minimum temperatures can be reconstructed for the period since the victim was missed.

If forensic entomological evidence is focused only on a certain part of the body a single representing collection of approximately 50 to 100 individuals of all sizes should be collected. If more than one region of the body is infested, separate collection of entomological evidence from the various regions should be made. Each collection should contain 50 to 100 individuals of all sizes.

One half of each collection should be killed immediately after crime scene investigation by pouring hot water over them. The second half has to be reared under well documented temperature conditions until a new stage in the insect's life (e.g. hatching from the egg, pupation, eclosion) can be observed.

If only eggs are found, this evidence must be reared under well-documented temperature conditions until hatching of the maggots. Larvae must develop until third instars, where species identification can more easily be done by microscope. Additional rearing until eclosion should be continued, to confirm with adults the results of species identification of maggots. Larvae or pupae recovered from the scene have to be reared at constant temperature until the next developmental stage can be observed.

Using PC-supported calculation of the accumulated degree-days for development of each species from oviposition to the next developmental step the time of oviposition can easily be calculated backwards along the reconstructed temperature curve from the crime scene.

When the microclimatic temperature records of the crime scene and the thermal constant of the recovered species and the developmental thresholds are known the time of oviposition can be calculated. Therefore, identification to the species level is an essential step in PMI estimation using insect development data. During the last decade forensic entomology working groups in different parts of the world have created a large amount of developmental information on their regional species, so that forensic entomologists can use this data to calculate larval ages, considering regional variation of thermal constants and thresholds.

Using identification keys, adults can be identified relatively easily to the species level. Identification of eggs or first and second instars is much more difficult. It is easier to analyse the species at the third instar level. When using morphological criteria in species identification, the forensic entomologist should verify his results by making photographs of the posterior view of the twelfth segment, the anterior spiracles and of the dissected mouthparts. For these investigations only the anterior and the posterior parts of a maggot are used, therefore the forensic entomological expert should store the other remains of each maggot separately in high concentrated ethanol (>80%), to enable complementary DNA-species analyses.

Each forensic entomological expert testament should contain

- the meteorological data from the nearest weather station for the period since the person was last reported,
- the meteorological data from the nearest weather station for 5 days after discovery of the body,
- the meteorological data from the crime scene of 3-5 days after discovery of the body,
- photographic documentation of the morphology of examined larvae, pupae or imagoes,
- the data of prospective rearings from the collected evidence and finally
- the calculation with respect of the thermal constants and the thresholds.

These procedures should provide a framework for the fundamental principles of guidelines in forensic entomological PMI estimation in future.

10:20-10:40 **Quality assurance in forensic entomology: Why, how and who?**

Emmanuel Gaudry[1], Laurent Dourel[1], Richard Zehner[2] & Jens Amendt[2]

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[2]Institute of legal medicine, Forensic Entomology, Frankfurt am Main, Germany

Quality assurance (QA), expressed by different levels of certification and accreditation, seems to intrude every area today. In fact it is already an autonomous business, also in Forensic Science, illustrated by the state of Wisconsin/USA or the french Institut de recherche criminelle de la gendarmerie nationale which employ a Quality Assurance manager. Therefore, Forensic Entomology, as an ambitious discipline in Forensic Science, has to face QC. Several lectures at the first meeting of the EAFE in 2003 highlighted already important proposals. But is there really a possibility for QA in Forensic Entomology?

Why QA in Forensic Entomology? Today, quality is common to all forensic investigative techniques. If Forensic Entomology want to keep scientific standards not just making entomological statements, it is reasonable to treat it like science, which means: working on a defined basis to a mutually agreed set of standards. Particularly the judges expect technical competency. Moreover there are already efforts underway e.g. in the United States and France. The specificity of each legal system should not be a drawback to finding a mutual basis for QA in Forensic Entomoogy.

How could QA be performed? There are different levels of quality and it has to be discussed how many levels should be or could be realised. Level 1 represents a minimum of quality and should lead to a framework of standards for the collection and documentation of entomological evidence in order to provide forensic entomologists, death scene technicians, forensic pathologists, but also the judge with a general, common and systematic approach, leading to an approved methodology. Level 2 deals with guidelines in making expertises (e.g. Best Practises Manual): Can we create minimum standards in writing an expertise and if yes, which minimum sets of data (e.g. accurate temperature data from the scene of death) are necessary? More important: Should these guidelines be an advice or mandatory? Level 3 is assumed to be the most controversial, when discussing the question: who is an entomological expert? A QA-system which comprises the accreditation of the forensic entomologists' expertise, with implementation of external supervision and blind trials. The organisation and implementation of Level 2 and 3 would be a great challenge and there is still the question whether a quality-process analogous to those of the other disciplines of forensic science is even possible in Forensic Entomology.

Who could perform QA in Forensic Entomology? Depending on the level of QA there are different answers. Realisation of level 1, establishing collection- and documentation guidelines, could be organised by the EAFE (best in agreement with other international associations like ENFSI[a] or ABFE[b]). Despite the problems discussed above, Level 2 could be also arranged by the EAFE. However, it is doubtful, whether the EAFE is the right institution especially for the implementation of Level 3. According to ISO/CEI 17025, only members (national accreditation bodies) of the EA (European co-operation for Accreditation) are the right institutions working with international standards.

