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Genetic population structure and microbiome of german cockroaches in urban environments

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ABSTRACT

GENETIC POPULATION STRUCTURE AND MICROBIOME OF GERMAN COCKROACHES IN URBAN ENVIRONMENTS

By
Xueyang Fan

Pests of human habitats may harbor and disperse pathogens that cause human disease. One such pest is the German cockroach (*Blattella germanica*), which is known to harbor numerous pathogens, including *Klebsiella* and *Pseudomonas*. The aim of this study is to reveal the importance of the German cockroach as a potential vector of human medically important diseases. To do so, this study investigates German cockroach population structure and their associated bacterial microbiome in urban residential environments. Ninety German cockroaches are collected from three residential apartment buildings in three New Jersey cities. Samples are caught by glue traps and stored at -20℃. DNA and RNA are extracted from cockroach samples and sent for Next Generation Sequencing. Single-Nucleotide Polymorphisms (SNPs) are the genetic markers used for the cockroach population structure analysis. Thirty samples of the same extractions are also used for bacterial genetic analysis. Phylogeny, Principal Component Analysis (PCA), and STRUCTURE analysis are used for characterizing the population structure of cockroaches, which reflects the dispersal ability and colonization history of the cockroach populations. Results show that population structure exists among the three buildings/cities and within each building and indicates limited gene flow among buildings/cities. Within buildings, genetic population structure indicates both dispersal within buildings and multiple colonization events within each building. 16S rRNA is studied for understanding the

bacterial microbiome community on cockroaches and is used to quantify the abundance of bacterial operational taxonomic units (OTUs) found on the cockroaches. Bacterial microbiome diversity and ordination of OTUs are used to characterize the bacterial microbiome among the 30 samples from the same three buildings/cities. The results show a low but significant differentiation of bacterial community among three buildings and within one building. To test whether the genetic distance of German cockroaches within and among the three buildings is correlated with community distance among bacterial communities on the cockroaches, a Mantel test is implemented. The result of this test is negative, which indicates the lack of correlation between cockroach populations and bacterial communities. A laboratory test of the bacterial dispersal ability of German cockroaches is done by infecting cockroaches with fluorescence-marked E. coli. This test shows the strong bacterial dispersal ability of cockroaches. In conclusion, German cockroach structure is shaped by geographic separation, which does not affect the bacterial community found on the same cockroach populations. These results have several important implications for control of cockroach infestations and for control of the spread of human disease. First, cockroaches can spread among within buildings and cockroaches also may colonize a building multiple time. This indicates that control efforts should aim to eliminate cockroaches from all apartments within a building to prevent recolonization from within. Residents should be informed of effective methods to prevent reintroduction from external sources. Second, cockroaches harbor several medically important human bacterial pathogens. Care should be taken not to interact with cockroaches to limit human infection. Third, because bacterial communities do not appear to be strongly shaped by cockroaches, researchers should investigate other mechanisms of bacterial dispersal.

GENETIC POPULATION STRUCTURE AND MICROBIOME OF GERMAN COCKROACHES IN URBAN ENVIRONMENTS

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APPROVAL PAGE

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Tears of sadness and the tears of joy.
After crying, they are all the same.

A work of science and a work of life.
They are the same to my young eyes.

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CHAPTER 1

INTRODUCTION

1.1 Background

Urbanization creates heterogeneous structures and artificial landscapes and habitats that are distinct from the natural world (Dawson *et al*. 2009, Cui and Shi 2012). Urbanization has increased substantially in recent decades: the urban human population is estimated to have doubled since 1957 and is predicted to reach 70% of the global population in thirty years (Dawson *et al*. 2009). One of the characteristics of urbanization is the built environment (Stokes and Seto 2019). Structures in the urban environment are developed in two and three dimensions (Zhou *et al*. 2017, Mahtta *et al*. 2019). In two-dimensional space, the distribution of buildings, streets, and green space creates a mosaic of habitat types. The built environment also reaches into the third - vertical - dimension as the shape, size, and inner structure of buildings varies greatly, further enriching the complexity of the urban environment (Stokes and Seto 2019).

The complexity of the urban environment directly affects animal species living in urban areas. For example, street arrangements affect how much residents utilize walking and bicycling as means of transportation and recreation (McCormack and Shiell 2011), which can increase human movements between locations within urban settings. The impact of urban structures is not only limited to human beings, but also to other species sharing the same environment (Lagucki *et al*. 2017). Moreover, the heterogeneous mosaic environment of urban structures may affect multiple species (McIntyre 2000, Egerer *et al*.

2017). A study about the northern dusky salamander (*Desmognathus fuscus*) in New York City showed a strong population differentiation in an urban environment, which indicates the limit of the range of salamander movement (Munshi-South *et al*. 2013). Similarly, a study on white-footed mouse (*Peromyscus leucopus*) in New York City found a rapid genetic differentiation in an urban environment that has more habitat fragmentation due to parks and buildings than rural environments (Munshi-South and Kharchenko 2010). It also indicated the range of white-footed mouse in New York City is limited by the separation of their habitats.

The urban effect on the active range of organisms is also found in arthropod species. The active range of bees can be reduced by urbanization (Egerer *et al*. 2017). The isolation of urban gardens impeded the movement of bees and limited their foraging range across gardens (Egerer *et al*. 2017). For spiders, urbanization may reduce their movement between source habitats and new habitats (Otoshi *et al*. 2015). The effect of urban fragmentation on the active range of organisms is not only negative. Multiple studies have shown that fragmentation may also have positive and neutral effects on the dispersal and diversity of arthropod pollinators (Kremen *et al*. 2007).

The role of many arthropod species as vectors of human pathogens makes their movement and distribution in urban environments important to understand thoroughly (Šrámová *et al*. 1992). Many arthropod species in urban environments are vectors of human pathogens. For example, the common housefly (*Musca domestica*) is a widespread vector of human diseases, including *Shigella dysenteriae*, the bacterium that causes dysentery, and *Eberthella typhosa*, the bacterium that causes typhoid fever (Sarwar 2015). The housefly carries these pathogens on their feet and mouthparts (Sarwar 2015). Pharaoh ants

(*Monomorium pharaonis*) carry multiple pathogens, such as *Pseudomonas* and *Salmonella,* in hospital environments (Beatson 1972). Researchers found human disease pathogens, such as *Klebsiella pneumoniae* and *Escherichia coli*, on German cockroaches (*Blattella germanica*) and American cockroaches (*Periplaneta americana*) (Pai *et al*. 2005).

Urbanization can alter the ability of human disease vectors to disperse human pathogens. Hemme and colleagues (2010) found that the urban landscape can affect mosquito (*Aedes aegypti*) population structure by creating geographical barriers. Another study showed the distribution of kissing bugs (*Triatoma infestans*) - a blooding-sucking insect species that is an important vector of *Trypanosoma cruzi*, the cause of Chagas disease - was strongly affected by the street layout in urban environments (Barbu *et al*. 2013). Therefore, understanding the effect of urban structure on the distribution of disease vector species is important for the effective management of public health.

Among many arthropod pests, the German cockroach is a common pest species in urban environments (Bonnefoy *et al*. 2008) and has been shown to vector multiple medically important human pathogens (Devi and Murray 1991). For example, *Enterobacter cloacae* and *Pseudomonas aeruginosa* have been detected from the gut parts of cockroaches in urban environments (Devi and Murray 1991). These same medically important human pathogens have also been detected on the external body surfaces of cockroaches (Kassiri and Kazemi 2012). Therefore, the German cockroach is a potentially important vector of multiple human bacterial diseases (Beatson 1972, Pai *et al*. 2005).

The distribution of the German cockroach is affected by the structure of the urban landscape. Building separation is one of the factors that can shape cockroach dispersal and distribution. For instance, Crissman and colleagues (2011) found distinct cockroach

populations among separate buildings within the same city. Another study showed evidence that cockroach distributions may be affected by human transportation between locations (Booth *et al*. 2011). The structure within buildings can also affect cockroach distributions. One mark-and-recapture study indicated that individual cockroaches have active ranges across multiple apartments (Owens and Bennett 1982). While genetic studies have shown differentiation of cockroach populations among apartments within buildings (Crissman *et al*. 2010), these prior studies were not able to elucidate the details of cockroach distribution and movement within buildings due to insufficient genetic specificity. With more sensitive genetic markers, such as single-nucleotide polymorphisms (SNPs) (Brookes 1999), we may be able to achieve a higher resolution pattern of the distribution of cockroach populations, which can help us better understand their dispersal patterns.

Furthermore, although multiple studies have found human bacterial pathogens on or in cockroaches (Devi and Murray 1991, Fotedar *et al*. 1991, Pai *et al*. 2005, Kassiri and Kazemi 2012), the impact of cockroaches on the dispersal of pathogens in general, and bacteria in particular, remains unclear. Some evidence shows that cockroaches can transmit gut bacteria from older individuals to nymphs (Carrasco *et al*. 2014). Another laboratory study showed that cockroaches can transmit bacteria among individuals within a population (Kopanic Jr *et al*. 1994). However, the evidence from urban environments shows that our understanding of the impact of cockroaches on the distribution of bacterial communities remains lacking. The correlation between cockroach population structure and bacterial communities also needs to be study further.

A higher resolution analysis of German cockroach distribution can be useful to provide more information about the connection and distribution pattern of cockroaches in local environments, such as population differentiation and gene flow among floors within a building. The more detailed understanding of cockroach movement and population structure within buildings will allow more efficient pest control, and therefore more effectively prevent the resurgence of cockroach infestations. Understanding the relationship between cockroach populations and human disease pathogens can also optimize the resource spending and strategy of pest control by targeting control on situations where cockroaches are more likely to harbor and spread medically important pathogens.

1.2 Research Questions

In this study, the main question is to understand the impact of the distribution of German cockroach (*Blattella germanica*) on the bacterial community harbored by German cockroaches in urban environments. To answer this question, we divided this research into three steps:

1. What is the genetic populational structure of German cockroaches in different locations in New Jersey both in a large range (among cities) and in a local range (within a building)?

2. Are bacterial communities found on German cockroach populations different from one another?

3. Is there a correlation between the German cockroach population structure and bacterial communities from the same cockroach populations at both large and local ranges in New Jersey.

1.3 Hypothesis and Predictions

Our main hypothesis is that German cockroach dispersal and distribution has a strong impact on bacterial communities found on the exoskeletons of these same cockroaches. We test this hypothesis with five key subsidiary hypotheses.

Hypothesis 1: Cockroach dispersal is limited among apartment buildings in different cities. Prediction: Cockroach genetic population structure will show clear population differentiation among cities. Rationale: If cockroach dispersal among cities is limited, then populations at the city scale will be genetically isolated.

Hypothesis 2: Cockroach dispersal within residential apartment buildings is not limited, with cockroaches able to disperse among apartments and among floors. Prediction: Cockroach genetic population structure will show little population genetic structure within residential apartment buildings. Rationale: If cockroaches disperse freely among apartments and floors within apartment buildings, the cockroach population within buildings will be panmictic and show little population structure.

Hypothesis 3: Bacterial communities found on cockroaches are primarily determined by the local environment and thus will vary substantially among apartment buildings in different cities. Prediction: Bacterial community composition will significantly differ among apartment buildings in three cities. Rationale: Bacterial communities found on cockroaches will be determined by local conditions, potentially including the local environment and/or dispersal by local cockroach populations.

Hypothesis 4: Cockroaches transmit bacteria to other individual cockroaches. Prediction: When bacterially infected cockroaches are introduced into bacteria-free cockroach populations, the initially bacteria-free cockroaches will acquire culturable bacteria on their exoskeletons. Rationale: If bacteria are effective dispersers of bacteria, they must be able to transfer bacteria from one individual to another.

Hypothesis 5: Cockroaches disperse bacteria and thereby structure bacterial communities. Prediction: Genetic distance among cockroach populations will be correlated with distance among bacterial communities harbored by these same cockroaches. Rationale: If cockroach are primary dispersers of bacteria then the relatedness of bacterial communities found on them will mirror the relatedness of the cockroaches themselves.

1.4 Objectives

As stated above, the distribution of arthropod species can be affected by habitat fragmentation and building isolation. Many arthropod species, such as German cockroaches, are vectors of medically important human pathogens. Therefore, we used German cockroaches (*Blattella germanica*) as our research organism to study the distribution pattern of vector species in urban environments and its relation to the bacterial community found on the vector species. This is also the first study to test the effectiveness of SNP markers in quantifying German cockroach genetic population structure. To approach this goal, the research is divided into five main parts:

Objective 1: Cockroach collection and DNA/rRNA extraction. Cockroaches will be collected from multiple residential apartment buildings within New Jersey. These samples will provide genetic material for both cockroach population genetic structure

(DNA) and for bacterial microbiome analysis (rRNA), which are necessary to test hypotheses 1, 2, 3, and 5.

Objective 2: Determine cockroach population genetic structure. Cockroach population genetic structure will be characterized using single nucleotide polymorphisms (SNPs) (Ahmadian *et al*. 2000). Cockroach DNA will be sequenced using NextRAD technology (Russello *et al*. 2015). Resulting SNPs will be analyzed with a variety of methods including phylogenetic, F_{st} (Holsinger and Weir 2009), Principle Components (Jolliffe and Cadima 2016), and STRUCTURE analyses (Pritchard *et al*. 2000, Falush *et al*. 2003, 2007, Hubisz *et al*. 2009). Cockroach population genetic structure will inform us as to whether cockroaches are able to disperse among buildings/cites and within buildings and thereby test hypotheses 1 and 2.

Objective 3: Characterize cockroach bacterial microbiomes. The bacterial barcoding gene 16S will be sequenced from rRNA extracted from the cockroach samples to quantify the abundance of bacterial operational taxonomic units (OTUs) found on the cockroaches (Nguyen *et al*. 2016). These OTUs will form the basis of the bacterial community analyses. To investigate differences among bacterial communities, and thereby test hypothesis 3, we will utilize diversity analyses, Principle Coordinates Analysis (PCoA) (Gower 1966), and Linear discriminant analysis Effect Size (Segata *et al*. 2011).

Objective 4: Experimentally test whether cockroaches can transmit bacteria to other cockroaches. To test hypothesis 4, that roaches can transmit bacteria to other cockroaches, we will experimentally inoculate the surfaces of cockroaches with *E. coli,* and then introduce these infected cockroaches to naïve cockroach populations. If the naïve

cockroaches are shown to have culturable *E. coli*, then this will demonstrate that cockroaches can transmit bacteria from one individual to another.

Objective 5: Test whether bacterial community relatedness is correlated with cockroach genetic relatedness. This objective tests our overall hypothesis and hypothesis 5, that cockroaches structure bacterial communities by dispersing bacteria among locations. We will test this hypothesis with a Mantel test (Mantel 1967) of the correlation between two distance matrices, one for cockroach genetic distance and one for cockroach bacterial microbiome community distance.

1.5 Study Species and Technologies

In this study, we used the German cockroach (*Blattella germanica*) and their associated bacterial microbiome as the model system. German cockroaches and their associated bacterial microbiome are an ideal model organism to investigate the impacts of pathogen vectors on human health. German cockroaches are a globally distributed pest species of multiple human-related locations such as human dwellings, hospitals, and business places (Devi and Murray 1991; Wang *et al*. 2008). The innate dispersal ability of German cockroaches is considered to be limited by building structure and is otherwise highly affected by accidental dispersal by human activity (Rust *et al*. 1995). Mark-and-recapture studies showed there were only 15% of individuals moved across different areas within an apartment unit (Rivault 1990). Another study showed 30% of studied cockroaches can move across apartments within a building (Owens and Bennett 1982). Therefore, on one side, individual German cockroaches can freely move and interact within a metapopulation (Rust *et al*. 1995) in one location. Furthermore, individuals from different families in a

metapopulation can aggregate, feed, and rest together (Lihoreau *et al*. 2016), which can create a strong gene flow among individuals. On the other hand, the limited dispersal ability of German cockroaches can create detectable population structure within and among buildings (Crissman *et al*. 2010).

As a common pest species, German cockroaches are considered to have important impacts on human health. Cockroaches produce allergens that are an important contributor to asthma (Do *et al*. 2016). Many human medically important pathogens are detected from German cockroaches from residential and hospital locations (Rivault 1993; Kassiri and Kazemi 2012; Salehzadeh 2007). German cockroaches are potential vectors of bacterial pathogens (Rivault 1993; Pai *et al*. 2005; Kassiri and Kazemi 2012; Salehzadeh 2007). Previous study indicated the strong ability of cockroaches to disperse and transmit bacteria among individuals in the laboratory environment (Kopanic Jr *et al*. 1994). However, the test of this dispersal ability in the urban environment has not been done yet. Therefore, understanding the distribution of German cockroaches and its impacts on the dispersal of bacteria in urban environment is important for human health.

In this study, we used male adult German cockroaches collected from three apartment communities in three cities (Paterson, Irvington, and Trenton) of New Jersey by setting glue traps in each apartment for fourteen days. The genome of German cockroaches was extracted from cockroach samples (with gut removed) for cockroach population study and the bacterial community study. Single-nucleotide polymorphisms (SNPs) markers were used in the cockroach population study. SNPs are commonly found genetic variations in organisms (Ahmadian et al. 2000), which can provide more genetic loci than microsatellites, and therefore potentially higher resolution for genetic population studies.

The bacterial community study is based on the 16S rRNA sequencing. 16S rRNA has nine variable regions and is commonly used as a barcode gene in the study of bacterial communities (Janda and Abbott 2007). Those genetic markers were also used in the study of the correlation between cockroach populations and bacterial communities. Jwax, a lab-reared German cockroach strain (Wang and Bennett 2006), was also used in this study for a laboratory experiment about the bacterial dispersal ability.

CHAPTER 2

GENETIC STRUCTURE OF GERMAN COCKROACHES IN THE URBAN ENVIRONMENT IN NEW JERSEY

2.1 Introduction

Chronic pest infestations in residential buildings is a common phenomenon in the urban environment (Runstrom and Bennett 1984; Saenz *et al*. 2012; Barbu *et al*. 2014; Wang *et al*. 2019). Even in the locations where extermination treatments are applied multiple times, pest resurgence can occur in treated households (Barbu *et al*. 2014). The pests in the environment can come from three sources: from within the local household when treatments were not completely effective, from adjacent households and endogenous pest movement, and from new colonizations from the external environment where movement is assisted by human action (Saenz *et al*. 2012). Evidence shows that the colonization of pests in urban environments occurs in adjacent locations (Runstrom and Bennett 1984; Cooper *et al*. 2015), indicating endogenous pest movement. Furthermore, human beings and pets can be the vector for pests among more distant locations as well (Szalanski *et al*. 2014). Pest recolonization results in wasted time, effort, and resources (Myers *et al*. 2000). However, population demographics of pest species are often poorly understood. A well-developed understanding of these source–sink dynamics and the population structures of pest species that result will allow for more effective pest control (Pulliam 1988).

Cockroach species (Order: Blattodea), as generalists (Dow 1986), have a good ability to survive with limited resources (Bell *et al*. 2007). Some species of cockroaches are pest species *as Periplaneta americana* (L)*., Blattella germanica, Periplaneta fuliginosa* (Serville)*, Periplaneta brunnea* (Burmeister), and *Periplaneta australasiae* (F.) (Mallis

1954) and can live in a wide range of habitats including hospitals, restaurants, and dwellings. In urban areas, cockroaches live on food resources that are provided by a variety of plant- and animal-based foods that are unintentionally provided by human beings (Bell *et al*. 2007, Kassiri and Mazemi 2012). Several commensal cockroach species are widely distributed in the world. They are able to disperse independently within buildings and have low motility among buildings without human assisted dispersal (Roth 1985). Together these characteristics lead to strong genetic structuring within and among cockroach populations (Vargo *et al*. 2014).

The German cockroach (*Blattella germanica*) is a globally distributed pest in human habitats (Devi and Murray 1991) and is the most common cockroach species in the U.S (Wang *et al*. 2008). *B. germanica* are known to harbor several medically important pathogens (such as *Enterobacter cloacae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Serratia marcescens*) both within their guts (Devi and Murray 1991) and also attached to their surfaces (Kassiri and Mazemi 2012; Rivault 1993; Salehzadeh, 2007). *B. germanica* also produces and spreads allergens that contribute to asthma and damage human health (Gore and Schal 2007).

