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ORIGINAL ARTICLE

Development and validation of a new technique for estimating a minimum postmortem interval using adult blow fly (Diptera: Calliphoridae) carcass attendance

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Abstract Understanding the onset and duration of adult blow fly activity is critical to accurately estimating the period of insect activity or minimum postmortem interval (minPMI). Few, if any, reliable techniques have been developed and consequently validated for using adult fly activity to determine a minPMI. In this study, adult blow flies (Diptera: Calliphoridae) of *Cochliomyia macellaria* and *Chrysomya rufifacies* were collected from swine carcasses in rural central Texas, USA, during summer 2008 and *Phormia regina* and *Calliphora vicina* in the winter during 2009 and 2010. Carcass attendance patterns of blow flies were related to species, sex, and oocyte development. Summer-active flies were found to arrive 4–12 h after initial carcass exposure, with both *C. macellaria* and *C. rufifacies* arriving within 2 h of one another. Winter-active flies arrived within 48 h of one another. There was significant difference in degree of oocyte development on each of the first 3 days postmortem. These frequency differences allowed a minPMI to be calculated using a binomial analysis. When validated with seven tests using domestic and feral swine and human remains, the technique correctly estimated time of placement in six trials.

Keywords Forensic entomology · Decomposition ecology · *Cochliomyia macellaria* · *Chrysomya rufifacies* · Pre-colonization interval · Postmortem interval

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Introduction

When an animal dies, it attracts a variety of scavengers that break down the body and return its nutrients to the soil [1]. The preeminent mechanism of that mechanical breakdown is the feeding of immature arthropods such as blow flies (Diptera: Calliphoridae). Although the mobile adult insects arrive before their progeny, blow fly larvae have predictable, thermally dependent development, which can be used to estimate a "postmortem interval" (PMI) [2]. Therefore, most existing research on insect/carcass interaction has focused on the immature stages in studies with human decedents [3, 4], dogs [5], and swine [6]. In contrast to a strictly developmental perspective, however, Tomberlin et al. [7] described the insect activity on a carcass as a series of physiological and behavioral responses of insects to specific cues provided by the carcass and its associated microbial flora. In this interaction-based framework, carrion use by insects is dictated by the process of neurosensory detection and behavioral activation, followed by searching and location of the carcass prior to colonization, defined as the period of insect activity (PIA) [7]. The portion of the PIA encompassed by the initial detection of carrion by an adult insect until colonization is defined as the pre-colonization interval (pre-CI). The pre-CI is little understood although recent work by several authors has shown that factors such as temperature, humidity, and wind speed can have a significant impact on the duration of the pre-CI in species such as blow flies [8–11] and beetles [12, 13]. Adult insects represent the most mobile phase of the arthropod carrion breakdown community, controlling overall geographic distribution, patch-based species interactions, and aggregation. Mobility also influences the speed at which individual insects can reach any given carcass and colonize it.

Adult insect behavior can be highly variable, based on species ecology and biology. From a decomposition ecology perspective, central Texas makes an excellent study system.

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The extreme high heat of the summer promotes rapid decomposition, while the mild winters prevent frozen preservation. Approximately ten species of Calliphoridae are known to inhabit carrion in Texas with variable seasonal occurrence [14]. Two particularly important summer-active species are *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae), the native secondary screwworm [15, 14], and *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae), the invasive hairy maggot blow fly. This species is facultatively predacious and cannibalistic as third instars, and its apparent intraguild advantage may be shifting the geographic distribution and population size of other native blow fly species [16–18].

In the winter, two commonly encountered blow flies are *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) and *Phormia regina* Meigen (Diptera: Calliphoridae). *C. vicina* is the most cold hardy of the local flies, active even in the depth of winter [15]. *P. regina* is a widely distributed blow fly, active in both spring and summer in colder climates. This species is one of the more generalist blow flies, accepting much older carrion as a colonization site than other species [15, 19, 20].

Because baseline pre-CIs are not known for local blow fly species, a primary purpose for this study was to document the duration of the location phase and the timing of initial appearance at a carcass. By allowing a better estimation of pre-CI for species encountered during casework, forensic entomologists can better calculate minimum postmortem intervals (minPMI), encompassing both the pre-CI and post-CI portions of the period of insect activity or minPMI [7]. A further purpose of documenting adult blow fly behavior was to record some of the physiological factors that underlie carcass attendance by blow flies. Once known, the patterns of carcass attendance might be linked with a deeper ecological explanation such as interspecific competition or pathogen avoidance [21]. Such underlying ecological, genetic, or fitness-based reasons stabilizing a behavior or biological process allow the behavior or process to be predictable within certain bounds. Finding predictable trends in carrion-associated insects and linking them to ecology is a great goal of forensic entomologists striving to meet the recommendations of the 2009 National Academy of Sciences/National Research Council (NAS/NRC) report on forensic science [7]. Of particular interest is the development of quantitative, stochastic, or probabilistic methods for estimating portions of the PMI. As adult blow flies typically represent the first insects to arrive at a fresh cadaver, their behavior could be useful in allowing the development of an analytical technique by which the pre-CI can be estimated. Aside from being a powerful technique in its own right, estimations of PIA based on adult blow flies would be useful for recently exposed cadavers, where the small size of larvae, or lack thereof, impedes identification and application in forensic casework [22]. As part of the development of any technique, particularly one used in forensic science, the useful limits, proper application, and validation must be investigated. Therefore, the third objective of this study was to test and validate a novel method of PIA estimation based on the ovarian physiology of adult blow flies.

