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## Archaeoentomological and archaeoacarological investigations of embalming jar contents from the San Lorenzo Basilica in Florence, Italy

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## ABSTRACT

Entomological investigations of material from embalming jars found in the San Lorenzo Basilica of Florence, Italy, reveals information concerning the mortuary and embalming practices of the Medici family during the 17th and 18th centuries. The analysis of samples from these jars demonstrated the presence of mid- to late colonizers, *Hydrotaea capensis* (Diptera: Muscidae) and *Conicera tibialis* (Diptera: Phoridae), among the human remains and embalming materials within the jars. The presence of puparia and absence of adult flies suggests that some of the jars may have been initially left open to the surrounding environment and later closed. The lack of dipteran remains from sepsids, piophilids, and fannids was not surprising as materials in the jars were not likely to attract these types of flies. Spider beetles, likely *Ptinus dubius* and *Ptinus subpilosus* (Coleoptera: Ptinidae), were recovered from the embalming jars, indicating that insects also had access to embalming jar contents after drying. The absence of dermestid beetles, which are extremely common on dried remains, supports the interpretation that these jars could have been made unavailable at some point after embalming. These analyses provide an interesting case for insect colonization into embalming jars and give more information regarding mortuary practices.

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## 1. Introduction

Entomology has long been applied to archaeological contexts to make inferences about a wide range of subjects (Smith, 1986; Kenward, 1975; Kenward, 1976; Osborne, 1983; Panagiotakopulu et al., 2007). The field of archaeoentomology applies entomological techniques to materials of human origin, such as mummies and coprolites (Nystrom et al., 2005; Couri et al., 2009; Huchet & Greenberg, 2010; Panagiotakopulu & Buckland, 2012; Huchet et al., 2013). Many invertebrates do not preserve well in archaeological contexts because of their soft-bodied nature. In contrast, arthropods, such as insects, have bodies comprised primarily of chitin, which make them more resistant to natural decomposition. Heavily sclerotized parts of insects, for example, the exoskeletons of adult insects or the puparia of holometabolous insects, are resistant to many taphonomic processes. This resilience makes certain insects, including flies (Order Diptera) and beetles (Order

Coleoptera), excellent sources of information when collected from archaeological contexts. The presence of insect remains within archaeological contexts reveals information regarding the environment and mortuary practices of peoples in antiquity (Gilbert & Bass, 1967; Teskey & Turnbull, 1979).

The types of information gathered from insects at archaeological sites mirror the types of information that can be revealed by insects during forensic investigations (Gennard, 2007). For example, the presence of different insect species can lead researchers to estimate the length of time that human remains were open to the environment prior to burial (Campobasso et al., 2004). In an archaeological setting, these kinds of data can be valuable for better understanding mortuary practices, recognizing curatorial issues, and as support for information gathered from historical records (Morrow et al., 2015; Giuffra et al., 2011a).

Taphonomic studies of insect remains found in archaeological context have primarily included identification of insects belonging to the taxonomic orders Coleoptera (beetles) and Diptera (true flies). The arrival of arthropods to corpses varies greatly with environmental factors such as ambient temperature, aridity, and season. Holometabolous insects, such as dipterans and coleopterans, experience a pupal stage as part of their life cycle. During this stage, cases known as “puparia” are

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formed inside of which the insects undergo metamorphic processes. These puparia are comprised of chitin, which is resilient enough to be recovered from archaeological contexts (Morrow et al., 2015; Morrow et al., 2016). The hardened exoskeletons of many arthropods, including beetles, grasshoppers, ants, and a wide range of other insects, also preserve well in archaeological contexts.

The corpocenos (community of organisms found in context with corpses) varies greatly by the geographic region, climate, and seasonality associated with the death and subsequent mortuary practices used on a body. Speaking generally, early stages of decomposition tend to be dominated by flies, which create an alkaline environment as they colonize a corpse. This makes the environment unsuitable for many beetles that feed on decaying material, but it may attract certain types of beetles, such as rove beetles (Staphylinidae) and carrion beetles (Silphidae), which are predators of maggots. Beetles belonging to an array of taxonomic families have been documented at carcasses and corpses during various stages of decomposition (Scott, 1998; Midgley et al., 2010).

