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The Development of an Insect Succession Model Suitable for Time-Since-Death Statistics

Anne E. Perez

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The Development of an Insect Succession Model Suitable for Time-Since-Death Statistics

Anne E. Perez

Dissertation submitted to the Eberly College of Arts and Sciences
at West Virginia University

in partial fulfillment of the requirements for the degree of

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2014

Keywords: forensic entomology, succession interval, postmortem interval, intercarcass distance,
accumulated degree hours, statistics
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ABSTRACT

The Development of an Insect Succession Model Suitable for Time-Since-Death Statistics

Anne E. Perez

For academic carrion insect succession studies to be applicable to forensic casework, the study must provide statistically defensible postmortem interval (PMI) estimates. Multiple statistical approaches have been used to describe insect succession, but no study to date has been able to determine a confidence interval about a succession based PMI estimate. The ability to reject PMI values, and thereby create a confidence interval is determined by statistical power, of which sample size is a positive correlate. A proposed model established prospective sample sizes for desired levels of statistical power, indicating a target sample size of ~50 carcasses for estimating PMI based on two carrion insect species (LaMotte and Wells 2000). A surrogate for human decomposition has been identified, the domestic pig, yet studies to date have failed to include more than 5 carcasses in a single treatment group. This lack of replication has led to strictly observational findings that are unsuitable for forensic use. As replication of carcasses increases, in an attempt to generate a suitable reference insect succession dataset, additional obstacles are encountered. Constraints of time and space, as well as development of an appropriate species list and sampling procedure are of specific concern. Throughout a century of research concerning insect succession on carrion, issues central to development of large datasets, including the effect of year, fine-scale spatial discrepancies, repeated sampling and intercarcass distance, remain largely unknown.

The use of temperature to describe succession is an additional area of succession research critical to casework application. Because temperature directly affects decomposition rates, insect development rates and insect activity, succession data collected across time and space (potentially experiencing differing temperature histories) may most accurately be described using a physiological time measure as compared to an absolute time measure. Further, succession described in physiological time may be more accurate for application to a case that occurred at a different time and location than the reference dataset. The proposed research aims to create a succession dataset that is suitable for statistical analysis and estimation of time since death by: (1) investigating current sampling methods as well as the assumption that succession is consistent among year and small-scale variation in location, (2) determination of minimum intercarcass distance to ensure independence of carcasses, (3) identifying candidate insect species appropriate for analytical methods prescribed by LaMotte and Wells (2000), and (4) assessing accuracy of PMI estimates using an absolute and physiological time measure.

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Chapter 1: General Introduction

Estimation of Time Since Death and Forensic Entomology

In criminal investigations, the amount of time elapsed since death, commonly referred to as the postmortem interval (PMI), is critical to establishing death case timelines. The appropriate approach to PMI estimation is context dependent. In the early postmortem interval, within ~24 hours (Cantenese et al. 2010), there are reliable techniques that utilize physical and physicochemical processes (Henssge and Madea 2007). During the subsequent portion of the postmortem interval, available techniques are less reliable and result in an estimation that widens as PMI increases. Forensic entomology can potentially be used for extended PMI estimations. While forensic entomology includes any intersection of insects and the law, the most common application is the estimation of PMI using the development of dipteran larvae (maggots) (Catts and Goff 1992). Under optimal circumstances, blow flies (*Calliphoridae*) and flesh flies (*Sarcophagidae*) will arrive at a corpse within minutes after death (Catts 1991, Catts 1992). Among these flies are gravid females that use the corpse as an oviposition site. Assuming that the deceased was not infested with maggots prior to death (myiasis) or maggots originating from a different oviposition site (e.g. adjacent garbage dumpster), the minimum PMI (PMI_{min}) can be determined by comparing attributes (i.e. length, weight) of a maggot collected from the corpse to experimentally generated development data for the same maggot species under similar environmental conditions (Williams 1984).

Forensic Insect Succession

A second, much less common approach, (Nichols et al. 2011) compares the set of insect species/life stages in the corpse to a model of postmortem insect succession (Wells and LaMotte 2010). Simply stated, succession is a change in community composition over time (Drury and Nisbet 1973). Insect succession on carrion is dominated by two orders, Diptera and Coleoptera that use a corpse as: (1) a source of nutrition, (2) a mating site, (3) an oviposition site and (4) hunting ground (by predators and parasitoids). As the pioneering Calliphoridae and Sarcophagidae larvae decrease in number, larvae of other, less studied, taxa including Piophilidae, Sepsidae, Muscidae, Fannidae, and Sphaeroceridae (Byrd and Castner 2010) are collected. The Coleoptera of noted importance are: Silphidae, Staphylinidae, Nitidulidae, Cleridae, Histeridae, Dermestidae, and Trogidae. Adult silphid beetles arrive comparatively early during decomposition (Sharanowski et al. 2008) because the adult beetles are carrion eaters as well as predatory on maggots (Steele 1927). Similarly, their larvae are scavengers and predators of carrion insects (Ratcliff 1996). Opportunistic predators on carrion insects are present throughout decomposition including staphylinids, clerids, and histerids. In general, the latest arrivers are dermestid and trogid beetles (Anderson and VanLaerhoven 1996, Kocarek 2003) that can utilize parts of the corpse other insects cannot digest (e.g. dried skin, hair, cartilage) (Ratcliffe 1980).

A succession model describes the species and development stages present as a function of the amount of time the corpse has been accessible to insects (or other taxa should they be used for this purpose) at that location (Anderson 2010). For brevity, this time period is called the

succession interval (SI) and it is equal to the actual PMI if an investigator is confident the remains were not significantly disturbed and the victim died where the remains were discovered. If there is indication that death and remains discovery occurred at disparate locations, the SI can be equal to the minimum PMI (PMI_{min}). Even though the term SI was not used, the modern forensic use of the concept is outlined by Schoenly et al. (1992). Training data or baseline fauna of a specific geographical region is stored in an occurrence matrix (Figure 1). Each column of the occurrence matrix represents a period of time (days) and each row represents a taxon. Each cell of the matrix contains a one or zero, indicating the presence or absence of a taxon on a corpse through time. A collection of corpse fauna is compared to the baseline occurrence matrix and the lower and upper limit of the SI are defined by the first time and last time all corpse fauna are present together in the training data. This approach assumes no random variation of the succession pattern in a given set of experimental conditions, resulting in an estimate without confidence intervals.

Forensic Science and Expert Testimony

For expert testimony based on forensic entomology research, or any other forensic discipline, to be accepted in the United States federal court system it must be “scientifically valid” and reliable (*Daubert v Merrell Dow Pharmaceuticals* 1993). These are not legal terms, but when scientists apply them to an analytical method, valid is generally synonymous with accurate, and reliable means that the method gives consistent results (Cole 2006). Furthermore, *Daubert* outlined four conditions to help define the acceptability of scientific testimony. Expert testimony must be based on science that: (1) has a testable and tested hypothesis, (2) is peer-reviewed/published, (3)

has known error rates, and (4) is generally accepted in the appropriate scientific community (a condition adopted from a previous decision, *Frye v. United States* 1923).

Even though nearly 20 years have passed since *Daubert*, many methods (including many based on forensic entomology) aimed at estimating PMI don't measure accuracy and precision and therefore fail to meet judicial standards (Madea 2005).

Trends in Forensic Insect Succession Literature

Studies repeatedly affirm that insect succession on carrion is “predictable” because of observed consistency of: (1) the sequence of carrion insect arrival (Anderson and VanLaerhoven 1996, Avila and Goff 1998, Arnaldos et al. 2005, Sharanowski 2008) (2) carrion insect arrival and departure times (Archer 2003), and (3) change in community composition (Catts 1992, Voss et al. 2009) on carrion exposed under similar conditions. Among similar and simultaneously exposed carcasses, observed variation in succession ranges from negligible (Anderson and VanLaerhoven 1996, Hobishack 1997, Leblanc and Strongman 2002) to considerable (Tantawi et al. 1996, Nelder et al. 2009). Minimal replication preventing the development of a forensically applicable succession dataset was noted (Voss et al. 2009) yet inadequate experimental designs persist (Michaud et al. 2012). It is not uncommon to have only one replicate per set of experimental conditions (Horenstein et al. 2010, Anton et al. 2011, Castro et al. 2012). While some authors used a much larger number of carcasses (i.e. 72 (Dillon 1997)), the carcass replicates per set of experimental conditions remained low (i.e. ≤ 5 carcasses (Dillon 1997, Wang et al. 2008)). The within group variance has to be quantified before the variance encountered among groups can be attributed to a studied factor. Another complicating factor is inconsistent presentation of data. Some authors pool data from multiple carcasses into a

“representative” figure of the observed succession for presentation in publication (Tabor et al. 2004, Eberhardt and Elliot 2008, Wang et al. 2008, Kelly et al. 2009). The literature on carrion arthropod succession reveals a lack of replication within single studies, resulting in purely descriptive and anecdotal knowledge of carrion insect succession that is inappropriate for forensic use.

Approaches to Estimate Time Since Death Using Insect Succession

In the past decade there has been an increase in studies attempting to describe variability associated with PMI estimates based on SI (Schoenly et al. 1996) or a portion of SI (i.e. pre-appearance interval (Matuszewski 2011, 2012)). Methods using resampling statistics, specifically jackknifing and bootstrapping, can be used to assign confidence intervals to PMI estimations (Schoenly et al. 1996), but application is limited if the collected data is atypical, an unsubstantiable distinction given universally low replication (≤ 5 carcasses). In addition, the probability of insect occurrence on a corpse can be described using logistic regression (Michaud and Moreau 2009). A possible flaw with these approaches is that they are based on datasets with potentially under-characterized variation because of extremely small sample sizes. Relative error can be used to assess the performance of models describing the pre-appearance interval of coleopteran species (Matuszewski 2011, 2012), but the overall predictive power of the model is not reported.

LaMotte and Wells (2000) proposed a method for attaching a confidence level to a succession-based PMI estimate. Unlike previous applications this model accommodates natural variation in

insect succession in the same set of experimental conditions. The proposed model is a day-by-day contingency table based on the presence/absence of selected categories of species. The model also shows that for a likely level of statistical significance and number of species, an experimental data set must be based on at least 50 carcasses in order to ever be able to exclude a PMI value in casework using the presence/absence combinations of two species. As the number of replicates increases in order to create a dataset suitable for generating a statistically supported estimation of time since death, many challenges arise concerning space and man-hours collecting and identifying specimens. Approaches to overcome these challenges include the determination of a meaningful subset of insect taxa, the effect of pooling data from carrion exposed across time, the effect of repeated sampling on one carcass, the appropriate inter-carcass distance to ensure independence of samples taken from different carcasses placed concurrently, and the appropriate implementation of a time measurement in an insect succession model.

Development of an Insect Succession Dataset

Surrogate Models of Human Decomposition

Human corpses for experiments are difficult to obtain, especially in high numbers, and require specific accredited locations for their outdoor exposure (Mann et al. 1990). Because of these barriers, researchers have chosen to use a range of carcass types including (e.g. guinea pigs (Voss et al. 2009), chickens, (Arnaldos et al. 2004a), cats (Early and Goff 1986), dogs (Reed 1958), goats (Wells and Greenberg 1994a), impalas (Ellison 1990) and alligators (Nelder et al. 2009)). Data collected from non-human decomposition models are used in forensic casework

(Goff and Flynn 1991, Turchetto et al. 2001, Arnaldos et al. 2004b, Olivia and Ravioli 2004), yet relevance of these data is tenuous. For small carcasses (20-100g) there is no effect of carcass size on community structure (Kuusela and Hanski 1982). However, studies with a wider range of carrion sizes have suggested otherwise (Denno and Cothran 1975, Braack 1987, Wells & Greenberg 1994a). There is observed resource partitioning in some carrion fly (Denno and Cothran 1975, Braack 1987) and burying beetle (Scott 1998) species, resulting in preferential colonization of a carcass type. Small carcasses can be dominated by the species that arrives to the corpse first, but as the carcass size increases, the number of species inhabiting the corpse increases (Kneidel 1984, Wells and Greenberg 1994b). Carcass size can effect community composition throughout succession, a species arrival time can be delayed, or duration increased on larger carcass (Wells and Greenberg 1994a).

The animal that has gained the most support as a model for human decomposition is the domestic pig, *Sus scrofa*. Pig carcasses are very attractive as a decomposition model because they are comparatively cheap and easy to obtain in high numbers (Catts and Goff 1992, Goff 1992). There is not an effect of pig carcass weight on the decomposition or fauna attracted within a limited weight range (Hewadikaram and Goff 1991). A 23 Kg pig is suggested as an ideal weight (Catts and Goff 1992) and in the only study to directly compare human and porcine corpses this size was supported as a model for human decomposition (Haskell et al. 2002). Initial comparisons have demonstrated similarity of arthropod taxa collected from human and pig cadavers, but very few comparisons have been made (Carvalho et al. 2000, Schoenly et al. 2007). While current work supporting the use of the domestic pig as a surrogate model for human

decomposition is less than conclusive, preliminary studies for the application of succession studies executed with pigs to forensic cases are promising.

Spatial and Temporal Variation

Investigators often measured the effect of season (Carvalho and Linhares 2001, Centeno et al. 2002, Tabor et al. 2004, Sharanowski et al. 2008, Voss et al. 2009, Matuszewski et al. 2010), geographic location (Anderson 2010), and habitat (Dillon 1997, Richards and Goff 1997, Davis and Goff 2000, Matuszewski et al. 2008, Voss et al. 2009) on succession pattern. Yet year-to-year and fine-scale spatial variation have been largely uninvestigated. The dependence of insect succession on year has important implications for applying baseline data from one year to casework in another. Inferring an effect from published studies is difficult because of insufficient replication (Tabor et al. 2004) and low occurrence resolution due to diffuse sampling schemes (Archer 2003, Voss et al. 2009).

There are observed differences among habitats within the same general geographic location (Davis and Goff 2000, Eberhardt and Elliot 2008, Voss et al. 2009), but it is unclear whether differences in succession arise between distinctly separate, but characteristically similar sites, within the same habitat and geographic location. This is a required area of further study not only due to the space requirements necessary for exposure of ~50 pigs, but also to justify the application of baseline data generated in disparate sites within a broader geographic area. If there is negligible variation in succession attributable to sites in the same geographical location, carcasses within a reasonably tight proximity could be pooled into the same treatment group,

allowing ample space for exposure sites. Considerable variation among characteristically similar exposure sites would suggest a need for an increased number of placement site location characterizations to compose a dataset reflective of inter-site variability.

Effect of Repeated Sampling

Commonly, insect succession studies involve repeated samples of a carcass over time. Destructive sampling, exhaustively characterizing all specimens on carcasses at varying days postmortem, presents major logistical challenges because of the large number of insects involved. Control carcasses, left un-sampled, appear to decay at the same rate and have similar insect activity as sampled carcasses (Anderson and VanLaerhoven 1996, Dillon 1997, Eberhardt and Elliot 2008, Sharanowski et al. 2008, Segura et al. 2009, Segura et al. 2011). Quantitative studies found no effect of repeated sampling on biomass of insect fauna or carcass (De Jong et al. 2011) or overall taxon number in rat carcasses (De Jong and Hoback 2006). However, an a posteriori power analysis of the models used by De Jong and Hoback (2006) revealed inadequate sample sizes to detect significant differences (Michaud and Moreau 2013). Intense repeated sampling of maggots (i.e. 45-55% reduction of larval masses per day) can slow physical decomposition and differentially affect abundance of adult taxa (Michaud and Moreau 2013). While most field studies do not quantify sampling intensity of “representative” samples (subsamples thought to include at least one individual of all taxa in the carcass) (Davis and Goff 2000, Eberhardt and Elliot 2008, Sharanowski et al. 2008) this sampling scheme seems to oversample in comparison to many succession studies (i.e. 50-100 maggots/sample (Tabor et al. 2004, Wang et al. 2008, Kelly et al. 2009). No effect of sampling on decomposition or selected

taxon abundance was observed for intentionally modest maggot sampling schemes (i.e. $\geq 5\%$ reduction of larval masses per day) (Michaud and Moreau 2013), but the effect of repeated sampling of a community, one that includes the collection of adults and immatures, remains uncharacterized.

Effective Intercarcass Distance

For an investigator, both space and time are optimized as the distance between corpses decreases, but increasing the distance between corpses presumably promotes independence of carrion insect activity. For within site placement, 100 m between carcasses was suggested (Schoenly et al. 1991) but commonly corpses are placed ≤ 50 m apart (e.g. Anderson and VanLaerhoven 1996, Avila and Goff 1998, Archer and Elgar 2003, Tabor et al. 2004, Eberhardt and Elliot 2008, Segura et al. 2009, Voss et al. 2009, Michaud and Moreau 2009, and Matuszewski et al. 2010). This distance seems sufficient to prevent cross-contamination by post-feeding larvae (Greenberg 1990, Lewis and Benbow 2011), but given the high mobility of carrion-seeking adult insects (Bishopp and Laake 1921, Gilmour et al. 1946, MacLeod and Donnelly 1963, Mayer and Atzeni 1993, Roslin 2000, Tomlin et al. 2006) a concern is that >1 corpse will “compete” for the same insects. The minimum intercorpse distance to ensure independent carcasses is an urgent area of study, as independence of experimental units is a common assumption of many statistical approaches and is yet to be tested (Michaud et al. 2012)

Determination of Useful Insect Taxa for an Insect Succession Model

Choosing which insects are the best forensic indicators is an arduous task due to the presence of 100s of species (Payne 1965) and 10,000s to 100,000s of individuals on any one corpse (Schoenly et al. 2007, Braak 1984). There are many ways to narrow datasets, including limiting taxa to those that are: sarcosaprophagous (Shahid et al. 2003), used to estimate postmortem intervals in previous studies (Schoenly et al. 2007), present as immatures, if the adults are not directly dependent on carrion (Kulshrestha and Satpathy 2001, Lefebvre and Gaudry 2009), reared from experimental carcasses (Carvalho et al. 2000), nonreoccurring taxa (Schoenly 1992), taxa with comparatively narrow PMI widths (Matuszewski et al. 2010) or by excluding insects that are thought to be collected simply by chance, i.e. not included if only collected $< 2x$ (Lefebvre and Gaudry 2009) or from too few carcasses (Archer 2003). For the intended analysis (LaMotte and Wells 2000) the most basic criteria are collection from practically every carcass exposed and nonreoccurrence.

Insect development and activity are temperature dependent. For carrion insects this is determined by ambient temperature, sunlight, and in some cases metabolic heat generated by a mass of larvae. This creates a problem when selecting a succession model if crime scene temperatures do not match the reference experiment, as they seldom do. One possible way to apply reference data to insects with a different temperature history is to measure development or succession as thermal units rather than absolute time. At intermediate temperatures insect developmental rates often approximate a linear function of temperature. Under those conditions a particular number

of accumulated degree hours (ADH) are required to reach a given developmental stage, and ADH can be calculated from any temperature profile (Higley and Haskell 2010).

