Ant predation of blow flies during decomposition and its potential impact on PMI estimations

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Ant predation of blow flies during decomposition and its potential impact on PMI estimations

A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science, John Jay College of Criminal Justice, City University of New York

by

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Ant predation of blow flies during decomposition and its potential impact on PMI estimations

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This Thesis has been presented to and accepted by the Office of Graduate Studies, John Jay College of Criminal Justice in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science.

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**Abstract**

When an organism dies, the process of decomposition begins and many insects are attracted to the decomposing remains, or carrion, as a prospective food source for themselves, or their offspring. Blow flies (Family: Calliphoridae), in particular, are one of the first insects to discover and colonize a dead body due to the ability to detect a decomposing resource from large distances. As a result, forensic entomologists take advantage of the behavior and developmental cycles of early colonizers to calculate the post-mortem interval (PMI) in order to determine the time since death. However, along with the insects that are attracted to the decomposing resource for food, other insect predators that prey upon these primary colonizers are also attracted. A common example of these predators includes the ubiquitous family of ants (Family: Formicidae, Order: Hymenoptera). Little is known regarding the impact that ant scavenging can have on the carrion insect community. To further understand the species interactions between blow flies and ants on carrion, two insect species were used: the green bottle fly, *Lucilia sericata* (Meigen), and the western harvester ant, *Pogonomyrmex occidentalis* (Cresson). Individual olfactory surveys were performed to determine if there was a preference to pork liver with *L. sericata* eggs. Results determined that there was a preference toward pork liver, regardless of the presence of *L. sericata* eggs ($\chi^2 = 9.071$, $p=0.010722$). Microcosm colony trials were also performed investigating the scavenging ability of ant colonies consisting of 30-40 workers. These results determined that *P. occidentalis* scavenging did not have a significant effect on pork liver (ANOVA $p=0.078$, Welch two-tailed $p=0.078$), but did have a significant effect on the removal of blow fly eggs (ANOVA: $F_{1,19}=6.359$, $p=0.021$; Welch t-test: $t_{11.089}=-2.522$, $p=0.028$). These results indicate that although *P. occidentalis* is attracted to fresh pork liver with or without the presence of *L. sericata* eggs, this species is more likely to prioritize blow fly eggs as a food source over fresh liver, exhibiting an average scavenging rate of 0.02149 grams of *L. sericata* eggs/ant/day. In forensic cases where ant populations are high in the vicinity of a corpse, ant scavenging on blow fly eggs could delay blow fly colonization. The results of this experiment help to further our knowledge regarding species’ interactions between ants and blow flies and how this interaction can ultimately effect PMI estimations.
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**Introduction**

Forensic science is the application of the natural sciences, such as physics, chemistry, and biology, to matters of the law. It is the job of the forensic scientist to recognize, collect, preserve, and identify evidence to be used in legal matters, whether it be regulatory, civil, or criminal (DeForest, Gaensslen, & Lee, 1983). In many criminal cases, such as a murder investigation, a deceased individual is discovered and it is in the interest of those involved in the investigation to determine when the person died. The time between the moment a person has died to the moment their body is discovered is called the postmortem interval, or PMI (Bautista, 2012). The PMI can be determined by a number of ways, like noting the stage of decomposition (Goff, 2009), using the temperature of the cooled body, *algor mortis* (Marshall, 1965), or the onset of rigidity due to chemical processes, *rigor mortis* (Krompecher *et al.* 2007). However, these estimations of PMI can be unreliable, particularly when the time of death exceeds 72 hours, as they can be affected by any number of factors, from the weather, pH, humidity, soil quality, changes in temperature, the amount of blood loss, and diseases the decedent acquired before death (Zhou and Byard, 2010). Fortunately, there are reliable means to determine the PMI that can be employed. These methods typically involve studying the organisms that utilize the decomposing remains, also known as carrion, as a food source. Thus, the carrion insect community can provide valuable information regarding the PMI when traditional, pathological means cannot be employed.
Entomology is a branch of zoology that studies insects and other arthropods, so it follows that forensic entomology is the application of such studies to legal matters, including criminal investigations (Hall, 2001). The earliest record of forensic entomology being used dates back to 13th century China when an investigator solved a murder by noting the appearance of blow flies congregating on a farm tool that could have only belonged to the murderer, who was confronted and confessed to the deed (Benecke, 2001). At the same time, the Bubonic Plague ravaged Europe, inspiring works of art and writings dedicated to the interest of the dead and the insects that colonize them. One recurring subject was the "Dance of Death" or Danse Macabre, an artistic allegory which demonstrated how death unites everyone, regardless of status or age. Wood-cuttings, frescos, and illustrations of decomposing bodies with skeletonized heads and snake-sized maggots writhing out of them, depicted the way how decomposition starts from the head downward, in the order of insects colonizing the head’s orifices and body’s soft tissues (Benecke, 2001). Poems like Charles Baudelaire's "Une Charogne" ("A Carcass"), contained in his collection Les Fleurs du Mal (1857), describes the sound of maggot masses "Like water and the wind running/Or corn that a winnower in rhythmic motion/Shake in their winnowing baskets."

Blow flies also played an important role in the development of the scientific method. Aristotle (1910 [c. 343 BCE]) and natural philosophers of his time believed maggots "spontaneously generated" from the secretions of organs of other animals. It was not until 1668 when Italian physician Francesco Redi demonstrated that rotting meat only produces maggots when exposed to air, and does not produce any when it is
sealed, debunking the two-thousand-year-old belief, and proving that life can only come from something that was already living, *Omne vivum ex vivo* (Barnett, 2011).

Insects were first used to estimate the PMI in 1855, by a French hospital physician named Bergeret d'Arbois. Although d'Arbois made many erroneous assumptions, like the metamorphosis of a fly taking a whole year, or laying eggs being limited to the summer season, he properly determined that there were at least 2 different times eggs were laid on the corpse: the first time when it was fresh, eggs were laid by flies, and a second time when the corpse was dried, eggs were laid by moths (Benecke, 2001). This represented one of the first documented works exploring the role of successional waves of insects during decomposition: how certain insect types are attracted to remains at specific points during the decomposition process. In 1894, Jean Pierre Mégnin published a book called *La Faune des Cadavres: Application de l’Entomologie à la Médecine Légale* (The Fauna of Corpses: Application to Forensic Entomology) that was the result of decades of experience studying insects and dead bodies, and contained anatomical information of various arthropod species related to 8 waves of succession during the decomposition of a body. This work was pivotal in the advancement of advanced forensic entomology and brought attention to the predictability of insect behavior and development during decomposition (Benecke, 2001).

When an organism dies, and the heart stops beating, it immediately begins the process of decomposition. The capillaries immediately cease providing blood to the
cells, causing the body to become pale, also known as *pallor mortis* (Schäfer, 2000). The body temperature begins to slowly match the ambient temperature, which is called *algær mortis* (Marshall, 1965). The body's muscle cells continue to use any remaining adenosine triphosphate (ATP) by anaerobic respiration until it is depleted, at which point cells are unable to break the bonds between actin and myosin fibers needed to allow for muscle relaxation (Goff, 2010). The muscles subsequently become stiff and begin to degrade themselves, which is called *rīgor mortis* (Goff, 2010). Without a functioning heart, blood begins to drain to the lower parts of the body due to gravity, discoloring them reddish to bluish-purple and is termed *livor mortis* or lividity (Janaway et al., 2009). Since the blood is unable to provide oxygen or remove carbon dioxide from the tissues, the body's pH becomes increasingly acidic due to accumulating partial pressure of carbon dioxide combining with water in the cytoplasm, creating carbonic acid (Johnson, 2008). The acid destroys the cell structures by denaturing the proteins and causes the release of enzymes that cause further cell destruction, called autolysis (Zapico, 2014). The anaerobic bacteria inside the body's intestines and lungs that were normally kept in check by the immune system, invade the surrounding tissues and begin to consume any sugars, fats, and proteins created by the cells that went through autolysis to reproduce and accumulate (Goff, 2010). Fungi and the aerobic bacteria on the skin start to proliferate because the skin has stopped growing and no longer sheds on its own (Janaway et al., 2009). As the bacteria grow, they expel waste in the form of gases like ammonia, methane, and most importantly, hydrogen sulfide, which can enter hemoglobin and turn it into the green colored sulfhemoglobin, which in turn causes the
appearance of the body to look green and marbled anywhere blood accumulates (Janaway et al., 2009). The gases also force liquids out of natural orifices and causes the body to bloat and become malodorous (Carter et al., 2007). The whole process that involves the destruction of cells, the proliferation of anaerobic and aerobic bacteria, combined with the eating of the flesh by animals, is called putrefaction (Goff, 2010). Decomposition continues until all the soft tissue is consumed by the bacteria, molds, fungi, and animals, and only a skeleton remains.

