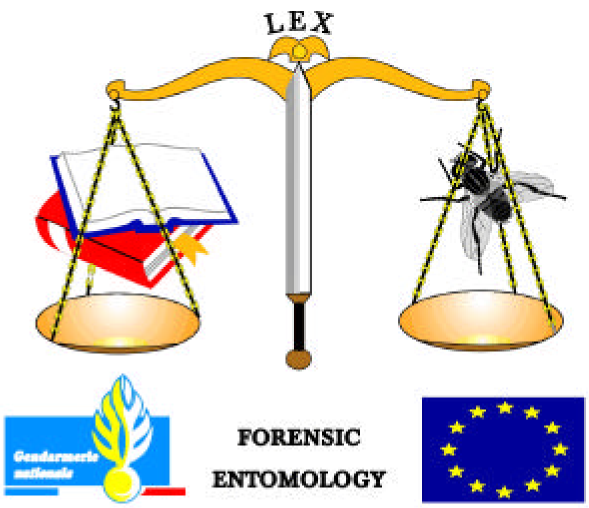


**The first European Forensic Entomology Seminar was financially supported by :**

- European union (OISIN Programme)

**Supports and helps from :**

- Direction Générale de la Gendarmerie Nationale :
    - Bureau Exécution du Budget
    - Division Relations Internationales
    - Service d'Information et de Relations Publiques des Armées de la Gendarmerie
- Centre Technique de la Gendarmerie Nationale :
    - Service de Traitement de l'Information de la Gendarmerie
    - Service Technique des Relations Publiques
- Commandement des Ecoles de la Gendarmerie :
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# CONTENTS

# L'émergence d'un nouveau département

Le hasard fait souvent bien les choses, notre éminent Directeur de l'Institut, le colonel Caillet, est certainement d'accord avec l'adage puisqu'il raconte que c'est en lisant une revue au cours d'un déplacement, qu'il a eu connaissance de l'entomologie légale. A la même époque, le Général Bedou, commandant alors le Centre de perfectionnement de la police judiciaire (actuellement Centre national de Formation de la police judiciaire), fait la même lecture. L'article "incriminé", signé d'un certain docteur Leclercq a été assez convaincant pour qu'il sollicite la mise en place d'une unité spécialisée en Entomologie légale au sein de l'IRCGN. C'est ainsi qu'en 1992 naissait notre département.

Dès cette année, le chef d'escadron Masselin, chef de la division criminalistique D, assisté de l'adjudant-chef Salon, a eu la lourde mission d'équiper le futur laboratoire et de poser la première pierre de l'édifice. C'est dans cette optique que le regretté et mémorable Jacques Salon est allé faire connaissance avec le monde fascinant des insectes, auprès des spécialistes du Muséum National d'Histoire Naturelle, notamment le professeur Menier et le professeur Matile, directeur de la section entomologie malheureusement trop tôt disparu. Une correspondance suivie avec le Dr Leclercq a permis d'obtenir de précieuses informations. Les premiers mois ont été consacrés aux stéréo microscopes, à la verrerie et autres installations nécessaires aux élevages entomologiques. Ce n'était pas chose aisée du fait qu'il n'existait pas de laboratoire de référence ; nous étions vraiment des pionniers en la matière ; cette "exception culturelle" au sein d'un laboratoire de criminalistique n'allait pas tarder à intriguer les médias.

L'année 1992 voit la consécration de cette nouvelle unité, dont le capitaine Ceccaldi prend la tête, assisté du gendarme Tabary, avec qui il va signer les premiers dossiers. La tâche n'est pas toujours facile : il faut se familiariser aux mises en élevage des spécimens immatures, à l'identification - tâche peu aisée surtout pour des personnels issus d'unité de terrain - à la datation à partir de l'étude de la biologie des diptères de la faune nécrophage.
En 1993, j'intègre en fin de deuxième escouade (les initiés apprécieront) le laboratoire au moment où débute une phase importante : la recherche, l'exploitation et le classement de la bibliographie, fruit de pérégrinations notamment à la bibliothèque centrale du Muséum national d'histoire naturelle, alors que les premières "Affaires" arrivent progressivement.

L'année suivante, nous accueillons trois nouveaux spécimens, les gendarmes Pasquerault, Myskowiak et Rocheteau. Le département prend alors son véritable essor. En 1995, le capitaine Vian en tient les rênes, avec le même essaim, jusqu'en 1998 où il sera secondé par un nouveau venu, le lieutenant Gaudry, tandis que le gendarme Rocheteau migre pour intégrer l'école des officiers à Melun. Pendant ce temps nous ont rejoints deux nouvelles recrues civiles : messieurs Lefebvre et Vincent, techniciens supérieurs d'études et de fabrications.

Le capitaine Vian est ensuite parti s'empuper sous le soleil calédonien, passant le flambeau pour quelques mois au capitaine Malgorn dont l'envol vers d'autres responsabilités laisse au lieutenant Gaudry la direction de la "caste".

Aujourd'hui, le département entomologie de l'institut de recherche criminelle fête ses dix ans d'existence, et fort de ses 400 dossiers, peut remercier l'ensemble des personnes figurant dans cette préface mais aussi bien d'autres, comme le chef d'escadron Nogues et le lieutenant-colonel Rouillon qui toutes ont contribué à son essor et conduit à ce qu'il est aujourd'hui : une entité novatrice et performante qui a fait ses preuves dans le monde particulier de la criminalistique.

L'adjudant Bernard CHAUVET
Département entomologie

The seminar is inaugured by Général LEPETIT, Major General, on behalf of the Director of French Gendarmerie, President of the Main Committee.

- Overall presentation speech by Major Général LEPETIT,

- Introduction of forensic entomology by Doctor LECLERCQ, Main President of the Seminar,

- Origin of the forensic entomology in the french gendarmerie by Général BEDOU, initiator of this field.

**Forensic entomology, toads and blowfly hearts**

M. BENECKE

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The organizers asked us to give a short introduction into historic aspects of forensic entomology. In our presentation, we will focus on:

Perception of animals related to real, observed decompositios vs. animals of symbolic meaning. Here, we try to differentiate between snakes and maggots in medieval pictures (Dances Of The Dead), as well as mentioning toads and moth. We also show an example where a renaissance artist substituted the heart of a decomposed body (ivory skeleton, „Tödlein“) by a blow fly.

Brief overview over early forensic entomology cases, their main misconceptions, and what we might learn from them. This will touch the very modern question of what makes a person a good expert witness, compared to a bad one.

**Keywords** : Forensic entomology, history, art

## Admissibility of Forensic Entomology Evidence in U.S. Courts

R.D. HALL

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In U.S. federal and state jurisdictions, evidence must be relevant before it can be considered by the trier-of-fact. Theories of logical and legal relevancy may be used to challenge admissibility. Questioning legal competency is the most common strategy to exclude forensic entomology evidence. Under the current *Daubert* standard, forensic entomology has been regarded as good science ; therefore, the focus has shifted to qualifications of the expert witness and how such science is applied to the fact pattern. In regard to the latter point, the entomologist may have failed to consider data that should have been considered, relied on data that should not have been considered, or relied on erroneous data. If it can be demonstrated that the entomologist's opinion does not rest on a reliable foundation, the opinion can then be shown to be untrustworthy, incompetent, and inadmissible as a matter of law. A frequent weak point of minimum postmortem interval estimations derived from the thermal developmental profiles of necrophilous Diptera is the use of retrospective temperature data accumulated at sites remote from where the decedent decomposed.

## L'entomologie légale en Belgique depuis 1947

M. LECLERCQ

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ABSTRACT

Forensic Entomology in Belgium since 1947
Faunal Succession on cadavers updated
Highest importance of the ecological categories

Il faut vous dire que notre "hobby entomologique" a débuté en 1939 avec l'aide d'entomologistes spécialisés dans les diptères...

Le 21 mai 1947 ; le cadavre d'un enfant est découvert derrière un fourneau dans les Ardennes belges : de nombreuses larves avaient rongé la face faisant disparaitre les yeux et la peau - pénétré dans les sinus frontaux - dévoré le cerveau - le cou et la partie supérieure des quatres membres et les viscères étaient très endommagés - le cadavre était entouré d'un linge où circulaient des larves en fin de croissance. On trouva aussi une femelle morte de *Calliphora vicina* R.-D. et une pupe récente de cette espèce mais en outre des pupes récentes de Phorides.

Confronté avec ces larves, nous avons constaté qu'elles étaient tout à fait identiques à celles d'un élevage expérimental que nous avions dans le Laboratoire de Biochimie de la Faculté de Médecine et qui plus est dans des conditions thermiques comparables à celles de l'endroit de la découverte du cadavre.

DISCUSSION :

1. Les larves correspondaient inconstestablement à la première escouade et à la première génération puisqu'il n'y avait aucune pupe vide.
2. Les pontes des oeufs ayant eu lieu dans le laboratoire au cours de la dernière semaine d'avril 1947, nous avons déclaré au Professeur de Médecine légale que le corps de l'enfant fut déposé à cette même période à l'endroit de la découverte, soit peu après le meurtre...

L'enquête criminelle a confirmé les conclusions de cette expertise rationnelle qui a permis de disculper deux des trois personnes qui étaient suspectées.

C'est cette première affaire qui a orienté mon "Hobby entomologique"

- Vers la faune des cadavres et l'application de l'Entomologie à la Médecine légale créée par Mégnin, Brouardel...
- Et notre association permanente avec les médecin-légistes de l'Institut médico-légale de l'Université de Liège...

Les progrès des connaissances sur la Systématique et la Biologie des espèces, ainsi que les résultats de nos expertises entomologiques nous ont amené à revoir le tableau de la sucession des 8 escouades d'insectes et d'acariens établit par Mégnin qui ne correspond plus à la réalité, par exemples :

- Mis dans la sixième escouade après 6 - 12 mois, les acariens se succèdent beaucoup plus tôt en plusieurs escouades spécifiques : probablement 4 : franchement aquatiques & semi-aquatiques & peu hydrophiles & milieu en voie de dessication ou desséché selon l'état de décomposition du cadavre... (Leclercq, M., Verstraeten, Ch., 1988, 1993).
- Mis dans la cinquième escouade après 4 - 8 mois, les Phorides se trouvent beaucoup plus tôt après 3 semaines et très tard jusqu'à plusieurs années après le décès (Leclercq, M., 1999)...

Il est donc préférable d'établir la chronologie de l'arrivée et de la succession des espèces suivant les 4 groupes écologiques :

- Nécrophages (attirés les premiers par le cadavre même à distance).
- Nécrophiles (prédateurs ou parasites des nécrophages).
- Omnivores.
- Opportunistes.

Ensuite, il faut préciser les périodes d'activité et d'inactivité, non seulement des espèces récoltées sur place mais aussi de celles qui sont absentes et auraient pu arriver logiquement sur le cadavre dans cette éventualité, on doit discuter toutes les hypothèses possibles qui rendraient compte de leur absence pouvant être due à l'action des coupables - on en arrive ainsi à admettre que l'endroit de la découverte du cadavre n'est pas celui de la mort de la victime...

Dans une enquête compliquée où nous n'avions qu'un seul Coléoptère Staphylinide : *Omalium rivulare* venant d'éclore comme indicateur, à la première expertise entomologique, Mr le Juge d'Instruction nous a demandé une deuxième expertise pour avoir le plus de probabilité ou de certitude sur l'absence des premiers Calliphorides nécrophages : réexaminer les pièces anatomiques du cadavre et autres prélèvements mis sous scellés.

Le résultat de cette nouvelle investigation fut très intéressant :

- Aucun représentant des premières mouches nécrophages malgré les conditions favorables allant du 18 août 1985 (disparition de la victime) au 15 octobte 1985.
- Les causes éventuelles pouvant justifier leur absence sur les restes du cadavre à l'endroit de la découverte (arthropodes nécrophiles, oiseaux et petits mammifères insectivores...) ayant été éliminées, on a pu conclure que la victime n'était pas décédée à l'endroit de la découverte...
- Dans le scellé contenant un fémur, un radius et de la terre, on trouva : des pupes vides, quelques unes non écloses, une larve au stade prépupal de diptères Piophilides. En outre, une larve d'*Omalium rivulare* se trouvait dans une pupe de Piophilide.
- En fonction des données météorologiques, nous avons présumé l'arrivée des Piophilides sur le cadavre pendant la première décade de septembre 1985...
- La date de la ponte des oeufs d'*Omalium rivulare* devait ainsi correspondre au début de septembre 1985 et la croissance des larves a duré tout ce mois la nymphose s'est bloquée par le froid à partir du 21 octobre 1985 et pendant la longue période hivernale de 139 jours (ce qui est rare chez nous) jusqu'au 21 avril 1986 et l'éclosion de l'unique *Omalium rivulare* a eu lieu le 29 avril 1986 (découverte des restes du cadavre).

Cette deuxième expertise entomologique demandée par le Juge d'Instruction a permis de :

- Démontrer l'utilité de revoir les pièces anatomiques un certain temps après la première autopsie puisque des insectes inapparents à ce moment peuvent se manifester ultérieurement.
- Retrouver les traces des Piophilides.

Ce travail d'équipe a permis :

- D'identifier la victime.
- Dater la mort dès la 18 août 1985 ou 1 à 2 jours après la disparition.
- Fixer l'endroit du crime qui ne correspond pas à celui de la découverte des ossements (29 avril 1986) et conclure à la manipulation et au transport du cadavre.

L'enquête judiciaire a suivi son court et les accusés, reconnus coupables, ont été condamnés à la plus lourde peine, à deux reprises en Cours d'Assises (Leclercq, M., Dodinval, P. Piette, P., Verstraeten, Ch, 1991).

Pour conclure, nous pouvons dire que chaque expertise entomologique est spécifique et pour être efficace, plusieurs conditions sont primordiales :

- Echantillonnage complet des insectes, acariens, à tous les stades de développement.
- Identification des espèces, informations sur leur biologie...
- Bilan de leur succession suivant les groupes écologiques, souvent, les nécrophages sont les meilleurs indicateurs, mais il ne faut pas exclure les nécrophiles comme nous l'avons démontré avec *Omalium rivulare* et au Royaume-Uni, Easton (1944) s'est fondé sur fondé sur *Lathrimeum atrocephalum*, autre coléoptère staphylinide, dans un problème médico-légal.
- Etude des conditions météorologiques et établissement d'un graphique des températures journalières (maximale, minimale, moyenne) pendant la période concernée et dans certains cas, il faut comparer les températures enregistrées à l'observatoire météorologique le plus proche avec celles de l'endroit de la découverte du cadavre.
- Participation du médecin légiste qui précise les modifications postmortem... et des enquêteurs qui peuvent donner des informations utiles...

Soulignons que les insectes nécrophages sont dotés :

- D'un système sensoriel hautement adapté à la détection d'un cadavre.
- D'un puissant dynamisme écologique pour aller vers leurs ressources alimentaires et la ponte des oeufs.
- Réagissant de façon assez spécifique aux températures, ils arrivent les premiers sur un cadavre, devenant ainsi des témoins stables et précieux dans le sens large... Ils n'ont pas fini d'étonner et d'être correctement exploités.

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**A short introduction to the Beetles, with emphasis on the species of forensic interest (Insecta : Coleoptera)**

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Lady-beetles, the sacred scarab of the ancient Egypt are Coleoptera, but there are so many more !

The name Coleoptera derives from the greek "koleos" ,"case" and "pteron", wings, meaning that the anterior pair of wings is transformed into elytra that cover and protect the fragile membranous folded flight wings. This is one of the major features of the beetles that give them considerable advantages compared to other insects, and most probably one of the reasons of their biological success.

Beetles are holometabolous insects that represent the most important group of living animals in terms of number of species, no only among insects but also among all other animals.
Almost 350 000 species of Coleoptera have been described compared to the just above one million of animals species recorded to day. This means that one animal species over four is a beetle.

Beetles live in every kind of habitats, except at the poles and at sea. They show an incredible diversity in size and colour and an outstanding number of morphological and anatomical adaptations related to their life though the general basic morphological groundplan remains the same.
Their diet covers a wide range from strict herbivory to predation mostly on insects, other arthropods and invertebrates. Some beetle which are of interest to us like the *Nicrophorus*, and species of hide and larder beetles, are scavengers and feed on dead matters, including corpses.

The conference will present the general features of the group as well as on the species of forensic interest. It will draw attention on
very particular characters : the antennal sensillae which are " the nose" of these insects and allow them to find decaying bodies, sometimes miles away. Original scanning electron microscope specially made for this seminar will be presented.

ABOUT BEETLES :

General : Crowson, R., 1981. The biology of the Coleoptera. Academic Press, 802 p. ISBN 0-12-196050-1.

Specialised : Beetles, p. 61 in : Byrd J.H. & Castner J.L., 2001. Forensic Entomology. The utility of Arthropods in legal investigations. CRC Press, 418 pp. ISBN : 0-8493-8120-7.

# The value of necrophagous species of fly (Diptera) in forensic entomology

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ABSTRACT

Necrophagous flies, in particular blowflies (family Calliphoridae), represent the most commonly studied insects in relation to forensic entomology because: (1) their larvae (maggots) are the insects that are most commonly associated with corpses; (2) they colonise the body most rapidly after death and in greater numbers than most other insect groups; (3) they usually provide the most accurate information regarding time of death, a major objective of forensic entomology. Blowfly involvement in forensic entomology is simply a reflection of their natural role as decomposers, a human body being at least as attractive as that of a dead animal. Other relationships of blowflies with humans include wound causing (myiasis) and the opposite, wound healing (maggot therapy).

In Europe, the most important forensic species is probably the urban bluebottle blowfly, *Calliphora vicina*, but many other blowflies can be involved at the death scene, mainly in the genera *Calliphora, Lucilia, Phormia, Protophormia* and *Chrysomya*. The species involved depends on the geographical location of the body and its specific location in respect to the immediate surroundings, e.g., indoors/outdoors, woodland/pasture, sunshine/shade and so forth. Hence, the species on a body can indicate something about the place of death and possible subsequent movements of the body.

Adult blowflies are attracted in large numbers by the odours of decay, often within a few hours of death. The attractive odours are mainly due to bacterial action on dead tissues. Odour location is very precise in blowflies, enabling them to locate bodies even in apparently hidden locations.

Another important family of flies that arrive soon after death is the Sarcophagidae, the fleshflies. Many other families can be involved. On buried bodies, species in the families Muscidae and Phoridae can be dominant, because these flies are not deterred by a covering of soil, which generally inhibits egg laying by blowflies.

The forensic value of flies is mainly in determining when flies first gained access to a body, which can provide an estimate of the minimum post-mortem interval. Because what is being determined is the time when flies gained access to a body, this may indicate timings other than death, e.g., the time when a body wrapped in plastic bin liners was dumped, so tearing the liners and enabling entry of flies that were previously excluded.

**Keywords :** Diptera, Calliphoridae, post-mortem interval

## INTRODUCTION

Along with other groups of insects, many fly species (Diptera) are saprophages, feeding as adults and, particularly, as larvae on decaying organic matter. Some saprophages are preferentially necrophagous, feeding on dead animals, especially vertebrates. Their carrion feeding behaviour has an important role in the environment, markedly accelerating the rate of decomposition of corpses and, ultimately, recycling the nutrients. Some of the genera of carrion feeding flies include species that have evolved to stay ahead of the natural competition and, rather than wait for an animal to die, they will lay their eggs or larvae on the dead or diseased tissues of still living animals. This infestation of living animals is called myiasis (Hall and Wall, 1995). In its most advanced forms, myiasis is caused by species that can only develop on living tissues and these can be serious parasites of humans and of domestic and wild animals. However, species that feed preferentially on dead tissues can, with care, be utilised in modern medicine for their wound healing properties (Sherman *et al*., 2000).

Although many insects are attracted to carrion, either to feed or to deposit eggs or larvae, the most important group in forensic terms are the blowflies (Diptera: Calliphoridae) because :

- Their larvae (maggots) are the insects that are most commonly associated with corpses.
- They colonise the body most rapidly after death and in greater numbers than most other insect groups.
- They usually provide the most accurate information regarding time since death, a major objective of forensic entomology.

All necrophagous Diptera have a similar lifecycle, which will be considered here by reference to blowflies. Blowflies have a life-cycle that begins with an egg stage, laid by gravid females. Very occasionally, live larvae may be laid, when one or more eggs become fertilised some time before egg-laying, at which time fertilisation normally occurs. Eggs hatch into larvae, which have three distinct growth stages, called instars (Figure 1). In between each instar a moult occurs in which the cuticle (skin) of the last larval instar is shed, enabling the larvae to grow rapidly and greatly in size. When the larvae have finished feeding, they leave the food source and search for somewhere to undergo pupariation. This may be near to or distant from the body, depending on the species and the environment in which the body is found. If outside, larvae frequently burrow a few centimetres into the soil or crawl under a log or rock. If indoors, larvae frequently crawl under the edges of carpets or furniture. They may also pupariate within the victims clothing. At the site for pupariation, the skin of the third instar larva hardens and darkens, forming a protective, barrel-shaped puparium around the larval tissues, which metamorphose inside into a pupa.

When development is completed, the adult emerges from the puparium, breaking out from the anterior end by using an eversible sac-like extension of the head, the ptilinum, as a battering ram. The ptilinum also helps adults emerging from buried puparia to force their way through soil to the surface. As the newly emerged adult extends and hardens its wings, the ptilinum is withdrawn into the head and becomes no longer eversible.

## OBJECTIVES AND OUTPUTS OF FORENSIC ENTOMOLOGY

As indicated above, a major objective of forensic entomology is to provide an indication of the time since death, the post-mortem interval. In reality, the most that forensic entomology can provide is the time that flies first found a body, which gives a minimum time since death. Depending on the particular case, the interval between when the victim died and when flies first gained access to the body, and so were able to lay their eggs or larvae, can be more or less difficult to estimate and it is an area deserving of much more intensive research.

Often it is not possible to provide an estimate of the post-mortem interval by an analysis of the ages of developing stages that were deposited on the body soon after death. This is usually when some weeks or months, possibly even years, have elapsed since death, and the first insects on the body have long since left. In such cases an estimate of time since death can be determined by reference to the community of necrophagous insects, including flies, that has developed on the body. The French entomologist, Mégnin was the first to develop the concept of insect succession on a body (Mégnin, 1894). He observed that as a body decomposes in a series of relatively well defined stages, so there is a series of relatively well defined assemblages of insect fauna that arrive to feed or breed on the body. By determining the faunal assemblage on a corpse it is, therefore, possible to estimate the period that has elapsed since death. Like other groups of insects, flies show a succession. The sequence of fly arrivals at a dead body follows this general order, although it cannot be considered inflexible:-

Blowflies (Calliphoridae) usually arrive first, followed closely by,
Muscidae and then by,
Fleshflies (Sarcophagidae), then by,
Piophilidae, Fanniidae, Drosophilidae, Sepsidae, Sphaeroceridae, Syrphidae and Ephydridae, then by,
Phoridae and Thyreophoridae.

Flies tend not to be attracted to an old and dried body, which is more attractive to various beetles (Coleoptera) and moths (Lepidoptera).

As well as time or season of death, other information that can be obtained from a study of necrophagous flies includes the manner of death, the geographical place of death and the means of death.

## IDENTIFICATION OF NECROPHAGOUS FLIES

Before any interpretation of insect evidence can be made, the vital first step is to identify the species involved. As with the number-plate of a car enabling access to a country's vehicle registration database, revealing a car owners' name, address and so forth, the name of a fly enables access to all of the recorded biological information on that species. There is still a great need for taxonomic study of insect fauna of forensic value, at both a morphological and molecular level. Despite what is already known about the taxonomy of flies of forensic value, further morphological studies are needed to refine identifications, especially of the immature stages (Smith, 1986, 1989). The greatest value of molecular studies might lie in being able to use DNA to identify geographical populations of flies (e.g. Hall *et al*., 2001) and, therefore, enable a taxonomist to comment on post-death movements of a body.

The most frequently encountered fly species at suspicious deaths of humans in Britain, and probably the rest of Northern Europe, is *Calliphora vicina*, the common urban bluebottle fly (Figure 2). In an analysis of 30 cases of suspicious death investigated at the Natural History Museum, London, specimens of species in the genus *Calliphora* were identified in 93% of them (28/30) and were the only species collected in 60% of them (18/30). Of the two common *Calliphora* species in England, there were six times as many cases involving *C. vicina* as involving *C. vomitoria*. Species in other genera or families were encountered much less frequently, e.g., *Lucilia* in 20%, *Muscina* and *Fannia* in 13%, Sphaeroceridae in 10%, Sarcophagidae, Phoridae, Psychodidae, Heleomyzidae and Scatopsidae in 7%, and Anthomyiidae, Syrphidae, Anisopodidae, Tipulidae and Piophilidae in 3% of cases. The groups of fly less frequently encountered were those that tend to be attracted to bodies in later stages of decomposition.

## BLOWFLIES AS INDICATORS AT A CRIME SCENE

### Location of bodies by blowflies

The high proportion of cases involving only *Calliphora*, indicated above, reflects the rapidity and accuracy with which these flies will locate dead bodies, often within only minutes after death. Their carrion location strategy is very similar to the manner by which fly agents of traumatic myiasis will locate a host (Hall, 1995). This involves activation, followed by upwind flight along the odour plume (orientation), followed by landing.

The attractive odours are mainly due to bacterial action on dead tissues and include hydrogen sulphide, ammonia and organic sulphur containing compounds, including methyl mercaptan, dimethyl disulphide and dimethyl trisulphide (Gill, 1982). The accuracy of blowfly location of an odour source is well illustrated by the accuracy with which New World screwworm flies, *Cochliomyia hominivorax*, will locate odour sources (Figure 3). In that case, the odour sources were two small bottles delivering a synthetic attractant, swormlure-4 (Mackley and Brown, 1984). Thus the major stimulus to the adult flies was an olfactory rather than a visual one. This demonstrates how blowflies can locate even well hidden sources of odour, such as a body under floorboards from which odours are dispersing to the outside through airbricks.