Accumulated Degree Hours

ADH is used in agriculture to predict phenology of both plants and their insect pests and has been proposed to estimate the time since death for many non-insect related postmortem processes that are temperature dependent, including: accumulation of chemical markers of decomposition (Vass et al. 2002), RNA degradation in tooth pulp (Young et al. 2013), and the physical decomposition process (Megyesi et al. 2005, Simmons et al. 2010, Michaud and Moreau 2011). Seminal work describing temperature dependent development of forensically relevant flies dates back to the 1950's (Kamal 1958) and use of ADH in forensic entomology to estimate time since death in the United States dates back to 1985 (Greenberg 1985). The use of ADH in estimating time since death from blow fly development is validated by one study (VanLaerhoven 2008) and showed utility in multiple case studies (Greenberg 1985, Lord et al. 1986a, Lord et al. 1986b). There is some support for the use of ADH to predict the occurrence of specific insect species on a corpse (Michaud and Moreau 2009, Matuszewski 2011, 2012), but ADH has not been used in place of absolute time in a succession model. While there are many thermal models an investigator could chose, an ADH model is a logical place to begin the assessment of temperature incorporation into a succession model.

Many avenues of insect succession research are continually investigated, but those areas at the heart of its practical application to forensic work remain largely unstudied. Questions addressed by this work will include (1) the effect of year, fine-scale spatial variation, and repeated sampling on succession, (2) determination of effective intercarcass spacing (3) identification of a manageable and meaningful subset of the carrion insect community and (4) implementation and assessment of ADH performance in an insect succession model. Given all of the above, and the possible impact it may have on future insect succession field studies, I completed a research project that examined insect succession on >50 replicate pig carcasses leading to the development of a reference dataset that can be used to estimate a statistically supported PMI.

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	Day	<u>Postmortem Interval</u>						
		1	2	3	4	5	6	7
Taxon A		0	0	0	0	0	1	1
Taxon B		0	0	1	1	1	1	1
Taxon C		1	1	1	0	0	0	0
Taxon D		0	0	1	1	0	0	0
Taxon E		0	1	1	0	0	0	0
Taxon F		0	0	0	1	1	1	0

Figure 1. Hypothetical occurrence matrix (adapted from Schoenly et al. 1992)

Chapter 2: Spatio-temporal and Sampling Effects on Carrion Insect Communities: Considerations for Generating a Large Forensic Dataset

Abstract

Frequently published descriptions of carrion insect succession in different geographical areas, habitats and seasons highlight large-scale, spatio-temporal variation in insect succession. Forensic investigators aim to use reference datasets that most closely mirror crime scene conditions, but application to casework lags because associated postmortem interval (PMI) estimates lack statistical support. Almost universally, insect succession studies contain low replication (≤ 5 carcasses/experimental conditions), undoubtedly hindering the use of models capable of attaching probabilities to PMI estimates. Larger datasets can be obtained if data are pooled from the same season of different years exposed in characteristically similar locations. This coupled with the common practice of repeatedly sampling the same carcass aids in overcoming constraints of space, time and carcass availability. Effects of interannual variation, variation among characteristically similar sites and repeated sampling on succession remain largely uncharacterized. A common metric of succession comparison among carcasses, the Jaccard similarity index, was used to compare insect communities of 53 repeatedly sampled pig carcasses, placed over 3 years in 3 characteristically similar sites. This study found significant variation in community similarity across years and sites, as well as a negative effect of repeated sampling on succession rate. This understudied spatio-temporal variation and effect of repeatedly sampling must be incorporated or addressed in reference datasets. Succession data collection for forensic application should span multiple years and sites as well as avoid comprehensive species collection, as crime scenes are not equal to experimental exposure sites in space and time.

Introduction

Most commonly forensic entomologists are employed to estimate time since death or the postmortem interval (PMI) (Catts and Goff 1992). There are two general entomological approaches to make this estimate: (1) development and (2) succession. Both are inferential approaches based on the comparison of collections made at a death scene to experimental data generated under similar conditions. Since the earliest arrivers to a corpse (blow flies and flesh flies) use it as an oviposition/larviposition site, physical aspects of the developing maggots (e.g. weight, length) are compared to species-specific laboratory generated development data to provide an estimate of the minimum amount of time elapsed since death or PMI_{min} (Williams 1984). Estimates using succession are based on the comparison of the insect community collected at a crime scene to experimentally generated data of insect community changes on a corpse through time (Wells and LaMotte 2010).

In comparison to development, noted advantages of using succession-based PMI estimations include: (1) encompassing the time elapsed before insect arrival, thereby estimating both a minimum and maximum PMI (given the corpse was not moved) and (2) the previous development of methods to provide statistically supported PMI estimates (LaMotte and Wells 2000). A large difference between development and succession studies, affecting succession study applicability, is the experimental location; developmental data are commonly acquired in a laboratory setting and succession data are obtained in the field. In succession fieldwork there are constraints of time, space, and corpse availability. To deal with these constraints it is common for investigators to: (1) repeatedly sample from the same corpse (De Jong and Hoback 2006,

Michaud et al. 2012, Michaud and Moreau 2013), (2) use non-human carrion subjects (Catts and Goff 1992), and (3) simultaneously expose a limited number of carcasses. But with each of these field-practical solutions comes analytical considerations.

Destructive sampling, exhaustively characterizing all specimens on carcasses at varying days postmortem, presents major logistical challenges because of the large number of insects involved (1,000s of individuals per carcass (Braack 1984, Schoenly et al. 2007) and 100s of taxa (Payne 1965)). Consequently, investigators commonly make repeated samples of a given carcass over time in order to describe succession. Unsampled control carcasses appear to decay at the same rate and have similar insect activity as repeatedly sampled carcasses (Anderson and VanLaerhoven 1996, Dillon 1997, Eberhardt and Elliot 2008, Sharanowski et al. 2008, Segura et al. 2009, Segura et al. 2011). Recent authors highlight the need for quantitative treatment of repeated sampling and demonstrate preliminary support for the current practice; among smaller carcasses (i.e. rat carcasses) there is no effect of repeated sampling on overall taxon number (De Jong and Hoback 2006) and removing $\leq 5\%$ of eggs and larvae present does not affect abundance of many carrion insects (Michaud and Moreau 2013). Using a community similarity index, like the Jaccard similarity index (JSI) is a common method of determining the similarity of succession among carcasses (Schoenly and Reid 1987, Schoenly 1992, Tabor et al. 2004, Shi et al. 2009) because it is based on presence/absence data (as opposed to abundance), and is easily calculable by dividing the total number of taxa shared by two corpses by the total number of taxa collected. The effect of sampling on succession rate can be determined from the JSI values of repeatedly sampled corpses compared to previously unsampled control corpses.

Because human corpses for experiments are difficult to obtain, especially in high numbers, and require specific accredited locations for their outdoor exposure, pig carcasses are commonly used as a decomposition model (Mann et al. 1990). Pig corpses are comparatively cheap, easy to obtain in high numbers, and there is preliminary experimental support for their use as a surrogate for human decomposition (Carvalho et al. 2000, Haskell et al. 2002, and Schoenly et al. 2007). However, the constraints of time, space, and corpse availability have collectively led to low sample sizes among studies using pig carcasses (at most a replication of 5 in an experimental treatment (Dillon 1997, Wang et al. 2008)). This lack of replication has led to only a preliminary understanding of the inherent variation in forensic insect succession.

In the literature, observations of little to no variation among carcasses placed at the same time (Anderson and VanLaerhoven 1996, Hobishack 1997, Leblanc and Strongman 2002), contrast against indications that succession on carcasses in the same conditions can vary widely (Reed 1958, Tantawi et al. 1996). In any analysis the within group variance has to be quantified before the variance encountered among groups can be attributed to a studied factor (e.g. wrappings, sunlight, or location). Variability in succession has not been empirically characterized for pigs exposed to similar conditions leaving many studies purely descriptive and anecdotal, as well as inappropriate for forensic use.

A possible avenue to obtain larger training datasets is to pool data collected from the same or similar locations exposed at similar times (i.e. same season of different years), a practice encouraged by recent authors to increase the inferential power of a succession dataset (Michaud et al. 2013). The effect of geographic location has been widely recognized to affect succession

patterns (Anderson 2001), but it is unclear whether differences in succession (and to what degree) arise between distinctly separate, but characteristically similar sites, within the same geographic location. This is a required area of further study not only due to the space requirements necessary for exposure of replicate pigs, but also to justify the application of baseline data generated in disparate sites within a broader geographic area. A succession model suitable for time since death statistics should be applicable: (1) to a defined area (e.g. an EPA-defined ecoregion) and (2) across years (given the same season in each year). In other words, carrion insect communities at any chosen, characteristically similar, site within the defined area should not significantly differ, regardless of year of exposure. If there are determined effects, this inter-site and/or inter-annual variation needs to be incorporated into a succession model to make it fit for forensic inference.

The aim of this work is to determine the effect of year, distinct yet similar locations and repeated sampling on succession in order to establish future protocols for the generation and application of datasets suitable for forensic use.

Materials and Methods

Data Collection

Daily insect collections from 53 experimental domestic pig carcasses were made during the summer months (June-August) of three consecutive years (2008-2010) in three mature oak woodlots (n=9, 36, and 8 for woodlots 1, 2, and 3, respectively). The order of daily collections was not randomized, but was purposely varied to avoid an effect of time of day on collection

composition (Appendix 1). See Chapter 4 Materials and Methods for additional details concerning the study site and methods of collection and identification.

Effect of Year

An occurrence matrix was created, for each exposed pig, with species as columns, days as rows and each cell containing either a zero (absence) or one (presence). Columns were summed and any species total greater than zero was scored as a one. The resultant row represents the presence/absence of all species collected during the entire 14-day exposure period and was used to calculate the JSI (see introduction) for all pair-wise comparisons of the 53 carcasses exposed. A JSI score ranges from zero to one; the closer the score is to one the more similar the insect communities. A Welch's one-way analysis of variance (ANOVA) ($p < 0.05$) was used to examine the average JSI of pair-wise comparisons of pigs exposed in the same year as compared to the average JSI of pair-wise comparisons of pigs exposed in different years. A Welch's ANOVA was used to test for equal means because this analysis is robust to heterogeneity of variance and unequal sample sizes. All analyses were performed using the statistical package JMP version 11.0.0 (SAS Institute Inc, Cary, North Carolina).

Effect of Placement Site

Pig placement sites were chosen in three woodlots, all within the same ~800 acre farm. The same JSI calculations described to test the effect of year were used to test the effect of woodlot. A one-way ANOVA was used to examine the average JSI of pair-wise comparisons of pigs exposed in

the same woodlot as compared to the average JSI of pair-wise comparisons of pigs exposed in different woodlots. JSI scores were arcsine transformed prior to analysis.

Effect of Repeated Sampling

In order to determine the effect of repeated sampling on succession, four control pigs (two in 2009 and two in 2010) were exposed during the same exposure periods and in the same manner as the 53 other experimental carcasses, but each control pig was sampled only on day seven. Mean JSI was calculated for each of multiple sets of pair-wise comparisons, including; (1) each control pig collection to all day four through eleven collections from experimental pigs, and (2) each experimental pig, day seven collection to all day four through eleven collections from experimental pigs. A range of experimental pig collections from days four through eleven was used for comparison to control pigs to elucidate any possible patterns that would indicate a decrease or increase in succession rate. Within pig comparisons (e.g. pig 1 day 7 collection versus pig 1 day 8 collection) were omitted from the analysis. A separate one-way ANOVA was used to determine the relationship of the average JSI of control comparisons and experimental comparisons. Box-Cox power transformations were necessary for multiple sets of data to meet the assumption of normality. In addition, whenever the assumption of homogeneity of variance was violated a Welch's ANOVA was used. In an attempt to control the family-wise error rate for these eight comparisons the critical p -value was lowered to $p < 0.00625$ ($0.05/8$).

Results and Discussion

Effect of Year

Jaccard similarity indices are significantly higher for pairwise comparisons of carrion insect communities in pig carcasses exposed in the same year than pairwise comparisons of carrion insect communities in pig carcasses exposed in different years ($F=266.6373$, $p<0.0001$) (Figure 1). The effect of year on community composition is likely the result of annual variation in abundance of carrion frequenting species with lower species abundance resulting in lower occurrence/collection. Annual variation in occurrence was observed for at least one forensically important species (case work examples include Greenberg 1985, Oliveira-Costa and Antunes de Mello-Patiu 2004, Huntington et al. 2007), *Cochliomyia macellaria*; in 2009 *C. macellaria* larvae were collected from 65% of exposed carcasses, in contrast to collection from 100% of exposed carcasses in 2010. Data collected solely over one year (2009 or 2010) would have lead to opposite conclusions concerning the relative utility of *C. macellaria* for use in succession casework.

Because it is only the very common species that are suitable for PMI estimation, it may be that species showing annual fluctuations would not directly affect casework analysis. It may be, though, that a fluctuating species could affect occurrence patterns of forensically important species. From this dataset, variation in occurrence rate of incidental species examples included: (1) the scarab beetle, *Dialytes truncatus*, was not collected in 2009, but was collected from 42% of exposed carcasses in 2010 (2) the tenebrionid beetle, *Uloma punctulata* was collected from

30% of carcasses exposed in 2009 but was not collected in 2010. In the absence of exhaustively characterizing how fluctuations in carrion insect abundance and interactions affect succession-based PMI estimations future succession studies should aim to span multiple years to incorporate more variation than single year studies, as suggested by previous authors (Michaud et al. 2012). In addition, succession data collected solely over one year could be inappropriate for extrapolation to casework from a different year. These data also stress the need to continually add to existing succession datasets and repeat field validation tests of succession datasets through time.

Effect of Placement Site

Jaccard similarity indices are also significantly higher for pairwise comparisons of carrion insect communities in pig carcasses exposed in the same woodlot than to pairwise comparisons of carrion insect communities in pig carcasses exposed in different woodlots ($F=76.1681$, $p<0.001$) (Figure 2). This variation occurs among pig placement sites in extremely close proximity (all located on the same ~800 acre farm), most likely closer than the distance between an experimental site used to generate a succession dataset and a crime scene. The effect of woodlot on community similarity is likely a result of spatial variation in abundance of carrion frequenting species. These differences could be a result of woodlot size and edge effects (Diaz et al. 2010), or the patchy distributions of insects that utilize ephemeral resources (Hanski 1987, Blackith and Blackith 1990), local variation in species visiting carrion is not always easily explained (Hanski 1987). A noted difference across woodlots included consistently higher occurrences of multiple rove beetle species in woodlot 1 (*Ontholestes cingulatus* (100%), *Philonthus varians* (100%),

and *Atheta sp.* (100%)) as compared to woodlot 2 (*O. cingulatus* (100%), *P. varians* (67%) and, *Atheta sp.* (66.6%)) and 3 (*O. cingulatus* (57%), *P. varians* (52%) and, *Atheta sp.* (52%)). To ensure that adequate variation is encompassed, forensic insect succession datasets need to include data collected over multiple characteristically similar sites spanning the entire area of desired forensic application.

Effect of Repeated Sampling

On day seven since exposure, repeatedly sampled carrion pairwise community comparisons (experimental) are not significantly more similar to each other, than they are to previously unsampled carrion pairwise community comparisons (control) ($F=6.9075$, $p=0.0087$) (Figure 3). While the comparison of day seven experimental and control collections does not indicate an effect of sampling, day seven experimental and control collection comparisons to preceding and subsequent days, do indicate an effect of sampling on succession. Jaccard similarities are not significantly different but are higher for control insect communities than experimental communities when compared to subsequent experimental day collections (days eight through eleven). Day seven experimental insect communities are significantly more similar to days prior to collection (days four through six) as compared to control insect communities (Figure 3). Contrary to previous observations (Anderson and VanLaerhoven 1996, Dillon 1997, Eberhardt and Elliot 2008, Sharanowski et al. 2008, Segura et al. 2009, Segura et al. 2011) this study suggests that repeated sampling retards succession.

During sampling, an effort was made to collect as few adult insects as possible, as well as minor subsets of immature insect species. The percentage of immature individuals removed was not quantified and it is possible that the same effect of sampling would not be observed with a sampling scheme that collected fewer immatures (Michaud and Moreau 2013). A potential caveat of smaller collections, could be an increase in false negative observations. For the collection of adults, an effect of sampling could be avoided if a small subset of forensically important insects is collected instead of all species present. Further, easily identified species could be recorded instead of collected (Michaud and Moreau 2009). A possible avenue of future research could be the development of a time scale correction factor to adjust for repeated sampling.

This study represents the largest examination of the effect of small-scale spatial variation, interannual variation and repeated sampling, on insect succession. Because crime scene locations are unequal in space and time, to experimental sites, the observed effect of all studied factors suggests that development of forensically relevant reference datasets should include data collected from multiple years, multiple characteristically similar sites, and from carcasses that are minimally sampled.

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Figure 1. Average Jaccard similarity index of within and among year pairwise comparisons

Figure 2. Average Jaccard similarity index of within and among woodlot pairwise comparisons

Figure 3. Pairwise comparisons of control and experimental, day 7 since exposure, insect collections to days 4-11. The control bars represent the average JSI for pairwise comparisons of the four control pigs to all repeatedly sampled pigs on days 4-11. The experimental bars represent the average JSI for pairwise comparisons of all day 7 collections from repeatedly sampled pigs, as well as all pairwise comparisons of the day 7 collections from repeatedly sampled pigs to collections from repeatedly sampled pigs on days 4-6 and 8-11.

