A peer-reviewed version of this preprint was published in PeerJ on 1 August 2017.

View the peer-reviewed version (peerj.com/articles/3506), which is the preferred citable publication unless you specifically need to cite this preprint.

https://doi.org/10.7717/peerj.3506
Use of necrophagous insects as evidence of cadaver relocation: myth or reality?

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The use of insects as indicators of postmortem displacement is discussed in many text, courses and TV shows, and several studies addressing this issue have been published. However, the concept is widely cited but poorly understood, and only a few forensic cases have successfully applied such a method. Surprisingly, this question has never be taken into account entirely as a cross-disciplinary theme. The use of necrophagous insects as evidence of cadaver relocation actually involves a wide range of data on their biology: distribution areas, microhabitats, phenology, behavioral ecology and molecular analysis are among the research areas linked to this problem. This article reviews for the first time the current knowledge on these questions and analyse the possibilities/limitations of each method to evaluate their feasibility. This analysis reveals numerous weaknesses and mistaken beliefs but also many concrete possibilities and research opportunities.
Use of necrophagous insects as evidence of cadaver relocation: myth or reality?

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Running title: Necrophagous insects evidence cadaver relocation

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Abstract

The use of insects as indicators of postmortem displacement is discussed in many text, courses and TV shows, and several studies addressing this issue have been published. However, the concept is widely cited but poorly understood, and only a few forensic cases have successfully applied such a method. Surprisingly, this question has never be taken into account entirely as a cross-disciplinary theme. The use of necrophagous insects as evidence of cadaver relocation actually involves a wide range of data on their biology: distribution areas, microhabitats, phenology, behavioral ecology and molecular analysis are among the research areas linked to this problem. This article reviews for the first time the current knowledge on these questions and analyse the possibilities/limitations of each method to evaluate their feasibility. This analysis reveals numerous weaknesses and mistaken beliefs but also many concrete possibilities and research opportunities.
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A. Introduction

A.1 Context

Insect analysis has been used in legal investigations for centuries in a practice now known as forensic entomology (18). Increased interest in this field since the late 20th century has resulted in more frequent use in investigations and the development of research on necrophagous species. Previous reviews have gathered and explained the aims and methods of forensic entomology (11, 28, 35, 154), but some fundamental questions remain unresolved, particularly the potential to use insects as evidence of corpse relocation.

Forensic taphonomy can include a variety of changes due to human activity, especially steps taken to hide a cadaver (77). Attempts to prevent discovery often include cadaver concealment, wrapping and displacement. Such post-mortem relocation can occur shortly after death or after days of concealment and can take place over a short distance (e.g., from the room where the death occurred to the garden of the house) or a longer distance. In most cases, the environment where the cadaver was hidden is very different from that of the place where death occurred (137). Forensic entomology manuals and courses often state that insects can be used as evidence of cadaver relocation (9, 28, 35, 89, 117, 126, 144) because the biology and ecology of necrophagous species can convey information on where and how insects live and thus may highlight inconsistencies regarding cadaver location and decomposition. However, while this idea is appealing, it may not reflect reality.

It may seem obvious that “if a body is discovered with insects restricted to a habitat or geographic region different from that in which it is discovered, this is an indication that the body may have been moved following death” (117). However, most, if not all, European necrophagous
species have large distribution areas covering many countries and hundreds of thousands of square kilometers, making the sampling of non-native species quite unlikely. While each species has an ecological niche (e.g., forest or synanthropic; sun or shady habitats), such preferences are not rules. Additionally, as some species can travel kilometers to find carrion, microhabitats are only relative concepts (22, 118). The long dispersal capability of most necrophagous species, especially blowflies, makes it difficult to relate a given species to a particular place or habitat and thus draw inferences regarding cadaver relocation (166).

Temporal separation is another characteristic of necrophagous species. The phenology (cyclic and seasonal phenomena) of blowflies is well known; some species are primarily active during hot weather, while others are well adapted to cold climates (157). Such seasonality could, under certain circumstances, contribute useful information regarding the chronology of cadaver decomposition. However, the presence of larvae of a summer species on a winter cadaver does not constitute indisputable evidence of cadaver relocation. Colonization time is also strongly dependent on the stage of decomposition. Although it is far more complex than chronological succession (94), succession on cadavers has been experimentally shown in several countries and under multiple conditions (1, 3, 5, 8). Divergence from known succession patterns such as the absence of certain species or unusual associations might indicate cadaver relocation or concealment. The presence or absence of some instars is also of great interest, especially with regard to wandering larvae or pupae of pioneer species (e.g., Calliphoridae flies), which pupate away from the cadaver and can thus be found after cadaver removal.

Advances in genetics also offer numerous opportunities. First, genetics make it possible to connect individuals to a local population or even sub-population. As noted by Tomberlin et al., such possibilities are of great interest in the context of cadaver relocation (154). More anecdotally,
the genetic analysis of gut content has interesting potential to indicate which cadaver larvae have
been feeding on (32, 34). This technique should be developed in the coming years and provide
new tools for forensic entomologists and crime scene investigations.

This article reviews the current knowledge and promise of each method and evaluates its
feasibility. This analysis reveals the weaknesses and mistaken beliefs regarding the use of forensic
entomology as evidence of cadaver displacement as well as many concrete possibilities and
development opportunities.

