Effects of burial at low temperature on the colonisation of pig cadavers by Scuttle flies (Diptera, Phoridae) during a British winter.

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Abstract

Twenty-eight neonatal pigs were buried in three different conditions (partial burial, burial at 0.5m and exposed) in a section of woodland in Ascot, UK during the winter of 2009-2010. Replicates were sampled and culled at 5 and 8 weeks. Several species of adult Scuttle fly were recovered from the cadavers as well as a number of larvae, the vast majority of which were from the genus *Triphleba*. This is the first time that some species from this genus have been recorded feeding on carrion in south east England. Colonisation of the cadavers was measured using a count of the larvae recovered from each replicate and burial condition was found to have a significant effect at 8 weeks ($F_{[2,11]}=8.1421$, $p<0.01$). Several problems were also encountered during this study and are discussed at length. Further research is recommended to investigate the use of the genus *Triphleba* as a forensic indicator.

Introduction

Temperature-based developmental and oviposition studies of certain Dipteran families are a staple of forensic entomology (Greenberg, 1991; Grassberg and Reiter, 2001; Wells and LaMotte, 2001). Such studies equip the field entomologist with the tools to estimate a Post Mortem Interval (PMI) but require detailed and accurate data sets. There are a large number of confounding factors with entomological evidence in homicide cases such as weather conditions, temperature, burial, access, food type and decomposition, all of which need to be taken into consideration for PMI (Kaneshrajah and Turner, 2004; Plessis and Meintjes, 2004).

Calliphorid flies such as *Calliphora vicina*, *C. vomitoria*, *Phormia regina* and *Lucilia sericata* are amongst those most typically associated with forensic cases in the UK. These flies are, however, known to cease oviposition and in most cases resort to diapause during cold weather (Mellanby, 1938; Ring, 1967; Saunders, 1976). In cases such as these, the forensic entomologist may have to turn to less well studied Dipteran families to produce meaningful entomological evidence.

Disney and Manlove (2005) reported three homicide cases during the winter months in Wales, where *Megaselia abdita* and *M. rufipes* were recovered. These were also cases where common carrion flies were unable to gain access to the corpses due to either burial or being located in a sealed room. Although the *Phoridae* family had previously been identified as forensically important (Greenberg, 1991; Greenberg and Wells, 1998) this was the first example of their use in a UK legal investigation.

Phoridae are one of the largest and most diverse families of Diptera, living in a broad range of habitats and consisting of over 22 genera globally with *Megaselia* being one of the largest in the animal kingdom with some 1400 described species (Disney, 1994). Around 300 species of Scuttle fly occur in Britain. The detailed natural history of the...
huge number of identified species of Scuttle flies remains largely unknown, although a large body of work by Henry Disney, and also the German entomologist Herman Schmitz, over the last century have given a general overview of the behavioural habits of a number of important and interesting species. Like many other Dipteran families, Scuttle flies are holometabolous, with three larval instars. The larvae generally possess tapered processes at the posterior as well as a number of spines across the cuticle and the pupariums are typically characterised by the presence of respiratory horns. Feeding and reproductive habits are extremely varied. Many Phorid larvae are known to be saprophagous, feeding on organic matter, faeces and vertebrate and invertebrate carrion and general refuse, some are known to be fungusfeeding, some plant-feeding and others are predators, parasitoids and parasitic on other invertebrates (Disney, 1994). No doubt the feeding and breeding niches occupied by these flies are as numerous as the species found in this fascinating family of Diptera.

*Megaselia abdita* and *M. scalaris* are the two species most encountered in forensic cases (Greenberg, 1991; Greenberg and Wells, 2001, Disney and Manlove, 2005, Manlove and Disney, 2008). *Megaselia scalaris* has also been identified as a useful laboratory species (Disney, 2008). Some species of the genus *Triphleba* have also been associated with human remains in the UK as well as the well known Coffin fly *Conicera tibialis*, which is known to breed on carrion in the UK (Coyler, 1954a; Coyler, 1954b)

