INTRASPECIFIC GENE FLOW AND VECTOR COMPETENCE AMONG *Periplaneta americana* COCKROACHES (BLATTODEA: BLATTIDAE) IN CENTRAL TEXAS

A Thesis

by

JENNIFER LYNNE PECHAL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2008

Major Subject: Entomology

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Approved by:

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	Jeffery K. Tomberlin
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	Leon H. Russell
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ABSTRACT

Intraspecific Gene Flow and Vector Competence among *Periplaneta americana* Cockroaches (Blattodea: Blattidae) in Central Texas. (August 2008)

Jennifer Lynne Pechal, B.S., Sam Houston State University

Co-Chairs of Advisory Committee: Dr. Roger E. Gold
Dr. Jeffery K. Tomberlin

One of the most overlooked areas in forensic entomology is urban, which applies to insects and their arthropod relatives that have interactions with humans, their associated structures, and companion animals. American cockroaches, *Periplaneta americana* (L.), are common pests of urban environments. Analyzing spatial distribution of *P. americana* populations in an artificial, outdoor environment provided insight of gene flow among populations collected in central Texas. This information provides for a better understanding of how and if populations were segregated, or if there was a single unified population. Populations can be genetically differentiated through determining variation of specific gene regions within populations. This study revealed a ubiquitous distribution of cockroach populations, and their ability to indiscriminately inhabit areas within an urban environment. Overall, cockroaches were identified from a large interbreeding population with no discernable relationship between genetic variation of *P. americana* and spatial distribution.

Identifying cockroach populations is relative to understanding the ability of surrogate species indirectly affecting man by their ability to transfer disease-causing

organisms including bacteria. This may have potentially deleterious health consequences on animal and/or human populations. There are several pathogens associated with cockroaches which are overlooked during diagnosis of sudden ailments with symptoms being similar to food-borne illnesses, including abdominal cramping, diarrhea, nausea, and fever. Analyzing spatial distributions of *Escherichia coli* and *Campylobacter* spp. in relationship to collected cockroaches allowed for prevalence of bacteria species to be identified among populations. The prevalence of bacteria isolated from total populations collected indicated a high prevalence (92.3%) of bacteria carried by the exoskeleton of *P. americana*. Gram-negative bacteria acquisition and dissemination of organisms such as *E. coli* was prevalent on campus. Screening for *E. coli* 1057:H7 and *Campylobacter* spp. resulted in no positive colony growth. The lack of *Campylobacter* spp. growth from cuticular surfaces may have resulted from undesirable conditions required to sustain colony growth. Data from this study corroborates the potential ability of cockroaches to mechanically transmit pathogens.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Forensic entomology is the study of insects and other arthropods as they pertain to legal proceedings. First documented in 13th century China, insects were used to identify a murderer whom committed a crime near a rice field. Ecological succession studies of forensically important species (i.e. Diptera: Calliphoridae) have been conducted since the mid 19th century (Benecke 2001). Species specific biology, ecology, and development data are vital pieces of information used throughout litigations.

Forensic entomology can be categorized into three areas, medical-legal, stored products, and urban (Smith 1986). One of the most overlooked areas in forensic entomology is urban, which applies to insects and their arthropod relatives that have interactions with humans, their associated structures, and companion animals. Formosan termites (*Coptotermes formosanus* Shiraki) (Isoptera: Rhinotermitidae) have been estimated to cost the Southern United States $1 billion/year (Pimentel et al. 2005). Red imported fire ants (*Solenopsis invicta* Buren) (Hymenoptera: Formicidae) have an estimated $300 million/year in damage with an additional $200 million/year allocated for control in Texas (Pimentel et al. 2005). Insects from Blattodea, Hymenoptera,

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This thesis follows the format of the Journal of Economic Entomology.

Coleoptera, and Isoptera are economically important in urban environments.

Damage caused by urban pests is difficult to assess because of additional costs incurred that are not included with pest control treatment estimates. Controlling economically damaging urban pests is a multi-billion dollar industry. One of the more important urban insects is the cockroach (Order: Blattodea) which resides both in and around homes.

Cockroach Biology

Approximately 4,000 cockroach species have been described world-wide (Yilmaz et al. 2004, Triplehorn et al. 2005). Cockroaches, as do termites (Order: Isoptera) date back 350-400 million years (Grimaldi and Engel 2005). The fossil record places these two groups back to approximately the same era (Thorne et al. 2000). Molecular work by Grandcolas and D'Haese (2001) determined that the order Isoptera may be a sister group to the order Blattodea. Inward et al. (2007) supported the previous study and have proposed termites as a clade within the primitive cockroach family, Cryptocercidae; thus, identifying *Cryptocercus* as a sister group to termites. The relatedness of these two groups could allow genetic information known about termites to be applied to the study of molecular variation of cockroaches.

Cockroach habitats are typically tropical; however, they can survive in subtropical and cooler zones so long as they remain indoors or are closely associated with humans. Cockroaches are gregarious insects that can reside in large numbers in small spaces within urban environments. Cockroaches have a paurometabolous

metamorphosis consisting of three stages, which are the egg, nymph, and adult. Food is essential for survival. An immature cockroach can survive approximately 10 d without food, while adults have been documented to last up to six weeks (Baumholtz et al. 1997). Moisture is also instrumental in the longevity of cockroaches, regardless of developmental stage. Adult cockroaches, depending on species, will die in one to four weeks without water. In contrast, they can live at least a year when adequate moisture is present (Baumholtz et al. 1997).

Cockroaches have omnivorous feeding behaviors and are indiscriminate towards sources of potential nutrients. They have been found to feed on feces, blood, and other fluids excreted by humans, prior to contacting human food thus raising concerns of deleterious health consequences for humans (Le Guyader et al. 1989). Cockroaches have been found to feed directly on human tissue as documented with incidences involving neglected and abused children (Denic et al. 1997).

Determining areas with high cockroach densities is medically important because of resulting health problems. Human hypersensitivities to cuticular artifacts and bites from cockroaches are associated with high infestation rates, as well as being instrumental in the vectoring of disease-causing pathogens (Brenner 2002). Asthma costs Americans approximately $12.7 billion annually (Gore and Schal 2007). Cockroach allergies related to skin and lung irritations are problems in low-income housing areas (Baumholtz 1997, Rauh et al. 2002). Allergens produced by cockroaches may lead to broad class allergies to crabs, dust-mites, lobsters, and shrimp (Brenner 2002). Also, in

homes with cockroach infestations, allergens are up to fifty times greater in the kitchen than in any other area of the house (Yin et al. 2001).

Non-physiological ailments may result from the presence of cockroaches. Psychological effects, including but not limited to phobia(s), social stigmas implying a lack of sanitation, and general anxiety may result from the presence of cockroaches (Rivault et al. 1994). Also, these insects are closely associated with animals which may be infected with medically important pathogens; *Blattella germanica* (Linnæus) (Blattodea: Blatteridae) have been found to harbor pathogens in swine production facilities (Lee et al. 2003, Zurek and Schal 2004).

American cockroaches, *Periplaneta americana* (L.) (Blattodea: Blattidae), are considered pests of urban structures (Benson and Zungoli 1997). These cockroaches are approximately 3.8 cm long with red-brown wings with light markings on their pronontum and thorax. The female produces an egg case (ootheca) with 6-14 eggs in parallel rows. A single female has the potential to produce between 210-1440 offspring. Oothecas are generally hidden in crevices in areas neighboring their foraging and shelter locations. Development to complete maturity for *P. americana* can take over a year with 13 molts. American cockroaches can live between two and four years under favorable conditions (Benson and Zungoli 1997). *Periplaneta americana* reside in moist climates and may have population surges after heavy rains (Benson and Zungoli 1997). Temperature plays a role in their activity level. Previous studies indicate cockroaches are suited for 28^{o}C, with a minimum threshold of 10–15^{o}C and a maximum threshold of 33–35^{o}C (Murphy and Heath 1983, Baumholtz et al. 1997).

Population Molecular Analyses

Molecular techniques can be used to identify insect species. Polymerase chain reactions (PCR) use a primer to selectively amplify a targeted sequence of DNA, which can act as a species-specific marker used for identifications. Amplification length and rate of success are based on quality and quantity of DNA extracted. Rates of PCR amplification dropped by 91% when medium-length sequences (300-400 bp) were amplified, versus short-length sequences (100-200 bp) (Franzten et al. 1998). Genetic material primed for amplification may undergo damage, degradation, or are completely unable to replicate during PCR due to small template DNA size, oxidative damage, and/or enzymatic breakdown of the sample (Taberlet et al. 1996, Franzten et al. 1998). Eukaryotic ribosomal RNA (rRNA) is arranged with genes being separated by internal transcribed spacer (ITS) regions, and non-transcribed spacer (NTS) regions. Genes usually occur in tandem repeating units and have NTS regions between repeating segments of RNA, while ITS regions separate genes within each unit. Despite looking at the lesser of the two variable spacer regions, ITS regions still can provide an ample amount of variation to reveal a relatively moderate level of gene flow amongst the given cockroach population in central Texas (Mukha et al. 2007).

Defining a population depends on several factors such as spatial distribution, structures from which collections were made, ecological niches occupied by a population or the general bias of the collector(s) may contribute to the definition of a given “population.” Populations can also be distinguished genetically by analyzing allelic frequencies present in varying populations. Hypothetically, genetic variability

decreases in populations secluded from other populations (Cloarec et al. 1999). In regards to cockroaches, isolated populations may have limited gene fluctuation because of minimal migration from outside populations contributed minimally to an isolated, non-diverse gene pool (Mukha et al. 2007).

Only a few cockroaches are needed to establish a new population in a given area. Mukha et al. (2007) studied *B. germanica* and identified three cockroach populations with substantial genetic differentiation, hence, isolated populations, separated between 15 and 115 km. Conversely, Cloarec et al. (1999) demonstrated limited genetic variation between *B. germanica* populations in two French cities (Rennes and Sète) approximately 900 km apart by analyzing isoenzymatic genetic markers. Previous studies are inconclusive as to whether or not populations analyzed over distances are homologous.

Cockroaches can passively and actively disperse to new locales (Jobet et al. 2000). Active movement appears to be confined to temperate climate zones when alternative ideal habitats are within close proximity (Cloarec et al. 1999). Schoof and Siverly (1954) indicated a lack of dispersal among *P. americana* populations through the sewer system in Phoenix, Arizona, USA. The inability to disperse may have resulted from sewer systems providing an ideal habitat for cockroaches, including ample amounts of water, food availability, and shelter. It appears that when the requisites for life are fulfilled the necessity to actively disperse reduces.

Genetic variation among dispersing populations may result from various genetic events. Such factors include genetic drift, founder effects, natural selection, migration, and gene flow (Jobet et al. 2000). Founder effects are thought to occur more frequently

in cockroach populations because of their ability to establish new populations with a limited number of individuals (Cloarec et al. 1999). Gene flow may be caused by long range passive travel, i.e. people moving location to location with boxes and other storage materials infested with cockroaches. Cloarec et al. (1999) suggested populations within a defined geographical area (i.e. a city) were more homologous than populations compared between greater distances (i.e. city to city). This similarity may result from increased movement of humans within cities compared to the movement of humans between cities and consequently the transfer of cockroaches from one site to the next (Cloarec et al. 1999). Populations separated by variable distances retaining similar allelic frequencies indicate a homologous correlation between populations, hence, gene flow (Cloareac et al. 1999).

Vector Competence of Cockroaches

Vectors are organisms that are capable of transmitting pathogens (Prescott et al. 2005). Arthropods are known to transmit medically important pathogens which have resulted in numerous diseases world-wide (Mullens and Durden 2002). There has been substantial work on the transmission of pathogens by biting arthropods (i.e. Diptera: Culicidae), but the role of non-biting arthropods has not been as thoroughly investigated (Healing 1995, Tatfeng et al. 2005). Vector competence is the capability of an organism (vector) to infect, replicate, and transfer pathogens (Bennett et al. 2002). There are several pathogens associated with cockroaches which are overlooked during diagnosis of

sudden ailments with symptoms being similar to food-borne illnesses including abdominal cramping, diarrhea, nausea, and fever.

Social insect populations can be distinguished by castes or spatial distribution. A structured population can impact the virulence of a pathogen (Fries and Camazine 2001). Small population sizes are more likely to carry pathogens with low virulence when compared to populations with higher numbers of individuals (Fries and Camazine 2001). Gregarious behaviors exhibited by cockroaches may also follow the pattern of reduced virulence due to increased pathogen exposure.

Multiple pathogen transmission routes may occur among populations with infected individuals. Vertical transmission occurs when an infected mother passes on the pathogen or disease to her progeny (i.e. generation to generation). Horizontal transmission occurs within a single generation in which infected individuals pass organisms to other members within the same population. In bees (Hymenpotera: Apidae), horizontal transmission of pathogens have been found to stem from drift, contact between various colonies when foraging, and/or environmental contamination such as water (Fries and Camazine 2001). Horizontal transmission has the potential to decrease virulence of transferred pathogens (Fries and Camazine 2001). Kopanic et al. (1994) determined cockroaches inoculated with a pathogen on their cuticle will transfer pathogens by walking on surfaces, regurgitation, or defecation. Horizontal transmission has been proven under laboratory conditions. Cockroaches inoculated with *Salmonella* transferred bacteria to uninfected cockroaches confined within the same region (Kopanic et al. 1994). The resulting amount of colony forming units transferred to uninfected

roaches varied throughout the study (Kopanic et al. 1994). German cockroaches have been shown to horizontally transmit *Metarhizium anisopliae* from contaminated to non-contaminated cockroaches under laboratory conditions (Quesada-Moraga et al. 2004). Vector-borne pathogens appear to be more virulent than directly-transmitted pathogens (Fries and Camazine 2001).

Cockroaches are important carriers of pathogens due to their unsanitary lifestyle. Cockroaches breed and forage in sewer systems, septic tank areas, garbage bins, and latrine pits (Vythilingam et al. 1997, Mpuchane et al. 2006b). They can then enter urban structures through sewage systems, steam tunnels, and manholes. Specimens collected near sewer covers had bacteria present on them, thus indicating acquisition through foraging in filth laden locations (Barcay 2004). Accessibility to human fecal matter within sewer systems can lead to further distribution of bacterial species via cockroaches (Schoof and Siverly 1954). Untidy residential areas are prime cockroach habitats because of the accessibility of food, water, and shelter. Urban environments are not the only areas susceptible to foraging and harborage of cockroaches. Confined animal facilities also provide ideal environmental conditions for populations to establish, thus creating the potential to spread disease-causing organisms (Fischer et al. 2003).

Cockroaches can transmit bacteria, viruses, protozoa, fungi, and helminthes resulting in food poisoning and a multitude of infections (Rivault et al. 1994). Bacteria accumulation can occur passively through cuticular contact with environmental objects in addition to oral ingestion of food sources containing pathogens (Rivault et al. 1994). Nymphal and adult stages of *P. americana* lack substantial titers of bacterial pathogens

under controlled settings (Barcay 2004). Le Guyader et al. (1989) displayed bacterial fauna similarities between adults and nymphs, thus indicating shared foraging and residential locations. Cockroaches can alternatively spread pathogens as their nymphal cuticle is ecdysed or as they lose body parts (Mpuchane et al. 2006a).

Cockroaches have the ability to carry and transmit pathogens both externally and internally. There is increased diversity of bacterial fauna in the stomach with declining fauna in the intestine, and the least amount of diversity occurring on external surfaces (Elgderi et al. 2006). Mpuchane et al. (2006a) determined *B. germanica* collected from kitchens in Botswana had an average bacterial load of $\log_{10}$ 5.8-7.4 colony forming units. Fischer et al. (2003) indicated a high rate of pathogen transfer during nocturnal periods, when the majority of cockroach species are most active.

There is a positive association between the bacterial fauna of an environment and the diversity of bacteria carried by cockroaches (Rivault et al. 1994). Additionally, Rivault et al. (1994) determined through mark and recapture experiments, the ability of cockroaches to move from floor to floor within an urban structure and from location to location within the same building. Population movements within a single structure has unknown contamination rates because the vector competence of cockroaches has yet to be fully determined. Microorganisms can affect humans in different ways depending on inoculating dose and health of the infected person. It may require from one hundred to thousands of cells for an adverse reaction to occur (Healing 1995).

Healing (1995) described pathogen associations of cockroaches studied in apartment complexes in Rennes, France. He determined comparable bacterial

composition within each apartment, but with low levels of species overlap between facilities (Healing 1995). The lack of species complexities could result from cockroaches traveling similar routes (i.e. sewers) to different dwellings, resulting in continued exposure to microbes already established in various residential areas and their cockroach populations. Also, once sufficient food and water sources have been established, cockroaches will not seek alternative locations, thus reducing bacterial diversity among populations.

In the Federal Territory of Kuala Lumpur, it was determined that *P. americana* had a higher prevalence of bacterial species than other cockroach species collected (Vythilingam et al. 1997). The high rate of prevalence may be indicative of *P. americana* cuticle being more conducive to carrying organisms. An alternative explanation might merely be that there were greater *P. americana* numbers than other cockroach species, resulting in a greater frequency of pathogens.

The decline in incidence of an illness and removal of potential arthropod vectors (i.e. cockroaches) from urban establishments indicates the capability of microorganism transmission through arthropods. Urban buildings can not be completely protected from cockroaches entering the premises, unless a comprehensive pest control program is implemented and maintained on a regular basis. Controlling populations and preventing future population surges is important in reducing the potential for vectoring pathogens. Mechanical exclusion, biological control, sanitation, and chemical controls such as pheromones, insect growth regulators, and pesticides can all be used to control cockroach populations (Benson and Zungoli 1997). Sanitation is an efficient way to

eliminate pest populations because it reduces food and water availability, hence forcing the insects to forage other locations for required nutrients.