Flies are not limited to the early stages of decomposition. Several dipteran families can be found in and on corpses during many stages of decomposition. Members of the family Calliphoridae (blow flies) are typically the first flies to arrive after death (Gennard, 2007). Blow flies may arrive within hours or even minutes of death given the correct circumstances. Members of this family are quickly followed by the arrival of flies belonging to families Muscidae (house/stable flies) and Sarcophagidae (flesh flies). All of these flies begin to infest a dead body while it is still in the fresh stage of decomposition (prior to bloating) (Van Laerhoven, 1996).

After the bloat stage of decomposition begins, these flies may be joined by cheese skippers (Family Piophilidae) and little house flies (Family Fannidae) (Payne, 1965; Grassberger & Frank, 2004). The larvae of all of these dipterans will pierce through the body as they feed and grow, which will eventually help to release the internal pressure created by gases during the bloat stage. This will lead to body deflation, which marks the beginning of the active decay stage of decomposition. This new stage often attracts black scavenger flies (Family Sepsidae) (Van Laerhoven, 1996; Payne, 1965). The final stage of decomposition is known as the dry stage. At this point, a corpse has been reduced to dry skin, cartilage, bones, and sometimes other desiccated tissues. Following the dry stage, an additional type of fly becomes attracted to the corpse. Coffin flies of the family Phoridae are tiny flies known to penetrate through earth and crawl into presumably sealed coffins to infest a corpse. The different behaviors and attributes of arthropods comprising the corpocenos allow for interpretations of their presence among human remains.

The insect remains recovered in the present study came from embalming jars containing tissues and materials used for the preparation of corpses belonging to members of the Medici family. The Medici gained prominence in Florence beginning in the late 14th century under the leadership of Cosimo the Elder, also known as the *Pater Patriae*. Though Florence prided itself on being a republic, the Medici family financed the majority of the city's government, which afforded them great political power. The family also funded many artists, giving birth to the Italian Renaissance. Being one of the most influential and wealthy dynasties of the time was reflected in Pope Pius II's comment that Cosimo di Giovanni de' Medici was "a king in all but name and ceremony" (Strathern, 2005). The power of the Medici family stretched well into the 18th century, despite years of fluctuating between ruling the city and being forced into exile at the hands of religious zealots and competing noble families.

The Medici family tree is split into two major branches deriving from the lineages of the sons of Giovanni di Bicci. The "senior" branch derives from Cosimo the Elder and the "junior" branch deriving from Lorenzo the Elder. The senior branch ended when Alessandro de' Medici was assassinated in 1537. The junior branch came to power under Cosimo I de' Medici, who became the first Grand Duke of Tuscany (Fornaciari et al.,

2008). The death of Gian Gastone de' Medici in 1737, who died without offspring, marked the end of Medici rule as the House of Lorraine seized control of Florence (Strathern, 2005).

The prominence of the Medici family led them to have funerary customs similar to those practiced for royalty in neighboring countries. Near the end of the Medici line, this would have involved procedures for surgical embalming, which was considered to be the best technique for reliable long-term preservation of corpses belonging to public figures until the early 19th century (Marinozzi, 2012). Some signs of embalming have been found in the remains for members of the Medici grand dukes, which has allowed for a better understanding of embalming procedures used on members of this family between the 16th and 18th centuries. Embalming jars containing tissues and materials used in the embalming process were interred within the Old Sacristy of the San Lorenzo Basilica in Florence, Italy. The Old Sacristy has been the place of interment for most members of the Medici family over the centuries. The present study examined insect remains collected from these jars.

The jars were exhumed in 2010 as part of a multidisciplinary project initiated in 2004 to study the burials of the Medici (Lippi, 2006; Villari et al., 2009). A large portion of the human remains examined in the larger study show evidence of embalming practices, even though the bodies have become skeletonized (Giuffra et al., 2011a; Fornaciari et al., 2008; Fornaciari et al., 2006; Fornaciari et al., 2007). The embalming jars contained the visceral remains of at least two prominent members of the Medici family along with materials, such as sponges and cloth, used to prepare their bodies after death (Fornaciari et al., 2008; Marinozzi & Fornaciari, 2005). The jars also contained the remains of various insects. The identification of these remains allowed researchers to discuss if and when the jars were closed to the exterior environment. These jars could have been individually sealed with cork or ceramic lids after they were used. At some point, all jars were placed into a small sub-floor space. A total of 10 samples were submitted for analysis to the University of Nebraska-Lincoln in 2012.