Methods

For each trial, three 60–80 kg white commercial swine (*Sus scrofa domesticus* L.) were obtained from a commercial abattoir. Each pig was killed by humane cranial trauma to avoid any tranquilizer effects [23] and to better mimic traumatic human death [24]. The Texas A&M University Institutional Animal Care and Use Committee required no animal use protocol, as the swine were deceased at the time of acquisition.

The experimental animals were killed at approximately 07:30 for the summer trials and 09:30 for the winter trials. Within 1 h of death, the swine were placed in rural pasture near Snook, Texas (30° 26′ 14″ N/96° 25′ 12″ W). Each pig was placed in full sun along a north/south line approximately 40 m apart. To prevent nocturnal vertebrate scavenging, carcasses were placed beneath a wire cage each evening. For the summer trials, trial 1 was conducted from 7 to 9 August 2008 and trial 2 from 5 to 7 September 2008. For the winter, trial 3 was conducted from 7 to 13 January 2009 and trial 4 from 24 February to 7 March 2010.

Following pig placement, blow fly collection was attempted at hourly intervals between sunrise and sunset [25] as both preliminary observations and other authors have found only negligible nocturnal fly activity before sunrise or after sunset [26]. Standard collections consisted of ten targeted sweeps of a 21-cm aerial net made within 30 s over each carcass. Flies thus collected were preserved immediately in ~80 % ethanol. Observations were continued 3–14 days postmortem until calliphorid third instars were observed on all carcasses, as this stage typically would be identifiable to species level by a forensic entomologist and readily applied to an investigation.

In the laboratory, individual flies were removed from the ethanol and externally dried before identification by species and sex. Those belonging to family Calliphoridae were weighed to 0.0001 g with an Adventure-Pro AV64 Ohaus scale (Pine Brook, NJ, USA). To assess physiological development state, the ovaries of female flies were dissected using Anderson's method under a Meiji Techno EMZ-8TR microscope (Santa Clara, CA, USA) [27]. One arbitrary oocyte from each fly was measured lengthwise to 0.1 mm while wet with ethanol. A single oocyte was chosen instead of the entire ovary, both to decouple the degree of yolk deposition from the positive effect of body size on overall ovary dimension [28] and to allow for a rapid dissection process. As 1.2 mm is a conservative estimate of the length for viable eggs of all three tested species [29], and all oocytes thus observed were clearly in the final stage of development prior to oviposition [30], flies with oocytes >1.2 mm were therefore classified as "gravid," cf. Gordh and Headrick [31].

Statistical analysis (SPSS 15.0 (SPSS Corp., Chicago, IL)) was used to investigate the relationship between fly arrival at a

carcass and physiological parameters. Body mass for each sex and each species was regressed against the PMI at which they were collected. ANOVA was used to compare female oocyte length between fly species, trials, positions, absolute PMI, and the number of days elapsed since death, with a post hoc Tukey's HSD test. As *C. macellaria* and *C. rufifacies* both showed a clear relationship between oocyte length and time of collection (Fig. 1), 95 % confidence intervals were constructed for the mean frequency of oocyte 1.2 mm or more in length on the first, second, and third day postmortem (Table 1). These frequency confidence intervals were then used as estimates of the true population frequencies in the validation tests. Intervals were not constructed for the winter-active *C. vicina* and *P. regina* because small sample sizes precluded meaningful statistical analysis.

Validation tests

Domestic pig (*Sus scrofa domesticus* L.), feral pig (*Sus scrofa scrofa* L.), and one human cadaver (*Homo sapiens sapiens* L.) were used in seven total validation tests. Specifics of species, size, location, cause of death, timing of death, exposure, and sampling are summarized in Table 2. For each validation test, a subject was exposed to outdoor

