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ABSTRACTS

Identification of true flies (Diptera, Brachycera) involved in forensic cases in Central Europe

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Numerous species of true flies (Diptera, Brachycera) are known to attack dead humans. Each species has a slightly different biology with the females laying eggs at different state of decomposition, at different localities on the body, and with the larvae having another cylce of development. It is therefore of great importance to identify correctly specimens found on dead bodies.

Six families of flies which are most important in forensic cases are presented: Phoridae, Piophilidae, Fanniidae, Muscidae, Calliphoridae, and Sarcophagidae. The most important morphological characters are shown, and differences between these families explained. Those species of the six families which are most often found on dead humans in Central Europe are selected and their identification is discussed. As a conclusion it will be seen that only well-preserved specimens allow a safe identification, because numerous structures on head, thorax, abdomen und the terminalia (especially of males) are needed for correctly naming a specimen at hand.

Differences in insect colonization and decomposition rates between carcasses placed inside and outside a suburban house in Canada.

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Homicide victims are frequently found inside residences, in urban and suburban areas. However, due to obvious logistical problems, most research in forensic entomology is conducted on carcasses placed outdoors in rural areas. In this study, carrion decomposition and faunal colonization was compared in two suburban regions of Edmonton, Alberta a large city in Western Canada.

Three freshly killed pig carcasses were placed inside a small, unoccupied house in a suburban area of the city. One carcass was placed in the bathtub, one in the bedroom and one in the living room. Three freshly killed pig carcasses were placed outside, in a suburban enclosure near the house. The outside pigs were placed directly on the grass. Temperature was recorded at each site on an hourly basis. The carcasses were examined, photographed and sampled on a regular basis for six weeks.

The indoor carcasses were colonized much more slowly than outdoor carcasses and were only colonized by Calliphoridae (Diptera), whereas outdoor carcasses were more immediately colonized and a normal sequence of Diptera and Coleoptera were observed. The number of insects on indoor carcasses appeared much greater than on outdoor carcasses. Diversity of Calliphoridae on the indoor carcasses was not restricted.

Forensic Entomology besides corpses and morgues; 2 new cases reported from the Basque Country (N. Spain)

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People use to identify Forensic Entomology with legal aspects referred to murderers, suicides, post-mortem intervals and other criminal circumstances related to human deceases. Nevertheless, some interesting aspects have sporadically been discussed in previous forensic meetings. Therefore, more legal aspects concerned to Forensic Entomology should be taken in consideration where no corpses or necrophilous insects must be necessarily involved. An important aspect related to our actual global developmental model refers to food imports where other species of insects can disturbed our standards of life and our economy. Related to this applied aspect of Forensic Entomology are 2 cases that should be evaluated last summer in Bilbao (Vizcaya, Spain) and required my services as Entomologist.

In the first case, an enterprise was affected due to the presence of a moth infesting a shipment of powdered cocoa with sugar. Larvae segregate silk filaments to get fixed to the internal walls of the containers, contaminating the product. Adults were also collected from the containers. Pheromone traps were installed to capture the adults and prevent potential contamination of the products. As the problem did not stop, they asked for my services in order to determine not only the species but the potential focus of the problem to prevent future contaminations of the product. Some moths and larvae were received at the laboratory and after 2 weeks, new adults were reared from the samples. Specific identification was confirmed by specialists* in the family Pyralidae (Lepidoptera) as the tobacco moth *Ephestia elutella* (Hubner, 1796). Although the life cycle may vary considerably depending on environmental conditions, a minimal of 4 weeks was reported from bibliography. As the product had recently arrived to the port of Bilbao (Northern Spain) it was concluded that the contamination took place in origin and that the moth developed inside the containers during the transportation.

The second case, also related to food pests, affected not only the economy of an enterprise but the quality of life of a neighbourhood in Bilbao. A week after the first case was reported, a neighbour contacted personally with me asking for my assistance referred to a massive invasion of a small beetle in a whole building. The beetle was identified as *Tribolium castaneum* and neighbours were invited to begin legal actions against importers and customer officers for negligence. The focus was a shipment of sunflower seeds abandon for more than a month in the custom dependencies close to the house.

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I would like to mention the kind contribution of Dr. Arbogast (Florida, USA) and Dr. Locatelli, (Milan, Italia) for the confirmation of *E. elutella* identification.

Beyond the blowfly; time for another trick

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Blowflies are the most important tool of the forensic entomologist. However, during the colder periods of the year blowflies constitute at most a minor part of the carrion-fauna. From forensic literature, one might get the impression that on carrion during the colder part of the year only very few insects can be found. This view needs some adjustment.

Dutch forensic samples from the colder half of the year contained quite a diversity of insect species. Interpretation of these samples proved difficult. The main problems are:

- Identification in general and of immature stages especially.
- Paucity of life cycle data due to limited field activity of entomologists.
- Obscurity of the literature concerned.
- Rearing of the immature stages: many species seem to be long-lived (i.e. almost a year) as larvae, and thus are prone to desiccation and/or mould during the rearing process.

Decomposition of carrion and development of the insect fauna in the colder seasons is very slow. It is not unusual to find larvae hatched from eggs that where deposited in different seasons on a body exposed for a longer period. In comparison with blowflies and other warm-season carrion insects, many of the autumn/spring species do have a rather short oviposition period. The presence of any immature stage is thus an indication of the availability of the body in the oviposition period of the particular species.

Forensic samples from bodies that were exposed in autumn, have the highest species diversity. Preliminary results suggest that autumnal victims can easily be recognised by several species which oviposit during that period. Most larvae of these species remain alive and some are even active during the coldest period of the year.

There are only few species that deposit eggs during the coldest period (winter). Examples of such really cold-adapted carrion-feeders in the Netherlands are several species in the genera *Trichocera* (Trichoceridae, winter gnats), *Neoleria* (Heleomyzidae, flies), and *Catops* (Cholevidae, beetles).

Until now, few data about the spring-breeders have been collected. In literature it is suggested that oviposition is in spring much more spread in time than in autumn. The reason given for this is that in general the stimulus in autumn is the change in daylight, while the emergence from the winter rest is regulated by a rise in temperature which is thus much more variable.

The present knowledge of the oviposition period of Dutch carrion insects makes it is possible to recognise whether a body was available to insects in autumn. Absence of these autumn breeders on a body found in the cold season suggests a much later availability. When more information becomes available about the lifecycle and larval development of the cold-season carrion feeders in relation with temperature, a much more precise estimation of the post-mortem period can probably be made.

Forensic entomology in Morocco: creation and development

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Science not in use in Morocco, forensic entomology was officially started in June 2001 in the LARATES. Up to this day and after three years of existence more than 40 investigations have been treated on human adult cadavers of which three cases of less than 13 years old children following a criminal act (sexual abuse and abandoned new-born). Insects found belong to terrestrial Diptera and Beetles.

Otherwise, result of a preliminary study of arthropod succession on pig carrion in a coastline city (north/west of Morocco) from 21th of February 2002 to 14th of January 2003, permitted to collect more than 45 species belonging to 21 families of necrophagous insects with an overlap of squads (Calliphoridae, Muscidae, Sarcophagidae and Dermestidae) during the first three months post mortem with a precocious apparition of acarine and adults of Sepsidae within the first month after the death.

In order to improve criminal investigations, a scientific research program in toxicology was launched.

Frequent mistakes in collection of entomological evidences in a crime scene

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Forensic Entomology is the science to determine the post mortem interval of the dead person precisely by investigating a range of insects, which lay their eggs on the corpse within a few minutes of death.
Insects and their eggs, which are collected from the crime scene and from the body during autopsy, will help to determine post mortem interval correctly. The most important factors are a thorough inspection of the crime scene and collection of the necessary clues to be able to determine the postmortem interval. However, there have been serious mistakes in collection of entomological evidence. Therefore, we will deal with the most frequent mistakes and our suggestion is that an entomologist should be invited to the crime scene to minimize the mistakes in collection of entomological evidences.

