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Forensic entomology when the evidence is "no insect." Best carrion fly species for predicting maximum postmortem interval in the United Arab Emirates

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ABSTRACT

The carrion insect species that most quickly deposit offspring on a corpse are, when available, likely to yield a more useful estimate of postmortem interval (PMI) compared to later arrivals. This is in part because the age of the oldest larva will be as close as possible to the PMI when doing a development analysis, and because the preappearance interval (PAI), the time the corpse was exposed before insect colonization, corresponds to the narrowest window of time since death for an insect-free corpse when doing a succession analysis. Given replicated training data a prediction of exposure time for a corpse can be in the form of a confidence set, and the maximum value of that set for an insect-free corpse is a probabilistic version of PAI. To discover the insect species likely to be useful in the early postmortem period in the United Arab Emirates we exposed 216 rat carcasses outdoors at two sites in Dubai over three-day periods during winter. Rats were sampled twice each day without replacement and kept in the lab to allow carrion insects to complete development to the adult stage. The fly species produced in this way were Sarcophaga dux, S. ruficornis, Wohlfahrtia nuba, W. indigens (Sarcophagidae), Chrysomya albiceps (Calliphoridae), and Musca domestica (Muscidae). To the best of our knowledge this is the first record of W. indigens larvae feeding on carrion. While adult C. albiceps and M. domestica were abundant on the carcasses, C. albiceps colonized too slowly to be useful for this type of succession analysis within this time frame, and M. domestica emerged from a single rat. The Sarcophagidae were rapid colonizers, and under these conditions the probability is >90% that a carcass would remain free of S. dux larvae not more than 57 h and free of W. nuba larvae for not more than 51 h.

1. Introduction

Forensically important insect succession patterns may depend on local conditions [1]. No succession or other forensic entomology experiment has been published from the United Arab Emirates (UAE), although the presence of some fly species commonly used in death investigation was documented [2–4]. Furthermore, extrapolation from data collected in nearby countries such as Saudi Arabia [5], Kuwait [6], and Iran [7] may be unwarranted given that the UAE biota is more characteristic of countries to the south [8]. Death investigation methods within the UAE might be improved if there were a body of forensic entomological literature focused within the country.

The utility of carrion insect succession is that it can be used to estimate the postmortem interval (PMI, [9]). Although many authors described succession patterns showing insect taxon relative abundance [10–15], most proposed methods for a succession-based PMI estimate can only take into account taxon and life stage presence or absence (=occurrence) [16–21].

Carrion insect succession research can be labor intensive, particularly when including adequate replication for associating a probability with a PMI-prediction [18,22]. For many carrion insect taxa, it

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is easier to collect and identify the adult stage, however the larval stage most commonly serves as evidence in a death investigation. Furthermore, at a given location some typically carrion-colonizing species may visit an item of carrion but not deposit eggs or larvae [23,24]. For that reason, some adult visitation patterns may not be relevant to casework practice.

A special case of succession analysis concerns a corpse not yet colonized by carrion insects when 1) the weather seems fine for insect activity and, 2) there is no barrier preventing access to the corpse. If death occurred at the site of discovery, the absence of insects can support an estimate of maximum PMI equal to the time period required for carrion insects to reach the corpse [25,26]. A formal term for the time elapsed between exposure of the corpse and insect arrival is the pre-appearance interval (PAI) [27]. Because PAI can refer to the time before the arrival of any insect, or to the first individual of a particular species, or to a single individual insect, its meaning should be clarified with each use. *Here we define PAI as the time between carcass placement in the field and the initial oviposition or larviposition on that carcass by a particular carrion insect species.*

Because PAI is a small portion of the total succession interval, it can be measured with less effort than a traditional succession experiment. Furthermore, species that more rapidly exploit a corpse are likely to be the most useful in casework because their absence implies a more narrow window of time since death when using succession analysis and because a development analysis is likely to yield the minimum PMI that is most close to actual PMI [26]. In order to identify UAE insects likely to be most useful for death investigation because they quickly colonize a dead body, we recorded PAI at two sites in Dubai during winter.

2. Materials and methods

2.1. Experimental carcasses

These protocols were approved by the Florida International University Institutional Animal Care and Use Committee, IACUC-18–064 and IACUC-15–024. Our experimental carcasses were commercial pet food rats (*Rattus rattus*, Kiezebrink Rodents, Putten, Netherlands), 250–350 g, killed by CO_2 asphyxiation, and immediately flash frozen. The rats were initially exposed to wild insects in the morning (see below). Because the probability of carrion fly colonization is much lower at night than during the day [28–31], at a death scene a corpse first available to insects in the morning could have been decomposing all night (killed just after sunset), without being colonized, or for very little time (killed just before sunrise). To investigate the effect of decomposition time prior to exposure, rats were thawed for either 24 or 16 h at room temperature prior to placement in the field.

