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CASE REPORT

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# Fly pupae and puparia as potential contaminants of forensic entomology samples from sites of body discovery

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**Abstract** Fly pupae and puparia may contaminate forensic entomology samples at death scenes if they have originated not from human remains but from animal carcasses or other decomposing organic material. These contaminants may erroneously lengthen post-mortem interval estimates if no pupae or puparia are genuinely associated with the body. Three forensic entomology case studies are presented, in which contamination either occurred or was suspected. In the first case, blow fly puparia collected near the body were detected as contaminants because the species was inactive both when the body was found and when the deceased was last sighted reliably. The second case illustrates that contamination may be suspected at particularly squalid death scenes because of the likely presence of carcasses or organic material. The third case involves the presence at the body discovery site of numerous potentially contaminating animal carcasses. Soil samples were taken along transects to show that pupae and puparia were clustered around their probable sources.

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## Introduction

Contamination of forensic entomology samples can occur when invertebrates collected from a body or body discovery site originate from a source other than the deceased. For example, forensic entomologists may inadvertently collect contaminating invertebrates from bodies in the mortuary due to accidental transfer of insects between corpses or infestation of bodies by insects living in the mortuary [4]. There is also a risk of contamination from the mortuary if invertebrates that have fallen from the autopsy table to the floor are collected, since these may be mixed with specimens from earlier cases that have escaped the mortuary cleaning process. Additional contamination can occur at body discovery sites when the invertebrates collected have originated from nearby animal carcasses or organic refuse [6, 24]. A search for carrion insect breeding sources often reveals potential contamination sources at death scenes; however, factors such as scavengers or floods may remove contaminants before a body is discovered. Furthermore, some body discovery sites are difficult or impossible to search fully because of dense and thorny undergrowth, convoluted topography (i.e. multi-storey houses) or dangerous terrain (i.e. cliff ledges, caves, mine shafts).

Estimates of minimum post-mortem interval (PMI) will be unaffected by contamination when contaminating species are ‘incidental’ invertebrates that use the body as shelter or as a hunting ground. However, estimates of minimum PMI can be distorted if evidence becomes contaminated by forensically important invertebrates such as maggots. For example, if contaminant maggots are older than the maggots actually originating from the body, the minimum PMI estimate will be erroneously lengthened. The problems arising from potential contamination at death scenes by feeding insects have been recognised [6, 24], and it is now possible to recover human DNA from the crops of maggots and beetles to verify that the insect has been

feeding on the body in question [8, 14, 24, 25]. However, scant attention has been paid to the implications of contamination by eclosed fly puparia or non-feeding life history stages such as fly pupae.

Fly pupae and puparia can provide valuable information to forensic entomologists but can prove misleading as contaminants. When live pupae of the first colonising generation are collected from a body, a key assumption in using juvenile growth rate data to age them is that these pupae fed on the body as larvae. If the contaminants are the oldest specimens collected, they can again erroneously lengthen the minimum PMI estimate, and the degree of error will depend not only on the age gap between the contaminants and the oldest specimens genuinely associated with the body but on previous environmental conditions as well. Contamination by eclosed puparia can cause the same problem if they are the oldest life history stage collected from the body discovery site. Additionally, their presence indicates that adults of the first generation of juvenile carrion insects have emerged, which means that less accurate succession data will then be used to age the body.

Contaminating pupae and puparia can also lead to other errors besides the miscalculation of minimum PMI. First, puparia can be extremely durable and may remain associated with a skeleton or decomposition site for months to years [12, 17, 20]. This is useful for seasonally dating the time of death if the puparium belongs to a seasonally active carrion insect species [3], but contaminants may provide a false seasonal estimate. Second, as with maggots [24], the presence of pupae or puparia at a site may be used to infer that a decomposing body has previously been located there, which is important where clandestine relocation of a body is suspected. However, contamination under these circumstances may result in a false conclusion if all pupae or puparia collected were derived from other sources.

The risk of body discovery site contamination by pupae and puparia is especially great due to their ability to accumulate in soil. First, the durability of emerged puparia means that they can build up over many years from background organic waste and animal carcasses. Second, live pupae that are overwintering may also accumulate in soil within a single year. The contamination potential of pupae and puparia is also high because they are likely to be distributed widely from their point of origin. When feeding concludes, wandering prepupae travel from their original feeding site, and while prepupae of many blow fly species remain within a few metres of their food source (e.g. [16, 17, 21]; personal observation), they have been known to travel up to 30 m if suitable pupariation sites are unavailable [13].