Urban landscapes present a complex environment for biological organisms (Melles *et al*. 2003). The complexity of food resources (Kwate *et al*. 2009; Rundle *et al*. 2009) and different economic conditions of residents (Corburn *et al*. 2006) likely modulates the distribution and abundance of cockroaches. In addition, differences in building configuration and function affects migration and distribution patterns of cockroaches in urban areas. For example, building layout and construction details, such as the separation of rooms, strongly affects the distribution of German cockroaches (*Blattella germanica*)

(Crissman *et al*. 2010). Other research shows that the movement of farm workers in an agricultural landscape can influence the distribution of cockroaches (Booth *et al*. 2011).

Cockroach related pest control costs ranked the 3rd among urban pests in the U.S. (IBIS-World 2011). Despite the economic impact of *B. germanica* our understanding of their patterns of colonization, control or eradication, and recolonization, remains unclear. Cockroach population genetic structure may be the best tool to understand these dynamics. The earliest population genetic studies of *B. germanica* in urban environments utilized allozyme loci and failed to reveal significant differentiation among populations between two cities in a range of 900 km in France (Cloarec *et al*. 1999). People used F_{st} to estimate the differentiation degree within a group (Holsinger and Weir 2009). Low F_{st} indicates a strong gene flow within a given population. More recently, Crissman *et al*. (2010) used microsatellite markers to show low yet significantly non-zero F_{st} values (range 0.014-0.028) and therefore some cockroach genetic structure within apartment buildings. However, their results were not able to define any population genetic relationships within complexes as neither the STRUCTURE algorithm nor neighbor joining phylogenetic trees provided resolution of genetic structure within or among apartment buildings, but did differentiate among apartment complexes (Crissman *et al*. 2010). In contrast, a similar microsatellites approach found strong *B. germanica* population differentiation among swine farms across a broader region in North Carolina (Booth *et al*. 2011).

In the present research, we used single-nucleotide polymorphisms (SNPs) to quantify the population structure of *B. germanica* at local scales within apartment buildings and at regional scales among cities within New Jersey. Our novel use of more than 2000 SNPs allows us to examine *B. germanica* population structure in more detail than

previously possible. A detailed understanding of *B. germanica* population structure is essential for understanding how cockroaches colonize apartment buildings and recolonize individual apartments after local eradication. In particular, we aim to determine here whether the population genetic structure of *B. germanica* supports singular colonizations of apartment buildings and local spread, or multiple colonizations of the same apartment buildings. We hypothesize that there are three separate populations among three buildings and separate populations among floors in each building.

2.2 Methods and Materials

2.2.1 Cockroach Sampling

B. germanica were collected from multiple apartment buildings in the New Jersey cities of Paterson (PB, April 2017), Irvington (IB, May 2017), and Trenton (TB, February 2018). The cockroaches were collected using glue boards (Trapper Monitor & Insect Trap, Bell Laboratories Inc., Madison, WI) placed in apartments for approximately 14 days. Three traps were placed in the kitchen and one trap was placed in the bathroom in each apartment. After collection, traps with cockroaches were sealed in the separate plastic bags and stored at -20°C. Each building has multiple floors and multiple apartments per floor. The building in Paterson (PB) was built in 1962, the building in Irvington (IB) was built in 1953, and the building in Trenton (TB) was built in 1965. The location and distances between the buildings are shown in Figure 2.1. These three buildings range from northern to central NJ. The distance between PB and IB is about 22.7 km. The distance between IB and TB is 71.3 km. The distance between PB and TB is about 93.6 km. Each of the three buildings received monthly pest control services hired by the management. The pest control company primarily relied on application of cockroach gel bait to control cockroaches. However, many infestations were skipped during monthly pest control service when residents were not home during the visit. The treatment was very brief and insufficient bait was used. Some residents used sprays on their own to suppress cockroach infestations. Most of the apartments had one bedroom or were studios. Less than 10% of them had two bedrooms.

2.2.2 DNA Extraction and Single Nucleotide Polymorphism Analysis

For population genetic analyses, we sampled only those apartments that had adult cockroaches on the traps. We selected 30 adult cockroaches from each of the three buildings, spread across 6-8 apartments and 4-6 floors. We generally sampled 5 cockroaches per apartment except in building TB where there were not sufficient adult roaches in each apartment. Sample collection information is shown in Table. 2.1.

Cockroach genomic DNA was extracted from the cockroaches (after the abdomen was removed) using DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA, USA) in July 2018. DNA samples were sent to SNPsaurus (Oregon, USA) for sequencing and single nucleotide polymorphism (SNPs) analysis. Based on the information they provided, NextRAD genotyping-by-sequencing libraries were built by SNPsaurus using the method described by Russello *et al*. (2015). The Nextera reaction was scaled for fragmenting 40 ng of genomic DNA, although 50 ng of genomic DNA was used for input to compensate for degraded DNA in the samples and to increase fragment sizes. Fragmented DNA was then amplified for 27 cycles at 74 degrees, with one of the primers matching the adapter and extending 10 nucleotides into the genomic DNA with the selective sequence GTGTAGAGCC. The nextRAD libraries were sequenced on a HiSeq 4000 with one lane of 150 bp reads. The genotyping analysis is done by using custom scripts from SNPsaurus. The vcf file (Danecek *et al* 2011) was filtered to remove alleles with a population frequency of less than 3%. After the SNPs analysis, the SNPsaurus provided vcf file that included the SNPs data of the whole genome of *B. germanica* and a phylip data file (Felsenstein 1989) for creating the phylogenetic tree in the following steps using the alignment with Mesquite (Maddison *et al*. 2007).

2.2.3 Cockroach population genetic analysis

The genetic population analysis was done by using SNPs data from the whole genome of *B. germanica* we received from the previous sequencing step. Phylogeny, Principal Component Analysis (PCA), and STRUCTURE analysis were used as follows for quantifying the population structure of the cockroaches in our study.

2.2.4 Phylogenetic analysis

To investigate the genetic relationships within and among the populations from the three apartment buildings, a phylogenetic tree was created with genotype data from the SNPs sequencing result using IQ-Tree (Nguyen *et al*. 2015; Trifinopoulos *et al*. 2016). We used the IQ-tree web server to generate the tree (Nguyen *et al*. 2015; Trifinopoulos *et al*. 2016) using the Ultrafast method (Minh *et al*. 2013) with 10000 bootstrap alignments and 1000 iterations. Based on BIC criteria, IQ-Tree chose a symmetric model with unequal rates but equal base frequencies (Zharkikh 1994) and a discrete Gamma model (Yang, 1994) with 4 rate categories. The final phylogenetic tree was presented via iTOL (Ciccarelli *et al*. 2006).

2.2.5 Principal Component Analysis

We conducted PCA analysis using the “SNPRelate" package (Zheng *et al*. 2012) in R (R Core Team 2019). We took a hierarchical approach, first grouping our data by each of the three apartment buildings, and second grouping by floors within each building. We used the “gdsfmt” package in R to manage and organize the snp data (Zheng *et al*. 2012). The data were first pruned of any loci with a linkage disequilibrium value (Zaykin *et al.* 2008) greater than 0.2 using the “snpgdsLDpruning” function from the “SNPRelate" package in R (Zheng *et al*. 2012), resulting in 2419 unlinked loci.We performed principal component

analysis (PCA) on the unlinked loci using the function “snpgdsPCA” from the “SNPRelate" package in R (Zheng *et al*. 2012). We calculated F_{st} with 95% confidence intervals among the three buildings and between floors within each building using the “snpgdsFst” function following the method of Weir and Hill (2002) from the “SNPRelate" package in R (Zheng *et al*. 2012).

2.2.6 STRUCTURE Analysis

We analyzed population structure using the STRUCTURE algorithm as implemented in STRUCTURE version 2.3.4 (Pritchard *et al*. 2000, Falush *et al*. 2003, Falush, *et al*. 2007, Hubisz *et al*. 2009) using the dataset pruned of linked loci. We first applied the STRUCTURE algorithm to the full dataset with all three buildings. Next, we applied the STRUCTURE algorithm to each of the three buildings individually. We used 50000 burn-in periods and 200000 repeats. We ran five replicates for each presumed number of population (K). We used an admixture model. To identify the number of populations (K) among the three buildings, we tested from K=1 to 6. Within each building, we tested from K=1 to 8. We identified the correct number of populations (K) following the Puechmaille Method (Puechmaille 2016) rather than the Evanno Method (Evanno *et al*. 2005) because the Puechmaille Method is more appropriate for unbalanced samples (Puechmaille 2016). We used the StructureSelector online service (Li and Liu 2018) to interpret the STRUCTURE result and estimate the population number with Puechmaille Method (Puechmaille. 2016). StructureSelector (Li and Liu 2018) is an online service that integrates the function to estimate the population number with multiple methods and plot the population composition graph.

Table 2.1 *B. germanica* Samples Collected from Apartment Buildings in Three New Jersey Cities

Building	Floors sampled	Apartments sampled per floor	Cockroaches sampled per floor
PB	7	1	5
	9	2	10
	10	2	10
	11	1	5
IB	2	1	5
	4	2	10
	7	2	10
	9	1	5
TB	3	3	3
	4	2	4
	5	1	1
	6	3	11
	7	1	1
	8	2	10

2.3 Results

2.3.1 Cockroach genetic population structure among buildings and cites

Among the three apartment buildings in three different New Jersey cities, the global F_{st} value of 0.103 was significantly greater than 0 (95% CI 0.0985-0.1075), and therefore shows substantial gene flow and yet some evidence of population differentiation (Table 2.2). Principal components analysis supports this conclusion, with clear clustering of samples within buildings (Figure 2.2). Phylogenetic analysis also supports this conclusion with each of the buildings forming distinct clades with high bootstrap support (IB = 100%, TB = 100%, PB = 99.6%, Figure 2.3). Branch lengths representing genetic distance revealed that IB is more genetically distinct from both PB and TB than they are from each other (Figure 2.3). Structure analysis also supports population differentiation among the three apartment buildings, as the Puechmaille Method indicates three populations (Table 2.2), with all samples from IB and PB falling in the same group and 23 of 30 samples from TB showing majority likelihood for the third population and plurality likelihood for 5 of 30.

2.3.2 Cockroach genetic population structure within apartment buildings

F_{st} values within each building are also significantly greater than zero, and y*et al*so relatively low, suggesting substantial gene flow within buildings with some limited population structure (Table 2.2). Within PB building, the F_{st} value 0.057 (± 0.0045 CI) is lower than in the other two buildings, suggesting greater gene flow within PB. In TB building, F_{st} is higher (0.119 ± 0.0049 CI) than global F_{st} (0.103 ± 0.0045 CI) (Table 2.2). However, within buildings, the PCA result didn't show a clustering pattern as obvious as that found between buildings -- some floors are clustered, but others are not (Figure 2.2).

The phylogenetic result showed an obvious genetic structure among the three buildings. Bootstrap support was strong for the three separate three building clades and for most of the other branches on the tree (See Figure 2.3). At the building level, the bootstrap value supported that most of the samples are clustered within the apartments where they were collected from. However, within each building, several samples were clustered with samples from different apartments. Generally, samples from the same or nearby floors did not tend to be clustered in closely related clades (Figure 2.3).

STRUCTURE analysis agreed with the differentiation among buildings, with three clusters (K=3) of the *B. germanica* population revealed by STRUCTURE analysis based on the Puechmaille Method (Puechmaille 2016). Within each building, the STRUCTURE analysis indicated three population clusters in PB and IB and five populations for TB (Figure 2.4 and Table 2.2).

Table 2.2 F_{st} Values Across Three Buildings and Within Each Building and Population Estimation from STRUCTURE Analysis

Locations	Number of samples	F_{st} (95% CI)	Number (K) of populations found by STRUCTURE
Within PB	30	0.057 ± 0.0045	3
Within IB	30	0.089 ± 0.0062	3
Within TB	30	0.119 ± 0.0049	5
Among three buildings	90	0.103 ± 0.0045	3

The number of population is interpreted by Puechmaille Method (Puechmaille 2006).

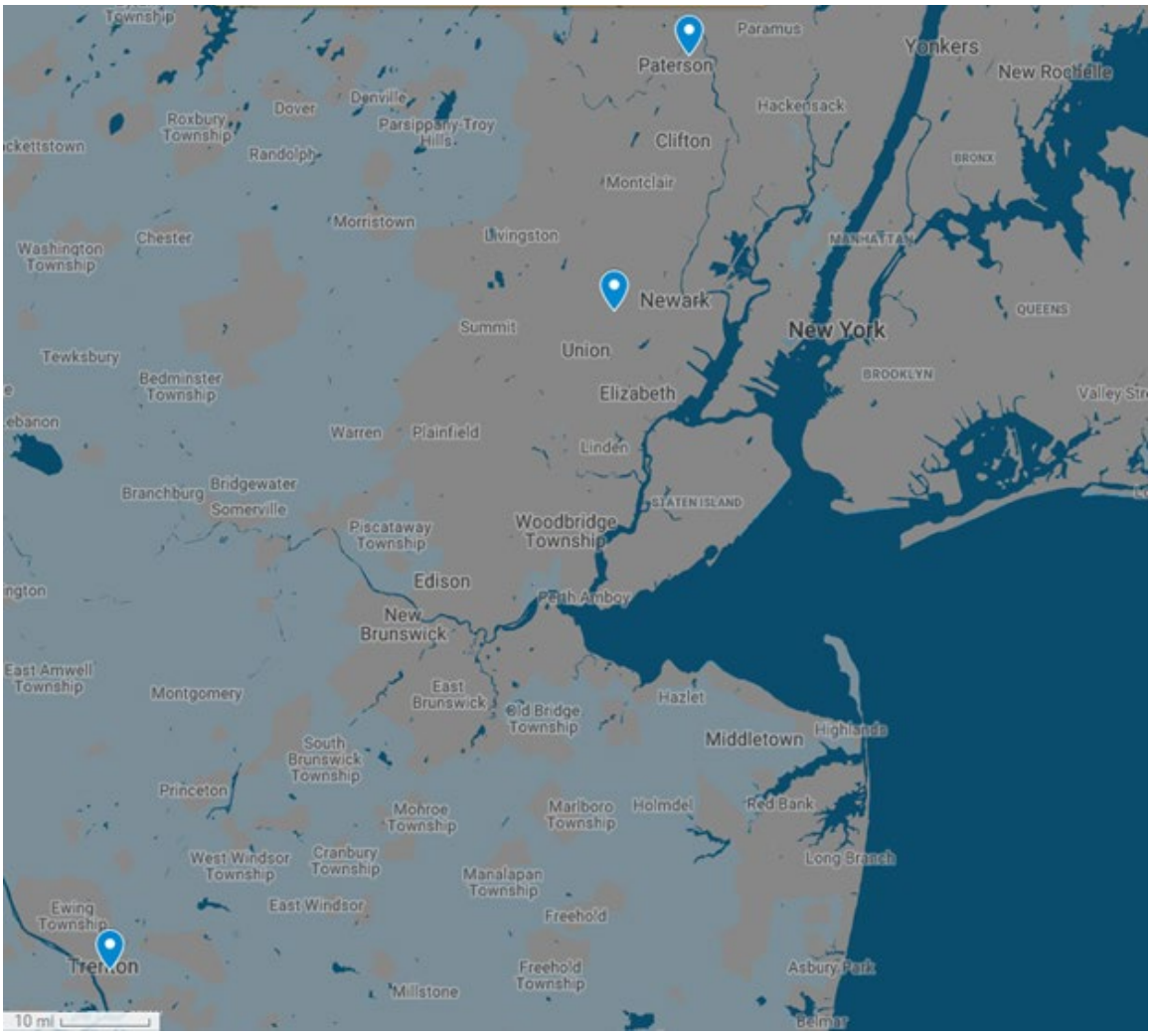
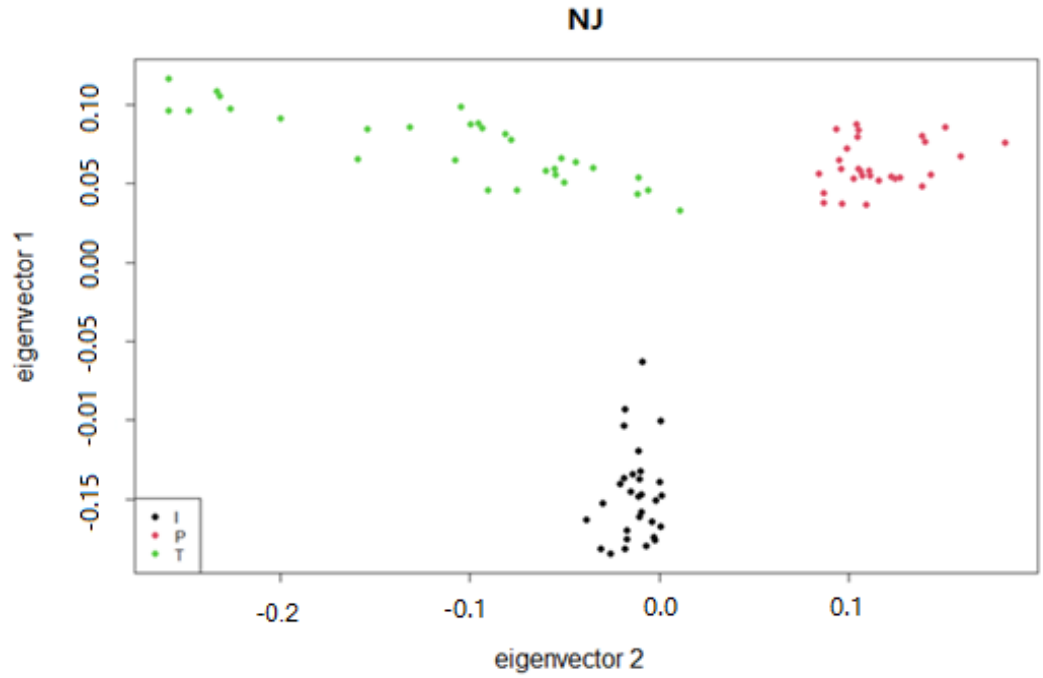


Figure 2.1 Locations of Three Buildings In New Jersey, USA, in the Cockroach Population Genetic Study.
NOTE: From top to the bottom are Paterson (PB), Irvington (IB), and Trenton (TB).