A.2 Survey methodology

The first phase of this survey was the identification of the the magnitude of this problem.
This step was addressed by searching in the main forensic entomology manual published in
English since these last 40 years if the question of corpse relocation was afforded. We found
references to this idea in most of them (9, 28, 35, 89, 117, 126, 144), but only a few case reports
(17, 67, 91). On the other side, we found several research article addressing this question as a main
goal or claiming it a potential application of their findings. Accordingly, use of insects to infer
corpse relocation appears being a complex and unstructured problem with numerous and disparate
information that deserved to be reviewed.

We first searched for the books and publication clearly addressing this question. From this
dataset, we listed the various facets of the problem and gathered them into four main concept:
spatial separation, behavior / development, phenology / colonization time and molecular analyses).
We then searched in the literature specific to each of these fields for data of potential use. This
data set was then analyzed to highlight discrepancies or spot methods with true potential
application.
**Spatial separation**

Only a few insect species are associated with cadavers, and even fewer are strictly necrophagous (requiring a cadaver to feed on during at least a part of their development) (144). Their diversity is visible in the variability in insect size, shape, behavior, ecological niche and distribution and reflects species-specific adaptations, which allow species to exploit different habitats and resources. Johnson defined four orders of habitat selection, from large geographical areas to local microhabitats (87). Furthermore, Matuszewski et al. defined species indicators of cadaver relocation as those that at least 1) have a strong preference for a given geographical area or habitat, 2) are resistant to relocation disturbance, 3) live on cadavers and 4) colonize cadavers shortly after death (112). Common species are also more likely to be found in association with criminal cases than are rare species. Unfortunately, the association with habitat appears to be more pronounced in the less common species than in those that are more common (99).

**B.1 Biogeography of European species of forensic importance**

According to the common definition, the distribution of a species is the geographical area within which that species is observed. Species may not be uniformly distributed in this area: variation in local density (e.g., clumped distribution) is common. However, individuals of a given species are not often observed outside of their distribution area. Online interactive maps can now be found on the web for most European taxa. Many of these databases mix old distribution data and modern records produced by amateur or professional entomologists (65). Such collaborative work is subject to information gaps and biases, particularly a lack of records. In particular, this
problem affects necrophagous species, which are infrequently sought out and are poorly known among entomologists. Accordingly, an apparently unusual/unexpected necrophagous species may detected simply because the site was previously unsampled (Figure 1).

Figure 1. The distribution of *Cynomya mortuorum* in Europe (source: www.gbif.org, 09/2016).

While it is not reported on the map/database, this species is also present in northern France (23):
To suspect cadaver relocation, it is necessary to find species that have restricted and well-established distributions. We listed here the few European necrophagous species complaining these criteria. Some interesting distribution areas can be observed for other necrophagous species, but most of these species are unusual, difficult to identify and poorly documented. Thus, while they are theoretically useable, these insects cannot be regarded as true indicators of cadaver relocation in terms of the criteria listed above.

Two common species of the genus *Cynomya* have restricted European distribution areas. *C. mortuorum*, a large, hairy bluebottle fly, can be found across the entire Palearctic region (25, 30) (Figure 1), but is rarely reported in central European countries, especially in a forensic context (25, 46, 139, 150, 164). Its distribution partially overlaps that of *C. cadaverina* (Robineau-Desvoidy, 1830), another cold-adapted species of forensic interest (92, 139).

Two other calliphorid flies, *Calliphora loewi* and *C. subalpina*, show a sub-alpine distribution (54, 139). *C. loewi* is present in the Holarctic and in a small area of Asia (139). In Europe, *C. loewi* is a forest species that is mostly found in northern and central Europe, from Siberia and the Caucasus to the Central European Territories (144). This limited distribution area could make it a good indicator of relocation, but its recent discovery in Madeira Island (Portugal) calls its relevance into question (135). Furthermore, while it has a large distribution, *C. loewi* is often recorded at low abundance (151). *C. subalpina* has a very similar distribution area and is subject to similar limitations (139).
Chrysomya albiceps is one of the few species that is, at least theoretically, usable as an indicator of cadaver relocation in Europe (70). The species is meridional, common and abundant in southern Europe and in most of the neotropical, Afrotropical and Oriental regions (70). However, while it is mostly found in southern Europe, this species has been observed migrating northward during the hot summer months (164). Additionally, while it is not a true invasive species, this fly has become more common in northern Europe. This northward expansion of its range during hot summers has the potential to cause confusion and precludes its use as evidence of cadaver relocation. Similar factors affect the use of C. megacephala, an Asian fly recently recorded in continental Europe and extending its distribution in the Mediterranean region (12, 51, 107, 134).

According to this review, the use of insect distribution area as evidence of long-distance cadaver relocation appears to be more a question for theoretical forensic entomologists than a forensic reality. Furthermore, the probability of someone transporting a cadaver inside a vehicle and traveling several hours across Europe to deposit it in the distribution area of a different species is likely low.

B.2 Species-specific habitats

Many forensic cases involve cadavers that have been transported several kilometers from the crime scene, especially to low-traffic areas such as forests, dumping sites, rivers or seashores (secondary decomposition sites) (112). As discussed above, such short-distance relocation cannot be elucidated using the presence of foreign necrophagous species. However, moving a cadaver can affect micro-environmental conditions such as climate (temperature, insulation, humidity),
synanthropy, vegetation and indoor/outdoor location. The population of necrophagous insects at
the secondary decomposition site may thus differ from that of the initial (primary) environment.