[a] European Network of Forensic Science Institutes; [b] American Board of Forensic Entomology

11:30-11:50 **Interpreting Meteorological Station Data**

Adrian Runacres

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As will be well known to the audience, temperature can be an important determinant factor in the growth rate of insects. However, the measurement of temperature and, importantly in terms of forensic investigations, its estimation, can be more complicated than one might immediately think. For example, air temperatures at exposed locations can be markedly different to those measured simultaneously at sheltered sites. Surface temperatures differ markedly from air temperatures and subsurface temperatures are different again, with this varying with depth. Crucially, the relationships between these variables can be complicated. They will change from site to site, with weather type and sometimes with the season. In some instances, it will be entirely appropriate to take measurements at an incident site, compare these against simultaneous measurements from a Met Office recording station, use a relatively simple regression analysis approach to determine the 'best fit' relationship, and then use this, coupled with data from the Met Office Station for an earlier time period, to estimate the temperatures at the incident site during that earlier period. However, in other instances, it will not be appropriate! This presentation will attempt to outline the pitfalls of temperature measurement and estimation, as well as discuss the sorts of instances where such an approach might be appropriate and where it would not.

Presenter - Adrian M E Runacres

Adrian heads the Advisory Services Department at TRL. Trained as a micro-climatologist and with extensive experience of working within the transport industry, Adrian provides specialist advice for incident investigations and transport related risk management commissions. He conducts research, including into such matters as the efficacy of ice detection and prediction systems, the accuracy of weather forecasts and maintenance management systems. Adrian regularly provides Expert evidence for Court proceedings and, in this regard, has acted in a number of highway related test cases and in non-transport related criminal investigations, where micro-climatological and meteorological expertise was required. Adrian also led the independent TRL investigation team appointed by the Highways Agency into the Selby Rail crash and provided evidence to the Railway Safety Inquiry, the Coroner's Inquest and the High Court considering the Civil Proceedings.

11:50-12:10 **Daily temperature fluctuation and the accumulation of day-degree fractions**

Richard Wall

School of Biological Sciences, The University of Bristol, UK

The use of a physiological time scale, such as accumulated day-degrees, is an important tool in the entomological estimation of post-mortem interval. However, there are numerous stages in the calculation of threshold temperatures and day-degrees where simplification may distort the estimates. One such important problem is the use of average daily temperatures rather than some measure of diurnal fluctuations, to calculate accumulated day-degree fractions. Here, the effects of incorporating a measure of daily temperature fluctuation into accumulated day-degree calculations is considered. This analysis shows that accumulated day-degree estimates, calculated by fitting a sine wave to the daily maximum and minimum temperatures, differ most markedly from the estimates obtained using average temperatures, when ambient temperatures are low and fluctuate across the lower temperature development threshold. In the UK, this is likely to be particularly important between autumn and spring. For the blowfly *Lucilia sericata*, for example, based on temperatures collected in spring 2003, the use of average temperatures gives considerably slower estimates of development than estimates based on the max/min temperatures.

12:10-12:30 **Study of decomposition rates and egg viability in a cold climate**

Amoret P. Brandt

Department of Entomology, The Natural History Museum
and School of Health and Life Sciences, King's College London

The estimation of PMI in countries with fluctuating temperatures, both seasonally and daily, is considerably more complicated than in those with fairly constant temperatures. Studies have therefore been carried out in Central London, at The Natural History Museum, to study the effect of cold winter temperatures on the decomposition rate of young piglets. Comparisons have been made between those laid outside on a roof, and those laid inside in an unheated, though warmer office, with limited access for insects. As a result of these studies, the most interesting aspect that arose was the viability of eggs. Although laid at the same time on both the inside and outside carcasses, those outside failed to hatch due to the cold temperatures, therefore limiting the decomposition rate considerably, whereas those inside hatched as expected. However, once the weather warmed up, the eggs laid outside hatched and the larvae continued their normal development. Laboratory studies were therefore carried out to assess how long the eggs of *Calliphora vicina* can remain viable in cold temperatures, and whether they are able to hatch into larvae once the temperatures rise, either due to improved weather conditions, or the removal of the body inside.

12:30-12:50 **Validation of post-mortem interval estimates using maggot age and development**

Sherah VanLaerhoven

University of Windsor, Rm 119 Bio, 401 Sunset Ave, Windsor, Canada

Much controversy exists in the North American forensic entomology community regarding methods for time of death estimates. The Westerfield case from the USA is a prime example of how these different methods result in a variety of time of death estimates. There is a need for blind validation studies to examine these methods in order to clear up the controversy. This talk will present the initial results of a recent blind validation study conducted in Ontario, Canada.

14:00-14:20 **Development data of the parasitoid wasp *Nasonia vitripennis* (Walker) Hymenoptera: Pteromalidae): Verification under field conditions**

Martin Grassberger[1,2], Christa Frank [2,3]

Institute for Legal Medicine and Forensic Sciences[1] (Medical University of Vienna), Institute for Anthropology[2] and Institute for Zoology[3] (University of Vienna)

Introduction: Parasitoid wasps, especially *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) have been the subject of numerous genetic, evolutionary, ecological and ethological studies, particularly of courtship behaviour, non-Mendelian inheritance, sex-ratio evolution and speciation.

Because parasitic wasps will only oviposit on fly pupae of a certain age, their temperature-dependent development can also be used to estimate an extended post-mortem interval (PMI). Although a few case reports have been published, neither detailed developmental data have been obtained in the laboratory nor have they been verified under field conditions.

Materials and Methods: Development times of the forensically significant parasitic wasp *Nasonia vitripennis* from oviposition to pupation, and from oviposition to adult emergence, were studied in the laboratory at temperatures of 15–35°C using host pupae of the blowfly *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae). The developmental data were then correlated with the observations made in a pig-carcass study.