*Megaselia scalaris* exploit a wide range of larval pabula and as such pose a considerable pest problem and disease vector as well as being of use in forensic investigations. Larvae have been found feeding on many insect and mollusc species, invertebrate faeces, eggs and carrion and also on rotting plant matter (Disney, 2008). Although some developmental data has been carried out, they have all been at temperatures exceeding 14°C (Prawirodisastro and Benjamin, 1979; Trumble and Pienkowski, 1979), without focusing on low temperatures and – certainly in the examples cited – with the larvae being reared on a synthetic diet. As such, these data cannot be used in the accurate estimation of a PMI. *Megaselia abdita* has been utilised to estimate PMI in the UK, however compared with *M. scalaris* it has received little attention in laboratory research. Greenberg and Wells (1998) carried out studies measuring the minimum development of *M. abdita* to each life stage at 10°C, 12.5°C and 23°C and *M. scalaris* in the same manner at 19°C, 22°C, 29°C and 35°C. Neither of these studies, however, considered time to oviposition or indeed minimum temperature of oviposition. As with many studies of this kind they lacked the detailed increments in temperature that would be of most use to forensic practitioners, in particular the low temperature measurements which could be of forensic significance in the higher latitudes.

*Triphleba nudipalpis* has recently been identified as a fly of forensic importance (Disney and Manlove, 2009). It was the only fly found on a murder victim who had been buried under heavy clay soil in the east of England. Similarly to the previous cases involving *Megaselia* the adult had dug down through the soil to feed on the cadaver and to oviposit. A few species are noted as carrion breeders in Disney (1983).

The current study aims to focus on the presence of Scuttle flies on pig carrion which has been either buried or exposed during the winter months of December and January in southern UK. It aims to observe how different burial conditions affect the colonisation of the cadavers by Scuttle flies as
some species, such as *Conicera tibialis*, are known to dig down to human remains which have been buried at a considerable depth and those flies that were used in the cases described by Disney and Manlove (2005) were found on remains buried over half a metre below ground. The presence of this family will be compared with the presence of Calliphorid flies and will investigate the effect of burial at low temperature on the presence of both families to assess their role as potential forensic indicators. Should both families be present and breeding on the carrion it may be possible to calibrate Phorid larvae against Calliphorid larvae, given what is already known about the development rates of certain species. This could have the potential of increasing the accuracy of PMI estimations. Since there have been no observational studies focussing on the presence of Scuttle flies it is hoped that there will be some findings which may help to outline potential further research with this family with regard to forensic science.

**Materials and Methods**

**Source of piglet carcasses**

The piglets used in this experiment were Large White domestic pigs (*Sus scrofa*) sourced from a local pigging farm. All piglets were naturally still-born, weighing between 0.5-1kg and were immediately double bagged and frozen in a sealed container to minimise exposure to any insect visitors. All piglets were thawed out for approximately 48 hours before being used and the maximum frozen period for any single piglet was no more than a calendar month.

**Methods of burial**

The burial site of all the replicates was in a sloping woodland at Silwood Park, Ascot around an 18m radius from BNG co-ordinates (494,282.894, 168,733.673) (Figure 1). The site was chosen primarily for the presence of thick shrubs and dense leaf litter which were suitable for the purposes of one of the experimental conditions. Secondarily, the experimenter considered the sloping nature of the site to be of importance as it would offer extra cover to a deserted carcass. The aim was to choose a burial site that a homicide perpetrator might choose for burying a corpse.

Piglets were placed at random in one of three conditions; exposed (10), partially buried (9) and buried (9). In the exposed condition (Figure 2), piglets were left above ground and uncovered. In the partially buried condition (Figure 3), piglets were buried under a layer of leaf litter and the gravesite disguised with Bracken which was growing naturally at the grave sites. In the soil burial condition (Figure 4), the piglets were buried in shallow graves of approximately 0.5m, this being the depth most often encountered in forensic cases (Shultz, 2008). Test runs using pig offal had shown that any meat buried in a non-secure manner was promptly dug up and carried away by larger vertebrates such as foxes, so in all conditions the piglets were placed inside mesh cages (50cm x 30cm x 20cm) designed to prevent disturbance. Figures 2-4 show the layout of the burials:
Figure 1: Map of the burial sites at Silwood Park, Ascot, showing the co-ordinates and maximum radius of graves from the central point.

Figure 2: Cross sectional diagram of the 'partially buried' condition; piglet is placed on top of the soil margin but underneath leaf litter and plant material which was naturally occurring at the site. It is also within a mesh security cage which is partially buried and pegged into the ground to prevent larger animals from disturbing the carcass.
Figure 3: Cross sectional diagram of the ‘soil buried’ condition; piglet is placed at a depth of 50cm at the bottom of the mesh security cage and covered completely with soil up to the normal soil margin and then leaf litter and plant material naturally occurring at the site.