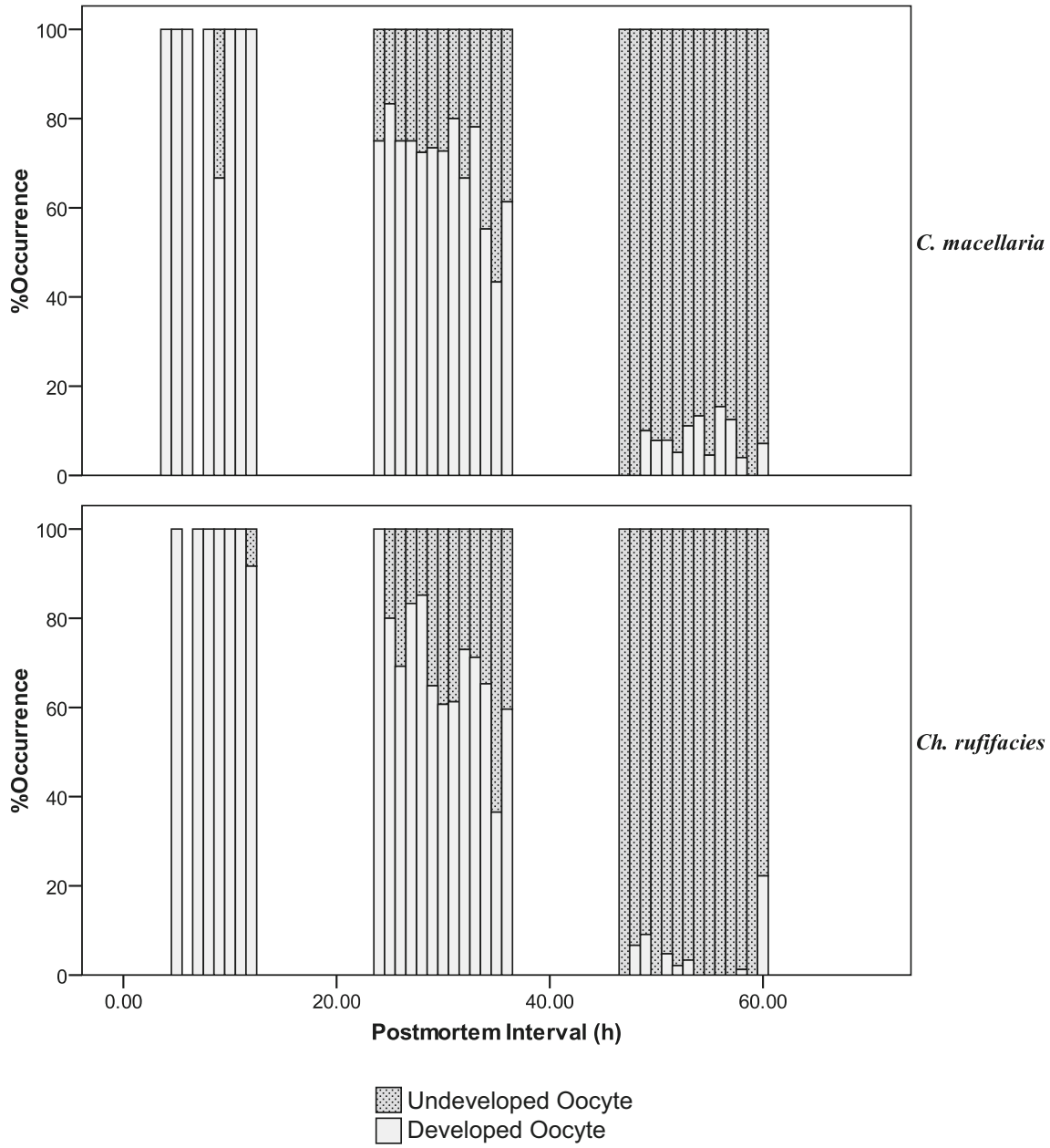

Fig. 1 Developed/non-developed oocytes. Daily binomial distribution of oocyte type for *Cochliomyia macellaria* and *Chrysomya rufifacies*, with "developed" being defined as oocyte length 1.2 mm or larger. The proportion of developed flies is significantly different between each day

Table 1 Binomial distribution for the population incidence of oocytes 1.2 mm or larger for each of the first 3 days postmortem

Species	Postmortem day	Mean Frequency	95 % CI	SEM
C. macellaria	1	0.96	0.89–1.04	0.04
	2	0.67	0.63–0.71	0.02
	3	0.08	0.05–0.11	0.01
C. rufifacies	1	0.98	0.93–1.03	0.02
	2	0.64	0.59–0.68	0.02
	3	0.02	0.01–0.03	0.01

insect activity. A co-author (JKT) then sampled adult blow flies on the subject and recorded time and date of collection, which were provided to the original experimenter (RMM), although the time of exposure was withheld. The collected blow flies were identified and dissected according to the protocol above, and a frequency of 1.2+mm oocytes was calculated for the species present in each sample. To predict the minPMI, the sample frequency was compared to the field population frequencies. For fly species collected in the validation tests, but not in the standard field collections, the profile of *C. macellaria* was used. If a sample frequency fell within a 95 % confidence interval for experimental postmortem day 1, 2, or 3, the test sample was predicted to have come from a carcass exposed for that many days. If a sample frequency fell outside a 95 % CI, a Bernoulli experiment was performed to determine the likelihood of finding exactly *n* ovarioles 1.2+mm in each sample of *x* flies (Table 3). The sample was then predicted to have come from the experimental day with the highest likelihood. Since the duration of the exposure phase was unknown [21], these estimates were expressed as minPMI.

Results

Over the course of this study, 2925 calliphorid flies were collected. Time of initial arrival on each carcass ranged from 4 to 9 h in the two summer trials, and 26 to 126 h in the two winter trials (Table 4). In summer studies, *C. macellaria* and *C. rufifacies* initially arrived within 1 h of one another: 9–10 h (first trial) or 4–5 h (second trial) postmortem (Table 4). Flies in trial 2 arrived a mean of 2.67 h before those in trial 1 (F=19.692, df=1, P=0.047). There was no significant difference in mean time of first arrival between species or between positions (F<12.538, df=1, P>0.050), nor were there any interactions between trial, position, and species (F<0.692, df=4, P>0.05). In winter, flies in trial 3 arrived a mean of 116 h before those in trial 4 (F=43.827, df=1, P=0.022). There were no differences between mean time of first arrival between species or position, nor were there any significant interactions between species, position, or trial (F<2.615, df=4, P>0.050).