A pathogen was once assumed to be viable based on the amount of viable DNA present in comparison to the known amount dead DNA within a cell (Jamil et al. 1993). Keer and Birch (2003) explained that mRNA was volatile, with a relatively short half-life, and that mRNA is a better molecular component to use for viability than DNA based on mRNA having a shorter half-life. Cells deemed viable had considerably less degraded DNA than cells known to be dead (Jamil et al. 1993). Determining a pathogenic organism's viability also requires analyzing the membrane integrity and metabolic activity (Keer and Birch 2003). Using the parameters established by Keer and Birch (2003) suggested the implementation of several molecular techniques to establish the viability of an organism.

Despite bacteria viability being based upon the amount of RNA present, cell death can affect the number and state of cellular components present (Keer and Birch 2003). There is not a single physiological characteristic which acts as good indicator of bacteria viability, and only after several different examination techniques can a proper estimate of viability be established (Lisle et al. 1999).

Colony growth determined by turbidity and/or colony formation is indicative of pathogen viability, given proper nutritional conditions. Pathogens unable to be sustained on media could be interpreted as negative results. However, non-culturable organisms can be possible health concerns, despite not being detected in clinical tests (Keer and Birch 2003).

Bacterial Pathogens Associated with Cockroaches

Cockroaches are known to carry pathogens naturally, as seen in Table 1, but are also known to transmit pathogens such as anthrax, cholera, diphtheria, pneumonia, tetanus, and tuberculosis (Baumholtz et al. 1997). All of these pathogens can be used as bioterrorism agents targeting animal or human populations.

Barcay (2004) implied that several disease outbreaks world-wide, such as dysentery, hepatitis, and gastroenteritis, could be contributed to the spread of pathogens through the environment by mechanical transmission of cockroaches. A few medically important pathogens that are carried by *P. americana* include *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Streptococcus* spp., and a protozoan pathogen, *Toxoplasma gondii* (Barcay 2004, Graczyk et al. 2005).

Cockroach cuticle can harbor several gram-negative bacteria in the group *Enterobacteriaceae* (Mpuchane et al. 2006b). Mpuchane et al. (2006b) suggested the lack of gram-positive bacteria present on the cuticle could result from cockroach secretions that inhibit gram-positive survival. Gram-negative bacteria fauna identified from cockroach cuticle are similar for cockroaches collected from hospitals and restaurant-type facilities (Elgderi et al. 2006). Fewer bacterial species and lower rates of positive prevalence were determined for roaches collected in a residential area (Elgderi et al. 2006). Fungi and yeasts (i.e. *Aspergillus* spp. and *Candida* spp.) have been found on cuticular surfaces of cockroaches collected from intensive care units of a Brazilian hospital (Lemos et al. 2006).

Table 1 – Naturally occurring pathogens (bacteria, fungi, and parasites) associated with cockroaches

Pathogen Classification	Species
Bacteria	*Aeromonas* spp.
	Campylobacter spp.
	Clostridium perfingus
	Enterobacter spp.
	Escherichia coli
	Klebsiella spp.
	Myobacterium leprae
	Pasterurella pestis
	Psuedomonas aerugionosa
	Salmonella oranienburg
	Salmonella bredengy
	Salmonella typhosa
	Shigella alkalescens
	Staphylococcus aureus
	Staphylococcus. spp.
	Streptococcus spp.
Fungus	*Aspergillus* spp.
	Candida spp.
	Penicillium spp.
	Rhizopus spp.
Parasite	*Cyclopsora cayentenensis* (oocysts)
	Entomoeba hystolitica (cysts)
	Hammerschmidtiella diesingi

Salmonella has been found on American cockroaches with up to 7–10 d after initial contact (Schoof and Siverly 1954). Under laboratory conditions, the pronotum of *P. americana* inoculated with *Salmonella enterica,* serotype Oranienburg, produced viable colonies up to 78 d after inoculation (Schoof and Siverly 1954). *Salmonella oranienburg* is also transferred by American cockroach feces to human food sources. Detection of the bacteria on food sources can last for several years after initial inoculation (Barcay 2004). Many of these pathogens can result in gastroenteritis along with other internal and external infections throughout the body, especially in areas with open wounds or other environments favorable for bacterial growth (Barcay 2004). It is evident that cockroaches provide a route of transmission for various pathogens.

Specific disease-causing pathogens commonly associated with cockroaches result in gastro-intestinal related illnesses. *Escherichia coli* and *Campylobacter* spp. transmission has been assumed to occur through mechanical transmission by cockroaches and result in ailments such as diarrhea, abdominal cramps, and fever (Altekruse et al. 1999, Zurek and Schal 2004).

***Campylobacter* species.** *Campylobacter* are microphilic, curved, gram-negative, non-spore forming motile bacteria (Yan et al. 2005). *Campylobacter fetus* (formerly *Vibrio fetus*) is differentiated into three subspecies: *C. fetus, C. interestinales,* and *C. jejuni* (Blaser et al. 1979). The last two subspecies have been detected in humans since 1947 with increasing annual frequency, but it was not recognized as a human pathogen until the early 1970's (Blaser et al. 1979, Butzler 2004). Laboratory tests perfected in 1973 differentiated between the three subspecies (Blaser et al. 1979). *Campylobacter*

spp. was not assumed to be a part of normal human bacterial fauna because it had only been found in patients displaying symptoms such as diarrhea and fever (Blaser et al. 1979). A small infective dose makes *Campylobacter* spp. difficult to isolate as the etiological agent for symptoms as common as fever and diarrhea. Campylobacter enteritis results from *Campylobacter* spp. infections and was characterized by diarrhea, abdominal cramps, malaise, fever, headache, and has a sudden on-set followed by a short duration period (less than a week) (Blaser et al. 1979). In human patients with symptoms of diarrhea, *C. jejuni* has been isolated as the etiological agent more than *Shigella* spp., *Salmonella* spp, or *E. coli* 0157:H7 (Blaser et al. 1979, Blaser 1997). Guillain-Barré syndrome, a demyelinating disease resulting in neuromuscular paralysis, pulmonary muscle deterioration, and death, has been linked to *C. jejuni* infections (Blaser 1997, Sahin et al. 2002).

Diseases associated with this microorganism commonly result from ingesting undercooked poultry, mishandling raw poultry, and cross-contamination of other surfaces (i.e. this bacteria has been found to survive in exposed environments containing oxygen on stainless steel and cotton dishtowel surfaces for over an hour), and survived in untreated water sources (Yan et at. 2005). Contact with infected children, consumption of unpasteurized milk and/or contaminated food products can result in the manifestation of symptoms related to *C. jejuni* infections. Most U.S. citizens become infected while traveling to foreign countries (Blaser et. al 1979, Blaser 1997).

Campylobacter jejuni is enteric in livestock such as cattle, swine, poultry, companion animals (i.e. dogs and cats), and wild animals such as rodents and raccoons

(Blaser 1997, Sahin et al. 2002). An earlier study indicated a relationship between the house fly, *Musca domestica* (L.) (Diptera: Muscidae), tenebrionid adults and larvae, and cockroaches as mechanical vectors of *C. jejuni* in poultry houses (Sahin et al. 2002). An additional link between the pathogen and humans is through cattle, sheep, and other livestock which ingest pathogens from contaminated water sources (Blaser et al. 1979). Consequently, human interactions with livestock increase the potential risk of contamination.

Similar strains of *Campylobacter* have also been found to infect humans and their companion animals, as evidenced by a Danish girl and her dog having the same strain of quinolone-resitant *C. jejuni* (Damborg et al. 2004). Transmission from humans to companion animals is demonstrated by the previous case discussed; however, the mode of pathogen transmission remains uncertain. Arthropods may play a vital role in the transfer of bacterial pathogens in such instances. Erythromycin is commonly used to treat infections with alternatives such as fluoroquinolenes and tetracyclines, but there is evidence that the usage of antibiotics in humans and animals used for consumption is increasing, hence pathogens are becoming more resistant to such courses of treatments (Blaser 1997).

Campylobacter jejuni is susceptible to oxygen in the atmosphere, which may limit grown in moist locations such as livestock feed and water (Sahin et al. 2002). Although, once chickens digest *Campylobacter* spp. and *E. coli*, the organisms may develop in the field better than under ideal laboratory conditions (McGee et al. 2004).

Campylobacter spp. colonization increased 10,000 times that of laboratory growth following expulsion from the digestive tract of poultry (McGee et al. 2004).

Escherichia coli. *Escherichia coli* are gram-negative, rod shaped bacteria with specific strains considered important pathogens occurring in humans and veterinary settings. The most common cause of enteric colibacillosis in piglets is *E. coli* (Zurek and Schal 2004). In human cases, there are several strains with varying effects ranging from mild fevers to hospitalizations and death, depending on the strain acquired. *Escherichia coli* titers in the environment denote the level of fecal contamination (Le Guyader et al. 1989, Rivault et al. 1994) Transmission of these organisms can follow an unsuspected fecal-oral interaction, such as using a contaminated hand towel and then touching food or the mouth area. One *E. coli* strain has been cited as one of the primary causes of Traveler's diarrhea for individuals visiting foreign countries lacking adequate sanitation facilities (Nataro and Kaper 1998).

Escherichia coli 0157:H7 is a medically important strain initially reported in 1982. It can cause bloody diarrhea, hemolytic uremic syndrome (HUS), kidney failure, and death (McGee et al. 2004). This strain of *E. coli* contains genes comparable to the Shiga toxin (Tarr 1995). *Escherichia coli* 0157:H7 has had reported outbreaks in the United States, Great Britain, and Canada with 20,000 infections and 100 deaths in the United States (Michino et al. 1999). Mead et al. (1999) estimated 73,480 *E. coli* 0157:H7 infections with an additional 61 deaths in the United States.

Cattle act as a primary reservoir of *E. coli* 0157:H7 with 2-24% of their fecal material contaminated with the pathogen (McGee et al. 2004). Cattle and other livestock

(i.e. turkeys) feces is contaminated with *Campylobacter* spp. and *E. coli* 0157:H7. Infected fecal material provided a breeding ground for other insects such as filth flies (*Stomoxys calcitrans* (L.) (Diptera: Muscidae), *Tabanus* spp. (L.) (Diptera: Tanabidae), and *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae) to acquire pathogens and therefore becoming mechanical vectors (Szalanski et al. 2004). Outbreaks of *E. coli* 0157:H7 may result from ingestion of contaminated beef or direct contact with contaminated cattle and/or their feces (McGee et al. 2004). The hide of cattle appears to harbor several pathogens, including *E. coli* 0157:H7, which can contaminate the carcasses of cattle (McGee et al. 2004). An *E. coli* 0157:H7 outbreak in Sakai City, Osaka, Japan in 1996 involved 9,451 cases with 12 deaths (Michino et al. 1999). The demographic of those infected was as follows: elementary school children; individuals at child care facilities, nursing homes; an industrial facility; and individuals who ingested a commercially prepared box lunch with unknown origins (Michino et al. 1999). This infection was the result of white radishes carrying the pathogen, which correlates with other studies indicating a presence of *E. coli* 0157:H7 on vegetables and fruits (Michino et al. 1999). A more recent outbreak occurred from July–October 2007 in 10 states (IL, KY, MO, NY, OH, PA, SD, TN, VA, and WI), resulting in 21 reported infections with eight hospitalizations and four HUS patients from ingestion of contaminated pepperoni on frozen pizza (CDC 2007).

Cockroaches could be possible mechanical vectors of nosocomial infections, especially to patients in neonatal units, intensive care, and who are immunocompromised patients (Fotedar et al. 1991, Gliniewick et al. 2003, Elgderi et al. 2006, Salehzadeh et

al. 2007). Nosocomial infections may result from pathogens on contaminated food, a contaminated water supply, and/or unsanitary facilities, like bathrooms (Lemos et al. 2006). *Supella supellectilium* (Serville) have been found to carry opportunistic bacteria species such as *Enterobacter agglomerans*, *Escherichia adecarboxylata*, *Serratia marcescens*, and *Serratia liquefaciens* which cause secondary infections in hospitals (Le Guyader et al. 1989). Salehzadeh et al. (2007) described hospitals infested with cockroaches contained higher bacterial counts than those found residential areas. This association of greater rates of bacteria may result from hospital environments being more conducive to bacterial acquisition from sources of contaminated water, food, and other objects along with safe harborage through contaminated areas. Multiple drug-resistant bacterial strains of medical importance have been isolated from cockroaches in many hospitals (Fotedar et. al 1991, Gliniewick et al. 2003, Elgderi et al. 2006, Salehzadeh et al. 2007). Understanding the nature of pathogen transmission from urban insect pests to humans could clarify the epidemiology of many unknown illnesses. The epidemiology of potentially fatal pathogens needs to be thoroughly examined as they relate to cockroaches.

Determining gene flow among populations collected in central Texas may allow for a better understanding of how and if populations are segregated, or if there is a single, unified population. Currently, a strong link between urban and forensic entomology does not exist. Cases involving abuse or neglect for young children or people in full-care facilities would rely on knowledge of both disciplines to successfully determine biology, development data, and foraging behaviors of alleged species under

investigation. Pathogens are important because they cause medically important infections and diseases within populations. Modes of transmission may be important in identifying sources and dispersal of pathogens by arthropods. Analyzing the pathogen fauna among cockroach populations collected in central Texas will help establish diversity of pathogens carried on their exoskeleton. Also, spatial distribution of bacteria species may indicate the origins of pathogens, acquisition by cockroaches, and distances cockroaches are capable of spreading viable organisms.

Therefore, the objectives and hypotheses of this thesis are:

1. Analyze the gene flow among *Periplaneta americana* cockroach populations in College Station, Texas (central Texas):

   H_o: There is no significant difference in the genetic makeup of field collected *P. americana* samples from discrete sites in central Texas.

   H_a: There is significant and measurable gene flow among field collected *P. americana* samples from discrete sites in central Texas.

2. Determine the epidemiology and/or spatial relationships of *Escherichia coli* and *Campylobacter* spp. associated through mechanical transmission by *Periplaneta americana* cockroach specimens in College Station, Texas (central Texas):

   H_o: There is no geographic relationship for bacteria recovered among field collected *P. americana* samples from discrete sites in central Texas.

   H_a: There are significant differences in the bacteria fauna among field collected *P. americana* samples from discrete sites in central Texas.

CHAPTER II

GENE FLOW AMONG *Periplaneta americana* (BLATTODEA: Blattidae) IN CENTRAL TEXAS

Introduction

Molecular techniques can be used to identify insect species. Polymerase chain reactions (PCR) use a primer to selectively amplify a targeted sequence of DNA, which can act as a species-specific marker used for identifications. Amplification length and rate of success are based on quality and quantity of extracted DNA. PCR amplification rates dropped by 91% when medium-length sequences (300-400 bp) were amplified, versus short-length sequences (100-200 bp) (Franzten et al. 1998). Genetic material primed for amplification may undergo damage, degradation, or are completely unable to replicate during PCR due to small template DNA size, oxidative damage and/or enzymatic breakdown of the sample (Taberlet et al. 1996, Franzten et al. 1998). Eukaryotic rRNA is arranged with genes being separated by internal transcribed spacer (ITS) regions, and non-transcribed spacer (NTS) regions. Genes usually occur in repeating, tandem units and have NTS regions between repeating segments of RNA, while ITS regions separate genes within each strand. Despite looking at the lesser of the two variable spacer regions, ITS regions still can provide an ample amount of variation to reveal a relatively moderate level of gene flow amongst the given cockroach population in central Texas (Mukha et al. 2007).

Defining a population depends on several factors such as spatial distribution, structures from which collections were made, ecological niches occupied by a population, or the general bias of the collector(s) may contribute to the definition of a "population." Differences in allelic frequencies may also be used to distinguish populations. Hypothetically, genetic variability decreases in populations secluded from other populations (Cloarec et al. 1999). In regards to cockroaches (Order: Blattodea), isolated populations may have limited gene fluctuation because of minimal migration from outside populations contributing to the non-diverse gene pool (Mukha et al. 2007).

Only a few cockroaches are needed to establish a new population in a given area. Mukha et al. (2007) identified three *Blattella germanica* (Linnæus) (Blattodea: Blattidae) populations with substantial genetic differentiation, hence, isolated populations separated between 15 and 115 km. In contrast, Cloarec et al. (1999) analyzed isoenzymatic genetic markers from *B. germanica* populations from two French cities (Rennes and Sète) approximately 900 km apart and demonstrated limited genetic variation. Consequently, due to contrasting results in previous studies it is inconclusive as to whether or not populations analyzed over distances are homologous.

Cockroaches can passively and actively disperse to new locales (Jobet et al. 2000). Gene flow may be caused by long range passive travel, i.e. people moving location to location with boxes and other storage infested with cockroaches. The similarity between populations may have resulted from the increased movement of humans within cities when compared to the movement of humans between cities and consequently increased transfer of cockroaches from one site to the next (Cloarec et al.

1999). Active movement appears to be confined to temperate climate zones when alternative, ideal habitats are within close proximity (Cloarec et al. 1999). Schoof and Siverly (1954) indicated a lack of dispersal among American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae), populations through sewer systems in Phoenix, Arizona, USA. This inability to disperse may have resulted from the ideal habitat a sewer system provided, including ample amounts of water, food, and harborage. It appeared that when requisites for life were fulfilled the necessity to actively disperse reduced.

Genetic variation among dispersing populations may result from various genetic events. Genetic drift, founder effects, natural selection, migration, and gene flow are some factors that might contribute to genetic variation (Jobet et al. 2000). Founder effects occur more frequently in cockroach populations due to only required a limited number of individuals to establish new populations (Cloarec et al. 1999). Cloarec et al. (1999) suggested that populations within a defined geographical area (i.e. a city) were more homologous than populations compared between greater distances (i.e. city to city). Populations separated by variable distances retaining similar allelic frequencies indicated a homologous correlation between populations, hence, gene flow (Cloareac et al. 1999).

The objective of this study was to determine gene flow among populations collected in central Texas. This information may allow for a better understanding of how and if populations were segregated, or if there was a single unified population.