Three case studies are presented from the Victorian Institute of Forensic Medicine (Australia), in which contamination of death-scene samples by pupae or puparia either occurred or was suspected. The impact of failing to exclude contaminants in the estimate of minimum PMI is also discussed. Suggestions are made for detecting contaminants and dealing appropriately with them in the analysis of entomological evidence.

## Case 1

The deceased was located in August (winter) in an abandoned and dilapidated building. An autopsy revealed that hypothermia was the probable cause of death, and no drugs or toxins were detected. The deceased had been missing for 30 days prior to discovery, although the minimal degree of decomposition indicated to the pathologist a period of approximately 7–10 days since death.

A light infestation of maggots and eggs of the blow fly *Calliphora vicina* Robineau-Desvoidy (Diptera; Calliphoridae) were the only insects located at autopsy despite a thorough examination of the body and clothing. Maggots of each larval instar were represented, and the oldest were third larval instar maggots. However, only seven specimens in this age group were located (mean length 14.6 mm, SE ±0.5, range 12–16 mm). The only carrion insect samples found at the body discovery site were nine eclosed blow fly puparia on the floor of an empty closet, within 1 m of the deceased's head. The proximity of these puparia to the deceased strongly indicated that they were associated with the body. A search was made in the house and grounds for vertebrate animal remains and other organic material supporting carrion insects, but none were found. However, there were large piles of hard rubbish in the area, making it impossible to exclude the presence of organic material.

Ambient temperature estimates for the body discovery site were obtained by performing scene and station correlation followed by regression correction of local weather station data [1]. Predicted temperatures at the body discovery site for the period the deceased was missing ranged between 7 and 12°C (mean 9°C, SE 0.1). A minimum PMI of 11–14 days was calculated from the 2,200–2,850 Accumulated Degree Hours (ADH) taken for Victorian *C. vicina* to reach the size range of the third larval instar maggots collected when reared at 10°C (M. Archer, unpublished data). However, the puparia were anomalous because at the death-scene temperatures, the time from egg to adult emergence for southeastern Australian winter-active blow flies would exceed the period the deceased was missing. This was also at odds with the apparently minimal decomposition of the body, even if the cold conditions were considered.

Puparia were identified as *C. augur* Fabricius (Diptera; Calliphoridae) using spine band patterns [23]. This species reproduces in southern Victoria only between September and April [3]; the puparia collected could therefore not have originated from the deceased given that the relatively fresh body was located in early August. Instead, these contaminating puparia probably originated from animal remains located in the area at an earlier time, and minimum death time was therefore determined using the third larval instar *C. vicina*. The reasons for excluding the contaminating puparia were set out in the report, and the excluded evidence was also listed and retained along with the primary evidence.

It is useful to extend this case study by examining some hypothetical scenarios that may have affected the outcome. For example, the eclosed puparia could be eliminated easily in this case using species activity times, but if they had been the winter-active *C. vicina*, the decision to eliminate them

would have been complicated. The period from egg to eclosion in local *C. vicina* is between 63 and 67 days (15,120–16,080 ADH), which is roughly twice the period since the last indisputable sighting of the deceased (e.g. on a security camera). Therefore, the contaminants could have been eliminated on the basis of this in combination with the relatively fresh state of the body.

A more complicated scenario would have been presented if the remains had been many months or years old and an estimate of season of death had been sought. Unfortunately, the entomological evidence would have provided an incorrect assessment that death had probably occurred between September and April. This underlines the need for caveats on unavoidable sources of error to accompany entomological opinions.

## Case 2

The severely decomposed body of an elderly man was located during winter in his room at a special-needs hostel, which had 22 residents. Welfare checks on the deceased were not undertaken, even though he had been seen daily until his disappearance approximately 3 weeks before his body was discovered. The hostel was squalid, and offensive background odours seemed sufficiently strong to mask the odour of the decomposing body. The window of the deceased's unheated room had been open.

The cause of death was ischaemic heart disease, but minimum PMI estimation was important because of potential duty of care issues. Therefore, entomological samples were collected from the body and clothing prior to autopsy, and an entomological examination of the body discovery site was conducted. Ambient temperature correlation was made between the death scene and the nearest weather station, and death-scene temperatures were estimated for the period between discovery and the last sighting of the deceased (following Archer [1]).