Results: Total developmental time of *N. vitripennis* from oviposition to adult emergence (mean± SD) was 43.5 ± 2.4, 22.5 ± 1.1, 14.8 ± 1.7 and 11.3 ± 0.9 days when reared at 15, 20, 25 and 30°C, respectively. At 35°C, *N. vitripennis* did not develop successfully. From linear regression of development rates, it was estimated that the minimum threshold (t_L) for total immature development was 9.8°C(˜ 10°C). Above this threshold, the overall thermal constant (K) for *N. vitripennis* was found to be 224.3 ± 1.7 degree-days. When compared to the results of a pig carrion study conducted under undulating temperatures the difference between observed and calculated time of oviposition for *N. vitripennis* was less than a day.

Discussion: Although the application of laboratory generated data to PMI estimation is straight forward and have proved accurate in our field study, several important issues have to be considered: Heavy rainfall, low temperatures and other environmental conditions may impede flight and drilling activity of female wasps. Furthermore, it is known that *N. vitripennis* is able to gain additional low temperature protection through acquisition of host cryoprotectants during larval feeding. Variation in the nutritive and biochemical qualities of the host and accumulation of drugs and toxic substances in the host tissues may also have influence on the rate of development, sufficient to alter estimates of the PMI. It should be noted that in the absence of certain conditions necessary for development, facultative diapause might be induced. Apart from misidentifications of specimen, a potential biogeographical variation in developmental times might also account for incorrect results in PMI estimation. Further laboratory and field studies are warranted to obtain a reliable PMI estimate when Pteromalid parasitoids are recovered with muscoid Diptera from the scene.

14:20-14:40 **A chemical analysis of odours associated with decomposition as well as an electrophysiological study of blowfly *Calliphora vomitoria* to understand its attraction to decomposing bodies**

Hélène N. LeBlanc

Insect Research Group, School of Environmental and Health Sciences, University of Derby, Kedleston Road, Derby, England DE22 1GB.

While it is known that certain specific insects are present on decomposing bodies, their attraction to these bodies has not fully been examined.

Research was conducted to determine fly succession on a decomposing body in Derbyshire, England and to determine the factors influencing their attraction to the carcass. The first step was to place three 50Kg pigs in a semi-open field. Throughout decomposition insect collections were made from pig 1, odour samples were taken from pig 2, and temperature recordings were made in and around pig 3. Observations and some insect collections were made from all three pigs.

The odour samples collected were analysed using gas chromatography (GC) and coupled GC-MS (mass spectrometry). These odour samples were then tested against female blowfly, *Calliphora vomitoria*, in electroantennogram (EAG) experiments. These tests were conducted to determine whether there was a relationship between the fly's attraction to the decomposing corpse and the change in the semiochemicals (behaviour-modifying chemicals) in the odour.

As decomposition progressed there were obvious changes in the composition of semiochemicals from the pig. Some of these changes could be linked with sudden large numbers of flies on the carcass. Electrophysiology work revealed that particular compounds elicited an electrophysiological response from *C. vomitoria*.

Work is also currently being done using Sepsids as they seem to show a preference to the later stages of decomposition. Behavioural work on *C. vomitoria* will hopefully reveal whether electrophysiological response to certain semiochemicals is due to an attraction or repulsion of the particular compound.

Results from these experiments and questions still remaining will be discussed.

14:40-15:00 **Heteroplasmy in the *Necrobia* genome (Coleoptera: Cleridae): Impact on DNA based species determination**

Richard Zehner, Marcus Häberle, Jens Amendt

Institute of legal medicine, Forensic Genetics, Univ ersity of Frankfurt, Germany

DNA analysis has become a widespread tool in species analysis. The mitochondrial encoded subunit 1 of the cytochrome oxidase (CO I) for example is a common marker used for species determination of necrophilous as well as other species. By comparison of the investigated sequences with reference sequences in many cases an unambiguous association of an unknown species to a known character can be achieved.

Although many species have been analyzed until today, reliable data are rare dealing with possible intraspecific variability. This knowledge is important in cases when the unknown subject does not match the reference sequence exactly. Do we associate this specimen to the nearest match, assuming intraspecific variability, or do we exclude it?

Our investigation on Clerid beetles indicate a relatively high degree of intraspecific variability within the CO I gene of several individuals of *Necrobia rufipes* and *Necrobia ruficollis*, respectively. The third common species of this genus, *Necrobia violacea* seems not to be affected in a similar extent.

The variability is expressed as a high degree of heteroplasmy, which is defined as the occurrence of different mitochondrial genomes within the same organism.

We present data and discuss possible impacts on sequence based species determination, which can be observed in wrong associations of sequences as well as RFLP patterns, if recognition sites of the enzymes applied are involved.

15:00-15:20 **The use of differential temporal gene expression to determine the age of *Calliphora vicina* pupae**

Carole Ames, Bryan Turner and Barbara Daniel

Department of Life Sciences and the Forensic Science Unit, King's College London

Calculation of the post-mortem interval of a discovered corpse often involves establishing the age of the insect species associated with the body. Larval length can be compared to an isomegalendiagram (Grassberger and Reiter, 2001) to discover age. This method is however restricted to larval stages only.

The aim of this current research is to try and determine the age of pupae using molecular techniques.

Throughout the developmental lifecycle of insects different genes will be expressed at specific time points. Once identified, these temporally expressed genes could provide markers as to the age/stage of development of an insect.

Total mRNA was extracted from *Calliphora vicina* pupae (which had been kept at 20°C since oviposition) at known time points. The extracted RNA was reverse transcribed to cDNA and initially amplified using actin primers. Actin was used to assess the viability of the cDNA and would later be used as an internal standard when comparing samples.