Materials and Methods

Sampling Technique for Cockroaches. *Periplaneta americana* (L.) were collected within 50 m of neighboring urban structures in College Station, Texas (Table A-1) and investigated for potential gene flow by phylogenetic analysis among the collected population(s). Collecting sites on campus were selected from locations with the highest cockroach populations based on preliminary trapping. Once locations were established, three collecting containers were placed within a 1.83 m^2 square at each trapping location. Coordinates of each site were determined with a Gormin eTrex® Vista Cx GPS unit (Garmin Ltd., Olathe, KS, USA). Additional samples from other following cities in Texas were obtained from the Texas A&M University Insects in Human Society (ENTO 322) Student Insect Collection including: Pleasanton, Del Rio, Bryan, and Hempstead, Texas. The cockroaches used from the Texas A&M University Insects in Human Society Student Insect Collection were preserved by pinning and stored in boxes turned by the students. Data points for all cockroaches collected were uploaded to Google Earth.

Containers used for collection were glass mason jars (430 ml) coated with Elmer's Acid Free Craft Bond (© Elmer's Products, Inc., Columbus, Ohio, USA) and rolled in Quickrete® Playsand (Quickrete® International, Inc., Atlanta, GA, USA), according to Granovsky (1983). The top 2 cm of the jar opening was lined with H-E-B brand petroleum jelly (H-E-B, San Antonio, TX, USA), and baited with approximately 51.76 ml beer (Miller Brewing Co., Milwaukee, WI, USA) and 7.04 g of H-E-B brand white bread (H-E-B, San Antonio, TX, USA) for specimen collections (Barcay 2004).

Baited containers were placed in the field immediately after adding the beer/bread mixture. Jars were set out prior to dusk and collected from the field after 8-12 h. Once jars were collected from the field, cockroaches were stored in the freezer.

Cockroaches were collected from each jar and stored in individual plastic bags (16.5 x 14.9 cm) with up to three plastic bags containing cockroaches from each site. Collected specimens were stored in a freezer at -20^{o}C until further analyses were conducted. This method should not negatively influence bacterial colony (Szalanski et al. 2004).

Molecular Analysis. Molecular probes were used to identify different haplotypes within each cockroach sample. The hind femur from each specimen was used for genetic analysis. The specific region providing the greatest amount of information about the genetic flow involved the ITS1 region which is located between the 18S and 5.8S gene. Fragments of both the 18S and 5.8S gene, and the entire IST1 region made-up the probe in identification of individuals and their genetic composition from the provided specimens and has been demonstrated in recent studies (Mukta et al. 2007).

A 562-bp section of the nuclear 3' portion of 18S rDNA, all of ITS1 region, and the 5' portion of 5.8S was amplified with the primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') and rDNA 1.58S (5'-GCCACCTAGTGAGCCGAGCA-3') with a thermal cycler profile consisting of 40 cycles of 94°C for 45 s, 53°C for 1 min and 72°C for 1 min as described by Szalanski and Owens (2003) (Vrain et al. 1992, Cherry et al. 1997). Amplified DNA from individual cockroaches was purified and concentrated with minicolumns according to

the manufacturer's instructions (Wizard PCRpreps, Promega). Samples were sent to The University of Arkansas Medical School DNA Sequencing Facility (Little Rock, AR, USA) for direct sequencing in both directions. Consensus sequences were derived from both of DNA sequences from an individual with Bioedit 5.09 to verify nucleotide polymorphisms (Hall 1999).

DNA sequences were aligned by CLUSTAL W (Thompson et al. 1994). The distance matrix option of PAUP* 4.0b10 was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980, Swofford 2001). Maximum likelihood and unweighted parsimony analysis on the alignments were conducted by PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing characters for all analysis. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings with the Branch and Bound algorithm of PAUP*. For maximum likelihood analysis, the default likelihood parameters were used (HKY85 six-parameter model of nucleotide substitution, empirical base frequencies with the exception of the transition/transversion ratio, will be determined). These parameters were used to carry out a heuristic search by PAUP* with a neighbor joining tree as the starting tree. Gene flow was evaluated applying Mitochondrial DNA haplotypes aligned by MacClade v4 (Sinauer Associates, Sunderland, MA). Haplotype distribution between populations, number of haplotypes, number of unique haplotypes, haplotype diversity (h), and nucleotide diversity (pi) was calculated with DNAsp v3.51 and Genealogical relationships among haplotypes were

constructed using TCS, with the method described by Templeton et al. (1992) (Rozas and Rozas 1999, Clement et al. 2000).

Results

DNA sequencing of the ITS1 region from 52 cockroaches samples (Table A-1) resulted an average size of 560 bp. There were 22 haplotypes observed from four Texas counties with the 3 haplotype being the most common (Table 2). There were 25 unique haplotypes. Del Rio, Texas is approximately 462 km from College Station; Pleasanton, Texas has a distance of approximately 274 km from College Station, Texas; TX; Hempstead, Texas is approximately 62 km away from College Station, Texas; Bryan, Texas is a sister city to College Station, Texas separated by approximately 8 km.

There were 41 polymorphic sites (Table 3). The average number of pairwise nucleotide differences was 3.992. Out of the 22 haplotypes there were 25 singletons or unique sequences. Nucleotide diversity, π, was 0.007, and the mean number of pairwise nucleotide differences between haplotypes, *k*, was 3.992.Tajima's D test of neutrality of mutations against excess of recent mutations were not significant (Table 4).

Applying P A U P * version 4.0b10 software, both Neighbor-Joining (NJ) and Maximum Parsimony (MP) analyses were conducted. Results of the NJ tree using uncorrected "P" distances is presented as an unrooted cladogram (Figure 1). For MP analysis, parametric bootstrapping (50% majority-rule) with a full heuristic search was employed for 1000 pseudoreplicates with a starting seed = 632095753. A total of 560 characters were evaluated with all characters equally weighted; 513 characters remained

constant and 20 characters were parsimony informative. Gaps in nucleic sequences were treated as “missing” with the starting tree(s) obtained via stepwise addition. The Branch-swapping algorithm: tree-bisection-reconnection (TBR) was employed. The sum of minimum possible lengths = 48; the sum of maximum possible lengths = 140. A single tree (Figure 1) was produced with length = 113, CI = 0.425 and RI = 0.293. Uncorrected (“P”) distances were used to construct the NJ tree. The distance matrix of 13 haplotypes (Table A-2) is a portion of all of the haplotypes determined from samples collected.

Phylogenetic trees were also obtained using a Bayesian analysis with the GTR+G model by applying Bayesian Evolutionary Analysis Sampling Trees (BEAST) version 1.4.7 software (Drummond and Rambaut 2007). For Bayesian inference, four Markov chains run for 10^6 generations with a burn-in of 2 $x10^4$ were used to reconstruct the consensus tree (Figure 2); MP branch support are presented above the major branches with posterior bootstrapping probabilities presented behind each node (Figure 2).

TCS spanning tree analysis reveled that haplotype 3 had the highest outgroup possibility for all of the 22 haplotypes (Figure 3)

Table 2. Sample sites and haplotypes frequencies from each collection site within Texas counties

City (County)	N	Haplotype (frequency)
Pleasanton (Atascosa)	1	17(1)
Bryan (Brazos)	2	1(1), 5(1)
College Station (Brazos)	48	1(10), 2(1), 3(14), 4(1), 6(1), 7(1), 8(1), 9(1), 10(4), 11 (1), 12(1), 13(1), 14(3), 15(1), 16(2), 18(1), 19(1), 20(1), 21(1), 22(1)
Hempstead (Waller)	1	1(1)

Table 3. Base pair differences between *P. americana* haplotypes from Texas

Haplotype	Nucleotide site															
	N	27	33	52	55	58	67	69	82	85	92	136	137	179	186	198
1	12	T	T	C	C	A	C	A	C	C	A	C	G	C	C	T
2	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
3	14	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4	1	*	*	*	*	*	G	*	*	*	*	*	*	*	*	*
5	1	*	*	*	*	G	G	G	*	*	*	*	*	*	*	C
6	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
7	1	*	*	*	*	*	*	*	*	*	C	*	*	A	*	*
8	1	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*
9	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10	4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
11	1	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*
12	1	*	*	T	T	*	G	*	T	T	C	T	*	A	T	*
13	1	*	*	T	*	*	*	*	T	T	*	T	*	*	*	*
14	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
15	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
16	2	*	*	*	*	*	G	*	T	*	*	*	*	*	*	*
17	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
18	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
19	1	*	*	*	*	*	G	G	*	*	*	T	T	*	*	*
20	1	*	*	T	T	*	*	*	T	T	*	T	*	A	T	*
21	1	*	*	T	T	*	*	*	T	T	*	T	*	A	T	*
22	1	*	*	T	*	*	*	*	T	T	C	T	*	*	*	*

Table 3. continued

Haplotype	Nucleotide site														
	N	199	225	239	264	272	303	314	355	366	437	463	488	514	515
1	12	T	A	G	G	G	G	A	A	G	C	A	C	A	A
2	1	*	*	*	*	*	*	T	T	*	T	*	*	C	*
3	14	*	T	*	*	*	*	*	*	*	*	*	*	*	*
4	1	*	T	*	*	*	*	*	*	*	*	*	*	*	*
5	1	*	T	*	*	*	*	*	*	*	*	*	*	*	*
6	1	*	T	*	*	*	*	*	*	*	*	*	*	*	T
7	1	*	*	G	*	*	*	*	*	*	*	*	*	*	*
8	1	*	*	*	C	*	*	*	*	*	*	*	*	*	*
9	1	A	*	G	*	*	*	*	*	*	*	*	*	*	*
10	4	A	T	*	*	*	*	*	*	*	*	*	*	*	*
11	1	*	*	G	*	*	*	*	*	*	*	*	*	*	*
12	1	A	*	G	*	*	*	*	*	*	*	*	*	*	*
13	1	A	*	G	*	*	*	*	*	*	*	*	*	*	*
14	3	*	*	G	*	*	*	*	*	*	*	*	*	*	*
15	1	A	*	*	*	*	*	*	*	*	*	*	*	*	*
16	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*
17	1	*	*	G	*	G	C	*	*	C	*	C	*	*	*
18	1	*	*	*	*	*	*	*	*	C	*	*	G	*	*
19	1	*	*	G	*	*	*	*	*	*	*	*	*	*	*
20	1	*	*	G	*	*	*	*	*	*	*	*	*	*	T
21	1	*	*	G	*	*	*	*	*	*	*	*	*	*	*
22	1	*	*	G	*	*	*	*	*	*	*	*	*	*	*

Table 4. Summary of statistics for rDNA genetic variation [a]

Sample	*n*	*h*	*s*	Hd	π (*k*)	θ_s	θ_g	D^{+}**	F^{+}*	D*
Texas	52	28	41	0.918 ± 0.025	0.007 (3.992)	0.017	9.29	-3.39	-3.41	-1.94

* $P < 0.05$; ** $P < 0.02$.

[a] *n* is the number of sequences, *h* is the number of haplotypes, *s* is then number of polymorphic sites, Hd is haplotype diversity ± SD, π is nucleotide diversity, *k* is mean number of pairwise nucleotide differences, θ_s is the theta per site, θ_g is theta per gene, D^{+} and F^{+} are statistics per Fu and Li, and F^{+} is Tajima D statistic.

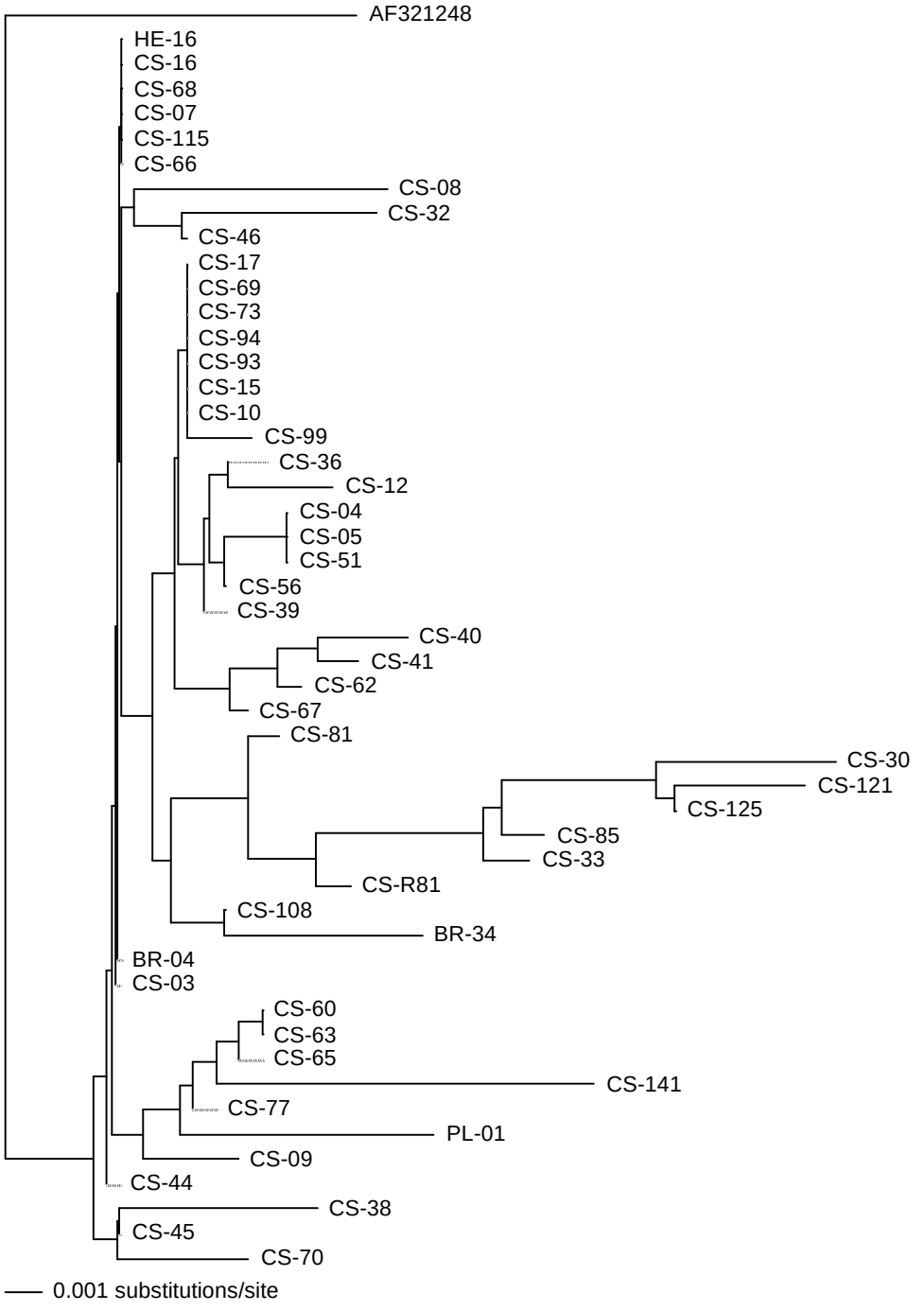

Figure 1. Phylogenetic relationship of *P. americana* rDNA ITS1 region. Neighbor-joining tree with a length = 113, CI = 0.425, and RI = 0.293 resulting from samples collected from quadrants on the Texas A&M University campus College Station, Texas, and from Bryan, Hempstead, and Pleasanton, Texas

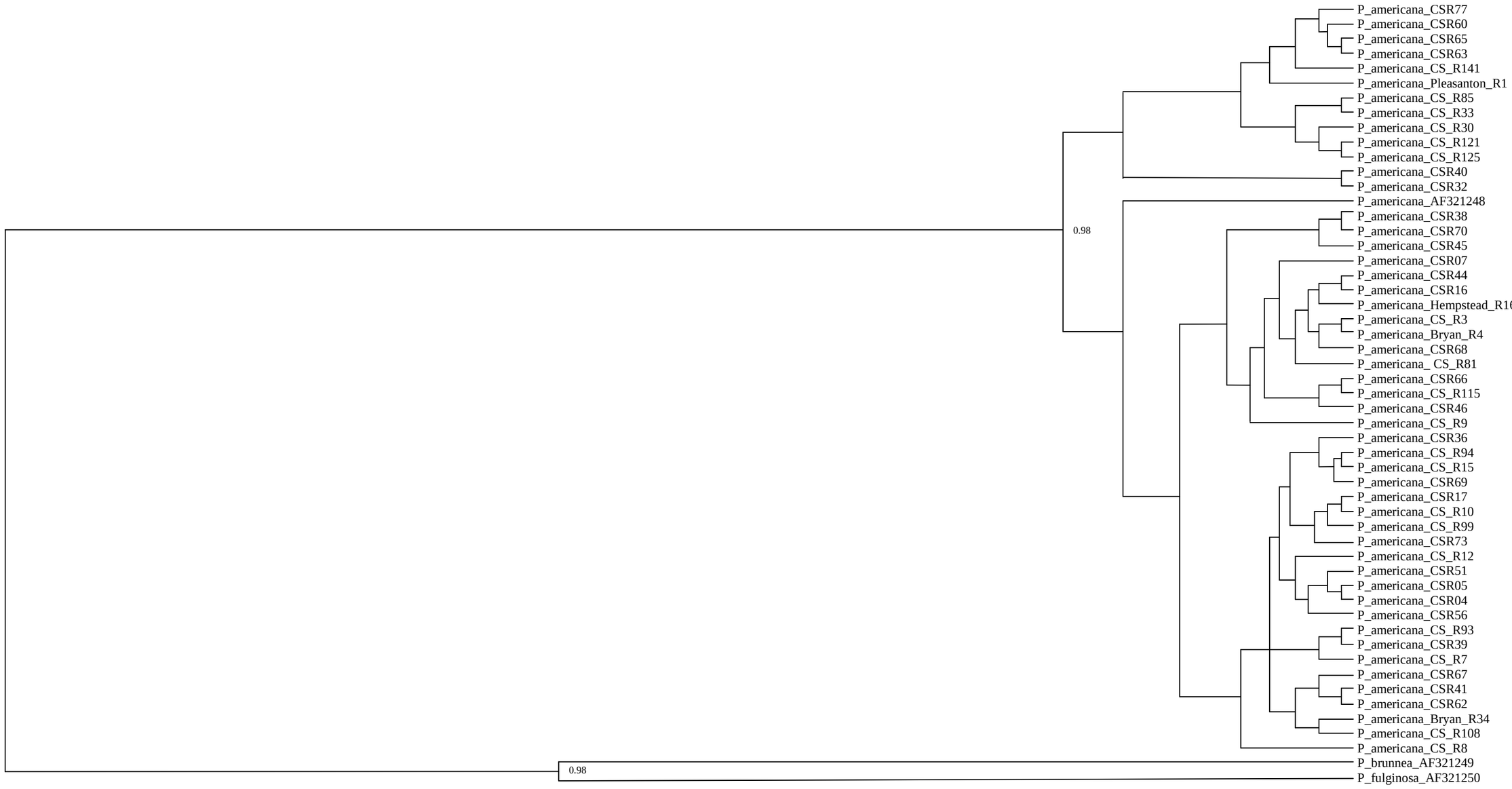

Figure 2. Phylogenetic trees using a Bayesian analysis with MP branch support are presented above the major branches with posterior bootstrapping probabilities presented behind each node for samples collected from quadrants on the Texas A&M University campus College Station, Texas, and from Bryan, Hempstead, and Pleasanton, Texas

