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Insect Signature Indicating Corpse Movement from Urban to Rural Areas of Northeast Ohio

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INSECT SIGNATURE INDICATING CORPSE MOVEMENT FROM URBAN TO RURAL AREAS OF NORTHEAST OHIO

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Bachelor of Science in Biology Hobart and William Smith Colleges May, 2006

submitted in partial fulfillment of requirements for the degree MASTER OF SCIENCE IN BIOLOGY at the CLEVELAND STATE UNIVERSITY September, 2010

This thesis has been approved for

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ABSTRACT

The distribution of insects geographically may provide evidence that indicates the movement of human remains from one location to another. The aims of this study were: (1) to observe insect succession in an urban and rural area in northeastern Ohio to document differences in the entomofaunal succession, and (2) to determine if there is an insect signature associated with a body moved from an urban to a rural area. It was hypothesized that there would be a difference in species composition between the urban and rural sites and the body moved would retain insect evidence indicating initial exposure to an urban insect community. The insect signature of a moved corpse should differ from that of the urban and rural corpses. Six 12-19 kg domestic pig carcasses were obtained and placed in the following locations: two in a rural area of Cuyahoga County, and four in an urban area on Cleveland State University campus. After 24 hours, two of the carcasses from the urban location were moved to the rural location. Each carcass was sampled by hand sorting, aerial sweep netting and pitfall traps from 16 June 2009 to 1 August 2009. Most of the specimens were collected within the first four weeks of the study and included both adult and larval samples. All three carcass types supported a similar array of blow flies (Diptera: Calliphoridae) and beetles (Coleoptera). The dominant calliphorid, *Phormia regina*, represented approximately 66% of all specimens collected and was similarly represented on all carcass types. Although there were a few species unique to the urban or rural treatments, statistically there was no significant difference in insect composition between the treatments. Our analysis revealed that

although species dominance and presence/absence of taxa may not indicate body movement in northeast Ohio, it does provide a database of forensically important insects which may be useful in future investigations.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 Forensic Entomology

Forensic entomology is the study of insects and their interaction with legal issues. This field has three categories: urban, stored product and medicolegal (Hall 1990; Catts and Goff 1992; Hall 2001). Urban forensic entomology involves insect pests that impact the human environment by becoming house or garden pests or causing property damage to structures. Also, this category encompasses agricultural concerns, including the misuse of pesticides and the insect pests associated with livestock facilities. Stored product entomology deals with insect contamination of commercial products. Contaminated food products range from insect parts in canned goods and maggots found in fast food sandwiches to insects in paper products (Hall 2001). The third type, medicocriminal forensic entomology is the most popularized category and involves the use of insect evidence in association with criminal investigations. This area of forensic entomology deals not only with insects involved in crimes such as homicide or suicide, but includes cases of neglect in hospitals and nursing homes, sudden deaths and traffic accidents

(Smith 1986; Hall 1990; Catts and Goff 1992). Medicocriminal entomology can be applied to a variety of investigations, ranging from human death investigations to poaching cases of endangered or protected animals (Anderson 1999).

The discovery of human remains raises questions about time, cause and location of death. Determining the postmortem interval (PMI), or the time between death and body discovery, has often been estimated using observations of decomposition and body temperature (Smith 1986), however, entomological evidence can also be a valuable tool for estimating the PMI in all stages of decomposition (Nuorteva 1977; Smith 1986; Anderson 1995). Numerous ecological investigations have been conducted in order to gather information about the insects associated with decomposition and their application to forensic entomology. The stages of decomposition, succession patterns of insects on carrion, differences in community structure of insects in various regions and the effect of temperature on insect development have all contributed significantly to the field of forensic entomology (Payne 1965; Johnson 1975; Kuusela and Hanski 1982; Mann et al. 1990; Anderson 2000; Campobasso et al. 2001; Carvalho and Lindhares 2001; Marchenko 2001; Bourel et al. 2003; Arnaldos et al. 2004; Carvalho et al. 2004; Grassberger and Frank 2004; Nabity et al. 2006; Sharanowski et al. 2008; Michaud and Moreau 2009).

1.1.1 History of Forensic Entomology

The earliest known use of forensic entomology was recorded by Sun Tz'u in his book entitled *The Washing Away of Wrongs* (translated by B.E. McKnight, 1981). Sun was an investigator and tells of a homicide in 1235 in a Chinese village. A corpse was

discovered in a field, slashed by a scythe. The magistrate, an observant man, had noticed that flies were attracted to fresh blood and asked all of the men to gather with their own scythes. Only one scythe attracted flies-the one with blood residue and tissue fragments from the victim that remained even after the blade was wiped clean. This evidence identified a suspect and when questioned, he confessed to the murder. Also, Sun discusses the activities of the blow flies (Diptera: Calliphoridae) in their attraction to tissue and the infestation of maggots in open wounds (cited in Smith 1986; Hall 1990; Hall 2001).

In the Western world, advances were made by Francesco Redi in 1668. Redi studied rotten meat that was exposed to adult flies and meat that was protected, finding that maggots hatched from eggs laid by flies on the exposed meat. This was a tremendous discovery and invalidated the previously believed theory of the spontaneous generation of maggots from rotten meat (Hall 1990).

Insects were used in an investigation in 1855 when the body of a mummified infant was found behind a chimney of a house being remodeled in Paris (Hall 1990; Amendt 2004). Dr. Louis François Etienne Bergeret performed an autopsy and employed forensic entomological techniques to determine that the child died in 1848. Bergeret used information obtained from flesh fly pupae and other insect taxa present on the corpse (Benecke 2001). Bergeret was able to estimate the PMI using the information provided by the insects and their life cycles. This led to the investigation of the previous occupants that lived in the house in 1848, who were later arrested and convicted of murder.

In the 1880's, Reinhard and Hofmann systematically studied forensic entomology in their use of exhumed bodies from which flies were collected and taxonomically

identified. Around this same time, Jean Pierre Mégnin was developing theories of the succession of insects present on corpses. Mégnin published numerous papers in the 1880's and 1890's, but his most important publication came in 1894 in a book entitled *La Faune des Cadavres: Application l'entomologie a la medicine legale* (Benecke 2001). Mégnin explained the successional waves of insects on human corpses and described their life stages, as well as the identification of insects based on anatomy. He included case reports, illustrating the use of entomology in criminal cases and this publication popularized the subject of forensic entomology and encouraged others to systematically study human corpses and the insect fauna present (Benecke 2001).

A surge in forensic insect studies followed in various parts of the world. One important contribution came from Eduard Ritter von Niezabitowski. He observed flies and beetles on the cadavers of cat, fox, rat, mole, calf and human aborted fetuses. In this experiment, he supported the theory that human and animal corpses share equivalent insect fauna (Benecke 2001).

Taxonomic keys were made available for common maggots and adult calliphorids during the 1930's and 1940's due to work of Knipling and Hall (Haskell et al. 1997). These publications allowed for more accurate identifications of the blow flies of North America. In the 1960's, the groundwork for forensic entomology began to develop. Bernard Greenberg, often called the father of forensic entomology, began studying blow flies (Goff 2000). His work on life cycles and the biology of blow flies provided a foundation for the field. Another important contributor was Jerry Payne; his work centered on the modern idea of insect succession. This involves the interactions between the organisms and the body they feed on, ultimately changing the body through an

orderly process, making each stage of decomposition attractive to a different group of organisms. Insect succession on a corpse is autogenic as the species present induce the changes to the physical environment (Smith 1996).

In a paper published in 1965, Payne explained the details of the successional changes that occurred during the decomposition of pig carcasses that were either protected or exposed to insects (Payne, 1965). Payne's studies on insect succession introduced a system which identifies six stages of decomposition, a system that is still used by most forensic entomologists. Also, Payne recorded over five hundred species, demonstrating the wide variety of organisms involved directly or indirectly in the decomposition process (Payne 1965).

Forensic entomology has only recently become widely accepted in criminal investigations as a valuable tool (Haskell et. al 1997; Anderson 2001). There has been a surge in publications from all over the world. Although there is a great deal of information available in terms of the species present in each region, more data are necessary in order to generate a much needed database of the succession of insects on carrion in various habitats and seasons for each major geographic region.

1.2 Ecology of Carrion

Decomposing animal remains, or carrion, are a short term habitat (days to a few years), offering a food source and shelter for a variety of associated decomposers and predators. Arthropods are a major constituent of this microcommunity, returning organic matter to the ecosystem (Johnson 1975; Tullis and Goff 1987). Insects are the primary arthropods present on carrion and demonstrate succession associated with the different

stages of decomposition (Greenberg 1991; Catts and Goff 1992; Anderson 2001; Amendt et al. 2004; Tabor et al. 2004). In a study of decay rates of pig carcasses due to insect activity, Payne (1965) discovered that the carrion exposed to insects showed a significant loss in tissue, with 90% of the tissue removed in 6 days, whereas the pigs protected from insects still had 20% of the carrion remaining after 100 days. Payne collected a total of 522 species, demonstrating the diversity of insects associated with decomposition (Payne 1965). Abell et al. (1982) also found a significant difference in decomposition of insectfree turtle carrion and carcasses exposed to insects. Turtle carrion protected from insects showed slow decomposition with no signs of decay while carrion in which insects had access resulted in accelerated decomposition.

