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Roles and interaction of blow flies (Diptera: Calliphoridae) and introduced fire ants (Hymenoptera: Formicidae: Solenopsis invicta and S. invicta x richteri) in carrion decomposition in the southeastern United States

Grant De Jong

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Invasive fire ants (*Solenopsis invicta* and its hybrid with *S. richteri*) have been reported from carrion in the southeastern United States and are considered a part of the succession community. Alteration of ecological processes by fire ants could affect forensic interpretation of entomological data; therefore, I conducted studies to investigate the relative roles and interactions of fire ants and blow flies in carrion decomposition.

The blow fly species composition in Mississippi has not been studied since 16 species were reported in 1983. Specimens from the Mississippi Entomological Museum were used to update the checklist of the blow flies of Mississippi and produce a photographic key to adults and third instar larvae. A total of 23 species of blow flies are now known or expected to occur in the state.

I conducted an experiment whereby portions of the succession fauna were excluded from access to carrion to study the relative effects of fire ants and blow flies on carrion decomposition and their interactions with each other. Fire ants made lesions in and partially buried carcasses, but their exclusion did not affect carrion decomposition rates; slightly affected the succession community; and strongly affected succession of blow flies, specifically.

Lastly, I collected fire ants from mounds at set distances from carrion and analyzed their guts for pig and blow fly DNA. The probability of detecting pig or blow fly DNA in ants collected directly from carrion increased with each succeeding day, and the probability of detecting either pig or blow fly DNA in ant guts decreased with increasing distance between carrion and the mound. Probability of detecting pig or blow fly DNA in ant guts from ants collected directly from the carcasses was 42% and 33%, respectively.

This study documented that fire ants scavenge on carrion, prey on other members of the succession fauna, and transfer acquired nutrients at least 3 m into the landscape. Thus, fire ants represent a barrier to normal faunal succession patterns on carrion and these delays should be considered by forensic entomologists for postmortem interval estimation.

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and continue there a year, and buy and sell, and get gain: Whereas ye know not what *shall be* on the morrow. For what *is* your life? It is even a vapour, that appeareth for a little time, and then vanisheth away. For that ye *ought* to say, If the Lord will, we shall live, and do this, or that" (Authorized King James Version, James 4: 13-15).

I especially thank my wife, Nikki, for allowing time for me to pursue my longtime dream of obtaining my doctoral degree, encouraging and cheerleading this endeavor. She and my two daughters, Abby and Katie, put up with piglets and DNA samples in the deep freezer (and sometimes the back yard). They also often faced long days and evenings in which I was locked in my office identifying insects in samples or writing and weeks when I travelled to MSU from Pensacola for classes and research. Hopefully, despite a few missed engagements, they realize that I love them and that I tried to make as much quality time as possible for them. ILYMoTTS, Honey!

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LIST OF SYMBOLS AND ABBREVIATIONS

ADD	*Accumulated degree day*
AFPFw2	*Ant, Fly, and Pig forward primer*
ANCOVA	*Analysis of Covariance*
ANOVA	*Analysis of Variance*
AntR2	*Ant reverse primer*
B-C	*Bray-Curtis Similarity Coefficient*
BLAST	*Basic Local Alignment Search Tool*
dATP	*Deoxyadenosine triphosphate*
dCTP	*Deoxycytidine triphosphate*
dGTP	*Deoxyguanosine triphosphate*
DNA	*Deoxyribonucleic acid*
dNTP	*Deoxynucleoside triphosphate*
dTTP	*Deoxythymidine triphosphate*
FIVS	*Forensic and Investigative Services*
FlyR1	*Fly reverse primer*
g	*Gravitational constant*
H'	*Shannon Diversity Index*
J_c	*Jaccard Similarity Coefficient*
MEM	*Mississippi Entomological Museum*

$MgCl_2$	*Magnesium chloride*
MSU	*Mississippi State University*
NWS	*National Weather Service*
PCC	*Pensacola Christian College*
PCR	*Polymerase chain reaction*
PigR1	*Pig reverse primer*
TAE	*Tris/Acetate/EDTA buffer solution*
TBS	*Total body score*
USDA	*United States Department of Agriculture*
USGS	*United States Geological Survey*

Greek

α	*Significance level*
β	*Model variable*
ρ	*Model variable*

CHAPTER I

BACKGROUND AND LITERATURE REVIEW ON CARRION ECOLOGY WITH SPECIAL EMPHASIS ON THE SOUTHEASTERN UNITED STATES

Carrion decomposition has been the subject of many ecological studies dating back to some of our greatest naturalists, fundamentally shaping much of our ecological knowledge. The objective of this chapter is to present as much information as possible on the current state of knowledge on the ecology of carrion decomposition and the individual roles and interactions of blow flies and fire ants in the ecology of carrion decomposition in the southeastern United States, and in Mississippi and the Florida Panhandle in particular.

Carrion as a Medium for Research

Ecologically, carrion, defined as the bodies of dead animals and humans, is a relatively small, discreet, usually unpredictable, nutrient-rich, depletable resource (Barton 2016), making it an ephemeral biogeographic "island" that is best studied from an interdisciplinary perspective. As a sink for energy and nutrients during life, carrion fits in the basal trophic level of "detritus" in many food webs (VanLaerhoven 2016). Coexistence of animal species, both intra- and inter-guild, as well as interactions between taxonomic kingdoms (Animalia, Fungi, Prokaryota, etc.) are easily studied on carrion as they link this resource to the rest of the ecosystem as part of the nutrient cycle (Crippen et al. 2016, Metcalf et al. 2016, Jordan et al. 2016). Competition, predation, and parasitism are all present as ecological constructs within the colonizing community (Merritt and De Jong 2016). Ethological, physiological, and developmental

adaptations of individual species to the relatively short time frame in which decomposing carrion is available and attractive can be studied from both temporal and spatial viewpoints (Tomberlin et al. 2016). Environmental and anthropogenic impacts on these processes can be investigated. Carrion deposited in aquatic biotopes can create a bridge between the terrestrial and aquatic realms, or between marine and freshwater realms.

Among the first naturalists to use carrion as an experimental medium was Francisco Redi (1626 – 1697), the "Father of Experimental Biology", who demonstrated that maggots on meat are derived from flies, disproving the vulgar theory of spontaneous generation at the macroscopic level in the 1600s (Redi 1668). Louis Pasteur (1822 – 1895), best known for disproving spontaneous generation at the microbial level using his famous swan-necked flasks and broth, also studied carrion (Schwartz 2001). [Incidentally, there persists in the 2020s a broadly accepted belief that spontaneous generation of living organisms, termed "abiogenesis" or "chemical evolution," occurred in the distant past by autocatalysis of amino acids, nucleic acids, and phospholipids at the molecular level and then self-organization at the organelle and eventual cellular level (Scharf et al. 2015), despite irreplicability of the process.]

Later studies in Pasteur's life included investigation of the role carrion plays in the epidemiology of anthrax (*Bacillus anthracis* Cohn), a "germ" which had only recently been isolated by the microbiologist Robert Koch (1843 – 1910). In a famous observation regarding "cursed ground", Pasteur found that earthworms in pastures where animals that had died of anthrax were buried had the potential to transmit spores to the ground surface where they could infect other organisms (Schwartz 2001). Similarly, the veterinarian Edmond Nocard (1850 – 1903), a contemporary of Pasteur, is credited with finding that anthrax spores could survive well

over a year in soil after infected carcasses were used as fertilizer (Schwartz 2001), which has led to current research on composting techniques suitable for dealing with disease (Xu et al. 2016).

Since the experimental repudiation of the spontaneous generation of maggots by Redi and of microbes by Pasteur, carrion decomposition and succession research has primarily been a component of hygiene and public health research (e.g., Pennington 1911, Payne 1965, Payne and Crossley 1966). Carrion is regarded as a potential reservoir of microorganism disease agents and as food for vermin that can spread disease. Other diseases potentially spread to humans from handling carrion include rabies virus, tularemia, *Salmonella* spp. and *Escherichia coli* (Migula) Castellani and Chalmers (Xu et al. 2016). Several organisms pathogenic to humans can persist in fecal material perimortally expressed from dead organisms; these include protozoans such as *Giardia lamblia* (Lambl) Kofoid and Christiansen and *Cryptosporidium parvum* Tyzzer or nematodes such as *Ascaris lumbricoides* L.*, A. suum* (Goeze), and *Baylisascaris procyonis* (Stephanski and Zernowski). Furthermore, ectoparasites, such as lice, fleas, or ticks, that were on the previously live organism may transfer to a new host after their host's death, bringing any additional pathogens with them (Morgan 2004). Nevertheless, unless there is considerable exposure and lack of basic health precautions, the risk of disease transmission from carrion to humans normally is quite low (Morgan 2004).

Other studies in carrion ecology have demonstrated the potential for so called "filth flies," comprising primarily the Calliphoridae, Muscidae, and Sarcophagidae, to be efficient vectors of disease (e.g., Bohart and Gressitt 1951). Several species of these flies (e.g., *Musca domestica* L. and *Chrysomya megacephala* F.) regularly breed in carrion and are known to be mechanical vectors of dozens of enteric diseases (Greenberg 1991) or to cause myiasis, the infestation of living tissue by maggots (James 1967).

Although the beginning of study in succession (the process of community change on a resource over time) is normally attributed to plant ecologists, Michaud et al. (2015) argued that the ecological study of succession may have actually had its start in carrion ecology. They pointed to the work of the French entomologist Jean Pierre Mégnin (1828 – 1905), who defined the concept of succession and used succession patterns in his work with medical examiners to help in forensic cases (Mégnin 1894). Since then, most ecological studies involving carrion in the context of forensic entomology have studied faunal succession patterns during decomposition (Benbow et al. 2016).

Several works (e.g., Johnston and Villeneuve 1897, Erzinçlioğlu 1983, Keh 1985, Greenberg 1991, Benecke 2001, Dadour and Harvey 2008) have chronicled the history of forensic entomology, in which carrion ecology is applied specifically for establishing legal witness at a crime scene, from an account in 13th Century China to Europe in the 1860s to a global interest in the present (Tomberlin and Benbow 2015).

Current research ranges from baseline studies in novel geographic regions to increasing accuracy of entomological evidence by examination of local population genetics of the carrion fauna (Tomberlin et al. 2011). Within the past 20 years or so, the field of forensic entomology has expanded from its traditional roots in research on decomposition and succession on human cadavers, investigating homicide, suicide, and accidental deaths, to research on wildlife carcasses to investigate poaching or animal cruelty cases, mass casualties, and entomotoxicological effects and fate of pharmaceutical agents and poisons in succession fauna (Tomberlin and Benbow 2015, Hocking and O'Regan 2016, Anderson 2016).

Human cadavers are generally unavailable for ecological decomposition research in the United States and, as of 2020, are limited to use in eight facilities, many of which are in the

southeastern United States (Wolff 2015, Wescott 2018). These facilities include the University of Tennessee at Knoxville Anthropology Research Center (Knoxville, Tennessee), the Western Carolina University Forensic Osteology Research Station (Cullowhee, North Carolina), the Texas State University Forensic Anthropology Research Facility (San Marcos, Texas), the Sam Houston State University Applied Anatomical Research Center (Huntsville, Texas), the Southern Illinois University Complex for Forensic Anthropology Research (Carbondale, Illinois), the Colorado Mesa University Forensic Investigation Research Station (Grand Junction, Colorado), the University of Southern Florida Forensic Institute for Research, Security, and Tactical Training (Land O' Lakes, Florida), and the Northern Michigan University Forensic Research Outdoor Station (Marquette, Michigan). Researchers utilizing these facilities conduct basic research on decomposition patterns, particularly relevant to their geographic setting, although some have also developed studies within a more focused research agenda, such as cadaver dog training, effects of wind, or interaction between insect and microbial succession fauna. Not all these facilities include entomology in their research programs.

Because experimental studies on human corpses have been limited to these few facilities, animal models have often been substituted. The most common animal model used is the domestic pig, *Sus scrofa* L. (Schoenly et al. 2007, Matuszewski et al. 2019), even though recent research (Wang et al. 2017, Dautartas et al. 2018, Steadman 2018) has identified minor differences in decomposition patterns between human cadavers and pig carrion.

Carrion Decomposition and Succession Studies in the Southeastern United States

Literature on carrion decomposition and insect succession studies conducted in the southeastern United States was found using internet searches, particularly using Google Scholar, EBSCO Search Academic Elite, and PubMed. Search terms included various combinations of

"insect succession" or "carrion decomposition" with the names of states in the southeastern United States (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas). Since carrion succession studies have also become a regular activity in undergraduate and graduate entomology and forensic science courses at major colleges and universities, electronic thesis and dissertation holdings in libraries of universities in the southeastern United States were searched using the terms "carrion," "decomposition", "succession", and "forensic", and combinations thereof. Because of the volume of work conducted in the southeastern United States on carrion decomposition, I opted, in this review, to be more comprehensive on when and where carrion ecology studies were done and who was conducting the research and less focused on the details of each study.

The eminent coleopterist Anne Howden (1927 – 2016) was among the first to study insect relations on carrion in the southeastern United States. She earned her Master of Science degree at North Carolina State College (now, North Carolina State University), investigating succession of beetles on carrion (Howden 1950), before spending the rest of her life working primarily in weevil taxonomy (Curculionoidea). Reed (1958) also pioneered research in insect succession on carrion in North America, with studies on the succession of arthropods on dog carrion in Tennessee. He reported 217 insect species.

Jerry Payne, long-time research entomologist with the United States Department of Agriculture (USDA) Southeastern Fruit and Tree Nut Research Station, Byron, Georgia, and colleagues conducted a series of experiments in the 1960s documenting the decomposition and insect succession on carrion in South Carolina. His project was conducted under the auspices of the U.S. Department of Energy to document what might happen to carrion after a potential nuclear weapon attack (Payne 1965, Payne and Crossley 1966) and is heralded as one of the first

modern detailed studies of insect succession in animal decomposition and the first to use the pig as a model. Although some carrion fauna members were collected by aerial netting or picking specimens from carrion, more complete census data were collected by sprinkling or painting carcasses with phosdrin. Payne and Crossley (1966) reported 522 animal taxa in the succession fauna collected from various stages of decomposition. Subsequent publications highlighted specific treatments, such as burial (Payne et al. 1968a) or submersion (Payne and King 1972), or members of certain taxonomic orders, such as Hemiptera (Payne et al. 1968b), Lepidoptera (Payne and King 1969), Coleoptera (Payne and King 1970), and Hymenoptera (Payne and Mason 1971). The latter publication notably did not include *Solenopsis* spp. among the collected fauna.

In 1971, William H. Bass, a forensic anthropologist at the University of Tennessee, started the Anthropology Research Center near Knoxville, Tennessee, as a research facility for examination of decomposition of donated human corpses. Bill Rodriguez's studies on insect succession on human corpses (Rodriguez and Bass 1983) was one of the first entomological projects to be conducted at the Center. Countless studies have been conducted since, many of which have remained unpublished (Bass and Jefferson 2003, 2007), and dozens of corpses are usually exposed at any given time. Data from multiple studies have been compiled into larger meta-analyses of succession, such as Shahid et al. (2003) and Schoenly et al. (2005), both investigating the hypothesis that the large number of carcasses at the Center affects the community composition of the carrion fauna relative to locations where carrion is less densely distributed.

C. Lamar Meek (1944-2000), entomology professor at Louisiana State University, conducted forensic entomology research in addition to his studies on mosquitoes. He published

over 20 papers on carrion-frequenting insects and participated in several legal cases on homicide and high-profile wildlife deaths. A few researchers carried on with decomposition and succession studies on wildlife carrion in the 2000s, aiming to aid state and federal game agencies with enforcement of wildlife poaching and animal abuse laws. Peters (2003) studied temperature variation in larval fly masses on black bear carrion in Florida; Watson and Carlton (2003) and Watson (2004) investigated insect succession on wildlife carcasses in Louisiana; Nelder et al. (2009) further researched carrion succession, particularly of flies, on alligator carrion in Mobile, Alabama; and Swiger et al. (2014) studied the interactions of *Chrysomya rufifacies* (Macquart) with other Calliphoridae on black bear carrion near Gainesville, Florida. Richards et al. (2015) studied arthropods on wildlife carrion specifically in salt marshes in southern Florida.

Current research programs at a few southeastern United States colleges and universities have assembled large teams of scientists (faculty, staff, graduate students, and undergraduate students) centered on forensic entomology. The two largest formal forensic entomology programs in the southeastern United States are at Texas A&M University and the University of Florida. Texas A&M University maintains a Forensic and Investigative Sciences (FIVS) Program associated with the Department of Entomology; thus, entomological studies in carrion decomposition are an integral and prodigious part of that program. Dr. Jeff Tomberlin, director of the FIVS program, has maintained a consistent presence in carrion research throughout the southeastern United States in Georgia (University of Georgia), South Carolina (Clemson University), and Texas. His research program has focused on the black soldier fly, *Hermetia illucens* (L.), in livestock waste management and the control of fly populations around confined animal facilities. This focus has led to considerable research into forensic entomology, as *H. illucens* is both necrovore and predator in the succession community. As of March 2019, he

had authored or co-authored 139 journal articles, 2 books, and 14 book chapters, most of which are on these topics.

Other carrion ecology studies conducted by entomologists associated with the Texas A&M FIVS Program included geographic distributions (Tenorio 2001), natural variation and ecology (Kirkpatrick and Olson 2002, Mohr 2012, Owings 2012, Liu 2014, Mohr and Tomberlin 2014, Pimsler 2015, Faris 2017, Kotzé and Tomberlin 2020, Lesne et al. 2020), thermal biology of carrion insects (Kirkpatrick 2004, Boatright 2009, Boatright and Tomberlin 2010, Ramos 2015, Thomas 2015, Cuttiford 2017), and interaction of carrion insects with microbes or other carrion fauna during succession (Brundage 2012, Pechal 2012, Tomberlin et al. 2012, Flores 2013, Heo 2016, McKenna 2017, Zheng 2017, Sawyer et al. 2020).

Another program is maintained at the University of Florida, where Dr. Jason Byrd works (current as of August 2020) as an Associate Professor in the Department of Pathology, Immunology and Laboratory Medicine and is currently the Associate Director for the William R. Maples Center for Forensic Medicine. Although Byrd and some students at the university have conducted basic research in carrion decomposition and succession, especially in regard to the effects of temperature on flies of forensic importance (e.g., Byrd 1995, 1998, Byrd and Butler 1996, Gruner 2004, 2014, Peters 2003, Sutton 2017, Swiger 2007), the University of Florida program appears to have become more applied in aiding law enforcement and less basic research oriented.

Sam Houston State University in Huntsville, Texas, maintains the Applied Anatomical Research Center, a research facility in which human decomposition studies can be conducted. Entomological studies conducted in the context of forensic entomology at this research center included Lindgren et al. (2010, 2015), primarily reporting unusual insect activity during

succession, such as partial burial of a corpse by fire ants, or the presence of Syrphidae, Psychodidae, Panorpidae, and Noctuidae on corpses.

At Louisiana State University, a formal forensic entomology program does not exist, but several graduate level forensic entomology studies have been conducted in the context of the Master of Arts (M.A.) degree in anthropology. For example, Watson and Carlton (2003) and Watson (2004), as mentioned above, examined insects associated with pig, black bear, deer, and alligator carcasses. Gremillion (2005) used 24-hour video surveillance to document insect succession and vertebrate scavenging on pig carrion near Quantico, Virginia. Pharr (2009) studied the effects of total concealment from insects, sealing pig carrion in 55-gallon barrels during decomposition. Jones (2011) studied primarily vertebrate scavengers on pig carrion but noted insect activity. Given that Louisiana has > 16,000 km^2 (>4,000,000 acres) of wetlands, representing ~40% of all the wetlands in the United States (Penland et al. 2002), it is natural that several studies at Louisiana State University were conducted studying decomposition and insect succession in aquatic habitats, including Hurst (2001), Renke (2010), Bangs (2014), Farris (2014), and Neuman (2017).

Several other colleges and universities across the southeastern United States have undertaken carrion decomposition and succession studies in the late 1900s despite not having formal forensic entomology programs in place, although some studies have been conducted independent of higher education facilities. Ted Adkins (1930 – 1989) from Clemson University and Edward Mondor from Georgia Southern University conducted carrion decomposition studies primarily in support of their forensic entomology case work (Lord et al. 1992). Other carrion researchers at Clemson University included De Lima Silva (1981), who studied effects and bioaccumulation of trace metals by carrion-attendant beetles at the Savannah River Site, South

Carolina, finding that they may act as sentinel species for habitat quality; Tomberlin and Adler (1998), who described terrestrial and aquatic decomposition of rat carcasses; and Cammack (2009), who investigated the effects of parasitism by the pteromalid wasp *Nasonia vitripennis* (Walker) and soil compaction on pupariation behavior in the blow fly *Lucilia sericata* (Meigen). Cammack continued with studies on decomposition and succession on small pig carcasses under a range of concealment at North Carolina State University (Cammack 2013, Cammack et al. 2016). Kneidel (1984a) reported an unusual occurrence of American dog ticks, *Dermacentor variabilis* (Say), attracted to rat and mouse carrion near Chapel Hill, North Carolina, although they were only common near selected carcasses, while Kneidel (1984b) studied small mammal and invertebrate carrion and its effects on community composition of the carrion-breeding Diptera. Goddard and Lago (1985), at the University of Mississippi, reported on ecological succession of blow flies on pig carrion in northern Mississippi. Benowitz (1990) studied behavioral variation in two species of *Nicrophorus* carrion beetles in Georgia. From 1985 to 1991, Dodd (1995) examined the taphonomic processes at work on turtle shells in north-central Florida, finding that shells underwent easily defined but unpredictable disarticulation stages. Shells remained intact for 12 to 30 months, depending on species, but were nearly always completely disarticulated by about 40 months. Tessmer et al. (1995) studied the circadian rhythms of carrion-frequenting flies in southern Louisiana.

After the turn of the century, Ulyshen and Hanula (2004) reported on diversity and seasonal activity of silphid beetles in northeastern Georgia. Tabor et al. (2004) reported the first study on insect succession on insect carrion from southwest Virginia, collecting 47 taxa in the spring and 33 taxa in the fall, with a statistically significant difference in the succession community between the two seasons. Barwary (2010) studied blow flies on chicken carrion at

various heights above ground in coastal Alabama. Goddard et al. (2012) documented succession on pig carrion at Mississippi State University as part of an undergraduate forensic entomology class. Lashley et al. (2017) used ~2,700 kg of feral swine carcasses to simulate a mass mortality event on forest land in Mississippi, finding large ecological effects from insect- and vertebrate-mediated dispersal of nutrients from pig carrion to the surrounding landscape. A study at North Carolina State University reported on a succession study on pig carrion, the effects of relocating decomposing carrion on the succession, and comparisons of various sampling techniques (Cruise 2017, Cruise et al. 2018a, b, c, 2020). Grow (2017) reported on succession fauna on 2 pig carcasses in northwest Florida. De Jong (2018) documented the presence of necrophagous insects in the diet of cottonmouth snakes (*Agkistrodon piscivorus* Lacépède) attracted to chicken carrion in coastal South Carolina swamps. Peoples and Payne (2018) reported on an undergraduate entomology class activity which followed the succession on 7 pig carcasses at the University of West Georgia. At Mississippi State University, Meyer et al. (2020) used time-lapse photography to document that fire ants (*Solenopsis invicta x richteri*) created lesions in the skin of decomposing pig carrion, and the lesions were subsequently used as oviposition and development sites for necrophagous Diptera. Lastly, a note was published on observations made tangential to the present study on unidirectional *en masse* migration of blow fly larvae from a pig carcass in Mississippi (Goddard et al. 2020).

Case studies of entomology used in a forensic context within the southeastern United States were uncommonly published unless they provided a novel observation. For example, Lord et al. (1992, 1994) described case studies from South Carolina in which non-calliphorid flies, such as *Synthesiomyia nudiseta* (Van der Wulp) or *H. illucens* were used as forensic indicators. Bucheli et al. (2009, 2010), reported a case where tineid moths comprised the majority of the

specimens collected on a human corpse and incorporated human hairs into their larval shelter; the hairs were sufficiently intact to extract and amplify mitochondrial DNA for identification purposes. Pimsler et al. (2016) reported an association of the mite *Myianoetus muscarum* (L.) on *S. nudiseta* on human corpses found indoors in Texas. The low number of published case studies is likely due in part to the legal status of each situation and because forensic entomology is becoming more commonly used in a routine manner without novel observations or insight.

The Process of Carrion Decomposition

Like many biological processes, carrion decomposition and succession have defined start and stop endpoints (death and "nothing remains", respectively), but the processes themselves are a continuum of changes (Schoenly and Reid 1987, Wescott 2018). Although, by definition, a continuum does not have start and stop endpoints for component seres or stages, the continuum identified for carrion decomposition and faunal succession has often been divided into recognizable stages for reference.

The first researcher to identify stages in carrion decomposition and succession was Yovanovitch (1888). Subsequent researchers have used various numbers of stages from two (Howden 1950) to eight (Mégnin 1894), but studies in the southeastern United States commonly range from four to six stages (e.g., Reed 1958, Payne 1965, Goddard et al. 2012, Cruise et al. 2018). The following descriptions of five stages (number based on preliminary work in Mississippi) roughly follows Keough et al. (2017), which used four stages, and apply specifically to the decomposition of pig carrion.

The "fresh stage" is characterized as the beginning of decomposition immediately following death. The fresh stage continues until the first signs of bloating begin. There are relatively few external changes occurring during the fresh stage, and the odor associated with the

remains is the natural smell of the body and may include some putrefactive odors. Livor mortis (the pooling of blood in inferior portions of the body), rigor mortis (the stiffening of limbs), and algor mortis (the cooling of the body to approximately ambient temperature) are processes of decay that occur during the fresh stage.

In the "bloating" stage, gases such as carbon dioxide, methane, hydrogen sulfide, and ammonia build up in the coelom and distend the body. Measurements of girth may be necessary to identify the beginning of this stage, as gross observation is often unable to detect the slight change in circumference. The skin begins to change color, often to green, and exhibits a marbling effect. During the bloating period, the body begins to purge decomposition fluids from natural orifices, and blood is sometimes released from the nose. Bloating can occur rapidly in warm temperatures and last 2-5 days, however, if the ambient temperatures are cooler, the carcass may experience numerous cycles of partial bloat and deflation.

After the bloat stage is the "active decay" stage, in which the skin cracks and abdominal gases release, decreasing the abdominal cavity in size. The skin will darken from the green hue to a brown and/or black appearance. Internal organs are generally unrecognizable as the decomposition and succession fauna liquify and mix the tissues. Superficial tissues of the limbs desiccate and have a leathery texture and appearance. Based solely on physical characteristics of the carcass, it may be difficult to recognize the end of this stage and the beginning of the next, but the "active decay" stage is generally regarded in Mississippi to end with the emigration of maggots from the carcass.

"Advanced decay" occurs when deflation has completely ended, superficial tissues sag, and the abdominal cavity has a sunken appearance. The tissues of the eyes and throat have a sunken appearance, and the skin takes on a "wet" appearance where the liquefaction and

disintegration of tissue begins. The abdominal cavity remains moist while other areas of the body such as the extremities may exhibit mummification or partial skeletonization, depending upon external environmental conditions. The skin may change colors periodically and range from dark colors such as brown, red, and black to light colors such as light brown, tan, and orange. The odor of decay during this phase is strong and putrid and can be detected over long distances.

The "skeletonized remains" stage occurs when the majority of soft tissue has decomposed or when mummified tissue begins to break down to reveal bone. Odor is minimal and takes on a musty, moldy, or ammoniacal smell. Middle phases of skeletonization include bones that are dry but retain grease and bones that are completely dry with little to no soft tissue adhering. Complete skeletonization can take place as quickly as two weeks in hot and humid environments and is characterized by the total lack of soft tissues. This stage ends as the bones themselves are dispersed and broken down.

Since the decomposition stages fall along a continuum, identification of the boundaries of each stage is obviously subjective. Protocols involving observable changes have been developed as a "Total Body Score" (TBS) to identify stages more objectively in human corpses and pig carrion (Galloway et al. 1989, Megyesi 2005, Myburgh et al. 2013, Chee Hau et al. 2014), Keough et al. 2017). These protocols generally include a process in which the condition of the carcass is scored according to multiple criteria to identify more objectively the stage to which a carcass has attained.

Although insect taxonomic groups are sometimes associated with particular stages, such as the arrival of blow fly larvae during the "fresh" stage, migration of blow fly larvae from the carcass at the end of the "active decay" stage, or arrival of trogid beetles during the "skeletonized

remains" stage, changes in the succession community may or may not correspond to stages during decomposition (Schoenly and Reid 1987).

The Carrion Succession Community

Organisms that make up the succession community can be as widely divergent as microbes (which can decompose a carcass by themselves, albeit incredibly slowly) and vertebrates (which can sometimes devour a carcass in its entirety within seconds, minutes, or hours), and these play important parts in the ecological succession on carrion resources. However, most press goes to the insect succession community, particularly the flies and beetles. I have elsewhere assembled a review of the primary groups of arthropods found on carrion in terrestrial habitats (Merritt and De Jong 2016), noting that the aforementioned flies and beetles comprise the primary groups that utilize carrion, but that there are also other groups such as bees, mites, parasitic Hymenoptera, and ants.

In most (but certainly by no means all) situations, blow flies (Calliphoridae) are the first and most important component of the succession community on a carcass. Blow flies can arrive within minutes or seconds after death (De Jong 1994). Furthermore, blow fly larvae usually consume most of the carcass (Putman 1978).

Blow fly Community of Mississippi and the Florida Panhandle

A checklist of 15 blow fly species known to occur in Mississippi was assembled by Goddard and Lago (1983), with only one species added since then (Goddard et al. 2015). Chapter II of this dissertation updates that checklist and provides identification keys to the adults and third instar larvae of all species known to occur or expected to occur in Mississippi. Based on

known distributions of blow flies across the United States (Whitworth 2006), a similar suite of species is known or expected to occur in the Panhandle region of Florida (Grow 2017).

Knowledge of the local blow fly fauna is important because they are primary decomposers of carrion in the southeastern United States (Reed 1958, Payne 1968, Goddard et al. 2012, Merritt and De Jong 2016). Blow fly larvae consume the majority of biomass in a carcass (Putman 1978), dispersing those nutrients into the landscape as they themselves migrate from the carcass, pupate, and eclose as dispersing adults (Goddard et al. 2020).

The bionomics and developmental rates of several common species of blow flies have been determined experimentally, often at a range of temperatures (e.g., Kamal 1958 and Anderson 2000 for *P. regina*, *Lucilia sericata* (Meigen), and *C. vicina*; Weidner et al. 2014 for *L. coeruliviridis* Macquart; Fisher et al. 2016 for *C. vicina*, *C. macellaria*, and *P. regina*). Thus, an estimate of the time required (in solar days or accumulated degree days [ADD] or another measure of time) for individuals of a species to have achieved a particular life stage (larval instars, pupa, adult) can be determined. Therefore, since blow flies can arrive at carrion within minutes of death, information that combines age of blow fly larvae on a carcass and ambient meteorological conditions is often used to estimate the "period of insect activity" or "post-colonization interval", which in turn can aid in estimation of the postmortem interval (PMI), or time since death (Anderson 2016).

PMI estimates can be affected by numerous factors. Being that blow flies are ectothermic organisms, temperature is a primary factor (Wardle 1930, Kamal 1958): blow fly larvae growth rates are generally proportional to the ambient temperature; however, use of ADD or ADH can help account for fluctuations or differences in temperature. Other factors usually involve restriction of access to carrion, such as burial of the carcass, enclosure of the carcass (e.g.,

wrapping in a blanket), submersion in water, etc. Such restriction of access delays oviposition by blow flies, so the "pre-colonization interval" is elongated and the PMI is subsequently also elongated (Anderson 2016). Determination of the PMI based solely on the development of blow fly larvae and not taking into account the possibility of restricted access would underestimate the true PMI.

Previous observations on fire ants and carrion are discussed below, but briefly, they can involve the buildup of soil on carcasses (effectively burying at least part of it) and preying on colonizing blow flies. Each of these activities has the potential to restrict blow fly access to carrion and, therefore, elongate the postmortem interval. Again, determination of PMI based on blow fly larval development would underestimate the true PMI if fire ant presence restricted blow fly access to carrion.

Ant Relations with Carrion

Ants in general

Fuller (1934) proposed that ants, in general, were not part of the normal carrion community, and their presence on carrion was only due to carrion incidentally being near ant mounds. Since then, ants have become recognized as common and often important members of the carrion community (Payne et al. 1968, Payne and Mason 1971, Anderson and VanLaerhoven 1996, Campobasso et al. 2009, Heo et al. 2009, Merritt and De Jong 2015, Neto-Silva et al. 2017, Mashaly et al. 2018). Eubanks et al. (2019) produced a literature review on 64 papers between 1956 and 2018 addressing ant relations with carrion, noting that they are generally considered to be directly feeding on carrion, altering the carrion to access liquids or prey items, or preying on other members of the succession fauna. Based on supplementary data in Eubanks et al. (2019), the number of publications discussing ants on carrion has approached an

exponential cumulative distribution curve (number of publications = $1x10^{-48}e^{0.0566 \times year}$, $r^2 = 0.982$) since 1956, the date of the first study considered in Eubanks et al (2019) (Figure 1).

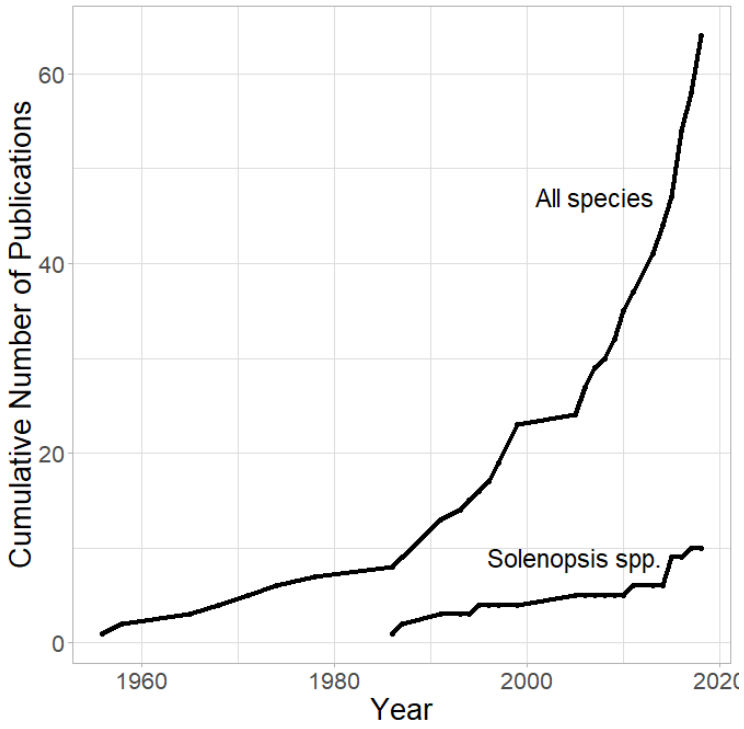

Figure 1 Cumulative number of publications between 1956 and 2018 in which ants were reported on carrion.

Drawn using data from Eubanks et al. (2019).

Ramón and Donoso (2015) briefly reviewed the forensic utility of ants, recognizing their importance as predators on other succession fauna, as agents of changes in decomposition processes, and even as agents of death themselves through stings. Byard (2004) and Heath and Byard (2014) presented case studies from Australia in which postmortem lesions caused by ants either did confuse or could have confused autopsy findings; species identity of the ants in those case studies was not reported.

Ants arrive early in the decomposition process (Goddard et al. 2012) and can overwhelm carcasses to the exclusion of other carrion fauna (De Jong and Hoback 2006, Lindgren et al. 2010, Merritt and De Jong 2016, Barton et al. 2017). However, ants can be found on carrion in all stages of decomposition (Eubanks et al. 2019). For example, Heo et al. (2009) found ants present at all stages of decomposition in Malaysia, where they captured and presumably fed on fly eggs, larvae, pupae, and adults. Paula et al. (2016) considered ants to be omnivores, necrovores, and predators on carrion-visiting insects in studies on the role of 33 species of Formicidae in carrion decomposition and their effects on blow fly succession in Brazil. They found that ants can significantly alter decomposition time, slowing it down by preying on other succession organisms, or alternatively, accelerating decomposition time by creating holes in the surface and entry points for other succession fauna or even by consuming all or part of the carcass themselves. Leaf-cutting ants in the genus *Atta* have recently been reported cutting clothing on a human cadaver in addition to producing lesions on the body in Brazil (da Fonseca de Souza et al. 2020). Several species of ants were recently reported from pig carrion in South Korea, with some preying on fly eggs and early instar larvae, as well as producing lesions or covering portions of the carcasses with soil (Park and Moon 2020a, b). In a laboratory-based study, Nappi (2018) found that the western harvester ant, *Pogonomyrmex occidentalis* (Cresson), prioritized predation on *L. sericata* eggs on pig liver, even though it was attracted to the liver with or without blow fly eggs present. In Mississippi, it has been thought that fire ants may accelerate decomposition through feeding on carrion and producing lesions that provide additional oviposition sites for blow flies (Meyer et al. 2020)

Fire ants

While ants have long been reported on many types of animal carcasses and in all stages of decomposition, introduced fire ants have only recently been associated with vertebrate carrion in North America (Stoker et al. 1995, Lindgren et al. 2010, Eubanks et al. 2019). *Solenopsis richteri* Forel, the black imported fire ant, was introduced to North America from South America apparently at the port of Mobile, Alabama, about the year 1918 (Tschinkel 2006). A second fire ant species, *S. invicta* Buren, the red imported fire ant, was introduced to North America at Mobile, Alabama, sometime between 1933 and 1945; a range of dates is given because of taxonomic confusion between the species that was finally resolved in Buren (1972). Both species have spread throughout the southeastern United States, with *S. richteri* found in 6 states from Arkansas and Mississippi to Virginia and *S. invicta* found in 16 states from California to Florida, north to Illinois, Missouri, Tennessee, and Virginia. A hybrid of *S. invicta* x *richteri* is distributed in an irregular, latitudinally dynamic band in central Alabama and Mississippi where populations of the two species overlap (Callcott and Collins 1996, MacGown and Forster 2005, Tschinkel 2006, MacGown 2016).

Two native species of *Solenopsis* exist in the southeastern United States, including *Solenopsis molesta* (Say), and *Solenopsis xyloni* McCook; these ants are sometimes referred to as "thief ants" (Tschinkel 2006). The Neotropical *Solenopsis geminata* (Fabricius) has also been introduced to North America, while approximately 190 other described, but taxonomically difficult, species of *Solenopsis* occur worldwide (Bolton 1994). It appears that the introduced fire ants may be ecologically displacing the native species of *Solenopsis* and other ant genera in some locales (MacGown 2016).

The potential forensic importance of introduced fire ants in carrion ecology was recognized first in the 1980s when Early and Goff (1986) and Tullis and Goff (1987) found contrasting effects of fire ants on carrion in the Hawaiian Islands (Table 1). Greenberg (1991) used those observations in a review of the use of Diptera as forensic indicators to report that fire ants aggressively prey on eggs and larvae of carrion flies. It is unknown why the presence of fire ants at carrion in the southeastern United States was not reported from their introduction in the 1930-40s to about 1990. Since then, however, introduced fire ants have come to be recognized as normal components of the carrion fauna in the southeastern US (Merritt and De Jong 2016), and reports of their presence at carrion has increased (Figure 1).

Table 1 Recent studies on fire ant (*Solenopsis* spp.) associations with carrion.

Citation	Location and carrion	Type of Study	Findings	Limitations
Early and Goff 1986	Hawai'i on pig	Unreplicated (3-pig) Experiment	*S. geminata* arrived d 1 and preyed heavily on other fauna and delayed "bloat" and "active decay" stages	*S. geminata*; distant location
Tullis and Goff 1987	Hawai'i on pig	Unreplicated (3-pig) Experiment	Few *Solenopsis*, late in succession, no significant effect	*S.* sp. B; distant location
Wells and Greenberg 1994	Texas on pig	Replicated Experiment	*S. invicta* increased gaps in which adult flies not found	Adult flies
Stoker et al. 1995	Mouse and chicken in Texas	Replicated Experiment	*S. invicta* completely excluded fauna from mouse carrion and reduced numbers of Diptera and Coleoptera on chicken carrion	Two cages fully loaded with carrion and individual carcasses removed daily; artificial substrate
Moura et al. 1997	Rat in Brazil	Unreplicated experiment	*Solenopsis* sp. chewed lesions into carcasses	Unreplicated; rat carrion; distant location
Lindgren et al. 2010	Human in Texas	Case study on experiment	*S. invicta* excluded fauna by burying large portions of corpse	Case study
Pechal et al. 2015	Human in Texas	Case study on experiment	*S. invicta* enlarged lesions made by a katydid	Case study
Maciel et al. 2015	Cat in Brazil	Case study	*S. saevissima* buried part of carcass, delaying Diptera succession and oviposition	Case study; distant location
Pereira et al. 2017	Cat in Brazil	Case study	*S. saevissima* buried part of carcass, delaying Diptera succession and oviposition	Case study; distant location
Mendonça et al. 2019	Opossum in Brazil	Case study	*S. saevissima* buried part of carcass, delaying Diptera succession and oviposition	Case study; distant location
Meyer et al. 2020	Pig in Mississippi	Replicated experiment	*S. invicta x richteri* made lesions in carcasses, which Diptera eventually used for oviposition	3 reps over 2 years; Diptera not identified

While many carrion succession studies merely mention the presence of introduced fire ants, and some authors speculate on their role as predators of other members of the succession fauna (e.g., Watson and Carlton 2003, Pérez et al. 2005, Goddard et al. 2012, Lindgren et al. 2015, Richards et al. 2015), only two studies have attempted to experimentally study the specific

effects that introduced fire ants have on the successional population dynamics of other species in North America.

After Greenberg (1991) cited personal communication from Jeff Wells regarding the presence of *S. invicta* on goat and rabbit carrion in Texas, Wells and Greenberg (1994) reported that *S. invicta* appeared to disrupt the normal succession of the blowflies *C. macellaria* and *C. rufifacies* by increasing the number of "gaps" in which the adult flies were not collected at carrion (which could, in turn, affect forensic interpretation of the entomological evidence). Although actual predation was not reportedly observed by Wells and Greenberg (1994), it was thought that ant predation during relatively vulnerable periods of larval life was, in part, how *S. invicta* affected succession patterns. VanLaerhoven (2016) considered the results from Wells and Greenberg (1994) to represent one of the few rare instances where direct predation by one species on other members of a community has produced a measurable effect on species diversity and dynamics of that community. Stoker et al. (1995) examined effects of *S. invicta* on the succession community by excluding fire ants from mouse (*Mus musculus* L.) and chicken (*Gallus gallus* L.) carrion. On mouse carrion, fire ants completely excluded all other succession fauna, while on chicken carrion, fire ants reduced numbers of adult Diptera and Coleoptera that were collected and also disrupted development of Diptera larvae late in succession. Sawyer et al. (2020) are currently conducting research on *Solenopsis* on carrion in Texas. Park and Moon (2020b) warned about the forensic implications of ant disruption in normal succession patterns.

Concerning feeding by ants on carrion, Moura et al. (1997) reported that fire ants were collected at rat carrion in Paraná, Brazil, and described that they had made post-mortem "artifacts" on the carcasses. Similarly, Pechal et al. (2015) reported that *S. invicta* constructed dirt mounds on a human corpse exposed experimentally in Texas and fed on tissues exposed by

feeding of *Peliodectes haldemani* (Girard), a katydid, on the corpse. This unusual activity was discussed in relation to post-mortem disturbances of carrion and potential misinterpretation of markings on a corpse. Lindgren et al. (2010) reported a case study in which a human corpse, also experimentally exposed in southeast Texas, was colonized by *S. invicta*, excluding normal carrion fauna by burying portions of the corpse and feeding on it. This was a novel observation in comparison to previously observed fire ant behavior at carrion in which they apparently preyed on other carrion fauna or just created small holes in the carcass surface.

Recent observations on a conspecific fire ant, *S. saevissima* (Smith), in Brazil also noted the behavior of partially burying carrion. Maciel et al. (2015), Pereira et al. (2017), and Mendonça et al. (2019) opportunistically found *S. saevissima* on individual carcasses of cat, *Felis catus* L., and big-eared opossum, *Didelphis aurita* Wied-Neuwied, each noting that that the ants' activity of burying portions of the carrion appeared to delay succession by necrophagous Diptera and decomposition of the carrion. Meyer et al. (2020) used time-lapse photography to document that fire ants (*Solenopsis invicta x richteri*) created lesions in the skin of decomposing pig carrion, and the lesions were subsequently used as oviposition and development sites for necrophagous Diptera.

Despite these observations, direct trophic evidence that fire ants feed on carrion itself or on other members of the succession fauna is specious. In fact, it is reasonable to presume that fire ants forage on carrion and prey on flies to provide protein for their larvae via trophallaxis. Vinson (1968) and Petralia and Vinson (1978) demonstrated that the only life stage of fire ants to consume solid protein was 4th instar larvae. Tschinkel (2006) reported that fire ants collect proteinaceous foodstuffs, such as dead insects, and stockpile them near the nest to dry and use as food later. Foraging adult ants did not include significant amounts of protein in their diets and

instead fed primarily on a liquid diet high in oils and sugars, while solid protein materials were filtered out in the infrabuccal cavity (Petralia and Vinson 1978).

The infrabuccal cavity is an invagination of the oral cavity of some Hymenoptera at the anterior end of the pharynx; it is lined with long hairs that filter solid ingested material > 0.88 μm in diameter and concentrates it in an infrabuccal pellet while liquid material is passed through the narrow pharynx to the crop and gut located in the gaster (Glancey et al. 1981, Vinson 1983, Hölldobler and Wilson 1990, Tschinkel 2006). It is possible that protein and other substances suspended in liquid form could pass on to the gut. The infrabuccal pellet is egested on a daily basis, presumably in the presence of 4th instar larvae (Hölldobler and Wilson 1990).

Goals for the Dissertation

Fire ants certainly are attracted to carrion, whether for feeding on the carcass or on prey items or to stockpile foodstuffs for later use or to deliver nutritive materials to their larvae. In this dissertation, I attempt to investigate and elucidate some of the roles that introduced fire ants play in carrion decomposition (i.e., their direct effects) in the southeastern United States, and secondly, their interaction with blow flies, which are often the most common and abundant members of the carrion succession fauna. This work involves an update to the checklist of the blow fly species known from or anticipated to occur in Mississippi, where much of this work was conducted; in addition to the checklist, identification keys to the adults and third instar larvae of those blow fly species is included in Chapter II. In Chapter III, I describe an experiment that selectively excluded members of the succession fauna (i.e., fire ants and blow flies) to identify potential roles of fire ants, looking specifically at their effects on physical decomposition, the succession community at large, and blow fly succession in particular. Lastly, in Chapter IV, I describe an experiment in which molecular techniques are used to detect pig and

blow fly DNA in fire ant gastrointestinal tracts to conclusively demonstrate that they are feeding on these organisms, as well as determining distances to which fire ants forage from their nests to access carrion or prey on carrion-attendant fauna.

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CHAPTER II

UPDATED CHECKLIST AND IDENTIFICATION KEYS TO THE BLOW FLIES (DIPTERA: CALLIPHORIDAE) OF MISSISSIPPI

Introduction

Calliphorid flies, also called "blow flies", are ecologically important (Merritt and De Jong 2016). In carrion decomposition, they are among the first arthropods to arrive after death of an organism and can be utilized by experts in establishing legal witness in a crime scene. In addition, blow flies are among the primary species causing myiasis in vertebrates and can mechanically spread several enteric disease pathogens, making them important in medical and public health entomology. *Pollenia* spp., which may occasionally be found at carrion as adults (Merritt and De Jong 2016), are obligate endoparasites of earthworms (Annelida: Oligochaeta) as larvae (Hall 1948). Correct knowledge of the local blowfly fauna is fundamental to correctly using these insects as entomological evidence in legal casework or addressing medical or public health concerns.

The Calliphoridae, as it is presently recognized, has been determined to be polyphyletic (Rognes 1997), and the phylogenetic relationships of the traditionally included subfamilies remains uncertain (Singh and Wells 2013). Each subfamily, however, appears to be monophyletic, with the exception of Melanomyinae possibly nesting within Calliphorinae (Singh and Wells 2013). Subfamilies that are most in question as to their phylogenetic relationship to Calliphoridae (e.g., Toxotarsinae, Bengaliinae) do not occur in the southeastern United States.

Nevertheless, I use the current classification system with Calliphorinae, Melanomyinae, and Chrysomyinae as subfamilies of Calliphoridae.

The blow fly fauna specific to Mississippi was first investigated by Goddard and Lago (1983), with 15 species reported, including the screwworm, *Cochliomyia hominivorax* (Coquerel), which had already been extirpated from the state, and an unnamed species of *Protocalliphora* Hough. Since then, Sabrosky et al. (1989) formally named the unnamed species of *Protocalliphora* as *Protocalliphora deceptor* Sabrosky, Bennett, and Whitworth, a species distributed across the southeastern United States north to Missouri, Ohio, and Connecticut. More recently, Goddard et al. (2015) reported *Chrysomya rufifacies* (Macquart) from the state of Mississippi. Barwary (2010) reported 11 of these species from neighboring coastal Alabama.

Most Nearctic calliphorid species are necrophagous, but a few species are only rarely or never found at carrion. Members of the *Pollenia rudis* group are parasites of earthworms but the adults are occasionally found near carrion; the reason for the association with carrion is unknown (Merritt and De Jong 2016). The *Protocalliphora* are parasites of birds and may be found with nestlings that had died as a result of an infestation (Sabrosky et al. 1989). The rarely collected members of Melanomyinae are suspected to be parasites of snails, based on known habits of Palearctic melanomyine species (Downes 1986).

Hall (1948) and Shewell (1987) provided identification keys to the Nearctic Calliphoridae; however, the identification keys in Hall (1948) are outdated and have proven difficult to use (Whitworth 2006), and Shewell (1987) only provided keys to the adults at the generic level. Whitworth (2006) and Jones et al. (2017) produced dichotomous identification keys to adults of 53 species and 41 species, respectively, of the 93 Nearctic species of Calliphoridae, including all species of forensic importance and all 22 species now known from or

suspected to occur in Mississippi (see Results). Additional useful key characters for determining many Nearctic species were included in Whitworth (2010). The most recent comprehensive identification keys for larvae are in Hall (1948); while many other published keys for larvae just treat selected taxa based primarily on their propensity to cause vertebrate myiasis or affiliation with carrion (e.g., Knipling 1936, 1939, Wells et al. 1999).

Identification keys that include only the Mississippi calliphorid fauna have not previously been produced. It is beneficial to have regional identification keys because they are more efficient for routine use by local naturalists, medical and veterinary entomologists, and public health investigators than comprehensive keys that include many more taxa than occur in the region. Therefore, the objective of this chapter is to update the checklist of the Calliphoridae of Mississippi and provide illustrated, dichotomous identification keys to adults and known third instar larvae.

It is understood that blow fly species not included in this list may expand their distributions into Mississippi. Restriction of the scope of the identification keys to include only species in the checklist may allow misidentification of adventive blow fly species. Users of these keys are encouraged to verify identifications with broader taxonomic works (e.g., Hall 1948, Sabrosky et al. 1989, Whitworth 2006, Jones et al. 2019), especially with questionable specimens.

Materials and Methods

Adult blow fly specimens in the Mississippi Entomological Museum (MEM) at Mississippi State University (MSU) were examined and identified using existing literature, primarily Hall (1948) and Whitworth (2006). The MEM includes the recently acquired insect collection from the University of Mississippi. Many specimens had already been identified by

Terry Whitworth, Puyallup, Washington, a recognized world expert on this group, or identified by Jerome Goddard and verified by Terry Whitworth or Raymond J. Gagné, USDA Systematic Entomology Laboratory, Washington, DC. Nevertheless, I reexamined all specimens in the MEM. Records were compiled to update the county-level distributional, phenological, and habitat data provided in Goddard and Lago (1983). Distributions were mapped to determine possible correlations with United States Geological Survey (USGS) Level IV Ecoregions (Appendix A). As of 1 August 2020, a total of 1,018 adult blow fly specimens from Mississippi was examined, and a list of all adult specimens examined is included in Appendix A. A total of 1,226 third instar larvae were also examined, but they represented only four species.

Key identifying characteristics of adults were photographed from specimens in the MEM; additional photographs are available in Jones et al. (2019). Key characteristics of third instar larvae were photographed if specimens were available from the author's personal collection, or published photographs were used with permission or through Creative Commons licensing if specimens were not available. It should be noted that not all characters were photographed, and most larval specimens that were photographed were not from Mississippi.

Lateral views of cephalopharyngeal sclerites were drawn from specimens in the author's personal collection. Reasons that larvae were not available included extirpation from the United States (*Cochliomyia hominivorax*), having a parasitic lifestyle (*Pollenia rudis*, *Protocalliphora deceptor*, and the Melanomyinae), or being naturally uncommon in the area (*Lucilia cuprina*). Figures were not provided to illustrate abdominal cuticular spine distribution patterns.

The photographs and drawings were incorporated into an illustrated, dichotomous key for identification of the species known or anticipated to occur in the state of Mississippi. The keys were primarily based on existing literature (Knipling 1936, 1939, Hall 1948, Kano and Sato

1952, Yahnke and George 1972, Downes 1986, Wells et al. 1999, Wallman 2001, Whitworth 2006, 2010, Tantawi et al. 2017, Jones et al. 2019) and available specimens, and key couplets for taxa for which specimens were not readily available were distilled from existing literature. The Melanomyinae are rarely collected, but identification keys for the Nearctic fauna are available in Downes (1986).

Figures 2 and 3 illustrate the diagnostic characters used in the key to adults. Photographs illustrating these characters are also available in Jones et al. (2019). Genitalic characters, while of great taxonomic value, were not incorporated into the key to adults to make the key easier to use for routine identifications.

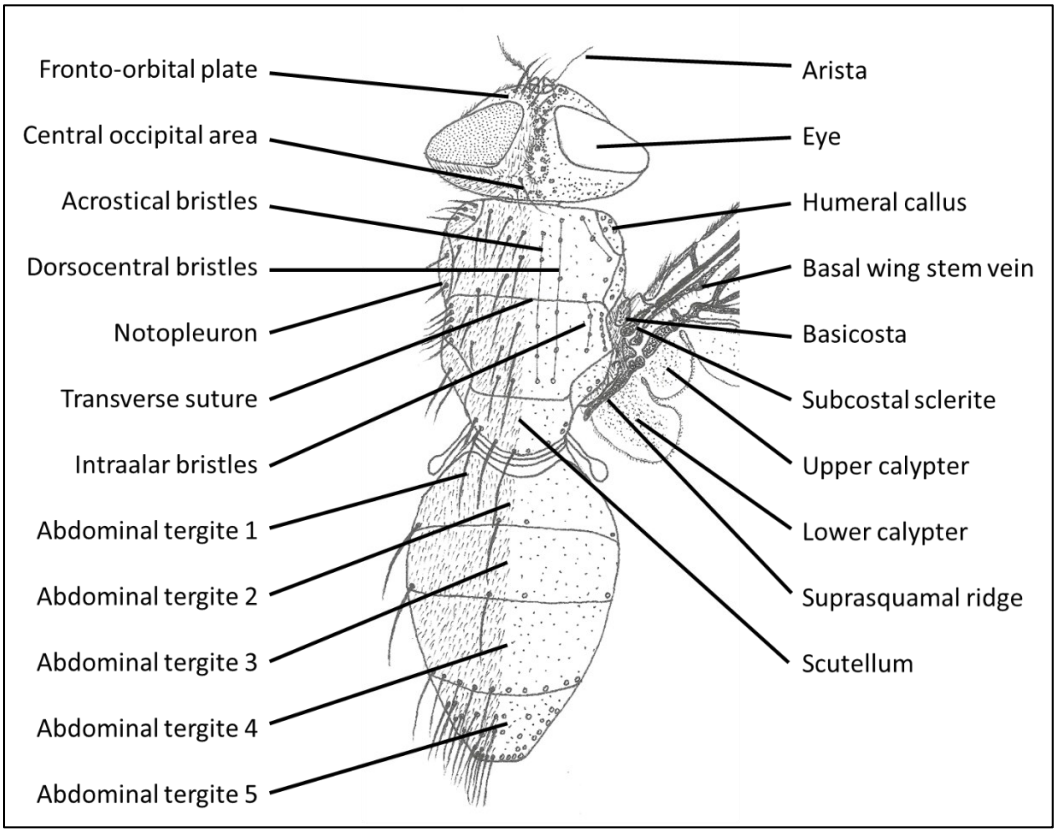


Figure 2 Diagrammatic dorsal view of generalized blow fly, with characteristics used in the key.