Body mass

Among summer flies, there was no difference in mean body mass between species, positions, or trials (F<0.039, df=4, P>0.843). Female flies collected on the first day postmortem weighed an average of approximately 0.006 g more than those on day 2 and/or day 3 of collection (F=9.591, df=2, P<0.001). Similarly, there was no difference in mean body mass (0.055 g) between species, positions, or trials for female winter flies (F=0.567, df=4, P=0.467).

Table 2 Descriptions of the specimens used in the validation trials. Subjects 1–6 were sampled by aerial net, subject 7 by sticky trap

	Specimen number						
	1	2	3	4	5	6	7
Site	Snook, TX	Snook, TX	CLL Airport	CLL Airport	Dayton, OH	Dayton, OH	San Marcos, TX
Species	Feral pig	Domestic pig	Domestic pig	Domestic pig	Domestic pig	Domestic pig	Human
Manner of death	Cranial trauma	Cranial trauma	Cranial trauma	Cranial trauma	Cranial trauma	Cranial trauma	Natural
Mass	10–20 kg	20–30 kg	20–30 kg	20–30 kg	6.8 kg	6.4 kg	47.6 kg
Time of death	Apr 2011	15 Sept 2011, 08:25	15 Sept 2011, 08:25	15 Sept 2011, 08:25	26 July 2011, 17:45	26 July 2011, 17:45	25 Oct 2011, 11:52
Storage, duration	Frozen until 12 June 2011	N/A	N/A	N/A	N/A	N/A	Chilled <5 °C
Time of exposure	13 June 2011, 15:30	15 Sept 2011, 09:53	15 Sept 2011, 09:19	15 Sept 2011, 09:21	26 July 2011, 18:27	26 July 2011, 18:27	2 Nov 2011, 15:00
Time of sampling	15 June 2011, 13:00	16 Sept 2011, 13:00	16 Sept 2011, 10:00	16 Sept 2011, 10:30	27 July 2011, 20:17	28 July 2011, 18:43	5 Nov 2011, 07:00–15:00
Average site temperature	30.9 °C	29.2 °C	29.2 °C	29.2 °C	25.1 °C	25.5 °C	11.0 °C

Table 3 Validation test results, using the binomial likelihood. Estimates were selected based on the number of individual with developed oocytes (n>1.2 mm) falling within the 95 % CI given by Table 1. If the confidence interval was invalid, the day with the highest species-appropriate binomial probabilities was chosen. For species without a known probability, *C. macellaria* was used

	Site	Species	Sample size	n>1.2 mm	CI match	Probabilities			Selected estimate	Actual exposure
						Day 1	Day 2	Day 3		
1	Snook, TX	*C. macellaria*	13	8	–	<0.01	0.21	<0.01	24–48 h[a]	46.5 h
2	Snook, TX	*C. rufifacies*	4	3	–	0.09	0.37	<0.001	24–48 h[a]	31 h
		C. macellaria	21	9	–	<0.01	0.01	<0.001	24–48 h[a]	
3	CLL Airport	*C. rufifacies*	3	2	Day 2	0.07	0.44	<0.01	24–48 h[a]	26 h
		C. macellaria	4	3	–	0.13	0.40	<0.01	0–24 h	
4	CLL Airport	*C. rufifacies*	3	2	Day 2	0.07	0.44	<0.01	24–48 h[a]	26.5 h
		C. macellaria	13	3	–	<0.01	<0.01	0.06	48–73 h	
5	Dayton, OH	*L. sericata*	5	4	–	0.15	0.33	<0.01	24–48 h[a]	26 h
6	Dayton, OH	*L. sericata*	1	0	Day 3	0.04	0.33	0.92	48–73 h[a]	48.5 h
		P. regina	1	0	Day 3	0.04	0.33	0.92	48–73 h[a]	
7	San Marcos, TX	*C. macellaria*	1	1	Day 1	0.96	0.67	<0.01	0–24 h	60–72 h
		P. regina	6	5	–	0.18	0.27	<0.01	24–48 h	
		Cy. cadaverina	12	10	–	0.06	0.12	<0.01	24–48 h	

[a] Indicates a correct estimate

Ovarian status

The ovaries of 2189 female flies were successfully dissected. In the summer trials, neither trial (F<0.175, df=1, P>0.676) nor position (F<0.272, df=2, P>0.762) had any significant effect on oocyte length, allowing oocyte development data to be pooled by species. Day of postmortem collection was the sole significant predictor of oocyte length (F=655.372, df=2, P<0.001), with day 1 having a mean length of 1.4 mm, day 2 a mean length of 1.0 mm, and day 3 a mean length of 0.3 mm. For both *C. macellaria* and *C. rufifacies*, each day was significantly different from the other two. For the winter species *C. vicina* and *P. regina*, none of the tested variables—trial, position, species, or day of collection—significantly explained oocyte length (F<7.047, df=4, P>0.053).