Table S1. Collection time and (daily collection order) of 53 experimental pigs

Date	Pig	2008		2009									
		1	2	3	4	5	6	7	8	9	10	11	12
2-Jul													
3-Jul													
4-Jul													
5-Jul													
6-Jul													
7-Jul													
8-Jul													
9-Jul													
10-Jul													
11-Jul		16:20 (1)		12:26 (5)		11:14 (1)	13:00 (7)	11:27 (2)	11:51 (3)	12:20 (4)	12:39 (6)		
12-Jul		16:00 (2)	12:30 (1)	9:30 (1)		11:12 (3)	11:21 (4)	10:30 (2)	12:00 (5)	14:31 (7)	12:16 (6)		
13-Jul		16:30 (2)	12:30 (1)	9:30 (1)		13:07 (7)	10:26 (3)	12:42 (6)	11:34 (5)	9:50 (2)	10:57 (4)		
14-Jul		16:30 (2)	12:10 (1)	9:35 (1)		12:34 (6)	10:34 (3)	13:07 (7)	11:26 (5)	10:05 (2)	10:58 (4)		
15-Jul		16:40 (2)	12:00 (1)	9:53 (1)		12:39 (7)	10:58 (3)	11:02 (4)	12:10 (6)	10:26 (2)	11:41 (5)		
16-Jul		12:00 (1)	16:00 (2)	9:21 (1)		11:37 (6)	10:09 (3)	11:55 (7)	10:56 (5)	9:45 (2)	10:27 (4)		
17-Jul		11:20 (1)	12:20 (2)	11:00 (1)		13:03 (7)	11:43 (3)	12:40 (6)	12:21 (5)	11:26 (2)	12:06 (4)		
18-Jul		13:30 (2)	13:00 (1)	9:02 (2)	10:38 (7)	13:32 (15)	9:46 (4)	13:43 (16)	13:21 (14)	9:26 (3)	12:17 (10)	14:06 (17)	12:31 (11)
19-Jul		13:00 (1)	13:30 (2)	9:01 (2)	10:32 (7)	12:21 (13)	9:35 (4)	13:35 (16)	13:09 (15)	9:19 (3)	11:12 (10)	14:53 (17)	11:37 (11)
20-Jul		14:00 (1)	14:40 (2)	9:10 (2)	10:27 (7)	13:51 (15)	9:42 (4)	14:05 (16)	13:37 (14)	9:24 (3)	12:55 (12)	14:17 (17)	13:19 (13)
21-Jul		11:40 (2)	11:00 (1)	10:57 (2)	12:32 (7)	15:09 (15)	11:36 (4)	15:21 (16)	14:51 (14)	11:12 (3)	14:23 (12)	15:47 (17)	14:39 (13)
22-Jul		12:00 (1)	12:30 (2)	11:08 (2)	13:14 (7)	16:17 (15)	11:45 (4)	16:30 (16)	16:03 (14)	11:21 (3)	14:52 (10)	16:55 (17)	15:09 (11)
23-Jul		14:30 (2)	14:00 (1)	10:45 (2)	12:18 (7)	14:45 (15)	11:20 (4)	15:06 (16)	14:17 (14)	10:55 (3)	13:17 (10)	15:33 (17)	13:25 (11)
24-Jul		11:20 (1)	12:00 (2)	11:39 (2)	12:51 (6)	17:10 (17)	15:35 (14)	16:19 (16)	12:12 (4)	11:57 (3)	14:28 (10)	15:50 (15)	14:40 (11)
25-Jul			12:30 (1)		13:36 (8)							11:43 (3)	15:27 (13)
26-Jul					15:29 (8)							16:48 (12)	14:46 (6)
27-Jul					13:28 (6)							12:01 (4)	15:22 (11)
28-Jul					12:08 (5)							11:55 (4)	15:00 (12)
29-Jul					9:07 (5)							8:52 (4)	11:36 (12)
30-Jul					17:00 (3)							16:46 (2)	18:20 (11)
31-Jul					16:00 (12)							16:15 (13)	13:49 (7)
1-Aug													
2-Aug													
3-Aug													
4-Aug													
5-Aug													
6-Aug													
7-Aug													
8-Aug													
9-Aug													
10-Aug													

Table S1. Continued

Date	Pig	2009										2010	
		13	14	15	16	17	18	19	20	21	22	23	24
2-Jul												14:16 (1)	
3-Jul												11:21 (1)	
4-Jul												9:27 (1)	
5-Jul												12:10 (1)	
6-Jul												13:57 (1)	
7-Jul												15:41 (4)	15:09 (3)
8-Jul												11:14 (1)	12:15 (3)
9-Jul												14:33 (9)	12:08 (4)
10-Jul												11:45 (5)	13:07 (8)
11-Jul												14:10 (8)	12:22 (4)
12-Jul												14:45 (10)	10:57 (4)
13-Jul												15:47 (8)	16:37 (10)
14-Jul												15:50 (16)	10:09 (3)
15-Jul												10:56 (3)	15:53 (12)
16-Jul													10:43 (4)
17-Jul													11:16 (6)
18-Jul		11:51 (8)	13:05 (13)	10:20 (6)	8:47 (1)	10:04 (5)	12:00 (9)	12:50 (12)					15:40 (20)
19-Jul		10:42 (8)	12:30 (14)	10:12 (6)	8:47 (1)	9:55 (5)	10:54 (9)	12:06 (12)					10:09 (4)
20-Jul		11:42 (8)	12:21 (10)	10:14 (6)	8:50 (1)	10:01 (5)	12:37 (11)	11:55 (9)					10:28 (6)
21-Jul		12:51 (8)	13:37 (10)	12:12 (6)	10:34 (1)	11:52 (5)	14:01 (11)	13:08 (9)					
22-Jul		14:19 (8)	15:46 (13)	12:24 (6)	10:47 (1)	12:00 (5)	14:38 (9)	15:31 (12)					
23-Jul		12:42 (8)	14:03 (13)	11:56 (6)	10:21 (1)	11:37 (5)	12:53 (9)	13:42 (12)					
24-Jul		12:27 (5)	13:48 (8)	14:59 (12)	11:20 (1)	15:20 (13)	14:09 (9)	13:27 (7)					
25-Jul		13:58 (9)	14:45 (11)	12:22 (5)	10:31 (1)	12:43 (6)	15:09 (12)	14:25 (10)	10:54 (2)	12:18 (4)	13:10 (7)		
26-Jul		15:09 (7)	13:03 (4)	15:50 (9)	11:26 (1)	16:05 (10)	14:20 (5)	12:32 (3)	12:00 (2)	17:10 (13)	16:30 (11)		
27-Jul		12:20 (5)	16:33 (13)	13:58 (7)	10:30 (1)	14:19 (3)	15:05 (10)	16:02 (12)	10:56 (2)	11:30 (3)	14:42 (9)		
28-Jul		14:40 (9)	15:30 (11)	12:21 (6)	10:24 (1)	12:42 (7)	14:50 (10)	15:20 (13)	11:00 (2)	11:40 (3)	13:09 (8)		
29-Jul		10:11 (9)	10:26 (10)	9:59 (8)	8:06 (1)	9:39 (7)	11:52 (13)	10:41 (11)	8:27 (2)	8:47 (3)	9:27 (6)		
30-Jul		17:26 (7)	17:42 (9)	17:05 (4)	18:37 (12)	17:15 (5)	17:53 (10)	17:33 (8)	18:49 (13)	16:30 (1)	17:21 (6)		
31-Jul		14:35 (9)	13:01 (5)	12:05 (3)	11:04 (1)	15:42 (11)	14:10 (8)	13:25 (6)	11:28 (2)	12:40 (4)	15:05 (10)		
1-Aug									15:17 (3)	14:53 (2)	14:30 (1)		
2-Aug									12:04 (1)	12:30 (2)	12:54 (3)		
3-Aug									13:30 (1)	14:20 (3)	13:55 (2)		
4-Aug									15:30 (1)	16:15 (3)	15:50 (2)		
5-Aug									16:00 (1)	16:26 (2)	16:50 (3)		
6-Aug									17:08 (1)	17:31 (2)	17:54 (3)		
7-Aug									10:55 (2)	10:23 (1)	11:22 (3)		
8-Aug													
9-Aug													
10-Aug													

Table S1. Continued

2010												
Date	25	26	27	28	29	30	31	32	33	34	35	36
Pig												
2-Jul												
3-Jul												
4-Jul												
5-Jul												
6-Jul												
7-Jul	14:40 (2)	14:02 (1)										
8-Jul	11:47 (2)	15:04 (4)										
9-Jul	11:45 (3)	11:04 (2)	14:05 (8)	10:35 (1)	14:55 (10)	13:11 (6)	12:39 (5)	13:38 (7)				
10-Jul	12:42 (7)	13:57 (10)	10:52 (3)	13:33 (9)	11:22 (4)	10:01 (1)	12:09 (6)	10:28 (2)				
11-Jul	11:55 (3)	11:14 (2)	13:45 (7)	10:44 (1)	14:30 (9)	14:55 (10)	12:48 (5)	13:20 (6)				
12-Jul	10:30 (3)	9:47 (2)	13:58 (8)	9:14 (1)	14:23 (9)	11:50 (6)	11:19 (5)	13:28 (7)				
13-Jul	16:16 (9)	10:35 (2)	15:02 (6)	10:05 (1)	15:26 (7)	14:16 (4)	13:45 (3)	14:38 (5)				
14-Jul	10:30 (4)	9:39 (2)	13:57 (12)	9:17 (1)	15:26 (15)	12:17 (8)	11:18 (6)	13:29 (11)	11:47 (7)	10:54 (5)	14:18 (13)	15:00 (14)
15-Jul	16:25 (13)	17:47 (16)	12:06 (6)	17:27 (15)	10:15 (2)	9:42 (1)	15:25 (11)	12:26 (7)	15:03 (10)	16:47 (14)	11:46 (5)	11:18 (4)
16-Jul	10:22 (3)	17:24 (21)	14:42 (14)	17:44 (22)	16:06 (18)	13:10 (10)	11:07 (5)	14:16 (13)	11:29 (6)	9:34 (1)	15:02 (15)	16:26 (19)
17-Jul	10:53 (5)	9:40 (2)	14:05 (13)	9:22 (1)	11:43 (7)	16:10 (19)	17:19 (22)	14:28 (14)	16:56 (21)	10:05 (3)	13:44 (12)	12:10 (8)
18-Jul	15:20 (19)	16:30 (22)	11:24 (8)	16:12 (21)	13:32 (14)	8:48 (1)	14:15 (16)	11:00 (7)	13:52 (15)	14:37 (17)	12:26 (11)	13:11 (13)
19-Jul	9:46 (3)	11:41 (8)	14:39 (16)	11:24 (7)	16:48 (22)	12:15 (9)	10:30 (5)	14:20 (15)	10:52 (6)	9:03 (1)	15:38 (19)	16:22 (21)
20-Jul	10:05 (5)	9:04 (2)	16:36 (19)	8:47 (1)	11:23 (9)	14:32 (15)	10:41 (7)	16:19 (18)	10:57 (8)	9:27 (3)	16:51 (20)	11:43 (10)
21-Jul			12:42 (10)	17:40 (22)	9:15 (1)	15:11 (17)	16:00 (19)	13:10 (11)	15:36 (18)	16:17 (20)	11:36 (7)	9:56 (3)
22-Jul			14:17 (13)	9:00 (1)	17:00 (21)	12:47 (9)	10:35 (4)	13:43 (12)	10:55 (5)	9:22 (2)	15:17 (16)	16:42 (20)
23-Jul									10:49 (3)	9:40 (1)	13:26 (9)	11:55 (5)
24-Jul									16:09 (14)	16:31 (15)	13:36 (8)	11:10 (2)
25-Jul									10:05 (3)	9:10 (1)	12:25 (9)	14:38 (15)
26-Jul									10:08 (3)	9:11 (1)	13:28 (12)	12:49 (10)
27-Jul									10:38 (3)	9:35 (1)	15:03 (11)	16:40 (16)
28-Jul												
29-Jul												
30-Jul												
31-Jul												
1-Aug												
2-Aug												
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5-Aug												
6-Aug												
7-Aug												
8-Aug												
9-Aug												
10-Aug												

Table S1. Continued

		2010											
Date	Pig	37	38	39	40	41	42	43	44	45	46	47	48
2-Jul													
3-Jul													
4-Jul													
5-Jul													
6-Jul													
7-Jul													
8-Jul													
9-Jul													
10-Jul													
11-Jul													
12-Jul													
13-Jul													
14-Jul		12:45 (9)	13:07 (10)										
15-Jul		14:36 (9)	14:15 (8)										
16-Jul		13:33 (11)	13:56 (12)	12:20 (8)	15:46 (17)	10:01 (2)	12:45 (9)	15:24 (16)	16:47 (20)	11:57 (7)			
17-Jul		15:08 (16)	14:48 (15)	15:29 (17)	13:02 (10)	10:29 (4)	15:49 (18)	13:23 (11)	12:38 (9)	16:31 (20)			
18-Jul		10:16 (5)	10:38 (6)	9:30 (3)	11:44 (9)	14:59 (18)	9:08 (2)	12:04 (10)	12:50 (12)	9:52 (4)			
19-Jul		13:37 (13)	13:58(14)	12:56 (11)	14:58 (17)	9:23 (2)	12:36 (10)	15:17 (18)	16:02 (20)	13:18 (12)			
20-Jul		14:51 (16)	15:07 (17)	13:55 (13)	17:27 (22)	9:48 (4)	14:12 (14)	17:08 (21)	12:00 (11)	13:05 (12)			
21-Jul		13:47 (13)	13:27 (12)	14:29 (15)	12:16 (9)	16:56 (21)	14:51 (16)	11:56 (8)	10:38 (5)	14:09 (14)	9:33 (2)	10:17 (4)	11:00 (6)
22-Jul		13:06 (10)	13:25 (11)	12:05 (7)	14:36 (14)	9:44 (3)	12:27 (8)	14:57 (15)	16:20 (19)	11:40 (6)	17:16 (22)	15:58 (18)	15:37 (17)
23-Jul		15:00 (13)	14:32 (12)	15:40 (15)	14:10 (11)	10:15 (2)	16:00 (16)	13:49 (10)	12:41 (7)	15:21 (14)	11:32 (4)	12:17 (6)	13:02 (8)
24-Jul		14:36 (10)	14:15 (9)	15:19 (12)	12:50 (6)	16:47 (16)	15:40 (13)	13:14 (7)	11:57 (4)	14:58 (11)	10:45 (1)	11:32 (3)	12:18 (5)
25-Jul		11:34 (7)	11:53 (8)	10:48 (5)	13:10 (11)	9:40 (2)	11:11 (6)	12:45 (10)	13:58 (13)	10:27 (4)	15:02 (16)	13:32 (12)	14:18 (14)
26-Jul		14:53 (16)	14:33 (15)	10:29 (4)	14:08 (14)	9:30 (2)	10:53 (5)	13:48 (13)	12:05 (8)	11:13 (6)	13:08 (11)	12:25 (9)	11:35 (7)
27-Jul		12:09 (7)	12:38 (8)	11:11 (4)	13:40 (9)	9:55 (2)	11:30 (5)	15:18 (12)	16:09 (14)	11:50 (6)	16:24 (15)	14:33 (10)	15:32 (13)
28-Jul				16:08 (13)	15:04 (8)	10:48 (2)	16:19 (14)	15:14 (9)	11:46 (5)	15:56 (12)	11:13 (3)	11:30 (4)	12:00 (6)
29-Jul				11:09 (5)	12:19 (10)	10:24 (2)	11:20 (6)	12:08 (9)	13:12 (14)	10:57 (4)	12:46 (12)	12:57 (13)	13:22 (15)
30-Jul											11:59 (7)	12:11 (8)	11:47 (6)
31-Jul											17:15 (6)	17:26 (7)	17:38 (8)
1-Aug											14:51 (7)	15:07 (8)	14:35 (6)
2-Aug											13:43 (8)	13:26 (7)	13:00 (6)
3-Aug											16:35 (8)	16:18 (7)	16:02 (6)
4-Aug													
5-Aug													
6-Aug													
7-Aug													
8-Aug													
9-Aug													
10-Aug													

Table S1. Continued

		2010				
Date	Pig	49	50	51	52	53
2-Jul						
3-Jul						
4-Jul						
5-Jul						
6-Jul						
7-Jul						
8-Jul						
9-Jul						
10-Jul						
11-Jul						
12-Jul						
13-Jul						
14-Jul						
15-Jul						
16-Jul						
17-Jul						
18-Jul						
19-Jul						
20-Jul						
21-Jul						
22-Jul						
23-Jul						
24-Jul						
25-Jul						
26-Jul						
27-Jul						
28-Jul		16:32 (15)	15:26 (10)	10:28 (1)	14:49 (7)	15:43 (11)
29-Jul		10:39 (3)	11:51 (8)	10:05 (1)	12:30 (11)	11:34 (7)
30-Jul		10:40 (2)	11:11 (4)	10:16 (1)	11:30 (5)	10:55 (3)
31-Jul		16:15 (2)	16:46 (4)	15:55 (1)	17:01 (5)	16:31 (3)
1-Aug		13:19 (2)	13:58 (4)	12:54 (1)	14:17 (5)	13:39 (3)
2-Aug		11:38 (2)	12:22 (4)	11:14 (1)	12:42 (5)	12:01 (3)
3-Aug		14:40 (2)	15:24 (4)	14:19 (1)	15:43 (5)	15:04 (3)
4-Aug		15:39 (4)	14:52 (2)	16:05 (5)	15:16 (3)	14:25 (1)
5-Aug		12:58 (2)	13:55 (5)	12:28 (1)	13:30 (4)	13:16 (3)
6-Aug		9:57 (2)	10:51 (4)	9:28 (1)	10:27 (3)	11:14 (5)
7-Aug		9:41 (2)	10:39 (4)	9:18 (1)	11:05 (5)	10:11 (3)
8-Aug		15:38 (2)	16:14 (4)	15:14 (1)	16:37 (5)	15:56 (3)
9-Aug		11:37 (4)	10:54 (2)	12:00 (5)	10:21 (1)	11:16 (3)
10-Aug		11:30 (2)	12:37 (5)	11:14 (1)	12:06 (4)	11:52 (3)

Chapter 3: Toward the Determination of Minimum Intercarcass Distance for Forensic Entomology Field Methodologies

Abstract

Forensic entomology field researchers commonly place experimental corpses ≤ 50 m apart. Experimental observations indicate that this intercarcass distance sufficiently prevents cross contamination by crawling larvae, but seems insufficient for highly mobile adult insects. Minimum intercarcass distance should ensure independence of corpses, an assumption of common statistical analyses. For 32 domestic pig carcasses exposed over two years (at a minimum intercarcass distance of 30 m), possible relationships between: (1) average distance to another carcass and aspects of the succession interval of forensically important species and (2) intercarcass distance and community similarity indices were determined with regression analyses. In addition, minimum intercarcass distance was investigated through semivariogram analyses of aspects of the succession interval as well as community similarity indices. Minimum succession interval was the only variable that displayed a consistent, and negative, relationship to distance. Semivariogram analysis suggests that commonly used intercarcass distances are satisfactory to ensure independence of carcasses.

Introduction

Insect succession on carrion can be used to estimate the postmortem interval (PMI) (Catts 1992). Forensic insect succession models describe the insect species and life stages present on a corpse over time as a function of multiple ecological factors (Anderson 2010). This period of exposure

to insects, termed the succession interval (SI) accounts for the time elapsed prior to presence and can therefore be used to estimate the minimum and maximum SI ($SI_{\min}$ and $SI_{\max}$). Forensic statistical methods should allow for probabilities to be associated with PMI estimations (National Research Council 2009). The only statistical method available for analyzing occurrence data from replicate carcasses (LaMotte and Wells 2000) suggests that $\approx \geq 20$ replicate corpses are necessary to be able to reject an SI value and thereby place a confidence interval about an SI estimate. Generating large succession datasets using human corpses is difficult because there are limited locations authorized for their outdoor exposure (Mann et al. 1990) as well as paucity in availability. As a result, succession researchers have most often used the domestic pig, *Sus scrofa*, as a surrogate for human corpses (Matuszewski et al. 2008, Sharanowski et al. 2008, Horenstein et al. 2010, Anton et al. 2011, Ortloff et al. 2012, Pastula and Merritt 2013), with initial support from the few studies to directly compare insect fauna and decomposition of pig (~23 kg) and human corpses (Carvalho et al. 2000, Schoenly et al. 2007).

Even when based on pig carcasses, generation of large succession datasets characterizing ecologically distinct regions presents tremendous practical difficulties (Matuszewski 2011, 2012) due to time and space constraints. For an investigator, both space and time are optimized as the distance between corpses decreases. But analytically, the spacing should ensure independence of corpses, the basis of true replication for statistical analysis. For within site placement, 100 m between carcasses is suggested (Schoenly et al. 1991) but commonly corpses are placed $\sim \leq 50$ m apart (e.g. Anderson and VanLaerhoven 1996, Avila and Goff 1998, Archer and Elgar 2003, Tabor et al. 2004, Eberhardt and Elliot 2008, Segura et al. 2009, Voss et al. 2009, Michaud and Moreau 2009, and Matuszewski et al. 2010). This distance seems sufficient to avoid cross-

contamination by crawling larvae (Greenberg 1990, Lewis and Benbow 2011), but given the high mobility of carrion-seeking adult insects (Bishopp and Laake 1921, Gilmour et al. 1946, MacLeod and Donnelly 1963, and Mayer and Atzeni 1993, Roslin 2000, Tomlin et al. 2006) a concern is that >1 corpse will “compete” for the same insects.