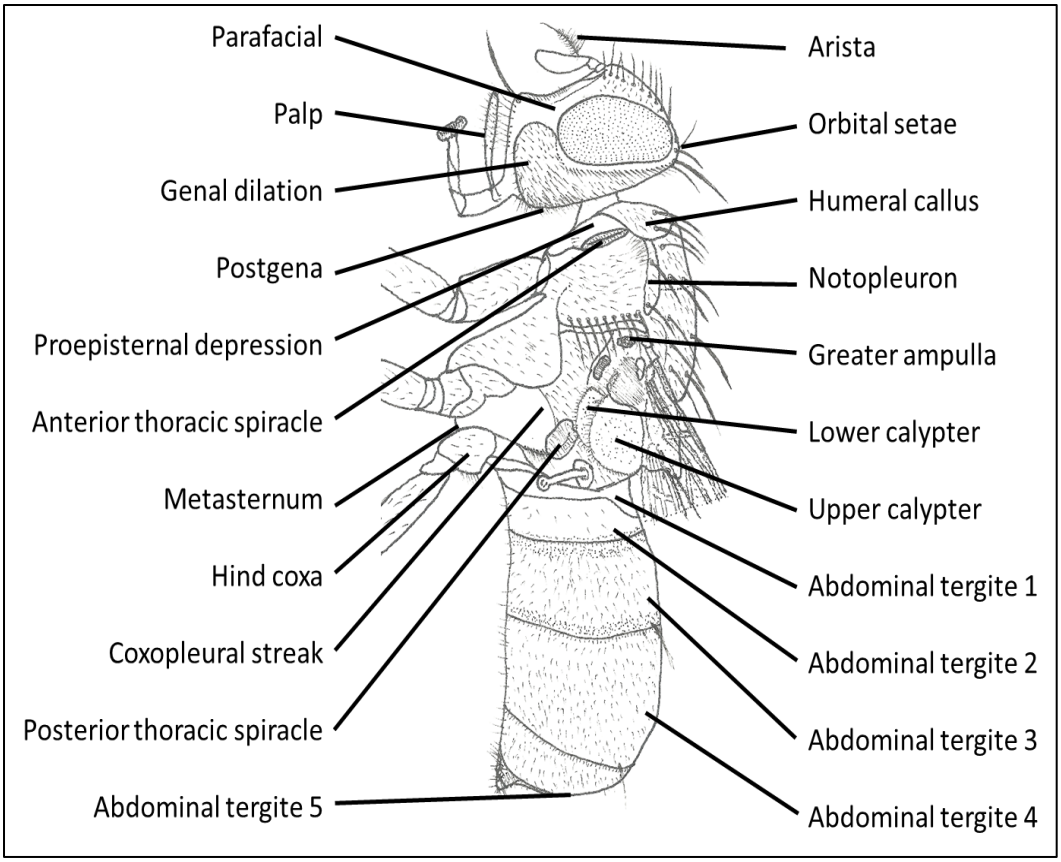


Figure 3 Diagrammatic lateral view of generalized blow fly, with characteristics used in the key.

Results and Discussion

A revised checklist of the 22 blow fly species known from or expected to occur in Mississippi is produced here. Of these, 21 species are known to occur in the state, and *Opsodexia grisea* is anticipated to occur there. Overall geographic distribution and nearby records of the "expected" species *O. grisea* are provided to justify inclusion in this list. Five of these species, known or expected to occur in Mississippi, are naturally rare species in the subfamily Melanomyinae and are not necrophagous. *Pollenia rudis* is not necrophagous as larvae but can be found at carrion as adults (Merritt and De Jong 2016). As noted in Goddard and Lago (1983), *Cochliomyia hominivorax* has been extirpated from the state, and records are historical only.

During the course of this study, I was unable to examine specimens of *Calliphora terraenovae*, *Lucilia silvarum*, and *Pollenia rudis* that had been collected in Mississippi, although these widespread species had previously been reported from the state (Goddard and Lago 1983) and reasonably could occur there. For example, Jewiss-Gaines et al. (2012) reported *P. rudis* as far south as Georgia and in neighboring Tennessee, while Whitworth (2017) reported *C. terraenovae* in North Carolina. Specimens of these uncommon species that were the basis of records in Goddard and Lago (1983) had been retained by specialists who confirmed those identifications (Jerome Goddard, personal communication).

The current total of 21 species known to occur in Mississippi includes the following five new state records.

Chrysomya megacephala, the Oriental latrine fly, is known from Alabama, Arkansas, Florida, Georgia, Louisiana, North Carolina, South Carolina, Tennessee, and Texas within the southeastern United States (Wells 2000, Cobb and Reeves 2005, Whitworth 2019), but is widespread throughout tropical regions worldwide. Despite its widespread presence in the southeastern United States, it appears to be generally uncommon, and only two specimens from Mississippi, both collected in 1999-2000 around Starkville, are housed in the MEM. The reason for the scarcity of specimens in Mississippi is unknown.

Angioneura flavescens, *A. obscura*, *Opsodexia bicolor*, and *O. nox* are members of the Melanomyinae. These species are often mistaken for Anthomyiidae (Whitworth 2017) and are generally widespread in the eastern United States (Downes 1986). All but three specimens of the Melanomyinae in the MEM were collected after the Goddard and Lago (1983) checklist was published. The three specimens that antedated that publication were collected in 1981, so they may not have yet been incorporated into the MEM for examination by Goddard and Lago (1983).

The other specimens were collected with various traps (black light, pan traps, interception traps) or sweeping through vegetation. One specimen of *A. flavescens* was noted as being collected on flowering *Prunus serotina* Ehrh. (black cherry).

Based on specimens in the University of Guelph Insect Collection, the collection of Terry Whitworth, the BOLD SYSTEMS database, and the Canadian National Collection of Insects, Jones et al. (2019) reported 3 additional blow fly species from nearby states that are not reported from Mississippi. *Calliphora alaskensis* Shannon was reported from specimens in Tennessee and North Carolina; *Lucilia illustris* (Meigen) was reported from the Carolinas and Georgia; and *Lucilia eximia* (Wiedemann) was reported from Oklahoma, Texas, Louisiana, and peninsular Florida. These species may eventually be found in Mississippi, but they are not imminently expected. Jones et al. (2019) provided keys to identify these species.

Records were insufficiently distributed around the state of Mississippi to allow assessment of correlations with USGS Level IV Ecoregions. At the county level, Oktibbeha County is the most speciose, with 14 species represented, followed by Lafayette County with 8 species, and Hinds, Pontotoc, and Tishomingo counties with 5 species each (Appendix A). All other counties were represented by ≤ 4 species, and, in fact, 39 counties do not have any records for blow flies present. The high species richness in Oktibbeha and Lafayette counties is likely due to the presence of the state's major universities (MSU and University of Mississippi, respectively) with their entomology students and entomology collections.

The 22 species (17.5 species/100,000 km^2) known from Mississippi is consistent with the number and density of blow fly taxa recorded for states across the contiguous United States (Table 2). For example, various states range from 14 to 34 species of blow flies, with taxa density ranging from 8 to 24 species/100,000 km^2. It is likely that more species than those

registered in the checklists from California, Missouri, and Virginia would actually be found in those states, given the age of the papers, increased emphasis on collection and taxonomy of blow flies, and the invasion of exotic *Chrysomya* species since publication (Wells 2000).

Table 2 Comparison of blow fly taxa richness and density in Mississippi and other states in which checklists have recently been produced.

State	Number of species	Species per 100,000 km^2	Reference
Virginia	14	12.6	Hall and Townsend 1977
Missouri	15	8.3	Hall 1979
South Carolina	20	24.1	Cobb and Reeves 2005
Mississippi	**22**	**17.5**	**This study**
Florida	24	14.1	Unpubl.
Idaho	33	15.2	Brothers 1999
Colorado	34	12.6	De Jong 1994
California	34	8.0	James 1955

Except for Mississippi, data not updated with individual state distribution records published since last checklist.

The identification keys produced herein should be of use to students and practitioners of public health, medical, veterinary, and forensic entomology in Mississippi. Given the similarity of the blow fly fauna in surrounding states with the fauna in Mississippi, these identification keys should also be useful for students and practitioners throughout much of the southeastern United States.

Checklist of the Blow Flies (Diptera: Calliphoridae) of Mississippi

Subfamily Calliphorinae

Tribe Calliphorini

Cynomya Robineau-Desvoidy 1830

Cynomya cadaverina (Robineau-Desvoidy, 1820)

Calliphora Robineau-Desvoidy 1830

Calliphora livida Hall, 1948

C. terraenovae Macquart, 1851

C. vicina (Robineau-Desvoidy, 1820)

C. vomitoria Linnaeus, 1758

Tribe Luciliini

Lucilia Robineau-Desvoidy 1830

Lucilia cluvia (Walker, 1849)

L. coeruliviridis (Macquart, 1855)

L. cuprina Wiedemann, 1826

L. sericata (Meigen, 1826)

L. silvarum (Meigen, 1826)

Subfamily Chrysomyinae

Tribe Chrysomyini

Chrysomya Robineau-Desvoidy 1830

Chrysomya megacephala (Fabricius, 1794)

Chrysomya rufifacies (Macquart, 1842)

Cochliomyia Townsend 1915

Cochliomyia hominivorax (Coquerel, 1858) - extirpated

Cochliomyia macellaria (Fabricius, 1775)

Tribe Phormiini

Phormia Robineau-Desvoidy 1830

Phormia regina (Meigen, 1826)

Protocalliphora Hough 1899

Protocalliphora deceptor Sabrosky, Bennett, and Whitworth, 1989

Subfamily Polleniinae

Pollenia Robineau-Desvoidy 1830

Pollenia rudis (Fabricius, 1794)

Subfamily Melanomyinae

Angioneura Brauer & Bergenstamm 1893

Angioneura flavescens (Reinhard 1929)

A. obscura (Townsend 1919)

Opsodexia Townsend 1915

Opsodexia bicolor (Coquillett 1899)

O. nox (Downes, 1986)

Not yet reported, but expected:

Opsodexia grisea (Coquillett, 1899) – known from Florida, Georgia, the Carolinas, and Illinois (Downes 1986). A photographed specimen from along the Dog River in Mobile County, Alabama, is published online at https:www.Bugguide.com; thus, this species is included. Multiple attempts to contact the photographer about the specimen did not yield additional information.

Key to the Adult Blow Flies of Mississippi

Following is a key to the adult blow flies of Mississippi, including *Opsodexia grisea*, which is expected to occur in the state); adapted from Downes (1986), Whitworth (2006, 2010), and Tantawi et al. (2017):

1. Basal section of wing stem vein setose above (Figure 4a). Subcostal sclerite setose (Chrysomyinae).....2

1’ Basal section of wing stem vein bare above (Figure 4b). Subcostal sclerite bare.....7

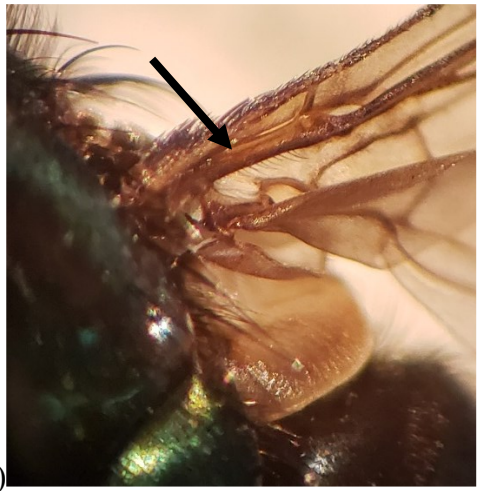
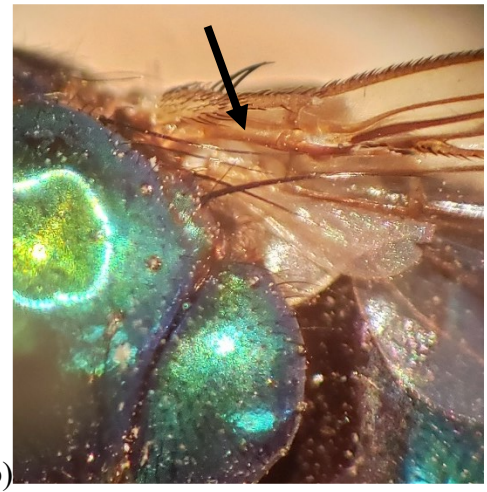
(a)  (b) 

Figure 4 Photographic comparison of adult Chrysomyinae and Calliphorinae.

Arrow is pointing at wing stem vein.
(a) Dorsal view of base of wing of *Cochliomyia macellaria*
(b) Dorsal view of base of wing of *Lucilia coeruliviridis*

2. Greater ampulla with stiff, erect setae (Figure 5a). Dorsum of first and second abdominal tergites black; posterior margins of third and fourth abdominal tergites black 3

2’ Greater ampulla bare or with fine, short setulae (Figure 5b). Abdominal color pattern not as above.....4

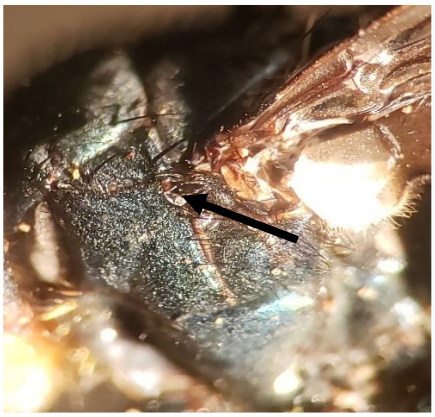
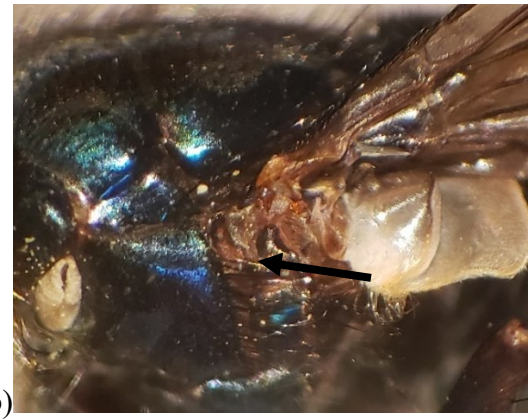
(a)  (b) 

Figure 5 Photographic comparison of adult *Chrysomya* and other Chrysomyinae.

Arrow is pointing at greater ampulla.
(a) Lateral view of base of wing and greater ampulla of *Chrysomya megacephala*
(b) Lateral view of base of wing and greater ampulla of *Cochliomyia macellaria*

3. Lappets of anterior thoracic spiracle dark brown or dark orange (Figure 6a); genal dilation with orange ground color with orange setae (Figure 6a); eye of male with upper facets enlarged and sharply demarcated from facets in lower third; male frons very narrow, eyes nearly touching, at narrowest about 0.01 head width; female frons at narrowest about 0.33 head width ..... C*hrysomya megacephala*

3’ Lappets of anterior thoracic spiracle pale or white (Figure 6b); genal dilation with various ground color, pale dusting, and mostly pale setae (Figure 6b); eye of male with upper facets not enlarged in comparison to those in lower third; male frons slightly broader, at narrowest about 0.05 head width; female frons slightly narrower, at narrowest about 0.30 head width ..... *Chrysomya rufifacies*

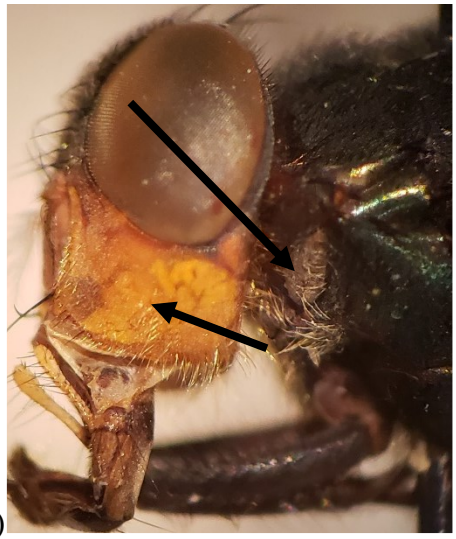
(a)  (b) 

Figure 6 Photographic comparison of adult *Chrysomya megacephala* and *Chrysomya rufifacies*.

Upper arrow is pointing at the anterior spiracle; lower arrow is pointing at the genal dilation.
(a) Lateral view of head and prothorax of *Chrysomya megacephala*
(b) Lateral view of head and prothorax of *Chrysomya rufifacies*

4. Genal dilation yellow or orange with mostly yellow setae (Figure 7a); head with predominantly yellow vestiture; posterior margin of hind coxa setose 5

4’ Genal dilation usually black with dark setae (Figure 7b); head with predominantly black vestiture; posterior margin of hind coxa bare or with weak setulae (Phormiini).....6

(a)  (b) 

Figure 7 Photographic comparison of adult Chrysomyini and Phormiini

Arrow is pointing to setae on genal dilation.
(a) Anterolateral view of head and prothorax of *Cochliomyia macellaria*
(b) Anterolateral view of head and prothorax of *Phormia regina*

5. Lower 1/2–1/3 of frontoorbital plate with pale setulae outside row of frontal setae (Figure 8a); polished central occipital area just below and behind the inner vertical setae black (Figure 8c); central polished stripe on dorsum of thorax long, extending beyond the transverse suture toward the anterior edge of the thorax; fifth tergite usually with pronounced ventrolateral areas of silvery microtomentum; postgenal setae usually pale yellow; female usually with yellowish brown basicosta (Figure 8e); usually with 2 pairs of proclinate orbital setae (sometimes one or both sides have only one) ....*Cochliomyia macellaria*

5’ Fronto-orbital plate with dark setulae outside row of frontal setae (Figure 8b); polished central occipital area just below and behind the inner vertical setae reddish (Figure 8d); central polished stripe on dorsum of thorax short, ending at about the transverse suture; ventrolateral areas of fifth tergite without pronounced silvery microtomentum; postgenal setae usually golden yellow; female with dark/black basicosta (Figure 8f); proclinate orbital setae absent. Eradicated from Mississippi; known from historical presence only .....*Cochliomyia hominivorax*

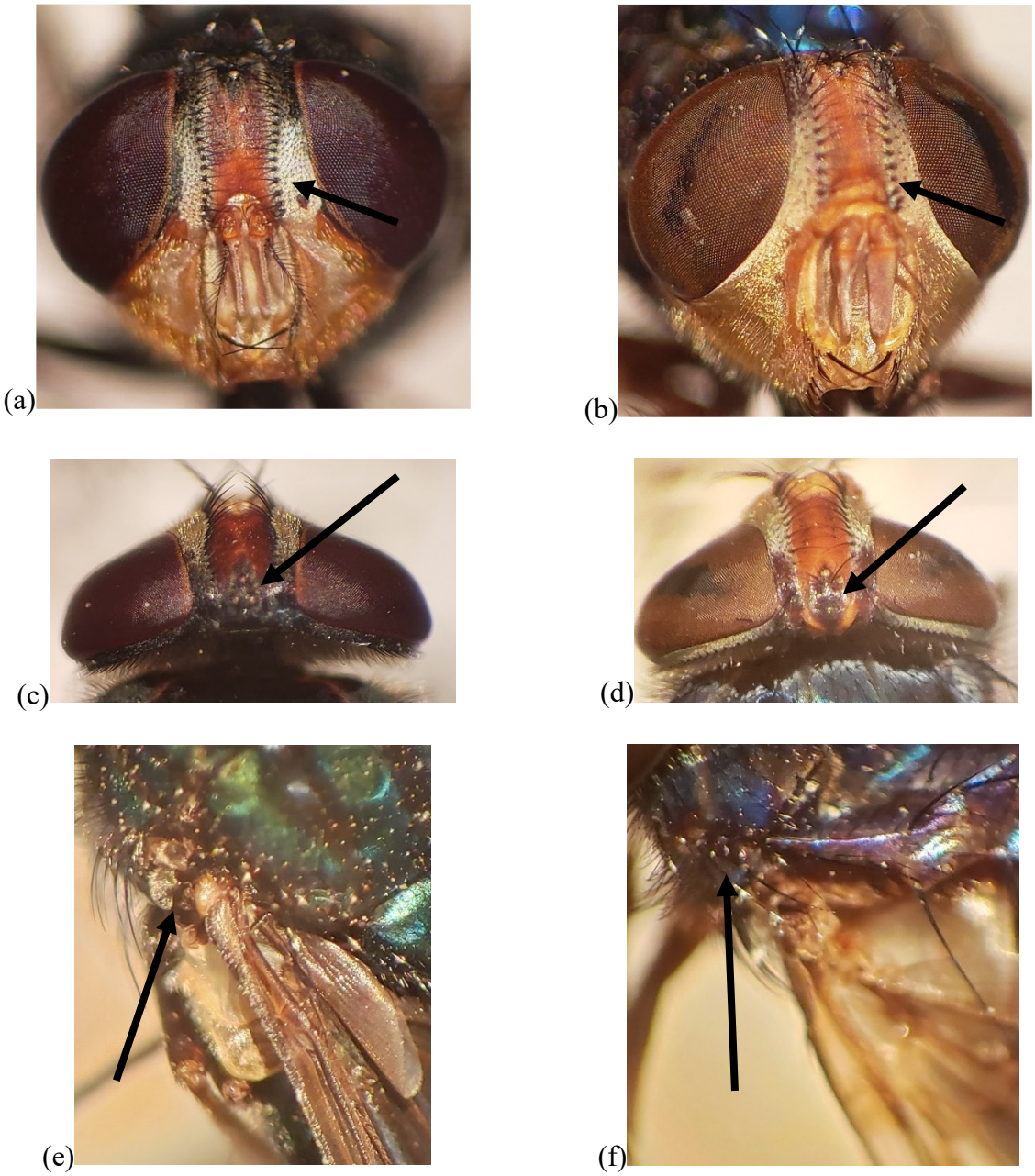


Figure 8 Photographic comparison of adult *Cochliomyia macellaria* and *Cochliomyia hominivorax*

(a) Frontal view of *Cochliomyia macellaria*
(b) Frontal view of *Cochliomyia hominivorax*
(c) Dorsal view of head of *Cochliomyia macellaria*
(d) Dorsal view of head of *Cochliomyia hominivorax*
(e) Dorsal view of base of wing of *Cochliomyia macellaria*
(f) Dorsal view of base of wing of *Cochliomyia hominivorax*

6. Anterior thoracic spiracle with bright orange setae (Figure 9a); two postsutural intra-alar setae (Figure 9c); basicosta orange; three or four notopleural setae present; anterior acrostichal seta moderate; thorax convex dorsocentrally. Scavenger species, not parasitic .....*Phormia regina*

6’ Anterior thoracic spiracle with dark brown setae (Figure 9b); three or four postsutural intra-alar setae (Figure 9d); usually only two notopleural setae; anterior acrostichal seta strong; thorax often more or less flattened dorsocentrally. Obligate parasite of nestling birds.....*Protocalliphora deceptor*

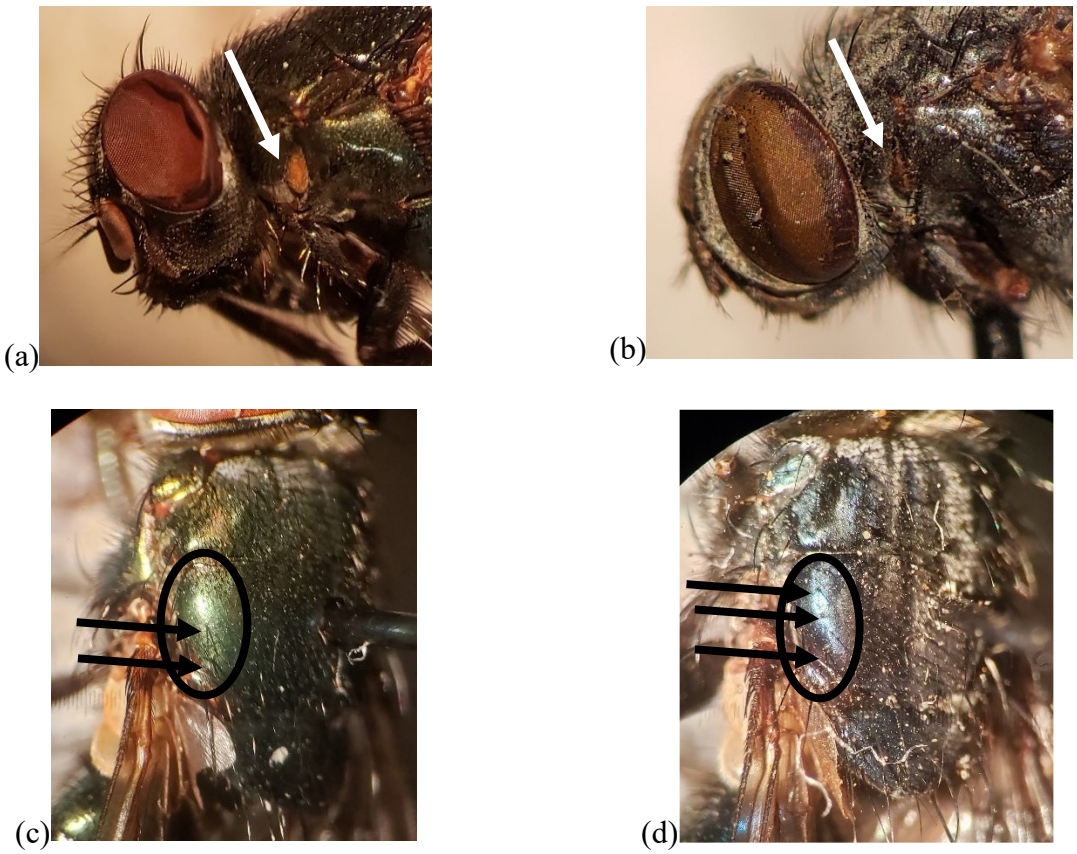


Figure 9 Photographic comparison of adult *Phormia* and *Protocalliphora*.

Arrows in (a) and (b) pointing at anterior spiracle; arrows in (c) and (d) pointing at post-sutural intra-alar setae.

(a) Lateral view of head and thorax of *Phormia regina*
(b) Lateral view of head and thorax of *Protocalliphora deceptor*
(c) Dorsal view of thorax of *Phormia regina*
(d) Dorsal view of thorax of *Protocalliphora* sp.

7. Middle of proepisternal depression bare, or if setose, then body dull black, not metallic blue or green (Figure 10a).....8

7’ Middle of proepisternal depression setose; body shining metallic blue, green, or bronze (Figure 10b), with sheen sometimes dulled by microtomentum (Calliphorinae) 13

(a)  (b) 

Figure 10 Photographic comparison of adult Polleniinae/Melanomyinae and Calliphorinae

Arrows pointing at proepisternal depression.
(a) Anterolateral view of head and prothorax of *Pollenia rudis*
(b) Anterolateral view of head and prothorax of *Lucilia coeruliviridis*

8. Body dull black; thorax with long, crinkly yellowish setae (Figure 11a); eyes small such that gena usually half height of eye or more (Figure 11c); coxopleural streak present; parafacial setose to lower eye margin; obligate parasites of earthworms (Polleniinae) .....*Pollenia rudis*

8’ Body yellowish or brownish; thorax without long, crinkly yellowish setae (Figure 11b); gena about one fourth height of eye (Figure 11d); coxopleural streak absent; parafacial bare on lower half or more; apparent obligate parasites of snails (Melanomyinae).....9

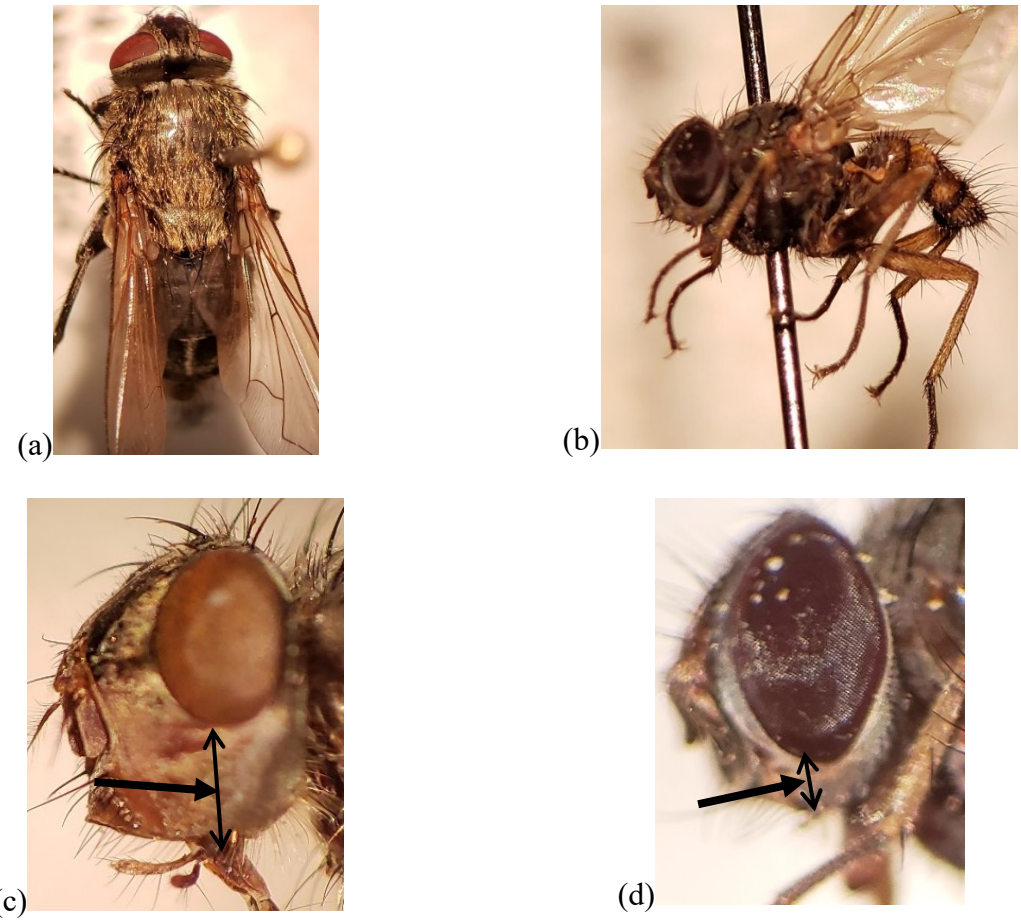


Figure 11 Photographic comparison of adult Polleniinae and Melanomyinae.

Arrows illustrating difference in height of genal dilation below the eye.
(a) Dorsal view of *Pollenia rudis*
(b) Lateral view of *Opsodexia bicolor*
(c) Lateral view of head of *Pollenia rudis*
(d) Lateral view of head of *Opsodexia bicolor*

9. Arista bare or with fine pubescence (Figure 12a); largest scutellar setae sublateral (nearer lateral margins than apex of scutellum, Figure 12c); metasternum with setulae.....10

9' Arista plumose (Figure 12b); largest scutellar setae subapical (nearer apex than lateral margins of scutellum, Figure 12d); metasternum without setulae.....11

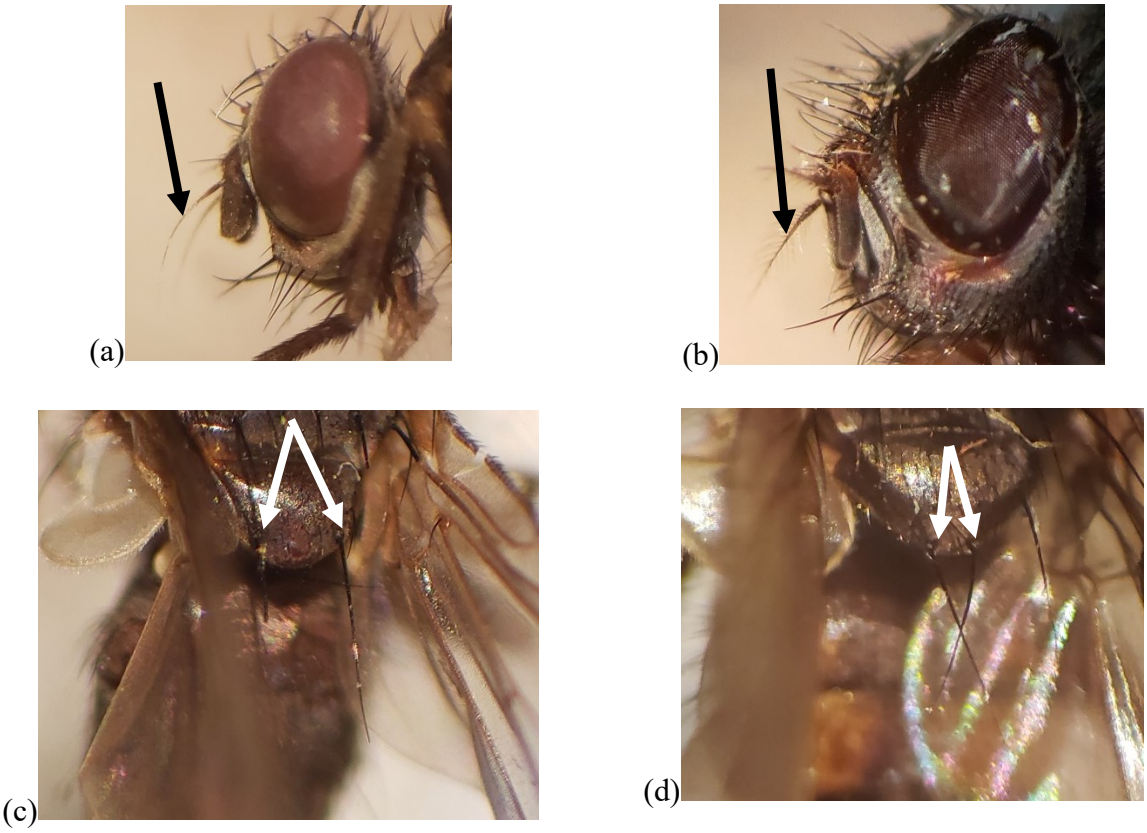


Figure 12 Photographic comparison of adult *Angioneura* and *Opsodexia*.

Arrows in (a) and (b) pointing to arista; arrows on (c) and (d) pointing to largest submarginal or subapical scutellar setae.
(a) Lateral view of head of *Angioneura obscura*
(b) Lateral view of head of *Opsodexia bicolor*
(c) Dorsal view of scutellum of *Angioneura obscura*
(d) Dorsal view of scutellum of *Opsodexia bicolor*

10. Second antennal segment dark brown (rarely orange); femora and tibiae darker brown, concolorous with mesonotum (Figure 13a) .....*Angioneura obscura*

10’ Second antennal segment, femora, and tibiae yellowish to orange, contrasting with mesonotum (Figure 13b)..... *Angioneura flavescens*

(a)  (b) 

Figure 13 Photographic comparison of adult *Angioneura obscura* and *Angioneura flavescens*.

(a) Lateral view of *Angioneura obscura*
(b) Lateral view of *Angioneura flavescens*

11. Abdomen largely yellow in ground color (Figure 14a) ..... *Opsodexia bicolor*

11’ Abdomen largely black or brown in ground color (Figure 14b).....12

(a) 

(b) 

Figure 14 Photographic comparison of adult *Opsodexia bicolor* and other *Opsodexia*.

(a) Posterodorsal view of *Opsodexia bicolor*
(b) Posterodorsal view of *Opsodexia grisea*

12. Apical posterodorsal bristle of hind tibia larger than apical dorsal bristle (Figure 15a); third abdominal tergum with median marginal setae (Figure 15c)..... *Opsodexia grisea*

12' Apical posterodorsal bristle of hind tibia not or scarcely separable from surrounding setulae (Figure 15b); third abdominal tergum without median marginal setae (Figure 15d) .....*Opsodexia nox*

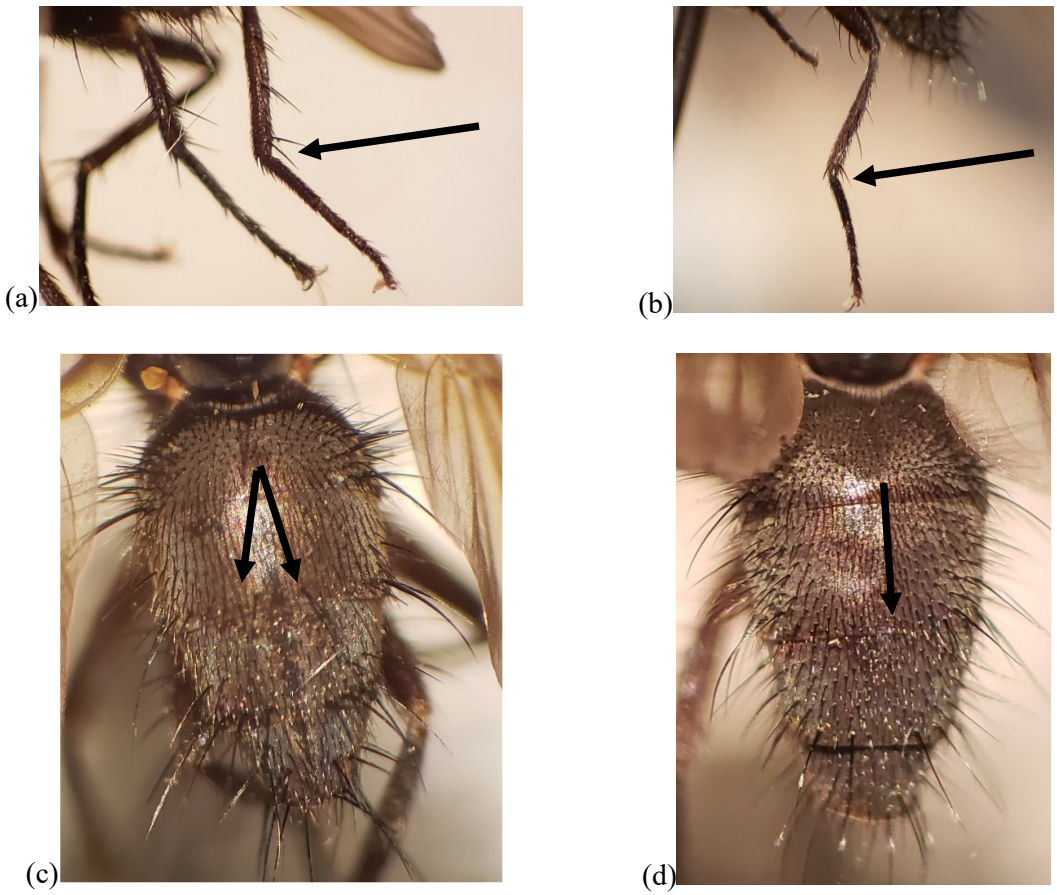


Figure 15 Photographic comparison of adult *Opsodexia grisea* and *Opsodexia nox*.

Arrows in (a) and (b) pointing to apicoposterodorsal bristle of hind tibia; arrows in (c) and (d) pointing to hind margin of third abdominal segment.
(a) Lateral view of hind leg of *Opsodexia grisea*
(b) Lateral view of hind leg of *Opsodexia nox*
(c) Dorsal view of abdomen of *Opsodexia grisea*
(d) Dorsal view of abdomen of *Opsodexia nox*

13. Thorax and abdomen usually shining green, blue, or bronze (Figure 16a) with microtomentum weak. Suprasquamal ridge with conspicuous cluster of setae near the base of scutellum; lower calypter bare above (Luciliini).....14

13’ Thorax usually dull, with more distinct microtomentum; abdomen usually metallic blue (Figure 16b) with whitish microtomentum (except *Cynomya*). Suprasquamal ridge bare or with inconspicuous fine setae; lower calypter setose above (Calliphorini).....18

(a)  (b) 

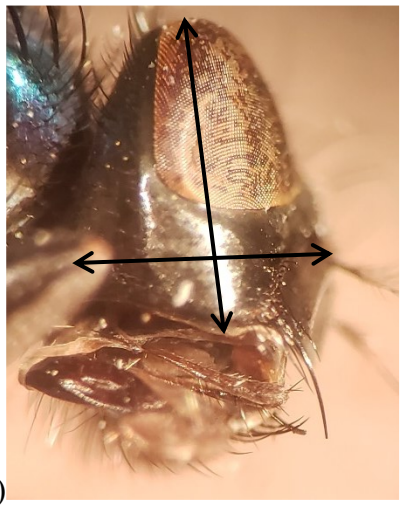
Figure 16 Photographic comparison of adult Luciliini and Calliphorini.

(a) Dorsal habitus of *Lucilia sericata*
(b) Dorsal habitus of *Calliphora* sp.

14. Palp dark brown to tan (Figure 17a, occasionally tip only may be dark); length of head at level of lunule more than half head height (Figure 17a); third abdominal tergite with one or two pairs of long, erect median marginal setae; basicosta dark brown or black; often parasitic on amphibians but can be associated with carrion ..... *Lucilia silvarum*

14' Palp pale orange to yellow (except dark in *L. cuprina*), not darkened apically (Figure 17b, palps of specimens that have been in liquid or stored improperly may be darkened); length of head at level of lunule less than half head height (Figure 17b), except in *L. sericata*; third abdominal tergite with marginal setae not especially strong or erect; basicosta can be dark brown, black, yellow, or orange 15

(a)



(b)

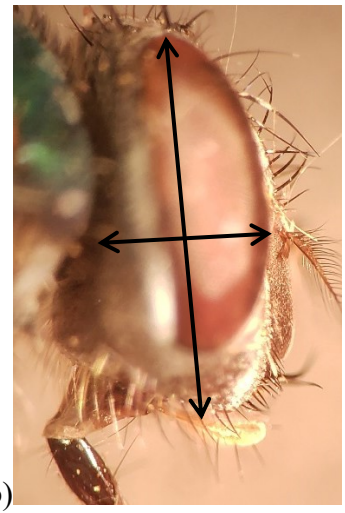


Figure 17 Photographic comparison of adult *Lucilia silvarum* and other *Lucilia*.

Arrows illustrating proportions of head.
(a) Lateral view of head of *Lucilia silvarum*
(b) Lateral view of head of *Lucilia sericata*

15. Two postsutural acrostichal setae (Figure 18a); abdomen usually uniformly metallic or microtomentose.....16

15' Three postsutural acrostichal setae (Figure 18b); abdomen with apparent mesal division in which one-half is microtomentose, the other half is shining.....17

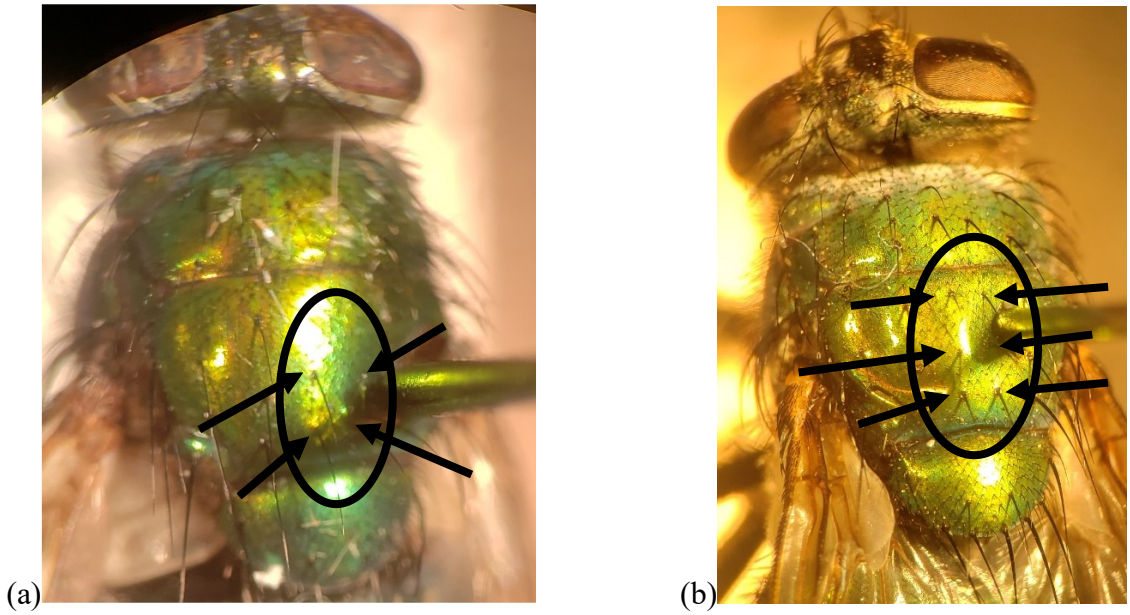


Figure 18 Photographic comparison of adult *Lucilia* species.

Arrows pointing at post-sutural acrostichal setae
(a) Dorsal view of thorax of *Lucilia coeruliviridis*
(b) Dorsal view of thorax of *Lucilia sericata*

16. Genal dilation entirely with dark setae and postgena with pale setae (Figure 19a). Frons of male with fronto-orbital plates almost touching, frons width at narrowest about 0.03 head width (Figure 19c); much less than width of first flagellomere; lower calypter tan. Female often with black setulae outside row of frontal setae on frontal plate; mature specimens longer usually 8–9.5 mm in length.....*Lucilia coeruleiviridis*

16’ Rear half of genal dilation and post-gena with pale setae (Figure 19b). Frons of male with fronto-orbital plates well separated; frons width at narrowest about 0.12 head width (Figure 19d); frons wider than the width of first flagellomere; lower calypter pale. Mature specimens smaller, 8mm length or less..... *Lucilia cluvia*

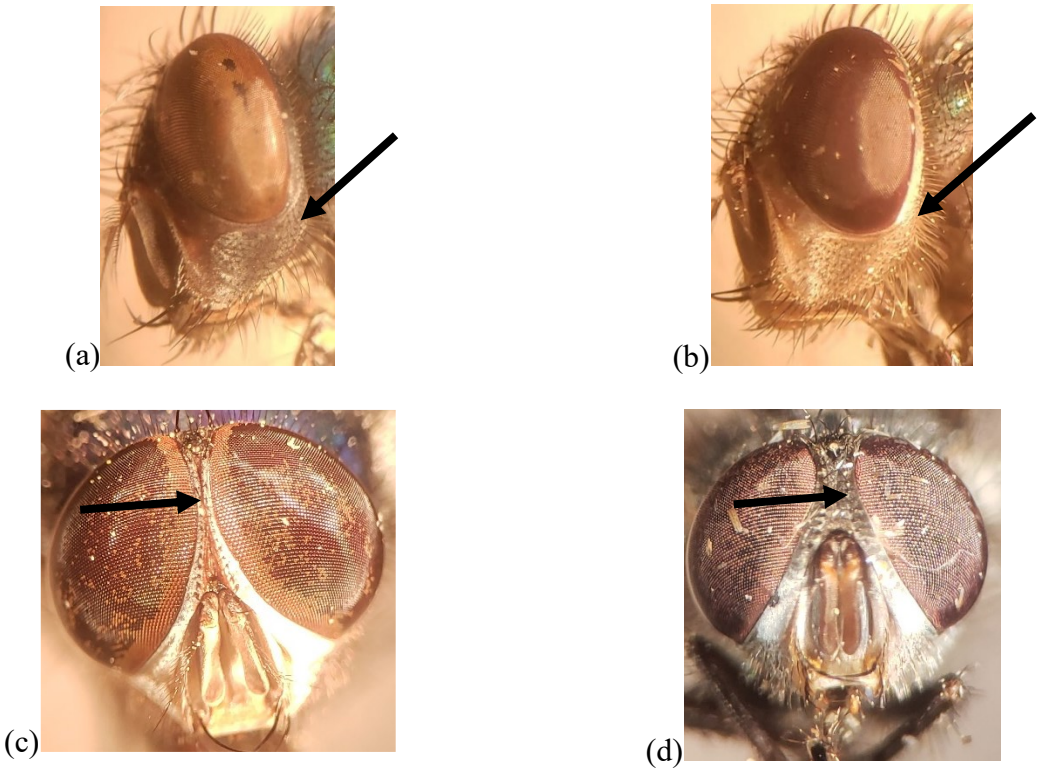


Figure 19 Photographic comparison of adult *Lucilia coeruliviridis* and *Lucilia cluvia*.

Arrows in (a) and (b) pointing at postgenal setae; arrows in (c) and (d) pointing at frons.
(a) Lateral view of head of *Lucilia coeruliviridis*
(b) Lateral view of head of *Lucilia cluvia*
(c) Frontal view of head of male *Lucilia coeruliviridis*
(d) Frontal view of head of male *Lucilia cluvia*

17. Central occipital area with group of two to five setae below inner vertical seta (Figure 20a); metasternum setose; abdomen usually bright green, occasionally shining coppery (Figure 20c); humeral callus with several setulae posteriad of the posterior humeral setae; notopleuron usually with five or more setulae on rear border; frons of male at narrowest about equal to width of parafacial at level of lunule, about 0.13 head width; frons of female at narrowest about 0.37 head width ..... *Lucilia sericata*

17' Central occipital area with single seta below inner vertical seta (Figure 20b); metasternum bare; abdomen dull coppery (Figure 20d); humeral callus posteriad of the humeral setae bare or with only two or three small setulae; notopleuron with only two or three small setulae on posterior border; frons of male at narrowest obviously much broader than width of the parafacial at level of the lunule, about 0.20 head width; frons of female at narrowest about 0.40 head width ..... *Lucilia cuprina*

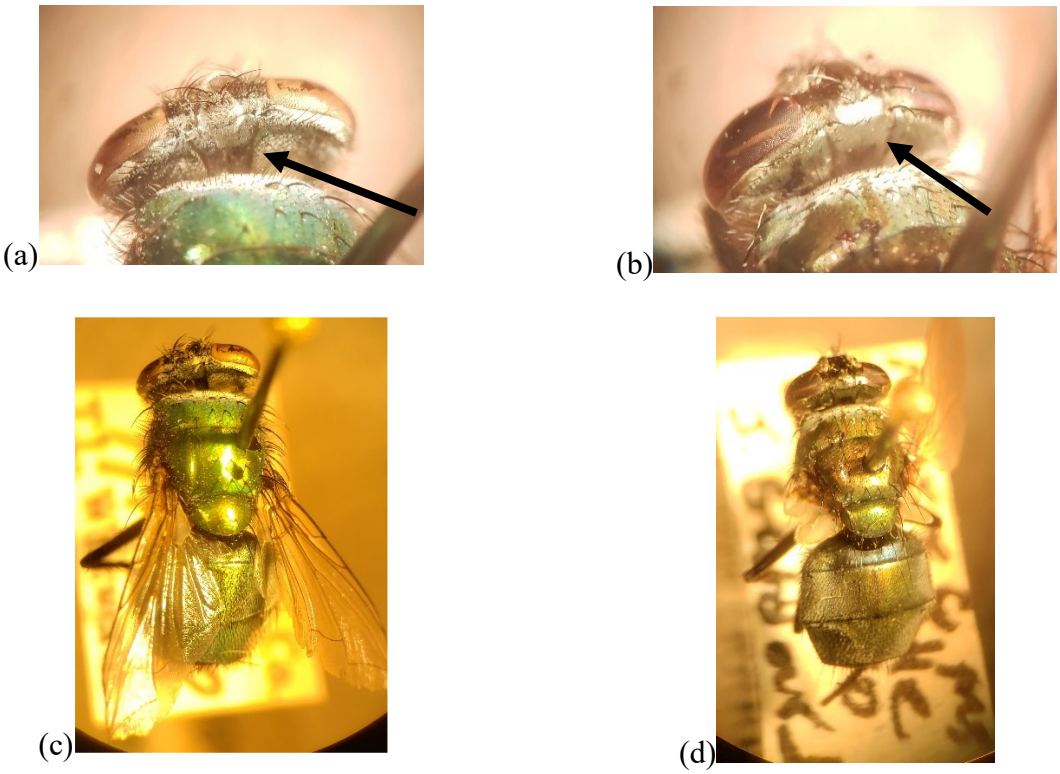


Figure 20 Photographic comparison of adult *Lucilia sericata* and *Lucilia cuprina*.

Arrows pointing at central occipital area.
(a) Posterior view of head of *Lucilia sericata*
(b) Posterior view of head of *Lucilia cuprina*
(c) Dorsal habitus of *Lucilia sericata*
(d) Dorsal habitus of *Lucilia cuprina*

18. Disc of upper and lower calypter (Figure 21a) white; rim of upper calypter usually tan; rim of lower calypter usually pale ..... *Cynomya cadaverina*

18' Disc of upper and lower calypter (Figure 21b) light to dark brown, rim of calypter pale or dark 19

(a) 

(b) 

Figure 21 Photographic comparison of adult *Cynomya cadaverina* and other Calliphorinae.

Arrows pointing to lower calypter.
(a) Dorsolateral view of wing base and calypters of *Cynomya cadaverine*
(b) Dorsolateral view of wing base and calypters of *Calliphora vomitoria*

19. Three postsutural intra-alar setae (Figure 22a) ..... *Calliphora livida*

19’ Two postsutural intra-alar setae (Figure 22b) 20

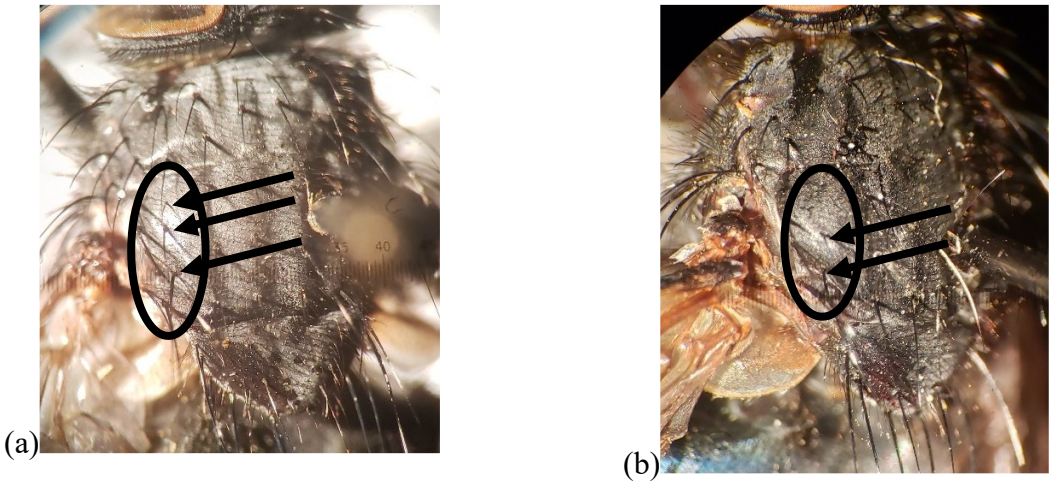


Figure 22 Photographic comparison of adult *Calliphora livida* and other *Calliphora*.