Validation tests

In six of the seven validations, the correct time of collection was estimated by the blind evaluator, based on either a consensus of species or by selecting the highest individual likelihood (Table 3). For the validation study that was not correctly predicted, temperature, time of year, and species of fly analyzed were different than the experimental design.

Table 4 True PMI, in hours, at which the first flies were collected from experimental swine carcasses for each position, trial, and fly species during each of four trials in an open field near Snook, Texas. Blow fly species with fewer than 14 total collected individuals are excluded

Trial	Species	Position		
		A	B	C
1	*C. macellaria*	10	9	9
	C. rufifacies	10	11	11
2	*C. macellaria*	4	12	5
	C. rufifacies	5	11	7
3	*C. vicina*	26	26	27
	P. regina	31	26	28
4	*C. vicina*	101	171	176
	P. regina	171	147	174

Discussion

Time of first arrival

These findings of rapid arrival are consistent with the characterization of *C. macellaria* as a primary colonizer [20]. The near synchronicity of *C. macellaria* and *C. rufifacies* is somewhat unusual, however, as *C. rufifacies* is broadly considered a secondary colonizer [16] and may actually gain a fitness advantage for its predacious/cannibalistic offspring by arriving secondary to *C. macellaria* [32]. The fast arrival of both species also runs contrary to studies which have not found the arrival of *C. macellaria* and *C. rufifacies* until the third postmortem day in the fall [33]. The answer most likely lies in the

high ambient temperature during the study, facilitating extremely rapid decomposition.

Unseasonably warm weather in the first 3 days of trial 3 is the probable cause of differences between the winter trials. The 20–30 °C temperatures and low wind speeds [11] were nearly optimal for bacterial growth, acceleration of decomposition, and volatile odor production [34]. In this study, *C. vicina* and *P. regina* always arrived within 48 h of one another. This pattern is broadly similar to one Louisiana study, where both *C. vicina* and *P. regina* were collected on day 2 postmortem [33]. However, in other literature, *P. regina* is described as a secondary colonizer, less cold hardy and preferring carcasses later in decay [15, 19, 20]. The difference is most likely due to climatic differences among studies, as winter in central Texas is generally mild, with daily temperatures averaging 5–18 °C [35].

Body size

In general, body size has a large impact on insect fitness [36] and strongly influences flight and locomotor ability [37]. In female blow flies, size directly affects reproductive output, with small fly ovaries having up to 80 % fewer ovarioles than those of average-size flies [28]. These smaller ovaries produce fewer eggs and lower overall lifetime reproduction [38]. In this study, body size did not affect the flies' response to the carcass, for either sex or species, indicating that small size was not a hindrance to carcass location and attendance.

Ovarian status

In most instances, female blow flies require a protein meal in order to complete oogenesis [39, 40]. They also need a carrion patch or carcass upon which to deposit their eggs/larvae. Gravid flies should therefore be under strong selection to also arrive at a carcass quickly, evaluate it, and either accept or reject it [41]. In this study, there was a very strong bias for gravid females to be the first arrivers but for the maturity profile to shift dramatically over the next 2 days to dominance by non-gravid females (Fig. 1).

This rapid change from carcass attendance by gravid vs. non-gravid females seems to indicate that these flies are sensitive to rapidly changing cues from the decaying carcass. Postmortem, cell lysis, and bacterial proliferation begin production, with an odor profile which changes substantially as decomposition proceeds [42, 43]. Vass et al. [44] found at least 478 volatile chemicals associated with decaying human cadavers. The bacteria-associated volatiles, hydrogen sulfide and ammonium carbonate, are known to attract *Lucilia sericata* at long range, while sulfide and indole-based putrefactive cues are probably close-range location and acceptance cues [45] Another possibility for the source of attractive volatiles is bacteria proliferating on the surface of intra- and interspecific eggs [46, 32]. These volatiles could only be produced following primary colonization, but they would affect later arrivers like the non-gravid flies of this study.

The complex relationship between species, gravidity, and responsiveness to carrion-associated volatiles might explain the high level of variation in the onset and duration of the PIA. In *L. sericata*, mid-oogeny females do not respond to a liver odor plume, while fully gravid and protein-starved/non-ovigenic flies are strongly attracted [47]. Similarly, in an experiment using raw liver exposed for a single day, 92 of 117 *C. rufifacies* females were in Spradbery stage X or fully gravid (equivalent to 1.3–1.5 mm ovariole length) [48]. Over an 18-day period, Mackerras [40] found the vast majority of flies caught had <50 % yolk deposition. The longer duration of that study suggests that after the first cohort of females oviposits, gravid flies are no longer attracted to a carcass in large numbers. Separation of reproductively developed from non-developed flies suggests that many blow fly females will use two separate carrion patches in their lifetime.