1.2.1 Decomposition

While many insects play important roles throughout decomposition, Diptera and Coleoptera are the two main groups present on carrion. The larvae of flies (maggots) have the ability to secrete ammonia and digestive enzymes while feeding, dissolving soft tissue and exposing the muscle fibers for consumption while also providing organic liquids for other organisms (Oldroyd 1965; Braack 1987). Maggots are primarily responsible for the consumption of most of the soft tissue, but also indirectly provide food for many other organisms due to the use of enzymes (Lord 1990). Carrion use by insects can be altered by a variety of factors such as geographic or topographic location, season and climate (Anderson 2001).

The number of decompositional stages is has been debated and reported to include anywhere from 2 to 8 stages; decomposition is most commonly divided into 4 stages

(Grassberger and Frank 2004) or 5 stages (Payne 1965; Tullis and Goff 1987; Goff 1993). For this study, I use 5 stages: fresh, bloat, decay, post decay and dry remains stages. The fresh stage begins immediately after death and ends when the first signs of bloating appear. During the bloated stage fluids begin to seep from body openings and putrefaction begins. Anaerobic bacteria metabolically produce gases which cause inflation of carrion. The activity of insects and putrefaction can result in an increase in the internal temperature during this stage (Tullis and Goff 1987). Decay is characterized by deflation of the carcass and an increase in the odors of decomposition due to the penetration of the skin by feeding larvae. Most adult calliphorid flies depart at this time and there is a steady decrease in carcass weight. The post-decay stage is marked by the departure of the large dipteran larvae and mostly bones, cartilage and small pieces of tissue are left behind along with the thick by-products of decay (BOD) (Tullis and Goff 1987). The dry phase begins when there is little decaying tissue left and ends when carrion insect activity ceases. During this stage the carrion may act as shelter for other organisms if there is enough dry tissue remaining (Reed 1958). Although decomposition is a continuous process there are separate stages that can be described, making it more convenient to discuss (Keh 1985). These stages are distinguished by the appearance of the carrion and the insects present, but the length of time during which each stage takes place is variable. Carrion use by insects can be altered by a variety of factors such as the geographic location, temperature, humidity, carcass size and type, and access to the body which may alter the attraction to and succession of the insects involved.

Studies in forensic entomology have used a variety of models to examine the decomposition process as well as insect succession on a wide range of animal models

from lizards and toads (Cornaby 1974) to squirrels (Johnson 1975), rabbits (Denno and Cothran 1976), pigs (Payne 1965; Tullis and Goff 1987; Hewadikaram and Goff 1991), dogs (Reed 1958), cats (Early and Goff 1986), alligator and deer (Watson and Carlton 2003, 2005). Few studies use human remains due to the difficulty in acquiring cadavers. The most prominent research involving human corpses was performed at the anthropological research facility at the University of Tennessee by Rodriguez and Bass (1983). The domestic pig, *Sus scrofa* L., is often used as a model for human decomposition due to their similarities in intestinal flora, omnivorous diet, skin composition and hair coverage (Anderson and VanLaerhoven 1996). These animals closely resemble humans in the pattern of decomposition and insect succession and are more easily attainable, relatively inexpensive and are more acceptable in the public eye (Catts and Goff 1992; Goff 1993; Dillon 1997; Campobasso et al. 2001). In Tennessee, a study comparing the insect community and decomposition of adult and infant human remains to a pig model found no significant difference in the insect community between the human remains and pig carcass (Haskell 1989 cited in Campobasso et al. 2001; Gruner 2004).

Aside from the animal model used in research, the size of the carcass may also have an influence on decomposition and succession. Komar and Beattie (1998) studied the effect of carcass size (small, medium and large) on the rate of decomposition in sun and shade environments, finding that small carcasses decayed faster than larger in both settings. Also, the carcass size affected the length of time spent in advanced decay, with small carcasses ranging from 2-3 days and large carcasses up to 21 days. Hewadikaram and Goff (1991) also studied the impact of carcass size and found similar insect

composition and succession patterns in two different size carcasses (large carcasses weighed 15.1kg and small carcasses 8.4kg), but decay occurred at different rates. The larger carcasses decomposed faster due to a greater number of adult flies arriving. This resulted in more maggots present on the larger carcasses and led to removal of the material more rapidly when compared to the smaller carcasses that supported a smaller maggot population (Hewadikaram and Goff 1991).

1.2.2 Succession

Decomposing remains provide a temporary resource acting as a rapidly changing habitat and food source for a variety of organisms, from bacteria and fungi to invertebrate and vertebrate scavengers (Early and Goff 1986). Many arthropod species, particularly the insects, arrive at remains in a predictable succession pattern. As the remains progress through the decompositional stages, a corpse changes physically chemically and biologically with each stage becoming attractive to a different group of insects (Anderson 2001).

Catts and Goff (1992) identified four categories of arthropods based on their ecological roles during decomposition. The first category is the necrophages, which includes species which feed and breed on the carrion tissue and are thought to be the most forensically important group. This category includes Diptera; primarily calliphorid flies as well as sarcophagids (Sarcophagidae), muscids (Muscidae) and piophilids (Piophilidae). Also included are Coleoptera, such as silphid (Silphidae), clerid (Cleridae) and dermestid (Dermestidae) beetles. Parasites and predators of the necrophagous species make up the second category. Included in this group are the histerid (Coleoptera:

Histeridae) and staphylinid (Coleoptera: Staphylinidae) beetles as well as the Hymenoptera parasites of the Diptera eggs, larvae and pupae. The third category consists of omnivorous species which feed on the carrion as well as the fauna associated with the corpse and this group includes wasps, beetles and ants. In some cases, large populations of omnivorous species may feed on the necrophagous species, impeding the rate of carrion removal (Early and Goff 1986). The final category is the incidental species which are present due to chance or use the corpse as an extension of their habitat. Such organisms include centipedes, isopods, spiders and springtails (Catts and Goff 1992).

One of the most important necrophagous insects to initiate carrion colonization is the blow fly. Blow flies can readily detect decomposition and adult females often oviposit within hours after the death (Nuorteva 1977; Rodriguez and Bass 1983; Smith 1986; Greenberg 1991; Dillon 1997; Anderson 2001). Visual and olfactory cues attract oviparous blow flies, which lay their eggs on moist tissues, primarily in natural body openings or open wounds (Amendt 2004). This is thought to be a strategy which may prevent desiccation and predation of the eggs and results in large maggot masses on the body (Byrd and Castner 2001).

The composition and succession of insect fauna which colonize carrion is influenced by many factors including geographic location of the remains, season and habitat. The geographic region in which the carrion decomposes is the most important factor because it is tied to the climate and soil type, which affects the type of insects present. Even though the same groups may occur during decomposition, the particular species that arrive as well as their arrival time during decomposition will vary depending

on the geographic location. Certain groups tend to colonize first, such as the calliphorids and sarcophagids (Anderson 2001).

Early and Goff (1986) studied succession in tropical O'ahu, Hawaii. Using the carcasses of domestic cats, they found that the groups of insects associated with carrion were similar to studies conducted in other regions, but with variation in the species present. Some of the families were completely absent, such as the silphid and nitidulid beetles, commonly found on carrion in other areas. The first colonizers were *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) and *Sarcophagula occidua* Macquart (Diptera: Sarcophagidae). Also, the total number of taxa collected from each site was lower when compared to other studies in temperate, continental regions. While studying the decomposition of pig carcasses in South Carolina, Payne (1965) collected a total of 422 insect species. The first colonizer was *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae), demonstrating that blow flies are often the first to arrive to carrion. In Tennessee, Reed (1958) found a total of 217 insect species associated with dog carrion, with the calliphorids *Phormia regina* (Meigen) and *Lucilia coeruleiviridis* (Macquart) (Diptera: Calliphoridae) among the first to arrive. In Saskatchewan, Sharanowski et al. (2008) found a variety of calliphorids (*Cynomya cadaverina* (Robineau-Desvoidy)*, Protophormia terraenovae* (Robineau-Desvoidy) and *Phormia regina*) arriving at pig carcasses in sunny and shaded areas in three different seasons.

The impact of geography on the species that arrive to carrion is a major factor which must be taken into consideration during an investigation. Data that are collected in one region should be cautiously applied in determining the postmortem interval in another region. Databases of insects associated with carrion should be compiled for each

region in which time of death will be estimated. Currently there are databases present for certain areas of the United States and Canada, including South Carolina (Payne 1965), Hawaii (Early and Goff 1986; Goff and Tullis 1987), Chicago (Baumgartner 1988), Missouri (Hall and Doisy 1993) Saskatchewan (Sharanowski 2008) Vancouver Island (Dillon and Anderson 1995; Dillon 1997), British Columbia (Anderson 1995; Anderson and VanLaerhoven 1996; Dillon and Anderson 1996) as well as the Iberian Peninsula (Arnaldos et al. 2004).

