

Effects of decomposition on carcass attendance in a guild of carrion-breeding flies

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Abstract. Many forensically important calliphorids, sarcophagids and muscids (Diptera) oviposit or larviposit on corpses only during the early stages of decomposition, yet individuals may attend bodies throughout decay. A field study was conducted to investigate how patterns of carcass use and attendance by some fly species are affected by decomposition. Five fly traps were placed in the forest and baited with whole, fresh piglet carcasses. Piglets decomposed in traps throughout the experiment, and all were skeletonized within 6 days. Flies were trapped at both early and late decomposition stages, and the species and population structures of trap catches were compared. More flies attended carcasses early rather than late in decay. For all species, flies attending early were mainly gravid females, but few gravid females attended late in decay. No females ovi- or larviposited late in decay, whereas females of all fly species deposited offspring early in decay. The number of males trapped of each species correlated positively with the number of females with eggs at early development stages. Observations were made of fly predation by European wasps *Vespula germanica* Fabricius (Hymenoptera, Vespidae) and jumper ants *Myrmecia pilosula* Smith (Hymenoptera, Formicidae) throughout the experiment. There was a higher risk for smaller fly species of being killed following predator attack. Ants and wasps attacked smaller fly species, whereas only wasps attacked larger fly species.

Key words. Calliphoridae, Sarcophagidae, Muscidae, carcass, decomposition, forensic entomology, Australia.

Introduction

There is incomplete understanding of the factors that attract carrion-breeding adult calliphorids, sarcophagids and muscids to carrion. However, it is known that different resource needs motivate males, gravid females and non-gravid female carrion flies to attend carcasses (Spradberry, 1979; Hayes *et al.*, 1999). Clarification of the factors governing carrion fly attraction to carcasses is important, because forensic entomologists may need to make assumptions about when females of carrion-breeding species will deposit offspring on carcasses. Some insect succession

studies also describe succession stages within which adult carrion flies occur (Payne, 1965; Anderson & VanLaerhoven, 1996; Archer, 2002). Additionally, guides to the collection of forensic entomology evidence advise that adult flies should be trapped at the body (Smith, 1986; Haskell *et al.*, 2001), and some workers may therefore use these flies to help determine the succession stage of the body. However, because carcasses are constantly changing throughout decomposition, it is important to assess how the nature of carcass use by forensically important species may change throughout their tenure. It is also important to assess whether the presence of adult carrion flies is likely to be a reliable indicator of corpse age.

Most field studies on fly attraction to carrion have been conducted using butchered meat baits rather than whole carcasses (Spradberry, 1979; Muirhead-Thompson, 1991; Fisher *et al.*, 1998; Hayes *et al.*, 1999). Catches of

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calliphorid species from meat-baited traps indicate that swarms of flies at carcasses comprise gravid and non-gravid females as well as males (Spradberry, 1979; Fisher *et al.*, 1998; Hayes *et al.*, 1999). There has been surprisingly little research on the population structure of flies attracted to whole carrion. However, Hall & Doisy (1993), working with whole chicken carcasses from which maggots were excluded, found that carcass age significantly influenced the species composition of attracted flies, although they did not examine within-species gender and female ovarian status.

The presence of feeding maggots affects the rate and nature of carcass decomposition (Payne, 1965; Putman, 1978), yet the effect on attracted fly populations of decomposition in tandem with maggot feeding has not been investigated. This is despite the fact that most bodies encountered in forensic entomology cases are maggot-infested.

Most carrion calliphorids, sarcophagids and muscids visit decomposing carcasses or bodies during early decay stages when the majority of flesh remains (Archer, 2002). However, observations of decomposing whole carcasses reveal that fly species usually associated with early decomposition may also occur at carcasses late in decay when they are nearly or completely skeletonized (Anderson & VanLaerhoven, 1996; Archer, 2002). Colonizing blow fly species are also often seen alighting on significantly decomposed human bodies during forensic scene investigations (personal observation). Flies are attracted to carcasses by volatile compounds emitted during decomposition (Ashworth & Wall, 1994; Morris *et al.*, 1998). There is presumably a great reduction in the volume of attractive chemicals released after flesh has decayed, but nevertheless, flies present at late decomposition stages may simply be responding to residual decay odours emanating from the carcass or body.

However, although residual odours may be partly responsible for attracting flies to decayed remains, this is unlikely to be the full explanation. Carcasses in early and late decay produce different chemicals. Sulphurous compounds, which are highly attractive to carrion flies (Ashworth & Wall, 1994), are released during the breakdown of proteins, and this occurs mainly during putrefaction while flesh is present (Gill-King, 1997). The profile of volatiles and gases changes during the concluding stages of decay (Vass *et al.*, 1992). It is therefore probable that flies can easily distinguish between chemical signatures of early and late decay carcasses, and are therefore not accidentally attracted during later stages.

Flesh on a carcass is a finite resource for which carrion fly larvae compete fiercely (Fuller, 1934; Uilyett, 1950). Larvae that fail to obtain enough food become small adults with reduced male mating success (Cook, 1992; Stoffolano *et al.*, 2000) and female fecundity (Webber, 1955). Thus, in order to maximize offspring growth and survival, gravid female carrion flies should be strongly selected to swiftly locate carrion suitable for ovi- or larviposition. For colonizing species, bodies become attractive almost immediately after

death, and the rapid colonization of fresh carrion (Anderson, 2001) demonstrates the efficiency with which females locate new ovi- or larviposition resources. It would therefore be expected that few of the colonizing fly species observed on extensively decayed remains are gravid females because visiting unsuitable remains requires energy and wastes search time.

