Forensic Entomology

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Arthropods and Corpses

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Summary

The determination of the colonization interval of a corpse ("postmortem interval") has been the major topic of forensic entomologists since the 19th century. The method is based on the link of developmental stages of arthropods, especially of blowfly larvae, to their age. The major advantage against the standard methods for the determination of the early postmortem interval (by the classical forensic pathological methods such as body temperature, postmortem lividity and rigidity, and chemical investigations) is that arthropods can represent an accurate measure even in later stages of the postmortem interval when the classical forensic pathological methods fail. Apart from estimating the colonization interval, there are numerous other ways to use

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arthropods as forensic evidence. Recently, artifacts produced by arthropods as well as the proof of neglect of elderly persons and children have become a special focus of interest. This chapter deals with the broad range of possible applications of entomology, including case examples and practical guidelines that relate to history, classical applications, DNA typing, blood-spatter artifacts, estimation of the postmortem interval, cases of neglect, and entomotoxicology. Special reference is given to different arthropod species as an investigative and criminalistic tool.

Key Words: Arthropod evidence; forensic science; blowflies; beetles; colonization interval; postmortem interval; neglect of the elderly; neglect of children; decomposition; DNA typing; entomotoxicology.

1. Introduction

Hundreds of arthropod species are attracted by corpses, primarily flies (Diptera), beetles (Coleoptera), and their larvae (Fig. 1). The animals feed on the body, and live or breed in and on the corpse, thus depending on their biological preferences, and on the state of body decomposition (Fig. 2).

Because arthropods are by far the largest and most important biological group on earth (they outnumber even plants), they can be found in a wide variety of locations including many crime scenes. This opens a wide range of applications of arthropod evidence to forensic entomologists (i.e., the use of insects recovered from crime scenes and corpses in an investigative or criminalistic context).

By calculating their developmental stage (Fig. 3), arthropods are useful in estimating the time since when a corpse was inhabited by the animals. This estimate is often referred to as the *postmortem interval* (PMI). One has to be well aware that this is, in technical terms, not a determination of the actual PMI in every case because the deceased may have been stored previously in an environment or under conditions that partially restricted access to insects (e.g., very cold, rainy, or tightly sealed environments). The term *colonization interval* is therefore more appropriate for forensic entomology purposes.

Insects are attracted to specific states of decomposition (e.g., bloated decay, fermentation, mummification, or skeletonization). Most species colonize a corpse for only a limited period of time. This change of insects over time is called *faunal succession*.

Together with the knowledge of larval growth rates (always depending on the specific environmental conditions), faunal evidence provides a method for estimating the time elapsed since death, but only if the biological observa-

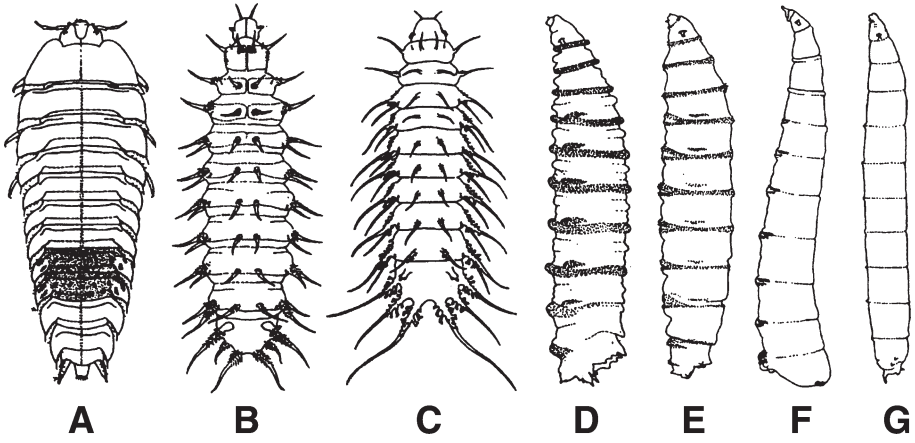

Fig. 1. Larvae of some forensically relevant insects. These insects are attracted by corpses at different yet overlapping times during the first months of decomposition of vertebrate corpses. **(A)** Staphylinid beetle larva (*Silpha* spec., length here: 17 mm). **(B)**–**(G)** fly larvae = maggots. **(B)** Small house fly *Fannia canicularis* (6 mm), **(C)** "bathroom fly" *Fannia scalaris* (5 mm), **(D)** blowfly *Phormia regina* (15 mm), **(E)** green bottle (fly) *Lucilia* spec. (15 mm), **(F)** house fly *Musca* spec. (11 mm), **(G)** cheese skipper *Piophila casei* (8 mm).

tions are set in relation to ecologic, criminalistic, and medicolegal clues *(1–30)*.

There are several other types of information that can be derived from arthropods found at a crime scene. For example, besides the estimation of the colonization time/PMI, the following information can be determined:

- Suspects have been linked to a scene of crime as a result of the fact that they had been bitten by arthropods specific to the vicinity *(119,120)*.
- Insects that live in restricted areas but are found on a corpse in a different area can prove that the body had been moved after death.
- Blowfly larvae can give information on how long children or elderly people were neglected by their relatives or nursing personel.
- Aspects of hygiene (e.g., appearance of larvae and flies in clean, empty rooms, or of maggots in food) can be explained by linking the entomological findings to known death cases or other environmental factors from the surroundings of the death scene *(30,31)*.
- A report describes that in ancient times a murder weapon was identified *(32)*.