Larvae feeding on different body regions of human corpses

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Comprehensive knowledge of insects' succession on decaying human corpses is required to detect abnormalities in the colonisation pattern indicating recent injuries of the skin and soft tissue.

In a prospective study 101 cases of human remains colonized by necrophagous insects in domestic situations were evaluated over one calendar year in a metropolitan area (Hamburg/Germany).

The most frequent fly species of which larvae were found actively feeding were blowflies: *Lucilia sericata* (52 cases) and *Calliphora vicina* (48 cases), *Calliphora vomitoria* (13 cases) and *Protophormia terranovae* (14 cases). Larvae of phorid flies (14 cases), *Sarcophaga* species (10 cases) and *Muscina stabulans* (6 cases) were not rare. Larvae of *Lucilia sericata* and *Calliphora vicina* were found earliest feeding mostly at the face or at the genital region, while the colonisation of the genital region depended on the accessibility (e. g. clothing, blankets). The axilla region was attractive after 3 days PMI. Larvae of *Calliphora vomitoria*, *Protophormia terranovae*, *Muscina stabulans* and *Sarcophaga* species were mostly detected after a PMI of 4 days. Interestingly larvae of Muscina stabulans were found feeding at the genital region in 4 out of 6 cases. After 4 to 7 days PMI and softening of the dermis due to putrefaction larvae were found feeding more and more frequently in all body areas. In some cases with a PMI up to 3 months maggots of blowflies or *Sarcophaga* species were detected at some parts of the corpse (mostly limbs or genital region) where the tissue had remained soft and greasy.

The colonisation pattern of fly larvae on human corpses is depending on the species and on the accessibility of the body region particularly in the first couple of days *post mortem*. During this period recent injuries might be detected by abnormalities in the colonisation pattern. With ongoing decay the pattern becomes less distinctive and feeding defects caused by larvae may impedingly be differentiated from vital injuries.

Variation of insect succession on cadavers: the effect of reduced accessibility

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Homicides and suicides continue to occur where corpses are left to decompose in places where insects have reduced accessibility.

The fundamental principles of forensic entomology are typically the succession of insects and other arthropods on decomposing bodies. Succession follows a prescribed sequence, whereby different species inhabiting the same ecological niche are attracted to the corpse at a certain stage of decomposition.

So what happens when the decomposing body is made less accessible to the insects ? Is the composition of the insect fauna and the sequence they visit the corpse disrupted ? What if the usual primary invaders, the Calliphoridae, are unable to access the body in the initial stages of decomposition and will this result in a miscalculation of the post-mortem interval ?

Two particular studies are used to illustrate this problem. Firstly, a series of experiments carried out in Western Australia where pigs have been killed with CO and decomposed in semi-sealed cars. Secondly, a human cadaver was left to decompose in a standard household plastic wheelie bin in Knoxville, Tennessee. In both studies, a comparison with control cadavers ie. pigs/human (not concealed) revealed significant disruption to the expected faunal succession.

The effect of larval mass on development rates of fly larvae feeding on human cadavers in Knoxville, Tennessee, and dispersal of post-feeding larvae

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In July 2004, the decomposition of a human cadaver was studied over a 2-week period. Twice a day, samples of the insects invading the body were collected, mainly Diptera larvae, but also Coleoptera, Hymenoptera and adult Diptera. The internal temperatures of the body (buccal, rectal and within the torso), together with the ambient temperature were recorded every 15 minutes by dataloggers using electronic sensor probes. Additional temperature readings were taken of the larval masses using a handheld infrared thermometer.

Mean larval lengths of each sample collected (~100 samples) were mapped against the larval mass temperatures from where they were taken, in order to try and ascertain to what degree the increased temperature of the larval masses affected the larval development rate. At present, most practicing forensic entomologists take larval masses into account when calculating the age of the collected larvae, but the calculations used tend to be instinctive and anecdotal, rather than based on published data. It is hoped that this study may give an indication of to what degree larval developmental rate is affected by larval mass temperature.

In addition, the dispersal of post-feeding larvae was observed from four different cadavers, with respect to time of day, number of days, direction, distance, speed and numbers. Despite certain commonly held assumptions, such as "larvae move up to 20ft/6m away" and "larvae always move downhill", considerable variation in larval behaviour was observed between the four cadavers.

What is the edge of a forest? A detailed diversity analysis of adult Diptera found on decomposing pigs in two areas of a Western German woodland.

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We tried to experimentally address a «simple» question that came up during a homicide trial in Aachen, Germany. Some dead adult flies of the genus *Bradysia* (Sciaridae) had been recovered from a corpse that was found on grassland close to a forest. *Bradysia* is known to live on the borders of forests. Relating to this expert witness statement, defense and prosecution asked: «Alright, but what *is* an edge of a forest?»

To determine if this question could be answered ecologically, we monitored all Diptera during decomposition of two pigs -- inside of the forest, and on the border of the forest. Over a period of six weeks in Autumn 2003, adult insects resting on the corpses were caught by hand/with a net. With the help of recent determination keys and the extensive collection of Museum Koenig in Bonn, Germany, we determined the following fly families by sex, and to the lowest taxonomic level possible (often to species level): Anthomyiidae, Calliphoridae, Dryomyzidae, Fanniidae, Muscidae, Heleomyzidae, Phoridae, Piophilidae, Scatophagidae, and Sepsidae.

On the inorganic side, the most obvious difference between both habitats was simply the outside temperature (mean $\Delta T = 0{,}9°C$, $p<0.001$). This is of course interconnected with outside humidity, and inside temperature of the two corpses (mean $\Delta T = 2{,}2°C$, max. ΔT 44.7°C vs. 35.7°C on 7th/8th day of decomposition).

Successional waves were represented not only by presence or absence but also by proportion of insect groups. These differences were of course dependent on the location of the pigs. For example, numerous Heleomyzidae were found during post-decay stage on both pigs. Piophilidae were found mostly after bloated decay inside the forest, but during three stages (bloated stage, decay stage, and post decay) on the pig on the border of the forest.

A much better definition of the classical successional waves was calculated out of the *relative number of adult individuals* on the corpses. Here, the relative decrease of Calliphorid individuals compared to the increase of Heleomyzids, Piophilids and Dryomyzids was one of the most prominent markers.

Whilst α-diversity (Shannon-Wiener index: 3.6 vs. 4; Evenness 0.7 vs. 0.7) and β-diversity (Renkonen Percentage Similarity Index: 70,2%) showed that both habitats were similar, i.e., no dominant insect species were present in general, this was not true for the fauna of the pigs. The decomposing pig on the border of the forest maintained a significantly higher number of different families and species of Diptera compared to the pig inside of the forest. This was not only due to an excess of a *Neoleria* (Heleomyzidae) species inside of the forest, but was a constant tendency that became more obvious the further decomposition progressed.

Several Diptera species had a clear preference for the border of the forest, e.g., *Parapiophila vulgaris* during post decay. Some 35.7% of the flies were found exclusively on the corpse of the pig at the border of the forest, whilst 10.7% were exclusively found on the corpse inside of the forest.

Differences in sexes of insects were very clear, too, but not as expected: Calliphoridae (23 ♂ / 301 ♀), Muscidae (4 ♂ / 89 ♀), and Fanniidae (1 ♂ / 87 ♀), yet Piophilidae: 116 ♂ / 41 ♀. This shows that male Piophilids -- in contrast to all other monitored flies -- try to establish a small, personal territory on the corpse whilst female Piophilids prefer to sit elsewhere, preferably in the sun. They only approach the corpse to lay their eggs, and probably to mate.