Non-gravid females of anautogenous fly species require protein to complete vitellogenesis (egg yolk formation) (Webber, 1958; Clift & McDonald, 1976; Belzer, 1978; Barton Browne & van Gerwen, 1992; Barton Browne, 1993), and these females form a proportion of carrion fly bait trap catches (Spradberry, 1979; Hayes *et al.*, 1999). Non-gravid females can presumably obtain protein from both early and late decay carcasses, although protein is possibly more abundant at fleshy early decay remains. It may also be quicker to obtain a protein meal at early decay remains because abundant liquid decomposition products are present. Thus, a feeding non-gravid female may be exposed to predator attack for less time at early decay remains. She may also be at greater risk of contracting a parasitic or fungal infection, such as *Entomophthora muscae* (Graham-Smith, 1916; Evans, 1989), if she attends carrion late rather than early because the risk of pathogen presence should increase with the number of previous visitors to the carcass. Therefore, although some non-gravid females may attend decomposed remains, the majority probably arrive during early decay.

Field bait trapping studies of several blow fly species also report comparatively high catches of non-gravid females in early stages of egg development compared with other non-gravid classes (Spradberry, 1979; Hayes *et al.*, 1999), and, presumably, protein requirements are fulfilled early in egg formation. It is therefore likely that non-gravid females visiting carrion will be mainly in the early stages of egg formation.

Male carrion flies attend carrion primarily to obtain matings (Shorey *et al.*, 1969), since many species can mature their testes without protein feeding. Some species of female muscoid flies are commonly receptive to males for only one, or rarely two, mating (Barton Browne, 1958; Reimann *et al.*, 1967; Guillot *et al.*, 1978; Merritt *et al.*, 1994), and field studies indicate that in blow fly species examined, all or most females are inseminated by the later stages of egg formation (Guillot *et al.*, 1977; Spradberry, 1979). Thus males of many carrion fly species are probably limited to copulating with non-gravid females in the early stages of egg development, and they should therefore be attracted to carcasses at the same time as potentially receptive females.

In the south-east Australian summer the most common dipteran species at decaying carrion in forests are five species of Calliphoridae [*Chrysomya varipes* (Maquart), *Chrysomya rufifacies* (Macquart), *Calliphora augur* (Fabricius), *C. stygia* (Fabricius) and *C. hilli* Patton], one muscid [*Hydrotaea rostrata* (Robineau-Desvoidy)] as well as sarcophagid species (personal observation). The species identities of these sarcophagids have not been determined here because the identification of sarcophagids to species

(especially females) requires considerable taxonomic experience (Smith, 1986). Male *Ch. rufifacies*, *C. augur* and *C. stygia* can mature their testes independent of protein (Mackerras, 1933; Roy & Siddons, 1939; Norris, 1959), although this has not been studied in the remaining species. Males of all species attempt to mate with females at carcasses; however, mating attempts are far more frequent in *H. rostrata* and *Ch. varipes* (personal observation). These flies are commonly preyed upon at carcasses by the jumper ant *Myrmecia pilosula* Smith (Formicidae, Hymenoptera) and the European wasp *Vespa germanica* Fabricius (Formicidae, Hymenoptera) (personal observation).

This study investigated the carrion fly population structures for various species present at whole, maggot-infested early and late decay carcasses. The numbers of flies trapped in each of the population groups (males, gravid females and non-gravid females) were compared between all guild members at early and late decay piglet carcasses. We also recorded maggot colonization patterns of carcasses to determine whether offspring deposition occurred at later decomposition stages. Finally, patterns of predation by wasps and ants were examined to determine the nature of risk to each fly species at different times during decomposition.

Methods

Field site and weather data

The study took place in the Coranderrk Bushland in Victoria, Australia (37°40'59 S, 145°31'07 E), a damp sclerophyll forest dominated by the Myrtaceae species *Eucalyptus cephalocarpa* (silverleaf stringybark), *E. aromaphloia* (scent-bark) and *E. obliqua* (messmate stringybark), and the Rubiaceae species *Coprosma quadrifida* (prickly currant bush).

Daily ambient temperature and rainfall data at Coranderrk Bushland were collected with a Weather Wizard II weather station (manufactured by Davis Instruments, Hayward, CA, USA) located approximately 1 km from Picaninny Swamp.

Trap design

Fly traps were 50-L green plastic rubbish bins with 100 mm deep vermiculite in the base. Traps were baited with fresh stillborn piglet carcasses (1210–1910 g, mean = 1506 g, SE = 140 g) that were allowed to decay throughout the experiment. Two 200 × 250 mm ventilation panels were cut from opposite sides of each bin and covered with open-weave dish cloths. This allowed air movement through the trap, and thus odour distribution, but excluded flies.

Bin lids were inverted when placed on traps. Circular holes (300 mm diameter) were cut from the centre of each lid, and a cotton bag was suspended below each with the base approximately 50 mm above the carcass. Thus, any carrion fly passing through the lid hole could enter the

bag, but was excluded from the carcass. A 400-ml pitfall trap was placed at the base of each bag. Pitfall traps contained a solution of ethanol (70% concentration) and glycerin (5% concentration). Flies were also trapped with an insect net, which was placed over the hole in the bin lid at standardized time intervals (see below). The bin was then tapped to agitate flies, which were captured and transferred to ethanol and glycerin solution.

Day 1 – observation/bait colonization

Five fly traps were placed 200 m apart at 09.00 h on February 7th, 2000. The traps, which were situated in clearings that experienced alternating periods of sun and shade throughout the day, remained in place for the duration of the experiment. No flies were trapped on day 1 because traps were left open for the first 24 h of the experiment to allow infestation of piglets (and thus relatively natural decomposition).