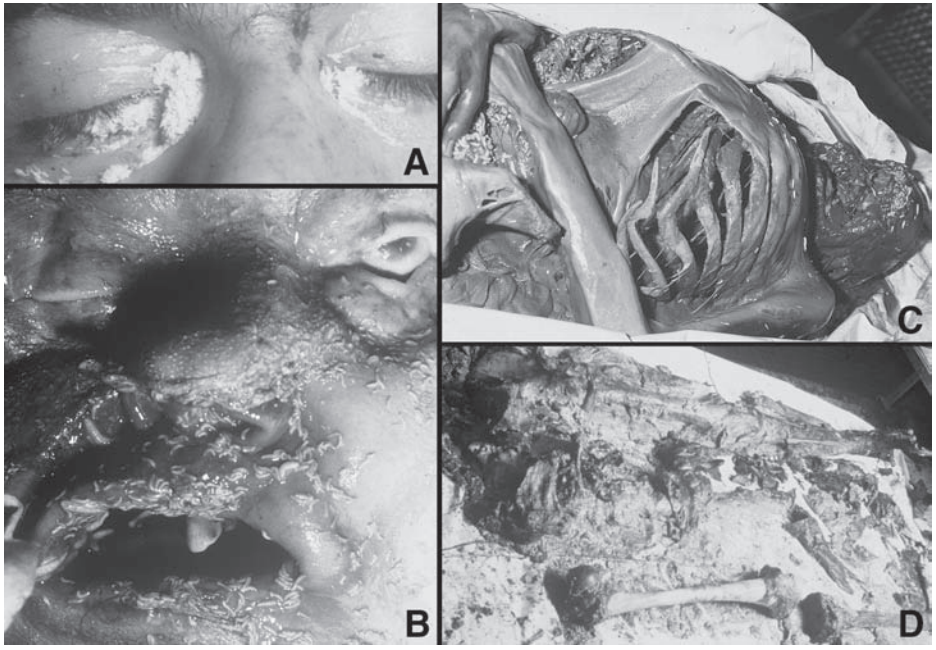

Fig. 2. Different stages of decay are associated with different types of insects and insect activity. Adult pregnant flies prefer to deposit eggs in the eyes, nose, mouth, and ears. **(A)** Patches of eggs (100–200 eggs each) from blowflies (few hours after death), **(B)** first instar larvae hatched and distributed over decomposing tissue (few days after death), **(C)** third instar larvae of flies produced severe tissue losses but left some skin relatively intact (few weeks after death), **(D)** most of the body tissue is vanished as a result of blowfly maggot interference; by now, cheese skipper larvae and adult beetles are present (few month after death). The exact determination of the colonization time ("postmortem interval") is only possible if weather conditions, especially the local environmental temperature, are well-known and documented. The corpses shown in (A)–(D) were found in warm months in Germany (Central Europe).

- Drugs that cannot be detected in severely decomposed tissue of a corpse may still be found in the insects that did feed on the corpse.
- The location of a stab wound can be determined by unusual feeding sites of beetles and maggots *(33)*.
- The question of whether a person was killed and brought outside during day or night time while it was raining or not may be scrutinized *(34–38)*.

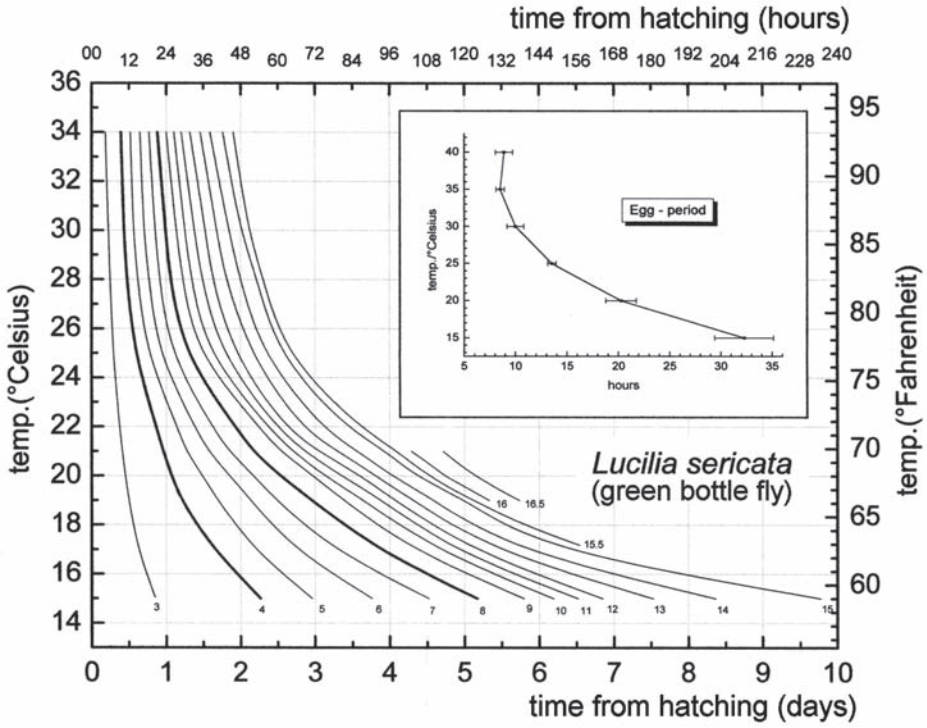

Fig. 3. Isomegale diagram as developed by Reiter and colleagues. Here: developmental times for the green bottle fly *Lucilia sericata,* a common early inhabitant of corpses. Note that the time inside of the egg (*egg period*) needs to be added (insert). (Modified according to ref. *10*.)

2. *History and Early Casework*

The acceleration of decomposition by insect interference has been well documented throughout the past centuries. In many cases, the decompositional stages seen in figurines, paintings, and drawings perfectly match with the influence of insects feeding on corpses (Fig. 4). What seems to be snakes are in many cases larvae of flies and beetles that are located at their preferred feeding sites: face (eyes, nose, ears, and mouth, where they are protected from drying up, rain, and cold) and the gut, after it becomes accessible. It is a phenomenon only of modern times that the actual view of decomposition becomes unusual even to medical doctors.

Correct artistic descriptions of insect activity, especially the early stages of infestation, are present in many sources including the following:

a
hic iacet : Robertus · toule ·
Expecto · resurrectionem · mortuorum ·
die mensis unii prat sibi deus :
b
c
d
e

- Paintings like "Les amants trépassés" from the Musée de l'Œvre Notre-Dame (Strasbourg) from about 1470 (Fig. 4A).
- German woodcuts of "Dances of the Dead" (in German called *Totentänze*) from the late 15th century (Fig. 4C,D) Apart from wars, the exposure to decomposing bodies was partially a result of the plague or a similar disease that killed one-third of all Europeans approximately between the years 1346 and 1351 (but also between 1896 and 1945 starting in Asia and spreading through rats on boards of ships, killing more than 12 million persons worldwide).
- The French poet's Charles Baudelaire's (1827–1876) poem "Une charogne" in the collection of poems *Les fleurs du mal.* It includes an accurate reference to the sound of maggot masses on corpses: "Like water and the wind running/Or corn that a winnower in rhythmic motion/Fans with fiery cunning" *(39)*.
- Sculptures like an ivory skeleton (Fig. 4E), in which the heart is substituted by a blowfly, or the very frequently found *Tödleins* (little skeletons in coffins) from the times of Renaissance and Romanticism.

Artwork such as these accurately depict the insect-mediated pattern of body mass reduction, particularly the skeletonization of the skull and the reduction of internal organs, with large parts of the skin left intact.

The first documented forensic entomology case is reported by the Chinese lawyer and death investigator Sung Tz'u in the 13th century in the medicolegal text book *Hsi Yuan Lu* (one possible translation: "The Washing Away of Wrongs") *(32,40)*. There, he describes the case of a stabbing near a rice field. The day after the murder, the investigator told all workers to put down their working tools (sickles) on the floor. Invisible traces of blood drew blowflies to a single sickle. So confronted, the tool's owner "knocked his head on the floor, and confessed to his crime."