Insects oviposit, or lay eggs on, a dead organism in a predictable sequence that can be correlated to decomposition (Anderson, 2001). Blow flies (Order Diptera, Family Calliphoridae) are usually the first insects to discover the remains within minutes of death due to their attraction to odor emitted from the body during early decomposition. Once remains are located, gravid females colonize by laying eggs typically in the natural orifices such as the eye sockets, ears, nose and mouth, anus and genital region, exposed body openings, and open wounds, (Byrd & Castner, 2001). The area where oviposition occurs is influenced by the presence of ammonia-rich compounds, moisture levels, pheromones, color, tactile stimuli, and by the presence of other species and other females' eggs (Anderson, 2001). The development of eggs and larvae on the body is highly dependent upon temperature because they are cold-blooded, or poikilothermic animals; higher temperatures result in faster growth, whereas cold temperatures slow or completely halts development (Higley & Haskell, 2001). The pattern of insect succession is dependent on many factors; such as the decomposition state, geographical location of the body (i.e. tropical vs. temperate climates, urban vs. rural), time of day,
season, if the body was indoors or outdoors, clothed or wrapped, submerged or floating in water, placed in a container, buried, burnt or hanged (Anderson, 2001). Each of the aforementioned factors may encourage or delay certain families of insects from colonizing a decomposing body.

The carrion insect community that develops during decomposition consists of many forensically important insects include blow flies (Family: Calliphoridae), flesh flies (Family: Sarcophagidae), house flies (Family: Muscidae), as well as various types of beetles (Order: Coleoptera), moths (Order: Lepidoptera) and wasps (Order: Hymenoptera, Superfamily: Chalcidoidea). Many blow fly species are considered to be necrophagous, meaning they feed directly on decomposing animal tissue for nutrients in order to complete their developmental cycles (Goff, 2010). Both flies and beetles undergo holometabolism, commonly known as complete metamorphosis, where a pupa (puparium for blow flies) is formed to protect the tissues while they are restructured from the larval to adult body form (Holometabolism, 2008). Once a decomposing food source is located, each female blow fly can lay approximately 250 eggs (Greenberg, 1991) which, dependent upon temperature and humidity, will hatch into larvae or "1st instars". However, some dipteran species, such as flesh flies, can retain the eggs in their ovipositor until they hatch, then deposit the larvae on the body (Castner, 2001). Fly larvae (also known as maggots), are tapered anteriorly and do not have any legs, but they have anterior and posterior spiracles and mouth hooks that can be used to differentiate between species and developmental stages (Smith, 1987). Though most blow flies are necrophagous, some species, like Chrysomya rufifacies (Macquart) are
known to be predacious and cannibalistic, feeding on other dipteran larvae as well as the decomposing tissue (Rosati, 2010).

The technique forensic entomologists use to estimate the development rate of insects with consideration to temperature is called accumulated degree hours (ADH), and the amount of ADH needed for a species of insect to reach certain developmental stages have been ascertained through experimentation (Amendt et al., 2007). After consuming enough nutrients and acquiring enough developmental time or ADH, the 1\textsuperscript{st} instar molts its skin and becomes a larger form of larvae called the 2\textsuperscript{nd} instar, followed by the 3\textsuperscript{rd} instar. In flies, the 3\textsuperscript{rd} instar stage can be divided into two phases: feeding and “wandering”. The first phase of the 3\textsuperscript{rd} instar stage is characterized by rapid feeding and high metabolic activity, which can generate enough heat to protect them from the effects of colder temperatures and may also affect their developmental rates (Villet et al., 2010). The second phase occurs when the larvae are no longer attracted to the food source, rather they leave the food source in search of a safe place to pupate or to allow the exoskeleton to harden. The larvae typically burrow down into the surrounding soil or in the folds of remaining skin and clothing and become immobile and form the \textit{puparium} (outer cuticle) or \textit{pupa}.

Forensic entomologists collect samples of the insect species that have colonized the body at various life stages. They also obtain valuable information, regarding the crime scene conditions with particular attention to any factors that could have affected decomposition and colonization (Campobasso \textit{et al.}, 2001) such as habitat
characteristics, site temperatures, weather station reports, in addition to pertinent case information relayed in reports investigators have made (Wells & LaMotte, 2001). With this knowledge, forensic entomologists can backtrack the observed degree hours of development the insects experienced back to the time of oviposition and convert that insect development to a time estimate. The minimum PMI ($PMI_{\text{min}}$) is then estimated by adding the interval between death and oviposition for the species in question (Higley & Haskell, 2001)(See Figure 1). However, as mentioned previously, the PMI may not always correspond directly to the time of oviposition, as there are outside factors that can drastically change the interval between the colonization even and the true time of death.

**Figure 1**: Timeline summarizing events in a generalized death investigation, and indicating maximum ($PMI_{\text{max}}$) and minimum ($PMI_{\text{min}}$ ) estimates of the postmortem interval. The grey boxes associated with each interval are their ‘windows’ of prediction. The spacing of the events is random; some events could be practically simultaneous. (Villet et al., 2010).
Decomposing remains not only attract necrophagous insects, but they also attract insects that prey upon those insects. These insects can be predatory or parasitic. The larvae and adults of beetle (Coleoptera: Silphidae, Staphylinidae, and Histeridae), and fly families (Diptera: Calliphoridae, Muscidae, and Stratiomyidae) are known to prey upon the necrophagous species feeding on the corpse. For instance, many adult beetles destroy eggs, larvae, and adults and take their remains back to nests to feed their own larvae (Bonacci et al., 2011). As mentioned previously, certain fly species, like *Chrysomya rufifacies*, now common in the US and southern region of Ontario (Rosati, 2010) are known to be predatory on other larvae during the later stages of their development (i.e. 2nd and 3rd instar stages) (Goff, 2010). Many wasps (Order: Hymenoptera, Families: Braconidae, Ichneumonidae, and Chalcididae) attack and parasitize dipteran larva and pupae in order to complete their own reproductive cycles (Gaudry, 2010). Other insects that play a role in the insect community that develops during the decomposition process include bees and wasps (Order: Hymenoptera; Families: Apidae and Vespidae), butterflies and moths (Order: Lepidoptera), which have been observed drinking the decomposition fluids from carrion (Smith, 1987). Other incidental insects have been documented as scavenging on human remains such as cockroaches (Order: Blattodea), and katydids (Order: Orthoptera) (Pechal et. al., 2015). If the corpse was found in a body of water, mayflies (Order: Ephemeroptera), stoneflies (Order: Plecoptera), caddisflies (Order: Trichoptera) and aquatic true flies, especially midges (Order: Diptera, Family: Chironomidae), would be considered forensically important, as their presence and life cycles would be used to determine colonization
events (Merritt & Wallace, 2001). Other arthropods like spiders, scorpions, and mites (Class Arachnida) can be found preying on insects or feeding on the corpse. For example, in terrestrial environments, the common woodlouse has been documented as feeding on the skin of decomposing remains (Subphylum Crustacea, Order Isopoda) (Pechal et. al 2015), while in aquatic environments, larger crustaceans like shrimp, crabs, and lobsters (Subphylum Crustacea) have been known to scavenge remains (Smith, 1987). Other insects can be both necrophagous and predatory, consuming from both the corpse and the colonizing insects, such as ants (Order: Hymenoptera, Family: Formicidae).

