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Annual variation in arrival and departure times of carrion insects at carcasses: implications for succession studies in forensic entomology

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Abstract

A succession of insect species associate with decaying bodies, and because of the relatively predictable arrival and departure times of many species, this process is routinely used to estimate minimum post-mortem interval. Corpse fauna are compared with baseline data on succession rates, which are usually taken from decomposing animal carcasses. Baseline data are traditionally collected over a single year only; however, annual differences in weather patterns and insect population parameters probably alter succession rates. Surprisingly, this potential source of error has not previously been examined. Therefore, a two-year succession experiment was performed to investigate annual variation in succession rates. Sets of five still-born piglet carcasses were placed in a forest each season of both years. Six insects that occur year-round were selected: adults of *Aleochara brachialis* Jekel, *Anotylus* spp., *Creophilus erythrocephalus* Fabricius (Staphylinidae: Coleoptera), *Ptomaphila lacrymosa* Schreibers (Silphidae: Coleoptera), *Pseudonemadus* spp. (Leiodidae: Coleoptera), and larvae of *Calliphora* spp. (Calliphoridae: Diptera). Arrival and departure weeks to and from carcasses were measured in each season of both years. Two-way ANOVAs revealed significant season and year interactions on the arrival weeks of *Anotylus* spp., *C. erythrocephalus*, and *Pseudonemadus* spp., and significant differences in seasonal and yearly arrival times for *A. brachialis*, and *P. lacrymosa*. For larvae of *Calliphora* spp., there was a significant seasonal effect on arrival times. A significant interaction between season and year affected the departure weeks of all taxa. These results show that error in the estimates of minimum post-mortem interval can result if baseline succession data are collected only over one year.

Introduction

Decomposing vertebrates are attractive to many invertebrates that use carrion as a food source, breeding medium or hunting ground. Decaying bodies and carcasses progress through a series of decomposition stages (Smith 1986), and a characteristic assemblage of invertebrates is associated with each stage (Payne 1965). The designation of decomposition stages is relatively subjective, and it is important to note that some invertebrates may vary in their stage associations. However, there is a definite succession of species arriving at, and departing from, decomposing bodies and carcasses until skeletonisation occurs (Bornemissza 1957; Early and Goff 1986; Anderson 2001).

The order of succession of carrion invertebrates, and the arrival and departure times of taxa involved, are potentially predictable (Smith 1986). Therefore, the minimum post-mortem interval can be estimated for forensic cases by using the invertebrate species composition on decomposing human bodies (Catts and Haskell 1990). Baseline invertebrate succession data for comparison with species present on corpses is usually collected from decomposing pig carcasses (i.e. Anderson and VanLaerhoven 1996; Lopes de Carvalho and Linhares 2001), and occasionally from human bodies (Rodriguez and Bass 1983), with a known death time. However, the timing and/or order of succession can be affected by burning, burial, habitat variation, sun exposure, or hanging (Payne *et al.* 1968;

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Shean *et al*. 1993; Avila and Goff 1998; VanLaerhoven and Anderson 1999; Shalaby *et al.* 2001). Therefore, a comprehensive picture of the succession of local carrion insects can be built only by collecting multiple sets of baseline succession data.

Succession patterns also differ greatly between seasons (Lopes de Carvalho and Linhares 2001; Archer 2002) since seasonal temperature differences affect decomposition rates, which can affect succession rates in turn (Archer 2002). Some carrion taxa may also be seasonally active (Anderson 1982; DeSouza and Linhares 1997; Piñero 1997; Tomberlin and Adler 1998; Davies 1999; Archer and Elgar 2003), whilst others may be active year-round (Archer 2002). Carrion taxa may also show seasonal variation in their abundance (Johnson 1974; Piñero 1997).

Additional complications may arise from variation between the same season in different years, both in climate and in the population parameters of the carrion invertebrates. Although the seasons show roughly the same broad climatic characteristics between years, unique weather patterns occur annually. Also, long-term ecological studies demonstrate that there may be between-year variation in the parameters of animal populations (Szaro *et al.* 1990; Keane and Morrison 1999; Martinez-Sanchez *et al.* 2000). The combined effect of yearly and seasonal variation on succession patterns has not yet been investigated by forensic entomology researchers: previously published forensic studies of succession on carrion have been conducted over one year only. It is therefore not known whether appreciable yearly differences exist in the arrival and departure times of carrion taxa. However, these differences may exist not only between seasons within the same year, but also between the same season in different years. This could be a critical source of error in succession-based estimates of the minimum post-mortem interval if baseline data are collected over one year only.