Unfortunately, the sex ratio of Heleomyzids (238 ♂ / 18 ♀) cannot be explained as of yet, because of a lack of ecological knowledge of this group.

An interesting observation was that *Lucilia ampullacea* arrived very early in and on the border of the forest, whilst *L. caesar* arrived 1-2 days later.

To answer our initial question from the trial: Yes, we did find marked ecological differences between both habitats. However, these differences can only be monitored by traps, or, like in our case, by catching adult flies by hand.

In this particular case, it took an ecologist, a taxonomist, a forensic entomologist, and a critical experimental investigation into one defined area over more than 600 working hours to determine these «simple» differences. However, some species do clearly prefer to live on the border between forest and grassland, and can therefore be used as a marker hinting towards this habitat.

Death and flies : to be there or not to be there !

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One of the main objectives of forensic entomology is to estimate post-mortem interval (PMI). Numerous case studies involving necrophagous flies have permitted the time of death to be established with great accuracy, within the confines of suitable meteorological conditions and ready accessibility to the body. In these cases PMI can be as precise as a few hours following death. But in our temperate countries, there are some periods (i.e. end of winter) where problems can occur.

In two case studies which took place in February and March 2004 at the location of two flats, PMI did not correspond to the time when the first flies arrived and laid eggs on the corpses. In the first case the victim wrote a dated letter before committing suicide, and in the second, other evidence from the crime scene (e.g. the mail was left in the mail box), suggested that death occurred a couple of days earlier.

In both cases, we found only *Calliphora vicina*. Rearing in the laboratory of material collected at the crime scene gave PMI estimates shorter than what was indicated from other evidence. But the most important point was that the egg-laying day of *C. vicina* coincided with local weather conditions. In both cases this was the only day where flies were able to fly outside and then penetrate the flat. Hence it is of primary importance to analyze very carefully data from weather stations in order to calculate PMI - even if these estimates do not fit with other evidence from the crime scene. Snow, rain and low temperatures will inhibit flies' activity and consequently the possibility for those flies to reach the corpse and to lay eggs. In our particular cases when daily mean temperatures were equal or above 10°C without rain or snow, flies would have been active.

Behaviour and development of *Calliphora vicina* at low temperatures and application of research data to a winter death in the UK

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In the UK, blowflies (Diptera: Calliphoridae) have a highly seasonal period of adult flight activity, being generally abundant in the summer months and absent in the winter months. However, the urban bluebottle blowfly, *Calliphora vicina*, is an exception to this general rule and can be found throughout the year, albeit at much lower levels in the winter months. Therefore, there is the potential for human bodies to become infested with larvae even if death and first exposure to flies occurs during the winter period. Such a situation arose in a case of suspicious death in northern England. The body was found in February 2004 but the victim was last seen alive in November 2003. The pathologist's initial report suggested that death had occurred no more than 2-3 weeks before the body was found. However, analysis of the insect evidence using data on larval growth available in the literature suggested that the larval infestation had occurred about 9 weeks previously, that is around the time of last sighting. This case highlighted areas for further study including the potential for diapause as a compounding factor in the estimation of larval age.

Two of the areas for further study are adult blowfly flight activity and reproductive condition during the winter months. To study these, sticky traps baited with liver and sodium sulphide were set up in a suburban garden and in London. Intermittent trapping was conducted in November and December 2004 followed by daily trapping of blowflies throughout January to March 2005. Trap catches were analysed in relation to a number of climatic parameters. Female flies were dissected to determine their mated status and the stage of egg development. From this, the liklihood of eggs being deposited on carrion exposed during the winter months was determined.

Enhanced standard operating procedures used under certain case scenarios in a forensic entomology laboratory

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Literature from several sources detail procedural guidelines that need to be established by a forensic entomology laboratory (Catts and Haskell, 1990; Byrd and Castner, 2001). The utilization of these guidelines creates a methodology for forensic entomology labs to process materials from cases whether they be collected by that laboratory or sent from a facility such as another entomologist or a law enforcement agency. This paper proposes additional procedures not yet addressed in the literature regarding specifically the receipt of evidence into the laboratory. The primary component of any laboratory revolves around documentation of how evidence is handled and processed. The conclusions derived from this evidence are deeply tied to this management of specimens and it is the documentation of this specimen management which substantiates the entomologists' claims.

The importance of the initial contact with the specimens in the forensic entomology laboratory is identified and addressed in this paper. Certain evidence accessioning procedures are discussed which add to the labs' ability to verify the work preformed and the conclusions ultimately reached. Paying attention to the details at the preliminary steps of evidence handling protects the forensic entomologist; these procedures further validate the scientific integrity of the methods employed and that of the practitioners conducting the analysis.

When receiving, processing, and returning evidence, it is possible for forensic entomology laboratories to have somewhat differing Standard Operating Procedures (SOP's); however the basic principles for handling this evidence is similar among laboratories. While all cases require adherence to these general SOP's, some cases may require enhancement of the established procedures. For instance, high profile cases or those that have deeply contestable points may need to be treated with extra caution by the receiving forensic entomologist. Recently, there have been instances where charges of felonious activity or gross negligence have been leveled at an opposing forensic entomologist. While these charges were totally untrue, the mere suggestion that a scientist who has worked for many years in the field could reach to such levels gives reason for great concern. How do you protect yourself from that happening again? Thus, when obtaining evidence from an opposing forensic entomologist, the receiving entomologist may be required to augment handling procedures to protect himself from such unfounded charges. Having a witness present during the initial inventory of the evidence, as well as thorough documentation with video, photographs and written descriptions all can provide protection from such accusations. It is paramount that the honesty, integrity, and ethics be maintained by the practicing forensic entomologist and false claims and slanderous accusations be thwarted from the outset.

A winter murder in Pennsylvania with recovery in early springtime: how the opposing forensic entomology experts disagreed

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The decomposing remains of a male subject were discovered in a gully of shallow water with and overstory of tall trees and thick underbrush on April 8, 1999 in the early evening hours in central Pennsylvania. The body was resting on the ground consisting of stones and fallen logs with the leg of the decedent submerged in water. The head was turned to the left side with the superior end of the body pointing upstream. At 23:30 hours on April 8, 1999, the body was transported to a funeral home and placed in a cooler. On April 10, 1999 at 04:30 hours, the body was transported to a forensic laboratory for autopsy which was conducted at 08:00 hours that same date. The decedent was identified and had been previously reported missing on or about the 22nd or 23rd of January of that year.

A forensic entomologist was contacted to recover the insect specimens seen on the remains. A number of specimens were collected by this forensic entomologist and were later identified. A post mortem interval estimation was attempted by the entomologist and the conclusions were presented in a submitted report to the prosecutor. At a later date, a suspect was arrested for the murder (originally the cause of death was ruled a drug overdose) of the decedent and was arraigned on capital murder charges. Trial was set for a future date.

In preparation for trial, the defense attorney contacted a forensic entomologist to review the forensic entomology report of the prosecution's witness. Upon study and reanalysis of the insect and climatological data, the second forensic entomologist reached several different conclusions and species identifications than what were stated by the prosecution's entomologist. The differences and opposing analyses will be discussed in the presentation. Comments and suggestions are welcomed from the attendees. The trial outcome will be presented at the end of the discussion period.

Living in squalor: when men and myiasis meet

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Forensic Alliance Limited

Forensic entomology has historically been known as an important tool to determine the Post Mortem Interval (PMI); however, this field had been diversifying and entomology's wide spread use in legal investigations is finally being considered.