In 1767, the biologist Carl von Linné made the observation that three flies destroy a horse as fast as a lion does, by producing large amounts of

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Fig. 4. (Facing page) Historical depictions correctly show the destruction pattern caused by arthropod activity. What seems to be snakes are larvae of flies and beetles in most portrayed cases. The insects are located at their preferred feeding sites: face (eyes, nose, ears, mouth where they are protected from rain, cold, and drying up) and gut after it becomes accessible. **(A)** Painting *Les amants trépassés*; ca. 1470, Musée de l'Œvre Notre-Dame, Strasbourg, France (Thank you to Burkhard Madea for bringing this to my attention) **(B)** "Here rests Robert Touse", "I await the resurrection from the dead"; most probably created in the 18th century (*118*), **(C,D)** "Dances of the Dead"; late 15th century, **(E)** ivory skeleton "Skelett in der Tumba"; 16th century, Schnütgen museum, Cologne, Germany; A blowfly substitutes the heart and maggots are visible on the skin.

maggots. During mass exhumations in France and Germany in the 18th and 19th centuries, medicolegal death investigators observed that buried bodies are inhabited by various arthropod species.

The first modern forensic entomology case report that included an estimation of the PMI was published by the French physician Bergeret in 1855 *(41)*. The case dealt with blowfly pupae and larval moths: "Within three years, four different tenants (i.e., families) lived in the flat. … The questions we had to deal with now were: (1) was the newborn delivered at an adequate age of gestation/born at the right time? (2) was it a live birth? (3) how long did the newborn live after confinement? (4) how did the newborn die? and (5) how long was the time interval between birth and death?" The first four questions were answered by means of classical forensic pathology; the fifth question was dealt with by calculating the colonization interval/PMI.

Estimation of the colonization interval of corpses started in the 19th century with the (popular) science book *La faune de cadavres* by Pierre Mégnin, which included already detailed case descriptions *(42,43)*. This entomological method then spread to Canada and the United States and then back to Europe. Starting in the 1920s, species lists and monographs on forensically important insects were finally published, putting the focus on ecology, metabolism, or anatomy. Both pest control and "maggot therapy" *(44–47,59)* received considerable attention during this period and many contributions stem from these fields, creating a major scientific source for interpretation of forensic insect evidence.

A detailed review on the early history of forensic entomology is given in ref. *48*.

3. Wound Artifacts and Unusual Findings

At the end of the 19th century, the bite patterns of cockroaches and ants became of interest. The abrasions of skin caused by these animals were sometimes mistaken for signs of poisoning (e.g., as sulphuric acid that had been running down the chin and neck) or wounds caused by appliance of external violence *(49–52)*.

Some researchers also focused on the influence of aquatic arthropods on corpses. *Gammarus pulex,* a freshwater crustacean, was found to produce large numbers of small needle-like lesions. Caddis-flies (Fig. 5) were also found to be of forensic relevance. They destroy skin layers of body parts that are not covered by clothing.

The casings of caddis-flies can also be used to determine how long a body had been in water (e.g., to answer the question when it was dumped in

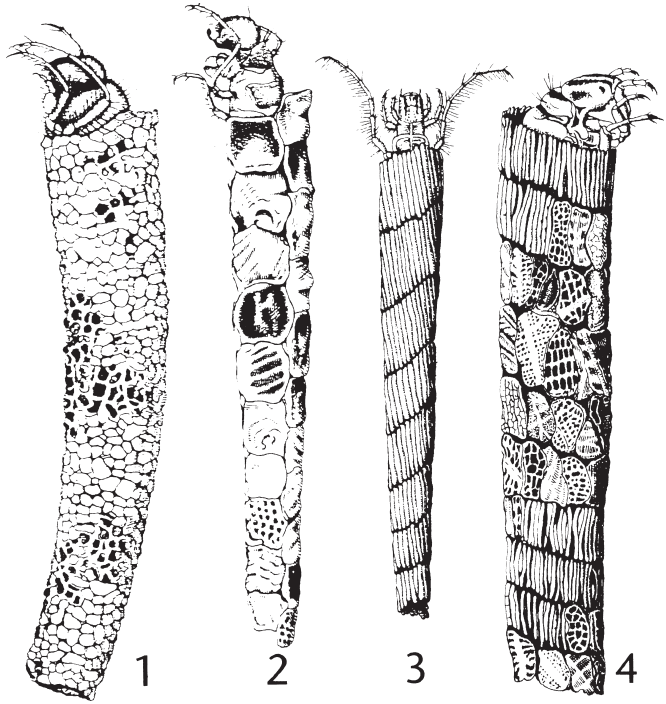

Fig. 5. Underwater (aquatic) arthropods can be used in forensic entomology, too, e.g., to estimate the colonization time of corpses by use of the aquatic arthropod's developmental stages or material used in casings. The animals do not necessarily feed on the corpse but can attach to clothing. Here: Caddis-flies (Tricheroptera) in their casings: **(1)** *Sericostoma* spec. (length of casing ≤15 mm), **(2)** *Lepidostoma hirtum* (≤18 mm), **(3)** *Triaenodes* spec. (≤30 mm), **(4)** *Phryganea* spec. (≤50 mm). Engelhardt in ref. *121*.

the water). In a case from the 1950s a caddis-fly casing (most likely of *Limnophilus flavicornis*) contained fibers of the red socks that were worn by the deceased. However, the fibers were only found at the very top and the very bottom of the casing, which meant that the fly had, for the most part, already built her casing and then finished it at the corpse (fibers on top), and after that attached it to the red sock (fibers on bottom). Because the attachment procedure takes at least a few days, it was estimated that the body had been in the water for at least 1 week *(53,54)*.

Another observation that was first reported in the 1930s were maggots entering the spongiosa of long bones to reach the bone marrow (intact skeleton, PMI 100 days, human remains found outdoors in Bavaria, Germany). It

was suggested that the animals crept through foramina nutritia, tiny gaps in the bones that allow blood vessels and nerves to enter the bones *(55)*. I saw the same phenomenon in an intact skeleton with the body tissues decomposed to a cream-like mass. A large quantity of cheese skipper larvae *(Piophila casei)* had entered the inside of the long bones and were still alive.

4. Exemplary Cases: Neglect of Elderly Persons and Children

Forensic entomology should be embedded in a criminalistic context. As a result of biological variations in growth rates, the influence of the environment can never be fully controlled under field conditions. It is important to give comprehensive answers to those questions that are actually asked by the court, the police, or other investigative authorities involved in a respective case. Therefore, instead of giving an extensive discussion of the determination of the PMI, the cases presented here are intended to show the wide range of possible applications of arthropods as forensic evidence.