*Calliphora* will arrive in large numbers and reduce the biomass of a corpse very rapidly. Therefore, there is soon little material remaining for other carrion insects that arrive later to feed on. The rapidity with which *Calliphora* arrive at a body is affected by many factors, including the degree of exposure of the body. If it is buried, shrouded in clothing or plastic bags, concealed indoors or otherwise inaccessible, then fly arrival will be delayed and access may be prevented. Because what is being determined in aging eggs, larvae or puparia on the body is the time when flies first gained access to a body, this may indicate timings other than death, e.g. :

- The time when a body wrapped in plastic bin liners was dumped, so tearing the liners and enabling entry of flies that were previously excluded.
- The time when a buried body was exposed, for example, by animal activity.

A body that is protected in some way from fly attack will retain its attraction to blowflies for longer than one that is accessible to flies from the moment of death. While blowflies might arrive at a scene of a suspicious death if they can detect the odour of decomposition, if they are unable to gain access to the body they will not usually lay eggs off the body.

Because blowflies will arrive at a body relatively quickly after death, their absence on a body can indicate something about the history of the body after death, e.g., their absence on a buried body can suggest that the body was buried relatively soon after death.

## SITES OF INFESTATION ON A BODY

When blowflies arrive and start to lay their eggs, the usual sites of infestation are the body orifices, such as the head orifices, the genitalia and the anus. These sites provide a more humid environment, with softer epithelial tissues than the skin surface.

The eggs and larvae are also somewhat protected from the attentions of predators or parasites, because they can crawl into the body for feeding and development. If maggots are found on a body at an early stage of decomposition in areas away from the body orifices, e.g., in a chest wound, this can be indicative of the fact that the person died as a result of some traumatic wounding to that region (e.g. by knife or gunshot), the wounds being attractive to the egg-laying female flies.

## AGING TECHNIQUES

Most techniques for aging maggots and other developmental stages require a detailed knowledge of the rate of development of the species at a range of temperatures together with as complete a picture as is possible of the temperatures to which the flies were exposed during their development on the corpse. The finest models for estimation of age as a function of temperature break-down if the temperature at the site of collection of larvae cannot be determined with accuracy. The most complete information on fly development is available for blowflies, reflecting their value as indicators of time since death. The available information, and descriptions of the techniques for handling it, have been summarised recently by Byrd and Castner (2001). Earlier information was considered by Smith (1985). The aging techniques will be dealt with by other speakers at this seminar and so will not be discussed further here.

## FACTORS AFFECTING RATES OF BLOWFLY DEVELOPMENT AND DIVERSITY OF FLY FAUNA

A range of factors can influence rate of fly development, and hence the interpretation of insect evidence, including temperature, season, maggot mass (larval density), toxins on the body and geographical location, including precise habitat at the location.

The geographical location of the site of discovery of a body can have a major effect on the diversity of insects found on it. For example, the composition of carrion fauna in the UK differs to that of Southern Europe, where *Chrysomya albiceps*, which is not found in the UK, can have a major impact. However, the composition of fauna can vary over a much shorter range of just a few metres, for example, when comparing the fauna of carrion placed in a hedgerow to that placed in the nearby open pasture or in woodland in the UK (Smith & Wall, 1997). Similar marked differences in diversity were found between hedgerow and pasture in Hungary (Table 1). The fly fauna on a body can indicate where the body was exposed to fly activity, especially if there is a suspicion that the body has been moved after death.

The development rate of larvae can also be different in different locations, mainly due to differences in temperature. For example, Shean *et al*. (1993) observed that development of larvae was more rapid on pig carcasses exposed to sunlight than on carcasses in the shade of trees and that, therefore, the cooler carcasses in shade took longer to decompose.

## FLIES ON BURIED BODIES

As mentioned above, burial can affect the colonisation of bodies by blowflies. And this burial need not be deep, even a shallow layer of soil of about 2.5 cm can be sufficient to deter oviposition by blowflies. This protective effect of soil does depend on the degree of compaction and particle size. Adult flies will penetrate loosely compacted soil and soil with deep cracks much more readily than well-compacted soil without cracks.

However, even if a body is buried under soil of sufficient depth and texture to prevent access by blowflies, other groups of fly are adept at finding and utilising buried bodies as a breeding media. Muscid flies, in the genus *Muscina*, are such a group, the females depositing eggs on the soil surface, with the emergent larvae burrowing down to find the body. Another group found on buried remains are flies in the family Phoridae, especially *Concicera tibialis*, the “coffin-fly”. In this case, it is the adults that burrow down and seek out the corpse rather than the larvae.

## CONCLUSION

There is a tremendous amount of information of forensic value that can be extracted from a study of the necrophagous flies found in cases of suspicious death. It may not be that fly evidence alone can categorically identify a particular individual as responsible for the death in a murder investigation. However, the estimation of a minimum time since death can help to direct a forensic investigation in the right geographical and temporal direction and thereby save a considerable amount of time and resources, financial, manpower and equipment. Fly evidence can also be viewed as a vital piece that adds to and completes the jigsaw puzzle of evidence against a suspect.

The considerable variation in each case of suspicious death necessitates considerable caution in the interpretation of fly evidence. Nevertheless, with increased research into important aspects of the taxonomy, physiology and ecology of flies of forensic importance, the value of forensic information from necrophagous flies is set to grow considerably in the next decade.

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**Table 1:** Total numbers of blowflies and fleshflies captured on adhesive sheets placed for eight 24-hour trapping sessions over a single dead rat. The rats were exposed in plastic trays protected by a chicken wire cover over which the adhesive sheet was placed. Rats were exposed either in a hedgerow or approximately 50 metres into a sheep pasture at Nagyegyháza, Hungary (Hall, Farkas and Wyatt, unpublished).

| SPECIES | RAT IN HEDGEROW | RAT IN PASTURE |
|---|---|---|
| ***Lucilia caesar*** | **8,447** | **1,306** |
| ***Lucilia sericata*** | **0** | **709** |
| ***Calliphora vicina*** | **20** | **33** |
| ***Calliphora vomitoria*** | **20** | **0** |
| ***Phormia regina*** | **14** | **240** |
| ***Protophormia terraenovae*** | **7** | **249** |
| ***Chrysomya albiceps*** | **0** | **66** |
| **Sarcophagidae** | **79** | **449** |

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**8 squadrons for one target : the fauna of cadaver described by P. Mégnin**

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ABSTRACT

When somebody dies, his body temperature falls to reach ambient temperature. *Livor mortis* and *rigor mortis* appear : the decay of the cadaver begins. At this moment no smell is yet perceptible (by a human being) but an army of insects has located its target.
During this natural process of decomposition, different species of insects, and more generally arthropods, come in succession in a chronological order on the cadaver.
Cadaver represents an ecosystem on which such organisms can either eat, mate, breed their progeny, find protection or even hunt. Indeed fauna cadaver is not especially necrophagous (using cadaver as nutritive substrate) but its presence is not a stroke of fortune.
How does the fauna cadaver locate its target ? They basically use their antennas as wonderful smell receptors that help them catch the olfactory *stimuli*. In their brain there is a sophisticated integration system that helps them stay in the most concentrated fluid zone to reach the origin of the odour : the corpse.
In the XIX$^{th}$ century, a French Army Veterinarian P. Mégnin observed on horses carcasses that the arrival of this little army is not anarchical. He reported in his book *La faune des cadavres* (1894) that eight waves of arthropods come in succession in chronological order on a corpse during the decay, from recent death up to skeleton stage. Each of them is attracted by a particular smell coming from the corpse that matches with a particular stage of the decay. The theory of eight waves was born.

**Keywords** : Fauna cadaver, eight waves, chronological succession

## INTRODUCTION

At the very beginning of the decay, just a few moments after the death, no odour is perceptible by a human being, but arthropods do smell it and locate their target in the minutes following the death, in optimal environmental conditions of humidity, temperature, sun exposure, and corpse localisation ( not buried, ...).

As the others, the first wave is composed of different species of insects. But what does a colonisation of a necrophagous army consist in ? When the corpse is reached, adults (essentially flies) fly above it. Gravid females lay their eggs especially in natural orifices and injuries. Adults suck liquids on the corpse whereas hatched maggots penetrate into the body to eat different tissues (muscle, liver,...) thanks to their mouthhooks. The maggots grow during three larval stages and, at prepupal stage, they leave the corpse and go underground to metamorphose in adults (pupal stage).

During this time, the decay continues, smell of the cadaver appears. Then little by little adults belonging to the first wave leave the corpse to be replaced by insects of the second wave. The same phenomenon is repeated for the other waves until the skeleton stage. The following list is not exhaustive but gathers the most common species.

## THE FIRST WAVE

Just a few minutes after the death and even when somebody is dying, flies are observed around the corpse. The first blow flies that lay eggs in the natural orifice and injuries are Diptera, Calliphoridae and Muscidae. In fact during the first two waves exclusively flesh flies are observed.

Calliphoridae family is represented by the genus *Calliphora*, namely big flies of a length of more than one centimetre long, with shining metallic exoskeleton. Physiologically active beyond 13°C, each female lays from 100 to 250 eggs.

Larvae are white, long and cone shaped, without fleshy protuberance or hairs except little tips in each segment to help in the locomotion. Their two mouthhooks help them for the locomotion and also to eat tissues they liquefy with bacteria and enzymes.

The species often observed at this stage are *Calliphora vomitoria* (Linné) and *Calliphora vicina* Robineau-Desvoidy.

*Calliphora vomitoria* is a fly with a hairy and black face, red at the third posterior part, black antennae with a blue black body. *Calliphora vicina*. has yellow antennae, black hairs on a clearly face and a blue black body.

Other Calliphoridae as *Protophormia* and *Phormia* are also observed. The first one, *Protophormia terraenovae* (Robineau-Desvoidy) is bigger (more than one centimetre). The other, *Phormia regina* (Meigen), is less than one centimetre long with a blue green metallic body. Their maggots have the particularity of becoming at the third larval stage predators of other species. The Calliphoridae family is also represented by species belonging to the genus *Musca* and *Muscina*. These flies are grey and smaller than *Calliphora*. Maggots are white, long and cone shaped with two fleshy horns. Maggots of the second genus become, at the third larval stage, predators of other species.

Females of *Muscina* lay between 150 to 200 eggs whereas *Musca* lay between 150 to 600 eggs**.**
The most common species of *Musca* is *Musca domestica* Linné**.** This fly is currently encountered in our homes on the windows.

## THE SECOND WAVE

When the cadaver smell is perceptible by a human being, appears another group, composed by the genus *Lucilia*, *Chrysomya*, *Cynomya* (Calliphoridae) and *Sarcophaga* (Sarcophagidae). These odours are less attractive for the species of the first wave that leave the corpse.
The flies belonging to genus *Lucilia* are always found in enlightened areas . They have a green metallic body and short abdomen. Females lay hundreds of eggs. Maggots are quite similar to those of *Calliphora*, but smaller. The most common species are *Lucilia caesar* (Linné), *Lucilia sericata* (Meigen).
*Chrysomya* are quite similar to *Lucilia* with typical black stripes on the abdomen and a white face. Maggots have a white body with particular fleshy protuberances all around. They are predators of other species.
*Cynomya* are often observed on big vertebrate cadavers. They are beautiful flies with a green and purple metallic body.

*Sarcophaga* are big flies with a long body. Their thorax has longitudinal black stripes and the abdomen has a draughtboard aspect. Females are viviparous: they lay directly grey and cone-shaped larvae. One of the most encountered species is *Sarcophaga argyrostoma* (Robineau-Desvoidy).

## THE THIRD WAVE

At this stage of decay, corresponding to fats impairment that emanate a particular smell, the first species of Coleoptera (genus *Dermestes*) and Lepidoptera (genus *Aglossa*) appear.

*Dermestes* are medium to small sized insects (6-7 mm) with an ovoid-shaped body greyish to yellowish. Adults and larvae eat meat. The body of the larvae has three pairs of legs and is covered by long hairs. They can have a cannibalistic behaviour when the food is missing. The most common species encountered are *Dermestes lardarius* Linné, *Dermestes frischii* Kugelann and *Dermestes undulatus* Brahms.

Genus *Aglossa* belongs to the family of the Pyralidae. They are little butterflies (2-3 cm) with yellow greyish wings that fly around the lights in the twilight. Caterpillars of *Aglossa* have a white cylindrical body and thanks to particular respiratory system, can live in fat. They have three pairs of anterior legs, and five posterior prolegs with a range of little hooks. *Aglossa pinguinalis* Linné is one of the species that is found near a cadaver.

## THE FOURTH WAVE

Caseic fermentation follows the fat fermentation, which emanates a particular smell, that attracts flies such as Piophilidae, Fanniidae, Anthomyidae and Coleoptera such as Cleridae (genus *Necrobia* and *Corynetes*).
Piophilidae (genus *Piophila*) are known to be found on old cheese. They are little black and shining flies. Their maggots are white and smooth with a particular hook at the extremity that enable them to jump in order to escape from predators like maggots of other species.
Fanniidae and Muscidae such as genus *Fannia* and *Hydrotaea* are grey to black flies with a common aspect. They have a rapid flight under the lights. Maggots are grey, flattened shaped with fleshy process that help them to float on the putrid liquid.

*Corynetes* and *Necrobia* are little Coleoptera with metallic coloured aspect (blue), having the same habits such as *Fannia* and *Piophila*. Maggots are predators of other species such as insects of the genus *Dermestes*. Their corpse is long (4-10 mm), dark with long hairs. Adults of *Corynetes* have little antennae with a club at the extremity. One of the most common species is *Corynetes rufipes* (Sturm).

## THE FIFTH WAVE

The next step of decay reached is characterised by the ammoniacal fermentation. At this moment there is a blackish liquefaction of tissues not consumed by previous waves. Most of the Diptera found at this stage of decay are not directly attracted by the corpse but above all by the fauna they hunt. The biggest are *Ophyra* (genus of Muscidae). There are also Phoridae and genus *Triphebla*, and seldom Thyreophoridae (not found since 1900 in Europe).
*Ophyra* are medium size flies (4-8 cm), black and shining. When at rest, wings of adults are disposed one under the other and help them penetrate into little interstices. Males have curved posterior tibia with hairs. Their maggots are quite similar to those of Muscidae.

At this stage of decay, most of the Coleoptera are Silphidae (such as genus *Hister*, *Necrophorus*, *Saprinus* and *Silpha*). They are the real "*gravediggers of nature*".
Most of them appear during the night and they dig the soil to bury their prey. Before leaving the corpse, females lay their eggs.
Insects of the Genus *Necrophorus* have long antennas (10 articles) and black elytrons with yellow stripes. One of them is *Necrophorus humator* Olivier.
*Silpha* adults are big insects (1.5-3 cm), with a black body, also have long antennae (11 articles) and edged elytrons. The larva is flattened and grows under the cadaver. *Silpha obscura* Linné is very common.
*Hister* are black and shorter than the previous genus. Posterior legs have two rows of spines. The larvae have a linear body and have the same habits as *Silpha*, such as *Hister cadaverinus* Hoffmann.
*Saprinus* are often found with *Hister* which shape and habits are quite similar to *Saprinus rotondatus* Kugelann.

## THE SIXTH WAVE

At this period, little arthropods take part in the desiccation of the corpse. They are not insects but dust mites (no eye, 8 legs and less than 1 mm long). Several species exist and are found on a cadaver after one year, even if their presence may be observed a few months after the death. They eat aqueous humour remaining in the different decomposed tissues. All specimen found belong to the Gamasidae family and genus *Uropoda*.

## THE SEVENTH WAVE

This wave appears when the tissues of the corpse are completely dry : the skin is parchment-like, ligaments are hard and similar to resin. The present fauna includes Dermestidae family (Coleoptera), and Micro-Lepidoptera such as genus *Aglossa* and genus *Tineola*.
Genus *Attagenus* belongs to this family of Coleoptera. Adults (2-5 mm) and larvae (with a row of hairs at the caudal extremity) look like small *Dermestes*. *Attagenus pellio* Linné can be observed at this stage of decay of the corpse.
Insects belonging to genus *Anthrenus* are smaller than the previous one and the body of the adults is round-like, such as *Anthrenus museorum* Linné.
*Aglossa caprealis* is a Micro-Lepidopetra (2 mm) found among this fauna. Adults and larvae look like those of *Aglossa pinguinalis* Linné (previously already described).
Other Lepidoptera are found such as species of Genus Tineola, the smaller of this group (about 1.5 mm wingspread). *Tineola biselliela* Hummel is a white insect with a row of red hairs on the head found on dried cadavers.

## THE EIGHTH WAVE

This wave includes only a few species of insects that come to eat remains unwanted by previous waves. One of these species belongs to the genus *Tenebrio* (Coleoptera). They are big insects (15-20 mm), black with small rough spots on the body. Larvae are long and yellow.
Larvae of *Tenebrio molitor* Linné are also found in flavour reserves.
Genus *Ptinus* includes small hairy insects (Coleoptera, 2 mm) with small rough spots on the body and long antennae. Species such as *Ptinus brunneus* are observed on old cadavers (2-3 years).

## CONCLUSION

All of the species described were observed on cadavers in the open air, more than one century ago. This is not an exhaustive list gathering all the insects encountered during the decay of the cadaver.
Geographical location of the corpse, environmental conditions, such as humidity, sun exposure and temperature, play a very important role on the decomposition and also have a great influence in the physiology of such cadaver fauna. A mummified corpse does not attract necrophagous squadrons in the same way as a putrefied one does.
Considering the 400 cases and more treated by the department, we can say that the first two waves seem to be a single one, excepted for the Diptera Sarcophagidae observed later than Calliphoridae : *Calliphora* and *Lucilia*.

The development of communications, commodity and merchandise exchanges, rules dealing with hygiene problems have had a great influence in the way of life of several species of insects. Moreover, species described such as Diptera included in the genus *Thyreophora* are not observed nowadays on big cadavers.

The major interest of this paper is to present the apparition on a cadaver, within hours or minutes of death, of a chronological and regular succession of arthropods, named by Mégnin *"Les travailleurs de la mort"* ("*The death workers* "or "*Gravediggers of nature"*).
This natural phenomenon can be a good help for magistrates and investigators in their inquiries.

## Process of body decay. Estimation of the time since death by the forensic pathologist

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The objectives of this presentation dedicated to forensic entomologists are double :
1 - To give up-to-date information on the process of decomposition of a human cadaver.
2 - To detail the different methods the forensic pathologist has at his disposal to estimate the post mortem delay.

The process of body decomposition is complex. After the phase of cell death with histological and histochemical changes, the body is submitted to an active organic decomposition depending of bacteriae and fungi. It is possible to distinguish three different phases : putrid emphysema, liquefaction of organs and progressive mineralization of organic matter. There are numerous physical, chemical and microbial reactions in the process of decomposition submitted to activating and inhibiting factors.
Of course, the different phases are variable in duration and intensity according to individual, climatic factors and the type of burial.
The chemistry of putrefaction will be explained and the different stages of body modifications leading to a skeleton will be describe using a large iconography.
The estimation of the post mortem delay can be an important question during the inquiries. Besides environmental and anamnestic evidence (conditions of clothing, eating habits, uncollected mail…), there are corporal evidence (rate of drying,
rigor, livor and algor mortis, decomposition) that are strongly influenced by temperature, humidity, location, clothing.
There are a lot of publications in the litterature describing technics to help the forensic pathologist but so far, the specialist can only give an approximation of the post mortem interval using biophysical and biochemical methods coupled to the results of the external examination of the body. The longer the period of time is, the less accurate all these methods are and the role of forensic entomology has to be considered rapidly.
The different methods used by the forensic pathologists and the results offered will be detailed.
Some information regarding the examination and autopsy of decomposed bodies will also be given in this presentation.

# Front-line experts in death investigation: the powerful link between the forensic entomologist and the forensic pathologist

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ABSTRACT

Every death investigation process requires the combined efforts of experts in different disciplines. The forensic pathologist normally has the legal authority to take charge of the dead body at a death scene and his primary functions are the exterior and interior examination of the cadaver by analyzing the extent of ante-mortem injuries and the post-mortem changes and the recovery of physical evidence. He is responsible for determining how, when and why of any death which is the result of violence, suspicious or unexplained circumstances, defending and explaining the reasons for making these diagnoses in a courtroom. In death cases where human remains are colonized by insects, the forensic entomologist may support the pathologist significantly. His principal role is to identify the arthropods associated with such cases and to analyze entomological data for interpreting insect evidence. He is responsible for determining the period of insect activity according to all the variables affecting insect invasion of remains and their development. The techniques devised recently can allow experts in this discipline to collect strong entomological evidence and provide useful information not only in a death investigation (including time, cause and manner of the investigated death, removal of the remains following death, time of dismemberment, post-mortem artifacts) but also at the scene (explaining alteration of bloodstain evidence) and even more in child neglect, sexual molestation and identification of suspects. Based on these experienced, the forensic entomologist should work with the forensic pathologist from the visual observations of the cadaver on the scene, through the collection of arthropods and temperature data at the death scene and at the autopsy, up to the final report with the interpretation of entomological evidence.

**Key words**: forensic entomology, forensic entomologist, forensic pathologist.

## INTRODUCTION

Every death investigation process requires the combined efforts and cooperation of experts in different disciplines: forensic pathologists, anthropologists, entomologists, death investigators, crime scene technicians. Unfortunately, not all these disciplines are always represented since they may become involved during the course of the investigation depending on the procedures adopted by the medicolegal jurisdiction, on the nature of the case and the need for verification of evidence [1].

Although death investigation reporting laws vary greatly around the world, in most countries the forensic pathologist has the legal authority to take charge of the dead body. His primary functions are the exterior and interior examination of the cadaver by analyzing the extent of ante-mortem injuries and post-mortem changes and the recovery of physical evidence [2]. He is responsible for determining manner, time and cause of any death which is the result of violence, suspicious or unexplained circumstances [3], defending and explaining the reasons for making these diagnoses in a courtroom.

When insects are found associated with the body, samples must be collected in conjunction with other biological evidence due to the important role of arthropods in human decay. In such cases the entomologist can provide invaluable aid to the pathologist. During the last three decades the utility of medicocriminal entomology in death investigations has been largely documented by several case studies both in Europe [4-7] and in US [8-10]. The techniques devised recently allow experts in this discipline to collect strong entomological evidence and provide very useful information not only in a death investigation including time since death [4-5, 8-9], season of death, geographical location of death [11], removal of the remains following death [12], time of decapitation and/or dismemberment [13-14], submersion interval [15-16], specific sites of trauma on the body [17-18], post-mortem artifacts [19], alteration of bloodstain evidence [20], use of drugs [21-22], and even more in linking a suspect to the scene of a crime [23], in child neglect [24-25], sexual molestation and identification of suspects [26-27].

Based on the above experiences the principal role of the forensic entomologist can be defined; it is to collect and identify the arthropods associated with such cases and to analyze entomological data for interpreting insect evidence. The forensic entomologist is responsible for determining the period of insect activity based on the composition of arthropod community found on the cadaver (as it relates to expected successional patterns) or on the age of developing immature insects according to all the variables affecting insect invasion of remains and their development [28]. Occasionally, the forensic entomologist can be also called upon as an expert witness to testify in a courtroom for defending and explaining his conclusions [29].

In our opinion, the complex ecology of the decay process and the role which insects play in the breakdown of corpses suggest that the collection and interpretation of entomological evidence associated with dead bodies should be done preferably by both the forensic entomologist and pathologist correlating circumstantial information and findings noted at the crime scene and at the autopsy. Only a multidisciplinary approach and the interaction of these different professionals can provide an high-quality level of death investigation.

## ENTOMOLOGICAL PROCEDURES AT THE DEATH SCENE AND AT THE AUTOPSY

Specific collecting techniques and standard procedures in different environvments at the death scene [1, 30], at the autopsy [31] and in the laboratory [32] have been developed to enhance the entomological evidence. The sequence of the entomological investigation at the death scene can be summarized in five stages [33]: assessment and notation of general scene characteristics; visual observations of insect infestation at the scene and on the body; collection of climatological and temperature data, collection of specimens from the body and from the surrounding before removal of the remains; collection of specimens from directly under and in close proximity to the remains after their removal. Haskell and Williams [30] suggest that at the scene the forensic entomologist should concentrate on collecting from around and under the corpse, since nothing must be moved or taken from the corpse without specific permission of the pathologist, except the insects which are on the surface and clearly visible. The forensic pathologist is usually responsible for moving the body in order to look underneath at the previously covered surface, recovering physical evidence vrom the body, seein that the body is properly transported to a mortuary without the loss of any interference [2]. At the autopsy, the entomologist should collect only those stages of insects which can be seen readily on the body, and use extreme caution when using forceps or other tools for collecting since post-mortem artifacts can be inflicted inadvertently (for instructions dealing with collection of insects from corpses see Lord and Burger [34]).

In our opinion, collection of insect evidence from the body and of temperature data should be done in conjunction with the forensic pathologist at the death scene and at the autopsy. These front-line experts can assist one another in their collection of evidence while performing together the recognition of decay stage and insect activity since they can support one another their own interpretation of findings.

## INSECT ACTIVITY, IRREGULAR DECOMPOSITION AND POST-MORTEM ARTIFACTS

At the recovery site and at the autopsy the main function of the pathologist is to look and observe, making such written notes and taking photographs for depicting the position of the body, the extent of ante-mortem injuries and the stage of decay. He has the responsibility of collecting environmental and body temperature (algor mortis) and other main post-mortem changes as stiffness (rigor mortis), settling of the blood (livor mortis or lividity) and the breaking down of the body's tissues (decomposition) useful for determining the time of death [35].

Between the post-mortem changes insect activity must be carefully noted, in our opinion, preferably by both pathologist and entomologist; in the absence of these experts death investigators must supplied in documenting the post-mortem changes relative to the decedent and the environment as recommended in the National Guidelines for Death Scene Investigation developed by the National Institute of Justice (U.S.A.) in 1999 [36]. Any region of the body where putrefaction is disproportionately advanced or associated with insects should be carefully examined due to the possibility that it could represent a site of ante-mortem injury. Since 1956 Moritz stated that it is a frequent mistake in forensic pathology to pass over such a region lightly, inasmuch as it seems to be particularly unfit for examination [37].