The effect of habitat on the abundance of certain species is well established (27, 84). However, published data regarding species-specific habitats vary, highlighting that such preferences are not rigid and often vary locally. Close inspection of the biology of necrophagous species exemplifies this complication. To perpetuate the species, adult females must find suitable carrion for their offspring. However, the occurrence of cadavers is by definition unpredictable because death is essentially temporally and spatially random. Accordingly, all necrophagous species have an efficient and highly selective olfactory sense that allows them to quickly detect cadavers. As noted by MacLeod and Donnelly, blowflies are powerful and active flies capable of dispersing over large distances (several kilometers per day) (99). It thus seems unlikely that a gravid fly living in a forested area will stop at the edge of a clearing if carrion is decomposing on the other side of that clearing (26).

Furthermore, many environmental parameters of the landscape or within a given habitat category can affect the abundance of species. Most studies in forensic entomology use simple categories (e.g., forest, sunny, indoor) without taking into account the surroundings and the variability within these categories (e.g., different types of forest or sizes of cities). Additionally, larger-scale effects and interactions within parameters (e.g., higher temperatures in large cities) are usually not considered (166). In a 1957 study, MacLeod and Donnelly clearly stated that “there is nothing to indicate whether the non-uniform distribution of the adult (flies) population is due to the faunal, floral, vegetation-structural or edaphic element of the environment, or to some combination of these” (99). More than fifty years later, Zabala et al. concluded that, except for the summer abundance of *C. vomitoria*, blowfly community composition cannot be used as evidence
of cadaver relocation, particularly in heterogeneous and densely populated areas (166). These authors also noted that any conclusion based on species-specific habitat preferences should be drawn only from local studies (5, 27, 43, 84). The following sub-section focuses on more particular habitat characteristics that may be of interest in determining the primary deposition site of a cadaver.

Indoor vs. outdoor

The question of the inside/outside location of a cadaver is a key point in many investigations (58) and access to the cadaver by necrophagous insects greatly affect its decomposition (33, 103). It has furthermore been proved that the location of a cadaver affects its colonization time (the pre-appearance interval, i.e., the time before insects reach the cadaver) and thus the PMI estimation (40, 132, 136). An indoor location also protects the cadaver from rain and is often associated with higher temperatures that can speed the development of the larvae.

The species associated with indoor locations have been investigated in many field studies and case reports. A pioneer study by Goff of 35 forensic entomology cases in Hawaii found that more insect species were found indoor (67). Centeno et al. also found two more species on carrion that was sheltered during the winter (36). But Anderson found the same species (except Lucilia illustris) on inside and outside cadavers (6). By contrast, Cainé et al. found more fly species on outdoor cadavers in Portugal (31), and Reibe and Madea also found greater species diversity in outdoor locations (136). In this last experiment, piglet carcasses located indoors (1st-floor room) were exclusively infested by C. vicina, while a variety of blowfly species (L. sericata, L. caesar, L. illustris, C. vicina and C. vomitoria) were found on the outdoor (garden) piglet carcasses (136). The importance of cadaver location for the abundance of larder beetles, which preferentially feed
and breed on dry material, was investigated by Charabidze et al. (39). While feeding larvae were more common in indoor forensic cases, no clear preference was observed in adults. These authors also found an effect of cadaver location on the presence of *N. littoralis* (41). However, they noted that this trend may be the result of the usually shorter PMI and low accessibility of indoor cases. Lastly, Leclercq reported Silphidae only from cadavers recovered from forest sites in Belgium (46), and Dekeirsschieter et al. did not identify any Silphidae species in cadavers found in urban Belgium (47). However, Chauvet and colleagues recorded the presence of *Nicrophorus spp.* on human cadavers discovered inside houses in France (42).

In accordance with these discrepancies, Frost et al. noted that although more species and specimens are often observed indoors compared to outdoors, this trend is not consistent (58). An extensive table summarizing the insect species reported from human remains found indoors can be found in their study (58). The authors clearly note that “none of the(se) listed insect species can be considered as exclusively indoors.” An example of the difficulty in formally linking the presence of a species to the inside/outside location was shown by Krikken et al. (91). From the numerous dead *L.* adult flies (no species name was reported) observed in an upstairs room with closed windows, the authors concluded that the body had first been outdoors in a warm, sunny environment and was later relocated into the room. However, this conclusion was only based on the supposed preference of *L.* to “oviposit on high temperature surfaces,” which the authors took to mean “outdoors,” a weak evidence in a forensic context.

In the future, mites may provide information regarding the location of the cadaver, but these species are relatively little known and are overlooked in forensic entomology. For further information, see Frost et al.’s above-mentioned review (58) and the work of Perotti (58, 128).
Open vs. forest and sunny vs. shaded places

The distinction between open and forest habitat is in itself not always clear: vegetation cover can vary according to season, but the exact location of the cadaver within any given open habitat is not always sunny (e.g., incised valleys). In a field study dating from 1957, MacLeod and Donnelly reported that *C. vomitoria* and *L. ampullacea* were abundant in regions of dense vegetation (i.e., forest habitats), whereas *L. illustris* and *L. sericata* were more common in open conditions (heliophilic species) (99). More generally, *L. sericata* is often found in bright sunlight (81), while *L. caesar* is associated with shade (121). However, despite evidence of the thermophilic character of some blowfly species, these preferences vary among local populations (85, 108). As an example, Joy et al. found the same species on sunlit and shaded pig carcasses in West Virginia, USA (90) and Hwang and Turner showed the ability of *C. vicina* populations to locally adapt their thermal requirements to suit their environment (85). Regarding coleopterans, Matuszewski et al. investigated species that colonized cadavers in open vs. forest habitats (112). They concluded that the presence of *Dermestes frischi, Omosita colon*, and *Nitidula spp.* could be used as evidence of relocation from rural open to rural forest habitat. In contrast, only *O. thoracicum* was classified as an indicator of relocation in the opposite direction. This conclusion is similar to that of Dekeirsschieter et al. (46, 47), who recorded seven Silphidae species in forest habitat (Belgium): all but *O. thoracica* were also caught in agricultural biotope (open habitat).