Season is another factor influencing succession of carrion. The abundance of various species of blow fly will change depending on the season. For example, in Florida *L. coeruleiviridis* was abundant year round, while *Calliphora livida* Hall (Diptera: Calliphoridae) was dominant from December to March and *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae) from June to September (Gruner et al. 2007). In Saskatchewan, adult blow fly activity varied by season and were the first colonizers. The greatest diversity occurred in the fall and in the spring when *Cynomya cadaverina, P. terraenovae* and *P. regina* were the most abundant blow flies whereas in the summer *C. macellaria* dominated. In the fall, *C. macellaria* and *P. regina* were co-dominant on the pig carcasses (Sharanowski et al. 2008).

1.2.3 Habitat and Movement of Carcasses

Local climate is affected by urbanization. More precipitation, lower wind speeds and higher ambient temperatures characterize urban areas. The increased temperature of an urban area is due to human activity and the reduction of natural surfaces is noticeable in densely populated areas and is referred to as the "urban heat-island effect" (Hwang and Turner 2009). The man-made environments provide a variety of substrates for many species of calliphorid fly as well as suitable conditions for growth and development of some species of blow fly.

Carrion flies partition resources in time and space. Although some species of blow fly are ubiquitous, certain species are narrowly indigenous. Different species of calliphorid are associated with different habitats with some in an urban or a rural area and others in both regions (Anderson 1995; Haskell et. al 1997; Grassberger and Frank 2004). Insect species associated with decomposing carrion have been studied in both urban and rural areas throughout the world in countries such as Brazil (Carvalho et al. 2000, 2004), Austria (Grassberger and Frank 2004), Columbia (Wolff et al. 2001), Argentina (Horenstein et al. 2007), Poland (Matuszewski et al. 2008), China (Wang et al. 2008) and Canada (Anderson 1995). In the United States studies have been conducted in Texas (Bucheli et al. 2009), Illinois (Baumgartner 1988), Tennessee (Rodriguez and Bass 1983), South Carolina (Payne 1965),Virginia (Tabor et al. 2004, 2005), Florida (Gruner et al. 2007), Louisiana (Watson and Carlton 2003, 2005), Hawaii (Early and Goff 1986) and West Virginia (Joy et al. 2006).

In a dense urban area of Chicago, Illinois, Baumgartner (1988) collected insects from exposed rat carcasses. Of the 12 species of blow fly collected, 92% of the specimens represented only 3 species: *C. cadaverina* (46%)*, Lucilia sericata* (Meigen) (29%) and *P. regina* (17%). These results contrast urban studies conducted in Indiana where *P. regina* comprised 59% of the blow flies collected at a city dump (Siverly 1970). Also, Baumgartner mentions a study in which *C. livida* is a dominant fly species in the spring and *C. cadaverina* is absent even though this study was conducted only 30 km

from the site used by Baumgartner (Johnson 1970 in Baumgartner 1988). In a decomposition study conducted in a rural area of northeast Ohio, Keiper (unpublished) found that 90% of the calliphorid larvae were that of *P. regina*. These results indicate that although the same species of blow fly may inhabit different areas, the dominance of the species may differ and should be explored to gain a greater understanding of the insect fauna occurrence and abundance in various regions.

In a study of blow flies in British Columbia, Anderson found partitioning of species in urban and rural areas (1995). *Protophormia terraenovae* and *Calliphora vomitoria* (Linnaeus) (Diptera: Calliphoridae) were found only in rural areas whereas *L. sericata* was found exclusively in urban areas. However, two species, *P. regina* and *Calliphora terraenovae* Macquart (Diptera: Calliphoridae) were found in both locations. Although these findings indicate that there may be a distinction between the species of blow fly which colonize carrion in different habitats, further investigation is necessary, especially in different geographic locations. It is crucial to determine the preferential habitat for the fly species in each region before any inferences can be made about body movement between urban and rural areas.

Information about the ecological and biological characteristics of a particular species of insect, such as the life cycle, seasonal and geographical distribution, can provide evidence about the general location of a corpse. The familiarity with local fauna is essential and is often believed to be useful in determining the movement of a body. For example, the presence of an urban species on a body discovered in a rural area may suggest the movement of the body after death. It can be inferred that the urban species were oviposited on the body before its movement to the rural area (Smith 1986; Anderson

and VanLaerhoven 1996; Haskell et al. 1997; Anderson 2001). Because bodies are often moved postmortem and concealed away from the primary homicide site (Smith 1986; Anderson 1995; Anderson and VanLaerhoven 1996; Haskell et al. 1997), a theory of the movement of remains can be supported with evidence obtained from insects. Given this information, it may be possible to identify the original location of the remains (Haskell et al. 1997; Greenberg and Kunich 2000).

1.3 Research Objectives and Hypotheses

My research examined decomposition and the ecology of carrion in two areas of northeast Ohio, urban and rural, using pig carcasses as a model for humans. The data generated from this project provides a database of forensically important insects which may be applied to forensic investigations of human death.

Aim 1: To observe insect succession in an urban and rural area of northeast Ohio. **Aim 2:** To determine if there is a difference in insect succession between the two locations. The insect community composition may represent a signature for decomposition in each area.

Hypothesis 1: There is a difference in species composition between the two sites. The insect signature on a corpse in an urban area is different from that of a corpse in a rural area.

Aim 3: To determine if there is a unique pattern of insect composition associated with a corpse moved from an urban to a rural area. Is there a characteristic pattern of succession associated with corpse movement from urban to rural areas?

Hypothesis 2: A corpse moved to a rural area will retain insect evidence indicating initial exposure to urban insects. The insect signature of a moved corpse differs significantly from both urban and rural signatures.

CHAPTER II

MATERIALS AND METHODS

2.1 Study Sites

Research locations in Cleveland and Hunting Valley, Ohio were chosen to provide urban and rural study sites for the project. Within each site an open, sunny habitat was selected. The experiment was conducted during 16 June – 1 August 2009.

The urban site was located at Cleveland State University (CSU), on the downtown campus in Cleveland, Ohio. The site was bordered on each side by public tennis courts and a gravel parking lot. The area was surrounded by a chain link fence which was secured to prevent public access.

The rural site was located at Squire Valleevue and Valley Ridge Farms (SVVF) in Hunting Valley, Ohio, a facility owned by Case Western Reserve University which is approximately 22.4 km east of the urban site. This farm is a 157 hectare property that serves as an educational and recreational area. The study site was located west of a research pond in an open field with herbaceous vegetation that is mowed annually. The

site was located in an area that is strictly for research purposes, limiting public access and reducing potential tampering with the experiments.

2.2 Experimental Design

Six domestic pig carcasses were used in the study, weighing between 11.8-19.5 kg (Table 1). All pigs were destined for euthanasia and were obtained from a private farmer in Marysville, Ohio on 13 June. The pigs were killed by a single shot to the head with a .22 caliber rifle. The carcasses were wrapped in plastic garbage bags, placed in containers with ice and transported to Cleveland State University where they were kept in a cold room at 4ºC. On 16 June the carcasses were placed at the study sites (4 at CSU and 2 at SVVF), 50 m apart under cages. The cages were constructed with a large wooden base, measuring 97 cm long by 74 cm wide by 12 cm high. Hexagonal wire mesh was attached to the base (Figure 1). The cages acted to prevent disturbance by large vertebrate scavengers, while still allowing insect access. A thin covering board was placed over each cage to limit excessive sun exposure and prevent the carcass from desiccating before multiple samples could be collected.

2.2.1 Pitfall Traps

Pitfall traps were used to collect crawling insects associated with the pig carcasses in each location as well as to document the movement of maggots off of the carrion. Four traps were placed around each carcass, one on each side (anterior, posterior, superior and inferior), 30 cm away from the pig (Figure 2).

Carcass ID	Treatment	Location	Length (cm)	Weight (kg)
	Rural	Squire Valleevue Farms	73	18.6
$\overline{2}$	Rural	Squire Valleevue Farms	71	16.3
3	Urban	Cleveland State University	61	11.8
4	Urban	Cleveland State University	82	18.1
5	$U \rightarrow R$	Squire Valleevue Farms	80	19.5
6	$U \rightarrow R$	Squire Valleevue Farms	77	18.1

Table I. Carcass information. Carcass identification number, treatment, final location, length and starting weight of each pig carcass.

Figure 1. Protective cage. The wooden base was attached to wire mesh and placed over each pig carcass to prevent disturbance by vertebrate scavengers.

Figure 2. Pitfall traps and covering board. Four pitfall traps (PF) were placed 30cm in each direction (N, S, E and W) from the carcass. The covering board (CB) acted to prevent immediate desiccation of the carcasses due to direct sunlight exposure.

The pitfall traps consisted of 946 mL plastic containers. The traps were buried so that the lip of the container was level to the ground surface and were filled partially with ethyl alcohol or soapy water to collect insects. The soap acted to reduce the surface tension of the water, ensuring that the insects captured in the traps sank to the bottom. Raised wooden covers were built for each pitfall trap and acted to reduce evaporation of the ethyl alcohol or soapy water in the traps. Insects from each trap were collected and strained into a container for preservation and identification.

2.3 Methods

On 16 June four pigs were placed in the urban site at CSU, two of which represented the urban treatment, and two pigs were placed in the rural site at SVVF, representing the rural treatment. All insect observations and collections from two carcasses in the same treatment were pooled and analyzed together. After 24 hours, two of the carcasses in the urban site were placed in plastic garbage bags and transported to the rural site, becoming the pigs in the urban to rural treatment. This simulated a homicide in an urban environment followed by the disposal of a victim in a rural setting.