The daylight hours of day 1 were divided into five observation periods. The first period commenced after traps were assembled, and each period lasted 90 min. Observation periods were separated by 40 min breaks. During observation periods, fly ovi- or larviposition, and ant or wasp predation on flies were recorded for 15 min at each carcass, and 3 min were allowed for movement to the next carcass.

The outcomes of attacks on flies initiated by *V. germanica* and *M. pilosula* could result in the following: death of the prey, the prey could escape without injury, or prey could escape with injuries. Attack outcomes were recorded separately for larger species between 6 and 11 mm body length (*C. augur*, *C. hilli*, *C. stygia*, *Ch. rufifacies* and sarcophagids) and smaller species between 3 and 6 mm body length (*Ch. varipes* and *H. rostrata*).

Days 2 to 6 – trapping and observation

Between days 2 and 6 we conducted twice daily observations at 08.00 h and 16.00 h. These observation periods were of the same duration as day 1 and the same data were recorded. Additionally, the decay stages of carcasses were described. Lids were replaced on traps at all other times between days 2 to 6 to enable fly sampling. However, flies were not sampled until 15 min after lid replacement to discourage accidental trapping of females that had oviposited or larviposited during the observation period. Ethanol traps were set 15 min after traps were closed, and flies were additionally captured by net every half hour from closed traps. Net and ethanol trap catches were combined to yield daily adult fly samples from each piglet.

Dissections

The species and gender of flies trapped at each carcass were identified and females were dissected to determine

ovarian status. The number of *Ch. varipes* females trapped was too high to permit dissection of all individuals and thus 30 females per sample were randomly selected (although all were dissected if less than 30 were trapped). Larviparous females were considered gravid if the uterus contained live maggots and oviparous females were considered gravid if the ovaries contained mature eggs. Non-gravid females were divided into early egg development (stages 0–II; Tyndale-Biscoe & Hughes, 1968) and late egg development (stages III–IV; Tyndale-Biscoe & Hughes, 1968) classes. The mating status of females was not determined because traps did not exclude them from mating between their arrival at piglets and capture.

Data analysis

Data were inspected for normality, log transformed where appropriate and analysed using Systat (version 9, Systat Software Asia Pacific, Bangalore, India). Data are means \pm SE.

Results

Weather

There was no rainfall during daylight hours. Daytime temperatures (recorded roughly every hour between 07.30 and 18.00 h) were: day 1, mean = 32.9°C (range = 35.7–28.9°C); day 2, mean = 33.2°C (range = 37.2–25.4°C); day 3, mean = 23.1°C (range = 25.9–20°C); day 4, mean = 22.9°C (range = 24.7–20°C); day 5, mean = 23.8°C (range = 28.1–17.1°C); day 6, mean = 25.7°C (range = 30.3–16°C).

Decomposition

The majority of flesh remained on all piglets by day 3 and all were bloated. Carcasses were largely or completely skeletonized by day 5 and all were skeletonized by day 6. Two separate periods of equal (2-day) duration were chosen and referred to as 'initial decomposition' and 'advanced decomposition'. These time periods provided two intervals of equal sampling effort, thus facilitating comparison between the parameters of fly samples trapped during each. Carcasses were designated 'initial decomposition' on days 2 and 3 and 'advanced decomposition' on days 5 and 6. During 'initial decomposition', plentiful flesh remained and the piglets supported large maggot populations. During 'advanced decomposition', there was little or no flesh remaining and carcasses provided little or no food for maggots. There was no significant difference between the mean daytime temperatures during the 'initial decomposition' and 'advanced decomposition' sampling periods (pooled $t = 1.5$, d.f. = 42, $P = 0.14$).

Carcass colonization

The viviparous *C. augur* and *C. hilli* were the sole species observed depositing offspring on day 1, although all study species were observed larvi- or ovipositing during the 'initial decomposition' period (days 2 and 3). No flies larvi- or oviposited on day 4, and no flies larvi- or oviposited during the 'advanced decomposition' period (days 5 and 6). No new eggs or first-stage larvae appeared on carcasses between days 4 to 6.

Predation

Both *V. germanica* wasps and *M. pilosula* ants attacked flies. Of the 71 attacks witnessed on 'large' species, all were initiated by *V. germanica*. Flies were killed on 20 occasions, escaped without visible injury on 50 occasions, and in one attack, the fly escaped with injuries. Of the 92 attacks witnessed on 'small' flies, 20 were initiated by *M. pilosula* and 72 were initiated by *V. germanica*. *Myrmecia pilosula* killed flies on 12 occasions and prey escaped without injury in eight attacks. *Vespula germanica* killed small flies in 60 attacks and prey escaped from 12 attacks without injury. Predation events were witnessed only between days 1 and 4.

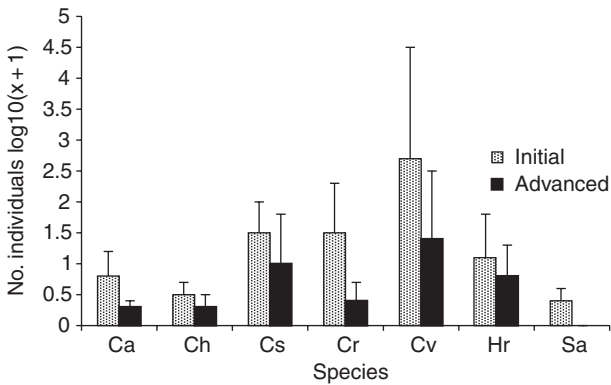
Species and gender effects on attendance

All members of the carrion-breeding fly guild active in summer at the field site were trapped (*C. stygia*, *C. hilli*, *C. augur*, *Ch. rufifacies*, *Ch. varipes*, *H. rostrata* and carrion-breeding sarcophagids). There was no correlation between the initial weights of piglets and the total number of flies trapped ($r = 0.11$, $P > 0.05$). Both sexes of all guild members were captured, except Sarcophagidae, for which only females were trapped. In all species, except *C. augur*, mean male numbers were lower at 'advanced decomposition' baits, and in all species, mean female numbers were lower at 'advanced decomposition' piglets (Table 1).