2.2. Field sites and carcass exposure dates

Rats were exposed at two sites in Dubai City. “Headquarters” (HQ) was adjacent to the General Department of Forensic Science and Criminology (25°16'45.7"N 55°21'22.7"E, Fig. 1). This was a barren dirt and paved field surrounded by a concrete wall and within a dense urban development with relatively little vegetation. “Police Academy” (PA) was a disused palm plantation adjacent to the Dubai Police Academy complex (25°07'05.8"N 55°11'19.1"E). Although the PA rats were on a patch of bare ground, this was surrounded by dense vegetation, much of it beneath shade screen.

Each rat was in a plastic food container (Fig. 2A). The container lid was not in place between sample times during the day, but to protect each carcass from vertebrate scavengers the lid was replaced on the container at 17:00 and opened the following morning at 08:00. Each lid had four holes approximately 12 mm in diameter to allow continued insect access. During the first trial, when the lids were fastened with rubber bands, overnight two containers were found opened in the morning and the enclosed rats were missing. After that the containers lids were secured by plastic cable ties each afternoon with no additional loss to scavengers. To maintain the original sample size, additional rats were exposed during a later trial (see Supplementary file).

Fig. 1. The Headquarters (HQ) field site. The arrows indicate rats not yet collected along one line of a grid pattern.

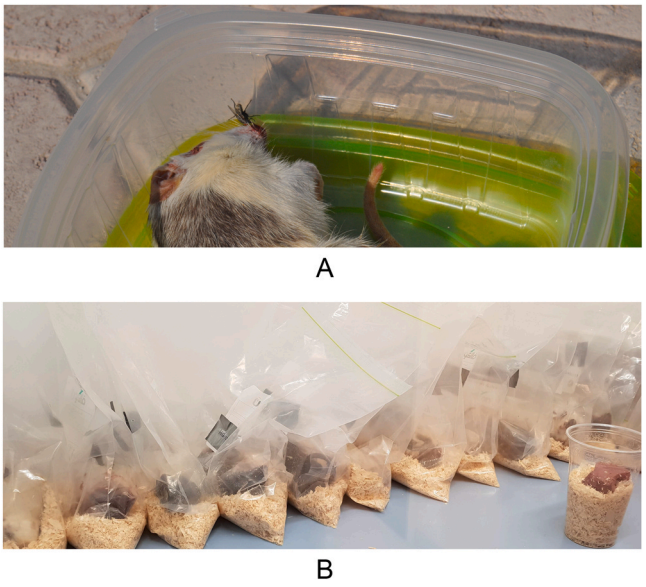

Fig. 2. A. A rat on Day zero visited by a fly in the genus *Wohlfahrtia*. The perforated lid was replaced on each container overnight to prevent vertebrate scavenging. B. Sampled rats were placed on sawdust in a perforated and sealed zip lock bag until larvae had become postfeeding. *Chrysomya albiceps* larvae, which are predaceous, were removed from the rats and reared in plastic cups on beef liver.

During an individual trial rats were first exposed to wild insects at about 08:00 on Day 0. Carcasses were arranged in a grid pattern spaced 12 m apart. Three randomly selected animals of each thawing period were then collected at 11:00 and 17:00 on Day 0, Day 1, and Day 2, at which time all carcasses had been retrieved. The two sites were used in an alternating pattern. The first day of exposure at HQ was December 12, 2018, and the final day of exposure at PA was January 1, 2019. No rats were exposed on December 15, 22, or 29. The total number of rats was 216 (2 thawing periods * 2 sites * 6 sample times/trial * 3 collected each sample time * 3 trials each site).

A sampled carcass was placed in the cut end of a plastic bag to contain decomposition fluid, then into a sealed perforated plastic bag and on a layer of sawdust to catch postfeeding larvae (Fig. 2B). Once it appeared maggots were no longer active in a carcass it was discarded, and the sawdust with live insects was moved to a plastic container with perforated lid to be held for adult emergence. Although we did not measure the amount of tissue consumed by the time all larvae became postfeeding, this appeared to be relatively little, so we have no reason to believe competition for food excluded any carrion insect species. Dark tuberculate larvae, likely to be *C.*

albiceps, were separated from other taxa and reared on beef liver to reduce predation on other maggot species (Fig. 2B). A small number of such individuals were confirmed to be *C. albiceps* when examined under a microscope [32].