2.3.1 Photographs

Photographs of the carcasses were taken on each sampling day to document the stage of decomposition and insect activity and arrival. This information was used to ascertain the pattern of insects arriving to the carrion throughout the study. At least 2 photographs were taken of each carcass on each occasion using an Olympus SP-550UZ digital camera. Observations were also noted on each sampling day to record ambient and

maggot mass temperature, amount of precipitation, percentage of cloud cover and descriptions of the carcasses to aid in determination of decomposition stage.

2.3.2 Temperature Data

Ambient and ground temperature readings were collected between 0700 and 1100 hours and were taken with a handheld thermometer and supplemented with data collected at the nearest field station for the urban and the rural area. Maggot mass temperature readings were also collected during the bloat and decay stages with a digital meat thermometer inserted directly into the mass on each carcass.

2.3.3 Sampling

Each carcass was sampled daily for the first 10 days to record the early decomposition processes and insect colonization. Due to inclement weather, collection was not possible in the rural site on day 8 of the study. From day 11 onward, specimens were collected every other day for one week, followed by sampling every five days for the next two weeks. Specimens were then collected once per week for the remainder of the study. The sampling intensity was more comprehensive than other studies published in which sampling was conducted for only 8 days and then ceased when advanced decay occurred. Carcasses were visited more frequently in the beginning of the study in order to observe the early colonization processes. When the carcasses reached advanced decay, sampling efforts were less frequent. Watson and Carlton (2003) follows a similar schedule of daily sampling, followed by sampling every other day and then sampling once a week.

To collect representative specimens at each sampling time, a variety of sampling methods were employed. Adult winged insects were obtained with an aerial sweep net. Crawling adults, egg and larval samples were collected manually with forceps from different areas of the carcass (oral/nasal cavities, abdominal/thoracic cavities and anal region). About 25 dipteran eggs and larvae were collected at each sampling occasion for the first seven days; half were placed directly into vials of 70% ethyl alcohol, while the other half were placed into vented rearing chambers, containing beef liver and saw dust to rear the larvae to adults for identification purposes. Adult specimens were placed on paper towel in a killing jar, a jar containing plaster of Paris soaked with ethyl acetate to kill the insects and maintain tissue flexibility. The insects were then transferred to vials to be pinned immediately or vials of 70% ethyl alcohol for preservation. This sampling procedure is similar to those described by Watson and Carlton 2003; Grassberger and Frank 2004; Tabor et al. 2004, 2005; Gruner et al. 2007).

Calliphoridae and Sarcophagidae larvae were examined under a binocular microscope to determine the instar based on the number of spiracular slits as well as identification to species if possible. The reared specimens, upon reaching adulthood, were placed in the freezer to kill the insects before representative of each species were pinned. All specimens collected were placed in the Invertebrate Zoology Collections of the Cleveland Museum of Natural History in Cleveland, Ohio.

2.4 Statistical Procedures

2.4.1 Species Accumulation Curves

Species accumulation curves were constructed in order to quantify and compare the richness of taxa accumulation and diversity between treatments. These curves plot the observed number of species as a function of the sampling effort needed to observe them, demonstrating an accumulation of individuals (Colwell et al. 2004). The curves read left to right. As more individuals are sampled, more species will be recorded, resulting in a curve that rises rapidly at first then slows as more rare species are added. When a clear asymptote has been reached, it indicates that no additional species will be added (Gotelli and Colwell 2001). The rate that new species are added gives a description of the species richness (Magurran 2004).

2.4.2 Rank-Abundance Curves

Rank-abundance curves were constructed for each treatment to compare the structure of the insect communities. The species are ranked and plotted on the x axis from most to least abundant, with their abundances in log scale on the y-axis. Abundance is in terms of relative abundance, so that all species combined equals 1.0 or 100%, and each species abundance is a proportion of the total. These plots are useful for contrasting species richness and differences in evenness among treatments. The shape of the rankabundance curve is used to describe the data. A steep slope indicates low evenness, where high ranking species have much higher abundances than low ranking species. A shallow slope gradient indicates high evenness, with similar abundances among different species (Magurran 2004). To compare the rank abundance plots, a Kolmogorov-Smirnov test was

used, which is a nonparametric test used to determine if two data sets have the same pattern of abundance, testing for significant differences in the species abundances between the two treatment assemblages (Magurran 2004). The software program PAST (version 2.00, Hammer et al. 2001) was used for this analysis.

2.4.3 Cluster Analysis

Cluster analysis was used to compare the differences among samples and communities, demonstrating similarities in species composition (Magurran 2004). Hierarchical clustering illustrates relationships among clusters, showing groups that are more similar to each other based on their similarities in species composition. This type of clustering produces dendrograms with branching structures and samples on one axis and a measurement of similarity between the clusters on the other axis (Gauch and Whittaker 1981).

Similarity measures are used to measure the distance between all pairs of treatments based on species composition, with the two most similar being grouped together into one cluster. The Jaccard coefficient uses presence/absence data to measure the differences in species composition by evaluating the similarity between treatments. This similarity coefficient combines three variables: a, the total number of species present in both treatments; b, the number of species present in only one treatment; and c, the number of species present in only the other treatment. The Jaccard coefficient is represented by the following equation: $C_J = a/(a + b + c)$. This value ranges between 0-1, where zero means that there is no common species between the two treatments and one means that the two treatments share all species (Magurran 2004).

A one way Analysis of Similarity (ANOSIM) was used to test for differences among the clusters in terms of species composition. This non-parametric procedure is a test for a significant difference between two or more groups, based on a distance measure with the null hypothesis that there is no difference in community composition among the treatments (Magurran 2004). An ANOSIM compares the distances between groups with the distances within the groups and the test statistic $R = (rb-rw) / (n (n-1)/4)$ where n is the number of samples being tested, rb is the average of similarities from pairs of replicates between different treatments and rw is the average of similarities among replicates within sites. The R value usually falls between 0 and 1; a high R value indicates dissimilarity between groups and a value of 0 suggesting that the null hypothesis is true and there is no difference between treatments (Clarke 1993). The software program PAST was used to construct all dendrograms as well as to perform the Analysis of Similarity.

2 .4.4 Shannon Index

The Shannon index is used to compare diversity among treatments. Species richness, or the number of species present and evenness, or the relative abundance that each species are represented in an area, is combined in the calculation of the Shannon index (Magurran 2004). The Shannon index (H') is represented by the following equation:

H' = \sum *ρi* ln *ρi* where *ρi* represents the proportion of species i relative to the total number of species. This index ranges from 0 to 4.6, with 0 representing every species in the sample being the same and 4.6 representing that the number of individuals are evenly
distributed among all species. Although the Shannon index considers species evenness, a separate evenness measure was also calculated to illustrate the evenness of species abundances among treatments (Magurran 2004). Evenness ranges between 0 and 1, where 0 indicates that the species abundances are not even and 1 indicates complete evenness. All Shannon index and evenness measures were calculated using the PAST software package (version 2.00, Hammer et al. 2001).

CHAPTER III

RESULTS

3.1 Decomposition

All carcasses experienced the same stages of decomposition, which are documented for the pigs in the urban-to-rural treatment (Figure 3).The decomposition rates were very similar among all carcasses and each carcass experienced the same 5 stages of decomposition (Figure 4). The rural carcasses decomposed similarly and entered each stage within 1 day of each other. The urban to rural carcasses entered the stages almost synchronously. Each carcass spent on average the same amount of time in each of the 5 stages of decomposition, moving into the dry remains stage after 15-17 days of placement in the study site.

Fresh Bloat

Decay Post Decay

Dry Remains

Figure 3. Stages of decomposition. Photographic representation of the 5 stages of decomposition as demonstrated by the urban to rural treatment carcasses.

Figure 4. Duration of decomposition stages. Length of each stage of decomposition for all carcasses in urban (U) , rural (R) and urban to rural $(U$ to $R)$ treatments.

3.2 Insect Activity

A total of 46 taxa were collected, representing 15 families (Diptera: Calliphoridae, Sarcophagidae, Piophilidae, Phoridae; Coleoptera: Anobiidae, Carabidae, Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae; Silphidae, Staphylinidae, Trogidae; Hymenoptera: Formicidae) (Table 2). The most common flies collected were the calliphorids *P. regina, Lucilia illustris* (Meigen) and *L. sericata.* The staphylinids *Creophilis maxillosus* (Gravenhorst), *Platydracus maculosus* (Gravenhorst) and *Hister* spp. were the most common beetles. Incidental species not associated with carrion were not included in this count.

The most numerous insects collected were the calliphorids (Table 3), which were represented by 7 species (*P. regina, L. illustris, L. sericata, L. coeruleiviridis, Calliphora* sp*.* and *P. terraenovae*).The most dominant blow fly to colonize the carcasses was *P. regina*, comprising 66% of the total specimens collected from all pigs (n=2883) and made up 64% of the rural specimens ($n=637$ of 990), 74% of the urban ($n=655$ of 884) and 60% of the urban to rural specimens (n=606 of 1009). Other species including *L. illustris* and *L. sericata* occurred less frequently at 4% (Table 2).