Ants are an important family of arthropods that comprise 15-20% of terrestrial biomass on average (Schultz, 2000). They are cosmopolitan in their distribution; and exist natively on all continents except Antarctica, as well as isolated islands in the Pacific like Hawaii, and other islands like Greenland and Iceland (Schultz, 2000). Ants live in a wide variety of habitats and have co-evolved and created commensal, mutualistic, parasitic, and mimetic relationships with other species (Wilson and Hölldobler, 2005). As social insects, ants live in colonies that can range from dozens to millions of individuals, depending on the species (Burchill & Moreau, 2016) and the season (Laskis & Tschinkel, 2009). Ants form nests at stable locations, like in the soil, dead wood, or a living tree (Wilson and Hölldobler, 2005), with few species like Army Ants being nomadic (Tschinkel, 2014).
Like flies and beetles, ants are also holometabolous with respect to their development which includes four to five instars while in the larval phase, and as a pupa, they can either form a "naked" imago-shaped pupa or spin a silken cocoon with a pupa inside (Tragust et al., 2013). The larvae are whitish in color, have no segmented legs, have highly developed heads are often covered in hairs or setae (Wheeler, 1918). The well-being of the larvae and pupae are handled by the workers; individuals carry larvae and pupae throughout the nest to optimize their environment as they grow (Wheeler, 1910). In addition, their diet is administered through communal regurgitation, known as trophallaxis until they become large enough to consume whole food (Wheeler, 1918). The egg laying is done by one or multiple gynes, commonly known as "Queens". The average lifespan of a worker can extend up to 3 years, depending on the species, while reproductive queens have considerably longer lifetimes, up to 30 years (Keller, 1998). The genders ratio of the offspring is highly skewed; all worker ants are female, however, some species' workers exhibit different body appearances, also known as polymorphism (Smith et al., 2008). A worker's role in the nest, or "caste", is determined by age, location in the nest (Tschinkel & Hanley, 2017), nutrition as larvae (Smith et al., 2008), and gene expression (Schwander et al., 2010). The gynes, also known as alates are reproductively viable adults that are highly mobile due to the presence of wings. These alates leave their nest and take flight in search of partners to create more colonies, also known as the nuptial flight (Smith, 2000). During the nuptial flight, virginal queens mate with several males to store large amounts of sperm to produce their colonies for the rest of its life. By using the sperm cells acquired during this nuptial flight, the queen can
choose to fertilize her eggs and control the gender of her offspring; fertilized eggs become diploid females and female larvae that are fed more nourishment than other larvae become female gynes (Smith et al., 2008), while unfertilized eggs become haploid male gynes (Van Wilgenburg, Driessen, & Beukeboom, 2006). Ants forage for food to keep the colony alive, akin to homeostasis in organisms; every individual, from the queen, to the larvae, the pupae, and the workers must be maintained or else they will not survive. By using chemical signals known as pheromones, ants can give each other directions that aid in their survival, from directing others to the location of food or to warn others of dangers like predators. Ants constantly renew a volatile trail of pheromones to a potential food source until the colony no longer needs it or if the food site is saturated, which signal the ants to stop renewing the chemical trail and allow it to disappear (Hölldobler and Wilson, 1990 A).

Despite their pervasiveness, ants' contribution to the field of forensic entomology is limited to a small number of case studies or reports noting them and/or their role as opportunistic scavengers that feed off corpses or prey on other insects at all stages of carrion decomposition (Byrd and Castner, 2001). However, there have been several studies that have demonstrated their significant role in the colonization and assembly of the carrion insect community.

The presence of ants has been noted to be able to delay the colonization of a corpse to such an extent that it can throw off the colonization estimate by days. Specifically, the acrobat ant (Hymenoptera: Crematogaster sp.), has been observed
consuming fly eggs and maggots, with the consumption rate so high that the initial colonization was delayed up to 3 days (Byrd and Castner, 2001). In studying the colonization of an adult male corpse with an abdominal wound, Lindgren et al. (2011) noted how the Red Imported Fire Ant (*Solenopsis invicta*, Buren) monopolized the body for 9 days and prevented blow fly colonization until the ants were ejected by the body bloating. Campobasso et al. (2009) noted ants removing eggs and larvae of colonizing flies and beetles, which can be the most populous arthropod feeding off the body. Campobasso lists one case when the colonization time of an ant species determined the PMI:

> In a case of human remains discovered in a metal toolbox, the minimum postmortem interval (PMI) was estimated based on the period required for establishing a colony by ants of the species *Anoplolepsis* (sic) *longipes* (Hymenoptera: Formicidae), and a minimum period of colony development of 12 months was indicated.

Wells and Greenburg (1994) experimented with the Red Imported Fire Ant using different non-human vertebrate carcasses and carrion-baited traps and discovered that their presence directly influenced the consumption rate of the Secondary Screwworm *Cochliomyia macellaria* (Townsend) by removing the superficial larvae leaving only the burrowed larvae to consume the tissue. The fire ants also prevented colonization by *Chrysomya rufifaces* for about 3 to 5 days. Paula et al. (2016) studied several ant species as they interacted with 4 pig (*Sus scrofa*, Linnaeus) corpses in Brazil and noted that the
presence of ants can simultaneously increase the PMI estimation by feeding on necrophagous insects and increase decomposition by feeding on the carrion and creating "wound-like patterns" to allow more insects to colonize. In addition, these artifacts and wounds may be misinterpreted forensically. Lastly, Byard (2005) reported 3 cases where he concluded that postmortem ant activity can mimic antemortem wounds, can modify or erase actual antemortem wounds and can cause extensive hemorrhaging, which in turn increases decomposition and insect activity. All these aforementioned examples illustrate that the role of ants within the insect community cannot be underestimated, particularly in regards to their influence on the colonization estimate and time of the death of a deceased individual.

Research on ant predation and scavenging on vertebrate corpses, especially human bodies, is limited, primarily to few case reports as previously mentioned. Within the field of forensic entomology, data is limited to records of postmortem wounds made by ants (Campobasso et al., 2009), behavioral observations on the aggressiveness of certain species, such as Red Imported Fire Ant, *Soleonopsis invicta* (Lindgren et al., 2011), or a simple compilation of ants encountered during insect successional studies (Maria Lopes de Carvalho and Linhares, 2001; Tabor *et al*., 2005; Bonacci *et al*., 2011; Wang *et al*., 2017). This study provides an important step towards more focused research on the feeding behavior of one of the most populous and widespread groups of scavengers, ultimately enabling forensic entomologists to more accurately determine the postmortem interval in cases where any activity is prevalent. The purpose of this study was to examine the foraging preferences of ants, specifically, if ants would utilize
carrion as a resource and if this varied with the presence of blow flies at various life stages. Study species included a forensically important blow fly species, the Common Green Bottle Fly *Lucilia sericata*, (Meigen) and the Western Harvester Ant *Pogonomyrmex occidentalis*, (Cresson), an ant found in the Western United States and Canada. *Lucilia sericata* is a species of blow fly named for its metallic green-exoskeleton, is cosmopolitan with a worldwide distribution, yet is found primarily in temperate and tropical habitats of the Northern Hemisphere (Byrd and Castner, 2001). This species is one of the first fly species to colonize carrion within hours after death, preferring to oviposit in natural orifices and wounds (Greenberg, 1991; Byrd and Castner, 2001). As with most blow flies, in the wild, females usually only lay eggs between sunrise and sunset (Campobasso et al., 2001). With respect to development, *L. sericata* exhibits optimal growth in regions and seasons where the temperature is above 15.8° C (60.44° F); with larvae entering into a diapause state with metamorphosis halted until the environment is better suited for development (Anderson, 2000). As a result of their developmental temperature thresholds, this species tends to have a higher abundance in warmer climates and in warmer seasons. *Lucilia sericata* has also gained interest due to their larvae being used to consume necrotic tissue from living patients, called "maggot therapy" (Sherman and Tran, 1995). This medical use has led to various protocols to raise them in sterile environments (Sherman and Tran, 1995).