A repeated measures ANOVA was performed with sex and species as factors, and numbers of flies trapped during initial and advanced decomposition as repeated measures. The difference across repeated measures was significantly influenced by species ($F_{6,56} = 12.6$, $P < 0.001$). Higher mean numbers of all species were captured at initial rather than advanced decomposition piglets. *Chrysoma varipes* was trapped in the highest mean numbers at piglets both in advanced and initial decomposition, and *C. stygia* was trapped in the second highest mean numbers at carrion in both states. However, *Ch. rufifacies* mean numbers were third highest only during initial decomposition, followed by *H. rostrata*, *C. augur*, *C. hilli* and Sarcophagidae. During advanced decomposition, *H. rostrata* were trapped in the third highest numbers, followed by *Ch. rufifacies*, *C. hilli* and *C. augur*. No sarcophagids were trapped during advanced decomposition (Fig. 1).

Table 1. Mean number (\pm SE) of male and female flies trapped at carcasses ($n=5$) during initial and advanced decomposition.

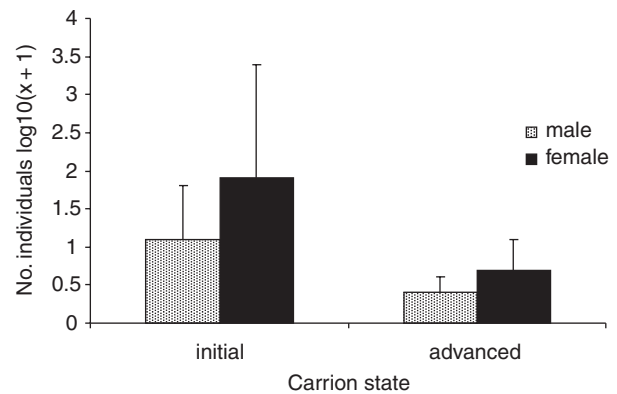
Species	Initial decomposition		Advanced decomposition	
	Female	Male	Female	Male
<i>C. augur</i>	11.0 (\pm 5.0)	0.2 (\pm 0.2)	1.0 (\pm 0.4)	0.4 (\pm 0.2)
<i>C. hilli</i>	4.0 (\pm 1.8)	0.6 (\pm 0.2)	2.0 (\pm 0.6)	0.6 (\pm 0.6)
<i>C. stygia</i>	30.2 (\pm 2.5)	0.8 (\pm 0.5)	7.4 (\pm 5.2)	0.2 (0.2)
<i>Ch. varipes</i>	490.2 (\pm 60.3)	66.8 (\pm 10.7)	17.8 (\pm 7.2)	7.0 (\pm 4.7)
<i>Ch. rufifacies</i>	26.2 (\pm 4.1)	2.4 (\pm 1.0)	1.4 (\pm 0.7)	0.2 (\pm 0.2)
<i>H. rostrata</i>	10.6 (\pm 2.9)	2.0 (\pm 0.8)	4.8 (\pm 2.2)	1.2 (\pm 0.5)
Sarcophagidae	1.4 (\pm 0.5)	0.0 (\pm 0.0)	0.0 (\pm 0.0)	0.0 (\pm 0.0)

**Fig. 1.** Mean numbers + SE of each species trapped at carcasses ($n=5$) during initial and advanced decomposition (both sexes combined). Ca = *Calliphora augur*, Ch = *C. hilli*, Cs = *C. stygia*, Cr = *Chrysomya rufifacies*, Cv = *Ch. varipes*, Hr = *Hydrotaea rostrata* and Sa = family Sarcophagidae.

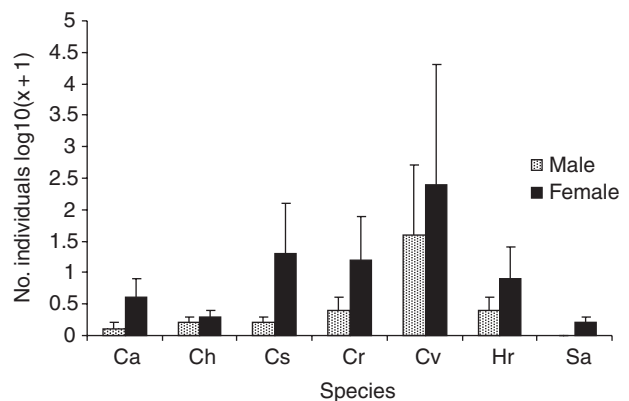
Sex also significantly influenced the difference in fly numbers across repeated measures ($F_{1,56} = 1.9$, $P < 0.001$). Mean male and female numbers combined between species were higher during initial decomposition; however, there was a greater reduction in mean female numbers than mean male numbers attending unsuitable carrion (Fig. 2). There was no interaction between sex and species across repeated measures ($F_{6,56} = 0.8$, $P > 0.6$).