Arrows pointing at postsutural intraalar setae.
(a) Dorsal view of thorax of *Calliphora livida*
(b) Dorsal view of thorax of *Calliphora vicina*

20. Basicosta yellow to orange (Figure 23a); genal dilation with reddish ground color on anterior 0.5 to 0.67, black posteriorly (Figure 23c); frons of male at narrowest about 0.08 head width..... *Calliphora vicina*

20' Basicosta dark brown or black (Figure 23b); genal dilation, when fully colored, entirely black (Figure 23d); frons of male at narrowest usually less than 0.07 head width.....21

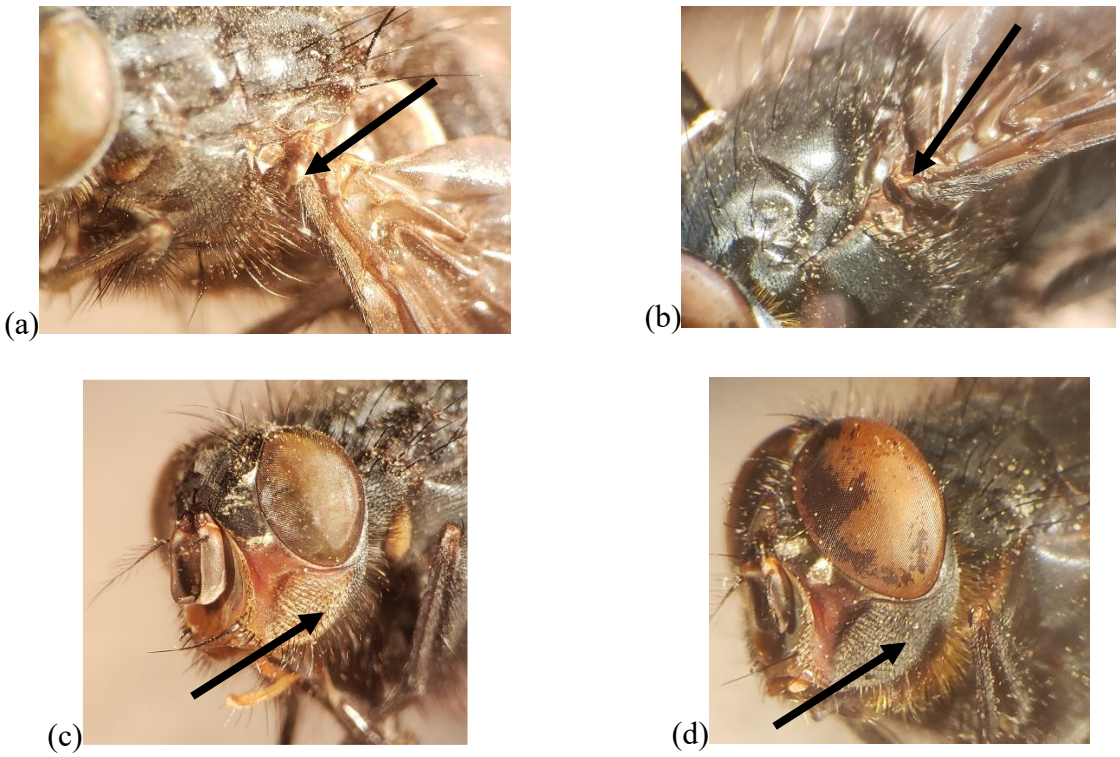


Figure 23 Photographic comparison of adult *Calliphora vicina* and other *Calliphora*.

Arrows in (a) and (b) pointing at basicosta; arrows in (c) and (d) pointing at genal dilation.
(a) Anterodorsal view of base of wing of *Calliphora vicina*
(b) Anterodorsal view of base of wing of *Calliphora terraenovae*
(c) Frontolateral view of head of *Calliphora vicina*
(d) Frontolateral view of head of *Calliphora terraenovae*

21. Postgena and lower posterior corner of genal dilation and back of head with long yellow-orange setae (Figure 24a), sometimes extending forward along edge of lower genal dilation; vestiture of the occiput below postocular setae primarily pale setae. Frons of male at narrowest about 0.04 head width; frons of female at narrowest about 0.34 head width ..... *Calliphora vomitoria*

21' Postgena and genal dilation with mostly dark or black setae (Figure 24b); vestiture of the occiput below postocular setae with three or more rows of black setae; other characters vary .....*Calliphora terraenovae*

(a)  (b) 

Figure 24 Photographic comparison of adult *Calliphora vomitoria* and *Calliphora terranovae*.

Arrows pointing at postgenal setae.
(a) Anterolateral view of head of *Calliphora vomitoria*
(b) Anterolateral view of head of *Calliphora terraenovae*

Key to the Third Instar Larval Blow Flies of Mississippi

Following is a key to the third instar larval blow flies of Mississippi. *Lucilia coeruliviridis* and *L. cluvia* are as yet undifferentiable as larvae, and Nearctic *Angioneura* and *Opsodexia* species are unknown in the larval stage. Couples addressing abdominal spine patterns do not have illustrations. As such, best practices would be to rear larvae to adult stage for which the key is more complete. Nevertheless, this key is adapted from Knipling (1936, 1939), Hall (1948), Kano and Sato (1952), Yahnke and George (1972), Wells et al. (1999), and Wallman (2001), with terminology based on Teskey (1981):

1. Peritreme of posterior spiracle incomplete (Figure 25a): either absent or without strong coloration in the vicinity of the ecdysial scar (= "stigmatal scar", "button").....2

1' Peritreme of posterior spiracle may be thick or thin, but is complete (Figure 25b), including in the vicinity of the ecdysial scar 7

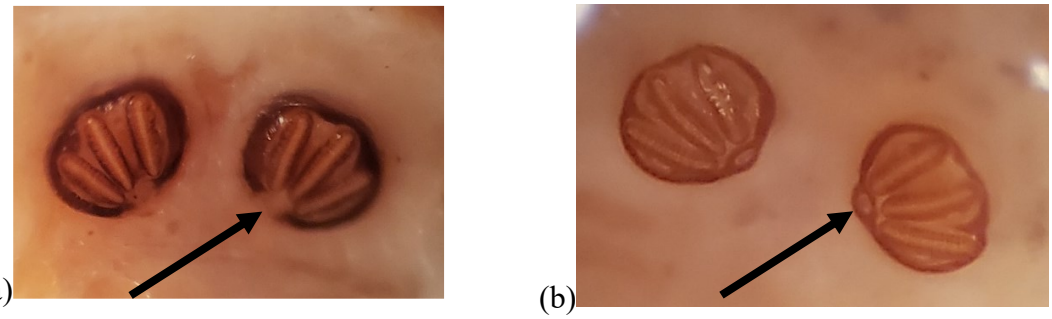


Figure 25 Photographic comparison of larval Chrysomyinae and Calliphorinae.

Arrows pointing at peritreme.
(a) Caudal view of posterior spiracles of *Cochliomyia macellaria*
(b) Caudal view of posterior spiracles of *Lucilia coeruliviridis*

2. First 7 abdominal segments with several pairs of digitiform papillae (Figure 26a; "hairy maggots") ..... *Chrysomya rufifacies*

2' First 7 abdominal segments not papillate (Figure 26b) 3

(a)  (b) 

Figure 26 Photographic comparison of larval *Chrysomya rufifacies* and other Calliphoridae.

(a) Lateral habitus of *Chrysomya rufifacies*
(b) Dorsal habitus of *Calliphora* sp.

3. Accessory oral sclerite divergent posteriorly and pigmented along posterior arms (Figure 27a) ..... *Chrysomya megacephala*

3' Accessory oral sclerite not pigmented, or if slight pigmentation, not along posterior arms (Figure 27b) 4

(a)  (b) 

Figure 27 Photographic comparison of larval *Chrysomya* and *Cochliomyia*.

(a) Ventral view of mouthparts of *Chrysomya rufifacies*
(b) Ventral view of mouthparts of *Cochliomyia macellaria*

4. Pigmented spines above anus forming a V-shaped pattern (Figure 28a); spines absent dorsally on posterior margin of segment XI 5

4’ Pigmented spines above anus forming a small oval cluster (Figure 28b); spines present dorsally on posterior margin of segment XI 6

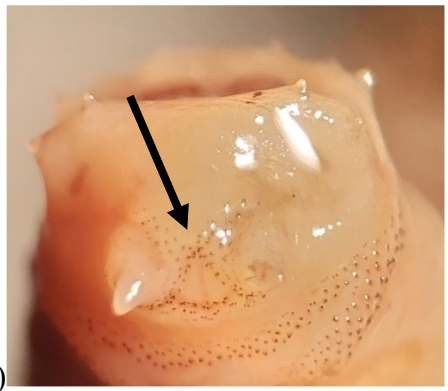
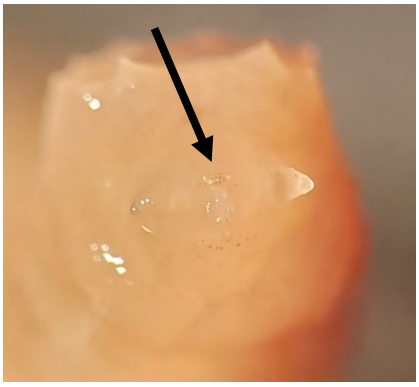
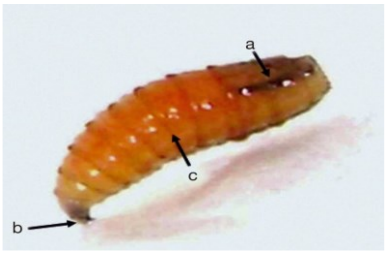
(a)  (b) 

Figure 28 Photographic comparison of *Cochliomyia* and Phormiini.

Arrows pointing at pigmented spines above anus.
(a) Posterior view of the pattern of spines on anal protuberance of *Cochliomyia macellaria*
(b) Posterior view of the pattern of spines on anal protuberance of *Phormia regina*

5. Tracheal trunks leading from posterior spiracles pigmented dark brown to black and visible laterally through cuticle (Figure 29a); extirpated from Mississippi..... .....*Cochliomyia hominivorax*

5' Tracheal trunks leading from posterior spiracles unpigmented and nearly indistinguishable through cuticle (Figure 29b) .....*Cochliomyia macellaria*

(a) 

(b) 

Figure 29 Photographic comparison of larval *Cochliomyia hominivorax* and *Cochliomyia macellaria*

(a) Lateral view of larva of *Cochliomyia hominivorax*, from Olea et al. (2014). "Third-instar larvae of *Cochliomyia Hominivorax*" [*sic*] by María Sofía Olea et al., Korean Society for Parasitology and Tropical Medicine (DOI: 10.3347/kjp.2014.52.1.89) is licensed under CC BY-NC 3.0 (https://creativecommons.org/licenses/by-nc/3.0/). Arrow "a" is pointing at tracheal trunks of posterior spiracles.
(b) Lateral view of larva of *Cochliomyia macellaria*

6. No prothoracic fringe of spines present; dorsal surface with bands of spines along anterior and posterior margin of most segments dorsally, absent posteriorly on segment X .....*Phormia regina*

6' Prothoracic fringe of longer spines present; dorsal surface densely covered with spines on most segments; obligate hematophagous parasitic in bird nests...*Protocalliphora deceptor*

7. Obligate parasite of earthworms and few other organisms. *Nota bene*: I have not seen this species as larvae, and the two drawings I have seen are vastly different. Hall (1948) and Yahnke and George (1972) provided drawings of the posterior spiracular plate with the spiracular slits either subparallel (Hall) or widely divergent apically (Yahnke and George); however, both show the peritreme surrounding the spiracular field forming a complete, dark, thin ring, including in the vicinity of the ecdysial scar .....*Pollenia rudis*

7' Not parasitic in earthworms; peritreme surrounding the spiracular field forming a complete, dark, thick ring which may in some species be paler in the vicinity of the ecdysial scar 8

8. Accessory oral sclerite absent (Figure 30a), do not confuse the mouth opening as the accessory oral sclerite 9

8' Accessory oral sclerite present (Figure 30b) 13

(a) 

(b) 

Figure 30 Photographic comparison of larval Luciliini and Calliphorini.

(a) Ventral view of mouthparts of *Lucilia coeruliviridis*
(b) Ventral view of mouthparts of *Calliphora livida*

9. Inner tubercles on upper margin of posterior cavity separated by a distance approximately equal to the distance between the inner and median tubercles (Figure 31a) .... *Lucilia sericata*

9' Inner tubercles on upper margin of posterior cavity separated by a distance approximately twice the distance between the inner and median tubercles (Figure 31b) 10

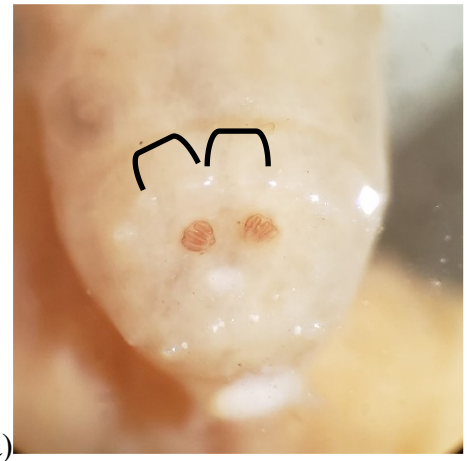
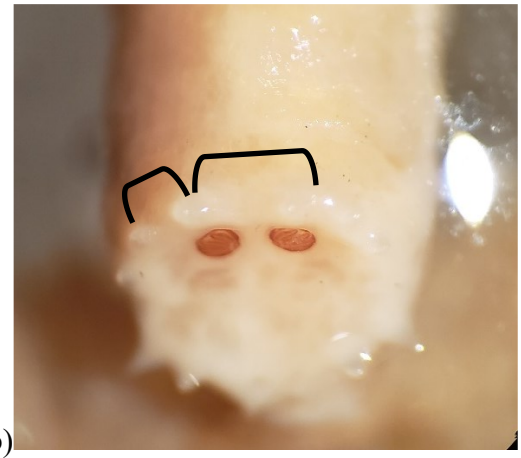
(a)  (b) 

Figure 31 Photographic comparison of larval *Lucilia sericata* and other *Lucilia*.

Brackets illustrating differences in distance between tubercles.
(a) Caudal view of *Lucilia sericata*
(b) Caudal view of *Lucilia coeruliviridis*

10. Inward projection of lateral margin of peritreme between outer and middle slits absent or at least not prominent (Figure 32a); 3-4 irregular rows of spines dorsally at posterior margin of segment XI; tubercles bordering stigma field small..... *Lucilia cuprina*

10' Inward projection of lateral margin of peritreme between outer and middle slits dark colored and prominent (Figure 32b); 6-8 irregular rows of spines dorsally at posterior margin of Segment XI; tubercles bordering stigma field large.....11

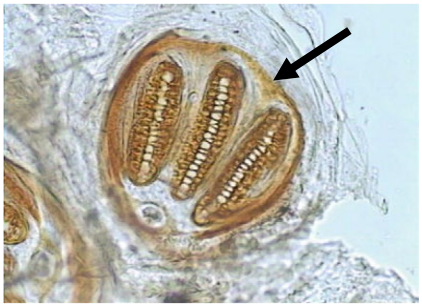
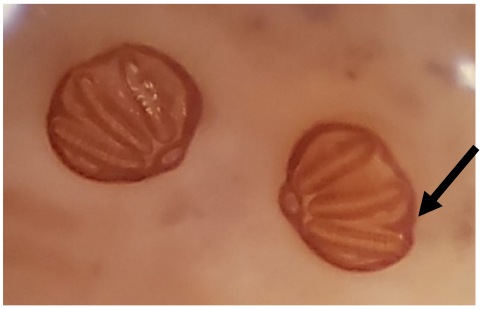
(a)  (b) 

Figure 32 Photographic comparison of larval *Lucilia cuprina* and other *Lucilia.*

Arrows pointing at inward projection of peritreme.
(a) Caudal view of posterior spiracle of *Lucilia cuprina*, from Pirali-Kheirabadi et al. (2010). "Posterior spiracles of *L. cuprina*" by Pirali-Kheirabadi et al. © The Author(s) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2837235/) is licensed under CC BY-NC 3.0 (https://creative commons.org/licenses/by-nc/3.0/).
(b) Caudal view of posterior spiracle of *Lucilia coeruliviridis*

11. Anterolateral margin of segment XI with 1-2 rows of irregular spines dorsally; dorsum of segment XII scabrous..... *Lucilia silvarum*

11' Anterolateral margin of segment XI devoid of spines dorsally; anterior half of segment XII smooth .....*Lucilia coeruliviridis/cluvia*

12. Segment IX bare anterodorsally.....*Calliphora livida*

12’ Segment II – IX spinose anterodorsally.....13

13. Segment IX bare posterodorsally; anterior spiracles with 10-11 rounded orifices .....*Calliphora terraenovae*

13’ Segment IX with a complete posterior band of spines; anterior spiracles with 7-9 rounded orifices.....14

14. Labial sclerite with apical toothlike portion subequal to width of basal portion (Figure 33a) ..... *Cynomya cadaverina*

14’ Labial sclerite with apical toothlike portion longer than the greatest width of the basal portion (Figure 33b).....15

(a) (b)

Figure 33 Photographic comparison of larval *Cynomya cadaverina* and other Calliphorini.

Arrows pointing at apical tooth-like portion of labial sclerite.
(a) Diagrammatic lateral view of cephalopharyngeal sclerites of *Cynomya cadaverina*
(b) Diagrammatic lateral view of cephalopharyngeal sclerites of *Calliphora vicina*

15. Spines generally small with acute tips, sometimes double-pointed..... *Calliphora vicina*

15’ Spines larger with broad tips, all single-pointed..... *Calliphora vomitoria*

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CHAPTER III

RELATIVE ROLES OF BLOW FLIES AND FIRE ANTS IN CARRION DECOMPOSITION AND EFFECTS OF FIRE ANTS ON BLOW FLY SUCCESSION

Introduction

The objective of this chapter is to elucidate relative contributions of blow flies and fire ants to the rate of decomposition of pig carrion in eastern central Mississippi and the Florida Panhandle. For the purposes of this study, "blow flies" refers to the balance of the succession community after ants are removed because it is assumed that blow flies (Calliphoridae) would be the predominant taxon.

Fuller (1934) proposed that ants, in general, were not part of the normal carrion community, their presence on carrion only due to the carrion incidentally being near ant mounds. Since then, ants have become recognized as common and often important members of the carrion community (Payne and Crossley 1966, Payne and Mason 1971, Anderson and VanLaerhoven 1996, Campobasso et al. 2009, Heo et al. 2009, Neto-Silva et al. 2017, Mashaly et al. 2018). Eubanks et al. (2019) produced a literature review on 64 papers addressing ant relations with carrion, noting that they are generally considered to be directly feeding on the carrion, altering the carrion to access liquids or solid material, or predating on other members of the succession fauna.

Ants can arrive early in the decomposition process (Goddard et al. 2012), and they can overwhelm carcasses to the exclusion of other carrion fauna (De Jong and Hoback 2006,

Lindgren et al. 2010, Merritt and De Jong 2016, Barton et al. 2017). However, ants can be found on carrion in all stages of decomposition (Eubanks et al. 2019). For example, Heo et al. (2009) found ants present at all stages of decomposition in Malaysia, where they captured and presumably fed on fly eggs, larvae, pupae, and adults. Paula et al. (2016) considered ants to be omnivores, necrovores, and predators on carrion-visiting insects in studies on the role of Formicidae in carrion decomposition and their effects on blow fly succession in Brazil. They found that ants can significantly alter decomposition time, slowing it down by preying on other succession organisms, or alternatively, accelerating decomposition time by creating holes in the surface and entry points for other succession fauna or even by consuming all or part of the carcass themselves. Leaf-cutting ants in the genus *Atta* have recently been reported cutting clothing on a human cadaver in addition to producing lesions on the body in Brazil (da Fonseca de Souza et al. 2020). In a laboratory-based study, Nappi (2018) found that the western harvester ant, *Pogonomyrmex occidentalis* (Cresson), prioritized predation on *Lucilia sericata* eggs on pig liver, even though it was attracted to the liver with or without blow fly eggs present. In Mississippi, it has been thought that fire ants may accelerate decomposition, although this has not been tested empirically.

While ants have been found on many types of animal carcasses and in all stages of decomposition, introduced fire ants have only recently been associated with vertebrate carrion in North America (Stoker et al. 1995, Lindgren et al. 2010, Eubanks et al. 2019). In addition to three native species of *Solenopsis,* two species were introduced to North America from South America in the first half of the 1900s (Tschinkel 2006). Both nonnative species have spread throughout the southeastern United States, along with a narrowly distributed hybrid (Callcott and Collins 1996, MacGown and Forster 2005, MacGown 2016).

The potential forensic importance of introduced fire ants in carrion ecology appears to have been recognized first in the 1980s when Early and Goff (1986) and Tullis and Goff (1987) found contrasting effects of fire ants on carrion in the Hawaiian Islands. Greenberg (1991) used those observations in a review of the use of Diptera as forensic indicators to report that fire ants aggressively prey on eggs and larvae of carrion flies. Since then, introduced fire ants have come to be recognized as normal components of the carrion fauna in the southeastern United States (Merritt and De Jong 2016), and reports of their presence at carrion has increased (Figure 1).

While many carrion succession studies merely mention the presence of introduced fire ant species, and some authors speculate on their role as predators of other members of the succession fauna (e.g., Watson and Carlton 2003, Pérez et al. 2005, Goddard et al. 2012, Lindgren et al. 2015, Richards et al. 2015), few studies have attempted to study the specific effects that introduced fire ants have on the successional population dynamics of other species. In that regard, fire ants were involved as one of the few rare instances where direct predation by one species on other members of a community has produced a measurable effect on species diversity and dynamics of that community (VanLaerhoven 2016).

Stoker et al. (1995) examined the effects of fire ants on the succession community by excluding fire ants. The ants altered carrion community composition in both scarce (small mice) and abundant (chicken) resource conditions. Likewise, Wells and Greenberg (1994) reported that fire ants appeared to disrupt the normal succession of the blowflies *Cochliomyia macellaria* (F.) and *Chrysomya rufifacies* (Macquart) by increasing the number of "gaps" in which the adult flies were not collected at carrion (which could, in turn, affect forensic interpretation of the arthropod evidence). Although actual predation was not observed by Wells and Greenberg (1994), it was

thought that ant predation during relatively vulnerable periods of larval life was, in part, how fire ants affected the succession patterns.

Concerning feeding on the carcass, Moura et al. (1997) reported that fire ants were collected at rat carrion in Paraná, Brazil, and described that they had made post-mortem "artifacts" on the carcasses. Similarly, Pechal et al. (2015) reported that *S. invicta* constructed mounds on a human corpse exposed experimentally in Texas and fed on tissues exposed by the feeding of *Peliodectes haldemani* (Girard), a katydid, on the corpse. This unusual activity was discussed in relation to post-mortem disturbances of carrion and potential misinterpretation of markings on a corpse. Lindgren et al. (2010) reported a case study in which a human corpse, also experimentally exposed in southeast Texas, was colonized by *S. invicta*, excluding the normal carrion fauna by burying portions of the corpse and feeding on it. This was a novel observation in comparison to previously observed fire ant behavior at carrion in which they apparently preyed on other carrion fauna or just created small holes in the carcass surface.

Recent observations on a conspecific fire ant, *S. saevissima* (Smith), in Brazil have also noted the behavior of partially burying carrion. Pereira et al. (2017) and Mendonça et al. (2019) opportunistically found *S. saevissima* on individual carcasses of a cat, *Felis catus* L., and a big-eared opossum, *Didelphis aurita* Wied-Neuwied, respectively, each noting that that the ants' activity of burying portions of the carrion appeared to delay succession by necrophagous Diptera. Conversely, Meyer et al. (2020) used time-lapse photography to document that fire ants (*Solenopsis invicta* x *richteri*) created lesions in the skin of decomposing pig carrion, and the lesions were subsequently used as oviposition and development sites for necrophagous Diptera. Despite these observations, direct trophic evidence that fire ants feed on the carrion itself or prey on other members of the succession fauna is specious.

I generated 23 specific hypotheses regarding the effects of fire ants on carrion decomposition, carrion fauna composition, and blow fly colonization and development. These hypotheses are formalized and enumerated below in the statistical analysis section of the Materials and Methods, providing an in-depth description of the statistics used to test each hypothesis.

Materials and Methods

Study Design

The study design was an experimental design involving inclusion or exclusion of members of the succession fauna onto decomposing pig carrion (Table 3). There were three treatments: 1) a control with no insects excluded, 2) a treatment with fire ants specifically excluded but blow flies with access, and 3) a treatment with blow flies specifically excluded but fire ants with access. A treatment in which both ants and flies were excluded was found to be untenable in practice and, in fact, unrealistic; therefore, this treatment was not used.

Table 3 Tabular summary of treatments.

Treatment	1 (control)	2	3
Succession taxa excluded	Neither ants nor flies	Ants	Flies
Succession taxa allowed	Both ants and flies	Flies	Ants
Net mesh	Coarse, with tears	Fine, with tears	Coarse
Pesticide	None	Pyrethroid	None

Pig carcasses in treatments 1 and 3 (in which ants were not excluded) were placed 1 to 2 m from a visible fire ant mound, while pig carcasses in Treatment 2 were located at least 5 m from a visible fire ant mound. Fire ants typically use underground foraging tunnels (Tschinkel 2006), so foragers may still easily find a potential food source such as carrion within the 5 m

distance, but it was still distant from the central portion of the nest and brood chamber. Treatments were usually spaced at least 15 m apart from each other to avoid non-independence of the succession data (Perez et al. 2016) and to assure that the proximal mound represented the nearest fire ant nest. Even though Shahid et al. (2003) and Schoenly et al. (2005) demonstrated that dense levels of carrion enrichment do not impact subsequent decomposition and succession studies, carcasses in this study were placed at different locations within each study area for each replicate to avoid any unintentional influences of previous replicates.

Study Areas

Two study areas were used, including the property of Jerome Goddard, approximately 7 miles (11.5 km) west of Starkville, and the Camp o' the Pines property of Pensacola Christian College near Molino, Florida (Figure 34).

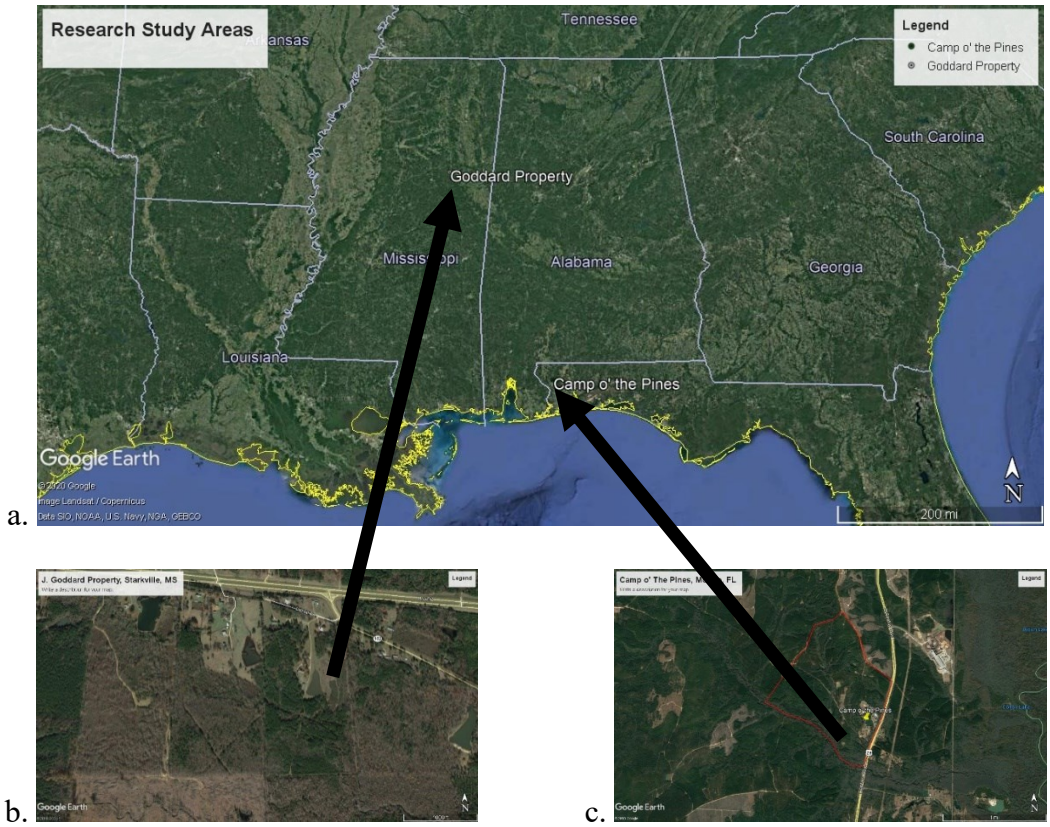


Figure 34 Google Earth images of study areas.

(a) Google Earth image of southeastern United States, showing locations of study areas.
(b) Google Earth image of Jerome Goddard property, Starkville, Mississippi.
(c) Google Earth image of Camp o' the Pines property, Molino, Florida.

The Goddard property (Figure 35) is located near Adaton, Mississippi, about 11.5 km west of the western edge of Starkville, Mississippi, near the intersection of U.S. Highway 82 and State Highway 182. Habitat is rural, and it is located in Ecoregion 65b, "Flatwoods, Blackland Prairie Margins." Elevation is 91 meters above sea level. The property comprises about 30 hectares, including woods, a 0.5 hectare pond, and a large meadow. Experimental plots were located in meadow habitat east of the pond, halfway between the pond and the mixed deciduous forest woods, at an elevation approximately 4 m upgradient from the pond. Based on Google Earth historical images, the meadow had been cleared from forest land at some point between

November 2012 and December 2015. GPS coordinates (WGS84 datum of World Geodetic Survey 1984) of the meadow are N33°29′44″ W88°57′32″.

Figure 35 Photograph of the Mississippi study site.

Common plants identified in the meadow and woods on the Goddard property in a quick survey on 5 May 2019 included grasses, herbs/shrubs, and tree species, including both upland and wetland species. Grasses included *Andropogon* spp. (bluestem grasses), *Carex* spp. (sedges), *Dicanthelium/Panicum* spp. (common grasses), *Eleocharis* spp. (spike rushes), *Juncus* spp. (rushes), *Poa* sp. (bluegrasses), *Scirpus* spp. (bulrushes), and *Setaria pumilla* (Poir.) Roem. & Schult. (yellow foxtail). Herbaceous or shrubby plants included *Allium* spp. (wild garlic), *Arisaema dracontium* (L.) Schott (green dragon), *Cirsium vulgare* (Savi.) Ten. (bull thistle),

Clematis spp. (clematis), *Glandularia* sp. (mock vervain), *Krigia cespitosa* (Raf.) K. L. Chambers (weedy dwarf-dandelion), *Pastinaca* spp. (wild parsnip), *Ranunculus* spp. (buttercups), *Salvia lyrata* L. (lyre-leaf sage), *S. urticifolia* (nettleleaf sage), *Scandix pecten-veneris* L. (shepherd's needle), *Smilax* spp. (greenbriars), *Taraxacum officionale* F. H. Wigg (common dandelion), *Trifolium repens* L. (white clover), *Vaccinium* sp. (blueberry), and *Vitis rotundifolia* Michx. (muscadine grape). Tree species, mostly located in the woods but with a few solitary individuals scattered in the meadow, included *Acer rubrum* L. (red maple), *Albizia julibrissina* Durazz. (mimosa), *Celtis occidentalis* L. (American hackberry), *Fagus grandifolia* Ehrh. (American beech), *Juniperinus virginiana* L. (eastern red-cedar), *Quercus* spp. (oaks), and *Salix* spp. (willows). This is not, nor is it intended to be, a comprehensive list of the flora on the property.

The Camp o' the Pines property (Figure 36) is located near Molino, Florida, 2.5 km west of the Escambia River along U.S. Highway 29, 5.5 km north of the intersection with Florida Highway 97 and about 55 km upstream of the mouth of the Escambia River into the Gulf of Mexico at Santa Rosa Island. It is in Ecoregion 65f, "South Pine Plains and Hills." Elevation is 16 meters above sea level. The property comprises about 272 hectares (675 acres), and experimental plots were located in an undeveloped area in a meadow surrounded by a mixed upland hardwood forest that is along the floodplain of Pine Barren Creek in the southwestern portion of the property. According to Google Earth historical imagery, the meadow land had been cleared from forest at some point between February 2008 and April 2010, and a deer feeder and hunting blind were installed. GPS coordinates of the meadow are N30°47'02" W87°20'41". This site is located 21 km north of the Roy Hyatt Environmental Center used by Grow (2017) in investigation of the carrion succession on 2 pig carcasses in spring season.

Figure 36 Photograph of the Florida study site.

Common plants identified in the meadow and surrounding woods on the Camp o' the Pines property in a quick survey on 18 May 2019 included grasses, herbs/shrubs, and tree species, including both upland and wetland species. Grasses included *Andropogon* spp. (blue stem grasses), *Carex* spp. (sedges), *Dicanthelium/Panicum/Paspalum* spp. (miscellaneous common grasses), *Eleocharis* spp. (spike rushes), *Juncus* spp. (rushes), *Rhynchospora* spp. (beaksedges), and *Scirpus* spp. (bulrushes). Herbaceous and shrubby plants included *Callicarpa americana* L. (American beautyberry), *Calystegia sepium* (L.) R.Br. (hedge bindweed), *Daucus carota* L. (wild carrot), *Equisetum* sp. (horsetail), *Eupatorium capillifolium* (Lam.) Small (dog fennel), *Ilex glabra* (L.) Gray (inkberry), *Ipomoea pandurata* (L.) G. F. W. Mey. (bigroot

morningglory), *Rhexia mariana* L. (Maryland meadow-beauty), *Rubus* spp. (wild blackberries and dewberries); *Rudbeckia laciniata* L. (cutleaf coneflower), *Smilax* spp. (greenbriars), *Solidago* spp. (goldenrods), *Trifolium* sp. (clover), and *Vitis rotundifolia* Michx. (muscadine grape). Tree species, mostly located in the woods but with a few solitary individuals scattered in the meadow, included *Diospyros virginiana* L. (American persimmon), *Ilex vomitoria* Sol *ex.* Aiton (yaupon), *Nyssa sylvatica* Marshall (black gum tupelo), *Pinus elliottii* Engelm. (slash pine), *P. palustris* Mill. (longleaf pine), *Quercus laevis* Walter (turkey oak), *Q. virginiana* Mill. (southern live oak), and *Triadica sebifera* (L.) Small (Chinese tallow tree). As with the list of plants at the Mississippi site, this is not, nor is it intended to be, a comprehensive list of the flora on the property.

Experiment Dates

Each carcass was visited daily through the first 10 days (approximately 240 hours of exposure), by which time most carcasses had attained the advanced decay or dry/skeletonized stages. Replicates were conducted in sequence for logistical reasons. Both study areas followed the same schedule. The first replicate was exposed May 13, 2019, finishing May 23; the second replicate was exposed May 24, finishing June 3; and the third replicate was exposed June 4, finishing June 14.

Environmental Variables

Both study areas are in the Köppen-Geiger Climatic Zone *Cfa*, (Humid Subtropical Climate). This climate zone is characterized by the following temperature and precipitation statistics: coldest month average temperature > 0°C, at least one month's average temperature >

22°C, and at least four month's average temperature > 10°C, no significant precipitation difference between seasons, and no dry months during summer (Peel et al. 2007).

Historical meteorological data were obtained from a nearby National Weather Service (NWS) weather station. For the Florida study site, the closest NWS station was Naval Air Station Whiting Field, located at N30°42'45" W87°01'06", 32.5 km east southeast of the study site (http://w1.weather.gov/obhistory/KNSE.html). For the Mississippi study site, the closest NWS station was the Columbus/West Point/Starkville, Golden Triangle Regional Airport, located at N33°27'00" W88°35'29", 35.0 km east southeast of the study site (http://w1.weather.gov/data/obhistory/KGTR.html).

To provide data specifically for each study area and to determine if the sites are relatively comparable from a thermal perspective, a continuously recording data logger (Onset Corp. HOBO MX2202 Pendant® Temperature Logger) was affixed 1.5 m above ground level to an isolated tree that was located centrally at each study area. Before deployment, the temperature loggers were synchronized to record temperatures hourly within 2 s of each other, beginning 13 May at 08:00 and ending 14 June at 12:00.

Accumulated degree day (ADD) data were calculated from the hourly data collected by the onsite data loggers. By definition, ADD at day 0 was 0. Thereafter, ADD for each successive day was calculated by averaging the lowest temperature and the highest temperature reported between sample events and adding to the ADD of the previous day; that is, ADD were not calculated on solar day temperature extrema from midnight to midnight. Even though the lower developmental thresholds for many summer-dominant blow flies is ~10°C, ADD was calculated using a 0°C baseline because the ADD calculations were used for decomposition stages and not blow fly development.

Carrion

Pig carcasses, frequently used as a model for carrion decomposition studies (Magyesi et al. 2005, Keough et al. 2017, Steadman et al. 2018), were used to study the decomposition and succession patterns. Neonate domestic pigs were obtained from a piggery where they had been culled due to not meeting commercial health criteria/benchmarks and had not been euthanized for the purposes of this project; therefore, MSU Institutional Animal Care and Use Committee (IACUC) permission was not necessary for this project. Pigs weighed between 2.08 to 5.70 kg (Table 4). Comparison with a standard growth table suggested that the pigs ranged from one to three weeks of age postpartum (https://midwestresearchswine.com/herd-health/growth-rate-chart/). All pigs were frozen shortly after death to halt decomposition processes until 2 days before they were exposed. For two days before exposure, the pigs were placed in an insect-proof room or refrigerator at 4°C to thaw, although they usually remained slightly frozen on Day 0. There was an attempt to expose similarly sized carcasses in each replicate.

Table 4 Mass (in kg, average ± standard deviation) of pig carcasses used in carrion decomposition study.

	Mississippi	Florida
Replicate 1	4.9 ± 2.5	2.3 ± 1.2
Replicate 2	2.7 ± 1.4	4.6 ± 2.4
Replicate 3	2.8 ± 1.4	2.2 ± 1.1

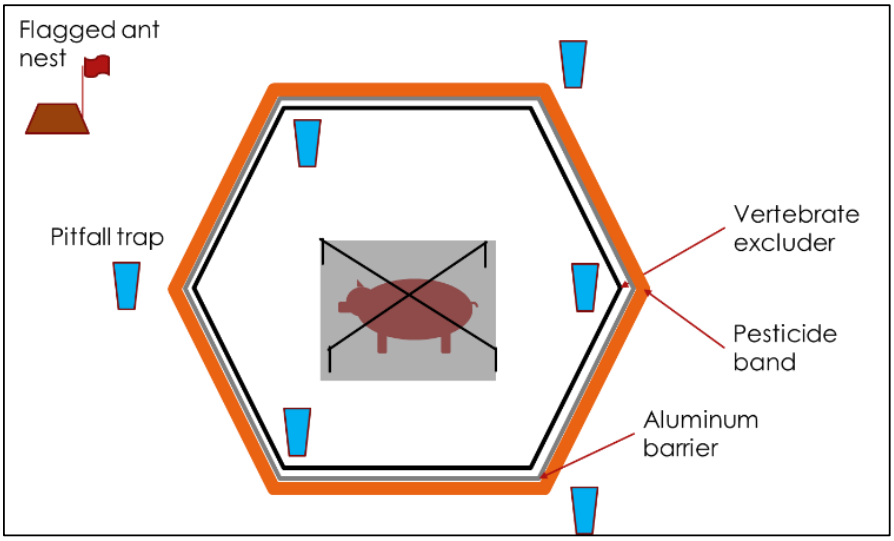
Field Methods

Carcasses were placed individually in bags made of white polyester netting to selectively exclude insects from carrion. The top of the netting was elevated by a frame so that it did not contact the carcass. Each bag had an open end to allow sampling access (similar to a pillowcase).

This opening was tied shut except during sampling events. Each bagged carcass was placed directly on the ground.

To discourage scavenging by terrestrial vertebrates such as coyotes (*Canis latrans* Say), foxes (*Vulpes vulpes* L.), domestic dogs (*Canis lupus familiaris* L.), and raccoons (*Procyon lotor* L.), carcasses were enclosed within hexagonal plastic pet cages (as in Meyer et al. 2020; perimeter ~ 5.5 m), surrounded by 2.5 cm mesh garden netting on the sides and top (Figure 37a, b). The top was also criss-crossed with clothesline rope that had orange flagging hanging from it to deter scavenging by vultures (primarily *Caragyps atratus* (Bechstein)). It was recognized that this protective caging and mesh bags provided scattered shading for the carcasses, which may have altered the succession pattern in comparison to being in open sun.

(a)



(b)

Figure 37 Equipment at study sites.

(a) Diagrammatic scheme of layout of site; aluminum barrier and pesticide band were present only in treatment 2, in which fire ants were being excluded
(b) Photograph of site with cage present

Fire ants were excluded in Treatment 2 by fine mesh netting and by a band of Suspend® CM (Bayer Corporation; active ingredient: 4% deltamethrin, a water-based pyrethroid insecticide, diluted 1.5-2.0 ounces/gallon (= 11.7-15.6 mL/L) per label instructions for "perimeter treatment") as in Meyer et al. (2020). A barrier of 12.5 cm tall aluminum flashing was installed about 10-15 cm outside the cage and sunk into the ground about 2.5 cm (Figure 37a). One gallon (3.8 L) of the insecticide was poured in a 50 mm wide band just outside the aluminum flashing with the intent that the aluminum flashing would prevent the pesticide from flowing laterally and onto or under the pig carcass. Therefore, the insecticide was at least 0.8 m away from the pig carcass and separated from it physically by the aluminum flashing and the cage. The pesticide also likely seeped vertically into the ground a few centimeters to cut off potential underground fire ant foraging tunnels.

Blow flies were excluded in Treatment 3 by a slightly coarser mesh netting which allowed fire ant access. Bricks were placed on loose netting to keep the sides taut and to prevent flies from crawling under the mesh where liquids had seeped from the carcass.

Since it was anticipated that the netting around the carrion in treatments 2 and 3 may have influenced microclimatic conditions, Treatment 1 likewise had the coarse netting, but with large holes cut into it to allow access to succession fauna and with no pesticide applied either on the ground or on the netting. Charabidze et al. (2015) demonstrated that Diptera can access concealed carrion even with extremely limited access, so the holes provided ample access to succession fauna while maintaining the netting microclimate.

It is understood that the presence of netting in all treatments and the aluminum barrier and pesticide band in Treatment 2 may have excluded more succession fauna than just the target insects; however, it was assumed that fire ants and blow flies were the most common and were

the focus of the study rather than the entire community. Although the entire community that was collected was primarily assessed in this study in light of fire ant presence/absence, the equality of the treatments in putting up barriers to the succession fauna controlled this aspect of the study. Nevertheless, it is likely that the barriers excluded fauna other than the target insects.

Carcass Assessment

During each daily carcass visit, the carcass was scored according to a modified version of the rubric in Keough et al. (2017) to obtain a Total Body Score (TBS) and objectively determine stage of decomposition (Appendix B). For this assessment, the head and neck, trunk, and limbs were scored independently using characteristics such as odor, color and texture of exposed skin, degree of bloating, exposure of bones, and insect activity. The maximum score of the head and neck region and the trunk region was 13, representing total skeletonization of the carcass with only dry bones remaining. The maximum score for the limbs was 11. The average decomposition stage of the three body regions, determined using the rubric, was considered the stage of decomposition for the carcass.

In addition to the scores, photographs were made of each carcass on each day, particularly of specific parts of the carcass, postmortem artefacts caused by the ants, maggot masses, etc. The frame and bag made photographs from directly above the carcasses difficult to obtain. Detailed notes on decomposition and insect succession patterns were recorded for the carcasses in Florida.

Girth of the abdominal region was measured with a >60 cm length of vinyl measuring tape situated transversely under each carcass when it was exposed. Daily measurements of girth ceased after bloating was complete and girth had returned to the girth reported on Day 0. Finally,

carcasses were weighed on Day 0 at placement and on Day 10 upon retrieval. The proportion of ending mass to initial mass was calculated.

Faunal Sampling

At each daily visit to the carcasses, samples of the succession fauna were collected using various techniques. Schoenly et al. (2007) established that a combination of aerial netting and pitfall trapping provided the most complete catch of forensically important fauna, and this two-method combination was used to assess the potential impact of fire ant presence on community ecology. Random and targeted samples of maggots were collected directly from the carcasses to assess potential impact of fire ant presence on blow fly larval development. Details of each of these methods is described below.

Pitfall traps.

Six pitfall traps were located at each carcass, with three pitfall traps deployed about 15 cm inside of the cage and 3 pitfall traps deployed about 15 cm outside the cage or aluminum flashing (total of 6 pitfall traps/carcass). Pitfall traps were deployed with the jar filled to ~2.5 cm deep with soapy water. To avoid rainfall possibly flooding the collection jar, a small (15 x 40 cm) board was placed over each trap. Material from the three pitfall trap samples located inside the cage was composited into a single sample on each day for each treatment; likewise, material from the three pitfall traps located outside the cage were composited into a single sample on each day for each treatment. Thus, there were two pitfall trap samples per day per treatment per replicate. Collection jars in the pitfall traps were replaced with soapy water daily.

During the time frame of Replicate 2 (24 May to 3 June), a suite of three pitfall traps was deployed at the Florida study site in a location > 50 m from the pig carcasses to provide

background information on community composition. These pitfall traps were treated similar to those around the pig carcasses, with a 15 x 40 cm cover board, daily collections with replacement of 2.5 cm soapy water, and compositing of the samples into a single sample. These background samples were not included in the original research proposal.

Aerial/Sweep sample

When present, adult flies and other flying insects were collected with an aerial net by sweeping; collected material was preserved in 70% ethyl alcohol. The only attempt to standardize the collection was to sample for approximately 5 minutes.

Random maggot sample

For the random maggot sample, a grid made of hardware cloth was held over the carcass to superimpose the grid onto the carcass (Figure 38). Grid cell size was 1 inch x 1 inch (2.54 x 2.54 cm). Cartesian coordinates (x, y) were chosen at random using a cell phone app to identify a location on the body from which maggots would be collected. A 37 mm internal diameter round plastic cylinder was centered on the random location and used to delimit an area of 10.7 cm^2 on the carcass. Using a plastic spoon, all fly eggs, maggots, and other succession fauna in the area delimited by the cylinder were collected. Samples were preserved in 70% ethyl alcohol.

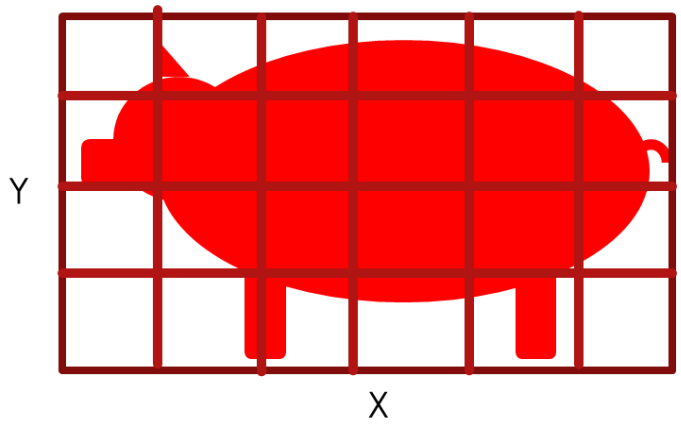


Figure 38 Diagrammatic representation of grid map for random collection of maggots directly from each carcass.

Actual grid size was 2.5 cm.

Targeted maggot sample

When present, a sample of some of the largest maggots was collected from the carcass, focusing the search on the orifices. If collection of the sample was estimated that it would remove over 10% of the maggots on the carcass, then the condition was noted and a sample was not collected so as not to adversely affect the population. Exemplars of other succession fauna present (e.g., beetles) were also collected into this sample but added to the aerial/sweep samples for analysis so that this sample would be comprised solely of maggots. Samples were preserved in 70% ethyl alcohol.

Targeted maggot samples are often collected in experimental forensic entomology for the purpose of PMI estimation under the assumption that the largest individuals are the oldest individuals (Anderson 2016, but see Moffatt et al. 2015). Although the data from these samples may eventually be used to investigate the PMI based on larval size, these samples were primarily collected for this research project to supplement the random maggot sample data.

Specimen identification and voucher specimens

All organisms were identified to the lowest practical taxonomic level, depending on age and condition of specimens and availability of relevant taxonomic literature and other resources. All organisms were identified using a Bausch + Lomb Stereo Zoom 7® stereo microscope at magnifications from 7x to 70x. Adults and third instars of blow flies were identified to the species level; eggs, first instars, and second instars of blowflies (except *Chrysomya rufifacies*) were identified to the family level. Fire ants in the Florida Panhandle study area were *Solenopsis invicta*, while fire ants from the Mississippi study area were *S. invicta* x *richteri* hybrids. Most taxa not normally of forensic importance were identified to the family level.

Representative specimens of all taxa were curated as appropriate (pinned or in alcohol) and vouchered. Voucher specimens were distributed first to the author's personal collection and second to the MEM. Some voucher specimens were distributed to taxonomic specialists (see Acknowledgements) for identification or for verification of my identifications.

Statistical Analysis

Because the background samples were not replicated, no statistical analyses were conducted to compare the experimental treatments with background conditions, although some qualitative comparisons were made in terms of species presence or absence. Additionally, throughout the balance of this chapter, so as not to continually make qualifying statements about whether or not the background sample data are included, discussion of results excludes data from background samples unless specifically stated.

Community ecology parameters

The sampling methods described above provided data to calculate several community ecology parameters for analysis. Basic community ecology parameters included total number collected, taxa richness, and the Shannon Diversity Index (H′). The H′ was calculated as

$$H' = \sum_{i=1}^{n} \rho_i ln \rho_i \,, \tag{1}$$

where ρ_i = the proportion of organisms in the ith taxon.

Two community similarity indices were also calculated from the data. The Jaccard Similarity Index (J_c) compares two samples to quantify the number of taxa that are common to both samples. It is calculated as

$$J_c = a/(a + b + c), \tag{2}$$

where *a* is the number of taxa common to both samples, *b* is the number of taxa collected only in the first sample, and *c* is the number of taxa collected only in the second sample (Ludwig and Reynolds 1988). Samples are compared in pairwise fashion.

The Bray-Curtis Similarity Index (*B*-*C*) compares the proportions of taxa common between two samples. It is calculated as

$$BC = \sum_{i,j=1}^{n} \frac{1}{2\left(minimum\ of\ \rho_i, \rho_j\right)}, \tag{3}$$

where ρ = the proportion of the community of samples i and j composed of each taxon, (Ludwig and Reynolds 1988). Like the J_c, *B-C* is compared between samples in pairwise fashion. Pitfall traps located near fire ant mounds can become inundated by fire ants (Joe MacGown, personal communication), overwhelming the community, so, to avoid skewing the calculations due to excessive numbers of that single taxon, *B-C* was calculated after removing fire ant count data from the community data.

Both indices are relative, with the J_c ranging from 0 (no taxa in common) to 1 (all taxa in common), and the *B-C* ranging from 0 (no taxa in common) to 1 (all taxa in common at identical proportions). Standard thresholds do not exist to differentiate subjective categories of "high" or "low" similarity for either index.

Blow fly larval development

The number of blow fly larvae in each developmental stage was determined and compared between Treatments 1 and 2. Treatment 3 was not considered because blow flies were excluded and there was no larval blow fly development. Data from the random and targeted maggot collection methods were used in this analysis, pooling data from all three replicates.

Statistical Hypotheses

Numerous hypotheses were tested since there were multiple stages and milestones to be reached. Many hypotheses were tested both between and within study areas for comparable data sets; if there were no or few statistically significant differences between study areas, the data were combined to increase number of replicates and statistical power.

Site temperatures

Although temperature was not measured as a part of the decomposition process, ambient temperatures from the on-site data recorders were analyzed to determine if the sites were relatively similar thermally. The null hypothesis (H_{o1}) is as follows:

H_{o1}: There is no statistically significant difference in ambient temperatures between the study areas.

The alternative hypothesis is that there are statistically significant differences in temperatures between the study areas. Because the temperature loggers were programmed to record within 2 s of each other, a paired *t*-test was used to determine if statistically significant differences in temperature existed between the study areas. A statistically significant result would indicate a temperature difference between the two study areas and could help explain potential differences in fire ant effects on carrion decomposition or the carrion community.

Physical decomposition parameters

Hypotheses regarding physical decomposition parameters included time to successive decomposition stages, time to bloating milestones, and percent loss of mass over 10 days. Specifically, the following seven related null hypotheses for each analysis, as well as the statistical test used to identify if there were any significant differences, are as follows:

H_{o2}: There is no statistically significant difference in the time (in ADD) required for carcasses to achieve the "bloat" stage, as measured by the modified Keough standards, among treatments.

H_{o3}: There is no statistically significant difference in the time (in ADD) required for carcasses to achieve the "active decay" stage, as measured by the modified Keough standards, among treatments.

H_{o4}: There is no statistically significant difference in the time (in ADD) required for carcasses to achieve the "advanced decay" stage, as measured by the modified Keough standards, among treatments.

H_{o5}: There is no statistically significant difference in the time (in ADD) required for carcasses to achieve the "skeletonized remains" stage, as measured by the modified Keough standards, among treatments.

The alternative hypothesis for these last four null hypotheses is that there are statistically significant differences in the time required for carcasses to achieve each successive decomposition stage among treatments. Analysis of variance (ANOVA) tests were used to identify if statistically significant differences existed among treatments. Dunnett's test is specifically designed to make multiple comparisons against a single control, so it was conducted *post hoc* when $p < \alpha$ in the ANOVA test to determine specifically which Treatment(s) were statistically different (MacDonald 2014).

H_{o6}: There is no statistically significant differences in the time (in ADD) required for the abdominal circumference of carcasses to achieve maximum bloat among treatments.

H_{o7}: There is no statistically significant differences in the time (in ADD) required for the abdominal circumference of carcasses to return to initial abdominal circumference among treatments.

The alternate hypothesis for these last two null hypotheses is that there are significantly significant differences in the time required for carcasses to achieve each successive bloating milestone among treatments. ANOVA tests were used to identify if statistically significant differences existed among treatments. Dunnett's test was conducted *post hoc* when $p < \alpha$ to determine specifically which Treatment(s) were statistically different (MacDonald 2014).

H_{o8}: There is no statistically significant difference in the percent mass loss of the carcasses over ten days among treatments.

The alternate hypothesis for this last of the seven related null hypotheses is that there are statistically significant differences in the percent mass loss of the carcasses over ten days among treatments. ANOVA tests were used to identify if statistically significant differences existed among treatments. Dunnett's test was conducted post hoc when $p < \alpha$ to determine specifically which Treatment(s) were statistically different (MacDonald 2014).

A statistically significant result for hypotheses Ho2 through Ho8 would indicate that either exclusion of fire ants or exclusion of blow flies has an effect on the decomposition rate of the carrion. A statistically significant result for the post hoc Dunnett's test would indicate which treatment was different, and, therefore, identify which component of the community, when excluded, created the effect found when the hypothesis was tested. Using mass loss over 10 days

as an example, if the average value for Treatment 2 is statistically higher than the average values for the other two treatments, it would indicate that exclusion of fire ants allows more mass to be retained, and, therefore, that presence of the fire ants (as in the control) allows more mass to be decomposed – fire ant presence would be a significant factor in accelerating decomposition.

Community composition parameters

The basic community composition parameters (number of organisms, taxa richness, and H′) were tested for similarity of treatments over time. The following three related null hypotheses for analysis of the statistically tested community composition parameters are as follows:

H_{o9}: There is no statistically significant difference in the intercept and slope coefficient estimates for a regression equation describing the number of organisms collected in the community composition samples over time, among treatments.

H_{o10}: There is no statistically significant difference in the intercept and slope coefficient estimates for a regression equation describing the taxa richness collected in the community composition samples over time, among treatments.