Rural vs. urban

The term “synanthropic” is used to characterize species that live near humans and benefit from them and the artificial habitats they create. Cities, and more generally human activities, are also often associated with the production of meat waste that can attract necrophagous insects. In
addition to these direct modifications of the environment, urbanization affects the climate, resulting in local warming (161). As ambient temperature is of prime importance for insect activity and development, heat islands such as those observed in large cities can offer thermal refuges for several species.

Although it is present in both rural and urban habitats, *C. vicina* tends to be found predominantly in shady and urban areas (14, 52, 71). In contrast, *C. vomitoria* is often described as a more rural species that avoids cities (84, 85, 120, 133, 144). *C. loewi* and *C. subalpina* are also known to avoid urban areas (120, 139, 155). In an extensive 2014 study examining a 7,000 km² landscape in Spain, Zabala et al. found a significant relationship between summer abundance of *C. vomitoria*, distance to urban areas and degree of urbanization (166). This pattern was especially clear during summer, when *C. vomitoria* was significantly more abundant at points far from urban areas. However, for the nine other calliphorid flies they investigated (including *C. vicina* and *L. sericata*), no clear synanthropic relationship was found.

Several comparative studies on local blowfly populations have also been performed in the UK (44, 86, 98-101, 143). Using meat-baited bottle traps, Hwang and Turner described three groups of necrophagous flies corresponding to three habitat types (85): the urban habitat was characterized by *C. vicina*, *L. illustris* and *L. sericata*, while rural grasslands were inhabited by *L. caesar* and rural woodlands were inhabited by *C. vomitoria*. Wyss also reported that in Switzerland, *L. argyrostroma* was found in urban areas, while *C. mortuorum* avoided them (163). Souza and Von Zuben found significant differences in the synanthropy of some Calliphoridae and Sarcophagidae flies in Brazil (147, 148). But in southern Africa, Parry et al. observed that species assemblages present in human-disturbed areas were very similar to those recorded in natural habitats (124).
There are few data on necrophagous coleopterans, likely due to the under-representation of these insects in anthropized environments. Due to their large size and low agility in flight, many Coleoptera of forensic interest appear to be poorly suited to urban conditions. In 2011, Dekeirsschieter et al. recorded seven Silphidae species in a Belgian forest environment, six in agricultural biotopes and none in urban locations (47). According to these results, silphid beetles may be good indicators of cadaver relocation between rural and urban habitats (11, 111).

However, most, if not all, species of forensic interest also show inconsistencies or exceptions in their habitat-association patterns. As an example, many authors have found that *L. sericata* is associated to urban habitats (57, 57, 84, 86, 120). A study from Germany even found that *L. sericata* had the highest Synanthropy Index (SI) of all blowfly species they studied (Steinborn, 1981 in 134). Another German study reported *L. sericata* and *C. vicina* as the only blow fly species caught indoors (141). *L. sericata* was also classified by Greco et al. as the most synanthropic blowfly in Italy (71). However, *L. sericata* was also recorded in natural open habitats in Poland and in open pasture in England (44, 111, 143). Similarly, Greco et al. (71) observed a preference of *L. caesar* for wild and rural habitats, a trend supported by some former studies (15, 71, 84) but in opposition to the findings of Fisher (57). Thus, while their presence reflects ecological preferences, necrophagous insects are not sufficiently clearly repartitioned between urban and rural areas, and it currently appears that their distribution is too variable to be used as evidence of corpse relocation in a forensic context.

**Others specific locations**

**Water**
The simplest change is the relocation from water to open air. In such a case, the presence on the cadaver of any aquatic invertebrate could be used as evidence of cadaver relocation. In contrast, the finding of the usual necrophagous species on an immersed cadaver may be more challenging to interpret. Four sequential steps have been used by Merrit and Wallace to describe changes in body position in water over time: 1) the body sinks to the bottom; 2) there is horizontal movement at the bottom; 3) the body floats to the surface; 4) surface drift occurs (115). The discovery of a cadaver in water during the initial steps is characterized by the absence of the usual necrophagous species (e.g., Calliphoridae) and the presence of ubiquitous aquatic invertebrates (e.g., Chironomidae larvae, snails, etc.) (115). During the first 2 steps of immersion, the cadaver is fully immersed and the presence of any terrestrial larvae on the cadaver would indicate they were laid before immersion. This possibility is especially interesting because blowfly larvae can resist submersion in water and stay alive for several hours (2, 138). However, the finding of the same species on a floating cadaver (steps 3 and 4) would yield less if any information, as many fly species can lay eggs on the emerged parts of a floating cadaver (13, 153).

The presence of Coleoptera would be more questionable. The larvae of most Silphidae species live underneath cadavers and dig pupation chambers into the soil for nymphosis. Thus, these larvae should not be observed on floating cadavers. Furthermore, large adults are less agile in flight than are flies and thus avoid landing on small surfaces surrounded by water. Barrios and Wolff did not observe any necrophagous Coleoptera species on pig cadavers placed in two freshwater ecosystems, even during the floating phases (13). However, Tomberlin et al. observed many small staphylinid beetles on rat carcasses in water and even found single adults of the silphid beetle *Necrophila americana* and the dermestid beetle *Dermestes caninus* (153). As dermestid
beetles usually colonize and feed on dry materials (37, 140), such a finding is a good example of
the risk associated with drawing conclusions on cadaver relocation from general trends.

