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BENECKE M, SEIFERT B (1999) Forensische Entomologie am Beispiel eines Tötungsdeliktes. Eine kombinierte Spuren- und Liegezeitanalyse. (Forensic Entomology exemplified by a high profile homicide. A combined stain and postmortem time analysis (postmortem interval, PMI)) (Archiv für Kriminologie 204:52-60)

[Forensic entomology exemplified by a homicide. A combined stain and postmortem time analysis].

[Original Article in German]

Benecke M, Seifert B.

Abstract (Medline / Pubmed)

The combined analysis of both ant and blow fly evidence recovered from a corpse, and from the boot of a suspect, suggested that an assumed scenario in a high profile murder case was likely to be true. The ants (Lasius fuliginous) were used as classical crime scene stains that linked the suspect to the scene. Blow fly maggots (Calliphora spec.) helped to determine the post mortem interval (PMI) with the calculated PMI overlapping with the assumed time of the killing. In the trial, the results of the medico-legal analysis of the insects was understood to be crucial scientific evidence, and the suspect was sentenced to 8 years in prison.

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Introduction

The arthropods living on corpses can support death investigations by the various conclusions derived from them. In addition to the determination of the post-mortem interval of even skeletal bodies (e.g. Lord et al. 1994), a plethora of further conclusions are possible, e.g. on the post-mortem relocation of a corpse and toxification of a body (Goff & Lord 1994), if necessary, even after skeletonization has already taken place. Arthropodological investigations have also been successfully applied in work processes (Nuorteva 1977), cases of child neglect (Lord 1990), questions of hygiene and legal medicine (Benecke, submitted) and in the investigation of perpetrators living far from the scene of the crime (Webb et al. 1983, Prichard et al. 1996). In the meantime, in addition to the necessary growth curves of the animals (Reiter 1984, Nishida 1984, Smith 1986), many of the possible deviations have been statistically recorded (Schoenly 1992, Schoenly et al. 1996, Introna et al. 1989).

While police entomological departments have already been established in the Franco-Belgian area and in the U.S.A. (among others at the French Gendarmerie Nationale and the FBI; see also Leclercq et al. 1973, Leclercq et al. 1988, Lord 1990, Nuorteva 1977, Héduin et al. 1996, Catts & Goff 1992), there is often a lack of awareness or technical equipment, at least among scientists in the German-speaking countries, to make use of the enormous possibilities of forensically applied entomology and arachnology.

The decisive step before the animals are expertly identified is the correct preservation of the animal material. Only proper storage will enable the entomologist to correctly identify the species by one or more of the following characteristics of the animals, each of which can decide on a case-by-case basis whether identification is possible or not: size, body attachments (bristles, antennae, spiracles), color, degree of hardening of the body shell. In addition, in order to take account of possible natural variations, a sufficient number of animals of the same species must be collected from a corpse (and, if crime scene work is possible, from the surrounding area). Furthermore, a good photographic documentation of the findings on the corpse is desirable. Experience teaches us that at the site of discovery, usually no macro photographs are taken with simultaneously photographed millimeter scale, or that the photographer does not focus on the arthropods but on the areas underneath them. Although it is almost impossible to determine insects reliably on the basis of photographs, in individual cases, good photographs allow statements to be made on highly restricted but nevertheless relevant issues such as the approximate time of transfer of corpses to the open air (Benecke, unpublished). Good overview photos can also document the location of the animals.

This location information can help to present arthropod-accessible and -inaccessible surface areas of a corpse.

Collection and Storage Methods

a. General Information

The basically correct way to store each arthropod for forensic-entomological examination is to store it in a jar with a snap-on lid filled with 70% ethanol. Transparent containers for 35 mm film or screw-on plastic test tubes with round bottoms have also proved to be very useful. 1.5 mL Eppendorf reaction tubes and centrifuge tubes are difficult to use because of the tapered bottom, in which parts of the animals are often held by adhesive forces.

