COMPARISON OF MOSQUITO COMMUNITIES COLLECTED FROM DIFFERING LIVESTOCK LOCATIONS AND AN EXAMINATION OF PHENOTYPIC TRAITS EXHIBITED BY FOUR LATITUDINALLY SEPARATED POPULATIONS OF *AEDES ALBOPICTUS* (SKUSE)

A Dissertation
presented to
the Faculty of the Graduate School
at the University of Missouri-Columbia

In Partial Fulfillment
of the Requirements for the Degree

Doctor of Philosophy

---

by

MARGO L. MIRE

Dr. Richard Houseman, Dissertation Supervisor

Dr. Jennifer Benne, Dissertation Supervisor

DECEMBER 2013

The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

COMPARISON OF MOSQUITO COMMUNITIES COLLECTED FROM DIFFERING PRIMARY LIVESTOCK LOCATIONS AND AN EXAMINATION OF PHENOTYPIC TRAITS EXHHIBITED BY FOUR LATITUDINALLY SEPARATED POPULATIONS OF *AEDES ALBOPICTUS* (SKUSE)

Presented by Margo L. Mire

A candidate for the degree of doctor of philosophy,

And hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Richard Houseman, Dissertation Supervisor, Div. of Plant Sciences

---

Dr. Jennifer Benne, Dissertation Supervisor, Div. of Plant Sciences

---

Dr. Deborah Finke, Div. of Plant Sciences

Dr. Qisheng Song, Div. of Plant Sciences

Dr. Mark Ellersieck, Div. of Animal Sciences

DEDICATION

I would like to thank my husband, Phillip Mire, for helping me set shepherd's hooks at 15 trapping locations, for his willingness to chauffer me to differing 'latitudinal locations' and for his overwhelming faith in me during my educational journey. He has earned his own doctoral degree right along with me. I also want to express my thanks to my mother and my children for living with insects in the freezer and learning more about mosquito biology than they really wanted to know. My family has been more than great, they have been true blessings. This group of people has listened to me explain methods of carrying out genetic analysis (and acted interested), listened to me memorize taxonomical relationships (and acted interested) and they have loved me through it all (and acted interested). My family has carried larger, heavier loads because of my involvement with school, and have never complained. I love each of you with all of my heart, and cannot thank you enough.

ACKNOWLEDGEMENTS

I would like to thank my Ph.D. advisors, Drs. Richard Houseman and Jennifer Benne for the support they have provided me during my graduate school journey. I would also like to express my appreciation and sincere thanks to my committee, Drs. Finke, Song and Ellersieck. Each of my committee members have always given me support and assistance at any time that I needed it. These professors have been willing to give me their time and attention, of which I am eternally thankful. I especially would like to thank Drs. Finke and Houseman for their open-door policies and willingness to discuss various aspects of insect ecology and statistics with me.

The next group I would like to acknowledge includes the many livestock owners and producers that have allowed me to set mosquito traps on their properties. Locating landowners that maintained livestock that fit the parameters I had set and that were willing to allow me access to their property was something that I will always be thankful for. I am also grateful to the Department of Natural Resources in Waycross, Georgia and to the Florida Medical Entomological Laboratory in Vero Beach, Florida for the efforts made in setting oviposition traps and collecting egg papers for me.

I must also acknowledge the help I have received from other labs at University of Missouri and Lincoln University of Missouri. Michael Tarka, from GIS lab at Lincoln University, has provided me with aerial photographs and maps of trapping locations. I also have received much guidance from Dr. Lori Eggert's

lab at the University of Missouri. Without Dr. Eggert and her lab, my journey into microsatellite analysis would have been a very painful experience.

I would like to thank Dr. Dayananda Chandrappa for sharing his knowledge of the world, people and molecular methods with me. I would also like to give a very special thanks to my friends Mervat, Tamee and Lauren. They have made me feel like part of a group, even though my lab work kept me in a different city from them for years. I cannot thank them enough for their support and friendship.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

Table Page

ABSTRACT

Mosquito presence and fitness play key roles in the spread of zoonotic pathogens affecting human as well as livestock and wild populations of vertebrates. This study examined the (1) differences between mosquito communities collected from mid-Missouri agricultural locations having differing primary livestock hosts and (2) genetic differences between populations of *Aedes albopictus* (Skuse) collected from four differing latitudes. Adult mosquito collections from 15 trapping locations that maintained 5 different primary livestock groups were made during 2009 and 2010. MRPP analysis indicated a difference between the mosquito community collected from the bovine trap sites and the community collected from the capine trap sites. An indicator species analysis found three particular mosquito species that may indicate the presence of bovine livestock in the environment.

The ability of female mosquitoes to transmit pathogens among vertebrate populations has a direct relationship with her fitness as an adult. During the summer of 2010 four populations of *Ae. albopictus* were collected and used for comparisons of phenotypic traits expressed under the same environmental conditions. Florida larvae developed faster than Ohio and Georgia larvae in the same environment. During interspecific competition, Georgia larvae developed slower than Tennessee and Ohio larvae. Microsatellite analysis found genetic differences between the Ohio and Florida populations, but none due to geographic separation. These latitudinally separated populations of the invasive *Ae. albopictus* have exhibited genetic differences in body size that may

significantly influence their success in disease transmission. This study adds more information concerning the presence of genetic differences of populations from differing climates that influence body size and female mosquito fitness.

Chapter 1

Introduction

Mosquitoes (Family Culicidae, Order Diptera) are blood-sucking insects whose biology facilitates transmission of various pathogens and allows them to function as vectors of pathogens such as Rift Valley Fever, malaria and Yellow Fever (Chaves-Carballo 2005, Nelms et al. 2013, Ross 2002, Smithburn, Haddow and Gillet 1948). The overall fitness level of the adult blood-seeking mosquito has a direct relationship with her ability to transmit disease pathogens from host to host (Haramis 1985).

1

Because populations are able to adapt over time to differing temperatures as well as photoperiods, these mosquito species are capable of carrying out geographic range expansion into various latitudinal clines (Bradshaw 2001, 2006). The life history of mosquitoes is often affected by environmental conditions such as temperature (Clements 1992, Sibly and Atkinson 1994) and nutritional resources (Merritt et al 1992). Water sources utilized by mosquitoes for oviposition and larval development can vary from permanent water sources, such as ponds or salt water marshes, to more ephemeral water sources such as tree holes, puddles or any type of natural or artificial container that collects and holds water in the environment. Container breeding mosquitoes are especially

affected by these environmental changes in temperature and resource availability due to the smaller size of the aquatic habitat.

Many of the temperature and resource-affected life history traits affect the mosquito's ability and success as a disease vector (Alto et al 2008, Grimstad and Walker 1991). Further research into the relationship between mosquito phenotype and genotype will be beneficial in providing more efficient prediction of disease outbreak geographically and perhaps influence the allocation of resources to mosquito control.

Mosquito life cycle

Compared to other animals that avoid harsh environments, many arthropods utilize diapause as an escape strategy from unfavorable environmental conditions. Diapause is a state of dormancy that many mosquitoes experience that is initiated by changing environmental cues. In temperate climates, diapause is generally initiated by photoperiod changes associated with differing times of year (Hawley, Reiter and Copeland et al. 1987). This strategy maintains populations, even when environmental characteristics such as temperature of water or nutrient availability are sub-optimal for the mosquito. Many genera of mosquitoes in temperate climates express the ability to undergo diapause, whether as adults, by overwintering, or as eggs, which are highly resistant to desiccation (Clements 1992). Seasonal photoperiod and temperature changes initiate and end diapause, providing an efficient escape strategy from poor environmental conditions.

The life cycle of mosquitoes involves both aquatic and terrestrial environments. Aquatic habitats vary from ephemeral water supplies, such as tree holes and refuse containers, to permanent stands of water, like ponds or saltwater marshes. These water sources are utilized as habitats during the immature life stages and also provide suitable locations for gravid females to lay eggs. Eggs are laid either singly or in groups, depending on the genus. Members of the *Anopheles* and *Toxorhynchites* genera oviposit singly on the water's surface by flying low and, in a dipping motion, dropping eggs onto the water. Members of *Culex, Culiseta, Uranotaenia* and *Coquillittidia* lay their eggs closely together so they form rafts which float on top of water. Females of these genera land on top of the water and use their legs to position eggs carefully into groups.

3

The genera *Aedes, Psorophora* and *Orthopodomyia* lay their eggs singly on a substrate at or near the water's edge where seasonal flooding immerses the eggs and triggers hatching.

Mosquito larvae breathe by obtaining air through a siphon structure located on the posterior end of the body. While most larvae use their siphon to break the surface of the water for the intake of air, in some cases, as with the genera *Mansonia* and *Coquillitidia,* the siphons are utilized to cut into aquatic foliage and obtain oxygen from the submersed roots of plants (Bosak and Crans 2002). Mosquito larvae, depending upon species, utilize varying strategies for obtaining nutrients from the environment. Some species of larvae are filter feeders, obtaining microbial nutrients from the water column, while others scrape

bacteria from substrates, and yet others, like some *Toxorhynchiti* larvae, are predaceous on other aquatic organisms present in the habitat.

After completing four larval instars, the holometabolous mosquito enters a pupation period. This pupal stage of the mosquito life cycle is a mobile, non-feeding stage. Mosquito pupae rely solely on the energy reserves obtained in the larval stage that play critical roles in adult body condition and survivorship (Lucas and Romoser 2001). The mosquito larvae molt into pupae that are covered with a transparent pupal skin. Siphon-like structures, pupal horns, are used for air intake during this stage of mosquito development. Before eclosion from the pupal covering, the adult mosquito's wings, legs and mouthparts can be seen through the skin. Mosquito pupae spend most of their time at the water surface and dive

4

in a tumbling manner in response to stimuli in the environment. Interestingly, the pupae have been shown to exhibit some defensive behaviors toward aerial predators in their environment (Rodriguez-Prieto, Fernandez-Juricic and Martin 2006). These behaviors involve sinking to the bottom of the habitat when shadows are detected at the water surface.

Relationships between larval food resources, adult fitness and the capacity to transmit infection have been identified through laboratory studies, with reduced food availability producing smaller adult females and suggestions that smaller females are more competent disease vectors than larger females Smaller females generally are more susceptible to pathogen infection than larger more fit adult females (Fish and Carpenter 1982, Grimstad and Haramis 1984,

Haramis 1985, Hawley 1985, Paulson and Hawley 1991). In general larval mosquitoes do not feed directly on the detritus material found in the habitat, but instead consume the microbial flora that grows on the surface of the detritus (Merritt, Dadd and Walker 1992), with microbial growth being supported by both plant and animal detritus (Yee and Foster 1992). The quantity of these microbes present in the environment affects competition among larval mosquitoes in the environment for this nutritional resource (Walker et al. 1991, Yee, Kaufman and Juliano 2007).

Independent of life history, most mosquito species require blood meals obtained from vertebrate hosts for egg production. Body size of the female mosquito has some influence on the number and size of blood meals the females need. Smaller females often require more than one blood meal to acquire the proteins necessary for ovary maturation (Clements 1992, Hawley 1985) demonstrating the relationship between smaller body size and the need for more blood meals (Scott et al. 2000). The relationship between smaller body size due to environmental factors and the need for more blood meals can be a dangerous one. Alto et al (2005) has shown that when *Ae. albopictus* females are smaller in body size due to interspecific competition, they exhibit a higher titer and infection rate after being infected with arboviruses. A smaller female that was nutrient stressed as a larva often is associated with increased infection and transmission (Grimstad and Haramis 1984, Grimstad and Walker 1991 and Nasci and Mitchell 1994) that may be due to a thinner basil lamina within the mosquito midgut

(Grimstad and Walker 1991), possibly allowing easier escape of the pathogen into the body giving opportunity for replication and dissemination.

Most adult mosquitoes, all males and most females, must utilize carbohydrates obtained from plant sugars as energy sources (Clements 1992). Mosquitoes have been shown to have definite plant species preferences for sugar-feeding in the environment (Gouagna et al. 2010, Manda et al. 2007). While both sexes utilize carbohydrate resources, only female mosquitoes are known to feed on blood to obtain protein used for ovary maturation (Clements 1992). Usually 2-3 days following emergence, females of anautogenous species will engage in host-seeking behaviors in search of vertebrate animals present in the mosquitoes' environment (Clements 1992). The quantity of blood proteins

6

necessary for ovary maturation and egg development may vary depending on the life history of the female (Nasci 1986).

Various cues within the environment initiate host-seeking behavior. Vertebrate hosts emit compounds through respiration and other metabolic processes which create odor plumes. Of these compounds, carbon dioxide has been found to be an attractant for all biting flies, and by definition, attraction implies activation of host location behaviors (Clements 1999, Sutcliff 1987). Using carbon dioxide as a long-range attractant, the mosquito moves into close proximity of the potential host and once the mosquito is close to the host, odor, visual cues (including size, shape, color and movement), heat and water vapor

provide mid- to short-range stimuli that the female uses to locate a feeding site (Mc Iver 1982).

A review by Tempelis (1975) describes nine host-feeding patterns exhibited by mosquitoes. These patterns are: (1) feed almost exclusively on birds, (2) feed almost exclusively on mammals, (3) feed readily on birds and mammals, (4) feed almost exclusively on amphibians, (5) feed almost exclusively on reptiles, (6) feed exclusively on fish, (7) feed readily on all four classes of terrestrial vertebrates, (8) feed preferentially on birds in spring then shift to mammals seasonally and (9) feed exclusively on birds in one geo-region and on mammals in a different region. Examples of mosquito species and their preferred feeding patterns are: *Culex pipiens* (Linnaeus) and *Culex restuans* (Theobald) that are shown to be primarily avian host feeders (Kilpatrick et al 2006, Suom et al 2010). *Aedes sollicitans* (Walker), *Mansonia perturbans* (Walker) and *Anopheles quadrimaculatus* (Say) that are shown to be predominantly mammalian feeders (Crans 1963).

7

Mosquito-host interactions

After a host has been located, the mosquito-host relationship begins. Host activity has been demonstrated to have more influence than attractiveness on the feeding success of the mosquito (Edman and Webber 1974). The more physically active the host, the less likely the mosquito will be to feed long enough to obtain a complete blood meal; therefore, mosquitoes are more likely to bite

hosts that show little or no defensive behaviors (Day and Edman 1984, Edman and Kale 1971).

The availability of particular hosts in an environment influences the range of hosts that particular species of mosquitoes utilize for feeding, producing mosquito feeding patterns that are determined through repeated contact with a particular host instead of fixed-feeding behaviors (Edmund, Webber and Kale 1972). During the decision-making process involved in host choice, mosquitoes have been shown to return to hosts that require less expenditure of energy and over time have definite preferences for these host on which they have been maintained (Mwandawiro et al. 2000). This physiological process, or behavioral conditioning, implies host imprinting contributes to host preference. More

information regarding the environmental influences that play roles in the host-choice decision making process would be helpful in the understanding of disease transmission.

Disease transmission cycles

The abundance, host preferences and host-seeking behaviors of mosquitoes are all integral parts of the disease transmission cycle (Dia et al. 2009). Although some mosquito species are more attracted to non-human hosts, there does exist a potential for human infections with a zoonotic pathogen when the more attractive hosts are unavailable. The female mosquito ingests the pathogen during a blood-meal, and if the pathogen is able to pass through the

mosquito's midgut barrier, it will replicate and reach the salivary glands, enabling the female to transmit the pathogen to the next host (Grimstad and Walker 1991).

Disease transmission cycles vary depending upon the pathogen. Horizontal transmission cycles require a host that is able to maintain the pathogen and a vector that is capable of transmitting the disease to new hosts. For example, eastern equine encephalitis (EEE) maintains a stable transmission cycle with this virus being maintained in passerine bird populations and vectored by the mosquito *Culiseta melanura* (Coquillett) to other species of host. This ornithophilic mosquito transmits the virus among birds and rarely bites humans, which serve only as a reservoir host. Mosquito genera that are generalist feeders, such as some *Aedes, Ochlertotatus* and *Coquillettidia,* are able to

acquire the virus from infected hosts in the avian population. These mosquitoes then serve as bridge vectors, transmitting a pathogen from one species to another, by infecting humans, horses, pigs or game birds. Hosts that are able to maintain the virus without becoming viremic, or infective, are considered to be dead-end hosts as they do not amplify the pathogen, ending the transmission cycle. Viral disease transmission by mosquitoes may also occur vertically, as infective females pass the viruses to their progeny (Unlu, Mackay and Yates 2010). In these instances the female mosquito may serve as the reservoir host by maintaining the virus during overwintering and in the spring introducing the virus to new hosts. The vertical transmission of West Nile virus by *Culex tarsalis* (Coquillett) has already been demonstrated in the laboratory (Reisen et al. 2006).