The relocation of a cadaver from freshwater to a marine environment (and the inverse) can
also occur, especially in the case of floating cadavers, which can be carried by tides. As most
aquatic species are limited to a restricted salinity range, the presence of a given species outside of
its range may be useful as evidence of cadaver relocation. Detailed data on species associated with
marine and freshwater environments can be found throughout the literature (4, 146). Cadaver
relocation can also occur within the same aquatic environment. In freshwater, the species
distribution depends on the physico-chemical attributes of the water (oxygen, pollutants, turbidity),
and in running freshwater, there is a succession of habitats and biotopes from source to estuary.
As an example, Ephemeroptera and Trichoptera larvae are found only in clean and well-
 oxygenated water, while Eristalidae are found in water with a high organic load (110). Abundant
literature can be found on this topic, especially with respect to bioindicators (122).

Insects of buried/concealed cadavers

A relatively common method of cadaver concealment is burial, which greatly affects
carrion decomposition and access by entomofauna (142). Deep burial and/or protection of the body
by a coffin limit but do not prevent postmortem colonization of the body. Experiments on buried
pig carcasses and insect sampling during exhumations have shown the presence of many
necrophahous species (24, 61, 94, 125, 144, 156). Although no necrophagous species appear to be
restricted to buried cadavers, their relative abundance and diversity often vary compared to
exposed cadavers (25). Thus, the absence of the expected species (e.g., calliphorid flies) and the
presence of many concealment-related species (e.g., Phoridae) may indicate that a cadaver had been previously buried (82, 83).

*C. tibialis* is one of the main species found on concealed cadavers; several authors have reported that this small fly occurs frequently and in large numbers (24, 109, 116). The regular occurrence of *C. tibialis* on buried cadavers is linked with its specific behavior: females can burrow through the soil to a depth of 2 m to oviposit, and larvae can crawl even deeper (117). *Megaselia scalaris* is also often found. This fly is a warm-climate species, but it has been carried around the world by humans and has been associated with indoor forensic cases in temperate regions (50). However, these two species are also present in other environments, including indoors and in the open air (49), and their presence cannot be considered to constitute definitive evidence of burial.

More interestingly, Szpila et al. demonstrated the ability of *Phylloteles pictipennis* and *Eumacronychia persolla* (Diptera: Sarcophagidae) to reach deeply buried animal remains and breed on this food source (152). As noted by the authors, both of these species develop exclusively on buried food resources, making them potential indicators of cadaver relocation.

By contrast, common blowflies and muscid flies have limited abilities to colonize buried resources, as shown by Gunn and Bird (74). But *Muscina stabulans* and *M. prolapsa* have colonized remains buried up to 40 cm deep (74). As noted by the authors, the presence of large numbers of larvae of a given species feeding on bodies buried deeper than indicated by their species-specific limitations may be an indication that the body had been exposed above ground for sufficient time for eggs to be laid. Indeed, larvae laid before burying are able to fully develop on cadavers that were subsequently buried (10, 74). Lastly, Gunn and Bird showed the ability of wandering larvae that have grown on a buried cadaver to reach the surface and pupate (74). According to this finding, the presence of pupae on the soil above the grave does not indicate that
the cadaver was buried after the pupal stages emerged. Other inferences can be drawn from the absence or presence of specific instars, as described in more detail in the section of this review focusing on larval behavior.

Mariani et al. reported the use of an unusual biocenose as evidence of post-exhumation entomological contamination (105). Entomological investigation revealed the presence on exhumed remains of numerous necrophagous insects as well as omnivorous and storage pests (Dermestidae, Nitidulidae and Tenebrionidae beetles; Tineidae moths; and cockroaches). As none of these insects are able to burrow as adults or as larvae, their presence provides evidence of contamination during storage in the cemetery after exhumation.

B. 3 Conclusions related to species-specific habitats

As described herein in detail, various species-specific habitats can be used as evidence of cadaver relocation from one habitat to another (Table 1). However, most of these trends are not rules and thus are not restrictive enough to formally demonstrate that a cadaver was moved. Accordingly, while entomological evidence related to species-specific habitats may help support hypotheses regarding cadaver relocation, strong inferences are usually not appropriate.

Finally, other insects (non-necrophagous species) could provide evidence of cadaver relocation. As reported by Goff, “If a body is outdoors near or under vegetation, it is possible for insects associated with that vegetation to move onto the body, although typically not to feed or lay eggs” (66). However, as these insects are not directly linked to the cadaver, it would be difficult to prove they were moved together with it. Furthermore, the probabilities of 1) having a non-necrophagous species crawling on a cadaver, 2) moving this insect with the cadaver, 3) sampling
and identifying it at the secondary site and 4) that species being located outside its natural range are likely very low. We have found no report of any such case in the forensic literature.

Table 1. Summary of the use of spatial characteristics of necrophagous insects as evidence of cadaver relocation. The first location is shown in the column, and the second (final) site is shown in the row. The availability and strength of each clue are modulated by the length of time the cadaver remained in each place. More details for each scenario can be found in the main text.