The Western Harvester Ant (*Pogonomyrmex occidentalis*) is located in the western United States and Canada (Janicki et al., 2016). The name "*Pogonomyrmex*, Greek for "bearded ant", refers to the psammophore structure on the ant's head that
looks like a beard of long, curved hairs; its purpose is to aid the ants in carrying large loads of small seeds, eggs, and dry sand, as they build nests in areas with loamy or sandy soil (Lavigne, 1969). In general, harvester ants are named due to their mutualistic relationship with plants and their seeds; they collect seeds with lipid-rich tissue called elaiosomes and store them in their nest as a food source for the colony, which, in turn, allows the plant to disperse their seeds and germinate underground out of the reach of seed predators (Lengyel et al., 2010). Aside from eating seeds, harvester ants have been documented collecting flowers, fruits, leaves, twigs, mosses, lichens, dead invertebrates, and vertebrate feces (Pirk & Lopez de Casenave, 2006). The *Pogonomyrmex* genus is also known for having highly toxic and painful venom compared to other ants; on average, they have an LD$_{50}$ = 0.66 mg/kg, which is over 4 times more lethal than the common honey bee *Apis mellifera* at 2.8 mg/kg (Meyer, 1996). *Pogonomyrmex occidentalis* was selected as a study species due to its distribution, wide range of food preferences and ease of accessibility.

The experiment was carried out in two parts: 1) an olfactory test to determine if *P. occidentalis* responds to pork liver, *Sus scrofa domesticus*, (Erxleben) with or without the presence of *L. sericata* eggs and 2) microcosm experiments on the scavenging rates of *P. occidentalis* on pork liver and on *L. sericata* eggs over a 24-hour period.

With respect to the olfactory trials, if there is no preference for carrion, then it is hypothesized that *P. occidentalis* would not respond favorably or would show an aversion to the presence of pork liver (H$_0$). Alternatively, if there is a preference for
carrion, then individuals would show a biased response towards the presence of pork liver (H_{1A}). In addition, the presence of *L. sericata* eggs on the pork liver could act as an attractant (H_{2A}) or deterrent (H_{2B}) for *P. occidentalis*. With respect to the scavenging trials, if the masses of the liver and *L. sericata* eggs do not show any significant difference (p > 0.05) after 24 hours, then it is hypothesized that *P. occidentalis* colonies do not affect *L. sericata* colonization (H_{0}). Alternatively, if there is a significant amount (p < 0.05) of mass loss from both liver and *L. sericata* eggs, then the ants do affect *L. sericata* colonization (H_{1}). In addition, the colonization can also be affected if only the liver loses significant mass and not the eggs (H_{2}), or if only the eggs lose significant mass over the liver (H_{3}).

**Materials and Methods**

**Colony Conditions**

Wild-type *Lucilia sericata* (Meigen) adults were collected in the Manhattan area using wasp traps baited with fresh pork liver. The wasp traps were placed in multiple locations around Central Park and John Jay College (524 West 59th Street, New York, NY). Traps were emptied and separated by species, then placed in cages kept at John Jay College in New York, NY.

Adult *L. sericata* were kept in a 45 cm x 45 cm x 45 cm cage with a 12 L: 12 D diel light cycle and were fed granulated sugar and powdered milk *ad libitum* in 100 mm x 15 mm Petri dishes. Water was provided in a 250 mL Erlenmeyer flask plugged with a sponge and cheesecloth to prevent flies from entering the flask and drowning. The
rearing cage was maintained at approximately 22°C and between 15-20% humidity (See Image 1).

*Pogonomyrmex occidentalis* was acquired by purchasing them online through the vendor "Ants Alive" (www.antsalive.com) located in Utah. Workers were divided amongst six (6) 7-1/8" x 4-3/8" x 5-1/2" (LxWxH) plastic containers filled halfway with sandy soil. Each cage had approximately 30 to 40 workers. Ants were fed a diet of sugar and water *ad libitum* by depositing a few milligrams of sugar on the soil and using a spray bottle to dispense water to allow ants to drink water beads from the soil and cage walls. To prevent escape, the top of the cages was secured with mosquito netting and the cages were kept in a Rubbermaid tote box tub (50.8cm x 38.1cm x 12.7cm [LxWxH]) filled with 3 cm of soapy water to collect any escaping individuals. The cages were maintained at approximately 22°C and between 15-20% humidity (See Image 2).

*Image 1*: Rearing cage that held a large number (>100) *L. sericata* adults. The cage bottom is lined with brown paper towels to allow ease of cleaning. The fluorescent lights are attached to a timer that maintains a 12L: 12D diel cycle.
Rearing cage that held about 30-40 *P. occidentalis* worker ants. A mosquito net was fitted underneath the lid to prevent escape. A warning label was applied to advise caution to those unfamiliar with this species of ant whose sting was reported to be very painful.

**Image 2**

**Acquisition of *L. sericata* eggs**

Given the experimental design, *L. sericata* eggs were required as a resource. In order to collect eggs from adult female *L. sericata*, fresh pork liver (*Sus scrofa domesticus*) was placed on a 100 mm x 15 mm petri dish within the cage to provide an oviposition medium. Due to the relative size, diet, gut fauna and skin characteristics, the domestic pig is considered to be an acceptable model species for human decomposition (Schoenly *et al.*, 2006) and is commonly used as a standard resource for forensic entomology research. The liver was left in the adult fly cage for several hours (1-3) until gravid females laid enough eggs on the resource to accommodate the experimental trials. When a large enough number of eggs were deposited (approx. 2000+), the liver and eggs were removed from the cage (See Image 3).
**Image 3:** Example of Pork (*Sus scrofa domesticus*) liver with eggs deposited by *L. sericata*.

**L. sericata Colony Maintenance**

Any excess eggs that were not used for the experimental trials were either disposed of by freeze-killing (placing eggs in a freezer at approximately -20°C for 48 hours) or used to maintain the colony. Colony eggs were placed on fresh pork liver surrounded by a wet paper towel to maintain moisture. This was placed in a 1 L mason jar with 9 cm pine wood shavings to provide a suitable pupation medium for developing larvae. The mason jar was covered with black landscape tarp (14 cm x 14 cm) held with a metal lid ring to prevent escape and to allow adequate air flow (See Image 4a). The liver provided a food resource for the larvae to consume after hatching from the egg stage (See Images 4b and 4c). Rearing jars were kept in a room with an average temperature of 21° C and 30-40% humidity. Larvae were provided liver *ad libitum* to ensure maximum size and proper development. If the liver dried out or was completely consumed, it was replaced with a new piece of fresh liver and moist paper towel. Once the larvae reached the late 3rd instar phase of development and began to wander into
the wood shavings to form puparia, the food resource was removed. When adult flies emerged, the jar was placed in the colony cage and opened to release the newly emerged adults until the jar was mostly vacated, approximately 1-3 days. The jar was then emptied of its contents, cleaned, and reused for additional rearing. The procedure was repeated and the colony was maintained for the duration of the trials.

**Image 4a:** Rearing jar for *L. sericata.*

**Image 4b:** 1st instar *L. sericata* larvae emerging from eggs. (50 x Magnification)
Image 4c: Several 1st instar *L. sericata* larvae on the surface of pork liver. (50 x Magnification)

**Olfactory Survey**

Olfactory experiments using a typical Y-tube set-up have been used to determine the behavior of animals by giving them a binary choice between a control (blank) and a target resource (Bohbot et al., 2013; Bora, Deka, and Sen, 2016; Cooperband et al., 2008). In this experiment, three choices were included to determine if *P. occidentalis* demonstrated a preference/avoidance for carrion (represented through the use of pork liver) [Choice 1] or a preference/avoidance for carrion with *L. sericata* eggs [Choice 2]. A control or blank was also used for each trial [Choice 3]. Given the experimental design included three choices instead of two, as in a typical Y-tube experiment, a new device was constructed to provide an individual worker ant with an equal amount of access to
three choices. This device was constructed using a PVC pipe connector with “W”-shaped, which was purchased from a local hardware store (see Figure 5a). The connector was labeled a "Double-Wye" fitting, and it had four (4) 1.5" circular openings; when held, one opening was toward the holder, and the three other openings included one straight down the pipe fitting and two adjacent paths that veered 45 degrees from the center. This fitting was cut in half and a plastic plane was hot-glued on to the cut edge to allow for observation (see Figure 5b). Sponges were cut and placed in the holes to allow for air flow while also blocking the passageway so that the ants were contained within the device. The main opening, or entry passage, had a hole in the sponge to allow the insertion of tubing or container for the test subject to enter (See Figure 2).