Some of these samples could be linked to specific Medici family members. Sample 2 contained dried intestinal tissues linked to Anna Maria Luisa de' Medici (1667–1743) and Sample 10 was composed entirely of visceral fragments belonging to Vittoria della Rovere (1622–1694), who was the wife of Ferdinand II de' Medici. However, two other jars were reported to belong to the same individuals (Lippi, 2006). The remaining samples were not labeled, but were linked to members of the Medici family because they were recovered within the same context. Samples 3, 6, and 9 were comprised of textile, vegetal, and/or sponge fragments that had been used to clean the corpses. Samples 4, 5, and 8 contained cleaning materials mixed with desiccated human tissues. Samples 5 and 8 appeared to have tiny particles of intestinal contents (digesta), perhaps from food. Sample 7 was almost entirely comprised of insect remains and small fragments of cotton-like material.

Arthropod remains from these jars were examined in the present study. Herein, we describe the types of remains recovered, identify those with enough morphological evidence to be identified, and interpret the significance of finding these remains within the embalming jars. These analyses were conducted to better understand the archaeological corpocenos and to investigate the archaeoentomology of historic embalming jars. Through these data, we are able to better understand mortuary practices relating to members of the Medici family.

## 2. Methods and materials

Samples were collected from the 10 embalming jars and submitted to the Pathoecology and Palynology Laboratory in the School of Natural Resources at the University of Nebraska-Lincoln for analysis. The samples were first weighed using an electronic balance, accurate to 0.01 g. This was done for later quantification of microfossils, such as mites.

Samples were rehydrated in 0.5% trisodium phosphate for 48-h. The samples were subsequently disaggregated, treated with *Lycopodium* spore tablets dissolved in hydrochloric acid, and screened through a 250- $\mu$ m mesh.

Macroscopic remains (material larger than 250  $\mu$ m) were dried on filter paper overnight. Each sample was then sorted for arthropod remains using an Olympus model SZ40 dissecting microscope. The arthropod remains were individually removed from the samples and placed into vials. The remains were further separated by taxon for counting and each group of insect remains was weighed. The count was done by weighing out a gram of the specimens and then counting the number of puparia in the gram. This count was then multiplied by the total grams of insects from each sample.

Microscopic remains (microfossils smaller than 250  $\mu$ m) were scanned for mite eggs, larvae, nymphs, and adults. Whole and fractured eggs were counted. Only mite capitula, or mite fragments including capitula, were counted to prevent over-estimating mite densities in the samples. Quantification analysis was conducted utilizing *Lycopodium* spore counts in conjunction with mite capitula and mite egg counts. Microfossil concentration values are calculated using the following formula: microfossil concentration =  $[(f/m) \times a]/w$ , where  $f$  is the number of microfossils counted,  $m$  is the number of marker grains (*Lycopodium* spores) counted,  $a$  is the number of marker grains added to the sample, and  $w$  is the total weight of the sample prior to rehydration. Utilizing this system for quantification allowed us to estimate the numbers of microfossils (mites and mite eggs) present per gram of the sample.

Insect identifications were made using light microscopy (10–80 $\times$ ). Because samples typically consisted of a combination of intact insects and insect parts, some counts were based on a morphological feature unique to an individual, usually heads. References for identification and ecology included 1, 7, and 31. Most specimens could be determined to the species level based on morphological features, but definitive identifications of *Ptinus* specimens to the species level was not possible. Data analysis for insect counts utilized basic arithmetic.

### 3. Results

Of the ten samples, six (Samples 4–7, 9, and 10) contained insect remains. Significantly, the intestinal sections represented by Samples 1, 2, and 3 contained no insects. While several different types of insects were found within these samples, most of the insect remains were whole or fragments of puparia from members of the Muscidae and Phoridae. Puparia were found in both pre-eclosure (puparium containing pupa) and post-eclosure (puparium opened after adults emerge) stages. The presence of pupae within puparia varied greatly: ranging from fewer than 30% with pupae (commonly encountered) to 100% with unopened puparia (rarely encountered). Muscidae remains were frequently observed to have the puparia flattened or crushed.