One such example of this is in cases of abuse and neglect of vulnerable persons. As in the estimation of the PMI, the time since an event can be determined and provide useful information regarding the care of the elderly and the young.

In this presentation a case involving severe neglect of a child will be examined and issues relating to raising the awareness of hospital and police officials to these types of cases will be discussed.

Accelerated skeletonizing of a human body in 10 days by larvae of burying beetles (Coleoptera: Silphidae)

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Decomposition of cadavers underlies a typical sequence of stages. The different stages in decomposition of a cadaver are fresh, bloat, active decay and advanced decay/dry. Duration of each phase is depending on environmental conditions. Beetles are not the first insects using a cadaver for feeding, predation or reproduction. Normally beetles are most present at a cadaver during the bloat phase. Burying beetles (family Silphidae) are one of the dominant beetle families from the beginning of the bloat stage until ending of active decay stage. Early occurrence of is reported in tropical regions.

In summer 2003 in a rural area of Lower Saxony, Germany, a completely skletonized body was found in a brushwood. At the time of discovery masses of silphid larvae of all stages were present while fly larvae were absent. Fly pupae could only be found very rarely on the skeletal remains and in the ground samples from the surroundings. The corpse was lying completely dressed in a face down position. Around the skeletonized neck, a bootlace-like rope was found. Identification was possible by odontology (dental charts). The man with previous suicidal tendencies was missing for two weeks before discovery.

Occurrence of so many larvae of burying beetles in the early postmortem interval is astonishing. An explanation may be the hot and dry summer in Northern Germany in the year 2003.

Behavioral analysis of the repellent effect of various substances on necrophagous insects using olfactometry.

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The aim of the experiments was to study the repellent effect of some products able to be poured on corpses using olfactometers. This study follows previous observations carried out from 1999 to 2003 with rats cadavers disposed on the flat roof of the Forensic Institute.

In a first step, four species of Diptera (*Calliphora vicina, C. vomitoria, Protophormia terranovae* and *Lucilia sericata*) and one Coleoptera (*Dermestes haemorroidalis*) were tested.

The chemical substances tested were bleach, paradichlorobenzene, lime, soda, hydrochloric acid, citronella, perfume, petrol and insecticide.

Two distinct methods were used :
- flight behaviour was studied with a olfactometer in which insects can fly horizontally toward the treated bait or flee it,
- walk behaviour was analyzed in a tube in which insects move longitudinally. An air stream was sent through the part containing the treated bait,

Time response and type of effect (attraction or repulsion) were recorded for each individual. Detailed methods will be presented as well as the first results, which will be compared with previous results obtained in field tests. All data collected will be then the subject of statistical analysis: they should permit to characterize the effect of the products and insect responses and consequently to allow a more rational estimation of the post-mortem interval in the case of a body sprinkled with repulsive substances. Moreover, the simplicity of material and protocol will allow to extend this study to other substances and/or species.

Detection of methadone in necrophagous blow fly larvae *Calliphora vicina* after feeding on minced meat with different concentrations

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In the case of heavy maggot infestation extensive skeletonization of a dead body may occur within a couple of days. In these cases drug analysis in blowfly larvae which had been feeding on the body might be an alternative for toxicological investigation. While there are first studies about determination of opiates and benzodiazepines in blowfly larvae (1;2), investigation of methadone have not been established yet. Regarding the relevant numbers of methadone associated deaths it should be demonstrated that methadone can also be detected in blow fly larvae, feeding on substrate containing this drug.

Minced meat with a methadone concentration of 0,5 µl/g, 1,0 µl/g and 10,0 μg/g served as model for a distinct concentration of methadone in a dead body. After oviposition, the blow fly eggs were distributed and raised at room temperature (18 - 20 °C) to 3rd instar until peak feeding.
Methadone concentrations in whole *larvae*, in larval skins and larval internal organs were analysed with high-performance liquid chromatography wit UV detection at 210 nm (HPLC/DAD). For sample preparation the respective material (1 g) was extracted with chloroform (pH 7) with an ultra-turrax. After centrifugation, the organic solvent was evaporated to dryness and the methadone concentration was detected with HPLC-DAD.

In each group methadone was detected in whole *larvae*, in larval skins and larval internal organs. However, the concentration of methadone detected in the samples were only a small fraction of the concentration in the minced meat.

Our results indicate that the detection of methadone in larvae feeding on a dead body is in certain situation suitable for conclusions on methadone consumption of the deceased. However no strictly correlation between the concentration in minced meat and larvae was obtained.

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(2) Sadler D.W, Chuter G, Seneveratne C, Pounder D.J. 1997. Barbiturates and analgesics in *Calliphora vicina* larvae. *J. Forensic Sci.* 42:1214-1215.

Molecular identification of forensically significant calliphorids: Can nuclear DNA tell us more than mitochondrial DNA?

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Molecular-based identification has become a major research focus in forensic entomology in the last ten years. The ability to identify immature insects accurately and efficiently based on characteristic regions of DNA has been applied by many entomologists.

Research has focused mainly on the use of the cytochrome oxidase I (COI) encoding region of mitochondrial DNA (mtDNA) and its ability to successfully discriminate between the majority of forensically important Calliphorid species. However, there remain some closely related species that are not convincingly separated using this region of DNA.

COI sequence data also provides little scope for intraspecific study. Low levels of variation prevent critical analysis of species status, an important factor in forensic entomology. Consideration of species status issues is vital to ensure application of relevant developmental data in estimation of time since death and reduce the potential for error in our estimates.

The research discussed considers study of a region of nuclear DNA. The internal transcribed spacers ITS1 and ITS2, and the 5.8S ribosomal RNA gene that lies between the spacers were sequenced and screened for inter- and intraspecific distinction potential. Flies were sequenced from Australia, New Zealand, Thailand, England, the United States and southern Africa. The ability of this region to distinguish between species is discussed, as well as the identification of population level variation in preliminary work. The potential of the ITS1-5.8S-ITS2 fragment of DNA is compared to that of the currently preferred COI, with implications for the future of molecular-based identification in this field.

www.eafe.org: two years after

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Two years ago, the website of the EAFE went online. The goal of this website is to be a link with all the EAFE members, to collect information and events in FE world and to gather all the people who are involved with FE. Last year a new presentation was elaborate to be more attractive and convenient.

The EAFE members and associate members are invited to give their contribution by sending news references of FE papers, meeting announcement, FE event, and else.

Today, 99 members or associate members, who come from 25 countries, are registered in the association. With about 800-1500 connection per a month the website runs in a good way.

For the moment information are sent only by few people and often the same one. The aim of this communication is to foster members and associate members to give their contribution to the benefit of everyone involved or interested in Forensic Entomology.

Poster abstracts

Differences in Sizes of Diptera Larvae on Dressed and Undressed Pig Carcasses

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The most important criteria used to determine postmortem interval are where the corpse is, whether it is dressed or undressed and all ecological conditions. For example, the temperature, humidity and air currents affect Diptera.

The aim of this study was to determine differences in duration required for transition from larvae to pupae between dressed and undressed pig carcasses. To this aim, we put two pig carcasses, one dressed and one undressed, 60 m away from each other on a field on Anadolu Bulvarı, Ankara, Turkey. We identified Diptera families, measured lengths of Diptera larvae collected from the carcasses and determined the differences in their lengths and time from the larval to pupae stage.
As a result, there was no difference in time from the larval to pupae stage between dressed and undressed carcasses, but the lengths of larvae collected from the undressed carcass were longer.

A number of studies revealed that entomological examinations are reliable compared to forensic investigations and autopsy findings. Therefore, it is required that entomological examinations should be carried out and incorporated into crime scene investigations.