Michaud et al. (2012) suggested possible analytical approaches to determine minimum intercarcass distance for such experiments. These included the use of a geostatistical analysis, with a history of ecological use (Carroll and Pearson 1998, Crist 1998, King et al. 2010). The semivariogram tests for spatial autocorrelation of carcasses separated by varying distances. Generally, the observation that a variable measured at two distances are more, or less, similar than would be expected from a random pair of samples indicates positive, or negative, spatial autocorrelation, respectively (Legendre 1993). A semivariogram depicts spatial autocorrelation by fitting a model to a plot of the average variance of the measured variable as a function of pairwise distance (Figure 1). Each data point represents the variance of data in a specified lag class. Lag classes define the distance intervals in which pairs of observations are grouped. The range of the semivariogram is the distance where variance peaks or flattens and represents the minimum distance for which two observations are not spatially autocorrelated (Figure 1).

Presumably, if there is an effect of intercarcass distance it might be detected in terms of $SI_{\min}$, SI width, and community similarity indices. However, the aspects of carrion insect biology that might cause non-independence of carcasses suggest potential contradictory effects of intercarcass distance. Two corpses might “compete” for the same adult insects (Shahid et al. 2003), a process that would presumably retard succession, causing an increase in $SI_{\min}$ and/or width when

intercarcass distance is reduced. The nearby presence of a carcass installed earlier might “prime” the carrion insect population (Conley 1982), that is, increase the local pool of colonizing insects. This would result in a decreased $SI_{\min}$ for latter-placed carcasses. Another plausible mechanism for non-independence could be simply very local differences in carrion insect activity rather than any “interaction” of carcasses. This mechanism might produce a negative relationship between intercarcass distance and an index of community similarity of forensically important species.

The Jaccard similarity index (JSI) is commonly used in forensically focused studies to measure carrion insect community similarity across carcasses (e.g. Schoenly and Reid 1987, Schoenly 1992, Tabor et al. 2004, 2005, De Jong and Hoback 2006, Shi et al. 2009, Bunchu et al. 2012) and is attractive for use because it is based on presence/absence data, is easily calculable by dividing the total number of taxa shared by two corpses by the total number of taxa collected, and is suggested for use in comparison to other occurrence-based approaches (Janson and Vegelius 1981). The JSI ranges from zero to one, representing no species in common to complete overlap of species from pairwise carcasses. Less often, forensic entomology studies use abundance-based similarity indices, like Morisita’s metric (Schoenly and Hall 2002), likely because abundance measures are not always possible given common sampling schemes. An abundance based similarity index could identify differences/similarities not able to be detected with occurrence-based approaches (Chao et al. 2008). Morisita’s metric is advocated in comparison to other abundance-based indices because of demonstrated attractive statistical qualities (i.e. robust to effects of sample size and diversity (Wolda 1981)). Morista’s metric (C_D) (Morisita 1959) is :

$$C_D = \frac{2 \sum_{i=1}^S x_i y_i}{(D_x + D_y)XY}$$

where x_i and y_i are the number of times a species i occurs in the total sample X and Y, respectively. The product of x_i and y_i are summed for each species (total S) in either of the two samples. Simpson's diversity index (Simpson 1949) for each of sample D_x and D_y is calculated with the formula below.

$$D_x = \frac{\sum_{i=1}^S x_i (x_i - 1)}{X(X - 1)}$$

The Simpson's diversity index gives the probability that two random individuals drawn from the same sample belong to the same species ranging from zero to one. The closer the value is to one, the lower the species diversity. Morisita's metric ranges from zero to slightly over one: a zero value represents no species overlap, a value of ~1 indicates complete overlap of species occurring in the same proportions.

This study aims to estimate the minimum effective distance to ensure independent carcasses for forensic insect succession studies. Because intercarcass distance plausibly affects aspects of both carrion insect population and community dynamics, four variables (SI_{min} , SI length, and two community similarity indices) were utilized to: (1) test for a relationship to intercarcass distance through regression analysis and (2) determine minimum effect distance through semivariogram analysis. These relationships and distances were investigated for the same season of two different years with the expectation of similar trends/values across years.

Material and Methods

Data Collection

A total of 51 pigs were each exposed for a 14-day period during the summer months (July and August) of two consecutive years (20 pigs exposed in 2009, 31 pigs exposed in 2010) in Rensselaer, Indiana, USA. Because of limited pig availability, few of the pigs shared exactly the same exposure period. Those pigs sharing the exact same exposure period were considered to be in the same batch. Only pigs with similar distances across years were included in further analyses, resulting in 16 replicates per year (Figure 2). The placement site of each pig was located in the full shade of mature oak woodlots fragmented across corn and soybean fields. No two pigs shared the exact same pig placement site (across years) and each pig placement site within year was separated by at least 30 meters. Placement sites and the distance between placement sites were determined and recorded with an eTrex Summit HC handheld GPS receiver (Garmin Ltd, Olathe, Kansas). Pairwise distance was determined by plotting pig placement sites on a Cartesian coordinate system and calculating the Euclidean distance separating each pair of points.

Each pig weighed $16 \text{ kg} \pm 2.5 \text{ kg}$ and was protected from large vertebrate scavengers by a hardware cloth cage staked to the ground. Daily collections, for each of the 14 days consisted of a sweep net sample (eight rapid passes of a net directly over the body) and three handpicked samples; (1) maggots preserved on site, (2) maggots reared to adulthood for ease of identification, and (3) all other adult and immature insects in the immediate vicinity of the

corpse. To ensure consistent sampling across pig carcasses, all handpicked samples were collected in exactly 20 minutes. Handpicked and sweepnet samples have been documented to yield the highest percentage of forensically important insects (Schoenly et al. 2007).

Similarity Indices

An occurrence matrix was created, for each exposed pig, with species as columns, days as rows and each cell containing either a zero (absence) or one (presence). Columns were summed and any species total greater than zero was scored as a one. The resultant row represents the presence/absence of all species collected during the entire 14-day exposure period and was used to calculate the JSI (see Introduction) for all pair-wise comparisons of the 16 carcasses exposed in each year. Daily abundance counts for only adult dipteran species collected by sweepnet were summed for the entire exposure period and used to calculate Morisita's metric (see Introduction) for all pair-wise comparisons of the 16 carcasses exposed in each year.

Regression Analyses

To visualize the relationship between intercarcass distance and community similarity, four regression analyses and t-tests of zero slope were performed for each year of placement and both similarity indices. For each of eight species, previously determined to be abundant and nonreoccurring (*Necrodes surinamensis* (adult), *Necrophila americana* (larva), *Creophilus maxillosus* (adult), *Necrobia ruficollis* (adult), *Fannia scalaris* (larva), *Cochliomyia macellaria* (larva), *Phormia regina* (larvae), and *Lucillia illustris* (larva)) (Chapter 4), three separate

regression analyses and t-tests of slope were performed to determine the relationship of intercarcass distance (recorded for each pig carcass as average distance away from any other carcass placed in the same year) to $SI_{\min}$, SI width. In the same manner, a possible effect of priming was explored by determining the relationship of $SI_{\min}$ to order of carcass exposure (relation to first batch). For the 16 pigs per year determined to have similar placement site distances, in 2009, there were three different batches and in 2010 there were five different batches (recorded as 1-5). Because of the similarity of time elapsed between placements, the three batches of 2009 were recorded as one, three, and five. All regression analyses and t-tests of regression line slope were performed in Excel 2007 (Microsoft Corporation, Redmond, Washington). In an attempt to control the family-wise error rate the critical p -value was lowered to $p < 0.0021$ ($0.05/24$).

Semivariogram Analyses

Jaccard and Morisita's metric as well as the $SI_{\min}$ of *P. regina* and *L. illustris* were analyzed as Z-values for each of the two years. The $SI_{\min}$ semivariograms were restricted to two species, *P. regina* and *L. illustris*, because they were present on all carcasses included in the study resulting in the use of the same lag classes and number of lag classes. All metrics were log transformed prior to analysis. Isotropic semivariogram analyses were performed using the geostatistics software, GS+ Version 9 (Gamma Design Software, LLC, Plainwell, Michigan). Model type (linear, spherical, Gaussian, or exponential) varied by analysis and was determined by the GS+ software according to best fit. Custom lag classes were used for each year so that each lag class represented at least 30 pairs of points (Journal and Huijbregts 1987), resulting in a total of 6 lag classes. The active lag distance was set to the largest distance between any two exposed pigs in

order to include all measured comparisons. Semivariogram models can deteriorate when maximum distances are included because of fewer observations, but this was not observed as the active lag distance was manipulated between 80% (GS+ default setting) and 100% of the largest distance.

Results and Discussion

Regression Analyses

All four regression analyses, resulting from two years of data and two different community similarity indices, failed to detect a relationship of community similarity and intercarcass distance (Figure 3, Table 1). There was no consistent effect (positive or negative) of average distance to other carcasses on SI width or of batch on SI_{min} (Figure 4). However, a negative relationship between average distance to other carcasses and SI_{min} was observed for all 8 species analyzed (Figure 4, Table 2). Although, this relationship was not statistically significant, the consistent effect of distance on SI_{min} would be an intriguing avenue of future research in investigations of minimum effective distance.

From an ecological perspective, dependence of community or population characteristics (e.g. abundance (Michaud et al. 2012) or similarity indices) on intercarcass distance can serve as an indication of community and metacommunity dynamics. From a purely forensic standpoint, an effect on community is of interest because of the potential influence on aspects of the SI of forensically important insect species, which can be measured directly. Average distance to other carcasses potentially results in training data underestimating PMI_{min} . Given the large sample

sizes necessary to execute the previously proposed methodological approaches (Michaud et al. 2012) (≥ 16 carcasses) less labor intensive data collection and variable calculation are valuable conditions for future investigators. Practically, the focus of forensic investigators should be independence of a metric (like $SI_{\min}$) that directly affects PMI estimates and is the likely variable subject to statistical analysis.

Semivariogram Analyses

Many semivariogram analyses were best described by horizontal, linear best fit lines, indicating the independence of distance and the measured variable at sampling intervals $\geq 30\text{m}$, resulting in uninformative range values ($SI_{\min}$ of *L. illustris* both years and Morisita Index 2010) (Figures 5 and 6). Although half of the semivariograms were best described by spherical and exponential models (resembling “textbook” semivariograms (Figure 1) and providing ranges spanning 25.5 to 75.8 m), r^2 values were extremely low (Figures 5 and 6). In addition, these variograms contained no discernible correlation between distance and dissimilarity at low lag values (Figures 5 and 6). In sum, the semivariogram analyses suggest commonly used experimental distances are sufficient to ensure independence of measurements taken from experimentally exposed carcasses.

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Figure 1. A hypothetical semivariogram (modified from figure 2 of Karl and Maurer 2010)

Figure 2. Frequency distribution of intercarcass distance for years a) 2009 and b) 2010

Figure 3. The absence of a relationship between insect community similarity index and distance of pair-wise comparisons of pig carcasses. See Table 1 for associated r^2 , linear equations, and p -values.

Figure 4. Relationship of SI_{min} to distance and batch, as well as SI_{width} to distance for 8 common and nonreoccurring species. See Table 2 for associated r^2 , linear equations, and p -values.

Figure 5. Community similarity (measured as either a Jaccard similarity index or a Morisita's index) semivariograms for two years of data. Range distance and r^2 for each semivariogram: Jaccard 2009, $r^2=0.000$, range=41.1; Jaccard 2010, $r^2=0.105$, range=74.3; Morisita 2009, $r^2=0.000$, range=25.5; Morisita 2010, $r^2=0.008$, range=292.4.

Figure 6. SI_{min} semivariograms for two selected forensically important species for two years of data. Range distance and r^2 for each semivariogram: *P. regina* 2009 $r^2=0.076$, range=75.8, *P. regina* 2010 $r^2=0.112$, range=305.04; *L. illustris* 2009 $r^2=0.445$, range=264.23 *L. illustris* 2010 $r^2=0.000$, range= 305.0.

Table 1. The coefficients of determination, equations and p -values of $H_0:\beta_1=0$ for community similarity indices vs. average pairwise distance

	Total		2009		2010	
	Jaccard	Morisita	Jaccard	Morisita	Jaccard	Morisita
r^2	0.0066	0.0000001	0.0056	0.0264	0.0026	0.0013
p -value	0.2113	0.9851	0.4068	0.0714	0.5793	0.6968

Table 2. The coefficients of determination and p -values of $H_0:\beta_1=0$ for $SI_{\min}$ vs. distance, $SI_{\min}$ vs. carcass exposure order (Priming) and SI width vs. distance

		<i>N. surinamensis</i>	<i>N. americana</i>	<i>C. maxillosus</i>	<i>N. ruficollis</i>	<i>F. scalaris</i>	<i>C. macellaria</i>	<i>P. regina</i>	<i>L. illustris</i>
$SI_{\min}$	r^2	0.2389	0.0315	0.0435	0.1471	0.0630	0.1246	0.2381	0.0884
	p	0.0083	0.3391	0.2519	0.0483	0.1731	0.0906	0.0046	0.0983
Priming	r^2	0.0039	0.0052	0.1434	0.0101	0.0025	0.0048	0.0068	0.0059
	p	0.7530	0.7007	0.0326	0.6187	0.7881	0.7488	0.6538	0.6765
SI Width	r^2	0.0247	0.0470	0.0003	0.2163	0.0051	0.0001	0.0348	0.0305
	p	0.4242	0.2412	0.9344	0.0145	0.7036	0.9914	0.3068	0.3391

Chapter 4: Evaluating the utility of hexapod species for calculating a confidence interval about a succession based postmortem interval estimate*

Abstract

Carrion insect succession patterns have long been used to estimate the postmortem interval (PMI) during a death investigation. However, no published carrion succession study included sufficient replication to calculate a confidence interval about a PMI estimate based on occurrence data. We exposed 53 pig carcasses (16 ± 2.5 kg), near the likely minimum needed for such statistical analysis for two taxa, at a site in northcentral Indiana, USA, over three consecutive summer seasons. Insects and Collembola were sampled daily from each carcass for a total of 14 days, by which time each was skeletonized. A life stage of a given species was judged to be potentially useful for succession-based PMI estimation if it was 1) nonreoccurring (one period of presence on a corpse), and 2) found in a sufficiently large proportion of carcasses to support a PMI confidence interval. For this dataset that proportion threshold is 45/53.

Of the 266 species collected and identified, the larval forms of *Necrophila americana*, *Fannia scalaris*, *Cochliomyia macellaria*, *Phormia regina*, and *Lucilia illustris* satisfied these two criteria. Adults of *Creophilus maxillosus*, *Necrobia ruficollis*, and *Necrodes surinamensis* were common and showed only a few, single day gaps in occurrence. *C. maxillosus* adults, *P. regina*, and *L. illustris* (both as larvae) displayed exceptional forensic utility in that they were observed

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on every carcass. Analyses of occurrence matrices for each of these species alone or in two-species combinations suggest that a failure to record a taxon is a less reliable observation (i.e. by producing a false negative) than is a record of presence. Also, inverse prediction of the duration of insect succession, which we term the succession interval (SI), was more precise when based on two species rather than one species.

Introduction

The succession of insect species on a corpse is a biological clock that can be used to predict some portion of the time since death [1]. Specifically, a succession model describes the species and development stages present as a function of the amount of time the corpse has been accessible to insects (or other taxa should they be used for this purpose) at that location [2]. For brevity we designate this time period the succession interval (SI). If an investigator is confident the victim died where the remains were discovered and was not subsequently disturbed in a way that altered succession, the SI equals the actual postmortem interval (PMI). If death occurred elsewhere, as is often the case [3], it may be better to think of SI as a minimum postmortem interval (PMI_{min}).

Carrion insect succession has been investigated for many years [4-8] and the biology of carrion frequenting insects has been characterized in habitats across the world [2]. However, many published studies had a descriptive ecological rather than forensic focus and common data presentation methods, such as an aggregated general succession pattern rather than what occurred in any individual corpse, are not very useful for SI estimation in casework [9].

Another common practice of dubious forensic entomological utility has been the division of corpse decomposition into discrete categorical stages characterized by general appearance (e.g. fresh, bloat, etc.). Although these stages reflect real phenomena, such as the bloating of a corpse, they are subjectively defined and incorrectly thought to correspond to shifts in the insect fauna [10].

Schoenly et al. [11] proposed an explicit analytical framework for SI estimation and developed software for data storage and analysis. Their succession model is “baseline” data recorded as an occurrence matrix showing presence or absence (indicated by a 1 or a 0, respectively) of species/stages for each known SI value. Faced with a corpse of unknown SI, the set of insects on the corpse is compared to a model considered to be appropriate for the death scene. Model SI values with the same set of species as observed on the corpse are considered plausible for that particular case (see Fig. 1 of Schoenly et al. [12]).

Schoenly et al. [11] stated that taxa that “leave and reappear” during the course of succession can “distort” a PMI estimate, and recently this property of a taxon has been used to empirically assess the utility of insect species for this application [13]. It seems to us that the real problem with such a reoccurring taxon [14] is not the fact that it could yield an SI estimate that is not a continuous interval per se. Instead, gaps in occurrence may be a sign of unpredictability, either in actual occurrence or because individuals on a corpse are more likely to be missed by the collector compared to other taxa. The possibility of a false negative observation highlights the fact that specimen collection protocol is as much an experimental condition as are the environmental setting, SI, etc, for any carrion insect occurrence reference data [14-16].

LaMotte and Wells [17] developed statistical methods for inverse prediction of SI from categorical (e.g. occurrence) data. As with Schoenly et al. [11] the succession model of LaMotte and Wells [17] is an occurrence matrix with column values for each observed SI, but a cell contains a count of the number of training corpses observed to have a particular set of species rather than showing the presence or absence of a single species. A p-value is calculated for each possible SI for a corpse in a death investigation, and those SI values that are not rejected define a confidence interval. This requires “training” data from replicated experimental corpses. Without sufficient replication when producing training data the statistical test cannot reject any potential SI value and the casework situation is, in practical terms, as if every carrion insect species were present at all times during decomposition.

Probably more than 50 experimental corpses for a given set of conditions are needed for an investigator to ever be able to reject a possible SI value at typical levels of statistical significance when using ≥ 2 taxa (see Table 3 in LaMotte and Wells [17]). Furthermore, for such an SI estimation the species used would have to be quite common, i.e. observed at one time or another on almost all of the training corpses (see Discussion). Some forensic entomology researchers have exposed many more than 50 pig carcasses, for example one of us (NHH) has employed more than 600 for experiments and workshops (unpublished). However, despite sometimes including a high total over long periods, published succession datasets were based on carcasses installed < 50 at a time and under a variety of environmental conditions (e.g. Dillon [18]), and therefore they do not represent sufficient replication for the statistical methods of LaMotte and Wells [17].