The integration of optimal oviposition and optimal foraging theory begins to explain how a relatively short-lived fly would make use of two ephemeral resources in its lifetime to bear the highest number of offspring while also finding an easy meal. The first time flies use a carcass, they exploit it as a non-gravid protein consumer. Corpse fluids produced during active decay provide a ready meal for sponging mouthparts [42], and even dry carcasses in late decay provide appropriate protein [49]. The experience with carcass location may also serve a learning purpose, improving gravid flies' ability to detect and evaluate a subsequent patch [50], which fits the optimum oviposition theory [41]. The need to disconnect foraging and oviposition is reduced in a cooler winter environment, however. With decomposition occurring at a much slower pace, and a smaller overall population, winter-active flies may experience less selective pressure to arrive rapidly when gravid. The slower pace might allow winter-active flies to both feed and oviposit on a single carcass, as it would last long enough to support both activities.

Validation tests

In the seven validation studies, *C. rufifacies* was collected three times, and in each case, the number of days post-exposure was correctly estimated using their data. When both *C. macellaria* and *C. rufifacies* were considered together, the binomial likelihood was higher for *C. rufifacies* than for *C. macellaria*, giving it greater weight as a predictor. Using *C. macellaria* as a predictor was only correct two of five times, and very close to accurate in a third (Table 3). One confounding factor for tests 3 and 4 may be the locations of the pigs. Although placed less than 50 m apart, exposed 2 min later, and sampled 30 min later, the flies sampled from the two carcasses had a dramatically different composition. The pig in

test 4 was deeper into the woods, which may have delayed initial attraction and oviposition, which was then compounded by preferential attraction to the odor of eggs.

The validation test data from Dayton, Ohio, shows that the technique applies to multiple species of summer-active flies. Although the true population frequencies of developed/non-developed ovaries for *L. sericata* and *P. regina* are unknown, use of the *C. macellaria* frequencies yielded correct prediction of minPMI, albeit with a caveat of adequate sample size. Using a binomial experiment and the values given in Table 1, it would be mathematically impossible to differentiate between day 2 and day 3 estimates. The rule of thumb to avoid that kind of skewed results suggests that sample size (n)× likelihood of outcome (p) should be greater than 10 [51]. Based on the mean p from Table 1, a sample size of 16 should be sufficient to produce robust results.

Test 7 differed from the experimental setup in terms of carrion species, environment, and temperature. While the generated minPMI was not accurate, it does bear out some useful findings. Both *P. regina* and *Cynomya cadaverina* Robineau-Desvoidy (Diptera: Calliphoridae) showed similar patterns of ovarian development and predicted the same minPMI. This result indicates that physiological age structuring holds across more species than the four tested. The cold temperature at the cadaver probably caused failure to correctly predict minPMI. While the cadaver had been exposed to insects for 60 h when the sticky trap was placed, the average temperature for the previous 2 days had been cold (a low of 2.0 °C) and windy (gusts up to 16.5 m/s) (NOAA [52]), two factors known to delay blow fly arrival. The sample collector also reported negligible blow fly activity on the cadaver during the first day of exposure. Therefore, the estimates were correct in the sense that they predicted the PIA as opposed to the minPMI. They also illustrate that use of this technique under cases of unusual weather conditions, particularly extreme temperatures, could cause a significant underestimation of the minPMI. Further research is needed to confirm the environmental parameters under which this technique is viable.

Conclusions

Understanding the ecology of adult blow fly interactions with carrion allows them to serve as predictors of minPMI in the absence of corresponding larvae, an important step forward in estimating the complete PIA [7]. Although some authors have advocated collection of adult flies at scenes [22, 53], these specimens largely serve to validate larval identifications. As a result, only the post-CI of the PMI is typically calculated.

Even if it is not yet possible to estimate how long it takes flies to find a given corpse, using their ovarian status, the length of the adults' association can be estimated using the Bernoulli distribution and known population oocyte lengths. This technique has much potential, as the use of objective measurements and numerical probabilities means that it should meet the Daubert standard for scientific evidence [54]. Adult blow flies could be particularly useful as indicators in cases where the body is very fresh, as eggs and first instars can be difficult for field investigators to locate and preserve [22]. Success of the Dayton, Ohio, trial and the partial success in San Marcos, Texas, also imply that this technique may be applicable for a variety of species and locales, though further research into species and geographic variation would be required. However, the method is imperfect. Although six of the seven validation trials did correctly estimate a minPMI, one underestimated it by 48 h (Table 3). The lack of ovarian status data for species other than *C. macellaria* and *C. rufifacies* means that error rates and probabilities are unknown when generating estimates for other species. And the apparent effect of weather in test 7 indicates that environmental factors cannot be ignored completely.

Further population surveys are necessary to understand how the behavior of the adult blow fly relates to carrion. Validation, particularly with non-Texan populations, is also a necessity before the use of the ovarian-based minPMI estimate can be widely adopted. As a rapid, ready estimate of PIA, however, this adult-based technique shows great potential to facilitate the practice of forensic entomology.