Molecular variablility of populations of *Calliphora vicina* (Robineau Desvoidy) within UK geographic populations.

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Wild *Calliphora vicina* were collected in different geographical locations in the UK (Rhyl, Uppingham, Leeds) in July 2004. A newly designed trap was constructed, which was a modification of a "hanging bait trap" as described in A Dipterist's Handbook (Stubbs and Chandler, 1987) leading to increased retention of live flies in the upper collecting chamber.

Adult *C. vicina* were stored at -20^{o}C and total protein extracted from 5 flies from each geographic location. Four legs from each fly were chosen for processing to minimise possible contamination of protein extracts with proteins ingested by the fly or from other sources including eggs.

Leg samples were cleaned in absolute methanol, dried, ground up following treatment with liquid nitrogen and incubated at 37^{o}C overnight in sample preparation buffer (8M urea; 1% Triton-X 100; 2% DTT.) Extracts were diluted 1:4 with loading buffer (0.5M Tris-HCl pH6.8; glycerol; 10% SDS; 2-mercaptoethanol; 1% bromophenol blue; deionized water) and heated at 95^{o}C for 4 minutes. Samples were run on pre-cast polyacrylamide gels (200 volts for 45 min) on a mini Protean II (Biorad) to separate proteins and results analysed by Gel Compar II (Applied Maths).

The degree of variability in banding profiles produced from the individual flies was assessed. Initial analyses showed greater variation within the flies from Uppingham than between the three groups of flies from other locations.

Effects of constant temperature regimes between 9.8 °C and 32°C on development rates of *Protophormia terraenovae* (R-D) in British Columbia, Canada

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In the first days and weeks after death, forensic entomology is a valuable tool in determining elapsed time since death. Blow flies (Diptera: Calliphoridae) are the first to colonize a corpse, and develop in a predictable manner. However, in order to determine time since death, detailed developmental data are required for any species found on a body.

Protophormia terraenovae (R-D) is a very common blow fly species in Canada, being found in more than 80% of forensic entomology cases in BC in 2004. *P. terraenovae* is an abundant species in colder regions and is considered to have a Holarctic distribution. It is commonly found in higher altitudes in BC and is well established in the Coastal Western Hemlock biogeoclimatic zone of North America. However, very little work has been done on the developmental rates of this forensically important species. Also, evidence suggests that the same species from different geographical regions, may exhibit different developmental rates. Therefore, local data should be obtained for use in local forensic entomology analyses, as results for *P. terraenovae* in British Columbia may differ from results of *P. terraenovae* found elsewhere.

Eggs were collected from *P. terraenovae* colonies and raised to adulthood at constant temperatures of 9.8, 11, 13, 15, 20, 25, 28, 30, and 32 ºC. Development did not take place at 9.8 ºC and stopped at third instar at 11 ºC. *P. terraenovae* completed development at all other temperatures and reached the adult stage with a minimum of 52, 35, 20, 14, 11.5, 10, and 11.5 days, respectively. A linear relationship only exists in the central temperature range of development.

First observations on *Callíphora vicina* development and other Calliphorids collected in an area surrounding the University Campus of Leioa (Bizkaia, North of Spain)

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Preliminary results referred to Calliphoridae presence in an area surrounding the University Campus of Leioa (Bizkaia, North of Spain) are presented. We achieved this first research in the main seasons of the year and in the main habitats of the area; Oak forest, Eucalyptus forest, urban and a suburban area, being the last one the campus itself and a garden close to it. We would like to estimate the main species of forensic interested inhabiting the area and the potential differences referred to the distribution and fly patterns of this family. Following Kaneshrajah and Turner design, trap attraction fed with pig viscera, mainly liver and kidney, were placed in the different ecosystems since oviposition took place, mainly few hours (1-3 h.) after the trap was placed. Only in winter, oviposition delayed for 1-2 days due to low temperatures. At least 3 different species have been collected and reared during this year, *Calliphora vicina, Lucilia illustris* and *L. sericata*. Adults of *C. vomitoria* were sporadically collected but *C. vicina* was first in eggs deposition and dominant during the whole year, except for the summer months, when greenbottle flies arrived first and their presence dominated in the cultures. Therefore, we have focused our first efforts in the development times of the species under controlled conditions. Results indicate that the larval development scarcely changes with places, but there are some differences between different seasons and laboratory temperature. As expected, development has been faster during the warmer months (June-September) than in the cooler months (October-April).

For the biometric study of the maggots reared, a variable number of specimens (10-20) were boiled every 10-12 hours approximately. Maggots were placed in the boiling water for a minute approximately following Adams and Hall (2003). Great differences were observed between *Lucilia* and *Calliphora* species. Good results have been obtained just for *C. vicina*. *Lucilia* spp. used to relax badly and did not elongate adequately for a standardised biometric analysis and specific identification. We expect than other greenbottle fly species may have been reared but inadequate preservation of maggots difficult an adequate specific identification. Therefore, we will get only focused on the results obtained for *C. vicina* in the 3 periods of the year when this species was collected and reared; spring, autumn and winter. We will focus our future efforts in a more profound research referred to the better relax method before preservation for *Lucilia* species as we need to develop it in order to establish adequate standards for technicians responsible of future recollection of entomological evidences in forensic research in our country. All the specimens have been labelled and kept with 80º ETOH in our personal collection and are available for future comparative studies. A sample of reared adults has also been preserved dried for specific identification.

Maggot measurement was carried out with a calibre; this tool reduces personal artefacts and gives us a precision of two tenths. An average related to maggot age and season is presented for the different sampled habitats.

Adams Z.J.O. and M.J.R. Hall. 2003. Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. *Forensic Science Int.* 138: 50-61.
Kaneshrajah G. and B.Turner 2004. *Calliphora vicina* larvae grow at different rates on different body tissues. *Int. J.l of Legal Medicine* 117: 242-244

What does attract blowflies (Diptera, Calliphoridae) in a trap ? Preliminary investigation

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Blowflies are the first organisms to colonize corpses. They are of great forensic importance as they are sometimes the only way to estimate PMI (post-mortem interval).They could also potentially permit in some cases to detect if the corpse has been moved from one area to another one if species distribution is known in details. Hence it is important to map the geographical distribution of necrophagous flies (Calliphoridae). This implies to trap, as exhaustively as possible, quantitatively and qualitatively the species in numerous localities. An efficient trap is obviously necessary. While it is known that certain specific species are present on decomposing bodies, their attraction to these bodies has not fully been examined. Several investigations of chemical analysis of odours associated with decomposition are in progress (i.e. see work of Hélène LeBlanc). An odour bait of liver plus sodium sulphide has been shown to very attractive for *Lucilia sericata* (Hutchinson, 2000).To increase the efficiency of our traps, we decided to test two attractive baits: pig's liver and liver mixed with Na_2S (which produces H_2S in contact of the air's humidity).

For each experiment, 8 Upton's traps, modified by Faucherre and Cherix (1998), were placed in a meadow of the University Campus of Lausanne on two rows set 25 m apart. Four of theses traps were filled with 120 g of fresh mixed pig liver whereas the four others were filled with a mix of 25 g of fresh pig liver and 30 ml of Na_2S 10%. The two types of attractive traps were alternated. Twenty trapping days were conducted between May and July 2004. Each day, at 5 pm, flies were collected, killed with ethyl acetate, and mounted for identification.