Wounds of living persons are potential targets for the same flies that live or feed also on dead bodies during the early PMI *(56)*. This may lead to complications in the estimation of the colonization time. With the field of forensic entomology, and forensic entomologists being more present around the world, even "lower profile cases" (referring to those case in which no external violence has been applied to the victim), like the neglect of older people, come to our attention. Our population becomes older and the increase in the number of elderly people requiring residential care is rising. This leads also to an increased awareness of malpractice and neglect in the professional residential aged care setting *(57)*.

In specific cases, forensic entomology may help one to better understand the circumstances of death and especially those circumstances preceding death. From actual casework we get the impression that nursing injuries of elderly people currently become a severe problem positively correlated with the continuous aging of our society. From a juridical standpoint it is, and will continue to be, very difficult to judge if the nursing personnel in charge is guilty of misconduct. Forensic entomology can give important insights into the dynamics, amount, and final state of bodily care that was given to the respective person in need of care. Forensic entomology may also help to exonerate caregivers (e.g., when maggot infestation of a person's wounds occurred during a normal interval of nonvisits).

It is important that the forensic entomologist should attend the scene because the notice of complex environmental interactions, as well as the col-

Fig. 6. Case of neglect. Elderly woman surrounded by dead stable flies *Muscina stabulans*. Arrow points to intact eyes as an indication for the absence of blowflies that usually colonize corpses. Presence of dead *M. stabulans* strongly hints toward neglect with larvae feeding on excrements of the woman, but not on tissue. No entering/activity of blowflies because windows were closed.

lection of dead animals and pupae, can be problematic for police personnel unfamiliar with the discipline of forensic entomology.

4.1. Clean Apartment With Dead Stable Flies

In October 2002, an elderly woman was found dead in her third-floor apartment in Cologne, Germany. Her skin was mummified, but her eyes, for the most part, were still intact (Fig. 6). The apartment was very clean except for the bathroom, where the bathtub was filled with water and clothing. Apart from larvae, exclusively dead adult flies of the species *Muscina stabulans* were found scattered on the floor and on a window that pointed to the northwest (the apartment had no windows southward). No blowflies in the zoological sense of the word were present in any developmental stage. We decided to base our statement on the fact that all adult flies had already emerged from the pupae. We used the developmental data reported earlier *(12,15)* at a range of reasonably possible room temperatures:

Marchenko (*15*)	19°C	22.8 days
	20°C	21.0 days
	21°C	19.5 days
Nuorteva (*12*)	about 16°C	26–28 days

An approximate minimum interval of around 3 weeks would have been a culpable flaw of the paid professional caregiver who was supposed to look after the woman once every week. The caregiver, however, claimed that she had called the woman about 2 weeks prior, but the elderly woman allegedly rejected any visits. This possibility could not be ruled out because the old woman was known to be healthy, but mentally unstable and displaying "difficult" behavior.

This case shows the importance of a death scene visit by the forensic entomologist: the insects would not have been collected by the police investigators because they did not appear to be feeding on the corpse. They were considered just to "lay around by chance." In marked contrast to the entomological findings, it was assumed that the caregiver had tried her best and no criminal or civil accusation followed.

4.2. Deep Tissue Loss at Wrapped Foot

In September 2002, an elderly woman was found dead in her apartment in an urban town in Germany. Her one foot was wrapped in a plastic bag (Fig. 7). Inside the plastic bag, numerous larvae of *Lucilia sericata* were found. Inside of the flat, the police explicitly stated the absence of adult flies. However, the apartment was in bad shape and even the landlord had noted in January 2002 that renovations were urgently necessary as a result of wet spots in the walls. He also had noted the presence of "small flies." The women did not clean her toilet appropriately, and wet clothing was found in the washbowl. Therefore, a fly population could have been established even without injuries to the woman. To everybody's surprise, the caregiver openly stated that "it is well possible that the foot of the person was wrapped in a plastic bag and that maggots may have been present inside of the plastic bag during the lifetime of the woman." The general practitioner estimated the PMI as more than 2 days. The age of the maggots was estimated from their size (11 mm) as approx 4 days (4 × 24 hours) at a recorded environmental temperature of 20°C.

However, judging by the deep tissue loss of the woman's foot, it was decided that most likely the maggots had been feeding on the living woman for at least 1 week while she was still alive, but then the maggots left the bag to pupate elsewhere. The apartment could not be checked for pupae, however.

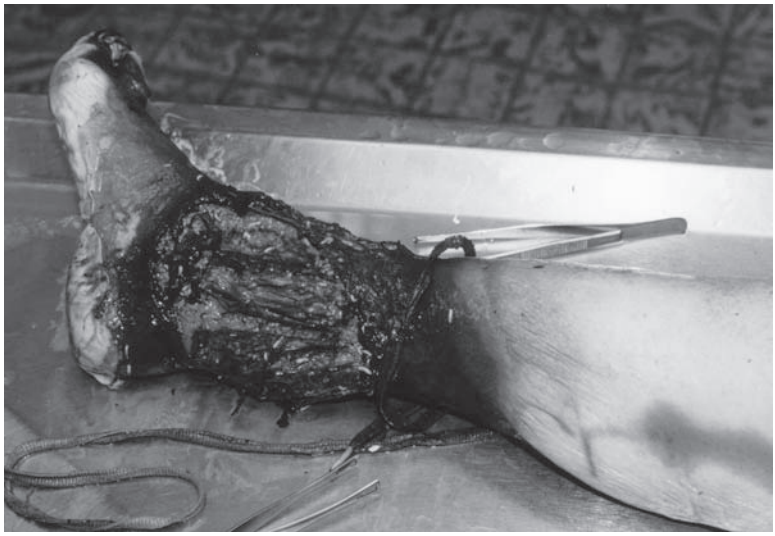

Fig. 7. Deep tissue loss on foot with the remainder of the body intact. Maggots fed on the wound even though the woman was still alive. Lace was used to close a plastic bag around the foot "so that the maggots could not crawl out any more." (Thank you to the Institute of Legal Medicine, Dortmùnd.)

4.3 Dead Mother on Couch

In March 2002, the corpse of an elderly woman was found in her apartment in an urban environment in Germany. The apartment was untidy in non-organic terms, but no rotten organic matter was present. The following insects were found on the corpse: larval *Fannia canicularis* house flies, larval *Muscina stabulans* stable flies, and adult *Dermestes lardarius* larder beetles. These insects are known to build up populations inside of human housings *(37,58)*, but the presence of *Fannia* frequently hints toward the presence of feces and urine (e.g., in cases of neglect).

In this case, further evidence of neglect of the living person was apparent as a result of the fact that the (obviously alive) skin was not fed on by the larvae and that pressure spots (Fig. 8) had formed. The eyes of the corpse were intact. Pupae of an unknown species were mentioned in a police report but they had not been collected. This led us to conclude that the corpse was not inhabited postmortem. If the eggs would have been deposited after the death of the woman, there would have been at least a minimal presence of eggs or larvae in the region of the eyes, ears, or nose because these are, together with wounds, preferred spots for colonization.