<table>
<thead>
<tr>
<th>Duration on 2nd deposition site</th>
<th>Duration on 1st deposition site</th>
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<td>Days</td>
<td>Weeks</td>
<td>Months/Years</td>
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<td>too short to get insects from the 1st location</td>
<td>various species - 1st site only</td>
<td></td>
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</tr>
<tr>
<td>Weeks</td>
<td>empty pupae of non-wandering Calliphoridae species from the 1st site</td>
<td>mostly late colonizers from 1st site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months/Years</td>
<td>traces² of various species from 1st site + late colonizer from 2nd site</td>
<td>late colonizers from both sites</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Behavior and development of instars

Extensive knowledge of the behavior of necrophagous insects is often key to interpreting forensic entomology. Knowledge of when adults are attracted by cadavers, how they colonize them and how their larvae grow allows forensic entomologists to elucidate a coherent post-mortem chronology. However, this component of the analysis is often underrated. First, only a few studies focusing on the behavior of necrophagous insects have been published. Furthermore, most of the available data are descriptive, consisting of field observations or trends rather than quantitative experiments (154). In a forensic context, these restrictions make it difficult to draw conclusions:
C.1 Adult behavior: colonization and egg laying

Egg laying depends on climatic conditions as well as species behavior and the accessibility of the cadaver. Indirectly, these parameters could theoretically be used as evidence of a displacement between places with different climactic conditions. An average species-specific minimum temperature is required for egg laying (54, 80). Krikken et al. reported a neat but questionable example of this phenomenon (91). Building on the disputable idea that L. “oviposit on high temperature surfaces,” the authors concluded that a cadaver discovered in a room had first been located outdoors in a warm and sunny place. However, while L. sericata is indeed heliophilic, this species can still lay eggs indoors. Many other weather parameters such as sun, wind and rain can also have an impact (38, 162). Furthermore, as fly displacement and egg laying mostly occur during the daytime, the presence of numerous egg batches on a cadaver located in a dark place could be suspicious (17, 73, 162). However, Gemmellaro et al. recently demonstrated the ability of some calliphorid flies, especially C. vicina, to reach meat-baited traps placed inside volcanic caves (62).

Some species are also known to oviposit in specific areas: calliphorid flies preferentially deposit egg batches on the face (nostrils, mouth, eyes), while most silphid beetles lay their eggs underneath cadavers (144). However, such behaviors are strongly affected by cadaver decomposition, wounds, presence of other larvae and species, collective behavior (egg aggregation) and the environment (41). More striking evidence is provided by the presence of eggs, especially those of large Calliphorids, in inaccessible places, especially underneath a
cadaver. Such a case was analyzed in 2013 in France (unpublished data): the presence of numerous
*L. sericata* and *C. vicina* egg batches in the folds of clothing underneath a cadaver served as
evidence of the secondary reversal of the cadaver by drug addicts searching for money.

C.2 Larval development, wandering larvae and pupae

Fly larvae live on the cadaver and are thus quite resistant to cadaver relocation. On the
opposite, the probability of transferring the insects present in soil or under the cadaver (wandering
larvae, pupae and most silphid larvae) along with the cadaver is very low. During the post-feeding
stage, larvae of several blowfly species (with the notable exception of Chrysomyinae) start
migrating from the cadaver to pupate away from predators in a protected location (68). Greenberg
(72) observed that more than 80% of the post-feeding larvae of *L. sericata* and *C. vicina* moved
out of the cadaver and were observed up to 8 m away. By contrast, only 2% of *P. regina*, 10% of
*M. stabulans*, and 16% of *C. rufifacies* larvae moved away. Due to the robustness of this behavior,
a good deal of information can be derived from the presence and location of the wandering larvae
and pupae/puparia around the cadaver.

The presence of necrophagous blowfly pupae and puparia (or dead adult flies) can be used
as evidence of the former presence of a cadaver. Genetic analysis can formally link these insects
to the victim (see the molecular analysis section of this review) (34). Such entomological evidence
was recently used during the famous Casey Anthony Trial (USA) (96), in which a first forensic
entomologist relied on the presence of numerous *M. scalaris* larvae, pupae and adults in a car trunk
as evidence of the former presence of a cadaver (7). However, an expert witness for the defense
showed that the same insects could also have come from a trash bag discovered in the trunk. As
the gut contents of insect samples were not tested for DNA, there was no evidence to support the
assertion that the insects originated on human remains. Mariani et al. (2014) also observed that in blowfly and muscid species, buried larvae ultimately left their food source to move to their usual pupariation depth. According to the authors, the presence of large numbers of post-feeding blowfly larvae without a cadaver in the vicinity could therefore indicate that a body may have been buried nearby rather than relocated.

On the opposite, the lack of pupae/puparia of Calliphoridae, together with the presence on a cadaver of non-wandering species (e.g., Chrysomyinae) may suggest the relocation of the cadaver after the wandering larvae had left. Such a case involving cadaver relocation in a car trunk after the larvae have moved was described by Benecke (19). Krikken et al. also reported a case of a skeleton found during winter in a small forest with “numerous empty pupal cocoons of *P. terraenovae* under the bones” (91). From these puparia, the authors concluded that the whole decomposition process had taken place in that same spot. However, as this species can pupate in the clothes or even on decomposing tissues, it was also reasonable to hypothesize that pupae had been moved together with the cadaver.