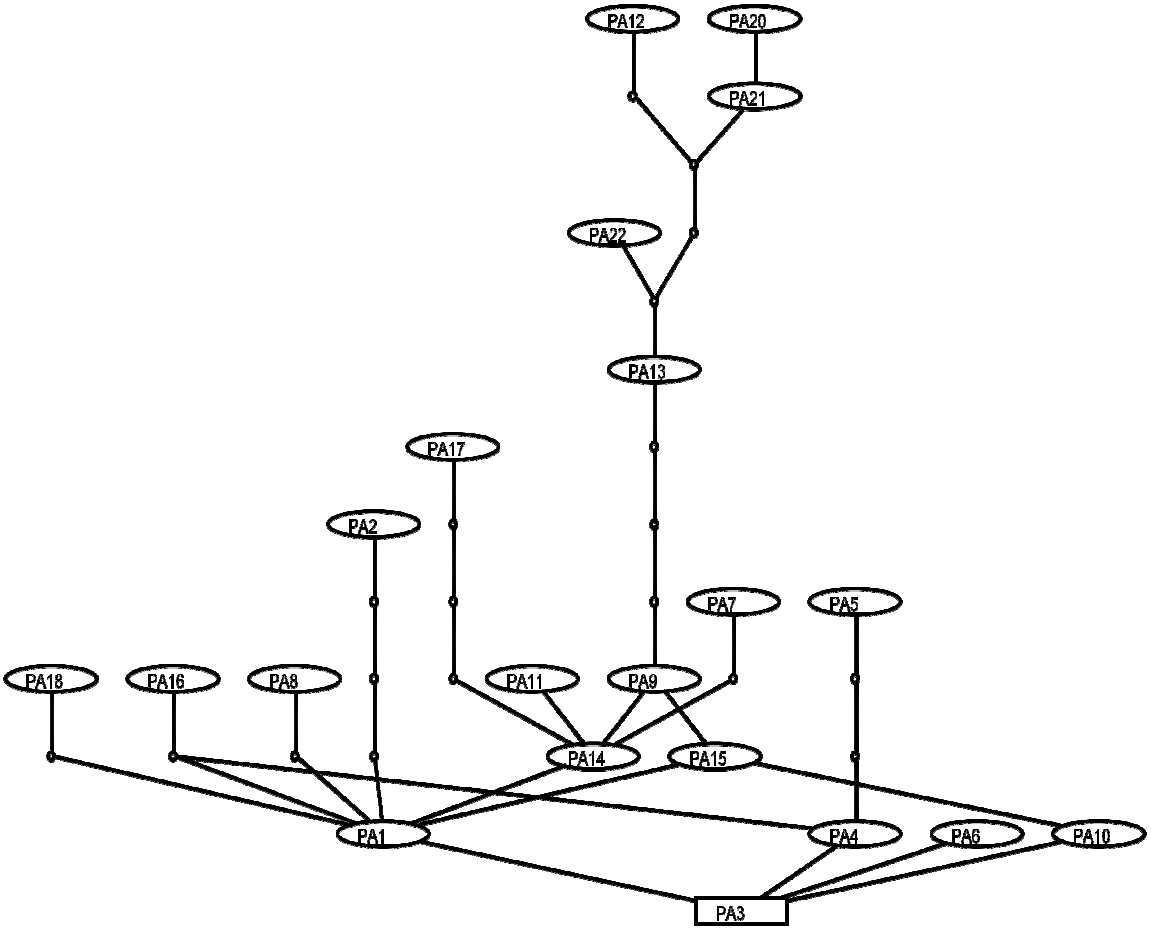

Figure 3. Genealogical relationship among haplotypes of *P. americana* estimated by TCS. The square is the most baysesian haplotype among the collected populations in Texas. Ovals are haplotypes not observed and each branch represents a single mutation

Discussion

The purpose of this study was to analyze the spatial distribution of *P. americana* populations in an outdoor, urban environment and to determine the extent of gene flow among the populations. This study attempted to determine genetic variability among *P. americana* collected on Texas A&M University in College Station, Texas.

Genetic differentiation occurs between populations in diverse locations for all organisms (Austin et al. 2004). Inward et al. (2007) suggested both the orders Isoptera and Blattodea are related, thus their genes would coalesce to a single common ancestor. It can be assumed that the individual lineages would comprise of similar genetic material, thus specific gene regions would be applicable for amplification purposes in both orders. Phylogenetic studies and population genetics performed on termites commonly used the 16S region of the gene for amplification. The 16S region of the gene was initially chosen as the amplification site in this study to determine variability among cockroach populations collected on campus. During this study, the 16S gene region amplification protocol commonly used in termite studies failed to amplify cockroach DNA. Differing genetic compositions of the 16S gene region selected may have resulted from evolution of separate ordinal lineages over time. The universal primers that annealed for termite DNA simply would not work for cockroach DNA and/or the annealing temperature may have been to low thus inhibiting annealing or too high which would damage the primers or DNA. No matter the cause, there was no successful amplification of the 16S gene region, so the ITS1 region was chosen for amplification

because of the availability of comparable sequences available on Genbank (National Center for Biotechnology Information).

The ITS1 region functions in primary rRNA processing and has a higher rate differentiation than the 18S gene region of rRNA (James et al. 1996). Mukha et al. (2007) reported rRNA genes as being the most conserved among populations, while non-transcribed spacer regions have the most variation, and transcribed spacer regions between the two extremes. There are conflicting results when analyzing the ITS1 region for genetic variability in insect populations. Szalanzski et al. (2008) determined a lack of diversity in the nuclear gene region (ITS1 region) with high levels of differentiation when examining the mitochondrial DNA region (16S gene) in *Cimex lectularius* (L.) (Hemiptera: Cimicidae). The ITS1 region may have indicated low levels of diversity in this species at this specific loci (Szalanzski et al. 2008). When the ITS1 region was used, it failed to determine phylogenetic relationships between *Reticulitermes* termites (Tripodi et al. 2006). On the other hand, there was sufficient variability in the ITS1 region used to identify diversity among *Diabrotica* (Coleoptera: Chrysomelidae) species (Szalanski and Owens 2003). Additionally, Szalanski et al. (2000) demonstrated differentiation between *Nicrophorus americanus* (Olivier) (Coleoptera: Silphidae) based on results from the ITS1 region. The current study may have demonstrated biotic homogenization within populations of *P. americana* based on data from the ITS1 region (McKinney and Lockwood 1999).

Haplotypes are defined by at least a single nucleotide difference within the same gene region between sequences thus identifying unique genes. Haplotype diversity is the

number of haplotypes compared to their relative frequency and determined the probability of two sequences chosen from a population being different (Austin et al. 2004).) Tajima's D is a statistical determination of the neutral mutation hypothesis in natural populations (Tajima 1989). Positive values of D indicate population bottlenecks while negative values of D suggest expansion of a population (Tajima 1989). Nucleotide diversity (Pi) in populations assumed neutrality based on the infinite alleles model (Austin et al. 2004).

Among the 52 sampled there were 22 haplotypes indicating a high amount of variation in the population. TCS spanning tree analysis defined lineages from nuclear markers which implied populations moderate levels of gene flow. The lack of isolated populations was reconfirmed by maximum likelihood and Baysian phylogenetic analyses.

Periplaneta americana samples from Bryan, College Station, Hempstead, and Pleasanton, Texas were in a single clade, including *P. americana* sequence obtained from Genbank (AF321248). Sequence comparisons reconfirmed speciation and revealed moderate interbreeding between *P. americana*. The Smokey Brown cockroach (*Periplaneta fuliginosa*) (Serville) and Brown cockroach (*Periplaneta brunnea*) (Burmeister) were chosen as outliers because their sequences were available on Genbank, AF321250 and AF321249, respectively, and are members of the genera as American cockroaches. Comparing various species allowed a broader analysis of *P. americana* to varying genetic sequences as a result of speciation within the same genera. Comparing the 52 sequences amplified to 22 haplotypes suggested a moderate amount of

variation in the population based on nuclear markers. The lack of isolation indicated interbreeding populations on campus. Differentiation of genetic variation based on spatial distribution of *P. americana* populations indicated the success and ability of breeding with independence among various populations.

Cockroaches might be capable of traveling between collecting sites through various migration methods such as walking, climbing, dispersal via steam tunnels and sewer systems throughout campus, and/or depositing their ootheca on materials transferred by humans. Individuals from one collecting site were able to migrate to other sites through any of the previously mentioned methods feasibly because the greatest distance between collecting sites was 1.44 km. Migration of individuals to new locations provided an opportunities for new genetic material to be introduced into a population thus increasing some haplotypic diversity. Szalanski and Owens (2003) suggested lack of variation among southern corn rootworm resulted from motility or population expansion. Diversity among populations collected on campus most likely resulted from the ability of cockroaches to travel successfully in urban environments and breed effectively with cockroaches from other areas. Thus contributing to a constant influx of genetic material into various populations. It remains unknown what degree of genetic variability is observed among other cockroach species.

Genetic variability in populations can be achieved through genetic drift, genetic flow, natural selection, and founder effects (Slatkin 1987). Genetic drift can affect nuclear genes though the fixation of loci in various locations, but gene flow can impede the permanent fixation of the alleles (Slatkin 1987). Lenormand (2002) determined gene

flow limited adaptation of genes to specific locations because new genes from outside sources prevent loci from becoming fixed in the environment. Gene flow can prevent speciation because introduced genetic material can be adapted for survival in a particular environment differing from the population in which it emigrated (Slatkin 1987). Gene flow is an indirect method of determining movement within a population. Bossart and Prowell (1998) indicated the only method that definitively determined gene flow among a population was through the use of genetic tags used to track movement which has had successful in marine organisms. Cloarec et al. (1999) defined gene flow as the movement of cockroaches over long distances by passive transportation, thus increasing the rate of homogenization among the genetic material between populations. Results found in the current study correlated with Cloarec et al. (1999) when they determined German cockroach populations were not isolated in two French cities 900 km apart. Mukha et al. (2007) determined three *B. germanica* populations found in farms separated by 10-100 km and had three populations differentiated by rDNA markers, but they were still not completely isolated. Species, including highly mobile organisms such as cockroaches, disperse through an environment until geographical structures such as oceans, deserts, and mountains impede expansion (Slatkin 1987).

Pesticide use is a common method implemented for supressing cockroach populations. Although increased and prolonged use of the same pesticides can lead to resistance. Lenormand (2002) suggested increased gene flow prevented resistance to pesticides. Introduced genetic material may not have been exposed to similar classes of pesticides thus specimens would be susceptible to novel pesticides locations. Natural

selection differs from genetic drift because not all alleles in different populations are effected the same and gene flow has no consequence on the outcome of genetic variation (Slatkin 1987). Founder effects display a portion of variation existing in the entire population because it comprises of a small number of individuals that colonized a new area (Cloarec et. al 1999 and Mukha et al. 2007).

To date, this study is the first using rDNA markers to identify spatial relationships and gene flow among *P. americana* populations in the United States. Future studies may analyze a broader range of genes including mitochondrial DNA to determine if there are lineages formed by maternal genetic material. Also, analyzing gene flow at several differing sequences within DNA may determine a more comprehensive evolutionary lineage of divergences in cockroach populations.

CHAPTER III

EPIDEMIOLOGY AND SPATIAL RELATIONSHIPS OF BACTERIAL SPECIES ASSOCIATED THROUGH MECHANICAL TRANSMISSION BY *Periplaneta americana* (BLATTODEA: BLATTIDAE) IN CENTRAL TEXAS

Introduction

Arthropods transmit medically important pathogens to humans (Mullens and Durden 2002). Cockroaches (Order: Blattodea) are important vectors of pathogens due in part to their unsanitary lifestyle. Cockroach cuticle can harbor several *Enterobacteriaceae* species (Mpuchane et al. 2006b). A few medically important pathogens harbored by the American cockroach, *Periplaneta americana* (Linnæus) (Blattodea: Blattidae) include: *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Toxoplasma gondii* (Barcay 2004). Cockroaches also reported to transmit pathogens such as anthrax, cholera, diphtheria, pneumonia, tetanus, and tuberculosis (Baumholtz et al. 1997). All of which could be used as bioterrorism agents targeting animal or human populations.

Cockroaches could be competent carriers of nosocomial infection agents, especially to patients in neonatal units, intensive care, and who are immunocompromised (Fotedar et al. 1991, Gliniewick et al. 2003, Elgderi et al. 2006, Salehzadeh et al. 2007). Nosocomial infections may result from pathogens in contaminated food or water, and/or unsanitary facilities, like bathrooms (Lemos et al. 2006). Salehzadeh et al. (2007)

described cockroaches collected in hospitals to have greater bacterial counts than found in residential areas. The association of higher bacteria rates results from hospital environments being more conducive to bacterial acquisition from numerous sources such as water, food, and/or harborage with contaminated objects. Multiple drug-resistant bacterial strains of medical importance have been isolated from cockroaches in several hospitals (Fotedar et. al 1991, Gliniewick et al. 2003, Elgderi et al. 2006, Salehzadeh et al. 2007). Understanding the nature of pathogen transmission from urban insect pests to humans could clarify the epidemiology of many unknown illnesses. The epidemiology of potentially fatal pathogens needs to be thoroughly examined as they relate to cockroaches.

Certain disease causing pathogens commonly associated with cockroaches resulted in gastro-intestinal related illnesses. Pathogens, such as *E. coli* and *Campylobacter* spp., commonly transmitted by cockroaches may be overlooked during diagnosis of sudden ailments with symptoms being similar to food-borne illnesses, including abdominal cramping, diarrhea, nausea, and fever.

Campylobacter are microphilic, curved, gram-negative, non-spore forming motile bacteria (Yan et al. 2005). *Campylobacter* spp. are not part of a normal bacterial fauna in humans but has been found in individuals displaying symptoms such as diarrhea and fever (Blaser et al. 1979). In human patients with symptoms of diarrhea, *C. jejuni* has been isolated to cause diarrhea-like symptoms more than *Shigella* spp., *Salmonella* spp., and *E. coli* 0157:H7 (Blaser et al. 1979, Blaser 1997).

Diseases associated with *Campylobacter* spp. result from ingesting undercooked poultry, mishandling raw poultry, and the cross-contaminating of surfaces, for example this bacteria has been found to survive in exposed environments containing oxygen on stainless steel and cotton dishtowel surfaces for over an hour, and can survive in untreated water sources (Yan et at. 2005).

Campylobacter jejuni is enteric in livestock such as cattle, swine, poultry, companion animals (i.e. dogs and cats), and wild animals such as rodents and raccoons (Blaser 1997, Sahin et al. 2002). *Campylobacter jejuni* is atmospheric desiccation and oxygen which inhibits growth in moist locations such as livestock feed and water (Sahin et al. 2002). Consequently, human interactions with livestock increase the potential risk of contamination.

Escherichia coli are gram-negative, rod shaped bacteria with specific strains considered important pathogens of humans and animals. In human cases, there are several strains that produce varying effects, ranging from mild fevers to hospitalizations and death depending on the strain acquired in its host. *Escherichia coli* titers in the environment corresponded with levels of fecal contamination (Le Guyader et al. 1989, Rivault et al. 1994). Transmission of these organisms can follow an unsuspected fecal-oral interactions, such as using a contaminated hand towel and then touching food or the mouth area.

Escherichia coli 0157:H7 is a medically important strain initially reported in 1982 (McGee et al. 2004). It can cause bloody diarrhea, hemolytic uremic syndrome (HUS), and death (McGee et al. 2004). *E. coli* 0157:H7 had reported outbreaks in the

United States, Great Britain, and Canada, with 20,000 infections and 100 deaths in the United States (Michino et al. 1999).

Pathogens are medically important because of resulting infections and diseases associated with human and/or animal populations. Modes of transmission are important in identifying sources and dispersal of pathogens, such as dissemination by arthropods. Bacterial strains identified were spatially analyzed in this study by determining where various pathogens were in relationship to different cockroach populations. Analyzing the pathogen fauna among populations in a given location could help establish the pathogen diversity cockroaches carry on their exoskeleton based on locations. Also, spatial distribution of bacterial fauna may indicate acquisition locations and distances cockroaches are capable of spreading viable organisms.

The objective of this study was to analyze spatial distributions of *E. coli* and *Campylobacter* spp. in relationship to different cockroach populations. This information may determine the spatial distribution of bacterial fauna and identify locations with high bacterial titers.

Materials and Methods

Sampling Technique for Cockroaches. *Periplaneta americana* (L.) were collected within 50 m of neighboring urban structures in discrete locations in College Station, Texas (Table A-1). Collecting sites on campus were selected from locations with the highest cockroach populations during preliminary trapping. Once locations were established, three collecting containers were placed within a 1.83 m^2 square at each

trapping location. The north quadrant was approximately 0.29 km^2. The central quadrant was approximately 0.40 km^2. The south quadrant was approximately 0.32 km^2, and the west quadrant had an area of approximately 0.58 km^2. Coordinates of each site were determined with a Gormin eTrex® Vista Cx GPS unit (Garmin Ltd., Olathe, KS, USA) and data points uploaded to Google Earth.