Still-born carcasses are effective models for examining seasonal and yearly differences in succession patterns. Still-born piglets are cheaply and ethically obtained, and are therefore ideal for a highly replicated, large-scale investigation into seasonal and yearly effects on carrion succession. A two-year, all-season study of carrion insect succession in southern Victoria was performed using still-born piglet carcasses. A forest environment was chosen because of its attractiveness as a covert dumping area for bodies, and as a secluded location for suicide (personal observation). Arrival and departure times to and from carcasses were examined for selected taxa active year-round on carcasses.

Methods

The field site was Coranderrk Bushland, Healesville, Victoria. Coranderrk Bushland is a fully fenced 144-ha reserve with no public access. Work was conducted in damp sclerophyll forest in an area dominated by silverleaf stringybark (*Eucalyptus cephalocarpa*), scent-bark (*E. aromaphloia*), messmate stringybark (*E. obliqua*) and prickly currant bush (*Coprosma quadrifida*).

Still-born piglets between 880–2020 g (mean = 1553, s.e. = 47) were used. The carcasses, which were frozen within hours of death, were placed in the field freshly thawed and free of external defects. One set of five carcasses was placed in each season during both 1999 (summer: 12 February; autumn: 12 April; winter: 11 June; spring: 22 October) and 2000 (summer: 4 January; autumn: 11 April; winter: 9 July; spring: 14 October), comprising a total of 40 carcasses in eight sets of five.

Carcasses were enclosed in scavenger-proof Weldmesh™ carcass cages (25-mm mesh) that prevented disturbance by vertebrates while allowing access to invertebrates. These cages could also be lifted to allow access to invertebrates underneath carcasses. Carcasses were always placed between 1300 and 1400 hours, and were placed at least 50 m apart on the west sides of mature trees. Each carcass was visited at least once weekly throughout the study, and the total number of visits made to each carcass was the same. Invertebrate samples from the skin surface and soil surface underneath carcasses were preserved in 70% ethanol. Two soil samples (1 cm^3) were also taken from under the head and abdomen and placed in 70% ethanol. Samples were identified to the lowest taxonomic rank to which they could confidently be assigned.

Adults of *Aleochara brachialis* Jekel, *Anotylus* spp., *Creophilus erythrocephalus* Fabricius (Staphylinidae: Coleoptera), *Ptomaphila lacrymosa* Schreibers (Silphidae: Coleoptera), *Pseudonemadus* spp. (Leiodidae: Coleoptera), and larvae of *Calliphora* spp. (Calliphoridae: Diptera) were selected for comparison of arrival and departure times between season and year. These taxa conformed to a set of criteria: they were sampled from all eight carcass sets (two years, all seasons), they occurred on at least three out of the five carcasses in each set, and they occurred continually throughout their tenure (did not appear and reappear at carcasses). They were also not cryptic, and were therefore reliably sampled if present. *C. erythrocephalus* and *P. lacrymosa* are large, conspicuous, and completely unlike any other species present. They were therefore identified by eye, and were not sampled. *A. brachialis*, *Anotylus* spp., *Pseudonemadus* spp., and *Calliphora* spp. were sampled, and were identified using a microscope.

Data analysis

Data on arrival and departure weeks of taxa were inspected for normality and log-transformed where appropriate. These data were analysed using Systat Version 9. A series of two-way ANOVAs, with season and year as factors, were performed on the arrival and departure weeks of the selected taxa.

Results

A series of two-way ANOVAs, with season and year as factors, revealed a variety of effects on arrival and departure times of various taxa (Table 1).

Arrival weeks

There were significant interactions between season and year for the arrival weeks of *Anotylus* spp., *C. erythrocephalus* and *Pseudonemadus* spp. (Table 1.). Arrival at carcasses was more rapid for most species on carcasses in 2000 than in 1999. However, there was also great seasonal variation in arrival times over both years (Fig. 1*a–c*). There were significant differences both in seasonal and yearly arrival times for *A. brachialis* and *P. lacrymosa* (Table 1). For both, arrival at carcasses was usually more rapid in 2000 than in 1999. For both, arrival was most delayed in winter, followed by autumn, spring and summer (Fig. 1*d*, *e*). For larvae of *Calliphora* spp., there was a significant seasonal effect on arrival times, but no significant year effect (Table 1); arrival occurred during the first week in all seasons of both years, except winter, when arrival took longer in both years (Fig. 1*f*)

Departure weeks

A significant interaction between season and year affected the departure weeks of all taxa (Table 1). Generally, departure from carcasses was more rapid in 2000 than in 1999, and took longer in winter and autumn than in spring and summer. However, there was some variation in this pattern (Fig. 2*a–f*).