The pathogens vectored by mosquitoes to livestock animals are often multi-host infections that are transferred from wildlife populations. These zoonotic pathogens, existing in more than one taxa, are found in multiple species of animals (Taylor, Latham and Woolhouse 2001). All multi-host pathogens can be classified as zoonotic agents, or pathogens that can infect more than one taxon. These pathogens that infect multiple taxa of wildlife pose a greater risk to disease outbreak than species-specific pathogens (Cleaveland, Laurenson and Taylor 2001).

Genetic comparisons

Various biotic and abiotic characteristics of an environment are able to influence life history traits of many organisms by affecting growth, survivorship and disease transmission (Alto et al. 2005, Nasci and Mitchell 1994, Sibly and Atkinson 1994). When trait expression has been environmentally influenced and more than one form of the trait is present in a population, there is indication of genetic diversity within the population, and this diversity enables the population to survive across a variety of environments. (Scheiner 1993, West-Eberhard 1989).

Genetic diversity among populations may be influenced during periodic environmental stress which can affect the gene pool of the population. Repeated exposure to these periods of environmental stress has the ability to influence the allelic diversity for a particular trait within the population. Individuals lacking the ability to survive stressful environmental conditions are not capable of

contributing genes to the population. Over time the allelic diversity of the population can become structured according to traits of the contributing individuals, since genes that contribute to the survival of organisms remain in the gene pool. In these ways the effects of environmental stress can influence the evolutionary rate of the population by maintaining, decreasing or adding to the genetic variability within the population. A significant amount of genetic variability in a gene pool allows the population to be less affected by adaptation limits (Badvaev 2005, Hoffmann and Hercus 2000).

Insects with aquatic life stages are especially sensitive to changes in their developmental habitat. Aquatic habitats are inherently more susceptible to desiccation of habitat, overcrowded populations and competition for nutritional resources. Body size is one trait that responds to differing environments, and is often analyzed in mosquitoes. These measurements are used to determine the effects of various life history traits on the fitness of mosquito adults (Nasci 1986). A lack of nutrients, overcrowding due to high population densities, or both during the larval life stage can reduce body size of adults, shorten lifespan, and leave mosquitoes more susceptible to infection with arboviruses (Alto et al. 2005, Alto et al. 2008, Hawley 1985). Because smaller females have more frequent biting patterns during gonotrophic cycles, an increased susceptibility to infection enhances their capability to be pathogen vectors (Maciel-De-Freitas, Codeco and Lournco-De-Oliveira 2007). Using body size as a method for measuring genetic variation, it has been noted that field caught mosquitoes generally are smaller in

body size than mosquito populations that have been reared in the lab. These wild populations maintain a higher genetic variability for body size than the lab populations when both populations are experimentally exposed to a range of environmental conditions (Schneider et al. 2010). Because lab-reared populations are typically maintained at optimal conditions there is no selective pressure on the same traits that wild populations depend on for survival. Under these conditions allelic diversity may not be as high in lab populations of mosquitoes as it is for wild populations (Schneider et al. 2010) that are continuously exposed to changing environmental characteristics.

Developmental time, or time required for larvae to mature, is another phenotypic trait measured when examining environmental effects on mosquitoes. The effects of varying environmental temperatures on larval populations have been studied, using developmental times, adult body sizes, longevity and disease vector competence as methods of measurement of effect (Padmanabha, Lord and Lounibos 2011, Dodson, Kramer and Rasgon 2012).

Along with the measurement of phenotypic traits, such as body size and developmental time, molecular methods are often used for comparing genetic diversity among and within populations. Microsatellite analysis is one method that is used to answer questions involving population origins, genetic distance and for comparing genetic distinctness between individuals or groups. Microsatellites are short, non-coding, repeating sections of DNA involving simple motifs, usually 1-5 base pairs long. These motifs may be repeated up to approximately 100 times in

the genome. Microsatellites are utilized for genetic analysis and comparisons because they are highly polymorphic, abundant and evenly distributed throughout the genome. These characteristics allow them to be useful in describing genetic variation among populations and in assessing the degree of genetic distance in relation to geographic distance between populations (Kothera 2009). These markers are also easily amplified by polymerase chain reaction (PCR), are co-dominant in nature and there is typically a high allelic diversity and differing loci. Microsatellites have been used for measuring genetic variability among many types of organisms spanning a wide range of taxa (Hankison and Ptacek 2008, Kawka et al. 2007). Microsatellites have recently been used, for example, in the population structure analysis of *Aedes albopictus* (Skuse), *Aedes aegypti* (Linnaeus) and various *Anopheles* species (Brown et al. 2013, Deitz et al. 2012).

Study Purpose

This research attempts to answer two questions by examining the effects that environmental factors have on mosquito communities and on the genetic differences exhibited by differing populations of the invasive mosquito, *Aedes albopictus*. The first question asks if primary livestock species present at agricultural locations influence the mosquito community structure at a particular location. We predict a difference will be found between the communities collected at these differing livestock locations given previously collected information concerning mosquito host choices and the factors that influence those choices.

These differing livestock groups span a large range of body sizes, a range of defensive behaviors and are well established in the collection areas.

The second question asks if there is a difference in phenotypic trait expression between geographically separated populations of *Ae. albopictus*, and if there is, can this difference be correlated to the geographic distance separating the populations. We are looking for information that will either support or reject our hypothesis that there are genetic differences between latitudinally separated populations of *Ae. albopictus* that are responsible for differences phenotypic trait expression.

In order to answer these questions, several objectives must be met by carrying out multiple studies. These objectives are: a) to determine the influence

that primary livestock in agricultural settings may have on mosquito community structure at that location, b) to compare phenotypic differences exhibited by geographically separated populations of *Ae. albopictus* when populations are exposed to the same environmental conditions, and c) to look for a relationship between geographic and genetic distance between populations. Information from these studies will be relevant to our knowledge concerning mosquito ecology, the prediction of livestock disease outbreak and mosquito control efforts.

Chapter 2

Comparison of the composition of mosquito communities collected from locations with differing primary livestock species

Introduction

The impacts these mosquito-borne diseases potentially have on animal production industries could affect the agricultural economy in the United States. Livestock production is an important segment of the United States' national economy. According to the 2007 Census of Agriculture, livestock sales accounted for $153.6 billion (52%) of the nation's total market value of products (Edwards and Massey 2011). The state of Missouri is one of the leading livestock producing states in the United States, with bovine (calf and beef), swine (hogs) and poultry (broilers and turkey) operations (Missouri Dept. of Economic Development 2008).

In 2011, a case study was carried out in three Missouri counties to examine the impact that livestock production has on the economy (Edwards and Massey 2011). Animal production in each of the three counties was compared, with results indicating two counties had increases in animal production while one did not. This study gave solid indication that the total value of agricultural products sold increased in two of the counties primarily due to an increase in livestock production.

Investigating the specific species of mosquitoes that share an environment with particular livestock animals will provide a first step in examining the potential for disease outbreak among livestock populations. This study looks for effects of differing prevalent livestock hosts as environmental variables on the composition of the mosquito community present in the agricultural environment. The pathogens transmitted by mosquitoes to livestock animals are often multi-host infections that are transferred from wildlife populations. These zoonotic pathogens, existing in more than one taxa, are found in multiple species of animals (Taylor, Latham and Woolhouse 2001). Multi-host pathogens that can be classified as zoonotic agents are pathogens that can infect more than one taxon. These pathogens that infect multiple taxa of wildlife pose a greater risk to

disease outbreak than species-specific pathogens (Cleaveland, Laurenson and Taylor 2001). Many zoonotic pathogens are included in the arbovirus groups that are transmitted by arthropods to vertebrate hosts. These animals act as reservoirs of the pathogen or virus, giving it an environment in which to multiply (Calisher and Karabasos 1988). While many diseases are of wildlife origin, the zoonotic potential of these pathogens are of great concern. For example, Taylor, Latham and Woolhouse (2001) found that of 1,415 identified pathogens spanning 313 genera, 61% of them are not only zoonotic, but are capable of infecting multiple animal species (Taylor, Latham and Woolhouse 2001). Multi-host pathogens that are capable of infecting more than one taxonomic order present

higher risks of outbreak in the environment than species-specific pathogens (Cleaveland, Laurenson and Taylor 2001).

While many genera of mosquitoes are opportunistic in their feeding patterns, some species have preferred host preferences that have been influenced by host availability in the environment and or defensive behaviors exhibited by the host (Day and Edman 1984, Edman and Kale 1971, Edmund, Webber and Kale 1972). Studies have shown that these preferences may change such as bringing mosquitoes from the wild and maintaining them in laboratories, they have been shown to lose the preference for feeding on a particular host species, for example an anthropophilic mosquito species that is maintained on small laboratory animals for many generations (Gillis 1964, Laarman 1958). Mwandawiro et al (2000) demonstrated that when mosquitoes were given a host choice by being released into a net holding both cows and pigs, the mosquitoes showed a tendency to feed on the same host that they had been maintained on in a previous experiment. The offspring of the pig-fed mosquitoes, however, did not show any tendency toward one host or the other, indicating there were no genetic predispositions for host choice. This behavioral conditioning has been shown by mosquitoes that return to hosts that allow them to expend less energy while obtaining a blood meal, and these mosquitoes have shown preferences for the hosts on which they have been maintained (Mwandawiro et al. 2000).

17

By examining mosquito communities present at differing livestock locations, we were able to test our hypothesis that the presence of primary livestock found in agricultural locations influences the composition of the mosquito communities in the environment. We predict there will be differences between the mosquito communities' composition because of the established presence of the livestock species. With consistent presence and availability of the livestock species in the agricultural environment, mosquito species have the opportunity to become conditioned to these species as hosts. Agricultural locations with aquatic habitats, plants for carbohydrate resources and available hosts possess all of the necessities for mosquito population establishment. Mosquito species that share the environment with and show a preference for these primary livestock species will become part of the community at that location. The information gathered from this and future research into the structure of mosquito communities associated with various agricultural animal industries will not only be medically beneficial to human and livestock populations, but economically beneficial as well. Investigations in this area will allow mosquito control effort to be allocated in specific and effective ways.

Methods

Study sites

Adult mosquito collections for this study were carried out over six counties in mid-Missouri during the summers of 2009 and 2010. Fifteen agricultural locations were used in the survey, with latitudes and longitude coordinates

recorded for each (Table 1). All of the areas surveyed were rural agricultural environments with one primary, but not exclusive, species of livestock on the premises. For this study the term ‘livestock group’ will be used to describe the primary livestock species maintained at the location. Locations that produced more than one type of livestock were excluded as possible collection sites when used for comparison between those livestock types in the study. For example, if the landowner maintained goats (capine) and horses (equine), then this location was not used for analyses which compared capine livestock and equine livestock as main hosts. This method of choosing collection locations attempted to maintain the integrity of data collection and avoid any possible confounding or misinterpretation of results.

19

The landscape features of all study sites are typical of rural mid-Missouri. All trapping locations were similar in that there were rolling pastures edged by heterogeneous wooded areas. The pastures were comprised of cool-season grass which compromises 55% of Missouri’s native grass species (Navarrete-Tindall 2010). These agricultural locations are suitable for grazing ruminants such as horses, cows and goats. The same types of landscapes are utilized for hog and poultry facilities. Any subtle differences between the locations used for trapping mosquitoes were not fully described, nor were they investigated in detail in the field. All locations were chosen based on the similarity of landscape features as determined by visual observation. Although aquatic habitats were not always visually observed at the specific trap location, GIS technology was used

for landscape characterization and revealed multiple water sources at each site (Table 2, Figures 1 through 15). Water sources listed in the table are permanent as they are visible in the aerial photographs. Ephemeral water sources such as tree holes are assumed to be present in the environment, even though not visually confirmed.

Mosquito collections were made over a two year time period, with the first season's (May through September of 2009) collections being carried out from a total of nine locations: three with capines, three with equines and three with bovines as primary species. The second season of collections (June through September of 2010) were made from a total of six locations, three with poultry (two turkey farms, one chicken yard) as main hosts and three with swine.

20

The collection sites that maintained turkeys as a main host reared and maintained the turkeys in 'grow-out houses'. The collection site that maintained chickens reared them in a penned area using a chicken coop for shelter. One swine population was maintained in a nursery barn. Each of the enclosure styles, the grow-out house and nursery barn, had many large, mesh covered windows that allowed the mosquitoes easy access to the livestock inside. Due to bio-safety regulations for these collection sites, CDC light traps were placed outside the structure, either immediately beside or as close as possible to the structure near open windows. Once a trap was installed for the collection season it was not moved.

Miniature CDC light traps were used for mosquito collection, with one trap being set at each collection site. These traps are built with a fan apparatus on the top side of the trap. The suction created by the fan pulled mosquitoes that were attracted to the trap by carbon dioxide. Dry ice was placed in a cooler and allowed to sublime releasing CO_2 at the light trap fan (Figure 16). The light trap and cooler were hung on a shepherd's hook that stayed in the same location throughout the collection season. Mosquitoes exhibiting host-seeking behaviors were attracted to the bait (dry ice) and pulled by the fan into a collection cup attached to the trap.

During both collection seasons the traps were set in late afternoon and picked up in the morning of the next day to coincide with the feeding times of grazing livestock and the crepuscular/nocturnal feeding behaviors of mosquitoes makes them more likely to be trapped during their preferred feeding times. Each week collections were brought to lab and killed by freezing then keyed to species using Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico (Darsie and Ward 2004).

Data Analysis

Species abundance, evenness and richness were quantified and the Shannon Index of the mosquito community was calculated for each trapping site. Species richness values indicate the number of species present in the collections made at a particular trapping site and evenness gives a measurement of the relative frequency of individuals per species present in the trap collection. The

Shannon Index measure of diversity was used (Shannon and Weaver 1949) as it reflects the number of species collected at a trapping location, while taking into account how evenly the numbers of individuals are distributed among the species. Diversity within a community will rise with higher richness (more species present) and evenness values (how equal the abundance of species are) (Jost 2010). These measures were then compared using analysis of variance (α = 0.05) to locate any significant differences between mosquito communities associated with primary livestock in terms of species diversity, species evenness, species richness and abundance. When ANOVA found the overall F statistic significant, indicating at least one mean was different between the means of livestock groups, a Tukey-Kramer Honestly Significant Difference (HSD) (α = 0.05) means separation test was carried out. Tukey-Kramer HSD compares the specific treatment means to one another in paired comparisons.

Differences in the composition of mosquito communities collected from each livestock group were visualized using non-metric multidimensional scaling (NMDS) ordination using PC-ORD (Kruskal 1964, Mather 1976). For the NMDS analysis, the Sorensen (Bray-Curtis) distance measure was used to determine dissimilarity distances of the samples and then starting coordinates used for analysis were randomly chosen by the program. NMDS, non-metric multidimensional scaling, determines the ease with which communities would naturally separate into the pre-set livestock groupings. NMDS is a convenient method for allowing the visualization of grouping differences on a

multidimensional plane. Each trapping site was ranked according to a Bray-Curtis metric, NMDS ordination ranked the sites, and goodness-of-fit was evaluated using an associated ‘stress’ value designated with each iteration of values moving toward community dissimilarities. These values are used to measure the amount of stress on data sets that would be necessary to infer differences in the compared community data. Smaller values indicate more community differences and less risk of misinterpretation of results (Clarke 1993). Stress values of < 0.05 give a good representation of data with no prospect of misinterpretations, <0.1 are values that have little risk of misinterpretation, stress values of < 0.2 can give usable representations however values at the upper end of this range are dangerous to interpret and values of >0.2 yield plots that are dangerous to interpret (Kruskal and Wish 1978).

23

Multi-response Permutation Procedure (MRPP) was used to compare differences in composition between groups of mosquito communities collected during the study. This nonparametric method of analysis was used to test for significant differences between mosquito community compositions. Communities were grouped according to the primary livestock collection sites. If differences between communities resulted in statistical significance, then indicator species analysis was carried out to determine which mosquito species was responsible for the separation of groups. PC-ORD software (McCune and Mefford 2011) was used, following the method of Dufrêne and Legendre (Dufrene and Legendre

1997) which relies on the fidelity and abundance of a species to be an indicator of a particular habitat, or set of environmental conditions.