When trap catch numbers from 'initial' and 'advanced' decomposition were combined to give overall catches of each species, there was an interaction between sex and species ($F_{6,56} = 8.8$, $P < 0.001$). The highest mean numbers of males and females trapped were *Ch. varipes*. The second highest mean numbers of males trapped were *H. rostrata*, followed by *Ch. rufifacies*, *C. hilli*, *C. stygia*, *C. augur* and Sarcophagidae. The second highest mean numbers of females trapped were *C. stygia*, followed by *Ch. rufifacies*, *H. rostrata*, *C. augur*, *C. hilli* and Sarcophagidae (Fig. 3).

There was a significant difference between species in the median proportion of males present during initial decomposition (Kruskal–Wallis $T = 12.7$, d.f. = 6, $P < 0.05$). *Calliphora hilli* males were present in the highest proportion, followed by *H. rostrata*, *Ch. varipes*, *Ch. rufifacies*, *C. augur*, *C. stygia* and Sarcophagidae (Fig. 4). It was not

**Fig. 2.** Mean numbers + SE of male and female flies trapped at carcasses ($n=5$) during initial and advanced decomposition (all species combined).

possible to test for differences in the proportions of males at unsuitable carrion because low trap catches resulted in many missing data values.

**Fig. 3.** Mean numbers + SE of males and females of each species trapped at carcasses ($n=5$) with initial and advanced decomposition samples combined. Ca = *Calliphora augur*, Ch = *C. hilli*, Cs = *C. stygia*, Cr = *Chrysomya rufifacies*, Cv = *Ch. varipes*, Hr = *H. rostrata* and Sa = family Sarcophagidae.

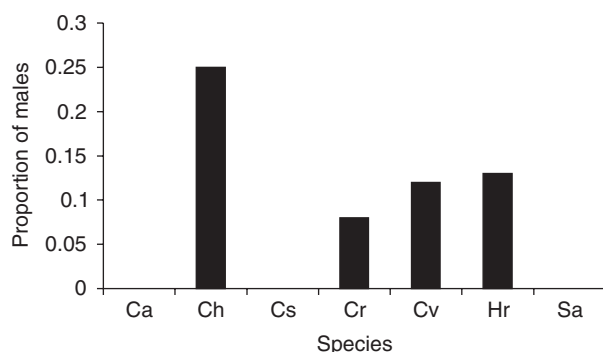


Fig. 4. Median proportions of males of each study species found at carcasses ($n = 5$) during initial decomposition. Ca = *Calliphora augur*, Ch = *C. hilli*, Cs = *C. stygia*, Cr = *Chrysomya rufifacies*, Cv = *Ch. varipes*, Hr = *Hydrotaea rostrata* and Sa = Sarcophagidae.

Ovarian status effects on attendance

All trapped sarcophagids were gravid females, but both gravid and non-gravid females were trapped in all other species (Table 2). Repeated measures ANOVA was used to investigate the influence of species and ovarian status (gravid or non-gravid) on differences between numbers of females trapped during initial and advanced decomposition. An interaction between ovarian status and species significantly influenced the difference in female numbers across repeated measures ($F_{6,56} = 4.1$, $P < 0.005$; Table 2). There was a decrease in the mean numbers of gravid females of all species at carrion between initial and advanced decomposition, and there was also a decrease in the mean numbers of non-gravid females between initial and advanced decomposition samples in all species except *C. augur*. However, the magnitudes of these decreases across samples varied between species.

There was also a significant difference between species in the median proportions of gravid females present during initial decomposition (Kruskal–Wallis $T = 24.2$, d.f. = 6, $P < 0.001$, Fig. 5). Gravid *C. augur* and sarcophagids were present in the highest proportions, followed by *C. stygia*, *Ch. varipes*, *Ch. rufifacies*, *H. rostrata* and *C. hilli*. It was not possible to examine proportions of females present during

advanced decomposition because low trap catches resulted in many missing values.

Multiple correlations using a Bonferroni correction revealed that when species catches were combined between initial and advanced decomposition, there was a significant positive correlation between the number of males and 'early egg development' females of each species ($r = 0.97$, $n = 7$, $P < 0.05$), but not between the number of males and 'late egg development' females ($r = 0.65$, $n = 7$, $P > 0.05$), or between the number of males and gravid females ($r = 0.80$, $n = 7$, $P > 0.05$).

Numbers of non-gravid females that were either 'late egg development' or 'early egg development' varied between species and carrion decomposition stage (Table 2). A repeated measures ANOVA with species and egg stage (early and late development) as factors and initial and advanced decomposition as repeated measures revealed a significant difference in the mean (transformed) numbers of non-gravid females trapped in initial decomposition (untransformed mean = 32 ± 5) and advanced decomposition (untransformed mean = 21 ± 8) samples ($F_{1,48} = 11.8$, $P < 0.005$). This variation in the numbers of non-gravid females was not influenced by egg stage ($F_{1,48} = 0.2$, $P > 0.05$), species ($F_{5,48} = 1.8$, $P > 0.05$) or an interaction between the two factors ($F_{5,48} = 0.3$, $P > 0.05$). However, a significant interaction between egg stage and species ($F_{5,48} = 7.2$, $P < 0.001$) influenced combined numbers of non-gravid females trapped at the two bait stages; there were more early egg development than late egg development females trapped for all species, but the magnitudes of differences varied between species (Fig. 6).

Discussion

Forensic entomology texts advise that collection of adult flies associated with bodies should be made at death scenes (Smith, 1986; Haskell *et al.*, 2001). However, although this procedure gives a complete inventory of taxa associated with the corpse, caution should be employed in using this evidence to make inferences about the minimum post-mortem interval. Our results demonstrate that gravid female carrion flies may be present at carrion no longer used for offspring deposition. Also, colonizing species were attracted

Table 2. Mean number (\pm SE) of gravid and non-gravid females, as well as numbers of early egg development (early) and late egg development (late) females trapped at carcasses ($n = 5$) during initial and advanced decomposition.