Calliphorids arrived within 15 minutes of carcass placement at both sites, but oviposition was not observed until the following day. Blow fly eggs were deposited in similar areas on all carcasses. Clusters of eggs were observed in moist areas such as the eyes, ears, nose, mouth, anus and genital openings as well as the bullet entrance wound, the skin folds and the area between the ground and the carcass.

The average ambient temperatures were similar among the treatments with the urban site having consistently higher ambient temperatures (Figure 5). When maggot

masses were available on each carcass, representing L2-L3, maggot mass temperatures were recorded. The maggot mass temperatures were much higher than ambient with the highest maggot mass temperature recorded 13.3º C above the ambient temperature (Figure 6).

Table III. Abundance of species collected. List of collected insect species arranged in decreasing order of abundance values recorded from urban to rural treatment. Abbreviations are as follows: Ab (abundance), R-A (relative abundance).

Figure 5. Average ambient temperature. Temperature readings for all treatments recorded on each sampling interval.

recorded on each sampling interval with (**----**) maggot mass and (smooth line) ambient temperature.

3.3 Succession

The times of insect arrival, duration and diversity appeared to vary slightly by location (Figures 7-10). The fresh stage was dominated by adult calliphorids and ants. The bloated stage consisted mostly of Diptera, including calliphorids and sarcophagids as well as Coleoptera, including Staphylinidae and Silphidae. During the third stage, decay, the dominant beetle families were Staphylinidae, Silphidae and Histeridae, all of which were still present through the post decay phase as well. Cleridae, Scarabaeidae and Trogidae also arrived during the fourth stage, post decay. The last stage, dry remains, consisted of the families Piophilidae, Cleridae, Phoridae and Nitidulidae.

3.3.1 Urban Treatment

The pattern of succession for the urban treatment is summarized in Figure 7. During the fresh stage green bottle flies were observed within minutes of placement of the carcasses. *Lucilia illustris* was the first species of blow fly captured from the urban treatment followed by *P. regina*, both of which remained on the carcasses through the bloated-post decay stages of decomposition. Ants were also present in large numbers, and gathered blow fly eggs and fed on fluids, particularly on the eyeballs. The bloated stage lasted 4 days for pig 3, but 7 days for pig 4. During this time, calliphorids were the dominant taxa present, but towards the end of the bloated stage beetles began arriving, including *C. maxillosus* (Coleoptera: Staphylinidae) and *Hister* spp.

The decay stage supported numerous species, including the calliphorid *Cochliomyia macellaria*, *O. noveboracense* (Forster) (Coleoptera: Silphidae) along with *Hister* and staphylinid beetles. As the carcasses progressed into the post decay stage of

decomposition, larvae began wandering off to pupariate, and many larvae were collected from pitfall traps including calliphorid and sarcophagid maggots. The post decay stage supported the greatest diversity of species in the urban treatment (Figure 8). Several species of beetles arrived for the first time in the urban site, including the families Cleridae, Dermestidae, Anobiidae, Scarabaeidae. The last of the calliphorids collected in the urban site were adult *P. regina* and *L. sericata* that had recently emerged from puparia surrounding the cages in the early dry remains stage. The species attracted to the carcasses during this time included *M. scalaris* (Loew) (Diptera: Phoridae), dermestid (Coleoptera: Dermestidae) and checkered beetles (Coleoptera: Cleridae).

Figure 7. **Succession on urban treatment.** Succession table indicating Diptera and Coleoptera species present at each sampling interval collected from the urban treatment. Stages of decomposition are represented by: (F) Fresh, (B) Bloated, (D) Decay, (PD) Post Decay and (DR) Dry Remains.

Figure 8. Number of species collected. Total number of species collected on each sampling day from each treatment.

3.3.2 Rural Treatment

The pattern of succession for the rural treatment is summarized in Figure 9. June 16, 2009 marked the start of the fresh stage of decomposition. From hand picked larvae and adult aerial sweep net samples, the dominant calliphorid species was *P. regina.* Clusters of blow fly eggs were present on the face and in the skin folds of the hind legs. Egg cluster size increased as more adult calliphorids laid eggs, which soon hatched out as early instars on the face on day 2. *Phormia regina* was present during the entire bloated stage, but other blowflies appeared later, such as *Lucilia* spp. and *Calliphora* spp. By day 3, staphylinid beetles had arrived to feed on the maggots and were present consistently throughout the bloated stage. As the carcasses became more bloated, other beetles arrived, such as silphids (*O. noveboracense*), dermestid and *Hister* spp. At the end of the bloated stage, scarab and other silphids (*N. americana* (Linnaeus) and *N. surinamensis* (Fabricius)) arrived. The end of the bloated and early decay stages supported the greatest diversity of insect species (Figure 8). Calliphorid larvae were still present at the beginning of active decay, but eventually wandered off to pupariate in the surrounding soil. Most of the same species present during the bloated stage were also present throughout the decay and post decay stages, but new species arrived including flesh flies and beetles of the family Cleridae. The last of the calliphorids were collected as larvae in the pitfall traps in the very beginning of the dry remains stage. Staphylinid and silphid beetles were collected through the first half of the stage. Species that only occurred in the dry remains stage included beetles in the family Nitidulidae (*Omosita colon* (Linnaeus)), other species of checkered beetles and *M. scalaris*.

Figure 9. Succession on rural treatment. Succession table indicating Diptera and Coleoptera species present at each sampling interval collected from the rural treatment. Stages of decomposition are represented by: (F) Fresh, (B) Bloated, (D) Decay, (PD) Post Decay and (DR) Dry Remains.

3.3.3 Urban to Rural Treatment

The succession for the urban to rural treatment is summarized in Figure 10. During the fresh stage of decomposition the urban to rural treatment carcasses were exposed in the urban site. They remained in this site for approximately 24 hours, during which time they attracted insects. Judging from the eggs collected for rearing, the urban to rural carcasses were utilized by *P. regina* as well as *Lasius neoniger* Emery (Hymenoptera: Formicidae), an ant species that is common in urban areas in the Midwestern United States. This ant species was only collected in the urban site and on the urban to rural carcasses during the first 2 days of the study. During the bloated stage other calliphorid species were collected including *L. illustris, L. sericata*, *P. terraenovae* and *Calliphora* spp. *Calliphora* spp. were only collected in the rural site from both rural treatments and the urban to rural treatments. Flesh flies and staphylinid and silphid beetles began arriving during the bloated stage, followed by *Hister* beetles, arriving toward the end.

The greatest diversity of insects was supported by the carcasses during the decay stage, with a total of 19 different species collected with pitfall traps and by hand during this stage (Figure 8). Many different species of beetle began arriving at this time, such as beetles of the families Silphidae, Scarabaeidae and Trogidae, some of which were only collected during the decay stage (*Achenomorphus corticinus* (Gravenhorst) and *N. surinamensis*). As the carcasses progressed into the post decay stage, the calliphorid larvae began to wander off and other beetles arrived to utilize the carcasses as they dried out, such as *Necrobia rufipes* (De Geer) and *Dermestes pulcher* LeConte. The carcasses continued to desiccate into the dry remains stage and the last of the calliphorids were

collected as larvae in the pitfall traps during the early part of this stage. Flesh fly larvae were also collected from pitfall traps, but at the end of the stage. *Hister* and clerids were the last beetles to leave the carcasses. However, the last insects collected were cheese skipper larvae (Diptera: Piophilidae). On the last sampling day, these larvae were found only on the urban to rural treatment.

Figure 10. Succession on urban to rural treatment. Succession table indicating Diptera and Coleoptera species present at each sampling interval collected from the urban to rural treatment. Stages of decomposition are represented by: **(F)** Fresh, **(B)** Bloated, **(D)** Decay, **(PD)** Post Decay and **(DR)** Dry Remains.

3.4. Species Richness

According to the species accumulation curves constructed, all three treatments showed a tendency toward leveling off. After 16 days, 83%, 81% and 88% of species were collected from the urban, rural and urban to rural treatments, respectively. The curve illustrates the rapid increase in species richness in the beginning of the study as a variety of insect species are attracted to the carcass and then an eventual plateau is reached as fewer species become attracted to the carcasses. The urban to rural treatment had the greatest number of species (33), while the urban treatment had the fewest species (24) and the rural treatment had an intermediate number of species (31) (Figure 11).

Figure 11. Species Richness. Observed insect species richness as a function of time collected from all treatments.

3.5 Community Composition and Structure

3.5.1 Rank-Abundance Curves

When plotted, the rank-abundance curves among the 3 treatments revealed similar slopes (Figure 12). The steep slope gradient in all 3 plots indicates high dominance, reflecting the dominance of *P. regina*. This species was the most abundant and represented 74% (N = 884), 65% (N = 990) and 60% (N = 1009) among urban, rural and urban to rural treatments, respectively (Table 2). No significant differences were found in the rank abundance distributions between the treatments when compared with the Kolmogorov-Smirnov test for the rural versus urban ($D = 0.213$, $p = 0.209$), urban versus urban to rural ($D = 0.234$, $p = 0.131$) and rural versus urban to rural ($D = 0.085$, $p =$ 0.994) treatments.