Adult Calliphoridae and *Musca domestica* were identified using morphological keys [33,34]. Not every fly with the general appearance of *C. albiceps* was examined to be sure it was not *C. rufifacies*, but we confirmed *C. albiceps* presence by positive identification and we confirmed absence by the lack of any similar-looking insect. A small number of adult Sarcophagidae were identified by comparison to figures of the male genitalia [4], but most were identified using cytochrome c oxidase subunit one sequence [35]. Not every sarcophagid specimen in the samples was identified to species (see next paragraph), but because the two genera could be distinguished with the naked eye based on a darker gray color and a checkered abdomen (*Sarcophaga*) or a lighter gray color and a spotted abdomen (*Wohlfahrtia*), it could be obvious that a species was absent because that genus was absent.

When 200 sarcophagid specimens had been identified by haplotype the counts were: *Sarcophaga dux* = 52, *S. ruficornis* = 36, *Wohlfahrtia nuba* = 108, *W. indigens* = 4. From then on, sequencing or examination of male genitalia of each genus morphotype continued until an individual of the more common species of a genus was confirmed, as occurred for every set of *Sarcophaga* or *Wohlfahrtia* adults from a given rat (some yielded no Sarcophagidae or only one sarcophagid genus). Therefore, the presence or absence of *S. dux* and *W. nuba* among the adult flies produced from each rat was confirmed, while occurrence information on the other Sarcophagidae species is potentially incomplete and not reported here.

2.3. PAI prediction tables

The statistical procedure was that of [18] (see also [22,36]). A "mystery" corpse, one for which the absence of the insect species is known but exposure time is unknown, is separately compared to the training data at each known exposure time. A contingency table analysis is used to reject or fail to reject a hypothesis of exposure time, and unrejected values are the prediction of exposure time in the form of a confidence set, e.g., a 95% confidence set if rejecting for $p \leq 0.05$. The hypothesis is rejected if the training data value for the taxon combination of the mystery corpse is low enough, a value provided by Table 3 in [18] (Fig. 3). For a single carrion insect species

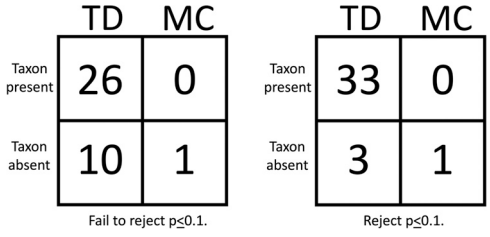

Fig. 3. Examples of the contingency table approach [18] used to test the hypothesis that a hypothetical casework "mystery corpse" (MC), not yet colonized by that insect taxon, would match the training data (TD) carcasses for a given exposure time. The TD values are those observed for *S. dux* on Day 2 at 11:00 (left) and Day 2 at 17:00 (right) in Table 2. The hypothesis is rejected if the number of uncolonized TD carcasses is so few that the same exposure time for the MC is untenable. Unrejected TD exposure times are the prediction for the exposure time of the MC in the form of a confidence set. If, as emphasized in this paper, the occurrence is "absent," this confidence set describes the pre-appearance interval for that species. For our TD data pooled by species as in Table 2 (n = 36 for each exposure time period), the hypothesis is rejected at the 5% level if the value in the relevant TD cell is ≤2, and the hypothesis is rejected at the 10% level if the value in the relevant TD cell is ≤5. For our data separated according to location and carcass thaw time as in Table 1 (n = 9 for each exposure time period), the hypothesis is rejected at the 5% level if the value in the relevant TD cell is 0, and the hypothesis is rejected at the 10% level if the value in the relevant TD cell is ≤1 [18].

this is a 2 × 2 contingency table, and PAI is the maximum value of the prediction of exposure time for a mystery corpse not yet colonized by that insect species.

3. Results

The weather was stable during the experiment, with zero precipitation and a gradual relatively steady decrease in the daily average temperature at Dubai International Airport from 23.8 °C on December 12 to 22.9 °C on January 1 (Supplementary file).

We identified five Diptera species that fed on the rat carcasses as larvae. These were *Chrysomya albiceps* (Wiedemann) (Calliphoridae), *Sarcophaga* (=*Liosarcophaga*) *dux* Thompson, *S.* (=*Parasarcophaga*) *ruficornis* Fabricius, *Wohlfahrtia nuba* (Wiedemann), *W. indigens* Villeneuve (Sarcophagidae), and *Musca domestica* Linnaeus (Muscidae). To the best of our knowledge this is the first published report of *W. indigens* reared from carrion.