Image 5a: "Double-Wye" PVC pipe fitting, as found in a hardware store.
**Image 5b:** Olfactory maze, testing a *P. occidentalis* colony's willingness to enter using tubing extending to their cage.

**Figure 2:** Illustration of an example olfactory survey trial with the maze. This is a top-down view of the maze with sponge barriers, livers and eggs drawn in. The placement of the test livers was randomly placed in two of the three paths for every set of 10 ants tested.
The experiment consisted of 9 repetitions of 10 trials using 10 individual ants, (N=90). Individual ants were separated from their colony and kept in holding containers and starved for 24 hours. After 24 hours, the ants were introduced individually to the olfactory maze through the single path end. The ant was provided with three choice pathways: one path led to the control (blank), one path led to the fresh liver, and the third path led to liver and *L. sericata* eggs. The placement of these three choices was randomized for each ant and the equipment was rinsed between trials. A stopwatch was used to quantify the amount of time each ant took to make a choice. A choice was determined when an ant maintained their position in a pathway and/or interacted with the resource for over 10 seconds. If an ant chose the blank path and stayed in it for over 10 seconds, or if they could not decide any path within 15 minutes, the ant was removed and it was concluded that the ant was not interested in any of the resources. Any interesting observations that affected the decision making were also noted.

**Scavenging Rate Trials**

Scavenging trials were conducted to determine the rate at which *P. occidentalis* removed *L. sericata* eggs and/or pork liver as a resource. Five ant colonies were created in the method described in the Colony Conditions section on page 17 and are visually depicted in Figure 3. Each colony was used for 2 trials each, replicating the experiment 10 times (N=10). For each trial, a colony of ants was to be exposed to a mass of pork liver (1.722±0.168 g) and *L. sericata* eggs (0.1898 ± 0.1009 g) that were weighed and recorded by analytical balance before the 24-hour period (See Images 6 and 7). The liver
and eggs were placed on a piece of weighing paper and deposited into each of the 5 cages (See Image 8). After 24 hours, the masses of the pork liver and eggs were taken again and recorded (See Images 9A and B). The difference of mass due to the combined efforts of ants, natural moisture loss and the eggs hatching was converted to a fraction of mass loss by using the equation \[(\text{Original mass} – \text{new mass})/\text{Original Mass} = \text{Fraction of mass lost}\]. To account for the fraction of mass lost due to environmental conditions, a control condition was incorporated by placing 10 similar masses of liver and eggs into separate containers without ants for a 24 hour period.

Figure 3: Illustration of a scavenging trial. The white shape represents the weighing paper used to handle the liver after measuring the mass on an analytical balance. The waxy paper prevented soil from sticking to the liver to ensure the accuracy of subsequent mass measurements.
**Image 6:** Fresh pork liver measured out for ant colony cage "A" on December 8th, 2017.

**Image 7:** Total egg mass collected from liver on December 8, 2017. This egg mass was divided into 5 smaller masses of similar size to distribute among the 5 ant colonies. (refer to Appendix Table 5a).
**Image 8:** *P. occidentalis* Colony A after 24 hours exposure to the liver and *L. sericata* eggs. The liver is considerably darker and smaller than seen in Image 6. Note that eggs were removed from the weighing paper and into the upper right corner of the cage.

**Image 9:** Close up of the liver in Ant Colony Cage A after 24 hours exposure. Loss of moisture caused the liver to shrink in area and volume, and darken in color (A). The picture on the right is the egg mass removed from the liver (B).
**Statistical Analysis**

The olfactory survey was analyzed using a Pearson's Chi-square test with a significance level of $p < 0.05$ to determine if there was a significant difference between the counts of *P. occidentalis* choosing the control, the pork liver, and the pork liver with *L. sericata* eggs. The olfactory maze allowed for 3 different choices of equal access, therefore the number of degrees of freedom was 2. For the scavenging trials, the statistical tests used were one-way Analysis of Variance (ANOVA) with Welch Two-Sample t-test assuming variances unequal, the two-tailed hypothesis for liver values and a hypothesis for egg values using a significance level of $p < 0.05$. The analysis was done in regards to the percentage of mass loss between the control liver, the control *L. sericata* eggs and those resources simultaneously exposed to ants. Additionally, in the Welch t-test for *L. sericata* egg scavenging, variances were not assumed equal due to the Levene's test calculating $F_{(1,18)} = 33.665$ based on the mean, making $p < 0.0001$, lower than the significance level of $p = 0.05$. All statistics were calculated using IBM SPSS (Version 24). To calculate the rate of scavenging of eggs, the values for the egg masses removed were divided by the number of the ants in that colony. The number of ants was based on the number initially placed in the colony, which was adjusted with an average 20% mortality die-off rate, which is considered to be reflective of the overall mortality rate experienced by the colonies during the experimental period.
Results

Olfactory Survey

Out of the 90 *P. occidentalis* worker ants selected, 6 of them died during the 24-hour starvation period, thus only 84 trials were recorded. These trials were carried out over a 9-day period (See Appendix, Table 1). Out of the 84 ants that took the survey, 15 chose the control, 34 chose the liver, and 35 chose the liver with *L. sericata* eggs on them (See Figure 4)( See Appendix, Table 2). There was a significantly higher number of ants drawn to the liver, with or without *L. sericata* eggs present ($\chi^2_{2,84} = 9.071, p = 0.011$)(Appendix, Table 4). As a result, $H_0$, which stated that there was no significant preference/aversion to liver, was rejected and $H_1$, that there was a significant preference toward liver regardless of *L. sericata* egg presence, was supported. The ants also did not show any preference toward liver based on the presence of eggs, thus $H_{2A}$ and $H_{2B}$ are rejected.

*Figure 4:* Bar graph of Olfactory Survey Trials. There was a significant preference for liver ($\chi^2_{2,84} = 9.071, p = 0.011$). Each value is the number of ants that chose that option in that specific trial.
Scavenger Trials

To determine biomass loss due to environmental conditions, measurements were taken for the control Liver and *L. sericata* eggs as well as the experimental liver and *L. sericata* eggs (See Appendix, Tables 5a, and 5b, respectively). With respect to the liver resource, it was determined that there were no significant effects of *P. occidentalis* scavenging on the liver, meaning that the biomass loss of the liver during the 24-hour experimental period was similar between the liver exposed to *P. occidentalis* and the liver that was not exposed to *P. occidentalis* (ANOVA: $F_{1,19} = 3.471, p = 0.079$; Welch t-test: $t_{18}=1.863, p=0.079$)(See Figure 5)(See Appendix Table 6 and Table 7, respectively). Therefore, the null hypothesis $H_0$ could not be rejected and it was concluded that *P. occidentalis* did not have a significant effect on the removal of liver tissue.
Figure 5: Box plot of the fraction of mass lost for control and experimental liver groups. The minimum and maximum values for the control liver group are 0.436 and 0.599, respectively. The minimum and maximum values for the experimental liver group are 0.389 and 0.531, respectively. The variances for both groups were similar (Control: $\sigma^2=0.00249$, Experimental $\sigma^2=0.00195$). There was no significant effect of *P. occidentalis* on the removal of liver tissue (ANOVA: $F_{1,19}=3.471$, $p=0.079$; Welch t-test: $t_{18}=1.863$, $p=0.079$).