Species	Initial decomposition				Advanced decomposition			
	Gravid	Non-gravid	Early	Late	Gravid	Non-gravid	Early	Late
<i>C. augur</i>	5 (± 1.7)	0 (± 0)	0.6 (± 0.4)	0 (± 0)	0 (± 0)	0.4 (± 0.2)	0.4 (± 0.4)	0 (± 0)
<i>C. hilli</i>	0.6 (0.4)	1.2 (0.4)	1 (± 0.3)	0.2 (± 0.2)	0 (± 0)	0.6 (± 0.4)	0.6 (± 0.2)	0.2 (± 0.2)
<i>C. stygia</i>	26.8 (± 2.8)	3.4 (± 0.9)	2 (± 1)	1.8 (± 0.4)	6.4 (± 5.4)	1.6 (± 0.5)	1 (± 0.6)	0.8 (± 0.4)
<i>Ch. rufifacies</i>	19 (± 2.3)	7.2 (± 2.5)	4.8 (± 2.4)	2.6 (± 0.9)	0.4 (± 0.2)	0.8 (± 0.4)	0.6 (± 0.4)	0.2 (± 0.2)
<i>Ch. varipes</i>	47.8 (± 4.8)	12.2 (± 1.5)	9.6 (± 1.1)	2 (± 0.3)	4.8 (± 4.1)	10.8 (± 3.3)	12.2 (± 4.7)	2.2 (± 1.5)
<i>H. rostrata</i>	3.4 (± 1.2)	7.2 (± 2.7)	5.4 (± 2.2)	1.8 (± 0.8)	2.4 (± 1)	2.4 (± 1.2)	1.4 (± 0.7)	1 (± 0.4)
Sarcophagidae	1.4 (± 0.5)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)

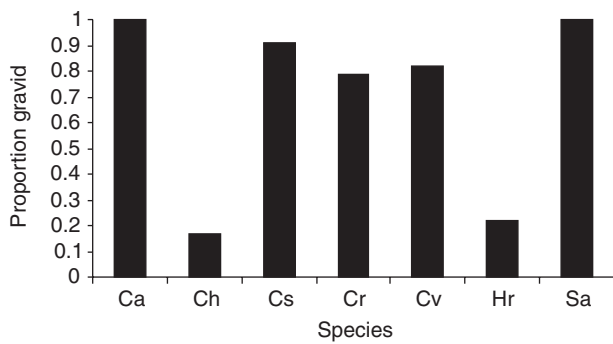


Fig. 5. Median proportions of gravid females for each study species found at carcasses ($n=5$) during initial decomposition. Ca = *Calliphora augur*, Ch = *C. hilli*, Cs = *C. stygia*, Cr = *Chrysomya rufifacies*, Cv = *Ch. varipes*, Hr = *Hydrotaea rostrata* and Sa = Sarcophagidae.

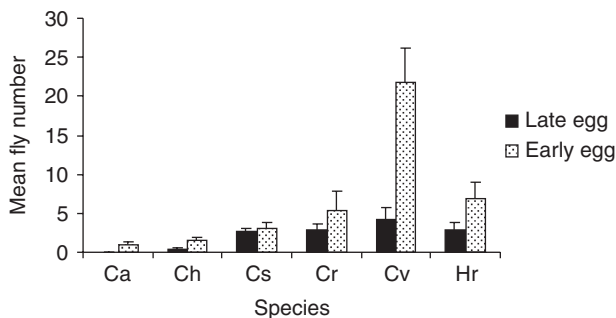


Fig. 6. Mean numbers of 'early' and 'late' egg development females trapped at each carcass ($n=5$) during initial and advanced decomposition combined. Ca = *Calliphora augur*, Ch = *C. hilli*, Cs = *C. stygia*, Cr = *Chrysomya rufifacies*, Cv = *Ch. varipes* and Hr = *Hydrotaea rostrata*.

late in decay, thus demonstrating the need for caution when using adult flies in succession-based estimates of post-mortem interval.

Far fewer flies attended piglets in advanced decomposition compared with those in initial decomposition. This was probably due to a combination of altered chemical cues emitted by baits as well as a reduced volume of attractive chemicals emitted later in decay. However, substantial numbers of carrion flies were still trapped at piglets that were almost, or completely, skeletonized. It is surprising that gravid females of several species were among these flies since no larvi- or oviposition occurred after day 3.

The three most common species trapped during advanced decomposition were *Ch. varipes*, *C. stygia* and *H. rostrata*, and these species also had the highest numbers of gravid females captured during advanced decomposition. The larvae of these species can occur early in succession, but may also be found later in succession than those of other guild members; *H. rostrata* maggots in particular are very resistant to the drier, food-depleted conditions occurring on old remains (Fuller, 1934). It is therefore possible that adults of these species can orientate to chemicals produced

during advanced decay, and that residual flesh and body fluids on advanced decomposition piglets produced small amounts of attractive chemicals. Gravid females attracted may then need to land on the remains to assess their suitability for larvae. Alternatively, gravid females may have arrived at piglets in search of carbohydrates, which are required for normal metabolic activity (Webber, 1957).

The majority of females attracted during advanced decay were non-gravid, although these females were in the minority during initial decay. There was a reduction in the numbers of non-gravid females of all species trapped during advanced decomposition, and this may be due to lower levels of attractive emissions from carcasses. However, the quality of the protein meal that can be obtained during initial decay may be much higher than that of a meal obtained during advanced decay, and part of the reduction in numbers of non-gravid females during advanced decomposition may therefore have been due to these females searching for higher quality protein sources than those potentially offered by late decay carcasses.