NJ
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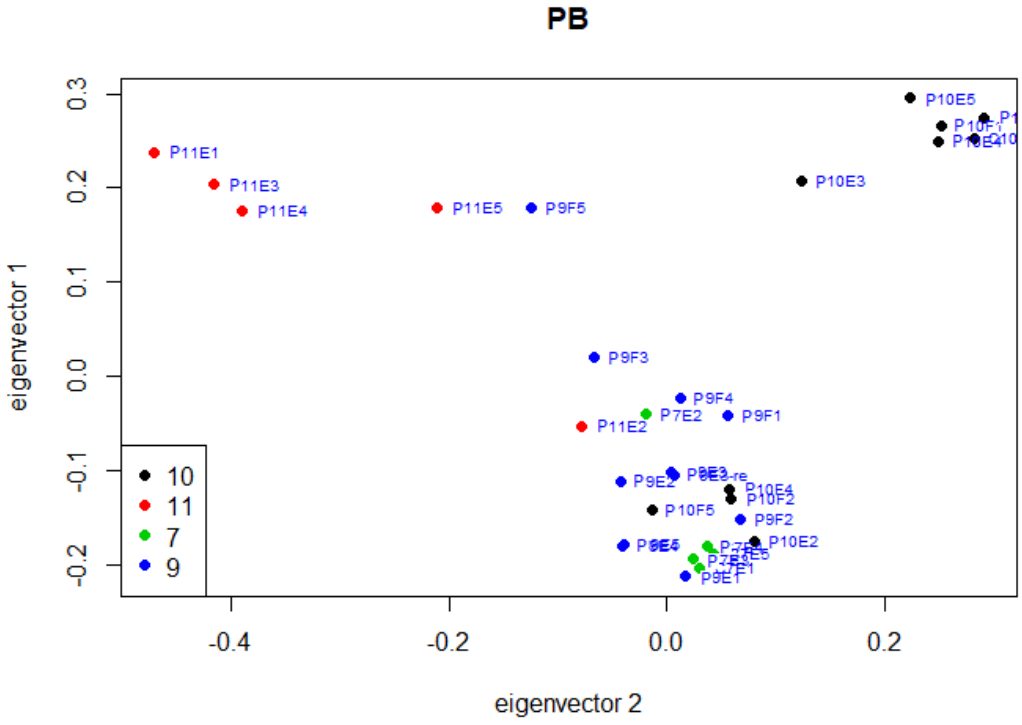
eigenvector 2
0.10
0.05
0.00
-0.05
-0.01
-0.15
-0.2
-0.1
0.0
0.1
I
P
T


PB
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eigenvector 2
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
P10E5
P11E1
P11E3
P11E4
P11E5
P9F5
P10E3
P9F3
P9F4
P7E2
P9F1
P11E2
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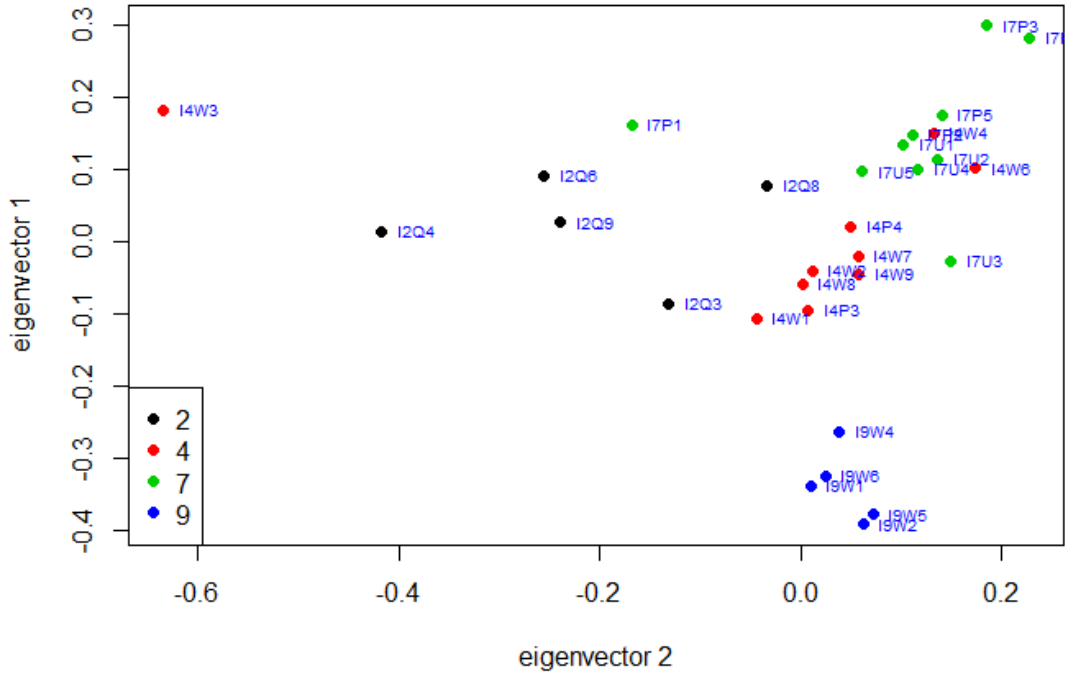
10
11
7
9

IB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eigenvector 2
2
4
7
9
I4W3
I7P1
I2Q6
I2Q8
I2Q4
I2Q9
I4P4
I4W7
I4W8
I4W9
I7U3
I2Q3
I4W1
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I9W6
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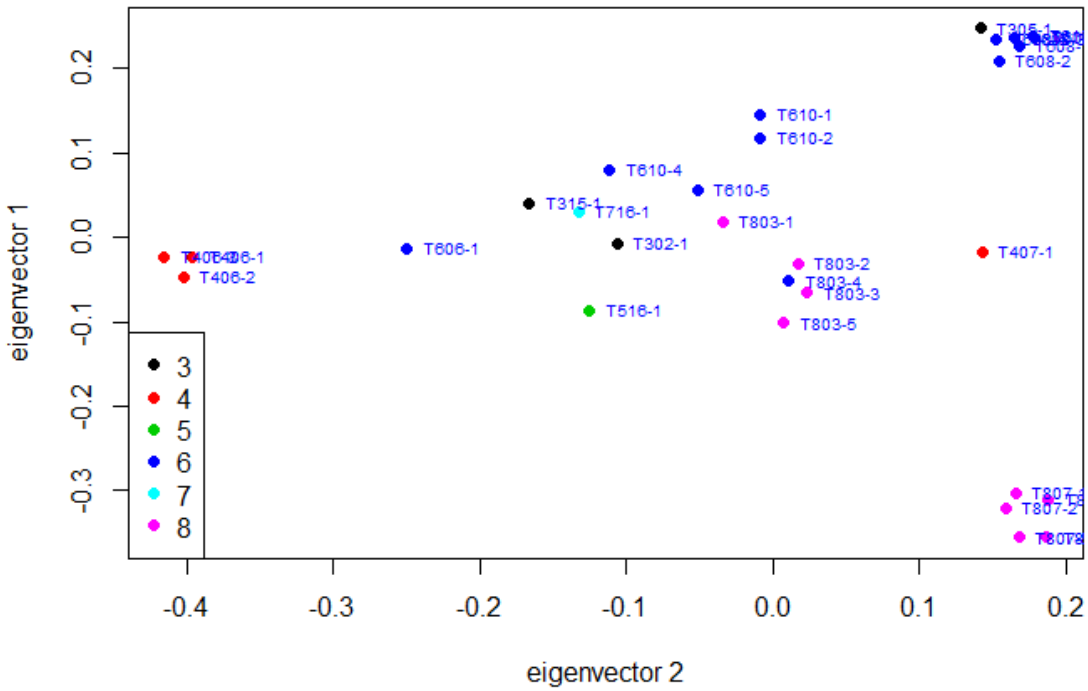
I9W5
I9W2
I7P3
I7P5
I4W6

TB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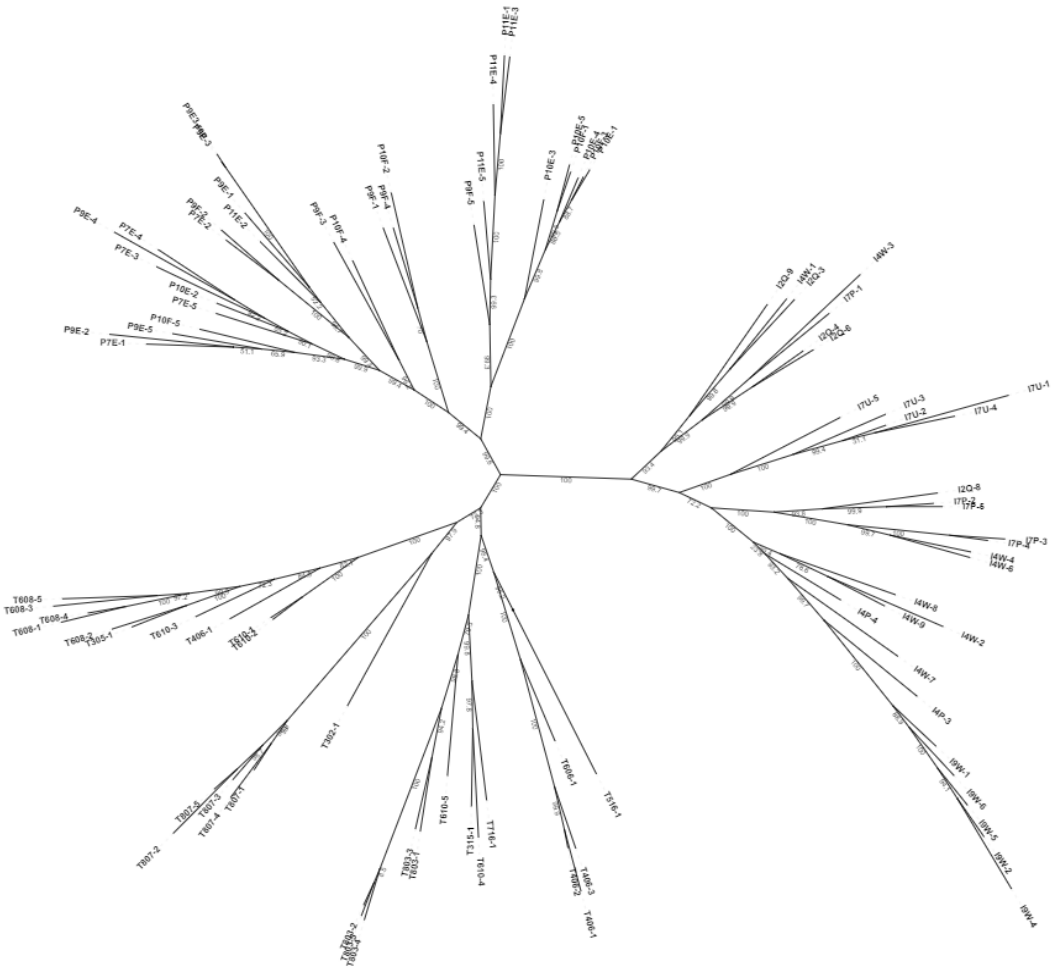
eigenvector 2
3
4
5
6
7
8
T608-2
T610-1
T610-2
T610-4
T610-5
T315-1
T716-1
T803-1
T606-1
T302-1
T803-2
T803-4
T803-3
T407-1
T406-2
T516-1
T803-5
T807-2

Figure 2.2 Principal Component Analysis results based on the cockroach SNPs data among and within three apartment buildings in three New Jersey cities.
NOTE: “NJ”.includes samples from all three buildings/cities. Colors indicate the locations (buildings) of the populations. “I” means “IB”. “P” means “PB”. “T” means “TB”.“PB”, “IB” and “TB” panels represent the populations within each building. Colors indicate locations (floors). The label of each sample follows the pattern: Building code (letter)-Apartment code (letter/number)- Replicate code (the last number). For example, I2Q9 means the sample 9 from 2Q apartment in the building IB. T606-1 means the sample 1 from the apartment 606 in the building TB.

NJ


P11E-1
P11E-3
P11E-4
P11E-5
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P10F-2
P9F-2
P9F-1
P9F-4
P9F-3
P10F-4
P9E-1
P11E-2
P9E-2
P11E-2
P9E-4
P7E-4
P7E-3
P10E-2
P7E-5
P10F-5
P9E-5
P9E-2
P7E-1
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I4W-1
I2Q-3
I4W-3
I7P-1
I2Q-7
I2Q-6
I7U-5
I7U-3
I7U-2
I7U-4
I7U-1
I2Q-8
I7P-5
I7P-3
I7P-4
I4W-4
I4W-6
I4W-8
I4P-4
I4W-9
I4W-2
I4W-7
I4P-3
I9W-1
I9W-6
I9W-5
I9W-2
I9W-4
T608-5
T608-3
T608-1
T608-4
T608-2
T305-1
T610-3
T406-1
T610-1
T610-2
T302-1
T807-5
T807-3
T807-4
T807-1
T807-2
T610-5
T516-1
T606-1
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T606-2
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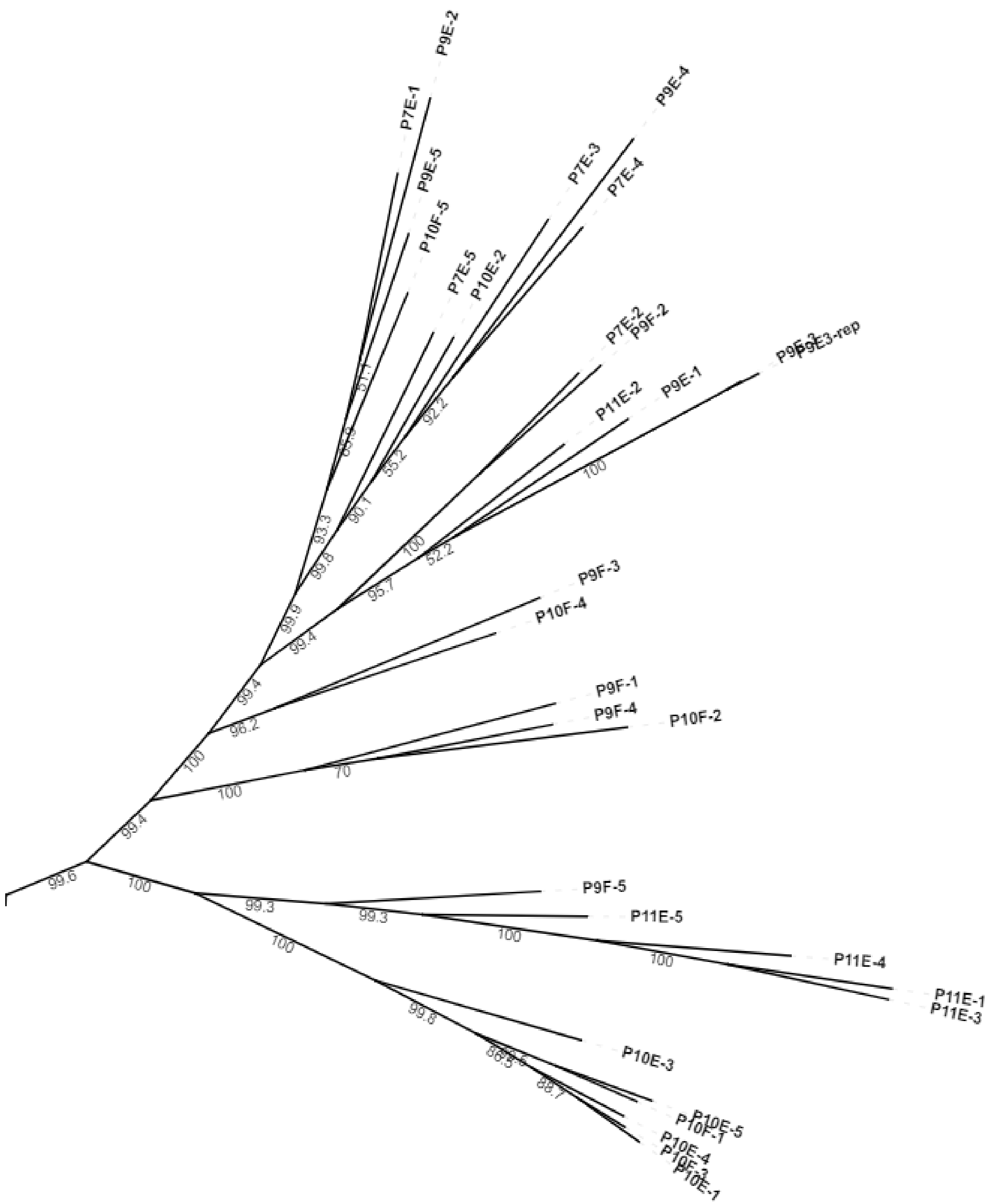
T303-3
T303-1
T610-4
T512-1
T716-1

PB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P9F-2
P11E-2
P9E-1
P9E3-rep
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P9F-1
P9F-4
P10F-2
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P11E-5
P11E-4
P11E-1
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P10E-5
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92.2
55.2
93.3
90.1
99.8
100
52.2
99.9
95.7
99.4
99.4
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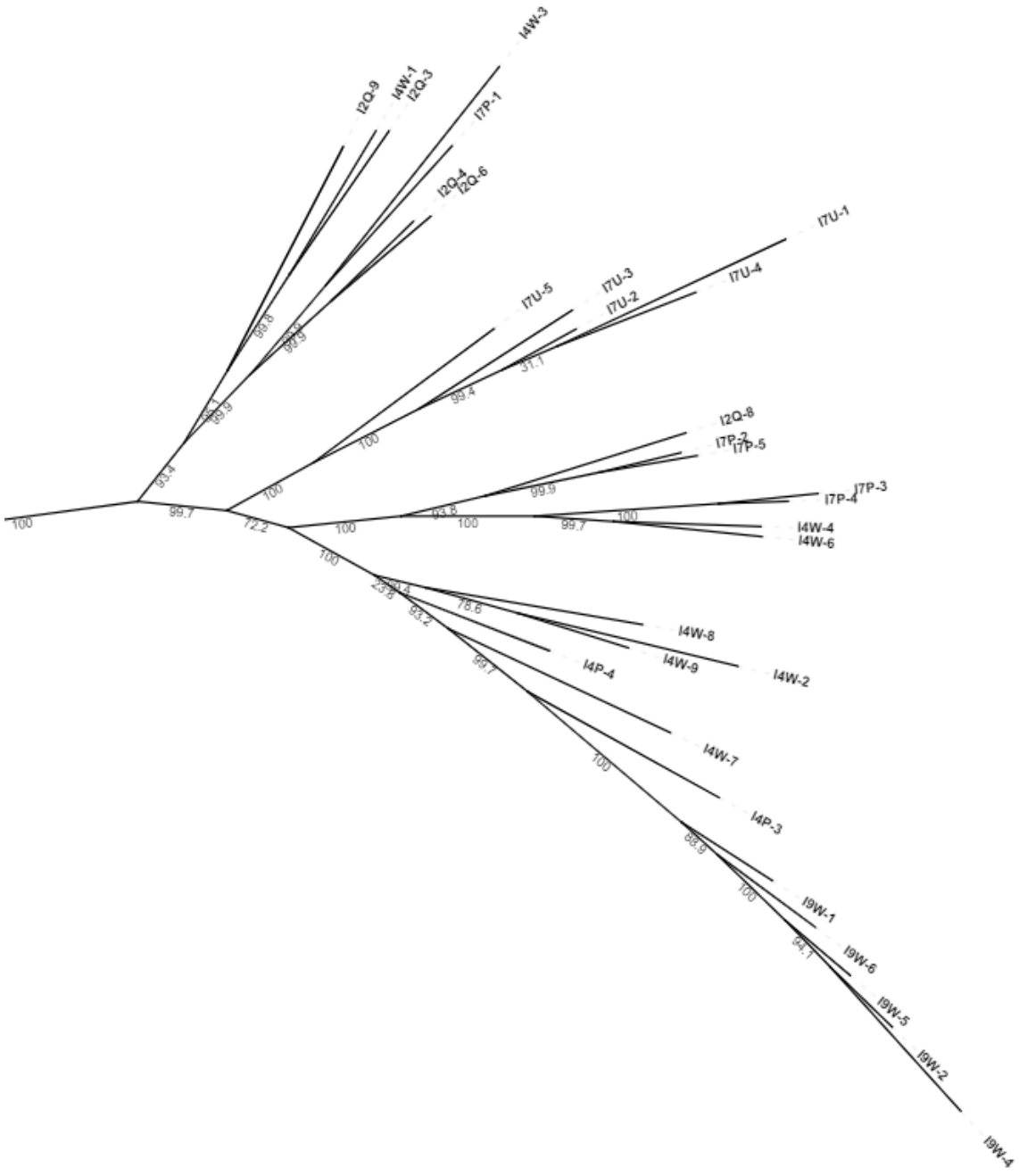
99.3
100
100
100
99.8
88.7