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References

1. Carter DO, Yellowlees D, Tibbett M (2007) Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften 94(1):12–24. doi:10.1007/s00114-006-0159-1
2. Greenberg B (1991) Flies as forensic indicators. J Med Entomol 28(5):565–577
3. Motter MG (1898) A contribution to the study of the fauna of the grave. A study of one hundred and fifty disinterments, with some additional experimental observations. JN Y Entomol Soc 6(4):201–231
4. Rodriguez WC, Bass WM (1983) Insect activity and its relationship to decay rates of human cadavers in East Tennessee. J Forensic Sci 28(2):423–432
5. Reed HB Jr (1958) A study of dog carcass communities in Tennessee, with special reference to the insects. Am Midl Nat 59(1):213–245
6. Payne JA (1965) A summer carrion study of the baby pig *Sus scrofa* Linnaeus. Ecology 46(5):592–602. doi:10.2307/1934999

7. Tomberlin JK, Mohr R, Benbow ME, Tarone AM, VanLaerhoven SL (2011) A roadmap for bridging basic and applied research in forensic entomology. Annu Rev Entomol 56(1):401. doi:10.1146/annurev-ento-051710-103143
8. George KA, Archer MS, Toop T (2013) Abiotic environmental factors influencing blowfly colonisation patterns in the field. Forensic Sci Int 229(1–3):100–107. doi:10.1016/j.forsciint.2013.03.033
9. Matuszewski S, Szafałowicz M (2013) Temperature-dependent appearance of forensically useful beetles on carcasses. Forensic Sci Int 229(1–3):92–99. doi:10.1016/j.forsciint.2013.03.034
10. Matuszewski S, Szafałowicz M, Grzywacz A (2013) Temperature-dependent appearance of forensically useful flies on carcasses. Int J Legal Med:1-8. doi:10.1007/s00414-013-0921-9
11. Mohr RM, Tomberlin JK (2014) Environmental factors affecting early carcass attendance by four species of blow flies (Diptera: Calliphoridae) in Texas, USA. J Med Entomol 51:702–708. doi:10.1603/ME13149
12. Matuszewski S (2011) Estimating the pre-appearance interval from temperature in *Necrodes littoralis* L. (Coleoptera: Silphidae). Forensic Sci Int 212(1–3):180–188. doi:10.1016/j.forsciint.2011.06.010
13. Matuszewski S (2012) Estimating the preappearance interval from temperature in Creophilus maxillosus L. (Coleoptera: Staphylinidae). J Forensic Sci 57(1):136–145. doi:10.1111/j.1556-4029.2011.01958.x
14. Tenorio FM, Olson JK, Coates CJ (2003) Decomposition studies, with a catalog and descriptions of forensically important blow flies (Diptera: Calliphoridae) in Central Texas. Southwest Entomol 28(1):37–45
15. Deonier CC (1940) Carcass temperatures and their relation to winter blowfly populations and activity in the Southwest. J Econ Entomol 33(1):166–170
16. Baumgartner DL (1993) Review of *Chrysomya rufifacies* (Diptera: Calliphoridae). J Med Entomol 30(2):338–352
17. Wells JD, Greenberg B (1994) Resource use by an introduced and native carrion flies. Oecologia 99(1–2):181–187. doi:10.1007/Bf00317099
18. Holt RD, Polis GA (1997) A theoretical framework for intraguild predation. Am Nat 149(4):745–764. doi:10.1086/286018
19. Gruner SV, Slone DH, Capinera JL (2007) Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida. J Med Entomol 44(3):509–515. doi:10.1603/0022-2585(2007)44[509:FICDAW]2.0.CO;2
20. Hall RD, Doisy KE (1993) Length of time after death—effect on attraction and oviposition or larviposition of midsummer blow flies (Diptera:Calliphoridae) and flesh flies (Diptera: Sarcophagidae) of medicolegal importance in Missouri. Ann Entomol Soc Am 86(5):589–593
21. Tomberlin JK, Benbow ME, Tarone AM, Mohr RM (2011) Basic research in evolution and ecology enhances forensics. Trends Ecol Evol 26(2):53–55. doi:10.1016/j.tree.2010.12.001
22. Catts EP, Haskell NH (1990) Entomology & death: a procedural guide. Joyce's Print Shop, Inc., Clemson, S.C
23. Patrican LA, Vaidyanathan R (1995) Arthropod succession in rats euthanized with carbon dioxide and sodium pentobarbital. J N Y Entomol Soc 103(2):197–207
24. Schoenly KG, Haskell NH, Hall RD, Gbur JR (2007) Comparative performance and complementarity of four sampling methods and arthropod preference tests from human and porcine remains at the Forensic Anthropology Center in Knoxville, Tennessee. J Med Entomol 44(5):881–894. doi:10.1603/0022-2585(2007)44[881:Cpacof]2.0.Co;2
25. USNO-AAD (2011) Complete sun and moon data for one day. US Naval Observatory Astronomical Applications Department. http://aa.usno.navy.mil/data/docs/RS_OneDay.php. Accessed 28 September 2011
26. Berg MC, Benbow ME (2013) Environmental factors associated with Phormia regina (Diptera: Calliphoridae) oviposition. J Med Entomol 50(2):451–457. doi:10.1603/me12188
27. Anderson JR (1964) Methods for distinguishing nulliparous from parous flies and for estimating the ages of *Fannia canicularis* and some other cyclorraphous Diptera. Ann Entomol Soc Am 57(2):226–236
28. Bennettova B, Fraenkel G (1981) What determines the number of ovarioles in a fly ovary? J Insect Physiol 27(6):403–410
29. Liu D, Greenberg B (1989) Immature stages of some flies of forensic importance. Ann Entomol Soc Am 82(1):80–93
30. Vogt WG, Woodburn TL, Tyndale-Biscoe M (1974) Method of age-determination in *Lucilia cuprina* (Wied) (Diptera: Calliphoridae) using cyclic changes in female reproductive system. Bull Entomol Res 64(3):365–370
31. Gordh G, Headrick D (2001) A dictionary of entomology, 2nd edn. CABI, New York City
32. Brundage A (2011) Fitness effects of colonization time of *Chrysomya rufifacies* and *C. macellaria*, and their response to intra- and inter-specific eggs and egg-associated microbes. Texas A&M University, College Station, TX
33. Watson EJ, Carlton CE (2005) Insect succession and decomposition of wildlife carcasses during fall and winter in Louisiana. J Med Entomol 42(2):193–203. doi:10.1603/0022-2585(2005)042[0193:isadow]2.0.co;2
34. Campobasso CP, Di Vella G, Introna F (2001) Factors affecting decomposition and Diptera colonization. Forensic Sci Int 120(1–2):18–27. doi:10.1016/S0379-0738(01)00411-X
35. NESDIS (2011) Climatography of the United States No. 84, 1971-2000: Station #411889. National Environmental Satellite, Data, and Information Service
36. Davidowitz G, D'Amico LJ, Nijhout HF (2003) Critical weight in the development of insect body size. Evol Dev 5(2):188–197
37. Hocking B (1953) The intrinsic range and speed of flight of insects. Transactions of the Royal Entomological Society of London 104 (223-345)
38. Wall R (1993) The reproductive output of the blowfly *Lucilia sericata*. J Insect Physiol 39(9):743–750. doi:10.1016/0022-1910(93)90049-w
39. Roy DN, Siddons LB (1939) On the life history and bionomics of *Chrysomya rufifacies* Macq (Order Diptera, Family Calliphoridae). Parasitology 31(4):442–447
40. Mackerras MJ (1933) Observations on the life-histories, nutritional requirements and fecundity of blowflies. Bull Entomol Res 24(3):353–362
41. Jaenike J (1978) Optimal oviposition behavior in phytophagous insects. Theor Popul Biol 14(3):350–356. doi:10.1016/0040-5809(78)90012-6
42. Archer MS, Elgar MA (2003) Effects of decomposition on carcass attendance in a guild of carrion-breeding flies. Med Vet Entomol 17(3):263–271
43. LeBlanc HN, Logan JG, Amendt J, Goff ML, Campobasso CP, Grassberger M (2009) Exploiting insect olfaction in forensic entomology. In: Current concepts in forensic entomology. Springer Netherlands, pp 205-221. doi:10.1007/978-1-4020-9684-6_11
44. Vass AA, Smith RR, Thompson CV, Burnett MN, Dulgerian N, Eckenrode BA (2008) Odor analysis of decomposing buried human remains. J Forensic Sci 53(2):384–391. doi:10.1111/j.1556-4029.2008.00680.x
45. Ashworth JR, Wall R (1994) Responses of the sheep blowflies *Lucilia sericata* and *L. cuprina* to odor and the development of semiochemical baits. Med Vet Entomol 8(4):303–309. doi:10.1111/j.1365-2915.1994.tb00093.x
46. Lam K, Babor D, Duthie B, Babor EM, Moore M, Gries G (2007) Proliferating bacterial symbionts on house fly eggs affect oviposition behaviour of adult flies. Anim Behav 74:81–92. doi:10.1016/j.anbehav.2006.11.013