If no 70% ethanol is available at the place where the corpse was found, rum, clear liquor or the like can be used for a few days in any closable container. 70% alcohol both prevents fungi, yeasts, and other insects from destroying the evidence, as well as keeps the animals flexible. The mobility of body and leg limbs, which is rapidly lost during drying, is important in forensic entomology, for example, to check the existence of hypopleural bristles of flies located laterally at the back of the ribcage. This bristle fringe (Fig. 1) is often difficult to see, but allows a reliable distinction to be made between blowflies (Calliphorids) and Muscids, two fly families common on corpses, which sometimes look deceptively similar on the outside, but can represent different growth rates and ecological environments. Alcohol is (in addition to deep-freezing the animals in situ) also a suitable storage medium in order to possibly later produce DNA profiles for species identification (Post et al. 1993, Sperling et al. 1994). In the author's investigations, the following formula has also proved to be a useful storage liquid for invertebrates in connection with DNA analysis:

100 mM trishydroxymethylaminoethane (Tris)
100 mM ethylenediaminetetraacetic acid (EDTA)
2% sodium dodecyl sulfate (SDS)
10 mM saline (NaCl). (*)

If desired, maggots can be killed by pouring hot water (80°C) over them before transfer to alcohol. The brown shell husk remaining after hatching can be stored in a dry place, but in practice, the use of alcohol has proved to be effective here too, because the broken egg tops are held in the egg by electrostatic attraction when dry.

b. Collection

Maggots can be transferred with rubber gloved hands or tweezers into any clean sealable container. In most cases, it is not necessary to separate the maggots from different areas of the corpse, as many of the maggots have to be screened anyway and species differences will be apparent. Nevertheless, it should be noted that female flies lay their eggs as egg sacs in one or a few places, and therefore, maggots from the same parents are initially found in patchy distribution. It may facilitate subsequent identification if the animals of each spot are placed in separate containers.

Besides maggots, hatched animals should also be collected. These are easier to determine and quickly give first indications of the expected corpse fauna. Adults, especially beetles, are usually found under drier areas of the corpse, often in or under locks of hair. Flies can often be caught by hand or in an upside-down jar at temperatures below 12°C–15°C, when they rest on a part of the corpse's skin or fall into the onset of cold rigidity.

As a rule, adult animals found dead can be just as identifiable as freshly collected ones, since the anatomically significant features are preserved, although shrinkage (drying artefact) is possible overall. This also applies to freshly dead maggots (Fig. 2). In the case of adult animals, especially those that have already died, spring steel tweezers and a brush (or similar) should be used to pick them up so that the animals do not break or lose relevant body attachments. However, the rule applies that even a small fraction of an animal can still be sufficient for successful identification (e.g. a single wing: Benecke 1996b). The abandoned shell husks of animals hatched from the pupa can also be used to determine what they are (e.g. Reiter & Wollenek 1983).

Labelling—the more detailed the locations and times of the animals are recorded, the more precisely the analysis can be carried out later. While in the dissection room, it may be sufficient to write down the body number with a waterproof felt-tip pen on all the vessels used—detailed information can also be found later in the files, in the field, it is necessary to document the date, weather, place of discovery, and name of the examiner. In entomological practice, it is common practice to dispense with the felt markers, which are often not solvent-resistant, and instead add a small slip of paper, clearly legibly written in pencil, to the animals stored in alcohol. Animals speared on an insect needle and stored in the insect box must be marked by means of small inscribed cardboard labels which are held in place under the animal with the same needle.

All jars, needles, cardboard labels, and boxes required for storage are available from specialist mail order companies (e.g. K&K GbR, Ratingen).

c. Breeding

A proportion of maggots, after their length and/or individual weight has been recorded (Cf. Wells & LaMotte 1995), should be kept in alcohol, and another proportion should be reared as adults. During storage, the following basic conditions must be observed—without going into further details at this point:

Moisture—most use of insect and spider breeds are unsuccessful because the animals dry out. It is essential to bring a piece of damp paper (Kleenex, handkerchief) or damp earth into the cultivation vessel. Although moisture is absolutely necessary for the development of the animals, especially pupated animals should not come into direct contact with water. It is therefore advisable to stick the damp cloth under the lid of the vessel with a narrow strip of adhesive tape or to use the apparatus shown in Fig. 3.