IB


I4W-3
I2Q-9
I4W-1
I2Q-3
I7P-1
I2Q-4
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I7U-5
I7U-3
I7U-2
I7U-4
I2Q-8
I7P-2
I7P-5
I7P-3
I7P-4
I4W-4
I4W-6
I4W-8
I4W-9
I4W-2
I4P-4
I4W-7
I4P-3
I9W-1
I9W-6
I9W-5
I9W-2
I9W-4
100
99.7
72.2
93.4
99.8
99.9
100
99.4
31.1
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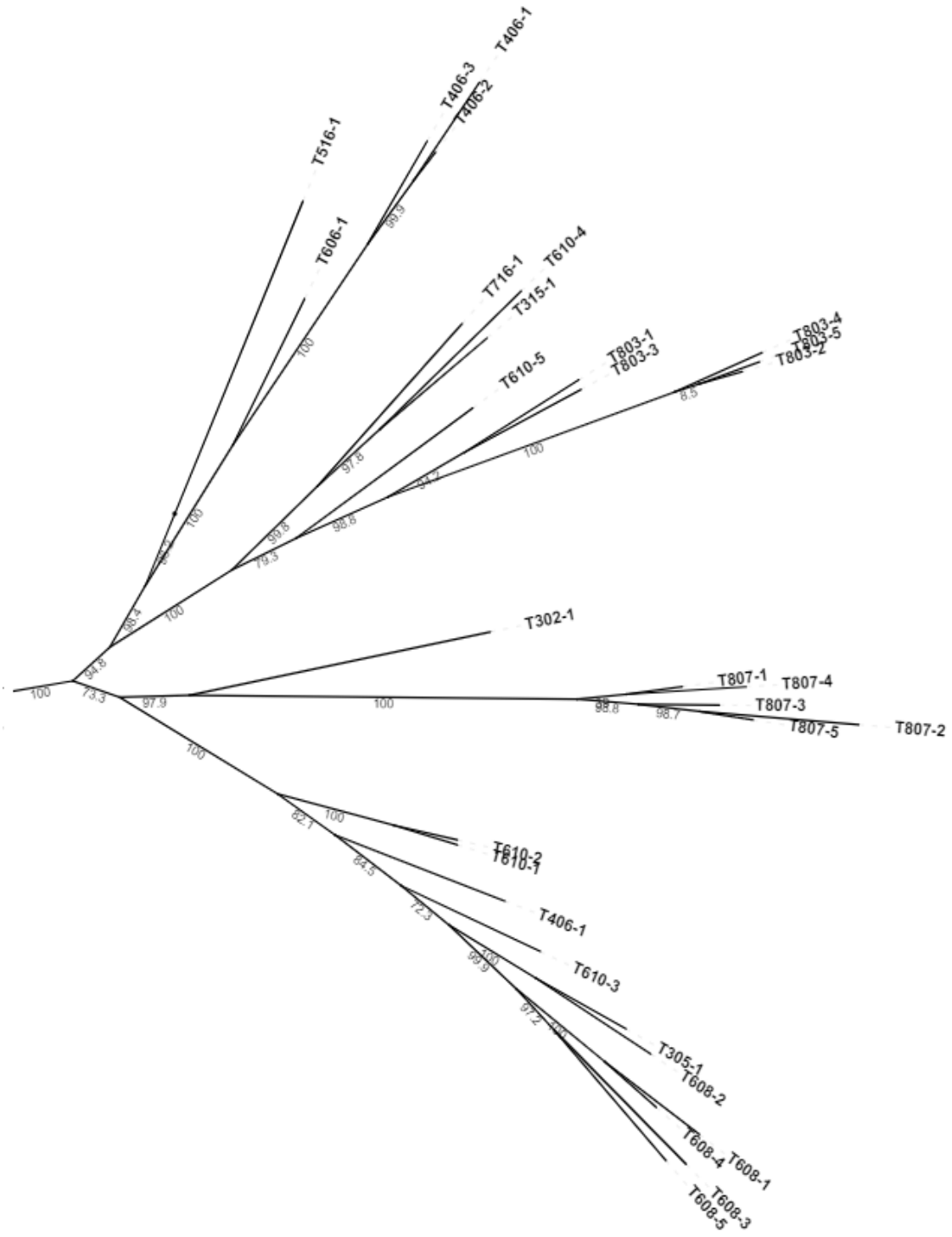
93.2
99.7
100
88.9
100
84.1

TB


T406-1
T406-3
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T516-1
T606-1
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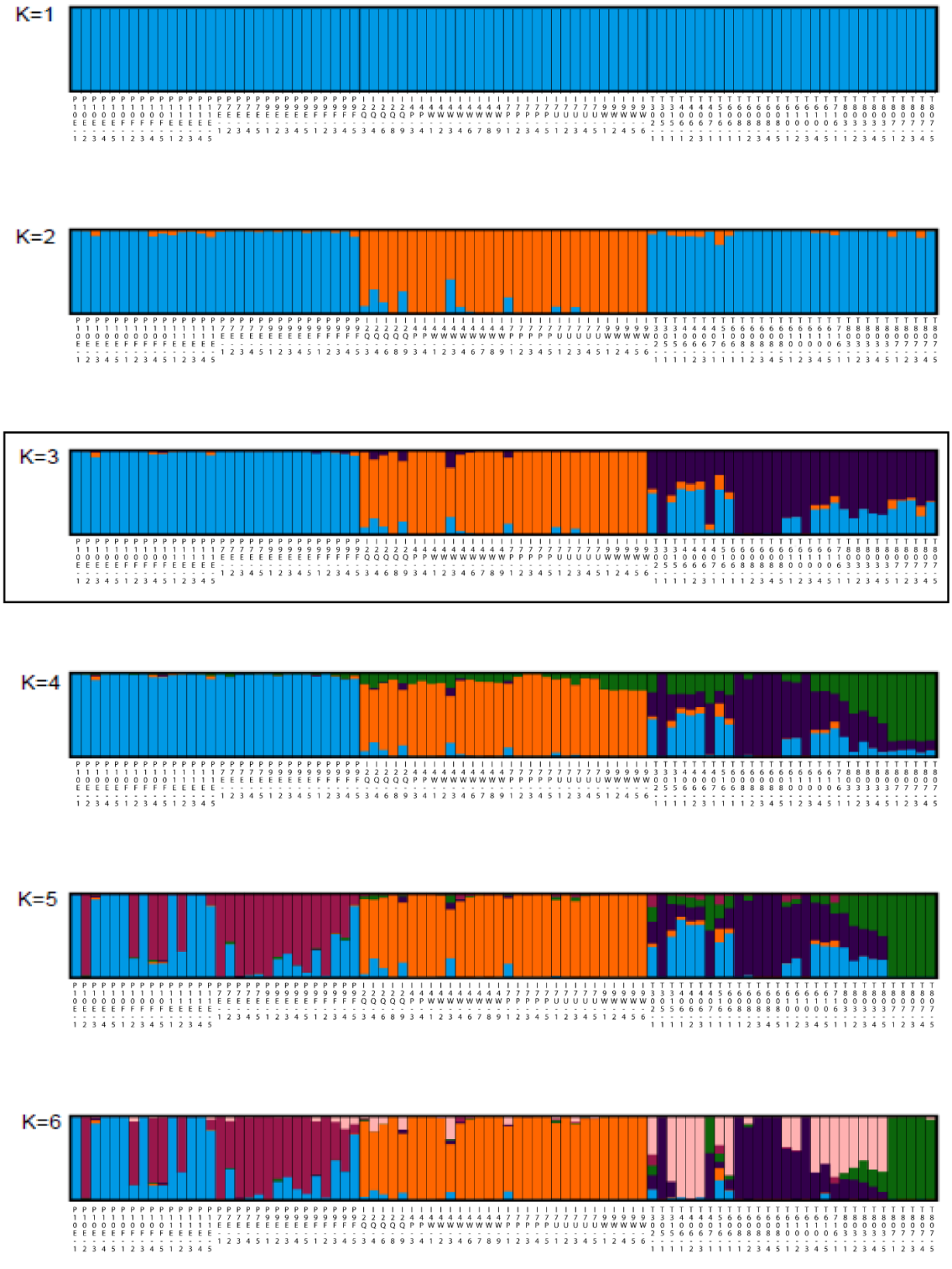
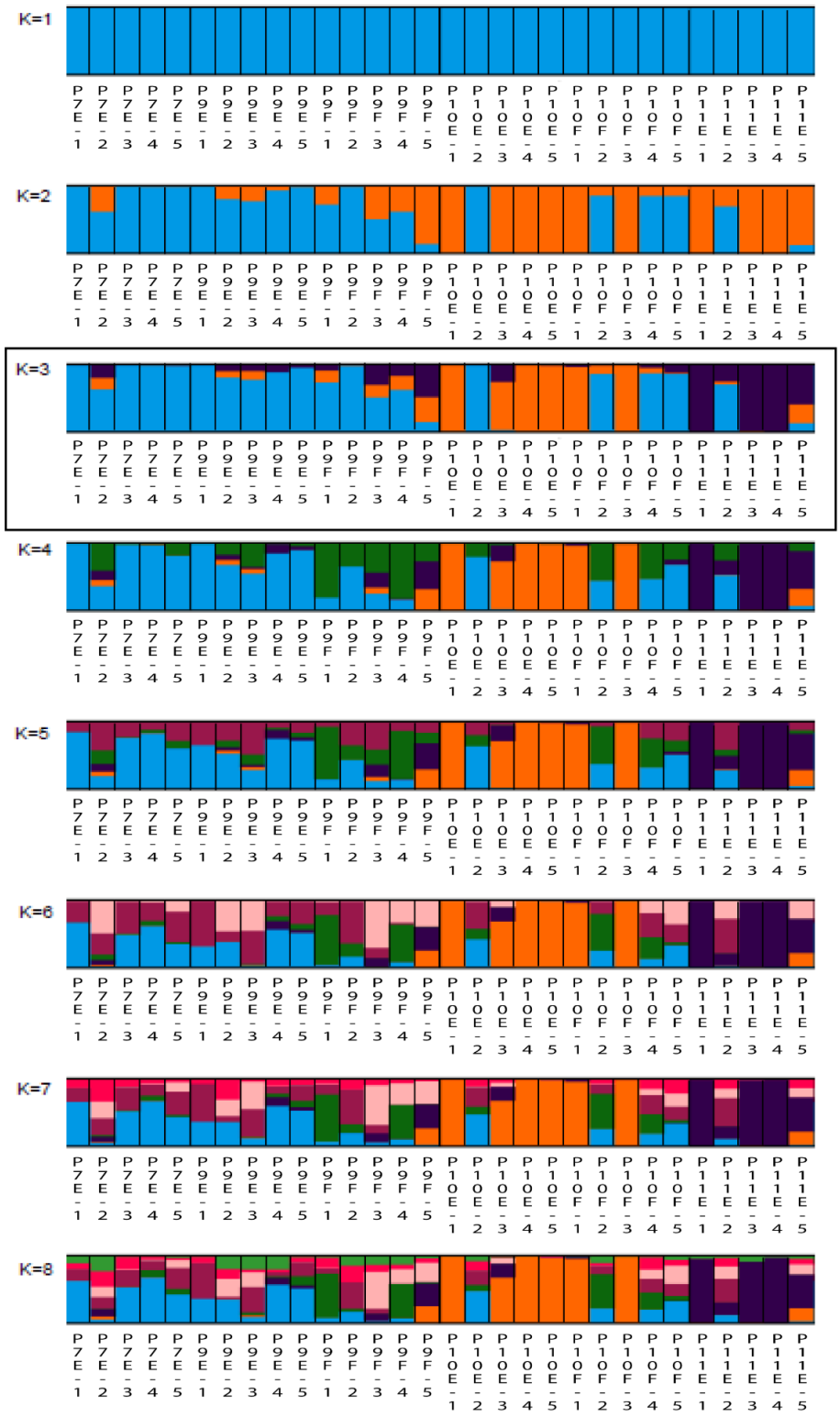
T610-2
T610-1
T406-1
T610-3
T305-1
T608-2
T608-4
T608-1
T608-3
T608-5

Figure 2.3 Unrooted phylogenetic tree from whole genome SNP data.
NOTE: The three main clades represent populations from the three apartment buildings in three New Jersey. The label for each leaf indicates Building-Floor-Apartment-Replicate.

NJ



PB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K=2
K=3
K=4
K=5
K=6
K=7
K=8
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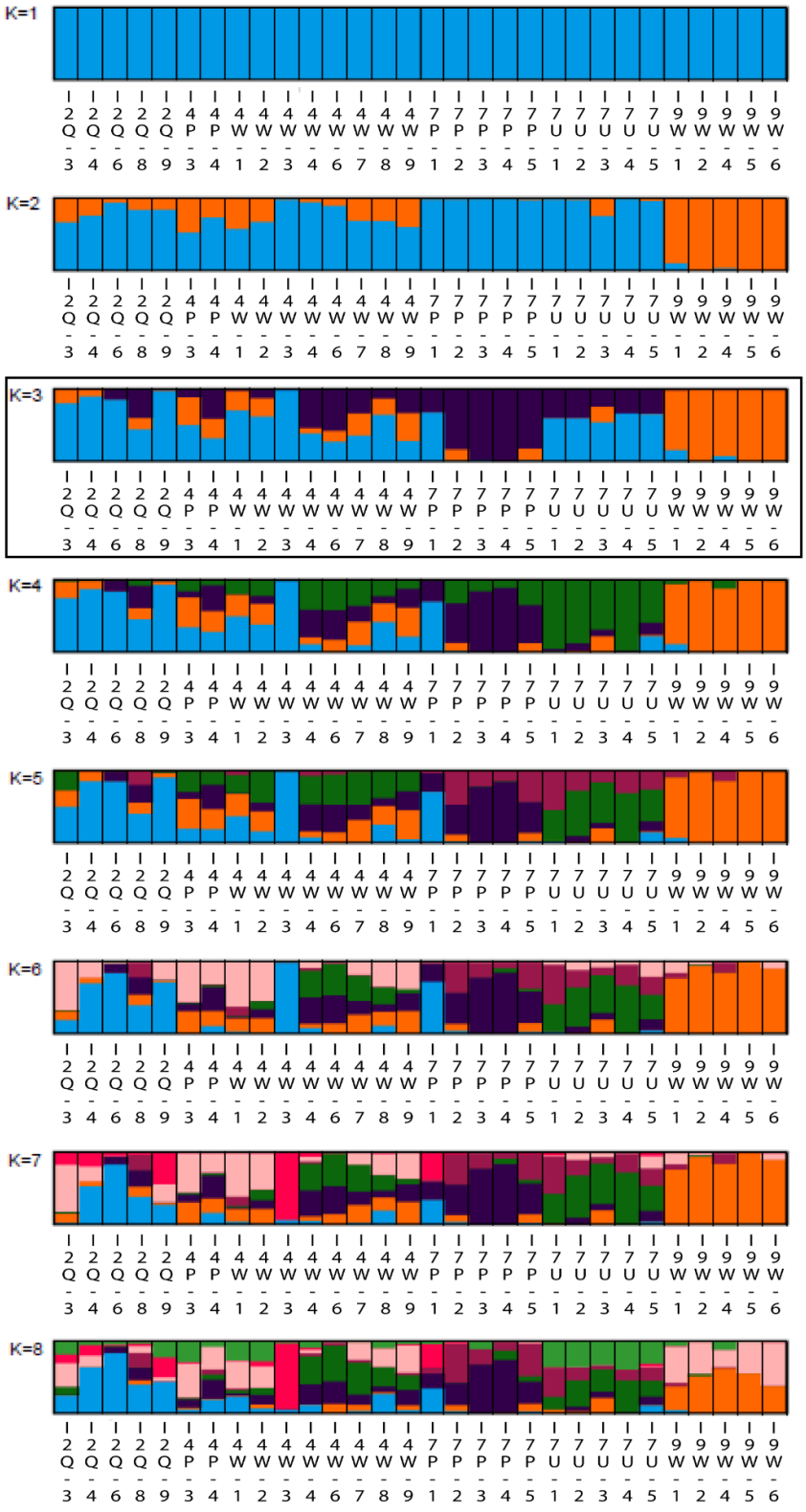
IB


K=1
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K=6
K=7
K=8
12Q-3
12Q-4
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14W-4
14W-6
14W-7
14W-8
14W-9
17P-1
17P-2
17P-3
17P-4
17P-5
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19W-4
19W-5
19W-6

TB

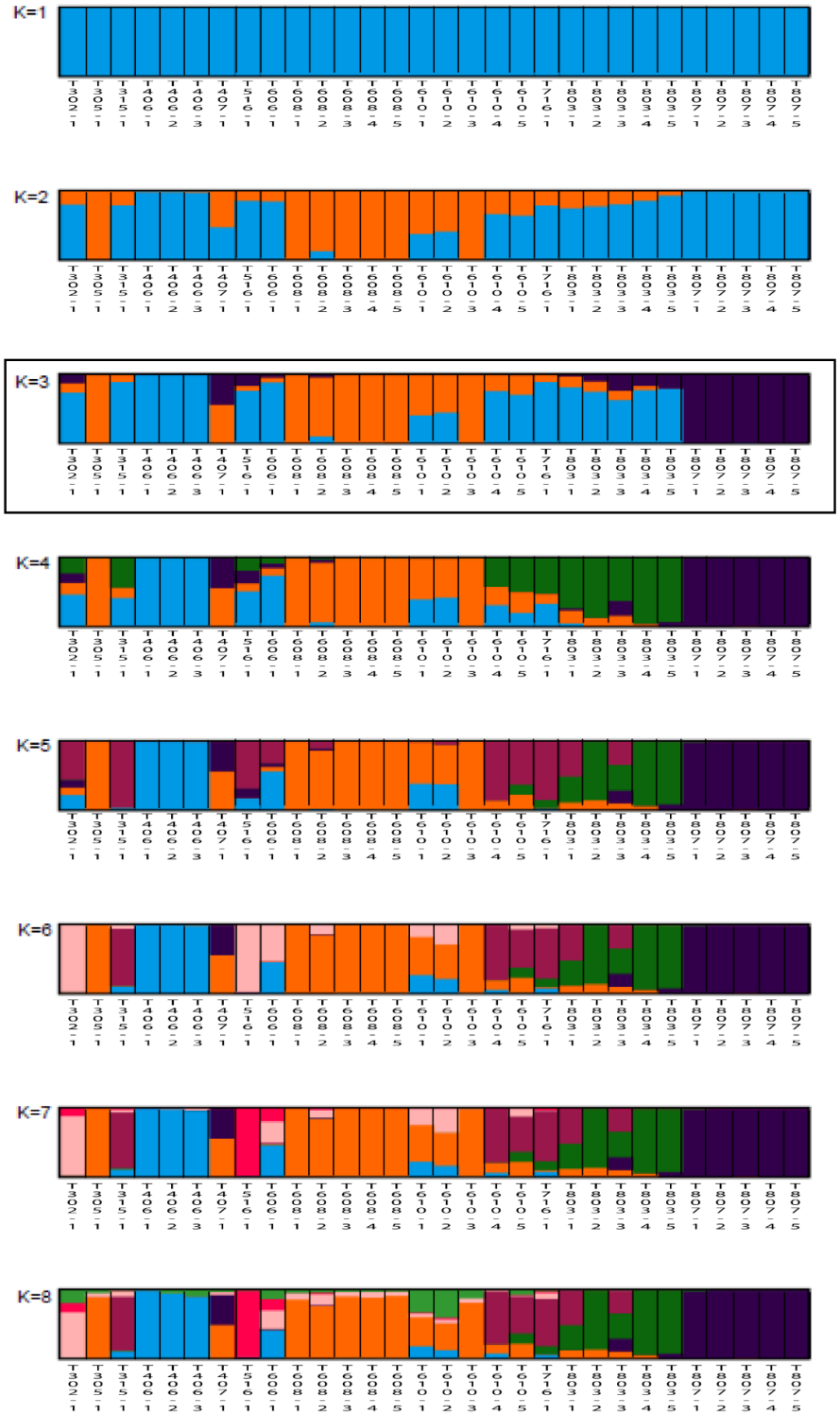


Figure 2.4 STRUCTURE results show the population estimates from SNPs data.
NOTE: Each column represents one sample genotype. Colors within samples show the estimated population ancestry of each genotype. The label for each sample is coded as "Building-Floor-Apartment-Replicate". The best fitting number of the population is indicated by the rectangle.

2.4 Discussion

This study used SNPs as genetic markers and F_{st}, PCA, phylogenetic, and STRUCTURE analyses to investigate the population structure of the German cockroach (*B. germanica*) in an urban environment at different geographic scales. At the regional scale, we compared populations among three buildings, one in each of one New Jersey city. At the local scale, we examined cockroach populations within each of the three buildings. We found that SNP genetic data show clear differentiation of German cockroach populations among the three buildings/cities. Within apartment buildings, we found that populations are less structured, with evidence for multiple colonizations and dispersal and gene flow within buildings.

Compared with the previous approaches to estimate the population structure of *B. germanica* (Cloarec *et al*. 1999; Jobet *et al*. 2000; Crissman *et al*. 2010) by using Random Amplified Polymorphic DNA or Microsatellite DNA, this study is the first to use SNPs, which provide more genetic loci and alleles that provide more parsimony informative characters, and hence more information and resolution to the analysis. For instance, Crissman and colleagues (2011) used 8 loci with 7.68 mean alleles per locus. In contrast we were able to utilize more parsimony informative characters with 2419 polymorphic loci (typically with 2 alleles per locus).