In traps filled baited with liver, 54 individuals of 4 species of Calliphoridae (*Calliphora vicina, Lucilia sericata, L. illustris and L. ampulacea*) were caught. In traps filled with the liver plus sodium sulphide, 34 specimens of 5 species (*C. vicina, L. sericata, L .illustris, L. ampulacea and L. caesar*) were collected. The low number and diversity of blowflies caught, compared to a previous study (Von Aesch *et al*., 2003), could be explained by the heat wave of summer 2003. This heat wave may have implied a food shortage or a very low reproduction success of blowflies consequently reducing drastically their populations. We first noticed that more individuals of Calliphoridae were collected in traps using only liver as bait. Another tendency is the preference of *C. vicina* towards the bait liver plus sodium sulphide. *Lucilia* species, on the other side, were preferentially captured in traps filled with liver only. But due to the very low number of blowflies caught, these results should be considered with caution.

Faucherre, J. & Cherix D. 1998. Contribution à la connaissance des Diptères nécrophages du Jorat (Vaud, Suisse). *Mitt. Schweiz. Ent. Ges.* 71: 211-217.
Hutchinson, R. A. 2000. Some behavioural responses of *Lucilia sericata* (Meigen, 1826) (Diptera, Calliphoridae) to three odour baist using sticky boards and electrified screen. *Studia Depterologica* 7: 233-240.
Von Aesch L. J. Pellet, D. Cherix & C. Wyss. 2003. Activity and behaviour of blowflies on pig liver baits in spring. *Mitt. Schweiz. Ent. Ges*. 76: 201-206.

A preliminary contribution to the distribution of necrophagous flies in the canton de Vaud (Diptera Calliphoridae)

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Time elapsed since death, or post-mortem interval (PMI) is a matter of crucial importance in the investigations of homicide and other untimely deaths. Even when the cause of death is natural, time of death can have important implications. The only way to estimate PMI when death antecedes by more than 72 hours, is the presence of necrophagous flies (Calliphoridae). Due to the lack of specimens in museum collections in our country we were interested in getting more data on the distribution and phenology of these flies. Generally, habitats, altitude, season, have a significant influence on insects' distribution. Therefore we decided to study the distribution of necrophagous flies (mainly Calliphoridae) in the three main biogeographic areas of the canton de Vaud.

We selected three study sites from the canton: Jura Mountains, Low Land, and Alps. In each study site we selected two types of forest (spruce forest and beech forest), one pasture and one urbanized habitat. Each biogeographical area is, notably, characterized by its altitude. The stations were chosen specifically to take into account this attribute: Low Land; stations between 400 m to 700 m, Jura Mountains; stations between 700 m to 1000 m, Alps; stations between 950 m to 1200 m. Three attractive traps were used in each selected habitat. The traps were installed at 9 am, left in place the whole day and collected the same day at 5 pm. The flies were first anaesthetized with CO2, then killed with ethyl acetate and rapidly mounted for identification. The experiment was repeated once a month, during seven months (May 2004 to November 2004) in each station. We put in place a temperature sensor in each habitat (e-button).

The preliminary results of this survey are as follows. We collected about 1000 specimens. The following species of Calliphoridae have been identified: *Cynomya mortuorum, Calliphora vicina, C. vormitoria, C. subalpina, Lucilia sericata, L. illustris, L. caesar.* Each site is characterized by a dominant species: *L. sericata* in Jura Mountains and Low Land, *C.vicina* in the Alps. On the other hand some species are not present in all sites such as *L. caesar* in the Alps, *C. vomitoria* and *C. mortuorum* in the Low Land.

The surprisingly low number of specimens caught could be a consequence of the heat wave during summer 2003. Data from the Swiss meteorological Institute indicated a very low pollen index in 2003. This could have implied a food shortage for the adult flies and a consequently low reproduction success.

Early colonisation on different bait by sarcosaprophagous Diptera in southeastern Spain[1]

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In order to evaluate the trophic and reproductive preferences of the commonest and most significant sarcosaprophagous species in every season, a sampling was carried out during autumn and will be continued throughout the four different seasons.

Bottle traps were used. They had a blind end and an inverted funnel at the other end to allow the adults access to the bait. Different baits were selected: minced pork and beef meat, fish (*Sardina pilchardus*), pork liver, both two cut in 2-3 cm portions, and human faeces homogenised with distilled water. Traps were placed in the Agricultural and Forestal Experimental field station in the University of Murcia, within the University Campus in Espinardo, 2 meters away one from each other. Traps remained in place until egg-laid or larval activity was observed on the baits.

The trap exposure needed to obtain eggs or larvae was 1-2 days. Minced meat and fish (up to 5 days for minced meat) needed the longest exposure. A clear preference was shown by Muscids for human faeces and by Calliphorids for liver. Muscids were able to develop the whole life cycle on this substrate, adults emerging 11-20 days after egg laying. Calliphorids were also able to develop on liver; emerging adults were obtained in 11-18 days. Sarcophagids were collected on all bait but, while they laid larvae on liver in less than 24 hours, the larviposition was delayed by between 3 and 5 days in fish, minced meat and faeces. Sarcophagids showed a clear preference for the liver, where the larvae number was higher. Adults emerged in 12-22 days.

These results are useful to evaluate the fauna associated with corpses found in different circumstances, e.g., in a rubbish environment, with open wounds or traumas, with viscera outside, ...

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Initial Studies on Insect Succession on Pig Carrion in the Centre of Iberian Peninsula.

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Forensic Entomology is a relatively new science in Spain. Investigations carried out in different countries, especially USA and Europe, have allowed Forensic Entomology to achieve a greater international recognition as an integrated discipline in criminal police investigations.

This discipline begins to be applied nowadays in Central Spain and the previous investigations in this respect are very scarce across the country: only carrion succession and sarco-saprophagous fauna studies have been developed in the NE and SE of the Peninsula respectively. Therefore it is necessary to carry out experiments about the basic forensic fauna and succession patterns in the centre of Iberian Peninsula, because the majority of the available information is useless to be applied in areas like Madrid, one of the cities with the biggest homicide indexes. The use of arthropods in forensic cases requires a detailed knowledge of the species biology, ecology, physiology, systematics, behavioural sequence in which they appear etc.

We present the project in which we are working, entitled : "Forensic Entomology: study of insect inhabiting decompositing remains to help police investigations" whose priority is the study of the fauna of Arthropods that invade human remains, using for it domestic pigs exposed outdoors in different annual stations during two years.

Moreover, this study will allow us to reach other objectives like:
- describing the developmental rates of Diptera larvae at different temperatures, to compare them with the results obtained for populations from other geographic areas,
- describing larvae stages of those Coleoptera species implicated in carrion succession,
- carrying out a faunistic study of major species of potential forensic interest across the central region of the Iberian Peninsula (Madrid area), using for it baited traps.

The project started in June of 2004, so we present some preliminary results of the samplings done until the present date.

Discrimination of the larvae of the bluebottle blowflies *Calliphora vicina* and *C. vomitoria* (Diptera: Calliphoridae)

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In the UK, the bluebottle blowflies *Calliphora vicina* and *C. vomitoria* (Diptera: Calliphoridae) are generally the most abundant species on human remains. It would be easy to think that discrimination of the larval instars of these common species was straightforward, because full descriptions of their larvae and a number of identification keys have been published. However, there can be difficulties in discriminating even these well studied species due to local variations in morphology and variation in some of the morphometric characters with larval length. This study examined the mouthparts, the shape and size of the spines and spine band characters of larvae of *C. vicina* and *C. vomitoria* as well as two morphometric characters, the width of the posterior spiracle and the spiracle distance factor (distance between posterior spiracles divided by the greatest diameter of one spiracle). It concluded that the best discriminators for first and second instar larvae were the mouthparts, while for third instar larvae they were the size and distribution of the cuticular spines and the greatest diameter of the posterior spiracle.