Corpses may be more decomposed in areas of the body where there are injuries which result in the exposure of blood and underlying tissues thus providing a portal entry for carrion insects and bacteria [38]; in fact, flies but also animal scavengers are usually attracted to blood and can produce a quick consumption of soft tissues in that region. Diptera larvae, in particular, can alter or destroy indications of the cause and manner of death or create post-mortem artifacts. For example, a knife or bullet wound can be distorted, enlarged or made unrecognizable by feeding larvae or other insects as ants [18]. Autopsy findings such as extravasation of blood through the underlying tissues at the wound location or penetrating injuries of the internal organs with haemorrhages may confirm the diagnosis of antemortem trauma.

Discriminating post-mortem insect artifacts from ante- or peri-mortem injuries requires a considerable experience from both forensic entomologist and pathologist. They can avoid confusion between shotgun wounds and entry holes in skin made by maggots, between peri-mortem sexual assault and insect clothing displacement [39], between rat droppings and fly puparia often overlooked on the recovery site, between blood stains and pseudo-blood spatters left by insects coming from a body or a large pool of blood [19-20], between "translucent red fibers" and Chironomidae larvae such as a case illustrated by Hawley *et al*. in 1989 [40].

## HEAT GENERATED FROM MAGGOT MASS: AN EMERGENCY IN DEATH INVESTIGATION

Changes in ambient temperature between the crime scene and the autopsy room must be considered by both the forensic pathologist and entomologist. Often human remains are stored from several hours to few days in coolers or refrigeration units prior to autopsy. The forensic entomologist should be aware of the total time the body was cooling and the temperature of cold storage. In cases where the corpse is heavily infested with Diptera larvae, temperature of maggot mass should be recovered as soon as possible before the body could be stored in coolers. The heat generated from larval aggregations has very important forensic implication since it can prevent the cooling effect both outdoors [41] and in refrigeration units.
Heat generated by larvae due to their frenetic activity and fast metabolism essentially depends on two main factors, density of larval mass [42] and size (dimensions and weight) of the carcass [43]. Temperatures of 27-37°C were recorded by Haskell [19, 31, 33] in aggregations of Diptera larvae feeding on corpses held under refrigeration prior to autopsy (1-4°C). In our experience we have frequently observed in coolers the heat produced by maggot mass as steam vapours rising from the body as described by Bass [44] and Diptera larvae actively feeding on. The forensic entomologist and pathologist should aware that these findings mean the maggot mass was well established prior to the body being placed in the cooler and the refrigeration units do not stop the feeding activity of large maggots clustered in dense masses or their development. Hence, since Diptera larvae could destroy the residual part of physical evidence still present on the body, corpses heavily colonized by maggots should be considered a forensic pathology emergency and the autopsy should be performed as soon as possible. These cases can be easily recognized only by a previous examination of insect activity on the body at the death scene and measurement of maggot mass temperatures.

## VISITING THE DEATH SCENE IN THE ABSENCE OF A CADAVER

A death scene properly processed can yield information useful to reconstruct events and circumstances, link a suspect to the victim or scene, establish the credibility of the statements made to investigators by witnesses [1].
In a death investigation it would be extremely useful to determine if carrion-fly larvae have been feeding on human tissue since Diptera larvae can be discovered in the absence of human remains at a location where the body is suspected to have been [45]. The presence of live maggots in the absence of a dead body at a location is almost certain evidence that some kind of corpse has been removed from the scene.

There was a case from southern Italy (Brindisi County) where a police informant identified a cellar in an isolated farmhouse where a murder had occurred and the body had been left. It was later learned that the criminal organization responsible for the murder learned of this “betrayal” and removed the body in an effort to discredit the informant. Police investigators recovered only a cluster of *Chrysomya albiceps* larvae, and no other biological evidence from the cellar. Our research work has demonstrated the possibility of extracting, amplifying and sequencing human mitochondrial DNA from Diptera larvae thus associating maggots and their last source of food [45].

## POSTMORTEM INTERVAL (PMI) ESTIMATION

The assessment of death chronology is always difficult for the forensic pathologist, especially on badly decomposed corpses or skeletonized human remains. Analyzing the successional patterns of arthropods found on the corpse or nearby or simply from their stage of development the forensic entomologist can estimate the period of insect activity only [28]. Based on the stage of decay, period of insect activity and the multitude of factors affecting the rate of decomposition and insect colonization the forensic pathologist should be able to determine a minimal and maximal probable time interval between death and corpse discovery.

On badly decomposed corpses or skeletonized human remains infested with insects, where no classical post-mortem changes in soft tissues (hypostasis, cadaveric rigidity, body cooling) are available, only the entomological evidence can provide invaluable aid to the forensic pathologist for predicting the PMI. In similar cases warm temperatures and the activity of animal predators (mainly flies but also mammalian carnivores such as dogs, rodents, birds) can reasonably explain the faster degradation of a cadaver. We observed a case of pre-skeletonisation as soon as 10 days in a body of a young male found in September (mean temperature fluctuating between 20-26°C), in a rural area of Lecce (southern Italy) close to agricultural fields, covered by stones. The head, the trunk and the arms were largely skeletonized and infested by Diptera larvae. The stones preserved the body from large animal scavengers but not from *Chrysomya albiceps* (Diptera) which were found in dense masses inside body cavities. In this case the post-mortem interval was determined by aging the oldest specimens of *Chrysomya albiceps* (puparia) sampled on the crime scene according to the weather record.

Compared with shallow graves, deep burials or aquatic environments significantly reduce the rate of decomposition resulting primarily from cooler temperatures and inhibition of insect activity [18]. Rodriguez and Bass [46] observed that Diptera could not colonize bodies buried deeper than 30 cm (1 ft) and at greater depths they did not find signs of relevant carrion-insect activity. Based on their experimental studies a complete skeletonization of a buried corpse takes approximately 2 to 3 years.

Like burials the aquatic environments also restrict the access of many carrion insects to the bodies, delaying corpse degradation. In similar cases some aquatic insects can be associated with bodies immersed in water and they can be used to determine submersion intervals [15-16] while terrestrial insects associated with bodies floating can indicate the length of time the body resurfaced [18].
Another challenge for the forensic pathologist is PMI estimation in burnt bodies or bodies completely carbonized. Only in cases where the human remains are infested with larvae the probable time interval between death and corpse discovery can be determined essentially based on the period of insect activity. In Southern Italy criminal organizations use to to cover up the murder or the identity of the victim burning out the bodies inside their vehicles [47]. Based on our experience, adult blowflies and fleshflies are soon attracted in large numbers by the smell of inner viscera widely exposed by fire consumption of the head, thorax and abdomen walls. Depending on the extent of burn injury Diptera can have easy access to the body inner cavities and are very skilfull getting to the uneven internal recesses laying eggs or depositing larvae soon after the fire has gone out and the body has cooled enough [47]. The prompt invasion of Diptera flies on burnt cadavers have been also confirmed by experimental studies on animal carcasses [48].

## USE OF DRUGS: ENTOMOTOXICOLOGY SUPPORTING PATHOLOGIST’S FINAL DIAGNOSIS

The forensic entomologist can support the pathologist also on his diagnosis of narcotic or drug intoxication since insects may serve as reliable alternate specimens for toxicological analyses [21]. A thorough toxicological analysis of post-mortem specimens may be crucial to the proper determination of death. In mummified bodies, skeletonized remains or bodies in advanced decomposition more traditional sources, such as blood, urine or internal organs are usually not available or the recovery and quantitation of drugs can be severely affected by tissue-decomposition products [49-50]. In similar cases the entomological evidence can provide a potentially valuable source of toxicological information and a more suitable specimen with less decomposition interference.
Several studies have described correlations between drug concentrations found in human tissues and in developing Diptera larvae [51-53], in puparial cases and insect fecal material [54]. However, the precipitous decrease in drug concentrations observed by Sadler *et al*. [55] in post-feeding larvae and at pupariation suggests that only larvae actively feeding on a corpse and fully developed should be sampled for toxicological analysis.
The interaction between the forensic pathologist and entomologist is advisable for interpreting the results of toxicological anlysis. The forensic pathologist should be aware that the absence of a drug in feeding larvae does not necessarily imply its absence in the food source [56-57].

Before using maggots for PMI determination the forensic entomologist should be aware of the toxicological results to evaluate the effect of drugs and toxins on the rate of Diptera development [21] or on the delayed invasion of tissues [58].

## CONCLUSION

The appreciation of the full significance of the autopsy findings and entomological data often depends on evidence that may be obtained only at the scene and before the body has been moved. Where insects are found associated with corpses, the forensic entomologist can reasonably support the pathologist's final determination, especially on badly decomposed corpses, burnt or skeletonized human remains. In our opinion the forensic pathologist should work with the forensic entomologist on death investigation cases, from the external examination of the cadaver, through the collection of arthropods and temperature data both at the death scene and at the autopsy, up to the final report with the interpretation of entomological and other physical evidence. Only a strict cooperation between various professionals can get an overall appreciation of the circumstantial information and findings noted at the death scene and at the autopsy and only collecting insect evidence at each step of the investigation can provide the full potential of medicocriminal entomologist and support admissible forensic evaluations [31].

Unfortunately the attendance of both these front-line experts at the scene of the death may vary from place to place; it is usually dependent on the suspects of the police officer or of the prosecutor. Not so many medicolegal jurisdictions are aware of the considerable advantages to having those professionals visiting the body at the scene of recovery or cooperating together at the autopsy [2]. In several cases, the initially suspicious circumstances may be easily dispelled mainly discriminating post-mortem artifacts from ante or peri-mortem injuries according to the characteristics of the environment.

Since entomological evidence is becoming admissible in many countries, it is no longer acceptable that law enforcement officers, death investigators or pathologists could be sceptical or could not appreciate the value of insects as forensic indicators [4, 59-60]. Even if a permanent status in criminal laboratories for forensic entomology was just promoted by Nuorteva [61] in 1988, in Liege (Belgium) at the XIVth Congress of the International Academy of Legal and Social Medicine, actually, to the best of our knowledge, except for several crime labs in US and for the Gendarmerie Nationale in France, there are not so many forensic entomologists permanently on duty in law enforcement agencies.

The reason why the entomologist is still not often included in death investigation from the beginning and he is rarely called upon to visit the crime scene can be due to different reasons [62] but mainly in some jurisdictions having an entomologist as a routine member of the investigation team could not prove practical since he should be available and ready to get the scene or the autopsy room 24 h a day. In the absence of the forensic entomologist, investigators or pathologists could supplied with collection of temperature data and of specimens from the corpse [28]; however, once collected the insect specimens should be brought to experienced forensic entomologists for identification and determination of elapsed time of insect development. The forensic entomologist should have access to information that may be essential in establishing the period of insect activity such as circumstances of body recovery, death environment and temperature data, presumptive cause and manner of death. Only this information can be correctly used to provide supportive elements, along with the pathologist’s report and crime lab evidence, in determining time, cause and manner of death. For the above reason, we strongly encourage the interaction between pathologists and entomologists in death cases where insects are associated with the remains.

As forensic scientists we should encourage local jurisdictions to spend some resources adopting different professionals on death investigations (entomologists familiar with cadavers, investigators or pathologists familiar with insects) and training them to recognize and properly preserve entomological evidence. Seminars and workshops on the applications of medicocriminal entomology should be arranged to assist the personnel working for law-enforcement agencies, including forensic pathologists and entomologists, in the proper collecting and processing of entomological evidence. In 1998 for the first time in Europe an international three-day seminar in forensic entomology was held in Bari (Italy) with Lee Goff as instructor. New efforts in this direction will be welcomed.

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# Les insectes necrophages au service de la justice : Entomologie forensique en suisse-romande

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## RESUME

Science méconnue en Suisse, l'entomologie forensique a été développée depuis 1993 en Suisse romande. Les insectes utilisés dans cette discipline appartiennent principalement aux ordres des Diptères et des Coléoptères. Une présentation des principales familles et leur biologie permet de comprendre l'utilité de ces espèces dans le calcul de l'intervalle post-mortem et leur utilisation potentielle dans les affaires criminelles.

## INTRODUCTION

Agréables ou désagréables, les insectes dominent dans la majorité des écosystèmes terrestres et aquatiques à l'exception des mers et des océans. Si l'on a décrit aujourd'hui plus d'un million et demi d'organismes, plus de la moitié sont des insectes. A titre de comparaison, nous ne connaissons guère plus de 4500 espèces de mammifères à la surface de la terre.
En Suisse, sur les quelque 40'500 espèces (animaux et végétaux) connues, les insectes représentent 30'000 espèces. Si les insectes dominent par leur diversité, ils dominent aussi par leur densité ou biomasse. Personne ne niera cette affirmation lorsque l'on sait qu'une invasion de criquets migrateurs peut comporter plus de 10 milliards d'individus ! D'autres exemples montrent que la biomasse des vertébrés de la forêt tropicale humide représente moins de la moitié de la biomasse des insectes. Plus près de nous, le Jura vaudois et ses pâturages boisés possèdent plus de 2000 fourmis des bois au mètre carré, ce qui dépasse largement le poids des chevreuils lorsqu'on étend cette surface à l'échelle de l'hectare. Si la diversité et la biomasse des insectes sont gigantesques aujourd'hui, il ne faut pas oublier non plus que l'histoire des insectes remonte à plusieurs centaines de millions d'années pour les plus anciens, ce qui n'est guère comparable avec les quelques millions d'années de l'espèce humaine. Survivants des grands cataclysmes terrestres, responsables notamment de la disparition des dinosaures, le succès des insectes est lié à leur exploitation extraordinaire de la majorité des habitats terrestres et à leur facilité d'adaptation au cours de ces temps géologiques. Ce succès est lié pour partie à la diversité de leurs régimes alimentaires et leurs places dans les différents niveaux trophiques des écosystèmes.

On distingue habituellement 5 types généraux d'alimentation, les détritivores ou saprophages, les carnivores, les fongivores, les herbivores et pour finir les mangeurs d'algues et de mousses. Si aujourd'hui les herbivores comprennent plus de la moitié des espèces d'insectes, les autres groupes sont néanmoins très importants, et comme nous le verrons, recèlent des applications tout à fait étonnantes pour l'homme.

## LES INSECTES NECROPHAGES

Il y a deux groupes majeurs d'insectes attirés par les cadavres ; les Diptères et les Coléoptères, mais seul un nombre restreint d'espèces joue un rôle d'éboueur. Cela est suffisant pour « nettoyer » le milieu naturel des carcasses d'animaux.
Ces deux groupes appartiennent aux insectes à métamorphose complète. Leur développement peut se résumer de la manière suivante : pontes d'œufs, éclosion de l'œuf qui donne naissance à une larve, laquelle subira plusieurs mues lui permettant d'augmenter sa taille. Après un nombre déterminé de mues larvaires, l'insecte subit une transformation qui l'amènera au stade de nymphe. Ce stade, le plus souvent immobile, précède la dernière mue appelée mue imaginale qui donnera un imago ou insecte parfait. Ainsi, les larves de mouches appelées asticots ressemblent à de petits vers blancs sans pattes, tandis que les larves de Coléoptères se distinguent notamment par la présence de trois paires de pattes.

Il existe des nymphes nues (Coléoptères) ou des nymphes enfermées dans la dernière enveloppe larvaire que l'on appelle pupe (Diptères). Cette pupe forme un tonnelet qui ne permet pas de distinguer la nymphe.

La durée du développement complet dépend en grande partie de la température. Le nombre de mues larvaires est quant à lui fixé pour chaque espèce.

Les Diptères nécrophages appartiennent à plusieurs familles dont les plus importantes sont les Calliphoridés, les Sarcophagidés, les Muscidés, les Fanniidés, les Piophilidés et Phoridés. Chez les Coléoptères, les principales familles sont les Sylphidés, les Histéridés et les Dermestidés.

### <u>Les Calliphorides</u>

Cette famille de mouches de taille moyenne compte plus de 1000 espèces connues au monde mais seulement 52 en Suisse. Ce sont les mouches qui présentent un intérêt majeur en entomologie forensique permettant une estimation parfois très précise de l'intervalle post-mortem. Les adultes ont une taille comprise entre 6 et 14 mm et la majorité des espèces ont une apparence métallique avec des couleurs allant du vert au bleu ou simplement noir.

Les larves ont une taille allant de 8 à 23 mm, elles sont habituellement de couleur blanche ou crème. Le segment terminal du corps possède 6 petits tubercules. Ce segment contient aussi les spiracles postérieurs, qui permettent à la larve de respirer. Habituellement ce sont les premières mouches arrivées sur place et qui colonisent un cadavre humain. Dans certains cas leur arrivée est une question de minutes. La femelle possède des segments télescopiques au bout de son abdomen qui forment un ovipositeur. Une femelle peut pondre plusieurs centaines d'œufs. *Calliphora vicina* est probablement l'espèce la plus commune dont la distribution est quasi mondiale aujourd'hui. Mouche d'assez grande taille (10 à 14 mm de longueur), elle se reconnaît à ses joues jaunes, à sa couleur métallique bleue et à sa pilosité abondante.

## Les Sarcophagides

Ces mouches, souvent désignées sous le terme de mouches à viande ou mouches à damiers, comprennent quelque 2500 espèces à la surface de notre planète dont 119 en Suisse. Les adultes se rencontrent souvent sur les fleurs où ils viennent se nourrir de nectar. Les larves de ces espèces se nourrissent de toutes sortes de matières animales en décomposition et d'excréments. De nombreuses espèces sont parasites d'invertébrés et de vertébrés. De taille petite à grande (2 à 14 mm), les adultes se reconnaissent au dessin à damier sur l'abdomen, mais ne possèdent jamais de couleur métallique.
Du point de vue systématique, la détermination d'un Sarcophagidé est difficile et les larves doivent être élevées jusqu'au stade adulte pour être identifiées. Parmi les particularités de cette famille notons que les femelles ne pondent pas d'œufs, mais donnent naissance à des larves. Elles arrivent souvent juste après les premières Calliphoridés.

## Les Muscides

Cette famille comprend plus de 200 espèces en Suisse et environ 550 en Europe. De nombreuses espèces sont ubiquistes. Il suffit simplement de penser à la mouche domestique qui voyage depuis plusieurs siècles avec l'homme. Selon les espèces, les larves de Muscidés se rencontrent dans des milieux les plus variés: substances animales et végétales en décomposition, champignons, litière, etc. Elles sont saprophages, coprophages ou prédatrices. D'une taille plus modeste, (3-10 mm) les adultes sont relativement discrets du point de vue coloration (gris foncé) et très rarement avec une coloration métallique.

Les Fanniides

Longtemps classées parmi les Muscidés, les Fanniidés sont des mouches de taille petite à moyenne (3-9 mm). Elles se rencontrent principalement dans l'hémisphère Nord. Elles fréquentent de préférence les régions boisées et sont plus rares en milieu ouvert. Quelques espèces seulement sont nécrophages.

Les Piophilides

Les Piophilides sont des petites mouches de couleur sombre, mat ou brillantes, (2-6 mm). Les adultes volent près du milieu où se développent leurs larves. On rencontre des espèces floricoles, saprophages ou coprophages et souvent synanthropes. La mouche du fromage, autrefois très commune autour des fromages, reste une nuisance sérieuse dans l'industrie alimentaire. Mais cette espèce se rencontre aussi fréquemment sur des cadavres humains. L'une des caractéristiques de sa larve est de progresser en sautant. Ces larves peuvent produire occasionnellement chez l'homme une myase intestinale. On n'en rencontre que 14 espèces en Suisse.

Les Phorides

Les Phoridés sont de petites mouches brunes, noires ou jaunes ne dépassant pas 8 mm de longueur. Les femelles de nombreuses espèces sont aptères. Les représentants de cette famille se reconnaissent à leur thorax bossu et à leurs pattes très développées, ce qui leur permet une course rapide et saccadée très caractéristique. Elles se nourrissent de substances végétales et animales en décomposition. La femelle de la mouche des cercueils (*Conicera tibialis*) (taille 2 mm) peut traverser plusieurs dizaines de centimètres de sol pour aller pondre sur des cadavres.

Les Sylphides

Cette famille de Coléoptères comprend plus de 1500 espèces distribuées à la surface de la planète. D'une taille moyenne à grande (10-35 mm), ils sont à la fois prédateurs et nécrophages. Ils se reconnaissent par leurs élytres souvent courtes qui ne recouvrent pas complément l'abdomen. Certaines espèces prélèvent de la matière en décomposition, l'enfouissent dans le sol et pondent leurs œufs dans ce substrat.

Les Histerides

Ces insectes ne dépassant que rarement 10 mm de longueur, sont de forme ovoïde, souvent noirs brillants ou parfois vert métalliques. Actifs de nuit, ils se nourrissent souvent d'œufs et de larves de mouches nécrophages. Lorsqu'ils sont dérangés, ils se contractent et cachent leurs appendices en simulant la mort.

Les Dermestides

Les Dermestes sont des Coléoptères de taille moyenne (3.5-10 mm) dont le corps est couvert de poils courts ou d'écailles. Ils se nourrissent de toutes sortes de matières organiques sèches. Certaines espèces se rencontrent parfois dans les musées et sont capables de faire de gros dégâts dans les collections d'insectes. Ces espèces sont utilisées pour nettoyer les os de petits mammifères ou d'oiseaux pour des préparations muséologiques. Leur importance en entomologie forensique n'est pas encore complètement développée, mais leur présence est fréquente.

## L'ENTOMOLOGIE FORENSIQUE

Jusqu'au XVIIème siècle, on croyait que la présence d'asticots dans un cadavre était due à la génération spontanée. C'est en 1668 que Franscisco Redi démontra par une série d'expériences que ces larves provenaient d'œufs déposés par des mouches sur le cadavre. L'une des premières publications en Europe d'entomologie médico-légale remonte au siècle passé (1855) et relate l'autopsie faite par le Dr M. Bergeret d'Arbois (Jura français). Il s'agissait du corps d'un nouveau-né découvert derrière le manteau d'une cheminée. Bergeret trouva lors de la levée de corps des mouches à damier (Sarcophagidés) mortes, ainsi que des chenilles de Lépidoptères . Il estima que la mort devait remonter à 1848, se basant sur le fait que deux générations d'insectes annuelles s'étaient succédées et que les occupants de la maison à cette époque étaient coupables. Compte tenu des connaissances actuelles, l'estimation faite par Bergeret était probablement erronée. Toutefois le véritable premier cas d'entomologie forensique remonte au XIII ème siècle, reporté dans un ouvrage de médecine-légale chinois. Suite au meurtre d'un fermier dans une rizière, meurtre réalisé à l'aide d'une serpe, tous les membres de la communauté furent rassemblés et durent poser leur outil devant eux. Seule une serpe intéressa des mouches, attirées par l'odeur du sang ou des fragments de tissus. Son propriétaire fut ainsi confondu et condamné. L'auteur de cet ouvrage, un certain Sung Tz'u, rapporte aussi avoir vu des quantités de larves sur un cadavre et pense que leur présence pourrait être utile pour une investigation.

Cependant il faudra attendre la fin du XIXème siècle avec les travaux de Mégnin (1894) pour que la première base scientifique de l'utilisation des insectes nécrophages soit publiée. Au cours du XXème siècle de nombreuses contributions apportent des éléments utiles à la médecine légale dans l'utilisation de ces insectes pour donner une estimation du moment de la mort.

C'est en 1986 que K.G.V. Smith du département d'entomologie du British Museum of natural history de Londres publie le premier manuel d'entomologie forensique. Il se base notamment sur les travaux d'autres entomologistes comme M. Leclercq en Belgique, P. Nuorteva en Finlande et M. Marchenko en Russie pour ne citer que les plus importants. Il faudra encore attendre quelques années pour que l'entomologie forensique fasse son entrée dans des Institutions comme le FBI aux USA ou la gendarmerie nationale en France.

C'est en 1993 que l'entomologie forensique a débuté à la police criminelle du canton de Vaud sous la responsabilité de Claude Wyss, en collaboration avec les Instituts universitaires d'écologie et de médecine légale de l'Université de Lausanne et le Musée cantonal de zoologie.

Dès que la mort survient, un cadavre attire très rapidement un certain nombre de Diptères (Calliphoridés) qui viennent pondre leurs œufs. La ponte intervient dans les premières minutes ou heures qui suivent la mort. Les pontes sont déposées dans ou à proximité immédiate des orifices naturels. A partir de cet instant, le développement de l'œuf sera dépendant de la température ambiante. Dès lors, connaissant le temps de développement de l'œuf jusqu'à l'insecte parfait, à différentes températures, nous sommes en mesure de calculer précisément le jour, voire l'heure à laquelle les mouches ont pondu. Compte tenu de ce qui précède, on définit l'intervalle post-mortem (IPM) comme étant l'intervalle entre le moment de la mort et la découverte du cadavre. Il est donc indispensable de connaître la durée de chaque stade (œuf, larve nymphe) à différentes températures afin de pouvoir déduire le moment où les œufs ont été pondus.

Chaque espèce d'insectes et plus particulièrement de mouches possède une durée de développement qui lui est propre et dépendante de la température. D'autre part, il est important de connaître les limites vitales du développement afin de pouvoir intégrer ces données lorsque les conditions saisonnières changent rapidement. Ainsi, les larves de *Calliphora vicina*, espèce relativement commune sur les cadavres humains, stoppent leur développement lorsque la température s'abaisse au dessous de 2°C environ. En revanche, les espèces du genre *Lucilia* (mouches vertes de la famille des Calliphoridés) ont une limite de développement larvaire beaucoup plus élevée, de l'ordre de 9°C.

Depuis 1993, de nombreuses expériences menées conjointement sur les principales espèces de mouches nécrophages permettent de disposer de tables de développement indispensables à toute utilisation pratique. Une série d'expériences comparant l'influence d'une température constante par rapport à des températures fluctuantes, mais donnant une moyenne identique, a montré qu'il n'y avait aucune différence sur la durée du développement. Dès lors, le climat régnant à proximité immédiate du cadavre est un élément majeur de toutes investigations utilisant des insectes pour déterminer l'intervalle post-mortem.