One obstacle to the generation of sufficient replication in succession datasets is that such research can be very labor intensive. A single roughly human-size carcass can yield >100 species [16,19] and 10,000s of individuals [16,20]. If an investigator were to target only a small subset of the carrion insect fauna, one composed of the species and life stages most valuable for SI estimation, then the smaller amount of time spent per carcass should allow much greater replication in succession experiments. The purpose of this study was to identify carrion insect species that are useful for SI estimation according to the criteria of nonreoccurrence and commonness, and within the context of a training dataset large enough for the methods of LaMotte and Wells [17]. The training data were obtained from domestic pig carcasses, a typical experimental surrogate for a human corpse [16, 21, 22].

Materials and Methods

A total of 53 pigs (16 ± 2.5 kg) were exposed during the summer months (June-August) of three consecutive years (2 during 2008, 20 during 2009, 31 during 2010) in mature oak woodlots (approximately 56,200 m², 30,000 m², and 27,000 m² in area) within an approximately 3.34 km² corn and soybean farm in Rensselaer, Indiana, USA. Each experimental pig was to be discarded from a local pig industry rearing facility, because it was either growing too slowly for commercial purposes or because it displayed behavior suggesting an infectious disease, and died as a result of cardiac arrest stunning no more than 12 hours prior to exposure. Pigs (obtained in sets of 1-10 at a time) were placed immediately upon receipt after a visual inspection to ensure no previous insect colonization, under an open-bottomed weld mesh cage, secured to the ground

with stakes. Placement sites were in shade and at least 30 meters from the nearest pig. Daily insect collections were made from each pig over 14 consecutive days. All collections occurred between 0730 and 1830 hrs. The time of collection for each pig was not randomized, but was purposely varied from one day to the next to avoid a consistent effect of time of day on collection composition. Each collection included a series of 4 samples: a sweep net sample (8 rapid passes of the net directly above the carcass), a hand-picked sample of maggots preserved upon sampling, a hand-picked sample of maggots reared to adulthood, and a hand-picked sample of any other adult and immature insects on or immediately surrounding (<30 cm) the carcass. Each collection spanned 20 minutes per carcass to ensure consistent sampling across carcasses.

All individuals collected were identified using the help of taxonomic experts and/or published dichotomous keys (indicated for each species in supplemental material). An occurrence matrix was generated for each pig. Each column, representing a taxon, was summed for each pig and any value over 0 was scored a 1. Column totals were summed across pigs, and any score over 45 (see Discussion) was analyzed for reoccurrence.

Results and Discussion

For our sample size of 53, a taxon/stage must be observed on at least 45 of the experimental carcasses in order to potentially support a 90% confidence interval on PMI when using ≥ 2 taxa [17]. Of the 266 insect species we observed (see supplemental material), 17 were collected from 45 or more of the 53 carcasses exposed (Table 1).

Every common insect species displayed at least one occurrence gap in the collection data from at least one carcass. However, as stated in the Introduction, a gap could be a false negative. Eight of the 17 common species (*Necrodes surinamensis* adults, *Necrophila americana* larvae, *Creophilus maxillosus* adults, *Necrobia ruficollis* adults, *Fannia scalaris* larvae, *Cochliomyia macellaria* larvae, *Phormia regina* larvae, and *Lucilia illustris* larvae) showed a gap(s) of not larger than one sample day on an individual carcass. For the larvae we can be sure that each of those gaps is a false negative. *C. maxillosus* adults, which showed gaps on four carcasses, were visually observed on two of those carcasses on a day when no specimen was collected. Given that our sample effort was deliberately less for adults (i.e. avoided taking multiple individuals of a morphospecies to avoid depleting adult populations that were less abundant compared to larvae), our sampling strategy may also have been biased toward easily distinguishable species. More cryptic species may have been under sampled due to the co-occurrence of multiple species that lack macroscopic morphologically distinguishing characteristics (e.g. Histeridae), potentially creating spurious gaps in presence.

Three kinds of carrion insect, *C. maxillosus* adults, *P. regina* larvae, and *L. illustris* larvae, displayed exceptional forensic utility in that they were observed on every carcass and only two one-day gaps in *C. maxillosus* occurrence were not known to be a false negative. Further research on these species would be especially likely to be of practical value for death investigation, and concentrating on only these species and stages would be a logical way to manage the task of developing greater statistical power for SI estimation. Although training data from >120 experimental corpses are probably needed to place confidence limits on an SI estimate using three insect species [17], based on our experience a succession experiment of that

size targeting only those three species/stages would probably require less effort than the present study.

Sixteen of the 17 abundant species have previously been identified as forensically important (see Table 1 for citations). The exception is the chloropid, *Liohippelates bishoppi*, a common human and livestock pest (eye gnats and yaw flies) [23] that to our knowledge has not previously been classified as a forensic indicator. Because soil samples were not collected certain taxa or life stages, e.g. microphorine larvae, were probably excluded from the data.

Although beetles are certainly used to estimate PMI (e.g. [24-26]), most forensic entomology casework is based on maggots of the families Calliphoridae and Sarcophagidae (e.g. [21, 27-29]). These results show that, at least for our study site, beetles have potential forensic utility roughly comparable to that of Diptera.

Even though carrion insect occurrence depends on many environmental factors, (e.g. geographic location and season) we believe these results are relevant to investigators in many locations. The species identified as useful have wide ranging distributions (Table 1), with multiple being cosmopolitan (i.e. *C. maxillosus*, *N. ruficollis*, and *F. scalaris*) and/or listed in case studies in countries outside of the United States (i.e. *Stearibia nigriceps* in Spain [30]).

95% confidence intervals about SI based on either single species or pairwise combinations of *C. maxillosus* adults, *P. regina* third instar larvae, and *L. illustris* third instar larvae (Tables 2 and 3) suggest that an analysis based on a taxon not being observed is more likely to produce a

discontinuous SI prediction compared to a prediction based on all species (one or two, depending on the succession model being used) being observed. We suspect that this reflects the relatively lower reliability of not observing a species/life stage compared to observing one when the carcass is not exhaustively sampled.

It also appears that using combinations of two taxa observed to be present produces a more precise SI prediction compared to an analysis based on a single species present. Single species prediction intervals were 4923, 4376, and 3829 ADH₁₀ in width (Table 2), while pairwise combination prediction intervals were all 3829 ADH₁₀ in width (Table 3).

Finally, we note that although one purpose of this paper is to promote the use of multiple species and increased experimental replication for PMI estimation, the statistical analysis of LaMotte and Wells [17] can be applied to data for a single species, for which fewer training replicates are needed compared to multiple species. Furthermore, although the inverse prediction methods we developed for placing statistical confidence limits on a PMI estimate based on continuous, quantitative or categorical data were illustrated using insect data, they are equally suitable for any type of postmortem measurement variable such as the level of a volatile organic compound [31], amount of bone weathering [32], or tissue color and degree of RNA degradation [33].

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Table 1. Distribution and published forensic utility of carrion insects that were observed on a high enough proportion of carcasses (>45/53) for placing confidence limits on an estimation of postmortem interval.

Order	Family	Species	Life Stage	# of Pigs	Distribution	Forensic Importance
Coleoptera	Histeridae	<i>Euspilotus assimilis</i> ^a	Adult	53	Nearctic [34]	Watson and Carlton [49]
	Silphidae	<i>Necrodes surinamensis</i>	Adult	45	Nearctic [35]	Byrd and Castner [50]
		<i>Necrophila americana</i>	Adult	49	Nearctic [36]	Byrd and Castner [50]
		<i>Necrophila americana</i>	Larva	48	Nearctic [36]	Byrd and Castner [50]
	Staphylinidae	<i>Creophilus maxillosus</i> ^b	Adult	53	Cosmopolitan [37]	Byrd and Castner [50]
		<i>Creophilus maxillosus</i>	Larva	50	Cosmopolitan [37]	Byrd and Castner [50]
		<i>Philonthus politus</i> ^b	Adult	48	Holarctic [38]	Michaud et al [51]
		<i>Philonthus sericans</i> ^b	Adult	49	Nearctic and Neotropical [39]	Tabor et al [52]
	Cleridae	<i>Necrobia ruficollis</i>	Adult	45	Cosmopolitan [40]	Goff et al [53]
	Nitidulidae	<i>Omosita colon</i>	Adult	52	Holarctic [41]	Byrd and Castner [50]
Diptera	Piophilidae	<i>Prochyliza xanthostoma</i>	Adult	53	Nearctic [42]	Byrd and Castner [50]
		<i>Stearibia nigriceps</i>	Adult	50	Holarctic [43]	Salona et al [30]
	Sepsidae	<i>Meroplius stercorarius</i>	Adult	53	Holarctic [44]	Michaud et al [51]
		<i>Sepsis punctum</i>	Adult	46	Holarctic, Neotropical, Oriental [45]	Byrd and Castner [50]
	Chloropidae	<i>Liohippелates bishoppi</i> ^c	Adult	52	Nearctic [46]	Not previously
	Fanniidae	<i>Fannia scalaris</i>	Adult	53	Cosmopolitan [47]	Byrd and Castner [50]
		<i>Fannia scalaris</i>	Larva	51	Cosmopolitan [47]	Byrd and Castner [50]
	Calliphoridae	<i>Cochliomyia macellaria</i>	Adult	50	Nearctic and Neotropical [48]	Byrd and Castner [50]
		<i>Cochliomyia macellaria</i>	Larva	46	Nearctic and Neotropical [48]	Byrd and Castner [50]
		<i>Phormia regina</i>	Adult	53	Holarctic [48]	Byrd and Castner [50]
		<i>Phormia regina</i>	Larva	53	Holarctic [48]	Byrd and Castner [50]
		<i>Lucilia illustris</i>	Larva	53	Holarctic [48]	Byrd and Castner [50]

Table 2. Contingency tables based on the presence/absence of *C. maxillosus* (Cm) adults, *P.regina* larvae (Pr) and *L. illustris* larvae (Li). The absence of a species in a given category is indicated with an apostrophe following the species name abbreviation (ex. Pr' = *P. regina* not observed). Column values are the start of each time interval as measured in accumulated degree hours above 0°C. The grey, bracketed area is the confidence interval about a PMI estimation, at the 95% confidence level for each scenario of presence/absence.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
Cm	1	[5	31	50	51	47	33	21	12	6]	2	1	0	0	0	0	0	0
Cm'	52	48	22]	3	2	[6	20	32	41	47	51	52	53	53	53	53	53	53
	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
Pr	0	2	[18	46	52	52	47	40	19	5]	2	2	0	0	0	0	0	0
Pr'	53	51	35	7]	1	1	[6	13	34	48	51	51	53	53	53	53	53	53
	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
Li	0	0	[19	47	51	46	31	19	8]	1	1	0	0	0	0	0	0	0
Li'	53	53	34	6]	2	[7	22	34	45	52	52	53	53	53	53	53	53	53

Table 3. Contingency tables based on the presence/absence of all possible two species combinations of *C. maxillosus* (Cm) adults, *P. regina* larvae (Pr) and *L. illustris* larvae (Li). The absence of a species in a given category is indicated with an apostrophe following the species name abbreviation (ex. Pr' = *P. regina* not observed). Column values are the start of each time interval as measured in accumulated degree hours above 0°C. The grey, bracketed area is the confidence interval about a PMI estimation, at the 95% confidence level for each scenario of presence/absence.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
Cm Pr'	1	[5	20	5]	0	1	[2]	1	[6	5	2]	1	0	0	0	0	0	0
Cm' Pr	0	[2	7]	1	1	[6	16	20	13	4	2	2]	0	0	0	0	0	0
Cm Pr	0	0	[11	45	51	46	31	20	6]	1	0	0	0	0	0	0	0	0
Cm' Pr'	52	46	15	2]	1	0	[4	12	28	43	49	50	53	53	53	53	53	53

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
Cm Li'	1	[5	20	5	2	7	12	11	9	6	2]	1	0	0	0	0	0	0
Cm' Li	0	0	[8	2	2	6	10	9	5]	1	1	0	0	0	0	0	0	0
Cm Li	0	0	[11	45	49	40	21	10	3]	0	0	0	0	0	0	0	0	0
Cm' Li'	52	48	14]	1	0	0	[10	23	36	46	50	52	53	53	53	53	53	53

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
Pr Li'	0	[2	6	5	2	6	17	23	13	5	2	2]	0	0	0	0	0	0
Pr' Li	0	0	[7	6]	1	0	1	[2	2]	1	1	0	0	0	0	0	0	0
Pr Li	0	0	[12	41	50	46	30	17	6]	0	0	0	0	0	0	0	0	0
Pr' Li'	53	51	28]	1	0	1	[5	11	32	47	50	51	53	53	53	53	53	53

Table S1. List of beetle species collected from 53 pig corpses and reference used for identification

Family	Subfamily	Species	Identification Reference
Carabidae	Harpalinae	<i>Amara familiaris</i> ^d	Lindroth [1]
		<i>Amara littoralis</i> ^d	Lindroth [1]
		<i>Apristus subsulcatus</i> ^j	Lindroth [1]
		<i>Chlaenius tricolor</i>	Lindroth [1]
		<i>Harpalus indianus</i> ^j	Lindroth [1]
		<i>Lebia analis</i> ^j	Lindroth [1]
		<i>Stenolophus ochropepus</i> ^j	Lindroth [1]
Trogidae	Troginae	<i>Omorgus suberosus</i>	Vaurie [2]
		<i>Trox unistriatus</i>	Vaurie [2]
		<i>Trox spinulosus</i>	Vaurie [2]
Geotrupidae	Geotrupinae	<i>Geotrupes splendidus</i>	Howden [3]
Scarabaeidae	Scarabaeinae	<i>Onthophagus hecate</i>	Howden & Cartwright [4]
		<i>Onthophagus orpheus</i>	Howden & Cartwright [4]
		<i>Onthophagus pennsylvanicus</i>	Howden & Cartwright [4]
	Aphodiinae	<i>Dialytes truncatus</i> ^e	Gordon & Skelley [5]
Silphidae	Melolonthinae	<i>Macroductylus angustatus</i>	Horn [6], Katovich [7]
	Silphinae	<i>Necrodes surinamensis</i> ^k	Ratcliffe [8], Ratcliffe [9]
		<i>Necrophila americana</i> ^k	Ratcliffe [9]
		<i>Oiceoptoma noveboracense</i> ^k	Ratcliffe [9]
	Necrophilinae	<i>Nicrophorus marginatus</i>	Ratcliffe [9]
		<i>Nicrophorus orbicollis</i>	Ratcliffe [9]
		<i>Nicrophorus tomentosus</i>	Ratcliffe [9]
		<i>Bisnius blandus</i> ^b	Smetana [10]
Staphylinidae	Staphylininae	<i>Creophilus maxillosus</i> ^{b,k}	Brunke et al. [11], Kasule [135]
		<i>Ontholestes cingulatus</i> ^b	Brunke et al. [11]
		<i>Philonthus</i> ^k <i>caeruleipennis</i> ^b	Smetana [10], Kasule [135]
		<i>Philonthus</i> ^k <i>cruentatus</i> ^b	Smetana [10], Kasule [135]
		<i>Philonthus</i> ^k <i>politus</i> ^b	Smetana [10], Kasule [135]
		<i>Philonthus</i> ^k <i>rectangulus</i> ^b	Smetana [10], Kasule [135]
		<i>Philonthus</i> ^k <i>sericans</i> ^b	Smetana [10], Kasule [135]
		<i>Philonthus</i> ^k <i>varians</i> ^b	Smetana [10], Kasule [135]
		<i>Platydracus fossator</i> ^b	Brunke et al. [11]
		<i>Anotylus</i> sp. ^b	Arnett & Thomas [12]
	Oxytelinae	<i>Oxytelus</i> sp. ^b	Arnett & Thomas [12]
		<i>Belonuchus rufipennis</i> ^b	Expert Identification
		<i>Lathrobium</i> sp. ^b	Arnett & Thomas [12]
	Paederinae	<i>Rugilus</i> sp. ^b	Arnett & Thomas [12]
		<i>Aleochara</i> sp. ^b	Arnett & Thomas [12]
		<i>Aleochara</i> sp. ^b	Arnett & Thomas [12]
	Aleocharinae	<i>Atheta</i> sp. ^b	Arnett & Thomas [12]
		<i>Falagria</i> sp. ^b	Hoebeke [13]
		<i>Prionochaeta opaca</i>	Arnett & Thomas [12]
	Chlovinae	<i>Sciodrepoides terminans</i>	Hatch [14]

Table S1. continued

Family	Subfamily	Species	Reference
Hydrophilidae	Sphaeridiinae	<i>Cercyon praetextatus</i>	Smetana [15]
		<i>Cryptopleurum subtile</i>	Downie & Arnett [16]
		<i>Sphaeridium bipustulatum</i>	Downie & Arnett [16]
Histeridae ^k	Dendrophilinae	<i>Carcinops pumilio</i> ^a	Bousquet & LaPlante [17], Chu [110]
	Histerinae	<i>Atholus sedecimstriatus</i> ^a	Bousquet & LaPlante [17], Chu [110]
		<i>Hister abbreviatus</i> ^a	Bousquet & LaPlante [17], Chu [110]
		<i>Hister depurator</i> ^a	Bousquet & LaPlante [17], Chu [110]
		<i>Hister servus</i> ^a	Expert Identification, Chu [110]
		<i>Margarinotus hudsonicus</i> ^a	Bousquet & LaPlante [17], Chu [110]
	Saprininae	<i>Geomysaprinus moniliatus</i> ^a	Bousquet & LaPlante [17], Chu [110]
		<i>Euspilotus assimilis</i> ^a	Bousquet & LaPlante [17], Chu [110]
		<i>Saprinus lugens</i> ^a	Bousquet & LaPlante [17], Chu [110]
Curculionidae	Ceutorhynchinae	<i>Curculio orthorhynchus</i>	Gibson [18]
	Entiminae	<i>Calomycterus setarius</i>	Arnett et al. [19]
		<i>Cyrtopistomus castaneus</i>	Arnett et al. [19]
		<i>Otiorhynchus ovatus</i>	Bright & Bouchard [20]
Cerambycidae	Lamiinae	<i>Oplosia nubile</i>	Arnett et al. [19]
Chrysomelidae	Criocerinae	<i>Oulema sayi</i>	Richard [21]
	Eulopinae	<i>Paria quadrinotata</i>	Wilcox [22]
	Galerucinae	<i>Phyllotreta robusta</i> ^j	Howard [23]
Meloidae	Meloinae	<i>Epicauta funebris</i> ^j	Werner [24]
Tenebrionidae	Tenebrioninae	<i>Uloma punctulata</i>	Horn [25]
Cleridae	Korynetinae	<i>Necrobia</i> ^k <i>ruficolis</i>	Kim & Jung [26], Boving & Champlain [136]
		<i>Necrobia</i> ^k <i>rufipes</i>	Kim & Jung [26], Boving & Champlain [136]
		<i>Necrobia</i> ^k <i>violacea</i>	Kim & Jung [26], Boving & Champlain [136]
Nitidulidae	Carpophilinae	<i>Epuraea obtusicollis</i>	Parsons [27]
	Cryptarchinae	<i>Glischrochilus fasciatus</i>	Parsons [27]
		<i>Glischrochilus quadrisignatus</i>	Parsons [27]
	Nitidulinae	<i>Omosita colon</i>	Parsons [27]
		<i>Pallodes pallidus</i>	Parsons [27]
		<i>Stelidota octomaculata</i>	Parsons [27]
Monotomidae	Monotominae	<i>Monotoma picipes</i> ^j	Bousquet & Laplante [28]
Coccinellidae	Coccinellinae	<i>Harmonia axyridis</i>	Gordon & Vandenberg [29]
Dermestidae	Dermestinae	<i>Dermestes</i> ^k <i>ater</i>	Downie & Arnett [16], Rees [137]
		<i>Dermestes</i> ^k <i>caninus</i>	Downie & Arnett [16], Rees [137]
		<i>Dermestes</i> ^k <i>maculatus</i>	Downie & Arnett [16], Rees [137]
Buprestidae	Agrilinae	<i>Agrilus</i> sp. ^j	Arnett et al. [19]
Cantharidae	Chauliognathinae	<i>Chauliognathus marginatus</i> ^j	Fender [30]
Elateridae	Elaterinae	<i>Melanotus gradatus</i> ^j	Quate & Thompson [31]
		<i>Parallelostethus attenuatus</i> ^j	Arnett et al. [19]

