

TECHNICAL NOTE**PATHOLOGY/BIOLOGY***J Forensic Sci*, 2016

doi: 10.1111/1556-4029.13121

Available online at: onlinelibrary.wiley.com

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Cleaning Puparia for Forensic Analysis

ABSTRACT: We tested procedures for removing adipocere from insect samples to allow identification. An acceptable procedure was determined: (i) Samples were sorted in petri dishes with 75% alcohol to remove any larvae, adult insects, or other soft-bodied material. (ii) Samples of up to 24 puparia were placed in a vial with 15 mL of 95% acetone, capped, and vortexed for a total of 30–90 sec in 10- to 15-sec bursts. This step removed large masses of adipocere or soil from specimen. (iii) Specimens were removed from acetone and placed in a vial of 15 mL of 2% potassium hydroxide (KOH) and vortexed in 10- to 15-sec bursts until all puparia appeared clean (with our samples this required a total of 60–120 sec). (iv) Specimens were removed from the 2% KOH, placed in 75% ethanol, and examined microscopically. (v) Material was stored in 75% ethanol for identification and long-term preservation.

KEYWORDS: forensic science, taphonomy, forensic entomology, puparia, adipocere, blow fly, Calliphoridae

Among the most common forms of insect evidence at homicides with bodies with advanced decomposition are insect puparia: the last larval skin inside of which some types of flies (e.g., Calliphoridae, the blow flies; Sarcophagidae, the flesh flies; Muscidae, filth flies) form the pupal stage. Because blow flies are typically the first flies to arrive at a dead body, identification of blow fly species can be invaluable for establishing time of death and potentially other information like location or manner of death. Even after adult emergence, the puparia may persist if protected from weathering, even hundreds of years (as illustrated by the recovery of insect puparia at archeological sites) (1).

Because the puparium is, literally, the last larval skin (specifically, the exoskeleton of the third-stage maggot), in principle, the morphological features of that stage should allow identification of the insect species. In practice, the puparium is a distended, mahogany-colored, barrel-shaped version of the third-stage maggot, and these developmental changes can obscure some features of larval morphology. Moreover, because larval features may be obscured, the condition of the puparium is extremely important. Most commonly, puparia at forensic scenes are either clean or dirt-covered. Most dirt is easily removed in water or 75% ethanol; however, other coatings may require special attention.

In most instances, cleaning insects prior to identification is not necessary. However, some insect sampling methods lead to potential problems through removal of identifying features after trapping (e.g., beetles caught in moth traps destroying wing features, or scales obscuring beetle features) or through the trapping

medium itself (e.g., insects caught on sticky traps). Less commonly, the medium in which the insect occurs may itself present a barrier to identification. Usually, insect sampling ensures that the insect is removed from the medium, as occurs when sampling soil and aquatic insects. However, with insects associated with organic decay of feces and of plant or animal tissue, the medium itself can pose a problem. In these instances, material on the insect can be removed by one or a combination of three processes: mechanical removal, simple washing, or soaking/agitation in a solvent. In all approaches, the two key criteria are efficiency in processing and avoiding damage to specimens.

With most forensic samples, insects either remove themselves from decaying tissue (e.g., mature blow fly maggots typically migrate away from their larval hosts to move to pupation sites) or do not interact with decaying tissues in such a way as to pose a cleaning issue (1). Even maggots collected from within decaying matter typically have little or no decompositional material adhering to their exoskeletons. However, when insects occur in the advanced stages of decomposition, there is the possibility that they may be caught in adipocere, the wax-like material formed through the (typically) anaerobic bacterial hydrolysis of lipids during decomposition (2).

Various procedures have been developed for removal of adipocere (and other tissues) in the cleaning of bones for osteological analysis. Typically, these involve the use of sodium hydroxide or similar strong bases (e.g., 3). However, we are unable to find any comparable procedure for cleaning other types of materials, particularly insects. In a recent case, we encountered puparia that were so heavily covered with adipocere that the removal of adipocere was essential for further analysis. Here, we report the rapid procedure we used to remove adipocere from insect samples.

Materials and Methods

Soil and leaf litter samples were collected by the Nebraska State Patrol beneath and away from the remains of a 20-year-old

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Received 31 July 2015; and in revised form 10 Nov. 2015; accepted 23 Nov. 2015.

female, a homicide victim (Fig. 1). These samples were provided to our laboratories for analysis. Additionally, a dessicated human tissue and bone sample with embedded puparia was provided for removal of insect evidence and osteological analysis. Various entomological samples were recovered from the soil and leaf litter material, including many puparia. Puparia also were recovered from the human tissue sample.

All recovered puparia were extensively covered in particles or layers of adipocere, sufficient to obscure morphological features necessary for identification. Besides this difficulty, the amount of adipocere on the puparia presented potential health hazards and difficulties in processing because of the strong decomposition odors.