H_{o11}: There is no statistically significant difference in the intercept and slope coefficient estimates for a regression equation describing the Shannon-Weaver diversity of organisms collected in the community composition samples over time, among treatments.

The alternate hypothesis for each null hypothesis is that there are statistically significant differences among treatments in either the intercept or parameter coefficient estimates for the parameters calculated from the community composition samples. Multiple linear regression methods using dummy variables (Kleinbaum et al. 1998; also known as Analysis of Covariance or ANCOVA) were used to derive a single regression equation, given that

$$y = \beta_0 + \beta_1 x + \beta_2 z_1 + \beta_3 x z_1 + \beta_4 z_2 + \beta_5 x z_2 + \varepsilon, \quad (4)$$

where y = the estimated parameter (number of organisms collected, taxa richness, or H'), x = day of succession, $\beta_{0 \text{ through } 5}$ represent the regression coefficients, z_1 is a dummy variable with value of 1 if Treatment 2 and value of 0 if otherwise, z_2 is a dummy variable with value of 1 if Treatment 3 and value of 0 if otherwise, and ε = statistical error. Essentially, the ANCOVA model tests whether the regression lines derived from the data are coincident or not, using days as the independent variable and treatment as the covariate.

Use of ANCOVA to test for differences between treatments is preferred over individual comparison of regression line slopes and intercepts and regular ANOVA methods (Kleinbaum et al. 1998). The α value can remain uncorrected since only a single test for coincidence is generally conducted instead of multiple tests of intercepts and slopes. Furthermore, the resulting equation reduces to the regression for the control treatment if the regression coefficients for the dummy variables = 0 (i.e., $\beta_2 = \beta_3 = \beta_4 = \beta_5 = 0$). When a nonsignificant result ($p > \alpha$) for intercepts and slopes of the control variables and the dummy variables is found, it indicates that the corresponding treatments are statistically similar to each other, since the lines are statistically coincident.

A statistically significant result ($p < \alpha$) for the partial F test on β_0 is indicative that the regression for Treatment 1 has an intercept not equal to 0, a statistically significant result for the partial F test on β_1 is indicative that the regression for Treatment 1 has a slope not equal to 0. A statistically significant result for the partial F test on β_2 or β_3 is indicative that the regression for Treatment 2 has an intercept or slope, respectively, that is not equal to 0. A statistically significant result for the partial F test on β_4 or β_5 is indicative that the regression for Treatment 3 has an intercept or slope, respectively, that is not equal to 0. When there are differences in statistical significance between regression coefficients for different treatments, then it is an indication that there is potentially a difference between those treatments: opposite signs (+ or -) for the regression coefficient would clearly indicate differences between treatments, while additional analysis directly comparing slopes (Kleinbaum et al. 1988) would be required if the signs of the regression coefficients were the same.

Community similarity parameters

Community similarity indices J_c and *B-C* were based on pooled data from replicates to better characterize the fauna at each treatment and sample day. Because there is no defined threshold for similar/dissimilar for either J_c or *B-C*, the results were verbally described in terms of percent similarity. To identify potential temporal trends, the community similarity parameters were tested statistically, with the following two related null hypotheses (H_o) for each analysis of the statistically tested community similarity parameters:

H_{o12}: There is no statistically significant difference in the intercept and slope coefficient estimates for regression equations describing the Jaccard similarity coefficient on community composition samples over time, among treatments.

H_{o13}: There is no statistically significant difference in the intercept and slope coefficient estimates for regression equations describing the Bray-Curtis similarity coefficient on community composition samples over time, among treatments.

The alternate hypotheses are that there are statistically significant differences among treatments in either the intercept or parameter coefficient estimates for the community. ANCOVA methods were used to identify if statistically significant differences existed among the comparisons of treatments. For this test, the comparisons were against Treatment 1 (as the control), so only one dummy variable was needed and reassigned as follows: $z_1 = 1$ if the comparison for the similarity involved Treatment 1 and Treatment 2, and $z_1 = 0$ if the comparison for the similarity involved Treatment 1 and Treatment 3. Because of slight dissimilarities in the community composition between Mississippi and Florida, these hypotheses were tested for the data from each state individually.

For this analysis, a statistically significant result ($p < \alpha$) for the partial F test on β_0 is indicative that the intercept of the regression describing similarity between Treatment 1 and Treatment 2 is not equal to 0 and is irrelevant for determining differing effects of the treatments. A statistically significant result for the partial F test on β_1 is indicative that the slope of the regression describing similarity between Treatment 1 and Treatment 2 is not equal to 0; here, a positive sign on the coefficient means that the communities are becoming more similar over time and a negative sign on the coefficient means that the communities are becoming more dissimilar over time. Likewise, a statistically significant result for the partial F test on β_3 is indicative that the slope of the regression describing the similarity between Treatment 1 and Treatment 3 is not

equal to 0, with a positive coefficient indicating more similarity between the communities over time and a negative coefficient indicating less similarity between the communities over time. A lack of statistically significant results ($p > \alpha$) indicates that the communities are similar to the control over time, and the exclusion of fire ants or blow flies has no discernable effect on the community composition over time.

Blow fly larval development

Hypotheses regarding blow fly larval development included the proportion of blow fly larvae in each instar at each successive sample date among treatments. Even though there was a mix of blow fly species, data from all calliphorid species were combined, since it was often difficult to reliably and consistently separate most species at the earlier instars. Specifically, ten individual null hypotheses ($H_{o14\text{-}23}$), one for each day of succession, are each as follows:

H_{o14}: There is no statistically significant difference in the relative proportions of blow fly maggots in each immature life stage (egg, first, second, and third instars) on day one among treatments.

Only H_{o14} is presented above; $H_{o15\text{-}23}$ are identical except in the day of succession being two, three, four, . . ., ten. The alternate hypothesis is that there are statistically significant differences in the relative proportions of blow fly maggots in each immature life stage at each successive sample date among treatments. Fisher's exact test for independence (Agresti 2007, McDonald 2014) was used to identify if statistically significant differences existed between treatments on each day of succession. The Fisher's exact test was chosen over the more conservative Pearson's χ^2 test because there were numerous structural and sampling zeros in the

dataset which could not be resolved and which would result in a division by zero error in the χ^2 test. Because of slight dissimilarities in community composition between Mississippi and Florida, these hypotheses were tested for the data from each state individually. A statistically significant result for this test would indicate that the proportions of each life stage present on a given day (one, two, three, . . . , ten) are different between Treatment 1 and Treatment 2, suggesting an effect due to the presence/absence of fire ants.

When possible, all variables were tested for the assumptions associated with each statistical test using a relevant statistic (e.g., Shapiro-Wilks W for normal distribution, Levene's test for homoscedasticity, Durban-Watson test for independence, visual plots for linearity, actualization of random sampling, mutually exclusive results, etc.). When necessary and possible, data were mathematically transformed to meet or approximate assumptions associated with the statistical tests. If assumptions were greatly violated (> 2 assumptions violated), non-parametric tests were also used to compare treatments; however, if the decision regarding statistical significance ($p \leq \alpha$ or $p > \alpha$) based on the non-parametric test was the same as for the parametric test, then only the results of the parametric test were reported. When there were no or few statistically significant differences between study areas for a given set of hypotheses, the data were pooled from both study areas for comparison among treatments, resulting in a larger number of replicates and greater statistical power.

An *a priori* significance level of $\alpha = 0.05$ was used for all tests, including for tests of assumptions. For multiple comparisons within a family of similar statistical tests (e.g., tests of assumptions, *t*-tests of parameter values between states, Fisher's exact tests), the Holm's Sequential Bonferroni correction adjustment (Holm 1979) was applied to the α level. The Holm-Bonferroni correction factor was applied by ranking the derived p-values and sequentially testing

the p-values from smallest to largest against progressively less strict corrected α values, designated as α_{HB} values. In practice, the smallest p-value was tested against α/k, where k = number of within-family statistical tests that were conducted. Subsequent, larger p-values were then sequentially tested against α/(k-1), α/(k-2), . . ., α, stopping at the last p-value comparison or when a p-value was greater than its α_{HB} value. If a p-value was greater than its corresponding α_{HB} value, then all subsequent tests with larger p-values were considered to be statistically non-significant. The dichotomy of significance/non-significance based on an *a priori* α value was used despite concerns about the misuse of p-values in statistical inference (Wasserstein and Lazar 2016, Wasserstein et al. 2019); however, statistical tests in which p-values were near the α_{HB} threshold were interpreted cautiously. To aid in interpretation, p-values were reported to the third decimal point, except when $p < 0.001$, in which case p-values were reported to the fourth decimal point.

All statistical tests were conducted in R (R Core Team 2019) using the R Commander GUI and associated packages (Fox 2005, 2017, Fox and Bouchet-Valat 2019) or the R Companion package (Mangiafico 2019).

Results and Discussion

General Description of Carrion Decomposition

The pig carcasses decomposed along the same stages as expected but the timing of each stage differed by treatment (Figure 39; note that it was frequently difficult to get good photographs because of the netting). Additionally, there was limited variation in the timing of each stage among replicates. Observations are provided below without interpretive discussion.

Figure 39 Photographic progression of decomposition of pig carrion in Florida.

Treatment 1 = control; Treatment 2 = fire ants excluded; Treatment 3 = blow flies excluded.

Figure 39 (continued)

Treatment 1 (Control)

The "Fresh" stage of decomposition in Treatment 1 lasted generally through Day 1. No physical changes were noted on any carcasses, except where ants had begun to produce small lesions. Adult flies were uncommon in the vicinity, while ants were scattered all over the carcass.

In Treatment 1, the "Bloat" stage of decomposition was characterized by the beginning and continued bloat of the carcasses, often with marbling or shiny appearance of the skin, especially on the abdomen. This stage generally lasted through Days 2 and 3. Flies were often abundant in the vicinity, and blow fly eggs were usually present in large batches on the face, between the legs, and along the carcass/ground interface. Ants usually constructed thatch coverings over the face and anal regions. In one case, a narrow column of ants was observed traversing between the nearest mound and the carcass.

In Treatment 1, the "Active Decay" stage generally occurred on Days 3 and 4. Portions of the head, especially the eyes and mouth, and often the hind limbs, were completely exposed and obliterated by maggots, while the rest of the carcass was filled with maggots which may or may not breach the skin. Thatch coverings from the ants were limited to areas where the carcass contacted the ground and not onto upper surfaces. Flies tended to be common in the vicinity, while ants were sometimes observed carrying small maggots.

The "Advanced Decay" stage in Treatment 1 generally occurred on Days 5 and 6. The carcasses were usually partially skeletonized with large portions of exposed, drying skin holding the general shape of the carcass. Most maggots had migrated from the carcasses, occasionally disarticulating the carcasses and displacing the bones by their movements. Flies and beetles were present but not as abundant as on previous days, possibly because of spiders which had become

quite numerous. A few ants were seen on the surfaces of the carcasses, but they were more frequently observed attacking migrating maggots.

By Day 7, the carcasses in Treatment 1 had reached the "Skeletal Remains" stage. The carcasses were mostly skeletonized with only small bits of dried skin attached. Movements of migrating maggots continued to displace and scatter the drying bones. Occasionally, spots of mold were observed on skeletal and dried skin surfaces around Day 10. Very few flying insects were observed in the vicinity throughout the stage.

Treatment 2 (fire ants excluded)

In Treatment 2, the "Fresh" stage lasted through Day 1. No physical changes were observed on the carcasses, and there were few flies in the vicinity.

The "Bloat" stage of Treatment 2 occurred on Day 2 and 3. Usually, the characteristic elevation of the legs due to the bloat was observed while the skin o the abdomen took on either a marbled, greenish appearance or became glossy. Early in the stage, batches of fly eggs were present on the face, in the ears, between the legs, and around the groin areas. Later in the stage, early instar maggots completely obliterated portions of the face, legs, and groin.

The "Active Decay" stage in Treatment 2 was only observed on Day 4 when the entire, deflated carcass appeared to be filled with maggots. Portions of the carcasses were often obliterated by maggots, revealing the skeleton; other portions of the carcass were covered by the translucent skin so that the masses of maggots were visible underneath.

In Treatment 2, the "Advanced Decay" stage lasted from Days 5 to 6. The carcasses were completely deflated and partially skeletonized, with remaining skin turning black and beginning to dry. Maggots completed their migration from the carcasses, often displacing bones as they moved, although most appeared to perish at the pesticide barrier. Few large flying insects were

observed, and ants were observed outside the pesticide barrier attacking maggots that had migrated beyond it.

The "Skeletal Remains" stage in Treatment 2 began by Day 7 of exposure. Large portions of the skeleton was exposed and disarticulated, with remaining skin black, drying, and curling. Spots of mold were observed on Day 10. A few maggots finished their migrations, and large flies were generally uncommon in the vicinity. In one replicate, eclosion of the adult flies occurred on Day 10 so that teneral flies were observed on nearly every vertical surface in the vicinity.

Treatment 3 (blow flies excluded)

The "Fresh" stage in Treatment 3 occurred on Days 0 and 1. No physical changes were observed on the carcasses, although there was sometimes a noticeable fetid odor. Ants were observed scattered all over the carcasses, but not in large groups.

In Treatment 3, the "Bloat" stage occurred on Days 2 through 4. Skin on the bloated abdomen turned greenish black or took on a marbled, sometimes glossy, appearance. Ants built thatch coverings on the face and groin areas of the carcasses; in one carcass, they obliterated many underlying tissues including the tongue and snout.

The "Active Decay" stage in Treatment 3 began about Day 5. In some carcasses, it was difficult to determine if the stage had even ended by Day 10, although in one replicate, the stage was done by Day 7. The carcasses deflated, although not to the same degree as the other carcasses. Generally, the skin remained unbroken except where ants had produced lesions in the skin. The thatch coverings produced by the ants remained in place on the face and groin regions of the carcasses and often were expanded along the front legs, the belly, and back where the carcasses contacted the ground. Below the thatch coverings, the ants frequently obliterated large portions of muscle tissue, such as along the hindquarters.

The "Advanced Decay" stage in Treatment 3 was reached in only one replicate, beginning about Day 7. Generally, the completely deflated carcasses experienced drying of the exposed skin over muscular areas such as the head, neck, and legs, while skin covering the abdominal region became moist and greasy. The skin and tissues in contact with the ground under the carcass decomposed completely. Although the odor was extremely strong, there was very little insect activity; even ants were uncommon on the exposed surfaces.

The carcasses in Treatment 3 did not attain the "Skeletal Remains" stage by Day 10.

Temperature

Temperature data for the two study areas indicated some minor (1 – 4°C) differences between average high and low temperatures between study sites, both historically (Table 5) and contemporaneously with the experiment (Figure 40). A paired *t*-test indicated that there was a statistically significant difference in hourly temperatures between the study sites ($p = 0.0018$, $t = 3.1528$, $df = 772$), based on the on-site temperature loggers. This result rejects null hypothesis (H_{o1}) that "*there is no statistically significant difference in ambient temperatures between the study areas.*" This result is likely a function of the disjunct geographic location of the study sites; however, it is possible that the result may also be a function of the very large number of observations (n=774), substantially increasing the statistical power of the test.

Table 5 Historical and actual temperature data for the study areas.

	Starkville, Mississippi	Molino, Florida
Historical		
Average High Temperatures	27.4 – 31.4°C	28.2 – 31.4°C
Average Low Temperatures	14.8 – 19.4°C	18.7 – 22.5°C
Actual		
High Temperatures	29.5 – 39.6°C	24.9 – 38.9°C
Low Temperatures	9.0 – 21.6°C	9.9 – 23.0°C

Historical temperature data for the study areas for 13 May – 14 June, based on data from data from 1945 – 2018 from the closest NWS station. Actual on site temperature data for the study areas for 13 May – 14 June 2019 was measured by data logger.

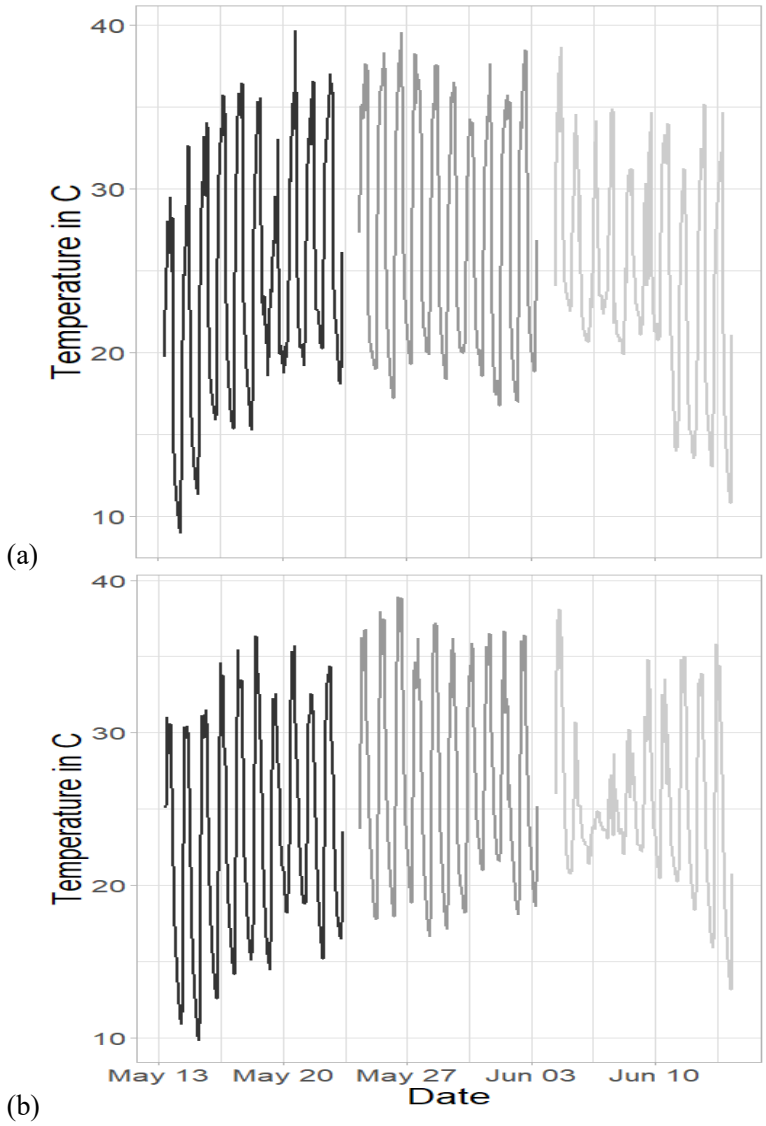


Figure 40 Ambient temperatures during pig carcass exposure.

Replicate 1 (black line) = May 13 – May 23; Replicate 2 (dark gray line) = May 24 – June 3; Replicate 3 (light gray line) = June 4 – June 14, 2019.
(a) Mississippi
(b) Florida

Final ADD values on Day 10 ranged from 240 to 281 ADD (Table 6). Given that the average temperature was about 20°C, differences between Mississippi and Florida temperatures were generally less than a full day for any replicate. Replicate 1 was a full day (26 degree days)

warmer than Florida, whereas both states had approximately the same ADD (<8 degree days) for Replicates 2 and 3.

Table 6 Accumulated degree days (ADD; baseline = 0°C) for each replicate in Mississippi and Florida.

	Mississippi			Florida		
Day of Succession	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Day 0	0	0	0	0	0	0
Day 1	22	28	28	21	27	29
Day 2	47	57	55	41	55	56
Day 3	72	86	83	63	84	80
Day 4	98	114	109	87	111	106
Day 5	125	142	137	113	138	132
Day 6	151	169	161	138	165	159
Day 7	181	196	183	163	193	186
Day 8	209	222	207	191	222	213
Day 9	237	251	230	215	250	238
Day 10	266	281	255	240	277	262

Fire Ant Effects on Physical Decomposition Parameters

Statistical tests on the data from the physical decomposition parameters regarding the assumptions of the statistical tests (Table 7) indicated that normality was the only assumption that was regularly violated ($p < 0.033$). The regression used in ANOVA is known to be relatively robust to violations of normality, especially when the distribution is identical for the groups being tested (MacDonald 2014). The assumption of homoscedasticity of data was not violated ($p > 0.10$), while the assumption of independence was violated only once ($p = 0.027$; otherwise $p > 0.670$). Based on these results, I chose to continue with the use of parametric statistics for analysis.

Table 7 Results of statistical tests of assumptions for ANOVA of physical decomposition parameter data.

	Normality		Homoscedasticity		Independence	
	Shapiro-Wilks W	p-value	Levene test	p-value	Durban-Watson test	p-value
Time (ADD) to achieve maximum bloat	0.765	**<0.001**	1.667	0.222	2.552	0.770
Time (ADD) to return to original girth	0.843	**0.007**	2.389	0.126	2.653	0.836
Time (ADD) to achieve "Bloat" stage	0.786	**0.001**	0.952	0.400	1.383	**0.027**
Time (ADD) to achieve "Active Decay" stage	0.708	**<0.0001**	0.727	0.500	2.424	0.670
Time (ADD) to achieve "Advanced Decay" stage	0.886	**0.033**	1.462	0.263	2.673	0.847
Time (ADD) to achieve "Skeletonized Remains" stage	0.834	**0.005**	2.010	0.169	2.565	0.778
Mass loss (%) over 10 days	0.817	**0.003**	2.695	0.100	2.674	0.848

Statistically significant results in **bold** font.

Preparatory *ad hoc* analyses using Student's *t*-tests indicated that physical decomposition parameters for each treatment generally did not differ between the Florida and Mississippi study sites (Table 8). In only one comparison (days to achieve "bloat" stage in Treatment 3) were statistically significant results found ($p = 0.016$, although using the Holm-Bonferroni correction factor where $\alpha/k = 0.002$, this result would not be statistically significant), but all other results were clearly not statistically significant ($p \geq 0.070$). Because the results did not differ between study areas for these treatments, all replicates were used from both study areas for further statistical analysis of the physical decomposition parameters.

Table 8 p-Values from Students *t*-tests comparing physical decomposition parameters between results in Mississippi versus results in Florida.

	Treatment 1	Treatment 2	Treatment 3
Time (ADD) to achieve maximum bloat	1.000	0.609	0.795
Time (ADD) to return to original girth	0.349	0.561	0.874
Time (ADD) to achieve "Bloat" stage	0.116	0.230	**0.016**
Time (ADD) to achieve "Active Decay" stage	0.251	0.101	0.155
Time (ADD) to achieve "Advanced Decay" stage	0.070	0.251	1.000
Time (ADD) to achieve "Skeletonized Remains" stage	0.132	0.539	0.374
Mass loss (%) over 10 days	0.731	0.801	0.238

Statistically significant results in **bold** font.

Exclusion of succession groups significantly affected the time in which the carcass remained bloated over original girth ($p = 0.002$, Table 9, $\alpha_{HB} = 0.007$). This result rejects null hypothesis (H_{o7}) that "*there is no statistically significant difference in the time (in ADD) required for the abdominal circumference of carcasses to return to initial abdominal circumference among treatments."* However, the lack of a statistically significant difference in time to achieve maximum bloat ($p = 0.175$) supports the related null hypothesis (H_{o6}) that "*there is no statistically significant difference in the time (in ADD) required for the abdominal circumference of carcasses to achieve maximum bloat among treatments."*

Table 9 p-Values for ANOVA tests comparing all three treatments, with Dunnett's test

	All Treatments (ANOVA)		Dunnett's post-hoc comparison	
	p-value	$F_{2,15}$ value	p-value (Treatment 2 v. 1)	p-value (Treatment 3 v. 1)
Time (ADD) to achieve maximum bloat	0.175	1.959	n/a	n/a
Time (ADD) to return to original girth	**0.002**	9.913	0.809	**0.005**
Time (ADD) to achieve "Bloat" stage	0.728	0.324	n/a	n/a
Time (ADD) to achieve "Active Decay" stage	0.209	1.740	n/a	n/a
Time (ADD) to achieve "Advanced Decay" stage	**0.008**	6.908	0.485	**<0.001**
Time (ADD) to achieve "Skeletonized Remains" stage	**0.004**	7.931	0.311	**0.001**
Mass loss (%) over 10 days	**<0.0001**	76.35	0.615	**<0.0001**

Statistically significant results in **bold**. Dunnett's test conducted as a *post hoc* multiple comparison test only when ANOVA indicated statistically significant results (otherwise, n/a = not applicable). All 6 replicates from Florida and Mississippi included.

Likewise, the times required to transition into the "Advanced Decay" stage ($p = 0.008$) and the "Skeletonized Remains" stage ($p = 0.004$), and percent mass loss over 10 days ($p < 0.0001$; Table 9) were significantly different among treatments. For those particular parameters, null hypothesis (H_{o4}) that "*there is no statistically significant difference in the time (in ADD) required for carcasses to achieve the 'advanced decay' stage among treatments"* and null hypothesis (H_{o5}) that "*there is no statistically significant difference in the time (in ADD) required for carcasses to achieve the 'skeletal remains' stage among treatments"* are both rejected. Conversely, null hypothesis (H_{o2}) that "*there is no statistically significant difference in the time (in ADD) required for carcasses to achieve the 'bloat' stage among treatments"* and null hypothesis (H_{o3}) that "*there is no statistically significant difference in the time (in ADD) required for carcasses to achieve the 'active decay' stage among treatments"* are both supported.

For each of the parameters with statistically significant differences, Dunnett's test (Table 9) indicated that the exclusion of blow flies was the factor that significantly increased the decomposition rates and significantly decreased the mass loss over 10 d ($p < 0.005$), while exclusion of fire ants did not significantly increase or decrease decomposition rates ($p > 0.311$). Exclusion of insects, particularly blow flies has been elsewhere demonstrated to be a major factor in decomposition rate (Simmons et al. 2010).

Although fire ant presence or absence did not statistically affect the carrion decomposition rates based on the physical decomposition parameters I chose to measure (time to bloat milestones, time to decomposition stages, and mass change over ten days), there were physical changes observed. For instance, fire ants were observed chewing lesions into the carcasses (Figure 41) and building a "thatch" covering on the face, anal regions of the carcasses (Figure 42), and other places of contact with soil. When ants were excluded, these lesions and thatch coverings were not present. The postmortem lesions may have served as additional openings for oviposition by blow flies and other succession fauna (Meyer et al. 2020, Park and Moon 2020a), but they did not appear to accelerate or decelerate the rate of seral decomposition changes.

Figure 41 Postmortem artifacts (lesions in thoracic and abdominal skin) from *Solenopsis* on a pig carcass.

(a) 

(b) 

Figure 42 Postmortem artifacts (thatch on hindquarters and tissue destruction) from *Solenopsis* on a pig carcass.

(a) Day 3, Treatment 3, Replicate 1
(b) Day 9, Treatment 3, Replicate 1

Similar reports of soil or thatch coverings by ants on carcasses have been noted elsewhere. Maciel et al. (2015), Pereira et al. (2017), and Mendonça et al. (2019) incidentally found the Neotropical fire ant *S. saevissima* on cat and big-eared opossum carcasses, noting that that the ants' activity of burying portions of the carrion appeared to delay succession by necrophagous Diptera and decomposition of the carrion. Lindgren et al. (2010) reported S. *invicta* burying large portions of an experimentally exposed human cadaver, including filling in postmortem wounds with soil, and delayed colonization of the corpse by several days. Park and Moon (2020b) opined that similar behaviors by *Tetramorium tsushimae* Emery in South Korea could delay succession of other fauna and affect forensic interpretation of entomological evidence at cadavers.

Synopsis of Faunal Sampling

Excluding the background pitfall trap samples, a total of 23,737 organisms was collected and identified, with the majority being collected in the pitfall traps (Table 10). There were 153 taxa represented (Appendix C), although there may have been some minor overlap between taxonomic names for larvae and adults for some taxa (only Carabidae, Calliphoridae in eggs, first instar larvae, and second instar larvae, and Sarcophagidae). Insects were taxonomically represented by 12 orders and 51 families, and other invertebrates (spiders, myriapods, mollusks, segmented worms, etc.) were represented by 8 orders and 14 families (Appendix C).

Table 10 Number of organisms collected in the vicinity of pig carcasses in Mississippi and Florida, May 13 – June 14, 2019.

	Mississippi		Florida	
	Number Collected	Taxa Richness	Number Collected	Taxa Richness
Aerial Samples	127	16	267	47
Inner Pitfall Traps	1,942	49	6,436	77
Outer Pitfall Traps	3,300	40	6,176	77
Random Maggot Collection	568	3	3,759	5
Targeted Maggot Collection	389	4	773	5
Background Pitfall Traps	--	--	1,171	49
Total (excluding Background Pitfall Traps)	6,326	71	17,411	116

An additional 1,171 organisms were collected in the background pitfall traps in Florida, representing 49 taxa (Table 10), for a grand total of 24,908 individuals in 156 taxa. Only three taxa were collected in background samples that were not also collected in the treatment samples: *Reticulitermes flavipes* (Kollar), *Camponotus* sp., and an unidentified Mutillidae (Appendix C). Conversely, there were 110 taxa that were found in the treatment samples and not in the background samples.

About 60 of the taxa collected from carrion are from insect families commonly associated with carrion decomposition (Reed 1958, Payne and Crossley 1966, Merritt and De Jong 2016). There were some generalist predators (e.g., the cicindelid tiger beetles *Cicindela punctulata* Olivier and *Tetracha carolina* L., some unidentified robber flies in the family Asilidae, and 12 taxa of spiders; Appendix C), in addition to predators already known to frequent carrion (e.g. *Hydrotaea aenescens* (Wiedemann), staphylinid beetles; Farkas and Jantnyik 1990), as well as some apparently incidental taxa.

General Description of Insect Succession Patterns

The Diptera and Coleoptera are usually the most obvious succession fauna encountered during carrion decomposition (Reed 1958, Payne and Crossley 1966, Merritt and De Jong 2016), and this was the case with the pig carcasses exposed for this study.

Diptera

In Mississippi, 2,098 individuals and 12 distinct taxa of flies were reported (Figure 43). In Treatment 1, Calliphoridae arrived during the "Fresh" stage, as expected. Phoridae, Sarcophagidae, and Psychodidae also arrived during the "Fresh" stage. Some of those families tapered off in the later stages of decomposition. In Treatment 2, only Calliphoridae and Phoridae were present in the "Fresh" stage and persisted through the decomposition stages. In Treatment 3, only the Phoridae were present across all stages. These flies are small and capable of passing through the mesh that excluded larger flies. Other Diptera were present in single stages (sometimes only on a single day, suggesting that they were merely adventitious species) in various treatments (Figure 43).

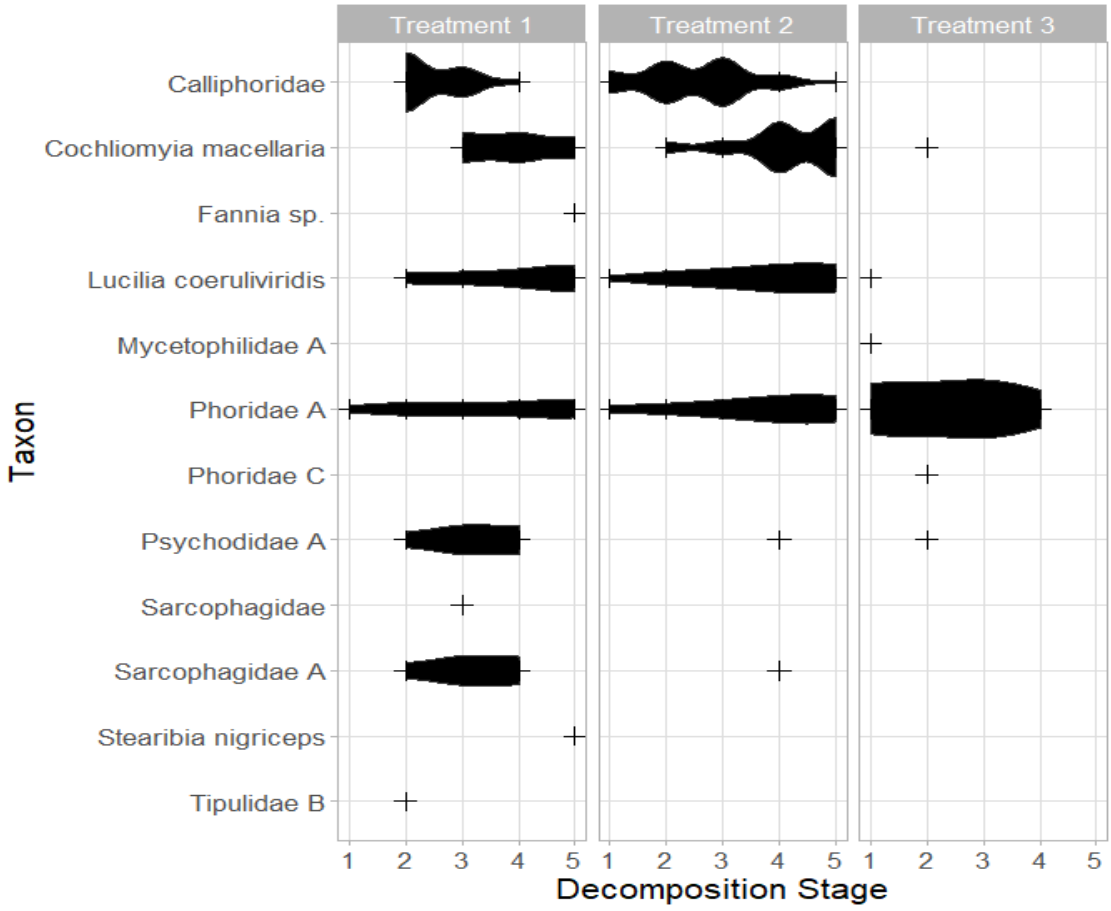


Figure 43 Temporal distribution of Diptera on pig carrion in Mississippi.

Decomposition stages are 1 = "Fresh", 2 = "Bloat", 3 = "Active decay", 4 = "Advanced decay", and 5 = "Skeletonized remains". Plus symbol (+) indicates presence only in that stage; line thickness indicates relative abundance of the taxon.

In comparison, 20 fly taxa (6,284 individuals) were collected at the carrion in Florida (Figure 44). Along with *Calliphora vicina* Robineau-Desvoidy, the same suite of blow fly taxa was collected by Grow (2017), except in different relative abundances of each taxon due to sampling at a different time of year (March-May). Among the Diptera, phorids were also present during the "Fresh" stage of decomposition in both Treatment 1 and Treatment 2, persisting through all of the decomposition stages. When ants were excluded, most Diptera arrived during the "Bloat" stage and then persisted. As expected, larger Diptera, including Calliphoridae, were

absent in Treatment 3. Notably, fly taxa that were collected in only on decomposition stage were found mostly in the "Skeletonized" stage in Treatment 1, but in the "Active decay" stage in Treatment 3 (Figure 44). A few taxa, such as Tipulidae and the bibionids *Dilophus* sp. and *Plecia nearctica* Hardy, are likely accidental.

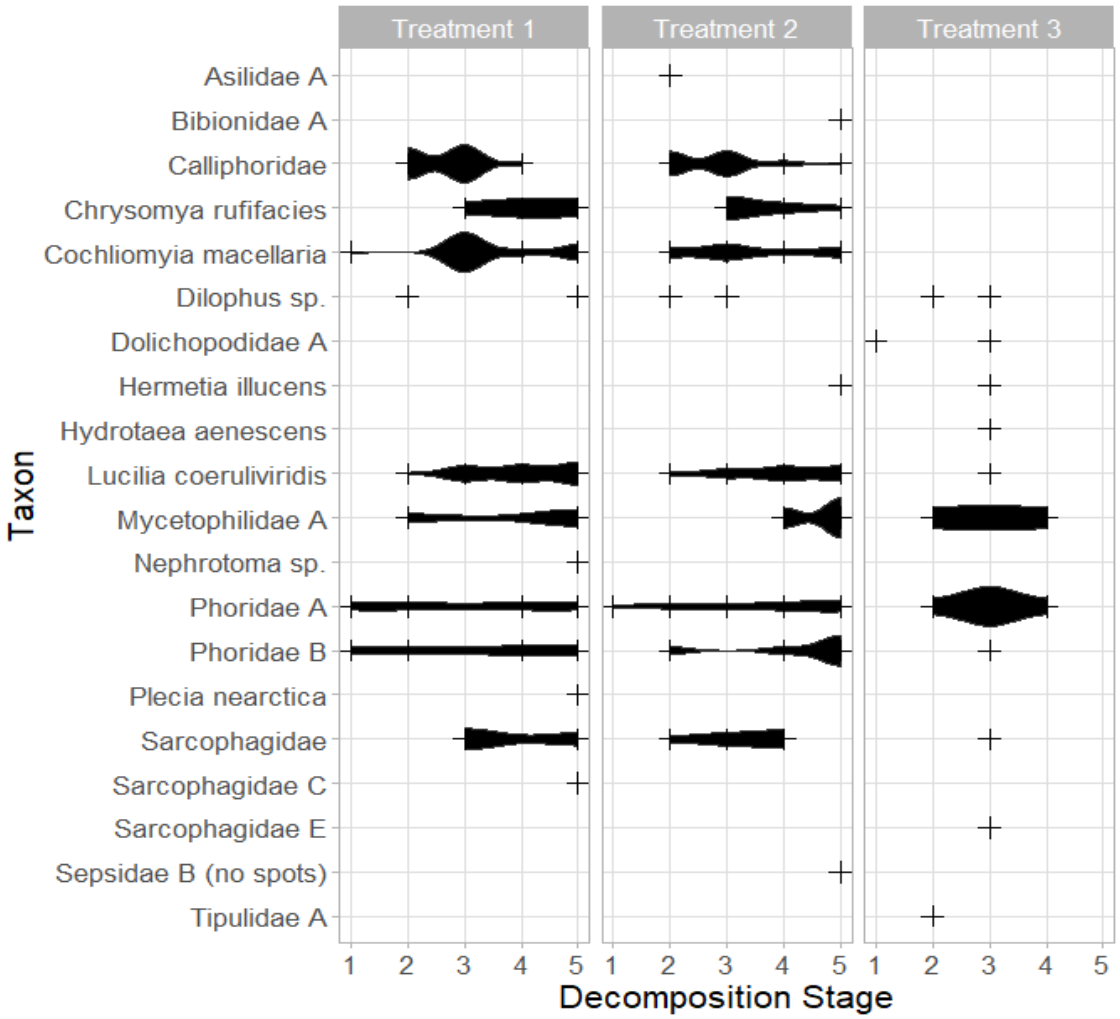


Figure 44 Temporal distribution of Diptera on pig carrion in Florida.

Decomposition stages are 1 = "Fresh", 2 = "Bloat", 3 = "Active decay", 4 = "Advanced decay", and 5 = "Skeletonized remains". Plus symbol (+) indicates presence only in that stage; line thickness indicates relative abundance of the taxon.

Coleoptera

There were 80 individuals and 23 distinct taxa of beetles represented on the pig carrion exposed in Mississippi (Figure 45). Of these, 9 taxa were representatives of the Staphylinidae. Only one taxon, “Staphylinidae A”, was present throughout the succession; this beetle arrived in small numbers during the “Fresh” stage and remained through the “Skeletonized” stage in Treatments 1 and 2. All other beetle taxa were present only within single stages across all three treatments (Figure 45).

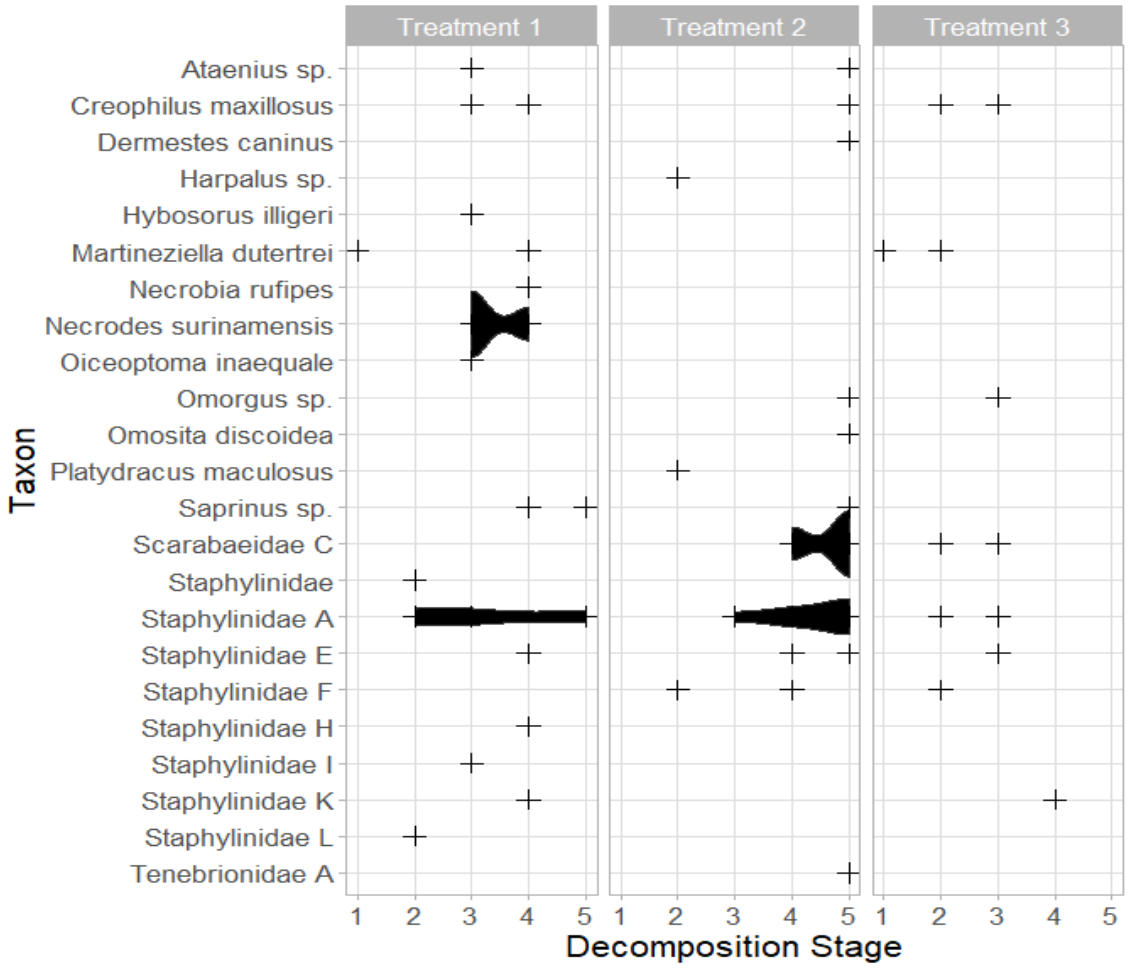


Figure 45 Temporal distribution of Coleoptera on pig carrion in Mississippi.

Decomposition stages are 1 = “Fresh”, 2 = “Bloat”, 3 = “Active decay”, 4 = “Advanced decay”, and 5 = “Skeletonized remains”. Plus symbol (+) indicates presence only in that stage; line thickness indicates relative abundance of the taxon.

In Florida, there were 36 taxa of beetles collected (633 individuals), including 10 taxa of staphylinid beetles (Figure 46). As in Mississippi, staphylinid beetles tended to arrive early and persist through all stages of decomposition. In addition to the staphylinid beetles, other taxa that remained at carrion through at least two decomposition stages included the nitidulid *Stelidota coenosa* Erichson, the histerid *Saprinus* sp., the anthicid *Acanthinus argentinus* (Pic), and carabid larvae (Figure 46). Most species were normally associated with carrion, but a few (e.g.,

the coccinellid *Coleomegilla maculata* (DeGeer) and the scarabaeid *Euphoria sepulchralis* (F.)) are likely incidental.

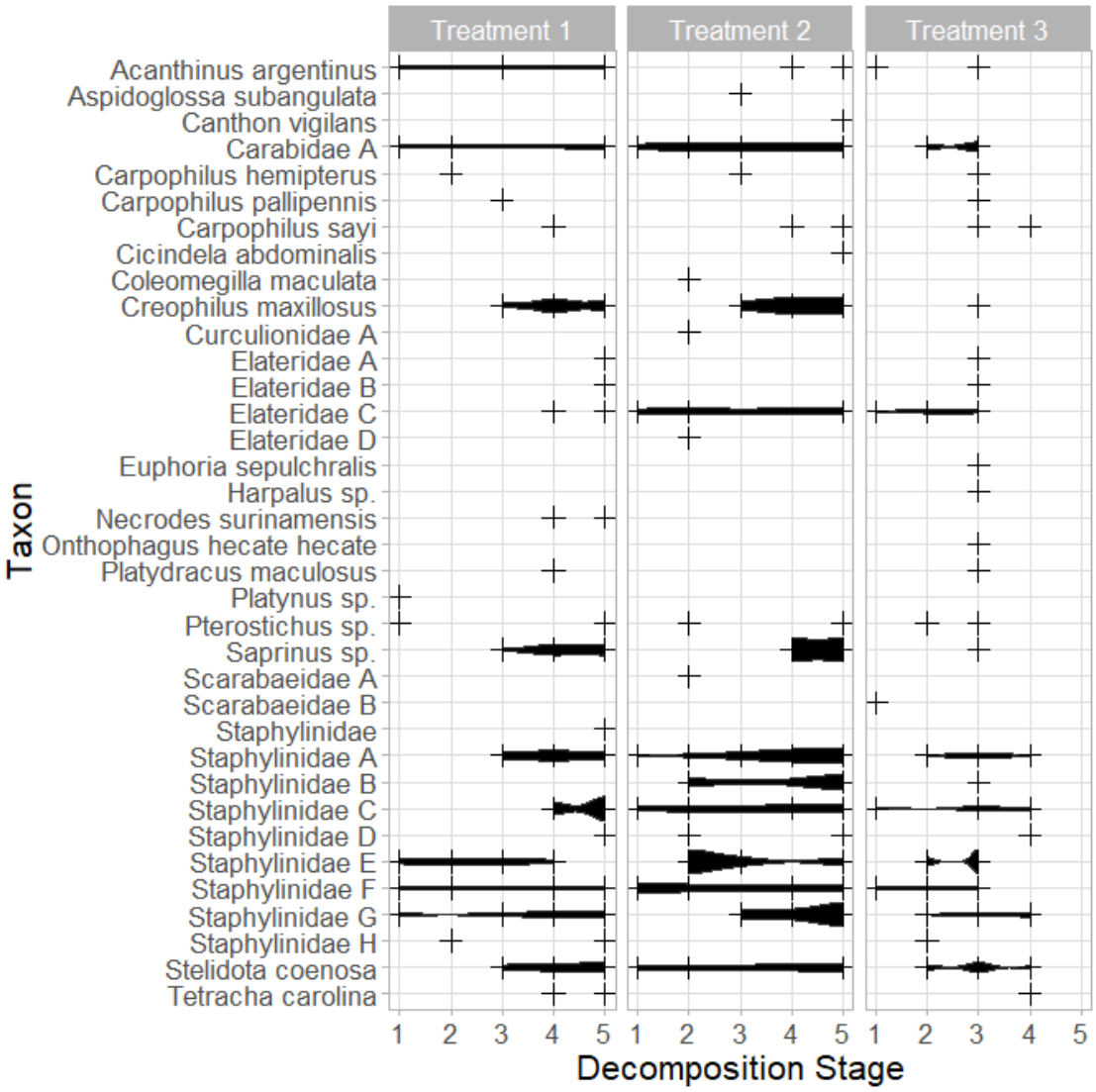


Figure 46 Temporal distribution of Coleoptera on pig carrion in Florida.

Decomposition stages are 1 = “Fresh”, 2 = “Bloat”, 3 = “Active decay”, 4 = “Advanced decay”, and 5 = “Skeletonized remains”. Plus symbol (+) indicates presence only in that stage; line thickness indicates relative abundance of the taxon.

Formicidae

As expected, ants were overwhelmingly represented by fire ants, with 3,883 individuals of *S. invicta* x *richteri* in Mississippi and 8,821 individuals of *S. invicta* in Florida. Other ant species collected included *Forelius mccooki* (McCook), *Hypoponera opacipes* (Mayr), and *Paratrechina longicornis* (Latreille) in Mississippi (total of 13 individuals), and *Brachymyrmex patagonicus* Mayr, *Crematogaster cerasi* Fitch, *Cyphomyrmex rimosus* Spinola, *H. opaciceps*, and *Odontomachus haematodus* L. in Florida (total of 423 individuals). All these species, except *F. mccooki* and possibly *H. opaciceps*, are nonnative in the southeastern United States (Fisher and Cover 2007).

In the background samples in Florida, 7 species were encountered, including one specimen of *Camponotus* sp., a taxon not found in the treatment samples. *Crematogaster cerasi* was far more abundant in the background samples than in the treatment samples, likely because members of this genus are not predators but are scavengers on dead insects and plant materials (Fisher and Cover 2007), while *H. opaciceps* and fire ants were far more abundant in the treatment samples than background (Appendix C).

Solenopsis species were found on pig carrion in all stages of decomposition in both Mississippi and Florida. In Treatment 2, where ants were specifically excluded, they were apparently still attracted, since they were found in large numbers in pitfall traps outside of the cage and pesticide band. A few fire ants were found in the inside pitfall traps, likely because underground foraging tunnels typical of *Solenopsis* spp. (Tschinkel 2006) extended into the caged area; however, the pesticide likely cut off the ants from the main part of the nest, since it reduced or completely excluded fire ants in all 10 days of succession in Mississippi (*ad hoc* two-way ANOVA, $p = 0.009$, $F = 9.04$, $df = 1$) and in the first 7 days in Florida ($p = 0.787$, $F = 0.07$,

df=1). After several days, ants breached the pesticide and aluminum flashing barriers; however, by that time, the carcasses had attained a later stage of decomposition and ants were likely not attracted to the carcasses themselves but probably to the migrating Calliphoridae, as evidence by the fact that ants were rarely seen on or collected from the carcasses.

Other Succession Fauna

Besides the Diptera, Coleoptera, and Formicidae, probably the most conspicuous group at carrion was the Aranaea, many of which were present throughout succession. In Mississippi, 8 taxa of spiders (115 individuals) were collected, and in Florida, 9 taxa of spiders (595 individuals) were collected. Most of the spiders were from geophilic families, such as Anyphaenidae, Corinnidae, some Linyphiidae, Lycosidae, and Salticidae (Dondale 1990, Ubick et al. 2005). *Falconina gracilis* (Keyserling) is a suspected associate of *S. invicta* (Ubick and Richman 2005), and 37 individuals were collected throughout succession in Florida but only two individuals were collected in Mississippi. Seven of the nine taxa of spiders collected in the treatment samples were also found in the background samples in Florida; their abundances were relatively comparable between treatments and background.

Taxa other than Diptera, Coleoptera, Formicidae, and Aranaea were rarely collected at carrion in Mississippi, with less than 26 individuals collected in each taxon. In Florida, *Allonemobius socius* (Scudder), the southern ground cricket, was represented by 92 individuals. This cricket was most commonly found in pitfall traps, possibly attracted by a new refugium provided by the cover board, but it was also found inside the bags, suggesting that it might have been attracted to decomposing carrion. Entomobryid collembolans were abundant in pitfall traps (411 individuals) but never directly on carrion. All other taxa in Florida were represented by less than 40 individuals in total.

Forty-six taxa were common between the background samples and the treatment samples in Florida, including ants, spiders, crickets, and collembolans; however, this fact alone does not indicate that they may have been adventive on carrion. Although I counted the specimens in the background samples, they were not replicated, rendering it impossible to determine if there were statistically significant differences in number of organisms.

Fire Ant Effects on Community Ecology

In the community ecology samples (pitfall traps plus aerial samples), 5,369 organisms, representing 71 distinct taxa, were collected in Mississippi, and 12,879 organisms, representing 110 distinct taxa, were collected in Florida. The inner and outer pitfall traps each collected about half of the organisms. Because aerial samples generally collected only a few representative adult flies or beetles, they did not comprise a large proportion of the community ecology samples in terms of number of organisms collected but did contribute over 10% of the taxa richness. In Mississippi, nine taxa (12.7%) were collected only in the aerial samples, while in Florida, twelve taxa (10.9%) were collected only in the aerial samples.

Assumptions

Assessment of the statistical assumptions associated with linear regression in both the Florida and Mississippi community composition and community similarity data (Table 11) showed that most parameters met all assumptions or failed to meet only one assumption, so parametric statistics were considered to be acceptable for use. Because data from Mississippi on number of organisms badly violated two assumptions, I chose to continue data analysis with logarithm-transformed data on number of organisms collected.

Table 11 Results of statistical tests of assumptions for community composition and community similarity data.

	Normality		Homoscedasticity		Independence	
	Shapiro-Wilks W	p-value	Levene test	p-value	Durban-Watson test	p-value
Florida						
Number of organisms	0.383	**<0.0001**	0.825	0.442	1.847	0.290
log(number of organisms)	0.975	0.073	0.288	0.751	1.274	**<0.0001**
Taxa richness	0.987	0.498	0.263	0.770	1.600	**0.048**
Shannon Index	0.982	0.249	0.582	0.561	1.333	**<0.0001**
Jaccard Index	0.938	0.223	**0.013**	0.911	2.164	0.832
Bray-Curtis Index	0.934	0.181	0.188	0.670	2.482	0.360
Mississippi						
Number of organisms	0.513	**<0.0001**	1.343	0.278	1.147	**0.010**
Log_{10}(number of organisms)	0.982	0.869	2.036	0.150	0.838	**0.002**
Taxa richness	0.932	0.054	0.053	0.948	1.687	0.338
Shannon Index	0.964	0.397	0.838	0.443	1.086	**0.008**
Jaccard Index	0.980	0.817	0.401	0.674	1.044	**0.004**
Bray-Curtis Index	0.887	**0.024**	0.501	0.488	0.312	**<0.0001**

Statistically significant results in **bold** font.

Community composition parameters

There were obvious differences between dates in the collection of organisms regardless of treatment (Figure 47a, 47b), usually with larger numbers of insects collected in the pitfall traps on Day 1 in some treatments, followed by lower numbers of individuals collected on subsequent days. This may have been due to insects attempting to take advantage of a new refugium (the cover board) and predators searching the new habitat for prey.

On Days 7 and 8, there tended to be a spike in number of organisms collected (up to 850 individuals), corresponding to large maggot migrations from the carrion. Number of taxa collected ranged from 4 to 19 per day, with most values between 10 and 15 (Figure 47c, 47d).

Shannon-Weaver diversity values ranged from 0.1 to 2.8, with apparently slightly lower values in Mississippi than in Florida (Figure 47e, 47f).