The samples varied greatly in their numbers of insect remains. Remains within samples ranged from fewer than 50 individual specimens to >20,000 specimens. Additionally, the samples varied in the diversity of insects found. Typically, only a single taxon of dipteran was present inside each sample. However, Sample 6 contained both muscids and phorids. Muscids were also found in Samples 4 and 9, while phorids were found in Samples 5, 7, and 10 (Table 2). Spider beetles (Coleoptera: Ptinidae) were recovered in two of the samples: Sample 5 (Fig. 3 and Table 2) and Sample 4 (Fig. 4 and Table 2).

Mites (Class Arachnida, Subclass Acari) were observed during the analysis of microfossils. Mites and/or mite eggs were encountered in Samples 4–7, 9, and 10. These mites were never found as whole specimens, and identification to family was not possible given the poor preservation state of the recovered organismal fragments. For this reason, mite densities are represented by counts of mite capitula rather than by whole specimens (Table 1).

**Table 1**

Sample contexts and total mite densities from Medici embalming jars.

Sample #	Context	Mite density
1	Intestine section	No mites present
2	Intestine section	2 capitula observed
3	Packing material and textiles	121 mites/g
4	Viscera, insects, and textiles	10,783 mites/g
5	Soft tissue, insects, and vegetal remains	5125 mites/g
6	Packing material, textile, insects and sponge fibers	5723 mites/g
7	Fiber and insects	9191 mites/g
8	Soft tissue and vegetal remains	No mites present
9	Packing material, textiles, and insects	20,574 mites/g
10	Intestine fragments and insects	1300 mites/g

### 4. Discussion

The results of the entomological analysis reveal information regarding the mortuary techniques practiced during the 17th and 18th centuries in Florence. The bodies were processed carefully as shown by the large variety of organic remains that were placed within the body cavity (Giuffra et al., 2011a; Giuffra et al., 2011b). The precise history of these embalming jars is ambiguous, making it difficult to know how anthropogenic manipulations have affected insect communities within them over time. It has been reported that these jars were inspected a few times prior to the current study (Lippi, 2006).

A total of 6 out of 10 samples from the embalming jars contained insect remains. Some jars were sealed with corks, which were presumably used given their ability to seal the internal contents from the outer environment while allowing for internal gases to escape through pores. There was also an absence of adult flies within the samples. Some insects, such as many of the dipterans, are capable of depositing eggs into areas where the adults themselves are not able to enter. However, if flies had deposited eggs into the jars that were sealed, then when the fly pupae eclosed (emerged), the adults would not have been able to escape the jars and their carcasses would have been recovered by our analysis. Thus, these jars might have been closed after being infested and after adult flies had emerged from their puparia.

Samples from Jars 1, 2, 3, and 8 contained no insect remains (Table 2). These jars held intestinal sections and other soft tissues as well as packing textiles and vegetal remains (Table 1). Jars 1 and 8 contained no mites. It is likely that these jars were closed to the external environment quickly after they were filled during the embalming process, effectively preventing infestation of insects and potentially of mites as well. A lack of arthropods might also imply that embalming or other factors made the tissues inside of these jars unattractive to these organisms.

Puparia of *Hydrotaea capensis* (Diptera: Muscidae; formerly *Ophyra capensis*) (Fig. 1) were collected from jars 4, 6, and 9 (Table 2). These jars contained viscera and textiles and were infested with mites ranging from 5723 mites/g to 20,574 mites/g (Table 1) (Fig. 5). Jars 4 and 9 both lacked phorid puparia, suggesting that they were closed to the external environment prior to the arrival of these tiny flies, but after *Hydrotaea capensis* had reached their pupal stages. The small number of muscids recovered from jar 6, along with an increasing number of phorids, is curious. We cannot find a satisfying explanation of this arthropod assemblage.

Phorid puparia (Fig. 2) were collected from jars 5, 6, 7, and 10 (Table 2). Jars 5, 7, and 10 contained bodily tissues, vegetal remains, fibers, and many phorid puparia in addition to mites ranging from 1300 mites/g to 9191 mites/g (Table 1). The extremely high number of phorids from jar 10 could indicate that the intestinal fragments dried quickly upon removal from the body and thus attracted phorids rather than *Hydrotaea capensis*. This jar also contained a low concentration of mites, which supports a rapid drying hypothesis.