Third larval instar *Fannia canicularis* Linnaeus (Diptera; Fanniidae) and *C. vicina* blow fly eggs as well as larvae of all three larval instars were collected from the body in the mortuary. Two live *C. vicina* prepupae with pale and recently formed puparia as well as one puparium containing a decomposed pupa were collected from the inner surface of the deceased's clothing. It was considered highly likely that these originated from the deceased because of their intimate association with the body and because large numbers of *C. vicina* prepupae were found under the deceased's garments.

The deceased's room was filled with rubbish to a depth of up to 1 m in places, which was composed mostly of food wrappers, books and paper. No decomposing organic material or animal carcasses were located, although there were large accumulations of mouse droppings and a strong odour of rodent urine in the room. It was therefore likely that small animal carcasses were present in the roof and wall spaces as well as in the other rooms in the hostel. Approximately 50 *C. vicina* prepupae were located amongst the rubbish at varying distances from where the body had lain. Three eclosed *C. vicina* puparia were also located in a cluster approximately 2 m from the body.

The eclosed puparia were excluded from the analysis of minimum PMI due to the risk of their being contaminants. A combination of factors casts unassailable doubt on the origin of these puparia from the deceased: the nature of the surroundings, the low numbers of eclosed puparia located, and the lack of pupae at ages between eclosed and newly pupariated. Although fairly compelling in combination, each factor in isolation would not necessarily provide sound evidence of contamination. For example, discreet age cohorts of juvenile insects are often associated with bodies in colder months when there is sporadic occurrence of weather suitable for blow fly oviposition. Puparia may also be located in unexpectedly low numbers, even when associated with old remains.

The recently pupariated prepupae and dead pupa were deemed the oldest life history stage that could be associated strongly with the body. The minimum PMI of 12–18 days was therefore calculated from the ADH (3,960–5,400) taken for Victorian *C. vicina* to reach pupariation when reared at 15°C (M. Archer, unpublished data). The minimum PMI would have been approximately 31–34 days (11,160–12,240 ADH) if the eclosed puparia had been included in the analysis. This exceeds by about 1 week the last reported sighting of the deceased, but the informant was uncertain of the precise date of the last sighting, so this estimate would also have been plausible.

## Case 3

The deceased was last seen alive in July and was located in November (spring) of that year, partially submerged in the mud of a coastal salt marsh. The likely cause of death was blunt trauma to the head. The exposed upper body of the remains was mostly skeletonised, while the buried portion was inaccessible to insects and almost fully fleshed. An entomological examination both for the death scene with the remains in situ and for the remains and clothing at autopsy was carried out.

All larval instars of the flies *Hydrotaea rostrata* Robineau-Desvoidy (Diptera; Muscidae), *Calliphora hilli* Patton (Diptera; Calliphoridae), and *Piophila casei* Linnaeus (Diptera; Piophilidae) were feeding at autopsy. They were also present in the mud at the death scene, within approximately 3–4 m of the body. Numerous eclosed and uneclosed puparia of these species were also found in the clothing and the mud surrounding the body. *Dermestes maculatus* DeGeer (Coleoptera; Dermestidae) larvae and adults were found at autopsy on the body and clothing.

A partial sheep carcass was located approximately 5 m north of the body. This was in a much later decomposition stage than the deceased, and only wool and bleached bones remained. The soil underneath this carcass and in the surrounding 1 m radius (depth of approximately 10 cm) was searched for invertebrates, but none were found. The wool was also searched for invertebrates, and two dead *Calliphora stygia* Fabricius (Diptera; Calliphoridae) puparia were

collected. A rat carcass was located approximately 25 m north of the body. This was partially skeletonised, and while dipteran maggot activity had ceased, it was infested by adults and larvae of the hide beetle *D. maculatus*. The soil underneath this carcass and in the surrounding 1-m radius (depth of approximately 10 cm) was searched for carrion invertebrates, but none were found. Numerous bleached skeletal elements of small vertebrates, such as rabbits, were located for several square metres surrounding the rat carcass. Soil in this area was excavated, but no carrion invertebrates or their remnants were found.