^{a,b,d,e} Indicates identification assistance of a taxonomic specialist thanked in the acknowledgements

^j Indicates a taxon collected a total of one time

^k Indicates a taxon collected as both immatures and adults, identification reference is listed second (if needed)

Table S2. List of fly species collected from 53 pig corpses and reference used for identification

Family	Subfamily	Species	Reference
Chironomidae	Chironominae	<i>Chironomus</i> sp.	Oliver [32]
		<i>Polypedilum</i> sp. ^j	Oliver [32]
Culicidae	Culicinae	<i>Aedes cinereus</i>	Siverly [33]
		<i>Aedes triseriatus</i>	Siverly [33]
		<i>Aedes vexans</i>	Siverly [33]
Cecidomyiidae	Cecidomyiinae	<i>Dasineura</i> sp. ^j	Gagne [34]
Sciaridae	Sciarinae	<i>Schwenkfeldina dolens</i> ^j	Steffan [35]
	Cratyninae	<i>Epidapus</i> sp. ^j	Steffan [35]
Mycetophilidae	Mycomyinae	<i>Mycomya</i> sp. ^j	Vockeroth [36]
Keroplatidae	Macrocerinae	<i>Macrocera</i> sp. ^j	Vockeroth [36]
Rhagionidae	Chrysopilinae	<i>Chrysopilus quadrates</i>	Hardy [37]
Tabanidae	Chrysopsinae	<i>Chrysops univittatus</i>	Thomas & Marshall [38]
Empididae	Empidinae	<i>Empis clausa</i>	Coquillett [39] & Melander [40]
Hybotidae	Hybotinae	<i>Syndyas lustricola</i>	Teskey & Chillcott [41]
	Tachydromiinae	<i>Crossopalpus septentrionalis</i>	Melander [42]
		<i>Platypalpus flammifer</i>	Chillcott [43]
		<i>Platypalpus</i> sp. ^j	Steyskal & Knutson [44]
Dolichopodidae	Sciapodinae	<i>Condylostylus furcatus</i>	Curran [45]
		<i>Condylostylus patibulatus</i>	Curran [45]
		<i>Condylostylus siphon</i>	Curran [45]
	Dolichopodinae	<i>Dolichopus scapularis</i>	Van Duzee & Curran [46]
	Medeterinae	<i>Medetera aberrans</i>	Bickel [47]
		<i>Medetera apicalis</i>	Bickel [47]
		<i>Medetera veles</i>	Bickel [47]
Phoridae	Metopininae	<i>Megaselia scalaris</i>	Peterson [48]
		<i>Megaselia morphoserries-1</i>	Peterson [48]
		<i>Megaselia morphoserries-2</i>	Peterson [48]
		<i>Megaselia morphoserries-3</i>	Peterson [48]
		<i>Megaselia morphoserries-4</i>	Peterson [48]
	Phorinae	<i>Dohrniphora incisuralis</i>	Peterson [48]
		<i>Dohrniphora mississippiensis</i> ^j	Peterson [48]
Syrphidae	Syrphinae	<i>Toxomerus marginatus</i>	Vockeroth [49]
		<i>Toxomerus politus</i>	Vockeroth [49]
Ulidiidae	Ulidiinae	<i>Euxesta notata</i>	Curran [50]
		<i>Euxesta stigmatias</i>	Curran [50]
Platystomatidae	Platystomatinae	<i>Rivellia quadrifasciata</i>	Namba [51]
Piophilidae ^k	Piophilinae	<i>Prochyliza xanthostoma</i>	McAlpine [52], Smith [138]
		<i>Stearibia nigriceps</i>	McAlpine [53], Smith [138]
		<i>Protopiophila latipes</i>	McAlpine [53], Smith [138]
Lauxaniidae	Homoneurinae	<i>Homoneura philadelphica</i>	Shewell [54]
	Lauxaniinae	<i>Camptoprosopella</i> sp. ^j	Shewell [55]
		<i>Melanomyza</i> sp.	Shewell [55]
		<i>Minettia lupulina</i>	Malloch & McAtee [56], Shewell [54]
		<i>Minettia magna</i>	Malloch & McAtee [56]

Table S2. Continued

Family	Subfamily	Species	Reference
Sepsidae ^k	Sepsinae	<i>Meroplius stercorarius</i>	Steyskal [57], Smith [138]
		<i>Sepsis punctum</i>	Ozerov [58], Smith [138]
		<i>Sepsis neocynipsea</i>	Ozerov [58], Smith [138]
Chloropidae	Oscinellinae	<i>Apallates particeps</i> ^c	Sabrosky [59]
		<i>Apallates</i> sp. (undescribed) ^{c,j}	Expert Identification
		<i>Conioscinella triorbiculata</i> ^c	Sabrosky [60]
		<i>Hippelates nobilis</i> ^c	Sabrosky [59]
		<i>Hippelates plebejus</i> ^c	Sabrosky [59]
		<i>Liohippelates bishoppi</i> ^c	Sabrosky [59]
		<i>Liohippelates pallipes</i> ^c	Sabrosky [59]
		<i>Olcella provocans</i> ^c	Sabrosky [61]
		<i>Rhopalopterum carbonarium</i> ^c	Expert Identification
		<i>Tricimba trisulcata</i> ^{c,j}	Sabrosky [62]
		<i>Lotophila atra</i> ^j	Marshall & Richards [63]
		<i>Coproica ferruginata</i> ^f	Richards [64]
		<i>Leptocera caenosa</i> ^f	Buck & Marshall [65]
Sphaeroceridae	Copromyzinae	<i>Leptocera erythrocerata</i> ^f	Buck & Marshall [65]
		<i>Spelobia bifrons</i> ^f	Marshall [66]
	Limosininae	<i>Spelobia clunipes</i> ^f	Marshall [66]
		<i>Ischiolepta pusilla</i> ^f	Han & Kim [67]
		<i>Leucophenga morphoserries-1</i>	Wheeler [68]
		<i>Leucophenga morphoserries-2</i>	Wheeler [68]
		<i>Drosophila morphoserries -1</i>	Wheeler [68]
Drosophilidae	Steganinae	<i>Drosophila morphoserries-2</i>	Wheeler [68]
		<i>Drosophila morphoserries-3</i>	Wheeler [68]
	Drosophilinae	<i>Drosophila morphoserries-4</i>	Wheeler [68]
		<i>Scaptomyza</i> sp. ^j	Wheeler [68]
		<i>Diastata</i> sp.	McAlpine [69]
	Anthomyiinae	<i>Anthomyia oculifera</i>	Huckett [70]
		<i>Anthomyia procellaris</i>	Huckett [70]
Diastatidae	Diastatinae	<i>Hylemyza partita</i>	Huckett [71]
		<i>Emmesomyia socialis</i>	Steyskal [72]
	Pegomyinae	<i>Pegomya</i> sp. ^j	Huckett [71]
		<i>Fannia canicularis</i> ^k	Chillcott [73]
Fanniidae	Fanniinae	<i>Fannia scalaris</i> ^k	Chillcott [73]

Table S2. Continued

Family	Subfamily	Species	Reference
Muscidae	Azeliinae	<i>Hydrotaea^k dentipes</i>	Huckett [74], Skidmore [78]
		<i>Ophyra^k aenescens</i>	Huckett [74], Skidmore [78]
		<i>Ophyra^k leucostoma</i>	Huckett [74], Skidmore [78]
		<i>Muscina^k assimilis</i>	Snyder [75], Skidmore [78]
		<i>Muscina^k levida</i>	Snyder [75], Skidmore [78]
		<i>Muscina^k pascuorum</i>	Snyder [75], Skidmore [78]
		<i>Muscina^k stabulans</i>	Snyder [75], Skidmore [78]
	Coenosiinae	<i>Neodexiopsis ovate</i>	Snyder [76], Skidmore [78]
	Muscinae	<i>Eudasyphora cyanicolor</i>	Cuny [77]
		<i>Morellia micans</i>	Skidmore [78]
		<i>Musca domestica</i>	Sabrosky [79]
		<i>Stomoxys calcitrans</i>	Huckett & Vockeroth [80]
	Mydaeinae	<i>Myospila meditabunda</i>	Huckett & Vockeroth [80]
	Phaoniinae	<i>Phaonia fuscana^j</i>	Malloch [81]
Sarcophagidae ^k		Morphoserries-1	GE Shewell [82], Smith [138]
		Morphoserries-2	GE Shewell [82], Smith [138]
		Morphoserries-3	GE Shewell [82], Smith [138]
		Morphoserries-4	GE Shewell [82], Smith [138]
		Morphoserries-5	GE Shewell [82], Smith [138]
Tachinidae	Exoristinae	<i>Winthemia</i> sp. ^j	Wood [83]
	Tachininae	<i>Epalpus signifier</i>	Wood [83], O'Hara & Wood [84]
		<i>Genea brevirostris^j</i>	James [85]
		<i>Gnadochaeta^j</i>	Wood [83]
		<i>Siphona intrudens</i>	O'Hara [86]
Calliphoridae	Calliphorinae	<i>Calliphora vicina</i>	Whitworth [87]
		<i>Calliphora vomitoria</i>	Whitworth [87]
	Chrysomyinae	<i>Phormia regina^k</i>	Whitworth [87], Wells et al. [139]
		<i>Cochliomyia macellaria^k</i>	Whitworth [87], Wells et al. [139]
	Luciliinae	<i>Lucilia coeruleiviridis^k</i>	Whitworth [87], Liu & Greenberg [140]
		<i>Lucilia illustris^k</i>	Whitworth [87], Liu & Greenberg [140]
		<i>Lucilia sericata^k</i>	Whitworth [87], Liu & Greenberg [140]
	Polleniinae	<i>Pollenia rudis^j</i>	Whitworth [87]

^{c,f} Indicates identification assistance of a taxonomic specialist thanked in the acknowledgements

^j Indicates a taxon collected a total of one time

^k Indicates a taxon collected as both immatures and adults, identification reference is listed second (if needed)

Table S3. List of non-fly and non-beetle species collected from 53 pig corpses and reference used for identification

Order	Family	Subfamily	Species	Reference
Collembola	Entomobryidae	Lepidocyrtinae	<i>Lepidocyrtus curvicolis</i>	Arnetti [88] Scott [89]
Orthoptera	Gryllidae	Oecanthinae	<i>Neoxabea bipunctata</i> ^j	Walker [90]
Psocodea	Psocidae	Amphigerontiinae	<i>Blastopsocus lithinus</i>	Moeford [91]
		Psocinae	<i>Cerastipsocus venosus</i> ^j	Moeford [91]
Hemiptera	Aphididae	Aphidinae	<i>Aphis</i> sp. ^j	Wilson [92]
	Cicadellidae	Deltocephalinae	<i>Amblysellus curtisii</i> ^j	Delong & Hamilton [93]
			<i>Latalus sayii</i> ^j	Beirne [94]
			<i>Osbornellus auronitens</i>	Beamer [95]
			<i>Paraphlepsius</i> sp. ^j	Oman [96]
			<i>Scaphoideus</i> sp. ^j	Oman [96]
			<i>Texananus</i> sp. ^j	Oman [96]
		Typhlocybinae	<i>Alebra</i> sp.	Oman [96]
	Membracidae	Smiliinae	<i>Vanduzea</i> sp. ^j	Dennis [97]
			<i>Heliria cristata</i> ^j	Dennis [97]
	Flatidae	Flatinae	<i>Metcalfa pruinosa</i> ^j	Dozie [98]
			<i>Ormenoides venusta</i>	Dozie [98]
	Cydnidae	Cydninae	<i>Pangaeus bilineatus</i>	Froeschner [99]
	Thyreocoridae	Thyreocorinae	<i>Galgupha nitiduloides</i>	Hart [100]
	Pentatomidae	Pentatominae	<i>Cosmopepla lintneriana</i> ^j	McPherson [101]
	Berytidae	Metacanthinae	<i>Jalysus</i> sp. ^j	Henry [102]
	Rhyparochromidae	Rhyparochrominae	<i>Myodocha serripes</i> ^j	Peredo [103]
			<i>Scolopostethus thomsoni</i>	Slater & Brailovsky [104]
	Miridae	Bryocorinae	<i>Pycnoderes quadrimaculatus</i>	Blatchley [105]
		Cylapinae	<i>Fulvius imbecilis</i> ^j	Henry et al. [106]
		Orthotylinae	<i>Halticus bractatus</i>	Henry [107]
	Tingidae	Tinginae	<i>Corythucha arcuata</i>	Gibson [108]
Neuroptera	Chrysopidae	Chrysopinae	<i>Leucochrysa</i> sp. ¹	Strange [109]
Hymenoptera	Tenthredinidae ¹			Chu [110]
	Formicidae	Formicinae	<i>Camponotus americanus</i>	Wheeler [111]
			<i>Camponotus novaeboracensis</i>	Wheeler [111]
			<i>Camponotus pennsylvanicus</i>	Wheeler [111]
			<i>Formica fusca</i> grp.	Creighton [112]
			<i>Formica pallidefulva</i>	Trager et al. [113]
			<i>Lasius niger</i>	Wilson [114]
			<i>Lasius umbratus</i>	Wilson [114]
			<i>Prenolepis imparis</i>	Fisher & Cover [115]
		Myrmicinae	<i>Aphaenogaster fulva</i>	Creighton [112]
			<i>Aphaenogaster tennesseensis</i>	Creighton [112]
			<i>Myrmica americana</i>	Weber [116]
			<i>Myrmica punctiventris</i>	Weber [116]
	Megachilidae	Megachilinae	<i>Megachile</i> sp.	Mitchell [117]
	Halictidae	Halictinae	<i>Augochloropsis sumptosa</i>	Campbell [118]
			<i>Lasioglossum (Dialictus)</i> sp.-1	Gibbs [119]
			<i>Lasioglossum (Dialictus)</i> sp.-2	Gibbs [119]

Table S3. Continued

Order	Family	Subfamily	Species	Reference
Hymenoptera	Braconidae	Alysiinae	<i>Aphaereta pallipes</i>	Wharton [120]
		Cheloninae	<i>Ascogaster</i> sp. ^j	Shaw [121]
		Helconinae	<i>Diospilus</i> sp. ^j	Sharkey [122]
		Microgastrinae	<i>Apanteles</i> sp.	Whitfield [123]
			<i>Microgaster</i> sp.	Whitfield [123]
			<i>Microplitis</i> sp. ^j	Whitfield [123]
			<i>Mesochorus</i> sp. ^j	Wahl [124]
		Mesochorinae		
		Cynipinae	Morphoserries- 1	Ritchie [125]
		Eucoilinae	Morphoserries- 1	Ritchie [125]
	Ichneumonidae	Eucoilinae	Morphoserries-2	Ritchie [125]
			<i>Basalys</i> sp. ^j	Masner & Garcia [126]
			<i>Brachyserphus</i> sp. ^j	Masner [127]
		Entedoninae	<i>Chrysocharis</i> sp. ^j	Hansson [128]
			<i>Hyssopus rhyacionae</i> ^j	Schauff [129]
		Pteromalinae	<i>Nasonia vitripennis</i> ^j	Darling & Werren [130]
		Toryminae	<i>Torymus</i> sp.	Vere Graham & Gijswijt [131]
Lepidoptera	Arctiidae	Arctiinae	<i>Estigmene</i> sp. ^{j,l}	Wagner [132]
	Geometridae	Ennominae	<i>Lomographa</i> sp. ^{j,l}	Wagner [132]
			<i>Plagodis</i> sp. ^{j,l}	Wagner [132]
			Individual ^{j,l}	Wagner [132]
	Lycaenidae	Polyommatainae	<i>Celastrina</i> sp.	Belth [133]
	Noctuidae	Acontiinae	Individual ^{j,l}	Wagner [132]
		Catocalinae	<i>Catacola</i> sp. ^{j,l}	Wagner [132]
			<i>Phoberia</i> sp. ^{j,l}	Wagner [132]
			<i>Zale</i> sp. ^{j,l}	Wagner [132]
		Plusiinae		
	Nymphalidae	Heliconiinae	<i>Boloria</i> sp. ^{j,l}	Belth [133]
	Tineidae	Tineinae	<i>Tinea</i> sp.	Dietz [134]
			<i>Tineola</i> sp. ^j	Dietz [134]

^j Indicates a taxon collected a total of one time

^k Indicates a taxon collected as both immatures and adults, identification reference is listed second (if needed)

^l Indicates a taxon only collected as immature

Table S4. Reoccurrence frequency (Reoccurrence Carcasses/# of Pigs (from table 1)) for each carrion species that was observed on a high enough proportion of carcasses (>45/53) for placing confidence limits on an estimation of postmortem interval.

Species	Gap >1 day	Reoccurrence Carcasses	Reoccurrence Proportion (%)
<i>Euspilotus assimilis</i> (A)	Yes	23	43.4
<i>Necrophila americana</i> (A)	Yes	14	28.6
<i>Creophilus maxillosus</i> (L)	Yes	27	54.0
<i>Philonthus politus</i> (A)	Yes	29	60.4
<i>Philonthus sericans</i> (A)	Yes	37	75.5
<i>Philonthus</i> sp.(L)	Yes	30	61.2
<i>Omosita colon</i> (A)	Yes	40	76.9
<i>Prochyliza xanthostoma</i> (A)	Yes	26	49.0
<i>Stearibia nigriceps</i> (A)	Yes	16	32.0
<i>Meroplius stercorarius</i> (A)	Yes	38	71.7
<i>Sepsis punctum</i> (A)	Yes	22	47.8
<i>Liohippelates bishoppi</i> (A)	Yes	27	51.9
<i>Ophyra</i> sp.(L)	Yes	21	43.8
<i>Fannia scalaris</i> (A)	Yes	37	69.8
<i>Cochliomyia macellaria</i> (A)	Yes	16	32.0
<i>Phormia regina</i> (A)	Yes	27	50.9
<i>Nicrodes surinamensis</i> (A)	No	5	11.1
<i>Necrophila americana</i> (L)	No	11	22.9
<i>Creophilus maxillosus</i> (A)	No	4	7.5
<i>Necrobia ruficollis</i> (A)	No	9	20.0
<i>Fannia scalaris</i> (L)	No	12	23.5
<i>Cochliomyia macellaria</i> (L)	No	6	13.0
<i>Phormia regina</i> (L)	No	1	1.9
<i>Lucilia illustris</i> (L)	No	14	26.4

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Chapter 5: Precision and Accuracy of Succession Based Postmortem Interval Estimates Using a Physiological and an Absolute Time Measure

Abstract

Although insect development and activity are temperature dependent, carrion insect succession has been historically recorded in absolute time. Recent studies have suggested the utility of accumulated degree hours (ADH), a physiological time measure, for improved prediction of succession events across carcasses exposed to variable temperature histories. The generation of a succession training dataset large enough to statistically support succession interval (SI) estimates most likely requires pooling of data developed at unequal temperature histories, suggesting utility of a physiological time measure. Preliminary support and identification of useful species for ADH- described succession can be gained through observations of a comparative increase of insect occurrence overlap and decrease of relative presence period. These characteristics should result in more precise (determined by a decreased SI width) and accurate SI estimations. The presence/absence of eight, previously determined forensically important, species collected from 53 pigs exposed over three years with varying temperature histories were used to determine both ADH and absolute time measured: (1) occurrence overlap, (2) relative presence period, (3) SI width and (4) accuracy of statistically supported SI estimates (determined using the approach of LaMotte and Wells (2000)). Two different ADH ranges were used to determine SI width and accuracy, in order to explore any effect of binning. Comparatively, the use of ADH increased occurrence overlap and decreased SI width, but did not affect relative presence period or SI accuracy. Equivalent accuracy of a more precise SI indicates utility of ADH in succession models, but limitations of ADH use are highlighted.