2.4.1 Among buildings

F_{st} values among and within each building are higher than 0 but less than ~0.12(Table 2.2). This indicates there is slight but significant genetic differentiation at these scales. F_{st} values at building level and city level (0.103) are higher than one of the previous researches using the Microsatellite DNA markers (overall F_{st} values= 0.048. Crissman *et al*. 2010) while

lower than another study (overall F_{st} values= 0.171. Booth *et al*. 2011) on all the scales. The range of the studies may explain this difference among researches. In our study, the range of buildings was about 80 km. In Crissman *et al*. (2010), cockroach samples were collected from buildings in a range smaller than 10 km, while the range was extended to 150 km (and focused on hog farms) in Booth *et al*. (2011). If the distance among buildings may affect the genetic differentiation of *B. germanica,* that may explain the difference among the F_{st} values in these three studies. Human dispersal of cockroaches is likely to be reduced with greater distance. (Booth *et al*. 2011),

The PCA (Figure 2.2), phylogeny (Figure 2.3) and STRUCTURE (Figure 2.4) results all suggest clear genetic differentiation among the three buildings/cities. The bootstrap values of the phylogeny are nearly 100% and thus support the hypothesis that the buildings/cities are more effective at genetically isolating cockroach populations compared to floors within the same building. From the PCA and STRUCTURE results, we can see the same separation of populations. This result agrees with the conclusion of the previous studies that there is genetic structuring and limited gene flow among apartment buildings both within and among cities (Booth *et al*. 2011; Crissman *et al*. 2010). Even at these different scales, buildings are likely to be a barrier to *B. germanica* populations and prevent the gene flow among populations. STRUCTURE and phylogeny of the samples showed that the samples from TB and PB are genetically more related than the samples from IB. This showed that the cockroach genetic distance is not correlated to the geographic distribution of populations because IB is geographically in the middle of the location between TB and PB. Other research has shown that the distribution of cockroaches can be more related to the traffic between locations than the simple geographic distance between

them (Booth *et al*. 2011). Therefore, the genetic population connection between TB and PB may be the result of human assisted dispersal of cockroaches.

The long genetic distance of IB population to PB and TB on the phylogeny shows that among the samples from the three cities, PB and TB are more closely related than IB. From the STRUCTURE result, we can observe the unique population composition of IB compared to other two buildings. IB building was built in 1953 which makes it has the longest history among the three buildings. Therefore, it is possible that the structure of the populations represents a different time window of cockroach colonization of different buildings.

2.4.2 Within buildings

Within buildings, F_{st} values (0.057~0.019) are all higher than previous studies conducted at the same spatial scale (F_{st} values= 0.014~0.028. Crissman *et al*. 2010). Phylogeny and STRUCTURE results indicate multiple populations can be defined within each building (Table 2.2, Figure 2.3 and 2.4). PB and IB have three populations in each building. TB has five populations according to the STRUCTURE algorithm. Many but not all apartment samples are clustered, suggesting that gene flow is strong within apartments but weak among apartments and floors. Based on the phylogeny, PCA result, and the STRUCTURE result, we found some clustering patterns of German cockroaches within each building. In PB, three populations were formed. In general, samples from the seventh and ninth floor formed one population. Samples on the tenth floor form the second population. The third population was formed in the apartment on the eleventh floor. Samples in apartment 11E are all closely related except one sample, which was clustered with most of the samples on the seventh and the ninth floor and with four samples from the tenth floor. This indicated

that cockroaches in this building are stable while a migration and gene flow can still occur across floors. For IB, a similar pattern presented. However, in this building, samples from the ninth floor are very isolated to other populations, suggesting an independent colonization event. Among the second floor, fourth floor and the seventh floor, gene flow happened more widely, while samples from apartment P on the seventh floor are more unique than other lower floors. This indicated a second independent colonization in this building. TB showed a more complicated pattern than other buildings. Samples from apartment 07 on the eighth floor, apartment 08 on the sixth floor, and apartment 06 on the fourth floor are unique. Other samples in this building formed two uncertain populations. The increased genetic variability and reduced genetic relatedness in TB may be the result of more frequent colonizations from outside the building.

On the other hand, results showed the gene flow generally happened within each building. Individuals from one apartment shared genetic similarity to individuals from other apartments in all three buildings. In PB, there is no unique population in any of the apartments, which suggests a high level of gene flow in that building. The F_{st} value of PB supports this conclusion, and it is the lowest F_{st} among the three buildings. This indicates that after cockroaches colonize one apartment in the building, they may move to other apartments in the same building across different floors. In other words, the individuals in one apartment may be migrants or descendants of migrants from multiple sources within the same building. In TB, there are five populations defined by STRUCTURE. A relatively high F_{st} value in that building indicates populations in TB are more isolated than in the other buildings. The lower gene flow in TB may be the result of multiple colonization of

cockroaches in a short period. However, this cannot be confirmed without more information such as the movement history of human residences in the buildings.

In this study, we used SNPs as markers to investigate the genetic population structure of cockroaches distributed across three buildings in three New Jersey cities. On a large scale, SNP data show a clear genetic differentiation among the three buildings in NJ. The F_{st} among the three buildings is close to the average F_{st} values in the United States (Vargo *et al*. 2014). The genetic distance of populations from three buildings may reflect cockroach colonization history in the research area. In previous research, microsatellite markers revealed population differentiation among apartment buildings and apartment complexes within one US city (Crissman *et al* 2010). However, the microsatellite approach didn't provide a sufficiently resolved phylogeny with high bootstrap value that can be useful to show population relatedness within buildings (Booth *et al*. 2011; Crissman *et al*. 2010). This may be the result of a lower number of parsimony informative characters provided by only eight microsatellite markers. With our more detailed phylogeny based on ~2500 SNPs, we can identify relationships among individuals within buildings with greater certainty and thus identify patterns of colonization and dispersal within buildings.

Cockroaches are known vectors of human pathogens (Rivault 1993; Salehzadeh, 2007). It is important to understand the movement of cockroaches in both large and small scales. Locating the source of cockroaches helps in pest prevention and control. Combining the result from the SNPs analysis of cockroaches with surveys such as extermination history and movement of residents will inform pest control strategies and maximize the efficacy of control efforts.

CHAPTER 3

BACTERIAL MICROBIOMES ON GERMAN COCKROACHES IN URBAN RESIDENTIAL ENVIRONMENTS IN NEW JERSEY

3.1 Introduction

Arthropods are an important vector of medically important human pathogens (Šrámová *et al*. 1992). These vectors span many taxa including beetles, cockroaches, and ants that can carry gram-negative bacteria including *Enterobacter* sp*., Serratia* sp. and *Pseudomonas* sp. (summarized by Šrámová *et al*. 1992). Therefore, control of these pests is an important element in efforts to limit the spread of pathogens to human beings (Debboun and Strickman 2013). Many pest control methods and strategies have been developed (Rust *et al.* 1995). The cost of pest management has increased in the US from $7.8 billion in 2015 to $9 billion in 2018 (Pest Control Technology 2019). Scientific research on the role and impact of arthropods in the dispersal of human diseases is essential to the development of cost effective and efficacious extermination programs (Lounibos 2002; Tatfeng *et al*. 2005).

The German cockroach (*Blattella germanica* L.) is a globally distributed and common pest of human dwellings, health care facilities, and places of business (Devi and Murray 1991). Scientists have investigated the presence of particular bacteria in the gut and on the surface of *B. germanica*. In one study, 99.4% of cockroaches from the hospital environment and 94.2% of cockroaches from the residential environment were shown to carry medically important bacteria, which indicates that *B. germanica* is a potential human disease vector (Fotedar *et al.* 1991). Pathogens, for example *Enterobacter cloacae, Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Serratia marcescen*, were isolated from the gut (Devi and Murray 1991) and surface (Kassiri and Kazemi 2012) of *B.*

germanica. Microbial diversity and communities are associated with many environmental factors (Dunn *et al*. 2013, Kumari and Choi 2014, Curl 2016, Dannemiller *et al*. 2016), that may create variation in bacterial communities among locations. In spite of the known importance of *B. germanica* in spreading pathogens that cause human disease, the importance of *B. germanica* in shaping bacterial communities remains unknown.

Bacterial communities associated with *B. germanica* vary across space and time. In one study, Carrasco *et al*. (2014) found that bacterial communities in the guts of *B. germanica* shifted through different life cycle stages when bacteria are transmitted from adults to juveniles via feces. Another study about the relationship between different cockroach habitats and the bacterial community of cockroaches showed that the bacterial community from cockroaches in commercial locations are different from other types of locations such as hospital environments (Xue, *et al.* 2009). Furthermore, in the same study (Xue *et al*. 2009), some bacterial species such as *E. coli* were surprisingly absent from cockroach surfaces, as *E. coli* has been found to be common on cockroaches in other studies (Pai *et al*. 2005). This suggests that bacterial communities on cockroaches in one population may be unique compared to those from other locations. However, most prior studies of bacterial communities associated with *B. germanica* focused on particular bacterial species rather than their complete bacterial microbiome. Some research has investigated the bacterial community among different strains of cockroach but only in the gut of artificially treated cockroaches in the lab (Zhang and Yang 2019). Our focus is to reveal the bacterial microbiome on cockroaches in urban environments.

In the present reserach, we sampled bacterial microbiomes of *B. germanica* populations from multiple apartments within one apartment building in each of three New

Jersey cities. We hypothesized that the bacterial microbiomes from *B. germanica* will differ among cities due to geographic separation. We further hypothesized that bacterial microbiomes from *B. germanica* will differ within apartment buildings. To test these hypotheses, we analyzed the bacterial microbiome identified from 16s rRNA of cockroaches collected from three locations in NJ. This study is the first to directly compare bacterial communities of *B. germanica* among different residential locations.

3.2 Methods and Materials

3.2.1 Overview

To compare bacterial microbiomes among different cockroach populations we utilized multiple statistical and hierarchical approaches. We tested whether bacterial diversity is correlated with cockroach population density. We conducted ordination analysis to determine whether bacterial community composition varied within or among buildings. Finally, we conducted Linear discriminant analysis Effect Size (LEfSe) to determine whether particular bacterial taxa characterize microbiome differences among the three apartment buildings.

3.2.2 Cockroach collection and bacterial community extraction

In this study we used a subset of the cockroach samples described in Chapter 1. The cockroach samples were collected from residential apartment buildings in Paterson (PB, April 2017), Irvington (IB, May 2017), and Trenton (TB, February 2018) , New Jersey, by placing four glue traps in the kitchen and bathroom of each apartment. The traps were retrieved after approximately 14 days. We selected ten adult male cockroach samples distributed across four floors in each building for bacterial microbiome analysis (Table 3.1).

To obtain rRNA for this study, we used the same DNA/RNA extraction from the cockroach population analysis described in Chapter 1. DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA, USA) were used for the extraction from abdomen-removed cockroaches. Previous research demonstrated that DNeasy Blood & Tissue Kits are more effective for bacterial DNA extraction compared to other specific DNA extraction kits

(Evans *et al*. 2018). Cockroach population density per apartment was calculated by averaging the number of cockroaches on the four traps in each apartment.

To investigate the bacterial community, 16S rRNA was used because this gene is a genetically conserved characteristic and exists in all bacteria. 16S rRNA is often used as a barcode gene for bacterial species identification (Janda and Abbott 2007). Sequences of 16S rRNA with 97% or greater similarity were grouped into operational taxonomic units (OTUs). The abundance of bacterial OTUs have been used in a variety of analyses such as diversity studies and clustering studies of bacterial communities (Nguyen *et al*. 2016).

Sequencing of the 16s rRNA was done by MR DNA (Shallowater, TX, USA) with miSeq technology. 30 cycles of PCR with HotStarTaq Plus Master Mix Kit (Qiagen, USA) was used on the PCR primers 515/806 designed for 16S rRNA gene V4 region with barcode on the forward primer. After the PCR amplification, sequencing was performed at MR DNA on a MiSeq. Sequence processes were conducted using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against database from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu) by MR DNA (Shallowater, TX, USA).

3.2.3 Bacterial Community Analysis

To quantify differences among bacterial communities found on cockroaches within and among the three buildings, we primarily utilized the “phyloseq” package (McMurdie and Holmes 2013) in R (R Core Team 2019). We deleted OTUs within the *Blattabacterium* genus because these bacteria are endosymbionts associated only with *Blattella*. The OTU data were rarified with the function “rarefy_even_depth” in the “phyloseq” package to

normalize bacterial counts among samples. To compute the coverage (the percentage of target regions that have been sequenced at least once (Sims *et al*. 2014), we used the "phyloseq_coverage" function from the "metaMisc" package (Chao and Jost 2012; Mikryukov 2020;). We calculated the relative abundance of the bacterial community using all the species except *Blattabacterium* sp. We present the most common phyla and genera to broadly characterize the bacterial community.

Diversity analysis

We calculated and plotted OTU richness, Simpson index, and Shannon index as measures of diversity across the three buildings by using the "plot_anova_diversity" function from "microbiomeSeq" package in R (Ssekagiri *et al.* 2018). We conducted a pairwise ANOVA test of differences between buildings of OTU richness, Simpson index, and Shannon index using the "plot_anova_diversity" function from "microbiomeSeq" package in R (Ssekagiri *et al.* 2018).

We tested the effect of cockroach population size on the bacterial diversity on the cockroaches. The cockroach population size was estimated by averaging the number of cockroaches among all of the traps from the apartment from which they were collected. We used the "aov" function in the "stats" package in R (R Core Team 2020) to test for differences in the natural log of cockroach population density among three buildings. We tested for a correlation between cockroach population size and OTU diversity with linear mixed model analysis as implemented in the "lme4" package (Bates *et al*. 2014) in R (R Core Team 2020). We tested for significant effects of 'building' on bacterial diversity (Shannon index) using the "mixed" function from the "afex" package using Kenward-Roger's F test (Kenward and Roger 1997; Singmann *et al*. 2015). We treated buildings as

a random factor, then compared with and without "cockroach population" with a likelihood ratio test (Bates *et al* 2014) to test for an effect of cockroach population on Shannon diversity.

Ordination analysis

To test for similarity of bacterial communities among the three buildings and among floors in each building, we used Principal Coordinate Analysis (PCoA) methods (Gower 1966) from the "phyloseq" package with the "ordinate" function in R (R Core Team 2019). To test for similarity among buildings, we first compared samples using PCoA from all three buildings, and then conducted pairwise comparisons to further elucidate differences found among all three. For the analysis within buildings, we removed one singleton sample (the only sample appearing on a single floor) for each building, and then conducted PCoA with samples grouped by floor. By doing this, every floor across all three buildings had three samples each.

We calculated distance among the bacterial communities with the "Bray-Curtis" method (Bray and Curtis 1957). We then tested for differences of bacterial communities among buildings and floors with Permutational Multivariate Analysis of Variance Using Distance Matrices (Adonis) (Anderson 2001) y using "adonis" function in "vegan" package (Oksanen *et al*. 2013) in R (R Core Team 2019).

Linear discriminant analysis Effect Size

To test if there are differentially abundant bacterial taxa (species or genera) that characterize the three buildings, we used Linear discriminant analysis Effect Size (LEfSe) (Segata *et al*. 2011) as implemented on the Huttenhower Galaxy Server (http://huttenhower.org/galaxy/). After formatting the data sheet with the function on the

website, we set the p-value at 0.05 for the factorial Kruskal-Wallis test (Kruskal and Wallis 1952) from the highest taxonomy level. Then the pairwise Wilcoxon test (Wilcoxon 1992) between lower taxonomy levels with the result from Kruskal-Wallis test with p-value at 0.05. The threshold on the logarithmic LDA score (Fisher 1936) for discriminative features is 2.0 based on other bacterial research and the LEfSe default setting (Segata *et al*. 2011; Nirmalkar *et al*. 2018).

3.3 Results

A total of 1707 OTUs were identified across the 30 samples. Among them, 147 OTUs were identified as *Blattabacterium* sp., which were removed from all following analyses because these bacteria are endosymbionts associated only with *Blattella*, leaving 1560 OTUs. The coverages of the sequence are all above 90% among all the samples, which showed a good depth of sequencing. Nineteen phyla were identified. The three phyla representing more than 5% bacterial abundance were Proteobacteria (60%), Firmicutes (18%), Bacteroidetes (16%) (Table 3.1 and Figure 3.1). At the genus level, 398 genera were identified. The five most abundant genera are Enterobacter (13.1%), Pseudomonas (12.4%), Serratia (11.4%), Desulfovibrio (6.1%), and Bacteroides (5.6%) (Table 3.1).

3.3.1 LEfSe Analysis

The LEfSe analysis did not identify any species or genera that significantly characterize differences among the buildings.

3.3.2 Diversity Analysis

Neither bacterial OTU richness, nor Simpson's diversity, nor Shannon's diversity differed among buildings (Figure 3.2). Cockroach population density in the apartments from which we collected bacterial samples was lower in TB than in either IB or PB (Figure 3.3). Cockroach population density in our collection is significantly lower in TB than in other buildings. The linear mixed model analysis showed cockroach population density does not predict Shannon's diversity of the bacterial community (Figure 3.4; $F_{1,17.67} = 0.15$; $p = 0.709$).

3.3.3 Ordination Analysis

Among buildings

PCoA and Permutational Multivariate Analysis of Variance Using Distance Matrices (Adonis) showed that the bacterial communities differ significantly among the three buildings ($R^2=0.096$; $p = 0.022$, Figure 3.5 A). Pairwise Adonis analysis showed that PB and TB showed significantly different clustering patterns ($R^2=0.093$; $p = 0.011$, Figure 3.5 D), while PB-IB ($R^2=0.073$; $p = 0.094$, Figure 3.5 B) and IB-TB ($R^2=0.056$; $p = 0.332$, Figure 3.5 C) did not.

Among floors

PCoA and Permutational Multivariate Analysis of Variance Using Distance Matrices (Adonis) showed that the bacterial communities differ significantly among floors in TB ($R^2=0.372$; $p = 0.006$, Figure 3.6 C), while Adonis did not show significant differences in bacterial composition among floors in PB ($R^2=0.304$; $p = 0.098$, Figure 3.6 A) or IB ($R^2=0.229$; $p = 0.067$, Figure 3.6 B).

3.4 Discussion

Blattella germanica is a common pest species and a potentially important bacterial vector. We detected multiple medically important bacteria on cockroaches sampled in an urban environment across three apartment buildings, one in each of three New Jersey cities. Previous research has shown that the bacterial community from cockroaches can change with the life cycle of cockroaches (Carrasco *et al*. 2014). It differs among environments that have different functions, such as market, medical and residential buildings (Xue *et al*. 2009). Therefore, there is a possibility that *B. germanica* is a potential vector species that can transmit bacteria to the environment and other individual cockroaches in the local population.

Our results demonstrate that characterizing bacterial communities isolated from *B. germanica* using rRNA with miSeq technology is an effective approach for understanding bacterial community diversity in different cockroach populations. The coverages (the percentage of target regions that have been sequenced at least once) of all of the 30 samples are above 91% which shows that our sequencing is reliable and accurately reflects the actual relative abundances in the bacterial communities (Good 1953). In this study, we used the DNA extraction from abdomen-removed cockroach samples for the 16s rRNA analysis. Therefore, the DNA extraction included the surface microbial organism and organism from the cockroach body materials. Although we used DNA extraction from the abdomen-removed cockroaches, there is evidence that the bacterial community from inside and outside of cockroaches are not significantly different (Xue *et al*. 2009). In our study, we want to investigate the loading of bacteria on the surface of cockroaches to understand the way cockroaches spread bacteria.

Among the three buildings, the five most dominant genera are *Enterobacter* (13.1%), *Pseudomonas* (12.4%), *Serratia* (11.4%), *Desulfovibrio* (6.1%) and *Bacteroides* (5.6%). *Enterobacter* and *Pseudomonas* were found on *B. germanica* at the similar frequency compared to previous research (Pai *et al.* 2005; Xue *et al*. 2009). However, *Klebsiella* was not found in our study while it has been widely found on roaches in previous studies (Pai *et al*. 2005; Xue *et al*. 2009;) This pattern has been commonly found in other studies with other bacteria (Xue *et al*. 2009). It is possible that the bacterial community of cockroaches differs at large scales. This may explain why certain bacterial species appear in some cockroach microbiomes and not in others, as was the case with *Klebsiella* not appearing in our samples.

Our ordination results show that the bacterial microbiome communities in cockroach populations from these three buildings are significantly different, with differences between PB and TB being particularly pronounced. Research has shown that cockroaches can potentially receive bacteria from other individual cockroaches (Carrasco *et al*. 2014). Therefore, the clustering pattern among buildings may be explained by exchanges of a limited part of the bacterial community among cockroaches within buildings and limited exchange among buildings in different cities, which is not surprising. Differences among buildings in bacterial communities may be associated with different environmental aspects such as cleaning frequency and pet abundance (Dunn *et al*. 2013).

Within buildings, communities among floors of TB show an obvious clustering pattern. This may be the result of the instability of the cockroach population in TB. The cockroach population is relatively smaller than other buildings in our studies, which may indicate the frequent rotation of cockroach colonization in TB. This could cause

heterogenic bacterial distribution in TB. However, our results should be interpreted with caution because of our limited sample size. Outliers, such as samples in T315 (Figure 3.6 C) may substantially alter the clustering result. Further research with more samples will be required to fully elucidate these patterns.