Contamination was a distinct possibility, given the abundant animal carcasses in the vicinity of the deceased. It was therefore necessary to establish that carrion invertebrates collected from the human remains most likely originated from them. Samples from the general area were collected to investigate whether puparia were clustered around carcasses and the deceased in a patchy distribution or whether there was a more continuous distribution between them. In addition to the excavations already made around each set of remains, 15 control soil excavations (each 30 $cm^2$ and 10 cm deep) were made along a line transect extending approximately 30 m north of the body (with the body at the south end of the line). The sheep and rat carcasses both lay within 3 m of the line. Each excavation was spaced at approximate 2 m intervals along the transect. The excavated soil was searched for carrion invertebrates in the same manner as the soil surrounding both the body and carcass. No carrion invertebrates, pupae or puparia were located in any control samples, which strongly suggests that these species were distributed in concentrated clusters associated with each set of decomposing remains. Actual translocation of invertebrates between carcasses cannot be excluded with this technique, but the findings provide evidence against the presence of large accumulations of puparia throughout the general area.

The minimum PMI was not estimated in this case because the presence of eclosed puparia associated with the body demonstrated that a succession-based PMI estimate was required. Unfortunately, there are no rigorous local succession and decomposition data available for decomposition in this environment.

## Conclusions

These case studies highlight some issues associated with forensic entomology sample contamination by pupae and puparia. In cases 1 and 2, including the contaminants or suspected contaminants in the evidence analysis could have extended the estimate of minimum PMI by roughly 7 or 2 weeks, respectively. It can therefore be seen that pupal and puparial contamination is an important problem, and that appropriate steps should be taken to detect and eliminate potential contaminants from body discovery site samples.

In some cases, it is possible to positively identify contaminants and eliminate them from samples. Case 1 demonstrates that seasonal distributions of blow flies can be used to detect and eliminate contaminants. Emergence holes in puparia left by seasonally active parasitoid wasps, such as chalcids [3], can also allow elimination of contaminating puparia. However, a potential area of difficulty with using insect activity times to eliminate contaminants is that this method requires certainty that the remains cannot have been exposed during the last activity period of the species in question. This can be easily judged when the deceased is in the initial decomposition stages, but this becomes more subjective as decomposition advances. Decomposition is variable [22], and while there are many decomposition timeline guides for particular geographic areas and seasons (e.g. [2, 9, 11, 15]), the timing of later decomposition changes can fall well outside what the current literature would predict.

In addition to seasonal distributions, knowledge of species natural history may allow their detection as sample contaminants. For example, many calliphorids and sarcophagids are obligate parasites of other invertebrates, such as earthworms, and some are obligate myiasis agents [7]. It is therefore important that the status of a species as a carrion breeder is established if it is used forensically.

Toxicological indicators may also help to assess the likelihood that a pupa or puparium originated from a given set of remains. Various toxicological substances have been isolated from dipteran puparia [5, 10, 18], and their presence would provide strong evidence of origin from human remains. The absence from a pupa or puparium of toxicological substances found in the body should not, however, be used to identify it as a contaminant because it has been shown that levels of certain drugs can drop precipitously in pupariating *C. vicina* [19].

In many cases, it will be impossible to detect pupal or puparial contaminants, although there may be strong reason to suspect their presence. In such cases, an assessment of the contamination risk as well as a decision whether to eliminate potential contaminants from analysis must be made. Alternatively, two minimum PMI estimates could be provided: one that includes and one that excludes the suspected contaminants. However, presentation of a single estimate is preferable because it ensures that the entomologist, rather than a lawyer or police officer, makes the decision about which estimate is the most scientifically sound.

Control soil sampling can also be helpful for assessing contamination risk because it allows systematic investigation of the overall distribution of pupae and puparia in the surrounding area. Control sampling procedures are best suited to open and accessible death scenes where samples can be taken over large sites and are also particularly useful for body discovery sites rich in organic matter, such as refuse dumps, farms, abattoirs and bird or bat roosts. These techniques are now used wherever possible in Victorian forensic entomology casework, whether or not contamination is suspected. Ideally, samples from several transects are required to determine rigorously whether the distribution of pupae is clustered around the body.

Extensive sampling at sites of body discovery may reveal the nature of the distribution of pupae and puparia, but case 1 demonstrates that the proximity of puparia to re-

mains does not necessarily mean they are associated. However, large numbers of puparia in close association with remains greatly increase the probability that they are not contaminants, and this is more compelling if control excavations along transects demonstrate that the soil concentration of pupae or puparia decreases with distance from the body.