Total mRNA will then be subjected to differential display. Differential display is a method that detects and characterises changes in gene expression between samples. It involves random amplification of mRNA to produce various cDNA fragments which can be visualised on a gel. Any differences between fragment gel patterns between time points will be highlighted. These genes can then be sequenced, identified and used as markers for pupal development.

15:20-15:40 **The importance of histology in determining the age of postfeeding *Calliphora vicina* larvae (Diptera: Calliphoridae): preliminary results**

K. Pien[1], T. Boonen[2], K.Vits[2], P. Grootaert[3], M. Pipeleers-Marichal[1]

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Introduction

The minimal post-mortem interval (PMI) can be estimated by measuring the degree of development (length and/or weight) of larvae found on a cadaver. To achieve this, the time of oviposition is deduced from the degree of insect development. The growth curve of the *Calliphora vicina* maggot is "S" shaped, displaying slow growth during the first two larval instars and a slow decrease in size between cessation of feeding by the third instar and the onset of pupariation (postfeeding larvae). Since the postfeeding stage may take as long as the rest of larval development and lacks abrupt changes in appearance, a precise PMI estimate during this period is difficult. The degree of crop emptying may give an indication about the time the larva ceased feeding, because during the first postfeeding day, the crop shrinks from 7 mm to 3 mm, from the second day until pupariation however the decline in crop length is too gradual to be useful.

In order to reach the adult fly stage, the larvae have to pass through changes of form, which are collectively called "metamorphosis". Metamorphosis starts in the postfeeding third instar larva and is accompanied by physiological and biochemical changes, some of which can be detected by light microscopy and may therefore, be of interest to determine the age of the postfeeding larvae.

Furthermore, as *Calliphora vicina* is a holometabolous insect, the process of histolysis of tissues and replacement by adult fly structures may also be useful to determine the age of the developing pupae.

The aim of this study is to develop a method that allows for an easy way to determine the age of the postfeeding larvae and pupae by light microscopy and could therefore be used to determine the corresponding minimal PMI.

Materials and methods

Flies and larvae were from a stock colony of *Calliphora vicina* reared in an environmental chamber, with 16 h of daylight (24°C) and 8 h darkness (18°C). 30 postfeeding larvae were collected from day 4 till day 8. Length and weight were measured daily. The larvae were sacrificed by boiling in water and conserved in a mixture of ¾ ethanol (70%) and ¼ acetic acid (99%). The second to last abdominal segment of 15 larvae was dissected and embedded in paraffin, 5 µm serial sections were cut, stained with hematoxylin-eosin-saffranin (HES) and examined using a Leica light-microscope. The other 15 larvae were conserved for further investigations.

Results

The fat body is composed of rounded polyhedral cells (trophocytes) and like the vertebrate liver, it synthesizes, stores and mobilizes lipids, proteins and glycogen. In this study, only fat body cells with a prominent nucleus were studied. Distinct histological changes like the

formation of granules around the nucleus and in peripheral regions of the cells were observed. Changes in the cuticle thickness and lysis of the gut epithelium were seen.

Conclusions

The macroscopic changes of the postfeeding larvae of *Calliphora vicina* are of little use to determine the minimum PMI, especially when a 95 % confidence interval is calculated. In this study, we have shown that the microscopic changes occurring in the fat-body and other organs of *Calliphora vicina* larvae can be used to determine the larval age and hence the PMI.

16:10-16:30 **The Ageing of Ovine Psoroptic Mange (Sheep Scab)**

Peter G. Bates

Parasitology Section, Scientific Services Unit, Veterinary Laboratories Agency (Weybridge), Addlestone, Surrey, KT15 3NB, United Kingdom.

Domestic sheep can be attacked by a number of ectoparasites, all capable of causing considerable distress and possible death of the host. All UK sheep farmers should be aware of the Code of Recommendations for the Welfare of Livestock: Sheep (DEFRA 2000) which emphasises the prevention and treatment of ectoparasites. Effective chemical treatments are available so a stockowner could face prosecution for animal cruelty (under the Agricultural (Miscellaneous Provisions) Act 1968) if he neglects to prevent or treat for ectoparasites.

Ovine psoroptic mange (sheep scab), caused by the non-burrowing mite *Psoroptes ovis*, is a form of debilitating allergic dermatitis resulting in wool loss, intense irritation, epileptiform seizures, scab formation and death. In addition to the standard animal welfare legislation the disease also falls under the Sheep Scab Order (1997). The Order is intended to give Local Authorities (LAs) the means to improve the control of sheep scab and prosecute when owners of infested sheep do not take appropriate measures voluntarily.

Ageing the duration of infestation (and therefore the period of neglect) is crucial to a successful prosecution. Unfortunately this is not that easy. Early lesions are undetectable and a "sub-clinical" phase (characterised low mite numbers and small lesions) can last for a matter of days, weeks, months or even years before the lesion progresses into the active phase, eventually covering the whole of the sheep. Sheep with sub-clinical disease can look perfectly healthy and can unknowingly be introduced to a flock and thus be responsible for the spread of infestations within flocks and between properties.

A number of laboratory or field studies were undertaken to investigate the potential factors that could influence the duration of the sub-clinical phase and consequent ageing of infestations. The duration of the sub-clinical phase was found to be extended through a number of host modulated factors, (open fleeced breeds of sheep, low individual susceptibility to infestation, natural challenge on body areas less susceptible to colonisation and on sheep previously exposed to infestation), parasite modulated factors (mite populations of low virulence or small numbers of challenging mites) or factors modulated by previous chemotherapeutic agents (eg. the use of synthetic pyrethroid pour-ons or endectocides administered orally as anthelmintic drenches). Thus the animal with the largest lesion is not necessarily the animal with the oldest lesion.