**Table 2**

Insect remains recovered from the Medici embalming jars. For Diptera remains were puparia (mostly fragmentary) and for Coleoptera, remains were of adults (mostly fragmentary, primarily elytra). Counts represent number of individuals based on unique posterior spiracular plate for puparia, and on paired elytra for beetles. Species followed by \* indicate that morphological characters are consistent with this species designation, but a definitive species id is not possible.

Jar #	Context	Order	Family	Species	#	References
1	Intestine section				0	
2	Intestine section				0	
3	Packing material & textiles				0	
4	Viscera, insects, & textiles	Diptera	Muscidae	<i>Hydrotaea capensis</i>	>20	(Smith, 1986; Couri et al., 2009)
5	Soft tissue, insects, & vegetal remains	Diptera	Phoridae	<i>Conicera tibialis</i>	>100	(Smith, 1986)
6	Packing material, textile, insects & sponge fibers	Coleoptera	Ptinidae	<i>Ptinus dubius</i> *	4	(Arnett et al., 2014)
		Coleoptera	Ptinidae	<i>Ptinus subpilosus</i> *		(Arnett et al., 2014)
		Diptera	Muscidae	<i>Hydrotaea capensis</i>	2	(Smith, 1986; Couri et al., 2009)
		Diptera	Phoridae	<i>Conicera tibialis</i>	>50	(Smith, 1986)
7	Fiber and insects	Diptera	Phoridae	<i>Conicera tibialis</i>	>500	(Smith, 1986)
8	Soft tissue & vegetal remains				0	
9	Packing material, textiles, & insects	Diptera	Muscidae	<i>Hydrotaea capensis</i>	>100	(Smith, 1986; Couri et al., 2009)
10	Intestine fragments & insects	Diptera	Phoridae	<i>Conicera tibialis</i>	>2000	(Smith, 1986)

Beetle remains were only encountered in Jars 4 and 5 (Table 2). These were spider beetles (Coleoptera: Ptinidae) (Fig. 3), and morphological features on material were consistent with *Ptinus dubius* (Jar 4) and *Ptinus subpilosus* (Jar 5) although we cannot be definitive regarding these identifications. Spider beetles are associated with decomposition of dried tissues, but are typically less common than other insects on dried remains.

A diversity of mites was recovered in this study. Though our research team did not possess the expertise to identify these mites taxonomically, we were able to quantify the number of mites that were observed within the samples. This was done using the *Lycopodium* quantification technique often used for palynological and archaeoparasitological analyses. In the present study, this technique was applied to quantify the mites and mite eggs present in the Medici embalming jars. We are not aware of previous use of this approach with arthropods, but our results demonstrate the technique's potential usefulness as a tool for measuring the presence of such organisms within archaeological contexts.

Some of the jars may not have been closed immediately to allow for the release of decomposition gases produced by internal microbiota. However, the embalming process itself is designed (in part) to avoid this sort of microbial decomposition. Additionally, the contents of these jars (e.g., sponges and fabric used in embalming) argue against the development of decomposition gas being a major issue affecting the timing of jar sealing.

These analyses provide additional data regarding the corpocenos as related to historic human remains in Italy. The assemblage of insects



**Fig. 2.** Puparium belonging to a coffin fly, *Conicera tibialis* (Diptera: Phoridae) from a Medici embalming jar. (Image credit: J.J. Morrow, 2014).

recovered from these jars is most often associated with mid-late stage decomposition, which provides information regarding mortuary practices. While the role of insects at corpses has been well-documented, we know little about the role of mites in corporeal environments. The mite data from this study may prove useful in future archaeocarological studies.

The location of different insect species by jar and a knowledge of insect biology allows us to interpret our findings relative to embalming and decomposition. First, none of the jars showed any evidence of species associated with early decomposition, and, in particular, no blow fly (Diptera: Calliphoridae) remains were discovered. Typically, the calliphorids are the first insect species to arrive at a dead body, but they are not typically associated with later stage decomposition, particularly after soft tissues have decomposed. Second, all of the arthropod species recovered in this analysis are associated with mid to late stage



**Fig. 1.** Posterior spiracles of a puparium of *Hydrotaea capensis* (Diptera: Muscidae) from a Medici embalming jar. Scale = 0.2 mm. (Image credit: J.J. Morrow, 2014).