Results

A total of 6890 mosquitoes, 34 species in six genera, were collected during the spring and summer of 2009 and 2010 among all trapping locations (Table 3). Values for diversity indices were calculated for mosquito communities collected from livestock group locations collected during 2009 and 2010 (Table 4).

There were significant means differences among Shannon Diversity Index values ($p \leq 0.0049$) (Table 5) of the the main livestock groups, with swine groups (2010) being less diverse than bovine groups (2009) ($p \leq 0.00062$), capine groups (2009) ($p \leq 0.0092$) and equine groups (2009) ($p \leq 0.042$). There were no differences in comparisons between bovine, capine and equine groups, all collected in 2009 (Table 6). The poultry group (2010) did not differ significantly in diversity from any other host groups (Figure 17).

24

There were no significant differences in evenness among the livestock groups from both collection years (Table 7, Figure 18).

Significant differences among richness values ($p \geq 0.0154$) for livestock groups were uncovered. Comparisons of mosquito species richness for each livestock group indicated that mosquito species richness was significantly lower for swine (2010) than for bovine groups (2009) ($p \leq 0.0187$). Although equine

(2009) richness value was greater than swine (2010), the difference was only nearing significance ($p \leq 0.0609$) (Table 8, Figure 19).

In NMDS analysis, fifty runs with real data and fifty runs with randomized data were used to determine that two dimensions best suited the data set for the final solution. The final stress of 5.5 was calculated in sixty-five iterations, and groups were plotted onto a two dimensional axis (Figure 20). The value of 5.5 assigned to the process of iterations required to fit the individuals into host groups indicates the amount of stress it required to assign the mosquito species to pre-determined livestock group. The final value of 5.5 suggests that there is a difference in mosquito communities as grouped by host and little effort was required to group them as such.

25

MRPP indicated an overall difference among the mosquito communities ($p \leq 0.046$) (Table 9). Pair-wise comparisons between mosquito communities were carried out to determine between which groups significant differences were to be found. The communities collected at capine host locations and bovine host locations were significantly different from each other ($p \leq 0.049$) (Table 10). An indicator species analysis was carried out to identify specific differences between communities, at the species level. Species identified in this analysis with a significant probability of being a species indicator for an environment maintaining bovines as main-hosts were *Ochlerotatus triseriatus* (Say), *Ochlerotatus fulvus palens* (Ross) and *Culex pipiens* (Linnaeus) (Table 11). There were no other

species that showed significant results for being an indicator of particular host presence in a location.

Discussion

Agricultural environments naturally provide all of the resources necessary for the establishment of mosquito populations, such as water sources (permanent or ephemeral) for reproduction, plants that serve as carbohydrate sources for adults, and vertebrate hosts that provide blood-meals necessary for egg maturation. Particular species of mosquitoes have been shown to be influenced by the availability of specific hosts in the environment as this produces host-feeding patterns. These patterns are determined through repeated contact with a certain host, instead of a fixed-feeding behavior (Edman 1974). This

26

behavioral conditioning may influence mosquitoes to orientate to particular hosts, as mosquitoes have shown definite preference for the types of blood-meals they have already fed upon (Hii et al. 1991, Mwandawiro et al. 2000).

This study utilized five different vertebrate hosts, over 2009 and 2010 collection seasons, as environmental variables in a comparison of mosquito communities associated with each host habitat.

Although not every collection location was identical in landscape features such as the number of buildings and microhabitats, all were representative of typical agricultural environments for each of the livestock types. For example, locations used in the second year of collections, 2010, maintained swine and poultry as primary livestock and typically had more out buildings than the capine,

equine or bovine locations collected from during 2009, the first year of the study. Each of the locations were typical for the type of livestock maintained there. Agriculture environments may provide habitats for unique mosquito species, potentially influencing the types of mosquitoes found in each environment. Container breeding mosquitoes for example, exhibit breeding habitat preferences that were likely to be more common in a typical poultry or swine area (Adebote et al. 2006, Yan and Zhong 2005).

Our results suggest that there may be some influence of primary livestock species on some aspects of the composition of mosquito communities collected from typical mid-Missouri agricultural environments Communities collected in 2010 from locations with swine as primary species were less diverse than communities collected in 2009 at equine, bovine and capine locations.

27

One possible explanation for this difference is a temporal influence. Differences in collection years make it problematic for all possible comparisons to be made equally between communities. Annual temperatures and precipitation differences could have great impacts on mosquito species presence or absence. For example, container breeding genera such as *Aedes*, *Ochlerotatus* and some *Culex* are sensitive to annual rainfall amounts because of the nature of their breeding habitats. A dry year could decrease the number of species present in the environment, even for just one collection season.

When comparing the 2010 swine communities with the 2009 communities outside of a time reference, we should point out the availability and kinds of

breeding sites available at these livestock collection locations could have also influenced the presence or absence of certain mosquito species. The mosquitoes collected at the swine livestock locations were not as diverse as a group as the communities collected from other livestock locations, however the evenness of species present was not significantly different. This would imply sufficient numbers of mosquito species individuals for the species to be well established in the environment. Although there was no significant difference in the distribution of individuals from species present from the other livestock locations, a decrease in species number created a significant difference in community diversity. With the necessity of more outbuildings in close proximity to the livestock at swine and poultry locations, the influence of breeding habitats resulting from the presence of these structures could have impacted the species presence at these collection locations.

The species collected in the CDC light trap were exhibiting host-seeking behaviors in close proximity to the swine individuals. If another host species, avian species in the tree canopy for example, were able to provide a blood meal that required less energy cost from the mosquito and were abundant in the environment at that time, some mosquito species would not have been represented in the collection trap.

MRPP analysis determined a difference between the mosquito communities collected at the bovine group collection locations and the communities collected at the capine livestock group locations. Importantly, these

collections were made in the same collection year, 2009. Although there were not differences in diversity, evenness or richness between the livestock group mosquito communities, when species to species comparisons were carried out, there were some significant differences located. Host body size differences and host defense behavior differences play roles in blood-feeding success for mosquitoes (Day and Edman 1984, Edman and Kale 1971, Edman and Webber 1974). Bovine are considerably larger than capine which could account for differences in mosquito attraction in the environment due to differences in amount of body heat which in turn affects the dispersal of these olfactory cues for host-seeking behaviors into the environment (De Jong and Knols 1995, Olanga et al 2010).

The presence of mosquitoes in the environment is influenced by many factors, such as availability of aquatic habitats for breeding, availability of plants for energy resources and host availability for blood-feeding. The landscape characteristics among all collection sites used in the study, such as grassy areas and tree lines, provide natural habitats for vertebrate and plant species. These same landscape features also provide breeding habitats for many genera of mosquitoes. The diversity and abundance of mosquito species give structure to the community composition.

There are approximately 3200 species of mosquito species that have been described worldwide (Darsie and Ward 2004). Missouri has about 50 species of mosquitoes that have been identified throughout the state (Missouri

Department of Conservation), some of which are competent vectors of diseases such as Rift Valley Fever (RVF), West Nile virus (WNV) and Avian Malaria (Plasmodium spp.), which may infect a wide range of domestic livestock species. The seven most abundant species collected during study were *Culex restuans* (Theobald), *Culex pipiens* (Linnaeus), *Aedes vexans* (Linnaeus), *Ochlerotatus hendersoni* (Cockerell), *Ochlertotatus triseriatus* (Say), *Psorophora columbiae* (Dyar and Knab), *Culex erraticus* (Dyar and Knab) species (Figure. 2-21). Out of these seven, *C. restuans, A.vexans, C. pipiens* and *O. hendersonii* were found among all host groups. Of these four species, three species, *A.vexans, C.restuans* and *C.pipiens*, have been shown to vector Rift Valley fever, West Nile virus, and Avian Malaria (Anderson et al. 2004, Anderson et al. 1999, Ejiri et al. 2011, Miller et al. 2002, Turell et al. 2001). Rift Valley fever and West Nile virus are zoonotic pathogens that have been problematic to human and livestock populations (Meegan 1979, Anderson et al.1999)

30

While mosquito-borne pathogens could have huge impacts on domestic livestock populations in the U.S. and greatly affect state and local economies, there is little information available on the specific roles that these livestock hosts play in mosquito community composition. Livestock animals have been used in efforts to prevent disease by diverting the mosquitoes from humans, however for this to be effective in mosquito control, there would require at minimum some level of host preference by the mosquito, whether innate or conditioned by the environment (Yakubu and Singh 2008).

Mosquitoes' spatial preferences within a habitat seem to play a role in disease transmission to domestic or wild birds, which in turn can affect human and other livestock populations. For example, Darbro and Harrington (Darbro and Harrington 2006) were able to distinguish that *C. pipiens* showed no preference for the tree canopy unless the trap was baited with a chicken or sparrow, whereas *C. restuans* had a definite preference for forest canopy even in the absence of an avian baited trap. The forest canopy is an example of an environment that is home to a prevalent type of 'main-host', and the species of mosquitoes found in this environment are primarily ornithophilic in feeding behaviors (Cerný, Votýpka and Svobodová 2011). Perhaps agricultural settings that have well established livestock hosts provide environments suitable for the conditioning of mosquito species blood-feeding behaviors. Seasonality may also be responsible for many mosquitoes exhibiting a switch in host preference when the availability of main host in the environment changes (Chandler, Parsons and Boreham 1977, Nasci and Edman 1981), which by implication could associate main-hosts in an environment with the composition of mosquito communities located in the same place.

Table 1 Latitude and longitude coordinates for the fifteen mosquito collection locations used during 2009-2010 collection seasons.

Host Trap	Year of Collection	Easting	Northing	Missouri County
Bovine 1	2009	554160.6	4273041.7	Cole
Bovine 2	2009	565865.27	4262144.2	Cole
Bovine 3	2009	542169.59	4284244.9	Moniteau
Capine 1	2009	574976.39	4264441	Cole
Capine 2	2009	559309.57	4294690.6	Boone
Capine 3	2009	553289.16	4251911.4	Cole
Equine 2	2009	564167.25	4287856.7	Boone
Equine 1	2009	574280.82	4280346.3	Callaway
Equine 3	2009	554588.13	4273335.4	Cole
Poultry 1	2010	584841	4272197	Callaway
Poultry 2	2010	592308	4264813	Osage
Poultry 3	2010	591357	4262897	Osage
Swine 1	2010	585395	4276034	Cole
Swine 2	2010	595437	4259128	Osage
Swine 3	2010	594919	4257328	Osage

Table 2 Landscape characteristics and water source information based on 2008 aerial photographs. Photographs have a two foot resolution. A circle with one-half mile diameter centered on sample site was used to estimate the vegetative cover and distance from water.

Trap ID	Pasture Type	Forest Type	Water Sources	Description of Water Source and Distance from Trap
			Collection Year 2009	
Bovine1	80-90% cool-season grassland	10-20% mixed species forest	5	Pond 425' to the south Pond 750' to the south Pond 600' to the northeast Pond 1,000' to the north Pond 1,200' to the north
Bovine2	60-70% cool-season grassland	30-40% mixed species forest	2	Pond 490' to the southeast Pond 1210' to the south
Bovine3	70-80% cool-season grassland	20-30% mixed species forest	4	Pond 770' to the northeast Pond 1,090' to the southeast Stream 585' to the northwest running in a southwest to north direction Stream 665' to the east running in a north-south direction
Capine1	90-95% cool-season grassland	5-10% mixed species forest	2	Pond 470' to the southwest River 750' to the south running in an east-west direction
Capine2	40-50% cool-season grassland	60-70% mixed species forest	5	Pond 1,020' to the west Pond 810' to the north Pond 830' to the east Pond 1,060' to the southwest Stream 215' to the west running north-south and returning to the south 345' to the east
Capine3	90-95% cool-season grassland	5-10% mixed species forest	3	Pond 1,130' to the northeast Pond 900' to the north Pond 1,015' to the northwest
Equine1	10-20% cool-season grassland	80-90% mixed species forest	6	Pond 240' to the north Pond 450' to the northeast Pond 780' to the northeast Pond 1,040' to the northeast Pond 890' to the northwest Stream 870' to the south running in an east-west direction
Equine2	90-95% cool-season grassland	5-10% mixed species forest	4	Pond 265' to the south Pond 1,120' to the southwest Pond 700' to the west Pond 850' to the southeast
Equine3	50-60% cool-season grassland	40-50% mixed species forest	3	Pond 1,030' to the west Pond 1,110' to the southwest Stream 340' to the west running in a north-south direction

Collection Year 2010				
Poultry1	40-50% cool-season grassland	50-60% mixed species forest	3	Pond 175’ to the west Pond 1,150’ to the west Pond 910’ to the south
Poultry2	60-70% cool-season grassland	30-40% mixed species forest	3	Pond 460’ to the south Pond 920’ to the southeast Stream 570’ to the north running in an east-west direction
Poultry3	80-90% cool-season grassland	10-20% mixed species forest	6	Pond 710’ to the south Pond 128’ to the northwest Pond 1080’ to the north Pond 724’ to the south Stream 1150’ to the west running in a north-south direction Stream 565’ to the north running in an east-west direction
Swine1	25-35% cool-season grassland	65-75% mixed hardwood	5	Pond 175’ to the west Pond 600’ west-southwest Pond 257’ to the north-east Stream 250’ to the east running in a general north-south direction Stream 990’ to the north running in a general east-west direction turning south
Swine2	80-90% cool-season grassland	10-20% mixed species forest	4	Pond 95’ to the north Pond 770’ to the south-east Pond 224’ to the south-west Pond 610’ to the west-southwest
Swine3	50-60% cool-season grassland	40-50% mixed species forest	7	Pond 280’ to the south Pond 140’ to the north-east Pond 255’ to the north-east Pond 1030’ to the west-southwest Pond 938’ to the south Pond 856’ to the southeast Pond 507’ to the southwest

Table 3 Mosquito species and quantities collected for each livestock group. Capine (goat), bovine (cow) and equine (horse) were the primary livestock at collection locations carried out in 2009, while swine (hogs) and poultry were the primary livestock at collection locations during the 2010 collection season.

	2009			2010	
Mosquito Species	***Capine***	***Bovine***	***Equine***	***Swine***	***Poultry***
Aedes aegypti (Meigan)	*1*	*0*	*0*	*0*	*0*
Aedes albopictus (Skuse)	*6*	*4*	*5*	*0*	*1*
Aedes vexans (Linnaeus)	*75*	*468*	*159*	*6*	*430*
Anopheles barberi (Coquillett)	*0*	*0*	*1*	*0*	*1*
Anopheles crucians (Wiedeman)	*0*	*0*	*0*	*1*	*0*
Anopheles freeborni (Aitken)	*0*	*3*	*2*	*1*	*32*
Anopheles punctipennis (Say)	*4*	*16*	*15*	*0*	*4*
Anopheles quadrimaculatus (Say)	*1*	*0*	*3*	*0*	*4*
Coquillittidia perturbans (Walker)	*7*	*9*	*30*	*0*	*1*
Culesita impatiens (Walker)	*0*	*0*	*0*	*1*	*0*
Culex erraticus (Dyar andKnab)	*26*	*82*	*102*	*11*	*733*
Culex peccator (Dyer and Knab)	*0*	*1*	*0*	*0*	*0*
Culex pipiens (Linnaeus)	*147*	*500*	*283*	*42*	*30*
Culex reeveesi (Wirth)	*0*	*1*	*0*	*0*	*0*
Culex restuans (Theobald)	*345*	*897*	*804*	*504*	*274*
Culex salinarius (Coquillett)	*16*	*35*	*16*	*0*	*0*
Culex tarsalis (Coquillett)	*10*	*9*	*9*	*0*	*16*
Culex territans (Walker)	*1*	*1*	*0*	*0*	*0*
Culiseta inornata (Williston)	*5*	*3*	*1*	*0*	*0*
Ochlerotatus canadensis canadensis (Theobald)	*1*	*0*	*0*	*0*	*0*
Ochlerotatus fulvus pallens (Ross)	*1*	*6*	*0*	*0*	*2*
Ochlerotatus hendersoni (Cockerell)	*19*	*157*	*9*	*0*	*0*
Ochlerotatus sollicitans (Walker)	*1*	*0*	*3*	*3*	*15*
Ochlerotatus thibaulti (Dyer and Knab)	*0*	*3*	*0*	*0*	*0*
Ochlerotatus triseriatus (Say)	*8*	*107*	*9*	*1*	*1*
Ochlerotatus trivittatus (Coquillett)	*0*	*5*	*3*	*0*	*0*
Ochlertotatus taeniorhynchus (Weidemann)	*0*	*3*	*0*	*0*	*0*
Orthopodomyia signifera (Coquillett)	*0*	*0*	*1*	*0*	*0*
Psorophora ciliate (Fabricius)	*0*	*5*	*1*	*0*	*6*
Psorophora columbiae (Dyar andKnab)	*8*	*55*	*19*	*24*	*190*
Psorophora cyanescens (Coquillett)	*3*	*9*	*3*	*5*	*0*
Psorophora discolor (Coquillett)	*1*	*0*	*0*	*0*	*0*
Psorophora howardii (Coquillett	*0*	*3*	*0*	*0*	*0*
Psorophora signipennis (Coquillett)	*0*	*5*	*0*	*0*	*0*
Totals by Host	*686*	*2387*	*1478*	*599*	*1740*
Total Mosquitoes Collected					*6890*