Cadaver relocation can also be characterized by discrepancies between local temperature and larval development. As an example, a finding of third-instar *L. sericata* on a cadaver in a cold location (e.g., a cellar with a constant 9-10°C temperature) would be suspicious. However, this discrepancy often results from the on-site microclimate (e.g., direct sun exposure), larval-mass effect or conservation of the cadaver or samples (e.g., high temperature during transport) rather than relocation. Cadaver relocation should be considered only in the absence of these biases. Lastly, a less formal but striking clue regarding cadaver relocation is the presence of crushed pupae/imago on or under the cadaver. We observed the presence of flattened pupae or newly
hatched flies (flat, dry individuals with a still-visible ptilinum) directly under the cadaver in several forensic cases. Relocation of the cadaver likely occurred after numerous flies had started to emerge, and some specimens were compressed under the cadaver during or after moving. If such specimens are observed on site (before the cadaver was moved by the forensic team), relocation after larval pupation can be suspected.

C. Phenology and colonization time

The temporal activities of flies vary due to intrinsic rhythms (e.g., life history, reproductive cycle, development time, etc.) and extrinsic seasonal effects (e.g., temperature, photoperiod and availability of resources) (166). Accordingly, species-specific phenology can be an indicator of the season of death and, at least theoretically, of cadaver relocation (90). For example, the finding of only “late” colonizers on a cadaver and no traces of pioneer species should be suspicious and suggests that the cadaver was not accessible to insects (e.g., concealed or hidden under inclement weather conditions) during the first stages of decomposition. An example is given by Krikken et al.: only a small number of insect eggs (attributed to blowflies) were found on a cadaver discovered during a warm summer (90). Considering the total absence of maggots from the body and the post-mortem interval calculated by the pathologist, the authors concluded that the body must have been sheltered, delaying colonization by blowflies.

Mądra et al. (102) observed clear seasonality trends for 9 Staphylininae species and concluded that they are good candidates as indicators of cadaver relocation. The results for flies are far more divergent. In Spain, Zabala et al. observed that L. sericata, L. illustris and Ch. albiceps were clear indicators of summer (166), while C. vicina and C. vomitoria were common year round with maximum abundance in the spring. However, due to the wide variability in these results
according to landscape, the authors concluded that they cannot be used as evidence of cadaver relocation. The same conclusion can be drawn from the results obtained by Greco et al. in Italy (71). The authors showed differences in the abundances of Calliphoridae by month of sampling. However, this effect was also strongly dependent on trap location. For example, C. vicina was observed throughout the sampling period (except from June to September) in rural and urban areas but was absent during the cooler months (November to January) in the wild area. This interaction of phenology and spatial distribution clearly prevents the use of these species as evidence of post-mortem displacement.

This question is also linked to the effect of time on colonization by necrophagous insects. This subject is widely studied and debated within the forensic-entomology community. It is widely understood that some species are early colonizers, while others are observed during later stages of decomposition (144). However, the colonization period of a given species varies depending on many parameters, including climate, season, geographic area, local environment, insect populations, and other factors (33). These points must be carefully examined before any attempt to use unusual succession as evidence of cadaver relocation. Furthermore, open habitats allow easy access to the cadaver for predators or parasites such as wasps, Silphidae and Cleridae. They can decrease the number and diversity of Diptera larvae, especially if predation occurs during the early developmental stages (e.g., egg removal by wasps). Thus, the absence of some pioneer species does not imply cadaver concealment during the first stage of decomposition.

Finally, duration is fundamental in considering cadaver relocation. Different amounts of time spent in the first location, during transportation and in the secondary decomposition site are associated with different types of evidence. Table 2 summarizes the overall scenarios and
corresponding timeframes for “simple” cases. However, the problem of time must be considered in each particular context (Table 1 and above in this review).

Table 2. Effects of time spent in the first (columns) and secondary (lines) decomposition sites on the necrophagous entomofauna. According to the time spent in each location, different species and developmental instars may be found on the cadaver, affecting the interpretation of entomological samples as evidence of cadaver relocation. More details on the entomological phases of the colonization process can be found in Tomberlin et al. (154).

<table>
<thead>
<tr>
<th>1 - First location</th>
<th>Indoor (closed)</th>
<th>Outdoor</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rural</td>
<td>Urban</td>
<td>Forest</td>
</tr>
<tr>
<td>Indoor (closed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>Mainly Phoridae, few Calliphoridae (no wandering larvae / only Chrysomiinae pupae)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Freshwater (immersed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freshwater species</td>
<td>Lot of Phoridae with only a few other terrestrial species - no large species</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Salted water (immersed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salted water species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burried/Concealed</td>
<td>Calliphoridae, Sarcophagidae, Muscidae (possibly other) - all developmental stages</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X = Not possible   Questionable   Blank: Unknown / Not enough data
D. Contribution of molecular analyses

E. 1 Cuticular hydrocarbons

The ability to identify forensic species at different developmental stages and to link them to local populations can be crucial in determining whether a body was moved from the crime scene. Simple molecular analyses concern cuticular hydrocarbon profiles. This thin epicuticular layer of wax consists of free lipids, a class of compounds that includes hydrocarbons, alcohols, fatty acids, waxes, acylglycerides, phospholipids and glycolipids (64). This phenotype is biologically very stable and almost entirely determined by genotype (127, 158). Byrne et al. (29) demonstrated that the cuticular hydrocarbons of three geographically distinct populations of *P. regina* are differentiable. However, some local populations can interbreed with adjacent populations, and the minimal interval over which adjacent populations can be considered distinct is still unknown. Accordingly, this method could be used as evidence of the presence of a non-local population on a cadaver (which suggests cadaver relocation) but will not yield results in the case of short-distance relocation (29). More research on this promising topic should be conducted in the future (154).