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## References

1. Archer MS (2004a) The effect of time after body discovery on the accuracy of retrospective weather station ambient temperature corrections in forensic entomology. J Forensic Sci 49(3): 553–559
2. Archer MS (2004b) Rainfall and temperature effects on the decomposition rate of exposed neonatal remains. Sci Justice 44 (1):35–41
3. Archer MS, Elgar MA (2003) Yearly activity patterns in southern Victoria (Australia) of seasonally active carrion insects. Forensic Sci Int 132:173–176
4. Archer MS, Ranson DL (2005) Potential contamination of forensic entomology samples in the mortuary: a case report. Med Sci Law 45:89–91
5. Benecke M (2004) Arthropods and corpses. In: Tsokos M (ed) Forensic pathology reviews, vol II. Humana, Totowa, pp 207–240
6. Christopherson C, Gibo DL (1997) Foraging by food deprived larvae of *Neobellieria bullata* (Diptera: Sarcophagidae). J Forensic Sci 41:71–73
7. Colless DH, McAlpine DK (1991) Diptera. In: Naumann ID (ed) The insects of Australia, vol II. Melbourne University Press, Melbourne, pp 717–786
8. DiZinno JA, Lord WD, Collins-Morton MB, Wilson MR, Goff ML (2002) Mitochondrial DNA sequencing of beetle larvae (Nitidulidae: *Omosita*) recovered from human bone. J Forensic Sci 47:1337–1339
9. Early M, Goff ML (1986) Arthropod succession patterns in exposed carrion on the Island of O'Ahu, Hawaiian Islands, USA. J Med Entomol 23:520–531
10. Gagliano-Candela R, Aventaggiato L (2001) The detection of toxic substances in entomological specimens. Int J Leg Med 114:197–203
11. Galloway A, Birkby WH, Jones AM, Henry TE, Parks BO (1989) Decay rates of human remains in an arid environment. J Forensic Sci 34:607–661
12. Gilbert BM, Bass WM (1967) Seasonal dating of burials from the presence of fly pupae. Am Antiq 34:534–535
13. Green AA (1951) The control of blowflies infesting slaughter houses. I. Field observations of the habits of blowflies. Ann Appl Biol 38:475–494
14. Linville JG, Hayes J, Wells JD (2004) Mitochondrial DNA and STR analyses of maggot crop contents: effect of specimen preservation technique. J Forensic Sci 47:1055–1059
15. Mann RW, Bass WM, Meadows L (1990) Time since death and decomposition of the human body: variables and observations in case and experimental field studies. J Forensic Sci 35:103–111
16. Nuorteva P (1977) Sarcosaprophagous insects as forensic indicators. In: Tedeschi CG, Eckert WG, Tedeschi LG (eds) Forensic medicine: a study in trauma and environmental hazards, vol II. Physical trauma. Saunders, Philadelphia, pp 1072–1095
17. Nuorteva P (1987) Empty puparia of *Phormia terraenovae* R-D (Diptera, Calliphoridae) as forensic indicators. Ann Entomol Fenn 53:53–56
18. Pien K, Laloup M, Pipeleers-Marichal M, Grootaert P, De Boeck G, Samyn N, Boonen T, Vits K, Wood M (2004) Toxicological data and growth characteristics of single post-feeding larvae and puparia of *Calliphora vicina* (Diptera: Calliphoridae) obtained from a controlled nordiazepam study. Int J Leg Med 118:190–193
19. Sadler DW, Fuke C, Court F, Pounder DJ (1995) Drug accumulation and elimination in *Calliphora vicina* larvae. Forensic Sci Int 71:191–197
20. Teskey HJ, Turnbull C (1979) Diptera puparia from pre-historic graves. Can Entomol 111:527–528
21. Tessmer JW, Meek CL (1996) Dispersal and distribution of Calliphoridae (Diptera) immatures from animal carcasses in southern Louisiana. J Med Entomol 33:665–669
22. Ubelaker DH (1997) Taphonomic applications in forensic anthropology. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC, Boca Raton, pp 77–90
23. Wallman JF (2001) Third instar larvae of common carrion-breeding blowflies of the genus *Calliphora* (Diptera: Calliphoridae). Invertebr Taxon 15:37–51
24. Wells JD, Introna F, Di Vella G, Campobasso CP, Hayes J, Sperling FAH (2001) Human and insect mitochondrial DNA analysis from maggots. J Forensic Sci 46:685–687
25. Zehner R, Amendt J, Krettek R (2004) STR typing of human DNA from fly larvae fed on decomposing bodies. J Forensic Sci 49:337–340