Table 4 Richness, evenness and Shannon Diversity Index values for each of the trapping sites used in 2009 and 2010. Richness describes the number of species present at a site, evenness value describes the relative number of individuals per species at a site and Shannon Index reflects the number of species collected at a site, while taking into account how evenly the numbers of individuals are distributed among the types

Trap ID	Richness	Evenness	Shannon Index
Collection Year 2009			
Bovine 1	24	0.46	1.461
Bovine 2	18	0.679	1.961
Bovine 3	12	0.6	1.492
Capine 1	13	0.694	1.781
Capine 2	13	0.567	1.455
Capine 3	14	0.577	1.524
Equine 1	16	0.504	1.398
Equine 2	15	0.5	1.355
Equine3	16	0.522	1.447
Collection Year 2010			
Poultry 1	15	0.55	1.489
Poultry 2	6	0.599	1.073
Poultry 3	5	0.537	0.864
Swine 1	7	0.476	0.926
Swine 2	7	0.251	0.488
Swine 3	4	0.62	0.859

Table 5 Results from ANOVA carried out on diversity values between mosquito collections from differing livestock groups. Differences between livestock groups were found to exist in richness and Shannon Index diversity values. (α = 0.05)

	DF	F Value	Pr > F
Richness	**4**	5.24	**0.0154**
Evenness	**4**	0.98	0.4595
Shannon Diversity Index	**4**	7.37	**0.0049**

Table 6 Tukey-Kramer HSD for Shannon Diversity Index means between livestock groups. The swine livestock group collections of mosquitoes were less diverse than all other livestock groups except poultry, which may have been influenced by the landscape structure of swine production locations or differences in host group collection years. It should be noted that the swine and poultry groups of mosquitoes were collected in a different year from bovine, capine and equine groups.

Mean Shannon Index Values	Collection Year/ Livestock Group	2009/ Bovine	2009/ Capine	2009/ Equine	2010/ Poultry	2010/ Swine
1.64	**2009/Bovine**		0.9986	0.7188	0.1377	**0.0062**
1.59	**2009/Capine**			0.8544	0.2045	**0.0092**
1.40	**2009/Equine**				0.6593	**0.042**
1.14	**2010/Poultry**					0.3171
0.76	**2010/Swine**					

Table 7 Tukey-Kramer HSD for evenness means between livestock groups. No specific differences between mean evenness values were found in livestock group comparisons.

Mean Evenness Values	Collection Year/ Livestock Group	2009/ Bovine	2009/ Capine	2009/ Equine	2010/ Poultry	2010/ Swine
0.31	**2009/Bovine**		0.97	0.9707	0.7883	0.9039
0.37	**2009/Capine**			0.7419	0.9835	0.9989
0.26	**2009/Equine**				0.4561	0.6013
0.42	**2010/Poultry**					0.9987
0.39	**2010/Swine**					

Table 8 Tukey-Kramer HSD for richness means between all livestock groups. The swine group had significantly less species of mosquitoes collected than the bovine group. Differing years of host group collections and landscape characteristics of the swine and poultry collection locations may have played roles in significant differences in richness values between groups.

Mean Richness Values	Collection Year/ Livestock Group	2009/ Bovine	2009/ Capine	2009/ Equine	2010/ Poultry	2010/ Swine
18.00	**2009/Bovine**		0.5691	0.9354	0.0721	0.0187
13.33	**2009/Capine**			0.9354	0.5691	0.1922
15.67	**2009/Equine**				0.2243	0.0609
8.67	**2010/Poultry**					0.9005
6.00	**2010/Swine**					

Table 9 Euclidean distance averages of mosquito communities for each livestock group. MRPP analysis found differences between communities of mosquitoes collected from differing livestock locations. Euclidean distance was used in MRPP analysis for determining average distances for groups. Permutations of randomly assigned species to pre-determined livestock groups were used to determine

Livestock Group	Group identifier	Group Size	Average distance
2010/Poultry	3	3	0.63611111
2010/Swine	2	3	0.54722221
2009/Capine	1	3	0.1861111
2009/Bovine	4	3	0.36944444
2009/Equine	5	3	0.26388888

Test statistic: T = -1.8483716

Observed delta = 0.40055555

Expected delta = 0.50000000

Chance-corrected within-group agreement, A = 0.19888890

Probability of a smaller or equal delta, p = **0.04637305**

the probability of finding an equal or smaller delta. P value indicates there is a significant difference between the mosquito communities according to livestock groups. It should be noted that although all communities are being compared, the swine and poultry host group communities were collected in a different year than the bovine, capine and equine groups.

Table 10 Pair-wise livestock group comparisons carried out in MRPP analysis. The capine and bovine communities are significantly different from each other (*p* = 0.04977), and the swine and bovine communities are approaching significant difference (*p* = 0.090647). Note: p values were not adjusted for multiple comparisons. Significant values in bold and *

Livestock Groups Compared	Collection Year	P value
Capine vs. Poultry	2009 vs. 2010	0.116212
Capine vs. Swine	2009 vs. 2010	0.194951
Poultry vs. Bovine	2010 vs. 2009	0.148634
Poultry vs. Equine	2010 vs. 2009	0.121496
Swine vs. Bovine	2010 vs. 2009	0.090647
Swine vs. Equine	2010 vs. 2009	0.164614
Bovine vs. Equine	2009 vs. 2009	0.462212
Capine vs. Bovine	2009 vs. 2009	**0.04977***
Capine vs. Equine	2009 vs. 2009	0.161596
Swine vs. Poultry	2010 vs. 2010	0.659493

Table 11 Species indicator values for each of the mosquito species collected from livestock locations during 2009 and 2010. A Monte Carlo test for significance was performed on the observed indicator values of mosquito species using 4999 random permutations. *Culex pipiens* (Linnaeus), *Ochlerotatus triseriatus* (Say) and *Ochlerotatus fulvus palens* (Ross) were found to have significant indicator values for the bovine livestock group. Significant values in bold type and have *.

Mosquito species	Livestock Group	Observed Indicator value	Indicator Value from Randomized Groups	Standard Deviation	P value
Culex pipiens (Say)	bovine	49.9	32.6	6.74	**0.0136***
Ochlerotatus fulvus palens (Ross)	bovine	66.7	30.7	13.54	**0.0598***
Ochlerotatus triseriatus (Say)	bovine	84.9	59.4	18.15	**0.0194***
Culex salinarius (Coquillett)	bovine	52.2	34.3	11.64	0.1032
Coquillittidia perturbans (Walker)	equine	63.8	39.1	15.43	0.121
Ochlerotatus thibaulti (Dyar and Knab)	bovine	66.7	28.9	15.89	0.1504
Ochlerotatus hendersoni (Cockerell)	bovine	84.9	55.7	20.15	0.1556
Culex tarsalis (Coquillett)	poultry	36.4	32.8	7.71	0.3487
Anopheles punctipennis (Say)	bovine	41	34.4	12.57	0.3621
Ochlerotatus trivittatus (Coquillett)	bovine	41.7	32.3	16.4	0.4381
Aedes albopictus (Skuse)	bovine	31.2	32.9	11.45	0.4427
Culiseta inornata (Williston)	bovine	22.2	31.8	16.32	0.4673
Culex erraticus (Dyer and Knab)	poultry	76.8	70.5	13.1	0.4889
Aedes vexans (Linnaeus)	bovine	41.1	41.6	11.59	0.4953
Anopheles quadrimaculatus (Say)	equine	37.5	32.3	16.71	0.5399
Psorophora ciliate (Fabricius)	bovine	27.8	31.8	16.62	0.6159
Culex restuans (Theobald)	bovine	31.8	34.1	5.7	0.6257
Ochlerotatus sollicitans (Walker)	poultry	45.5	43.4	18.27	0.6441
Psorophora cyanescens (Coquillett)	bovine	30	33.2	14.41	0.6631
Psorophora columbiae (Dyer and Knab)	poultry	42.8	59.2	14.54	0.9678
Aedes aegypti (Linnaeus)	capine	33.3	33.3	0.47	1
Anopheles barberi (Coquillett)	poultry	16.7	24.1	17.76	1
Anopheles crucians (Wiedemann)	swine	33.3	33.3	0.47	1
Anopheles freeborni (Aitken)	poultry	28.1	49.7	20.07	1

Mosquito species	Livestock Group	Observed Indicator value	Indicator Value from Randomized Groups	Standard Deviation	P value
Culesita impatiens (Walker)	swine	33.3	33.3	0.47	1
Culex peccator (Dyar andKnab)	bovine	33.3	33.3	0.47	1
Culex reeveesi (Wirth)	bovine	33.3	33.3	0.47	1
Culex territans (Walker)	capine	16.7	23.6	17.28	1
Ochlerotatus canadensis canadensis (*Theobald)*	capine	33.3	33.3	0.47	1
Ochlertotatus taeniorhynchus (Weidemann)	bovine	33.3	33.3	0.47	1
Orthopodomyia signifera (Coquillett)	equine	33.3	33.3	0.47	1
Psorophora discolor (Coquillett)	capine	33.3	33.3	0.47	1
Psorophora howardii (Coquillett)	bovine	33.3	33.3	0.47	1
Psorophora signipennis (Coquillett)	bovine	33.3	33.3	0.47	1
Averages		41.2628	36.92	9.92	0.6389

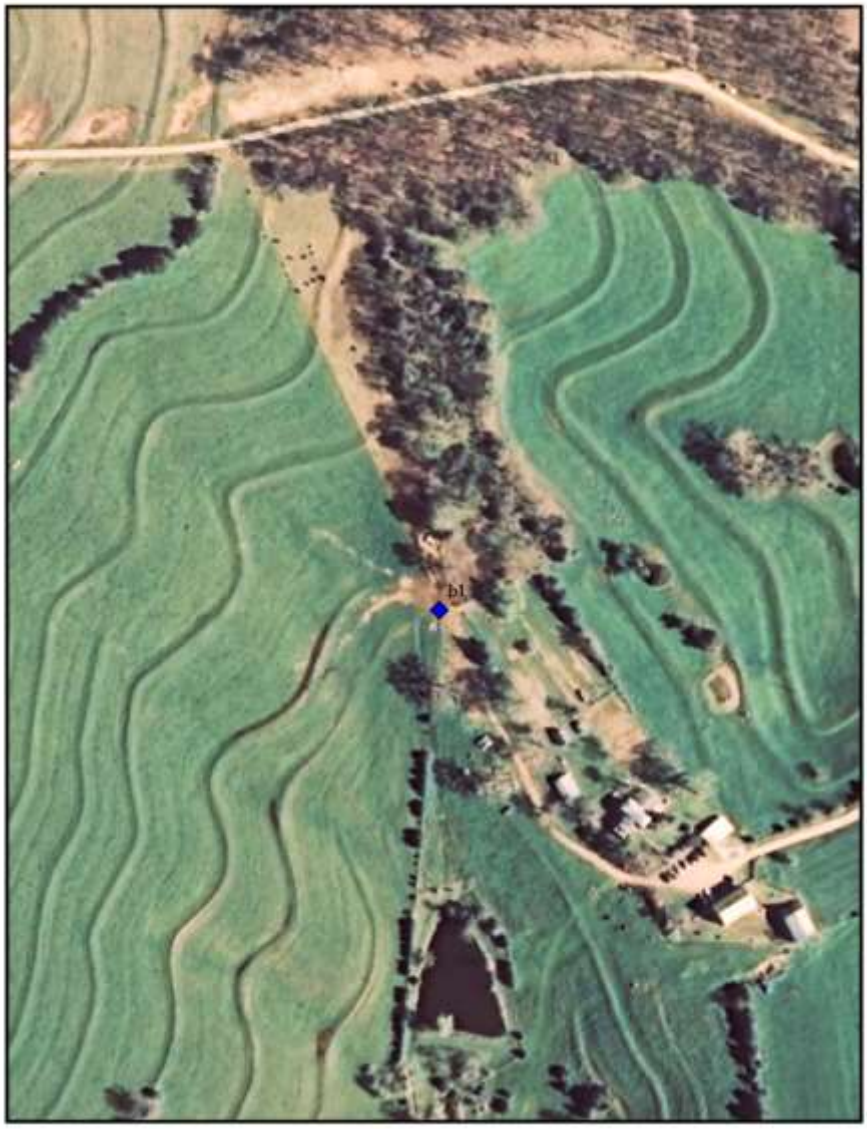

Figure 1 Aerial photograph of trap site Bovine 1. UTM coordinates 554160.604m E 4273041.723m N, located in Moniteau County, Missouri

Figure 2 Aerial photograph of trap site Bovine 2. UTM coordinates 565865.27m E 4262144.195m N, located in Cole County Missouri

Figure 3 Aerial photograph of trap site Bovine 3. UTM coordinates 542169.592m E 4284244.912m N, located in Calloway County, Missouri

Figure 4 Aerial photograph of trap site Capine 1. UTM coordinates 574976.39m E 4264440.983m N, located at Carver Farm, Lincoln University, in Cole County, Missouri

Figure 5 Aerial photograph of trapping site Capine 2. UTM coordinates 559309.566m E 4294690.577mN located in Boone County, Missouri

Figure 6 Aerial photograph of trapping site Capine 3. UTM coordinates 553289.161m E 4251911.397m N, located in Cole County, Missouri

Figure 7 Aerial photograph of trapping site Equine 1. UTM coordinates 574280.822m E 4280346.328m N, located in Callaway County, Missouri.

Figure 8 Aerial photograph of trapping site Equine 2. UTM coordinates 564167.246m E 4287856.733m N, located in Boone County, Missouri.

Figure 9 Aerial photograph of trapping site Equine 3. UTM coordinates 554588.132m E 4273335.428m N, located in Cole County, Missouri.

Figure 10 Aerial photograph of trapping site Swine 1. UTM coordinates 585395m E 4276034m N, located in Cole County, Missouri.

Figure 11 Aerial photograph of trapping site Swine 2. UTM coordinates 595437m E 4259128m N, located in Osage County, Missouri.

Figure 12 Aerial photograph of trapping site Swine 3. UTM coordinates 594919m E 4257328m N, located in Osage County, Missouri.

Figure 13 Aerial photograph of trapping site Poultry 1. UTM coordinates 584841m E 4272197m N, located in Calloway County, Missouri.

Figure 14 Aerial photograph of trapping site Poultry 2. UTM coordinates 592308m E 4264813m N, located in Osage County, Missouri.