Most non-gravid females trapped at both decomposition stages were in early egg development, which supports the idea that late egg development females are less likely than early development females to attend carrion, since a higher proportion of them will have satisfied their protein and mating needs. Many early development females, by contrast, may still be protein-hungry and sexually receptive.

Chrysomya varipes and *H. rostrata*, which mate frequently on carcasses, had the highest carcass attendance by early development females. They were also the two species for which the highest numbers of males were captured. Our results also suggest that males increased their attendance at carrion when they were more likely to encounter females that were non-gravid and in the early stages of egg development. These females are probably more likely to be receptive if virgin. Norris (1959) determined that sex ratio and overall trap catch numbers were positively correlated in several species of Australian blow fly, including members of this guild. He suggested that males may be less likely to leave favoured habitats than females, thus visiting traps in lower numbers (Norris, 1959). He did not, however, examine male numbers in relation to the number of potentially receptive females.

Males of many calliphorid species, including *C. stygia* of this guild, set up 'mating stations' at nectar and honeydew sources that attract females (Norris, 1959, 1964). Males perch on surrounding vegetation and fly out to intercept potential mates (Norris, 1964; Guillot *et al.*, 1978). There is evidence that males are more common at carbohydrate sources than proteinaceous ones (Graham-Smith, 1916; Guillot *et al.*, 1978), and this may be because the chance of an encounter with a receptive female is higher. However, carbohydrate sources flux and protein sources decompose, so their relative value as mating stations will vary. Thus, it would benefit males to assess the value of individual mating stations by the attendance levels of potentially receptive females. They may do this using female sex pheromone concentration. Receptive females of the carrion-breeding

blow fly *Lucilia cuprina* Weideman (Diptera: Calliphoridae) release an airborne sex pheromone when receptive (Bartell *et al.*, 1969), and females of this guild may behave similarly.

All species attracted to piglets experienced predation risk, but the nature of this risk varied between different sized flies. *Myrmecia pilosula* ants attack only small species, whereas *V. germanica* wasps attack both large and small flies. Both ants and wasps usually killed the small flies they attacked, although the probability of attack for individuals was likely to be reduced because higher numbers of small flies were present. Large flies usually escaped from wasp attacks without visible injury. Nevertheless, flies that escape their skirmish with predators may still experience wing fray (Allsopp, 1985) or sustain damage to sensory structures. Flies that attended late decay carcasses encountered no predation risk, but such flies are probably still at risk of contracting a disease from previous visitors.

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References

- Allsopp, R. (1985) Wing fray in *Glossina morsitans centralis* Machado (Diptera: Glossinidae). *Bulletin of Entomological Research*, **75**, 1–11.
- Anderson, G.S. (2001) Insect succession on carrion and its relationship to determining time of death. *Forensic Entomology: the Utility of Arthropods in Legal Investigations* (ed. by J. H. Byrd and J.L.Castner), pp. 143–175. CRC Press, Florida.
- Anderson, G.S. & VanLaerhoven, S.L. (1996) Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences*, **41**, 617–625.
- Archer, M.S. (2002) The ecology of invertebrate interactions with vertebrate carrion in Victoria, with reference to forensic entomology. PhD Thesis, The University of Melbourne.
- Ashworth, J.R. & Wall, R. (1994) Responses of the sheep blowflies *Lucilia sericata* and *L. cuprina* to odour and the development of semiochemical baits. *Medical and Veterinary Entomology*, **8**, 303–309.
- Bartell, R.J., Shorey, H.H. & Barton Browne, L. (1969) Pheromonal stimulation of the sexual activity of males of the sheep blowfly *Lucilia cuprina* (Calliphoridae) by the female. *Animal Behaviour*, **17**, 576–585.
- Barton Browne, L. (1958) The choice of communal oviposition sites by the Australian sheep blowfly, *Lucilia cuprina*. *Australian Journal of Zoology*, **6**, 241–247.
- Barton Browne, L. (1993) Physiologically induced changes in resource-oriented behaviour. *Annual Review of Entomology*, **38**, 1–25.
- Barton Browne, L. & van Gerwen, A.C.M. (1992) Volume of protein meals taken by females of the blowfly, *Lucilia cuprina*: ovarian development related and direct effects of protein ingestion. *Physiological Entomology*, **17**, 9–18.
- Belzer, W.R. (1978) Patterns of selective protein ingestion by the blowfly *Phormia regina*. *Physiological Entomology*, **3**, 169–175.
- Clift, A.D. & McDonald, F.J.D. (1976) Some relationships between diet and ovarian development in *Lucilia cuprina* (Wied.) (Diptera: Calliphoridae). *Australian Journal of Zoology*, **24**, 87–93.
- Cook, D.F. (1992) The effect of male size on receptivity in female *Lucilia cuprina* (Diptera, Calliphoridae). *Journal of Insect Behaviour*, **5**, 365–374.
- Evans, H.C. (1989) Mycopathogens of insects. *Insect–Fungus Interactions* (ed. by N. Wilding, N. M. Collins, P. M. Hammond and J. F. Webber). Academic Press, London.
- Fisher, P., Wall, R. & Ashworth, J.R. (1998) Attraction of the sheep blowfly, *Lucilia sericata* (Diptera: Calliphoridae) to carrion bait in the field. *Bulletin of Entomological Research*, **88**, 611–618.
- Fuller, M.E. (1934) The insect inhabitants of carrion, a study in animal ecology. *Bulletin of the Council for Scientific and Industrial Research*, **82**, 5–62.
- Gill-King, H. (1997) Chemical and ultrastructural aspects of decomposition. *Forensic Taphonomy: the Postmortem Fate of Human Remains* (ed. by W. D. Haglund and M. H. Sorg), pp. 93–108. CRC Press, Boca Raton.
- Graham-Smith, G.S. (1916) Observations on the habits and parasites of common flies. *Parasitology*, **8**, 440–544.
- Guillot, F.S., Coppedge, J.R., Goodenough, J.L., Adams, T.S. & Ahrens, E. (1977) Behavior and reproductive status of native female screwworms attracted to a host. *Annals of the Entomological Society of America*, **70**, 588–590.
- Guillot, F.S., Brown, H.E. & Broce, A.B. (1978) Behaviour of sexually active male screwworm flies. *Annals of the Entomological Society of America*, **71**, 199–201.
- Hall, R.D. & Doisy, K.E. (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. *Annals of the Entomological Society of America*, **86**, 589–593.
- Haskell, N.H., Lord, W.D. & Byrd, J.H. (2001) Collection of entomological evidence during death investigations. *Forensic Entomology: the Utility of Arthropods in Legal Investigations* (ed. by J. H. Byrd and J. L. Castner), pp. 81–120. CRC Press, Florida.
- Hayes, E.J., Wall, R. & Smith, K.E. (1999) Mortality rate, reproductive output, and trap response bias in populations of the blowfly *Lucilia sericata*. *Ecological Entomology*, **24**, 300–307.
- Mackerras, M.J. (1933) Observations on the life-histories, nutritional requirements and fecundity of blowflies. *Bulletin of Entomological Research*, **24**, 353–362.
- Merritt, D.J., Yin, C.M. & Stoffolano, J.G. (1994) Copulatory apparatus and deposition of male accessory secretion in *Phormia regina* (Diptera, Calliphoridae). *Annals of the Entomological Society of America*, **87**, 97–103.
- Morris, M.C., Woolhouse, A.D., Rabel, B. & Joyce, M.A. (1998) Orientation stimulants from substances attractive to *Lucilia cuprina* (Diptera, Calliphoridae). *Australian Journal of Experimental Agriculture*, **38**, 461–468.
- Muirhead-Thompson, R.C. (1991) *Trap Responses of Flying Insects: the Influence of Trap Design on Capture Efficiency*. Academic Press, London.