Fig. 8. Eyes intact, matching pressure points on the neck. The presence of larval *Fannia canicularis* house flies, larval *Muscina stabulans* stable flies, and adult *Dermestes lardarius* larder beetles strongly hinted toward neglect of this woman (i.e., colonization of the body while she was still alive).

The woman's son was prosecuted for neglect of his mother. He claimed that he fed his mother the evening before she died and that she was well at that time. Referring to the entomological findings and the pressure spots, his statement was not believed by the court.

We could not answer whether or not the woman had suffered from pain by larvae living on her body. From physicians performing maggot therapy *(44–47,59–61)* it is known that blowfly maggots inside of wounds may cause no pain at all, but may also cause severe pain.

4.4. Time of Neglect vs Time of Death

Close cooperation between forensic scientists, medicolegal investigators, and police forces have made it possible to estimate not only the PMI but also the amount of time a child was left neglected. In one particular case, on the skin surface under the diaper of a dead child (anogenital region), third instar larvae of the false stable fly *Muscina stabulans* and the lesser house fly *Fannia canicularis* L. were found. *F. canicularis* adults are attracted to both feces and urine.

From the face, larvae of the bluebottle fly *Calliphora vomitoria* were collected. *C. vomitoria* maggots are typical early inhabitants of corpses. From the developmental times of the flies it was estimated that the anogenital region

of the child had not been cleaned for about 14 days (range: 7–21 days) and that death had occurred only 6–8 days prior to the finding of the body. This led to a conviction not only of the mother but also of the welfare workers involved. The court decided that the time span between the onset of neglect and death of the child was long enough to seek medical advice and help, thus the child´s life could have been saved *(62–64)*.

4.5. Maggots in Only One Eye Socket of a Dead Person

A 41-year-old physician was found dead on his bed. The body was partially mummified and parts of the hip region were skeletonized as a result of maggot activity. In the face, blowfly maggots (*Lucilia [Phaenicia] sericata*) were found exclusively in one eye socket (Fig. 9). This is a very unusual occurrence because on that side a bedlight (40 W light bulb) had been switched on during the 7-week duration of the PMI. All other lights in the apartment were switched off, and no direct sunlight could enter the room where the body was found (only a TV set had been running all the time, about 2 m away from the head, at the foot of the bed). Obviously, the maggots, which usually flee light, had used the one eye that was further away from the light at the bed as the primary feeding source. Because continuing mummification led to a substantial restriction of the feeding material, the maggots finally switched to the eye on which the light was shining *(65)*.

4.6. Credit Card Fraud and Forensic Entomology

In November 2000, a decomposed woman's corpse was found in an apartment in a town in Central Germany. Because the doors were regularly closed, police assumed that the dead person was the tenant. As a result of the severity of decay, the PMI could not be determined by means of classical forensic pathology. On the other hand, determination of the PMI was important in this case because a credit card of the woman had been used, possibly after her death. At the death scene, numerous larvae of the "fly of the dead," *Cynomya mortuorum,* were found.

C. mortuorum larvae are known to normally feed on decomposing animal tissue. In this case, *C. mortuorum* could outcompete other fly species because of the closed rooms/restricted access. According to Nuorteva and Stærkeby *(2,7),* who found that at 15–16.6ºC the developmental time from egg to adult for *C. mortuorum* takes at least 26.2 days (maximum 31 days), we gave a similar estimation of colonization time. It was therefore possible that the bank card was used after the death of the woman *(62)*.

Fig. 9. Partly mummified body. In the face, blowfly maggots of the green bottle fly *Lucilia sericata* were found exclusively in one eye socket. See text for further details on this case.

4.7. Absence of Pupae as an Indication That a Corpse Was Moved

The corpse of a man was found in the trunk of his car. The body was partially decomposed. Because blood was found at the scene where the man was suspected to be killed, and as a result of witnesses' observations, it was assumed that (a) the man had been killed several days before in his car, then had been stored somewhere else and then was moved back or (b) he had been stored all the time in the trunk of the car.

About 1 year later, we were asked for an entomological expert opinion. The car was still in police custody and therefore could be examined. We found that no pupae had entered the gaps between the trunk and the back seats. This was unusual because maggots prefer to pupate in hidden places. Furthermore, the temperature had fluctuated heavily at one point so that maggots were expected to hide from the cold and/or to enter diapause. Apart from species determination of maggots and pupae that had been collected by the police the year before, we delivered the opinion that most likely the person was colonized by maggots at one point and then stored somewhere until many larvae went into a postfeeding or diapause state. Afterward, the corpse was moved back into the trunk of the car where only a few maggots were left on the corpse. Of those few, none entered the gaps. This clue became of great interest for the police and the district attorney's (prosecution's) office. Since this incident, the district attorney makes out search warrants for suspects' houses in respective cases to search them for matching pupae.

5. Collection of Arthropod Evidence

Under real casework conditions it might be necessary to adapt the collection procedure of arthropod evidence to the given situation or to local procedural regulations (e.g., chain of custody regulations). However, for training of federal agents of the Federal Bureau of Investigation (United States of America) and Bundeskriminalamt (Germany) we developed the following guidelines *(66,67)*.

5.1. Ten Golden (and Easy) Rules for Collection of Arthropod Evidence

1. Take very good close-up photographs of all locations from where animals are collected. The state of insect-aided decomposition can severely change within days, even under cool conditions, and even when the body is stored in a cooling apparatus (Fig. 10). Also, bites of mites should be documented on living persons (e.g., possible offenders; Fig. 11).
2. Photograph without a flash. Maggots will "flash out," which means they become "just white nothings," especially on digital photographs.
3. A metric *and* an inch scale should always be used on every single picture (Fig. 12).
4. Collect one spoon full of insects from at least three different sites of the corpse and the crime scene in three different, clearly labeled jars.
5. Put half of the insects in 98% ethanol. Cheap ethanol (i.e., methylated spirit for camping purposes) can be used without any problems. Neither isopropyl alcohol

Fig. 10. Effects of storage on corpses. **(A)** Body found at a crime scene in the very hot summer of 2003 in Western Germany: maggot length did not exceed 5 mm, eyes intact. **(B)** Three days later, at autopsy: the skin is dried up, both eyes are destroyed by the maggots; marked contrast to appearance of the corpse when it was found at the scene of crime.

("hand cleaning alcohol") nor formalin should be used! Killed insects can be stored frozen with or without ethanol.