Discussion

Seasonal variation in succession and decomposition patterns has been documented (Mann *et al.* 1999; Lopes de Carvalho and Linhares 2001), but it has not been shown previously that inter-year variation in arrival and departure times of carrion taxa can occur. However, these results are unsurprising given that temperature patterns vary annually, and that temperature has a known influence on decomposition rate (Mann *et al.* 1999; Archer 2002). Temperature and rainfall affected the rate of piglet decomposition in this study, and both higher temperature and rainfall increased the rate of mass loss of the carcasses and also the rate of progression through decomposition stages (Archer 2002, 2004). It is also known that population parameters of carrion insects are annually variable (Johnson 1974; Piñero 1997) and this may also affect arrival and departure times of taxa. Population levels of carrion taxa may cycle due to disease, predation pressure, competition or climate variation. Also,

Table 1. Results of ANOVAs performed with season and year as factors on arrival week and departure week of taxa/life-history stages that were collected year-round on 3–5 carcasses

*, $P < 0.05$; **, $P < 0.005$

Taxon	Arrival week Season	 Year	 Season × Year interaction	Departure week Season	 Year	 Season × Year interaction
Coleoptera						
Staphylinidae						
Aleochara brachialis	$F_{3,28} = 36.0$, $n = 36$**	$F_{1,28} = 6.0$, $n = 36$*	$F_{3,28} = 1.2$, $n = 36$	$F_{3,28} = 19.8$, $n = 36$**	$F_{1,28} = 16.2$, $n = 36$**	$F_{3,28} = 11.4$, $n = 36$**
Anotylus sp.	$F_{3,27} = 50.2$, $n = 35$**	$F_{1,27} = 0.9$, $n = 35$	$F_{3,27} = 10.8$, $n = 35$**	$F_{3,27} = 21.5$, $n = 35$**	$F_{1,27} = 13.3$, $n = 35$*	$F_{3,27} = 3.3$, $n = 35$*
Creophilus erythrocephalus	$F_{3,28} = 24.4$, $n = 36$**	$F_{1,28} = 1.3$, $n = 36$	$F_{3,28} = 4.4$, $n = 36$*	$F_{3,28} = 52.5$, $n = 36$**	$F_{1,28} = 30.7$, $n = 36$**	$F_{3,28} = 15.1$, $n = 36$**
Silphidae						
Ptomaphila lacrymosa	$F_{3,25} = 32.8$, $n = 33$**	$F_{1,25} = 7.1$, $n = 33$*	$F_{3,25} = 0.5$, $n = 33$	$F_{3,25} = 15.1$, $n = 33$**	$F_{1,25} = 12.4$, $n = 33$*	$F_{3,25} = 3.1$, $n = 33$*
Leiodidae						
Pseudonemadus spp.	$F_{3,31} = 7.6$, $n = 39$*	$F_{1,31} = 1.0$, $n = 39$*	$F_{3,31} = 3.9$, $n = 39$*	$F_{3,31} = 36.1$, $n = 39$**	$F_{1,31} = 7.2$, $n = 39$*	$F_{3,31} = 3.5$, $n = 39$*
Diptera						
Calliphoridae						
Calliphora larvae	$F_{3,32} = 16$, $n = 40$**	$F_{1,32} = 0.01$, $n = 40$	$F_{3,32} = 0.01$, $n = 40$	$F_{3,32} = 48.5$, $n = 40$**	$F_{1,32} = 16.3$, $n = 40$**	$F_{3,32} = 10.7$, $n = 40$**