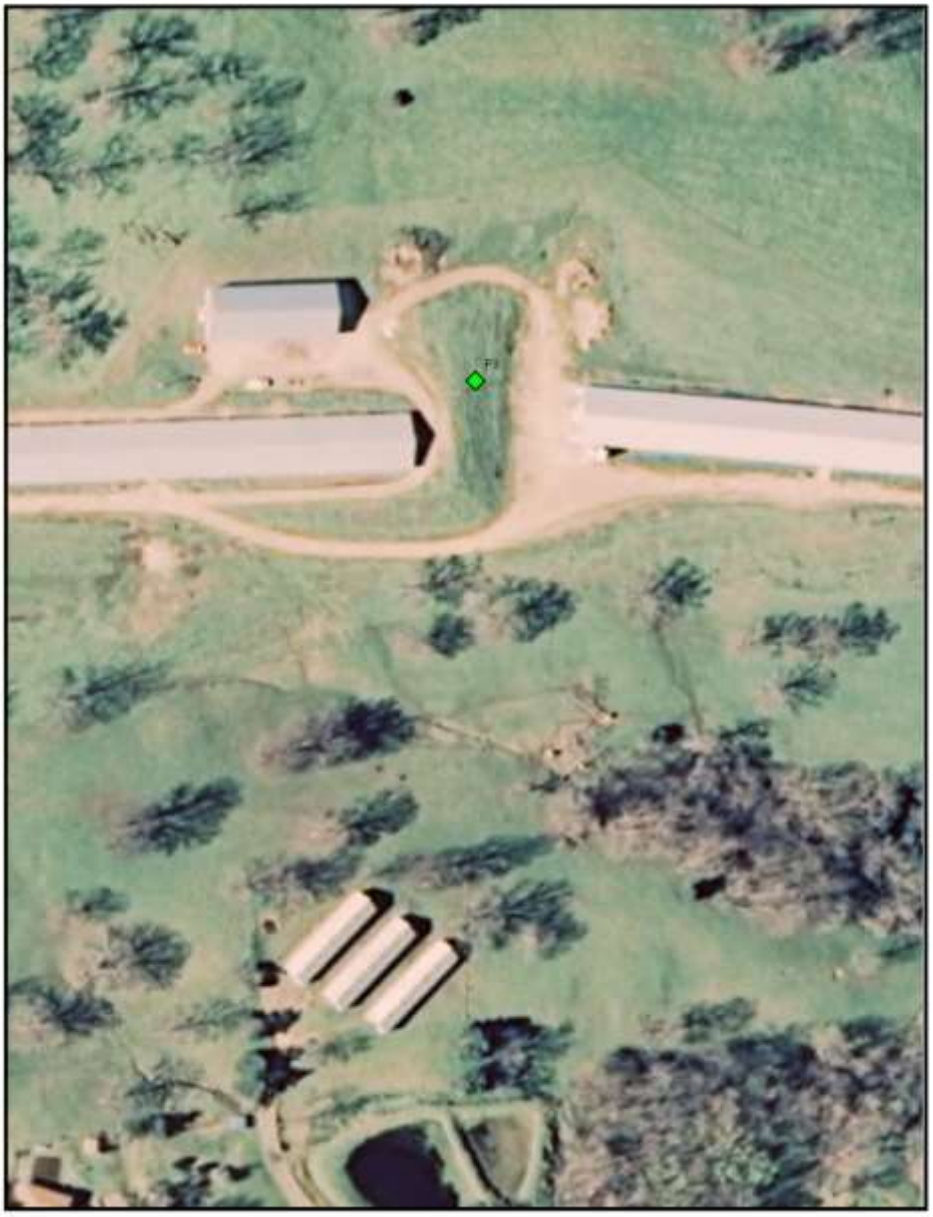

Figure 15 Aerial photograph of trapping site Poultry 3. UTM coordinates 591357m E 4262897m N, located in Osage County, Missouri.

Figure 16 CDC Miniature light trap used at all collection sites. Dry ice was used as an attractant for mosquito collection. Host-seeking females were drawn into the collection cup when attracted to carbon dioxide emitted from hose.

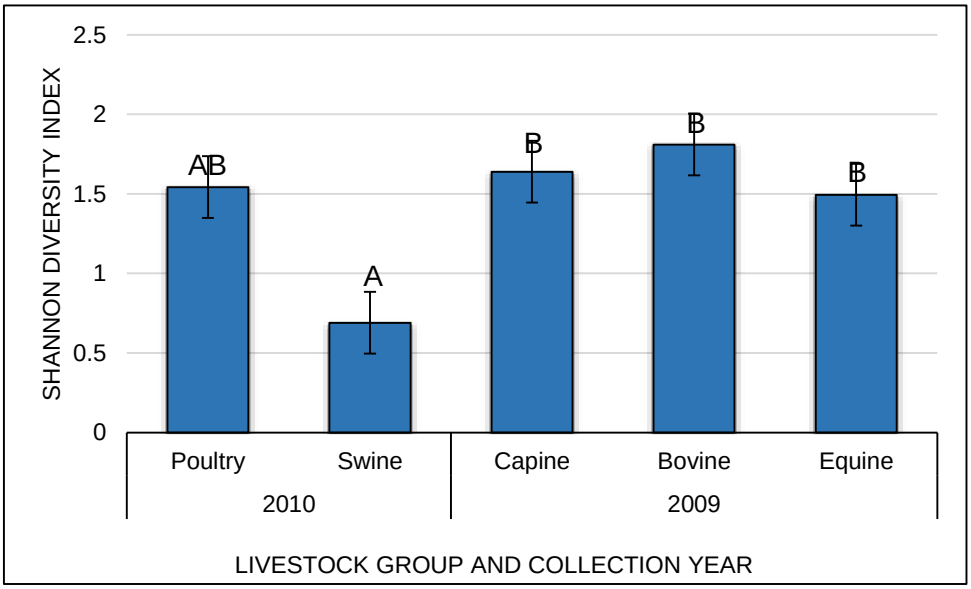

Figure 17 Shannon Diversity Index values for five different livestock collection groups of mosquitoes carried out during 2009 and 2010. Analysis of variance (α=0.05) indicated a significant difference in diversity among the mosquito populations collected from differing livestock collection groups (p<0.0049). Means (± SE) with different letters are significantly different (Tukey-Kramer HSD, p<0.05).

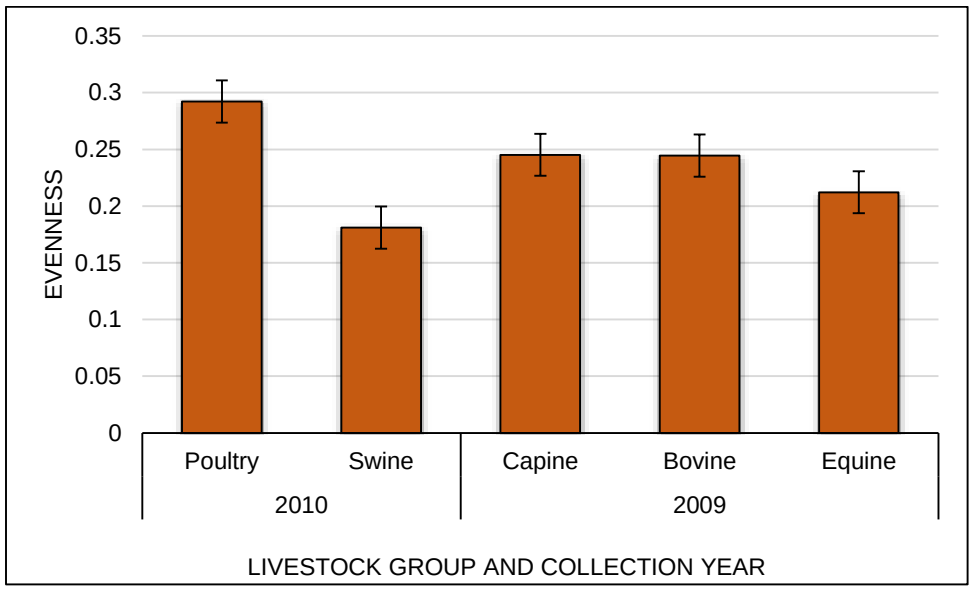

Figure 18 Evenness values for five different livestock collection groups of mosquitoes carried out during 2009 and 2010. Analysis of variance (α=0.05) indicated no significant differences (p > 0.4595) in evenness means (± SE) between any of the host groups.

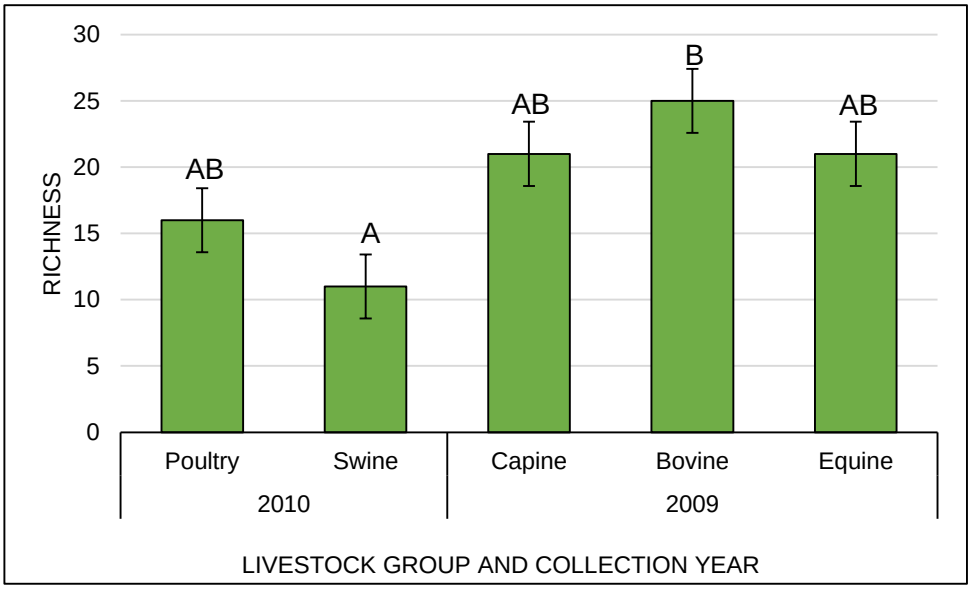

Figure 19 Richness values for five different livestock collection groups of mosquitoes carried out during 2009 and 2010. Analysis of variance (α=0.05) indicated a significant difference (*p*>0.0154) in species richness among host groups. Means (± SE) with different letters are significantly different (Tukey-Kramer HSD, *p*<0.05).

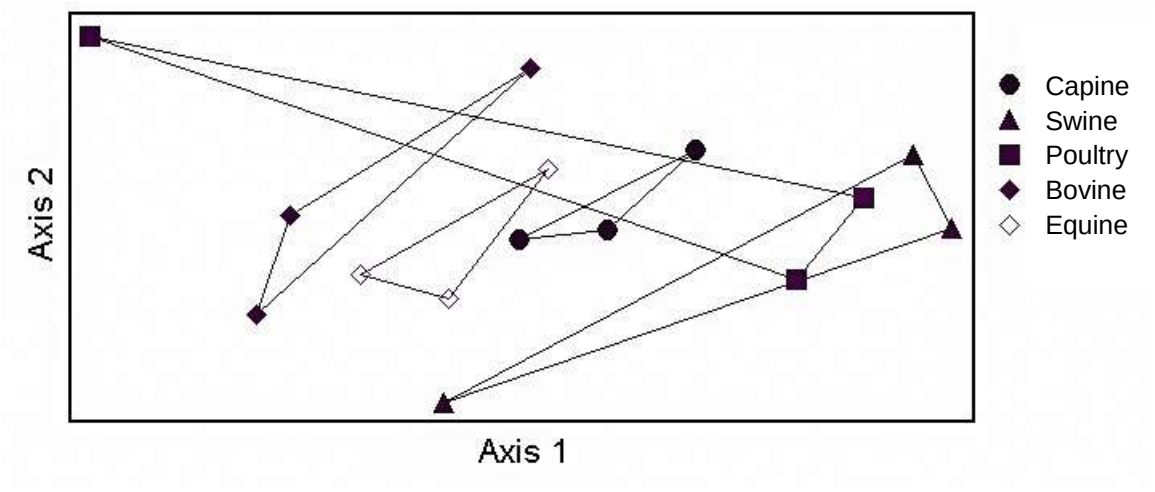

Figure 20 Results from NMDS analysis indicating separation of mosquito communities collected in 2009 and 2010 from five differing livestock locations. Non-metric multidimensional scaling was applied to all host data using pre-defined groups according to livestock species. The stress required for data to separate into these groups was calculated to be 5.5. This value indicates there is little chance of misinterpretation of these results. The poultry livestock group includes portions of each of the other host groupings, which seems to confirm the lack of significant difference between this group and the other livestock groups.

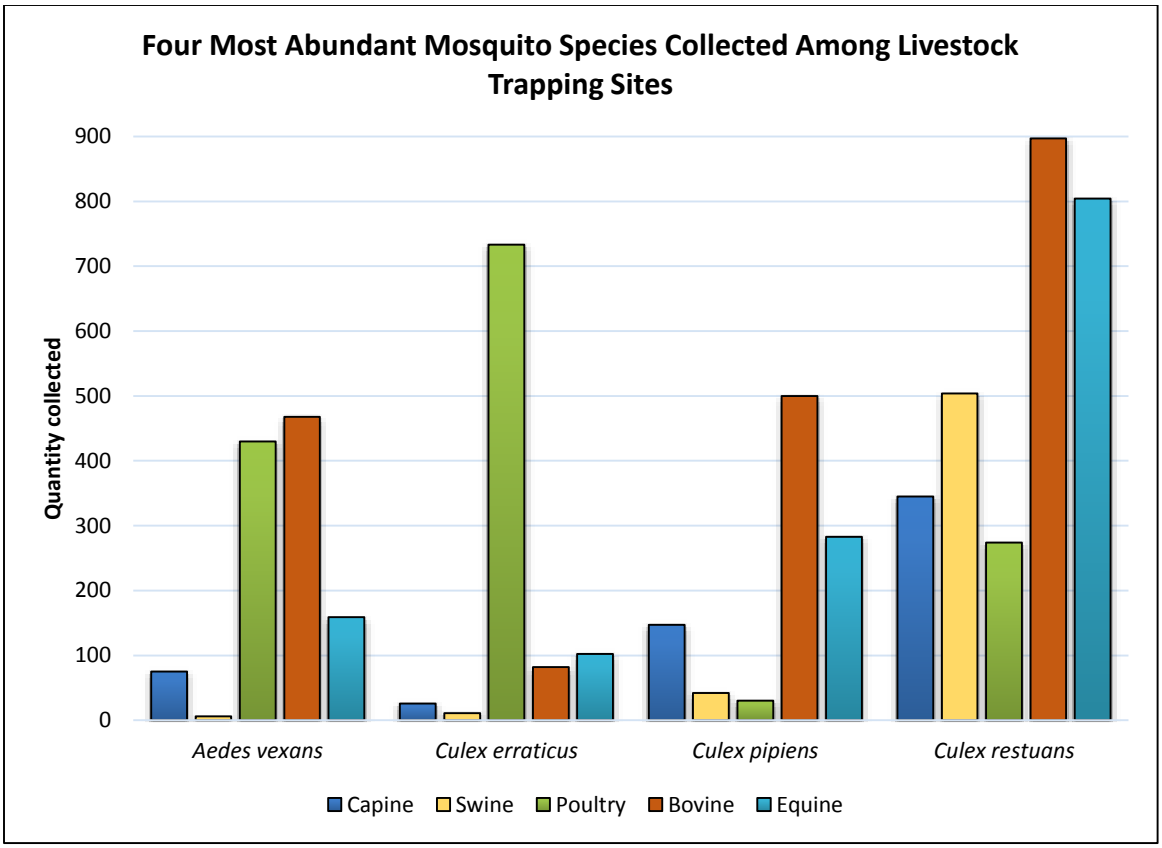

Figure 21. Four most abundant mosquito species collected among all livestock trapping locations during 2009 and 2010. *Culex restuans* (Theobald), *Culex pipiens* (Linnaeus), *Aedes vexans* (Linnaeus) and *Culex erraticus* (Dyar andKnab) species were found among all host groups.

Chapter 3

Phenotypic trait and genetic differences compared among three populations of *Aedes albopictus* (Skuse) distributed between two latitudinal clines

Introduction

Aedes albopictus is indigenous to Southeast Asia and has played a role in the recent outbreaks of dengue fever (DEV) and Chikungunya virus (CHIKV) around the world. The role this mosquito has played in these events has brought it to the forefront of global awareness as a health threat (Paupy et al. 2009, Rezza 2012). Commonly known as the Asian Tiger mosquito, this mosquito has been established in the United States since 1985 (Hawley et al. 1987). *Aedes albopictus* is currently the most invasive mosquito worldwide (Bonizzoni et al. 2013). During the recent history of only 30-40 years, *Ae. albopictus* has become established in every continent but Antarctica (Benedict et al. 2007, Caminade et al. 2012). The rapid range expansion that this medically important mosquito has undergone allows for a unique opportunity to examine the phenotypic and genetic differences among latitudinally separated populations of this tropical species in more temperate climates.

Phenotypic plasticity, or the ability of one genome to vary gene expression in response to changing environmental conditions, plays a role in the survival or range expansion of many organisms exposed to changing environmental conditions. This plasticity is adaptive when these traits contribute to the fitness of an organism and are elicited by a particular environmental

condition that occurs among diverse environments (Newman 1992). Phenotypic plasticity has been well documented in mosquito species, such as larval response when exposed to differing environmental temperatures (Haramis 1985, Sibly and Atkinson 1994). These changes may affect an organism's behavior, physiology or morphology, providing coping mechanisms that assist in the survival of the species. Insects with aquatic immature stages often rely heavily on phenotypic plasticity in an attempt to maintain optimal fitness in varying environments. Genomes that are able to produce more than one phenotype in varying conditions maintain the phenotypic diversity in the species across a wide range of environments (Scheiner 1993, West-Eberhard 1989).