While the clustering analysis demonstrated significant differences in community similarity among and within buildings, the LEfSe analysis demonstrated that no particular OTUs were responsible for these effects.

Although cockroach population density in the apartments that we sampled in TB is significantly lower than in other buildings, bacterial diversity was not affected by cockroach population density. This indicates that bacterial diversity may remain more stable when they are carried by cockroaches. Research about microbial diversity in urban environments has shown that the microbial diversity and community composition in the environment can be affected by many characteristics such as air conditioner use, pets (Dannemiller *et al*. 2016), and seasons (Kumari and Choi 2014). The cockroach samples we collected are from different buildings and seasons. The lack of bacterial diversity dissimilarity among cockroach populations may be the result of a homogenized environmental bacteria community among buildings, or the preferred environment provided by cockroaches.

This study directly compared bacterial microbiome communities from cockroaches among different residential buildings. We found the possible differentiation of bacterial communities among the three buildings and among floors in one building. Nevertheless, these results should be interpreted with caution. This dissimilarity of bacterial microbiome communities can be the result of multiple effects including the different environment

among buildings, the effects of distribution by cockroaches, or the limited samples among floors, among other potential factors. Many studies have focused on the bacterial community loading especially in hospital environments (Fotedar *et al.* 1991; Kassiri and Kazemi 2012; Fakoorziba *et al*. 2014). Results from this study can be correlated with cockroach population structure to reveal the potential important role of cockroaches as a vector species.

Table 3.1 *Blattella germanica* Sample Collection in Three Buildings for Bacterial Microbiome Analysis

Building	Floors sampled	Apartments sampled per floor	Cockroaches sampled per floor
PB	7	1	3
	9	1	3
	10	1	1
	11	1	3
IB	2	1	3
	4	1	3
	7	2	1
	9	1	3
TB	3	3	3
	4	1	1
	6	1	3
	8	1	3

Table 3.2 The Most Five Common Phyla/Genera of Bacteria on Cockroaches by Relative Abundance Summed across Samples and Buildings

Phylum	Percentage	Genus	Percentage
Proteobacteria	58.99%	*Enterobacter*	13.06%
		Pseudomonas	12.38%
		Serratia	11.44%
		Desulfovibrio	6.09%
Firmicutes	17.96%		
Bacteroidetes	15.97%	*Bacteroides*	5.63%
Acidobacteria	2.96%		
Actinobacteria	2.46%		

The five most abundant phyla and five most abundant genera were listed on this table.

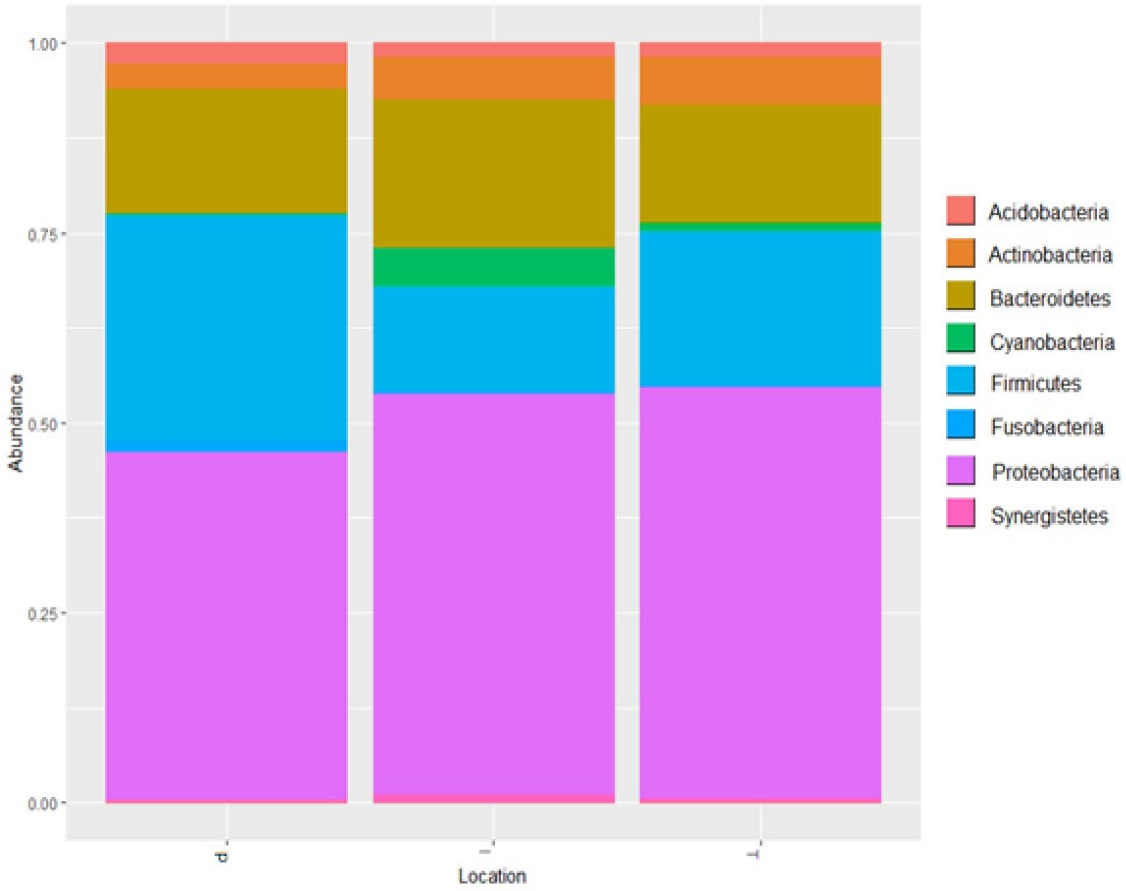


Figure 3.1 The composition of the bacterial microbiomes from cockroaches at the phylum level.
NOTE: large relative abundance of Cyanobacteria in Irvington.

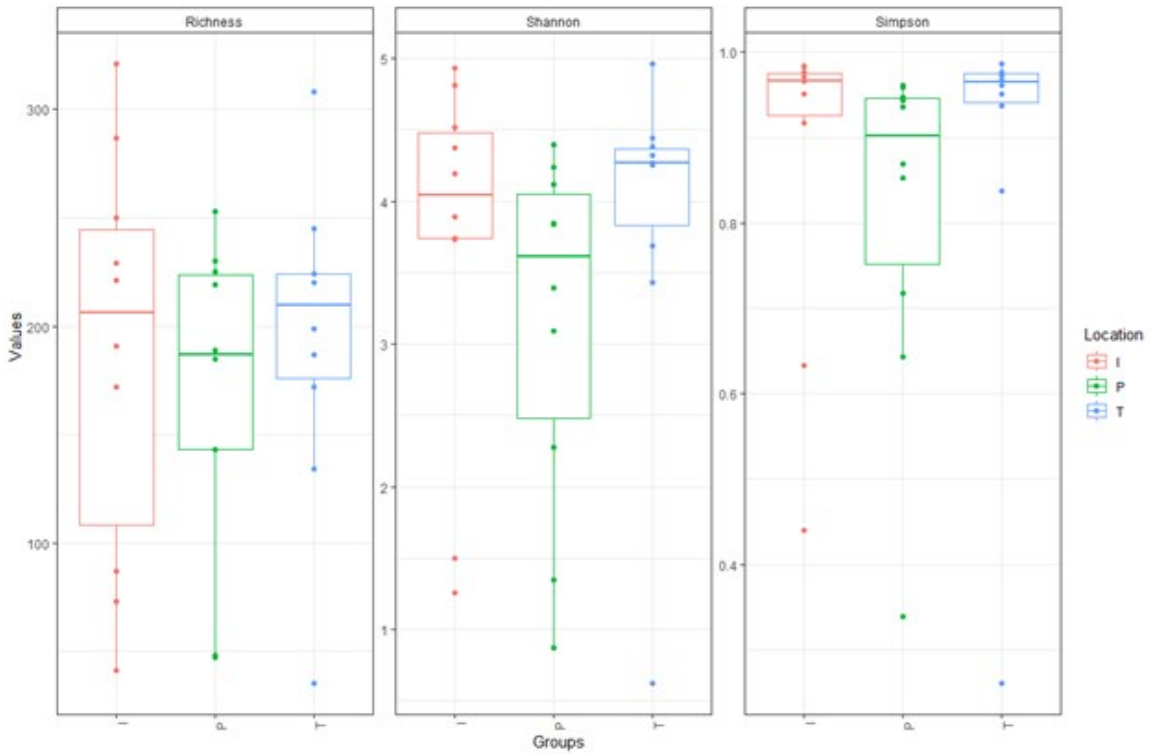


Figure 3.2 Bacterial OTU richness and Simpson diversity index within cockroach microbiomes among three apartment buildings in New Jersey.
NOTE: The difference among three buildings is not significant ($p>0.05$).

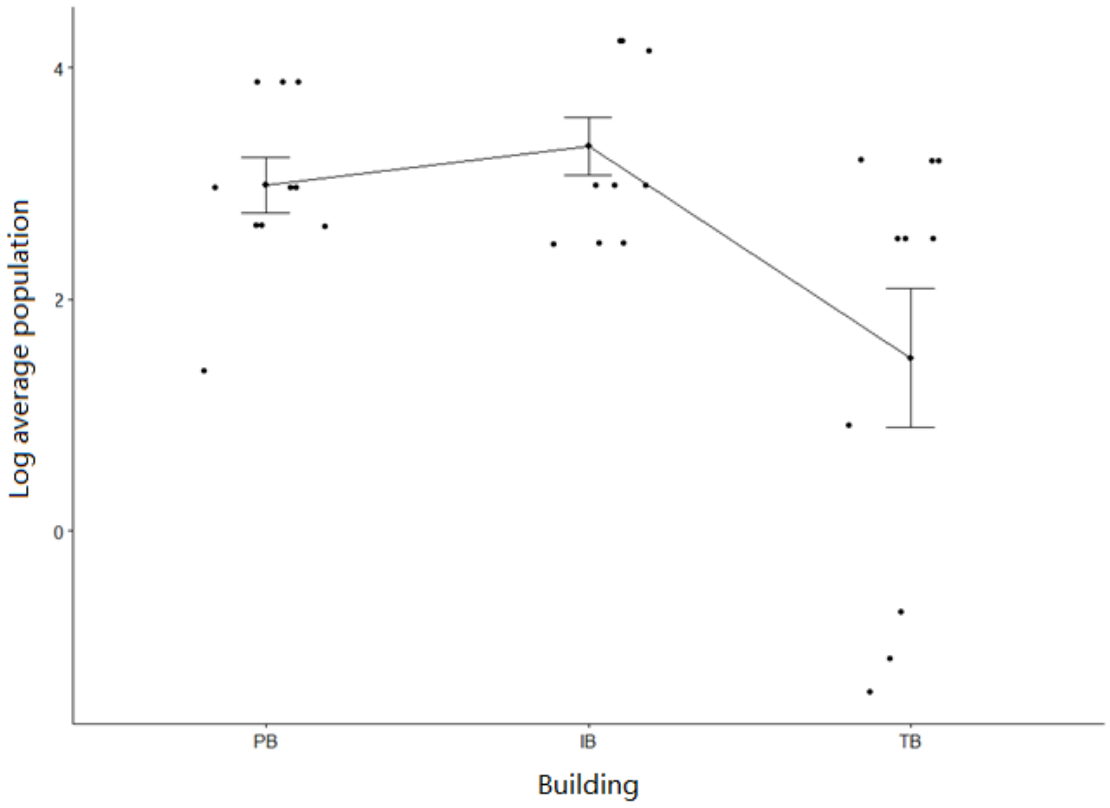


Figure 3.3 Mean of cockroach population density among three buildings (± standard error).
NOTE: Log scale of y axis. Each point represents the mean the number of cockroaches per sticky trap per apartment.

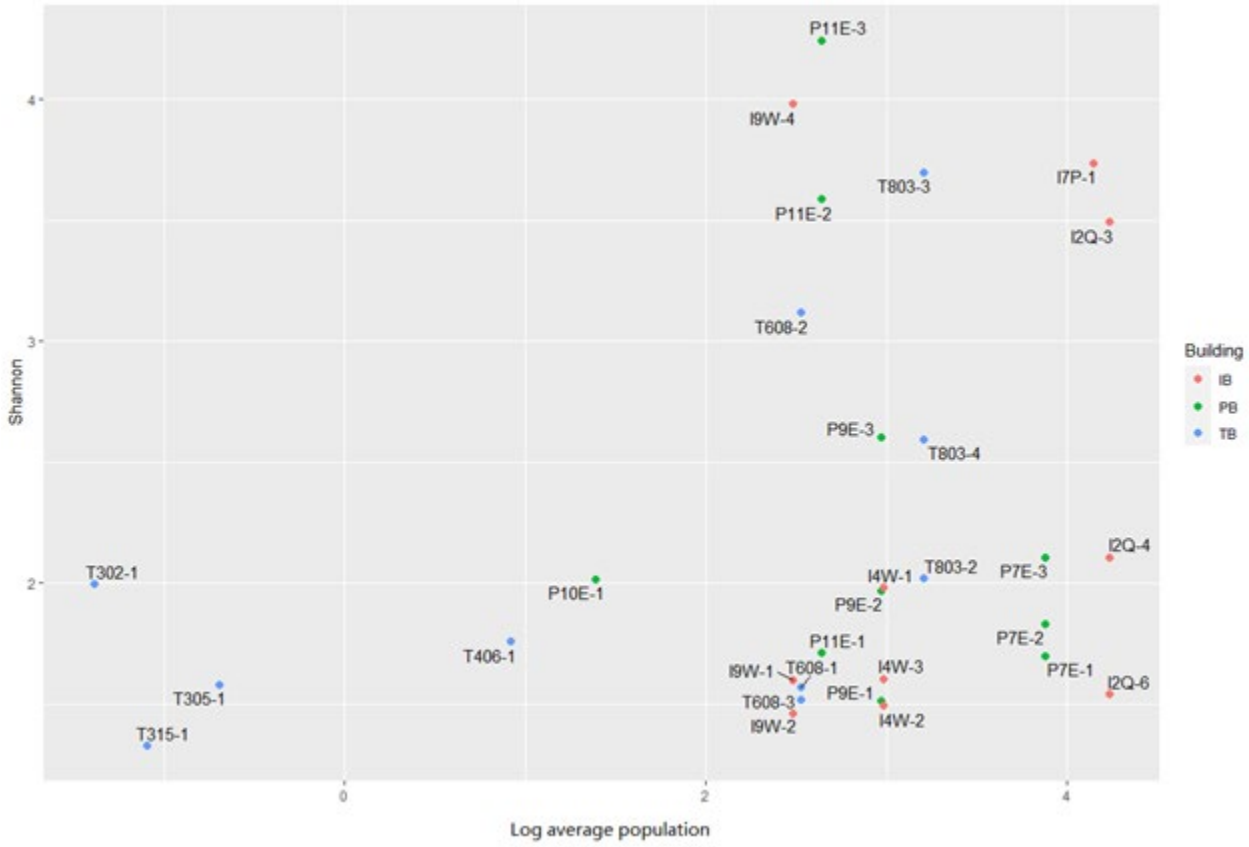
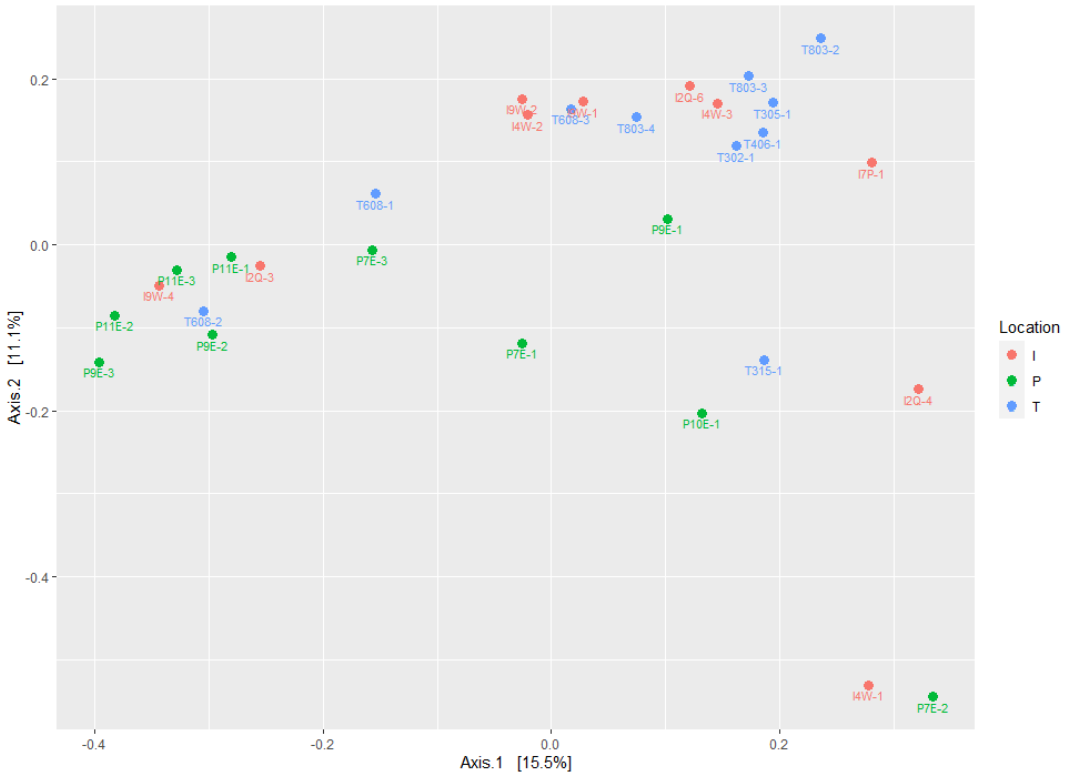
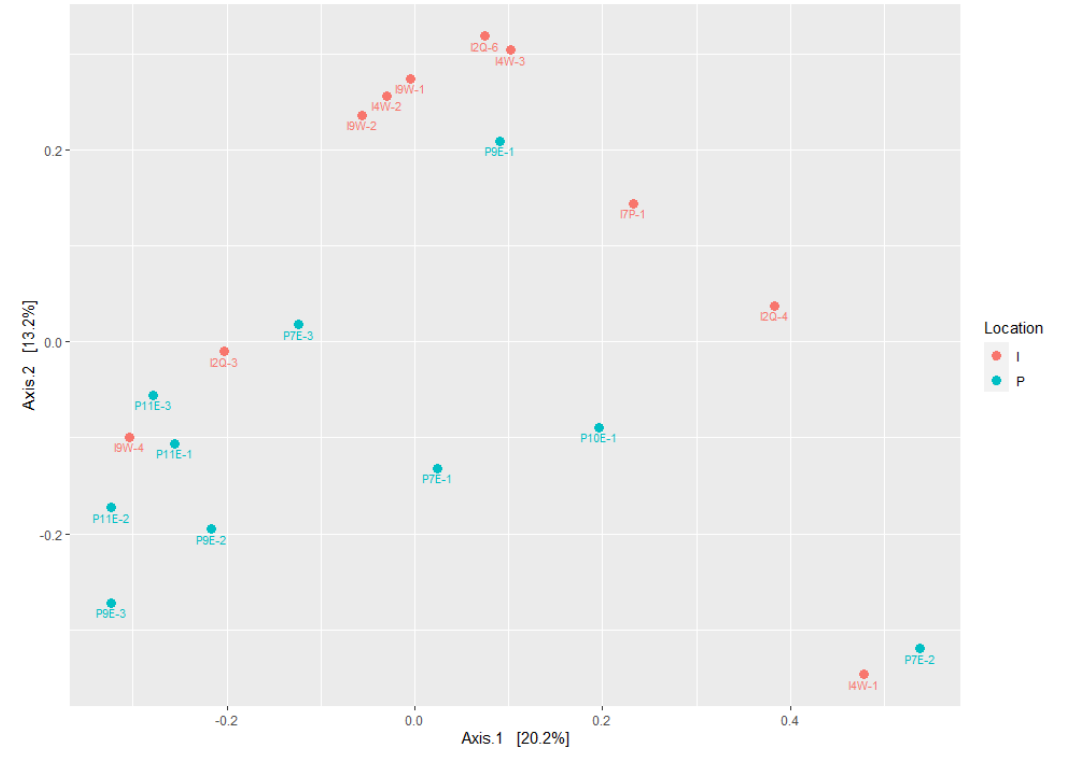


Figure 3.4 Plot of average population densities of cockroaches and Shannon values. There is no significant difference between them.