3.5.2 Cluster Analysis

There is virtually no similarity (0-8%) between the treatments in the fresh stage and low similarity in the dry remains stage (19-39%). The active decay stages of decomposition, bloated, decay and post decay, demonstrate the most similarity (23-53%) (Table 4). When comparing the treatments based on compositional similarity, the urban treatment becomes less similar to the urban to rural treatment, whereas the urban to rural treatment becomes more similar to the rural treatment. This illustrates that the moved carcasses become more similar to the rural treatment over time in terms of species composition.

Figure 12. **Rank-abundance plots.** Rank-abundance plots for species collected from each treatment.

Table IV. Jaccard's coefficient. Calculated Jaccard's coefficient values of similarity comparing each treatment against one another in each stage of decomposition.

When comparing the 3 treatments in terms of overall species composition, the cluster analysis reveals strong similarities between the urban to rural and rural treatments, but separates the urban treatment (Figure 13). The urban treatment has a Jaccard's coefficient of about 0.35, or a 35% overlap of species with the rural and urban to rural treatments. Urban to rural pig 6 has a 45% similarity with the other carcass in its treatment and one of the rural pigs (pig 1). Rural pig 2 and urban to rural pig 5 are the most similar, with a Jaccard's coefficient of 0.55, indicating a 55% similarity in the species collected from those carcasses.

Cluster analyses were also constructed to compare each treatment in terms of similarity in species composition throughout the different stages of decomposition. In the early phases, the urban treatment separated into one group, while 1 rural and 1 urban to rural carcass clustered together (Figure 14). Over time, the urban carcasses form their own group, while the rural and urban to rural treatments become more similar and cluster together. The dendrogram divided the carcasses into 2 groups in the bloated stage and rural pig 1 formed 1 group while all other carcasses formed a larger group. Within the larger group, the urban carcasses clustered together, while the other rural and the urban to rural carcasses shared a similarity of about 0.35 with each other and 0.32 with the urban treatment (Figure 15). In the decay stage the urban carcasses clustered to form 1 group with a 0.40 similarity, while the rural and urban to rural treatments clustered to form a second group (Figure 16). A sub group was formed with urban to rural pig 5 and rural pig 1, demonstrating a high degree of similarity of around 0.63. The dendrogram constructed for the post decay stage illustrates a similar trend, with the urban carcasses composing 1 group with a similarity of 0.40 and the rest of the carcasses showing a similarity of about

0.33 in the other group (Figure 17). In the dry remains stage dendrogram, 2 groups are formed, with the urban carcasses making up 1 group, sharing 0.35 similarity in species. The other group contains the rural and urban to rural carcasses, with no clear separation between these treatments (Figure 18). Using Jaccard's similarity metric, the Analysis of Similarity (ANOSIM) indicated no significant difference in species composition between the urban, rural and urban to rural treatments when all stages of decomposition were combined (R = 0.472, p = 0.202).

3.6 Diversity

The urban treatment demonstrated lower diversity compared to the other two treatments, especially in the decay and dry remains stage where the Shannon index value was up to two times greater for the rural and urban to rural treatments. There was an overall increasing trend in diversity for the rural and the urban to rural treatment carcasses. The rural treatment demonstrated lower species evenness $(0.35 \pm 0.06SE)$ compared to the other treatments (Urban, $0.36 \pm 0.09SE$; Urban to Rural, $0.44 \pm 0.12SE$) (Table 5).

Figure 13. Dendrogram of similarity of carcass treatments. Dendrogram for hierarchical clustering of carcass treatments according to similarities in insect species composition calculated with Jaccard's coefficient of similarity (U=Urban, R=Rural, U to R= Urban to Rural).

Figure 15. Dendrogram of similarity in bloated stage. Dendrogram for hierarchical clustering of carcass treatments in the bloated stage of decomposition according to similarities in insect species composition calculated with Jaccard's coefficient of similarity (U=Urban, R=Rural, U to R= Urban to Rural).

Figure 17. Dendrogram of similarity in the post decay stage. Dendrogram for hierarchical clustering of carcass treatments in the post decay stage of decomposition according to similarities in insect species composition calculated with Jaccard's coefficient of similarity (U=Urban, R=Rural, U to R= Urban to Rural).

Figure 18. Dendrogram of similarity in the dry remains stage. Dendrogram for hierarchical clustering of carcass treatments in the dry remains stage of decomposition according to similarities in insect species composition calculated with Jaccard's coefficient of similarity (U=Urban, R=Rural, U to R= Urban to Rural).

Table V. Shannon Index and evenness. Calculated Shannon Index of Diversity and evenness values for all treatments in each stage of decomposition.

CHAPTER IV

DISCUSSION

The main objectives of my study were to observe insect succession in an urban and rural area of northeast Ohio, in order to see if there is a different pattern of insects associated with a simulated corpse moved from the urban to the rural area. My results add to the knowledge of forensically important carrion associated insects of Ohio.

A total of 46 taxa representing 15 different families of Diptera, Coleoptera and Hymenoptera were collected and identified. A variety of necrophagous, predaceous and omnivorous species were found. Other researchers have reported 37-522 arthropod species during their decomposition studies (Reed 1958; Payne 1965; Early and Goff 1986; Watson and Carlton 2003; Grassberger and Frank 2004; Sharanowski et al. 2008). The differences in the numbers of taxa may have resulted from factors such as climate and regional biodiversity, carcass size duration of study and also the inclusion of incidental and adventive species in the count as well (Reed 1958; Payne 1965; Early and Goff 1986).

The succession of insects observed in my study follows a similar pattern observed in temperate and tropical areas by Early and Goff (1986), Grassberger and Frank (2004), Payne (1965) and Tabor et al. (2004). Calliphorids were the first to arrive and were dominant on the carcasses during the early stages of decomposition. Predators of the necrophages, including ants and some beetles (Coleoptera: Silphidae, Staphylinidae and Histeridae) arrived in response to the large number of blow fly eggs and larvae present on the carcasses. As decomposition progressed in the decay stages, the principal species became Silphidae, Staphylinidae and Scarabaeidae. The later stages, advanced decay and dry remains, attracted beetles such as Cleridae, Dermestidae and Nitidulidae.

In my study, there were differences in the taxa involved in decomposition and associated with pig carrion due to the location of the carcasses, however there were similarities in the arrival and duration of certain species. The findings of my study are similar to others in terms of blow fly species collected. Tabor et al. (2004) reported similar findings of the dominance of *P. regina*, with 90% of all specimens collected in Virginia from late April through June belonging to this species. Dillon (1997) recorded similar abundances of *P. regina* in British Columbia, finding that it was often the codominant species collected from carrion in spring and summer. Gill (2005) also collected *P. regina* from carrion, finding that it was the dominant species during the summer in rural Manitoba, while *C. vicina, L. sericata* and *P. terraenovae* were collected less frequently.

Kuusela and Hanski (1982) found that small communities tend to display a broken stick distribution with 1-2 species dominating the carcasses and comprising up to twothirds of the total number of individuals. They suggested that species that develop at a

faster rate have an advantage in excluding other species and leading to an overall decrease in species diversity. The results of my study are consistent with this, with the dominant species *P. regina* making up approximately two-thirds of the total number of individuals collected from all carcasses. The reason for the clear dominance of this species may lie in the faster development rates at warm temperatures, giving it an advantage and resulting in the exclusion of other species. In my study, the urban site had consistently higher ambient temperatures and the urban to rural carcasses demonstrated a larger difference between maggot mass and ambient temperatures. These increased temperatures, due to either ambient or maggot masses may have resulted in faster development for the maggots present, most of which were *P. regina*.

Kamal (1958) found that the development time for *P. regina* was much more rapid than other calliphorid species. *Phormia regina* spent on average 11 days in the immature stage, a shorter time than other species (12-23 days). Not only did *P. regina* develop more quickly, but also had a longer life span and was more adaptable to fluctuating environmental conditions, such as food deficits, crowding and low humidity. Kamal (1958) suggested that due to the adaptability and more rapid development time, *P. regina* can occur in high abundances. Anderson (2000) reported similar observations of the rapid development of *P. regina* at high temperatures when compared to other calliphorid species. Temperature clearly affects insect development and must be taken into consideration in an investigation. If movement is suspected, any temperature related effects, such as corpse being exposed in an urban area with higher ambient temperatures initially, must be accounted for. Insects that may have developed more quickly, at higher temperatures, may lead the investigator to an underestimation of the postmortem interval.