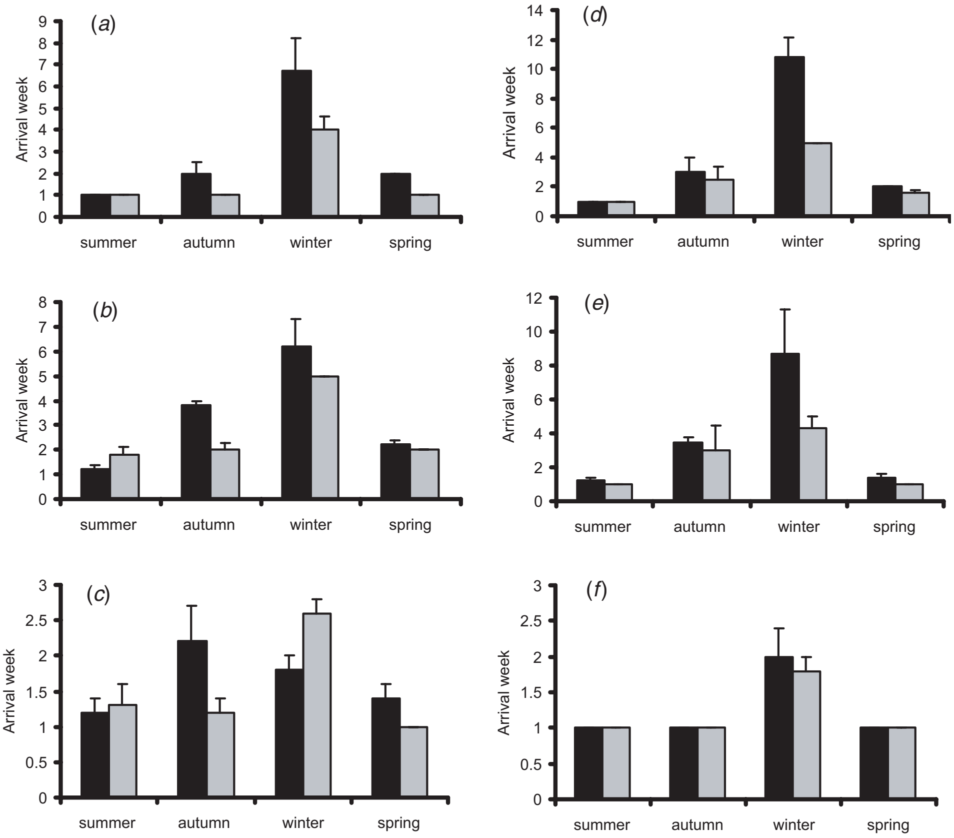

Fig. 1. Arrival weeks of (*a*) *Anotylus* spp., (*b*) *Creophilus erythrocephalus*, (*c*) *Pseudonemadus* spp., (*d*) *Aleochara brachialis*, (*e*) *Ptomaphila lacrymosa* and (*f*) *Calliphora* spp. larvae at each carcass set for 1999 (black bars) and 2000 (grey bars). Values are means + s.e.

animals may be distributed in their environment according to availability of resources (Harper 1982), thus carrion use may vary stochastically in response to the availability of alternative resources, such as other carcasses, dung or fungus.

These results are of great forensic significance: if succession patterns are investigated using only a single set of carcasses, an incomplete picture of succession patterns can emerge. Errors in estimates of succession-based minimum post-mortem intervals can then flow from this if incomplete results based on a single year are extrapolated to future years. Still-born piglets were used here, and it can be expected that there will be differences in the decomposition and succession patterns between these and adult pigs or humans. Therefore these data are not intended for use in forensic cases. Instead, it is hoped that they will inform the design of forensic succession studies that use model carcasses of an appropriate size. These succession studies should be conducted over more than one year to measure potential variation in the arrival and departure times of carrion invertebrates. Additionally, it would be advantageous to place carcasses several times within a season since there can also be potential variation within each season.

Adult blow flies were generally the first insects to arrive at piglets in this study. Carcasses were also rapidly infested with their maggots once the minimum temperature for blow fly oviposition was reached, which was approximately 15°C during this study (Archer