Introduction

Ambient temperatures affect insect development and activity levels. Because of this relationship, it is common to describe insect development and phenology with a model that measures time in heat units as opposed to absolute time (Damos and Savopoulou-Soultani 2012). Physiological models are commonly used in agriculture to predict emergence times/critical life stages of target pests (Stevenson et al. 2008, Nowatzki et al. 2002). For similar reasons, a physiological time measure is attractive for modeling postmortem decay, because rarely do temperatures at a crime scene mimic experimental conditions.

Current forensic studies aimed at estimating the postmortem interval (PMI) commonly use a thermal accumulation model (Vass et al. 2002, Megyesi et al. 2005, Simmons et al. 2010, Michaud and Moreau 2011), expressing physiological time as accumulated degree hours or days (ADH/ADD). This model utilizes an assumed positive linear relationship between temperature and development rate at intermediate temperatures and has the advantage of being calculable from any temperature profile (Higley and Haskell 2010). Given a curve representing temperature history (i.e. temperature versus time), the area underneath the curve and above a lower developmental threshold (base temperature) for a specific period of time, equals the amount of ADH experienced. This area can be approximated by summing the average temperatures for a specified period of time (e.g. hourly) above a base temperature (Σ temperature recorded – base temperature). Forensic entomologists use ADH to describe the development of forensically relevant flies (e.g. Williams 1984, Anderson 2000, Nabity et al. 2006) and beetles (e.g. Richardson and Goff 2001, Midgley and Villet 2009, Velasquez and Vilorio 2009). The minimum

time elapsed since death can be determined by comparing attributes (e.g. length, weight) of maggots collected from the corpse to experimentally generated development data for the same maggot species under similar conditions (Williams 1984).

Support for the use of ADH to calculate specimen age comes from both successful use in case reports (Lord et al. 1986a, Lord et al. 1986b, Oliveira-Costa and Mello-Patiu 2004) and a validation study (VanLaerhoven 2008). Noted advantages to using a linear model, as opposed to some other positive function, (for examples of nonlinear models see table 1 of Kontodimas et al. (2004)) include comparative simplicity of concept and calculation, and examples of comparable performance for agriculturally important insect species (Higley and Haskell 2010). Even though the relationship between development rate and temperature is curvilinear at temperature extremes (i.e. high and low temperatures), to my knowledge there has not been a comparison of predictive performance of linear and nonlinear development models for any forensic insect. While the use of ADH to describe forensic insect development is widespread, the assumption that development is a linear function of temperature has seldom been tested (Nabity et al. 2006).

If development is linear, individuals of a species should reach a developmental landmark (e.g. pupariation) at the same ADH value (thermal constant) regardless of temperature history (as long as fluctuations stayed within the linear temperature range). In other words, a zero-relationship between thermal constants and rearing temperature distinguishes data appropriate for a linear model (Arnold 1959). This assumption could be preliminarily investigated for any experiment, rearing the same species at more than one temperature, by testing for significant differences in thermal constants across temperature treatments.

A second, time-dependent process that is used to estimate time since death compares the set of insect species/life stages in the corpse to a model of postmortem insect succession (Wells and LaMotte 2010). Succession encompasses the time elapsed both pre- and post- colonization, enabling the estimation of both a minimum and maximum times since death (assuming the victim died where she or he was found). Commonly, succession data are recorded as an occurrence matrix, displaying presence or absence of species/life stages for each known value of a succession interval (SI). Insects collected on a corpse with an unknown SI are compared to a succession model generated under similar conditions. Model SI values with the same species composition as collected from the corpse are included in a PMI estimation (Schoenly et al. 1996). Since forensic insect succession is an ecological process that involves both insect activity and development, it is not surprising that there is support for a positive relationship between succession rate and temperature (Archer 2003, Archer and Elgar 2003, Michaud and Moreau 2009, Michaud and Moreau 2011) though historically succession patterns have been described in absolute time (i.e. days) (Anderson and VanLaerhoven 1996, Tabor et al. 2004, Voss 2008).

Intuitively, a succession pattern would be harder to model, as compared to development, because it involves multiple species, most likely with both different base temperatures and development rates. In addition, for some species (e.g. *Ontholestes cingulatus*) a model that incorporates ADH does not increase the predictability of occurrence on carcasses (Michaeud and Moreau 2009).

The greater complexity of a succession pattern as compared to a single-species growth curve suggests that something other than a simple landmark must be used to determine the appropriateness of an ADH model. While the synchronization of development can be shown by a small number of developmental milestones, synchronization of two or more succession data sets should be evaluated according to some more complicated measure of overlap. Complete overlap

of two succession datasets results from synchronous arrival and departure of a species from both corpses. An increase in single species succession pattern overlap on multiple carcasses when measured in ADH as compared to absolute time can be used to preliminarily assess a species future use in a succession model described in physiological time.

Establishing the exact form of the relationship of succession rate and temperature is beyond the scope of this paper. Instead, I will evaluate the performance of carrion insect species for SI prediction using an ADH model. Previous authors have identified predictability and narrow presence period as the most important measurable features of a carrion insect species that leads to a precise PMI estimation (Matuszewski et al. 2010). Given a practical measure of physiological time, a candidate species should display an increase in temporal overlap across carcasses indicating predictability. This increase in temporal overlap should result in a decreased SI width, and a decreased SI width equals a more precise PMI estimate (Schoenly et al. 1996).

In addition to being common (Chapter 4) and nonreoccurring (Schoenly 1992), a species that demonstrates both an increase in temporal overlap among carcasses and decrease in relative presence period in each carcass, when measured in ADH, exhibits utility for succession models that incorporate temperature. The implementation of a physiological time scale into a succession model is not straightforward because temperature history is a continuous variable and succession data are typically recorded in discrete ranges representing columns in an occurrence matrix. For multiple species of forensic importance, our objectives are to: (1) document statistically supported SI widths (made possible by the approach of LaMotte and Wells (2000)) resulting from different column values (both days and ADH ranges) to give an initial evaluation of the practical

implications of pooling ADH in a succession model, (2) determine the relationship of the relative presence period and occurrence overlap to statistically supported SI widths and (3) assess the predictive performance (accuracy) of SI widths resulting from different column values.

Materials and Methods

Temperature Comparison Among Years

Daily insect collections from 53 domestic pig carcasses were made during the summer months (June-August) of three consecutive years (2008-2010). Because of limited pig availability, few of the pigs shared exactly the same exposure period, therefore few had the same ambient temperature history. Those pigs sharing the exact same exposure period were considered to be in the same batch. See Chapter 4 Materials and Methods for details concerning experimental methods and the study site. Temperature was measured hourly throughout the entire experimental period with a HOBO Pro series weatherproof data logger (Onset Cape Cod, Maine). The data logger was secured to a tree, at chest level (~1.22 m), in full shade. The amount of ADH (Σ temperature recorded– base temperature) in each 24 hour period starting at the time of pig placement was calculated using three different commonly used base temperatures (0, 6, 10 °C) (VanLaerhoven 2008). Possible differences in total heat input to each carcass were investigated by comparing the average ADH per day experienced by each batch of pigs using a one-way analysis of variance (ANOVA) in the statistical package JMP version 8.0.1 (SAS Institute Inc, Cary, North Carolina). A separate ANOVA was run using ADH calculations with each of the three base temperatures to determine if significant differences in average ADH are dependent on

base temperature. An *a posteriori* Tukey test for multiple comparisons of means was used to identify which exposure periods significantly differed ($p < 0.5$).

Occurrence Overlap and Relative Presence Period

The calculated ADH of each daily occurrence for each species previously described as abundant and nonreoccurring (*Necrodes surinamensis* adults, *Necrophila americana* larvae, *Creophilus maxillosus* adults, *Necrobia ruficollis* adults, *Fannia scalaris* larvae, *Cochliomyia macellaria* larvae, *Phormia regina* larvae, and *Lucilia illustris* larvae) (Chapter 4) were used to determine occurrence overlap and relative presence period. Both average metrics were calculated twice, once using an absolute (hour) and once using one physiological (ADH) time measure. The proportion of occurrence overlap was calculated for each pairwise comparison on the 53 pigs using one of four equations (Figure 1) depending on the nature of the overlap. Relative presence period was calculated by dividing the length of occurrence of each species by the total length of the sampling period for each pig carcass exposed. This calculation is the same as the previously described relative length of the presence period (Matuszewski et al. 2010).

For both overlap and relative presence period calculations, the comparison of hour and ADH calculations for the same instance was facilitated by scoring each comparison as either a 1 or 0. A score of 1 indicates the calculation using hours is greater, while a score of 0 indicates the calculation using ADH is greater. Average scores provide a numerical means to describe trends in the data not apparent from average relative presence period or overlap calculations. An average comparison score > 0.5 , indicates absolute time calculations were more often higher. Conversely,

an average comparison score <0.5 , indicates physiological time calculations were more often higher.

Comparison of SI Widths Using Hours and ADH

Separate, single-species, contingency tables (as described by LaMotte and Wells (2000)) were created from occurrence matrices of the eight identified species. For six of the eight species, (*N. surinamensis*, *C. maxillosus*, *N. ruficollis*, *C. macellaria*, *P. regina*, and *L. illustris* larvae) three statistically-supported SI widths, were calculated using three separate contingency tables, each with different column values: (1) absolute time in days, (2) ADH ranges representing the average daily ADH (547 ADH), and (3) ADH ranges representing the minimum ADH experienced in a 24-hour period (428 ADH) (Appendix 1). Two different ADH ranges were used to explore a possible effect of binning. Because a fourteen day collection period did not encompass the entire SI for two species (*N. americana* and *F. scalaris*), the time elapsed before the first SI value (SI_{min}) was calculated in the same manner as SI width. A longer time to SI_{min} presumably shortens the SI width. The SI values calculated were based on the scenario of presence of the studied species at $\alpha=0.05$ (any day with more than four carcasses observed to have the species present was rejected from the PMI estimation) (See table 3 of LaMotte and Wells (2000)).

To facilitate the comparison of all three measurements, the SI widths calculated from absolute time column values were converted to ADH by multiplying the number of days included in the width or pre- SI_{min} interval by the average ADH experienced over the entire experimental period. In changing column values of occurrence matrices, periodically zeros were created due to non-

collection as opposed to an observed absence. If this non-collection zero occurred between two observations of presence, a presence was recorded in the cell with no experimental observation. Otherwise, cells with no experimental observation were recorded as absences.

Succession Interval (SI) Performance

The same 24 contingency tables created for determining SI widths (three contingency tables, each with different column values, for each of eight species) were also used to assess the performance of SIs. In the same manner, two additional sets of contingency tables were created solely from either 2009 (n=20 pig corpses) or 2010 (n=31 pig corpses) corpse collections for each of eight species. Observations were made for three general situations: a corpse collection compared to a SI established by a contingency table resulting from training data with either a (1) dissimilar, (2) similar, or (3) varied (both similar and dissimilar) temperature history. Each SI was calculated based on the scenario of presence of the studied species at $\alpha=0.05$. See table 3 of LaMotte and Wells (2000) for all maximum observed frequency values (x) of species presence for which a specified time period since death can be rejected from a PMI estimation given a desired confidence level and number of categories (e.g. for the presence/absence of one species the number of categories is two). The frequency with which each single observation of presence as well as the entire relative presence period of an experimental corpse was encompassed (i.e. smaller than or equal to) by the SI was recorded.

Dissimilar Temperature History Comparisons

The performance of dissimilar temperature comparisons was evaluated by determining the number of times the entire presence period and each single observation of presence on experimental carcasses exposed in 2009 were encompassed by the 2010 training data SI (20 comparisons) and vice versa (31 comparisons).

Similar Temperature History Comparisons

The performance of similar temperature comparisons was evaluated by removing a random subset of corpses from the training data used to create a contingency table for a particular year (three of 20 removed from 2009 and five of 31 removed from 2010). The removal of three and five corpses, respectively, from the 2009 and 2010 contingency tables was chosen in order to remove a similar proportion of corpses in each year, while maintaining a training dataset capable of determining a SI. These subsets of corpses were compared to the reduced dataset from which they were removed to determine the frequency of entire presence period and single occurrence observations encompassed by the reduced dataset SI. This procedure was performed ten times and the average was recorded.

Varied Temperature History Comparisons

A contingency table including data collected from all three years contains experimental corpses with varied temperature histories. Like the similar temperature history comparisons, a random

subset of corpses from the entire training dataset was removed (eight of 53), removing a similar proportion of carcasses as for 2009 and 2010. These subsets of corpses were compared to the reduced dataset from which they were removed to determine the frequency of entire presence period and single occurrence observations encompassed by the reduced dataset SI. This procedure was performed ten times and the average was recorded.

Results and Discussion

Temperature Comparison Among Years

There are significant differences in accumulation of heat units per day across exposure periods when calculated with each of the chosen base temperatures, 0°C ($F=11.1326$, $p < 0.001$), 6°C ($F=11.6022$, $p < 0.001$) or 10°C ($F=11.2703$, $p < 0.001$). Separate Tukey tests for multiple comparisons displayed the same relationship among exposure periods regardless of base temperature used to calculate average ADH. Exposure periods during 2008 and 2010 were significantly warmer than exposure periods in 2009 (Figure 2). These observed differences are important for exploring the effect of using a physiological time measure and can be used to interpret occurrence overlap, relative presence period, SI width and performance results. A base temperature of 0°C was used for all subsequent ADH calculations.

Occurrence Overlap and Relative Presence Period

For seven out of eight species, occurrence overlap was greater when measured in ADH as compared to hours (Table 1). Both an increase in ADH-measured average occurrence overlap and low unlike temperature comparison values, indicate utility of ADH for describing insect succession for most of the studied species. Average occurrence overlap comparisons of corpses experiencing like-temperatures are centered on 0.5 (Table 1), indicating equal likelihood that occurrence overlap will be higher when calculated with either ADH or hours for corpses experiencing very similar temperature histories.

Relative presence periods were very similar whether calculated using hours or ADH, the average difference between measurements is less than 0.01 (Table 2). The similarity of measurements can be, at least partially, attributed to the inconsistent effect of time measure on relative presence period across years. Compared to absolute time, ADH-measured relative presence periods were more often wider in cooler years, and more often narrower in warmer years, leading to very similar overall relative presence period averages (Table 2). Average occurrence overlap does indicate a temperature effect on succession but the minimal increase in occurrence overlap (average increase is 3.43% for the seven affected species) and inconsistent effect of temperature on relative presence period suggests that this effect is not linear and/or there is an inherent difference in succession between a cold and warm year.

One species, *Necrophila americana* (all instars), did not exhibit the same response in occurrence overlap to temperature as observed in the seven other species studied, instead ADH measured

overlap decreased by one percent (Table 1). The tendency toward comparatively narrower ADH-measured relative presence period during warm periods and wider ADH-measured relative presence period during cooler periods was not observed for two species, *F. scalaris* and *N. americana* (Table 2). These observations may be explained by the length of the collection period (14 days). After the first summer of exposures, (two pig corpses) a two week collection period was chosen because no carrion insects were observed after that time. During collections in subsequent summers, this collection period length did not include the entire SI for two species (*N. americana* and *F. scalaris*). Recording the last day of presence as the last day of collection (when possibly it was several days later) could superficially increase absolute time-measured overlap because all corpses were exposed for very similar amounts of time but differed in ADH experienced.

Comparison of SI Widths Using Hours and ADH

SI width was narrower calculated in ADH compared to absolute time. For four of six species SI width was narrowest with minimum ADH column values (Table 3). For the remaining two species, both the SI_{min} was always longest with minimum ADH column values, indicating a possible shortening of SI width (Table 3). Using ADH, and presumably other physiological time measures, decreases SI width for succession data collected across years with varying temperature histories, resulting in more precise SI estimations.

Relationship of Overlap and Presence Period to SI Width

Insect species with smaller relative presence periods commonly have lower overlap measurements (Tables 1 and 2). Both metrics are used for assessing the relative usefulness of carrion species (Matuszewski 2011), but they can give contradictory indications. Species with smaller relative presence periods are attractive for use in estimating a precise SI but low overlap across corpses can result in a wider statistically supported SI than the average relative presence period indicates. As an example, *N. surinamensis* has the shortest average relative presence period, but this does not equate to the narrowest PMI width (*C. macellaria*), possibly due to a comparatively lower occurrence overlap. The species with the highest occurrence overlap measurements, consistently have larger relative presence periods and wider SI estimations, suggesting that narrow relative presence period is a better predictor of narrow SI width (Tables 2 and 3). In the evaluation of relative usefulness of carrion insect species, overlap and relative presence period can be used as preliminary indicators, but only after species-specific statistically supported SI widths are created can the relative utility of a species be determined.

Succession Interval (SI) Performance

An ADH-described SI is, on average, more accurate than an SI described in absolute time (Table 4). Further, among ADH-described SIs those binned by average ADH values are more often most accurate (Table 4). This observation is incongruous with the observation of narrowest SI widths when ADH minimum bins are used, evidence that in some cases, procedures aimed at shortening

SI widths may result in less accurate SI estimations. The largest disparity between average absolute and physiological SI performance occurred among dissimilar temperature history comparisons, with improved SI accuracy when ADH was used. Although SI performance improved, the average dissimilar comparison performance is substantially lower than both similar and varied temperature history comparisons. In a few cases (e.g. *C. macellaria* 2009 to 2010 and *N. surinamensis* 2010 to 2009) dissimilar comparisons led to gross underperformance regardless of the time measure. The underperformance and large fluctuations in accuracy of dissimilar temperature comparison performance makes SI estimation using succession data with experimental temperature history differing from case temperature history inappropriate, whether described in days or ADH.