With respect to *L. sericata* egg masses, it was determined that *P. occidentalis* had a significant effect on the number of eggs removed from the pork liver (ANOVA: $F_{1,19}=6.539$, $p=0.028$, Welch t-test: $t_{11.089}=-2.522$, $p=0.028$) (See Figure 6) (See Appendix Tables 8 and 9), and enforces the idea that the null hypothesis $H_0$ can be rejected and hypothesis $H_3$ was proven: the scavenging rate of only *L. sericata* eggs by *P. occidentalis*
was statistically significant to affect colonization. The scavenging rate for each colony ranged from 0.01227 to 0.02778 g/ant/day (See Figure 7). The mean scavenging rate was determined to be 0.02149 +/- 0.00809 g of *L. sericata* eggs per ant per day.

**Figure 6:** Box plot of the fraction of mass lost for control and experimental egg groups. The minimum and maximum values for the control liver group are 0.231 and 0.567, respectively. The minimum and maximum values for the experimental liver group are 0.294 and 1.000, respectively. The variances for both groups differed greatly (Control: $\sigma^2=0.00978$, Experimental $\sigma^2=0.8309$). There was a significant effect of *P. occidentalis* on the removal of *L. sericata* eggs (ANOVA: $F_{1,19}=6.359$, $p=0.021$; Welch t-test: $t_{11.089}=-2.522$, $p=0.028$).
Figure 7: Bar graph of *L. sericata* Egg Scavenging Rate per trial. Each value represents the number of grams of *L. sericata* eggs an ant in that trial moved off the pork liver within 24 hours.

**Discussion**

Ants are ubiquitous insects due to their adaptation to almost every biome in the world, including those that have undergone development by humans. In order to survive in those environments, each species of ant have developed their own diet, which can be general or extremely specialized, including feeding only upon fungi that they cultivate within their nests (Hölldobler and Wilson, 1990 B). Additionally, the food an ant species may forage for varies greatly and can range from harvesting honeydew from insects, collecting and storing seeds, raiding other insect nests for eggs and larvae, preying on larger animals, and scavenging (Hölldobler and Wilson, 1990 C). However, within the field of forensic entomology, their impact on the succession of the carrion
insect community has been widely underestimated. This study provides one of the first lab-based experiments examining the behavior of an ant species and its potential to utilize carrion as a resource and the species interactions between ants and blow flies. In this particular study, the olfactory survey demonstrated that even a species called a "harvester ant" which collects and stores seeds as food over winter is opportunistic enough to become predacious on other living insects and can even exhibit necrophagous feeding habits. In this study, *P. occidentalis* was attracted to pork liver as a food resource, but the presence of *L. sericata* eggs and 1st instar larvae (which inevitably hatched from the eggs within the 24-hour sampling period) did not appear to influence the choice of an individual worker ant. With respect to their behavior, although they were attracted to the liver, the ants seem to be repelled by liver with a high moisture content, or liver with an excess of blood. During preliminary trials, however, ants were observed blood directly from the liver like a sponge. If ants were allowed to interact with the liver for a period of time, some ants would get stuck in the pool of blood surrounding the liver and would stop moving. This marked decrease in ant activity may have been due to the effect of coagulating blood, which may have interfered with their breathing by obstructing their spiracles. Given that harvester ants are relatively small in size and commonly occur in regions with sandy soil, excessive moisture and viscous fluids would be disadvantageous to their survival. That being said, this species may readily use carrion as a food source, however, they may tend to avoid areas or entire carcasses should excessive pooling of blood be present.

With respect to the scavenging trials, though the mass loss was not statistically
significant, the experimental liver lost less mass than the control group. A possible hypothesis to explain this discrepancy is that the presence of *L. sericata* eggs in the experimental group may have mitigated the evaporation of moisture by being on top of the liver, or the eggs themselves contributed to a regaining of moisture in the liver they were placed on over the 24-hour period when they hatched. The amount of moisture lost could also be due to the amount of surface area the liver exposed, which may have been reduced due to the presence of the eggs for a period of time. The mass of the maggots themselves may have contributed to the increased mass of the livers in the experimental group if they were hidden inside when the livers were weighed. Regardless of the reason, the statistics show that if the ants contributed to the loss of mass in the livers, it is extremely negligible, at least in the presence of *L. sericata* eggs.

There was also a loss in the *L. sericata* egg masses in both the control and experimental groups. In the control group, the decrease in mass was possibly due to moisture loss since at the time of the control group testing, the humidity was relatively low (15-20%). Under magnification, most of the eggs were still intact, except the ones that were broken when they were being taken apart to be weighed before dividing them among the colonies. Previous research (Kranz *et al.*, 2017; Wall *et al.*, 2001; Davies, 1948) has demonstrated that blow flies, like *L. sericata*, prefer and develop quickly in humid environments, as their mortality increases in drier ones. However, this may be less of a concern considering the immediate area characteristics of the oviposition sites, which are typically much more humid than that of the environment (i.e. bloody liver, open wounds, orifices, etc.). As a result, female blow flies will ovipositz
their eggs in less humid environments because the immediate area has an increased and suitable moisture content for the development of eggs and the subsequent larvae.

When the eggs were exposed to ant scavenging, however, there was a significant loss in egg mass due to two primary factors: the eggs were physically removed from the liver in addition to desiccation effects. It is known that ants are relatively strong for their size; one study (Nguyen et al., 2014) showed the Allegheny mound ant (*Formica exsectoides* Forel) can withstand a tensile strength about 5,000 times its own weight. Upon oviposition, blow fly females also release secretions that will harden and assist in attaching the eggs to the surface of the oviposition medium and will also attach the eggs to each other (Greenberg, 1991). However, when considering the species interactions that can occur between flies and ants, this egg laying strategy may work against them; the ants can remove large clumps of eggs off the liver and back to the nest with little effort. In this particular study, one experimental trial had nearly 100% of the eggs that were placed on the liver removed, leaving only a small number of individual eggs that were physically adhered/embedded into the liver. Considering this experiment used small colonies of 30 to 40 ants, and the egg mass size used was also relatively small (0.1898+/−0.1009 g), it is reasonable to conclude that a wild *P. occidentalis* colony numbering in the thousands could remove a significant number of blow fly eggs, potentially delaying colonization and causing inaccuracies in the colonization estimates, and ultimately the PMI estimation.

In addition to these experiments, additional scavenger trials were conducted with more mature forms of *L. sericata*. Though these data were preliminary and lack the
appropriate number of replications, some important observations were noted. Personal observations indicated that *P. occidentalis* would attack any insect they perceived as an intruder, and when adult flies were on the grounds near their colony they would react by tearing the body into pieces. In one scavenger trial with 3rd instar larvae, 10 larvae were placed in one ant colony, and only one was left after the 24-hour period. The larva was presumed to be dead until it was observed under the microscope where the internal tissue and respiratory tract was still functioning (see Image 11). It was hypothesized that the larva's muscles may have been paralyzed due to the potent and pain-inducing venom the *Pogonomyrmex* species uses as a defense mechanism. Hypothetically, a single worker ant can sting as many targets as it can to paralyze them and collect them at a later date. Thus, *P. occidentalis* can have a significant effect on not only the egg stage, but on the subsequent larval stages as well, and may alter the wandering behavior of the larvae as they enter into the late 3rd instar stage.
Image 11: *Lucilia sericata*, 3rd instar larva, mouth hooks are clearly visible. The larva was unable to make any large movements at the time when this picture was taken, allowing ease of handling and posing. (75 x Magnification).

**Future Research**

For the olfactory survey, the error could have come from several sources, particularly during the starvation period when a number of ants died. Improved protocols could be implemented to ensure the ant’s survival during the 24-hour starvation period. Obviously, further development of the improvised maze and creating more improvements could be made to the olfactory set-up, such as the implementation of a sealed environment which would be beneficial because it would increase accuracy and precision while decreasing bias and error due to uncontrollable factors like
vibrations, outside odors, or residues that may interfere with an ant's senses. Increasing the number of colonies as to prevent using ants a second time for the survey would prevent any biases from those already exposed to the liver and blow fly eggs, although that information could have been shared by the workers when they were returned to their respective colonies.