D'autres facteurs vont influencer la colonisation d'un cadavre : le milieu. Ainsi certaines espèces sont plutôt liées au milieu forestier, tandis que d'autres sont ubiquistes. Un travail réalisé dans les environs de Lausanne (Faucherre et Cherix, 1998) d'avril à octobre à l'aide de pièges a permis de récolter 17 espèces de mouches nécrophages appartenant aux familles des Calliphoridés (10 espèces), Sarcophagidés (3 espèces) et Muscidés (4 espèces). On remarque que 14 espèces sont présentes en prairie et 11 seulement en milieu forestier, mais 8 espèces sont communes aux deux milieux. Ce travail apporte des éléments importants dans le cadre d'expertises en entomologie forensique. En effet il est indispensable, lors de la découverte d'un cadavre d'être particulièrement attentif à différents aspects comme la saison et l'altitude qui vont affecter la phénologie des espèces ainsi que du type de milieu. La spécificité des Diptères nécrophages rencontrés dans un milieu ouvert ou fermé peut en effet apporter des renseignements complémentaires sur un éventuel déplacement de cadavre. Certaines espèces n'étant jamais ou que très rarement présentes dans un milieu ouvert, comme la prairie, ou réciproquement, dans un milieu forestier.

De plus, la connaissance du comportement de chaque espèce est indispensable à l'interprétation d'une analyse. En effet, suivant de nombreux auteurs, les Diptères nécrophages ne volent pas la nuit ni dans des lieux sombres ou encore cessent toute activité en dessous de 10-12 degrés Celsius.

Or un cas récent, pour lequel une véritable expérimentation a été mise en place a permis de montrer qu'il n'en était rien et que sous certaines conditions bien particulières, *Calliphora vicina* pouvait se déplacer dans l'obscurité et pondre ses œufs à une température constante de 5° C (voir Faucherre et. al, 1999).

L'utilisation judicieuse de ces connaissances permet de calculer un intervalle post-mortem. La méthode consiste à récolter le matériel entomologique existant sur les lieux de la découverte macabre. Le matériel récolté est ensuite mis en élevage, à une température donnée et l'on surveille l'apparition des adultes. A partir de ce moment, compte tenu de la dernière partie connue et maîtrisée du développement, il s'agit de reconstruire la première partie à l'aide de données météorologiques ayant régné les jours ou les semaines précédent la récolte du matériel.

Pour les calculs, on utilise la notion de degré/jour qui peut se résumer de la manière suivante : Si un insecte a besoin de 100 degrés/jours pour passer de l'œuf à l'adulte, en théorie cela représente 10 jours à 10° C ou 5 jours à 20° C. Bien évidemment, il faudra tenir compte de la température minimale de développement de chaque espèce.

Il existe plusieurs moyens d'obtenir des informations météorologiques en Suisse, notamment grâce aux stations automatiques de l'Institut suisse de météorologie ou alors en installant sur place un thermographe pendant une certaine durée. Les données relevées permettent d'établir une comparaison a posteriori avec la station météo la plus proche, puis de reconstruire le jeu de données durant la période nécessaire.

## ANALYSE DE CAS

En octobre, un crâne humain est repéré dans la litière d'une forêt d'altitude. Nous trouvons le corps à proximité, dans un talus, caché sous des branches de sapin. L'endroit est passé au peigne fin. Nous récoltons plus de cinquante pupes pleines de Diptères, des prépupes, des larves et quelques Coléoptères nécrophages. Ce matériel entomologique est mis en élevage au laboratoire. Les Coléoptères sont identifiés à l'espèce et mis en collection. Peu après la récolte des pupes pleines, on constate l'émergence de mouches nécrophages qui sont identifiées. Les relevés météorologiques de trois stations entourant l'endroit de la découverte sont demandés pour les mois de juin à octobre. Divers calculs sont effectués pour donner une estimation des températures du site la plus exacte possible. Sur la base de la moyenne journalière des températures du site et en comparant avec les tables de développement de l'espèce de mouche découverte, nous avons déterminé l'intervalle post mortem, remontant au mois d'août de la même année. S'agissant d'un meurtre, la date de la mort a été d'une importance capitale pour la suite de l'enquête.

## RECHERCHES EN COURS

Afin de pouvoir disposer de données de terrain complètes, plusieurs expériences ont été menées en utilisant des porcs comme substrat. Ces études nous permettent de suivre heure après heure durant les premiers jours, la colonisation des insectes nécrophages. Elles permettent la récolte d'un abondant matériel dont l'identification est indispensable à la connaissance des insectes utilisant ce substrat.

A ce jour plusieurs milliers de spécimens ont été montés, identifiés et étiquetés et servent de référence indispensable dans ce domaine. Par exemple, deux nouvelles espèces de mouches pour la Suisse ont été ainsi récoltées et identifiées. D'autres expériences ont été effectuées pour comprendre le comportement des mouches nécrophages et traitent du vol et de l'attraction du substrat.

## CONCLUSION

A ce jour, plus de huitante cadavres humains ont fait l'objet d'une analyse d'entomologie forensique en Suisse romande et apportent des données originales et indispensables aux calculs de l'intervalle post-mortem. De telles données présentent un intérêt non négligeable dans des affaires à caractère criminel, le jour du décès pouvant être déterminant dans l'inculpation d'un coupable.

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## Techniques of Sampling of Forensic Entomological Evidences used by the French Nationale Gendarmerie

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**Keywords :** Forensic entomology, sampling techniques

### INTRODUCTION

The use of entomology, as a forensic science, requires a good quality of samples during crime scene investigations and autopsy.
This paper propose to describe the techniques of forensic entomological evidences collection used by the crime scene technicians of the French National Gendarmerie.

### OBJECTIVES

* The objectives of entomological sampling in a forensic way is to:

- obtain representative samples of necrophagous and local faunae;
- collect all the species present;
- collect the oldest development stage for each species.

* Techniques must be realized easily by crime scene investigators.

It is possible to reach theses objectives with the collection of the **maximum** of Insects; but it is **impossible** with a choice on insects or about the location of samples during collection.

### MATERIALS AND METHODS

ENTOMOLOGICAL SAMPLES:

Materials :
*Light-tension pliers, laboratory spoon, different size of jars, bottle of alcohol (Ethanol 70%)



Techniques :
On the crime scene and during post-mortem operations.
Collection of a "maximum" of crawling specimens (eggs, maggots, beetles and else) everywhere on the corpse and around the corpse.

- Dipterian immature stages:
  Half of them killed in Ethanol 70%

Half of them kept alive in jars after making several small holes in the top.

It is better to use several jars with few maggots inside than only one with too many maggots to avoid predator behaviour, competition and else.

- All Insects adults (Diptera, Coleoptera,…):
  Killed in Ethanol 70%.
- Other immature stage than Diptera:
  Killed in Ethanol 70%.

SOIL SAMPLES:

Materials:
Screen, plastic bags and shovel.

Techniques:
Samples must be realized all under and around the corpse until about 1 meter far and 10 cm deep. All the soil samples are then gathered in the same bag.

PACKAGING, PRESERVATION and TRANSPORT

Forensic Entomologists or crime scene technicians work only in a judicial environment. This point is very important for the packaging of samples (officially closed before transport by putting under seal) in the way to avoid judicial problems during trial testimony.

Before be sending to the laboratory, in case of delay in the process, samples can be preserved in a refrigerator, several hours (no more than 1 week), between 4° to 7°C.

Transport of samples from crime scene to laboratory must be done as fast as possible (these evidences are also living organisms) by air, train, road (by territorial units; special transporters exist)

## TEACHING ACTIVITY

Magistrates and investigators can call us to move on crime scene and help the first ones either during technical investigations and post-mortem operations either to give technical information to the second ones.

But it is not possible for all the cadaver discovered in France so, entomological evidences are mainly collected by crime scene technicians who are especially trained in this way, during their school time in Fontainebleau at the National formative school in Judicial process.

An important part of our activity consists is the teaching of sampling techniques to such technicians and to promote this field to others person involved in criminal investigations (magistrates, forensic pathologists).

The department Forensic entomology participates to the elaboration of technical practice book of Crime Scene Investigations.

## CONCLUSION

A good method of sampling is essential for forensic entomologists, as we are, to give back accurate post-mortem interval and reply to the mission we are in charge. Gestures must be complete, accurate and easy to be used in good conditions by crime scene technicians.

# Using rates and colonization patterns of insect succession on a body to determine time since death

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ABSTRACT

Forensic entomology is the study of the insects associated with a dead body, primarily to determine time since death. Insects are the first witnesses to a crime, usually arriving within minutes of death if conditions are suitable. They are attracted primarily to wound sites and natural orifices. There are two major ways of using insects to determine time since death.

Analyzing the Successional Colonization Patterns of The Insects Over Time.

Analyzing Dipteran Larval Development Rates, or maggot development

The first method, using successional patterns of insect colonization, is used when someone has been dead for about a month up to a year and sometimes much longer than a year. It is based on the fact that, as the body decomposes, it goes through relatively rapid biological, physical and chemical changes and each of these decompositional stages is attractive to a different species, or group of species, of insects. These insects are attracted in a predictable sequence. Some are attracted within minutes of death, others several days later, other not until several weeks have passed. This sequence of insect colonization is greatly affected by biogeoclimatic zone, season and habitat, but within those parameters, it is predictable. So an analysis of the insects present on the body at the time of death, together with evidence of insect species whose tenure on the body is complete, will determine the window of time in which death must have taken place. This technique will be explained using case examples. Limitations of the technique will be discussed.

The second method is usually used when someone has been dead for a matter of hours, up to several weeks after death. It is based on the predictable development of insects over time, dependent primarily on temperature and species. If the temperature, species and oldest stage of insect on the body are known, then the minimum time it takes that species to reach that stage, under those conditions, can be determined. This will give the minimum time since death. This talk will concentrate on the first technique and the parameters that impact insect colonization of a body. My colleague, Dr. Reiter, will speak on the second technique

**Keywords** : Time of death, insects, succession

Forensic entomology is the study of insects associated with a dead body, primarily to determine time since death and is a very valuable tool in homicide investigations worldwide. Insects are usually the first to arrive on a body after death, and they colonize in a predictable sequence (Anderson 2001). There are two methods for determining time of death: using Dipteran larval development, which is of most value from hours to weeks after death, and analyzing the successional colonization of insects over time, which is of most value from weeks to months to years after death. This session will concentrate on the latter.

When remains are found weeks, months, or even years after death, insect evidence is the most reliable and often the only method available to reliably determine time of death (Anderson 2001). A dead body is a food resource for a great many organisms and it supports a large, and rapidly changing ecosystem as it decomposes. As the body decomposes over time, it goes through a rapid and predictable sequence of decompositional stages, from fresh to skeletal. During this decomposition, it goes through dramatic physical, biological and chemical changes (Van Den Oever 1976; Coe and Curran 1980; Henssge *et al*. 1995). Each of these stages of decomposition is attractive to different groups of carrion insects.

Insects colonize in a predictable sequence, with some species being attracted to the remains immediately when the body is fresh, others attracted some days or weeks after death, and still others being attracted when there is little but sinew and bones left. Insects are attracted to the body to feed directly on the remains, to feed on other insects, to lay their eggs and for shelter.

When species of insects colonize a body, they remain on the body for a period of time, until decomposition reaches a stage that is no longer attractive to them. As the insects leave the remains, they invariably leave evidence of their presence behind, such as cast larval skins, empty pupal cases or peritrophic membrane. Meanwhile, decomposition is continuing and the body, while becoming unattractive to some species is becoming attractive to other, later colonizers. Therefore, when remains are found, the forensic entomologist will study the insects that are present on the remains at the time of discovery and the evidence left behind by earlier colonizers. As well, they will note the species that are absent, but normally present in the colonization sequence. From this, an accurate time of death can be established.

When we look at the insect evidence on a body, we look first at the species that are present now, when the body is recovered. These are usually a diverse range of species and will, together, indicate a wide range of time. So alone, they might indicate that death could have occurred anything from, for example, six to ten months ago. However, insects leave evidence of themselves behind, so by studying this evidence, such as empty pupal cases or peritrophic membrane, we can determine which species were present, but are no longer associated with the body, as the body has deteriorated past the stage they prefer.

This narrows our time frame as it indicates that it is past the time that such species feed on the remains, so now we might narrow the time frame to, for example, eight to ten months.
Finally, we look at the insect assemblage and look at the species that have NOT yet arrived. So therefore, the body has not yet reached the stage to which these later stage insects are attracted. This narrows our time frame so that we can come up with a window of time in which death must have taken place. For instance, in this example, we might say, that death took place between eight and nine months ago.
Although the sequence of colonization of insects on the body is predictable, it is impacted by many different factors such as geographical location, meteorological conditions, habitat, season *etc.* Therefore, the colonization will be different in France as opposed to Switzerland, or Australia or China. It will be different if the body is found in a forest or on a mountain, or in shade, or sunlight, or buried or above ground *etc*. However, when the sequence of insects colonizing carrion is known for a given area and set of circumstances, an analysis of the arthropod fauna on a carcass can be used to accurately determine the time of death.
A brief sequence of insect colonization on a body will give an example of this colonization. This sequence uses a pig as a human model and comes from a police training exercise in Ottawa, Ontario, Canada. The fresh pig had a large wound in the throat, and this rapidly attracted blow flies, which laid eggs. Within 24 h, large masses of maggots were observed in the throat and mouth. As the days progress, the tissue is rapidly removed. A few days after death, beetles arrive to feed on the maggots. Eventually, only skin an bone are left, with many species feeding on the body over time.

***Case Example 1***. A young teenage girl went missing in March in Northern Canada. She was last seen alive in the presence of her boyfriend. The police strongly suspected that her boyfriend, who was about 16 yrs old, had killed her, but they had no evidence to prove this. The following year, in May, the suspect's father decided they were going to move from the townhouse in which they lived, and told his son to pack up his bedroom for the move. The suspect panicked. He went into his room and locked himself in. The room was in the basement of the townhouse and was the only finished room in the basement. He was, like many teenage boys, very protective of his personal space, so had a lock on his door and no family member had been in his room for years! The father heard lots of hammering noises coming from the boy's room. After awhile, the boy ran away. The father was concerned so broke open the door and entered the bedroom. Inside, the room was almost entirely taken up by a large home-made waterbed frame. The mattress itself which would contain water, had been removed, but the frame and the plastic liner remained. The father noticed that the boy had been trying to pry away the baseboard of the bed, but had not been strong enough. The father was stronger and so pulled the board back and looked under the bed. The body of the girl was revealed.

The police arrived and investigated the scene. Insect evidence was collected from the scene, and the body was shipped down to Vancouver for autopsy, where I collected further evidence.

The body was very well preserved by mummification. The forced air heating we use in North America is extremely good for preserving bodies! Most of the tissue was intact and mummified, although there was some insect activity. The insect evidence included species such as Dermestidae and Piophilidae which are species that colonize a body weeks and months after death, and can be found up to a year or more after death. So their presence indicates a relatively long elapsed time since death. However, this body was found up north in Canada – where it is extremely cold for many months of the year. In May, when the body was found, it was still dropping below $0^{o}C$ at night, so the insect season had not yet begun. Therefore, the insects could not have colonized her in the present year. The fact that there were any insects at all on the body indicated that the body must have been present in the room during the previous summer.

However, the most interesting factor about the case was the *lack* of a certain group of insects – the blow flies or Calliphoridae. These insects colonize immediately after death, laying their eggs on the body. These eggs hatch into maggots which feed voraciously on the remains, removing the bulk of the tissue. They then leave the body and pupate, forming a hard outer cocoon or pupal case. Within this pupal case, they metamorphose or change into the adult fly which flies away, leaving behind the empty pupal case, as evidence that this cycle has taken place. In this case there were several lines of evidence that indicated that no blow flies had fed on the remains. First of all, the remains were intact. A body colonized by blow fly maggots, will be skeletonized very rapidly. Second, if blow flies had been present, their empty pupal cases would have been found in the carpet under the bed. An extensive search did not reveal any pupal cases. Finally, when a body is attractive to blow flies, it is going from the fresh stage into putrefaction, which results in an intense odour. Had the body undergone such decomposition in a house, then all the family, and probably the neighbours, would have noticed! Therefore, blow flies had not colonized the remains. The body at discovery was certainly at a stage that was too advanced for blow fly attraction, but the evidence indicated that blow flies had never been attracted. This proved that the victim had died before the insect season began the previous summer. Not only had she died before the previous summer, she must have died and had time to mummify before the insect season began in late May/early June, in order for her body to be mummified and no longer attractive to blow flies. It was however, still attractive to later colonizing insects. This is entirely consistent with death occurring the previous March when she was last seen alive. The boy later confessed that he had strangled her in March and put her under the bed, as he did not know what else to do with her!

One of the greatest caveats in this sort of analysis is that insect succession on carrion is very dependent on the external environment. Many factors affect the body's decomposition and this impacts the sequence of insect colonization. Insects that colonize a body 6 weeks after death in one region, may not colonize a body in another region until 3 months after death. Therefore, you can not take data from one region and apply it to a different region.

***Case Example 2***. Highly decomposed, partially skeletonized human remains were found in a park, in late August in Southern British Columbia. They were found in a depression beneath a fallen tree and had been lightly covered with soil. The body was partly exposed and in a heavily shaded area. I collected insects from the body and the scene.

Pupae and empty pupal cases belonging to *Phormia regina* (Meigen) (Diptera: Calliphoridae) were present, indicating that these flies had been attracted to the remains and had laid eggs which had developed into maggots, pupae and eventually adult flies. This gave a minimum elapsed time since death of 25-29 days, under the crime scene temperatures (Anderson 2000). However, this is only a bare minimum as empty puparia may survive in soil for some time, so emergence could have occurred days, weeks or months prior to discovery. Therefore, in a case with many later colonizing species present, blow flies can give a minimum elapsed time since death, but that is all.

Blow fly development is primarily temperature dependent, so data can be transferred from one region to another, as most developmental data is lab generated. However, many other species were present that indicated a later elapsed time since death and their colonization times depend very much on the biogeoclimatic zone, season, and habitat (Anderson and Vanlaerhoven 1996; Anderson 2001). Other species associated with the body included *Fannia* larvae (Diptera: Fanniidae), Piophilidae larvae (Diptera) and various Coleoptera species, both adult and larvae, including *Carpophilus,* sp (Nitidulidae), *Nicrophorus* sp. (Silphidae), and several Staphylinidae species. Due to research on insect succession on carrion in British Columbia conducted by myself and my graduate students, data were available for this biogeoclimatic zone, season and habitat.

*Fannia* species larvae are later colonizers and are predominantly found on bodies in wet areas. The actual time of arrival of these species varies dramatically with season and conditions. On exposed pig carrion, in shaded conditions, in summer, in this region of British Columbia, they are first found 72 days after death (Dillon and Anderson 1995). This fits with the scenario for this body. However, if the body had been found earlier in the season, such as spring, the arrival time would have been different (Dillon and Anderson 1995). If the body had been more completely and more deeply buried, the arrival time would have been much more rapid (Vanlaerhoven and Anderson 1999). Colonization times would have also been different had the body been found in a different area of British Columbia (Dillon and Anderson 1996) or the world (Smith 1986).

Piophilidae larvae are also later colonizers and were first collected from shaded regions of this region of British Columbia 48 days after death, irrespective of season (Dillon and Anderson 1995). This fits the scenario of this case. However, had the body been in bright sunlight, the interpretation would have been different, as we have recorded them on pig carcasses in open pastureland as early as 29 days after death (Anderson and Vanlaerhoven 1996). In other regions of the temperate world, Piophilidae are usually much later colonizers, and are reported to be found three to six months after death (Leclercq 1969; Smith 1986). Piophilidae remain attracted to remains for many months after death (Anderson and Vanlaerhoven 1996).

*Carpophilus* larvae (Nitidulidae), were first found in pig carcasses a minimum of 64 days after death in this geographic region and *Nicrophorus* sp. (Silphidae) were found between 21-167 days after death.

The victim was found partially buried in shaded conditions in late summer. Both early and late colonizers were collected from the remains. The presence of later colonizers such as Piophilidae, Fanniidae and certain species of Coleoptera, indicate a minimum elapsed time since death of 64-72 days. This is a minimum as many of the later stage insects will continue to be attracted to the remains for some time. Therefore, death probably occurred at least 2.5-3 months before discovery and possibly longer. However, some soft tissue was still present so it is unlikely that death occurred before early summer, nor in the previous year, as the soft tissue would have been removed by maggot activity. This case remains unsolved at this time.

In conclusion, insect colonization of a dead body occurs in a predictable sequence, therefore, forensic entomology is an excellent method for determining time since death in the later postmortem interval. However, in order for this technique to be viable, reliable data must be available for local conditions, including biogeoclimatic zone, season, and habitat. Data from other regions or conditions should not be applied, as arrival times and tenure on the body of many insect species, are strongly impacted by biogeoclimatic zone, season and habitat. Research is required in all biogeoclimatic zones in succession of insect species is being applied to death investigations.

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## Post-mortem interval estimation using insect development data

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### TIME SINCE DEATH – THE POST-MORTEM INTERVAL

Estimating time since death of an individual is frequently of paramount interest in medico legal investigations and may considerably assist the investigators efforts. Therefore, the reliability of such an evaluation is most essential. It has been known since antiquity that immediately after death muscular rigidity develops, which in turn passes off as the signs of decomposition appear. The timing of this sequence of events is so variable, however, that it is a poor indicator of the time since death.

Other physiological changes used for post-mortem interval estimation, like body cooling, potassium concentration in vitreous humour, stomach emptying, mechanical excitability of skeleton muscle or chemical excitability of the iris are either inaccurate or only useful in the early post-mortem period.

After 48-72 hours, forensic entomology is often the only method for determining the post-mortem interval. Since Mégnin (1894), synanthropic flies, particularly calliphorids, are recognised as the first wave of the faunal succession on human cadavers. They are therefore the primary and most accurate forensic indicators of time of death.

### BLOWFLY DEVELOPMENT

The female flies lay eggs on the fresh corpse (or even on a debiliated live victim), choosing wounds or moist areas such as the eyes, lips, nostrils, and genitalia. Successive waves of eggs are laid, producing new generations of maggots as the post-mortem interval progresses. The hatched maggots secrete digestive fluids with proteolytic enzymes that help disintegration of the tissues. The growing larvae molt twice (second and third instar) until fully grown. After "peak feeding" the maggots enter the migrating stage, where most species begin to wander restlessly away from the corps to find a dry and dark spot for pupariation. Inside the pupal shell metamorphosis takes place at the end of which the new (teneral) adult fly emerges (Figure 1). After some time beetles and many other types of insects and arthropods join in the decomposition process.

When estimating the PMI from maggot length it is regarded as common practice to measure the largest individuals, since they are the oldest before peak feeding (Figure 2). When larvae enter the migratory phase, length is no longer an indicator of age, since body length and weight begin to decrease. It is also vital to have accurate data on the weather, especially the ambient temperature and rainfall during the period that the body was lying there, as the oviposition behaviour of the adults and development of immature insect stages is markedly altered by climatic conditions.

The development of insects is temperature dependent and can easily be visualised in growth- and other development-curves for various constant temperatures. Since blowflies arrive on the body soon after death, estimating the age of the insects will also lead to an estimation of the time of death.

## THE TEMPERATURE SUMMATION MODEL

Mathematically the temperature dependent development of insects is widely described using the temperature summation model, which is valid for the linear proportion (Range B) of the sigmoid development curve (Figure 3).

In this model the total amount of heat required, between the lower and upper thresholds, for an organism to develop from one point to another in its life cycle is calculated in units called degree-days (Figure 4). These degree-days are the accumulated product of time and temperature between the developmental thresholds for each day. The lower developmental threshold for an organism is the temperature below which development stops. The lower threshold is determined by the organism's physiology and can be established either experimentally or mathematically. It is independent of the method used to calculate degree-days.
The upper developmental threshold is the temperature above which the rate of growth or development begins to decrease or stop. The upper developmental threshold of an organism can only be determined experimentally and may be neglected in most forensic PMI estimations.
One degree-day is one day (24 hours) with the temperature above the lower developmental threshold by one degree. For instance, if the lower developmental threshold for a species is 15°C and the temperature remains 16°C (or 1° above the lower developmental threshold) for 24 hours, one degree-day is accumulated. If the maximum temperature for the day never rose above the development threshold the no degree-days were accumulated. Unfortunately temperatures do not stay at one fixed level for 24 hours – they fluctuate.

Each species of necrophagous flies requires a different but relatively constant amount of degree-days for total immature development (=thermal constant) (Figure 5). Therefore the identification to the species level is a vital step in PMI estimation using insect development data. When the microclimatic temperature records at the crime scene, the amount of degree-days for the recovered species and the developmental thresholds are known, the time of oviposition can be calculated.

Calculating degree-days for the daily temperature fluctuations that occur in nature is more difficult. Several methods are used by the forensic entomological specialist to estimate degree-days through the use of daily minimum and maximum temperatures. All are approximations of the actual number of degree-days accumulated for a given set of daily temperatures and developmental thresholds, and therefore do not provide the exact degree-day values.
It is important to know that as the PMI increases, the less accurate or wider ranging the PMI becomes.

## RETROSPECTIVE DETERMINATION OF THE TIME OF OVIPOSITION

Entomological evidence found on and around the corpse should be collected and preserved according to medico-legal standard procedures. On site microclimatic temperatures prevailing in the maggots' immediate environment have to be recorded for at least 3-5 days and correlated retrospectively with the air temperature records from the nearest weather-station. Obtaining accurate weather data is vital for estimating the PMI.
Assuming an average constant temperature, as is the case with corpses found indoors, larvae or pupae recovered from the scene, should be stored at the established constant temperature, until they pupate or the first adults emerge. Their age can then be determined retrospectively, using the isomorphen-diagram. Where temperature is variable an age range can be estimated between the points where the observed morphological change (pupariation or eclosion) cuts the graph at the maximum and minimum temperatures recorded. If the temperature is roughly constant, as is the case with corpses found indoors, the use of the growth curve and the Isomorphen-diagram could provide a quick and precise minimal estimate for the PMI.
It has to be emphasized that the use of the temperature summation model will yield the more accurate results. If the data for calculating development are limited to daily maximum and minimum temperatures, then estimates should be limited to degree-days. If hourly temperature data are available, then degree-hours may be calculated. See figure 6 for PMI estimation using the degree-day model.

## MAGGOT MASS

It is important for the entomologist to visit the crime scene or at least to obtain detailed photographs to see whether maggot mass formation occurred during decomposition. The metabolic heat generated by a mass of maggots on dead tissue can be sufficient to raise the temperature of the mass well above ambient temperatures and can exceed 45°C. In conditions under developmental minimum, maggot mass temperatures may be sufficient to allow continued development.