Figure 4: Cross sectional diagram of the ‘exposed’ condition; piglet is placed on top of naturally occurring leaf litter and plant material within mesh security cage. The cage is partially buried and pegged into the ground to prevent larger animals from disturbing the carcass.

Temperature data loggers [Thermochron ‘iButtons’; model DS1921G, Maxim Integrated Products, Inc. (California, U.S.A.)] were buried within each piglet carcass in a small incision made on the uppermost side behind the ribs and also in the surrounding soil or leaf litter, depending on the condition of either partially buried or soil buried, respectively. Ambient air temperature for the exposed condition was collected from the Silwood Park weather station.

Sampling methods

Piglets were left undisturbed in each condition for a total of 8 weeks, with half the replicates being culled and examined after 5 weeks. At the point of sampling the piglet was extracted directly from the grave site and immediately bagged in separate sealed plastic bags which were secured and transferred to the laboratory for examination. Once at the laboratory, each piglet was unsealed and processed individually to avoid cross-contamination.

The process of examination consisted of an initial search of the carcass lasting a minimum of 15 minutes, examining ears, eyes/eye sockets, mouth, anus, vagina and the wound opening holding the data logger. Any live larvae, eggs and other insects were
immediately fixed in 70% alcohol and some eggs and larvae were also transferred to sample tubes containing paper towel and a small piece of liver and kept for incubation. Any excess soil was searched thoroughly and the bags which the pigs had been sealed in following exhumation were also thoroughly searched. Once the processing was complete the carcases and any packaging and soil were disposed of in clinical waste bins.

The larvae held for incubation were then transferred to containers secured with an elastic band around entomological net with a piece of dry tissue, a small amount of wetted fish food, dog food and sugar water. These containers were held in a controlled temperature room within a sealed tank ventilated to the outside of the building at a temperature of 22°C/70% humidity.

All recovered insects were sorted to order in the first instance. Following this, all Diptera specimens were identified down to genus level and Phorids and Calliphorids were further identified down to species. Phorids were separated and slide mounted for the purposes of identification. This was carried out in line with the instructions laid out by Disney (1983). Several Phorid larvae were also slide mounted for the purposes of identification to family.

Several pitfall traps and Irwin traps baited with fish heads or liver were set in the surrounding area which could be used as control collections and to get a better idea of the local insect fauna.

Colonisation of each cadaver was measured by the number of Phorid and Calliphorid larvae present. Statistical data analysis was carried out using ‘R’ (v.2.9.0; The R Foundation for Statistical Computing, 2009) where a one-way ANOVA was calculated for each time period, 5 and 8 weeks. A generalised linear model with a poisson error was also used to assess the effect of burial type on colonisation across the entire experiment. Observational data were also collected regarding the range of diversity of insect visitors and more specifically, species of Phorids and Calliphorids.

Results

Colonisation of pig cadavers

After 5 weeks, analysis of variance showed that there was no significant effect of burial condition on the colonisation of the cadavers ($F_{[2,11]}=0.7969$, p<0.5).

After 8 weeks, however, analysis of variance showed that there was a significant effect of burial condition on the colonisation of cadavers ($F_{[2,12]}=8.1421$, p<0.01).

A generalised linear model was used to assess the effect of burial condition across the entire experiment, not accounting for time period. No significant differences were found using poisson error (partial $F_{[25,17]}=0.964$, buried $F_{[25,27]}=0.971$ and exposed $F_{[25,27]}=0.968$) and the data was highly over dispersed (Null deviance: 2195.1 on 27d.f.), this is most likely due to the relatively small data set used. A quasipoisson error was tested to try and reduce the over dispersion, however this also returned non-significant results (partial $F_{[25,17]}=0.995$, buried $F_{[25,27]}=0.996$ and exposed $F_{[25,27]}=0.996$) and the over dispersion remained the same.

The most larvae were found at the 8 week sampling point and the vast majority of these were in the partial condition. No larvae were found at 5 or 8 weeks in the buried condition. Arithmetic means of the total amount of larvae found on each pig in each condition were: Partial (week 5 = 1.4, week 8 = 136.75), Buried (week 5 = 0, week 8 = 0),
Exposed (week 5 = 2, week 8 = 7.4). Figure 5 summarises the totals of larvae found:

![Graph showing number of larvae found across burial conditions and time periods.]