E. 2 Genetics of insect populations

If post-mortem changes are suspected, relocation can be shown by determining the relationships between insects sampled at the initial and secondary sites (129). Several studies have highlighted significant genetic differences between populations of the same species on different continents (21, 48, 145) but also across a continent (78, 97). To identify genetic variations between populations, methods such as simple conformation polymorphism strand (SSCP) analysis and AFLP (amplified fragment length polymorphism) are available. However, all kinship analyses
require a solid genetic database including the variability among geographical sites, the
development of which requires thorough field samplings (93), precise morphological identification
and complete genetic characterization of each collected individual.

DNA-based identification has been shown to be a valuable tool with which to identify adult
insects of forensic interest as well as immature stages and fragments of cuticles or puparia (75, 76,
88, 95, 113, 114, 123, 145, 149, 160, 165, 167, 168). These DNA-based identifications can be used
to create a reference library of identified specimens. On this basis, databases such as GenBank and
the Barcode of Life Data Systems (BOLD) have been used to detect genetic variation within local
populations of the same species (158). Using SSCP analysis, inter-population differences have
been detected between the African and North American populations of the common housefly,
*Musca domestica* (106). Harvey et al. also found differences in the COI gene between South
African and Australian populations of two species of forensic interest, *Chrysomya rufifacies* and
*L. cuprina* (79). Furthermore, Desmyter and Gosselin (48) and Boehme et al. (20) found sequence
differences between *Phormia regina* specimens from North America and Europe (Belgium, France
and Germany) (20, 48). Jordaens et al. confirmed this divergence in the COI with newly sequenced
material (88). However, sequence divergence within each continent was only ca. 0.4%, making
 genetic differentiation of local strains difficult. New scientific projects dedicated to building
datasets that reflect the diversity of necrophagous entomofauna at the European scale are currently
expanding and should address this question in the near future (63, 145).

Using AFLP surveys, Picard and Wells observed that groups of adult *L. sericata* and *P.
regina* trapped together on a bait were predominantly composed of related individuals, with a
genetic diversity lower than that observed at a larger scale (130, 131). This pattern also holds true
for gravid females and therefore probably for larvae, suggesting that the population genetic
structure of adults could be extended to the larval population growing on a cadaver. If so, this result might support the use of genetic tests to infer post-mortem relocation of a cadaver by connecting a larva found in one location to the larval population growing on a cadaver in a second location. Faulds et al. confirmed the validity of this AFLP method: kinship testing based on AFLP data yields adequate kinship estimates with limited error (55). As noted by the authors, this type of analysis can be performed on any life stage of the insect and on any species. Regarding species of interest in forensic entomology, AFLP data are already available for \( P. \text{regina} \), \( L. \text{sericata} \) and \( C. \text{megacephala} \) (12, 130, 131). These results support the idea that AFLP analysis for full sibship is a promising method for the detection of postmortem relocation.

**E. 3 Identification of human DNA**

Another contribution of molecular analyses is the identification of human DNA in the digestive tract of the larvae. This method can be used to determine the genetic profile of the victim (16, 33, 160) and can be used in the absence of a cadaver as well as after its relocation (60). Indeed, the presence of necrophagous larvae or pupae in an empty place can suggest the former presence of a cadaver. If genetic analysis of the gut content reveals the victim’s DNA, entomological evidence can be used as evidence of relocation (96, 159). In 2001, Wells et al. demonstrated that mitochondrial DNA sequences can be obtained from the dissected gut of a maggot that had fed on human tissue. In 2012, Chaves-Briones et al. reported the first forensic case of victim identification from human DNA isolated from the gastrointestinal tract of necrophagous larvae (45). Still more striking evidence of the potential of this method was provided by Marchetti et al. (104). In this study, the authors used short tandem repeat (STR) analysis to extract and type human DNA from empty puparia collected in two forensic cases. As puparia cases are highly durable, they offer a
unique opportunity to indicate cadaver relocation a long time after the event. Njau et al. also demonstrated that DNA analysis could be used to determine whether the larvae sampled on a cadaver were introduced from an alternative food source (e.g., a dead animal or a trash can near the cadaver) (119). However, due to the rapid degradation of DNA by gut digestive enzymes, such analyses are limited to two days post-feeding (39, 130).

E. Conclusion

1/ The question of cadaver relocation has arisen in many forensic cases, but it has received little attention in the forensic science literature, except in forensic entomology.

2/ Even if some species are preferentially found in some biotopes, most are not sufficiently geographically restricted to serve as evidence of cadaver relocation.

3/ Only field studies performed at a local scale and focusing on a clear question (e.g., differences between rural and urban areas) should be used as references. 3/ Time is a key point: a cadaver that remained in the first location for too short a time is not likely to have been colonized by local insects, while any that remained too long would likely have been abandoned by the insects before cadaver relocation.

4/ Specific sets of circumstances allowing inference of corpse relocation from cadaver entomofauna are:

- relocation from open air to an aquatic environment (and the converse),
- relocation from open air to a grave or burial site (and the converse),
- removal from an indoor location if some larvae or pupae remain in the first location,
- evidence of cadaver relocation with the support of molecular analysis.

Analyses can be performed only by trained forensic entomologists and require early discussion with investigators, extensive on-site sampling, the conservation and analysis of relevant samples, and a considerable amount of chance.

We recommend that forensic entomologists perform experiments *a posteriori* to comply with the circumstances of a given forensic case and not rely on general trends or previous results at a broader scale.
F. References


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