Containers used for collection were glass mason jars (430 ml) coated with Elmer's Acid Free Craft Bond (© Elmer's Products, Inc., Columbus, Ohio, USA) and rolled in Quickrete® Playsand (Quickrete® International, Inc., Atlanta, GA, USA), according to Granovsky (1983). The top 2 cm of the jar opening was lined with H-E-B brand petroleum jelly (H-E-B, San Antonio, TX, USA) and baited with approximately 51.76 ml beer (Miller Brewing Co., Milwaukee, WI, USA), and 7.04 g of H-E-B brand white bread (H-E-B, San Antonio, TX, USA) for specimen collections (Barcay 2004). Baited containers were placed in the field immediately after adding the beer/bread mixture. Jars were set out prior to dusk and collected from the field after 8-12 h. Once jars were collected from the field, cockroaches were stored in the freezer.

Cockroaches were collected from each jar and stored in individual plastic bags (16.5 x 14.9 cm), with up to three plastic bags containing roaches from each site. Collected specimens were stored in a freezer at -20^{o}C until further analyses were conducted. This method should not negatively influence bacterial colony (Szalanski et al. 2004).

Screening for *Escherichia coli* Activity. Media used for screening *Escherichia coli* followed the manufacture's recipe of 32.6 g /L of CHROMagar™ ECC media

(CHROMagar, Paris, France). *Escherichia coli* 0157:H7 specific media was made using CHROMagar™ 0157 (CHROMagar, Paris, France) at a 29.4 g/L ratio.

Agar was poured into petri dishes (100 x 15 mm, VWR International, West Chester, PA, USA) making approximately 20 petri dishes/500 ml media. Petri dishes were divided into thirds and appropriately labeled for the specimen. Working under sterile conditions, forceps were flame sterilized using 95% ethanol (EtOH) and cooled prior to touching the cockroach to be plated. Dorsal and ventral sides of each cockroach were plated within their designated areas. Once the cockroach was plated it was moved to an isolated area, the forceps were sterilized using the aforementioned flaming technique. The process was repeated for all *P. americana* collected.

Escherichia coli samples plated on CHROMagar ECC and CHROMagar 0157 were incubated in a Percival Environmental Chamber Model I36LLVL (Percival Scientific, Inc., Perry, IA, USA) at 37^{o}C for 24 – 48 h. *Escherichia coli* colonies were counted by placing each plate on a white sheet of paper (21.6 x 27.9 cm) after incubation. Blue colored colonies were identified as *E. coli*, red colonies were coliform forming bacteria, and colorless colony forming units were non-coliform forming gram-negative bacteria and counted. Screening for *E. coli* 1057:H7 followed the same technique, but with positives indicated by a mauve coloration

Colonies that were positive for *E. coli* were stored in sterile 1.5 ml microtubes with snap caps (VWR International, West Chester, PA, USA) in a 60% Tryptic soy agar (Fisher Scientific, Pittsburg, PA, USA)/40% glycerin (Fisher Scientific, Fair Lawn, NJ, USA), and frozen at -80^{o}C, according to Hanahan et al. (1995).

Screening for *Campylobacter* species Activity. *Campylobacter* specific media was made using the following recipe: 25 ml of defibrinated sheep blood (Colorado Serum Co, Denver, CO, USA), 1 tube of antibiotic premix, 21.5 g BBL™ Brucella agar (BD, Becton, Dickinson and Co., Sparks, MD, USA), and 500 ml distilled water. Antibiotic premix was made by suspending 159.0 mg Cephalothin (MP Biomedicals, LLC., Solon, OH, USA), 50.0 mg Trimethoprim Lactate (Research Products International Corp., Prospect, IL, USA), 100.0 mg Vancomycin hydrochloride (Acros Organics, Morris Plains, NJ, USA), 3.22 mg Polymyxin B (InvivoGen, San Diego, CA, USA), and 20.0 mg Amphotericin B (Acros Organics, Morris Plains, NJ, USA) into100 ml distilled, sterile water. The total antibiotic premixture was divided into 20 tubes each containing 5 ml aliquots, covered with parafilm (American National Can™, Greenwich, CT, USA), and stored in a -20^{o}C freezer.

Agar was poured into petri dishes (100 x 15 mm, VWR International, West Chester, PA, USA) making approximately 20 petri dishes/500 ml media. Petri dishes were divided into thirds and labeled for the appropriate specimen. Working under sterile conditions, forceps were flame sterilized using 95% ethanol (EtOH), and cooled prior to touching the roach to be plated. Dorsal and ventral sides of each cockroach were plated within their designated areas. Once the cockroach was plated it was moved to an isolated area, the forceps were sterilized using the aforementioned flaming technique. The process was repeated for all *P. americana* collected.

Campylobacter spp. specific media was grown in an anaerobic environment for 96 h prior to checking for growth. An anaerobic environment was achieved by placing a

BD BBL ™ CampyPak ™ Plus Microaerophilic system envelope with Palladium catalyst (Becton, Dickinson and Company, Sparks, MD, USA) in an acrylic canister (17.8 x 12.7 cm, Oggi Co., Anaheim, CA, USA) with a chrome locking clamp with a silicone gasket that sealed air tight. *Campylobacter* spp. selective media were removed from the anaerobic environment after 96 h followed by identification and prevalence of colonies.

Campylobacter spp. colonies were frozen at -80^{o}C in a Tryptic soy agar and 15% (wt/vol) glycerin solution in sterile 1.5 ml microtubes with snap caps following the methods of Wasfy et al. (1995).

Koch's Postulates Experiments. Field collected cockroaches were plated on CHROMagar ECC and CFU counts were made after 24 h. Cockroaches were sterilized by shaking individuals in 20 ml of 95% ethanol for 2 min; the cockroaches were plated on CHROMagar ECC and CFU counts were made after 24 h. *Escherichia coli* ATCC25923 cells suspended in sterile saline underwent a 10-fold serial dilution until log-5. Cockroaches were inoculated with 1 ml aliquots and plated. CFU counts were made after 24 h.

Statistical Analysis. JMP Statistical Discovery software version 5.1 (SAS Institute Inc., Cary, North Carolina) was used for the analysis of all results. Oneway ANOVA, $\alpha = 0.05$, was performed to analyze the mean total population numbers collected and quadrant counts. A linear regression, $\alpha = 0.05$, was performed to determine the correlation between mean temperatures and mean population totals collected. Oneway ANOVA, $\alpha = 0.05$, was performed to analyze the mean number of bacteria

colony forming units for *E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative, and quadrant counts. Oneway ANOVA, $\alpha = 0.05$, was performed to analyze the mean number of bacteria colony forming units for *E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative; population stage of development; and quadrant counts.

Results

Cockroaches (N =687) were collected from four designated areas, north, central, south and west, from the Texas A&M University campus College Station, Texas, Figure 4. The mean number of cockroaches collected from Jan–May 2008 was 3.67 ± 4.23 total (3.10 ± 3.31 nymphs and 0.56 ± 1.73 adults). The north quadrant had the lowest mean of cockroaches collected with 1.86 ± 1.60 total (1.86 ±1.25 nymphs and 0.00 ± 0.65 adults). The central quadrant had a mean of 2.21 ± 1.13 cockroaches with 2.14 ± 0.88 nymphs and 0.07 ± 0.46 adults. The south quadrant had a mean of 4.05 ± 0.94 total (3.25 ± 0.74 nymphs and 0.80 ± 0.39 adults). The mean number of cockroaches collected in the west quadrant was 7.29 ± 1.60 total (5.86 ± 1.25 nymphs and 1.43 ± 0.65 adults). There was no significant difference ($F = 2.746$; df = 4, 160; $P = 0.0542$) between population means within quadrants (north, central, south, and west). The mean number of total cockroaches from each location can be seen in Table 5.

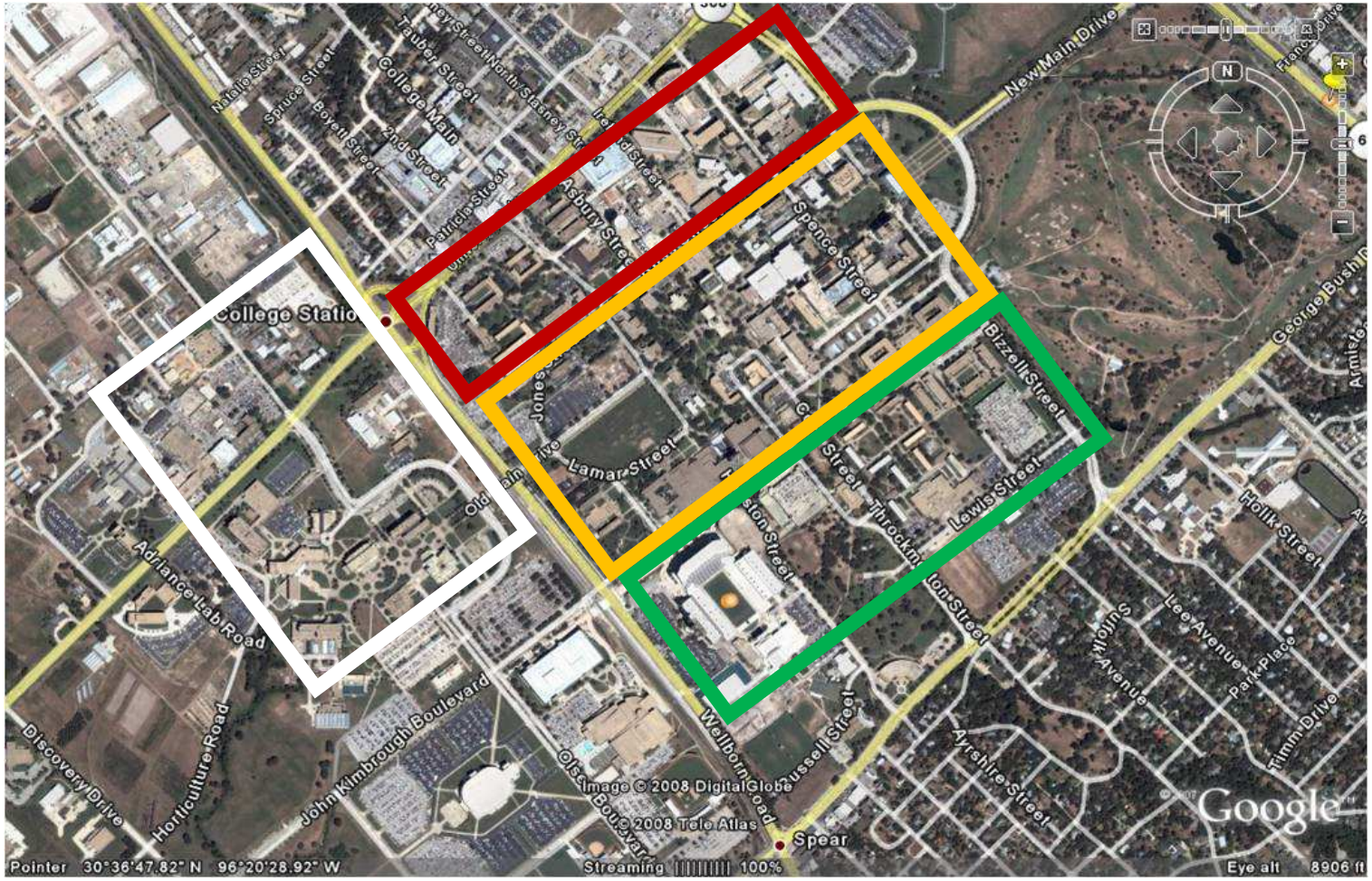

Figure 4. The Texas A&M University campus, College Station, Texas, divided into four areas, north (red), central (orange), south (green), and west (white), used for sampling cockroach populations. Images taken from Google™ Earth Plus v. 4.3

Table 5. Mean number of total cockroach population collected in each quadrant (north, central, south, and west) of the Texas A&M University campus, College Station, Texas

Quadrant	N	Mean ± Std Error [a]	95% Mean	
			Upper	Lower
North	35	1.86 ± 1.60 a	-1.36	5.08
Central	35	2.21 ± 1.23 a	-0.06	4.49
South	78	4.05 ± 0.94 a	2.15	5.95
West	17	7.23 ± 1.60 a	4.07	10.50

[a] Same letters following means within the column were not significantly different ($P < 0.05$, Tukey-Kramer HSD).

Table 6. Positive rates of bacterial (*E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative) prevalence for *P. americana* populations collected on the Texas A&M campus, College Station, Texas, as categorized by building function

Building Type	Cockroach Population [a]
Administration	427/687 (62.2%)
Lecture Building	103/687 (15.0%)
Dining Hall	2/687 (0.3%)
Water Tower	75/687 (10.9%)
Garage	80/687 (11.6%)

[a] Percentages based on the number of cockroaches collected at each building type compared to the total number of cockroaches collected from Feb 2007-May 2008.

There were five categories (Table 6) for the building and/or structures from which cockroach populations were collected adjacent to: administration (primarily offices, some classrooms, and vending machines); lecture buildings (primarily lecture or research areas, some offices, and vending machines); dining halls (food establishments on campus with the primary purpose of food and beverage distribution); water tower; and garage. The prevalence of bacteria on cockroaches for each building type indicated that administration buildings had the highest positive rate of cockroaches, while the dining hall maintained the lowest rate of prevalence on *P. americana* populations.

Comparing the temperature to number of cockroaches collected indicated no significant difference ($F = 0.383$; df = 5, 333; $P = 0.5372$) between the total population means and the mean temperature (Table 7). There was no correlation between mean temperature and total cockroach population means collected for College Station, Texas locations including the quadrants designated on the Texas A&M University campus. R^2 values for north, central, south, west and College Station, Texas populations were, 0.14, 0.18, 0.07, 0.10, and 0, respectively (Table 7).

Koch's postulates tested during this experiment resulted in a R^2 value of 0.932. The prevalence of the bacteria isolated from total populations collected indicated a high prevalence (92.3%) of bacteria on the exoskeleton of *P. americana* (Figure 5). Bacterial screening for *E. coli* resulted in a significant difference ($F = 2.468$; df = 4, 694; $P = 0.0437$) between quadrants (Figure 6). There were also cockroaches that after plated had too many bacterial colony forming units to count. The north quadrant had 1 *E. coli*,

Table 7. A linear regression determined the correlation between total cockroach populations compared to the mean temperature of collection dates, for all quadrants on the Texas A&M University campus, College Station, Texas in addition to undisclosed locations from College Station, Texas not found on campus

Location [a]	N	Mean ± SE [b]	Slope	R^2
North	35	3.34 ± 3.44 a	y = 0.03x + 0.15	0.14
Central	35	4.29 ± 5.04 a	y = -2.04x + 0.29	0.18
South	78	4.47 ± 3.93 a	y = 3.18x + 0.06	0.07
West	17	4.41 ± 6.23 a	y = 5.04x - 0.03	0.10
College Station	37	1.00 ± 0.00 a	y = 1x + 0	0

[a] Collections from north, central, south, and west were quadrants on the Texas A&M University campus, College Station, Texas, and College Station specimens were from undisclosed locations in College Station, Texas.

[b] Same letters following means within the column were not significantly different ($P < 0.05$, Tukey-Kramer HSD).

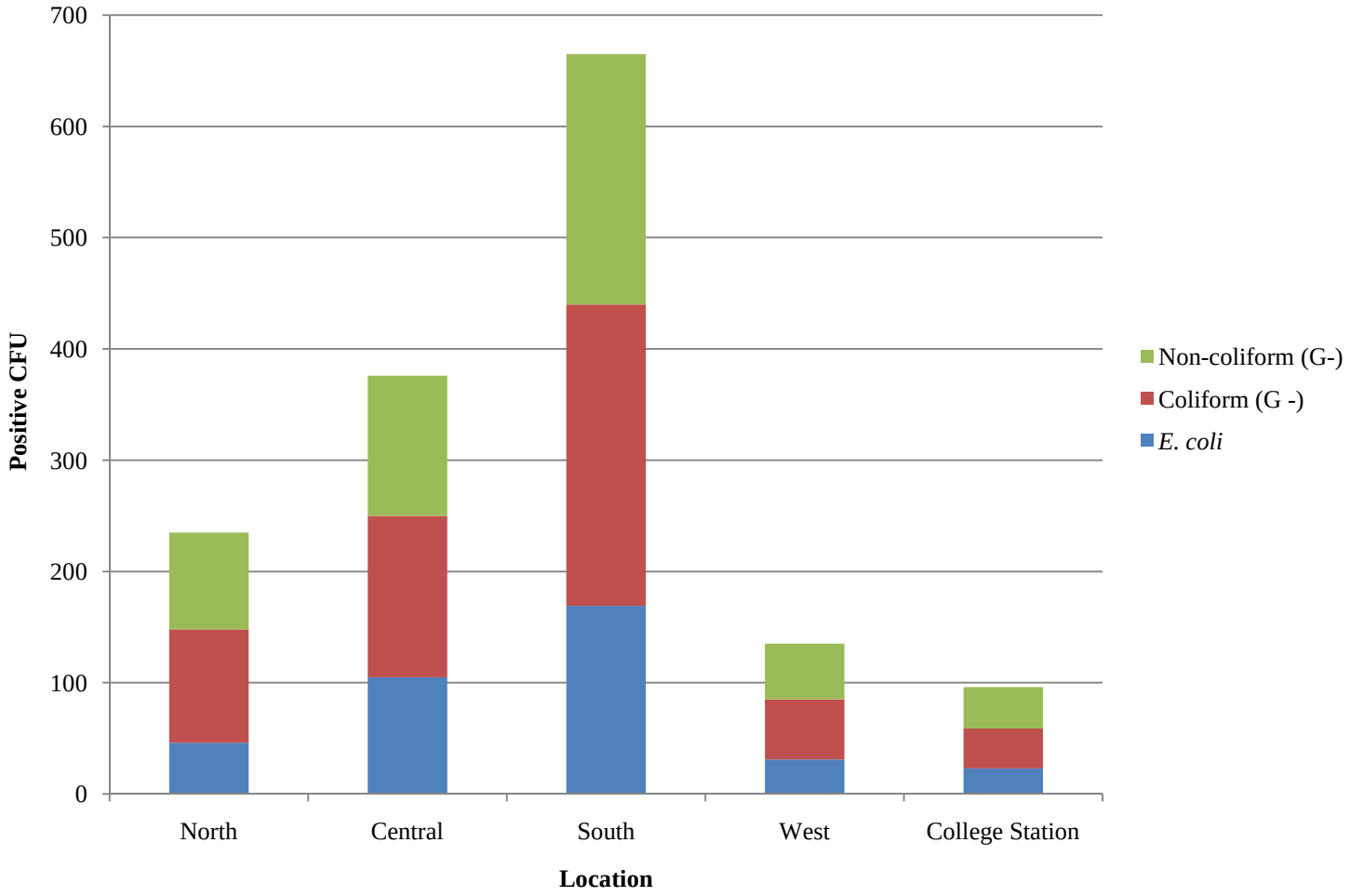

Figure 5. Prevalence of bacteria (*E. coli, c*oliform forming gram-negative, and non-coliform forming gram-negative) from the total cockroach population collected on the Texas A&M University campus, College Station, Texas