16:30-16:50 **The Ageing of Ovine Traumatic Myiasis (Sheep Blowfly Strike) Infestations**

Peter G. Bates & Mark R. Rankin

Parasitology Section, Scientific Services Unit, Veterinary Laboratories Agency (Weybridge), Addlestone, Surrey, KT15 3NB, United Kingdom.

Blowfly strike (traumatic myiasis) is a widespread problem in sheep throughout the UK and all other sheep rearing countries. The principal strike species in the UK is Lucilia sericata. Blowfly strike is of economic significance through its damaging effects on fleece and hide, but more importantly it is considered a major sheep welfare problem with clinical signs ranging from local discomfort and irritation in smaller lesions, to serious loss of condition, debility and death. In a survey of sheep farmers in England and Wales, 80% reported at least one case of blowfly strike in their flocks, with an estimated half a million sheep struck annually.

All UK sheep farmers should be aware of the Code of Recommendations for the Welfare of Livestock: Sheep (DEFRA 2000) which emphasises the prevention and treatment of strike. Effective chemical treatments are available so a stockowner could face prosecution for animal cruelty (under the Agricultural (Miscellaneous Provisions) Act 1968) if he neglects to prevent or treat for strike.

Research has been carried out at the Veterinary Laboratories Agency (VLA) investigating the ageing of blowfly strike (and therefore the period of neglect) by comparing the length of infesting larvae against standard larval growth curves for varying temperature ranges (between 8°C and 40°C), for L sericata. If the sheep body temperature, strike lesion temperature, ambient temperature and larval length and species are known, their approximate age can be extrapolated from the growth curves, as well as identifying secondary strike lesions by the presence of multiple larval stages. It is very important that the larvae are submitted for ageing in a standard manner, fully extended and all parameters such as temperatures are recorded in detail. Ageing strike lesions by comparing the plasma concentrations of larval metabolites (namely ammonia and blood urea nitrogen) with time following infestation were also investigated, but only larval morphometry was shown to be a valid method.

Following this basic research a service was made available to the VLA Regional Laboratories (on behalf of the Local Authorities and the RSPCA) in 1996 to aid prosecutions for animal cruelty. Since the service became available larvae have been examined from 57 welfare cases. The majority of cases concerned eye, foot, wound or body strike in live sheep and a number of case studies will be discussed. A number of investigations have also been carried out for other host species involved in cases of animal cruelty (eg. dogs, cats, poultry, swine and wildlife), although accurate data is not currently available for these host species.

16:50-17:10 **Ageing blowfly strike lesions by larval morphology**

Mark R. Rankin & Peter G. Bates

Veterinary Laboratories Agency (Weybridge), Addlestone, Surrey.

One tool available to Forensic Entomologists for producing estimates of the age of blowfly strike lesions or post-mortem interval (PMI), is the application of baseline data of key dipteran species in faunal succession of ovine blowfly myiasis. *Lucilia sericata* is a key species responsible for the over half a million cases annually in England and Wales.

Baseline data was generated from *in-vitro* culture of *L. sericata* larvae taken from a case of ovine myiasis (VLA isolate). Growth curves of the larvae were produced for a range of temperatures between 8°C and 40°C. The results were used to construct an isomegalendiagram used to give a reliable scientific estimation of the age of the larvae from their length and the environmental temperature.

Endothermic heat generated within a larval mass has been reported to raise the temperature of the mass above the ambient air and carcass temperatures. *In-vitro* studies of the effect of larval cohort size, air and larval substrate temperatures upon the temperature of larval masses were undertaken. Data from these studies indicated the effect of larval cohort on larval mass temperature became significant at the highest cohort size investigated (10,000) and when the larvae reached third instar. Heat production was more dependent on larval instar than cohort size, once the cohort was above 400 larvae. When cohorts of 10,000 larvae were subject to a variable incubation temperature regime, the temperatures of the cohorts indicated endothermic heat to be semi-independent of environmental air temperature. In the context of estimating age of strike lesions, the influence of endothermic heat is considered significant only when there are dense aggregations of larvae in late second to third instar. At larval incubation temperatures above 15°C, elevations of temperature due to endothermic heat do not lead to significant increases in growth rate. The age of a strike lesion can therefore be made on the basis of larval length and ambient air temperature, at air temperatures above 15°C.

Estimates of *L. sericata* include the period of egg development prior to hatching. *In-vitro* studies of the effect of incubation temperature and duration of egg development were carried out using the VLA isolate. The data produced concurs with published data, when compared at the same incubation temperatures, despite the use of eggs of different isolates of *L. sericata*.

The *in-vitro* growth curves of *L. sericata* larvae have been partially validated by production of larval growth curves from *in-vivo* studies. Data was generated from seven sheep, which received artificial challenges of first instar larvae of the VLA isolate. Larval growth rates *in-vitro* did not differ significantly with larval growth rates *in-vivo*, within the temperature range of 25°C to 35°C. This temperature range is within the physiological temperature range (22°C to 38°C) recorded from areas of skin of several live animals. These areas correspond to blowfly predilection sites on sheep e.g. withers.

Interpolation of estimates of larval age from the *in-vitro* model is considered valid because it has been shown to give accurate estimations of age of larvae in-vivo. These are considered accurate estimates of the minimum of the range of age of the strike lesion and an estimate of the minimum period of neglect for *L. sericata*-struck sheep.

P1 Entomological Sampling Kit. (A little step for the crime scene technician, a bigger one for the Forensic Entomologist?)