In Cuyahoga County, *P. regina* was the most abundant species and was collected first from the rural and urban to rural treatments. On day 1, *P. regina* was collected from the rural and urban site as eggs and reared to the adult stage. *Phormia regina* is a primary blow fly species collected and utilized in forensic cases, occurring in both rural and urban areas (Anderson 1995). Baumgartner (1988) reported the two most abundant blow fly species as *P. regina* and *L. sericata* in a dense urban area of Chicago, and found that other species such as *C. vicina, L. illustris, P. terraenovae* and *L. coeruleiviridis* were rare. In California, Denno and Cothran (1975) found that *L. sericata* and *P. regina* were the dominant calliphorids from June to September. Joy et al. (2002) reported that *P. regina* was dominant on carrion in late spring. *Phormia regina* was also the dominant calliphorid collected by Dicke and Eastwood (1952) in Madison, Wisconsin from April through August, comprising 51% of the blow flies captured. In my study, the remaining calliphorids (*C. macellaria*, *C. vicina, L. illustris, L. sericata*, *L. coeruleiviridis* and *P. terraenovae*) were captured during the first 8 days of the study. *Lucilia* spp. arrived after *P. regina* for the urban to rural and rural treatments. In the urban treatment, *P. regina* was not collected as adults or larvae until day 3. *Calliphora* arrived later, appearing on day 4 in the rural site and then again on days 6 and 7, but was never collected in the urban site. *Calliphora* spp. typically prefer shady areas and arrive after *Lucilia*, but before *Sarcophaga* (Smith 1986). A similar pattern of arrival occurred in my study and it was not surprising that very few of this species was collected from my carcasses, which were placed in direct sunlight. *Calliphora* species are commonly considered an urban species (Byrd and Castner 2001, Anderson and VanLaerhoven 1996, Smith 1986), however, were only collected in the rural site in my study. This may be due to the close proximity of the

rural site to human dwellings. Generalizations are often made based on observations of fly populations in certain regions; however, caution must be applied as these generalizations are not always accurate for every geographic area.

Protophormia terraenovae arrived in the end of the bloated stage for both urban and urban to rural treatments. Gill (2005) reports that *P. terraenovae* did not arrive to carrion until 9 days after death, but both *P. regina* and *L. illustris* arrived within the first 3 days. *Cochliomyia macellaria* was collected only in the urban site, in the decay stage of decomposition, although this taxon is widespread (Byrd and Castner 2001).

Sarcophagidae were collected in low numbers in my study. This may be due to interspecific competition between calliphorids and sarcophagids. Denno and Cothran (1976) observed that high numbers of calliphorids limited the numbers of sarcophagids on rabbit carcasses in California. Although Calliphoridae typically are smaller than Sarcophagidae, they must proceed through an egg stage, whereas sarcophagids are ovoviviparous, depositing larvae that are able to feed immediately on carrion. This strategy results in fewer, but larger, sarcophagids and more numerous but smaller calliphorids. However, Calliphoridae are able to compensate for the delay in larval activity by arriving to carrion first. Their presence in large numbers results in calliphorid larvae diminishing the food source relatively quickly, therefore, outcompeting the sarcophagids.

The difference in the arrival time of the species in different sites is not unexpected. Bourel et al. (1999) noticed differences in the patterns of colonization of rabbit carcasses year to year in the same location. In the first year of the study, the first calliphorids to colonize were *C. vicina* and *C. vomitoria*, but they occurred on day 3 and
did not lay eggs until day 5. In the second year of the study, these same species arrived on the day 1 and immediately began to lay eggs. Anderson and VanLaerhoven (1996) observed *P. regina* on carcasses during the fresh stage, but only *L. illustris* immatures were collected at this time. Denno and Cothran (1976) report that *P. regina* is not typically a pioneer species, instead arriving later to colonize the remains, whereas *L. sericata* was the first calliphorid to be collected. In my study, *P. regina* was collected as eggs in the urban and rural sites on the first sampling day, indicating that not only did they arrive quickly to the carcasses, but also utilized the carcass earlier than previously reported.

Stearibia nigriceps Meigen (Diptera: Piophilidae) arrived to the urban to rural carcasses on the last sampling day, during the dry remains stage of decomposition. There are a variety of reports of this species arriving at different times. Dillon (1997) observed adult Piophilidae arriving during the bloat and decay stages, but larvae only in the remains stage. Anderson and VanLaerhoven (1996) collected the larvae 29 days postmortem, much earlier than previously reported. Smith (1986) reported that piophilid larvae usually arrive during the later stages when the carcass dries out, but some adults have been collected much earlier (4 days postmortem). Smith (1986) also noted that even the occurrence of the adults early on does not necessarily indicate that oviposition occurs and the larvae are often found on a corpse 2 months postmortem. The adults often feed on fluids in the early stages of decay, but prefer to oviposit in advanced decay (Tabor et al. 2004). These observations are similar to those described by Nuorteva (1977), Reed (1958) and Johnson (1975) all of which depict the attraction of Piophilidae to mummified carcasses during the dry decay stage.

Predators of the necrophages arriving to the carcasses included Hymenoptera and were most numerous through day 7 of the study. Ants arrived rapidly after carcass placement and were observed on the face and especially on the eyeballs, feeding on the fluids and the eggs and larvae of the blow flies. In the urban site, *Tetramorium caespitum* (L.) was collected on day 1 only, and *Lasius neoniger* Emery and *S. molesta* (Say) were collected throughout the study. The large number of ants present on the urban carcasses, in particular pig 4, strongly influenced the rate of decomposition. The ants were strong predators and removed a large number of eggs and larvae from the carcass in the early stages of decomposition, which reduced the number of blow fly larvae available to feed on the soft tissue. This activity prolonged the stages of bloat and decay for pig 4 (Figure 3).

Early and Goff (1986) observed similar activity in O'ahu, Hawaii and reported significant predation by fire ants (*Solenopsis geminata* (Fabricius)) throughout the decay stage, up to 15 days postmortem. The predation reduced the rate of carcass tissue removal and the duration of the bloat and decay stages of decomposition. Anderson and VanLaerhoven (1996) also collected ants on all carcasses throughout decomposition, indicating that they are common carrion insects.

In the rural site, *L. alienus* (Foerster) and *Myrmica* sp*.* were collected on sampling day 1. A total of four species were collected from the rural carcasses and were most numerous in the early decay stages, with few found after the decay stage. For the urban to rural treatment carcasses, six different species of ant were collected. *Lasius neoniger* were collected on sampling day 1, while the pigs were in the urban site, and then were

collected the following day at the rural site, after the pigs had been moved from urban to rural locations.

The arrival of beetles (Insecta: Coleoptera) in response to the Diptera larvae present on the carcasses included the families Silphidae (carrion beetles), Staphylinidae (rove beetles) and Histeridae (clown beetles). The most dominant beetle species collected were *Creophilis maxillosus*, *Platydracus maculosus* and *Hister* spp. Rodriguez and Bass (1983) reported the arrival of carrion, clown and rove beetles during the bloated stage, but the greatest number of these species arrived during the decay stage. In my study, the first beetles arrived on day 3, but the majority was observed during the end of bloat and throughout the decay stages. The early arrivers included *C. maxillosus*, *Platydracus* spp., and *Hister* spp*.* in the rural treatment and *Playdracus* spp. and *Necrophila americana* in the urban to rural treatment. In the urban treatment, the first beetle to arrive was *C. maxillosus* on day 6. These results confirm previous studies in which *C. maxillosus* and silphids were among the first to arrive shortly after death (Anderson and VanLaerhoven 1996). Clearly, the arrival of these beetles is rather unpredictable, limiting their value as PMI indicators.

Four species of Silphidae were collected throughout the study; *Oiceoptoma noveboracense* was collected from all 3 treatments, but the other species (*Necrodes surinamensis*, *N. americana* and *O. inaequale* (Fabricius)) were only observed on the rural and urban to rural treatments, arriving on day 3 of the study. The larvae of Silphidae were observed much later in the advanced stages of decomposition, at the end of post decay. Few *N. surinamensis* were collected in this study. A possible reason for this lies in the primarily nocturnal behavior of this species, whereas other carrion beetles (*N.*

americana) are diurnal and were collected more often (Byrd and Castner 2001). Early and Goff (1986) observed that Silphidae were completely absent from their decomposition studies in Hawaii, indicating that the arrival of certain species depends on geographic location.

A similar pattern was observed among the Staphylinidae and 4 species were collected; *C. maxillosus* was observed on all treatments, but the other species (*Platydracus* spp*., Aleochara* sp., and *Achenomorphus corticinus*) were only collected in the rural site on the rural and urban to rural treatments. Early and Goff (1986) noticed similar patterns of staphylinid abundance, with an insignificant number of *C. maxillosus* arriving to one study site, but a large number acting as a primary predator of dipteran larvae at the other site. Staphylinids can arrive at various times during decomposition. In my study, *C. maxillosus* was collected during the bloated, decay and post decay stages from all carcasses. *Platydracus* spp. shared a similar pattern, however, were not collected in the urban site. Gill (2005) reported that *C. maxillosus* arrived in the first 8 days of the summer study and 22 days after carcass placement in the fall. Staphylinids arrived on day 3 of a study conducted by Watson and Carlton (2003) and were abundant throughout the study. Anderson and VanLaerhoven (1996) observed Staphylinidae during the bloated stage (days 2-10) and then again in advanced decay and dry remains stages.

Necrobia spp. were collected in my study from all three treatments. Adult *Necrobia rufipe*s and *N. violacea* Linnaeus occurred on all 3 treatments, but *N. ruficollis* Fabricius was only collected from the rural and urban treatments. In the urban site, the clerid beetles began arriving in post decay and were present throughout dry remains. In the rural treatment, *Necrobia* species arrived in decay (*N. ruficollis*) and were present

throughout dry remains. For the urban to rural treatment *N. rufipes* arrived in post decay and *N. violacea* in the dry stage. *Necrobia* spp*.* are different than others in the family Cleridae, feeding on skin and bones (Smith 1986). These observations confirm previous publications in which *Necrobia* were collected mostly in the dry remains stage although a few did arrive earlier in decomposition (Rodriguez and Bass 1983; Anderson and VanLaerhoven 1996; Grassberger and Frank 2004; Gill 2005).