Three forensic cases involving *Hydrotaea capensis* (Diptera)

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Comisaria General De Policia Cientifica, Seccion De Antropologia

We are reporting on three case studies involving concealed corpses. They were studied at the Forensic Entomology laboratory in the Department of Anthropology of the General Department for Forensic Sciences (Madrid, Spain). These cases have a number of features in common as regards their geographic location: Alcoy (Alicante), Estepona (Málaga) and Melilla (north of Morocco).
We would like to highlight the importance of:
- databases with information relating to cases analysed in the laboratory.
- the identification of every specimen collected at the scene of the crime and at the autopsy.

Case 1. The corpses of a woman and a man were found on 25th March, 2003. They were buried in a bungalow basement in a village called Alcoy (Alicante), situated on the east coast of Spain. The couple had disappeared in 2002. It seems that they had been kidnapped and murdered. The grave was 1.25 metres deep, 2.70 metres long and 0.82 metres wide. The bodies were covered by sand and a 10 cm layer of concrete. The assailants had hidden the bodies, burying them together. The first body, the man, was placed at a depth of 0.62 metres and the second, the woman corpse, under the first one at 1.05 metres depth. The man was partially mummified, but the woman was better preserved due to the protection given by the man corpse and being wetter than the man. Fauna collected at the scene of the crime and at the autopsy consisted of: larvae, pupae and adult specimens of *Hydrotaea capensis*, Wiedemann, 1818 (Diptera: Muscidae); one adult of *Laemostenus (Pristonychus) terrícola*, Herbst, 1784 (Coleoptera: Carabidae); one larva of *Blaps* (Coleoptera: Tenebrionidae) and pupae of Diptera Phoridae. An IPM of about 6 months was calculated.

Case 2. A male corpse was discovered on 1st April, 2003, inside a boat anchored at the "Sea Club" in Estepona village (Málaga), on the south coast of Spain. The body showed evidence of violence: his hands were tied and he had a rope around his neck. The corpse was wrapped in a sleeping bag. The body was in an advanced stage of decomposition with skeletonised parts. Fauna collected at autopsy consisted of: larvae, pupae and adult specimens of *Hydrotaea capensis*. An IPM of about 1 year was calculated.

Case 3. On 14th June 2004, a male corpse was exhumed at Melilla in the north of Morocco. Apparently, his wife had tried to poison him with the medicine called "COLME" (Cyanamide, calcic carmicide) six months before the date of exhumation. Fauna collected during the exhumation of the corpse consisted of: larvae and pupae of *Hydrotaea capensis* and pupae of Diptera Phoridae.

All three cases deal with human remains that were discovered in an advanced state of decomposition.The dead bodies were concealed. The offences took place in the same geographic region: the Palaearctic region, Mediterranean sub-region. No blowflies of the family Calliphoridae (Diptera) were present on the corpses, and so we concluded that they had not been exposed to the air prior to their concealment.

(Thanks to Dr. Adrian Pont who kindly identified the *Hydrotaea capensis*)

Comparison between forensic entomological and anatomo-pathological exams to determine the burial time of swine carcasses in a fraudulent case.

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Post mortem evaluation for a number of death pigs was compared to entomological exams in order to determine the burial time. In the denounced land were made some holes in places choosen arbitrarily. Entomological material was found in areas with carcasses in colliquative rotten stage and the checked fauna was composed by Diptera and Coleoptera. Where there were only bony tissues remains we observed the absence of entomological reports. In one case by use of entomological exam was possible to identify a vertical burial stratification. The comparison between entomological evaluation and anatomo-pathological exam in veterinary medicine is a useful tool to investigate the illegal disposal of carcasses.

Sun versus shade: sarcosaprophagous Calliphoridae in central Portugal

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Studies of Calliphorid sarcosaprophagous community are very scarce in the Iberian Peninsula (Goff *et al*., 2004): This work will increase the knowledge of said group and it will be very useful in order to establish a forensic data base.

To study the Calliphorid community two traps, as designed by Schoenly (1981), were used, baited with piglets of 5 kg weight. One of them was sited in a sunny area and the another in a shady one. The experiment was conducted in the Botanic Garden of the University of Coimbra within the University Campus, in the centre of Coimbra City. This site is representative of a semi natural place with a climate of Mediterranean and Atlantic influences. 56 samples from each trap were obtained between May and September 2004.

12 different Calliphoridae taxa were collected: *Lucilia silvarum, Calliphora vicina, Calliphora vomitoria, Chrysomya albiceps, Lucilia ampullacea, Lucilia caesar, Lucilia illustris, Lucilia sericata, Protophormia terranovae, Stomorhina lunata* and *Pollenia sp.* Their presence in the two environments (sun and shade) as well as their abundance and distribution throughout the sampling period are presented and discussed.

A comparison of the composition of the found community with those belonging to other areas of the Iberian Peninsula (Arnaldos *et al.*, 2001, 2004, Castillo Miralbes, 2002) is also presented. This study reinforces the need for local studies of entomo- sarcosaprophagous fauna so results can be applied to actual forensic cases.

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The succession and development of insects on pig cadavers and their significances in estimating PMI in south China

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The determination of the time after death is very important in criminal investigation for it is the gist to determine investigation direction and scope, to ascertain and exclude suspect. For centuries it remains a difficult problem. This actuality was altered by the swift development of forensic entomology in last century. In most area of the world forensic entomology has been carried out to ensure the demand of the determination of PMI. The study of the activity and succession of insects on cadavers and corpses and its relationship with PMI is the key research in forensic entomology. In this paper our research on this aspect will be reported.

In different months the pig cadavers were placed in different environment to observe their decomposition process and insects' activities and succession on them. They were observed twice every day till the cadavers completely decomposed. Each time the cadaver's decay phenomena and insect were noted and sampled. The temperature and humidity were automatically recorded once every hour by electronic thermo hygrometer. Each fly species were observed as followed: 1) when adult arrive cadaver; 2) when ovulation; 3) when incubation; 4) the larval body-length change pattern; 5) when mature maggot start crawl away from cadaver; 6) when massive crawl away; 7) when start pupation; 8)when massive pupation; 9) Morphogenesis of pupae; 10) when blooming; 11) when massive blooming.

1. Insects species: There were about 47 insects species found on cadavers. The species quantity was less than other researches due to human activity's influence upon biodiversity. The species were as follows: *Chrysomya megacephala, Achoetandrus rufifacies* (Macquart), *Ceylonomyia nigripes* Aubertin*, Chrysomya pinguis* (Walker), *Lucilia sericata* (Meigen), *Lucia cuprina* (Wiedemann), *Lucilia (Luciliella) bazini, Parasarcophaga ruficornis* (Fabricius), *Parasarcophaga crassipalpis* (Macquart), *Parasarcophaga albiceps* (Meigen), *Musca domestica* Linnaeus*, Musca ventrosa* Wiedemann*, Hydrotaea (Ophyra) spinigera leucostoma,* Sepsidae *(unidentified),* Horidae *(unidentified), Hermetia illucens (L.), Saprinus splendens, Merohister jekeli* (Marseul), *Saprinus optabilis* Marseul*, Necrobia rufipes* (Degeer), *Necrobia ruficollis* (Fabricius), *Dermestes maculates* Degeer*, Creophilus maxillosus, Platydraus sp,* Aleocharinae*, Diamesus sp.* Geotrupidae*, Onthphagous taurinus* White*, Conocephalum sp., Anisolabis maritina (*Gene*), Vespa bicolor bicolon* F., *Vespa affinis affinis* (L.), *Vespa velutina nigrithorax* Buysson*, Paravespula flavices flaviceps,* Formicidae*, Harpegnathos venator* (F.Smith), *Pheidologeton affinis* (Jerdon), *Camponotus variegates* (F. Smith), *Acarina (*3 species).