Three potential solvents were evaluated for removing adipocere without damaging puparia: 75% ethanol, 95% acetone, and 2% potassium hydroxide. Solvents were tested with single puparia, and microscopically assessed after each step. A Vortex-Genie II (Scientific Industries, Inc., <http://www.scientificindustries.com>) adjustable speed vortexer was used on a medium setting (eight) for all vortexing steps. Samples were vortexed in 3-dram glass vials (Bioquip <http://www.bioquip.com>) with neoprene stoppers. Once an initial procedure was developed that provided good results, the procedure was repeated with increasing numbers of puparia per sample. In total, the final procedure was tested with six subsamples with approximately 24 puparia per subsample.

Safety

The solvents we used (ethanol, acetone, and potassium hydroxide) all present potential health hazards and must be used in accordance with local regulations. In the United States, Material Data Safety Sheets provide relevant hazard data for these

compounds, including appropriate protective clothing (lab coat, gloves, eye protection, and possibly respiratory). Because adipocere qualifies as human tissue, we did our work in a laboratory certified (by the University of Nebraska-Lincoln) as Biological Safety Level (BSL) two, with all associated procedures and requirements. All used solvents (with or without adipocere) were placed in used solvent containers for disposal by the UN-L Environmental Health and Safety (in accordance with their waste management procedures).

Ethical and Legal Considerations

Because methods reported here were developed in association with an open (but inactive) death investigation, prior to submission, this manuscript was reviewed by the Nebraska State Police and the Pierce County (Nebraska) Attorney's Office and approved for publication.

Results and Discussion

Initial examination indicated that most puparia were covered with adipocere that obscured morphological features (Fig. 1). Additionally, the samples had objectionably strong decomposition odors. Mechanical removal of material with microprobes (e.g., minuten pins on stick handles) was possible, but was only partially effective, had the potential of damaging specimens, and was labor intensive. Consequently, chemical removal of adipocere seemed a preferable procedure.

Our criteria for an acceptable cleaning procedure was removal of most adipocere (>90%) from puparia, removal of adipocere in the posterior groove of sarcophagid puparia (where spiracular plates occur), efficiency (minimal labor requirements and short processing times), and avoiding any damage to puparia or other forensically relevant materials in a sample.

Soaking in solvents alone was insufficient to clean puparia. However, through trial and error, an acceptable procedure was determined, as follows:



FIG. 1—Two puparia covered in adipocere, in situ, away from remains of a homicide victim.



FIG. 2—The posterior of a puparium after cleaning to remove adipocere which had obscured morphological features.

- Samples were sorted in petri dishes with 75% alcohol to remove any larvae, adult insects, or other soft-bodied material. A snorkel hood with negative pressure was used to mitigate odors while sorting samples (working in a fume hood might be an acceptable alternative.)
- Samples of up to 24 puparia were placed in a vial with 15 mL of 95% acetone, capped, and vortexed for a total of 30–90 sec in 10- to 15-sec bursts. This step removed large masses of adipocere or soil from specimen.
- Specimens were removed from acetone and placed in a vial of 15 mL of 2% potassium hydroxide (KOH) and vortexed in 10- to 15-sec bursts until all puparia appeared clean (with our samples, this required a total of 60–120 sec).
- Specimens were removed from the 2% KOH, placed in 75% ethanol, and examined microscopically. If insufficient cleaning occurred, specimens were set aside for repeated vortexing in 2% KOH, or if adipocere was adhering to the posterior groove of sarcophagid puparia, a probe was gently used to dislodge material.
- After all cleaning steps, materials were stored in 75% ethanol for identification and long-term preservation.

Figure 2 shows the posterior of a puparium after cleaning. This technique provides a reliable, rapid method for rapidly cleaning a large number of puparia or pupa. We limited our vortexing steps to 24 puparia to avoid potential damage from puparia collisions. In microscopic examinations, we did not notice increased fragmentation or specimen damage after vortexing in KOH, probably because most material we used were whole puparia (both eclosed and unclosed). Similarly, we did not see any damage to other insect evidence (elytra, head capsules, etc.) in our samples, although clearly fine material (legs and antennae) probably should be separated if possible before vortexing. After cleaning, pupae were stored in vials with ethanol (to the top of the neoprene stopper to avoid air space); ethanol is not necessary for preserving puparia, but the liquid storage does help avoid damage to puparia as they are moved.

Prolonged soaking in KOH or heating in KOH could be used as an alternative to vortexing, and might avoid the potential for damage to delicate material. In our example, specimens were not delicate, and we chose vortexing because it was fast and gave us greater control over the degree of processing needed. In particular, rapid removal of adipocere from puparia seems to require both a solvent and mechanical disturbance. By vortexing puparia in short bursts and examining specimens immediately thereafter, we could achieve adequate cleaning without prolonged labor or the longer processing time associated with soaking.

It is worth noting that finding puparia with extensive adipocere coverage is unusual and can be of forensic significance. In this instance, the location of adipocere covered puparia away from the body, coupled with the occurrence of other insects associated with advanced decomposition (Piophilidae larvae), and indicated the location where the body was discovered was a secondary crime scene (i.e., the body was moved well after decomposition had begun). This homicide remains unsolved.

Acknowledgments

We appreciate the assistance of the Nebraska State Patrol with recovery of evidence for analysis.

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