6. Attempts should be made to kill the animals with hot water ("tea water") before placing them in ethanol.
7. If possible, put half of the insects alive in a refrigerator (not a freezer). Put fabric on top so the insects can breathe. Maturing might become an issue, so forward the animals to a biologist within 1 or 2 days. Keep white larvae separate from brownish larvae and separate larvae from adults if possible.
8. Label excessively: location, exact time, date, initials.
9. If questions arise during collection, a forensic entomologist should be called.
10. Determination (i.e., identification of the arthropod species) *must* be performed by an experienced entomologist using keys that can be applied to the local fauna (e.g., *68–76)*. However, for many regions of the world, appropriate keys are not yet available. Some forensic entomologists determine third instar larvae of known maggot species by the characteristics of the maggot's mouth parts (Fig. 13 *[5,36,74,77,78])*.

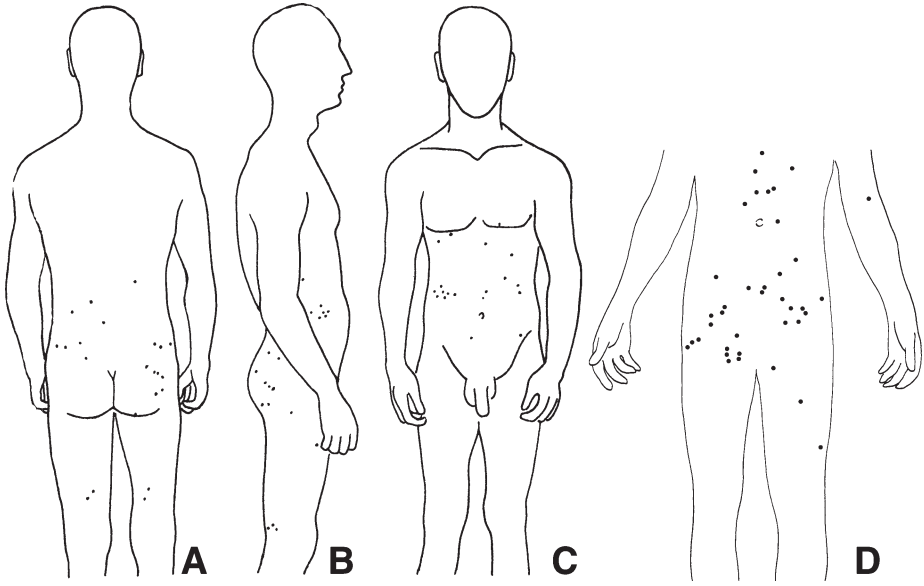

Fig. 11. Photographic documentation of the bite pattern of mites that led to a life sentence for homicide in this case. **(A)–(C)** The pattern of *Eutrombicula belkini* mite bites on the offender was similar to the pattern seen on the body of a police investigator who attended the crime scene. Development of reddening (swelling and shrinking) allowed to match time and location of the offender at the crime scene. **(D)** Self-test of this author in summer 2004 with *Neotrombicula autumnalis* leads to a similar pattern of bites. (For details see refs. *119,120.)*

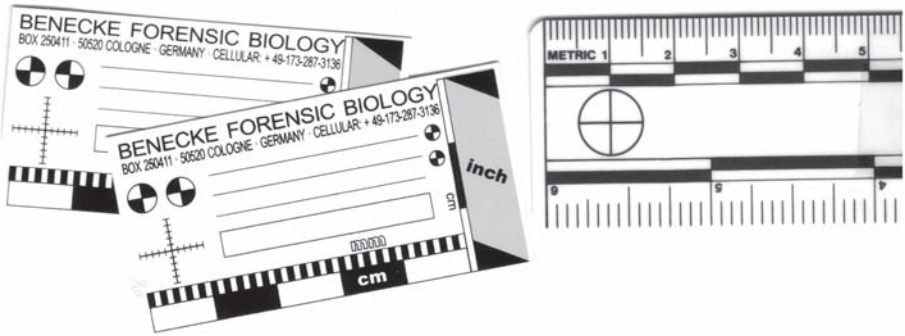

Fig. 12. Use of scales is essential to determine the length of larvae on photographs. International scales should be used as a result of different units of measurement in different countries. (From ref. *66.*)

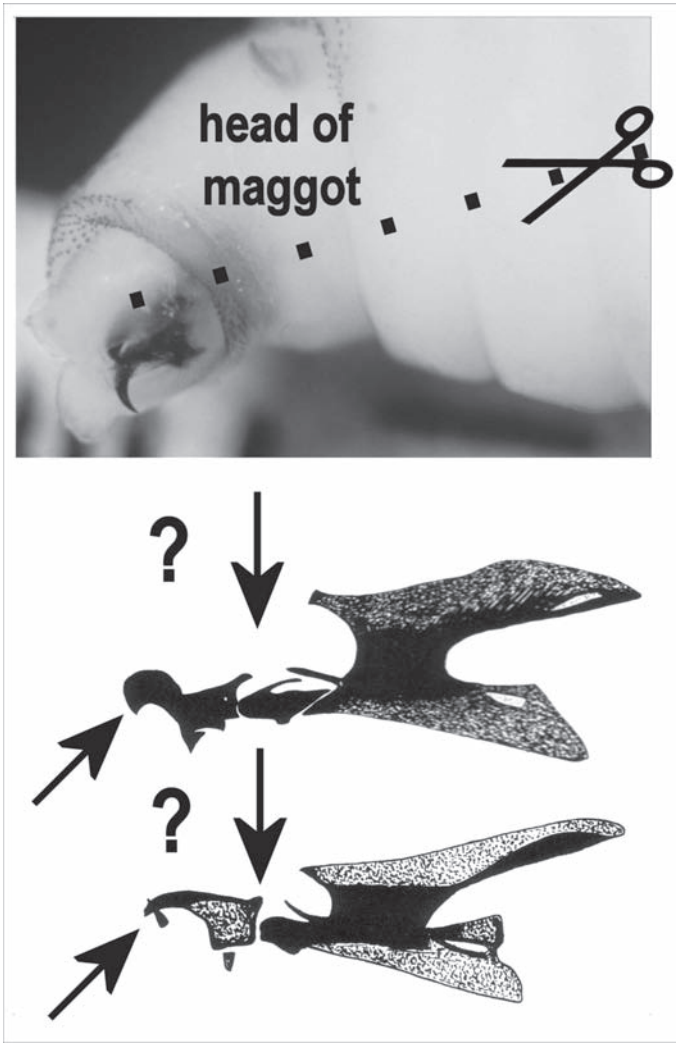

Fig. 13. Out of maggots that are stored in 98% ethanol (no isopropanol, never formalin), mouth hooks (cephalo-pharyngeal skeleton) can be extracted even after years. This may allow determination of species by comparing morphological features like the presence of an anterior apical sklerite (see question marks in drawing), and the shape and coloration of the structures. (From ref. *66*.)