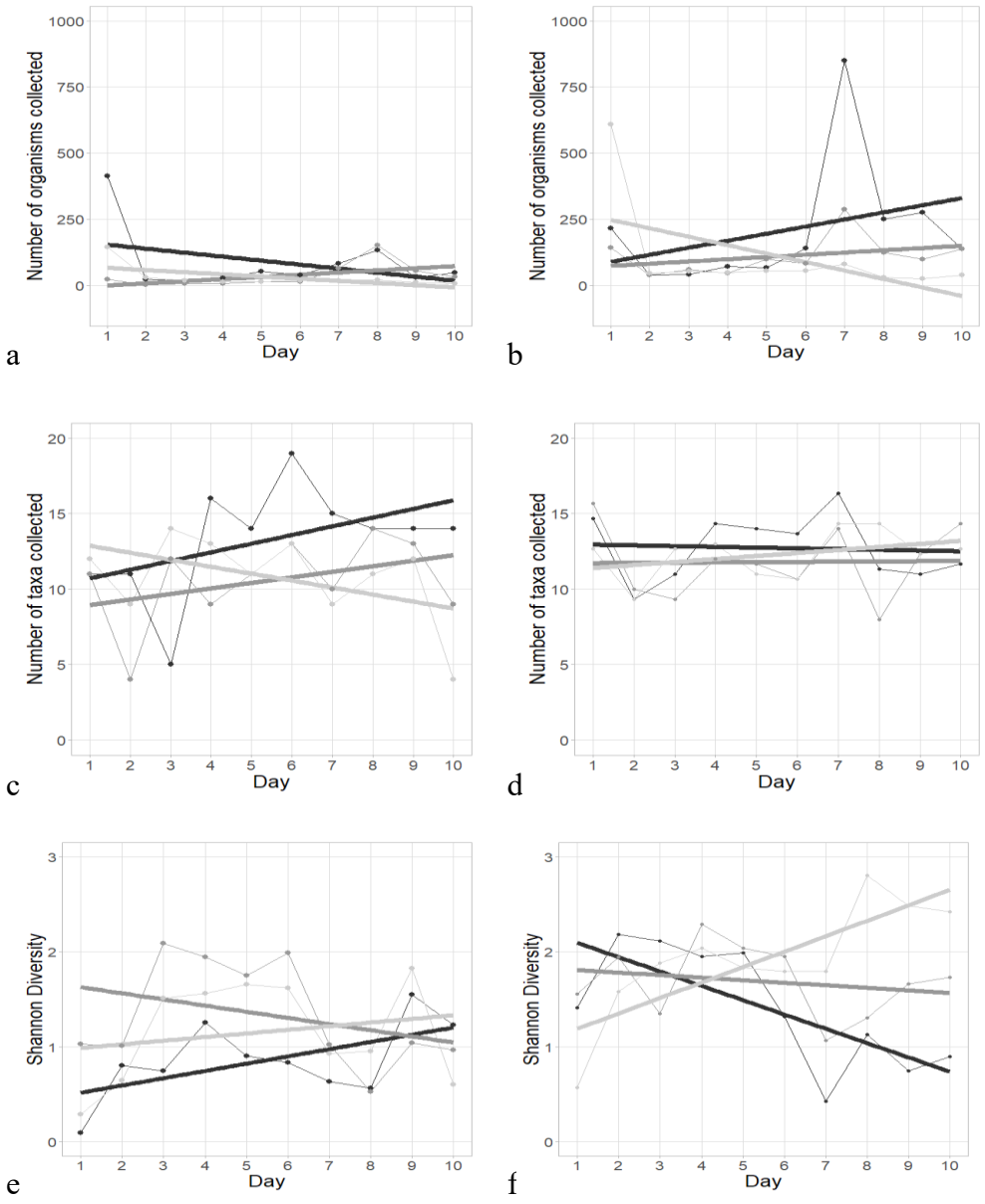


Figure 47 Basic community ecology parameters for samples collected at pig carrion in Mississippi and Florida, May-June 2019.

Data from pitfall trap samples plus aerial samples. Lines represent treatments as follows: black = Treatment 1 (control), dark gray = Treatment 2 (fire ants excluded), and light gray = Treatment 3 (blow flies excluded); thin = average of three replicates; thick = regressions for ANCOVA.

a) Average number collected per three pitfall traps in Mississippi
b) Average number collected per three pitfall traps in Florida
c) Taxa richness collected in pitfall traps and aerial samples in Mississippi
d) Taxa richness collected in pitfall traps and aerial samples in Florida
e) Shannon Diversity Index values for pitfall traps and aerial samples in Mississippi
f) Shannon Diversity Index values for pitfall traps and aerial samples in Florida

Regression lines describing the number of organisms collected in the community composition samples over time were not coincident, with Treatment 2 different from the other treatments in Mississippi (ANCOVA, $p < 0.010$) and Treatment 3 different from the other treatments in Florida ($p = 0.005$; Table 12). The support or rejection of null hypothesis H_{o9} that *"there is no statistically significant difference in the intercept and slope coefficient estimates for a regression equation describing the number of organisms collected in the community composition samples over time, among treatments"* appears to be somewhat geographically influenced. In Mississippi, the statistical difference of Treatment 2 from the other treatments appears to be driven primarily by the number of organisms collected on the first day. An average of only 22 organisms was collected in the community composition samples on Day 1 in Treatment 2, while an average of 143 – 550 organisms (of which 79.1 – 99.3% were fire ants) were collected in the other two treatments on that day.

Table 12 ANCOVA of community composition parameters over time.

	Mississippi		Florida	
Y	Regression equation	Partial F-test p-value	Regression equation	Partial F-test p-value
Log_{10} (number collected)	$y = (1.7123 \pm 0.1877)$ $- (0.0052 \pm 0.0302)x$ $- (1.1999 \pm 0.2654)z_1$ $+ (0.1124) \pm 0.0428)xz_1$ $- (0.0713 \pm 0.2654)z_2$ $- (0.0766 \pm 0.0428)xz_2$ Overall ($F_{5,84} = 9.179$)	**<0.0001** 0.865 **<0.0001** **0.010** 0.792 0.077 **<0.0001**	$y = (1.6965 \pm 0.1891)$ $+ (0.0509 \pm 0.0305)x$ $- (0.0737 \pm 0.2675)z_1$ $- (0.0060 \pm 0.0431)xz_1$ $+ (0.3160 \pm 0.2675)z_2$ $- (0.1232 \pm 0.0431)xz_2$ Overall ($F_{5,84} = 3.92$)	**<0.0001** 0.098 0.783 0.889 0.241 **0.005** **0.003**
Taxa richness	$y = (4.5556 \pm 0.8879)$ $+ (0.3232 \pm 0.1431)x$ $- (1.4667 \pm 1.2557)z_1$ $- (0.0182 \pm 0.2024)xz_1$ $+ (1.5556 \pm 1.2557)z_2$ $- (0.5677 \pm 0.2024)xz_2$ Overall ($F_{5,84} = 4.45$)	**<0.0001** **0.027** 0.246 0.929 0.219 **0.006** **0.001**	$y = (13 \pm 1.97)$ $- (0.0485 \pm 0.3171)x$ $- (1.2889 \pm 2.7825)z_1$ $+ (0.0647 \pm 0.4484)xz_1$ $- (1.8 \pm 2.7825)z_2$ $+ (0.2485 \pm 0.4484)xz_2$ Overall ($F_{5,84} = 0.190$)	**<0.0001** 0.879 0.644 0.886 0.519 0.581 0.966
H’	$y = (0.4846 \pm 0.1958)$ $+ (0.0585 \pm 0.0316)x$ $+ (0.3368 \pm 0.2769)z_1$ $- (0.0409 \pm 0.0446)xz_1$ $+ (0.3695 \pm 0.2769)z_2$ $-0.0438 \pm 0.0446)xz_2$ Overall ($F_{5,84} = 1.031$)	**0.015** 0.067 0.227 0.363 0.186 0.329 0.405	$y = (1.7745 \pm 0.2046)$ $- (0.0931 \pm 0.033)x$ $- (0.2396 \pm 0.2894)z_1$ $+ (0.0488 \pm 0.0466)xz_1$ $- (0.7355 \pm 0.2894)z_2$ $+ (0.2027 \pm 0.0466)xz_2$ Overall ($F_{5,84} = 6.155$)	**<0.0001** **0.006** 0.410 0.298 **0.013** **<0.0001** **<0.0001**

Coefficient estimates (β) ± standard error of the estimates and p-values for the partial F-test for each parameter estimate to test for coincidence of treatments. y = community composition parameter, x = day of succession, and z = dummy variables as described in text. Statistically significant results in **bold** font.

Likewise, in Florida, an average of 608 organisms (of which 79.2% were fire ants) were collected on Day 1 in Treatment 3, with an average of 142 - 215 organisms collected in the other treatments on that date. However, about Day 7, large maggot migrations occurred in Treatments 1 and 2, greatly increasing the number of organisms collected in the pitfall traps (Figure 46b). In Treatment 1 on Day 7, fire ants accounted for 93.9% of the organisms collected, probably captured while predating on the migrating maggots.

For taxa richness, in Mississippi, Treatment 1 had a significant increasing temporal trend ($p = 0.027$), Treatment 2 had no temporal trend ($p = 0.929$), and Treatment 3 had a significant decreasing temporal trend ($p = 0.006$). Conversely, there were no temporal trends ($p > 0.581$) in the Florida data (Table 12). The support or rejection of null hypothesis H_{o10} that *"there is no statistically significant difference in the intercept and slope coefficient estimates for a regression equation describing the taxa richness of organisms collected in the community composition samples over time, among treatments"* thus appears to be geographically influenced. In Mississippi, it may be that the exclusion of fire ants or blow flies may prevent subsequent succession taxa from arriving and being collected on later dates; this will be investigated further in the community similarity data, below.

In Mississippi, regression lines describing H' over time were coincident for all three treatments ($\beta_2 = \beta_4 = \beta_5 = 0$, $p > 0.0673$), supporting null hypothesis H_{o11} that *"there is no statistically significant difference in the intercept and slope coefficient estimates for a regression equation describing the Shannon-Weaver diversity of organisms collected in the community composition samples over time, among treatments"*. Conversely, regression lines describing H' over time in Florida were different among all three treatments (Table 12), with a decreasing temporal trend in Treatment 1 ($p = 0.006$), a non-significant temporal trend in Treatment 2 ($p = 0.298$), and a strongly increasing temporal trend in Treatment 3 ($p < 0.0001$), rejecting null hypothesis H_{o11}. The decreasing trend in Treatment 1 may be an artefact of the large number of fire ants collected on Day 7 (93.9% of the organisms collected) and subsequent days, decreasing diversity. The increasing trend in H' values in Treatment 3 in Florida, may be due to lower numbers of fire ants collected in later days when maggots were not available.

The equivocal effects of fire ants on the community composition is in accordance with theoretical considerations from Kneidel (1984) and considered by Stoker et al. (1995). These studies found that smaller carrion, such as mouse carcasses, are utilized by a much less diverse community, while the community on larger carcasses is naturally more diverse and less subject to influence. Furthermore, they inferred from those observations that disturbances of other fauna may not effect changes in the community composition on larger carcasses.

As mentioned above, the aerial samples generally collected only a few representative adult flies or beetles. While they did not comprise a large proportion of the community ecology samples in terms of number of organisms collected, they did contribute over 10% of the taxa richness. An *ad hoc* ANOVA test indicated that the increase in taxa richness due to the aerial samples was evenly distributed across treatments ($p = 0.818$ and $p = 0.302$ for data from Mississippi and Florida, respectively), so it is unlikely that further standardization of the aerial samples might have changed these results.

Community similarity parameters:

Jaccard similarity index values (J_c) ranged from 0.07 to 0.77; however, in general, communities were less than 55% similar to each other, considering only presence and absence of individual taxa (Figure 48a, 48b). Bray-Curtis similarity index values (*B*-*C*), which also considers the relative contribution of each taxon to the community, ranged from 0 to 0.66, again with communities generally less than 55% similar to each other (Figure 48c, 48d). Negative slopes for most J_c and *B*-*C* comparisons indicated that the communities in the separate treatments tended to become more dissimilar to each other over time (Figure 48).

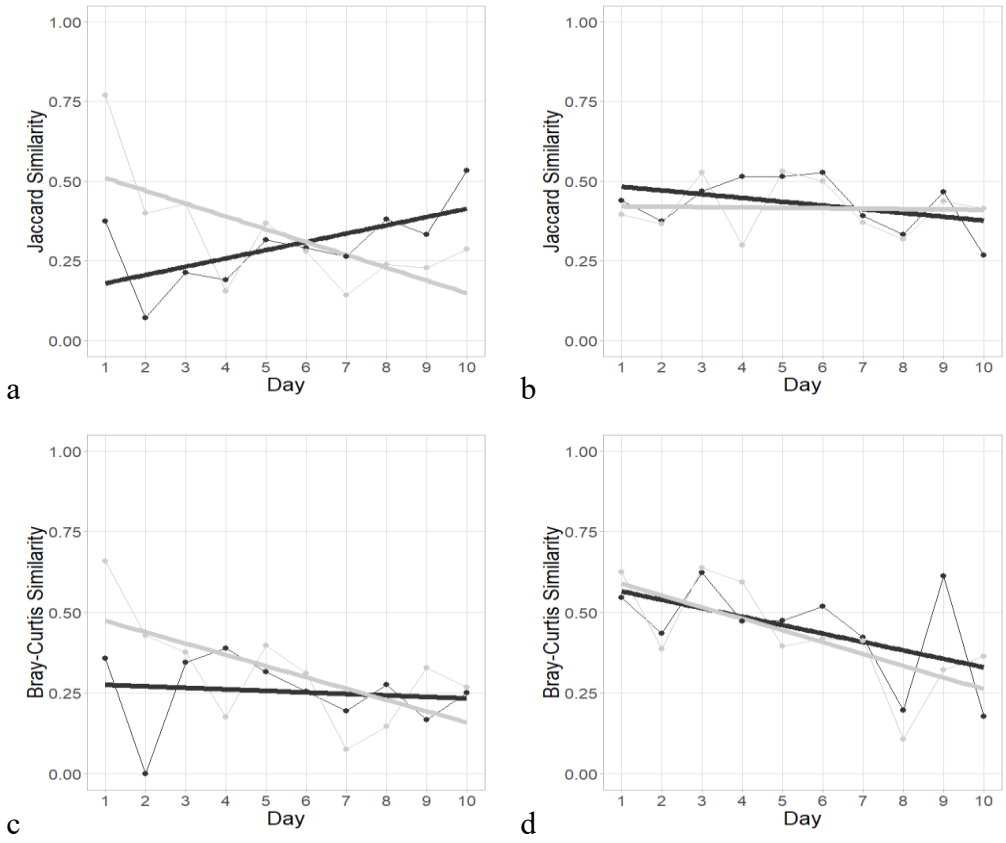


Figure 48 Jaccard Similarity Coefficient and Bray-Curtis Similarity Coefficient over time for community composition samples collected at pig carcasses in Mississippi and Florida, May-June 2019.

Black line represents paired comparison of Treatment 1 versus Treatment 2; gray line represents paired comparison of Treatment 1 versus Treatment 3; thin = average of three replicates; thick = ANCOVA regression line.
a) Jaccard Similarity Coefficient values for samples collected in Mississippi
b) Jaccard Similarity Coefficient values for samples collected in Florida
c) Bray-Curtis Similarity Coefficient values for samples collected in Mississippi
d) Bray-Curtis Similarity Coefficient values for samples collected in Florida

J_c values comparing Treatments 1 and 2 increased over time in Mississippi while J_c values comparing Treatments 1 and 3 over time decreased, indicating that the ANCOVA regression lines were not coincident (Table 13, p = 0.024). The J_c values representing the comparison of taxa composition between Treatments 1 and 2 dropped from a value of 0.38 on

Day 1 to 0.07 on Day 2, and then increased from there to end at 0.53 on Day 10 (Figure 48a). Likewise, there was initially high similarity (J_c = 0.77) in taxa composition between Treatments 1 and 3 that dropped quickly to < 0.50, remaining between 0.14 and 0.43 through the rest of the 10-day exposure (Figure 48). For Mississippi data, these results reject null hypothesis H_{o12} that "*there is no statistically significant difference in the intercept and slope coefficient estimates for regression equations describing the Jaccard similarity coefficient on community composition samples over time, among treatments."* As mentioned above, these results may be because later colonizing taxa may be dependent on changes effected by earlier colonizing taxa. It is also possible that the consistent decreasing similarity between Treatments 1 and 3 may be a methodological effect due to the mesh bag excluding more taxa than just blow flies.

Table 13 ANCOVA of community similarity parameters over time.

	Mississippi		Florida	
Y	Regression Equation	Partial F-test p-value	Regression Equation	Partial F-test p-value
J_c	y = (0.1541 ± 0.0855)	0.090	y = (0.4947 ± 0.0585)	**< 0.0001**
	+ (0.0260 ± 0.0138)x	0.078	- (0.0118 ± 0.0094)x	0.229
	+ (0.3965 ± 0.1209)z_1	**0.005**	- (0.0721 ± 0.0828)z_1	0.397
	- (0.0662 ± 0.0195)xz_1	**0.004**	+ (0.0106 ± 0.0133)xz_1	0.440
	Overall ($F_{3,16}$ = 4.139)	**0.024**	Overall ($F_{3,16}$ = 0.570)	0.643
B-C	y = (0.8382 ± 0.0866)	**<0.0001**	y = (0.5915 ± 0.0901)	**<0.0001**
	- (0.0156 ± 0.0139)x	0.279	- (0.0261 ± 0.0145)x	0.091
	- (0.3291 ± 0.1224)z_1	**0.016**	+ (0.0346 ± 0.1274)z_1	0.790
	- (0.0193 ± 0.0197)xz_1	0.341	- (0.0102 ± 0.0205)xz_1	0.625
	Overall ($F_{3,16}$ = 22.21)	**<0.0001**	Overall ($F_{3,16}$ = 3.214)	0.051

Coefficient estimates (β) ± standard error of the estimates and p-values for the partial F-test for each parameter estimate. y = community similarity parameter, x = day of succession, and z = dummy variables as described in text. Statistically significant results in **bold** font.

In Florida, regression lines describing J_c comparing Treatments 1 and 2 and comparing Treatments 1 and 3 over time were coincident (table 13, $\beta_2 = \beta_3 = 0$, p = 0.643). J_c values started

at 0.39 – 0.44 on Day 1 and tracked consistently with each other through Day 9 (Figure 48b), except for Day 4, when J_c for the paired comparisons differed substantially. These results support null hypothesis H_{o12} in Florida.

Regression lines describing *B-C* comparing Treatments 1 and 2 and comparing Treatments 1 and 3 over time in Mississippi were different in intercept ($p = 0.016$, Table 13), indicating that the lines were statistically parallel but not coincident ($p < 0.0001$). The *B-C* values started separately on Day 1 at 0.37 – 0.65 and then rarely diverged more than 0.2 apart the rest of the time (Figure 48c). For Mississippi data, these results reject null hypothesis H_{o13} that "*there is no statistically significant difference in the intercept and slope coefficient estimates for regression equations describing the Bray-Curtis similarity coefficient on community composition samples over time, among treatments. B-C* values in the Florida data set tracked with each other within 0.25 over the entire study (Figure 48d) and were statistically coincident ($\beta_2 = \beta_3 = 0$, $p = 0.051$, Table 13), supporting null hypothesis H_{o13} in Florida.

It appears that the effect over time of fire ants on community similarity with communities in which specific faunal elements are excluded is equivocal. Although the general trend appeared to be toward dissimilar communities, it was only for J_c values describing the comparison between the control and the community in which blow flies were excluded that a statistically significant difference was observed. This may possibly be due to fire ants continuing to exert predation pressures on the community in the control treatment and when blow flies are excluded, whereas blow flies do not exert the same predation pressures, so the effect is not observed when they are excluded. As mentioned above, it is also possible that it is a methodological effect, wherein the netting on Treatment 3 excluded more of the fauna than just blow flies.

To investigate if presence of taxa unique to a particular treatment helped to explain the minor differences among treatments, I conducted an *ad hoc* ANOVA test to compare treatments. Even though in Mississippi there were some differences in community similarity over time (measured as J_c) and there were more unique taxa (16 taxa) in Treatment 1 than in Treatments 2 (10 taxa) and 3 (7 taxa), there was not a statistically significant difference in the number of taxa unique to any given treatment based on averages of the replicates ($p = 0.965$, Table 14). In the Florida data set, there were more unique taxa in Treatment 2 (18 taxa) than in Treatment 1 (9 taxa) and Treatment 3 (12 taxa), and again there were no significant differences in the number of taxa unique to any given treatment based on averages ($p = 0.358$, Table 14).

Table 14 Number of taxa unique to each treatment.

	Mississippi		Florida	
	Average ± S.D.	Total	Average ± S.D.	Total
Treatment 1	9 ± 5	16 (31.4%)	10 ± 8	9 (19.6%)
Treatment 2	10 ± 7	10 (23.8%)	13 ± 3	18 (33.3%)
Treatment 3	8 ± 7	7 (17.1%)	9 ± 6	12 (24.5%)
ANOVA p-value	0.965	--	0.358	--

Reported as average ± standard deviation of the three replicates and as the total community; proportion of the total number of taxa in that treatment indicated in parentheses.

Based on data from the physical decomposition parameters (Table 9), exclusion of blow flies slowed decomposition and therefore delayed succession, while exclusion of fire ants did not change the normal decomposition rate and succession. In Mississippi, taxa that normally arrive later in succession failed to arrive in sequence when blow flies were excluded in comparison to the control when all fauna had access to the carrion. This may have been because they were dependent on changes to the carrion effected by the blow flies (e.g., destruction of tissues, the change from the "active decay" to "advanced decay" stages, etc.) or due to the presence of netting.

Fire Ant Effects on Blow Fly Larval Development

In Mississippi, 568 individuals were collected in random maggot collection samples, representing 3 taxa, and 389 individuals were collected in targeted maggot collection samples, representing 4 taxa (Table 10). Taxa represented in these samples included Calliphoridae (unidentified eggs, first instar larvae, and second instar larvae), *Cochliomyia macellaria*, *Lucilia coeruliviridis*, and unidentified Sarcophagidae. As observed with Wells and Greenberg (1994), taxonomic lumping of early life stage Calliphoridae may have decreased successional variation, so species-specific influences on blow flies by fire ants remain unknown.

In Florida, 3,759 individuals were collected in random maggot collection samples, representing 5 taxa, and 773 individuals were collected in targeted maggot collection samples, representing the same 5 taxa. Calliphoridae (unidentified eggs, first instar larvae, and second instar larvae), *C. macellaria*, *L. coeruliviridis*, and unidentified Sarcophagidae were also collected in Florida, along with *C. rufifacies*. Sarcophagidae was represented by a total of 14 individuals in the Mississippi data (1.5% of all individuals) and 44 individuals in the Florida data (1.0% of all individuals). Sarcophagidae data were excluded from further analysis to focus on the blow flies.

The random maggot collection samples appeared to be consistently more successful in collecting blow fly larvae of some life stages on some days than the targeted maggot collection samples. This was expected, given that the protocol for the targeted maggot collection samples indicated targeting larger individuals (Tables 15-16). The largest larvae present are often assumed to be from the earliest colonizing cohort, and, therefore, also assumed to represent the oldest individuals (Anderson 2016, but see Moffatt et al. 2015).

Table 15 Number of blow flies from each life stage in random maggot collection samples from Treatments 1 and 2 on the first ten days of exposure of pig carcasses in Mississippi.

	Treatment 1				Treatment 2			
	Egg	L1	L2	L3	Egg	L1	L2	L3
1	--	--	--	--	--	--	--	--
2	--	--	--	--	**16**	3	--	--
3	68	26	--	--	--	46	2	8
4	--	--	**7**	1	**1**	**3**	3	1
5	--	--	1	3	--	152	19	12
6	--	--	5	11	--	**5**	42	23
7	--	--	**3**	39	--	**1**	**7**	25
8	--	--	--	20	--	--	--	5
9	--	--	--	1	--	--	--	4
10	--	--	--	2	--	--	--	3

All replicates combined. **Bold** font indicates that no specimens of that life stage were collected on that day in the targeted maggot collection samples.

Table 16 Number of blow flies from each life stage in targeted maggot collection samples from Treatments 1 and 2 on the first ten days of exposure of pig carcasses in Mississippi.

	Treatment 1				Treatment 2			
Day/Stage	Egg	L1	L2	L3	Egg	L1	L2	L3
1	--	--	--	--	**32**	--	--	--
2	--	--	--	--	--	2	**2**	--
3	12	1	--	**3**	**8**	107	30	14
4	--	--	--	6	--	-	2	15
5	--	--	1	7	--	1	5	14
6	--	--	3	11	--	--	18	29
7	--	--	--	4	--	--	--	7
8	--	--	--	9	--	--	--	11
9	--	--	--	3	--	--	--	4
10	--	--	--	4	--	--	--	1

All replicates combined. **Bold** font indicates that no specimens of that life stage were collected on that day in the random maggot collection samples.

In Mississippi, there were 8 instances in which a particular life stage was collected on a certain day in random maggot collection samples when the same life stage and day had no specimens collected in the targeted maggot collection samples, but only 4 instances where blow

fly larvae of specific life stages were collected in targeted maggot collection samples and not in the random maggot collection samples (Tables 15-16). Similarly, In Florida, there were 9 instances in which a particular life stage was collected on a certain day in random maggot collection samples when the same life stage and day had no specimens collected in the targeted maggot collection samples, but only 5 instances where blow fly larvae of specific life stages were collected in targeted maggot collection samples and not in the random maggot collection samples (Tables 17-18).

Table 17 Number of blow flies from each life stage in random maggot collection samples from Treatments 1 and 2 on the first ten days of exposure of pig carcasses in Florida.

	Treatment 1				Treatment 2			
	Egg	L1	L2	L3	Egg	L1	L2	L3
1	--	--	--	--	26	--	--	--
2	21	**6**	--	--	--	**556**	65	6
3	9	**2**	12	281	**25**	239	487	306
4	**1**	**41**	**56**	501	--	11	122	736
5	--	--	2	103	--	**3**	12	113
6	--	--	--	3	--	--	--	--
7	--	--	--	6	--	--	--	--
8	--	--	--	**2**	--	--	--	4
9	--	--	--	--	--	--	--	1
10	--	--	--	--	--	--	--	--

All replicates combined. **Bold** font indicates that no specimens of that life stage were collected on that day in the targeted maggot collection samples.

Table 18 Number of blow flies from each life stage in targeted maggot collection samples from Treatments 1 and 2 on the first ten days of exposure of pig carcasses in Florida.

	Treatment 1				Treatment 2			
Day/Stage	Egg	L1	L2	L3	Egg	L1	L2	L3
1	--	--	--	--	13	**3**	--	--
2	20	--	--	--	--	--	15	10
3	23	--	27	50	--	26	92	20
4	--	--	--	65	--	3	4	58
5	--	--	2	45	--	--	5	58
6	--	--	--	15	--	--	--	**12**
7	--	--	--	14	--	--	--	**20**
8	--	--	--	--	--	--	--	25
9	--	--	--	**8**	--	--	--	8
10	--	--	--	**4**	--	--	--	--

All replicates combined. **Bold** font indicates that no specimens of that life stage were collected on that day in the random maggot collection samples.

Overall, patterns from the random and targeted maggot collection samples were similar. Therefore, to provide a more complete data set with fewer structural and sampling zeros, the random and targeted maggot collection sample data were combined for further statistical analysis (Tables 19-20). Assumptions associated with Fisher's exact test include the fixity of row and column totals, random and independent sampling, and mutually exclusive observations; all assumptions were met as much as was possible.

Table 19 Number of blow flies from each life stage in Treatments 1 and 2 on the first ten days of exposure of pig carcasses in Mississippi.

	Treatment 1				Treatment 2				Fisher's exact test
	Egg	L1	L2	L3	Egg	L1	L2	L3	p-value
1	--	--	--	--	32	--	--	--	n/a
2	--	--	--	--	16	5	2	--	n/a
3	80	27	--	5	8	153	32	22	**< 0.0001**
4	--	--	7	9	1	3	5	16	0.215
5	--	--	2	10	--	153	24	27	**< 0.0001**
6	--	--	8	25	--	5	60	52	**0.006**
7	--	--	3	48	--	1	7	32	0.063
8	--	--	--	30	--	--	--	16	n/a
9	--	--	--	4	--	--	--	8	n/a
10	--	--	--	6	--	--	--	4	n/a

Data from random and targeted maggot samples combined. Shading of cells in Treatment 2 indicates presence of life stages absent in Treatment 1. Statistically significant results in **bold** font.

Table 20 Number of blow flies from each life stage in Treatments 1 and 2 on the first ten days of exposure of pig carcasses in Florida.

	Treatment 1				Treatment 2				Fisher's exact test
Day/Stage	Egg	L1	L2	L3	Egg	L1	L2	L3	p-value
1	--	--	--	--	39	3	--	--	n/a
2	41	6	--	--	--	556	80	16	**< 0.0001**
3	32	2	39	331	25	275	579	327	**< 0.0001**
4	1	41	57	587	--	14	126	800	**< 0.0001**
5	--	--	4	148	--	3	17	178	**0.013**
6	--	--	--	18	--	--	--	12	n/a
7	--	--	--	20	--	--	--	20	n/a
8	--	--	--	2	--	--	--	29	n/a
9	--	--	--	8	--	--	--	9	n/a
10	--	--	--	13	--	--	--	--	n/a

Data from random and targeted maggot samples combined. Shading of cells in Treatment 2 indicates presence of life stages absent on the same day in Treatment 1. Statistically significant results in **bold** font.

Presence of fire ants in Treatment 1 appeared to have a significant effect on blow fly larval development on pig carcasses during specific times in succession. On days 3, 5, and 6 in Mississippi, there was a statistically significant difference in proportions of blow fly life stages

present (Table 19, $p < 0.006$), rejecting null hypotheses H_{o19}, H_{o21}, and H_{o22} that *"there is no statistically significant difference in the relative proportions of blow fly maggots in each immature life stage (egg, first, second, and third instars) among treatments."* Similarly, on days 2 – 5 in Florida, there was a statistically significant difference in proportions of blow fly life stages present (Table 20, $p < 0.013$), rejecting null hypotheses H_{o18}, H_{o19}, H_{o20}, H_{o21}, and H_{o22} in the state. Only on days 4 and 7 in Mississippi was the null hypothesis (H_{o20} and H_{o23}) supported due to no statistically significant differences in the proportions of blow fly life stages present between treatments ($p > 0.063$).

Zero-inflated data sets on Days 1 – 2 and 8 – 10 in Mississippi and Days 1 and 6 – 10 in Florida, plus the consequent inability to test the results statistically, rendered no decision on null hypotheses H_{o17}, H_{o24}, H_{o25}, and H_{o26} in both states, null hypothesis H_{o18} in Mississippi, and null hypotheses H_{o22} and H_{o23} in Florida. Nevertheless, the pattern of presence of several blow fly life stages in Treatment 2 and absence (or at least indetectable presence) of blow flies in Treatment 1 illustrates an obvious difference between the two treatments on the first 2 days in Mississippi and the first day in Florida. (Conversely, the pattern in which only the same life stages are present in both treatments, as in Days 8 - 10 in Mississippi and Days 6 – 9 in Florida, indicate an obvious lack of effect due to exclusion of fire ants, despite an inability to test the results statistically.)

Specifically, in both random and targeted maggot samples, no blow fly eggs or larvae were collected on Day 1 in Treatment 1, while eggs and sometimes first instar larvae were collected on Day 1 in Treatment 2 (Tables 19-20). On the subsequent four to five days, first and second instar larvae were scarce in Treatment 1, third instar larvae being abundant, while all instars were commonly collected in Treatment 2 (Tables 19-20). In targeted maggot samples on late days of exposure, a few pupae were also collected, although they were not collected in

random maggot samples. In both Mississippi and Florida, these effects delayed the succession of several flies (including blow flies and flesh flies) past the “fresh” decomposition stage into the “bloat” decomposition stage (Figures 43 and 44).

It appears that fire ants impeded colonization of blow flies on carrion by apparently preying on eggs and larvae. Figure 49 shows fire ants preying on second instar larvae in Mississippi on Day 4 at the 3 cm mark and to the left of the 5 cm mark of the vinyl measuring tape. Fire ants on the left side of Figure 49 are preying on blow fly eggs.

Figure 49 Ant predation on eggs and early instar blow fly larvae.

Photo credit: Jerome Goddard.

It is possible that fire ants somehow made the carcasses unsuitable for colonization – possibly even by their mere presence. Some organisms are deterred by the odors of potential predators, and this may be the case with blow flies and fire ants. For example, several studies have demonstrated that some blow flies actively avoid ovipositing on substrates where predaceous blow flies (*Chrysomya albiceps* (Wiedemann) and *C. rufifacies*) were present or likely to occur (Wells and Greenberg 1994, Yang and Shiao 2012, Galindo et al. 2016). Nevertheless, this reason seems unlikely, since Meyer et al. (2020) demonstrated that blow flies use lesions caused by fire ants as oviposition sites.

Nevertheless, about Day 3 in Mississippi and Day 2 in Florida, blow flies were able to overwhelm the predatory ants in terms of biomass or numbers (as in Meyer et al. 2020), growing quickly into older instars. I observed several instances of lethal mass attacks by fire ants on third instar blow fly larvae, but far less often than on eggs and earlier instars. In summer 2020, during the experiment described in Chapter IV, I also observed fire ants interrupting blow fly oviposition by chasing adult blow flies away almost as soon as they landed on carrion. Fire ants apparently continued to render carrion inhospitable for blow fly eggs and first instar larvae, as evidenced by the lack of these early life stages on later dates when ants were present (Days 4 - 7 in Mississippi and Day 5 in Florida, Tables 19-20).

Based on previous carrion succession studies in the same geographic region (Goddard and Lago 1985, Goddard et al. 2012), early instar blow fly larvae would not be expected after about Days 5 or 6. Once carrion achieves the "Active decay" stage, it is usually no longer attractive to blow fly oviposition (Benbow et al. 2016), and an entomological sign of onset of the "Advanced decay" stage is mass emigration of blow fly larvae (Goddard et al. 2020). Only late

instar larvae would be expected to be present on later dates, but exclusion of early instars by fire ants precedes these normal times in which carrion is no longer attractive for blow fly oviposition.

Presence of fire ants delayed most blow fly colonization by one to two days and limited the number of days in which eggs and early instar larvae could be present in large enough numbers to detect. The delay in colonization is similar to that described by Lashley et al. (2017), where large vertebrate scavengers appeared to delay blow fly colonization on pig carrion elsewhere in Mississippi. Sawyer et al. (2020) showed that fire ants can have the same effect in Texas. These results also appear to corroborate the hypothesis promulgated by Wells and Greenberg (1994) that predation (by both *C. rufifacies* and fire ants) on vulnerable early life stages of blow flies may shift succession of the blow fly community on carrion to a later time frame.

Importantly, delay in blow fly colonization due to presence of fire ants has implications in forensic science. There is an assumption in forensic entomology that blow flies begin colonization of carrion soon after death unless there is a barrier that prevents immediate colonization. Most barriers that have been studied are physical, such as wrappings or being buried or enclosed in a car or building (Pechal et al. 2014, Lutz et al. 2018, Malainey and Anderson 2020). This study demonstrates that presence of fire ants can function as a barrier that can delay colonization and requires consideration when encountered (Park and Moon 2020b). Further study could include larger carrion which may more effectively model adult human corpses to investigate if fire ants consistently present a barrier to blow fly colonization, in accordance with anecdotal evidence from Lindgren et al. (2010).

As discussed in Chapter I, Vinson (1968) and Petralia and Vinson (1978) demonstrated that only fourth instar larvae of fire ants consume protein, which would need to be provided via

trophyllaxis by foraging adults. Adults, on the other hand, feed primarily on liquids, while solid materials in their food is filtered in the infrabuccal cavity and expelled in the infrabuccal pellet (Vinson 1983, Hölldobler and Wilson 1990). Liquids are used both for nutrition of the individual insect and for trophallaxis with nest mates using the crop, which functions as a "social stomach" (Tschinkel 2006). Therefore, it is possible that fire ant foragers may have been ingesting liquids from carrion or blow fly eggs and larvae, as well as transporting solid material back to the nest for their larvae. Chapter IV of this dissertation will address ingestion of carrion or blow flies by foraging fire ants through searching for pig or blow fly DNA in their gut contents.

Conclusions

Fire ant presence did not statistically affect carrion decomposition rates, slightly affected the overall carrion community, and clearly affected colonization of blow fly larvae on pig carrion in this study. Some effects were geographically constrained to either the Mississippi or Florida study areas, but many were observed in both areas.

Fire ant presence did not statistically affect carrion decomposition rates based on the physical decomposition parameters I chose to measure (time to bloat milestones, time to decomposition stages, and mass change over ten days), rejecting null hypotheses for these parameters. Nevertheless, physical changes were observed among treatments. When fire ants were allowed access, they were observed making lesions into exposed surfaces of the carcasses and building a "thatch" covering on the face and anal regions of the carcasses, as well as other places of contact with the soil. When ants were excluded, these lesions and thatch coverings were not present. Postmortem lesions may have served as additional openings for oviposition by blow flies and other succession fauna, but they did not appear to accelerate or decelerate the rate of seral decomposition changes.

Similarly, presence of fire ants did not appear to consistently affect community composition, measured in number of organisms, taxa richness, or Shannon diversity. When treatments were compared, results varied by geographic region, but were generally relatable to the number of fire ants collected in pitfall traps on given days (particularly on Day 1 or Day 7 when >90% of the organisms collected were fire ants).

The effect of fire ants on community similarity between the control treatment with normal succession and the treatments in which faunal elements are selectively excluded appears to be somewhat geographically driven. In Florida, the presence or absence of fire ants or blow flies did not alter similarity of the community with the control treatment. Conversely, in Mississippi, exclusion of blow flies resulted in a community that diverged over time from similarity with the control in which both blow flies and fire ants were present. Exclusion of fire ants resulted in a community that was unchanged or became slightly more similar to the control community. Exclusion of blow flies had a stronger effect than exclusion of the fire ants. This is possibly because changes to the carrion effected by blow flies (e.g., tissue destruction or the seral change from the "active decay" stage to the "advanced decay" stage) were missing, and the carrion decomposition rates were retarded, maintaining the carrion in earlier decomposition stages. It is also possible the method of exclusion (i.e., netting) prevented fauna other than blow flies from arriving and colonizing. Either way, late arriving succession fauna may not have arrived as normal.

It appears that fire ants impeded colonization and larval development of blow flies, delaying successful colonization of blow flies on carrion by about two days in Mississippi and by one day in Florida. Within a day or two, however, blow flies were able to overwhelm and evade the predatory fire ants and then grow quickly into older instars. Fire ants continued to render the

carrion unsuitable for blow fly eggs and first instar larvae afterward, as evidenced by the lack of these early life stages on later dates when fire ants were present.

For students and practitioners of forensic entomology in the southeastern United States, these results have implications. The relative lack of strong effects on decomposition rates and community indicates that decomposition would be expected to continue as normal, although postmortem artifacts (lesions, thatch/soil buildup, and consumption of portions of the carrion) could affect interpretation of physical evidence. Care should be duly exercised. Furthermore, the effects of fire ant presence on colonization by blow flies and development of their larvae suggests that the post-mortem interval may be underestimated if it is calculated using development rates of blow fly larvae, as is commonly done. When forensic entomologists use developmental rates of blow fly larvae, it is assumed that the oldest larvae present were from eggs laid shortly after death. Delays in the successful colonization by blow flies because fire ants are preying on eggs and early instar larvae means that this assumption may be incorrect. Students and practitioners of forensic entomology should note the presence or absence of fire ants and be prepared to adjust estimates of the postmortem interval accordingly.

Caveat

The presence of netting and a ring of pesticide to exclude certain elements of the succession fauna are not likely to be encountered in a forensic casework setting. Therefore, although this study produced data that may be of interest to forensic investigators (Matuszewski et al. 2019), it 1) is designed to test the specific hypotheses proposed in this ecological study, 2) is not designed as a standard succession study, and 3) is not intended to produce succession data suitable as reference material for forensic casework, except under specific circumstances.

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CHAPTER IV

SPATIOTEMPORAL DISTRIBUTION OF FIRE ANT FORAGING ON PIG CARRION AND PREDATION ON BLOW FLIES

Introduction

Since 1970, numerous publications have noted the presence of fire ants at carrion (Figure 1; Eubanks et al. 2019). Researchers in many studies have noted predation by fire ants on the other members of the carrion fauna as well as apparent consumption of portions of the carcass (Table 1, e.g., Early and Goff 1986, Tullis and Goff 1987, Watson and Carlton 2003, Pérez et al. 2005, Goddard et al. 2012, Lindgren et al. 2015, Richards et al. 2015, Eubanks et al. 2019, Meyer et al. 2020). In most cases, this intuitive conclusion was based on observations of fire ants carrying off blow fly eggs or early instar larvae and previous knowledge that fire ants can subsist on a diet of invertebrate and vertebrate tissues (Gavilanez-Sloan and Porter 2013), as well as the characteristic postmortem lesions on carcasses. However, fire ant trophic biology suggests that fire ant workers are incapable of ingesting solid protein (Tschinkel 2006), so it is unclear if fire ants actually eat carrion and fly larvae or if they are merely transporting the materials elsewhere. One objective of this chapter is to use molecular techniques to identify if pig or fly DNA can be found in the guts of fire ants collected on pig carrion, conclusively demonstrating if the ants are feeding on the carcass and the attendant fauna.

Additionally, if fire ants ingest carrion or fly larvae, nutrients from these resources may be passed on to other members of the nest and dispersed into the landscape, so a second objective

of this chapter is, assuming that pig and/or fly DNA is detected in fire ant guts as evidence that they feed on carrion and its attendant fauna, to determine the spatiotemporal extent to which fire ants forage on carrion and its attendant fauna within the first 5 days of carrion succession.

Fire Ant Trophic Biology

In Mississippi and the Florida panhandle, fire ants live in visible mounds that extend above the ground surface and in nests that extend below the mound and laterally for a short distance. They manage a broad territory surrounding the central mound up to a radius of several meters using underground tunnels that radiate from the mound throughout the territory (Markin et al. 1975, Tschinkel 2006). At periodic distances, the tunnels open to the ground surface so that no place within their territory is farther than about a meter from an opening. Workers travel along the underground tunnels and scout within a 30 to 40 cm radius around surface openings for potential food. Once a food resource is discovered, the foragers mark the area with scent glands to recruit other foragers (Tschinkel 2006).

Foragers can quickly assemble at the new resource to transfer it to the nest (Tschinkel 2006). If a solid resource is small enough to be carried by individual workers, they carry these individual pieces back the nest and pass them off to other workers within the nest. If the resource is large, foragers cut it up and bring back pieces. Tschinkel (2006) and Gayahan (and Tschinkel (2008) report that these larger solid materials, such as dead insects, are generally stockpiled at the top or edge of the mound.

If the new food resource is liquid, foragers imbibe the liquid and bring it back to the nest to distribute by trophallaxis (Glancey et al. 1981, Tschinkel 2006). Suspended solid materials > 0.88 μm in diameter are retained in an infrabuccal pocket, while liquids are passed on to the crop. The crop serves as a social stomach, so that as a forager returns to the nest with a full,

distended crop, it regurgitates droplets from the crop and passes them to other ants in direct mouth-to-mouth contact. The recipient can then serve as a donor for other ants until the resource is adequately distributed. In the original forager, a minute amount of liquid food may be passed on to the midgut via the proventriculus for its own nutrition (Tschinkel 2006). Although dissolved carbohydrates are the most common nutrients passed in liquid form to the midgut, it is feasible that other dissolved nutrients, including proteins and nucleic acids, would also pass to the midgut in these workers.

In adult worker fire ants, the infrabuccal cavity is lined with long hairs that filter solid ingested material > 0.88 μm in diameter and concentrates it in an infrabuccal pellet (Glancey et al. 1981, Vinson 1983, Hölldobler and Wilson 1990, Tschinkel 2006). The infrabuccal pellet is egested on a daily basis, presumably in the presence of 4th instar larvae (Hölldobler and Wilson 1990).

As food is brought to the nest by individuals, nutrients are distributed among other members of the nest, including larvae, although organization of food sharing is dependent on the physical (liquid or solid) and chemical (carbohydrate, oil, or protein) type of food, life stage of the recipients (young instar larvae, last instar larvae, or adults), caste (workers or alates), season, and even recent climatic events (Tschinkel 2006). Foraging adult ants do not include significant amounts of protein in their diets and instead feed primarily on a liquid diet high in oils and sugars (Petralia and Vinson 1978). Conversely, using three specific morphological features, the 4th instar is the only life stage capable of ingesting solid food (Petralia and Vinson 1978). First, on the ventral thoracic region, they have a "food basket" of inward facing hairs that can hold small pieces of food in place; this structure is absent in earlier instars. Second, they have sclerotized mandibles capable of chewing, also absent in earlier instars. Third, their

proventriculus is about 45 μm in diameter, which allows them to swallow larger pieces of solid material than adults, whose proventriculus is restricted to < 0.9 μm by its passage through the petiole at the anterior end of the abdomen (Petralia and Vinson 1978, Tschinkel 2006).

Carrion and the carrion attendant fauna represent both liquid and solid food resources for fire ants, as well as oils and proteins, and it is clear from the literature and personal observations that fire ants somehow utilize carrion resources. However, given the inability of adult worker fire ants to ingest solid particles > 0.88 μm, it is unclear what the ants do with carrion. It is possible that dissolved protein and other substances suspended in liquid form could pass on to the gut and become available for food sharing with the rest of the nest through trophallaxis, Fire ants also carry off prey items such as blow fly eggs and early instar larvae, so it is possible that they stockpile these foodstuffs for later use (Gayahan and Tschinkel 2008) or to deliver nutritive materials to their larvae.

If fire ants feed on carrion and carrion fauna, then traces of DNA specific to those sources should be found in their alimentary system. Whether stored in the crop as a social stomach for later trophallaxis or passed on to the rest of the gut system within any individual ant, finding DNA from carrion or the attendant fauna would provide conclusive evidence that fire ants feed on the carrion and/or its attendant fauna. Therefore, the purpose of this study is to use molecular techniques to detect pig- or blow fly-specific DNA in the gut systems of fire ants collected from and near pig carrion.

DNA Barcoding

The method relied upon in this study is commonly called DNA barcoding. It is based on the concept that each species' DNA is unique and particularly variable in short segments of certain genes. The name "barcode" stems from the sequence uniqueness in the short segments

analyzed, although flanking regions may be highly conserved. The mitochondrial cytochrome oxidase I gene (COI) is an example of a gene used for barcoding in animals (Luo et al. 2011). Molecular biology methods are used to amplify and sequence the DNA from known/vouchered species, which is used to assemble a database of DNA sequences. Unknown DNA samples can be similarly amplified and sequenced and its DNA sequence is compared to the DNA sequences in the database. This may result in positive specific identification if the sequence is already in the database, or in a unique identification if the sequence is not yet in the database because the species has not yet been sequenced or possibly even described.

DNA samples can include isolated tissues of an organism or bulk samples from the environment. Identification of a species' DNA within an environmental sample provides evidence that the species is present, as in DNA metabarcoding of diatom communities in streams to assess water quality (Vasselon et al. 2017). When the contents of a gut are sampled, the discovery of a species' DNA within the sample suggests that the given species represents one of the food items of the organism whose gut was sampled (e.g., Pompanon et al. 2012, Fayle et al. 2015, Roslin and Majaneva 2016, Nielsen et al. 2017). In this research, I am looking in the gut of fire ants for DNA sequences specific to pig and blow fly as evidence of scavenging and predation, similar to the study by Fayle et al. (2015), which demonstrated predation on termites by 14 species of ants.

Polymerase Chain Reaction (PCR)

In the present study, DNA extracted from ant guts was amplified using the PCR method (Sambrook and Russell 2001) to identify, specifically, pig and blow fly DNA. The PCR method was developed by Kary Mullis (1944 – 2019) for which he won the Nobel Prize in chemistry in 1993 (shared with Michael Smith). The basic concept is that sequences of DNA can be copied

exponentially in vitro. Amplified DNA from the cytochrome C oxidase I subunit gene (CO1) can be used as a species-specific marker. A short, single-stranded DNA sequence, or a "primer", designed to bind the CO1 sequence, directs a polymerase enzyme to synthesize the complementary DNA strand. The process is cycled n times (usually 25 to 35 times) to generate approximately 2^n copies of the DNA strand in a process called "amplification". PCR allows detection of those specific DNA sequences that may be present in tiny amounts in the original sample.

PCR is conducted in the presence of several essential reagents that enable the in vitro amplification of DNA. A thermostable DNA polymerase catalyzes the reaction. The *Taq* polymerase was isolated from the bacterium *Thermus aquaticus* (Brock and Freeze) and is commonly used in PCR applications. It can survive the high temperatures needed to denature (or separate) the two strands of the DNA molecule and still retain its enzymatic activity.

A pair of primers identify the portion of the DNA molecule to be amplified. Primers are either obtained commercially or designed for each application. A forward primer is designed to bind to the 3′ end of the target DNA sequence to be amplified, while a reverse DNA primer is designed to bind near the 5′ end of the sequence to be amplified. The forward and revers primers bind to complementary strands of the same DNA molecule.

A few PCR primers for pig DNA have been published and produced for commercial (food contamination) use or for use in carrion decomposition studies (e.g., Tajima et al. 2002, Michaud and Foran 2011). Despite the design of multiple primers for various Calliphoridae species, there has been little success in differentiating among species (e.g., Yusseff-Vanagas and Agnarsson 2017, Bortolini et al. 2018), especially recently diverged species. However, since the purpose of this experiment was to differentiate blow fly DNA from pig and fire ant DNA and not

to differentiate species of blow flies, a single primer was deemed sufficient for identification of blow fly DNA. Dr. Florencia Meyer, MSU, designed a forward primer universally for pig, blow fly, and fire ant DNA, as well as reverse primers that would specifically bind to pig and blow fly DNA. The DNA sequence amplified from pig is larger than that amplified from blow fly, allowing clear separation of amplicons by gel electrophoresis. Preliminary optimization experiments demonstrated that these primers have the ability to amplify the target DNA in the fire ant gut environment at a sensitivity sufficient to detect samples of pig DNA as small as 50 pg and can detect pig DNA diluted with fire ant DNA down to a ratio of 1:500 (Karson Pettit, Brooklyn Thompson, Jerome Goddard, and Florencia Meyer, personal communication, 22 Nov 2019).

Free nucleotides must be available for building new strands of DNA. These are supplied by a solution containing deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP), collectively termed deoxynucleoside triphosphates (dNTPs). Buffers are added to maintain pH or provide anions and cations. Divalent cations, particularly magnesium (Mg^{2+}), are required by most thermostable DNA polymerases, including *taq* polymerase.

The antennae, crop, and intestinal tissues of some ant species [*Tetramorium caespitum* (L.), *Camponotus floridensis* (Buckley), and, to a lesser degree, *S. invicta*] are known to inhibit PCR analysis of midgut contents (Penn et al. 2016); however, in preliminary optimization experiments, *S. invicta* exhibited minor inhibition of PCR analysis only when whole body samples were examined, and dissection of the gut appeared to eliminate the inhibition. I therefore used dissected guts for all PCR analyses described in this chapter.

Under the assumption that ants were feeding on the pig carrion and the attendant carrion fauna (Chapter II), I generated 12 specific hypotheses regarding spatiotemporal distribution of this behavior during the first 5 days of succession, using the presence or absence of pig and fly DNA in the guts as evidence. These hypotheses are formalized and enumerated below in the statistical analysis section of the Materials and Methods, providing an in-depth description of the statistics used to test each hypothesis.

Materials and Methods

Carrion

Carcasses were placed on the ground at a set distance near fire ant mounds. Distances between carcasses and fire ant mounds were 0.5, 1.0, 2.0, and 3.0 m. Each carcass-mound combination was unique such that there were no instances in which a carcass was exposed within 10 m of 2 or more mounds, nor were there any instances in which a mound was within 10 m of 2 or more carcasses (Figure 50). Neonatal pig carcasses were similar in size to those used in the exclusion experiment described in Chapter III (Table 21).

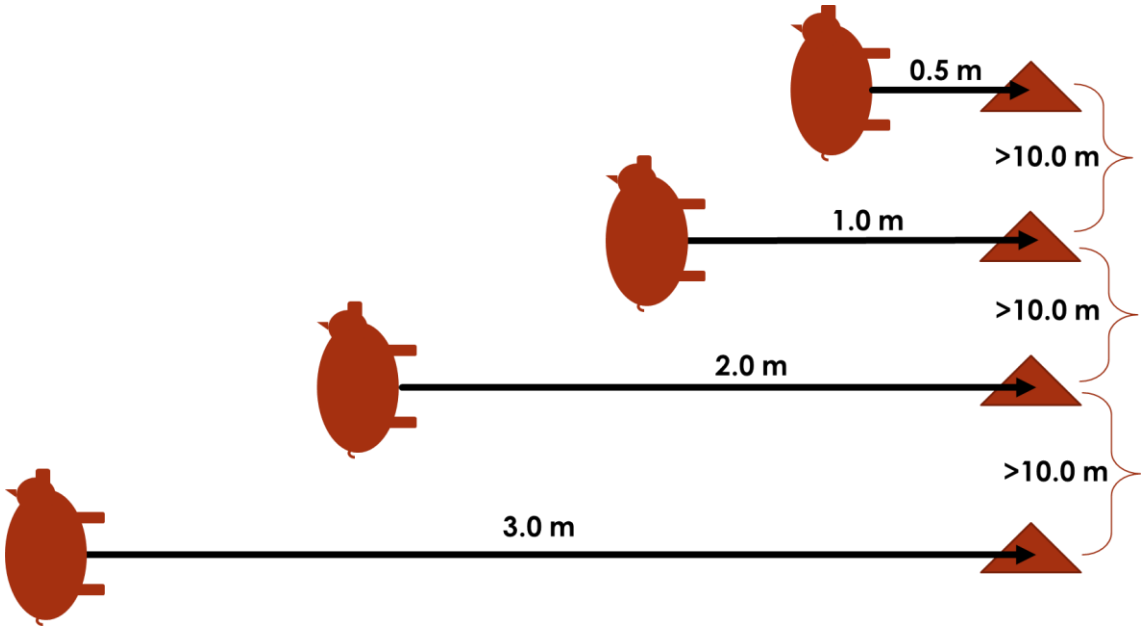

Figure 50 Diagrammatic scheme of layout of site.

Table 21 Mass (in kg, average ± standard deviation) of pig carcasses used in carrion decomposition study.

	Mississippi	Florida
Trial 1	Not measured	3.6 ± 1.5
Trial 2	4.6 ± 2.4	3.9 ± 0.5

Sample Sites and Dates

Preliminary exposure of pig carcass material was made in a residential property in Pensacola, Florida, using pork neck meat obtained from a butcher and exposing it 0.5 m from a fire ant mound. Ants were collected from the meat itself on 15 February. Similarly, on 15 April, a neonate pig carcass was exposed 0.5 m from a fire ant mound, and samples of ants were collected from both the mound and the carcass daily for 5 days as described below. These ants were used for preliminary assessment of DNA extraction and amplification methodology.

The same Mississippi study area (Goddard property near Adaton, Mississippi) as was used in the exclusion experiment (Chapter III) was used for this experiment (Figure 51). In the months prior to the experiment, portions of the meadow were plowed and planted with *Panicum ramosum* Arechav. (browntop millet). Because of an abundance of vertebrate scavengers in the Mississippi study area, the plastic pet cages, clothesline ropes, and orange flagging were again used, but there were no mesh bags, aluminum flashing, or pesticide used. Pig carcasses were exposed 13 July 2020 and 22 July 2020 in the Mississippi study area.

In Florida, the West Campus of Pensacola Christian College was used (Figure 51). This rural, recreational property is located 20 km west of Pensacola, Florida, 1 km south of U.S. Highway 98 along the Perdido River. It is in Ecoregion 75a, "Gulf Coast Flatwoods." Elevation is 3 m above sea level. The property comprises about 105 hectares (260 acres), and experimental plots were located in an undeveloped meadow surrounded by pine flatwoods and oak/saw palmetto scrub in the southern portion of the property. GPS coordinates of the meadow are N30°23'33" W87°24'28". The only precaution against scavenging was the presence of a green plastic garden fence laid over the carrion and staked to the ground.