Beetles of the family Nitidulidae were first collected in the dry remains stage for the rural treatment and at the end of the same stage for the urban to rural carcasses. No nitidulids were collected from the urban site. These beetles are commonly referred to as sap beetles for their preference for sap and fruit juices, but a few are predacious on carrion (Smith 1986). *Omosita colon*, the species collected in my study, typically arrives during the advanced stages of decomposition with Dermestidae, but prefers flesh with more moisture (Smith 1986; Byrd and Castner 2001). Rodriguez and Bass (1983) found that this species was most abundant in the dry stages and Reed (1958) collected nitidulids as early as the decay stage. My results are similar to those of Anderson and VanLaerhoven (1996) who reported *O. colon* arriving 22 days after death, during the advanced decay and dry remains stages.

Dermestidae, a family of beetles that is commonly referred to as hide and larder beetles, were collected from all 3 treatments. In the urban site these beetles were more abundant than the rural site and were collected from post decay through dry remains stages. In the urban to rural treatment, *Dermestes pulcher* was collected from post decay to early dry remains and in the rural treatment adults were only collected during the bloated stage, but larvae were observed on the carcasses during more advanced stages of

decomposition, toward the end of dry remains. Dermestids often arrive during the dry and skeletal stages and may increase their activity during the warmer months. They are often observed arriving at the same time as the Nitidulidae, but instead preferring much drier skin (Byrd and Castner 2001). Smith (1986) reported that dermestids typically arrive 3-6 months after death when the carrion is extremely dry, indicating that in my study this species had an early time of arrival. However, previously published work demonstrates similar observations of dermestids occurring earlier. Dillon (1997) reports their appearance in the bloated stage, while 21 days after death dermestids first arrived, with the majority occurring on day 43 for Anderson and VanLaerhoven (1996). In Hawaii, these beetles arrived within 3-5 days postmortem, with adults and larvae collected by day 10 (Hewadikaram and Goff 1991).

Adult scarabs (Coleoptera: Scarabaeidae) were first collected at the end of the bloated stage, 6 days after placement of the rural carcasses. These beetles remained on these pigs throughout the dry remains stage. For the urban to rural treatment, *Onthophagus hecate* (Panzer) arrived during decay through early dry remains. In the urban site, however, the only scarabs collected was *Serica sericea*, during the post decay stage. Beetles of the family Scarabaeidae, especially the genus *Onthophagus* are commonly found on carrion and often construct tunnels under carcasses (Smith 1986). These beetles have been collected at varying times during decomposition. Dillon (1997) reported that in Canada *Onthophagus* arrived during bloat and post decay, while Rodriguez and Bass (1983) only observed scarabs during the dry stages in Tennessee.

Other beetles that were less frequently encountered were *Xestobium rufovillosum* (De Geer) (Coleoptera: Anobiidae) and *Trox unistriatus* (Beauvois) (Coleoptera:

Trogidae). *Xestobium rufovillosum,* the deathwatch beetle, was only collected in the urban site at the end of post decay, and is known to prefer mummified skin (Smith 1986). *Trox unistriatus*, a hide beetle, was collected from the urban to rural carcasses from the decay through early dry remains stages. The beetles of the family Trogidae are typically collected much later in decomposition, in advanced stages (Gill 2005). One possible explanation for not capturing this beetle from other carcasses lies in their deceptive nature. The adults often cover their body with mud and animal tissue, camouflaging themselves to look like debris, making them difficult to detect (Byrd and Castner 2001).

The presence of fewer beetles than flies is not unexpected. Smith (1986) points out that due to greater mobility of Diptera, flies often reach carrion much faster and in larger numbers than beetles. It is also advantageous that fly larvae have shorter development time, allowing for the utilization of the food source much more rapidly. Calliphorid larvae also produce ammonia, a compound which is toxic to most carrion beetles, providing yet another advantage for Dipteran larvae. On the other hand, beetles have better sensory capabilities than flies, allowing for the selection of more favorable feeding sites even though they occur at lower abundances (Smith 1986). In terms of collecting these organisms, beetles are much more difficult to procure, due to their lower abundances and mobility, whereas flies and larvae are much easier to capture.

Habitat did not appear to influence the types of blow flies associated with carrion. Although the numbers of certain blow fly species did vary by habitat, such as *L. sericata* and *L. illustris*, they were found in both urban and rural sites (Table 3). Other species, such as *C. macellaria* and *Calliphora* sp. were only collected in the urban and rural site,

respectively, but due to low abundances these species cannot be considered indicators of habitat type.

Overall, the urban treatment demonstrated lower species richness and lower diversity when compared to the rural and urban to rural treatments. Evenness was low in all treatments due to the clear dominance of *P. regina* on all carcasses. Over time, diversity generally increases; a trend that is apparent especially for the rural and urban to rural treatments. These carcasses demonstrate a much higher diversity in the dry remains stage, in particular. This pattern is due to not only the longer time span of the dry remains stage, but also the numerous beetle species present in the rural site throughout this stage.

Although the general trend of diversity increases over time, evenness fluctuates throughout decomposition. In the fresh stage, evenness is highest among all treatments due to the presence of few species, with individuals more evenly dispersed among these species. Evenness does decrease and is especially noticeable in the decay stage for all three treatments, in which there are more species present, but the large abundance of *P. regina* results in a less even distribution of individuals within these species. However, in the post decay and dry remains stages, evenness increases once again. Once the maggots wander off of the carcasses, individuals representing the remaining species are more evenly distributed, which increases the evenness value.

The similarity in insect composition is illustrated in the cluster analyses. In the fresh stage, the urban treatment clustered together due to the presence of the pavement ant on both carcasses. Surprisingly, one rural and one urban to rural carcass also grouped together due to the collection of *P. regina* from both treatments. In the fresh stage, *P. regina* was collected in the rural as well as the urban site, however, in the urban site, this

species was only collected from the urban to rural treatment carcasses. As demonstrated in the cluster analyses, urban and urban to rural carcasses formed one group in the bloated stage, while one rural carcass formed a separate group. The reason for the clear separation of the rural carcass lies in the presence of 5 species of beetle that were not collected from any other carcass during this stage. However, even in these early stages, the urban carcasses were more similar to each other than to other treatments. The urban to rural treatment formed a group within this cluster with one rural carcass, indicating a stronger similarity between the urban to rural carcasses with the rural site than the urban, a pattern that is repeated throughout the later stages of decomposition as well. These similarities between the rural and urban to rural treatments in the bloated stage lies in the presence of similar beetles, ants and blow fly species, whereas the urban treatment differs in ant species present.

In the decay stage, a similar pattern is expressed in which the urban treatment forms one cluster, while the rural and urban to rural cluster together. The rural and urban to rural carcasses share similarities in the large numbers of staphylinid beetles present on both treatments as well as the carrion and silphid beetles not found in the urban site. As decomposition continues, the urban to rural carcasses become more similar to the rural treatment and less similar to the urban treatment, a pattern clearly demonstrated by the cluster analyses from the decay through the dry remains stages of decomposition.

The calculated Jaccard's coefficient of similarity indicates that in the fresh stage there is very low similarity between the treatments, while there is low similarity during the dry remains stage of decomposition. However, in the more active stage of decay, bloated, decay and post decay, more decomposing tissue is present, resulting in a large

population of maggots, most of which represented the species *P. regina*. The dominance of this species along with its equal abundance on all three treatments results in the higher degree of similarity during these stages. These analyses do not indicate a strong pattern of similarity, even though the carcasses were sampled more intensively than practical in an actual investigation.

A possible explanation for the decline in similarity between rural and urban to rural treatments during the post decay and dry remains stage may lie in the presence of fewer maggots at this phase of decomposition and the appearance of a variety of different beetle species, causing the degree of similarity between the treatments to decrease. The similarity between the urban and rural treatments is most likely due to the proximity of the rural site to residential properties; it is probable that synanthropic species (those associated with human refuse) are found in the urban site as well as the rural.

These emerging patterns indicate that in northeast Ohio, when a corpse is moved from an urban to a rural area, the moved corpse quickly begins to mimic rural insect composition and succession and does not bring with it an insect signature. One species of ant was collected in the urban site and is believed to have been transported with the carcasses when moved to the rural location. The presence of this species in both sites may represent a temporary indicator of corpse movement and a short term signature. A low number of species were available to utilize the corpse in the urban site during the first 24 hours, which resulted in no insect signature present. This suggests that the presence of an insect signature may be not be a clear indication of corpse movement in this area of Ohio, although it has been suggested in previous literature. Generalizations made about the use of insects for particular aspects of a forensic investigation must be used with caution.

Using an insect signature to infer movement must be conservative and highlights the need for region specific studies of arrival times and occurrence of insect fauna. Information provided by such studies can be utilized as a database of forensically important insects in various regions and are invaluable to the field of forensic entomology. However, in the application of such information to criminal investigations, although previously suggested, may be difficult here in northeast Ohio. Patterns that arise, such as the dominance of particular species in both the urban and rural settings, would be difficult to present to a jury or to drive a forensic investigation.

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