6. DNA

6.1. DNA Typing

Identification of arthropod species found at a crime scene or on a corpse is essential but can be difficult on the morphological level. At the same time, the number of experts for morphological identification of arthropods is most dramatically decreasing since the 1990s.

Since 1985, DNA typing of biological material has become one of the most powerful tools for identification purposes in the field of forensic medicine and in criminal investigations *(79)*. The advantages of using DNA are as follows: (a) it provides a huge amount of diagnostic information compared to some older techniques (such as blood-group typing), (b) it is present in all biological tissues (except of red blood cells), and (c) it is much more resistant to environmental degradation than most other biological molecules (e.g., proteins).

Another benefit of DNA typing is that many loci for polymerase chain reaction (PCR) analysis are less than about 350 bp in length, allowing the use of sample DNA that is strongly degraded and broken into short pieces.

It would be helpful if DNA laboratories of medicolegal institutes were able to support forensic entomologists with DNA typing ("genetic fingerprints") of arthropod specimens. Because these laboratories are used to working with human DNA, such a service can frequently not be provided. Currently, one of the main goals in typing forensic DNA of arthropods is to find suitable PCR primers or sequencing sites for identification of arthropods on the species (not on the individual) level. Possible targets are all types of repetitive DNA like random amplified polymorphic DNA (RAPDs), short tandem repeats (STRs), and all types of minisatellite DNA, as well as nonrepetitive but unique sites on mitochondrial DNA (mtDNA *[80–94]*).

Invertebrates have a noncoding mtDNA region that contains a high proportion of adenine and thymine bases and might be useful for DNA typing in forensic entomology questions. There is a great deal of basic biological information available concerning fly mtDNA. This makes it relatively easy to design PCR primers and to interpret the results of any study on a new fly species. For insects, the base sequence of protein-coding genes like cytochrome oxidase subunits I and II (COI+II) may also help in determining species.

Closely related species can often be separated using a relatively short region (around 300 bp) that can even be obtained from degraded DNA. However, because of intraspecific variation in mtDNA haplotypes, two samples from the same species may not match exactly. Only experience with the taxo-

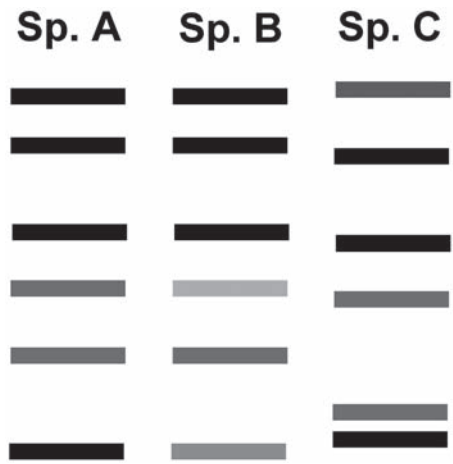

Fig. 14. To determine arthropod species, instead of morphological methods, the banding patterns as produced by separation of (randomly) amplified DNA stretches or cleavage with one or multiple restriction enzymes can be used. Both methods produce a DNA type or "genetic fingerprint" that looks like in this schematic presentation. Comparing species A, B, and C can still be difficult because point mutation may already have severely altered the pattern. Phylogenetic trees derived from restriction experiments/restriction site maps can be helpful if the actual local ecological parameters are known. In the future, direct sequencing may provide a better source for species identification. Still, only experience with the taxonomic group in question will allow an investigator to know if the differences observed between two samples fall within the range of normal variation for that zoological species, genus, or order.

nomic group in question will allow an investigator to know if the differences observed between two samples fall within the range of normal variation for that species *(93–96)*. In RAPDs, a single point mutation can eliminate a restriction site, so a reliable PCR restriction fragment length polymorphism analysis (performed after PCR) test would have to utilize restriction sites that are fixed or nearly fixed for the respective species (Fig. 14 *[92,97–99]).*

For an overview of DNA typing in forensic entomology see ref. *80*.

6.2. Non-Insect DNA

To date, there are only a few published reports on the use of DNA techniques by forensic entomologists. Because it is also possible to identify the gut contents (including human sperm and blood) of carrion-feeding arthropods and, thereby, relate an insect to a living person or a deceased, even when

contact between the two has not been observed, further developments in this field can be expected soon. Insect specimens can thus be a source of non-insect DNA (e.g., that of the organism on which they have fed). Recently, human mtDNA was successfully determined from the gut contents of insects *(100,101)*. Additionally, the gut contents of blowfly maggots were DNA typed as well as successfully tested for prostate-specific antigen that is present in sperm *(102)*. Such analyses may prove to be crucial evidence in the creation of victim/suspect associations. Investigators who find maggots but no corpse now have the potential to identify the insect's "last meal." There are also occasions, particularly if the scene has been disturbed, where both maggots and a corpse are present but not in physical contact. DNA analysis of maggot gut contents provides an independent means for relating larvae to a potential victim. This possibility also may prove useful in cases of multiple homicides or mass burials.

6.3. Collection for DNA Typing

For DNA typing purposes, freezing at –20°C or deeper temperatures as well as preserving in 98% ethanol is strongly recommended. These methods of storage allow one to undertake molecular genetic studies when necessary or to make this material available to others who are typing the DNA for identification purposes even after decades have passed. The old-fashioned method of drying the specimens on a needle may preserve the DNA for years, but storage as suggested above has been shown to be much more efficient *in praxi*.

6.4. Case Example: Maggots From Body Bag

As with all other criminalistic and forensic methods of investigation, DNA typing should not be understood as the ultimate method. For this reason, the German High Court even ruled out the use of DNA evidence as the *only* basis for conviction of a suspect. The following case illustrates that DNA typing has to embedded into the course of biological investigations and interpreted in the light of the actual findings at the scene.

In October 1997, a body that was in a state of severe decay was scrutinized for insect colonization to determine the colonization interval/PMI. Hundreds of maggots of an average size of 9 mm were found on the corpse that was stored inside a closed body bag. Additionally, numerous maggots were found on the outside of this body bag. The question was whether the maggots on the outside had squeezed themselves through tiny holes of the body bag to find a place for pupation, or if a second oviposition had taken place after the body was stored in the bag. Additionally, pupae were found on the floor beneath

the corpse. Because pupae represented the oldest developmental stage of arthropod infestation in this case, the next question was whether these pupae had fallen down from the corpse or whether they had fallen down from other corpses that had been stored in the same cooling room earlier.

Different fly species develop within different times. Therefore, estimation of colonization time is only possible if the insect species is known. In such cases, the insects can be used to estimate the time elapsed since death. The species of maggots, especially that of younger ones, is difficult to identify. For this reason, a quick inexpensive and reliable DNA test by use of RAPDs was applied in the aforementioned case. With it, at least the distinction of different maggot species was possible. The pupae found on the floor beneath the corpse were not related to the maggots found inside the body bag. Their developmental stage was of no relevance in this case. The age of the maggots was calculated and used for estimating the colonization time *(97)*.