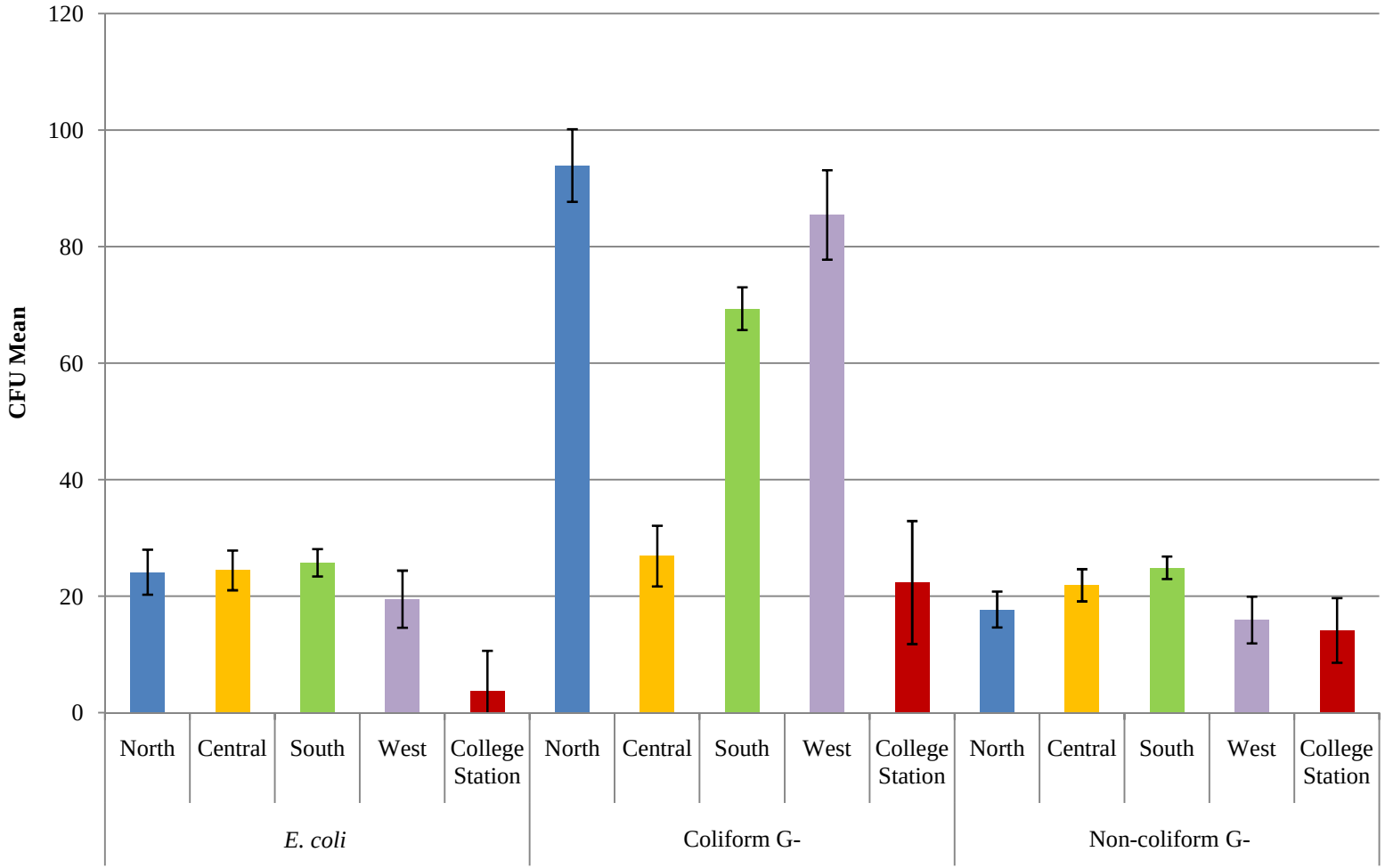

Figure 6. Bacterial counts from cockroach populations screened from each quadrant on the Texas A&M University campus, College Station, Texas

7 coliform forming colonies, and 0 non-coliform forming colonies. Central quadrant had 4 *E. coli*, 2 coliform forming colonies, and 5 non-coliform forming colonies. The south quadrant had the most with 28 *E. coli*, 14 coliform forming colonies, and 11 non-coliform forming colonies. The west quadrant had 0 *E. coli*, 2 coliform forming colonies, and 1 non-coliform forming colony. Various locations in College Station resulted in 0 plates with too many to count (Table 8). Coliform forming bacteria were significantly different ($F = 24.728$; df = 4, 665; $P < 0.001$) between quadrants, while non-coliform forming gram-negative bacteria had no significant difference ($F = 2.0573$; df = 4, 680; $P = 0.0848$) (Figure 6).

There were no significant difference ($F = 0.0420$; df = 2, 205; $P = 0.8379$) between adult and nymph stages of cockroaches collected compared to number of bacterial colony forming units of *E. coli* (Table 11). There were no significant difference ($F = 3.0748$; df =2, 216; $P = 0.0809$) between adult and nymph stages of cockroaches collected compared to number of bacterial colony forming units of coliform forming bacteria. There were no significant difference ($F = 0.0003$; df = 2, 216; $P = 0.987$) between adult and nymph stages of cockroaches collected compared to number of bacterial colony forming units of non-coliform forming bacteria plated (Figure 7).

Screening for *E. coli* 1057:H7 and *Campylobacter* spp. yielded no positive colony forming units for all of the samples screened (N = 724).

Table 8. Prevalence of cockroach specimens plated for *E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative that resulted in too many bacteria colony forming units to count for cockroaches collected on the Texas A&M University campus, College Station, Texas and various undisclosed locations in College Station, Texas

Location [a]	*E. coli* [b]	Coliform (G-)	Non-coliform (G-)	Total
North	1/104 (.009%)	7/104 (.067%)	0/104 (0%)	8/104 (.077%)
Central	4/155 (.026%)	2/155 (.013%)	5/155 (.032%)	11/155 (.071%)
South	28/354 (.079%)	14/354 (.040%)	11/354 (.031%)	53/354 (.150%)
West	0/74 (0%)	2/74 (.027%)	1/74 (.014%)	3/74 (.041%)
College Station	0/37 (0%)	0/37 (0%)	0/37 (0%)	0/37 (0%)
Total	33/724 (.046%)	25/724 (.035%)	17/724 (.023%)	75/724 (.102%)

[a] Collections from north, central, south, and west were quadrants on the Texas A&M University campus, College Station, Texas, and College Station specimens were from undisclosed locations in College Station, Texas.

[b] Percentages based on the number of specimens with too many bacteria colony forming units to count compared to the total number of specimens collected from each location.

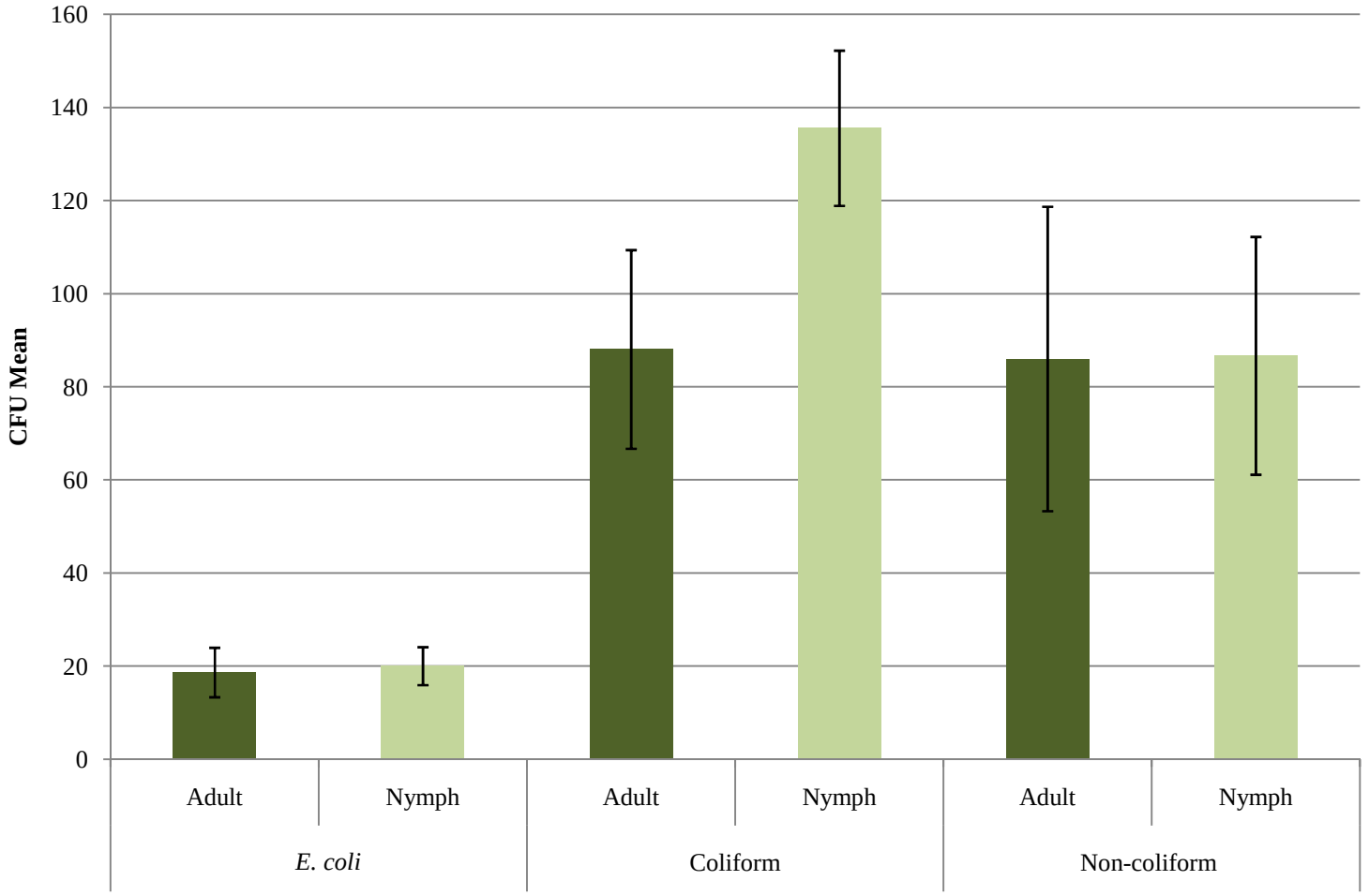

Figure 7. Comparison of bacteria counts for adults and nymphs in all quadrants collected on the Texas A&M University campus, College Station, Texas

Discussion

The purpose of this study was to determine the amount and viability of bacteria harbored by *P. americana* in an outdoor, urban environment by observing commonly occurring and ubiquitous examples such as *E. coli* and *Campylobacter*. The Texas A&M University campus provided an ideal location to conduct this experiment due to the familiarity of the structures, buildings, and roadways. Outdoor collecting sites on campus provided insight into American cockroach population within an artificial environment. Outdoor locations were chosen because American cockroaches are considered peridomestic pests and traveled freely between indoor and outdoor locations. Also, limited building access at times when cockroaches were most active (at night) made it difficult to maintain a regular collecting schedule of indoor facilities.

Cockroaches have increased activity when most of the buildings on campus were either unoccupied or closed. This implies that cockroaches can move within a building with limited restrictions, including foraging areas that are important in food preparation and handling. The south campus was interesting to note because it was the only quadrant that had sampling near a dining hall and as seen the density of cockroaches was the lowest at this location. The low population numbers could result from effective and well-maintained control strategies, or the facilities indoors provide adequate food, shelter, and water thus eliminating the need for cockroaches to forage in outdoor locations.

This study is the first to focus on population densities and bacteria associated with *P. americana* on a major university campus in the United States. Numerous studies have collected cockroaches in urban situations, but typically inside schools, hospitals,

and homes. Granovsky (1983) suggested trapping rates increased when the external surfaces of the collecting jar were coated with sand. Collecting containers were placed in discrete locations and areas that could not be easily seen by the public, because the disturbance of collecting jars could alter the population numbers. During the course of this study, only a single site lost a jar by the following morning, because they were too visible and accessible to the public due to their location next to a busy roadway. Also, there were times when a single jar was knocked down at a site because of via weather, wildlife or other unforeseen forces. A single jar being knocked over occurred nine times over the duration of this study.

Other factors that were not considered prior to collecting was the presence of feral animals on campus, including but not limited to frogs, snakes, cats, skunks, and opossums, with the latter three possibly consuming bread from the collecting jars. The only known incidence of wildlife having a known impact on the number of cockroaches collected occurred on west campus at the Koldus drain (north) collecting site. A skunk or skunk surfeit had burrowed under the concrete slab near the collecting location. It is uncertain how long the animal(s) resided at this location, but on an early collecting trip on 18 April 2008, their presence was made aware. After that day, the number of cockroaches from that site declined. Skunks are known carnivores but have been found to feed on insects when available (Crooks and Van Vuren 1995). It was not known what caused the decline in the population. It may have been the skunk which had access to a steady supply of cockroaches as a food sources, or it may have been a normal population fluctuation due to the season and weather variability.

Collecting jars were also placed near areas which maintained requisites of life such as food, water, and shelter. Jars were typically placed adjacent to a concrete or brick barrier such as a wall, stairs, or a structure enclosing a flowerbed. When foliage, typically ivy, was present, the collecting jars were placed below the surface of the foliage and adjacent to the structures. Observations made throughout the study indicated a high occurrence of cockroaches in ivy beds. The masonry structures coupled with ivy or other plant life may have provided adequate coverage for cockroaches to move without the threat of predation and with increased rates of foraging success. Also, the masonry and concrete structures may have provided an artificial heat source; thus, cockroaches could move next to buildings and forage for extended periods of time when temperatures were less than ideal than if they were foraging in exposed environments. Lin et al. (2007) demonstrated in Taiwan that heat output by concrete is stronger in the winter months, with a mean temperature of 14-28^{o}C, but the surface temperature of the material correlated with ambient temperature. The winter months in Taiwan are representative of the subtropical climates which are much higher than the temperatures of winter period in a temperate area such as Texas. Based on observations during this study, populations collected in areas with a concrete barrier and foliage maintained higher population than those sites without such structures.

Over the duration of this study, it was interesting to note the lack of cockroach species diversity being attracted to the collecting traps. There were three *Periplaneta fuliginosa* (Serville) (Blattodea: Blattidae) collected amongst other *P. americana* species. There were four *B. germanica* collected during the study near a residential

building on campus, but they were not intermingled with American populations. This was unusual, since German cockroaches are typically indoor pests. It is possible that there was a large population surge that could no longer be maintained by the area, or that there were not enough food resources in the building. Either situation could lead cockroaches to forage greater distances, thus going outside to find adequate food sources. It is unknown which situation forced German cockroaches outdoors, but it was unusual for *B. germanica* to forage outdoors.

There was no significant difference between collecting sites in each quadrant and population of *P. americana* collected. Specific areas of campus did appear to yield higher populations based on observational experience. Categorizing the collection sites on a university campus into specific types such as food establishments, residential or a hospital areas is difficult because most buildings include various types of establishments within a single structure. Buildings on campus usually include food resources like vending machine, coffee bar, full-scale dining area, lecture hall, and residential areas. Residential buildings on campus contained food because they serve as the primary living quarters for many people on campus.

The administration buildings maintained the highest outdoor populations, while dining halls had the least number of cockroaches collected. The dining halls may have had adequate control methods in effect to efficiently reduce population numbers. Alternatively, there may have been sufficient resources inside the buildings which failed to drive populations outside to forage. Control strategies may be less stringent in areas where food preparation is not the primary focus, such as in administration and lecture

buildings. Haines and Palmer (1955) determined that *P. americana* was a predominant species in sewer systems with low population densities indoors and around the home; although the restrooms of indoor facilities maintained the highest population numbers. Overall, the building type does not play a significant role in the population densities of cockroaches. The assumption can be made that the same applies for an area such as a university where cockroaches were ubiquitous in the environment.

Pai et al. (2003) determined that adult populations of *P. americana* and *B. germanica* were significantly higher than nymph populations collected in hospitals, which fails to correspond with data found in this study. There were no significant differences between adult and nymph populations collected around campus. This difference between studies may result from a difference in collection techniques or that the Pai et al. (2003) study was conducted indoors, from a single structure (hospitals) type, while the current study exploited various collecting locations and their outdoor structures. No significant difference between adult and nymph collections may have indicated a well-established population on campus, as well as a lack of control methods to reduce nymphal population numbers, or there may be a naturally higher frequency of nymphs during the collecting periods.