For many insects, body size and time required for development are traits easily influenced by temperature (Clements 1992), inter- intraspecific competition (Alto et al. 2005) and nutrient availability (Merritt, Dadd and Walker 1992). Developmental plasticity is a strategy utilized by insects to reach adulthood quicker (Nylin and Gotthard 1998). Speedier maturity may be beneficial for gaining a reproductive edge or survival. Container breeding habitats are especially sensitive to environmental change due to their relatively small size. These mosquitoes notably rely on plasticity in developmental time to cope with increased temperatures of the habitat, larval competition and lack of food resources. Under these circumstances genetic variation exhibited by different ranges of phenotypic plasticity in the population is often beneficial for survival and can also produce many changes in life history traits of the

organism, since natural selection does not typically select for single traits. Body-size in mosquitoes, for example, is closely related to fecundity, disease vector competence as well as lifespan of the insect (Haramis 1985, Hawley 1985, Nasci 1986).

Many environmental factors play a role in influencing adult mosquito body-size, such as temperature, resource competition (whether interspecific or intraspecific), larval density and predation (Alto et al. 2005, Kirby and Lindsay 2009, van Uitregt, Hurst and Wilson 2012). With this in mind, examination of natural populations of mosquitoes compared with laboratory maintained populations have been carried out, and in general it has been found that field-collected mosquitoes are smaller in body size than their counterparts that have been lab reared (Grimstad and Walker 1991). A difference in body-size is evidence that nutritional resources do in part have influence over the size of adult mosquitoes (Grimstad and Haramis 1984, Nasci and Mitchell 1994).

Measuring genetic variation

Morphological traits are often measured as expressions of genotypic differences within a population. For example wing length and length of developmental period are often used as measureable characteristics that are correlated to life history traits of mosquitoes. Wing length has direct correlations with mosquito body size, and body size is often used as a measure of fecundity, fitness and competence as a disease vector. It is generally accepted that larger females are typically more fecund and have longer life spans due to

greater fitness, whereas smaller females are less fit resulting in shorter life spans and surviving as more competent disease vectors (Alto, Reiskind and Lounibos 2008, Muturi et al. 2011, Nasci 1986, Paulson and Hawley 1991).

Genetic differentiation among populations may be measured using a variety of molecular methods including restriction fragment length polymorphisms (RFLP) (Severson 1995), randomly amplified polymorphic DNA (RAPD) markers (Apostol et al. 1996) and microsatellite analysis (Wang 2001). Microsatellite analysis is often used by molecular ecologists to examine population differentiation at varying spatial scales. This method of genetic examination depends on the presence of different alleles at the same microsatellite locus, and uses these differences to identify structuring within or among populations. Identifying genetic variances at the molecular level allows for determining the amount of genetic differences between populations that are more geographically separated than dispersal alone would explain. Isolation by distance can prove two populations are genetically differentiated based on the premise that geographically closer populations are more genetically similar and those farther apart are more genetically different. Microsatellite analysis has been used to analyze population structure of several mosquito species including *Ae. albopictus* (Kamgang et al. 2011), *Aedes aegypti* (*Stegomyia aegypti*) (Rasheed et al. 2013) and *Anopheles funestus* (Temu, Hunt and Coetzee 2004). In this study, we plan to test our hypothesis that the same species of mosquito are genetically differentiated as a result of geographic

separation and climatic differences. Phenotypic traits were examined using three geographically separated populations of *A. alboptictus* and molecular analysis using microsatellite markers was used to examine genomic variation and isolation by distance between geographically separated populations of *Ae. albopictus* from Ohio and Florida.

Methods

Mosquito populations

Mosquito populations used in these studies were collected during the summer of 2011 from three locations: Springboro, Ohio (39.5639° N, 84.2281° W) , Waycross, Georgia (31.2133°N, 82.3542° W) and Vero Beach, Florida (27.6383° N, 80.3975° W). Because the collection sites are geographically diverse, each population has acclimated to different average seasonal temperatures (Figure 22) with Ohio being the coolest average high temperature and Florida being the warmest. Adult mosquitoes were collected through human landing catches in Springboro, OH, while egg papers were collected from the field in Waycross, GA and Vero Beach, FL.

Mosquito rearing

Adult *Ae. albopictus* from OH were maintained on human blood in laboratory cages until they produced eggs. The egg papers were then collected, dried and stored prior to hatching. Upon hatching these egg papers produced the field generation from these two collection locations. Egg papers

collected from GA and eggs collected from the OH adults were hatched independently. Mosquito larvae from each state were reared in deionized water with ground Tetramin© (Blacksburg, VA) fish food ad libitum. Upon pupation, adults from each state were placed into separate cages to establish field generation adult populations to be used for egg collection.

All populations of field generation adults were maintained under insectary conditions of 23.3°C ±1 and relative humidity of 79.4% ± 6 for multiple generations, allowing all colonies the opportunity to grow in number. Populations were provided cotton balls soaked in a 10% sucrose solution daily and given weekly access to human blood for the collection of eggs on paper towels.

Experimental Design

Experimental design similar to that used by Agnew et al. (2002) was adopted to accommodate the small number of individuals in each geographic population. This experimental design uses fewer organisms than traditional population density studies and has successfully examined the influence of density-dependent factors on the life history traits of various mosquito species (Agnew et al. 2002, Agnew, Haussy and Michalakis 2000, Bedhomme et al. 2005, Koenraadt, Kormaksson and Harrington 2010).The progeny used in our study were generationally close enough to the wild parent populations to maintain genetic diversity. This diversity allowed us to measure phenotypic traits of these populations from the wild in a lab setting.

Eggs from Ohio and Florida (F_3 generations) and Georgia (F_4 generation) populations were flooded with deoxygenated water and first instar larvae from each population were counted into two density treatments. Larvae were placed into four ounce glass jars filled with deionized water to which two nutrient treatments using ground Tetramin® (Blacksburg, VA) fish food were added. Food treatments were labeled high (6mg ground Tetramin) and low (3mg ground Tetramin). Microcosms were then randomly selected and placed into one of two environmental temperatures (20° C and 25 °C), producing a factorial design of 3 states X 2 densities X 2 nutrient levels X 2 temperatures.

In total four treatment sets were used, with each set consisting of density 1/low food, density 1/high food, density 3/low food, density 3/ high food. Microcosms were randomly placed on the same shelf in each environmental chamber. Three environmental chambers were used for microcosms with each chamber used twice, once for 20°C treatments and once for 25° C.

Upon pupation, each pupa was placed into a *Drosophila* vial one half filled with deionized water. The day of pupation was recorded, and upon eclosion each adult was collected and frozen for future wing measurement. Wing measurements were taken from the axillary incision to the apical tip of the wing, excluding wing fringe, on each mosquito. Mean wing lengths and developmental times for all treatments were recorded and means for these variables were calculated (Table 12).

DNA Collection

After wing length measurements were taken from the adults collected at eclosion from the study, a random number generator, Random.org, was used to randomly select adults from the FL and OH treatments. Genomic DNA was collected from each of twenty-seven adults from the FL population (13 female, 14 male) and thirty adults from the OH population (15 female, 15 male) for individual analysis. Whole bodies were used and DNA was extracted with Illustra Nucleon Genomic DNA Extraction Kits. Upon collection, genomic DNA was purified with GeneJET Purification Kits to ensure removal of resin and impurities from DNA samples. Genetic polymorphism was analyzed at six microsatellite markers: AealbA9 (NED), AealbB6 (VIC), AealbB51 (VIC), AealbB52 (NED), AealbF3 (PET) and AeaslbD2 (6FAM). These markers have been previously shown to be polymorphic and useful for population analysis (Porretta et al. 2006) (Table 13). Microsatellite markers were used in 25µl multiplex PCR reactions containing 5µl Multiplex PCR 5X Master Mix, 3.75 µl Primer Stock, 2µl 1mM MgCl2, 2 µl BSA and 1µl 10 ng genomic DNA. Nuclease-free water, 11.25 µl, was added to bring solution to 25 µl volume. Two reaction mixtures were used, reaction 1 (AealbB51and AealbB52) and reaction 2 (AealbA9, AealbB6, AealbF3, and AealbD2) were optimized independently. Reactions were subjected to thermo cycler settings of 95° for 10 minutes, 40 cycles of 95°C for 1 min, either 51°C for reaction 1 or 54.3°C for

reaction 2 for 1 min, 72°C for 1 min followed by 10 minutes of 72°C. PCR product was then analyzed with an ABI 3730xl DNA Analyzer.

Data analysis

Statistical analysis was carried out on each sex independently, as mosquitoes are sexually dimorphic as adults. Data for each sex was tested for normality, and data that did not meet this distribution, was $\log_{10}$ transformed. Analysis of variance (ANOVA) ($\alpha = 0.05$) was used to compare the effects of and interactions between (1) state of collection, (2) density, (3) food level and (4) temperature on the wing length and developmental time from larval hatch to pupation. Wing lengths are commonly used as body-size indicators for mosquitoes in lab environments (Koella and Lyimo 1996, Siegel et al 1992) and development time (days from hatch to pupation) were analyzed for each sex separately. Any significant influences from variables or interactions of variables were further analyzed using Tukey-Kramer HSD procedure. Tukey-Kramer carries out pairwise means comparisons between treatments, adjusting for multiple comparisons. Means of wing lengths or developmental days means are shown back transformed as inverse logs.

GeneMarker © software was used to analyze the results of microsatellite amplifications carried out during multiplex PCR. Alleles, or different forms of the same gene, are recognized by the differing numbers of base pairs that make up the length of the DNA fragment. Since microsatellites are repeating sequences of DNA and are known to be polymorphic among populations, these markers

are useful population analysis tools. Allelic differences between populations were examined and recorded at each microsatellite marker. All genetic summary statistics and isolation by distance tests were calculated using GENEPOP v.4.2 (Raymond and Rousset 1995, Rousset 2008).

Results

Wing Length

The interaction between the amount of food present and larval density had significant effects ($p \leq 0.0128$) on the wing lengths of female *Ae. albopictus* (Table 14). The 1 larva/ High food (6mg) treatment produced females with significantly smaller wing sizes than the 1 larva/ Low food ($p \leq 0.0066$), 3 larvae/ High food ($p \leq 0.0213$) and 3 larvae/ Low food ($p \leq 0.0473$) treatments (Table 15). There were no significant influences of larval density or state of origin on the wing length, or body size of male *Ae. albopictus*. Temperature did have an effect of the wing length and developmental times of the males. Warmer rearing temperatures of 25°C produced significantly smaller males ($p = 0.03$) with mean wing lengths of 2.21 mm and cooler rearing temperatures of 20°C produced larger males with mean wing lengths of 2.45 mm.

Developmental Time

The four independent variables that were manipulated in the study were environmental temperature (temp), amount of nutritional resources (food), state of population collection (st) and population density (dens). Analysis of variance ($\alpha = 0.05$) found that the interaction between state of collection and

environmental temperature had a significant influence over the development time for the female *Ae. albopictus* ($p < 0.0151$) (Table 14). The temperature portion of the interaction of variables seemed to play a large role in the time required for larval development. In all but one the pairwise comparisons, the 25°C temperature treatment produced adults quicker than the treatments with a 20°C temperature. The only treatment pair from the pairwise comparisons in which both treatments were reared in the same temperature and produced a significant difference in developmental time means ($p = 0.0154$) was FL25 vs. OH25 pair. With the only difference in treatment variables being the state of origin, the OH females significantly faster in 7.41 days than the FL females that required 8.07 days when reared in the same temperature (Table 15).

Lastly, temperature had a significant influence on developmental time for male *Ae. albopictus* ($p < 0.001$), with the warmer temperatures of 25°C speeding up the mean developmental time to 7.45 days and cooler temperatures of 20°C slowing the developmental time to a mean 11.56 days.

Microsatellite analysis

All of the markers used in the study were polymorphic, with the number of alleles for each locus ranging from two to seven alleles. The Hardy-Weinberg (HWE) test for equilibrium was carried out for each locus separately, as well as for all loci collectively, and they did not deviate from the expected equilibrium. When all loci together were tested for each population, the OH and FL populations were found to be within equilibrium ($p < 0.0012$ and $p < 0.0008$

respectively). Fixation coefficients (FST) were calculated for each of the loci. These values estimate the amount of fixed genetic variation between individuals or populations. The Inbreeding Coefficient was estimated (FIS), quantifying the level of inbreeding in the population (Table 17). Exact G, or log-likelihood, tests were used for genetic differentiation between populations for each locus separately and for all loci. In total there were significant differences between the two populations across all loci collectively (p= 0.000027). Not every locus was significantly different between the OH and FL groups, however there were significant differences at AealbB51 (p= 0.02928), AealbB52 (p= 0.01722) and AealbA9 (p= 0.00011) (Table 18).

The presence of isolation by distance was tested between the two populations. This test seeks a correlation between the geographic distance between populations and genetic variation between them. A Mantel test with 1000 permutations was conducted. A one-tailed p-value of Pr (correlation >observed correlation) = 0.209 was calculated under the null hypothesis of no correlation existing between these values and could not be rejected.

Discussion

The success that *Ae. albopictus* has demonstrated as an invasive species is enhanced by the adaptability this mosquito has shown to varying environmental temperatures. This ability to undergo long-term environmental adaptation has allowed this species to become established across a latitudinal cline. Phenotypic plasticity is associated with range expansion as a strategy for

survival in changing environmental conditions. In our study we examined three populations of *Ae. albopictus*, each collected from geographically separated locations, each being found on a differing latitude. When each of the populations were exposed to the same environmental conditions, stressful and optimal, we found differences in expression of body size and the time required for development. The state of collection seemed to play some role in the phenotypic differences exhibited by the geographically separated populations. These findings would indicate a difference in genetics between the populations that could be attributed to long-term environmental adaptation, since the states represented environmental differences. Long-term exposure to different environmental temperatures seems to have affected female developmental times and body size of our experimental populations. Perhaps these particular expressions of these traits have become fixed in the populations' genome.

Our results did support previous knowledge that the amount of nutritional resources available in the environment has significant effects on the body size of the female adult mosquitoes (Merritt, Dadd and Walker 1992). When mosquito larvae are exposed to an insufficient amount of nutrients, plasticity expressed during development provide the larvae strategies to survive through quicker developmental times which result in smaller adult body size.

Variances in environmental temperature can also affect the body size of the adult mosquito (Clements 1992). It is difficult to discuss the effects of temperature on the development of immature mosquitoes, or any insect for that

matter, without acknowledging Bergmann's rule (Bergmann 1847), that points out the size differences among organisms can be correlated to environmental temperatures, and discussing the temperature-size rule. Bergmann's rule recognizes the negative relationship between temperature and body size the temperature-size rule promotes the idea that warmer, hotter temperatures will produce smaller insect adults (Atkins 1994, Kingsolver and Huey 2008). Because environmental temperatures follow a gradient with cooler temperatures in the north and the warmer temperatures being farther south the body size of many insects have been noted as to follow a latitudinal cline as well. These environmental gradients have been often replicated in laboratory settings, inducing phenotypic plasticity among populations of insects. These artificially induced traits are often measured and shown to follow a latitudinal cline and the temperature-size rule is inferred into natural environments (Belk and Houston 2002).

When subjected to poor larval environments, such as those with increased temperatures, the body size of the adult female is affected with a reduced size. These smaller females often have shorter lifespans and are more susceptible to infection with arboviruses (Alto et al. 2005, Alto et al. 2008, Hawley 1985). Because smaller females have been shown to have a more frequent biting pattern during gonotrophic cycles, the increased susceptibility to infection improves their competence as pathogen vectors (Maciel-De-Freitas, Codeco and Lourenco-De-Oliveira 2007).

The absence of a significant isolation by distance indicates the presence of gene flow along the latitudinal gradient we examined. The presence of genetic diversity among a population allows for species survival in different climates. Our most geographically separated populations showed phenotypic trait differences when reared under same environmental conditions. This phenotypic difference when there was no induced plasticity present gave evidence of genetic differences between the populations. We have demonstrated the presence of differences in phenotypic expressions of traits that can only be explained by genetic differences between populations.