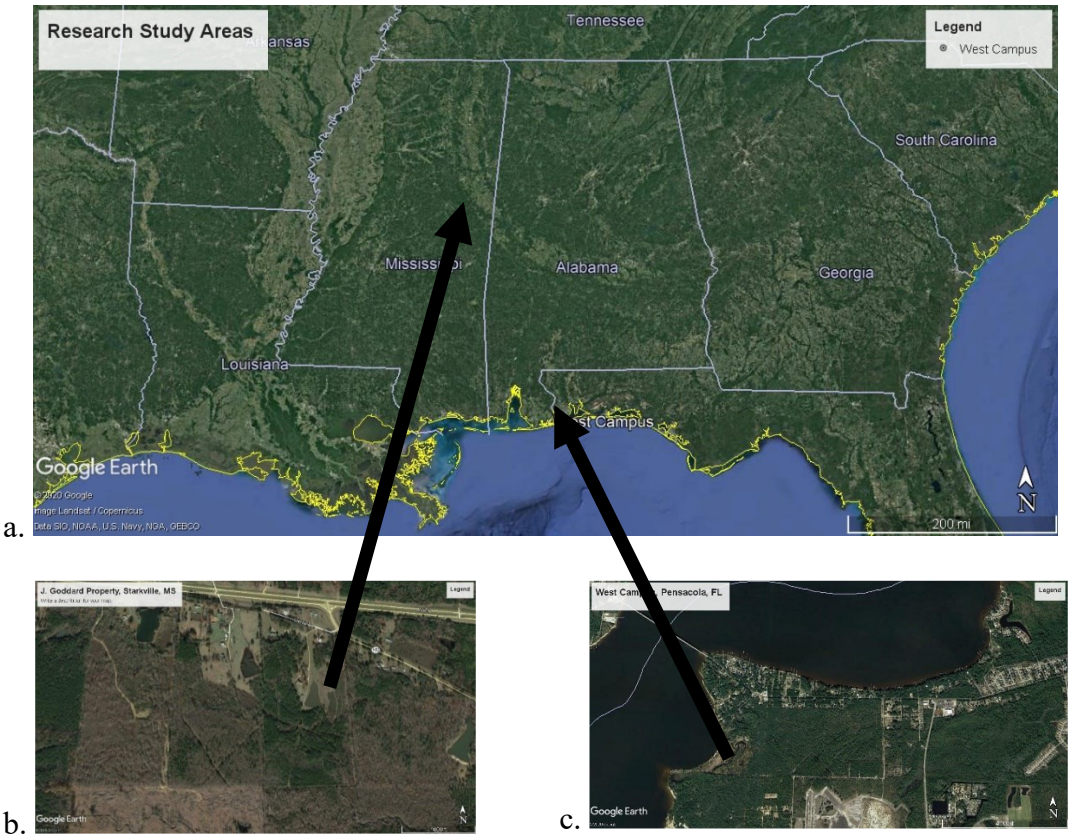

Figure 51 Google Earth images of study areas.

(a) Google Earth image of southeastern United States, showing locations of study areas.
(b) Google Earth image of Jerome Goddard property, Starkville, Mississippi.
(c) Google Earth image of West Campus property, Pensacola, Florida.

Common plants identified in the meadow and surrounding woods at the West Campus property included grasses, herbs/shrubs, and tree species, including both upland and wetland species. Grasses include *Andropogon* spp. (bluestem grasses), *Aristida stricta* Michx. (wiregrass), *Cyperus entrerianus* Boeckeler (woodrush flatsedge), *Fuirena squarrosa* Michx. (hairy umbrella-sedge), *Juncus* spp. (rushes), *Panicum* spp. (common grasses), *Rhynchospora* spp. (beak-sedges), and *Xyris elliottii* Chapman (Elliot's yellow-eyed grass). Forbs and shrubs

include *Carphephorus odoratissimus* (Gmelin) H.J.-C. Hebert (vanillaleaf), *Ceratiola ericoides* Michx. (Florida rosemary), *Cyrilla racemiflora* L. (leatherwood), *Eriocaulon compressum* Lamarck (flattened pipewort), *Eupatorium leptophyllum* de Candolle (false fennel), *Hypericum hypericoides* (L.) Crantz (St. Andrew's cross), *H. fasciculatum* Lamarck (sandweed), *Hydrocotyle bonariensis* Lamarck (largeleaf marshpennywort), *Ilex glabra* (L.) Gray (inkberry), *I. vomitoria* Sol ex. Alton (yaupon), *Lycopodiella* spp. (clubmosses), *Opuntia humifusa* (Raf.) Raf., (eastern prickly pear), *Pteridium aquilinum* (L.) Kuhn var. *pseudocaudatum* (tailed bracken), *Rubus trivialis* Michx. (southern dewberry), *Serenoa repens* (Bartram) Small (saw palmetto), *Smilax* spp. (greenbriers), *Solidago* spp. (goldenrod), *Vaccinium corymbosum* L. (highbush blueberry), and *Vitis rotundifolia* Michx. (muscadine grape). Trees include *Magnolia virginiana* L. (sweet bay), *Persea palustris* (Raf.) Sarg. (swamp red bay), *Pinus palustris* Mill. (longleaf pine), *P. elliottii* Engelmann (slash pine), *Quercus* spp. (oaks), *Taxodium* spp. (cypress), and *Triadica sebifera* (L.) Small (Chinese tallow tree). This is not, nor is it intended to be, a comprehensive list of the flora on the property.

Pig carcasses were exposed in the Florida study area on 19 June and 16 July 2020.

Field Methods

Samples of 20 to >50 fire ant workers were collected from the mound on the day of carrion exposure (Day 0) and from both the carcass and its corresponding mound each subsequent day for up to 5 days after initial exposure on the carcass. Ant specimens were collected directly from carcasses using forceps; often, several ants could be collected at once by tweezing the forceps parallel to the skin surface near an ant-formed lesion. Ants were placed in a plastic jar with 95-100% ethyl alcohol. Ant specimens from the mound were collected by inserting a wood dowel about 3 cm into the target mound and removing it a few seconds later

after ants had climbed onto the rod. The rod was then tapped on the edge of a large plastic bucket, shaking the ants loose into about 150 mL ethyl alcohol. Occasionally, ants ignored the wooden rod and had to be collected individually with forceps. Ants were subsequently placed into plastic containers in 95-100% ethyl alcohol until analysis.

On some dates, ants were not collected on the carcasses because fire ants were not present or for some reason very uncommon; however, ants were collected from the mounds on every date.

DNA Extraction

Ants were dissected to remove the gut. Prior to dissection, ant bodies were decontaminated of any potential surficial DNA by soaking in an aqueous 5% sodium hypochlorite solution for 60 seconds, then rinsed in 70% ethyl alcohol (Kwok and Higuchi 1989, Linville and Wells 2002), in accordance with best workflow practices for reliable molecular biology results (Murray et al. 2015). Dissection instruments were also dipped in aqueous 5% sodium hypochlorite solution between samples.

Dissection of ant guts was performed under a Bausch + Lomb Stereo Zoom 7® dissecting microscope at ~10x magnification. As described in Fisher and Cover (2007), "The true first abdominal segment is permanently fused to the thorax, where it is termed the propodeum. Since ants are petiolate, the petiole is the true second abdominal segment; if the petiole is 2-segmented, the next segment, or postpetiole, is the true third abdominal segment. Everything beyond the petiole (or petiole plus postpetiole) comprises the gaster." Therefore, in *Solenopsis*, which has a 2-segmented petiole, the suture between the 4th and 5th abdominal segment appears as the suture between the 1st and 2nd segment of the gaster. While the base of the gaster was held with forceps, the abdomen was split between the 4th and 5th segments with a teasing needle (Figure 52). A

second pair of forceps or a teasing needle with the tip bent into a hook was used to reach into the base of the split abdomen to sever the esophagus anterior of the crop as well as the mesentery tissues around the inner perimeter of the gaster. The gut material, comprised of the crop, stomach/midgut, Malpighian tubules, and hindgut, was then extracted from the gaster.

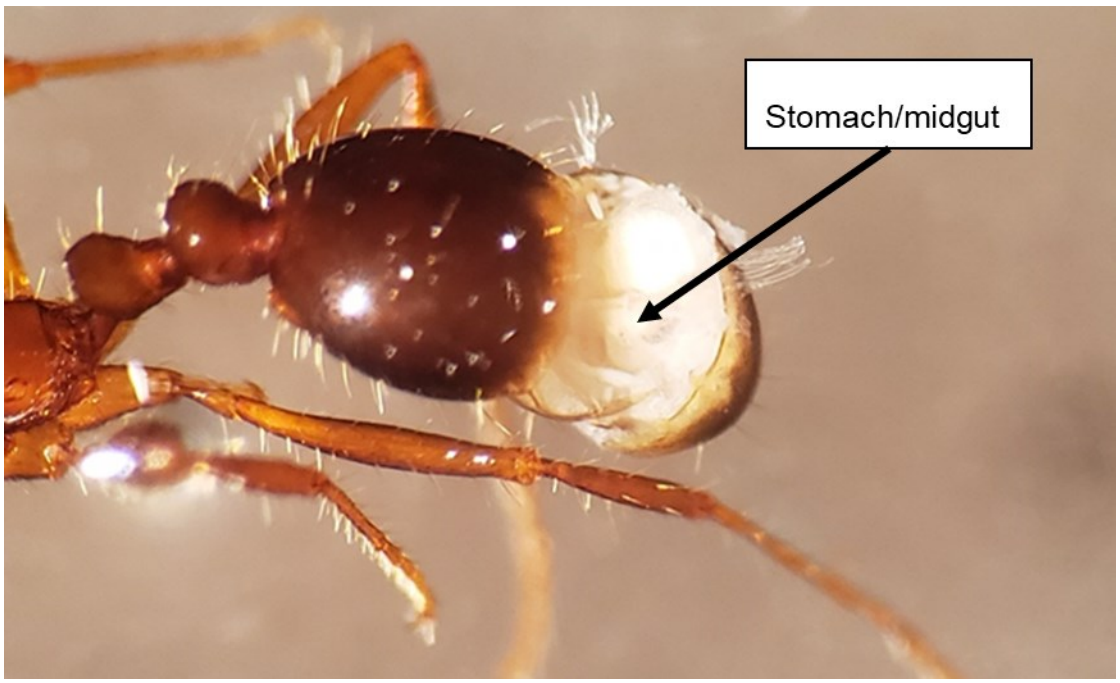

Figure 52 Dissected ant abdomen with stomach/midgut visible in 5th abdominal segment.

The gut material was placed in a sterile 0.9% phosphate-buffered saline solution in a 1.5 mL microcentrifuge tube. For the first set of samples from Florida, 20 ants were dissected and composited for each sample; for the second set of samples from Florida and both sets of samples from Mississippi, 40 ants were dissected and composited for each sample. A total of 5,940 ants was dissected.

DNA was extracted from ant guts using an IBI Scientific™ Genomic DNA Kit, following kit instructions and accompanying reagents. This spin column-based nucleic acid purification

technique involved five steps: tissue dissociation, lysis of cell membranes, binding of DNA to silica in a glass fiber column, washing extraneous material away, and eluting the DNA from the glass fiber column. Each step is described briefly below.

To dissociate the tissues, ant guts were placed in a 1.5 mL microcentrifuge tube to which 200 µL dissociation buffer and 20 µL proteinase K were added. This solution was incubated at 60°C for 30 minutes, agitating gently every 5 minutes. Since the tissues involved unsclerotized gut material, physical maceration was unnecessary. Lysis of the cell membranes was performed with 200 µL lysis buffer. The solution was incubated at 60°C for 20 minutes, agitating gently every 5 minutes. Occasionally, insoluble material remained at this step, so the solution was centrifuged at 16,000 x g for 2 minutes. The supernatant was transferred to a clean tube to which 200 µL absolute ethanol was added, followed by vigorously agitation for 5 s. This solution was applied to a glass fiber spin column and forced through by centrifugation at 16,000 x g for 2 min. The DNA-bound glass fiber spin columns were washed sequentially with 400 µL wash buffer solution and 600 µL wash buffer with ethanol, each followed by centrifugation at 16,000 x g for 30 s, to remove free nucleotides and other impurities. The column was transferred to a clean 1.5 mL microcentrifuge tube, and DNA was eluted from the glass fiber spin column using 50 µL pre-heated elution buffer (60°C). After 5 min, the column was centrifuged at 16,000 x g for 30 s to elute the purified DNA.

Quantification of purified DNA was determined by absorbance at 260 nm, measured with a NanoDrop™ 2000 spectrophotometer. The ratio of absorbance at 260 nm: 280 nm (A_{260}/A_{280}) was also measured to determine the "purity" of each sample in regard to impurities such as phenol, guanidine, proteins, RNA, and stray oligonucleotides that also absorb at 260 nm. An

A_{260}/A_{280} ratio near 1.8 to 2.0 is considered to be relatively pure and should provide good quality DNA for downstream procedures such as PCR (Manchester 1995).

Polymerase Chain Reaction

Purified DNA extracted from ant guts was amplified using standard PCR methods (Sambrook and Russell 2001) to identify, specifically, pig and blow fly DNA. In samples for which pig DNA was to be amplified, PCR was carried out in 25 µL reactions containing 1 mM 10x amplification buffer, 3.6mM $MgCl_2$, 1.08 – 1.20 µM concentration of a universal forward primer (AFPFw2, described below), 0.4 mM concentrations of mixed dNTPs, and 0.5 U *taq* polymerase, along with 1.08 – 1.20 µM concentration of pig reverse primer (PigR1, described below). In samples in which fly DNA was to be amplified, the same master PCR mix was used except that I used the universal forward primer AFPFw1 (described below) and the fly reverse primer FlyR1 (described below).

Each PCR reaction was done with 50 ng of template DNA, except in a few samples in which DNA concentration < 4 ng DNA / µL elution buffer. No PCR reaction had less than 15.6 ng of template DNA.

The forward primer for pig DNA amplification was AFPFw2 and for blow fly DNA amplification was AFPFw1. The reverse primers were PigR1 and FlyR1, respectively. All primers were designed by the Meyer lab at MSU (Table 22).

Table 22 Primer sequences and melting temperatures (T_m) for the primers used in this study.

Primer	5′ to 3′ sequence	T_m (°C)
AFPFw1	5′-TGCTTTTATTATAATTTTCTTTATAGT-3′	51.3
AFPFw2	5′-CTATTTTCAACAAATCACAAAGA-3′	51.7
FlyR1	5′-CAAATGTAATTCCTGTAGATC-3′	52.0
PigR1	5′-GGTTCTTTTTTACCTGAATAG-3′	52.0

Reverse primers were designed to specifically bind pig and blow fly DNA to generate different sized amplicons, allowing easier visualization when used in gel electrophoresis, as the smaller DNA fragments will travel farther within the gel than larger DNA fragments.

Preliminary optimization experiments that had previously been conducted in the Meyer lab indicated that the PigR1 reverse primer had a sensitivity sufficient to detect samples of pig DNA as small as 50 pg and could detect pig DNA diluted to 1:500 with ant DNA (Karson Pettit, Brooklyn Thompson, Jerome Goddard, and Florencia Meyer poster, 22 Nov 2019). Optimization experiments indicated that the FlyR1 reverse primer had a sensitivity sufficient to detect samples of fly DNA as small as 0.5 ng.

PCR was conducted using an Eppendorf® Mastercycler® Pro S thermal cycler under identical conditions for each batch. In optimization experiments, it was determined that the pig and fly reverse primers required different annealing temperatures, so PCR reactions occurred separately. Initial denaturing of DNA was conducted for 3 min at 95°C. Each sample was subjected to 40 cycles of denaturing for 45 s at 95°C, annealing for 45 s at 40°C (PigR1) or 42°C (FlyR1), and extension/elongation for 45 s at 72°C (Figures 53 - 54). After cycling, the samples were held at 4°C until electrophoresis.

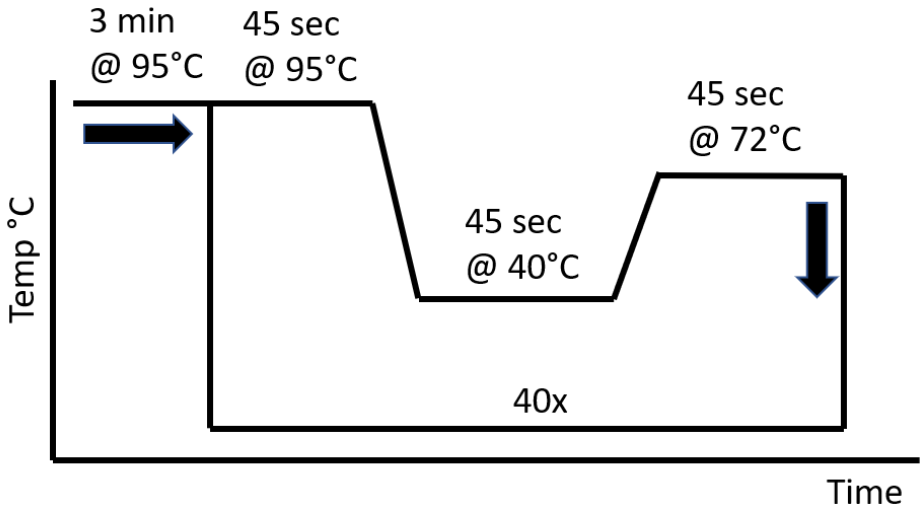

Figure 53 Schematic of PCR conditions for samples in which pig DNA was amplified.

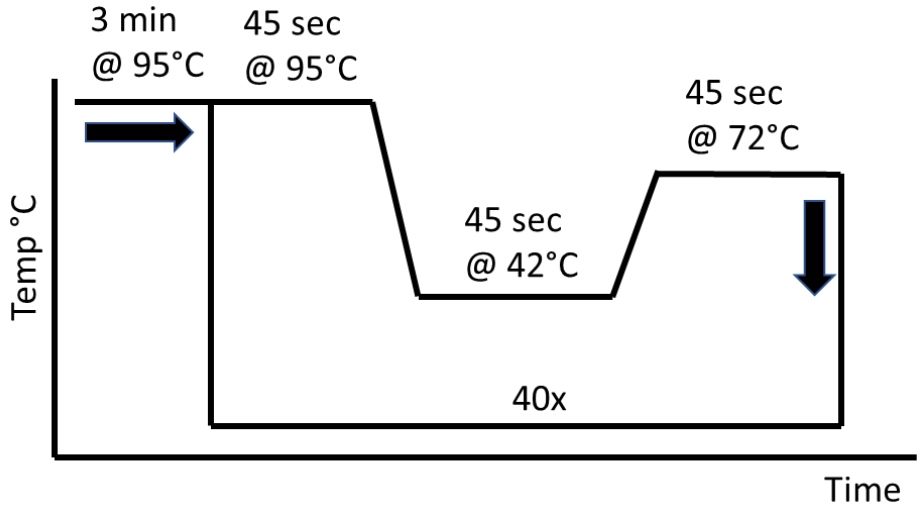

Figure 54 Schematic of PCR conditions for samples in which fly DNA was amplified.

Electrophoresis

Amplified DNA was separated using gel electrophoresis. Electrophoresis gels were made with a 5% agarose solution in Tris/Acetate/EDTA (TAE) buffer (40 mM Tris, 20 mM acetic acid, 1mM EDTA in water). Ethidium bromide stain (10 mg/mL), which fluoresces under

ultraviolet light when linked to DNA, was added to the gel, mixed at a rate of 1.6 µL ethidium bromide stain / 100 mL agarose solution.

Positive controls included 1 ng pig DNA and 1 ng blow fly DNA. Negative controls included samples from the nest on Day 0 when ants were assumed to be naïve to pig or blow fly DNA. A 100 bp DNA ladder served as a molecular-weight size marker. All DNA samples were mixed with 5 µL reaction buffer to allow visual monitoring of migration progress. Electrophoresis conditions were 100 volts at 80 mA for ~55 min or 115 volts at 80 mA for ~40 min. The final gel was visualized under ultraviolet light, and photographs were made of the gel using a Bio-Rad® ChemiDoc imager.

Successful amplification of DNA, as evidenced by a fluoresecent band in alignment with the positive control, indicated presence of the species' DNA and was recorded in the data set as "1". Absence of a fluorescent band in alignment with the positive control indicated that results were below the detection limits. Any result below detection limit was recorded as "0" in the data set. Non-specific bands (those present but not aligned with the positive control band) were ignored if the positive control yielded positive results.

Statistical Analysis

The methods described above resulted in binary positive or negative identification of pig or blow fly DNA in fire ant guts. As mentioned above, I developed 12 hypotheses regarding the presence or absence of pig DNA at distances from pig carrion. Specifically, six individual null hypotheses ($H_{o27\text{-}31}$), one for each of the first 5 d of succession and a sixth hypothesis in which data from all days are pooled (H_{o32}), are as follows:

H_{o27}: There is no significant relationship between the probability of detecting pig DNA in fire ant guts on day one and distance from carrion.

Null hypotheses $H_{028\text{-}31}$ are identical as above but substituting two, three, four, or five for day one. Null hypothesis H_{o32}, in which data are pooled across days, is as follows:

H_{o32}: There is no significant relationship between the probability of detecting pig DNA in fire ant guts and distance from carrion.

The alternate hypothesis is that there is a significant relationship between presence or absence of pig DNA and distance from the carrion. A binary logistic regression model was used to compare positive identification of pig DNA with distance of the collected ants from the carrion.

Likewise, six individual null hypotheses ($H_{o33\text{-}37}$) , one for each of the first 5 d of succession and a sixth hypothesis in which data from all days are pooled (H_{o38}), are as follows:

H_{o33}: There is no significant relationship between the probability of detecting blow fly DNA in fire ant guts on day one and distance from carrion.

Null hypotheses $H_{o34\text{-}37}$ are identical as above except with days two, three, four, and five substituted for day one. Null hypothesis H_{o38} in which data are pooled across days would be formally worded as follows:

H_{o38}: There is no significant relationship between the probability of detecting blow fly DNA in fire ant guts and distance from carrion.

The alternate hypothesis for each null hypothesis is that there is a significant relationship between presence or absence of blow fly DNA and distance from the carrion. A binary logistic regression model was used to compare positive identification of blow fly DNA with distance of the collected ants from the carrion.

A Wald test was calculated on the z value for each logistic regression model to test for significance of the slope. A statistically significant result for a hypothesis would indicate that fire ants forage at carrion more (positive slope) or less (negative slope) on a given day as distance to the carrion from their mound decreases. Exponentiation of the statistical estimates of the regression coefficients yielded probabilities of detection of pig or ant DNA in ant guts.

Assumptions associated with logistic regression analysis were checked as appropriate (e.g., whether dependent and independent variable data types were binary and ordinal, respectively; whether observations were collected independently; whether there was linearity of relationship between independent variables and the log odds; and if there was a sufficient sample size). An *a priori* significance level of $\alpha = 0.05$ was used for all tests, including for any statistical tests of assumptions. Since there were 12 regressions, the Holm's Sequential Bonferroni correction adjustment (Holm 1979) was applied to the α level, as described in Chapter III. Again, the dichotomy of significance/non-significance based on an *a priori* α value was used despite concerns about the misuse of p-values in statistical inference (Wasserstein and Lazar 2016, Wasserstein et al. 2019); however, statistical tests in which p-values were near the α_{HB} threshold

were interpreted cautiously. All statistical tests were conducted in R (R Core Team 2019) using the R Commander GUI and associated packages (Fox 2005, 2017, Fox and Bouchet-Valat 2019).

Results and Discussion

Temperature

Temperatures in both study areas did not vary considerably, probably due to the shorter time frames for each exposure. High temperatures in Mississippi ranged from 31.1°C to 36.1°C, and low temperatures ranged from 22.2°C to 24.4°C. In Florida, high temperatures ranged from 30.0°C to 34.4°C, and low temperatures ranged from 21.7°C to 26.1°C.

Community Composition

In the initial trial exposure in the Florida study area in February, only fire ants were collected. In the April and July exposures in the Florida study area, other members of the ant community were collected. Three other ant species collected at pig carrion were *Brachymyrmex patagonicus*, *Odontomachus haematodus*, and *Crematogaster cerasi*. In the second exposure in Florida, *C. cerasi* replaced *S. invicta* at the pig carcass located 3 m from a fire ant mound, and by 48 hours after exposure was the only ant species found on the carrion.

Blow flies observed at carrion in Mississippi during this study included *Chrysomya rufifacies*, *Cochliomyia macellaria*, and *Phormia regina*. Blow flies observed at carrion in Florida included *C. megacephala*, *C. rufifacies*, *Lucilia coeruliviridis*, and *L. cuprina*, although *C. megacephala* and *L. cuprina* were uncommon (<2 specimens observed). DNA from common blow fly species (*P. regina*, *L. coeruliviridis*, *C. macellaria*, and *C. rufifacies*) was successfully amplified using the FlyR1 reverse primer (Figure 55).

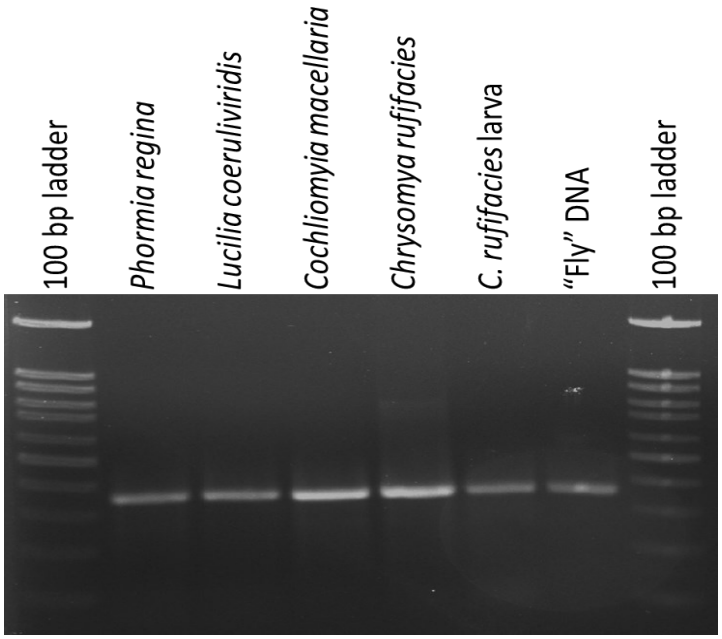

Figure 55 Gel electrophoresis indicating specificity of primers for amplification of DNA from common blow fly species.

"Fly" DNA indicates the DNA used as positive controls for sample electrophoresis. 100 bp ladder, molecular weight marker.

The DNA target sequence for FlyR1 was searched in the GenBank database, retrieved online using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi; Tajima et al. 2002), and it indicated agreement with *Chrysomya rufifacies*. Phylogenetic divergence of blow flies has historically been insufficient to allow consistent species-level identification among blow flies (e.g., Sonet et al. 2013, Googe 2014, Yusseff-Vanegas and Agnarsson 2017) unless multiple gene sequences are used (Zaidi et al. 2011). Based on the positive results, this target sequence was deemed adequate to separate pig DNA from blow fly DNA at the phylum level, since species-level identification was not necessary.

General Description of Fire Ant Succession and Carrion Decomposition

Fire ant colonization on carrion was slightly different between Mississippi and Florida. In Mississippi, fire ants quickly arrived at carrion at all distances from the mounds and produced characteristic lesions and thatch buildup. During the first exposure in Mississippi, carrion < 2 m from the nests was so overwhelmed by ant presence that blow flies were completely unable to colonize. Bloating and autolytic decomposition occurred normally, but without blow fly larvae, the carcass mostly desiccated. The skin was not decomposed or breached, leaving deflated, but recognizable, pig carcasses on Day 5 (Figure 56). Conversely, blow flies were able to colonize carrion ≥ 2 m from a fire ant nest despite presence of some ants, and decomposition proceeded as normal, with breakdown of all soft tissues, including skin, and with disarticulation, exposure, and dispersal of skeletal elements by Day 4 (Figure 56). In the second exposure in Mississippi, fire ants fully colonized carrion located 0.5, 1, and 3 m from the mounds, excluding flies to the extent that on Day 5, remains desiccated and were recognizable as pig carrion, as in the first exposure (Figure 57). The carcass exposed 2 m from the fire ant mound was colonized primarily by blow flies so that the carcass was nearly fully decomposed and the skeleton disarticulated and scattered by Day 5.

In both Florida exposures, fire ants quickly arrived at carrion at all distances from the nests, but blow flies were also able to colonize, including carrion within 0.5 m of a fire ant mound (Figure 58). With the exception of carcasses exposed 0.5 m from the ant mound, all carcasses decomposed to scattered bones and tiny scraps of skin remaining with ants absent by Day 5.

Figure 56 Photographs illustrating progression of decomposition in pig carcasses in Mississippi, exposed 13 July 2020, at distances of 0.5 to 3.0 m from fire ant mounds.

Photo credits (days 3 – 5): Florencia Meyer.

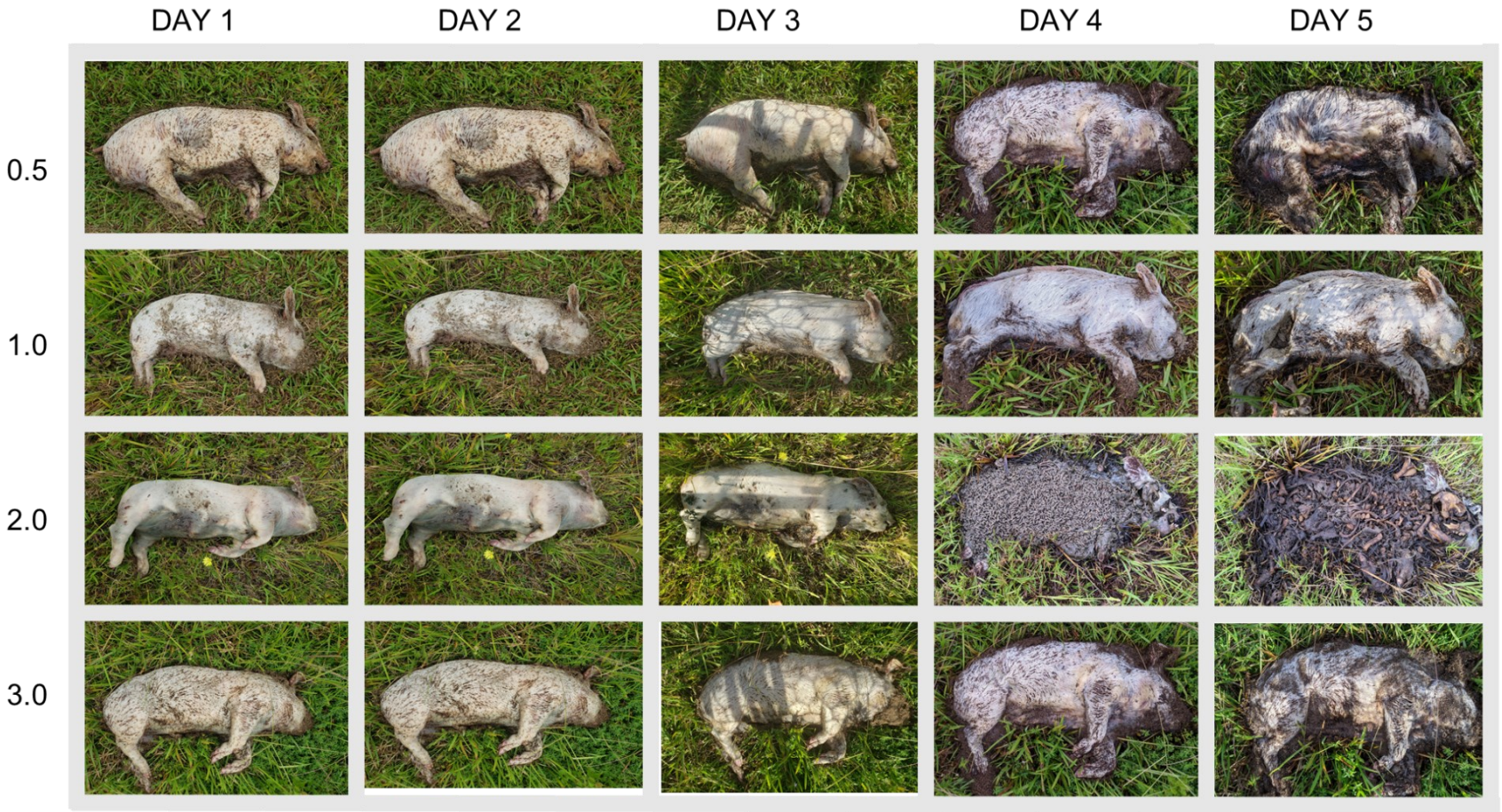

Figure 57 Photographs illustrating progression of decomposition in pig carcasses in Mississippi, exposed 22 July 2020, at distances of 0.5 to 3.0 m from fire ant mounds.

Photo credits (days 4 – 5): Florencia Meyer.

Figure 58 Photographs illustrating progression of decomposition in pig carcasses in Florida, exposed 16 July 2020, at distances of 0.5 to 3.0 m from fire ant mounds.

It is possible that these observations are due to increased aggression in the hybrid species found in the Mississippi study area. While *S. invicta* is known to be more aggressive as it invades new territory, including toward the *S. invicta* x *richteri* hybrid and other *Solenopsis* species (Fadamiro et al. 2009, Haight 2010), there is very little known about relative aggressiveness of fire ant species in the southern United States against invaders. In this study, it was noted that the hybrid seemed more aggressive, at least toward nest invasion: more workers climbed the dowel used to sample mounds and they climbed it more quickly in Mississippi, where the hybrid species occurred, than in Florida, where only *S. invicta* occurred.

On carcasses in which ants overwhelmed the blow flies in both Mississippi and Florida, adult flies landed on carcasses but were quickly chased away by ants such that they did not appear to have opportunity to oviposit. When fire ants did not completely overwhelm the blow flies, colonization by blow flies still appeared to be delayed, as described in Chapter II. The same observation was also made regarding other ant species by Park and Moon (2020) in South Korea.

Interestingly, the decomposition of carcasses was much more rapid than during the exclusion experiment in 2019. In the 2019 control treatment in which all fauna had access, carcasses did not reach the "Skeletonized remains" stage until Day 7, but in this experiment, the "Skeletonized remains" stage was reached by Days 3 to 5, depending on the proximity of fire ant mounds and overwhelming fire ant presence. This may have been in part because of higher daily low temperatures in 2020 in both study areas, despite similar daily high temperatures. It is also possible that the mesh bags in the 2019 exposures prevented soil bacteria in succession and insects from maneuvering bones into the soil during skeletonization.

DNA analysis

Collection and purification of DNA from ant guts collected in the first set from Florida ranged from 1.3 – 15.9 ng DNA / μL elution buffer and averaged 5.6 ± 3.3 ng DNA / μL elution buffer (mean ± s.d.). Collection and purification of DNA from ant guts collected in the other sets (both Mississippi and Florida) ranged from 2.9 – 114.9 ng DNA / μL elution buffer and averaged 22.0 ± 18.7 ng DNA / μL elution buffer, the overall larger values likely being a result of using twice as many ants for each sample. From all sets, A_{260}/A_{280} ratios ranged from 1.14 to 3.87 and averaged 1.97 ± 0.41 (Appendix D). These values indicate reasonably high quality DNA extraction, relatively uncontaminated by other organic molecules, acceptable for PCR procedures.

In some gels, positive identification bands were extremely light, but this study was not designed to quantify pig or fly DNA; rather, I was interested in showing presence or absence. Since neither pig nor fly DNA was found in ant guts from ants collected from mounds 3.0 m from the carcasses (see data below), data from ants collected at mounds >10 m away from a carcass were ignored.

Mississippi Set 1

Although ants were observed on all four carcasses in the first set of pigs exposed in Mississippi, pig DNA was detected in ant guts only on days 3 through 5 and only in ants collected from the mound located 0.5 m from the carcass (Figure 59). There was no pig DNA detected in ants collected from the carcasses or from the mounds more than 0.5 m from the carrion. Fly DNA was recovered more frequently from the fire ant guts than pig DNA in this set. Fly DNA was detected on days 3, 4, and 5 from ants collected directly from the pig carcass located 0.5 m from the mound and on day 3 from ants collected directly from the pig carcass

located 1.0 m from the mound (Figure 59). Fly DNA was also detected in ants collected from the mound located 0.5 m from carrion on days 2, 4, and 5, and from the mound located 1.0 m from the carrion on day 1. No fly DNA was detected in ants collected from the mounds located 2.0 and 3.0 m from the carrion (Figure 59).

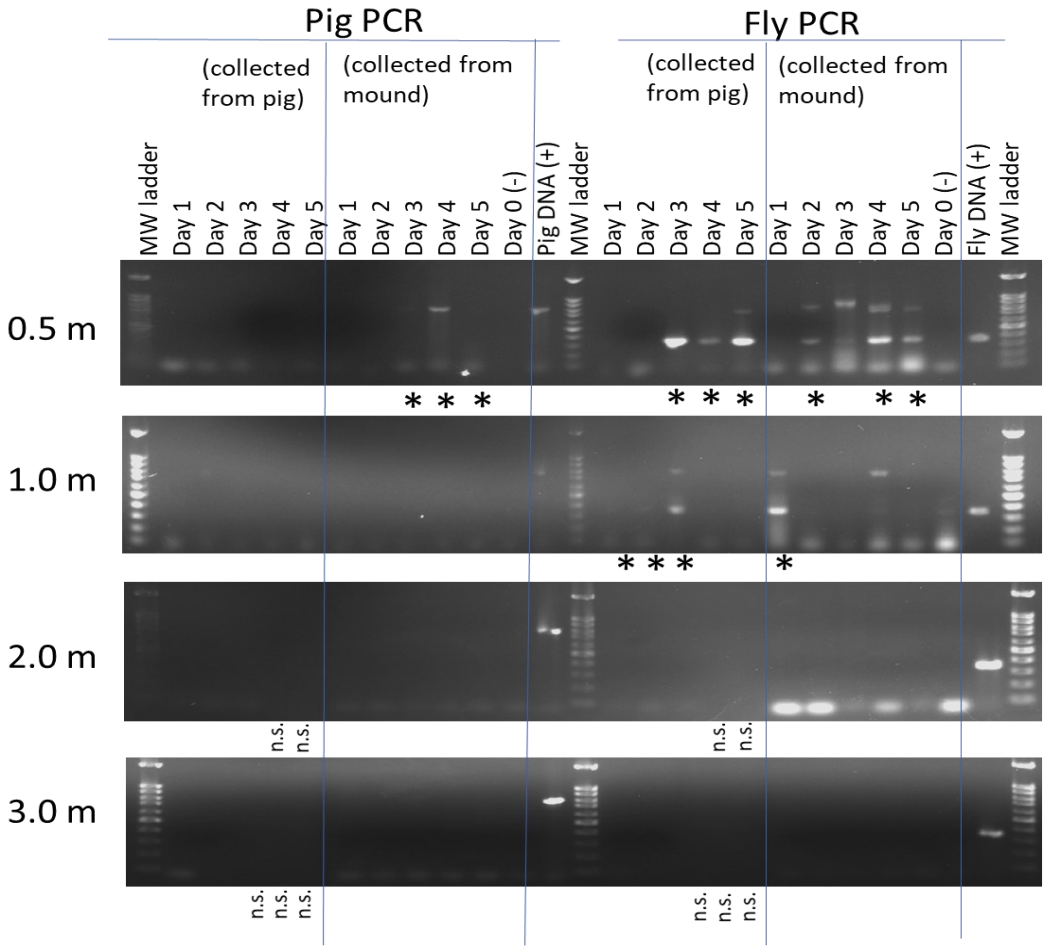

Figure 59 Gel electrophoresis separation of DNA fragments amplified from fire ant guts collected 14-18 July 2020 in Mississippi.

Fire ants were collected directly from four pig carcasses and from a corresponding mound located 0.5, 1.0, 2.0, and 3.0 m from the carcass. Pig and blow fly PCR reactions were conducted using different primers as described in the Methods section (Table 22). An asterisk (*) indicates positive identification of DNA. n.s. = not sampled due to absence of fire ants. MW ladder, molecular weight marker.

Mississippi Set 2

In the second exposure in Mississippi, pig DNA was detected in ant guts from ants collected on the carcass and mound located 0.5 m from each other. In ants collected directly from the carcass, pig DNA was detected on days 1, 3, 4, and 5; in ants collected from the mound, pig DNA was detected on days 2 through 5 (Figure 60). When the carcass was 1.0 m from the mound, pig DNA was detected in ants collected on the carcass on day 5 and in ants collected from the mound on days 3 and 5. No pig DNA was detected in ant guts from ants collected on the carrion and mound located 2.0 m from each other. When the carcass was located 3.0 m from the mound, pig DNA was detected in ant guts from ants collected directly on the carcass on days 3 and 5 but not from ants in the mound (Figure 60).

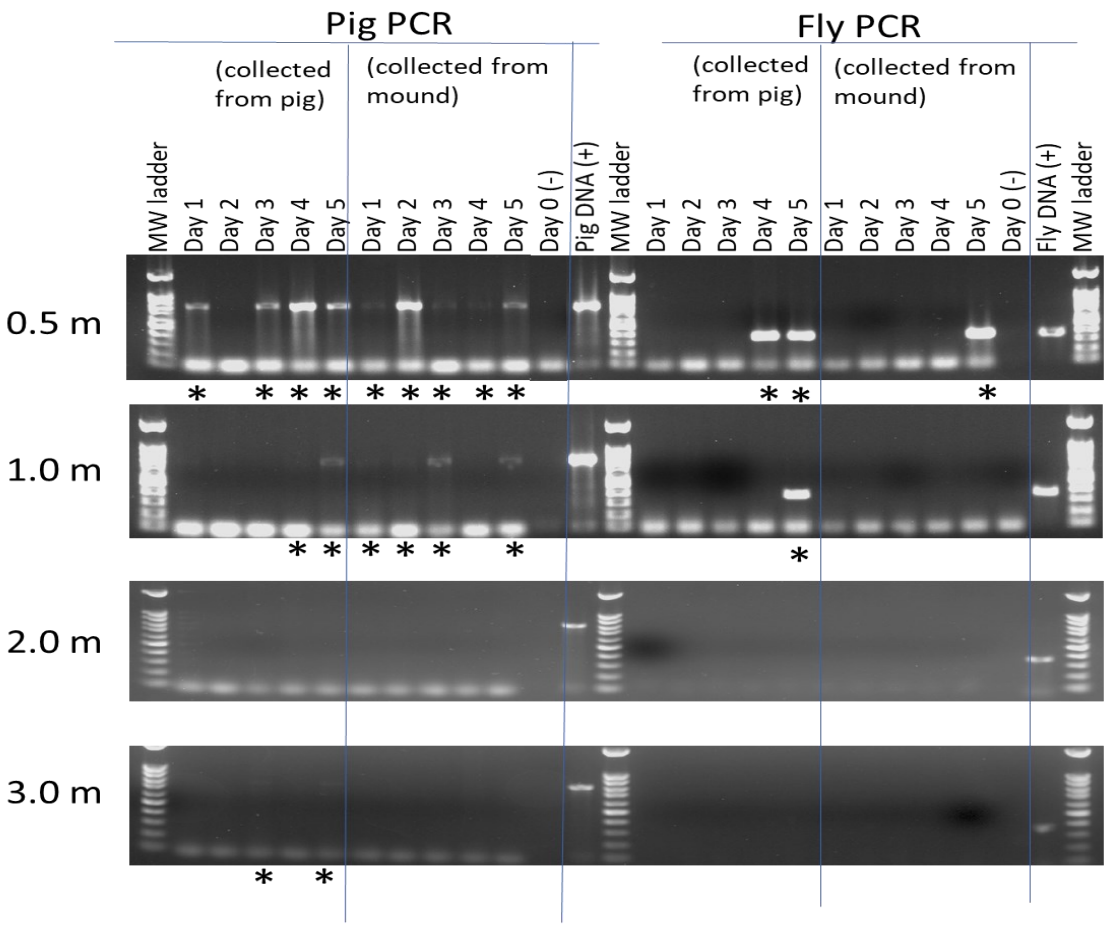

Figure 60 Gel electrophoresis separation of DNA fragments amplified from fire ant guts collected 23-27 July 2020 in Mississippi.

Fire ants were collected directly from four pig carcasses and from a corresponding mound located 0.5, 1.0, 2.0, and 3.0 m from the carcass. Pig and blow fly PCR reactions were conducted using different primers as described in the Methods section (Table 22). An asterisk (*) indicates positive identification of DNA. n.s. = not sampled due to absence of fire ants. MW ladder, molecular weight marker.

Florida Set 1

In the first Florida set, where the fire ant nest was 0.5 m from the carrion, pig DNA was detected in the guts of fire ants collected directly from the carrion on Days 1 and 2 (Figure 61). Pig DNA was not detected in guts of fire ants collected from the nest. Likewise, where the fire ant nest was 1.0 m from the carrion, pig DNA was also detected in the guts of fire ants collected

directly from the carrion on Days 1 and 2. However, pig DNA was detected in ant guts from ants collected directly from the nest on Day 2, but not on any other days. Pig DNA was not detected in ant guts from any sampled ants when the carrion and mounds were more than 1.0 m apart (Figure 61). Notably, the sample with the lowest concentration of DNA successfully amplified pig DNA: ant guts from ants collected on the mound 0.5 m from the carcass yielded only 1.3 ng DNA/μL elution buffer so that 12 μL of sample in the reaction provided 15.6 ng of template DNA).

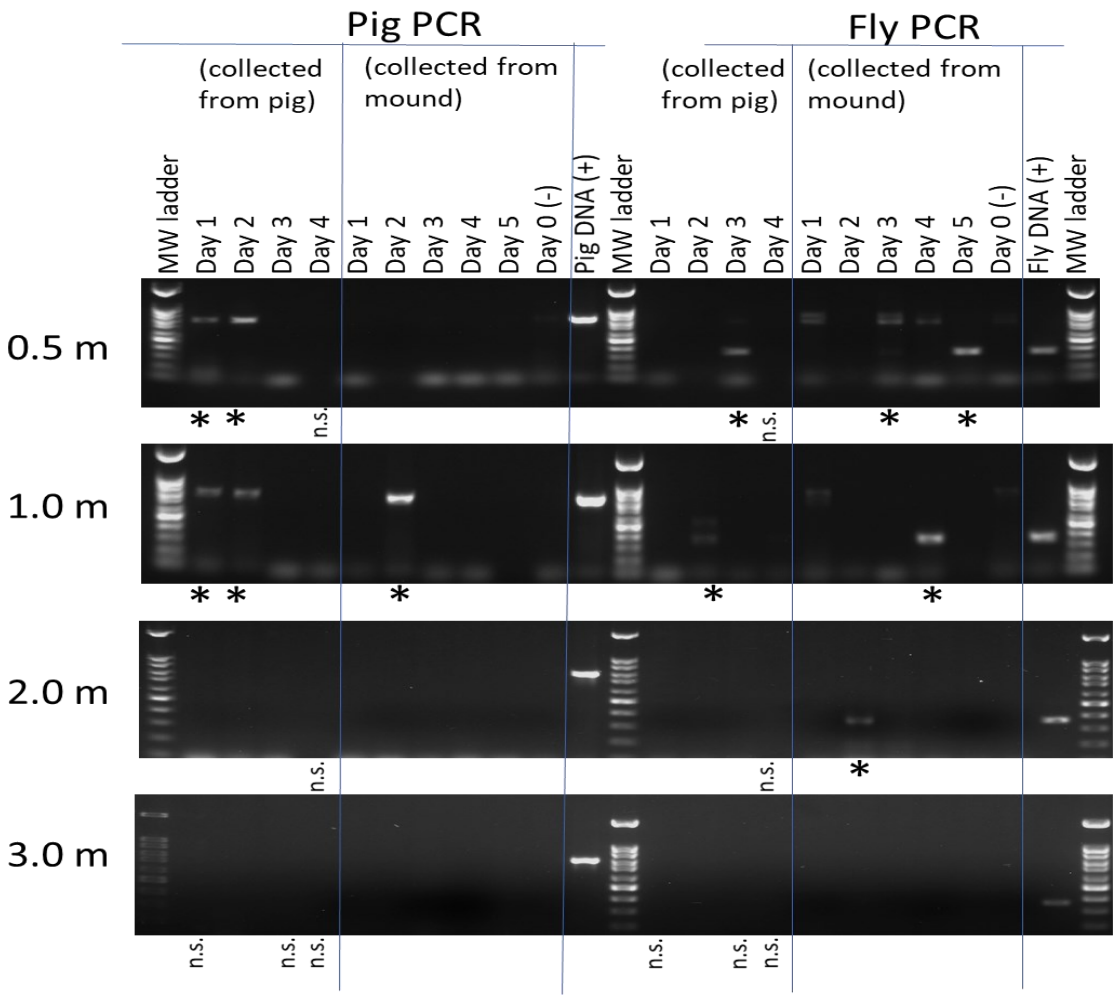

Figure 61 Gel electrophoresis separation of DNA fragments amplified from fire ant guts collected 20-24 June 2020 in Florida.

Fire ants were collected directly from four pig carcasses and from a corresponding mound located 0.5, 1.0, 2.0, and 3.0 m from the carcass. Pig and blow fly PCR reactions were conducted using different primers as described in the Methods section (Table 22). An asterisk (*) indicates positive identification of DNA. n.s. = not sampled due to absence of fire ants. MW ladder, molecular weight marker

Fly DNA was detected in ants collected directly from the carcass on day 3 and in ants collected from the mound on day 5 when the carcass and mound were 0.5 m from each other (Figure 61). Fly DNA was also detected in both the carcass (Day 2) and mound (Day 4) when the carcass was 1.0 m from the mound. The only instance of non-ant DNA detected in ant guts in

this study in which carcass and mound were 2.0 m from each other occurred on Day 2, when pig DNA was detected in ants collected from the mound. No fly DNA was detected in ant guts when the carrion or mound the ants were collected from were 3.0 m from each other (Figure 61).

Florida Set 2

When carrion was 0.5 or 1.0 m from the mound and ants were collected directly from the carcass, pig DNA was detected in ant guts on days 1 through 4 (Figure 62). Blow fly DNA was detected in ant guts on days 3 and 4 when carrion was 0.5 m from the mound and ants were collected directly from the carcass, but only on Day 2 when carrion was 1.0 m from the mound. For ants collected from the mound, pig DNA was detected in the guts of ants collected on days 3 through 5 when carrion was located 0.5 m from the mound and on days 1, 2, 4, and 5 when carrion was located 1.0 m from the mound. Fly DNA was detected in the guts of ants collected on days 2, 4, and 5 when carrion was located 0.5 m from the mound and on days 4 and 5 when carrion was located 1.0 m from the mound (Figure 62).

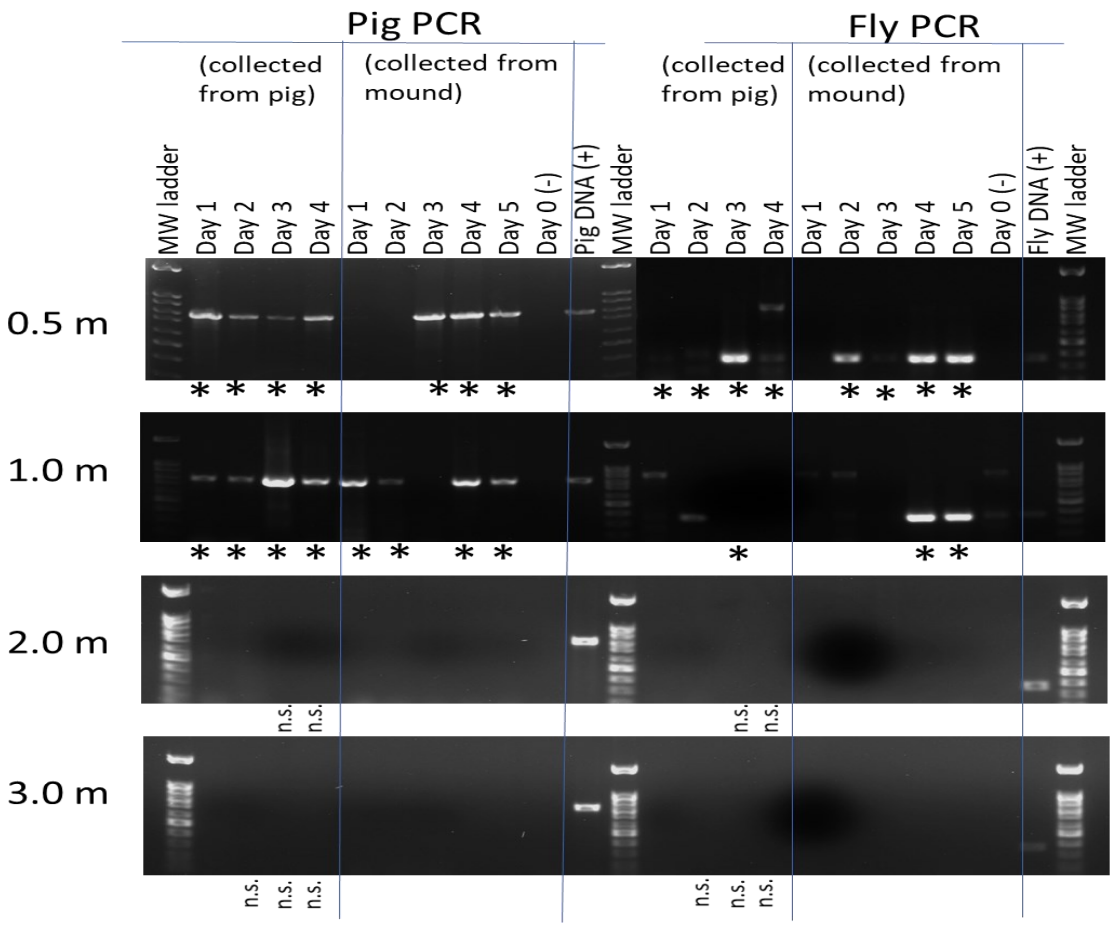

Figure 62 Gel electrophoresis separation of DNA fragments amplified from fire ant guts collected 17-21 July 2020 in Florida.

Fire ants were collected directly from four pig carcasses and from a corresponding mound located 0.5, 1.0, 2.0, and 3.0 m from the carcass in Florida. Pig and blow fly PCR reactions were conducted using different primers as described in the Methods section (Table 22). An asterisk (*) indicates positive identification of DNA. n.s. = not sampled due to absence of fire ants. MW ladder, molecular weight marker.

Conversely, neither pig nor fly DNA were detected in ant guts when carrion was located 2.0 or 3.0 m from the mound, either from ants collected on the carrion or on the mound (Figure 62). For carrion located 3.0 m from the mound, *Crematogaster cerasi* replaced *S. invicta* on Day 2 and was the only ant species found on that carcass through the rest of the experiment.

Crematogaster cerasi is an aggressive species that often makes inconspicuous nests in fallen logs

and stumps (Johnson 1988); the *C. cerasi* nest in a fallen log 1.5 m from the carcass was not noted when it was placed at this site 3.0 m from the *S. invicta* mound. Fly larvae completely decomposed this carcass, leaving scattered skeletal elements, between Day 2 and Day 3 (Figure 58).

Statistical analysis

The data set met assumptions associated with logistic regression analysis. Dependent and independent variable data types were binary and ordinal, respectively. Observations were collected independently. There was linearity of relationship between the distance from carrion and the log odds of detecting DNA in ant guts. On a day-by-day basis for each DNA target, there was not sufficient sample size for considerable statistical analysis, so 95% confidence intervals were not calculated. However, pooling of daily data yielded a sufficiently large sample size ($n > 30$), so 95% confidence intervals could be calculated.

There was not a statistically significant relationship between probability of detecting either pig DNA or fly DNA in fire ant guts as a function of distance from the mound on any of the first five days of succession ($p > 0.068$, Table 23). These results support the null hypotheses $H_{o27\text{-}31}$ and $H_{o33\text{-}37}$ which stated that *"there is no significant relationship between the probability of detecting [pig or fly] DNA in fire ant guts on [days one through five] and distance from carrion."*

Table 23 p-Values for logistic regression of the probability of detecting pig and fly DNA in fire ant guts on Days 1 through 5 of succession as a function of distance from pig carrion.