**Figure 5: Total number of larvae found on pig cadavers across the three burial conditions over two time periods**

**Observational data**

In total 9 invertebrate orders were recovered from the cadavers and pitfall traps, with 8 of these being recovered from the pigs themselves. Those recovered from the cadavers were Diptera (adults and larvae), Coleoptera (adults and larvae), Collembola, Opiliones, Isopoda, Hymenoptera, Dermaptera and Arachnida.

The larger part of the adult Dipteran specimens were acalyptrates; Sphaeroceridae, Heleomyzidae, Dryomyzidae, Phoridae and Calliphoridae. There were also a large number of Nematoceran flies; Mycetophilidae, Trichoceridae and Psychodidae. Larval specimens also showed surprising diversity with Phoridae, Muscidae, Dryomyzidae, Dolichopodidae, Trichoceridae and Chironomidae being recovered as well as a small number of Dipteran larvae which could not be identified.

Of the adult Phorids collected the following species were identified: *Triphleba autumnalis* (♀), *T. intempesta* (♀♂), *T. hyalinata* (♀♂), *T. trinervis* (♂), *T. excisa* (♀) and *Megaselia longicostalis* (♀) were all recovered directly from the cadavers. All of the larvae recovered were identified as *Triphleba*, however it is not possible to identify these to species. Sadly, none of the incubated larvae were successfully reared so it is impossible to know which species in particular were breeding on the carrion.

Phorid adults were significantly more abundant than Calliphorid adults with only 4 adult Calliphorids being recovered, all from pitfall traps, and no Calliphorid larvae being recovered. All 4 adults were identified as *Calliphora vomitoria* (♀).

Table 1 summarises the findings:
Table 1: Summary of the insect groups recovered from the piglet carcass and pitfall traps and the condition of the piglet carcasses across all sampling periods. Only numbers of Diptera are shown. Key: [insect families] A - Arachnid; C – Coleoptera; Cal – Calliphoridae; Col – Collembola; D – Diptera; Der – Dermaptera; DL – Diptera larvae; H – Hymenoptera; I – Isopoda; L – Lepidoptera; O – Opiliones; P - Phoridae [conditions] MD – minor decomposition; SD – substantial decomposition; S – smells; A – adipocere; NB – no bloating; HS – hardening of skin; B – bloating; SS – strong smells; ND – no decomposition; NS – no smell; NA – no adipocere; M – moulds; P – putrefaction.

<table>
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<th>Condition of pig</th>
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<td></td>
<td></td>
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</tr>
<tr>
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Colonisation of Pig cadavers by Scuttle Flies

Temperature data across each condition shows an expected pattern. Temperatures in the buried condition (Figure 6) are the highest on average and at maximum are up to 20°C warmer than in the exposed condition (Figure 7). Ambient temperatures in the exposed condition are the lowest on average as would be expected. Temperatures in the partial condition fall roughly in between (Figure 8). Erratic temperature fluctuations become more common towards the surface of the soil as other weather conditions such as rain and wind affect the ambient temperature directly and temperatures in the partial condition show that even a very shallow amount of coverage can offer substantially warmer conditions for invertebrates.

Figure 6: Mean temperatures of soil and internal piglet temperature over a period of 34 days in the ‘buried’ condition.

Figure 7: Mean ambient air temperature and internal piglet temperature over a period of days in the ‘exposed’ condition.
Discussion

Significant effects of burial condition at low temperature were found after a period of 8 weeks, with cadavers being partially buried bearing the highest numbers of Phorid larvae. No significant effects of burial condition were found at the 5 week interval or across the experiment as a whole. Having found no evidence of pupation of the Scuttle flies or any other species on the cadavers it is assumed that these larvae were the first wave to arrive. There were also a limited number of larvae recovered from the piglets in the exposed condition, enough to be reasonably useful in a forensic case but not enough to show a statistically significant difference from control in the current study.

When collecting the pigs from partial and exposed sites for sampling, huge numbers of flies were gathered underneath the cadavers and generally within the leaf litter, particularly in the case of partial burial. No such colonies of flies were observed anywhere else in the locality so it is safe to assume that these flies were present to feed on the carcasses. Temperatures within the leaf litter were substantially warmer than ambient temperatures so it is no surprise that the flies found this a preferable site for feeding, mating and breeding.