**Fig. 3.** Spider beetle remains (Coleoptera: Ptinidae) from Medici embalming jars. (Image credit: K.J. Reinhard, 2012).



Fig. 4. Spider beetle remains (Coleoptera: Ptinidae) from de' Medici embalming jars. (Image Credit: K.J. Reinhard, 2012).

decomposition up to, and in the case of the spider beetles, including decomposition of dried tissues. Third, just as the absence of blow flies tells us that the contents of the embalming jars were not “fresh” tissue (which would have been attractive to early stage decomposers), so too does the absence of another species tell us that old, dry tissues were not accessible to insects. The most common and ubiquitous insect group associated with decomposition of dried tissues, hair, and skin is the hide or larder beetles, the dermestids (Coleoptera: Dermestidae). If contents of the embalming jars had been left open for an extended length of time (many months), then it would have been inevitable that once all materials became completely dry, dermestid adults and larvae would have infested those jars. Consequently, it is almost certain that jars were not accessible to arthropods for an extended period of time.

Analysis of the entomological evidence suggests that some embalming jars may have been left uncovered for a period of time after embalming took place. These jars could have then been closed, possibly at different time intervals, which may have changed the internal environment of the jars and effectively stopped the development of remaining insects prior to eclosure. Eventually, all of these jars were entombed beneath the floor. Differences in the diversity of insect remains within the jars indicate that the jars varied in their attractiveness to insects as a reflection of their contents. These differences may also be explained by taphonomic factors including differential chemical changes within the individual jars (e.g. the presence of residual vinegars, strong brandies, and/or plant extracts on textiles used to process the corpse that were deposited within the jars), some jars being closed at different times or seasons, and mite infestations (Morrow et al., 2016).

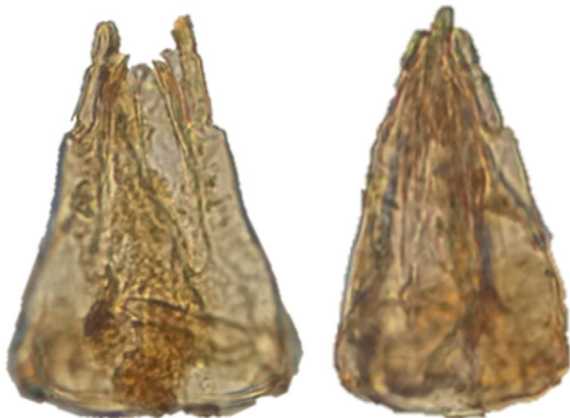


Fig. 5. Mite capitula recovered during microfossil examinations of Medici embalming jars. (Image credit: K.J. Reinhard, 2012).

Anthropophilic flies have been associated with human remains since the rise of civilization (Oldroyd, 1964). Keeping all flies away from a decomposing body is virtually impossible without metal screening and refrigeration, neither of which would have been used in the 15th to 18th centuries. Embalmers of the time period may have expected that flies would appear during body preparations and may have dealt with this reality by closing jars containing bodily tissues, such as intestines and other viscera, early. Perhaps they were less concerned with materials used in body preparation that were deposited in other embalming jars, possibly leaving those jars to be sealed later.

Adult flies would have been common during the embalming process in spring through fall, and the times of death for Vittoria della Rovere (March) and Anna Maria Luisa de' Medici (February), are at the edge of this period. Embalming Jar 2, containing remains of Anna Maria Luisa de' Medici, had no insects. This may be due to the absence of decompositional flies in the late winter-early spring time period. For other embalming jars containing remains not associated with a particular individual from the Medici family, we can say that these jars were likely open during the spring to winter period. This seasonal information may be helpful for future researchers attempting to associate the jars with specific deaths.

## 5. Conclusions

These interpretations suggest that the materials in the embalming jars were attractive to decomposers associated with mid- to late stage decomposition (which is true of all the species that were able to be identified from the jars), and that the jars were left uncovered for a sufficient length of time to allow for maggots to complete development and for adult flies to emerge, but not for so long a time that the jars' contents completely dried and could be infested by dermestid beetles.

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