Female body size is a fitness measurement among mosquito species that has been correlated with survival, fecundity and disease vector competence (Haramis 1985, Hawley 1985, Nasci 1986). The difference in fitness exhibited by the same species undergoing the same environmental stress should be carefully taken into consideration when vector competence and capability are in question. We have given evidence of genetic differences that seem to have been acquired over long-term environmental adaptations. This finding then begs the question, when traits are permanent and no longer just enhancing survival in a new climate, is the species is ready to survive another level of range expansion? If there are genetic differences for body size and optimal fitness among populations, does this imply different ranges of size for less fit adult female mosquitoes?

The successful invasion of *Ae. albopictus* into differing climates has given us the opportunity to investigate the differences in a species' phenotypic trait expression while it is responding to changing environmental conditions. It seems genetic differentiation between geographically separated populations may enhance *Ae. albopictus'* effectiveness in becoming established in differing climates. With world travel and trade on the increase, the opportunity for container breeding mosquitoes to become dispersed has become a very real problem. Our populations of *Ae. albopictus* were collected from varying latitudes and had maintained genetic diversity from the wild during our experiments. Our research has established a genetic difference in latitudinally separated populations that cannot be explained by phenotypic plasticity. More research in the area of genetic differences between populations will add to the understanding of invasive species ecology and the success of pathogen transmission among vertebrate host species.

Table 12 Mean wing lengths and developmental times in response to altered temperature, larval density and food availability of Ohio, Florida and Georgia populations of *Aedes albopictus* (Skuse). Not all treatment combinations are represented because of larval death.

Sex	State	Food	Density	Temperature °C	N	Mean Wing Length (mm)	Mean Developmental Time (Days)
F	FL	High	1	20	4	3.06	11.75
F	GA	High	1	20	4	2.82	11.60
F	OH	High	1	20	3	3.46	12.30
F	FL	High	3	20	3	2.81	11.67
F	GA	High	3	20	3	2.71	12.67
F	OH	High	3	20	9	2.55	11.67
F	FL	Low	1	20	2	2.48	12.50
F	OH	Low	1	20	4	2.85	7.50
F	FL	Low	3	20	4	2.93	11.00
F	GA	Low	3	20	2	2.83	12.50
F	FL	High	1	25	3	2.77	8.67
F	GA	High	1	25	2	2.94	8.00
F	OH	High	1	25	5	2.96	7.40
F	FL	High	3	25	8	2.58	8.75
F	GA	High	3	25	4	2.35	7.75
F	OH	High	3	25	9	2.54	7.89
F	FL	Low	1	25	3	2.41	7.67
F	GA	Low	1	25	1	2.33	9.00
F	FL	Low	3	25	5	2.84	8.60
F	GA	Low	3	25	5	2.82	7.80
F	OH	Low	3	25	3	2.55	7.30
M	FL	High	1	20	3	2.87	11.33
M	GA	High	1	20	1	2.41	12.00
M	OH	High	1	20	5	2.75	11.80
M	FL	High	3	20	12	2.58	11.00
M	GA	High	3	20	6	2.59	11.50
M	OH	High	3	20	8	2.59	11.50
M	FL	Low	1	20	4	2.59	11.50
M	GA	Low	1	20	3	2.04	13.33
M	OH	Low	1	20	4	2.23	7.25
M	FL	Low	3	20	2	2.51	12.00
M	GA	Low	3	20	2	2.27	11.00
M	OH	Low	3	20	7	2.50	10.71
M	FL	High	1	25	4	2.47	9.00
M	GA	High	1	25	3	2.31	7.30
M	OH	High	1	25	3	2.66	7.00
M	FL	High	3	25	7	2.35	7.57
M	GA	High	3	25	8	2.16	7.35
M	OH	High	3	25	9	2.33	7.11
M	FL	Low	1	25	4	2.24	7.50
M	GA	Low	1	25	5	2.30	7.20
M	FL	Low	3	25	4	1.97	8.25
M	GA	Low	3	25	5	2.00	7.20
M	OH	Low	3	25	10	2.15	7.60

Table 13 Influences of environmental variables on the developmental time and wing lengths of OH, GA and FL populations of *Aedes albopictus* (Skuse). Data was log_{10} transformed for normality. Bold type indicates significant *p* values. Significant effects (α=0.05) from variable interactions were recognized and took precedent over single variable effects.

Source		Female Wing Length		Female Developmental Time		Male Wing Length		Male Developmental Time	
	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
st	2	1.68	0.1887	2.81	0.0629	1.0	0.3720	1.49	0.2310
temp	1	5.79	0.0171	551.23	<.0001	4.77	**0.0313**	311.71	**<.0001**
st*temp	2	0.35	0.7041	4.29	**0.0151**	0.32	0.7266	1.88	0.1585
dens	1	1.58	0.2106	1.24	0.2661	1.07	0.3038	1.15	0.2870
st*dens	2	2.72	0.0681	0.06	0.9438	0.06	0.9437	0.34	0.7112
temp*dens	1	0.66	0.4193	2.74	0.0994	1.01	0.3167	1.12	0.2919
st*temp*dens	2	0.71	0.4925	0.11	0.8974	0.38	0.6854	0.92	0.4022
food	1	6.77	0.0100	0.04	0.8430	5.69	**0.0190**	0.04	0.8339
st*food	2	0.01	0.9917	0.59	0.5563	0.10	0.9083	0.00	0.9977
temp*food	1	0.09	0.7626	0.55	0.4611	0.00	0.9886	0.08	0.7815
st*temp*food	2	0.90	0.4100	1.80	0.1679	0.31	0.7310	1.48	0.2338
dens*food	1	6.32	**0.0128**	0.07	0.7889	0.30	0.5826	0.12	0.7328
st*dens*food	2	0.76	0.4713	1.44	0.2383	0.11	0.8956	1.68	0.1911
temp*dens*food	1	0.30	0.5838	2.92	0.0894	0.17	0.6807	1.47	0.2290
st*temp*dens*food	2	0.02	0.9798	0.39	0.6794	0.04	0.9622	0.05	0.9528

Table 14 Comparison of mean wing lengths resulting from the interaction between larval density and nutrient amount treatment effects. Tukey-Kramer HSD was carried out to look for the wing length treatment means that were significantly different from each other. Larval densities were 1 or 3/ food treatments were high (H) = 6 mg ground Tetramin or low (L) = 3 mg ground Tetramin. Significant p values are in bold type.

Mean Wing Lengths	Treatment Density/Food level	1/H	1/L	3/H	3/L
1.55	1/H		**0.0066**	**0.0213**	**0.0473**
2.34	1/L			0.7506	0.842
2.44	3/H				0.9999
2.44	3/L				

Table 15 Comparison of mean developmental times resulting from interaction between state and temperature treatment effects. Tukey-Kramer HSD was carried out to look for the developmental time treatment means that were significantly different from each other. Significant p values are in bold type.

Mean Developmental Days	Treatments	FL20	FL25	GA20	GA25	OH20	OH25
11.43	FL20		**<.0001**	0.6617	**<.0001**	1	**<.0001**
8.07	FL25			**<.0001**	0.1376	**<.0001**	**0.0154**
12.02	GA20				**<.0001**	0.6052	**<.0001**
7.5	GA25					**<.0001**	0.9941
11.48	OH20						**<.0001**
7.41	OH25						

Table 16 Five markers for microsatellite loci were used for determining genetic differences among populations (Poretta et al, 2006). Primers were fluorescently tagged for analysis of fragment size annealing temperature (Ta), number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O)

Locus	GenBank Accession no.	SSR motif	Primer sequences (5′−3′)	Ta (°C)	Clone size (bp)	N_A	H_O	H_E
AealbA9	DQ366022	AC)4GCAT(AC)2TC(AC)8CCAA(AC)2 CG(AC)GT(AC)C(AC)AT(AC)	F: TGGGACAAGAGCTGAAGGAT R: CTCGTTCTCTACTCTCTCCGTT	52	152	9	0.83	0.84
AealbB51	DQ366023	(AC)3T(AC)2AA(AC)AAA(AC)3 AA(AC)AT(AC)2T(AC)2	F: TCCACGTGGTATAACTCTGA R: GTAGTTGTCCAATTAACATCG	50	141	4	0.35	0.37
AealbB52	DQ366024	(AC)A(AC)A(AC)2 … (AC)6 … (T)3G(T)5G(T)4GGG(AC)3	F: GGGTCTAGAAGTAATAGCGATG R: GCATTCTTTGCTTCTGTTTGC	50	173	3	0.22	0.24
AealbB6	DQ366026	(AC)1AT(AC)7 GC(AC)2GCAT(AC)6AG(AC)	F: ATGAGGTGACCCTTTTGTGC R: 6-FAM_AAATTTTATAGGGCCCTCGG	50	139	4	0.32	0.35
AealbF3	DQ366027	(AC)6AT(AC)3AAAA(GC)2	F: CTCGTGAGTACGTTCCGTGA R: AGGGAAACAAGGACTTCATCA	53	247	4	0.53	0.47

Table 17 Genetic summary information for all loci used in population analysis. Expected heterozygosity (H_E), Observed heterozygosity (H_O) and *p*-values for Hardy-Weinberg equilibrium (HWE). Inbreeding coefficient (F_{IS}) and Fixation index (F_{ST}) are shown for each locus, not for each state population. Significant values are presented in bold type.

Locus	Population	H_E	H_O	HWE	Locus	F_{is}	F_{st}
AealbB51	FL	6.1429	5	0.3840	AealbB51	0.023	0.068
	OH	14.3091	15	**0.0012**			
AealbB52	FL	5.5098	4	0.2762	AealbB52	0.007	0.035
	OH	8.5789	10	1.0000			
AealbF3	FL	8.9302	12	0.1432	AealbF3	-0.168	-0.002
	OH	7.4	7	1.0000			
AealbB6	FL	19.5849	20	**0.0563**	AealbB6	-0.088	0.038
	OH	20.9057	24	0.3659			
AealbA9	FL	21.5686	15	**0.0006**	AealbA9	0.202	0.106
	OH	19.661	18	**0.0007**			
Mean	FL			**0.0012**			
	OH			**0.0008**			

Table 18 Exact G test for each *Aedes albopictus* (Skuse) population pair at each microsatellite locus. All but two loci showed significant differentiation between populations. In total, there were significant genetic differences between populations. Significant P values in bold type. Significant genetic differences were calculated using Fisher's method.

Locus	Population pair	P-Value	S.E.
AealbB51	OHM and FLM	**0.02928**	0.00238
AealbB52	OHM and FLM	**0.01722**	0.00105
AealbF3	OHM and FLM	0.41916	0.00452
AealbB6	OHM and FLM	0.15988	0.0053
AealbA9	OHM and FLM	**0.00011**	0.00008
Across all Loci	OHM and FLM	**0.000027**	

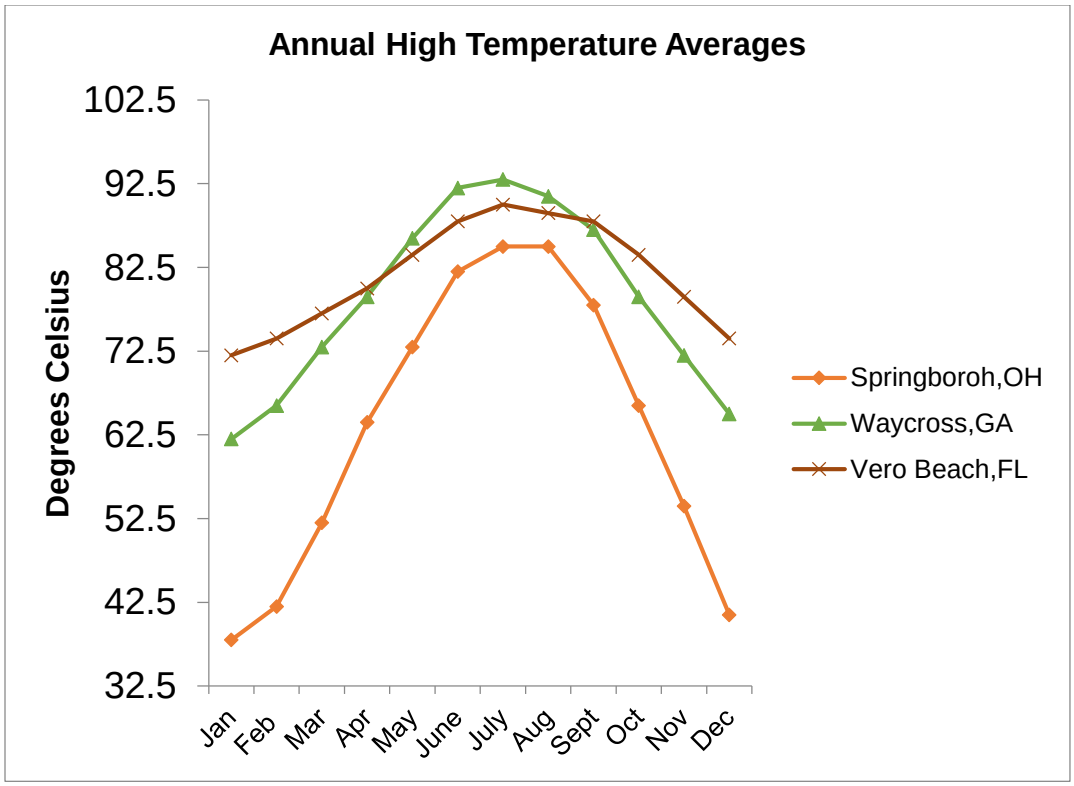

Figure 22 Annual average high temperatures for Springboro, OH, Waycross, GA and Vero Beach, FL. Temperature data collected from Weather Channel at http://www.weatherchannel.com. Temperature averages are continually collected, so values do not represent one specific year.

Chapter 4

Comparison of progeny body size when two geographically differing populations of *Aedes albopictus* (Skuse) are crossed

Introduction

Ae. albopictus has experienced a rapid range expansion during the last 30 to 40 years, becoming established on every continent but Antarctica (Benedict et al. 2007, Caminade et al. 2012). This invasive species, also known as the Asian Tiger Mosquito, has become established in the United States since 1985 (Hawley et al. 1987). Because this species is a vector of pathogens such as Dengue Fever and Yellow Fever (Bonizzoni et al. 2013, Paupy 2009) much attention has been drawn to the ecology and vector competency of this mosquito.

The plasticity of the mosquito genome allows it to express different traits in response to changing environmental characteristics, whether biotic or abiotic. Phenotypic plasticity has been well documented for mosquitoes when they are exposed to changing environmental conditions (Haramis 1985, Sibly and Atkinson 1994). These expressions can affect the insect's morphology, behavior and physiology either independently or in any combination, as the expression of these variable traits are the organism's strategy for coping with the environment. When these traits are contributing to the organism's fitness in the environment,

and are enhanced in a particular environmental condition, we can call them adaptive (Newman 1992).

Because *Ae. albopictus* has become adapted to differing climates within the U.S., we hypothesized that geographically separated populations of this species of mosquito would exhibit significantly different body sizes when subjected to the same environmental conditions. Using two populations of *Ae. albopictus*, each from a latitudinally differing location, we examined the effects of environmental temperature differences and location of population origin on body size.

Methods

Mosquito populations used to test difference of phenotypic traits were collected during the summer of 2011 from Springboro, Ohio (9.5639° N, 84.2281° W) and Waycross, Georgia (31.2133°N, 82.3542° W). Adult mosquitoes were collected through human landing catches in Springboro, OH, while egg papers were collected from the field in Waycross, GA. Adult *Ae. albopictus* from OH were given weekly access to blood for egg production. The egg papers were then collected and dried for approximately three days prior to flooding with deionized water. Upon hatching these egg papers produced the field generation from OH. Egg papers collected from GA and eggs collected from the OH population were hatched independently and mosquito larvae from each state were reared in deionized water with ground Tetramin© (Blacksburg, VA) fish food ad libitum.

Upon pupation adults from each of the states were placed into separate cages to establish field generation adult populations to be used for egg collection.

All populations of field generation adults were maintained under insectary conditions of 23.3°C ± 1 and relative humidity of 79.4% ± 6 for multiple generations, allowing the colonies an opportunity to grow in number, as well as allowing possible residual phenotypic effects from differing environmental conditions to be expressed. Populations were provided cotton balls soaked in a 10% sucrose solution daily and given weekly access to blood for the collection of egg papers.