47. Wall R, Warnes ML (1994) Responses of the sheep blowfly *Lucilia sericata* to carrion odor and carbon dioxide. Entomol Exp Appl 73(3):239–246
48. Spradbery JP (1979) Reproductive status of *Chrysomya species* (Diptera: Calliphoridae) attracted to liver-baited blowfly traps in Papua New Guinea. J Aust Entomol Soc 18:57–61
49. Huntington TE, Higley LG (2010) Decomposed flesh as a vitellogenic protein source for the forensically important *Lucilia sericata* (Diptera: Calliphoridae). J Med Entomol 47(3):482–486. doi:10.1603/me09052
50. Wajnberg E (2006) Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. Behav Ecol Sociobiol 60(5):589–611. doi:10.1007/s00265-006-0198-9
51. Peck R, Olsen C, Devore JL (2011) Introduction to statistics and data analysis. Duxbury Press
52. National Oceanic & Atmospheric Administration (2011) Quality controlled local climatological data (final) hourly observations table San Marcos Municipal Arpt (12979) San Marcos, TX (11/2011).
53. Smith KGV (1986) A manual of forensic entomology. Trustees of the British Museum (Natural History), London, England
54. Daubert v. Merrell Dow Pharmaceuticals, Inc (1993). US, vol 509. Supreme Court