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It is well known that a good sampling is a first guarantee to realise a good expertise. Improvement of the quality of the analysis is the aim of each analyst. It is particularly important in Forensic Science because results induce consequences for the inquiry and their interpretation has to be justified (testimony of experts during trials).

Since its creation in 1992, staff of the department of Forensic Entomology (Forensic Science Institute of the French Gendarmerie) has been training Crime Scene Technicians (CST) in the collection of insects at the crime scene and at autopsy. Nevertheless, during several years of activity, we faced the following situations. (i) Quality of the samples (alive and dead specimens) at their arrival at the laboratory was not constant. (ii) The collection was sometimes lacking (fauna not representative). (iii) Insects were sometimes not gathered at all (lack of specific material, an oversight?).

Several meetings with CSTs and the technical assistants who aid territorial units, on crime scenes, showed there was a real need to provide them with practical assistance when making a collection, in the form of a kit containing basic collecting materials, forms and guidelines.

In 2003, 391 entomological sampling kits (provided by us) were supplied to 220 Criminal Investigation Units (61% of total CIU).

One year after, we propose to draw up a balance sheet dealing with the setting up of such kit throughout the 22 French regions.

P2 Development of an Entomology Sampling Kit for the Belgian Crime Scene Investigation Teams

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Since October 2003, the Microtraces lab of the NICC has designed and developed an entomology sampling kit that was given to every crime scene investigation team throughout Belgium.

This sampling kit is designed to:
- Promote representative samplings, on crime scene as well as during the autopsy.
- Facilitate transport to the lab in a fast and safe way.

The sampling techniques that we propose should be compatible with all PMI-calculation methods.

This kit will be tested in Belgium during 2004 and will afterwards undergo an evaluation procedure.

P3 A comparison of variation in RAPD profiles for *Calliphora vicina* (Robineau-Desvoidy) populations from Aberdeen and Exeter

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Molecular differences in forensically important populations of Diptera are potentially of value to determine species and their origins from immature stages which are morphologically indistinct. The blowfly *Calliphora vicina* (Diptera: Calliphoridae) commonly occurs in urban areas and shows reported behavioural differences between different geographically located populations ie. northern populations diapause.

Stevens & Wall (1995) suggest the potential for RAPD analysis in global studies of intraspecific genetic variation in insects. RAPD-PCR is particularly suitable for insect molecular studies as only very small amounts of DNA may be available for analysis. RAPD analysis has previously been used for biological studies of taxonomy and population structure.

We have applied a Chelex® (BioRad) extraction method to reduce potential sample contamination and loss of DNA yield encountered with more conventional DNA extraction techniques.

Standardisation of RAPD-PCR using 'ready prepared beads' (Amersham Biosciences) reduces potential adverse effects on reproducibility of banding profiles. Primer '5' (5'd[AACGCGCAAC]-3') gave reproducible banding profiles for individuals collected from two geographical locations (Aberdeen and Exeter.) DNA was extracted from 10 individuals from each of f1 and f2 generations and characteristic band profiles were obtained by agarose gel electrophoresis. The degree of genetic variation was analysed using Gel Compar II software (Applied Maths).

P4 **A Case Description**

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INTRODUCTION: The decomposed remains of an unidentified man about forty years old were discovered hanging from a tree in a forested area in Aranda de Duero (north-west of Spain). The remains were badly decomposed. The dead body was shaded from direct sunlight for most of the day. The Judicial Authority asked for the estimation of the time of death; in order to focus the investigation.

MATERIAL AND METHODS: Insects were collected from the remains during autopsy by investigators on September 23, 2002, after the remains had been stored in a refrigeration cooler set at 2ºC from September 20th to the 23rd.

The insects collected, adults specimens of Coleoptera and Diptera larvae in different stages, were recovered from the body (head, natural orifices, trunk and extremities). Afterwards, in our lab belonging to the Department of Anthropology of the General Department for Forensic Science of the Spanish Police, Diptera larvae were measured and identified (Rognes K.,1991) and Coleoptera adults were also identified (Plata Negrache P. 1971, Outerelo R. *et al.* 1985).

Temperature data were obtained from the local weather station located 2 kms away from the crime scene and at approximately the same elevation. This occurrence provides greater accuracy in the estimation of the PMI.

RESULTS: Arthropod identification: 3rd. instar larvae Diptera:Calliphoridae; *Calliphora vicina* R-D, 2nd. instar larvae Diptera:Calliphoridae; *Chrysomyia albiceps* W, adult Coleoptera: Staphylinidae; *Philonthus curruscus*, adult Coleoptera: Dermestidae; *Dermestes frischii* K.

The degree days (DD) and accumulated degree day (ADD) were calculated. The lower limit temperature thresholds for development used for the two different species of Calliphoridae present were 10ºC for *Chrysomyia albiceps* and 6ºC for *Calliphora vicina*.

The oldest specimens recovered from the remains during autopsy was *Calliphora vicina* (3rd instard). Rearing and developmental data from a study by Marchenko on *C. albiceps* and by Byrd on *C.rufifacies* (a close sister of *C.albiceps*) and *Calliphora vicina* were used.

The presence of 3rd instar larvae of the group Diptera:Calliphoridae *Calliphora vicina* and 2nd instar larvae of *Chrysomyia albiceps* , both being consistent with the time of year the body was found. These two species of blow flies are common blow flies in this area of Europe during the summer and early autumn.

Between 30 and 40 ADDs (as calculated from the climatological data) are required for *C. albiceps* to reach into the middle of the 2nd instar, and between 100 and 160 ADDs to reach into the middle of the 3rd instar larvae for *Calliphora vicina*.