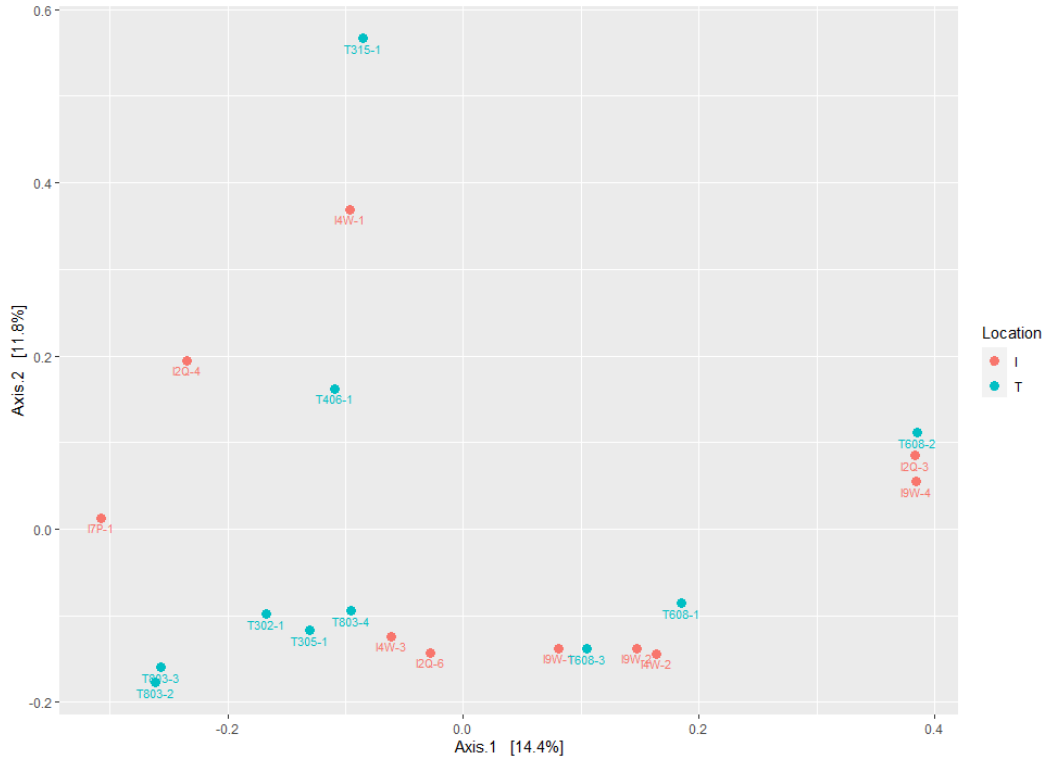
(A)


T803-2
T803-3
I2Q-6
I4W-3
T305-1
I9W-2
I4W-2
T608-3
I4W-1
T803-4
T406-1
T302-1
I7P-1
T608-1
P9E-1
P7E-3
P11E-1
I2Q-3
P11E-3
I9W-4
P11E-2
T608-2
P9E-2
P7E-1
T315-1
P9E-3
I2Q-4
P10E-1
I4W-1
P7E-2
0.2
0.0
-0.2
-0.4
Axis.2 [11.1%]
-0.4
-0.2
0.0
0.2
Axis.1 [15.5%]
Location
I
P
T

(B)


I2Q-6
I4W-3
I9W-1
I4W-2
I9W-2
P9E-1
I7P-1
I2Q-4
P7E-3
I2Q-3
P11E-3
P10E-1
I9W-4
P11E-1
P7E-1
P11E-2
P9E-2
P9E-3
P7E-2
I4W-1
0.2
0.0
-0.2
Axis.2 [13.2%]
-0.2
0.0
0.2
0.4
Axis.1 [20.2%]
Location
I
P

(C)


Axis.2 [11.8%]
Axis.1 [14.4%]
Location
I
T
T315-1
I4W-1
I2Q-4
T406-1
T608-2
I2Q-3
I9W-4
I7P-1
T608-1
T302-1
T803-4
T305-1
I4W-3
I2Q-6
I9W-
T608-3
I9W-
I4W-2
T803-3
T803-2

(D)

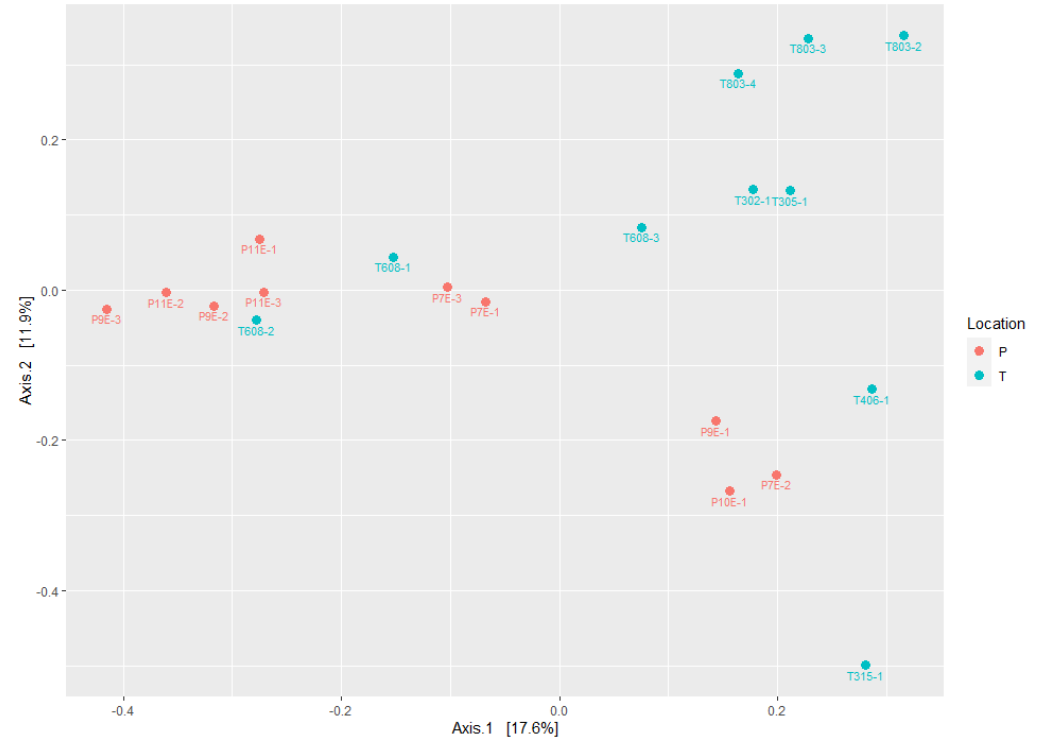
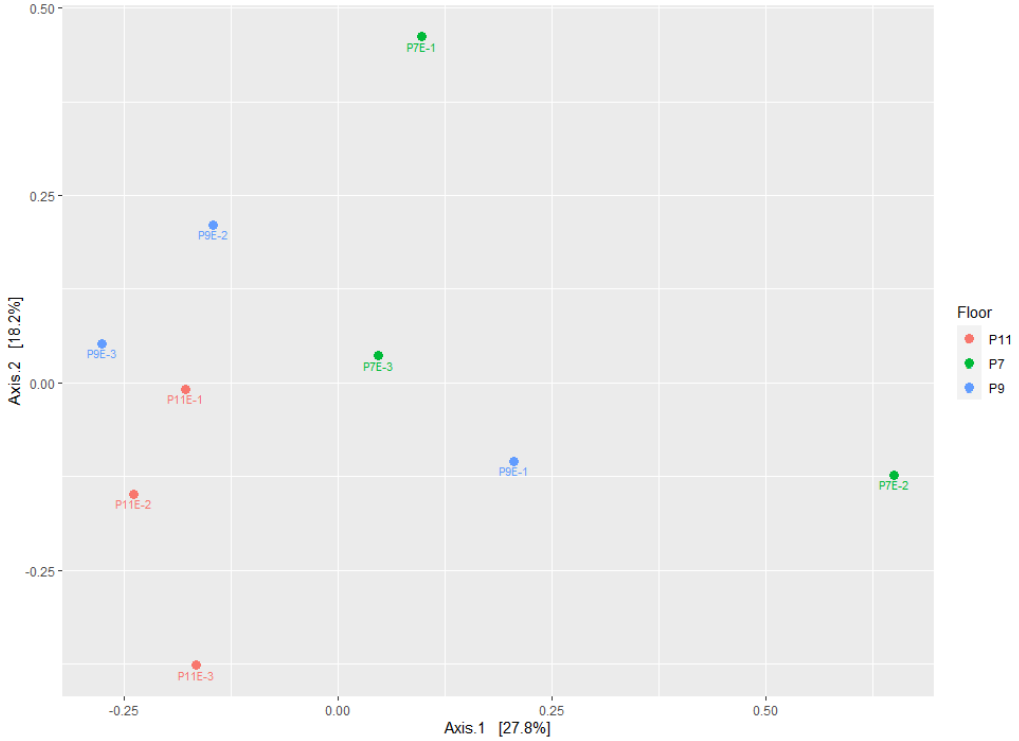

Axis.2 [11.9%]
Axis.1 [17.6%]
Location
P
T
T803-3
T803-2
T803-4
T302-1
T305-1
T608-3
P11E-1
T608-1
P7E-3
P11E-2
P11E-3
P9E-2
P7E-1
P9E-3
T608-2
T406-1
P9E-1
P7E-2
P10E-1
T315-1

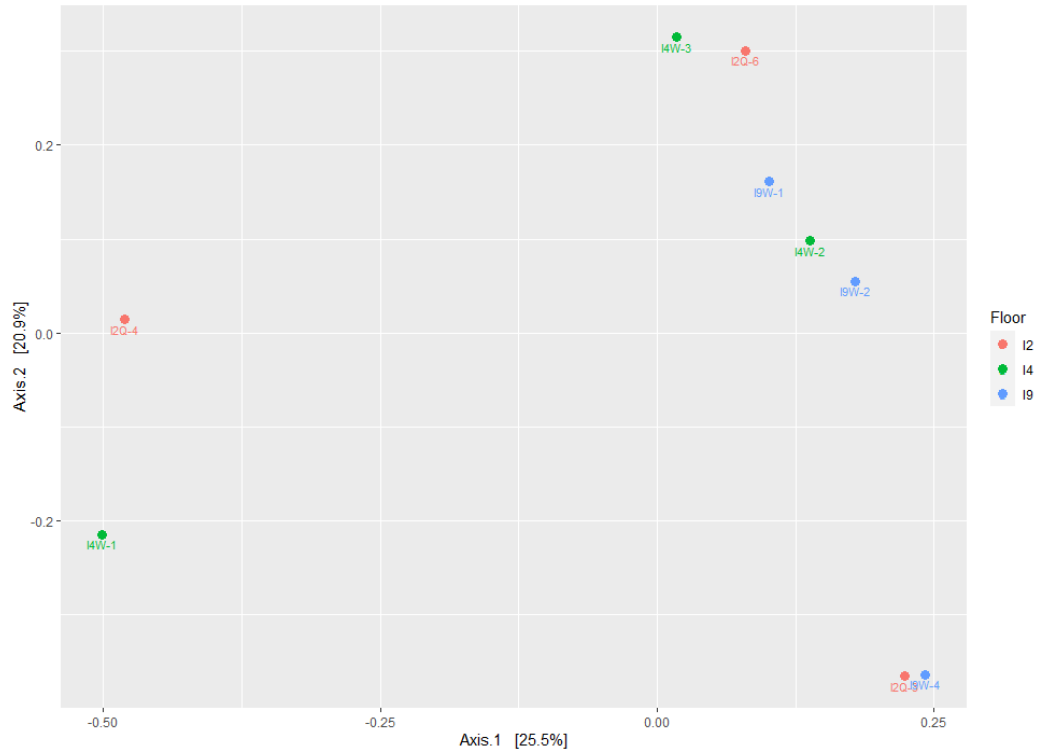
Figure 3.5 Ordination of cockroach microbiome samples among apartment buildings in NJ.

NOTE: The color indicates the buildings. The label of each sample follows the pattern: Building code (letter)-Apartment code (letter/number)- Replicate code (the last number). For example, I2Q-9 means the sample 9 from 2Q apartment in the building IB. T606-1 means the sample 1 from 606 apartment in the building TB. (A) Ordination of three buildings (IB, TB, and PB) in New Jersey. (B) Ordination of PB and IB. (C) Ordination of IB and TB. (D) Ordination of PB and TB.

(A)


0.50
0.25
0.00
-0.25
Axis.2 [18.2%]
P7E-1
P9E-2
P9E-3
P7E-3
P11E-1
P9E-1
P7E-2
P11E-2
P11E-3
-0.25
0.00
0.25
0.50
Axis.1 [27.8%]
Floor
P11
P7
P9

(B)


I4W-3
I2Q-6
0.2
I9W-1
I4W-2
I9W-2
0.0
Axis.2 [20.9%]
I2Q-4
-0.2
I4W-1
I2Q-3
I9W-4
-0.50
-0.25
0.00
0.25
Axis.1 [25.5%]
Floor
I2
I4
I9

(C)

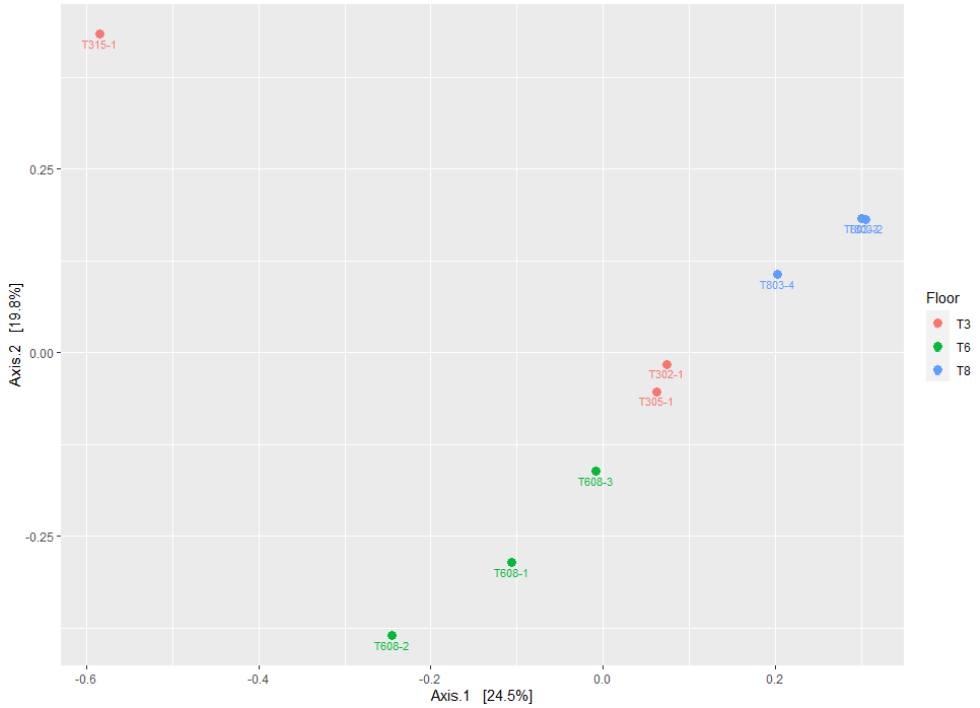


Figure 3.6 The ordination results of samples among floors within buildings.
NOTE: The label of each sample follows the pattern: Building code (letter)-Apartment code (letter/number)- Replicate code (the last number). For example, I2Q-9 means the sample 9 from 2Q apartment in the building IB. T606-1 means the sample 1 from 606 apartment in the building TB. (A) Ordination within PB. (B) Ordination within IB. (C) Ordination within TB.

CHAPTER 4

ABILITY OF GERMAN COCKROACHES TO DISPERSE BACTERIA

4.1 Introduction

Organisms such as parasites and pathogens rely on other species for dispersal, and the dispersal of vector species may strongly influence the genetic structure of the species dispersed (Brown *et al*. 2008). The impact of the environment not only affects individual species, but also closely related communities. This effect is most obvious in obligate parasite-vector relationships (Hafner and Nadler 1988; reviewed by Nieberding *et al*. 2004). Because vectors disperse only a subset of a parasite or pathogen population (Archie *et al*. 2008), we can predict that movement of vectors among localities can cause the genetic or community structure of pathogens to mirror those of the vectors. For example, research on fishes and parasites indicated that the genotype of parasites is correlated with the genotype of fish at a large scale (Criscione *et al*. 2006). For non-specific pathogen-vector relationships, scarce research has been reported. However, other research indicated that vector species can accumulate parasite diversity as they move among environments (Teitelbaum *et al*. 2018). In another study, the genetic diversity of *Escherichia coli* is positively correlated with the age of house mice, which is one of the vectors of *Escherichia coli* (Teitelbaum *et al*. 2018). Furthermore, after colonizing a new environment, a vector species can not only transmit parasites to other individuals, but also to the new environment (Keesing *et al*. 2006). Therefore, a migrating vector population with pathogens may have the ability to affect the genetic structure of pathogens in the new environment. Vector

populations that are larger and better connected are therefore likely to have larger and more diverse pathogen populations (Brown *et al*. 2008).

Animal population dynamics are shaped by habitat characteristics of locales that support individual populations and connectivity with other such populations. Beneficial locales support larger and more stable populations. Populations that are better connected are also less likely to experience population fluctuations and extinction. Therefore, populations in better conditions can be sources that persist and provide colonists to other populations. Conversely, sinks offer unfavorable conditions that would decline in size if not for migrants from source populations (Pulliam 1988). These source-sink dynamics affect pathogens as well as their vectors.

German cockroaches (*Blattella germanica* L.), as generalists (Dow 1986; Devi and Murray 1991), can live and move between human living areas. Cockroaches may disperse among adjacent human dwellings via their own motility, but they rely on human activity for long distance dispersal, for instance among buildings and cities (Booth 2011). Cockroaches are known to disperse medically important pathogens including *Enterobacter cloacae, Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Serratia marcescens* (Rivault 1993; Salehzadeh, 2007). Several laboratory experiments indicate that cockroaches can receive bacteria from food sources (Ash and Greenberg 1980). American cockroaches (*Periplaneta americana*) that were directly fed *Salmonella* were found to have transmitted *Salmonella* among cockroach individuals (Kopanic Jr *et al*. 1994). These results indicated the important role of cockroaches as vectors of pathogens. Therefore, in this study, we used the German cockroach (*Blattella* germanica) as a model species to understand the role of vector species on the dispersal of medically important pathogens.

Because bacterial pathogens may not be dispersed only by cockroach species, it can be difficult to determine the relative importance of cockroaches on the dispersal of pathogens. However, previous research on parasites and vector species may illuminate the way to test for the importance of cockroaches as significant vectors of pathogens. For instance, a study about mice and their gut bacteria indicated a correlation between the microbial community and genetic structure of mice (Suzuki *et al*. 2019). However, this relationship has not been found on German cockroaches.

Here, we describe the results of several experiments designed to test whether the German cockroach (*Blattella germanica*) is an effective disperser of bacteria in general and of medically important bacterial pathogens in particular. First, we test whether cockroach bacterial microbiome community similarity is correlated with cockroach population structure and genetic similarity with a Mantel test (Mantel 1967). Second, we test the ability of *Escherichia coli* to survive on fomites and on the surfaces of cockroaches. Last, but not least, we infected German cockroaches with *Escherichia coli* to test experimentally whether cockroaches are able to transmit bacteria to other cockroach populations. We hypothesize that there is a significant correlation between cockroach population structure and bacterial community similarity. We also hypothesize that German cockroaches can transmit bacteria within the population in the laboratory environment.

4.2 Methods and Materials

4.2.1 Correlation between cockroach genetic distance and cockroach bacterial community distance

At the city scale, we use a Mantel test (Mantel 1967) to test whether cockroach genetic similarity is correlated with bacterial community similarity from the same cockroach populations. The Mantel test tests the correlation between two matrices. In ecological studies, this test is widely used to compare spatial distances with bacterial community distances among samples (Wang *et al*. 2019). This method can also be applied with genetic distance among samples calculated with genetic markers such as single nucleotide polymorphisms (SNPs) (Sun *et al*. 2011; Baloch *et al*. 2017). In one study, matrices of genetic distance and bacterial communities from the same host population were compared by the Mantel test (Suzuki *et al*. 2019).