American cockroaches can take up to one year to reach maturity, and can live up to three years. Their indoor counterpart, the German cockroach, can reproduce several times a year, and has a lifespan of 200-300 d (Barcay 2004). It appears that integrated pest management strategies are not reaching breeding and/or foraging sites on a routine basis. The lack of a maintained control schedule could indicate why there were various

ages of cockroaches (nymphal instars to adults) collected on a consistent basis from all locations throughout the collection period.

The effect of temperature on population numbers was important to consider because it was generally assumed between 10-35^{o}C, resulted in a decline of cockroach activity, thus influencing the numbers acquired during collecting periods (Murphy and Heath 1983). There was no difference between mean temperatures and the total population means collected. This implied that overall temperature does not play a significant factor, population means collected but observation data suggested at lower temperatures (<10^{o}C) population means were different from populations collected at higher temperatures. This corresponded with Murphy and Heath (1983) concerning cockroach activity and temperature.

Prior to collecting, it was hypothesized that population means would increase as the temperatures rose. The lack of a relationship between temperature and population indicated another factor may be influencing populations that were not accounted for during this study. Cockroach populations, based on observational data decreased, when the minimum temperatures at night were cooler.

Ambient temperatures in Texas fluctuate between December and May because of seasonal transitions. The collecting period followed an erratic pattern with rising and falling temperatures, coupled with periods of intense rainfall. For example, during the last five days in February there was a day with a high of 29^{o}C followed by a low of -1^{o}C two days later. The rapidly changing temperatures likely influence foraging behaviors and overall activity. Traps used during this experiment were not designed to collect

specimens during rainy conditions, thus population numbers were minimal during these time periods. Based on observational data, days with rainfall had lower numbers of cockroaches collected than days without rainfall.

Spatial distribution of natural population is typically patchy. Resource levels fluctuate over time in individual locations, thus population numbers will also change over time indication a patchy distribution (Roughgarden 1977). Population fluxes are normal because collecting cockroaches from outside was coincidental with weather. Sometimes there is an abundance of cockroaches in a single location, and the next day there may be none at the same location. Population surges may result from rainfall, food availability, an overabundance of water in sewer systems, and/ or external weather conditions. There is limited data correlating the number of cockroaches collected from outside populations and weather conditions.

Establishing bacteria amounts carried by cockroaches is important because they can act as potential disease agent carriers. Lipsitch and Moxon (1997) defined virulence as the capability of organisms to infect or damage the host. Virulence and transmissibility has been theorized in vertebrate animals, including humans, in which virulence and transmissions of pathogens may not be directly related (Lipsitch and Moxon 1997). The pathogen may be in a different part of a host body than where symptoms are being displayed. Symptoms may result in parts of the body not in the path of the organisms, and symptoms may result from the immune response of the host to the threat of pathogens instead of the presence of the actual pathogen (Lipsitch and Moxon 1997). This theory of unrelated virulence and transmissibility may not apply to

invertebrates in the same methods as it applies to vertebrates. The exoskeleton of insects provided protection against invading organisms, but also provided surfaces on which pathogens may reside. Harboring pathogens on the exterior portion of the body may increase the potential of viability of pathogens, unlike vertebrates where pathogen entry into the body occurs with few barriers. It may be difficult to determine which cockroaches are diseased because pathogens have less opportunity to enter their body because of the exoskeleton, thus the physiology and behavior of the insect may not be influenced.

Escherichia coli and *Campylobacter* spp. are common bacteria that cause gastro-intestinal illnesses in humans. *Escherichia coli* was prevalent on surfaces contaminated by fecal matter and can last anywhere from 1.5 h-16 mo on dry, inanimate surfaces (Kramer et al. 2006). Scott and Bloomfield (1990) determined *E. coli* remained viable on laminate surfaces up to 4 h, and that the bacteria could transfer from contaminated surface to other objects such as fingertips, stainless steel, or cloth. Bacteria transfer occurred at the highest rate when a contaminated piece of cloth contacted fingertips, after contact *E. coli* was detected up to 48 h after initial contact (Scott and Bloomfield 1990). Kitchens maintained the highest numbers of *E. coli* resulting in part from poor sanitary habits after handling contaminated foods such as chicken. Cockroaches, if indoors, are prone to walk across surfaces that have been wiped down by a potentially contaminated cloth. In outdoor environments, *E. coli* has been found to survive up to 20 d the wood shavings of a farm structure (Bale et. al 1993). Indoors, the organisms were detected up to 21 h on a contaminated piece of paper (Bale et al. 1993). Due to their

ubiquitous nature, cockroaches can acquire bacteria from most surfaces in-or-out of doors, so long as bacteria are present in the environment. Cockroaches can act as potential carriers of pathogens in the surrounding areas.

Contamination rates of cockroaches compounded with their gregarious behavior could provide a mode for pathogens to spread to surfaces having direct contact with food. During this study, 51.7% of all cockroaches trapped were contaminated with *E. coli*. This was the lowest percentage of positive bacteria out of all the cockroaches screened for colony forming units. Despite having the lowest percentage of prevalence, one out of every two cockroaches on campus was carrying *E. coli*. A comparison was made to determine if the life stage (adult or nymph) made an impact on bacteria associations with the cockroaches and found there to be no significant difference.

Escherichia coli can be found on both internal and external surfaces of cockroaches (Rivault et al. 1994). The current study concurred with the Le Guyader al. (1989) study of gram-negative bacteria amounts not having a significant difference between adults and nymphs. Despite the stigma of cockroaches being filth laden, Bell et al. (2007) indicated cockroaches spending at least half of their time grooming and removing foreign objects from their body. The amount of time spent cleaning is inadequate because of contamination of the habitat and the capability to become re-inoculated with pathogens present in the environment. The ability to harbor bacteria on internal and external surfaces provides multiple means of pathogen transmission. In addition to direct contact with surfaces, cockroaches can disseminate internal organisms via defecation and/or regurgitation.

Compared to previous studies made indoors, the presence of bacteria on cockroaches appears to correlate with other studies with positives rates of bacteria in Ghana, France, and Taiwan (Agbodaze and Owusu 1989, Rivault et al. 1994, Pai et al. 2004). Overall, 92.3% of cockroaches collected from outdoor locations on campus carried gram-negative bacteria on their cuticular surfaces. When compared to another pest cockroach, the German cockroach, Pai et al. (2005) determined there was no significant difference between *P. americana* and *B. germanica* incident rates of positive growth of bacterial colonies on the integument and the gut. Although, *P. americana* had significantly higher rate of gram-negative colonies than *B. germanica* (Pai et al. 2005). *Periplaneta americana* can harbor more gram-negative bacteria from outside sources such as sewage, soil, contaminated water, and garbage, than *B. germanica* acquires from inside sources.

A previous study indicated cockroaches harbored bacteria present in the surrounding environment, as opposed to introducing new pathogens into the environmental fauna (Rivault et al. 1993). During this study, it was assumed cockroaches were mechanically transmitting pathogens obtained in the environment and were capable of traveling while harboring these bacteria. Koch's postulates were tested and demonstrated the ability of cockroaches to transfer bacterial species. This creates a public health concern if cockroaches inoculated with bacteria from outside migrated indoors and transmitted pathogens to sterile surfaces, such as areas in the kitchen. Chaichanawongsaroj et al. (2004) indicated *E. coli* levels on cockroaches coincided to *E. coli* levels in the environment. Buildings with the most to least amounts of bacteria were

as follows: hospitals, food establishments, and residential areas thus cockroaches in hospitals maintained the highest levels of *E. coli* (Chaichanawongsaroj et al. 2004). Rivault et al. (1993) discussed that not all bacteria would be able to survive on surfaces that a cockroach made contact with. Under proper conditions such as proper humidity, specific bacteria species can develop at successful rates. Specific food items can provide bacteria with enough nutritional resources and humidity to grow. This causes concern because food that is eaten raw such as fruits, vegetables, pastries, and breads may be cross-contaminated with disease-causing organisms. After retrieving traps, it was interesting to note the condition of the bread in each jar. Traps containing cockroaches usually had bread that appeared to be moldy or have black or green spots on it while the bread in jars without cockroaches lacked these discolorations.

Kopanic et al. (1994) determined a single cockroach contaminated with *Salmonella typhimurioum* could infect up to ten other cockroaches within a 24 hour period in a 1.1 L jar. Theoretically, a single cockroach can contaminate an entire area given an adequate period of time. In the current study, the time when each cockroach entered the collecting vessel is unknown, thus making the rate or occurrence of cross-contamination difficult to determine. Kopanic et al. (1994) previously studied cockroaches with known inoculated amount placed into a container which resulted in variable rates of cross-contamination. During this study, the collection jar was 2.3 times larger than the container used in the previous study, and cockroaches were in traps for half of the duration of the Kopanic et al. (1994) study. Therefore, cockroaches in this study may have all been exposed to limited cross-contamination. Data indicated bacteria

were present on a single roach while other cockroach(es) were absent of the bacteria within the same jar.

An alternative may be that under stress the cockroaches defecated or regurgitated food into the slurry of bread-beer mixture, thus providing a solution for the remaining cockroaches to become cross-contaminated with bacteria from other cockroaches. A downside to the methods used for this experiment was that all cockroaches from each individual jar at each site were placed in a single plastic container prior to freezing. This may have provided another means of cross-contamination. Potential spreading of pathogens among the specimens may have been prevented if each cockroach was stored in individual containers. It would be unrealistic for a single person to collect each cockroach individually as it was trapped at all of the locations. The methods described in this paper were sufficient to determine population numbers and bacterial counts with the least amount of cross-contamination possible.

Data indicated that collection locations were significantly different *E. coli*, coliform forming gram-negative bacteria were significantly different while there was no significant difference between non-coliform forming gram-negative bacterial species and collecting locations. It was interesting to note differences among collected populations and prevalence of bacteria, despite collecting sites being up to 1.44 km apart. A significant difference may indicate the environment of various collecting locations having differing compositions of bacteria. It is possible that the values for *E. coli* were not significantly difference for each quadrant even though the p-value indicated a significant difference. There were 75 specimens that resulted with too many bacteria

colony forming units to count. These numbers should not have affected the overall significant difference between populations, quadrants, and bacteria species because the number of too many to count bacteria colony forming units were proportional to initial rates of prevalence among populations collected on campus. Populations collected in the south quadrant had significantly different numbers of *E. coli* colonies when compared to other locations. It was interesting to note that this was the only location that was collected near a garage. No differences for non-coliform forming gram-negative bacteria among collected populations implies that cockroaches may have obtained bacteria from common means throughout campus, such as soil in the flowerbeds or a common water source any of which may have been contaminated with bacteria.

A common water source that may have been easily accessible to all specimens is through the sewer systems. *Periplaneta americana* may have traveled from one area of campus to another through various methods of transportation, including but not limited to steam tunnels, vehicles via infested materials, or physical movement by individual roaches. Cockroaches are capable of migration by ground movement, climbing vertical surfaces, swimming, and some limited flight capabilities (Bell et al. 2007). Jackson and Maier (1955) determined through capture and release experiments that cockroaches could travel through the sewer up to 107 m. Dispersal can occur rapidly because cockroaches are capable of traveling at speeds of 0.44-1.5 ms^{-1} on a horizontal plane and can become bipedal at their highest speeds (Full and Tu 1991). It is possible cockroaches remained in locations until resources were depleted and then dispersed in search of food.

Nocturnal habits of cockroaches allowed efficient ground movement but not without the threat of predation by nocturnal wildlife. Safer dispersal methods could be achieved by traveling through sewer systems. Incidentally, sewers are ideal locations for acquisition of pathogens by cockroaches. Also, students, faculty, staff, and visitors may unknowingly transfer cockroaches and/or ootheca to new locations from other residences or buildings. Increased populations resulted in an increased potential to infest new areas, hence establish new sites for pathogen acquisition and dispersal.

Several other studies agitated collected cockroaches in saline and used the solution to plate and determine bacteria numbers. Methods used during the current study directly plated cockroaches onto media. Humphrey et al. (1995) determined that directly placing samples on media would increase the rate of prevalence as opposed to placing the samples into a diluent. Direct plating may replicate what happens outside of laboratory conditions in a more realistic manner. It does not seem feasible that cockroaches will be shaken in a solution and then the solution be poured onto a food or food preparation area. Cockroaches typically walk over surfaces or may stop to feed on a food resource, thus inoculation periods vary from surface to surface. Direct plating may replicate *P. americana* cuticle indiscriminately contacting surfaces and with the possibly of pathogen transmission.

All specimens collected were negative for *E. coli* 0157:H7. Presence of this pathogen usually occurs in livestock area because cattle and sheep act as reservoirs for the pathogen (McGee et al. 1997). There were no locations on campus that housed livestock which were regularly sampled for cockroach populations. This may have

contributed to why there were no positives for *E. coli* 0157:H7. Although, *E. coli* 0157:H7 has been found on vegetables and soft cheese such as feta (Ramsaran et al. 1998, Michino et al. 1999). *Escherchia coli* 0157:H7 has also been found viable on dry surfaces such as stainless steel and was detected up to 60 d after inoculation (Maule 2000). Due to pathogenicity of the organism, negative results on all of the specimens tested were an optimal result.

Campylobacter spp. is normally found in the intestinal gut of animals and humans. It has microaerophilic properties which make growth of the organism susceptible to desiccation from oxygen. Altekruse et al. (1999) determined that survival of the organisms outside of the gut is poor. Unfavorable conditions can result in *Campylobacter* spp. to enter a stage where it is viable but nonculturable (Murphy et al. 2006). During this state, the organisms change their morphological characteristics from a spiral to a coccoid, but it can still result in infections and can colonize a host gut (Murphy et al. 2006). Growth in substances such as water, litter, and feed are typically not common in ambient temperatures because of the desiccation associated with being exposed to atmospheric oxygen (Sahin et al. 2002). Transmission of *Campylobacter* spp. to humans occured through consumption of under cooked meats such as beef, pork, and poultry or consumption of contaminated water sources or non-pasteurized milk (Sahin et al. 2002). The bacteria are sensitive to drying out and when suspended in a liquid that has been allowed to dry, *Campylobacter* spp., will not be detected after four hours (Humphery et al. 1995). Although, if the organisms is in a solution such as blood, it will test positive after four hours (Humphrey et al. 1995). Chynoweth et al. (1998)

determined *C. jejuni* could grow in sterile stream water under aerobic conditions for up to 55 d. *Campylobacter* spp. have been found on stainless steel, ceramic tile, cotton dishtowels, and other surfaces commonly associated with food preparation situations for over an hour (Yan et al. 2005). *Campylobacter* infections can result from surface water being contaminated by fecal material or through sewage contamination (Murphy et al. 2006).

The cockroaches in this study failed to have any positive rates of prevalence when screened for *Campylobacter* spp. The organism does not grow at temperatures below 30^{o}C, which could indicate why there were no positives (Park 2002). Susceptibility to cooler temperatures and exposure to oxygen makes it difficult for *Campylobacter* spp. to successfully grow outside of a host body, hence was a possibility as to why there was no positive colony forming.

Overall, this study displayed the wide distribution of cockroach populations on campus and their ability to indiscriminately inhabit areas within an urban environment. Pathogen acquisition and dissemination of gram-negative bacteria, such as *E. coli*, was prevalent on campus but without detection of the highly pathogenic strain of *E. coli* 1057:H7. Also, there was a lack of *Campylobacter* spp. growth from cuticular plating which may have resulted from undesirable conditions required to sustain colony growth.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The purpose of the first portion of this study was to analyze the spatial distribution of *P. americana* populations in an outdoor, urban environment and determine gene flow among the population. This study determined genetic variability among *P. americana* collected on a major university campus. The 16S region of the gene was initially chosen as the amplification site in identifying variability among cockroach populations. 16S gene regions are commonly amplified during termite studies failed to amplify and thus there was no discernable differentiation between individuals. The ITS1 region was chosen for amplification because there was no successful amplification at the 16S gene region. The current study demonstrated gene flow within populations of *P. americana* based on differentiation identified from the ITS1 region.

Among the 52 sequences amplified there were 22 haplotypes indicating a high amount of variation in the population. Haplotypes isolated during this study will be made available on Genbank. TCS spanning tree analysis identified discrete lineages from nuclear markers which demonstrates interbreeding of *P. americana* populations. The lack of population structure was reconfirmed by neighbor-joining and Bayesian phylogenetic analyses. *Periplaneta americana* samples from Bryan, College Station, Hempstead, and Pleasanton, Texas were in a single clade including *P. americana* sequence obtained from Genbank (AF321248). Samples of *P. fuliginosa* were in a

separate clade along with the sequences from Genbank for a *P. fuliginosa* (AF321250) and *P. brunnea* (AF321249) supports species identification with this marker. Comparing the 52 sequences amplified to 22 haplotypes suggests a high amount of variation in the population based on nuclear markers. Genetic variation based on spatial distribution of *P. americana* populations indicated the success and ability of breeding with independence with the individuals collected on campus, hence representing a free-living, interbreeding population.

Genetic variation of populations occurs through genetic drift, genetic flow, natural selection, and founder effects (Slatkin 1987). Lenormand (2002) determined gene flow limited adaptation of genes to specific locations because new genes from outside sources prevent loci from becoming fixed in the environment. Introduced genetic material adapted for survival in a particular environment can differ from the population from which it emigrated, thus preventing speciation via gene flow (Slatkin 1987). Gene flow acts as an indirect method of determining movement within a population.

This study is the first to date using nuclear markers in identifying spatial relationships and gene flow among *P. americana* populations on a university campus in North America. Future studies may analyze a broader range of genes including mitochondrial DNA to determine distinct lineages formed by mtDNA. Also, analyzing gene flow at various sequences may determine a more comprehensive evolutionary lineage of divergences in cockroach populations. Our study is a step towards the in-depth analysis of the phylogenetics of cockroaches. The data obtained during this experiment can contribute a small portion to the overall analysis of a comprehensive

phylogenetic study of cockroaches. There was a failure to reject the null hypothesis for the first objective of this study because there was no significant difference in the genetic make-up of field collected *P. americana* samples from locations in central Texas.