	Pig DNA	Fly DNA
Day 1	p = 0.143, -1.464, 30	p = 0.423, -0.802, 30
Day 2	p = 0.268, -1.109, 31	p = 0.299, -1.040, 31
Day 3	p = 0.111, -1.593, 28	p = 0.143, -1.466, 28
Day 4	p = 0.082, -1.741, 24	p = 0.170, -1.373, 24
Day 5	p = 0.089, -1.702, 22	p = 0.068, -1.824, 22
Days 1-5 combined	p = **0.0008, -3.365, 139**	**p = 0.0038, -2.892, 139**

Fire ants were collected on the carrion itself or on a corresponding mound 0.5 - 3.0 m away. Data presented as p-value, z-value, degrees of freedom. Statistically significant results in **bold** font.

Several of these p-values are fairly close to the α value of 0.05 and could reasonably be considered meaningfully significant. However, the α_{HB} value for the largest of these p-values that are close to 0.05 (p = 0.089 for detecting pig DNA in ant guts on Day 5 in Table 23) was α_{HB} = 0.006, and the p-values are considerably larger than their corresponding α_{HB} values.

The lack of statistical significance on individual days is likely due to the low recovery of pig and fly DNA from ant guts even when the ants were collected directly from the carrion itself (distance = 0 m). Probability of detecting pig DNA in fire ant guts when the ants were collected directly from the carrion ranged from 32.3 to 55.6% on days 1 through 5, individually (Figure 63). On each day, probability of detecting pig DNA decreased with increasing distance between the carrion and the mound.

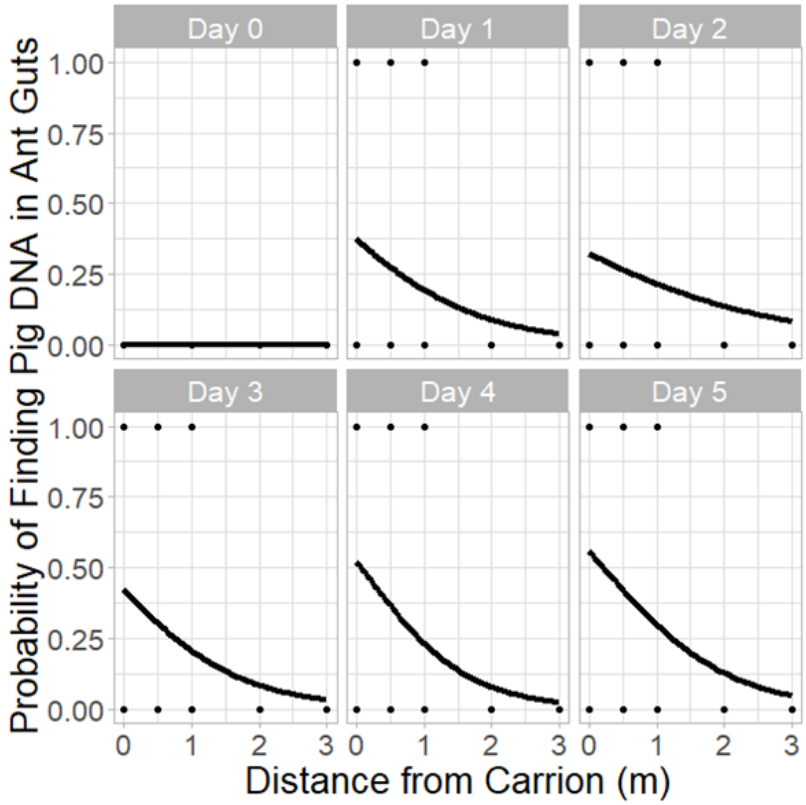

Figure 63 Probability of detecting pig DNA in ant guts from fire ants collected on Days 0 through 5 of carrion exposure, individually.

Ants were collected directly from carrion (0 m) and from corresponding mounds at distances 0.5 – 3 m from the carrion. Data from Mississippi and Florida combined.

Likewise, probability of finding fly DNA in fire ant guts when ants were collected directly from the carrion ranged from 13.9 to 58.8% on days 1 through 5, individually, with the level of probability increasing with each successive day (Figure 64). As with the pig DNA, probability of detecting fly DNA decreased with increasing distance between the carrion and the mound.

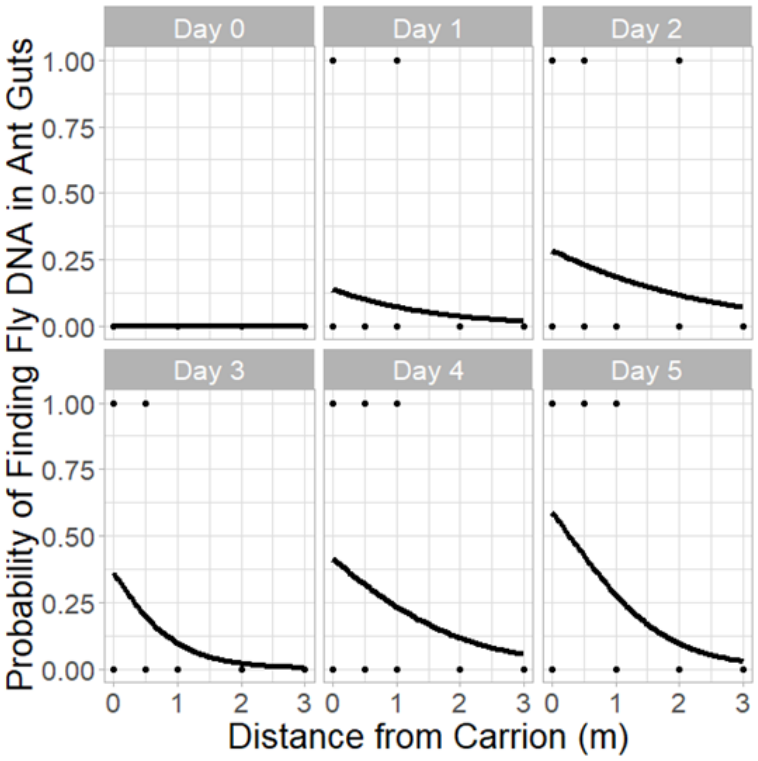

Figure 64 Probability of detecting fly DNA in ant guts from fire ants collected on Days 0 through 5 of carrion exposure, individually.

Ants were collected directly from carrion (0 m) and from corresponding mounds at distances 0.5 – 3.0 m from the carrion. Data from Mississippi and Florida combined.

Pooling of data from the first five days of succession shows a substantial decrease in the number of positive identifications of pig or fly DNA in the fire ant guts with distance. There were 37 positive identifications of pig and fly DNA from ants collected directly from the carcasses and no positive identifications from ants collected on mounds 3.0 m away from a carcass (Table 24). The number of positive identifications of pig DNA in ant guts at a distance of 0 m and the number of positive identifications of fly DNA in ant guts at the same distance were nearly identical.

Table 24 Number of positive identifications of pig and fly DNA in fire ant guts.

Distance from carrion (m)	Mississippi Set 1		Mississippi Set 2		Florida Set 1		Florida Set 2		Total
	Pig	Fly	Pig	Fly	Pig	Fly	Pig	Fly	
0.0	0	6	8	3	4	3	8	5	37
0.5	3	3	5	1	0	2	3	4	21
1.0	3	1	2	0	1	1	4	2	14
2.0	0	0	0	0	0	1	0	0	1
3.0	0	0	0	0	0	0	0	0	0

Ants were collected directly from pig carrion (0 m) and from corresponding mounds at distances 0.5 – 3.0 m from the pig carcasses.

Statistically, the probability of finding pig DNA or fly DNA in fire ant guts decreased significantly as distance between the carrion and the corresponding mound increased ($p = 0.001$ where the Holm-Bonferroni correction was $\alpha/k = 0.004$, and $p = 0.004$ where $\alpha/(k-1) = 0.005$, respectively; Table 24). These results reject both null hypothesis H_{o32}, which stated that *"there is no significant relationship between the probability of detecting pig DNA in fire ant guts on days one through five combined and distance from carrion"*, and null hypothesis H_{o38}, which stated that *"there is no significant relationship between the probability of detecting fly DNA in fire ant guts on days one through five combined and distance from carrion."*

Although the results for detecting pig DNA in ant guts are statistically significant, there is still, on average, only a 42.2% chance of detecting pig DNA and 33.0% chance of detecting fly DNA in fire ant guts when the ants are collected directly from the carrion itself (Figures 65 and 66). The probability of detection of either pig or fly DNA decreases to near 0% as distance between carrion and the mounds increases to 3 m.

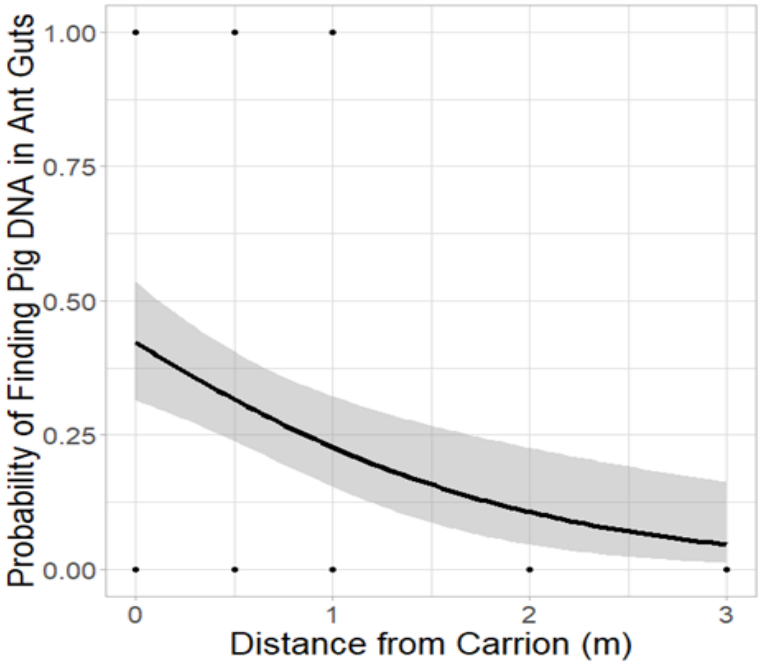

Figure 65 Probability of detecting pig DNA in ant guts from fire ants collected on Days 0 through 5 of carrion exposure, combined.

Ants were collected directly from carrion (0 m) and from corresponding mounds at distances 0.5 – 3 m from the carrion. Shaded area = 95% confidence interval.

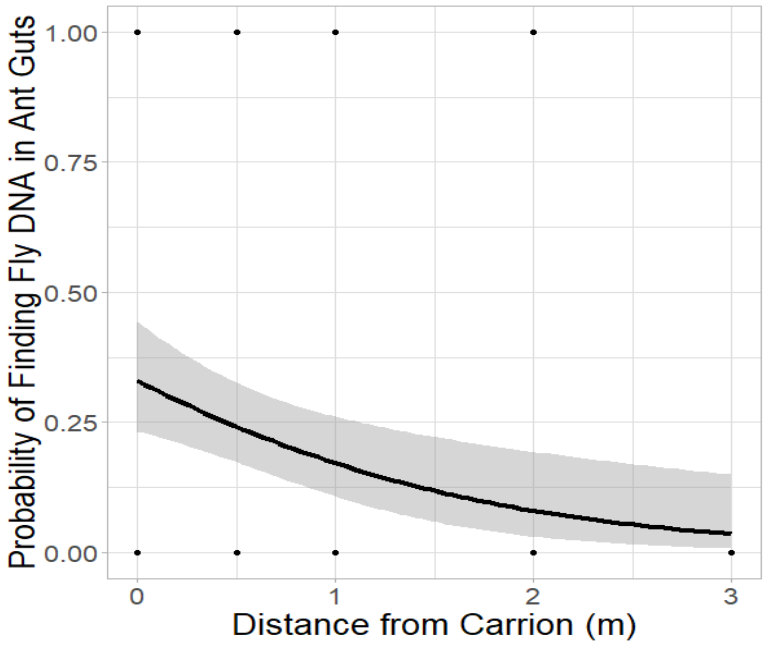

Figure 66 Probability of detecting fly DNA in ant guts from fire ants collected on Days 0 through 5 of carrion exposure, combined.

Ants were collected directly from carrion (0 m) and from corresponding mounds at distances 0.5 – 3 m from the carrion. Shaded area = 95% confidence interval.

In addition to the gradient of total number of positive identifications from 0 m to 3 m (Table 24), the number of positive identifications of pig and fly DNA in ants collected directly from the carcasses (i.e., at 0 m) also decreased as the distance between the carcass and the mound increased (Table 25). This may have been because of the proximity of the carrion to a larger proportion of the nest inhabitants or a more direct pipeline to food storage facilities in the nest (Tschinkel 2006, Gayahan and Tschinkel 2008). Nevertheless, physical appearance of lesions produced by fire ants did not appear to differ in size or shape with increasing distance from the mound.

Table 25 Number of positive identifications of pig and fly DNA in ant guts only from fire ants collected directly from pig carcasses.

Distance between the carcass and the mound (m)	Pig DNA	Fly DNA
0.5	10	10
1.0	8	6
2.0	0	0
3.0	2	0

It seems surprising that pig DNA was found in the guts of ants collected directly on pig carrion only about 42% of the time when it was clearly observed that the ants were chewing lesions into the pig carcasses. It is possible that larger pieces of solid material were carried away (Gayahan and Tschinkel 2008), ingestible solid material from the carcass was collected in the infrabuccal pocket, and that little liquid was immediately passed on to the gut (Petralia and Vinson 1978).

Similarly, fly DNA was found in the guts of a smaller proportion of ants than I expected based on the frequency with which ants were observed carrying fly eggs and larvae. It is possible that the eggs and larvae functioned as self-contained "packets" and were only ingested if the cuticle was punctured. Additionally, when the carcasses were ≤ 1.0 m from the mounds, fire ants tended to overwhelm the carcasses (Figure 54) to the extent that blow flies were nearly completely excluded. Ants were even observed disturbing flies so that they apparently were unable to oviposit, which would have reduced the opportunities for the ants feed on fly eggs or larvae.

There could be confounding effects that were not measured. For example, the age and size of the fire ant nests were not considered when choosing sites. This might be a confounding factor wherein older, larger nests may have had increased worker force to forage farther from the

nest and be able to access distant carrion; conversely, larger nests may have more workers that do not encounter resources from the carrion or it may be diluted as workers bring carrion back to the nest. Markin et al. (1973) showed that *S. invicta* nests can grow to >50,000 individuals in only three years, with underground tunnels extending up to 2 m from the mound. Nevertheless, given that these results are based off a sample of only 20 to 40 ants out of tens of thousands potentially inhabiting a nest, presence of pig and fly DNA in ant guts from fire ants collected from mounds is testament to the efficient distribution of nutrients in the ant nests through trophallaxis (Tschinkel 2006).

In forensic entomology, sampling for DNA in ant guts may perhaps allow reconstruction of a scene in which a cadaver had been near a fire ant mound and later removed; however, the result may be of more interest to trophic ecologists. Ecologists may find these results interesting in light of the flow of nutrients from carcasses into the surrounding landscape. Not only do blow fly larvae transfer nutrients into the landscape during postfeeding, prepuparial migration (Benbow et al. 2016, Barton et al. 2019, Goddard et al. 2020), but it appears that the fire ants are incredibly efficiently at transferring nutrients from both carrion and necrophilic flies at least 1 m into the landscape during the first days of carrion succession and can transfer those nutrients up to 3 m into the landscape.

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CHAPTER V

CONCLUSIONS

Chapter I

Carrion ecology has a rich history in the southeastern United States, boasting some of the earliest pioneering investigations into carrion decomposition and succession ecology that were conducted in the New World. A couple university-level programs have since been erected in the southeastern United States to study forensic entomology, an applied science within carrion ecology that relies heavily on insect succession patterns on carrion.

Ants, including the introduced fire ants *Solenopsis invicta*, *S. richteri*, and their hybrid, have become an expected member of the carrion succession fauna, with most current carrion ecology studies reporting their presence and mentioning an assumed ecological importance. Between 1984 and 2018, eleven published papers specifically addressed fire ants occurring on carrion. Most of those papers hypothesized the roles which fire ants play in decomposition and succession, such as feeding on carrion and its attendant fauna, citing the characteristic lesions and burial of portions of the carrion. Two papers described experimental work on the role of fire ants in carrion decomposition and succession; however, one was concerned only with the adult blow fly community and the other used non-standard model carrion. Both papers reported delays in decomposition and insect succession, attributed to fire ant presence.

Chapter II

Blow flies are the most prominent members of the carrion succession fauna in the southeastern United States. The first checklist of blow flies in Mississippi was compiled in 1983 with a total of 15 species known from the state. Two additional species have since been reported. Work conducted in this study, using specimens from the Mississippi Entomological Museum and some new collections, expanded that number to 23 species now known or suspected from the state. One species, the primary New World screwworm, *Cochliomyia hominivorax*, has been extirpated from the state, and another is known from an adjacent county in Alabama. The newly registered species include the forensically important Oriental latrine fly, *Chrysomya megacephala*, and four members of the subfamily Melanomyinae, which is suspected to be parasitic on snails in the larval stage and which do not frequent carrion as adults.

Further studies that could be conducted on the blow flies of Mississippi could involve collection in areas of the state that are poorly represented to better document distributions within the state, collection near the borders for adventive species, and rearing of immature stages for better characterization of those stages.

Chapter III

A controlled experimental study was conducted in central Mississippi and the Florida Panhandle in which fire ants and blow flies were selectively excluded from pig carrion to determine their relative roles in carrion decomposition and the effect their exclusion has on the rest of the carrion community. This was the first study to investigate the role of fire ants in carrion decomposition both by using a standard model organism and by looking at the entire succession community.

Exclusion of fire ants did not statistically affect carrion decomposition rates that I measured, although effects on carrion were noted, such as buildup of thatch along areas in which the carcasses contacted the soil and lesions chewed into the carcasses. Exclusion of fire ants slightly affected the overall carrion community in terms of number of organisms collected, daily taxa richness, and the Shannon diversity, as well as similarity between exclusion treatments and the control in which all fauna had access. Conversely, exclusion of blow flies resulted in significant effects to the decomposition rates, as well as more pronounced effects on the community composition and similarity to control than was seen with the exclusion of fire ants.

Exclusion of fire ants clearly demonstrated the effect they have on colonization of blow flies on carrion. Presence of fire ants was confirmed to delay successful colonization by blow flies by 1 day in Florida and 2 days in Mississippi, although blow flies did appear to overwhelm the fire ants by about Day 3 of succession and proceed with normal succession. This delay would need to be considered in a forensic entomology context should fire ants be found to have infested a corpse. This delay was also noted in the DNA study described in Chapter IV, showing that carcasses located close to fire ant mounds experienced shorter delays in blow fly colonization as distance between the carcass and the mound increased.

Improvements to this study could have included seasonal aspects, standardization of the distance between the carrion and the fire ant mound, and use of methods that are even more specific in their restriction of access (e.g., excluding only blow flies and not other large aerial succession fauna). This could perhaps be accomplished using baits specific to Calliphoridae to distract blow flies from succession on the carrion. Further research could include better characterization of the younger blow fly instars to be able to tease apart possible species-specific influences of fire ant presence.

Chapter IV

Assuming that fire ants were feeding on both pig carrion and fly larvae, an experiment was conducted to determine how far they forage from their nests to do so. Ants were collected from the carrion itself and from corresponding mounds located 0.5, 1, 2, and 3 m distant. Guts were dissected from the ants, and DNA extracted from the guts was amplified using PCR to detect pig and fly DNA.

Pig and blow fly DNA were detected in fire ant guts, being the first time that DNA from carrion and its attendant fauna has been reported in fire ant guts. On the first five days of succession individually, there was a decreasing effect of distance between carrion and the mound on the probability of detecting pig or fly DNA in ant guts. However, these relationships were not statistically significant, and probability of detecting pig or fly DNA in ant guts was less than 60%, even for ants collected directly from carcasses. Pooling of all data did result in a significant negative relationship between distance between carrion and the mound and probability of detecting both pig and fly DNA. Again, however, there was only a 42% probability of finding pig DNA and a 33% probability of finding fly DNA from ants collected directly on the pig carcasses, both decreasing to near 0% at a distance of 3 m between carcass and mound.

Refinements to this study could include more carefully choosing ants that had been present a longer time at the lesions and avoiding those that had more recently arrived, increasing specificity of the primers to separate blow fly species from each other, and increasing both time and distance factors to more fully explore limits of distribution. Further research could include collection of larvae from the ant mounds to determine if pig and fly DNA can be detected in their guts, which would clearly illustrate the trophallactic transfer of these nutrients into the mound. Additionally, it could be interesting to attempt amplification of bacterial genetic material on and

in the ants to determine if pathogens might also be transferred by the ants in similar fashion to the pig and fly DNA.

Overall, this study provides documentation that fire ants scavenge on carrion and prey on other members of the succession fauna, delay decomposition of carrion (especially when carrion is located close to their mounds), and transfer nutrients from carrion resources at least 3 m into the surrounding landscape.

APPENDIX A

LIST OF MISSISSIPPI BLOW FLY SPECIMENS EXAMINED FOR THE REVISED CHECKLIST OF CALLIPHORIDAE KNOWN FROM OR EXPECTED TO OCCUR IN MISSISSIPPI, WITH COUNTY-LEVEL DISTRIBUTION MAPS

This list contains the label data and distribution maps for the specimens that were examined in the course of the preparation of this dissertation. Most of the specimens are housed in the Mississippi Entomological Museum (MEM) at Mississippi State University. This collection also contains the recently acquired collection from the University of Mississippi. Other specimens were examined from student collections, the reference collection for the medical/veterinary entomology class, and the author's personal collection. Specimens are listed in order by county alphabetically, then by date. A county-level distribution map is provided for each species (Figures 67 – 74), and USGS Level IV Ecoregions are based on the map in Figure 75. Some labels represent multiple specimens, ranging from 1 to 55 specimens. As of 1 August 2020, a total of 1,018 adult blow fly specimens from Mississippi was examined.

Subfamily Calliphorinae

Tribe Calliphorini

Cynomya Robineau-Desvoidy 1830

***Cynomya cadaverina* (Robineau-Desvoidy, 1830)**

42 Specimens examined (figure 67a):

Mississippi: Lafayette Co., Oxford, 21 October 1980, Jerome Goddard, trap baited with liver; Oxford, 22 October 1980, Jerome Goddard, trap baited with liver; 9 mi. NE Oxford, 22 October 1980, Jerome Goddard, trap baited with liver; Oxford, 4 November 1980, Jerome Goddard, trap baited with liver; Oxford, 7 November 1980, Jerome Goddard, trap baited with liver; 9 mi. NE

Oxford, 7 November 1980, Jerome Goddard, trap baited with liver; 9 mi. NE Oxford, 6 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 20 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 21 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 22 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 23 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 24 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 25 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 26 March 1981, Jerome Goddard, trap baited with fish; 9 mi. NE Oxford, 27 March 1981, Jerome Goddard, trap baited with fish; T7S R2W Sec 34, 28 March 1981, Jerome Goddard, trap baited with mammal carrion; T7S R2W Sec 34, 30 March 1981, Jerome Goddard, trap baited with mammal carrion; 1 mi. E Oxford, 31 March 1981, Jerome Goddard, trap baited with mammal carrion; 1 mi. E Oxford, 2 April 1981, Jerome Goddard, trap baited with mammal carrion; T7S R2W Sec 34, 2 April 1981, Jerome Goddard, trap baited with reptile carrion; 1 mi. E Oxford, 4 April 1981, Jerome Goddard, trap baited with mammal carrion; 1 mi. E Oxford, 5 April 1981, Jerome Goddard, trap baited with mammal carrion; 1 mi. E Oxford, 6 April 1981, Jerome Goddard, trap baited with mammal carrion. **Oktibbeha Co.**, “A&M Coll”, 20 October 1914, E. G. Morgan; “Ag Coll Miss”, “N ‘14”, H. E. Weed; “Ag Coll Miss”, 11 April 1916, J. W. Newton; “Ag Coll Miss”, 28 March 1917, J. T. Sheton; “Ag Coll Miss”, 4 November 1917, T. A. Arnold; “Ag Coll Miss”, 5 March 1921, H. W. Allen; “Ag Coll”, 3 January 1923, [no leg.]; “St Coll Miss”, 3 April 1940, B. H. Buchanan; Starkville, 2 May 1975, W. H. Cross, swept from *Ligustrum vulgare* flowers; Craig Springs, 18 December 1979, G. C. Snodgrass, pitfall trap peripheral to cultured cotton; Craig Springs, 10 November 1981, W. H. Cross, interception trap-yellow; Craig Springs, 12 November 1981, white pan trap on ground; Craig Springs, 30 November 1981, W. H. Cross, interception trap-yellow; Starkville, 6 March

2018, Jacob Williams; Starkville, 24 March 2018, Sierra Bullock; Clay Lyle Building MSU, Starkville, 22 March 2018, E.B. Vernon; Starkville, 28 March 2018, Brady Thompson, ex. turkey; Starkville, 27 February 2018, Sierra Bullock. **Tishomongo Co.**, Eastport, 10 March 1980, Jerome Goddard, trap baited with fish. **Winston Co.**, 28 April 1971, H. R. Fulton.

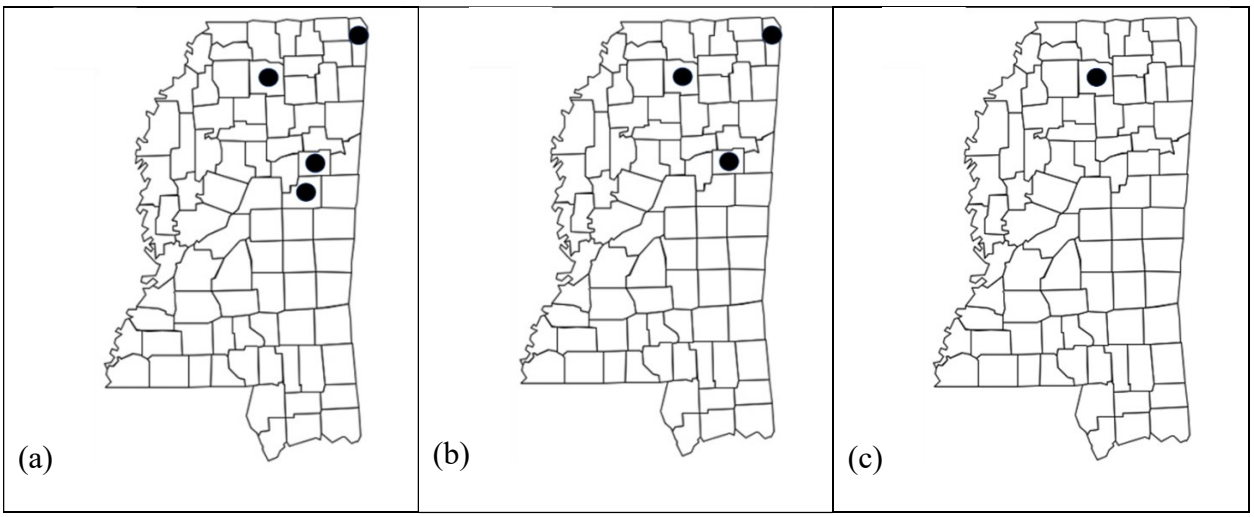

Figure 67 Distribution of blow fly species in Mississippi 1

(a) *Cynomya cadaverina;* Ecoregions 65a, 65b, 65d, 65e, and 65j
(b) *Calliphora livida;* Ecoregions 65a, 65b, 65e, and 65i
(c) *Calliphora terraenovae*; Ecoregion 65e

Calliphora Robineau-Desvoidy 1830

Calliphora livida Hall, 1948

32 Specimens examined (Figure 67b):

Mississippi: Lafayette Co., Oxford, 4 October 1980, Jerome Goddard, bird; Oxford, 21 October 1980, Jerome Goddard, trap baited with liver; Oxford, 4 November 1980, Jerome Goddard, trap baited with liver; 7 miles NE Oxford, 10 March 1981, Jerome Goddard, trap baited with fish; 7

miles NE Oxford, 12 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 13 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 14 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 16 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 21 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 22 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 24 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 25 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 28 March 1981, Jerome Goddard, trap baited with fish; T7S R2W Sec 34, 28 March 1981, Jerome Goddard, trap baited with fish; T7S R2W Sec 34, 29 March 1981, Jerome Goddard, trap baited with fish; 7 miles NE Oxford, 29 March 1981, Jerome Goddard, trap baited with fish. **Oktibbeha Co.**, "Ag Coll Miss", 2 March 1921, H. W. Allen; "Ag Coll Miss", 13 March 1921, H. W. Allen; Mississippi State University, 20 April 1970, Charles Bryson; Adaton, 15 March 1982, W. H. Cross; Co., 6 miles SW Starkville, 10-21 February 1985, R. L. & B. B. Brown; Starkville, T19N R14E Sec 34NE, 17 March 1992, Terry Schiefer. **Tishomingo Co.**, Natchez Trace mi. 298.9, N34°33'47" W88°16'02", 24-30 March 2003, Joe MacGown & T. Schiefer.

***C. terraenovae* Macquart, 1851**

0 Specimens examined (record based on Goddard and Lago 1983; Figure 67c).

Jerome Goddard, personal communication, graciously provided the following records:

Mississippi: Lafayette Co.: Oxford, 9 May 1980, Jerome Goddard, liver; 1.5 mi East of Oxford, 29 April 1980, Jerome Goddard, fish; 9 mi NE of Oxford, 20 March 1981, Jerome Goddard, mammal. Those identifications were confirmed by R. J. Gagné, U.S.D.A. Biosystematics Unit, Washington, DC.

***C. vicina* (Robineau-Desvoidy, 1830)**

85 Specimens examined (Figure 68a):

Mississippi: Attala Co., Kosciusco, 6 April 1936, D. W. Grimes; **Hinds Co.**, Clinton, 26 December 1971, Bryant Mather; Jackson, 14 April 1979, Bryant Mather; **Jackson Co.**, 1 mi. E Pascagoula on Hwy 90, 21 April 1990 A. Houston; **Lowndes Co.**, T17N R16E, Sec 34, Black Belt Prairie, 27 January 1993, D. M. Pollock; **Oktibbeha Co.**, "St Coll Miss", 16 May 1914, E. K. Dickey; "St Coll Miss", 16 May 1914, C.T. [illegible]; "Ag Coll Miss", 18 May 1914, R. C. Baylis; "Ag Coll Miss", 4 April 1915, J. G. Hensen; "Ag Coll Miss", ? April 1915, W. E. Warsham; "Ag Coll Miss", 2 May 1915, L. E. Mitchell; "Ag Coll Miss", 4 May 1915, W. T. Edwards; "Ag Coll Miss", 25 October 1915, J. E. Bonner; "Ag Coll Miss", [n.d.] 1915, E. Abbott; Starkville, 22 March 1919, W. Middlebrook; Starkville, 14 July 1919, W. Middlebrook; "Ag Coll Miss", 31 October 1924, Roy Melvis; Starkville, 22 March 1926, W. H. Cross, blacklight trap near edge of deciduous woods; "A&M C Miss", 9 April 1929, J. C. Young; "St Coll Miss", 7 March 1939, R. E. Taylor; "St Coll Miss", 22 June 1939, G.-C. Tatum; "St Coll Miss", 6 April 1941, C. B. Moswain; "St Coll Miss", 24 April 1941, R. Chance; "State College Miss", 19 April 1941, W. C. Walker; "Miss. St. Univ.", 22 March 1968, Charles Bryson; "Miss. St. Univ.", 29 March 1971, Charles Bryson; "Miss. St. Univ.", 1 May 1971, Charles Bryson; "Miss. St. Univ.", 16 December 1971, Charles Bryson; "St Coll Miss", 17 April 1973, G. Purser; Starkville, 4 March 1977, W. H. Cross; Starkville, 29 January 1981, W. H. Cross, in house; Starkville, 10 May 1981, W. H. Cross, white pan trap on ground; 3.2 mi. N Starkville, 31 March 1991, J. McCoy, in house; 3.2 mi. N Starkville, 31 March 1991, J. McCoy, on flowering *Photinia* x *fraseri*; Starkville, 8 April 1991, J. MacGown, on flowering *Photinia* x *fraseri*;

Starkville, 9 April 1991, T. L. Schiefer, on flowering *Photinia* x *fraseri*; Starkville, 9 April 1991, T. L. Schiefer & J. MacGown; Starkville, 17 March 1992 J. A. MacGown, on flowers of *Prunus carolinia*; Starkville, T19N R14E Sec 34SE, 17 March 1992, T. L. Schiefer, on flowers of *Prunus carolinia*; Starkville, 8 April 1994, T. L. Schiefer; Starkville, 11 April 1994, D. M. Pollock; Starkville, T19N R14E Sec 34SE, 10 April 1995, Terry L. Schiefer, on flowering *Photinia* x *fraseri*; Starkville, 22 March 2018, Sierra Bullock; Clay Lyle Building MSU, Starkville, 22 March 2018, E.B. Vernon; Starkville, 25 March 2018, Brady Thompson, ex. turkey; Starkville, 10 April 2018, Brady Thompson, ex. armadillo; Starkville, 10 April 2018, Nicholas Moses; Starkville, 23 April 2018, Jacob Williams; "Ag Coll Miss", W. E. Warsham (date illegible); "Ag Coll Miss", n.d., H. E. Weed; "Ag Coll Miss", 1 April [no year], C. B. Anders; **Pontotoc Co.**, 1 mi. SE Ecru, 21 May 1980, W. H. Cross, cotton; 1 mi. SE Ecru, 8 December 1980, W. H. Cross, *Arundinaria gigantea* litter peripheral to cotton field, 4784.2.

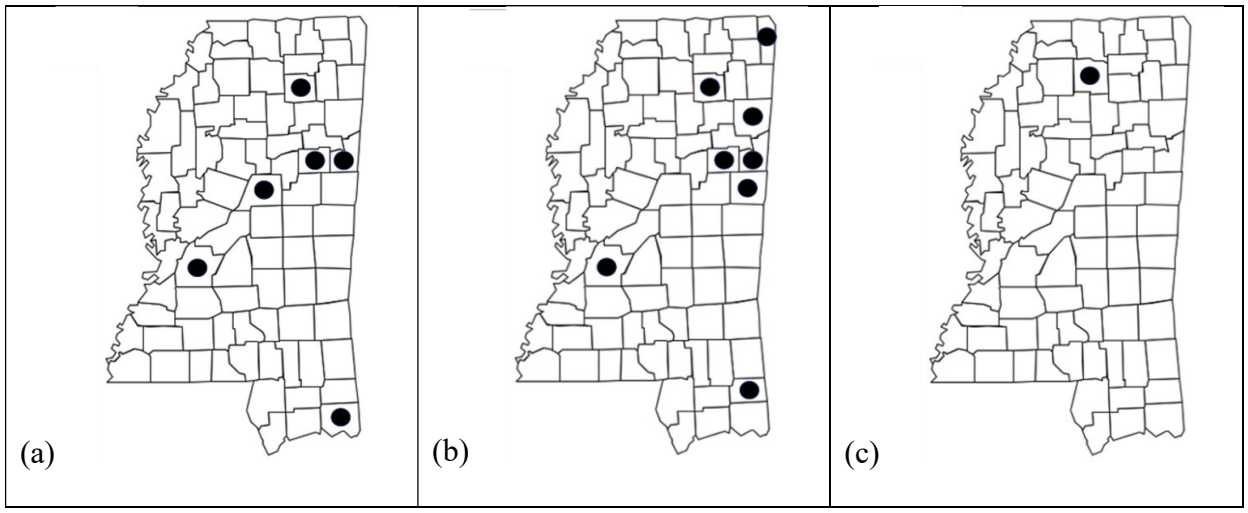

Figure 68 Distribution of blow fly species in Mississippi 2

(a) *Calliphora vicina*; Ecoregions 65a, 65b, 65d, 74b
(b) *Calliphora vomitoria*; Ecoregions 65a, 65b, 65j, 65p, 74b
(c) *Lucilia cluvia*; Ecoregion 65a

***C. vomitoria* Linnaeus, 1758**

22 Specimens examined (Figure 68b):

Mississippi: George Co., Nixon Lakes, 30°50;28”N 88°45’11”W, 8-10 April 1994, C. D. Prickett; **Hinds Co.**, Clinton, 26 December 1971, Bryant Mather; **Lowndes Co.**, T17N R16E, Sect 34, Black Belt Prairie, 10-12 February 1992, J. MacGown; T17N R16E, Sect 34, Black Belt Prairie, 16 March 1992, J. MacGown; T17N R16E, Sect 34, Black Belt Prairie, 27 January 1993, D. M. Pollock; **Monroe Co.**, 2 mi. NW Amory, T12S R8E Sec19W, 31 October 1990, T. L. Schiefer, *Aster* sp.; **Noxubee Co.**, 12 mi ESE Macon, T14N R19E, Sec10SW, 29 February 1992, T. L. Schiefer, on flowering *Prunus caroliniana*; Noxubee NWR, 33°16’44”N 88°46’39”W, 21 March 1997, T. L. Schiefer, on flowering *Prunus serotina*; **Oktibbeha Co.**, Noxubee NWR, 19 February 1985, R. L. Brown; Noxubee NWR, 5 March 1985, R. L. Brown; 6 mi SW Starkville, 10-21 February 1987, R. L. & B. B. Brown, malaise trap in mixed pine-hardwood forest; 6 mi SW Starkville, 4-9 March 1987, R. L. & B. B. Brown, malaise trap in mixed pine-hardwood forest; 6 mi SW Starkville, 23 April 1987, R. L. & B. B. Brown, malaise trap in mixed pine-hardwood forest; 1 mi SW Osborne, T19N R15E Sec 16, 22 February 1990, J. MacGown; Starkville, T19N R14E Sec 34NE, 17 March 1992, T. L. Schiefer, on flowers of *Prunus caroliniana*; Sessums, 33°23’31”N 88°42’40”W, 1 November 1998, J. A. MacGown; Sessums, 33°23’31”N 88°42’40”W, 18 January 1999, J. A. MacGown; Sessums, 33°25’31”N 88°42’49”W 1 November 1999, D. M. Pollock; Starkville, 26 March 2016, G. Batson; Clay Lyle Building MSU, Starkville, 2 April 2018, E.B. Vernon; **Pontotoc Co.**, 1 mi SE Ecru, 23 April 1980, G. L. Snodgrass, malaise trap in cultured cotton; **Tishomongo Co.**, Tishomongo S.P., 12 April 1986, D. Stout.

Tribe Luciliini

Lucilia Robineau-Desvoidy 1830

***Lucilia cluvia* (Walker, 1849)**

0 Specimens examined (record based on Goddard and Lago 1983; Figure 68c):

Mississippi: Lafayette Co., Oxford, 3 June 1977.

***L. coeruliviridis* (Macquart, 1855)**

330 Specimens examined (Figure 69a):

Mississippi: Bolivar Co., Rosedale, 20 June 1980, J. Goddard, "trap with mammal carrion"; **Chickasaw Co.**, Buena Vista, [no date], H. L. King; **Choctaw Co.**, 6 mi S Weir, 8 May 1980, P. K. Lago, "trap with reptile carrion"; Jeff Busby Park, 4 April 1992, R. L. Brown, "on *Crataegus* sp. flowers"; Ackerman, 9 April 1995, D. M. Pollock; **Claiborne Co.**, 3.6 mi W Port Gibson, N31°50'28" W91°02'37", 12 July 1993, T. L. Schieffer, "blacklight trap"; **DeSoto Co.**, Olive Branch, 4 April 2018, Sierra Bullock; **George Co.**, Mixon Lakes, N30°50'28" W88°45'11", 8-9 April 1994, R. L. Brown & D. Pollock, "on rotting crayfish"; Pascagoula WMA, N30°52'14" W89°02'38", 1 April 1995, D. M. Pollock, "sweeping"; Barton, N30°46'09" W88°31'55", 24 March 2006, J. G. Hill, "collected on cultivated blackberry flowers"; **Harrison Co.**, Tuxachanie Trail, T4S R11W Sec 29&30, 10 March 1991, T. L. Schieffer; D'Iberville, 2 April 1994, R. E. Seymour; **Jackson Co.**, T4S R8W Sec12, 11 April 1981, J. Goddard, "mammal carrion"; Gulf Coast Res. Sta., 10 May 1981, R. L. Brown; Gulf Islands National Seashore, 3-6 June 1984, R. Brown, G. Baker, and ent students; Gulf Islands National Seashore, 19-20 April 1985, R. L.

Brown; 6 mi N Ocean Springs, 20 April 1985, R. L. Brown; **Jefferson Davis Co.**, Morris, ? August 1916, B. A. Williamson; **Lafayette Co.**, Oxford, 20 October 1979, J. Goddard; Oxford, 24 April 1980, J. Goddard, "trap with mammal carrion"; Oxford, 6 May 1980, J. Goddard, "trap with mammal carrion"; Oxford, 3 June 1980, J. Goddard, "trap baited with fish"; Oxford, 1 July 1980, J. Goddard, "trap with mammal carrion"; Oxford, 12 July 1980, J. Goddard, "trap baited with fish"; Oxford, 21 October 1980, J. Goddard, "trap baited with liver"; **Lowndes Co.**, T17N R16E Sec34, Black Belt Prairie, 10 June 1993, D. M. Pollock; **Madison Co.**, Flora, 11 July 1987, Michael Ledlow; **Noxubee Co.**, Noxubee NWR, 33°16'44"N 88°46'39"W, 20 March 1997, T. L. Schiefer; **Oktibbeha Co.**, "St Coll Miss", 12 April 1911, M. Peeples; "A&M Coll", 20 April 1914, E. R. Raney; "Ag Coll Miss", 17 July 1914, C. C. Greet, "pecan"; "Ag Coll Miss", 30 April 1915, P. D. Davis; "Ag Coll Miss", 6 June 1915, R. H. Batty; "Ag Coll Miss", 17 April 1916, J. V. Pace; "Ag Coll Miss", 13 November 1915, R. C. Miner; "A&M Coll", 1 May 1916, F. W. Gardner; "Ag Coll Miss", 13 May 1916, J. S. Gray; "Ag Coll Miss", ? 1916, S. C. H. William; "Ag Coll Miss", 1 April 1917, J. I. Hurst; "Ag Coll Miss", 1 April 1917, J. F. Scoggin; Starkville, 30 April 1917, C. L. Chiles, "on dead cat"; "Ag Coll Miss", 30 September 1917, J. T. Shelton; "Ag Coll Miss", 22 April 1918, S. H. Livingston; "Ag Coll Miss", 23 April 1919, R. V. Miller; "Ag Coll Miss", ? May 1919, B. Donaldson; "St Coll Miss", 24 April 1924, E. Harrison; "A&M Coll Miss", 25 September 1924, Roy Melvia; "St Coll Miss", 28 April 1940, J. W. Murphy; "A&M Coll Miss", 1 September 1946, [no leg.]; Starkville, S end of Montgomery Extd., 17 July 1962, W. H. Cross, "Cultivated cotton"; Miss. State Univ., 24 April 1969, Charles Bryson; Miss. State Univ., 1 April 1970, Charles Bryson; Starkville, 26 April 1971, [no leg.]; Miss. State Univ., 3 May 1971, Charles Bryson; Miss. State Univ., 7 May 1971, Charles Bryson; Starkville, 9 April 1975, W. H. Cross, "blacklight trap near edge of deciduous woods";

Starkville, 21 August 1975, W. H. Cross, “blacklight trap near edge of deciduous woods”; Starkville, 31 August 1975, W. H. Cross, “blacklight trap near edge of deciduous woods”; Starkville, 5 October 1975, W. H. Cross, “blacklight trap near edge of deciduous woods”; Starkville, 6 October 1975, W. H. Cross, “blacklight trap near edge of deciduous woods”; Starkville, 17 October 1975, W. H. Cross; Starkville, 2 November 1975, W. H. Cross, “blacklight trap near edge of deciduous woods”; 13 April 1976, R. L. Combo, “sweeping crimson clover”; Dorman Lake, 28 April 1981, R. L. Brown, “malaise trap in hardwood forest”; Craig Springs, 30 October 1981, W. H. Cross, “interception trap – black”; Craig Springs, 1 November 1981, W. H. Cross, “interception trap – black”; Craig Springs, 9 November 1981, W. H. Cross, “interception trap – black”; Craig Springs, 9 November 1981, W. H. Cross; 6 mi SW Starkville, 11-17 July 1984, R. L. & B. B. Brown, “malaise trap in mixed pine-hardwood forest”; 6 mi SW Starkville, 11 August 1984, R. L., & B. B. Brown, “sweeping flowering *Cassia fasciculata*”; 6 mi SW Starkville, 27-29 April 1985, R. L. & B. B. Brown, “Malaise trap in mixed pine-hardwood forest” [this specimen with identification label of *L. eximia*, per T. Whitworth, but genae are black instead of golden-brown and the forewing costal vein basal to the costal break is not as pale as other *L. eximia* identified from Costa Rica and Puerto Rico]; Miss. State North Farm, 30 August – 1 September 1989, Jerry Baker, “yellow pan trap”; Starkville, 8 April 1991, J. MacGown, “on flowering *Photinia* x *fraseri*”; Starkville, T19N R14E Sec 34SE, 9 April 1991, T. L. Schieffer, “on flowering *Photinia* x *fraseri*”; Starkville, T19N R14E Sec 34SE, 11 April 1991, T. L. Schieffer, “on flowering *Photinia* x *fraseri*”; Starkville, T19N R14E Sec 34SE, 15 April 1991, T. L. Schieffer, “on flowering *Photinia* x *fraseri*”; Starkville, T19N R14E Sec 34NE, 17 March 1992, T. L. Schieffer, “on flowers of *Prunus caroliniana*”; Starkville, T19N R14E Sec34SE, 10 April 1995, T. L. Schiefer; Sessums, N33°23’31” W88°42’40”, 7 May

1996, J. A. MacGown, “on screen of screen porch”; Sessums, 33°25’31”N 88°42’49”W, 1 November 1999, D. M. Pollock; Sessums, N33°25’31” W88°42’49”, 1 November 1999, D. M. Pollack, “on dead mammal”; Starkville, N33°25’43” W88°52’16”, 30 May 2000, D. M. Pollack; Noxubee NWR, N33°18’01” W88°46’31”, 4 September 2009, J. G. Hill, “*Procyon lotor* carcass”; 3 mi E Starkville, N33°20’47” W88°44’46”, 6 November 2009, J. G. Hill & J. L. Seltzer, “on white aster flowers”; Starkville, 24 March 2018, K. Hamid, pig carrion; Miss. St. U., *ex*. dead cat, 9 August 2011, G. A. Awuni; Starkville, 28 March 2018, Brady Thompson, ex. turkey; Starkville, 3 April 2018, Jacob Williams, ex. salmon; Clay Lyle Building MSU, Starkville, 17 April 2018, E.B. Vernon; 10 mi. W Starkville, N33°29’44” W88°57’32”, 18 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 5 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 27 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 2 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 5 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 1 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 7 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 3 d; **Panola Co.**, 3 mi. SWS Sardis, 12 September 1979, W. H. Cross; 3 mi W Sardis, 12 September 1979, W. H. Cross, “Malaise trap in cultivated cotton”; **Pike Co.**, Summit, ? July 1915, K. L. Spurlock; **Pontotoc Co.**, 1 mi SE Ecru, 4 June 1980, G. L. Snodgrass, “malaise trap in cultivated cotton”; 1 mi SE Ecru, 6 June 1980, G. C. Snodgrass; 1 mi SE Ecru, 18 June 1980, W. H. Cross, malaise trap in cultivated cotton; 1 mi SE Ecru, 18 June 1980, G. L. Snodgrass, “malaise trap in cultivated cotton”; 1 mi SE Ecru, 20 June 1980, G. L. Snodgrass, “malaise trap in cultivated cotton”; 1 mi SE Ecru, 7 July 1980, G. L. Snodgrass, “malaise trap in cultivated cotton”; 1 mi SE Ecru, 9 June 1981, John MacDonald, “canopy trap”; **Quitman Co.**, 2 mi S Sledge, 10 June 1980, J. Goddard, “trap baited with fish”; **Scott Co.**, Forkville, July-August 1916, Jas. A. Price; 3 mi N Forest, 9 April 1981, J. Goddard, “bird carrion”; 1 mi. N. Raleigh,

14-15 June 1985, R. Brown & G. Burrows; **Warren Co.**, Boyina, 15 October 1976, Bryant Mather; Vicksburg, 24 May 1991, Bryant Mather; **Washington Co.**, Stoneville, Delat Experimental Forest, 18 October 1988 J. R. MacDonald; Co., Leroy Percy St. Park, N33°10'17" W90°56'10", 28 April 1993, R. L. Brown & D. Pollock, "blacklight trap"; **Winston Co.**, 13 mi S Starkville, 14-25 June 1982, N. Bedwell, "malaise trap in pine forest"; 4 mi N Hwys 15 x 25, 14 April 1995, D. M. Pollock 4 mi N Hwys 15x25, 14 April 1995, D. M. Pollock, "on dead opossum"; Tombigbee Nat. Forest, N33°10'31" W89°02'38", 3 May 1999, D. M. Pollock, "blacklight trap in mixed mesic forest".

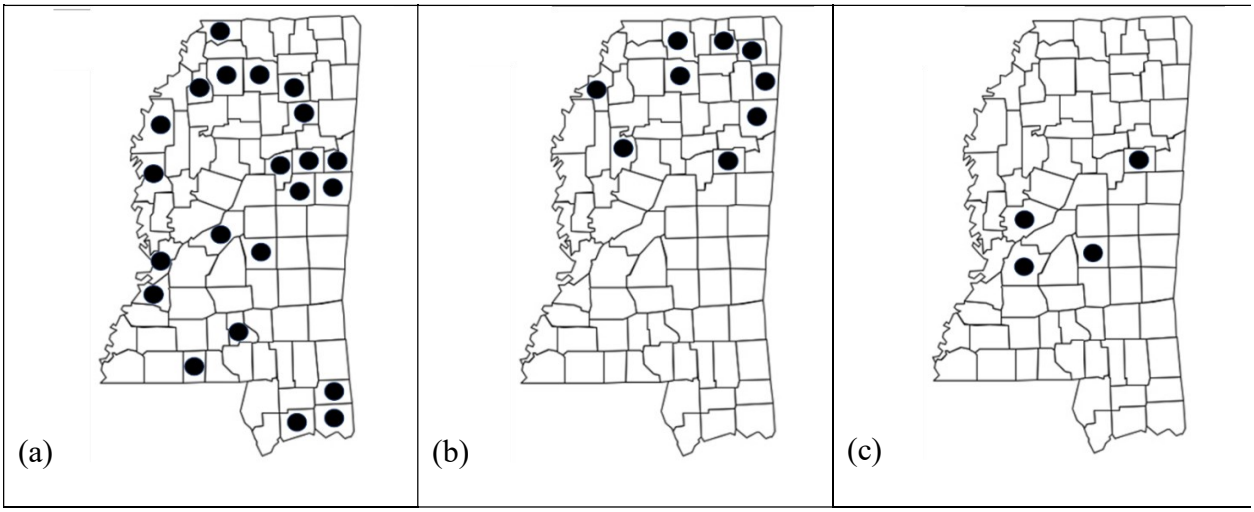

Figure 69 Distribution of blow fly species in Mississippi 3

(a) *Lucilia coeruliviridis*; Ecoregions 65a, 65b, 65d, 65e, 65f, 65p, 65r, 73a, 73d, 74a, 74b, 74c, 75i, 75k
(b) *Lucilia cuprina*; Ecoregions 65a, 65b, 65e, 65p, 73a, 74b
(c) *Lucilia sericata*; Ecoregions 65a, 65d, 74b

***L. cuprina* Wiedemann, 1826**

34 Specimens examined (Figure 69b):

Mississippi: Coahoma Co., 1 mi S. Clarksdale, 25 April 1981, Jerome Goddard; **Itawamba Co.**, 15 mi S. Fulton, 11 October 1980, Jerome Goddard; **Lafayette Co.**, Oxford, 1 October 1971, J. Baker; Oxford, 5 July 1979, Jerome Goddard; Oxford, 9 September 1979, Jerome Goddard; Oxford, 13 September 1979, Jerome Goddard; Oxford, 21 September 1979, Jerome Goddard; Oxford, 27 September 1979, Jerome Goddard; Oxford, 12 October 1979, Jerome Goddard; Oxford, 20 October 1979, Jerome Goddard; Oxford, 3 June 1980, Jerome Goddard; Oxford, 1 July 1980, Jerome Goddard; Oxford, 11 July 1980, Jerome Goddard; Oxford, 21 August 1980, Jerome Goddard; Oxford, 6 September 1980, Jerome Goddard; Oxford, 2 October 1980, Jerome Goddard; **LeFlore Co.**, 2 mi W Mintar City, 25 April 1981, Jerome Goddard; 3 mi W Rubeville, 25 April 1981, Jerome Goddard; **Marshall Co.**, T3S R3W Sec13, 14 September 1977, Sara Hurtle; **Monroe Co.**, Smithville, 6 July 1980, Jerome Goddard; **Oktibbeha Co.**, "Miss St Univ", 20 September 1971, Charles Bryson; Starkville, 4 October 1984, Steven MacDonald; Starkville*2, 3 October 1991, R. L. Brown; Starkville, 28 March 2018, Brady Thompson, ex. turkey; Starkville, 9 April 2018, Sierra Bullock; Starkville, 16 April 2018, Nicholas Moses; Starkville, 23 April 2018, Jacob Williams; **Pontotoc Co.**, 9.5 mi W Pontotoc, 14 September 1980, Jerome Goddard; **Prentiss Co.**, Bonneville, 29 June 1980, Jerome Goddard; **Tippan Co.**, 9 mi E Ripley, 19 September 1980, Jerome Goddard.

***L. sericata* (Meigen, 1826)**

21 Specimens examined (Figure 69c):

Mississippi: Hinds Co., Jackson, 20 June 1914, H. H. Leggett; **Oktibbeha Co.**, "St Coll Miss", 8 April 1913, "Pat"; "Ag Coll Miss", 17 April 1915, C. O. French; "Ag Coll Miss", 18 August 1915, E. K. Dickey; "Ag Coll Miss", 1 April 1916, G. W. Howard; "Ag Coll Miss", 1 May 1916,

L. J. Nettle; “A&M Coll Miss”, 28 April 1918, J. M. Sessions; “Ag Coll Miss”, 1 April 1919, J. T. Douglas; “Ag Coll Miss”, 20 April 1919, J. V. Vernon; “Miss St Coll”, April 1919, G. Gualace; “Ag Coll Miss”, 12 April 1921, A. McIntosh; “A&M Coll Miss”, 5 May 1925, Roy Melvin; “M.S.C.”, 5 May 1935, Dr. Miles; “Miss St Coll”, 5 May 1939, H. H. Boyd; Clay Lyle Building MSU, Starkville, 28 March 2018, E.B. Vernon; Starkville, 4 April 2018, Brady Thompson, ex. turkey; Clay Lyle Building MSU, Starkville, 17 April 2018, E.B. Vernon; Starkville, 20 April 2018; “Miss St Coll”, [no date], P. G. Bedenbaugh; **Scott Co.**, Harperville, MS, 19 May 1917, T. E. Hand; **Yazoo Co.**, Bentonia, MS, 30 March 1940, P. W. Graham.

***L. silvarum* (Meigen, 1826)**

0 Specimens examined (record based on Goddard and Lago 1983; Figure 70a):

Mississippi: Lafayette Co.: Oxford, 8 August 1978.