It is interesting that no larvae were found on any of the buried cadavers as Scuttle flies have previously been recovered from human remains in the UK, buried over half a metre below ground (Disney and Manlove, 2005). Since the cadavers in this experiment were buried for longer periods and at lower temperatures than in the cases laid out by Disney and Manlove (2005) - although internal pig temperature was highest in this condition across the whole experiment - it is considered that the soil type could be the limiting factor. Those victims described in the above paper are buried under heavy clay soil which would be very clumpy with large gaps in between allowing easy access for Scuttle flies. The soil under which the pigs were buried in this experiment was heavy and very sandy and probably left very little access to would-be visitors.

Figure 8: Mean temperatures of leaf litter and internal piglet temperature over a period of 34 days in the ‘partial’ condition.
Not all victims recovered from shallow graves have been buried deep in the ground (Cox and Hunter, 2005). Shallow graves are fairly common either because perpetrators are disturbed or become too tired to dig a deeper grave. If the soil is heavy enough, digging a grave can be quite a task on limited time. Based on the findings of this experiment, this potentially means that any remains found in a shallow grave, even during winter months, would bear heavy entomological evidence. The findings are limited to the habitat in this case. Colonisation may not have been as substantial in a more exposed area such as a field or moorland.

The observational data were particularly interesting in this project. There was a surprising amount of species diversity amongst the Scuttle flies. Some of these species had not been previously noted on carrion in southern UK. It is important to note that the vast majority of Phorid species, both adult and juvenile, present on the cadavers were of the genus *Triphleba* which has been documented as a potentially important forensic species but which has not been found in such diversity or in such great numbers. It was unfortunate that the *Triphleba* larvae kept for incubation died before pupation was reached. One or two adult *Megaselia longicostalis* were recovered from the cadavers but no *Megaselia* larvae were recovered.

There were several major issues affecting this study which resulted in some flawed data and overall a poorer outcome than was hoped. The snow put back the first sampling date by almost a whole week. Since time itself was not a factor in this study, it did not pose too much of a problem. It did, however, cause some freezing on the pitfall traps probably resulting in the loss of some possible insect samples. Related to this was the below freezing weather conditions as a whole – a milder winter was hoped for on the whole with more substantial colonisation. There is no demerit, however, in a forensic study at such low temperatures as crime is rarely dictated by weather conditions.

It was also found, shortly after putting the piglets out that distance between graves had not been considered as a limiting factor. Further reading and consultation found that this could affect the results as the olfactory sensors of visiting insects could become confused and mistake the multiple carcasses as a single one (Karg and Suckling, 1999). This was, unfortunately, unable to be rectified as the experiment was already a week in progress. The exposed condition piglets were much closer together (<0.5m) than the partially buried condition piglets (at least 1m) but they should really have been at least 15m apart. It was not felt that this would affect the soil buried piglets in the same manner and these were scattered in the same way as the partially buried piglets.

Further problems arose with the rearing of larvae which were recovered from the piglets. In hindsight not enough larvae were kept for incubation as the majority of them were killed as they were collected. All the containers with rearing larvae in were kept in a ventilated incubation chamber with two cultures of Scuttle flies (*Megaselia abdita* and *M. scalaris*) in containers which were thought to be sealed. When adults were finally discovered in the chamber they had escaped from their sealed containers and it was impossible to tell which had come from where. In any case the flies were temporarily paralysed with CO₂ and collected. All the adults were identified as *Megaselia abdita*, despite no *Megaselia* larvae being recovered from the cadavers. It is clear that some gravid females or perhaps some larvae escaped from the sealed culture containers and found their
way into the containers containing the *Triphleba* larvae, which had died. Ultimately this meant that there was no rearing data – no information was obtained about which species of *Triphleba* had been breeding and it is impossible to know if any of the *Megaselia abdita* adults had reared from larvae recovered from the piglets.

Despite all the problems, this study has managed to outline some areas which may be of interest in further research. *Triphleba* is potentially a very forensically important fly. Despite not having reared any adults, the larvae recovered from the piglets were all positively identified as *Triphleba*. It would certainly be interesting to discover which species had been breeding in the present study. Another possible area of research could be the effects of different soil types, and possibly pH on the ability of Scuttle flies to access cadavers. Given that this experiment was carried out in woodland it might also be interesting to investigate the differences in winter colonisation across different areas or habitats.

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References


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