To reduce error in the scavenger trials, more repetitions could have been incorporated into the experimental design. Also, using different types of prey, such as the 2\textsuperscript{nd} and 3\textsuperscript{rd} instar maggots, pupa, and adults, would increase the amount of information regarding species interactions and the scavenging effects of \textit{P. occidentalis} on \textit{L. sericata} over multiple life stages. Another improvement would be to prepare a colony that allows for higher visibility into the nest chambers to encourage more detailed observations. Finally, using a more temperature-controlled and humidity-controlled environment would definitely prevent some of the errors caused by having low humidity or unstable temperatures in both the olfactory survey and scavenger trials.

An important aspect to consider in the future would be to carry out similar experiments using other ant species to determine if they would also interact with liver and with blow fly eggs. Another aspect to consider would be to examine if the interactions between \textit{P. occidentalis} is similar when presented with different blow fly species or if some blow fly species are preferred over others. An additional aspect would be to increase the complexity of this experiment to evaluate the effect of ant/prey density on attraction or scavenging rates or to incorporate multiple species into the
experimental design. Last, but not least, other forensically important insect species and their various life stages (beetle eggs, larvae, other fly species) should be incorporated to see how they interact with ant species of interest with other forensically important species.
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## Appendix

**Table 1:** Olfactory Survey Data. Choice key is 0 = Control path, 1 = Liver path, 2 = Liver and *L. sericata* eggs path. Time is the approximate amount of time it took the ant to make the choice in minutes and seconds.

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<td>76</td>
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<td>00:23</td>
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<td>21</td>
<td>2</td>
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<td>79</td>
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<td>51</td>
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<td>03:37</td>
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<td>00:25</td>
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<td>1</td>
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<td>15-Nov-17</td>
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<td>82</td>
<td>1</td>
<td>00:37</td>
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<tr>
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<td>15-Nov-17</td>
<td>54</td>
<td>0</td>
<td>15:50</td>
<td>30-Nov-17</td>
<td>83</td>
<td>1</td>
<td>01:06</td>
</tr>
<tr>
<td>12-Oct-17</td>
<td>26</td>
<td>1</td>
<td>07:11</td>
<td>15-Nov-17</td>
<td>55</td>
<td>1</td>
<td>00:48</td>
<td>30-Nov-17</td>
<td>84</td>
<td>1</td>
<td>05:24</td>
</tr>
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<td>1</td>
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<td>16-Nov-17</td>
<td>56</td>
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<td>02:56</td>
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<tr>
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<td>16-Nov-17</td>
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<tr>
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<td>1</td>
<td>01:06</td>
<td>16-Nov-17</td>
<td>58</td>
<td>2</td>
<td>01:31</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2: Olfactory Survey Summary. Each trial lists how many ants participated and what decisions they made. Choice values totaled per trial and were summed at the bottom.

<table>
<thead>
<tr>
<th>Trial #</th>
<th># of Ants</th>
<th>Control</th>
<th>Pig Liver</th>
<th>Pig Liver with L. sericata eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
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</tr>
<tr>
<td>6</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>84</td>
<td>15</td>
<td>34</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 3: Three-way Olfactory Frequencies. SPSS Generated Table. Counts of choices per ant, and their expected numbers if choices were unbiased, and the amount each observation differed from the expected.

<table>
<thead>
<tr>
<th>Choice</th>
<th>Observed N</th>
<th>Expected N</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Choice</td>
<td>15</td>
<td>28.0</td>
<td>-13.0</td>
</tr>
<tr>
<td>Liver</td>
<td>34</td>
<td>28.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Liver and Fly Eggs</td>
<td>35</td>
<td>28.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Chi-Square Test of Olfactory Survey. SPSS Generated Table. The significance is .011, much lower than the expected significance level of 0.05. The null hypothesis is rejected: *P. occidentalis* ants were attracted to liver, and *L. sericata* eggs did not attract nor repel them.

<table>
<thead>
<tr>
<th>Chi-Square Test</th>
<th>CHOICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>9.071</td>
</tr>
<tr>
<td>df</td>
<td>2</td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td>.011</td>
</tr>
</tbody>
</table>
Table 5: Scavenger Trials. The first table (5a) lists the masses and fractions of mass loss over a period of 24 hours for liver and *L. sericata* eggs exposed to *P. occidentalis* worker ants. The second table (5b) lists the masses and fraction of mass loss over a period of 24 hours for control liver and *L. sericata* eggs. All statistics were carried out in SPSS using these values.

**Table 5a:** Experimental trials.

<table>
<thead>
<tr>
<th>Trial # (Colony Letter)</th>
<th># of Ants</th>
<th>Experimental Initial Liver Mass (g)</th>
<th>Exp. Final Liver Mass (g)</th>
<th>Exp. Fraction Liver Mass lost</th>
<th>Exp. Initial Egg Mass on Liver (g)</th>
<th>Exp. Egg Mass Removed from Liver (g)</th>
<th>Exp. Fraction Egg Mass Removed from Liver</th>
<th>Scavenge Rate (g/Ant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A)</td>
<td>33</td>
<td>1.341</td>
<td>0.629</td>
<td>0.5309</td>
<td>0.094</td>
<td>0.079</td>
<td>0.8404</td>
<td>0.02472</td>
</tr>
<tr>
<td>2 (B)</td>
<td>34</td>
<td>1.881</td>
<td>1.149</td>
<td>0.3892</td>
<td>0.329</td>
<td>0.171</td>
<td>0.5198</td>
<td>0.01575</td>
</tr>
<tr>
<td>3 (C)</td>
<td>24</td>
<td>1.805</td>
<td>1.025</td>
<td>0.4321</td>
<td>0.326</td>
<td>0.096</td>
<td>0.2945</td>
<td>0.01227</td>
</tr>
<tr>
<td>4 (E)</td>
<td>36</td>
<td>1.711</td>
<td>0.903</td>
<td>0.4722</td>
<td>0.299</td>
<td>0.299</td>
<td>1.0000</td>
<td>0.02778</td>
</tr>
<tr>
<td>5 (F)</td>
<td>28</td>
<td>1.888</td>
<td>0.988</td>
<td>0.4767</td>
<td>0.266</td>
<td>0.137</td>
<td>0.5150</td>
<td>0.01839</td>
</tr>
<tr>
<td>6 (A)</td>
<td>33</td>
<td>1.743</td>
<td>0.864</td>
<td>0.5043</td>
<td>0.116</td>
<td>0.108</td>
<td>0.9310</td>
<td>0.02738</td>
</tr>
<tr>
<td>7 (B)</td>
<td>34</td>
<td>1.793</td>
<td>0.997</td>
<td>0.4439</td>
<td>0.110</td>
<td>0.108</td>
<td>0.9818</td>
<td>0.02975</td>
</tr>
<tr>
<td>8 (C)</td>
<td>24</td>
<td>1.730</td>
<td>0.876</td>
<td>0.4936</td>
<td>0.118</td>
<td>0.037</td>
<td>0.3136</td>
<td>0.01306</td>
</tr>
<tr>
<td>9 (E)</td>
<td>36</td>
<td>1.799</td>
<td>0.912</td>
<td>0.4931</td>
<td>0.115</td>
<td>0.05</td>
<td>0.4348</td>
<td>0.01208</td>
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<tr>
<td>10 (F)</td>
<td>28</td>
<td>1.529</td>
<td>0.723</td>
<td>0.5271</td>
<td>0.125</td>
<td>0.118</td>
<td>0.9440</td>
<td>0.03371</td>
</tr>
<tr>
<td><strong>Averages</strong></td>
<td><strong>31</strong></td>
<td><strong>1.722</strong></td>
<td><strong>0.9066</strong></td>
<td><strong>0.4763</strong></td>
<td><strong>0.1898</strong></td>
<td><strong>0.1203</strong></td>
<td><strong>0.6775</strong></td>
<td><strong>0.02149</strong></td>
</tr>
</tbody>
</table>