## PITFALLS AND PROBLEMS

Neglecting the possibility of maggot-mass formation can lead to an overestimated post-mortem interval.
Insect activity at rain and at night (where most homicides occur) is still a subject in forensic research.
Time of death may not be equal to time of first oviposition.
Drugs, toxins and parasitoids can have marked influence on maggot development.
Short days and cooler temperatures may induce diapause (hibernation) in carrion associated insects, which makes PMI estimates difficult even for the specialist.

## GEOGRAPHIC DIFFERENCES IN INSECT DEVELOPMENT

It has to be borne in mind, that the developmental times from oviposition to adult eclosion might possibly differ in various regions of the world. This raises the question whether it is valid to assume that the thermal constant of a holarctic species is the same everywhere. However, larger differences do not necessarily have to be attributed to variation in experimental method (extrinsic factors). Geographic adaptation (intrinsic factors) could explain a difference in temperature dependent development. Consequently there is a continuing need to refine and improve insect development data. Precise values for developmental minima and degree-day estimates by stage are important areas of improvement.

## CONCLUSION

Assuming an average constant temperature, as is the case with corpses found indoors, the age of the recovered specimen can be read off instantly from development tables or graphs.

Where temperature is variable the degree-day model will yield more accurate results. The particular thermal constant, its variation and at least the lower developmental threshold for each criminolegal relevant species have to be generated in forensic entomological research laboratories. Since biological systems under field conditions are rarely predictable with the precision attainable in the laboratory, the greatest care must be taken in interpretation of the results.

As several records in criminolegal casework have shown, the use of entomological indicators in medicolegal death investigations promises to be a reliable technique for estimating the PMI in both early and advanced periods of cadaver decomposition. Insect thermal development is a powerful tool in the hands of experienced and well-trained crime scene investigators and additional research will improve its usefulness.

Figure 1. Blowfly lifecycle. The average duration from egg to adult is mainly dependent on species and temperature.

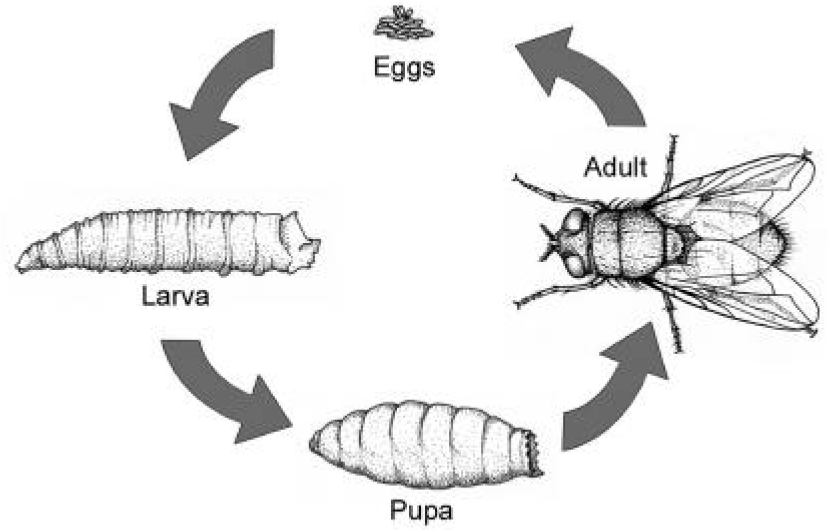


Figure 2. Growthcurves of blowfly larvae. Development from oviposition to pupariation at five different constant temperature regimes.

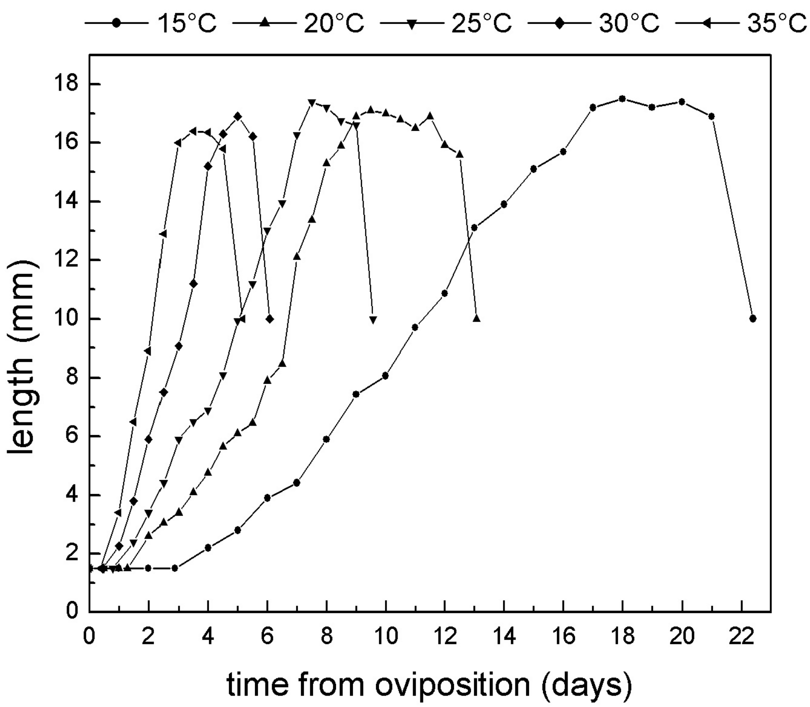


Figure 3. Generalized relationship between the rate of development and temperature, showing the non-linear portions A, C and the linear portion B, used to estimate the minimum development threshold ($t_L$) by extrapolation.

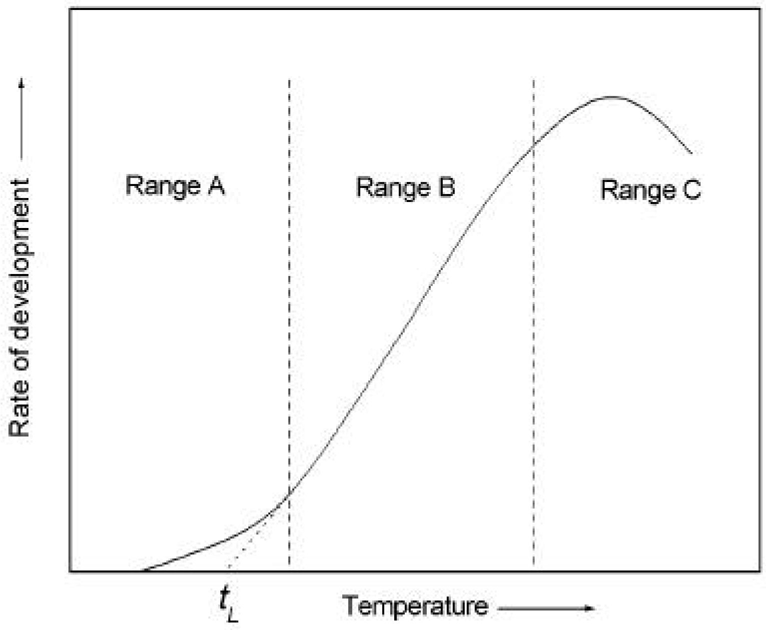


Figure 4. Accumulated degree-days between lower and upper developmental threshold. When calculating degree-days in forensic cases, the upper developmental threshold can usually be ignored.

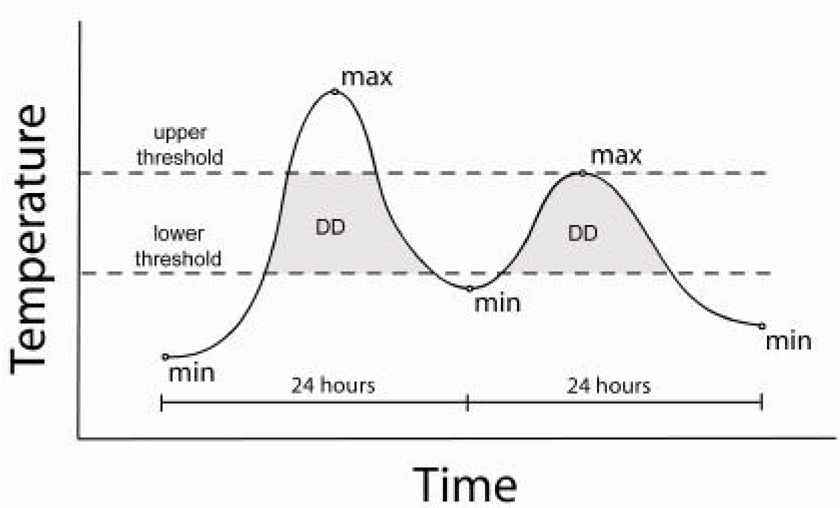


Figure 5. Isomorphen diagram for blowfly species X showing egg hatch, pupariation and eclosion at different temperatures. The areas (A1, A2, A3) represent the thermal constant from oviposition to adult eclosion. The thermal constant $K$ is calculated from the equation $K = y(t - t_L)$, where $y$ is the developmental time (days), $t$ is the rearing temperature (°C), and $t_L$ is the theoretical lower developmental threshold temperature (°C).

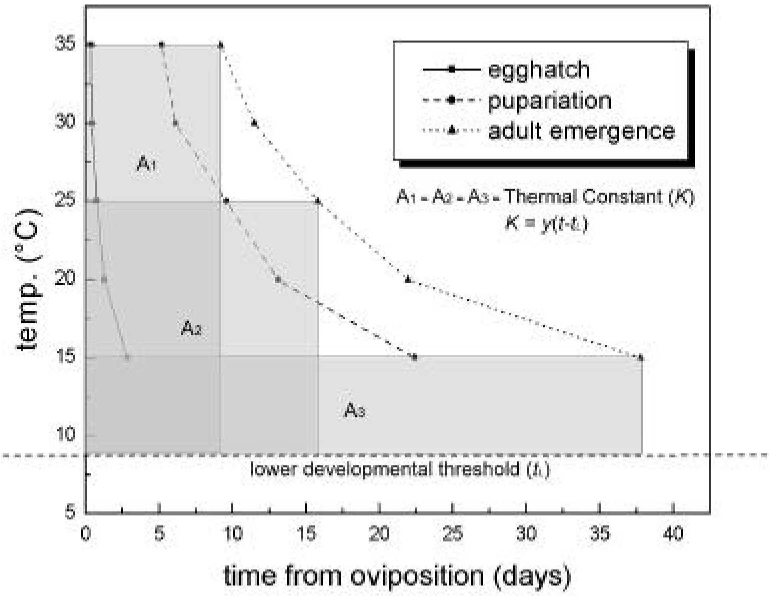
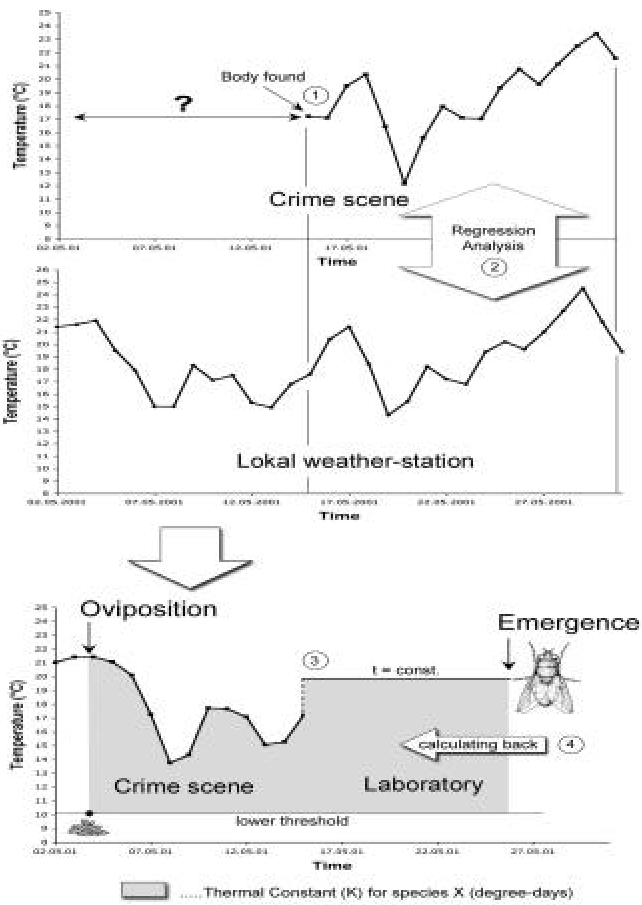


Figure 6. Estimating the PMI using the degree-day model. (1) Temperature data at the crimescene should be recorded for at least 3-5 days. (2) Recorded on-scene temperatures can then be correlated with meteorologic data from the nearest weather-station using regression analysis to estimate crime-scene temperatures retrospectively. (3) Specimen from the crime-scene are reared to pupariation or adult emergence under constant laboratory conditions. (4) When the amount of degree-days (thermal constant) for the recovered species is known, the time of oviposition can be calculated.



## The estimation of heat unit requirement of developing larvae using statistical regression of temperature measurements from a death scene

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The objective of this paper is to inform death scene investigators of an additional method or tool (statistical regression) in assessing temperatures at a death scene where micro-climatic conditions may have influenced those temperatures to deviate from what would be recorded at a National Weather Service Station.

Accurate estimation of energy heating units which drive the growth and development of life stages of blow flies used for establishing the PMI (postmortem interval) is essential when using these insects as evidence in a death investigation. The insects are cold-blooded (poikilotherm) creatures and thus, the speed of completing their life cycle is directly dependent on the temperature of the microhabitat in which they live. The microhabitat temperatures are generally estimated by using ambient temperatures from the closest National Weather Service (NWS) Station to the location where the remains are found. Diligence on the part of the scene investigator as to air temperature differences between the two locations (with extremes of either hot or cold temperatures) is extremely important when remains are found in environments atypical of sites where the NWS station has placed their recording instrumentation.

In a case from Indiana during July of 1993, the remains of a 10 year old male were found on July 21st under a heavily shaded concrete bridge lying in shallow water where the body rested at the bottom of an improved ditch embankment on large "rip-rap" limestone rocks. The boy was last seen riding his bicycle on the late afternoon of July 15. When investigators processed the body, they commented that it was cooler at the bottom of the embankment near the creek than in the open sunlit area at the top of the embankment and on the bridge.

The forensic entomologist and climatologist recommended that several temperature readings be taken above and below the bridge at intervals of 6 to 8 hours for approximately 5 days following recovery of the remains when synoptic weather conditions were similar to the period when the boy was missing. For the statistical regression proposed, it was necessary to take readings near the minimum temperature of the day, during the rise, near the maximum temperature, and during evening when temperatures were cooling. Air temperatures were recorded at three locations in the vicinity of the recovery : 20 feet north of the bridge at ca. 5 feet above the ground ; below the bridge ca. 4 feet above the location of the body ; and at ground level where the body lay.

Twenty data points were assessed on hourly temperature data corresponding to the same hourly recordings from the NWS station, Indianapolis (distance ca. 40 miles). A 2nd order polynomial regression yielded an $R^2$ value of .792, ($p = .0001$), and an equation to the line of $Y = -13.942 + 2.657X - .051X^2$, where X = hourly air temperature (°C) for Indianapolis NWS, and Y = hourly air temperature (°C) below the bridge at ground surface. This model suggested cooler temperatures at the scene in agreement with expectations based on general micro-climatic theory. When these hourly temperatures were used in estimating the development of the blow fly larvae [*Phormia regina* (Meigen)], a longer PMI resulted. These data concluded that insect colonization of the victim occurred prior to sunset on July 16 and that death would have occurred prior to sunset on July 15 and before sunrise on July 16. A confession later supported this estimate when the assailant stated that he killed the boy around 5 PM on July 15 and dumped the body over the bridge before dark.

## (Mostly) unpublished forensic arthropod cases from Germany

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We present four unusual cases that we encountered during our forensic entomology co-operations with several police departments in Germany.

1) Mass invastion of spiders and dermestid beetles into flats

- 1a) Examination of a nearly skeletonized, dried out corpse in the appartment where the person had been living did show masses of dermestid larva skins but nearly no blowfly larvae, pupae, or adults. Reason: An electrical heater dried out the body very quickly so that blowflies did not find a suitable habitat.
- 1b) In the same house, another (living) person was approached by us. Inside of the flat, hundreds of living spiders, and their spider webs were found. Reason: Person refused to use the toilet, or to clean his rooms -- excrements attracted large numbers of flies -- flies attracted numerous spiders.

2) The face of a dead person had maggots in only one eye socket. Reason: A light was switched on on that side of the body. First, maggots may have used up the material in the eye socket further away from the light but then moved over to the other eye.

3) During a joint task force operation against 5000 motorcycle rockers in the federal state Brandenburg, German policemen of another, wealthier federal state complained for many days about the local food situation. On the last day of the operations, „maggots“ were found in the food. We concluded from photographs that (a) the head parts of the alleged maggots were darkened as in beetle, or butterfly larvae, and (b) the larvae had been dead (stretched shape). These observations made it likely that the food was neither spoilt nor rotten but that somebody had thrown in the insect larvae on purpose.

**Keywords** : Case reports, forensic entomology, arthropods

## Beyond the limits, the case of *Calliphora* species (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. If temperature is one of the most important factors affecting fly oviposition activity and rate of larval development, even more important is the arrival time. Blowflies are known to be the first colonizers and can be attracted within minutes following death (C. Wyss, unpublished) but they are diurnal species and usually rest at night. Moreover, it is generally accepted that activity of necrophagous flies stops below an air temperature of 12°C (Erzinçlioglu, 1996) or 10°C (Williams 1984). Moreover fly oviposition is strongly influenced by temperature and generally does not occur below 10°C unless the substrate temperature has been influenced by solar radiations.

We report here two cases; the first one dealing with darkness and low temperatures and the second one with winter season, snow and low temperatures. In the first case, the body of a 77 year old male was discovered in July in a 10 m deep cave located in a forest in the Swiss Jura mountains at an altitude of 1260 m. He was lying on his back, fully dressed without visible injuries. The location of the body in the cave was in total darkness with a constant ambient temperature (5°C). Several batches of eggs were found on the top of the head and in the mouth. Trasnferred in the laboratory at 20°C, the eggs hatched and reached the adult stage. They all belonged to *Calliphora vicina*. In order to demonstrate that blowflies were able to fly and lay eggs in this cave we placed fresh meat and found the first eggs 11 days later. All emerged adults belonged to *C. vicina* (see also Faucherre at. al., 1999).

The second case occured within the Swiss Alps at an altitude of 1820 m. In December, two young boys disappeared while praticing snow surf. Due to bad weather conditions (snow, fog) during the following days, the rescue was abandonned. The next year in May, at a time when the last snow patches were melting another rescue was ready to be launched, when blowfly activity in the middle of a 500 $m^2$ snowdrift was discovered. At a depth of 50 cm, following a small opening, the head of the first corpse appeared. Behind the left ear numerous blowflies and egg masses were found. The temperature measured was about 2.5°C. Adults were easily caught while coming out. Identification of collected specimens revealed the presence of *Calliphora vicina* and *C. vomitoria*. These cases bring new horizons to forensic entomology and suggest that at least some Calliphora species might go well beyond previously accepted boundaries.

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**Keywords** : *Calliphora vicina*, *Calliphora vomitoria*, low temperature, activity

## Les Phorides (Diptères) sur cadavres humains en Europe occidentale

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The firsts investigations from the Phoridae flies on human cadavers are very olds (REINHARD, 1882 ; HOFMAN, 1886 ; MEGNIN, 1887 ; 1894 and SCHMITZ 1928 ; …). Among the faunal succession on human corpses exposed, the Phoridae flies were considered formerly characteristic of the fifth wave at 4-8 months since the onset and the third wave on buried corpses (MEGNIN, 1894). It is now recognized that they may be associated with the previous waves and also with the last to eliminate the rest of soft tissues of a rotting body after several years.
In 1947, the first Phoridae on human cadaver are found in Belgium after a post-mortem delay of third weeks with maggots of *Calliphora vicina* (LECLERCQ).
In Belgium, four different Phoridae flies are discovered on human corpses since 1947 : *Conicera tibialis*, *Megaselia rufipes*, *Megaselia scalaris* and *Triphleba hyalinata*. *Conicera tibialis* also nicknamed coffin fly (REINHARD 1882). The species can crossed the ground down to two meters for lay eggs in the buried corpses. The midges may be present up to fifth years of interval post-mortem. The period of flight is April at November. *Megaselia rufipes* has a changeable biology. *Megaselia scalaris* is a species of tropical climates but she is frequently founded around the Mediterranean. The species feed on decomposed animal and vegetable matters. It is a parasite fly. *Triphleba hyalinata* is founded in hood coffins, near tombs and in Belgium caves. The adults hatch between October and March.
The species identification may be get with the adults by a specialist entomologist.

**Keywords** : Forensic entomology, Phoridae, Europe

Les premières investigations concernant les Phorides sur des cadavres humains sont très anciennes (REINHARD, 1882 ; HOFMAN, 1886 ; MEGNIN, 1887 ; 1894 et SCHMITZ 1928 ; …). Parmi l'entomofaune qui se succède sur les cadavres humains exposés, les Phorides ont été classés autrefois dans la cinquième escouade après un intervalle post-mortem de 4 à 8 mois et dans la troisième ecouade pour les corps enfouis (MEGNIN, 1894). A l'heure actuelle, il est reconnu qu'ils peuvent être associés aux escouades précédentes et même aux dernières pour éliminer les restes tissulaires putréfiés après un intervalle post-mortem de plusieurs années.

C'est en 1947 que le Docteur LECLERCQ découvrit pour la première fois des Phorides associés avec des larves de *Calliphora vicina* sur un cadavre humain après un délai post-mortem de 3 semaines.

Depuis 1947, on a découvert quatre espèces de Phorides sur des cadavres humains en Belgique : *Conicera tibialis, Megaselia rufipes, Megaselia scalaris* et *Triphleba hyalinata*. *Conicera tibialis* est aussi surnommée « coffin fly » (REINHARD 1882). Cette espèce peut creuser le sol jusqu'à deux mètres pour pondre dans les cadavres enfouis. Ces moucherons peuvent être présents jusqu'à cinq ans d'intervalle post-mortem.
La période de vol est d'avril à novembre. *Megaselia rufipes* a une biologie variable. *Megaselia scalaris* est une espèce des régions tropicales, mais elle est fréquemment trouvée autour du bassin méditerranéen. Cette espèce se nourrit des matières organiques décomposées d'origine animale ou végétale. C'est une mouche myiasigène. *Triphelba hyalinata* est trouvée dans des cercueils en bois, près des tombes et dans certaines grottes en Belgique. Les adultes n'éclosent qu'entre octobre et mars. L'identification des espèces peut être obtenue avec les adultes par un entomologiste spécialisé.

Mots-clés : Entomologie médico-légale, Phorides, Europe.

## 1. INTRODUCTION

Les premières investigations concernant les Phorides sur des cadavres humains, soit enfouis dans le sol puis exhumés, soit exposés, soit dissimulés ou soit mis dans des cercueils en bois, sont très anciennes. Ces investigations ont été effectuées en Allemagne par REINHARD (1882) et HOFMAN (1886), en France par MEGNIN (1887 et 1894) et en Hollande par SCHMITZ (1928).

Autrefois, les Phorides ont été classés dans la cinquième escouade après un intervalle post-mortem de 4 à 8 mois et dans la troisième pour les cadavres enfouis (MEGNIN 1894,…).

En réalité, ils peuvent être associés dans les 8 escouades classiques. Ces moucherons ont été trouvés après un intervalle post-mortem de 3 semaines avec des larves de *Calliphora vicina* (Robineau-Desvoidy) sur le cadavre d'un nouveau-né le 20 mai 1947 puis ultérieurement dans d'autres expertises après des intervalles post-mortem allant de 26 jours à plus de 3 ans.

2. CAS CONCRETS

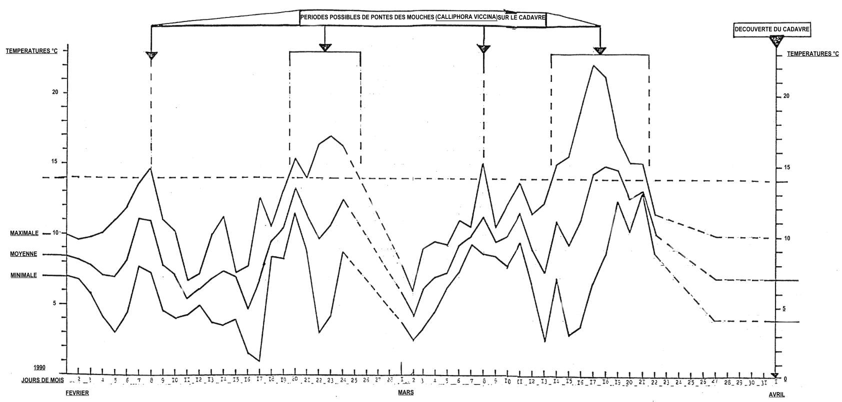
A. Le 29 décembre 1971, un cadavre a été découvert dans la nature. Après identification de la victime, l'enquête a déterminé que la personne était disparue depuis le 3 décembre 1971.

L'expertise entomologique a révélé entre autres des larves à maturité de *Protophormia terraenovae* (Calliphorides : Robineau-Desvoidy) ainsi que des larves et des adultes femelles de *Triphleba hyalinata* (Phorides : Meigen). Cette espèce de Phorides est inféodée aux cadavres. Elle a déjà été trouvée dans des cercueils en bois ainsi que dans les cimetières près des tombes. Elle a même été signalée dans certaines grottes en Belgique. Les adultes n'éclosent qu'en hiver avec comme dates extrêmes connues d'octobre à mars. Il n'y a qu'une génération annuelle. Concemant le cycle biologique, on peut dire que les deux premiers stades larvaires durent environ deux semaines et que les larves matures ne se transforment en pupes qu'au début de l'hiver.

B. Le 1er avril 1990, un cadavre a été découvert après un intervalle post-mortem qui était de 39-40 jours selon les données de l'enquête.

L'expertise entomologique a révélé des larves (13-14 mm) de *Calliphora vicina* (Robineau-Desvoidy) et des pupes pleines de Phorides. Après mise en élevage, on a constaté l'éclosion de moucherons du 25 avril au 28 avril 1990. Il s'agissait de *Megaselia rufipes* (Meigen).