Sample collection and sequencing

In this study, we used a subset of the cockroach samples described in Chapter 1. The cockroach samples were collected from Paterson (PB, April 2017), Irvington (IB, May 2017), and Trenton (TB, February 2018) residential apartment buildings in New Jersey by placing four sticky traps in the kitchen and bathroom of each apartment. The traps were retrieved after approximately 14 days. We selected ten samples from each building. These cockroach samples were from four floors of each building (Table 3.1). After being collected, samples were stored at -20 ℃ until being processed for the DNA/rRNA extraction in July 2018. To extract both cockroach DNA and bacterial rRNA, we used DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA, USA).

DNA samples were sent to SNPsaurus (SNPsaurus, Oregon, USA) for the cockroach SNP sequencing (Brookes 1999). SNPs are defined here as single nucleotide variation with a frequency of at least 1% among samples. They can be used in multiple research areas such as genetic population structure analysis or genetic analysis of diseases (Brookes 1999). Additional details can be found in Chapter 2 of this dissertation.

16S rRNA sequencing for the bacterial community analysis was processed by MR DNA (MR DNA, Shallowater, TX, USA). Bacterial Operational Taxonomic Unit (OTU) (Nguyen *et al*. 2016) abundance data was provided by MR DNA. OTUs are groups of sequences of the target gene with at least 97% similarity across samples. The abundance and variation of OTUs can be used in the studies of bacterial communities (Nguyen *et al*. 2016). Additional details can be found in Chapter 3 of this dissertation.

We tested for a correlation between cockroach genetic distance and bacterial community distance among the 30 samples with a Mantel analysis (Mantel 1967) in R (R Core Team 2019) using the "mantel.rtest" function from the "ade4" package (Chessel *et al*. 2004; Dray and Dufour 2007; Dray *et al*. 2007; Bougeard and Dray 2018). We created the cockroach genetic distance matrix with SNP data and bacterial community distance matrix using bacterial operational taxonomic unit (OTU) abundance. The cockroach population genetic distance matrix was built in R with "snpgdsIBS" function to calculate the "Identity by State" (IBS) (Luan *et al*. 2012) distance between each pair of cockroach samples. IBS is the proportion of shared alleles from a pair of individuals across loci. The distance is calculated as: "1-(Number of loci from individual sharing two alleles+ 0.5 × Number of locus from individual sharing one alleles) / total number of common, non-missing loci" (Anderson *et al*. 2010) The bacterial community distance matrix was created

with “distance” function with the “Bray–Curtis” method in R Base package (R Core Team 2019) (Bray and Curtis 1957). Finally, we tested whether roach genetic distance and cockroach bacterial community distance are correlated by conducting a Mantel test on these two matrices using the “mantel.rtest” function from the “ade4” package (Chessel *et al*. 2004; Dray and Dufour 2007; Dray *et al*. 2007; Bougeard and Dray 2018) with 999 permutations.

4.2.2 Bacterial Dispersal Ability of Cockroaches

To test directly the ability of cockroaches to disperse bacteria to other cockroaches, we first marked cockroaches with fluorescent *Escherichia coli* and introduced them into unmarked cockroach populations. Our purpose was to test the efficiency of cockroaches to transmit the arabinose-induced fluorescence transformed (pGLO) *E. coli* to other individuals in the population. After we prepared the *E. coli* laboratory strain we divided this experiment into three parts: 1. Testing the ability of pGLO *E. coli* to survive on fomites, 2. testing the ability of pGLO *E. coli* to survive on cockroaches without access to a food source, 3. testing the ability of cockroaches to disperse bacteria to other cockroaches.

Cockroach colonies

Living German cockroaches were used in this study. The Jwax strain cockroach colonies were obtained from the Dr. Changlu Wang lab at Rutgers University-New Brunswick. The Jwax strain was a laboratory strain maintained for over 30 years (Wang and Bennett 2006). 30.6-quart (19.70"L x 15.75"W x 7.75"H) plastic boxes were used to contain the populations. Three layers of stacked cardboard (6"L x 5"W x 2"H per layer) were provided for cockroach shelter. Water and Teklad rodent food (Indianapolis, Indiana, USA) were

provided everyone to two weeks, as needed. Boxes were cleaned weekly. Cockroach waste and dead cockroaches were removed by vacuum.

Fluorescent transformation of E. coli

We used a Bio-Rad pGLO Bacterial Transformation Kit (BiosRad, Hercules, California, USA) to insert the pGLO gene into a separate colony of HB101 K-12 *E. coli* so they could be identified from other *E. coli* by fluorescence. To transform the *E. coli*, we used heat shock methodology. First, we cultured the *E. coli* with 250 µl Luria broth at 37°C for 10 hours. Then we streaked the *E. coli* solution on 1 mg/ml arabinose agar plates and cultured it at 37°C for 10 hours. Next, we mixed 250 µl of $CaCl_2$ with 10 µl pGLO plasmid DNA to prepare the pGLO plasmid transformation solution. Then, we mixed the plasmid transformation solution with one colony of *E. coli* on the plate and incubated it on ice for 10 min. We heated the solution at 42°C for 50 seconds and immediately moved it back on the ice for 2 min. Finally, we mixed it with 250 µl LB broth and streaked it on an agar plate and incubated it at 37°C for 10 hours. We confirmed the successful transformation by streaking it on an agar plate with arabinose incubated for ten days at 37°C, and then observing the plate under fluorescent light. Arabinose-inducing is a well-developed method to control the gene expression with the regulation of arabinose (Siegele and Hu 1997). In this study, we used agar plates and liquid broth with the following contents: 20mg/ml LB, 50 µl /ml ampicillin, and 1mg/ml arabinose.

Testing the ability of E. coli to survive on fomites

We tested whether *E. coli* can persist on fomites (inanimate surfaces) over a period of 10 days. We prepared replicates of 1 cm x 2 cm flat plastic sticks as representative fomites. We prepared an *E. coli* solution with a concentration of cells of 2.2×10^5 CFU (Colony-

Forming Unit)/µl. The *E. coli* solution was made with a single *E. coli* colony from LB agar plate incubated for ten-hours in LB broth (20mg/ml LB with 50 µl/ml ampicillin) at 37°C. We used sterile LB broth for the control group.

To begin the experiment, we dropped 30 µl *E. coli* solution on 35 of the fomites and 30 µl sterile LB control on another 35 fomites. We then sampled 5 fomites per day per treatment for the first 5 days of the experiment and 2 fomites per day per treatment for culturable *E. coli.* To sample the fomites, we transferred each into 5 ml of LB broth and incubated them for ten hours at 37°C. Then the incubated solution was streaked on LB agar plate with 1 mg/ml arabinose to confirm presence or absence of *E. coli*.

Testing the ability of E. coli to survive on cockroaches without providing extra nutrition

To test the ability of *E. coli* to survive on German cockroaches, we collected 50 adult male cockroaches from our colony. We anesthetized the cockroaches with CO_2 and then dropped 30 µl *E. coli* solution (2.2×10^5 CFU/µl) on the wings of 25 cockroaches and 30 µl sterile LB control on the other 25, each in a separate Petri dish. From the first day to the fifth day, we transferred five cockroaches and submerged them individually into 5 ml of LB broth. We incubated them for ten hours at 37°C and streaked them on LB agar plates separately (20 mg/ml LB, 50 µl/ml ampicillin, and 1 mg/ml arabinose) and incubated them for hours at 37°C. We observed the plates under fluorescent light and recorded the result as *E. coli* "present" or "absent".

Testing the ability of cockroaches to disperse bacteria

After we understood the ability of *E. coli* to survive on different surfaces, we started the contamination experiment. Plastic boxes (19.70"L x 15.75"W x 7.75"H) were used as the experimental area for each replicate. In each box, 200 ml water and 5.5 g food were

provided. To inoculate the roaches with *E. coli,* we immersed the food into 5 ml of *E. coli* solution made by ten-hours incubated LB broth with a single *E. coli* colony from a single LB agar plate. Five adult male German cockroaches were placed in each box in the beginning of the experiment. We planned to add 25 uninfected adult male cockroaches into each box for each set on the days after the start of the experiment. We planned to test the cockroaches for *E. coli* contamination after five days from the start of the experiment. The testing of the presence of *E. coli* was planned to be conducted by emerging cockroaches in 5 ml LB and culturing it overnight before streaking on agar plates with 50 µl /ml ampicillin, and 1mg/ml arabinose. The control group used clean LB broth to replace the *E. coli* solution. However, the experiment ended when we found that the uncontaminated control colony of cockroaches was in fact subject to systemic and widespread contamination by our pGLO *E. coli*, in spite of what we believed to be strict control practices including housing the pGLO *E. coli* and the control colony in separate buildings and with sterile control techniques being used.

4.3 Results

4.3.1 Correlation Between Cockroach Genetic Distance and Bacterial Community Distance

The Mantel test showed that cockroach genetic distance is not significantly correlated with bacterial community distance among the 30 samples (r= -0.072, p =0.87, 9999 replicates).

4.3.2 Bacterial Dispersal Ability of Cockroaches

The transformation of fluorescence *E. coli* was successful. Glowing *E. coli* colonies were detected in the UV-light environment (Figure 4.1).

Figure 4.1 The *E. coli* were successfully transformed with pGLO plasmid.

Testing the ability of E.coli to survive on fomites

After ten days, all of the samples in the experimental group of fomites tested positive for *E. coli* from the third day to the tenth day. The control group did not show evidence for *E. coli.* (Table 4.1).

Testing the ability of E. coli to survive on cockroaches without providing extra nutrition

After ten days, all of the samples in the experimental group of cockroach surfaces tested positive for *E. coli* from the third day to the tenth day (Table 4.1).

Table 4.1 *E. coli* Survival Ability on German Cockroach Surfaces and Fomites

Days after experimental treatment	Positive samples			
	Control fomites	Experimental fomites	Control German cockroaches	Experimental German cockroaches
1	0/5	5/5	0/5	5/5
2	0/5	5/5	0/5	5/5
3	0/5	5/5	0/5	5/5
4	0/5	5/5	0/5	5/5
5	0/5	5/5	0/5	5/5
6	0/2	2/2		
7	0/2	2/2		
8	0/2	2/2		
9	0/2	2/2		
10	0/2	2/2		

Note: Each replicate was treated with either 30 µl of *E. coli* solution (2.2×10^5 CFU/µl) or 30 µl sterile LB

Testing the ability of cockroaches to disperse bacteria

In this experiment, contrary to our expectations, we detected *E. coli* from both control and experimental cockroach groups. To investigate potential contamination in the lab environment, we sampled our primary cockroach colonies to determine if the

contamination occurred at that level. We collected five cockroach samples from each of our four colony boxes for the test. All our cockroach populations were infected with the transformed *E.coli* (Table 4.2). We used five clean cotton swabs each to test the inside bottom surface of the boxes, the outside surface of boxes and the table around the boxes to test for environmental contamination. We found no transformed *E.coli* on these environmental surfaces (Table 4.2).

Table 4.2 Sample results for *E.coli* contamination in German cockroach colonies and nearby environmental surfaces

Sample locations	Positive samples per location
Roaches in Box 1	5/5
Roaches in Box 2	5/5
Roaches in Box 3	5/5
Roaches in Box 4	5/5
Bottom surface of boxes	0/20
Outside surface of boxes	0/20
Table surface around boxes	0/20

4.4 Discussion

In this study, we investigated the bacterial dispersal ability of German cockroaches at two scales – the correlation between roach genetic distance and microbiome community distance, and the direct lab experiment for transfer of *E. coli* among cockroaches. If cockroaches are important vectors of bacteria, we expected to see a correlation between cockroach relatedness and bacterial community relatedness, as well as evidence of the direct transmission of bacteria among cockroach individuals.

We found that the genetic distance among German cockroaches was not significantly related to bacterial community distance among the same cockroach population. Research has shown that this test can be applied to genetic data while it is more commonly used on spatial or community distance data (Hannelius *et al*. 2008, Diniz-Filho *et al*. 2013, Wang *et al*. 2019). Some studies demonstrated that this test can be applied to genetic distances as well (Baloch *et al*. 2017; Suzuki *et al*. 2019). Therefore, in our study, we compared the cockroach genetic distance to the bacterial community distance from cockroaches. Our result showed that there is no significant correlation between the cockroach genetic distance and the bacterial community composition distance from the same cockroach population. Based on the result from Chapter 3, we found that the bacterial communities did differ among buildings and among floors within buildings. It is possible that cockroaches received the bacterial community from the environment. By doing the Mantel test, we can confirm that cockroach population distribution is not likely to be a major factor that can shape the bacterial communities among our study sites. Research has shown that the bacteria from vector species can be determined by the vector distribution in a large range (Suzuki *et al*. 2019). In this research by Suzuki *et al.* (2019) about the beta

diversity of gut bacterial community and mice populations, a significant correlation was found across multiple states in the U.S. On the contrary, research within a relatively small range (within a city) leads to a similar culturable bacterial community among cockroach populations (Xue *et al*. 2009). However, our results show no such relationship among cockroaches and the bacterial communities they harbor. This difference in findings may be the result of local environmental effects on microbiota differentiation.

For the lab bacterial transmission experiment, *E. coli* presented a strong ability to persist on the surfaces of cockroaches and fomites (inanimate plastic surfaces) for up to ten days. Other research has shown that *E. coli* can survive on plastic surfaces for more than 16 days and can survive longer on organisms (Maule 2000), which makes cockroaches a potential vector species of bacteria. Our research confirms these findings.

The experiment to examine transfer among cockroaches was halted because of the detection of contamination of the pGLO-transformed *E. coli* in our source cockroach colonies. Tests of all four of our source cockroach colonies showed transmission of *E. coli* throughout the population had already occurred. This result, while unintentional, provides strong evidence for the ability of *E. coli* to be transmitted within and among cockroaches and cockroach populations.

A study investigating the ability of *E. coli* to survive on cockroaches in an agricultural environment showed that *E. coli* can maintain viability in cockroach feces for more than seven days after cockroach exposure to *E. coli* (Zurek and Schal 2004). Ash and Greenberg (1980) showed that cockroaches can receive bacteria from food sources. Although they only tested the gut and feces of cockroaches, another study (Kopanic Jr *et al*. 1994) directly tested the surfaces of cockroaches after they were fed bacteria

contaminated food in small groups (25 individuals) and large groups (100 individuals) and found their strong ability of carrying bacteria. In our experiments, it is highly possible that with the direct application of *E. coli* solution onto the cockroaches in our study, cockroaches will similarly transfer *E.* coli to and from their food sources. The contamination throughout all four boxes in our experiments indicates the strong ability of cockroaches as vectors to spread bacteria into new populations. Similar conclusions were drawn in the study of Kopanic's group investigating cross contamination among American cockroach (*Periplaneta americana*) populations (Kopanic Jr *et al*. 1994). Our studies indicate that cockroaches have a strong potential to be a vector of pathogens. However, we didn't detect any *E. coli* from the surfaces close to cockroaches after the contamination happened. Considering that *E. coli* can be more abundant on the surface of organisms than many abiotic surfaces, it is possible that the culturability of *E. coli* depends on the concentration of cells which can be higher on cockroaches than on environmental surfaces.

In our study, we investigated the correlation between German cockroach distribution and bacterial communities from the cockroach population in city and lab scales. Similar research about host-parasite interactions has been done in other vector species (Criscione *et al*. 2006; Suzuki *et al*. 2019). Our research filled a gap in the study of interactions between common bacterial communities and their cockroach vectors. At the city scale, we didn't find a significant correlation between the cockroach genetic distance and the bacterial community difference. However, in the lab scale, we found a strong ability for cockroaches to carry and transmit bacteria to other cockroaches.

CHAPTER 5

CONCLUSION

The goal of this study is to test the hypothesis that German cockroaches (*Blattella germanica*) are important dispersal vectors of bacterial communities. If cockroaches are important dispersers of bacterial communities, then we predict that the similarity of bacterial communities found on the surfaces of cockroaches will be correlated with the genetic distance among the cockroaches that harbor these bacterial communities. Because many bacteria found on cockroaches are medically important human pathogens, if true, this hypothesis has important implications for the control of these pathogens in particular and for human health and wellbeing in general.

This study was divided into three parts: 1) Investigation of genetic population structure of German cockroaches in New Jersey; 2) Investigation of bacterial communities found on these same German cockroaches in New Jersey; 3) Investigation of the correlation between the population structure and bacterial community of German cockroaches. Cockroach samples were collected from three apartment buildings located in three different cities of New Jersey (Paterson, Irvington, and Trenton). Single-nucleotide polymorphisms (SNPs) were used as genetic markers for the cockroach population study. Operational taxonomic units (OTUs) from 16S rRNA were used to study the bacterial community.

5.1 Genetic Population Structure of German Cockroaches

In the investigation of genetic population structure of German cockroaches (Chapter 2), a differentiation of German cockroach populations was confirmed among the three buildings. Principal component analysis (PCA), F_{st} analysis, phylogenetic relationships, and population analysis with STRUCTURE indicated there is substantial gene flow among the three buildings. However, three populations can still be identified among three buildings while each building has its own population. Within each building, there is a detectable differentiation among floors. This result also provided high resolution for detecting genetic similarity among individual cockroach samples. With the information PCA, F_{st} analysis, phylogeny, and STRUCTURE results provided, we can compare the similarity of single cockroaches to cockroaches from other populations, which can indicate the migration of that single cockroach.

This study also showed the potential use of SNPs as genetic markers in the study of German cockroaches and their ability to detect and resolve the genetic similarity of German cockroaches.

These findings show that cockroaches may migrate within apartment buildings and recolonize apartments where control had been successful. In addition, these results suggest that apartment buildings may experience multiple colonizations, also hindering pest control efforts. These two findings can inform and improve pest control strategies, emphasizing the need to first eliminate cockroach infestations building-wide to prevent recolonization from apartments within the building and also educating tenants to avoid introducing new cockroach colonists from outside the building.

5.2 Bacterial Community of German Cockroaches

In Chapter 3, we studied the bacterial community on the external body parts of German cockroaches. The diversity and ordination studies on these bacterial communities indicated that there is a moderate but statistically significant differentiation of bacterial communities among cockroach populations among these three buildings in New Jersey. Within buildings, we detected a slight differentiation of bacterial communities among floors in one of our studied buildings (Trenton). Results from this study showed German cockroaches carry different bacterial communities in different locations in both large (cities) and local scales (floors within buildings). However, the signal is not very strong in either of the scales, which indicates that the bacterial community on cockroaches may also be affected by other factors such as the local environment.

5.3 Correlation Between the Population Structure and Bacterial Community of German Cockroaches

In Chapter 4, we investigated the bacterial dispersal ability of German cockroaches in laboratory environments and in the urban environment. In the laboratory environment, the results from the bacterial infection test among cockroach populations with green fluorescent protein marked *E. coli* indicated a strong ability of German cockroaches to harbor and transmit bacteria. In the urban environment, our Mantel test found no correlation between the genetic distance among cockroaches and distance among the bacterial communities in the same cockroach populations. This result suggests that cockroaches may not be the most important factor for the dispersal of bacteria and the

maintenance of bacterial communities on German cockroaches, even though cockroaches have a strong ability to spread bacteria.

5.4 Importance and Future Steps

Our results in this study provided a higher resolution population structure of German cockroaches especially at the local scale (within buildings) than the previous studies (Booth 2010; Crissman 2011), and showed the advantage of SNPs as genetic markers in researches about cockroach genetic population structure. With the results about the similarity among different cockroach populations, people can make a more efficient pest management plan based on how different cockroach populations connect to each other. Our results about the bacterial community of cockroaches indicated that there are other factors that are responsible for the distribution of bacteria than cockroaches at a large scale (among cities), while cockroaches still can affect the bacterial community at a local scale (lab and floors). our results provide information for the evaluation of the bacterial dispersal ability of German cockroaches as a vector species.

Our results suggest a number of promising avenues for future research. A wider range of cockroach samples could tell us the gradational genetic change across different locations, which could provide more information about the migration pattern of cockroaches. A larger sample size for the bacterial community investigation could provide more reliable and potentially significant patterns especially at the local scale within a building. Collecting bacterial samples from the local environment could also reveal effects of the environment on the bacterial community of German cockroaches. In this study, we

developed methodologies and a workflow to study the relationship between cockroach and bacterial communities and tested the ability of different genetic markers in this type of study. Applying this approach with an extended sampling range and species will be helpful in the future.

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