7. *Forensic Entomotoxicology*

Insects that feed on tissue that contains substances relevant under toxicological aspects will, in many cases, ingest and store these toxicological-relevant substances in their own tissue. Extraction of the substance out of the insects was successfully used in cases in which the corpse was too decomposed to perform toxicological analysis on tissue samples:

- *Cochliomyia macellaria* blowflies were found on the corpse of a person who had bought phenobarbital the day before he was missing. They contained 100 μg phenobarbital per gram of larva.
- Arsenic, organophosphates, mercury, morphine (>10 μg/g in an empty pupa), cocaine, amitryptiline, 3,4-methylenedioxymethamphetamine, and nortryptiline were found in maggots and empty puparia up to 5 months after persons had died.
- In a suicide case, triazolam, oxazepam, phenobarbital, alimemazin, and clomipramin were detected 67 days postmortem in blowfly larvae recovered from the corpse but not in kidney and liver tissue of the decomposed body (Table 1 *[103–113]*).

It is difficult to calculate the amount of substances that were present in the dying person from the concentrations that are found in insect tissues. However, for qualitative purposes, the method does lead to useful results when embedded in the actual criminalistic context.

Table 1
Substance Concentrations in Tissue and Maggots That Were Found on a Decomposing Corpse (Concentrations in ng/g)

	Triazolam (benzodiazepine)	Oxazepam (benzodiazepine)	Phenobarbital (barbiturate)	Alimemazine (neuroleptic)	Cloripramin (tricyclic antidepressant)
Heart	398	1317	1391	318	2479
Liver	490	403	3630	368	433
Lung	173	1641	1233	344	455
Kidney	no result	286	1439	66	327
Maggot	204	153	761	22	28

Modified according to ref. *108*.

8. Further Artifacts Caused by Arthropods

8.1. Lesions

In contrast to blowflies, maggots, adult ants, cockroaches, and beetles can and will destroy layers of fresh or dried up (mummified) skin.

Dermestid beetles mostly feed on severely dried up corpses, whereas "bacon beetles" and "corpse beetles" (e.g., Silphid, Histerid or Clerid beetles like *Nicrophorus* spp., *Hister* spp., and *Necrobia* spp.) will cause lesions that may resemble close range or long range gun shot wounds (Figs. 15 and 16).

8.2. Blood Spatter

Adult blowflies can transfer actual blood (e.g., from a pool of blood) so that a fake blood-spatter pattern emerges (Fig. 17 *[114–117]*). The typical characteristics of fake blood spatter patterns caused by adult blowflies can be summarized shortly as follows:

1. Stains that have a tail-to-body (L[tl]/L[b]) ratio greater than 1.
2. Stains with a tadpole/sperm type structure.
3. Stains with a sperm cell-type structure that do not end in a small dot.
4. Any stains without a distinguishable tail and body.
5. Any stains with a wavy and irregular linear structure.
6. Any stains that do not participate in directional consistency with other stains that suggest a point of convergence at a point of origin.

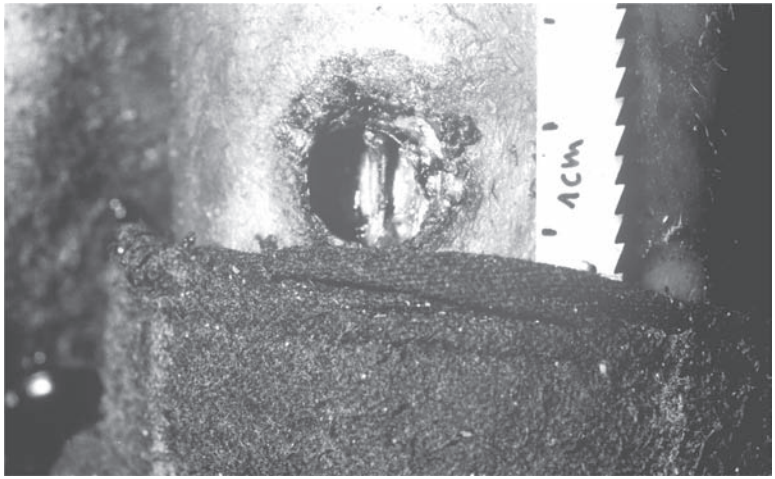

Fig. 15. Artifacts produced by beetles. Adult Histerid beetles produced a lesion that could be mistaken for a close range gunshot hole (partially mummified body found in summer in woodland in Cologne, Germany [Central Europe]).

Fig. 16. Artifacts produced by beetles. Adult Silphid beetles built breeding holes or removed tissue for breeding holes from a corpse that was put into a cooling apparatus during a very hot summer in Cologne, Germany (Central Europe). Maggots are present but did not cause the lesions. The author observed beetles feeding.

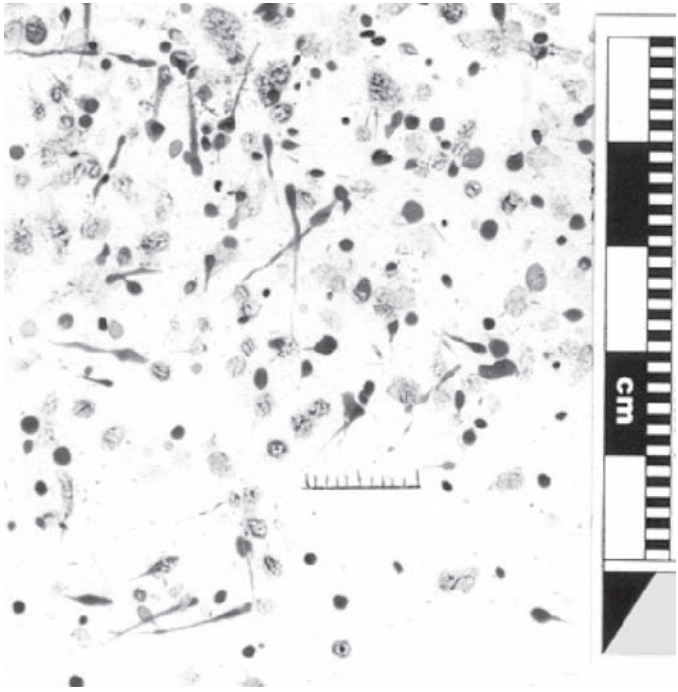

Fig. 17. Bloodstains as here produced by adult blowflies may have similarities with medium- or high-velocity blood spatter. Comparing the features of the fly spots (produced both by defecation and regurgitation) allows a distinction from true blood spatter caused by external violence. (From ref. *114*.)

Larger fly artifacts, within a group, will point in all directions. In contrast, trickling down of human blood or blood spread by violence will produce stains, within a group, that indicates a common general convergence point.

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