Oxygen—like humans, arthropods are dependent on oxygen for respiration. Breeding tanks must therefore always have breathing openings. However, the openings must not allow the passage of maggots (who intensively explore their surroundings). In the absence of air, the animals are not only suffocated, but mold also forms, which destroys the dead animals.

Food—maggots whose intestines have not yet been emptied (the filled intestine is clearly visible from above as a dark shimmer between the fatty tissue, Cf. e.g. Fig. in Reiter & Hajek 1984) should be fed with moist pieces of meat (for blowflies) or old cheese (for piophilids). Especially early corpse colonizers are not very demanding in their choice of food; however, it is recommended to feed younger animals (up to about 1 cm length) with autolytic moist meat, since they can absorb it better with their small jaws than fresh meat.

Maggots can prematurely pupate, especially in later stages of development, without additional feeding (e.g. Smirnov & Zhelochovtsev 1926), but in these cases, they show a smaller body size after hatching than an average member of the species. These sources of error — artifactual duration of development and body size — should be excluded in breeding, in order to allow a potentially desired recalculation of the time of death based

on the standard growth curves. Larger maggots, whose intestines have already been emptied, are not far from pupation and should be placed in a clean container containing only dry pieces of bark, dry leaves, a crumpled clean paper towel or similar: the animals like to hide in crevices where they pupate and finally hatch. Hatched animals still need some time to unfold their wings and harden their chitinous outer skeleton. To promote this phase (hardened animals usually do not break during identification), a few drops of sugar water should be dripped onto the bottom of the living vessel as the first meal after hatching.

Ambient conditions—a range between 18°C–28°C has proven to be the best breeding temperature in our laboratory. Important for the later recalculation of the time of death is the continuous recording of the temperature by means of a recorder or at least the reading and logging of the temperature over several days. For a continuous (and therefore better reproducible) development process, it is very helpful if there are no daily or other temperature fluctuations. While many corpse-associated flies reach their maximum growth speed at temperatures above 30°C (Schumann 1971, Reiter 1984), development below 12°C is extremely slowed or temporarily stopped (e.g. Hédouin et al. 1996). Details on the effect of cold phases and heat on the development of flies are found in Rosales et al. 1994 and Davison 1969, Turner & Howard (1992) investigated the heat release of maggot layers.

Disinfectant vapors and insecticides can inhibit or alter the growth of the animals. Therefore, only minimal amounts of these substances should be applied in the breeding area. (In our entomological laboratory, the tables and equipment are disinfected with 70% ethanol, the insect boxes for dried animals are lined with moth paper and placed in a closed cabinet to stop “museum beetles” (dermestids) which inevitably destroy any dry arthropod collection)

Under the above conditions, breeding is definitely successful if a maximum of ten larvae are placed in a fresh, clean container (Fig. 3) with their intestines emptied. There, the animals are left to their own devices—apart from the regular (e.g. two-day) moistening of the cloth. Younger animals must be raised with food (see above). In order to minimize the breeding effort, after the first larval molting of fly larvae, only about 10–30 animals of one egg sac or one corpse should be bred. A daily or weekly retention of maggots of different stages of development can be very helpful: If the breeding of the adult animals is unsuccessful, the maggots can often only be reliably identified as a certain species at a certain stage (e.g. larval stage 4) with the keys available today.

Protocol—in a breeding protocol, the developmental stage of the animals should be recorded daily, if identifiable. In the case of a developmental plateau, only the protocol can be used to identify factors to be taken into account in the evaluation. It is usually sufficient to document only the most obvious stages of development: Eating larva, intestines shimmer darkly through fatty tissue; white larva, close to pupation, intestine emptied; dark larva; light shell husk; dark shell husk; hatching; agile animal (Cf. Fig. 4). In individual cases, further stages of development can be shown, for example, the exact larval stage (see Smith 1986). Small, transparent breeding vessels (Fig. 2) facilitate the routine, recorded breeding of animals.

d. Gathering

In the dissection room, all animals can be put with tweezers into transparent plastic "histology trays" with a lid. Caution: maggots are very agile and usually climb over the edge of the vessel without difficulty! Adult beetles from a size of about 4 mm remain safely trapped in test tubes because they cannot climb the smooth walls.