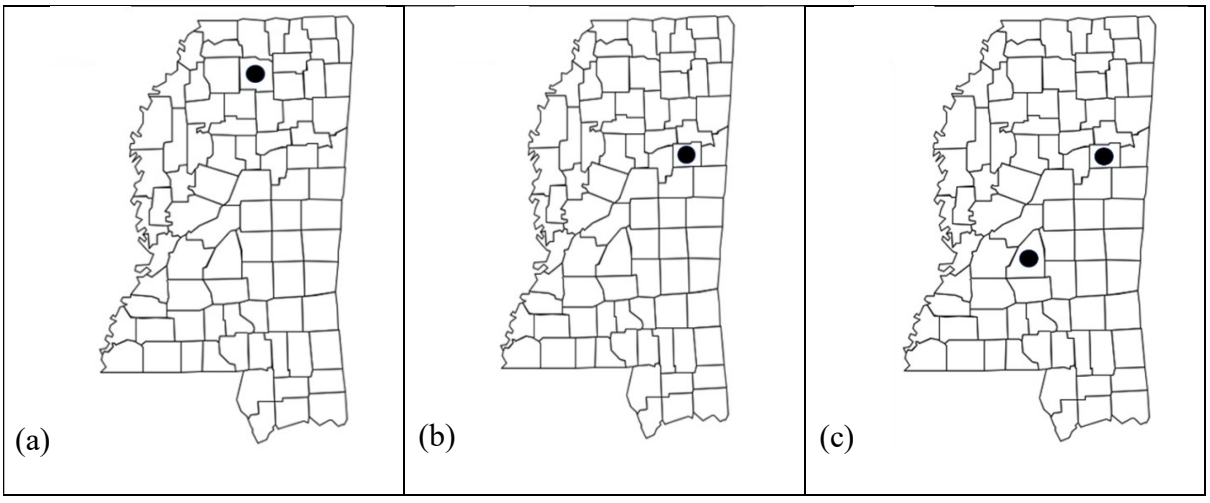

Figure 70 Distribution of blow fly species in Mississippi 4

(a) *Lucilia silvarum*; Ecoregion 65e
(b) *Chrysomya megacephala*; Ecoregion 65a
(c) *Chrysomya rufifacies*; Ecoregion 65a, 74b

Subfamily Chrysomyinae

Tribe Chrysomyini

Chrysomya Robineau-Desvoidy 1830

***Chrysomya megacephala* (Fabricius, 1794)**

2 Specimens examined (Figure 70b):

Mississippi: Oktibbeha Co., Sessums, 33°25’31”N 88°42’49”W, 1 November 1999, D. M. Pollock; Starkville, Oktoc Road, 33°22’02”N 88°45’32”W 17 October 2000, [no leg.]

***Chrysomya rufifacies* (Macquart, 1842)**

15 specimens examined (Figure 70c):

Mississippi: Oktibbeha Co., 10 mi. W Starkville, N33°29’44” W88°57’32”, 25 July 2020, G. De Jong, ex. pig exposed 3 d; **Rankin Co.**, Brandon, 7 November 2013, J. Goddard.

Cochliomyia Townsend 1915

***Cochliomyia hominivorax* (Coquerel, 1858) - extirpated**

1 Specimen examined (Figure 71a):

Mississippi: Warren Co., Vicksburg, 11 October 1946, Dr. Jones, ex. “nose of negro”.

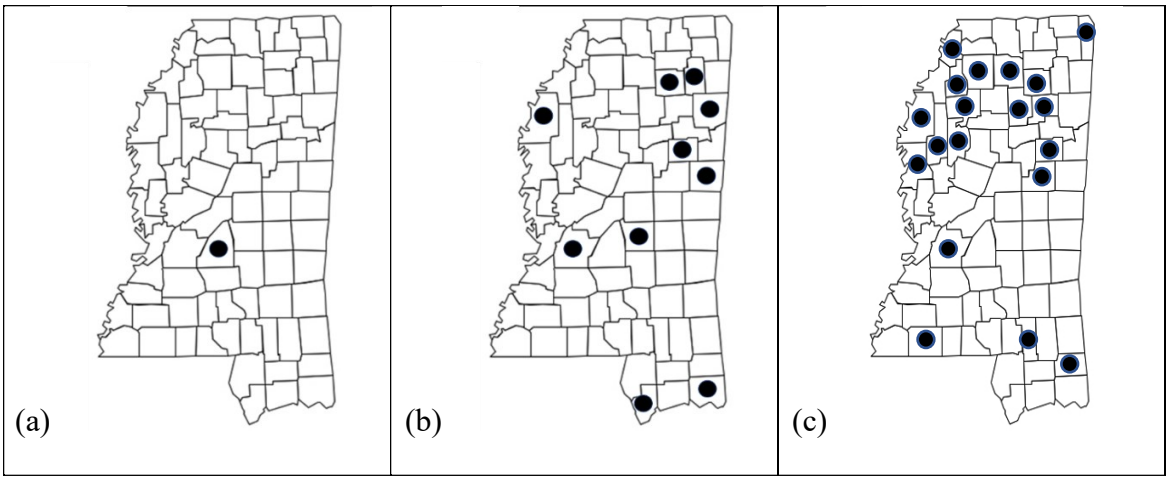

Figure 71 Distribution of blow fly species in Mississippi 5

(a) *Cochliomyia hominivorax*; Ecoregion 74a
(b) *Cochliomyia macellaria*; Ecoregions 65a, 65b, 65p, 65r, 73a, 75a, 75k
(c) *Phormia regina*; Ecoregions 65a, 65b, 65c, 65d, 65e, 65f, 65j, 65p, 73a, 73b, 74b, 74c

***Cochliomyia macellaria* (Fabricius, 1775)**

111 Specimens examined (Figure 71b):

Mississippi: Bolivar Co., nr. Cleveland, 4 August 1984, B. R. Farmer; **Hancock Co.**, 10 mi W Kiln, Texas Flat Road, 17 August 1984, P. R. Miller, ex. Armadillo; **Hinds Co.**, Jackson, 6 June 1914, H. H. Legett; **Jackson Co.**, Pascagoula, 4 September 1934, G. L. Bond; Bellefontaine Beach, 4 November 1990, T. L. Schieffer, "flowers of *Solidago pauciflosculosa*"; Greenwood Island, 4 November 1990, T. L. Schieffer, "flowers of *Haplopappus phyllocephalus*"; **Lee Co.**, Tupelo, 10 November 1985, C. T. Bryson; **Monroe Co.**, 2 mi. NW Amory, T12S R8E Sec19W, 31 October 1990, T. L. Schiefer; **Noxubee Co.**, 21 August 1973, M. F. Schuster; **Oktibbeha Co.**, "Ag Coll Miss"*2, September 1891, H. E. Wood; "Ag Coll Miss", 8 September 1912, C. F. Stiles, ex. Cowpeas; "Ag Coll Miss", 23 September 1917, T. A. Arnold, ex. Pear; "Ag Coll Miss", 10 April 1918, A. I. Coker; "Ag Coll Miss" 16 April 1918, L. F. Curl, "Bishopp No. 22390"; "Ag Coll Miss" 16 April 1918, L. F. Curl, "Bishopp No. 22384"; "Ag Coll Miss" 16

April 1918, L. F. Curl, “Bishopp No. 22387”; “Ag Coll Miss”. June 1920, H. W. Allen; Starkville, 9 June 1921, H. W. Allen; “State Coll Miss”, 7 August 1972, CAM; “Miss St. Univ.”, 4 September 1980, Charles Bryson; 2.3 mi N Oktoc, 17 July 1982, W. H. Cross; 6 mi SW Starkville, 11 August 1984, R. L. & B. B. Brown, “sweeping flowering *Cassia fasciculata*”; Starkville, T19N R14E Sec25SW, 21 July 1992, T. MacGown & T. L. Schieffer, “on *Ampelopsis arborea*”; Sessums, N33°23’31” W88°42’40”, 19 June 1995, J. A. MacGown, “on flowers of *Daucus carota*”; Sessums, N33°23’31” W88°42’40”, 25 June 1995, Mike MacGown; Sessums, N33°25’31” W88°42’40”, 21 July 1996, J. A. MacGown, “flowers of *Mentha* sp.”; Sessums, N33°23’31” W88°42’40”, 2 September 1998, J. A. MacGown; Sessums, N33°25’31” W88°42’49”, 1 November 1999, D. M. Pollock, “on dead mammal”; Starkville, Oktoc Rd., N33°22’02” W88°45’32”, 17 October 2000, D. M. Pollock, “dead red fox”; MSU South Farm, N33°26’20” W88°47’27”, 1-5 October 2005, J. G. Hill, “pitfall trap baited with chicken liver”; 3 mi E Starkville, N33°26’47” W88°44’46”, 6 November 2009, J. G. Hill & J. L. Seltzer, “on white aster flowers”; Starkville, 9 April 2018, Sierra Bullock; Starkville, 10 April 2018, Brady Thompson, ex. armadillo; 10 mi. W Starkville, N33°29’44” W88°57’32”, 17 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 4 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 18 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 5 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 19 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 6 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 20 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 7 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 21 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 8 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 22 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 9 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 23 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 10 d; 10 mi. W Starkville, N33°29’44”

W88°57’32”, 27 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 2 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 28 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 3 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 29 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 4 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 30 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 5 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 31 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 6 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 1 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 7 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 2 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 8 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 4 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 10 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 7 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 3 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 9 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 5 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 10 June 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 6 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 25 July 2020, G. De Jong, ex. pig exposed 3 d; **Pontotoc Co.**, 1 mi SE Ecru, 25 September 1980, G. L. Snodgrass; 1 mi SE Ecru, 26 September 1980, G. L. Snodgrass; 1 mi SE Ecru, 27 September 1980, G. L. Snodgrass; **Scott Co.**, Harrell Hill Prairie, T6N R9E Sec25, 16 August 1992, R. L. Brown, “sweeping flowering *Cassia fasciculata*”.

Tribe Phormiini

Phormia Robineau-Desvoidy 1830

***Phormia regina* (Meigen, 1826)**

201 Specimens examined (Figure 71c):

Mississippi: Amite Co., 5 mi WSW Liberty, 25 April 1973, W. H. Cross; **Bolivar Co.**, 2 mi W Rosedale, 20 June 1980, J. Goddard, "trap baited with fish"; **Calhoun Co.**, 10 mi NE Erma, 6 June 1980, J. Goddard, "trap with reptile carrion"; **Chickasaw Co.**, 5 mi N Houston, 4 June 1983, P. R. Miller, "on flowering *Castanea pumilla*; **Forrest Co.**, Hattiesburg, 17 February 1990, T. L. Schieffer, "on flowers of *Prunus* sp."; **George Co.**, Mixon lakes, N30°50'28" W88°45'11" 8-9 April 1994, R. L. Brown & D. Pollock, "on rotting crayfish"; Mixon lakes, N30°50'28" W88°45'11" 8-10 April 1994, C. D. Parker, "on rotting crayfish"; **Hinds Co.**, Clinton, 4 December 1979, Bryant Mather; **Lafayette Co.**, "U of M campus", 9 May 1980, J. Goddard, "trap with mammal carrion"; Oxford, 26 May 1980, "trap baited with liver"; Oxford, 3 June 1980, J. Goddard, "trap baited with fish"; Oxford, 12 June 1980, J. Goddard, "trap baited with fish"; Oxford, 1 July 1980, J. G., "trap with mammal carrion"; Oxford, 2 September 1980, J. Goddard; Oxford, 6 September 1980, J. Goddard; Oxford, 7 September 1980, J. Goddard; Oxford, 8 September 1980, J. Goddard; Oxford, 9 September 1980, J. Goddard; Oxford, 10 September 1980, J. Goddard; Oxford, 11 September 1980, J. Goddard, "trap baited with fish"; Oxford, 2 October 1980, J. Goddard; Oxford, 16 October 1980, J. Goddard; Oxford, 17 October 1980, J. Goddard; Oxford, 18 October 1980, J. Goddard Oxford, 19 October 1980, J. Goddard; Oxford, 21 October 1980, J. Goddard; Oxford, 22 October 1980, J. Goddard; Oxford, 8 November 1980, J. Goddard; Oxford, 13 November 1980, J. Goddard; T7S R2W Sec34, 6 April 1981, J. Goddard, "trap with reptile carrion"; **LeFlore Co.**, 2 mi. N Minter, 25 April 1981, J. Goddard, "trap with reptile carrion"; **Oktibbeha Co.**, "Ag Coll Miss", 10 April 1915, J. D. Maloney; "Ag Coll Miss", 5 May 1915, R. Bryant; "Ag Coll Miss", 1 April 1916, G. W. Howard; "Ag Coll Miss", 15 April 1916, A. McCluer; "Ag Coll Miss", ? September 1916, N. S.

Martin; “Ag Coll Miss”, 31 March 1917, E. Walduer; “Ag Coll Miss”, 15 April 1917, “O. F. Clark, “flying”; “Ag Coll Miss”, 22 April 1917, M. C. Guerry; “Ag Coll Miss”, 18 April 1918, E. C. Trissia; “Ag Coll Miss”, 26 April 1918, R. H. Pepper; “Ag Coll Miss”, 15 April 1919, E. E. Johnson; “Ag Coll Miss”, 19 April 1919, J. O. Williams; “Ag Coll Miss”, 3 May 1919, ? [illegible]; “Ag Coll Miss”, 14 August 1919, L. F. Curl; “Ag Coll Miss”, ? April 1920, W. E. Conn; “Ag Coll Miss”, 2 March 1921, H. W. Allen; “St Coll Miss”, 20 April 1939, L. Hinton; “Miss State Univ”, 20 April 1971, Charles Bryson; “Miss State Univ”, 21 April 1971, Charles Bryson; “Miss State Univ”, 22 April 1971, Charles Bryson; Starkville, 25 April 1971, Charles Bryson; “Miss State Univ”, 7 May 1971, Charles Bryson; “Miss State Univ”, 9 May 1971, Charles Bryson; “St Coll Miss”, 5 May 1972, M. Poole; “St Coll Miss”, ? September 1973, K. Chapin; “St Coll Miss”, 2 April 1974, K. Watts; Starkville, November 1974, W. H. Cross, “blacklight trap near edge of deciduous woods”; Sessums, N33°23’31” W88°42’40”, 28 March 1975, D. M. Pollock, “dead raccoon”; Starkville, 7 December 1975, W. H. Cross, “blacklight trap near edge of deciduous woods”, ; Starkville, 8 March 1976, W. H. Cross, “blacklight trap near edge of deciduous woods”; “Miss St Univ”, 1 March 1977, “P.P.”; “Miss State”, 1 August 1977, F. L. Hooper; Craig Springs, 5 December 1980, G. L. Snodgrass, “pitfall trap peripheral to cultured cotton”; Starkville, 17 July 1981, W. H. Cross, “white pan trap under blacklight”; Craig Springs, 5 November 1981, W. H. Cross, “interception trap-black”; Craig Springs, 13 November 1981, W. H. Cross, “on ground”; 3.2 mi N Starkville, 21 Mar 1991, J. McCoy, “in house”; Starkville, 8 April 1991, J. MacGown, “on flowering *Photina* x *fraseri*”; Starkville, 9 April 1991, J. Macgown &T. L. Schieffer, “on flowering *Photina* x *fraseri*”; Starkville, 15 April 1991, T. L. Schieffer, “on flowering *Photina* x *fraseri*”; Starkville, T19N R14E Sec34NE, 17 March 1992, T. L. Schieffer, “on flowers of *Prunus caroliniana*”; Starkville, T19N R14E Sec34NE, 11

April 1994, T. L. Schieffer, “on flowers of *Prunus caroliniana*”; Sessums, N33°25’31” W88°42’49”, 28 March 1995, D. M. Pollock, “dead raccoon”; John Starr Mem. Forest., N33°21’19” W88°51’13”, 7 June 1995, D. M. Pollock; Osborne, N33°30’41” W88°44’08”, 16 February 1999, J. A. MacGown, on dead dog in Black Belt Prairie”; Sessums, N33°25’31” W88°42’49”, 1 November 1999, D. M. Pollock, “on dead mammal”; MSU South Farm, “*ex. Sus scrofa domesticus*, advanced stage, day 24”, N33°25’21” W88°46’57”, 13 April 2012, C. Chesnut; Starkville, 15 March 2018, Nicholas Moses; Starkville, 25 March 2018, Brady Thompson, ex. turkey; Clay Lyle Building MSU, Starkville, 28 March 2018, E.B. Vernon; Starkville, 11 April 2018, Sierra Bullock; Pontotoc, 15 April 2018, Nicholas Moses; Starkville, 16 April 2018, Jacob Williams, ex. turkey carcass; 10 mi. W Starkville, N33°29’44” W88°57’32”, 16 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 3 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 17 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 4 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 18 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 5 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 21 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 8 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 22 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 9 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 28 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 3 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 20 May 2019, J. Goddard, F. Meyer, et al., ex. pig exposed 5 d; 10 mi. W Starkville, N33°29’44” W88°57’32”, 25 July 2020, G. De Jong, ex. pig exposed 3 d; **Panola Co.**, Sardis Dam, 17 April 1980, J. Goddard, “trap with mammal carrion”; Sardis Dam, 28 May 1980, J. Goddard, “trap baited with fish”; **Pontotoc Co.**, 1 mi SE Ecru, 18 December 1980, G. L. Snodgrass, “cultured cotton, 4780-12D”; 1 mi Se Ecru, 18 December 1980, W. H. Cross, “malaise trap in cultured cotton, 4779-33”; **Quitman Co.**, 2 mi S Sledge, 20 June 1980, J.

Goddard; **Sunflower Co.**, 1 mi W Ruleville, 25 April 1981, J. Goddard, “mammal carrion trap”; **Tallahatchie Co.**, 1 mi S Glendora, 25 April 1981, J. Goddard, “mammal carrion trap”; **Tishomingo Co.**, Eastport, 14 June 1980, J. Goddard, “trap with mammal carrion”; Eastport, 12 August 1980, J. Goddard, “garbage”; Eastport, 14 August 1980, J. Goddard, “garbage”; **Tunica Co.**, 3 mi S Tunica, 25 April 1981, J. Goddard, “mammal carrion trap”; **Washington Co.**, Stoneville, Delta Experimental Forest, 21 June 1988, J. R. MacDonald; **Winston Co.**, 8 mi S Louisville, 13 April 1994, C. D. Pickett; 4 mi N Hwys 15 x 25, 14 April 1995, D. M. Pollock, “on dead opossum”.

Protocalliphora Hough 1899

Protocalliphora deceptor Sabrosky, Bennett, and Whitworth, 1989

9 Specimens examined (Figure 72a):

Mississippi: Oktibbeha Co., Starkville, 11 May 1981, G. Hurst, “reared from pupae in Carol. chickadee nest”.

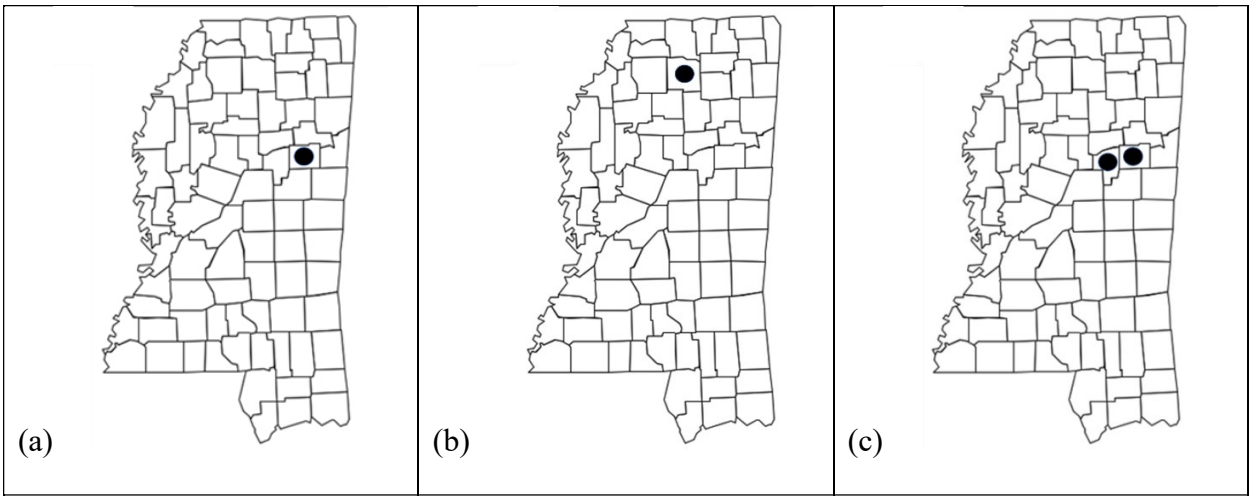

Figure 72 Distribution of blow fly species in Mississippi 6

(a) *Protocalliphora deceptor*; Ecoregion 65a
(b) *Pollenia rudis*; Ecoregion 65e
(c) *Angioneura flavescens*; Ecoregions 65a, 65b, 65d

Subfamily Polleniinae

Pollenia Robineau-Desvoidy 1830

Pollenia rudis (Fabricius, 1794)

0 Specimens examined (record based on Goddard and Lago 1983; Figure 72b)

Jerome Goddard, personal communication, graciously provided the following records:

Mississippi: Lafayette Co., Oxford, 10 November 1976, S. Merrell; 6 mi. N.W. Oxford, 26 January 1977, H. Schuster; Oxford, 3 February 1977, M. T. McCraine; Oxford, 9 February 1977, M. J. Chapman; Oxford, 12 February 1977, M. T. McCraine; Oxford, 22 February 1977, M. T. McCraine; 11 mi. N.W. Oxford, 5 March 1977, S.C. Elliot; Oxford, 7 March 1977, M. L. Baker; Oxford, 8 March 1977, D. F. Stanford; Oxford, 18 March 1977, G. Sparks; Oxford, 23 March

1977, S. C. Elliot; Oxford, 15 May 1977, P. K. Lago; Oxford, 25 May 1977, D. F. Stanford; Oxford, 26 May 1980, J. Goddard, liver; Oxford, 27 May 1980, J. Goddard, liver.

Confirmed by R. J. Gagné and later T. Whitworth.

Subfamily Melanomyiinae

Angioneura Brauer & Bergenstamm 1893

***Angioneura flavescens* (Reinhard 1929)**

4 Specimens examined (Figure 72c):

Mississippi: Choctaw Co., Choctaw Lake, 2 May 1995, D. M. Pollock, white pan trap under blacklight; **Oktibbeha Co.**, Starkville, 12 June 1981, W. H. Cross; Starkville, 13 April 1981, W. H. Cross; 2 mi. NE Craig Springs, T7N R13E Sec14SE, 21 March 1992, T. L. Schiefer, on flowering *Prunus serotina*.

***A. obscura* (Townsend 1919)**

1 Specimen examined (Figure 73a):

Mississippi: Noxubee Co., Noxubee NWR, 33°17’00”N 88°45’16”W, 11 May 1995, D. M. Pollock, sweeping.

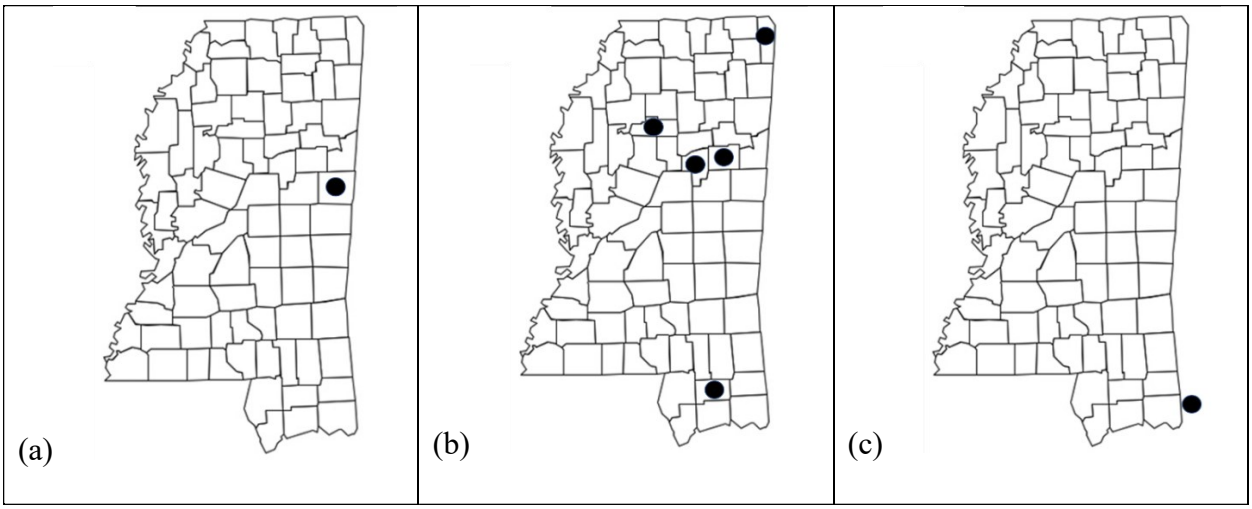

Figure 73 Distribution of blow fly species in Mississippi 7

(a) *Angioneura obscura*; Ecoregion 65b
(b) *Opsodexia bicolor*; Ecoregions 65b, 65d, 65f, 65j, 74b
(c) *Opsodexia grisea*

Opsodexia Townsend 1915

Opsodexia bicolor (Coquillett 1899)

5 Specimens examined (Figure 73b):

Mississippi: Choctaw Co., Choctaw Lake, 2 May 1995, D. M. Pollock; **Grenada Co.**, T21N R2E Sec12-13N and R3E Sec7S-18N, 14 May 1991, T. L. Schiefer; **Oktibbeha Co.**, Craig Springs, 4 November 1981, W. H. Cross, interception trap-yellow; **Stone Co.**, Sweetbay Bogs, T2S R13W Sec34SW, 12 March 1991, T. L. Schiefer, blacklight; **Tishomongo Co.**, Tishomongo S.P., 11 April 1986, K. Ward.

O. grisea (Coquillett, 1899)

No Mississippi specimens examined: record based on photographed specimen from Mobile Co., Alabama (Figure 73c).

***O. nox* (Downes, 1986)**

2 Specimens examined (Figure 74):

Mississippi: Winston Co., Tombigbee NF, 33°16’5”N 89°6’1”W, 17 May 1999, D. M. Pollock, blacklight trap in mixed mesic forest; Tombigbee NF, 33°16’5”N 89°6’1”W, 17 May 1999, D. M. Pollock, blacklight trap in sweet bay bog in mixed mesic forest.

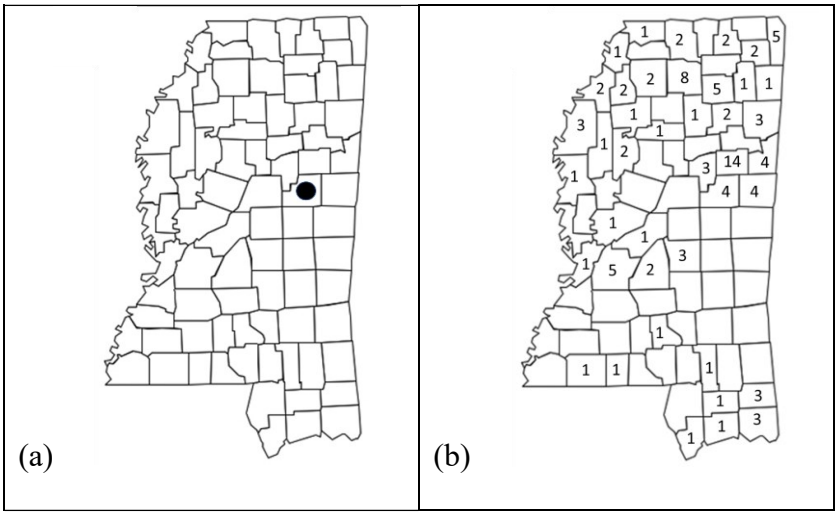

Figure 74 Distribution of blow fly species in Mississippi 8

(a) *Opsodexia nox*; Ecoregion 65b
(b) County totals for blow fly species represented

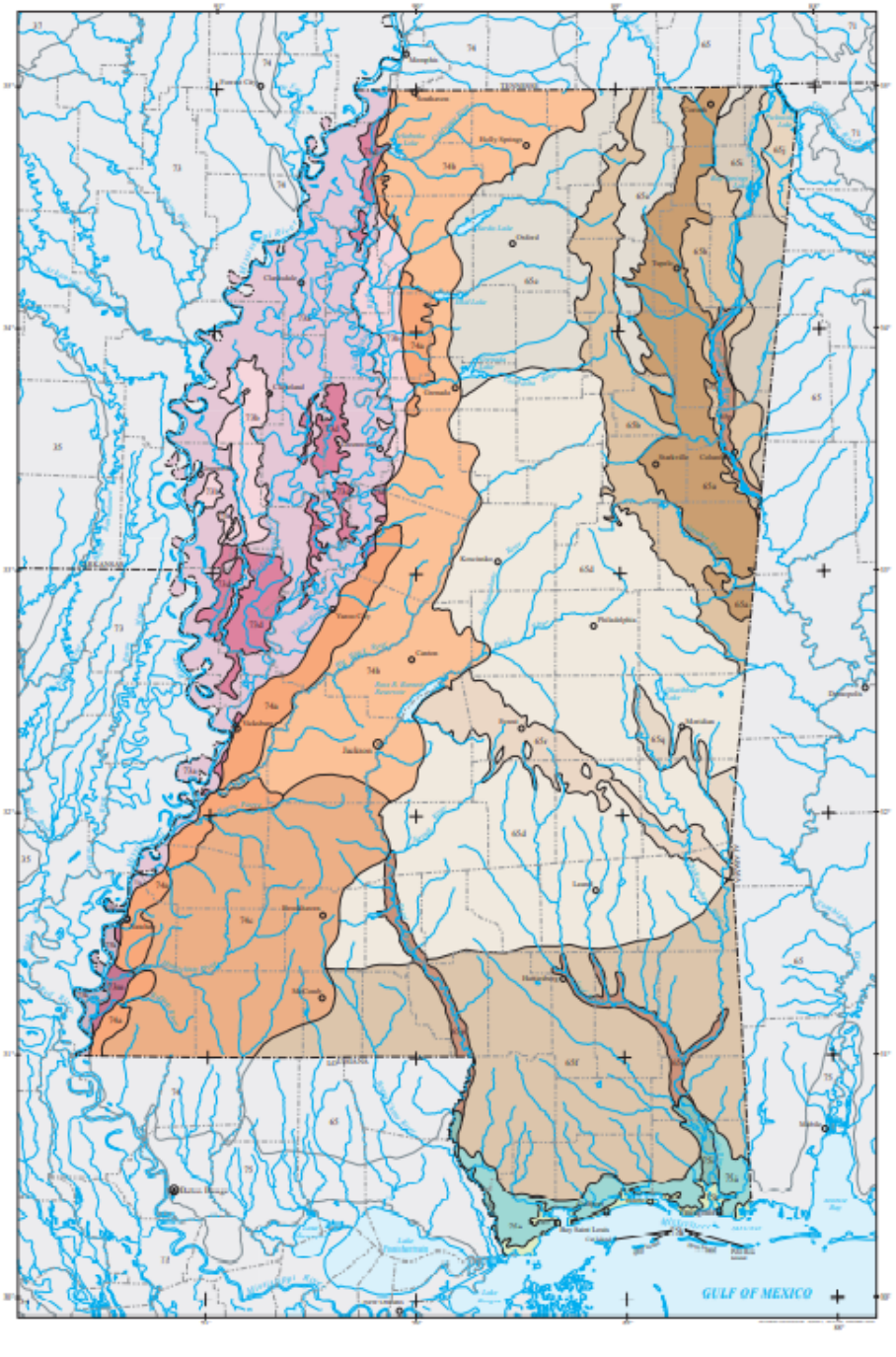

Figure 75 Ecoregions of Mississippi.

Chapman, S. S., G. E. Griffith, J. M. Omernik, J. A. Comstock, M. C. Beiser, and D. Johnson. 2004. Ecoregions of Mississippi (color poster with map, descriptive text, summary tables, and photographs). U.S. Geological Survey, Reston, Virginia. Public Domain.

APPENDIX B

MODIFIED TOTAL BODY SCORE FOR PIG CARCASS DECOMPOSITION

This modified Total Body Score (TBS) is a modification of the Megyesi et al. (2005) method, as amended by Keough et al. (2016) for scoring pig carrion decomposition. In this method, morphological conditions are observed in the head and neck region, the trunk region, and the limb regions, separately. Each region is assigned a decomposition score based on the observed condition. Modifications for the purpose of this dissertation include the description of additional characteristics, starting the scale at 0 instead of 1, and including 5 stages instead of four. Decomposition scores for each body region are described in Tables A-1 through A-3.

Table 26 Scoring of decomposition stages for the head and neck.

Stage	Score	Description
Fresh	0	Fresh, no discoloration; no odor
	1	Slight lividity (pink/red tinge); blow fly eggs may be present but no maggots; slight odor but not offensive
Bloat	2	Pronounced lividity (dark pink/red tinge); insect activity present; strong odor may be present
	3	Dark red discoloration with some flesh relatively "fresh"-looking; maggot colonization in orifices; initial bloating of neck with skin slippage; strong odor
	4	Discoloration and drying at edges of nose, ears, lips, eyes; prominent bloating in neck; maggot colonization in orifices; slight purging of fluids from mouth; strong odor
Active Decay	5	Purging of fluids from orifices; brown discoloration; hair loss and skin slippage; drying of lips, nose, and ears; extensive maggot colonization in orifices; strong odor
	6	Blackish discoloration of flesh; extensive maggot colonization in orifices; strong odor
	7	Collapse of bloat with caving in of tissues in neck and eyes; extensive maggot colonization in orifices; strong odor
Advanced Decay	8	Moist decomposition with bone exposure < 0.5 area being scored; nearly entire surface covered with maggots; strong odor
	9	Mummification with bone exposure < 0.5 area being scored; maggot migration; strong odor
	10	Bone exposure > 0.5 area being scored, with large areas of greasy tissues; maggot migration
	11	Bone exposure > 0.5 area being scored, with initial desiccation of mummified tissues ("soft jerky"); most maggots have migrated
Skeletonized remains/dry	12	Bone exposure > 0.8 area being scored or desiccation of mummified tissues ("dry jerky"); few if any maggots present; moderate odor of ammonia
	13	Dry bones; no odor or slight ammoniacal odor

Table 27 Scoring of decomposition stages for the trunk region

Stage	Score	Description
Fresh	0	Fresh, no discoloration
	1	Slight lividity (pink/red tinge); blow fly eggs may be present but no maggots; slight odor but not offensive
Bloat	2	Skin appears shiny/glossy and may show purple-black discoloration; initial bloating; insect activity present, particularly at ante- or post-mortem lesions; strong odor
	3	Gray-purple to green discoloration; abdomen attaining marbled appearance; bloated (maximum may be obtained); portions of surface may be covered with maggots; strong odor
	4	Purple-black discoloration; bloated (maximum may be obtained) purging of decompositional fluids from ante- or post-mortem lesions; skin slippage; hair loss; portions of surface may be covered with maggots; strong odor
Active decay	5	Gases beginning to purge with deflation of abdomen; extensive skin slippage; much of surface may be covered with maggots; strong odor
	6	Collapse of bloat with caving in of abdominal cavity; much of surface covered with maggots; strong odor
Advanced decay	7	Moist decomposition with bone exposure < 0.5 area being scored; nearly entire surface covered with maggots; strong odor
	8	Moist decomposition with bone exposure < 0.5 area being scored; maggots begin migration; strong odor
	9	Mummification with bone exposure < 0.5 area being scored; maggots in migration; strong odor
	10	Bone exposure > 0.5 area being scored, with large areas of greasy tissues; maggots in migration; strong odor
	11	Bone exposure > 0.5 area being scored, with initial desiccation of mummified tissues (“soft jerky”); maggots in migration; strong odor
Skeletonized remains/dry	12	Bone exposure > 0.8 area being scored or desiccation of mummified tissues (“dry jerky”); few or no maggots present; moderate ammoniacal odor
	13	Dry bones; no odor or slight ammoniacal odor

Table 28 Scoring of decomposition stages for the limbs

Stage	Score	Description
Fresh	0	Fresh, no discoloration
	1	Slight lividity (pink/red tinge); rigor mortis present; blow fly eggs may be present but no maggots; slight odor
Bloat	2	Pink/white appearance with initial bloat in proximal parts of limbs; insect activity; strong odor
	3	Grayish to green discoloration and mottled appearance; some skin slippage and hair loss; some maggots may be present; strong odor
Active decay	4	Discoloration to dark shades; desiccation of skin (starting distal to proximal); some maggots may be present; strong odor
	5	Complete brown/black discoloration; skin appearing leathery with dry margins; mostly without maggots; strong odor
Advanced decay	6	Moist decomposition with bone exposure < 0.5 area being scored; maggots, if present, spilling from other regions; strong odor
	7	Mummification with bone exposure < 0.5 area being scored; strong odor
	8	Bone exposure > 0.5 area being scored, with large areas of greasy tissues; maggots, if present, spilling from other regions; strong odor
	9	Bone exposure > 0.5 area being scored, with initial desiccation of mummified tissues (“jerky”); few if any maggots; moderate ammoniacal odor
Skeletonized remains/dry	10	Dry bones; few if any maggots; no odor or slight ammoniacal odor

APPENDIX C

LIST OF INVERTEBRATE TAXA COLLECTED AT PIG CARCASSES IN MISSISSIPPI AND FLORIDA, 13 MAY – 14 JUNE 2019

Table 29 List of invertebrate taxa collected at pig carcasses in Mississippi and Florida, 13 May – 14 June 2019. FL (Bk) = background samples).

Phylum/ Class	Order	Family	Taxon	MS	FL	FL (Bk)
Annelida	Haplotaxida	Lumbricidae	Lumbricidae A	0	13	3
Arachnida	Aranaea	Agelinidae	Agelinidae A	1	0	0
		Anyphaenidae	*Wulfina albens*	0	8	0
		Corinnidae	*Falconina gracilis*	2	37	4
		Linyphiidae	Linyphiidae A	0	30	8
			Linyphiidae B	5	0	0
			Linyphiidae C	7	183	29
		Lycosidae	Lycosidae A	57	33	9
			Lycosidae B	23	124	16
			Lycosidae C	18	176	15
			Lycosidae D	2	0	0
		Salticidae	Salticidae A	0	3	1
		Thomisidae	Thomisidae A	0	1	0
Hexapoda	Collembola	Entomobryidae	Entomobryidae A	25	411	57
		Sminthuridae	Sminthuridae A	0	6	2
Insecta	Blattodea	Ectobiidae	*Cariblatta lutea*	1	10	2
	Coleoptera	Anthicidae	*Acanthinus argentinus*	0	10	1
		Carabidae	*Aspidoglossa subangulata*	0	1	0
			Carabidae A	0	19	0
			Cicindelidia punctulata	0	5	0
			Harpalus sp.	1	2	9
			Platynus sp.	0	1	1
			Pterostichus sp.	0	7	10
			Tetracha carolina	0	6	0
		Cleridae	*Necrobia rufipes*	2	0	0
		Coccinellidae	*Coleomegilla maculata*	0	3	4
			Hippodamia convergens	1	0	0
		Curculionidae	Curculionidae A	0	1	2
			Curculionidae B	0	1	0
		Dermestidae	*Dermestes caninus*	1	0	0
		Elateridae	Elateridae A	0	2	1
			Elateridae B	0	2	0
			Elateridae C	0	15	4
			Elateridae D	0	1	1
		Histeridae	*Saprinus* sp.	4	49	0
		Hybosoridae	*Hybosorus illigeri*	1	1	0
		Nitidulidae	*Carpophilus hemipterus*	0	6	0

Table 29 (continued)

Insecta	Coleoptera	Nitidulidae	*Carpophilus pallipennis*	0	2	0
			Carpophilus sayi	0	6	0
			Omosita discoidea	1	1	0
			Stelidota coenosa	0	68	0
		Scarabaeidae	*Ataenius* sp.	2	0	0
			Canthon vigilans	0	2	0
			Euphoria sepulchralis	0	1	0
			Martineziella dutertrei	4	0	0
			Onthophagus hecate hecate	0	8	0
			Onthophagus pennsylvanicus	0	1	0
			Phanaeus vindex	0	1	0
			Scarabaeidae A	0	1	0
			Scarabaeidae B	0	1	0
			Scarabaeidae C	7	0	0
		Silphidae	*Necrodes surinamensis*	4	12	0
			Nicrophorus carolina	0	2	0
			Oiceoptoma inaequale	1	0	0
		Staphylinidae	*Creophilus maxillosus*	7	36	0
			Platydracus maculosus	1	2	0
			Staphylinidae	1	4	0
			Staphylinidae A	24	161	2
			Staphylinidae B	0	10	0
			Staphylinidae C	0	23	2
			Staphylinidae D	0	7	0
			Staphylinidae E	4	33	4
			Staphylinidae F	3	55	0
			Staphylinidae G	0	59	0
			Staphylinidae H	1	4	0
			Staphylinidae I	1	0	0
			Staphylinidae J	0	0	0
			Staphylinidae K	5	1	0
			Staphylinidae L	1	0	0
		Tenebrionidae	Tenebrionidae A	1	0	0
		Trogidae	*Omorgus* sp.	2	0	0
	Dermaptera	--	Unid. Dermaptera	0	1	0
	Diptera	Asilidae	Asilidae A	0	1	0
			Asilidae B	0	1	0
		Bibionidae	Bibionidae A	0	1	0
			Dilophus sp.	0	9	7
			Plecia nearctica	0	1	0
		Calliphoridae	Calliphoridae	531	1.834	0

Table 29 (continued)

Insecta	Diptera	Calliphoridae	*Chrysomya rufifacies*	0	58	0
			Cochliomyia macellaria	1,065	1,461	0
			Lucilia coeruliviridis	234	2,222	0
			Phormia regina	15	1	0
		Dolichopodidae	Dolichopodidae A	0	2	1
		Fanniidae	*Fannia* sp.	1	0	0
		Muscidae	*Hydrotaea aenescens*	17	1	0
			Musca domestica	4	0	0
		Mycetophilidae	Mycetophilidae A	1	16	2
		Phoridae	Phoridae A	81	66	0
			Phoridae C	1	0	0
			Phoridae B	0	48	0
		Piophilidae	*Prochyliza xanthostoma*	8	0	0
			Stearibia nigriceps	2	0	0
		Psychodidae	Psychodidae A	11	0	0
		Sarcophagidae	Sarcophagidae	0	51	0
			Sarcophagidae A	15	6	0
			Sarcophagidae B	0	2	0
			Sarcophagidae C	0	2	0
			Sarcophagidae D	0	1	0
			Sarcophagidae E	0	2	0
		Sepsidae	Sepsidae A	0	0	0
			Sepsidae B	0	0	0
		Stratiomyidae	*Hermetia illucens*	0	274	0
		Syrphidae	Syrphidae A	1	0	0
		Tachinidae	*Belvosia borealis*	1	0	0
		Tipulidae	*Nephrotoma* sp.	0	2	0
			Tipulidae A	0	1	0
			Tipulidae B	1	0	0
	Hemiptera	--	Unid. Hemiptera	1	13	1
		--	Unid. Homoptera	0	6	0
		Cicadellidae	Cicadellidae A	8	6	1
		Cydnidae	Cydnidae A	0	3	1
		Membracidae	Membracidae A	0	1	0
			Spissistilus festinus	0	1	1
		Pentatomidae	Pentatomidae A	0	3	2
		Psyllidae	Psyllidae A	1	0	0
		Reduviidae	*Apiomeris crassipes*	0	2	0
			Reduviidae A	8	3	0
			Reduvius personatus	0	1	0
			Rocconota annulicornis	2	0	0

Table 29 (continued)

Insecta	Hymenoptera	Chalcidoidea	Chalcidoidea A	7	0	0
		Formicidae	*Brachymyrmex patagonicus*	0	91	30
			Camponotus sp.	0	0	1
			Crematogaster cerasi	0	1	24
			Cyphomyrmex rimosus	0	85	12
			Forelius mccooki	1	0	0
			Hypoponera opaciceps	2	245	10
			Odontomachus haematodus	0	1	3
			Paratrechina longicornis	10	0	0
			Solenopsis hybrid	3,910	0	0
			Solenopsis invicta	0	8,820	792
		Mutillidae	Mutillidae A	0	0	1
		Pompilidae	*Anoplius americanus*	0	5	0
		Pteromalidae	Pteromalidae A	0	1	0
			Pteromalidae B	1	0	0
		Sphecidae	*Ammophila* sp.	0	1	0
		Torymidae	Torymidae A	36	9	0
	Isoptera	Rhinotermitidae	Reticulitermes flavipes	0	0	15
	Lepidoptera	--	Unid. Lepidoptera	1	0	0
		Arctiidae	Arctiidae A	0	1	0
	Orthoptera	Acrididae	Acrididae A	1	0	0
			Melanoplus sp.	0	1	0
		Gryllidae	*Allonemobius socius*	9	92	23
			Gryllus sp.	17	2	6
			Neonemobius cubensis	12	0	0
		Gryllotalpidae	*Neoscapteriscus borellii*	0	3	13
		Tetrigidae	Tetrigidae A	1	0	0
	Psocoptera	--	Unid. Psocoptera	0	2	0
	Thysanoptera	--	Unid. Thysanoptera	0	1	1
Chilopoda	Lithobiomorpha	Lithobiidae	Lithobiidae A	0	2	8
	Scolopendromorpha	Scolopendridae	*Scolopendra* sp.	1	0	0
Diplopoda	Polydesmida	Eurymerodesmidae	*Eurymerodesmus varius*	0	7	20
		Paradoxosomatidae	*Asiomorpha coarctata*	0	19	7
Mollusca	Stylommatophora	Zonitidae	*Ventridens* sp.	0	1	1
	Gastropoda	Limacidae	*Deroceras* sp.	5	0	0
Total				6,326	17,411	1,171

FL (Bk) = background samples in Florida.

APPENDIX D

STATISTICS ON DNA RECOVERY AND PURITY

Table 30 DNA recovery and A_{260}/A_{280} absorbance ratios.

Set	Pig #	Source	Day	DNA recovery (ng DNA / µL elution buffer)	A_{260}/A_{280} ratio
M1	a (0.5 m)	Mound	0	22.9	1.67
M1	a (0.5 m)	Mound	1	14.6	1.61
M1	a (0.5 m)	Mound	2	17.2	1.97
M1	a (0.5 m)	Mound	3	4.7	1.69
M1	a (0.5 m)	Mound	4	3.9	1.61
M1	a (0.5 m)	Mound	5	21.3	1.87
M1	a (0.5 m)	Pig	1	89.3	1.49
M1	a (0.5 m)	Pig	2	21.7	2.3
M1	a (0.5 m)	Pig	3	16.3	1.9
M1	a (0.5 m)	Pig	4	12.9	2.04
M1	a (0.5 m)	Pig	5	11.9	2.04
M1	b (1.0 m)	Mound	0	22.7	1.98
M1	b (1.0 m)	Mound	1	12.3	1.63
M1	b (1.0 m)	Mound	2	22.6	1.68
M1	b (1.0 m)	Mound	3	8.3	2.01
M1	b (1.0 m)	Mound	4	11.5	1.64
M1	b (1.0 m)	Mound	5	9.7	2.27
M1	b (1.0 m)	Pig	1	80	1.5
M1	b (1.0 m)	Pig	2	27.6	1.68
M1	b (1.0 m)	Pig	3	10.8	1.82
M1	b (1.0 m)	Pig	4	7.6	1.66
M1	b (1.0 m)	Pig	5	11.9	1.95
M1	c (2.0 m)	Mound	0	26.7	2.57
M1	c (2.0 m)	Mound	1	2.9	3.87
M1	c (2.0 m)	Mound	2	78	1.66
M1	c (2.0 m)	Mound	3	12.6	1.93
M1	c (2.0 m)	Mound	4	16.8	2.1
M1	c (2.0 m)	Mound	5	13.7	2.96
M1	c (2.0 m)	Pig	1	9.2	1.87
M1	c (2.0 m)	Pig	2	15.2	1.82
M1	c (2.0 m)	Pig	3	31.2	1.14
M1	d (3.0 m)	Mound	0	16.9	2.39
M1	d (3.0 m)	Mound	1	12.7	1.92
M1	d (3.0 m)	Mound	2	22.9	2.06
M1	d (3.0 m)	Mound	3	38.8	1.85
M1	d (3.0 m)	Mound	4	24.2	1.6
M1	d (3.0 m)	Mound	5	14.3	2.84

Table 30 (continued)

M1	d (3.0 m)	Pig	1	93.4	1.45
M1	d (3.0 m)	Pig	2	4.8	1.64
M1	n (>10 m)	Mound	0	48.6	1.52
M2	a (0.5 m)	Mound	0	4.4	1.65
M2	a (0.5 m)	Mound	1	17.3	2.07
M2	a (0.5 m)	Mound	2	9.1	1.97
M2	a (0.5 m)	Mound	3	18.6	1.75
M2	a (0.5 m)	Mound	4	8.3	2.3
M2	a (0.5 m)	Mound	5	25.8	1.66
M2	a (0.5 m)	Pig	1	11	1.88
M2	a (0.5 m)	Pig	2	22.6	1.98
M2	a (0.5 m)	Pig	3	17.1	1.59
M2	a (0.5 m)	Pig	4	10.1	2.04
M2	a (0.5 m)	Pig	5	6.3	2.1
M2	b (1.0 m)	Mound	0	15.6	1.6
M2	b (1.0 m)	Mound	1	12.9	1.83
M2	b (1.0 m)	Mound	2	16.4	1.81
M2	b (1.0 m)	Mound	3	4.2	1.56
M2	b (1.0 m)	Mound	4	10.3	2.04
M2	b (1.0 m)	Mound	5	12.6	1.85
M2	b (1.0 m)	Pig	1	10	1.74
M2	b (1.0 m)	Pig	2	15.8	1.89
M2	b (1.0 m)	Pig	3	23.3	1.71
M2	b (1.0 m)	Pig	4	14.5	1.84
M2	b (1.0 m)	Pig	5	17.4	1.8
M2	c (2.0 m)	Mound	0	15.7	1.73
M2	c (2.0 m)	Mound	1	14.3	1.92
M2	c (2.0 m)	Mound	2	15	1.71
M2	c (2.0 m)	Mound	3	36.9	1.31
M2	c (2.0 m)	Mound	4	13.9	2
M2	c (2.0 m)	Mound	5	47	2
M2	c (2.0 m)	Pig	1	54.2	1.59
M2	c (2.0 m)	Pig	2	114.7	1.53
M2	c (2.0 m)	Pig	3	16.4	1.91
M2	c (2.0 m)	Pig	4	14.7	2.01
M2	c (2.0 m)	Pig	5	15.9	1.74
M2	d (3.0 m)	Mound	0	18.3	1.87
M2	d (3.0 m)	Mound	1	40.6	1.8
M2	d (3.0 m)	Mound	2	11.5	1.97

Table 30 (continued)

M2	d (3.0 m)	Mound	3	11.7	2.06
M2	d (3.0 m)	Mound	4	11.8	1.82
M2	d (3.0 m)	Mound	5	13.3	1.91
M2	d (3.0 m)	Pig	1	15.5	1.74
M2	d (3.0 m)	Pig	2	16.4	2.1
M2	d (3.0 m)	Pig	3	11.8	1.98
M2	d (3.0 m)	Pig	4	66.7	1.56
M2	d (3.0 m)	Pig	5	10.8	2.05
M2	n (>10 m)	Mound	0	15.6	2.07
FL1	a (0.5 m)	Mound	0	2.9	1.95
FL1	a (0.5 m)	Mound	1	3.4	1.66
FL1	a (0.5 m)	Mound	2	2.5	2.58
FL1	a (0.5 m)	Mound	3	2.8	2.3
FL1	a (0.5 m)	Mound	4	1.8	--
FL1	a (0.5 m)	Mound	5	4.9	2.91
FL1	a (0.5 m)	Pig	1	5.5	2.15
FL1	a (0.5 m)	Pig	2	1.4	2.3
FL1	a (0.5 m)	Pig	3	1.3	2.92
FL1	b (1.0 m)	Mound	0	3.8	2.81
FL1	b (1.0 m)	Mound	1	6.8	1.94
FL1	b (1.0 m)	Mound	2	7.1	2.12
FL1	b (1.0 m)	Mound	3	6.8	1.9
FL1	b (1.0 m)	Mound	4	1.8	2.39
FL1	b (1.0 m)	Mound	5	2.9	3.08
FL1	b (1.0 m)	Pig	1	1.4	1.35
FL1	b (1.0 m)	Pig	2	7.7	2.52
FL1	b (1.0 m)	Pig	3	4.6	2.54
FL1	b (1.0 m)	Pig	4	4.4	3.77
FL1	c (2.0 m)	Mound	0	15.9	2.02
FL1	c (2.0 m)	Mound	1	11.9	1.89
FL1	c (2.0 m)	Mound	2	7.5	1.64
FL1	c (2.0 m)	Mound	3	4	1.88
FL1	c (2.0 m)	Mound	4	6.3	1.66
FL1	c (2.0 m)	Mound	5	7.5	1.65
FL1	c (2.0 m)	Pig	1	10.9	1.94
FL1	c (2.0 m)	Pig	2	5.5	1.8
FL1	c (2.0 m)	Pig	3	6.5	1.82
FL1	d (3.0 m)	Mound	0	4.7	--
FL1	d (3.0 m)	Mound	1	3.4	1.97

Table 30 (continued)

FL1	d (3.0 m)	Mound	2	5.4	1.78
FL1	d (3.0 m)	Mound	3	6.1	2.16
FL1	d (3.0 m)	Mound	4	4.9	1.51
FL1	d (3.0 m)	Mound	5	12.6	2.22
FL1	d (3.0 m)	Pig	2	4.2	2.26
FL1	n (>10 m)	Mound	0	8.9	1.53
FL1	n (>10 m)	Mound	1	2.9	2.51
FL1	n (>10 m)	Mound	2	5.9	2.1
FL1	n (>10 m)	Mound	3	4.1	2.54
FL1	n (>10 m)	Mound	4	4.1	2.52
FL1	n (>10 m)	Mound	5	3.4	1.47
FL2	a (0.5 m)	Mound	0	47.7	2.26
FL2	a (0.5 m)	Mound	1	47.4	1.98
FL2	a (0.5 m)	Mound	2	26.7	1.97
FL2	a (0.5 m)	Mound	3	24.7	1.48
FL2	a (0.5 m)	Mound	4	36.9	1.96
FL2	a (0.5 m)	Mound	5	8.5	1.98
FL2	a (0.5 m)	Pig	1	22.1	1.91
FL2	a (0.5 m)	Pig	2	26.1	1.45
FL2	a (0.5 m)	Pig	3	28.7	1.93
FL2	a (0.5 m)	Pig	4	25.1	1.88
FL2	b (1.0 m)	Mound	0	11.6	3.1
FL2	b (1.0 m)	Mound	1	38	1.91
FL2	b (1.0 m)	Mound	2	8.4	1.17
FL2	b (1.0 m)	Mound	3	10.8	1.92
FL2	b (1.0 m)	Mound	4	20.5	1.95
FL2	b (1.0 m)	Mound	5	18.3	2.03
FL2	b (1.0 m)	Pig	1	27.6	1.92
FL2	b (1.0 m)	Pig	2	10.1	1.87
FL2	b (1.0 m)	Pig	3	14.3	1.88
FL2	b (1.0 m)	Pig	4	10.7	1.81
FL2	c (2.0 m)	Mound	0	10.1	2.01
FL2	c (2.0 m)	Mound	1	15	1.91
FL2	c (2.0 m)	Mound	2	7.7	1.88
FL2	c (2.0 m)	Mound	3	13.8	1.88
FL2	c (2.0 m)	Mound	4	9.9	2.16
FL2	c (2.0 m)	Mound	5	40.3	1.93
FL2	c (2.0 m)	Pig	1	29.1	2.38
FL2	c (2.0 m)	Pig	2	30.7	1.79

Table 30 (continued)

FL2	d (3.0 m)	Mound	0	29.3	2.56
FL2	d (3.0 m)	Mound	1	30.1	1.98
FL2	d (3.0 m)	Mound	2	42.5	1.95
FL2	d (3.0 m)	Mound	3	14.4	2.01
FL2	d (3.0 m)	Pig	1	22.7	1.84
FL2	n (>10 m)	Mound	0	10.6	1.8