L'analyse du graphique des températures a permis de préciser les périodes d'activité et d'inactivité des *Calliphora vicina* et des *Megaselia rufipes*. Logiquement, le cadavre devait se trouver le 8 février 1990 à l'endroit où il a été découvert ce qui ne fixe pas la datation de la mort. En tenant compte des données météorologiques et de la biologie des *Calliphora vicina* et des *Megaselia rufipes*, le décès pouvait avoir eu lieu antérieurement pendant la huitaine de jours avant cette date du 8 février.



C. Le 26 décembre 1997, les policiers ont fait la découverte d'un cadavre à l'état squelettique très soigneusement emballé et dissimulé dans l'annexe d'une habitation.

L'expertise entomologique a révélé l'absence totale des représentants de la première escouade classique (Calliphorides,…) ce qui fait penser à une inaccessibilité permanente du cadavre pour ces mouches de grandes tailles ou au décès pendant une période hivernale où elles sont en hibernation et inactives.
Des pupes vides de *Fannia spp.* (Fanniides) et d'*Hydrotea (Ophyra) spp.* (Muscides) ont été trouvées permettant de dire qu'elles avaient terminé leur action. Une masse de Phorides a également été révélée par l'expertise. Cette masse comprenait des larves aux différents stades larvaires et des pupes pleines sur un reste de substrat en fermentation ammoniacale. Après mise en élevage expérimental à 18-20°C, le Docteur LECLERCQ a constaté des éclosions multiples de ces moucherons du 11 janvier au 2 février 1998. L'identification spécifique a été précisée par notre collègue R.H.L. DISNEY de Cambridge. Il s'agissait de *Conicera tibialis* (Schmitz).
Les données de l'enquête permettaient de dire que le décès avait dû avoir lieu au début de l'année 1993.

Les résultats de l'expertise entomologique ont permis de confirmer le décès durant la période hivernale et l'estimation de l'intervalle post-mortem de moins de 4 ans ; c'est-à-dire à partir de février-mars 1993 compte tenu de la biologie de *Conicera tibialis*.

*Conicera tibialis* est surnommée « la mouche des cercueils » ou « coffin fly ». REINHARD (1882) a trouvé des quantités énormes de pupes et des millions de ces moucherons sur des cadavres humains exhumés et sur un cadavre complètement gelé découvert dans un bois ; quatre ans et demi après l'enterrement, il a été exhumé et son crâne était couvert d'une épaisse couche de pupes ainsi que de ces moucherons en vie. Il a conclu que ces *Conicera tibialis* pouvaient être présent jusqu'à cinq ans d'intervalle post-mortem.

D'autres informations ont été publiées par COLYER (1954), SMITH (1986) et DISNEY (1994) :

- ces moucherons peuvent creuser un chemin sous terre vers des cadavres enfouis ;

- ils sont capables de faire le trajet inverse à travers le sol vers la surface ;

- les pupes vides démontrent qu'il y a eu des générations qui se sont développées au niveau du cadavre enfoui ;

- même après un intervalle post-mortem très long, des quantités très importantes de ces Phorides peuvent voler au niveau du sol à un endroit où un cadavre a été enfoui ce qui en fait des indicateurs naturels très précis.

D. Le 13 octobre 1999, le corps d'une femme âgée a été découvert au 6ème étage d'un immeuble. Les données de l'enquête ont permis de déterminer que cette personne était toujours en vie dans l'après-midi du 8 octobre 1999.

L'expertise entomologique a révélé la présence de moucherons et de petites larves au niveau des organes génitaux et des petites larves au niveau vaginal ainsi qu'une nymphe de blatte (*Blatella germanica*). Les larves ont été mises immédiatement en élevage expérimental. Leur transformation en pupes a débuté le 14 octobre 1999 et l'éclosion des adultes du 22 au 25 octobre 1999. Il s'agissait de mouches de la famille des Phorides appartenant au genre *Megaselia*. L'identification spécifique a été réalisée par R.H.L. DISNEY. Celui-ci a pu déterminer que les moucherons étaient *Megaselia scalaris*. Dans l'appartement, la température ambiante a pu varier aux alentours de 20°C. L'intervalle entre le 13 octobre (découverte du corps) et les 22-25 octobre 1999 (éclosion des mouches) est de 9-10 jours. C'est nettement trop court pour admettre un cycle biologique complet sur le cadavre. En effet, il faudrait au moins 30°C. constants pour rendre plausible l'intervalle comptabilisé. Dans ces conditions, on ne peut pas fixer la datation de la mort de cette femme avec les données entomologiques.

Tableau 1 :

| Température constante | Œufs Incubation | Larves | Pupes | Total Éclosion des mouches |
|---|---|---|---|---|
| 22°C. | 1,3 j. | 5,5 j. | 15,1 j. | 22 jours |
| 29°C. | 0,7 j. | 3,5 j. | 6,9 j. | 11,1 jours |
| 27°C.<br>75% HR.<br>Lumière continue | 12-16 heures | 4,6 j.<br>6,3 j. | 9,7 j.<br>9,8 j. | 14,3 jours pour les mâles<br>16,1 jours pour les femelles |

[Données précises sur la durée complète du cycle biologique de Megaselia scalaris en fonction des températures constantes.]

*Megaselia scalaris* est une espèce bien particulière. Elle est cosmopolite dans toutes les régions tropicales et subtropicales. Elle est devenue fréquente en bordure de la Méditerranée grâce à l'homme et aux moyens de transports. Dans les climats plus froids, elle se réfugie dans les immeubles pour échapper au gel. C'est la première fois qu'elle est trouvée en Belgique sur un cadavre. En outre, le premier cas en Europe a été constaté dans le sud de l'Italie sur un cadavre exhumé (Disney). Les femelles sont attirées par les odeurs nauséabondes : matières organiques décomposées (excréments, cadavres mêmes inhumés) ou végétales (fruits et aliments avariés). Elles concernent aussi la parasitologie. En effet, leurs larves se retrouvent dans des myiases cutanées, ophtalmiques, naso-sinusales, intestinales, uro-génitales, vaginales. Un premier cas de myiase vaginale avait déjà été observé au Texas. Dans le cas présent, il est logique d'admettre que la femme avait une myiase vaginale avant son décès et que la contamination a eu lieu vraisemblablement au début du mois d'octobre. Aux USA, elle a également été utilisée en toxicologie. Des pupes de *Megaselia scalaris* et des mues de larves *Dermestes maculatus* ont été retrouvées sur le cadavre d'une femme intoxiquée par des médicaments. De l'amitriptyline, de la nortriptyline et d'autres drogues ont été isolées à partir des pupes de *Megaselia scalaris* et des mues de *Dermestes maculatus*.

## 3. CONCLUSION

Jusqu'à présent, nous comptabilisons 4 espèces de Phorides trouvées sur des cadavres humains en Belgique : *Conicera Tibialis*, *Megaselia rufipes*, *Megaselia scalaris et Triphleba hyalinata*. En France et en Belgique, on comptabilise environ 300 espèces de Phorides. Les stades larvaires se trouvent dans les matières organiques d'origine animale ou végétale en voie de décomposition. Certaines de ces espèces se développent dans les champignons, d'autres sont prédatrices ou parasites. Le nombre des espèces attaquant les cadavres de vertébrés y compris les humains n'est pas encore établi.

Habituellement, les phorides ont été mis dans la cinquième escouade après un intervalle post-mortem de 4 à 8 mois. En réalité, selon l'espèce, elles peuvent se trouver dans les escouades précédentes « en parallèle ». Il est donc préférable de considérer d'abord les groupes écologiques : les nécrophages, les nécrophiles, les omnivores et les opportunistes sans oublier l'éventualité d'une myiase préalable au décès.

L'identification des espèces peut être obtenue avec les adultes, grâce aux ailes, par un entomologiste spécialisé. Il est donc nécessaire d'effectuer l'élevage expérimental des larves et des pupes. L'identification est indispensable puisque la biologie des espèces est variée ce qui est déjà évident pour les 4 espèces trouvées au cours de nos expertises entomologiques :

- *Conicera tibialis* peut traverser jusqu'à deux mètres de terre pour pondre dans les cadavres enfouis à condition qu'ils soient âgés d'au moins un an (Mattle, 1993) et la période de vol va d'avril à novembre.

- *Megaselia rufipes* a une biologie très variée.

- *Megaselia scalaris* est une espèce des climats chauds qui a été trouvée dans des immeubles en Belgique et au Japon. Elle réalise des myiases cutanées, ophtalmiques, naso-sinusales, intestinales, uro-génitales et vaginales.

- *Triphleba hyalinata* est inféodée aux cadavres. Elle a été trouvée près des tombes et dans certaines grottes. Les adultes n'éclosent que durant la période hivernale et les dates extrêmes connues sont octobre à mars.

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**Maggots on corpses – Who cares ?**

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Forensic Entomology has a long history in Europe, but just during the last decade there was a real breakthrough. During this period many *‚entomological cells‘* arose all over europe, of which the entomological department of the french Gendarmerie Nationale can be named in many respects exemplary and pathbreaking.

Yet in the german-speaking area, which will be described here exemplarily, forensic entomology shall be deemed even by many supporters to be an originally appendix, which based in fact on scientific fundamentals but is required very rarely in the *‚real world‘*. A charcteristic current quotation is the statement by the head of a german institute of legal medicine, who, after a lecture and inspiring discussion about forensic entomology, conclude: *‚Very interesting, but I think that I had needed this methods maybe two or three times during my thirty years old career‘*. Therefore it seems to be reasonible to clearify if it is worth to pay attention for maggots on corpses before discussing the cognizances of taking care for them. How far the quoted statement of the pathologist is warranted? The french colleagues confute that meaning impressivly, examine about 300 cases during a period of 6 years. Are the blowflies in germany demotivated? Is there any reason for a different situation in germany? An evaluation of about 6820 autopsies which were realized at the institute of legal medicine in Frankfurt am Main/Germany from 1993 – 2001 should verify the frequency of maggot-infested corpses. 314 (4,6 %) corpses showed maggots, that means an average of about 35 corpses per year (it is a remarkable fact that the mean number of ‚maggot-corpses‘ per year increased since the institute focused his research on forensic entomology, a clear indicator for the different kind of perceptions at the corpse and the importance of an entomological view). 22 of 314 (7,01 %) cases represent a homicide and entomological evidence would be very important for the criminal investigations. 101 (32,12%) cases showed unclear circumstances regarding the manner of death and thus a entomological examination could provide clues for a possible criminal investigations. After transfering this results to the official numbers of autopsies in germany we can face the following provoking data: In 1999 there were about 17000 autopsies in germany. That could mean a total of about 782 (= 4,6 %) corpses with maggots. About 55 (= 7,01 %) of this cases were maybe homicides and about 251 corpses with maggots (= 32,12 %) have an unknown postmortem interval in combination with a more or less obscure matter of death. Even these numbers don’t reflect a serious nationwide survey it is obviously that their is a big potential of entomological investigations in forensic science and that we are still facing an underestimated number of entomological expertises in germany and probably many parts of europe.

This fact reflecting eventually a gap and certainly leads to irreparable failures during criminal investigations. We are facing at the moment an enormous backlog regarding the evaluation of DNA-traces, which are assured many years ago by the police. Now, in this genetic age of PCR and other high sensible molecular techniques combined with a growing data-base of genetic fingerprints, the investigators can expect valuable results for even very poor traces. So, police clear out their evidences of the last decade, in hopes getting good results after all this time. Due to the popular presentation in the media (‚*Superintendent Blowfly*') many investigators finally know forensic entomology and sometimes they remember an old unsolved case, where maggots were observed. Usually a satisfying processing of that kind of cases is not possible because even such a magic weapon like forensic entomology needs evidences, namely maggots. But that kind of evidences were not collected during these days, instead we are confronted with pictures of the body and questions like: Is the victim four or six weeks dead ? 17 oder 21 hours? But of course that kind of detailed questions can not be answered only on the basis of pictures. All entomological fundamentals (which kind of species are on the corpse and at the surroundings, are all species and development stages on the picture, etc.) be absent and the chance for a good entomology expertise is give away. The question arise about the responsibilities and cognizances for that grievance, especially when we are thinking about a possible development on a european level. Who cares about the maggots ?

The french way seems to be very successful as mentioned already above and so it seems reasonable to put the police-officers, who are the first at the scene, in charge of the insect-collection. But depending on the interest and disposition of the detective, they will collect maggots or not, consult a forensic entomologist or not, ask the pathologist for his support or not. Yet, classical clues like the overflowed mailbox or an open TV-program are very important for the containment of the postmortem interval. And there is still the popular opinion that the estimation of time since death, the most important question in forensic entomology, is a important duty of legal medicine. This is illustrated by the response of the 16 german police state departments, which get an offer for entomological training and education. 12 departments even did not answer, just two departments use the offer. So, the institute of legal medicine is the right place for catching maggots? Certainly, sooner or later the maggoty body will reach the dissecting room. Of course the corpse can be examine much better on the table then in the field and insects, which live inside the cavity of the body, could be collected during autopsy. But for sure the imponderabilities are the same then for the police-detective, the quality of collecting depends strongly by the motivation of the collector, here the pathologist. And, much more important, without a precise frame of guiding principles and collecting-guidelines the quality of a collection will be unsteady. So we need a expert, a well-educated forensic entomologist? Of course this is true when we are thinking about the examination and evaluation of the collected data. But this expert is helpless if he get bad data and the forensic entomologist can't examine every corpse with maggots, he need support at the death scene.

CONCLUSIONS

- even forensic entomology is quite popular, police and prosecution still use this method rarely but **every** corpse which is infested by maggots should be examined under
- entomological guidelines. Not every collection have to lead inevitable to an expertise but if the maggots are wash away there is no way for compensation.We need a change of mind in the responsible institutions. Forensic entomology needs support!

Foundation of an european board of forensic entomology!?

- a profession needs professionals: the collection of entomological evidence should take place by an forensic entomologist where ever it is possible. If police-officer and pathologist have to take the samples, they need a standardized collection guide. This minimize the risk of making mistakes and moreover assure the quality of the samples: Standardization is important for a good expertise in front of the court. A narrow co-operation between police and pathology during collection in the field and in the dissecting room is very important.

Development of standards for collecting and preserving entomological evidence

# Poster Session

## Annual flight activity and reproductive status of blowflies in central London

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Trapping was carried out continuously in the period from January 2001 to March 2002, in the wildlife garden of The Natural History Museum, London, UK, using a 30 x 30cm board covered with a clear adhesive sheet, and odour-baited with minced liver and 10% sodium sulphide solution. The trap was positioned just inside an area of shrubbery, on a post approximately 30cm off the ground and fitted with a rain cover. *Calliphora vicina* adults were collected on the trap from March 2001 through to late December 2001, reappearing in February 2002. No flies were caught on the sticky trap during January and February 2001, and January 2002. Adults of *C. vicina* were present in the garden during this period but they were not responding to the odour-baited trap. In sunny weather, several flies were encountered resting in open areas and were collected with a hand net. *Calliphora vomitoria*, *Lucilia illustris*, *L. caesar*, *L. sericata* and *L. ampullacea* were also caught on the trap from late-April/early-May through to late-October/early-November 2001. No adults of these species were found overwintering at the trap site.

Quantitative samples of fly numbers were obtained by deploying a separate trap at the same site for a fixed period of 24hrs only, at regular intervals throughout the year. In those 24hr periods, the trap used for the “continuous” study was not deployed. *Calliphora vicina* comprised 86% of the flies caught, *C. vomitoria* just 0.5%, *L. illustris* 7%, *L. caesar* 3.5%, *L. sericata* 2% and *L. ampullacea* 1%. The dominance of *C. vicina* and very low numbers of *C. vomitoria* confirms their respective status as urban and rural species. Numbers trapped in a single 24-hour period ranged from just a single fly during the winter months up to 537 in the last week of June.

Samples of live female *C. vicina*, caught with a hand net during the winter and from the 24hr traps, were dissected to evaluate their reproductive status. Unmated flies were conspicuous by the absence of sperm in the spermathecae, and the developmental stage of the ovarioles was recorded. Overall body size was also estimated by recording the length of the wing cross vein cm-du. Females with mature eggs ready for oviposition were found at all times of year. Gamma correlation statistics were calculated and no significant relationships were found between any of the factors tested (body size, mating success, stage of ovarial development and month of capture).

**Keywords :** Blowflies, reproductive status, seasonal abundance

## Methods used for the killing and preservation of blowfly larvae and their effect on post-mortem length

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Length measurements taken from the largest larvae collected on a corpse can be used to estimate the age of the oldest larvae present and, therefore, give an estimate of minimum time since death. Consequently factors that affect post-mortem larval length will impact on any estimate of PMI derived from them. The methods used to kill and preserve larvae are known to affect their post-mortem length. This study looked at a number of factors: preservative, species, duration of storage in preservative prior to measurement and method of killing. Post-feeding third instar larvae of *Calliphora vomitoria* were either hot water killed or placed live into 80% ethanol or 10% formalin, and their lengths measured immediately after death (for the hot water killed samples), and again after 1, 7, 14 and 21 days storage in preservative. *Calliphora vomitoria* larvae placed live into 80% ethanol had a greater mean post-mortem length (15.2 ±1.0mm) than those placed live into 10% formalin (13.7 ± 0.9mm). Hot water killed larvae were also larger in 80% ethanol (18.2 ± 1.1mm) than in 10% formalin (15.9 ± 1.1mm). Overall length was much greater in hot water killed larvae compared with larvae placed live into the preservative.

To investigate the effects of varying the conditions used for hot water killing, post-feeding third instar larvae of *C. vomitoria* were immersed in water at 80 or 100ºC for 1, 30, 60 or 90s and their length recorded immediately after death and after 1, 7, 14 and 28 days storage in 80% ethanol. Using water at 100ºC, mean larval length increased with duration of immersion up to, but not beyond, 60s of immersion: 17.8 ±1.3 mm (90s ), 17.8 ± 1.2 mm (60s), 17.3 ± 1.2 mm (30s) and 15.7 ± 1.4 mm (1s). The range of immersion times examined with water at 80ºC did not give the large range in larval length seen with water at 100ºC, and there was no clear pattern of increasing length with increased immersion time.

Mean larval length showed an initial rapid increase during the first 24hrs of storage in preservative, from 16.9 ± 1.1 mm immediately after death to 17.4 ± 1.4 mm after 1 day in storage, followed by a slow shrinkage during subsequent storage: 17.3 ± 1.4 mm (day 7), 17.2 ± 1.4 mm (day 28). The period of increase in larval length during the first 24hrs in preservative was further examined by immersing larvae in water at 100ºC for 30s and recording their length immediately after death and after 3, 6, 9, 12, 24, 27, 30 and 33 hrs storage in 80% ethanol. The rate of increase in larval length was maximal during the first 3hrs in storage (maximum 0.2 mm/hr) and had returned towards zero after 12hrs storage.

It is clear from our results that, when using length measurements from preserved larvae as a basis for an estimate of minimum PMI, factors such as choice of preservative, method of killing, species involved, temperature of hot water used for killing, and duration of immersion in the hot water must all be considered.

**Keywords:** *Calliphora vomitoria*, larval preservation, post-mortem interval.

**How empty are empty fly puparia ? About the forensic use of dipteran remains**

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The estimation of the PMI (postmortem interval) by calculating the age of the necrophagous larvae on a corpse is most accurate 2-3 weeks after death by using the first colonization-wave, mainly blowflies. After the hatching of the first flies and the occurence of empty dipteran puparia the calculation of PMI is more or less a approximation: The date of hatching is unknown. But are these puparia really empty and useless for a forensic entomologist? Until today we don't have a chemical tool to date the age of a puparia e.g. by the degree of degradation of certain components. But the inside of the left shell could give another evidence.
Examining the inside of the puparia can reveal e.g. the existence of special parasitoids which fed and developed on the blowfly pupae. The finding of parasitoid remnants can reveal important parts of their biology (did they develop solitarious or gregarious? What kind of emergence hole they left?) and together with genetic and gas chromatographic methods it could be possible to assign these remnants to a certain parasitoid species. Sum up the development times of the host and its parasitoid can spread the window of time for a accurate mathematical estimation of the minimal PMI. But the empty puparia can also act as a vacancy for necrophilous tourists. There are clues that beetles of the genus *Necrobia* use abandoned puparia of blowflies as a place for their own pupation. Moreover we can present a case-history where abandoned puparia of the fly *Muscina stabulans* were used for pupation by the clothes-moth *Tineola bisselliela*. Evidences, beside some dead adults on the corpse, were different *Muscina*- puparias with the larval skins, silken cocoons and one dead adult of *T. bisselliela* in a "cocoon-puparia". The consideration of successional data combined with the development data of *Tineola bisselliela* aloud the calculation of a PMI up to six month.
The examples illustrate the need for collecting also obviously empty puparia as an entomological evidence.

**Keywords** : Empty fly puparia, PMI

## Estimation of postmortem interval by using parasitoids of necrophagous insects

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The estimation of the PMI (postmortem interval) is one the most important features in forensic entomology, the use of insects in criminal investigations. Most exact results are obtained 2-3 weeks after death by using the first colonization-wave of necrophagous insects, mainly blowflies. If there are longer periods of death, the methods for calculating the PMI are much weaker, e.g. using the successional stage of the fauna of the corpse. A specific group of insects is hardly used hitherto, despite the fact of their potential use for a exact estimation of longer PMI's: parasitoids.
Parasitoids, in contrary to real parasites, always kill their hosts. The main species belong to the Hymenoptera and Diptera, but the Coleoptera are also a important taxa. The parasitic habit is restrict to the immature stages, and the parasitoid female attack egg, larva or pupa of their hosts. The development is endo- and/or ectoparasitic. According to the parasitoid species there are many intermediate stages regarding the strategy of the parasitoid, e.g. the parasitoid can attack the egg of the host, but the parasitic larva starts its development just during the larval development of the host.
In europe the larvae and pupa of synanthropic flies are attacked by about 80 parasitoid species. Especially pupal parasitoids of blowflies could play a major role for the estimation of a PMI of about a period of several weeks. The time of parasitation is very often restrict to a small, very well defined window of time at the beginning of the pupal development. Moreover, the development times of many parasitoids are well examined due to the fact that they are important biological agent against syanthropic house-flies. After identification of the host and the parasitoid it is possible to sum up their development times. As a result, using these specialised guild of insects could prolongue the period, where we can determine a exact PMI. A case-history illustrate the potential use of parasitoids and the theoretical background.

**Keywords** : PMI, parasitoids

## The importance of death-scene investigations in forensic entomology

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The need for a professional collecting of insects at the death scene has pointed out many times and several authors provided detailed manuals for a suitable entomological data survey. Nevertheless we realize a great uncertainty among police investigators and forensic pathologists when working with insects on corpses. We present three cases which illustrate the importance of an accurate entomological death scene investigation and the need for a adequate communication between police officers, forensic pathologists and entomologists.
1. In July 1999 a 64 years old man was found stroke dead on the floor of his kitchen. Decomposition was in progress and the corpse showed heavy maggot infestation. There were no identication for the time of death and a forensic entomologist was called in. The examination of the entire first floor revealed no puparia, which seems not to be in coincidence with the stage of decomposition of the body. Finally in a last step the basement was checked and many puparia of *Lucilia sericata* were found on the floor of the cellar. Obviously the postfeeding maggots left the sun-exposed, dry kitchen and find a cool and shady place for pupation in the basement after crawling downstairs.
2. In August 1998 a 30 years old woman was found stabbed in her appartment. The forensic entomologist was called in for the autopsy and collected different larval stages of the blowflies *Lucilia sericata* and *Calliphora vicina*. After communicate with the police investigators a examination of the appartment was performed and revealed 6 more insect species and development stages. The most important findings were the pupa of *Calliphora vicina* behind the baseboard of the living room.
3. In May 2000 a 66 years old man was found stabbed in his appartment. Maggots of the blowfly *Lucilia sericata* were collected during autopsy by the forensic pathologists and transfered to the forensic entomologist the next day. During the examination of the appartment by the entomologist two days after the discovering of the body more maggots of the same species were found. The estimation of the postmortem interval via accumulated degree hours provided a period of four days, but the confession of the suspect, which was arrested later, revealed a PMI of five days. The pathologist, who examine the corpse in the appartment, remember a fan close by the body, who was at work and pointed directly on the body on the ground. Police Investigators were not able to confirm that findings exactly and pictures of the appartment did'nt clearify the situation. Nevertheless it has to take in account that the activity of the fan could has be manipulate the development of the blowfly-larvae by creating a desiccating atmosphere on the corpse.

**Keywords** : Death scene investigation, case-history

# Determining time of death using blow fly eggs in the early post mortem interval

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Forensic entomology is usually used to determine time of death in the later post mortem interval, when medical parameters are no longer of value. This usually occurs around 72 h or more after death. However, insects can be used to determine the minimum time since death as soon as the first blow fly (Diptera: Calliphoridae) eggs are laid, often very soon after death.

Although the pathologist usually determines time since death in the early post mortem interval, the opinion of an entomologist can also be very valuable. A case is presented which is interesting for two reasons; firstly, it uses egg development, but secondly, it involves a case which is over 20 years old, and was recently re-opened. The only entomological evidence was in the form of photographs. Usually, it is not possible to make any determinations from photographs alone, as species and age cannot be determined. However, this case was particularly unusual, as close up photographs taken by police at the crime scene showed the first egg eclosion. Weather records, developmental data and degree day accumulations, together with a knowledge of local species and their habitats, allowed the author to determine time since death. Time of death was affirmed when the defendant was convicted of first degree murder more than 23 years after the killing.