In addition to Diptera, the reared samples included at least two species of Hymenoptera, presumably fly pupal parasitoids. We have not yet identified them, but they also have the potential to be forensic indicators [37]. Adult Diptera that visited the rats but apparently did not oviposit included *C. marginalis* (Wiedemann) (Calliphoridae, PA site only) and individuals that resembled members of the muscid genus *Hydrotea*.

3.1. Carrion fly species utility during the first three days' exposure

S. dux and *W. nuba* both colonized rapidly enough to be used to predict PMI_{max} for an uncolonized carcass during the first three days' exposure (Tables 1 and 2, Supplementary file). By the third day of exposure the number of rats not yet colonized by one of these species (the number of carcasses in which the species was absent) was small enough that if these data were applied to the analysis of an uncolonized corpse, a hypothesis of the same exposure period would be rejected. If longer exposure periods are also rejected, and death occurred at the discovery site, this defines PMI_{max} because the probability the corpse could have been exposed for that or a longer period without colonization is too small.

Although there were slight differences in the colonization pattern comparing the two carcass thawing periods or exposure sites (Table 1), the potential effects of these treatments were inconsistent (e.g., *S. dux* colonization was more rapid than *W. nuba* for rats with a greater thawing period, but the opposite was observed for rats thawed for less time), and likely to be the result of random variation. Therefore, we consider the table of occurrence pooled by both treatments (Table 2) to be the best candidate method to validate for PAI prediction based on these sarcophagid species. To the extent that these data match a casework situation they support a 90% confidence set for a maximum exposure time of 57 h for a corpse not yet colonized by *S. dux* and 51 h for a corpse not yet colonized by *W. nuba*. Although we think that greater exposure times would similarly be rejected, that the number of uncolonized rats does not "rebound" on the fourth or subsequent days, this remains to be confirmed.

While adult *C. albiceps* were abundant at both sites, this species was too slow to colonize under these conditions to be used for PAI prediction. During the exposure interval of this experiment the number of rats that did not produce *C. albiceps* was never low enough to reject a hypothesis of exposure time. For the pooled data the lowest count observed was 17 out of 36 for the afternoon of Day 2 (see Supplementary file).

4. Discussion

Based on these results both *S. dux* and *W. nuba* have the potential to be highly valuable tools for UAE death investigations.

Table 1
Occurrence of two Sarcophagidae species larvae on rats exposed over a three-day period at two sites in Dubai and with two thawing intervals prior to placement in the field at 08:00 on Day 0. Rat carcasses were sampled without replacement and held for the insects to develop to the adult stage. Each cell value is the sum of three separate trials. Non-rejected exposure times form a confidence set for a mystery carcass not yet colonized by that insect species. See the text for additional details. (PA = Police Academy site. HQ = Headquarters site. Greater thawing period = 24.25 hours at room temperature. Lesser thawing period = 16.25 hours at room temperature.).

PA: greater thawing period					HQ: greater thawing period				
	Sarcophaga dux		*Wohlfahrtia nuba*			*Sarcophaga dux*		*Wohlfahrtia nuba*	
	Absent	Present	Absent	Present		Absent	Present	Absent	Present
Day 0 - 11:00	8	1	7	2	Day 0 - 11:00	9	0	9	0
Day 0 - 17:00	8	1	5	4	Day 0 - 17:00	7	2	6	3
Day 1 - 11:00	4	5	1	8	Day 1 - 11:00	7	2	6	3
Day 1 - 17:00	5	4	4	5	Day 1 - 17:00	3	6	3	6
Day 2 - 11:00	2	7	2	7	Day 2 - 11:00	1**	8	1**	8
Day 2 - 17:00	1**	8	2	7	Day 2 - 17:00	0*	9	2	7
PA: lesser thawing period					HQ: lesser thawing period				
	Sarcophaga dux		*Wohlfahrtia nuba*			*Sarcophaga dux*		*Wohlfahrtia nuba*	
	Absent	Present	Absent	Present		Absent	Present	Absent	Present
Day 0 - 11:00	9	0	9	0	Day 0 - 11:00	9	0	8	1
Day 0 - 17:00	6	3	4	5	Day 0 - 17:00	9	0	7	2
Day 1 - 11:00	7	2	3	6	Day 1 - 11:00	8	1	5	4
Day 1 - 17:00	4	5	4	5	Day 1 - 17:00	6	3	3	6
Day 2 - 11:00	3	6	1	8	Day 2 - 11:00	4	5	0*	9
Day 2 - 17:00	0*	9	0*	9	Day 2 - 17:00	2	7	1**	8

* Reject at 0.05 (and 0.1) level.
** Reject at 0.1 level.