The performance of physiological and absolute time measures within a similar or varied comparison type, were congruent although consistently, physiological time measures were slightly more accurate than the absolute time measure. These data indicate the practical utility of either training data with: (1) corpses that all experienced like-temperature histories to the case history or (2) corpses that experienced a wide variety of temperature histories that includes the case temperature history. The SIs of the above described training datasets did not display an obvious increase in accuracy given a time measurement type, but ADH-measured SI widths are consistently narrower as compared to absolute time-measured SI widths, and therefore more precise. Although minimum ADH yields a more precise SI, average ADH interval use is advocated because these intervals often increase precision with comparatively higher accuracy.

Also noted is a possible effect of sample size on SI performance. Prediction intervals derived from contingency tables with larger sample sizes (i.e. $n=31$ as compared to $n=20$) were more accurate. The similarity of the varied ($n=52$) and 2010 similar ($n=31$) temperature comparison indicates that the effect of sample size on SI accuracy may be restricted to sample sizes $\sim \leq 30$ and/or there is an interactive effect of sample size and temperature history on SI performance. The essentially equal performance of similar and varied temperature training datasets supports the practice of combining corpses with different temperature histories into a single training dataset in order to generate larger training datasets.

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Figure 1. Graphical representations and associated equations of four hypothetical types of occurrence overlap

Figure 2. Average Daily ADH for exposure periods, each experienced by a different batch of pigs. All daily ADH values were calculated using a 0° C base temperature. Differing shading of bars indicates pigs exposed in different years. Exposure periods sharing the same letter do not significantly differ in temperature accumulation rates and therefore represent batches with similar temperature histories. Exposure periods with differing letters significantly differ in temperature accumulation rates.

Table 1. Average occurrence overlap for absolute (hours) and physiological (ADH) time measures. Like temperature comparisons (i.e. % overlap hrs vs. %overlap ADH for within year and 2008 compared to 2010) and unlike temperature comparisons (i.e. 2009 compared to 2008 and 2010) range from one to zero. Values >0.5 indicate % overlap hours is more often greater; values <0.5 indicate % overlap ADH is more often greater.

Species	Life Stage	% Overlap	% Overlap	Like Temp	Unlike Temp
		Hrs	ADH	Comparison	Comparison
<i>N. surinamensis</i>	Adult	0.22	0.24	0.52	0.09
<i>N. americana</i>	Larvae (all instars)	0.48	0.47	0.61	0.63
<i>C. maxillosus</i>	Adult	0.47	0.52	0.46	0.07
<i>N. ruficollis</i>	Adult	0.21	0.24	0.46	0.17
<i>F. scalaris</i>	3rd instar larvae	0.33	0.38	0.47	0.29
<i>C. macellaria</i>	3rd instar larvae	0.30	0.31	0.63	0.37
<i>L. illustris</i>	3rd instar larvae	0.51	0.54	0.54	0.22
<i>P. regina</i>	3rd instar larvae	0.57	0.62	0.50	0.14

Table 2. Average relative presence period for absolute (hrs) and physiological (ADH) time measures. Column 2008&2010 represents the comparison of relative presence period measured in hours and ADH for each pairwise comparison in warm years. Column 2009 represents the comparison of relative presence period measured in hours and ADH for each pairwise comparison in the cool year. Values >0.5 indicate % overlap hours is more often greater; values <0.5 indicate % overlap ADH is more often greater.

Species	Life Stage	Relative Presence		2008&2010	2009
		Period Hrs	Period ADH		
<i>N. surinamensis</i>	Adult	0.1325	0.1321	0.74	0.39
<i>N. americana</i>	Larva (all instars)	0.3524	0.3525	0.30	0.56
<i>C. maxillosus</i>	Adult	0.1921	0.1932	0.64	0.35
<i>N. ruficollis</i>	Adult	0.2144	0.2155	0.57	0.39
<i>F. scalaris</i>	Larva (all instars)	0.2739	0.2747	0.27	0.39
<i>C. macellaria</i>	Larva (3rd instars)	0.1691	0.1677	0.82	0.50
<i>L. illustris</i>	Larva (3rd instars)	0.2495	0.2484	0.80	0.22
<i>P. regina</i>	Larva (3rd instars)	0.3227	0.3221	0.79	0.24

Table 3. Statistically supported SI widths calculated using one of three time measurement categories as column values in a contingency table; (1) days converted (# of days contained in SI width x average ADH experienced over the entire experimental period), (2) ADH mean (average ADH experienced over the entire experimental period and (3) ADH narrow (shortest ADH amount experienced in any 24 hour period over the entire experimental period)

	<i>Creophilus maxillosus</i>	<i>Necrobia ruficollis</i>	<i>Necrodes surinamensis</i>	<i>Necrophila americana*</i>	<i>Fannia scalaris*</i>	<i>Cochliomyia macellaria</i>	<i>Phormia regina</i>	<i>Lucilia illustris</i>
Days	10	8	6	5	4	6	9	8
Days								
Converted	5470	4376	3282	2735	2188	3282	4923	4376
ADH Mean	4923	4376	3282	3282	2735	2735	4376	3829
ADH Narrow	4708	3852	2996	3424	2996	2996	4280	3852

(*)Denote species with occurrences on the last day of collection, therefore all 4 associated values represent SI_{min} .

Table 4. The accuracy of succession intervals measured by the percentage of instances the SI contains either the entire presence period (E) or each single observation within a presence period (S) of an experimental pig used for comparison, at $\alpha = 0.05$. Comparisons belong to one of three categories: (1) dissimilar temperature comparisons (2009 to 2010 and 2010 to 2009), (2) similar temperature comparisons (2009 to 2009 and 2010 to 2010) and (3) varied temperature comparisons (2008-10 to 2008-10)

Species		<u>2009 to 2010</u>			<u>2010 to 2009</u>			<u>2009 to 2009</u>			<u>2010 to 2010</u>			<u>2008-10 to 2008-10</u>		
		Day	Min	Avg	Day	Min	Avg	Day	Min	Avg	Day	Min	Avg	Day	Min	Avg
<i>N. surinamensis</i>	E	93.55	93.55	96.77	45.00	45.00	70.00	86.67	86.67	86.67	86.00	84.00	88.00	91.25	90.00	95.00
	S	94.64	94.64	96.43	64.29	66.07	91.52	93.73	93.21	96.08	88.67	87.04	93.33	94.14	92.93	97.32
<i>N. americana</i>	E	96.77	16.13	35.48	95.00	90.00	90.00	90.00	90.00	86.67	96.00	88.00	96.00	95.00	95.00	95.00
	S	99.48	75.13	89.12	98.51	95.52	95.52	96.45	96.45	95.20	99.34	98.18	99.34	99.05	99.05	99.05
<i>C. maxillosus</i>	E	100.0	100.0	96.77	20.00	45.00	50.00	80.00	80.00	93.33	88.00	88.00	88.00	77.50	77.50	80.00
	S	100.0	100.0	99.22	70.90	80.60	84.33	97.00	97.00	99.02	96.57	96.18	97.09	94.46	95.13	95.20
<i>N. ruficollis</i>	E	87.10	87.10	96.77	45.00	50.00	50.00	83.33	76.67	76.67	90.00	88.00	92.00	88.75	87.50	95.00
	S	93.42	92.10	98.68	54.69	70.31	71.87	94.54	93.52	92.52	93.80	92.27	94.80	95.87	94.86	98.34
<i>F. scalaris</i>	E	74.19	54.83	77.42	100.0	100.0	95.00	86.67	86.67	90.00	96.00	96.00	96.00	86.25	90.00	83.75
	S	92.03	76.54	94.93	100.0	100.0	98.77	96.12	96.12	96.78	99.26	99.26	99.24	96.75	97.84	96.42
<i>C. macellaria</i>	E	6.45	41.94	38.71	70.00	90.00	100.0	90.00	86.67	88.57	98.00	98.00	98.00	100.0	92.50	90.00
	S	51.58	81.05	78.95	78.57	92.80	100.0	93.00	90.00	93.00	99.23	99.23	99.23	100.0	96.64	94.95
<i>L. illustris</i>	E	100.0	100.0	100.0	45.00	65.00	65.00	76.67	76.67	93.33	100.0	100.0	100.0	98.75	96.25	98.75
	S	100.0	100.0	100.0	83.01	91.51	91.51	95.90	95.90	98.91	100.0	100.0	100.0	99.77	99.07	99.77
<i>P. regina</i>	E	74.19	74.19	93.54	40.00	75.00	85.00	73.34	90.00	80.00	90.00	90.00	90.00	88.75	87.50	86.25
	S	94.26	95.34	98.45	82.02	93.20	94.56	91.96	96.47	92.99	98.06	98.06	97.30	96.68	96.46	96.02
Average	E	79.03	70.97	79.43	57.50	70.00	75.63	83.34	84.17	86.91	93.00	91.50	93.50	90.78	89.53	90.47
	S	90.68	89.35	94.47	79.00	86.25	91.01	94.84	94.83	95.56	96.87	96.28	97.54	97.09	96.50	97.13

Table S1. Contingency tables for all abundant and nonreoccurring insect species, each created with a different column value: (a) 1 day, (b) 428 ADH; the minimum ADH experienced in a 24 hour period over the entire experimental period and (c) 547 ADH; the average ADH for the entire experimental period. For tables (a) and (b) the column heading is the upper cut-off of the ADH range; the lower cut-off is 0.01 greater than the previous column heading.

Nicrodes surinamensis (adult)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	1	3	[22	31	27	13	11	6]	2	1	1	0	0	0
A	52	50	31	22	26	40	42	47	51	52	52	53	53	53

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	1	3	[14	23	36	23	12	12	6]	3	2	1	0	0	0	0	0	0	0	0
A	53	52	50	39	30	17	30	41	41	47	50	51	52	53	53	53	53	53	53	53	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9849
P	1	1	[9	24	35	19	13	7]	2	1	0	0	0	0	0	0	0	0
A	52	52	44	29	18	34	40	46	51	52	53	53	53	53	53	53	53	53

Table S1. Continued

Necrophila americana (larva)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	0	0	0	2	1	8	19	28	32	36	40	37	33	35
A	53	53	53	51	52	45	34	25	21	17	13	16	20	18

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	0	0	0	1	1	2	3	10	20	31	31	40	39	38	37	31	26	19	1	0
A	53	53	53	53	52	52	51	50	43	33	22	22	13	14	15	16	22	27	34	52	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9849
P	0	0	0	1	1	3	10	21	30	37	41	40	36	25	20	0	0	0
A	53	53	53	52	52	50	43	32	23	16	12	13	17	28	33	53	53	53

Table S1. Continued

Creophilus maxillosus (adult)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	3	[23	40	49	48	35	24	17	11	6	5]	1	0	0
A	50	30	13	4	5	18	29	36	42	47	48	52	53	53

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	4	[25	37	49	51	51	39	27	20	12	8	5]	1	1	0	0	0	0	0	0
A	53	49	28	16	4	2	2	14	26	33	41	45	48	52	52	53	53	53	53	53	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9849
P	1	[5	31	50	51	47	33	21	12	6]	2	1	0	0	0	0	0	0
A	52	48	22	3	2	6	20	32	41	47	51	52	53	53	53	53	53	53

Table S1. Continued

Necrobia ruficollis (adult)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	0	0	4	[17	23	26	23	17	13	10	8]	3	2	1
A	53	53	49	36	30	27	30	36	40	43	45	50	51	52

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	0	0	1	4	[17	27	25	27	22	16	11	9	7]	4	1	1	0	0	0	0
A	53	53	53	52	49	36	26	28	26	31	37	42	44	46	49	52	52	53	53	53	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9849
P	0	0	0	[8	21	27	28	22	17	11	9]	4	1	1	0	0	0	0
A	53	53	53	45	32	26	25	31	36	42	44	49	52	52	53	53	53	53

Table S1. Continued

Fannia scalaris (3rd instar larva)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	0	0	0	0	[5	7	20	28	30	39	34	28	20	12
A	53	53	53	53	48	46	33	25	23	14	19	25	33	41

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	0	0	0	0	0	4	[7	10	22	29	33	37	43	32	24	18	7	1	1	0
A	53	53	53	53	53	53	49	46	43	31	24	20	16	10	21	29	35	46	52	52	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9849
P	0	0	0	0	1	[5	9	22	32	38	44	33	23	13	2	0	0	0
A	53	53	53	53	52	48	44	31	21	15	9	20	30	40	51	53	53	53

Table S1. Continued

Cochliomyia macellaria (3rd instar larva)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	0	0	[17	30	31	21	18	8]	0	0	0	0	0	0
A	53	53	36	23	22	32	35	45	53	53	53	53	53	53

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	0	0	[9	18	33	30	22	20	14]	3	0	0	0	0	0	0	0	0	0	0
A	53	53	53	44	35	20	23	31	33	39	50	53	53	53	53	53	53	53	53	53	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
P	0	0	3	[20	35	30	23	14]	1	0	0	0	0	0	0	0	0	0
A	53	53	50	33	18	23	30	39	52	53	53	53	53	53	53	53	53	53

Table S1. Continued

Phormia regina (3rd instar larva)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	1	[9	42	49	52	50	44	25	12	6]	3	2	1	0
A	52	44	11	4	1	3	9	28	41	47	50	51	52	53

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	1	[9	27	45	52	53	48	45	37	22	7]	3	2	2	1	0	0	0	0	0
A	53	52	44	26	8	1	0	5	8	16	31	46	50	51	51	52	53	53	53	53	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9849
P	0	2	[18	46	52	52	47	40	19	5]	2	2	0	0	0	0	0	0
A	53	51	35	7	1	1	6	13	34	48	51	51	53	53	53	53	53	53

Table S1. Continued

Lucilia illustris (3rd instar larva)

a.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P	0	[8	43	43	49	30	26	10	7]	1	1	0	0	0
A	53	45	10	10	4	23	27	43	46	52	52	53	53	53

b.

	428	856	1284	1712	2140	2568	2996	3424	3852	4280	4708	5136	5564	5992	6420	6848	7276	7704	8132	8560	8988
P	0	0	[8	33	46	45	48	33	28	16	8]	2	1	0	0	0	0	0	0	0	0
A	53	53	45	20	7	8	5	20	25	37	45	51	52	53	53	53	53	53	53	53	53

c.

	547	1094	1641	2188	2735	3282	3829	4376	4923	5470	6017	6564	7111	7658	8205	8752	9299	9846
P	0	0	[19	47	51	46	31	19	8]	0	1	0	0	0	0	0	0	0
A	53	53	34	6	2	7	22	34	45	53	52	53	53	53	53	53	53	53

Chapter 6: Summary and Conclusions

In response to *Daubert v. Merrell Dow Pharmaceuticals* (1993) and/or a National Research Council report (2009), forensic entomology investigators have identified failures of current forensic entomology field research methodologies (Tomberlin et al. 2011a, 2011b, 2012, Michaud et al. 2012) including a lack of transparent/uniform data collection/presentation, (Tomberlin et al. 2012) inadequate sample sizes (Michaud et al. 2012) and description of successional variation (Tomberlin et al. 2011a, 2011b, Michaud et al. 2012) resulting in deficient inference strength (Michaud et al. 2012). Oddly, a second tendency of these authors, and others (VanLaerhoven 2010, Fiene et al. 2014), is to advocate an increase in basic ecological research, which will “...eventually produce guidelines that will meet the *Daubert* criteria (Tomberlin et al. 2011a)” instead of insisting on the timely development and use of forensic succession models that can statistically support PMI estimations.

For 14 years there has been a described novel theoretical model that can place a confidence interval about a succession interval (SI) estimate (LaMotte and Wells 2000), given the development of a succession dataset that is substantially larger than employed in any current study. The sheer amount of work required for this approach (exposure of >20, probably >50 carcasses) has likely resulted in this models disuse (Matuszewski 2011, 2012). In addition, the effect of many methodological practices necessary for generating a dataset of this magnitude have been, at best, understudied. Using the approach of LaMotte and Wells (2000) this work represents the first successful attempt to generate a succession dataset that can support an SI estimation at a common scientific level of confidence (95%). Examples of the model are demonstrated with insect species that display requisite (abundant and nonreoccurring) characteristics. Questions

central to succession data generation and application were characterized, including the effect of: repeated sampling, year, small-scale spatial variation, intercarcass distance, and measurement of time (absolute vs. physiological). This research provides many novel conclusions, but also provides valuable information to the field of forensic entomology in the context of statistical support of preliminary observations and intuitions of previous authors.

When using an insect succession approach, relatively few species are of true forensic utility (eight species of 266 collected). Admittedly, the species identified in this work will not be forensically important for every location, but many have wide ranging distributions and represent promising starting points for future investigators. The identification of a small, manageable, subset of the carrion insect community with exceptional forensic utility is a result valuable for many avenues of future research in forensic entomology. In order to use forensically relevant statistical models, sample sizes in carrion insect studies need to increase, substantially (i.e. at least 5x the average study). Evidence from this work suggests that researchers may benefit from sample sizes greater than 50 corpses. Focusing collection and identification efforts on a few species, as opposed to the entire carrion community, lessens researcher time and effort, leading to increased replication. The observed independence of intercarcass distance and community at the relatively narrow intercarcass spacing of >30 m contributes to optimizing researcher time as well as field space.

Pooling multi-year succession data also facilitates the generation of large succession datasets.

The demonstrated differences in insect communities as a function of both year and characteristically similar mature oak woodlots are of initial concern because the majority of forensic insect succession studies are executed within one year and succession characterization of

desired areas do not utilize more than one characteristically similar location. It would be exceedingly unlikely for a murder to occur in the same year and study site of a forensic insect succession study. Therefore, for a model to be forensically applicable, it needs to incorporate data collected from multiple characteristically similar sites over multiple years. Although community similarity is a common metric for studying successional variation, future studies could instead benefit from using the SI of forensically important insects as the dependent variable. This forensically targeted, not ecologically driven, approach can lead to results that are more directly applicable to casework.

The observed effect of sampling could possibly be avoided in future studies by collecting only those insects that are forensically useful. Further, many of these species are easily field identified and could be recorded/photographed (Michaud and Moreau 2009) as opposed to collected and preserved. This practice is impossible for most fly larvae, which must be collected for definitive identification. For larvae, a sampling effect may be avoided if relatively low rates of maggot removal ($\geq 5\%$ of total present) are used (Michaud and Moreau 2013).

A growing body of published work advocates the use of ADH-modeled succession (or portions of succession) for better prediction of species occurrence (Michaud and Moreau 2009, Matuszewski 2011, 2012, Matuszewski and Szafalowicz 2013). If multi-year succession includes carcasses exposed to significantly different temperature accumulation rates, it is expected that the use of ADH results in comparatively more precise and accurate SI estimations. Although ADH described succession models did not, generally, increase accuracy of SI estimations, use of ADH

did result in more precise SI estimations. But, the use of ADH described succession models has limitations.

Extremely poor prediction interval performance was observed when single year training data were used to predict the SI of data from a year with a distinctly different temperature history.

This observed underperformance is possibly due to the use of an inappropriate physiological model (i.e. the ADH model is probably not the most accurate description of the relationship of temperature and rate of insect succession) or possibly due to the sensitivity of insect communities and species-specific succession dynamics to non-temperature factors. Future succession models should contain carcasses exposed to similar temperature histories as compared to case temperature histories (either entirely or as part of a training dataset of carcasses with variable temperature histories) and should be described in ADH, although the use of a different physiological model may improve precision and accuracy of SI estimations.

Lastly, although a form of cross validation was performed with the developed training dataset, indicating adequate performance for targeted confidence intervals, external validation is a necessity prior to forensic application.

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