**Keywords** : Egg eclosion, Calliphoridae, case study

## Determining time of submergence in the marine environment using successional colonization patterns of aquatic fauna and decompositional rates of carrion

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The analyses of insects colonizing a body over time and their successional patterns are now a common and reliable method for determining time since death for victims found on land, providing that there are reliable data available on successional patterns for that biogeoclimatic zone, season and habitat. However, when a body is dumped in an aquatic environment, time since death is much more difficult to determine. This poster presents some results from our studies of carcass decomposition and colonization in the coastal marine environment surrounding the city of Vancouver, British Columbia, Canada. The work was conducted in two seasons, spring and fall, and two depths, 7.5 metres (“shallow”) and 15 metres (“deep”). Pig carcasses were used as these are considered to be excellent models for human decomposition in previous studies worldwide. Carcasses were placed at their sites by teams of divers from the Royal Canadian Mounted Police, Canadian Coast Guard, Canadian Amphibious Search Team and Vancouver Aquarium Marine Sciences Centre. Each carcass was previously killed by the butcher and immediately taken to its site by boat. The carcasses were tied with rope and attached to heavy, concrete anchors. When placed at their chosen site (shallow or deep), the anchors prevented the carcasses from being lost, but the rope allowed them approximately 3 metres of free movement, so that they sank or floated depending on decompositional stage. Divers with underwater still and video cameras observed, photographed and collected from the carcasses regularly. Typical decomposition characteristics associated with aquatic habitats were observed such as bloating, marbling, adipocere formation, algae accumulation and staining and disarticulation. Decomposition proceeded in a predictable pattern, but many stages, such as that of bloat, were much longer than seen on land. Differences were seen between seasons and depths, although the greater differences were seen between free-floating carcasses and those that became trapped against the sediment. Deterioration was more rapid in deeper carcasses at some times. Faunal scavenging occurred, but was not confined to wound sites. In the fall, fewer species seemed to be depth specific than in summer. This work is ongoing, expanding into freshwater lakes, in BC and Ontario, including the Great Lakes.

**Keywords** : Marine fauna, time of submergence, decomposition

## The value of forensic entomology in investigating wildlife crimes

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Forensic entomology is the application of the study of insects to law. Itis most commonly used to determine time since death in homicide investigations. However, it can equally well be used to determine the time since death of illegally killed wildlife and other animals, although most wildlife enforcement officers are not yet aware of this technology. Insects are attracted to animal remains and colonize them in the same manner and sequence as they do a human corpse. As well, insects can be used to determine many other factors in connection with a wildlife crime as well as time of death, just as they can in a human case. They can be used to determine whether a carcass has been moved, i.e. killed at one site, then parts of the carcass, for instance the hide or paws, moved to a second site. If the animal part has been moved to a different habitat, then insects on the remains may indicate the original kill site. Insects can be used to determine the position and presence of wound sites, as insects colonize a wound site first. Such wounds, such as those created by the removal of the gall bladder, may be missed in a highly decomposed carcass, unless the insects are studied. Wildlife crime often involves the removal of just a piece of the carcass, such as the paws, so insects could be used to determine the time of dismemberment. Some wildlife are poisoned and toxicological analysis of the insects can be used to determine the toxin, long after much of the animal's tissue has decomposed. Insects can also be used to determine the time of injury or entrapment, in cases in which the animal is wounded but not killed. Forensic entomology, therefore has a major role to play in wildlife crimes. Poaching is a very major crime, being considered second only to drug dealing in profit-margin in North America. A case is presented illustrating the use of forensic entomology in a poaching case in Canada, resulting in the resolution of the case, and the first sentence of jail-time in Canada for such a crime.

**Keywords** : Poaching, time since death, wildlife

**Larval development rates for two forensically important flies (Diptera, Calliphoridae) over a range of temperatures**

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Blowflies are commonly used in forensic work to determine the post-mortem interval (PMI). They are suited to this for two main reasons. Firstly, the adult female flies are able to locate corpses, often within hours and generally within 24 hours in the summer, and lay their eggs on them. Secondly, the maggots develop fast and at a predictable rate. It follows, then, that if maggots are recovered from a corpse, it is possible to calculate their age and therefore a minimum PMI. The picture is complicated somewhat due to the fact that a maggot's development is dependent on its thermal history, as well as time. That is, the higher the temperature, the faster the development. The larvae of different species, also, develop at different rates. Limited data exists for the development times of forensically important flies, particularly in the UK. Our work describes the growth rates for *Calliphora vicina* and, to a lesser extent, *C. vomitoria* over a range of temperatures typical of the UK's climate, from 4ºC to 30ºC. In all cases, the development curves show exponential growth to a maximum maggot length, followed by a decrease prior to pupariation. At all temperatures there are a number of individuals that grow more slowly – this appears to be inherent and not a result of competition. We will describe a revised model for larval growth based on the linear model of 'accumulated degree-hours', where development is regarded as a combination of temperature above the minimum developmental threshold multiplied by time. We will also discuss the evidence that biogeographically separate races of the same species have different development rates.

**Keywords :** Diptera, larvae, rates of development

## Diptera larvae biotest as alternative assay for detection of toxicity

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ABSTRACT

The biotest on diptera larvae of *Parasarcophaga argyrostoma* is a reliable technique because it can be used to detect toxicity of different types of compounds; it is an alternative method for the substitution of laboratory mammals that are used frequently for toxicity assays. In this work we have used, marine toxin (ciguatoxin), pesticides (carbofuran, aldicarb, methiocarb and chlorphenvinfos) and heavy metals (Cd, Se, Co and Pb). Ten larvae with synchronic growth are placed in plastic boxes with homogenized beef meat with different toxic compounds to be examined, at concentrations from 100 μg/g to 0.1 μg/g diet and lower if required. The larvae weight at different times compared with the toxic concentrations gives us the percentage of growth increase. To analyse the ciguatoxin (*Gambierdiscus toxicus*) we have used contaminated fish with growth inhibition at concentrations higher than 8 pg/g diet. The pesticides study shows that carbofuran is the most toxic, with growth inhibition at concentrations between 1-0.2 μg/g diet. Aldicarb and chlorphenvinfos show an acute lethal doses of 2.5 and 2.4 μg/g diet respectively. The least toxic is methiocarb with a lethal concentration of 6.25 μg/g. The results show that the most toxic metals were Se, Cd and Co whereas Pb is lower toxic, the NOAEL for both cations should be fixed at 10 μg $Me^{2+}$/diet. The acute lethal dose is established to 400 μg$Cd^{2+}$/diet. Growth inhibition occurs at concentrations of 10-100 μg/g diet.

**Keywords:** *P. argyrostoma*, pesticides, metallic ions, ciguatoxin, toxicity, alternative assay.

## INTRODUCTION

The biotest on diptera larvae (*Parasarcophaga argyrostoma*) used in forensic medicine to establish the time elapsed from death in order to fix the time of decease (Kintz *et al*., 1990), could be proposed as alternative method for toxicity, to establish toxicological end points and as assay for quality assurance in substitution of mammals. The presence of different heavy metals have been studied using diptera larvae (Nourteva *et al*., 1982) in the evaluation of mercury in fish using diptera larvae Sarcophaga. Rayms-Keller *et al*. in 1998, studied the biological effect of different metals during the growth of the larvae Aedes Aeg*ypty* (Diptera:Culicidae).
*Parasarcophaga argyr*os*toma* diptera larvae have been used to detect also marine toxins as ciguatoxin (Labrousse *et al*., 1996). The contamination of fish by ciguatoxin is frequent in the sea of Polynesia and in the Tropical Pacific and Caribbean Sea, because the intoxication of sees dinoflagellates. These enter in the trofic chain of different species of fish to mammal's even humans (Bagnis *et al*.1987; Bourdeau, 1992).
The other group of compounds studied is that of pesticides: carbofuran, aldicarb, methiocarb and chlorphenvinfos. The use of agrochemicals like crop protecting agents (carbamates), veterinary disinfectants and wood preservatives may result in (un)intentional exposure to the environment, of animals and man (Kuiper, 1996; Rouchaud *et al*., 1996). The pesticides studied in this work are carbamate insecticides, methiocarb has shown efficacy in repelling birds from a variety of crops, especially fruit; carbofuran produces behavioural and neurotoxic effects in fish (Saglio *et al*. 1996), and can also cause secondary poisoning of raptors several months after its application; aldicarb is used to control the insects and in the cotton crops and potato crops. Carbamate insecticides are illegally commercialised as rat poisons and commonly used for this purpose, these cause contamination of soil and food (Lima *et al*. 1995; Baron, 1994). Pesticides and heavy metals though occurring in low concentrations posed the threat of their being bioconcentrated and magnified in the higher trophic organism of the food chain (Rao *et al*., 1994).
In this work we also studied heavy metals as cadmium, selenium, cobalt and lead. Cadmium is a heavy metal of increasing prevalence in our environment due to the industrial production (Shaikh *et al*. 1980). Selenium toxicity has been studied because it has provoked ecological damage in several aquatic ecosystems, causing reproduction disorders and the elimination of fish communities (Cumbie *et al*., 1996; Nriagu *et al*. 1988). Lead is also an environmental pollutant, because the main cause of Pb pollution is due to the gasoline and some industrial activities. In the case of cobalt, animal tissues, ambient air, and drinking water are rarely sources of toxic levels of Co. Intoxication in man is usually a result of overzealous therapeutic administration or occupational exposure to various forms of Co. Other not published results on linearity, sensitivity and reproducibility of this assay were presented before (Ribas *et al*., 1999).

## MATERIAL AND METHODS

### Diptera larvae

The larvae of *Parasarcophaga argyrostoma* (Robineau-Desvoidy, Sarcophagidae) are obtained from the incubation of pupae, furnished by one of the authors, placed in a breeding cage at 30ºC with high degree of humidity, and the 12/24 h dark/light cycle was assured by a timing device.
The imagines are maintained in a box covered with tulle tissue and fitted out with a muff allowing access to the inside, a flask with cotton impregnated with water, and sugar in a Petri dish. The experiments are carried out using two cages of 40x40x40 cm, as described classically for their use in Entomology.

### Animal experimentation

Approximately two hundred imagines are obtained and fed “ad libitum”, the 10 first days after the eclosion of the pupae, till numerous mattings of the flies are realized after another 10 days. Biological cycle (ontogeny) of *P. argyrostoma* is between 25-30 days and easy to breed.
To induce lying after the flies have been matted, a piece of beef meat is placed in the cage, and in 20 minutes the fertile females layed their larvae. Larvae grow synchronically on meat overnight and can easily be observed without requiring optical instruments.

### Kinetics of larval growth

The growth test is realized by weighing the larvae one by one at time zero: 12 hours after lying on the fresh beef meat; this initial weight represents 100% of growth. The beef meat was first homogenized with a Waring Blendor and afterwards distributed in fractions of 9g into little plastic boxes, and each one newly homogenized with the concentration of the toxic compound, at 100, 75, 50, 25, 10 µg/g diet.

In this work we use as toxic agents: a.- marine toxin ciguatoxin; b.- pesticides: carbofuran, aldicarb, methiocarb, clorfenvinfos. c.- metals: $CdCl_2$, $Na_2 SeO_3$, $Pb(NO_3)_2$, $CoCl_2$. The plastic boxes are perforated, allowing an aerobic environment for the larvae growth. Then ten larvae with synchronic growth were laid on each one of the plastic boxes containing homogenized diet, and submitted to the before mentioned conditions.

The larvae are removed after 0, 24, 48 and 72 hours, and rolled on kleenex paper to clean them, and weighed again. The weight obtained at the beginning of the experiment is considered at time zero, the value is subtracted from 100, which corresponds to the initial weight of the larvae, to obtain afterwards the percentage of weight increase.

The value calculated in this manner for the control is taken as the 100% reference point (normal growth). All the values obtained on the series of samples are then compared to this standard and expressed as percentage of normal growth. The weight loss or a smaller increment of weight increase compared to a healthy sample indicates the degree of toxicity of the sample, establishing the kinetics of the poison and also the sensitivity of the test and the larvae for the toxic compound.

## RESULTS AND DISCUSSION

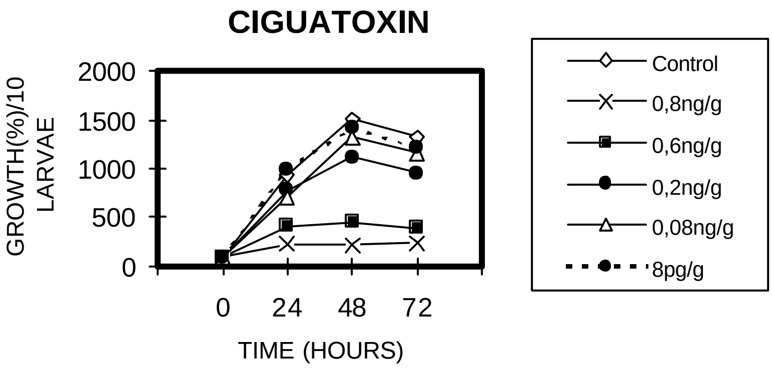
The scientific criticism of animal toxicity testing moved us to a non-animal testing strategy for toxicological design and diagnostic to biochemical and physiological effects of chemicals and toxins. The European Union Chemical Strategy will succeed in its current form if significant numbers of tests are proposed and suited to validation, drecreasing significantly animal testing, acceptable to scientific and public opinion.

The proposed bioassay is simple in its theory and practice. An important asset is the technical simplicity, which eliminates extraction and purification steps and electronic or expensive apparatus. The development of this technique does not require high-level training. It is very easy to perform and inexpensive because it does not need complex materials nor equipment, moreover it is easy and quick to breed the insects at low cost. This alternative assay with ten synchronic diptera larvae may produce better, more reproducible and faster results.

The larvae used in this assay can be fed easily with a wide variety of protein samples like meat, viscera, boiled eggs, shellfish and other (Labrousse and Matile, 1996). It does not require a special breeding, being very useful for toxicological experimentation. We intend to use it to detect poisons of mineral and organic compounds like pesticides and sea toxins, in different animal species like fish, birds and mammals. It would be especially interesting to detect hazard of poisoning in wildlife protected animals, because of the increasing use of pesticides and new synthetic compounds. We applied to detect toxicity in liver of birds of prey poisoned with pesticides applied in agriculture and used in Farms, before submitting the purified extract to instrumental analysis, in substitution of the biotest in mammals (not published results). There can be used a high number of larvae (140 or more) per experiment and each one with two assays, which render the results statistically more reliable and for some compounds more sensitive than other techniques.

The biotest, after validation, could also be used as an interesting alternative method for the substitution of laboratory mammals (mice, rats, rabbits and others) that are used frequently for animal experimentation to resolve some assays of toxicity. It is a versatile technique because different types of toxic can be detected. In this experiment we use a marine toxin (ciguatoxin), pesticides (carbofuran, aldicarb, methiocarb, chlorphenvinfos) and heavy metals (cadmium, selenium, lead and cobalt). To analyse the ciguatoxin (*Gambierdiscus toxicus*) we have used contaminated fish (moray: *Gymnothorax javanicus*) furnished by one of the authors; the ciguatoxin produces growth inhibition at concentrations greater than 8 pg/g diet and clear effects at 0.1-0.2 ng/g diet. The NOEL is 8pg/g diet (Figure 1).

*Figure 1*. Effect of ciguatoxin (ciguatoxin: *Gambierdiscus toxicus)* on the percent growth of the larvae *P. argyrostoma*.




PESTICIDES

The pesticides study shows that carbofuran is the most toxic, with an acute lethal dose of 1.5 µg/g diet, and with growth inhibition at concentrations between 1 µg/g diet and 0.2 µg/g diet (Figure 2). Methiocarb is the less toxic with an acute lethal dose of 6.25 µg/g diet (Figure 3).

*Figure 2.* Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with carbofuran.

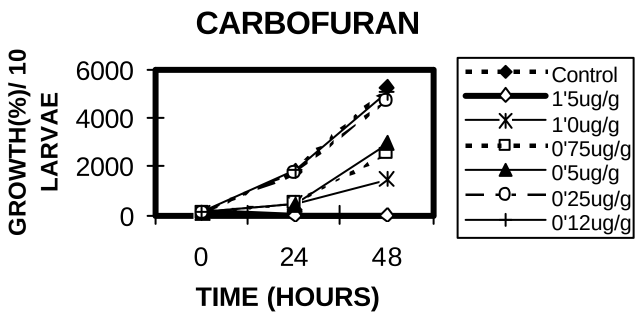
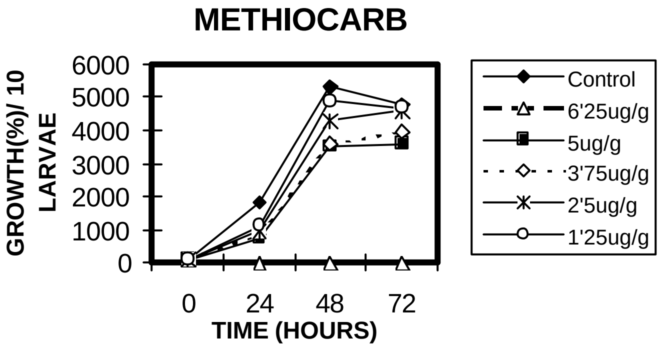



*Figure 3.* Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with methiocarb.



Aldicarb (Figure 4) and chlorphenvinfos (Figure 5) show acute lethal doses of 2.5 µg/g diet and 2.4 µg/g diet respectively.

*Figure 4.* Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with aldicarb.

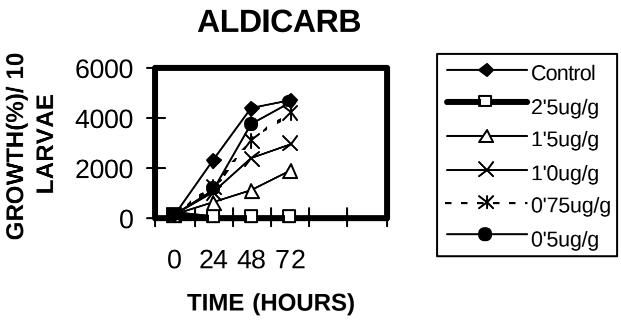
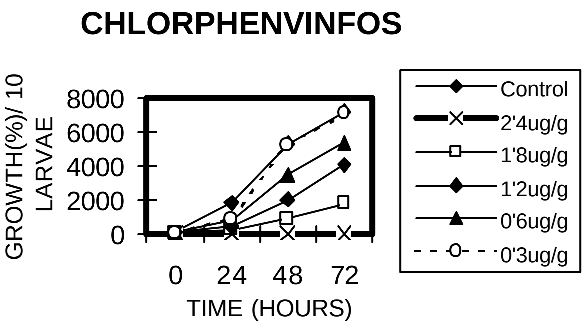



*Figure 5.* Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with chorphenvinfos.



HEAVY METALS

The results show that the more toxic metals, with lower larvae growth, are Cd (Figure 6) and Se (Figure 7). The growth inhibition for these metals occurs at concentrations between 10-100 μg/g diet. For Cd the acute lethal dose is established at 400 μg $Cd^{2+}$/g diet, and the NOEL is 10 μg $Cd^{2+}$/ g diet. After cadmium intoxication in humans, metallothionein concentration is increased in kidney, liver and pancreas, and it is encouraged its determination after extraction and submitted to capillary electrophoresis. In Forensic Medicine cadmium ions should be also measured by atomic absorption spectrophotometry or ICP-MS, very realisable techniques for this cation.

*Figure 6.* Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with cadmium.

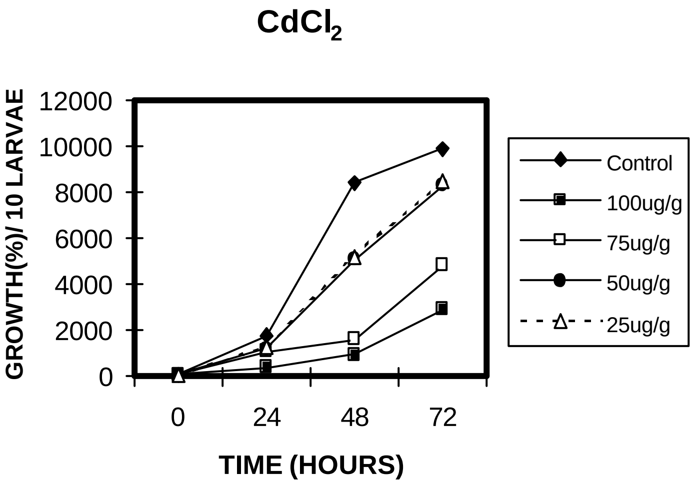


*Figure 7*. Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with selenium.

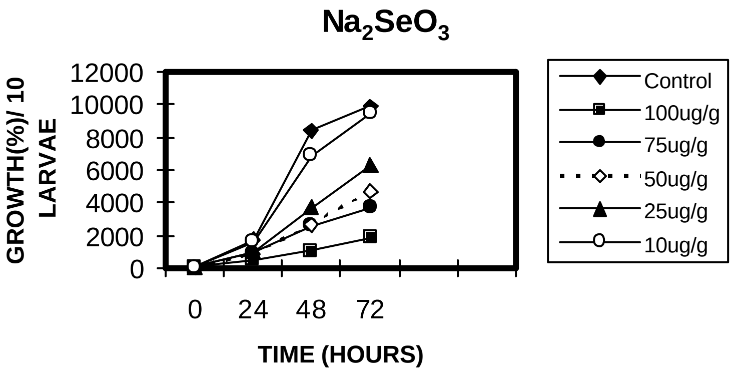



*Figure 8*. Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with cobalt.

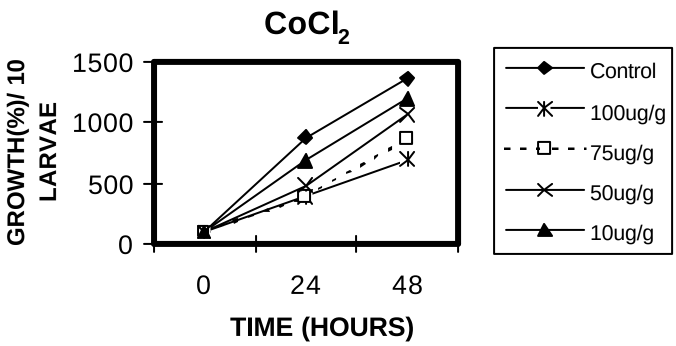
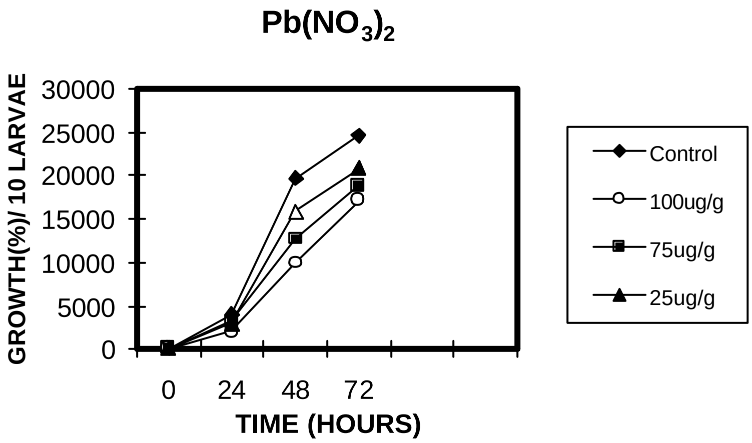


*Figure 9.* Kinetics of larval growth expressed as the percent of weight increase in control larvae and treated with lead.




Selenium is considered a cell protector agent against free radical attack and structural component of defence enzymes through the reduction processes like the selenium dependent enzyme glutathione peroxidase, but the recommended daily allowances (RDA) (70 µg/human adult/day) and the therapeutic doses are near, with the possibility to be a poison as high as 3 mg/day chronically, by its actual great use by the society as medicament. In the biosphere and earth-crust is not so common as lead, and other cations, which could be the cause of its toxicity by the induction of free radicals. On the other side cobalt is not so toxic as selenium, a diagnosis of toxicity should be due to the chronic ingestion between 90 and 225 mg cobalt, it is a structural element of cobalamine essential for iron absorption and induce the catalytic activity of important enzymes and proteins (Glucokinase and participating in the involvement of kidney in the glucose tolerance factor for insulin secretion and its regulation).

Lead is a heavy metal frequently polluted in environment and extensively used in metal alloys and industry after the decreased uses in gasolines. The effect of this toxic agent in blood of human beings for its metabolic action and by the lysis and its incidence to the erythrocytes membrane, induce to anaemia and it is accumulated into the kidney and brain. In this work it is used nitrate $Pb(NO_3)_2$ because its solubility and reliability by use in solution and accurateness for dose schedule in diet of larvae. This element with lower toxicity using the diptera larvae test, in comparison to other cations (Se, Cd and Co) may be due to the genetic habituation by the old presence of Pb in the earth-crust and the biosphere. The dose-response effect curve on the larvae weight show very close values with doses from 25 to 100 μg/g diet, and disturbing growth as it is seen in the before mentioned curve. To detect toxicity with diptera larvae in Forensic Medicine, ingested food and liver or kidney of poisoned subjects should be submitted to the test. After chronic exposure of lead, atomic absorption spectrophotometry and ICP-MS are the methods to apply in liver, bone and kidney.

This bioassay could be used in the future, to observe mutagenicity and genetic end points. It is thus necessary in toxicology to investigate chemicals with a test battery, which can detect all different genetic end points. The ontogeny of 30-40 days suggests using this diptera larvae to be submitted during several generations for genotoxic effects in a brief period of time. There can be observed the physiological alterations after the toxic effect during different generations. It must be assumed in certain cases, that a single molecule of a mutagen is enough to induce a mutation. The bioessay can be applied in order to identify mutagenic agents, and to establish the relationship between mutagenicity, carcinogenicity, teratogenicity and sterility. The results of these studies could furnish new data on the safeguard of the human gene pool and on the possible opposition to the genotoxic action of chemicals. This alternative assay is also useful to compare tissue drug levels with larval drug levels, and to detect the induction or inhibition of molecular biomarkers of exposition and/or environmental pollution.

**Acknowledgements**

This research was supported by the grant Nr 98/0050-02 Fondo de Investigación Sanitaria (FIS), Spain.

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**Atypical arthropod succession on pig carrion in a central european urban habitat**

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Forensic Entomologists frequently utilise insect succession data (sequence of arrival at the cadaver) to draw conclusions about the postmortem intervall and to determine wether remains have been moved after death. The estimation of time since death using succession data is mainly based on the assumption that insects colonize the remains in a predictable sequence. However, the species of insects associated with a corpse, and their times of arrival can vary considerably with geographic region due to many parameters. In addition, many if not all carrion succession studies involving larger carcasses have been conducted in a rural scenario because of their repulsive nature.

Since human cadavers have been repeatedly found in backyards in the City of Vienna, we conducted a carrion succession study within a restricted urban backyard from May to November 2001 to analyse sequence and composition of the local carrion visiting fauna.