2. Cadaver decomposition rate

In spring, summer and autumn, most of the cadavers decayed very quickly, the time needed from flesh to most of the bones bared (skeleton) was not over 20 days to one month, while in winter it needed a longer time, about several months. In the same season and resemble environment, decomposition rate of different cadavers was the same.

3. Insects succession pattern

Faunal colonization occurred in a regular succession pattern, the blow fly could only bred one generation before the flesh was eaten up. It showed the same pattern as other reports of India-Malaysia zoological region.

Although such kind of researches have been done in many areas throughout the world, but every region has its own entomography, and the succession and development of arthropods that colonize on the cadavers were somewhat impacted by the environment factors. Though our study we found that the flies can only breed one generation on the cadaver for the cadaver decomposed very quickly due to the hot weather and high humidity. This point is very useful for it can exclude the confusion that we don't know which generation the maggot or pupae is. In south China, the cadavers decomposed very quickly in hot weather. The cadavers can become highly putrefaction in about 2-4 days, and we cannot effectively deduce the PMI by regular pathologic means. Now with the better understanding of the flies' development, the PMI can be relatively precisely estimated within the first couple of weeks. For the cadavers we placed for a even longer time, the PMI can be estimated by the succession pattern of the insects colonizing on the cadavers. The entomology information is of great useful in forensic science, but we should know that the environment factors must be considered and the results must be interpreted with great care.

Carrion decomposition process and its temperature elevated by maggot colonization in east China

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The determination of postmortem interval of decay corpse, one of the most important question to solve for forensic investigator, remains the most difficult problem for centuries because a wide variety of techniques advocated based on forensic pathology just effort in the early postmortem period of the first day or two after death.

During the past decade forensic entomology develops very quickly, it has been regarded as the most accurate method to determine PMI of decay corpse. When using forensic entomology, the development situation and actual temperature insects experiences are the main factors employed. We carry out research to clarity the decay process and actual temperature of insect in corpse so as to convenient their application in the determination of PMI.

This research was carried out in Hangzhou. In different season the pig carrions were placed on grassland of campus to observe the carrion's decay process, temperature and insect's succession patterns. On the while new hatching larval were raised in the pig liver on constant temperature at the population density of 0,10,12,20,30 l/g respectively to observe the relationship between maggot density and liver temperature. Every day the maggot mass temperatures and development situation would be measured.

The results are as follows:

The decay process of pig carcass obeys the same pattern in different seasons. I.e.: first flesh, then rigor mortis, next bloat, when bloat to some degree, the corpse will break down. After that the corpse will turn into skeleton. In different season the time required from fresh corpse to skeleton is different, shorter in summer and longer in spring and autumn. The decay process was affected by temperature and rain drop, rain will delay the decomposition process. Insects such as flies will arrive at corpse immediately after death, first they arise in head and then in abdomen and thorax. The arriving time is different for different insect species. The larval migrate off the remains to pupate, in spring the mature maggot mass migrated off the remains twice, while in summer and autumn only once. In spring the first time maggot crawl off the remains is when the flesh in head is eaten up and another time is when flesh in abdomen and thorax was eaten up. In summer and autumn the mature maggot mass crawl away from carcass when most of the body was eaten up. Carcass temperature could be elevated in dense aggregation of larvae, in spring the carcass temperature could be elevated 8° while in summer up to 22°. In the first day the body temperature cool just as it's described by most of the forensic pathologist, after that carcass temperature keep the same as environment temperature for several days. Then it will increase greatly when the population of maggot is large enough. Then the temperature will keep in a high level for several days and did not change with environment temperature till most of the maggots crawl away.

Temperature change of pig liver infested with maggot also was observed. *L. sericata, a. grahami, c. megacephala* can elevate their food temperature several centigrade. The temperature increase with an increase in maggot density.

Food source location by *Dermestes maculatus* (Coloeptera:Dermestidae) DeGeer

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Previous studies indicate that *Dermestes maculatus* (Coleoptera:Dermestidae) DeGeer appear on decomposing remains, both human and animal, during the dry stage. However they do not give reasons as to how or why *D. maculatus* do this. This research seeks to investigate aspects of the preference for food source.

Ten adult males and ten adult females were given choices, using Y-tube assays, between pork fat and pork muscle in three different states of hydration – fresh, intermediate and dry, together with the presence and absence of other adult *D. maculatus*.
D. maculatus adults displayed significant preference ($P = 0.05$) within each choice combination offered. They chose the least hydrated option in 9 out of 12 choice combinations. Both males and females also displayed significant preference for the presence of other adults.

These results suggest that food odour, when combined with aggregation pheromones, are more attractive to *D. maculatus* than food odour, despite the state of hydration. They also suggest that D. maculatus can differentiate between odours of food sources in different states of hydration.

First forensic record of *Sarcophaga caerulescen*s in Switzerland

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On 8th July 2002, a corpse of a suicide victim was discovered hanging from a tree branch at the edge of a forest. His legs and knees were in contact with the ground. The altitude of the crime scene was 660 meters a.s.l. and the temperature at the time of discovery was 20.3°C. During our investigation at the crime scene, we collected several flying insects including blowflies. The following adult specimens of forensic importance were identified: *Lucilia caesar, Lucilia illustris* and *Calliphora vomitoria*. This material was found either on the body or within the immediate surroundings. Numerous batches of eggs and larvae were present on the corpse and within the clothes. No pupae were found even after an intensive local search.
Eggs and larvae were rapidly brought back to the laboratory to be reared under controlled conditions (23°C). They were fed with pig's meat. Their development was checked every day. On 19th July, the first adult flies emerged in our laboratory. These flies were identified as *Lucilia caesar*. Eleven days later (30th of July), a second species appeared belonging to the genus *Sarcophaga*, specifically *S. caerulescens*, Zetterstedt, 1838. Identification was based on male genitalia. This is the first time this species was discovered and identified on a corpse in Switzerland. No adults of this species were collected at the crime scene. Meteorological data related to the crime scene were obtained from a nearby weather station and used to determine the temperature at the crime scene before the corpse was found. According to our development tables with regard to *Lucilia caesar*, we could ascertain that the flies were first present on 1st July (+/- 24 hours) when they start to lay eggs. Other evidence from the investigation showed that this person disappeared on the afternoon of July 1st.

For the first time we reared a new species of *Sarcophaga* of forensic importance. In our case, with a mean temperature of 22.5°C, total development time was 30 days. If flies of this genus seem to be predominant on human bodies located in indoor habitats in the southeastern U.S. (see Byrd and Castner, 2002), they are rather rare or at least not frequently observed in Europe (see Introna et al., 1998 with a probable misidentification). Nevertheless Grassberger and Reiter (2002) were the first to provide valuable data on *Liopygia (=Sarcophaga) argyrostoma,* with a total development time of 31.3 days at 20°C. In all our case studies (152), we found flesh flies on only 30 occasions (about 20%). Their presence is restricted to summer months (June to September) with a peak in August (about 40%). Where flesh flies were collected, five cases are located in the countryside and 25 in houses. Identification of these specimens is under way.

There is a real need for detailed studies on development of these species (with correct identification) which could help investigators in determining PMI.

Grassberger M. and Reiter C., 2002, Effect of temperature on development of *Liopygia (=Sarcophaga) argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae) and its forensic implications. *J.Forensic Sciences* 47(6):1332-1336

Introna F, Campobasso CP, Di Fazio A, 1998. Three case studies in forensic entomology from Southern Italy, *J.Forensic Sciences* 43 (1): 210-214