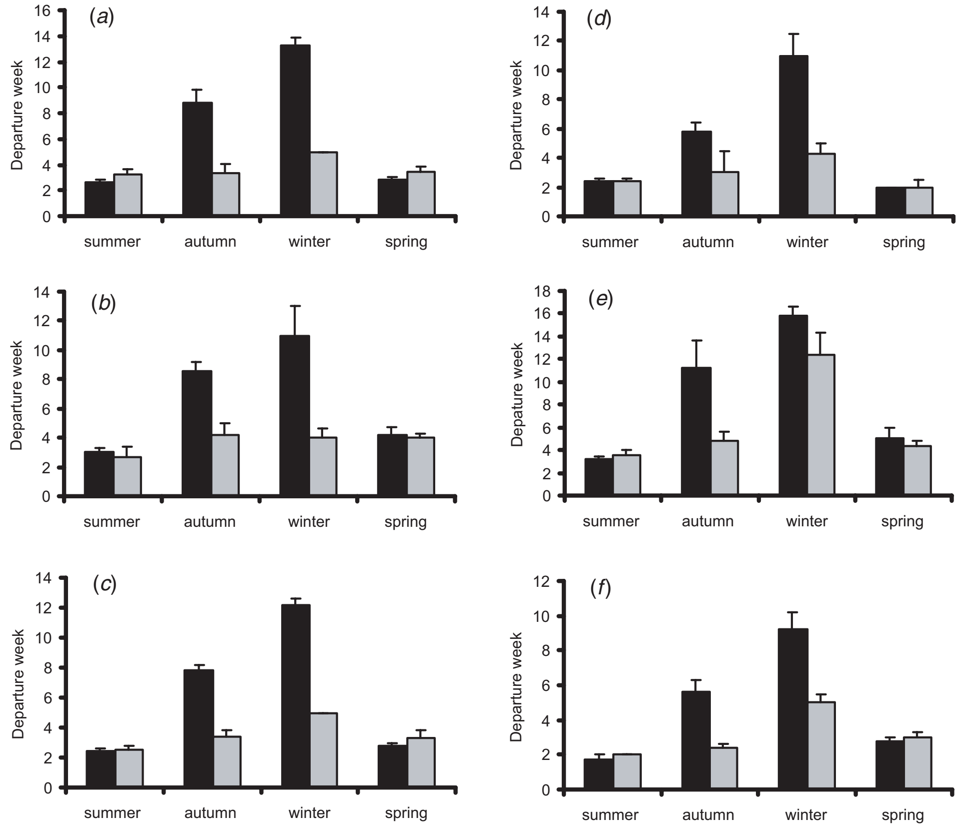

Fig. 2. Departure weeks of (a) *Aleochara brachialis*, (*b*) *Anotylus* spp., (*c*) *Creophilus erythrocephalus*, (*d*) *Ptomaphila lacrymosa*, (*e*) *Pseudonemadus* spp. and (*f*) *Calliphora* spp. larvae at each carcass set for 1999 (black bars) and 2000 (grey bars). Values are means + s.e.

2002). This was reflected in the low level of variation measured in the arrival times of blow flies at carcasses. The reliability of larval infestation by blow flies is important forensically in establishing minimum time since death because, in most environments, an exposed body will almost certainly be colonised within hours under suitable weather conditions (Greenberg 1991). Blow flies are fast and agile in flight, and many common carrion species occur at high population densities (Norris 1964), hence their efficiency at locating carrion.

The appearance time of later-arriving taxa was less predictable than that of blow fly larvae. Two main mechanisms could be involved. First, carcasses decomposed at different rates both between and within sets (Archer 2002). Carrion taxa are generally associated with carcasses at particular decomposition stages (Bornemissza 1957; Payne 1965), and the taxa investigated in this study also displayed these stage affiliations (Archer 2002). Several Victorian blow fly species are attracted to fresh carrion (Fuller 1934; personal observation); therefore, their maggots are the only taxon studied here that will arrive on fresh carcasses. Since all carcasses were placed in the field fresh, this can be expected to govern the predictability of arrival of blow fly maggots. Conversely, carcasses usually diverged in their decomposition stages with time after placement, hence there was variability in the arrival times of other taxa attracted at later decomposition stages, such as initial and active putrefaction. Second, adult taxa examined here are slower and more clumsy in flight than blow flies (personal observation). Therefore, it can be expected that they will not locate carcasses as efficiently as female calliphorids, which may cause additional variation in their arrival times.

There was a significant interaction between season and year for the departure times of all taxa. As with variation in arrival times, variation in departure times may be linked to differences between the decomposition rates of individual piglets, and piglet sets. As decomposition progresses, it can be expected that sources of variation affecting its rate will compound, and therefore differences between the decay states of carcasses will increase. If the departure times of taxa are influenced mainly by the carcass passing out of the suitable decay stage, then the departure times of carrion taxa should vary accordingly. Larvae of *Calliphora* spp. varied in their departure times along with other taxa. Therefore, while their appearance was correlated closely with the placement of carcasses, their disappearance was more variable. The completion of feeding probably influences departure times of blow fly maggots more strongly than does the decay state, and the time taken for maggots to complete feeding will depend critically on temperature (Kamal 1958; Byrd and Butler 1997; Anderson 2000).

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