CONCLUSIONS: Based upon insect development of the oldest specimens present (3rd instar larvae of Diptera: Calliphoridae; *Calliphora vicina*), the prevailing temperatures for the period, and the environment where the remains were recovered (hanging in a tree in shade), the remains

would have been exposed to colonization by *C. vicina* from the afternoon to early evening (prior to sunset) of September 12, 2002 to as early as late morning of September 10, 2002. This is provided the insects recovered are the oldest insects maturing on the remains. Death of the individual would have preceded the insect colonization.

Acknowledgements: We thank the very useful help from Dr. Haskell in order to calculate the PMI and elaborate this paper.

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P5 STR analysis of maggot crop contents

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DNA analysis of maggot crop contents can be useful in a forensic investigation to recognise the corpse a maggot has been feeding on, to help the identification of a missing person in the absence of a body or to prove that the victim has been removed from a crime scene.

In this study, the maggots fed on human tissue were collected during autopsy procedures and then preserved in pure ethanol at -80°C until needed. Human blood stains were also collected and DNA extracted using the Chelex® resin for further profile comparison.

The maggot crops were removed and human DNA was extracted using two combined methods (Chelex® resin and phenol-chloroform). Short Tandem Repeat analysis (STR) was made by the co-amplification of D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, Amelogenin, vWA, D8S1179, TPOX and FGA loci using the PowerPlex® 16 System (Promega). Reactions for multiplex PCR were prepared according to the manufacturer's recommendations and carried out in a thermocycler GeneAmp® PCR System 9700 (Applied Biosystems). The amplified products were detected and separated by capillary electrophoresis using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). The results were analysed with the Genescan® Analysis Software v. 3.7 and the typing was made by comparison with allelic ladders.

Comparisons of different experimental conditions have demonstrated that only some of them generated a complete STR profile. The genotypes matched those of the maggot's food source (human sample).

Keywords: Forensic Science, Forensic Entomology, DNA typing, short tandem repeat, death investigation, maggot crop contents.

P6 Insect activity on pig carcasses over winter in London

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An exploratory study was carried out to compare the decomposition rates and insect activity on indoor and outdoor piglet carcasses in Central London in winter, and to compare the fluctuating ambient temperatures with the internal body temperatures of the pigs.

Two young piglets were put out at The Natural History Museum, London in mid December, one on the roof and one in an unused office with a window left slightly open, at approximately the same height off the ground. Ambient temperature and internal body temperatures were monitored using Tiny Tag detectors. Egg masses were laid on both pigs by Day 2. Initially only those on the indoor pig hatched into larvae, but hatching was delayed on the outdoor pig until the weather improved. When egg masses were removed from the outdoor pig, they hatched within 24 hours in the lab, having lain dormant in the cold for up to 8 days previously.

The ambient indoor temperature ranged from 10ºC to 16ºC, and outdoor from -1ºC to 14ºC. By Day 27, the outdoor pig was still largely intact, due to lack of insect activity, and the body temperature had tracked that of the ambient temperature. However, by Day 27 the indoor pig was entirely desiccated due to extensive larval activity, and the internal body temperature had been 4ºC above the ambient temperature at times when maggot masses were most active.

This shows that even in low UK winter temperatures, flies are active and can lay eggs. However, the temperature must rise above a certain level before the eggs can hatch into larvae, a factor which could drastically affect the calculation of PMI by a number of weeks.

P7 Preliminary study of the sarcosaprophagous community in Córdoba (Argentina)

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It is well known that the arthropod community associated with a corpse varies between different regions. Due to the scarcity of this kind of study in Argentina (Centeno, 2000, Oliva, 2001, Centeno et al., 2002), a preliminary study on sarcosaprophagous fauna was conducted in the central area of Argentina Republic, the results of which are presented.

Study was carried out 5-km south from Córdoba, in an hemisynantropic Mediterranean area, belonging to the "espinal" environment, characterised by carob-trees (*Prosopis alba* and *P. nigra*). A modified Schoenly trap was used baited with plucked, beheaded chicken carcasses, with the skin, muscles and viscera in place. Samples were taken during November (austral spring), on alternate days, when collected specimens were taken away, and environmental conditions, as well as that from inside the trap and the bait, were registered.

3427 adults and 456 preimaginal individuals were collected. They belong to 11 arthropod orders. Diptera appeared as the most abundant group, representing the 79% of total captured specimens. The most numerous Dipteran family was the Calliphoridae (mainly *Phaenicia sericata* and *Chrysomya albiceps*), followed by Muscidae (mainly *Musca domestica*). Other abundant groups were Acarida, Hymenopteran Formicidae and Coleoptera (Cleridae, Staphylinidae and Dermestidae).

The usual decomposition stages were observed: fresh, bloated, decay, advanced decay and skeletonization. Fresh and bloated stages were characterised by Diptera, both adult and larvae, and Formicidae. During decay stage, Dermestidae adult and larvae were present. During the advanced decay, the corpse was newly hydrated as a consequence of rains, and many primary Diptera could be seen in the corpse, together with Dermestidae. Skeletonization was characterised by a very high abundance of Dermestidae.

P8 Effectiveness of a modified Schoenly trap for collecting sarcosaprophagous fauna

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The aim of this work was to compare the effectiveness in collecting adult sarcosaprophagous fauna of a modified baited Schoenly trap (BST) (Schoenly, 1981) versus the traditional collection (TradC), using the not baited Schoenly trap (UBST) as a control. 2,5 kg rabbits were used as animal model. Experiment was conducted in the countryside of Bogotá, at a height of 2600 m, in the Andean strip of Colombia (South America). The modified Schoenly trap was made in aluminium, in order to make it more resistant and was modified in certain other details (trap bottom, shape of collector funnels etc.).