Two medium sized pigs (*Sus scrofa* Linnaeus) weighing 44 and 37 kg, were killed with a shot in the head, clothed with jeans and shirt and placed at a secure research site (latitude of 48°12'N, longitude of 16°22'E) with moderate urban vegetation close to the city centre on May 2nd and August 20th, respectively. Carcass-, soil- and airtemperature were recorded continuosly during the experiment. Insect samples were collected once to twice a day.

In the study conducted Mai-June larvae and adults of *Calliphora vomitoria* outnumbered all other blowflyspecies, followed by *Protophormia terraenovae, C. vicina* and *Lucilia sericata*. Apart from *C. vicina* and *L. sericata*, which are commonly considered urban species, the high abundance of *C. vomitoria* was rather surprising, since this species was found almost exclusively in rural areas by other authors. In the study from August 20th large numbers of female adults of the non-indigenous blowfly *Chrysomya albiceps* began oviposition at day 3 after placement of the cadaver. The predatory second and third instars of *C. albiceps* larvae subsequently almost monopolised the cadaver. *C. albiceps* is generally described as tropical and subtropical species.

The observed northward expansion of its range beyond southern Europe obviously decreases the value of *Chrysomya albiceps* in estimating place of death, in that it is no longer exclusive to southern regions. Moreover, the aggressive feeding behaviour of second and third instar larve of *C. albiceps* on local carrion-breeding larvae could reset the post-mortem insect clock by clearing a corpse of all earlier arrivers. Our results clearly show, that caution must be used when drawing conclusions from succession data generated in different geographic areas.

**Keywords** : Forensic entomology, arthropod succession, urban ecology

Poster Session

**A novel approach on species identification of forensic relevant diptera by molecular means**

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Various different species of forensically important blowflies have been described and identified by means of morphological characters. The most commonly used families of saphrophagous blowflies in forensic analysis are *Calliphoridae* and *Muscidae* (i.e. *Calliphora vomitoria, Calliphora vicina, Lucilia sericata, Protophormia terraenovae, Phormia regina, Fannia scalaris and Mucscinia stabulans)*. These species of blowflies play an important role in the determination of post mortem intervals, manner and place of death. The prefered method to obtain the most precise oviposition time is to raise larvae found on corpse in the laboratory and determine the species of the adult fly after morphological criteria. Due to differences in the development of different flies the exact knowledge of the species is from high importance. Mitochondrial DNA analysis was successfully used to determine forensically important flies of various geographical regions. In this study we used a part of the mitochondrial gene encoding for cytochrome oxidase I (COI) for sequencing. Due to its large spectrum of substitution rates, this gene allows both intraspecific phylogenies and higher level systematics of diverged taxa. As discussed in previous studies the COI sequence data could differentiate the observed species successfully.
In our study the fly species under consideration were morphologically identified and their individual haplotype was determined. Flies, larvae and pupae from particular cases where also screened and their species was determined. The work presented here shows first sequence data from COI of Austrian blowflies from field collected material and laboratory lines. Here we examine intraspecific and interspecific mitochondrial DNA sequence variation of nine different necrophagous fly species commonly present in Austria.

Reconstructing phylogenies from intraspecific data such as mitochondrial DNA variation is often a challenging task because of large sample sizes and small genetic distances between the individuals. The resulting multitude of possible trees is best expressed by a network which displays alternative potential evolutionary paths in the form of cycles. Median joining networks successfully have been used for the genetic differentiation of human populations as well as particular fish and cattle groups. We used this phylogenetic approach of haplotype analysis to have a prompt and exact tool to verify species under consideration.

**Keywords**: Forensic entomology, mitochondrial DNA, haplotype network analysis

# *Thyreophora cynophila* (Diptera, Thyreophoridae) has disappeared from France ! What's the police doing ?

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To my knowledge, there are to-day only 2 species of insect of forensic interest which survival in nature is questioned.

*Nicrophorus americanus* a neartic species of Coleoptera Silphidae, for which american authorities have spent considerable amounts of money for its study, reintroduction or preservation in its original habitat. The reasons of the decline of this species are still questioned and many hypotheses have been put forward. *Nicrophorus americanus* exist only now in six states of the United States (Ratcliffe, 1999).

*Thyreophora cynophila*, of the dipteran family Thyreophoridae (close to the Piophilids), is known only from Western Europe. Following Séguy (1950) this species has not been collected, nor observed, since the middle of the XIXth century !

The species has been collected from carcasses of dogs ("*cynophila*"), donkeys, horses, in january and february, as mentioned by Séguy following Macquart.

Very little is known on the biology of *T. cynophila* It is supposed to have been found on large vertebrates bodies. My hypothesis is that its development occurs in the marrow of mammal bones.

*Thyreophora cynophila* is a scarce species in the collections, but it is probably to day an extinct species in the wild, at least in the western regions of Europe. One of the reasons is that no large vertebrates like horses or donkey are left dead in the open. Also, wild animals like boars, deer, …are not any more predated by wolves, thus leaving the long bones unopened, hiding the medullar canal and the marrow.

In Eastern countries of Europe, with large forest and a rather well preserved predator mammalian fauna (wolves), *Thyreophora cynophila* may still exist. This is to be verified.

**Determination of nymph development stages by observations of morphological characters *Calliphora vomitoria* (Linné) (Diptera: Calliphoridae) - Diptera of forensic importance.**

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## INTRODUCTION

The use of entomology, as a forensic science, needs a good knowledge of the development stage of Insects colonizing cadaver correlated with growth rates.

We propose to describe different development stages of the nymph (pupal stage) obtained by observations of morphological evolution after puparium's dissection.

## OBJECTIVES

Majority of growth rate tables used the relation "Time of development / Temperature" are valid for a total development (egg to adult) or larval stages (1st, 2nd, 3rd and prepupae). When Crime Scene Technician collects dead pupae, it is difficult to estimate, with an accurate method, the age of the death pupae. But pupae stage represent more than 50% of the total development of immature stages.

The aim of this study is to sequence the nymph evolution and described it in percentage of the global time of metamorphosis stage (pupa).

Percentage method allowed using these observations in each kind of climate condition or method in age estimating.

## MATERIALS AND METHODS

Insects are reared in incubator: 24°C ± 0,5 ; H.R. 75% ± 20, beef meat ad libidum and four daily observations).

Each day, only 2 hours-aged-pupae (colour of puparium nearly white) are collected and put in a box "J=0". Day after day this box become J+1, J+2,…J+n

Between J=0 to emergence, 15 pupae are extract every day and killed in alcohol 75%. Dissection of puparium and observations are used under LEICA stereomicroscope (X 40).

Morphological characters are chosen because of theirs discriminating capacity: legs segmentation, aspect of palps and proboscis, presence and colour of pilosity, eyes pigmentation, …

## RESULTS

Observations are reported in dichotomise identification table.

We can use the results with constant or fluctuant temperatures with ADD method or growth rates tables at constant temperature.

The different controls at 17, 30°C and external conditions show a real capacity to predict the age of the pupae with a good accuracy.

The same study has been done for *Calliphora vicina* R.-D., *Lucilia sericata* (M.) and *Protophormia terraenovae* (R.-D.) [Diptera ; Calliphoridae].

The use of this simple and accurate method in a forensic way can improve the Post Mortem Interval estimation.

# Effects of magnetic fields 50 hz 2.7 mt on physiological parameters during ontogeny of diptera *P. argyrostoma*.

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SUMMARY

Extremely low frequency magnetic fields (ELF-MF) 50 Hz, 27.5 Gauss (2.7 mT) are applied to study the potential biological effects on development during the ontogeny of diptera *P. argyrostoma*. Molecular changes of physiological parameters could help us to establish molecular markers for human and animals during the exposure to electromagnetic fields. With the aim of substitute laboratory mammals by diptera, we apply before validation, as "alternative assay" the diptera *Parasarcophaga argyrostoma*, to exposure to this fields and observe the possible molecular changes as biomarkers. One of our aims in other parallel experiments, is to observe the possible effects of magnetic fields on the electron flow mechanisms in animals, to establish the dose-response curve, and the synergic effect, with the participation of chemical environmental pollutants. The proposed brain tumours and leukaemia, as emerging effects in humans, are not yet scientifically justified. *P. argyrostoma* pupae, flies and larvae are exposed during its development cycle (ontogeny) of 25-35 days to ELF-MF (50 Hz, 2.7 mT) in comparison with others used as control. Experiments are carried out with groups of 300 larvae with synchronic growth, distributed in groups of 25, in plastic boxes with homogenized beef meat, and submitted in each phase (larvae, pupae, fly) to analysis of physiological parameters in comparison to the non exposed ones. Enzymes (GOT, GPT, GGT, CPK, LDH and Alc. phospatase) and other analytical parameters (glucose, albumine, creatinine, urea, uric acid, bilirrubin, Ca, P) are determined. It is showed after the exposure of pupae, that generally all physiological parameters are inhibited, but statistically significant only GOT, GPT and Alcaline phosphate. In the case of the fly stage, the enzymes CPK, alcaline phosphatase, total proteins and urea are statistically significant inhibited, whereas glucose is increased may be as energy pathway for ATP synthesis with regard to the control ones. Other measured parameters and metallic ions are not significative modified in flies by the presence of extremely low frequency magnetic fields of 50 Hz and 2.75 mT. The applied high intensity of 27.5 G, generally not done as environmental pollution, it is applied in this work because lower intensities do not induce any biological changes.

**Keywords** : ELF-MF, biomarkers, *Parasarcophaga argyrostoma*

## INTRODUCTION

Extremely low frequency magnetic fields (ELF-MF) of 50 Hz involve the high-tension power lines and electro-domestic devices. Actually they are submitted to scientific and social discussion because some opinions designed them to the possibility to be implicated to the potential injury on humans and of great interest in Public Health. Some studies provoke concern because the ELF-MF are ubiquitous and installed inside the great cities with high density of population. Epidemiological studies signalised a certain relationship between the exposition to ELF-MF of 50-60 Hz and the tumour induction in the brain and also leukaemia in children (Ahlbom 1997, Saffer et al. 1995, Savitz et al. 1988, Sancho et al. 1995).

Extremely low frequency magnetic fields (ELF-MF) of 50 Hz and intensity of 27.5 Gauss (2.75 mT) are applied to study the potential biological effects during the ontogeny of the diptera *P. argyrostoma* with the aim to establish some possible biomarkers. We apply the diptera *P. argyrostoma* (Labrousse and Matile 1996, Ribas et al., 1999) as “alternative assay” not yet validated in order to detect toxicity. The P. argyrostoma in its different stages of its biological cycle (fly, pupae, larvae) are submitted to magnetic fields and different molecules as enzymes and other physiological parameters are measured.

## MATERIAL AND METHODS

The larvae of *Parasarcophaga argyrostoma* (Sarcophagidae) are obtained from the incubation of pupae. The imagines are maintained in a box covered with tulle tissue and fitted out with a muff allowing access to the inside. The experiments are carried out using two cages of 40x40x40 cm, one of them is maintained inside the solenoid of magnetic fields (50 Hz, 27.5 G) (figure 1) and the second one as the control in one meter distance and the same conditions.

One cage is placed in the geometrical centre of the solenoid, inside the confined electromagnetic fields, meanwhile the other cage with the control animals is situated at 1 m distance, with the same experimental conditions. The first one is submitted to an electromagnetic field with the intensity of 27.5 Gauss (275 volts) generated by the solenoid conveniently evaluated and calibrated expressed thereafter through two graphics. The figure 1 show the solenoid inside of which is placed the box with the diptera in its different stages of its life cycle. Figure 2 show the calibration graphic of the magnetic field intensity.

Figure 1. Solenoid generating magnetic fields for the experiment to 50 Hz and 27.5 G.

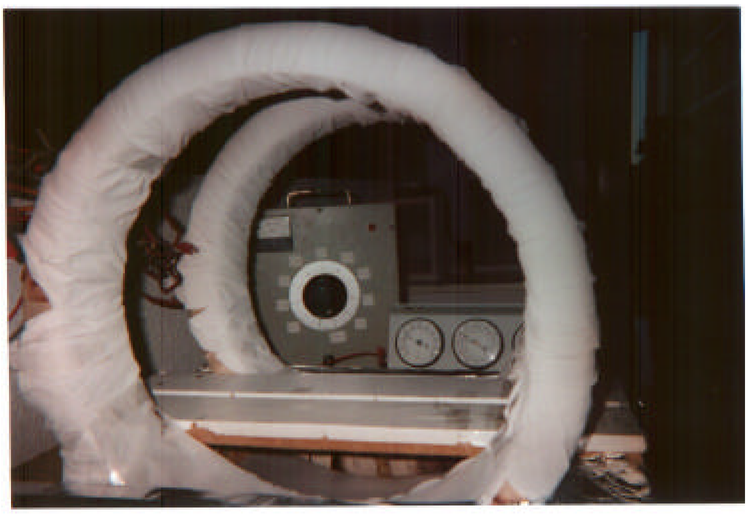


Figure 2 represents the graphic obtained after the calibration of the internal space of the two solenoids of copper wire, in function of the distance of separation of both, showing that the magnetic fields are confined inside the solenoid, within the space of 50 centimeters. Figure 3 shows the evolution of the magnetic field intensity in Gauss, with the current intensity increasingly with the current intensity (I) in amperes. The value of the field to which are submitted the animals is 27.5 (275 volts) and the frequency of 50 Hz.

Figure 2. Calibration of the magnetic fields within the solenoid space.

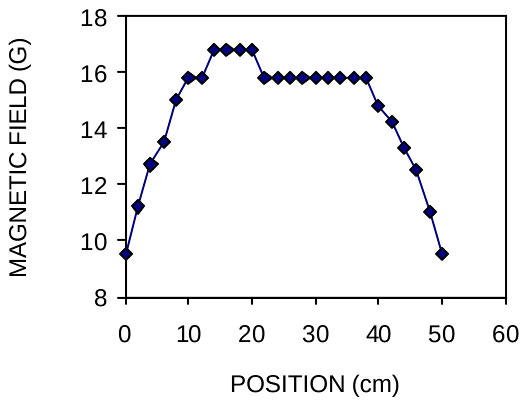
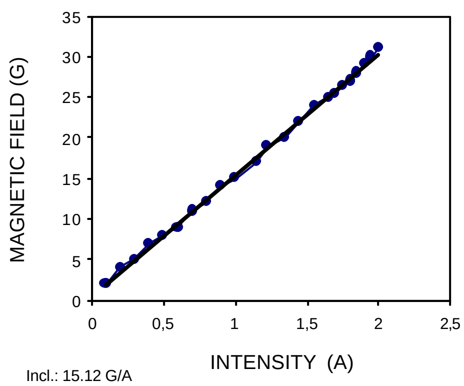



Figure 3. Distribution of the intensity of extremely low frequency electromagnetic fields within the solenoid space.



Biological cycle (ontogeny) of *P. argyrostoma* is between 25-30 days and easy to breed. *P. argyrostoma* pupae, flyes and larvae are exposed during is development cycle of 25-30 days to ELF-MF (50 Hz, 30 G) in comparison with others used as control. Experiments are carried out at the beginning with a group of 300 larvae with synchronic growth distributed in groups of 25, in plastic boxes with homogenized beef meat, and submitted each phase (larvae, pupae, fly) during four generations to magnetic fields. All along the exposition, we study the physiological and enzymatic parameters during ontogeny of *P. argyrostoma*. The analysis of physiological parameters as: enzymes (GOT, GPT, GGT, CPK, LDH and alcaline phosphatase) and another biochemical parameters (glucose, albumin, creatinin, urea, uric. acid, bilirrubin, Ca, P) in comparison with the non exposed controls, were realized after homogenization of the samples between 0.5 to 1 g weight 1:3 (w:v) NaCl 0.8 % with Ultra-Turrax and centrifugation to 14000 rpm. The supernatant is used to be submitted to the analysis with the autoanalyzer of biochemical parameters MEGA (Mitsubishi). The data obtained for larvae, pupae and flies, are expressed in the figures 4 to 9.

## RESULTS

### Pupae

It is shown after the exposure of pupae to magnetic fields with a frequency of 50 Hz, and an intensity of 27.5 G (2.75 mT), that generally all physiological parameters determined are inhibited, although the enzymes GOT, GPT and alcaline phosphatase shown statistically significant changes. This metabolic enzymes are connected to the transaminase activity necessary to the developmental biology during the metamorphosis from pupae to the fly.

Figure 4 . Biochemical parameters in pupae of *P. argyrostoma* exposed to ELF-MF, expressed in mg/g weight of pupae.

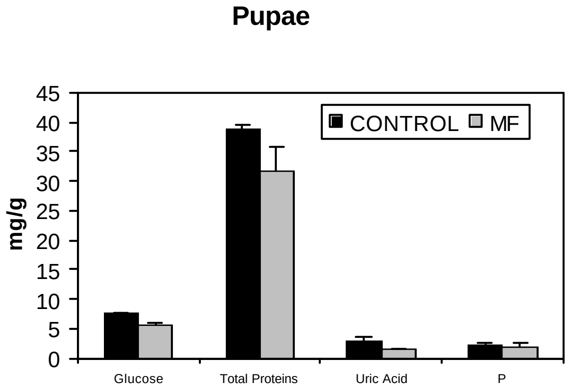



Figure 5. Biochemical parameters in pupae of *P. argyrostoma* exposed to ELF-MF, expressed as mg/g weight of pupae.

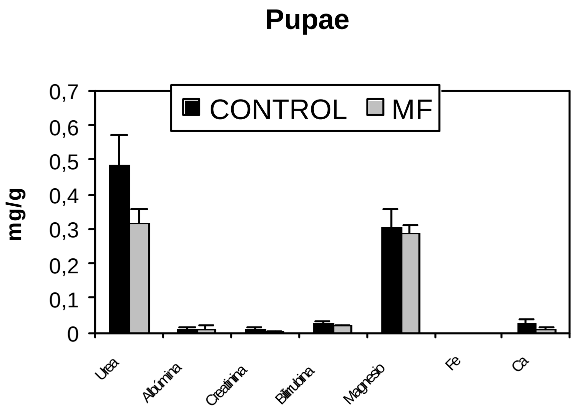
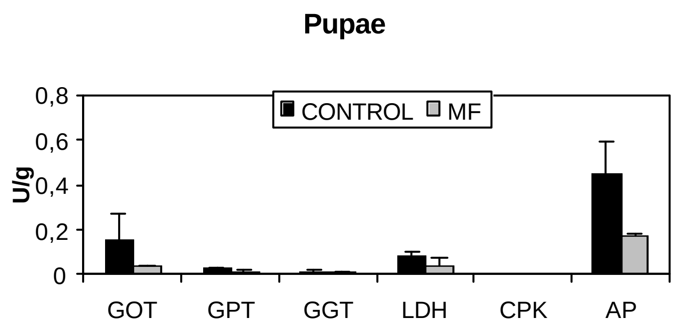


Figure 6. Enzymatic activities of several enzymes during the pupae stage of *P. argyrostoma*, exposed to ELF-MF and in control ones, expressed as Units/gram of pupae.




Fly

In the case of fly, the enzymes CPK, alcaline phosphatase, total proteins and urea are statistically significant inhibited, whereas glucose is increased may be as energetic substrate needed to the fly activity, like the pathway for ATP synthesis with regard to the control ones. Other parameters and metallic ions determined are not significantly modified in flies by the presence of extremely low frequency magnetic fields of 50 Hz and 2.75 mT. The applied high intensity of 2.75 mT, generally not done as environmental pollution, is applied in this work because lower intensities do not induce any biological changes. Mg, Fe and Ca are not modified in comparison to the control ones. There are low changes corresponding to the different ontogenic stage, principally for glucose much higher in the stage of fly, necessary for it activity.

Figure 7. Biochemical parameters in the fly stage of *P. argyrostoma*, expressed as mg/g body weight.

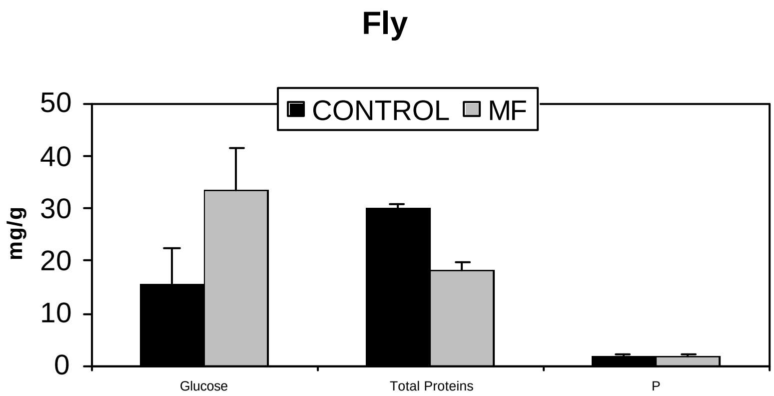



Figure 8. Biochemical parameters of the fly stage of *P. argyrostoma*, expressed as mg/g body weight.

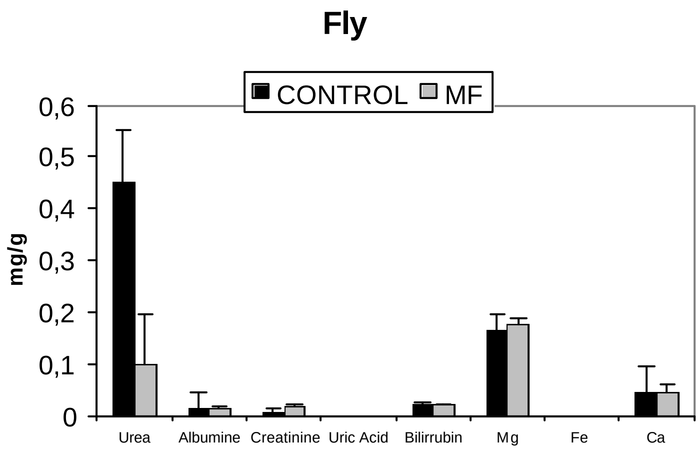
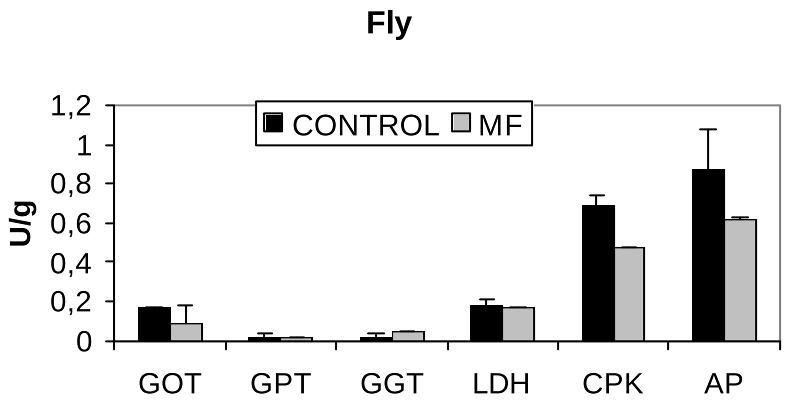


Figure 9. Enzymatic activities of several enzymes as biomarkers of the fly-stage during the exposure of *P. argyrostoma* to magnetic fields, expressed as Units/gram.




The enzymatic activity of the alcaline phosphatase, showed in figures 6 and 9, is inhibited during the exposure of pupae and fly to magnetic fields in comparison to the controls. This enzyme is a zinc-metalloenzyme, if the metallic ions are lost from the enzyme molecule, the enzymatic activity can also be lost under the effect of magnetic fields. It was demonstrated before by several authors (Ribas et al., 1976), that the loss of the structural zinc-ions of the alcaline phosphatase induce the loss of the enzymatic activity of the enzyme. This enzyme could be used as marker of the exposure to magnetic fields but must be corroborated to other animal species before to accept this result. In the fly stage the enzymes present higher activity than in other ontogeny stage because they have a high metabolic signification in adult age. Lactate dehydrogenase is higher in the fly stage by it reversible chemical reaction, because the pyruvate molecule is an important link in the energetic metabolism, necessary for the adult flies, which can be transformed to acethyl-coenzyme A by the pyruvate dehydrogenase and connected to the Krebs Cycle, or to give a glucose molecule through neoglucogenesis. Other enzymes there are not clearly and statistically significant modified.

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**Influence of fluctuating temperatures and population origins on the development of *Calliphora vicina* (Diptera, Calliphoridae)**

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Blowflies are among the first insects to discover and colonise in human corpses. Among the numerous species involved *Calliphora vicina* is the most frequent fly found in our research and is used to calculate post-mortem interval (PMI).
Since this species has been found in about 68% of investigated cases (C. Wyss, unpublished), detailed knowledge of length of development (measured in degree-days) is of prime importance in order to calculate PMI.
Time of development depends on temperature, but a great many factors can influence estimates of insect development. We studied two of them: the influence of fluctuating temperatures and the origins of populations on the development of *C. vicina* under laboratory conditions.
Gravid Females, from three different areas of our country (altitudinal gradient from lowland up to 1000 m a.s.l), were allowed to lay eggs. For each population, eggs were divided into 2 groups, the first one kept at a constant temperature, the second one submitted to fluctuating temperature (the mean corresponding to the constant temperature).
The different temperatures were chosen to reflect our annual local climatic conditions (7°C, 12°C, 18°C for the fixed temperatures and for the fluctuating temperatures the same ± 5°C over a 24 hours period).
Our results showed that at 18°C there were no differences in development time, but for lower temperatures (12°C) larvae reared under fluctuating temperature took more time to reach imago stage, but the opposite was observed at 7°C. This phenomenon is known as rate summation effect. Moreover fluctuating temperatures affect significantly the size of imagoes. We also observed differences in size (larvae as well as adults) between the three populations but this did not affect significantly the development time from egg to adult. We recommend one should be very cautious when using larval size only to determine PMI.
It has long been acknowledged that degree-days (accumulation from egg to adult emergence) is species specific and fairly constant at normal temperatures (that is normal range activity temperatures for this species). Our results nevertheless showed some significant differences form established findings (for *C. vicina*) suggesting strongly that PMI estimates should be done by using data obtained with indigenous populations.

**Keywords** : *Calliphora vicina*, fluctuating temperatures, development