The second objective of this study determined the amount and viability of bacteria harbored by *P. americana* in an outdoor, urban environment. There was no significant difference between collecting sites and population means of *P. americana* collected. Based on observational experience, specific areas of campus appeared to yield higher populations. No significant difference between adult and nymph populations may have indicated a population that is well established on campus, as well as the lack of control methods implemented to reduce population numbers. Cockroaches are virtually ubiquitous in urban environments. Overall, the function of buildings adjacent to collection locations did not play a significant role in population densities of cockroaches. There was no correlation between temperature and mean populations (total, adults, and nymphs) when analyzed. Thus affirming populations collected are not correlated to mean temperatures.

Establishing bacteria amounts carried by cockroaches is important because they can act as potential disease agent carriers. *Escherichia coli* and *Campylobacter* spp. are common causes of gastro-intestinal illnesses in humans. Despite the stigma of cockroaches being filth laden, Bell et al. (2007) implicated cockroaches spending at least half of their time grooming and removing foreign objects from their body. The amount of time spent cleaning is inadequate because of the pathogen contamination rates of in the areas cockroaches frequent and their ability to easily become re-inoculated.

Therefore, cockroaches can mechanically transfer pathogens present in the environment. The ability to harbor bacteria on internal and external surfaces provides multiple means of pathogen transmission. In addition to direct contact with surfaces, cockroaches can disseminate internal organisms via defecation and/or regurgitation.

Data indicated when collection locations were compared to bacteria amounts there were significant differences between locations and *E. coli*; there were significant differences between locations and coliform forming gram-negative bacteria; and there were no significant differences between non-coliform forming bacterial species and collecting locations. It was interesting to note both significant differences and no significant differences among collected populations and prevalence of bacteria, despite collecting sites being up to 1.44 km apart. No difference among populations could demonstrate cockroaches obtained bacteria from a universal substance used throughout campus, such as soil or a common water source, any of which may have been contaminated with fecal material. Sewer systems are easily accessible to specimens throughout campus and may have been a source of contamination.

Cockroaches are capable of migration via ground movement, climbing vertical surfaces, swimming, and they have some limited flight capabilities (Bell et al. 2007). *Periplaneta americana* may have traveled from one area of campus to another through various methods of transportation. Steam tunnels, automobiles via infested materials, or physical movement by individual roaches may have been a few methods of dispersal through the environment.

Despite having the lowest percentage of prevalence, one out of every two cockroaches on campus was carrying *E. coli* pathogens. All specimens collected were negative for *E. coli* 0157:H7. Due to pathogenicity of the organism, negative results on all of the specimens tested were an optimal result. Cockroaches collected and screened during this study failed to have any positive rates of prevalence of *Campylobacter* spp. Susceptibility to cooler temperatures and exposure to oxygen impedes *Campylobacter* spp. growth successfully outside of a host body, hence a possibility as to why there was no positive colony forming unit. Whole body extractions or fecal remains would be more likely be used to observe *Campylobacter* spp. in future studies.

Overall, this study displayed the ubiquitous distribution of cockroach populations on campus and their ability to indiscriminately inhabit areas within an urban environment. Gram-negative bacteria acquisition and dissemination of organisms, such as *E. coli*, was prevalent on campus but the highly pathogenic strain of *E. coli* 1057:H7 was not isolated. Also, there was a lack of *Campylobacter* spp. growth from cuticular plating which may have resulted from undesirable conditions required to sustain colony growth.

Data from this study suggested cockroach's ability to mechanically transfer pathogens. Insects are known to harbor and transfer pathogens in the environment, thus having potentially deleterious health consequences on animal and/or human populations. Dipteran species have been identified as mechanical vectors of pathogens. Houseflies, *Musca domestica* (L.) (Diptera: Muscidae) can carry *Vibrio chlorera*e, *E. coli*, and *Yersinia pseudotuberculosis* (Fotedar 2001, Zurek et al. 2001, De Jesús et al. 2004). In

1898, *Xenopsylla cheopis* (Rothschild) (Siphonaptera: Pulicidae) was reported as the vector of the etiological agent of plague (*Yersinia pestis*) (Burroughs 1947, Inglesby et al. 2000). The consequences of plague outbreaks throughout history are well known, but more current concerns associated with the disease involves aerosolation of the bacteria for use as a biological weapon (Inglesby et al. 2000). This technique could apply to other pathogens with numerous insects acting as mechanical transmitters and having successful rates of dissemination. *Stomoxys calcitrans* (L.) (Diptera: Muscidae), *Ades agypeti* (L.) (Diptera: Culicidae), and *Ae. taeniorhynchus* (Wiedemann) (Diptera: Culicidae) can spread *Bacillus anthracis* which is also a cause for concern if used as a biological weapon (Turell and Knudson 1987). The null hypothesis for the second study was rejected for *E. coli* and coliform forming bacteria because there was a significant difference between *P. americana* samples collected from various locations in central Texas. Analysis of non-coliform forming bacteria resulted in a failure to reject the null hypothesis because of there was no geographic relationship for bacteria recovered among field collected *P. americana* samples in central Texas.

Zurek and Schal (2004) suggested the capability of German cockroaches to mechanically transmit the porcine pathogen *E. coli* F18 through fecal material. Cockroaches also have been suggested as a vector in sever acute respiratory syndrome (SARS) which has reached epidemic levels in the past five years (Wu et al. 2004, Lau et al. 2005). SARS results from contact with individuals infected with a coronavirus, thus direct contact serving as the primary route of transmission (Wu et al. 2005). Although, cockroaches have been hypothesized to act as vectors when there was no contact

between infected and uninfected individuals (Wu et al. 2005). It is evident that cockroaches are capable of transmitting various disease-causing organisms by mechanical transmission. The absolute vectoring capability of cockroaches still remains unknown because experiments involving etiological agents occurring naturally or those which can be harmful to large populations, such as anthrax, have yet to be thoroughly tested.

There were limitations to information and implications resulting from data collected throughout the study. For example, populations collected for this study were only collected from selected areas of campus. The genetic data is consistent throughout the sequences amplified from the various locations on campus. The degree of variation of the population may be interpreted differently had other markers such as mtDNA or microsatellite segments been implemented to differentiate individuals. Also, *P. americana* were only collected in outdoor environments. It is possible that indoor populations have different bacterial faunas because of differing bacteria present in the environment. Future studies could include sampling larger areas for collecting, including health care facilities, like hospitals and nursing homes, or places associated with children, such as day cares and schools. It would be interesting to analyze diversity of bacteria from various locations including gram-positive bacteria, which were not screened for during this study. Also, identifying specific strains of pathogens through genetic analysis could allow for better mapping of distances and dispersal of disease-causing agents throughout the environment.

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APPENDIX

Table A-1. Collection sites with GPS for College Station, TX including the total number of cockroaches collected at each location

Quadrant	Location	GPS	Number Collected
North	W Blocker	30° 37'11.16" N / 96° 20' 33.99" W	29
North	E Water Tower	30° 36' 4.27" N / 96° 20' 35.75" W	75
North	S WERC	30° 37'13.73" N / 96° 20' 17.87" W	0
Central	S Arct Bldg B	30° 37' 8.44" N / 96° 20' 17.45" W	0
Central	W Arct Bldg C	30° 37' 8.84" N / 96° 20' 16.60" W	0
Central	NE Beutel (Dumpster)	30° 36' 52.95" N / 96° 20' 33.93" W	0
Central	S Beutel	30° 36' 55.36" N / 96° 20' 33.42" W	0
Central	W Bizzell Hall	30° 36' 49.98" N / 96° 20' 28.74" W	0
Central	W Board of Regents Annex I	30° 36' 42.39" N / 96° 20' 31.97" W	39
Central	W Board of Regents Annex II	30° 36' 42.78" N / 96° 20' 32.45" W	36
Central	S Board of Regents Annex	30° 36' 42.21" N / 96° 20' 30.78" W	80
Central	N Coke Bldg	30° 36' 52.85" N / 96° 20' 30.79" W	0
Central	S Geosci Bldg (Drain)	30° 37' 2.90" N / 96° 20' 11.58" W	0
Central	S Geosci Bldg (Dumpster)	30° 37' 3.02" N / 96° 20' 11.31" W	0
Central	W J.R. Thompson Hall	30° 37' 3.04" N / 96° 20' 27.38" W	0
Central	SE Langford Arct Bldg	30° 37' 7.67" N / 96° 20' 13.63" W	0
Central	E Langford Arct Bldg	30° 37' 7.83" N / 96° 20' 13.72" W	0
South	Bldg West of Duncan (Door)	30° 36' 42.14" N / 96° 20' 8.23" W	0
South	N Commons	30° 36' 57.00" N / 96° 20' 11.96" W	0
South	S Duncan (Trash compactor)	30° 36' 41.99" N / 96° 20' 6.97" W	0
South	S Duncan (Wall)	30° 36' 42.34" N / 96° 20' 5.93" W	0
South	S Duncan (Sewer cover)	30° 36' 42.62" N / 96° 20' 5.64" W	0
South	S Duncan (Walk-in)	30° 36' 42.93" N / 96° 20' 5.68" W	1
South	W Koldus (N)	30° 36' 41.11" N / 96° 20' 21.97" W	132
South	W Koldus (S)	30° 36' 41.86" N / 96° 20' 21.67" W	140
South	S Mosher - a	30° 36' 55.54" N / 96° 20' 5.12" W	1
South	S South Campus Garage (Wall)	30° 36' 47.33" N / 96° 19' 59.10" W	2
South	S South Campus Garage (Drain)	30° 36' 47.50" N / 96° 19' 58.81" W	78
West	E Borlog Center (Sewer cover)	30° 36' 30.19" N / 96° 20' 56.30" W	0
West	N HFS Bldg	30° 36' 33.85" N / 96° 21' 0.48" W	0
West	W Kleberg (Sewage cover)	30° 36' 36.11" N / 96° 20' 50.99" W	14
West	W Kleberg Drain (S)	30° 36' 36.57" N / 96° 20' 50.32" W	0
West	W Kleberg Drain (N)	30° 36' 36.70" N / 96° 20' 50.54" W	59
West	N Sat Utilities 1	30° 36' 30.36" N / 96° 20' 50.20" W	0

Table A-1. continued

Quadrant	Location	GPS	Number Collected
West	N Vet Med Sci (Glass enclave)	30° 36' 47.88" N / 96° 21' 8.62" W	0
West	N Vet Med Sci (Loading dock)	30° 36' 48.47" N / 96° 21' 7.48" W	0
West	N Vet Med Sci (Door-N)	30° 36' 49.09" N / 96° 21' 7.12" W	1
West	E Vivarium III (Corner)	30° 36' 51.28" N / 96° 21' 9.54" W	0
West	NE Vivarium III (Door-E)	30° 36' 51.6" N / 96° 21' 9.70" W	0
College Station, Texas (various locations)			37
Total			724

Table A-2. Uncorrected ("P") distance matrix of 13 haplotypes from populations collected all quadrants on the Texas A&M University campus, College Station, Texas in addition to undisclosed locations from College Station, Texas not found on campus

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12
8	0.01794	0.07034	0.06697	0.02882	0.00716	0.01269	0.00716	0.00896	0.01787	0.00897	-	
10	0.01622	0.06672	0.06524	0.02691	0.00536	0.01093	0.00536	0.00357	0.01607	0.00181	0.01073	0.00181
14	0.01441	0.06535	0.06346	0.02531	0.00181	0.00905	0.00181	0.00363	0.00896	0.00361	0.00721	0.00362
4	0.01622	0.06310	0.06160	0.02150	0.00357	0.01087	0.00357	0.00179	0.01429	0.00179	0.01075	0.00180
3	0.01619	0.06318	0.06163	0.02867	0.00714	0.01087	0.00714	0.00536	0.01786	0.00180	0.01250	0.00180
5	0.02162	0.06160	0.05976	0.01973	0.00898	0.01626	0.00898	0.00718	0.01971	0.00718	0.01619	0.00722
17	0.02170	0.07284	0.07078	0.03257	0.00908	0.01627	0.00908	0.01090	0.01624	0.01088	0.01447	0.01088
1	0.01260	0.06308	0.06158	0.02510	0.00000	0.00725	0.00000	0.00179	0.01071	0.00180	0.00716	0.00180
12	0.03259	0.05411	0.05445	0.03419	0.01967	0.02717	0.01967	0.02145	0.01964	0.02156	0.02693	0.02158
19	0.02533	0.06011	0.06173	0.03239	0.01445	0.01994	0.01445	0.01625	0.01980	0.01446	0.01986	0.01453
21	0.02717	0.05591	0.05625	0.03237	0.01430	0.02177	0.01430	0.01608	0.01786	0.01619	0.02156	0.01621
22	0.02350	0.05590	0.05618	0.02875	0.01074	0.01810	0.01074	0.01252	0.01430	0.01259	0.01797	0.01260
16	0.01813	0.05617	0.05458	0.01988	0.00543	0.01276	0.00543	0.00723	0.01618	0.00724	0.01266	0.00725

Table A-2. continued

Haplotype	13	14	15	16	17	18	19	20	21	22	23	24
8												
10	0.00893	0.00357	0.00358	0.00537	0.00714	0.00000	0.00000	-				
14	0.00542	0.00903	0.00182	0.00183	0.00361	0.00545	0.00545	0.00545	0.00362	0.00000	0.00723	0.00179
4	0.01071	0.00718	0.00360	0.00537	0.00536	0.00536	0.00536	0.00536	0.00357	0.00893	0.00540	0.01071
3	0.01429	0.00715	0.00536	0.00714	0.00893	0.00536	0.00536	0.00536	0.00357	0.00893	0.00715	0.01071
5	0.01615	0.01260	0.00903	0.01080	0.01077	0.01078	0.01078	0.01078	0.00898	0.01256	0.01083	0.01435
17	0.01272	0.01634	0.00908	0.00908	0.01088	0.01274	0.01274	0.01274	0.01089	0.00723	0.01452	0.00901
1	0.00714	0.00719	0.00000	0.00179	0.00179	0.00536	0.00536	0.00536	0.00357	0.00536	0.00539	0.00714
12	0.01967	0.02337	0.01978	0.02156	0.02145	0.02146	0.02146	0.02146	0.02325	0.02145	0.02518	0.02324
19	0.01805	0.01988	0.01271	0.01447	0.01623	0.01627	0.01627	0.01627	0.01445	0.01082	0.01814	0.01261
21	0.01788	0.02159	0.01440	0.01619	0.01608	0.01968	0.01968	0.01968	0.01788	0.01608	0.01980	0.01787
22	0.01432	0.01798	0.01081	0.01260	0.01252	0.01612	0.01612	0.01612	0.01432	0.01252	0.01621	0.01431
16	0.01260	0.01262	0.00545	0.00724	0.00722	0.00903	0.00903	0.00903	0.00722	0.00903	0.01084	0.01081

Haplotype	25	26	27	28	29	30	31	32	33	34	35	36
8												
10												
14	0.00000	0.00181	0.00544	0.00181	0.00363	0.00181	0.00363	0.00542	-			
4	0.00719	0.00360	0.00357	0.00357	0.00179	0.00357	0.00179	0.00903	0.00543	0.00359	0.00362	0.00362
3	0.00717	0.00360	0.00714	0.00714	0.00536	0.00714	0.00536	0.00899	0.00541	0.00718	0.00360	0.00360
5	0.01258	0.00900	0.00898	0.00898	0.00718	0.00898	0.00718	0.01442	0.01085	0.00901	0.00901	0.00901
17	0.00724	0.00907	0.01274	0.00908	0.01090	0.00908	0.01090	0.01269	0.00723	0.01269	0.00904	0.00904
1	0.00360	0.00000	0.00357	0.00000	0.00179	0.00000	0.00179	0.00542	0.00181	0.00357	0.00000	0.00000
12	0.01980	0.01987	0.02325	0.01967	0.02145	0.01967	0.02145	0.02171	0.01812	0.01618	0.01997	0.01997
19	0.01081	0.01263	0.01807	0.01445	0.01625	0.01445	0.01625	0.01804	0.01088	0.01265	0.01269	0.01269
21	0.01442	0.01450	0.01788	0.01430	0.01608	0.01430	0.01608	0.01996	0.01272	0.01439	0.01455	0.01455
22	0.01082	0.01086	0.01432	0.01074	0.01252	0.01074	0.01252	0.01629	0.00907	0.01078	0.01087	0.01087
16	0.00726	0.00548	0.00903	0.00543	0.00723	0.00543	0.00723	0.01089	0.00730	0.00182	0.00549	0.00549

Table A-2. continued

Haplotype	37	38	39	40	41	42	43	44	45	46	47	48
8												
10												
14												
4	0.00179	0.00179	-									
3	0.00536	0.00536	0.00714	0.00180	0.00536	0.00536	-					
5	0.00718	0.00718	0.00540	0.00720	0.00718	0.00718	0.01255	-				
17	0.01090	0.01090	0.01270	0.01086	0.01090	0.01090	0.01267	0.01809	-			
1	0.00179	0.00179	0.00357	0.00182	0.00179	0.00179	0.00714	0.00898	0.00908	0.00358	-	
12	0.02145	0.02145	0.01967	0.02175	0.02145	0.02145	0.02511	0.02520	0.02541	0.02353	0.01967	-
19	0.01625	0.01625	0.01445	0.01451	0.01625	0.01625	0.01627	0.01619	0.01816	0.01631	0.01445	0.02358
21	0.01608	0.01608	0.01787	0.01635	0.01608	0.01608	0.01974	0.02338	0.02000	0.01813	0.01430	0.00537
22	0.01252	0.01252	0.01431	0.01269	0.01252	0.01252	0.01613	0.01978	0.01632	0.01446	0.01074	0.00894
16	0.00723	0.00723	0.00542	0.00731	0.00723	0.00723	0.00908	0.01089	0.01464	0.00912	0.00543	0.01263

Haplotype	49	50	51	52	53	54	55
8							
10							
14							
4							
3							
5							
17							
1							
12							
19	-						
21	0.02177	0.01787	0.00358	-			
22	0.01990	0.01431	0.01073	0.00716	-		
16	0.01449	0.00903	0.01445	0.01084	0.00721	0.00720	-

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