In apartments, gaps in the concrete, cavities behind baseboards, picture frames, etc. should be searched, at least in the room where the corpse was found. Samples taken in the field from the surroundings of the corpse (e.g. fallen leaves, soil) within a radius of about one to two meters can help to discover animals that have migrated to pupate and also provide a first insight into the naturally occurring arthropod fauna at the place where the corpse was found. Beetles measuring only a few millimeters in particular are often only discovered in a second sampling of the soil/scattered leaves under bright lighting.

Flies from smaller breeding jars can be easily caught—as these are usually unwilling to fly due to lack of space—by placing a test tube over them with the opening facing down. The flies climb the inner vessel wall and can be immediately preserved in this way.

Stunning—bred arthropods can be temporarily stunned by the cold (refrigerator) or carbon dioxide (e.g. adding hydrochloric acid to lime and passing the resulting CO2 through a hose). In smaller containers, it is sufficient to shake the animals together with the container vigorously for five to ten seconds.

e. Species Identification

The final determination of the animals can only be carried out by an examiner familiar with the determination technique and equipped with the best possible determination keys. A good microscope and a preparation set are further requirements for entomological analysis. Without these aids, species identification is hopeless, and from experience, even skilled entomologists are wary of prematurely identifying families unknown to them right down to the species.

Adult animals, maggots, and (empty) shell husks are suitable for the determination (see also Fig. 2). For the sake of brevity, it is not possible to give details, but the following useful works should be pointed out: Smith 1986 (especially for the determination of corpse-associated flies (larvae)), Freude/Harde/Lohse 1964-1983, Koch 1985, Catts & Haskell 1990, Chinery 1993, Hoffmann/Wipking/Cölln 1996 (example of a detailed urban arthropod ecology), Schumann 1971, Reiter & Wollenek 1983 as well as the excellently illustrated determination volumes of the Royal Entomological Society (London). In the meantime, species identification is also possible on the basis of DNA profiles (e.g. Sperling et al. 1994, Guglich et al. 1994, Replogle et al. 1994). However, there is still a considerable need for research here.

In order to exclude an ecologically incorrect analysis, the animals must be additionally identified by a zoologist specialized in a certain group of animals (e.g. blowflies, lard beetles, and fur beetles, etc.) if there is the slightest doubt, and the results of the determination must always be compared with the local ecological conditions. The local fauna must therefore also be examined in relation to the arthropod families relevant for the study.

Further forensic-criminalistically useful basic information on the collection, preservation, and breeding of arthropods on land and in water can be found in Haskell & Williams 1990, Haskell 1990, Chinery 1973, Lord & Burger 1983, Basden 1947, Vance et al. 1995 and Borror 1981; many further details have been compiled in the excellent compilation on almost all questions of forensic entomology by Smith (1986).

Conclusion

The course of development of arthropod breeding from a corpse can contribute to the determination of the post-mortem interval; the determination of the fauna prevailing at the site where the corpse was found allows further conclusions about the course of

events. The preservation of the insect and spider material plays a decisive role. As a rule, adult animals should be preserved in 70% ethanol or 70% methylated spirits and stored in a cool place, while juvenile stages should be partly bred and partly also kept in alcohol. Two breeding apparatuses are presented, basic breeding instructions are given, and an overview of the existing literature sources is given.

Summary

Use of entomological evidence in medico-legal questions allows a wide range of applications, e.g. estimation of post-mortem intervals, investigation of taking away a corpse to another location and many others. An important point is to store and eventually breed carrion-associated arthropods in the right way. As a rule all arthropods should be stored cool in 70% ethanol at the crime scene to allow subsequent species determination. Basic breeding instructions are outlined, two breeding devices are described, and an overview of useful literature is given.

(*) By now, we use exclusively methylated sprits.

Addendum

Please note that this is a historic article.

The case was a high profile case ("Pastor Geyer case").

Methods and procedures changed since 1999.

— **MB, Feb 5, 2020**

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