Sampling was carried over 42 days. 11,847 individuals were collected, belonging to 99 species (97 in BST, 84 in UBST, 63 in TradC), from 43 families (43 in BST, 43 in UBST, 32 in TradC). They were classified in five ecological groups: necrophagous, predators, parasites, opportunists and accidentals. The abundance and diversity of the collected fauna were calculated using the Shannon-Weaver index, and its Dominance with the Simpson index, for every collecting method. Their values, as well as Richness Value, were compared using different tests: U of Mann-Whitney (for abundance and richness) and t-Student (for diversity and dominance).

Significant differences between BST and UBST in abundance ($U=1$; $n=3$; $p=0.04$) and diversity ($t=10.5$; $n=3$; $p<0.05$) and dominance ($t=11.831$; $n=3$; $p<0.05$) suggest the effectiveness of BST for collecting sarcosaprophagous fauna. Comparisons between TradC and BST indicated BST is the most efficient mechanism for collecting adult sarcosaprophagous fauna at light, with a significant differences in richness ($U=0$; $n=3$; $p=0.04$), total abundance ($U=0$; $n=3$; $p=0.04$), and differences between abundance and richness of the main sarcosaprophagous orders.

We conclude that the BST is more effective for differential collecting adult sarcosaprophagous fauna, and very superior to TradC. The community collected with BST displays a more representative overview of the structure and composition of the adult fauna associated to a decaying corpse.

P9 First approach to the effects of cocaine on development of *Sarcophaga tibialis* (Diptera, Sarcophagidae)*

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Sarcophaga tibialis (Macquart, 1850) is the most abundant Sarcophagidae species associated to corpses in south-eastern Iberian Peninsula. It appears during the first decomposition stages from spring to autumn, acting as the primary fly. Due to these characteristics, we investigated the effects of certain substances on its development, in order to apply it to forensic practice.
An experiment was conducted with four rabbits, three were administrated doses of cocaine (1/2 DL50; DL50; 2DL50); the fourth was used as a control. Cocaine was injected in isotonic saline directly into the vascular system through the auricular marginal vein. The control rabbit was injected with just isotonic saline solution. The rabbit livers were removed and presented to the *S. tibialis* larvae as food. Flies were from a stock colony started in 2000. A sample of 10 larvae of each colony was measured at 12 hours intervals. Pupae were also removed and observed at 12 hours intervals. Larval length has been used as variable for statistical analysis.
Preliminary results show that larval growth is affected by cocaine. Growth was slowed by the presence of cocaine, the control culture was the first to complete each larval instar, and were the first to pupate. The 2DL50 (double lethal dosage) colony was the slowest to attain maximum larval length.

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P10 Preliminary data about Sarcosaprophagous fauna in Portugal

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A first attempt to collect data about carrion fauna was made in Portugal (Coimbra) in Spring 2003. Our aim was to train and define the sampling protocol to be used in forthcoming studies. As there is a complete lack of knowledge, about carrion insects in Portugal, one of the objectives was to make a first assessment of this type of community. We used the domestic rabbit as our animal model.

The community evolution was followed until the establishment of the dry stage, when only Coleoptera individuals were found. A total of 18 insect species belonging to 3 orders and 10 families were collected. *Lucilia caesar* (Diptera, Calliphoridae) was the dominant species present during the study period.

We made a comparative analysis with the bibliographic data from other studies in the Iberian Peninsula along with the climatic factors, at the same time defining our main objectives in the following field experiments.

P11 Status and constraints in application of forensic entomology in Pakistan

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No organised system of research or routine examination on scientific lines is practised in Pakistan. On the basis of field investigation and considering the other studies we are confident to say that forensic entomology is truly an unexposed field. However in the present research paper social structure of the society and infrastructure of the judiciary chain is highlighted.

P12 The use of insects in attempts at determination of the time of death - case studies from Poland

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In Poland, until recently, forensic entomology has been a little used tool in attempts at determination of the time of death. Lately in Gdansk, the arousal of interest in this subject has been observed and co-operation between a medical expert and an entomologist takes place several times a year. This paper describes a number of local cases in which forensic entomology was involved during last year.

Case 1
On 31st May 2003, the corpse of a young, almost 30-year-old woman was found on a lake located in the village of Zawory near Gdansk. The body was partly skeletonized and a large number of different species of blow fly larvae were observed on the head and neck of the victim. A calculation of PMI was made with all species discovered on the corpse, and it was estimated that 24th May was the day of the appearance of the first egg-laying flies on the body. This result was consistent with the outcomes of the police investigation.

Case 2
On 13th October 2003, the remains of the body of a man were found by the Vistula River, in Pruszcz Gdanski, near Gdansk. The corpse was badly decomposed with internal organs and flesh in a creamy consistency. During the autopsy of the corpse, the larvae of Fannidae, Sepsidae, Piophilidae and imagines of Necrodes littoralis (Coleoptera: Silphidae) were collected. As these insects represent the 4th wave of faunal succession, we estimated the approximate age of the corpse to be 3-5 months. The result of our study was correct, as the police investigation proved that the body found belonged to a man who had gone missing in July.

Case 3
On 7th November 2003, during a forensic exhumation performed in the cemetery located in the village of Sztum, near Gdansk, living insect larvae were found on the corpse. These preimaginal stages belonged to Diptera: Sphaeroceridae and Coleoptera: Staphylinidae, which are typically associated with buried bodies and represent late waves of succession.