**Table 5b:** Control Trials.

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Control Initial Liver Mass (g)</th>
<th>Control Final Liver Mass (g)</th>
<th>Control Fraction Liver Mass lost</th>
<th>Control Initial Egg Mass (g)</th>
<th>Control Final Egg Mass (g)</th>
<th>Control Fraction Egg Mass Lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.046</td>
<td>0.820</td>
<td>0.5992</td>
<td>0.156</td>
<td>0.0940</td>
<td>0.3974</td>
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<tr>
<td>2</td>
<td>1.980</td>
<td>0.870</td>
<td>0.5606</td>
<td>0.159</td>
<td>0.0860</td>
<td>0.4591</td>
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<tr>
<td>3</td>
<td>3.122</td>
<td>1.548</td>
<td>0.5042</td>
<td>0.154</td>
<td>0.0920</td>
<td>0.4026</td>
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<td>4</td>
<td>3.995</td>
<td>2.252</td>
<td>0.4363</td>
<td>0.151</td>
<td>0.0960</td>
<td>0.3642</td>
</tr>
<tr>
<td>5</td>
<td>2.975</td>
<td>1.507</td>
<td>0.4934</td>
<td>0.150</td>
<td>0.0820</td>
<td>0.4533</td>
</tr>
<tr>
<td>6</td>
<td>2.105</td>
<td>0.909</td>
<td>0.5682</td>
<td>0.156</td>
<td>0.0930</td>
<td>0.4038</td>
</tr>
<tr>
<td>7</td>
<td>3.100</td>
<td>1.503</td>
<td>0.5152</td>
<td>0.156</td>
<td>0.1200</td>
<td>0.2308</td>
</tr>
<tr>
<td>8</td>
<td>2.667</td>
<td>1.277</td>
<td>0.5212</td>
<td>0.153</td>
<td>0.0660</td>
<td>0.5686</td>
</tr>
<tr>
<td>9</td>
<td>3.417</td>
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<td>0.113</td>
<td>0.0520</td>
<td>0.5398</td>
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<td>0.4570</td>
<td>0.120</td>
<td>0.0570</td>
<td>0.5250</td>
</tr>
<tr>
<td><strong>Averages</strong></td>
<td><strong>2.9429</strong></td>
<td><strong>1.4577</strong></td>
<td><strong>0.5156</strong></td>
<td><strong>0.147</strong></td>
<td><strong>0.0838</strong></td>
<td><strong>0.4345</strong></td>
</tr>
</tbody>
</table>
**Table 6:** Analysis of Variance (ANOVA) of Control and Experimental Liver groups. SPSS Generated Table.

Variance is based on the difference of masses between the Control Liver and Liver exposed to ants. F-test value is 3.471, and when calculated with df values 1 and 18, the significance level is $p = 0.079$, larger than the target significance of $p = 0.05$. The null hypothesis is retained: *P. occidentalis* ants did not affect the loss of mass of liver.

<table>
<thead>
<tr>
<th>ANOVA: Liver groups</th>
<th>The fraction of Liver Mass Lost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of Squares</td>
</tr>
<tr>
<td>Between Groups</td>
<td>.008</td>
</tr>
<tr>
<td>Within Groups</td>
<td>.040</td>
</tr>
<tr>
<td>Total</td>
<td>.048</td>
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</tbody>
</table>
**Table 7:** Welch Two-Sample Two-Tailed t-Test for Liver Groups. SPSS Generated Table calculated with Independent t-Test and one-way ANOVA. We assume variances are considered equal due to Levene’s Test calculating $F_{(1,18)} = 0.089$, making $p = 0.769$, larger than the significance of $p = 0.05$. Due to equal variances, the df = 18. Significance is $p = 0.079$, larger than the target significance of $p = 0.05$. The null hypothesis is retained: *P. occidentalis* ants did not affect the loss of mass of liver.

**ANOVA: Welch Two-Sample Two-tailed t-test**

<table>
<thead>
<tr>
<th>The fraction of Liver Mass Lost</th>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>3.471</td>
<td>1</td>
<td>17.739</td>
<td>.079</td>
</tr>
</tbody>
</table>

**Independent Samples t-Test: Liver**

<table>
<thead>
<tr>
<th>The fraction of Liver Mass Lost</th>
<th>Equal variances assumed</th>
<th>Equal variances not assumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levene's Test for Equality of Variances</td>
<td>$F$: .089</td>
<td>$F$: .089</td>
</tr>
<tr>
<td></td>
<td>$Sig.$: .769</td>
<td>$Sig.$: .769</td>
</tr>
<tr>
<td>t-test for Equality of Means</td>
<td>$t$: 1.863</td>
<td>$t$: 1.863</td>
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<tr>
<td></td>
<td>$df$: 18</td>
<td>$df$: 17.739</td>
</tr>
<tr>
<td></td>
<td>$Sig.$ (2-tailed): .079</td>
<td>$Sig.$ (2-tailed): .079</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>.0392416563009</td>
<td>.0392416563009</td>
</tr>
<tr>
<td>Std. Error Difference</td>
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<td>.0210644040811</td>
</tr>
<tr>
<td>95% Confidence Interval of the Difference</td>
<td>Lower: -.0050130144976</td>
<td>Lower: -.0050597318650</td>
</tr>
<tr>
<td></td>
<td>Upper: .0834963270993</td>
<td>Upper: .0835430444667</td>
</tr>
</tbody>
</table>
Table 8: ANOVA of Control and Experimental *L. sericata* Egg groups. SPSS Generated Table. Variance is based on the difference of masses between the Control Eggs and Eggs exposed to ants. F-test value is 6.359, and when calculated with df values 1 and 18, the significance level is p = 0.021, smaller than the target significance of p = 0.05. The null hypothesis is rejected: *P. occidentalis* ants did affect the loss of mass of eggs.

**ANOVA: *L. sericata* Egg Groups**

<table>
<thead>
<tr>
<th>The fraction of Egg Mass Lost</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>.295</td>
<td>1</td>
<td>.295</td>
<td>6.359</td>
<td>.021</td>
</tr>
<tr>
<td>Within Groups</td>
<td>.836</td>
<td>18</td>
<td>.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.131</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 9:** Welch Two-Sample Two-Tailed $t$-Test for egg groups. SPSS Generated Table calculated with Independent $t$-Test and One-way ANOVA. Variances are assumed unequal due to Levene’s test calculating $F_{(1,18)} = 33.665$, making $p < 0.0001$, smaller than the significance $p = 0.05$. Due to unequal variances, the degrees of freedom were calculated to be 11.089. Significance is $p = 0.028$, smaller than target significance of $p = 0.05$. To be a Right-Tailed $t$-Test, the significance is divided by two, making it $p = 0.014$, which is also smaller than $p = 0.05$. The null hypothesis is rejected: *P. occidentalis* ants did affect the loss of mass of eggs.

### ANOVA: Welch Two-Sample Two-tailed $t$-test

<table>
<thead>
<tr>
<th>Statistic</th>
<th>$df_1$</th>
<th>$df_2$</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>6.359</td>
<td>1</td>
<td>11.089</td>
</tr>
</tbody>
</table>

### Independent Samples $t$-Test: *L. sericata* Eggs

<table>
<thead>
<tr>
<th>Levene's Test for Equality of Variances</th>
<th>$F$</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.665</td>
<td>.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-test for Equality of Means</th>
<th>$t$</th>
<th>$df$</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal variances assumed</td>
<td>-2.522</td>
<td>18</td>
<td>.021</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>-2.522</td>
<td>11.089</td>
<td>.028</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-.2430102702456</td>
<td>.0963683743173</td>
<td>-.4454727118310 to -.0405478286602</td>
</tr>
<tr>
<td>-.2430102702456</td>
<td>.0963683743173</td>
<td>-.4549070892501 to -.0311134512411</td>
</tr>
</tbody>
</table>