Table 2
Table 1 data pooled by Sarcophagidae species.

	Sarcophaga dux		*Wohlfahrtia nuba*	
	Absent	Present	Absent	Present
Day 0–11:00	35	1	33	3
Day 0–17:00	30	6	22	14
Day 1–11:00	26	10	15	21
Day 1–17:00	18	18	14	22
Day 2–11:00	10	26	4*	32
Day 2–17:00	3*	33	5*	31

* Reject at 0.1 level.

Table 3
The number of rats in each sample of 36 that were not yet colonized by the larvae or eggs of any carrion fly species at the time of sampling. Initial exposure to potential insect colonization was at 08:00 AM on Day 0.

Day	Exposure period (hours)	No. rats/36
0	3	32
0	9	20
1	27	14
1	33	8
2	51	1
2	57	1

As described in the materials and methods, we did not document the complete occurrence pattern for *S. ruficornis* or *W. indigens*. Because the latter was so uncommon, we suspect that it is not useful in this context [22]. However, further investigation of *S. ruficornis* in the UAE is warranted.

Although *C. albiceps* colonized too slowly to be used for PMI_{max} estimation during the first three days it may be useful in this context for longer exposure times, and further research may show that *C. albiceps* in combination with one of the sarcophagid species can yield a more precise PMI estimate than one based on the individual insect species [22].

Musca domestica, despite being by far the most numerous adult fly species on and near the rats, was only reared from a single carcass. (HQ. Trial 1. Thawed 16 h. Day 1. 17:00) Under these conditions, at least, it will probably seldom be useful in a death investigation.

These results do not support the common claim that under field conditions such as these a corpse or carcass will be quickly colonized by at least one carrion fly species, and that an insect-free corpse must have died very recently [25]. By the afternoon of the second day of exposure the proportion of uncolonized rats was still 0.22 (Table 3).

Furthermore, we conclude that in locations such as Dubai, in which Sarcophagidae colonized before Calliphoridae, particular care should be taken when searching a fresh corpse for carrion insects. In our experience newly deposited Sarcophagidae larvae are difficult to notice because they are translucent and soon crawl out of sight. This contrasts with blow fly eggs, which are typically white in color, often arranged in clusters, remain where they were deposited, and the empty chorions may still be visible after the larvae hatch. If PMI is estimated based on the analyst detecting no sign of colonization in photographs of the victim [25], this might rule out the presence of immature Calliphoridae but not Sarcophagidae. Sampling an entire corpse population of carrion insects, such as was done in this experiment, would be impractical during most casework. More research into the amount of sampling effort necessary to declare an insect species absent is needed.

Obviously, there are substantial differences between a rat carcass and a human corpse. The utility of these, or any experiemental data for investigating a human death should be demonstrated by a validation experiment, i.e., by measuring PMI estimation performance under crime scene conditions [38]. Validation of PAI prediction based on these species under different ambient temperatures should include converting absolute time to accumulated degree hours [39].

Although in this paper we focused on PAI and the interpretation of insect absence, the statistical test can be similarly applied to presence data [22].

In summary, we found that that carrion insect colonization in Dubai during the early postmortem period was dominated by Sarcophagidae, and we conclude they are probably the most useful insect taxa for PMI estimation under these conditions. This pattern of sarcophagid colonization in the early postmortem period preceding that of calliphorids was also observed in Egypt [40] and differs from the pattern of calliphorid priority reported at many other locations around the world [1,41–44] including southwestern Iran [7].

CRediT authorship contribution statement

Jeffrey D. Wells obtained the funding, designed the experiment, participated in the data collection, performed the statistical analysis,

and prepared the first draft of the manuscript. **Amber E. MacInnis** participated in experimental design, supervised the fieldwork, and helped edit the manuscript. **Maurell A. Dsouza** helped conduct the fieldwork, performed the DNA analysis, and helped edit the manuscript. **Zain Ul Abdin** helped identify the specimens and helped edit the manuscript. **Sara Al Mughawi, Mohammad Al Khloofi, Mariam Sajwani, Maryam Al Maidoor, Ashwaq Saeed, Hamdan Ahli, Rawdha Al Shamsi, and Reem Al Mheiri** provided logistical support in Dubai.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2021.110999.

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