- Norris, K.R. (1959) The ecology of sheep blowflies in Australia. *Biogeography in Australia* (ed. by A. Keast, R. L. Crocker and C.S. Christian), pp. 514–544. Uitgeverij Dr W. Junk, Den Haag.
- Norris, K.R. (1964) The bionomics of blow flies. *Annual Review of Entomology*, **10**, 47–68.
- Payne, J.A. (1965) A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology*, **46**, 592–602.
- Putman, R.J. (1978) The role of carrion frequenting arthropods in the decay process. *Ecological Entomology*, **3**, 133–139.
- Reimann, J.G., Moen, D.O. & Thorson, B.J. (1967) Female monogamy and its control in the housefly, *Musca domestica* L. *Journal of Insect Physiology*, **13**, 407–418.
- Roy, D.N. & Siddons, L.B. (1939) On the life history and bionomics of *Chrysomya rufifacies* Macq. (Order Diptera, family Calliphoridae). *Parasitology*, **31**, 442–447.
- Shorey, H.H., Bartell, R.J. & Barton Browne, L.E. (1969) Sexual stimulation of males of *Lucilia cuprina* (Calliphoridae) and *Drosophila melanogaster* (Drosophilidae) by the odours of aggregation sites. *Annals of the Entomological Society of America*, **62**, 1419–1421.
- Smith, K.G.V. (1986) *A Manual of Forensic Entomology*. Cornell University Press, London.
- Spradberry, J.P. (1979) The reproductive status of *Chrysomya* species (Diptera: Calliphoridae) attracted to liver-baited blowfly traps in Papua New Guinea. *Journal of the Australian Entomological Society*, **18**, 57–61.
- Stoffolano, J.G., Gonzalez, E.Y., Sanchez, M. *et al.* (2000) Relationship between size and mating success in the blow fly *Phormia regina* (Diptera: Calliphoridae). *Annals of the Entomological Society of America*, **93**, 673–677.
- Tyndale-Biscoe, M. & Hughes, R.D. (1968) Changes in the female reproductive system as age indicators in the bushfly *Musca vetustissima* Wlk. *Bulletin of Entomological Research*, **59**, 129–141.
- Ulyett, G.C. (1950) Competition for food and allied phenomena in sheep-blowfly populations. *Philosophical Transactions of the Royal Society of London Series B*, **234**, 77–174.
- Vass, A.A., Bass, W.M., Wolt, J.D., Foss, J.E. & Ammons, J.T. (1992) Time since death determinations of human cadavers using soil solutions. *Journal of Forensic Sciences*, **37**, 1236–1253.
- Webber, L.G. (1955) The relationship between larval and adult size of the Australian sheep blowfly, *Lucilia cuprina* (Weid.). *Australian Journal of Zoology*, **3**, 346–353.
- Webber, L.G. (1957) Utilisation and digestion of carbohydrates by the Australian sheep blowfly *Lucilia cuprina*. *Australian Journal of Zoology*, **5**, 164–172.
- Webber, L.G. (1958) Nutrition and reproduction in the Australian sheep blowfly *Lucilia cuprina*. *Australian Journal of Zoology*, **6**, 139–144.

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