The second generation of OH *Ae. albopictus* and the third generation of GA *Ae. albopictus* were utilized as parental generations in cross breeding experiments. Parent mosquitoes were reared using the temperature of larval environment as an influence on adult body size. OH F2 generation eggs and GA F3 generation eggs were hatched in deionized water and populations of these larvae were reared independently in 25° C and 15°C environments using two environmental chambers. Larvae were provided a slurry of ground Tetramin© (Blacksburg, VA) fish food as needed. Upon reaching the pupal stage, these mosquitoes were transferred into containers partially filled with de-ionized water and allowed to eclose in separate cages. Eclosed adults were collected daily (< 24 hours old), sorted by sex, and put into one of eight cages. Cages were numbered 1-8, with each number representing a breeding pair of mosquitoes. Pairs were designed to ensure the pairing of all possible combinations of

temperature and state of origin (Table 19). Because the developmental time necessary for larvae to mature in the 15° C temperature is drastically different from developmental time required in a 25° C larval environment, varying numbers of adults were available for breeding purposes.

Individuals used to form breeding pairs were collected and provided cotton balls soaked in 10% sucrose solution. After collection of both sexes from each state, breeding pairs were arranged and maintained in cages for two to three days allowing time for copulation. Two blood meals were provided to each cage using membrane feeders filled with defibrinated sheep blood obtained from ©2013 Hemostat Laboratories. Oviposition cups lined with paper for egg collection were added to each cage three days after blood feeding. Egg papers were allowed to dry and then flooded with de-oxygenated water for hatching to be initiated. Eggs began hatching within 24 hours of being flooded.

After hatching began, larvae and egg papers were placed in 8x8 inch pans with approximately one liter of de-ionized water. The larvae were fed ground Tetramin ad libitum. Because none of the egg papers resulting from genetic crosses held large numbers of eggs, the larval pans used for rearing provided more than adequate amounts of space and plenty of nutritional resources for the larvae. This prevented negative effects on larval development and adult fitness that may arise from high larval density and low nutrient resource availability. The larvae were reared in an environmental chamber maintained at 20° C until pupation. Pupae were removed and adults were collected within 24 hours of

eclosion. Adults were then killed by freezing and stored in individual 1.5 ml tubes for further analysis. Adults from the parent generation were also killed by freezing and stored for further analysis. Wing length measurements from the axillary incision of the wing to the apical tip of the wing (excluding wing fringe) were taken on each mosquito.

Statistical Analysis

Parents

Due to wear and tear of daily existence in a cage, wing length measurements for parents were difficult to acquire. Measuring wings from the acriminal notch to the apical tip, excluding fringe, is a commonly used method of collecting wing length data (Nasci 1986). A very large portion of parent wing tips were damaged so that accurate measurements were impossible to make. Because the parental adults had unlimited access to a sucrose solution throughout the experiment, there was no method available to obtain the original weight at eclosion. Although the sample size of useable parent wing measurements was too small for meaningful analysis, wing measurements were taken whenever possible for anecdotal reference. Mean wing lengths were calculated from the measurements obtained in the parental groups of mosquitoes. Groups were separated by gender, state and rearing temperature. These mean measurements are for observation purposes only since sample numbers were very small for some state/temperature groups (Table 20).

Offspring

Wing measurements were taken from the axillary incision to the apical tip of the wing, excluding wing fringe, were made for each mosquito. Wing tip data were found to have normal distribution and analysis of variance ($\alpha \leq 0.05$) was carried out for each sex of mosquito independently as these insects are sexually dimorphic in size. When a significant effect of treatment was determined to exist on the wing lengths of offspring, a protected a Tukey-Kramer HSD comparison of means was performed to look for specific differences between each of the cross groups. This analysis looked for any significant treatment effect of breeding pair combination on groups of offspring.

Results

Specific breeding pairs did have a significant influence over the body-sizes for male and female F_1 offspring with $p = 0001$ for both sexes. Tukey-Kramer HSD analysis was carried out for each sex independently. There were significant difference between wing lengths of the female offspring from MGA15XPGA15 (wing length 2.47 mm) and MOH15XPOH15 (wing length 2.87 mm) offspring ($p = 0.0001$). With rearing temperature being the same, the state of parental origin may be the variable influencing the wing length differences between the females of these offspring groups. The male offspring from the MGA15 X PGA15 and MOH15 X POH15 crosses did not show a significant difference in body size ($p = 0.7784$). For both males and females, there were some significant differences in body sizes between multiple breeding pairs (Table 21 A and B).

Discussion

It is well known that female mosquito body size has a direct relationship with fitness which correlates with fecundity among other life history traits such as longevity and disease transmission (Alto, Reiskind and Lounibos 2008, Nasci 1986, Nasci and Mitchell 1994, Oliver and Howard 2011, Paulson and Hawley 1991). For female mosquitoes, larger body size indicates longer survivorship which in turn would give time for the completion of multiple gonotrophic cycles thus increasing the amount of progeny entering the environment. This study uncovered some body-size differences among offspring resulting from breeding parents from two geographically separated populations, OH and GA, of *Ae. albopictus*. We were looking for differences in body size that could be attributed to genetic differentiation between the OH and GA mosquito populations and not phenotypic plasticity alone. Several breeding pairs produced groups of female offspring that were significantly different in size from one another when the parental pairs crossed were only different in state of origin.

Ae. albopictus is an invasive mosquito that has successfully expanded its range world-wide. Our results indicate some genetic differences between these populations, and more research into these differences would be beneficial to the body of knowledge concerning female mosquito fitness and invasive species ecology.

Table 19 Description of rearing temperatures and quantities of *Aedes albopictus* (Skuse) adults used in breeding crosses. Number of females and males used in breeding pairs varied due to the differences in developmental periods required for differing environmental temperatures. The adults reared in 15° C temperature took much longer to eclose than did the adults reared in a 25° environment.

Cross Number	Maternal State	Rearing Temp °C	Qty	Paternal State	Rearing Temp°C	Qty
1	OH	25	14	GA	15	20
2	OH	15	20	GA	25	20
3	OH	15	12	OH	15	14
4	GA	15	8	GA	15	20
5	OH	25	20	OH	25	20
6	GA	25	20	GA	25	20
7	GA	15	9	OH	25	9
8	GA	25	20	OH	15	20

Table 20 Tukey-Kramer HSD cross offspring means separation of Ae.albopictus (A) female and (B) male wing lengths. Comparisons were carried out to look for treatment means that were significantly different from each other. P-values in bold type indicate significant differences between cross groupings.

(A)

Cross Pairings	MOH25 X PGA15	MOH15 X PGA25	MOH15 X POH15	MGA15 X PGA15	MOH25 X POH25	MGA25 X PGA25	MGA15 X POH25	MGA25 X POH15
(Female Mean Wing Length) (mm)	(3.29)	(3.27)	(2.87)	(2.47)	(2.92)	(3.23)	(3.07)	(3.27)
MOH25xPGA15 (3.29)		1	0.0713	**<.0001**	0.522	1	0.9934	1
MOH15xPGA25 (3.27)			0.1487	**<.0001**	0.6314	1	0.9965	1
MOH15xPOH15 (2.87)				**0.0001**	1	0.6676	0.9918	0.2304
MGA15xPGA15 (2.47)					**0.0071**	**0.0007**	**0.0517**	**<.0001**
MOH25xPOH25 (2.92)						0.9062	0.9994	0.6852
MGA25xPGA25 (3.23)							0.9996	1
MGA15xPOH25 (3.07)								0.9967
MGA25xPOH15 (3.27)								

(B)

Cross Pairings	MOH25 X PGA15	MOH15 X PGA25	MOH15 X POH15	MGA15 X PGA15	MOH25 X POH25	MGA25 X PGA25	MGA15 X POH25	MGA25 X POH15
(Male Mean Wing Length) (mm)	(2.66)	(2.62)	(2.38)	(2.31)	(2.52)	(2.55)	(2.58)	(2.69)
MOH25xPGA15 (2.66)		1	0.0487	**0.0025**	0.9398	0.9843	0.9995	1
MOH15xPGA25 (2.62)			**0.0376**	**0.0012**	0.9794	0.9979	1	1
MOH15xPOH15 (2.38)				0.7784	0.9497	0.5141	0.7767	0.2018
MGA15xPGA15 (2.31)					0.4896	0.0707	0.3008	**0.0253**
MOH25xPOH25 (2.52)						0.9999	0.9998	0.9642
MGA25xPGA25 (2.55)							1	0.9925
MGA15xPOH25 (2.58)								0.9997
MGA25xPOH15 (2.69)								

Chapter 5

Phenotypic effects of inter/intraspecific competition between *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) from differing latitudes

Introduction

The invasive species *Ae. albopictus* has been found to be a better larval competitor for resources in some studies (Ho, Ewert and Chew 1989, Juliano, Lounibos and O'Meara 2004, Lounibos 2001, Novak 1993), while others have indicated that there is no competitive advantage held by this species (Black et al. 1989). Competition within larval communities has been shown to have significant effects on the life history traits of the adults reared in these environments. Traits such as survivorship, developmental time and disease vector success are affected by larval competition in the environment. Competition may decrease developmental time required to reach adulthood providing the adult a type of escape strategy from a stressful environment. It may decrease adult survival as these adults are typically smaller and less fit, thus increasing success in disease transmission (Agnew, Haussy and Michalakis 2000, Armistead et al. 2008, Bevins 2008).

The recent invasion of the mosquito *Ae. albopictus* has provided an opportunity to examine the effects of competition on *Ae. albopictus* populations from geographically differing origins, specifically from differing climates. This invasive

species, also known as the Asian Tiger mosquito, as it is native to Southeast Asia and has become established in the United States since its arrival in 1985 (Hawley et al. 1987).

Methods

Ae. aegypti larvae from a long established lab colony competed for resources with *Ae. albopictus* collected from three different geographic locations in small microcosms. Mosquito populations used in these studies were collected during the summer of 2011 from three locations that are approximately equidistant apart: Springboro, Ohio (39.5639° N, 84.2281° W) , White Pine, Tennessee (36.1075° N, 83.2869° W) and Waycross, Georgia (31.2133°N, 82.3542° W). All populations of field generation *Ae. albopictus* adults were maintained under insectary conditions of 23.3°C ± 1 and relative humidity of 79.4% ± 6 for multiple generations, allowing all colonies the opportunity to grow numerically, as well as allowing possible residual phenotypic effects from differing environmental conditions to be expressed. Populations were provided cotton balls soaked in a 10% sucrose solution daily and given weekly access to human blood for the collection of eggs on paper towels.

The *Ae. aegypti* lab colony was maintained under the same insectary conditions, however blood meals were provided using a membrane feeders filled with defibrinated sheep blood obtained from ©2013 Hemostat Laboratories.

First instar (< 24 hours old) larvae from F_3 generations of OH and TN and the F_4 generation from GA *Ae. albopictus* populations were used. The larval density treatments were: 1 *Ae. aegypti* larva (A1), 10 *Ae. aegypti* larvae (A10), 5 *Ae.*

albopictus larvae from each individual state/5 *Ae. aegypti* larvae (G5, O5 or T5/A5), 10 *Ae. albopictus* larvae (G10, O10 or T10) and 1 *Ae. albopictus* larva (G1, O1 or T1). Densities were set at 10 larvae per treatment in attempts to maintain the same density effects among treatments. Each of the *Ae. albopictus* populations (OH, GA, and TN) were used separately and had four replicates of each treatment. Each microcosm containing one larva was filled with 17ml deionized water and 6mg Tetramin® (Blacksburg, VA) fish food. The microcosms containing 10 larvae were filled with 20ml DI and 3mg/larva ground Tetramin® (Blacksburg, VA). Differences in the amount of nutrient allowed for competition effects to be more evident.

Screw-on lids were applied to each jar and microcosms were randomly placed on a shelf in a 25°C environmental chamber and observed daily. Pupae were removed from microcosms and each was placed in a *Drosophila* vial half filled with de-ionized water and maintained in the 25°C environment until eclosion. Eclosed adults were collected daily and frozen for wing length measurement. Wing lengths are commonly used as a reliable measure of body size for mosquitoes. These measurements were taken for each sample of both species.

Data analysis

Statistical analysis was carried out on each sex independently for both species, as mosquitoes are sexually dimorphic by nature. Data were $\log_{10}$ transformed to meet the assumption of normality, and ANOVA ($\alpha = 0.05$) was carried out to determine the treatment effect on the variables wing length and number of days until pupation and number of days from pupation until eclosion for the

mosquitoes. Treatment combinations of geographically separated populations used in the intra- and interspecific competition study were the independent variables in this experiment. When a significant effect of treatment was determined to exist on the variable, a least squares means comparison was performed to determine the specific differences between treatments. A Tukey adjustment for multiple comparisons was used.

Results

Total numbers of adults collected did not equal the total numbers of larvae used in the experiment. Many of the individual treatments either did not produce a large enough sample set for one sex or both sexes to run solid statistical analyses. This lack of data does affect the results since sexes are analyzed separately; therefore, in some data sets the individual larval treatment is not present.

Aedes aegypti

There were no significant influences of treatment, whether single individual, multiple conspecifics or mixed species, on the wing length ($p = 0.8368$) of *Ae. aegypti* females. Treatments did have a significant effect on the number of days required from hatch to pupation ($p = 0.0013$) for these females as they developed faster when in a mixed species treatment than when reared with conspecifics. The *Ae. aegypti* males did not show any treatment effect for wing length ($p = 0.6229$), they did however exhibit significant influence from treatments on number of developmental days from hatch to pupation ($p < 0.0001$). (Table 22)

The females from the conspecific group of 10 *Ae. aegypti* pupated significantly later than the female *Ae. aegypti* in the mixed species treatments with *Ae. albopictus* from OH and TN. Although the *Ae. aegypti* females in the 5 GA *Ae. albopictus*/ 5 *Ae. aegypti* treatments were not significantly quicker in developing into pupae, the number of days were still visibly less than the number required for the conspecific 10 *Ae. aegypti* treatment (Table 23). Tukey-Kramer HSD comparisons of the treatment effect on developmental days to pupation also showed *Ae. aegypti* males pupating faster when in the presence of another species than when in a same species group (Table 23).

Aedes albopictus

Ae. albopictus males showed no effects from the varying treatments on wing length ($p > 0.38$) or developmental time from hatch to pupation ($p > 0.2766$).

Females displayed significant treatment effects on number of days from hatch to pupation ($p > 0.0003$), and approached significance on wing length ($p > 0.0992$) (Table 24). Paired comparisons were carried out with Tukey-Kramer HSD analysis for developmental time to pupation for female *Ae. albopictus* with significant differences being detected between several treatments (Table 25). The number of days necessary for the females in conspecific groups to pupate was less than the number of days required for members of the multispecies treatments. The treatments consisting of conspecific groups from differing state populations of *Ae. albopictus* did not show significant differences in development time between each other, however when each population was in a multispecies treatment there were

reportable differences. GA populations took longer to develop into pupae than the OH *Ae. albopictus* when both were in mixed species groups.

Discussion

Environmental characteristics are well known to influence life history traits in adult mosquitoes. Larval environments of container breeding mosquitoes are especially sensitive to biotic and abiotic changes, as typically these habitats are small and under threat of water loss, nutrient restriction and overcrowding of the aquatic community. Accelerated developmental times and smaller body size are two ways of coping with poor environmental conditions (Agnew, Haussy and Michalakis 2000, Alto et al. 2005, Haramis 1985).

In this study, *Ae. aegypti* males and females took longer to develop from hatch to pupation when sharing an environment with their conspecifics. In an intraspecific environment, the males required an average of 8.08 days to pupate and the females required an average of 9.33 days. These times are significantly longer than when they were experiencing interspecific competition with *Ae. albopictus*. *Ae. albopictus* populations actually developed faster in the presence of their conspecifics than when in competition with *Ae. aegypti*. Interestingly, the state of origin may have an effect on the *Ae. albopictus* developmental rate when sharing an environment with another species. The GA female population took significantly longer to develop than the OH female population and very close to significantly longer than the TN females.

The ability to develop faster when an environment has unsuitable conditions allows for one species to escape competition with the second species. In this case, the *Ae. aegypti* is pupating quickly and typically leaving the environment with a smaller wing length and less energy stores. Many studies have shown *Ae. albopictus* to outcompete *Ae. aegypti* as would be expected given the range expansion that *Ae. albopictus* has undergone recently. Both species being container breeding species, the invasive Asian Tiger mosquito has easily displaced many native populations because of the small size and risk of insufficient food resources that are inherent to container habitats.

These geographically separated populations did express plasticity in time required for larval development. In mosquitoes, shortened developmental time usually predicts a smaller less fit adult body size. Body size is a very plastic trait that reflects changes in nutrient amount (Fish and Carpenter 1982, Grimstad and Haramis 1984, Nasci and Mitchell 1994) and nutrient amount in the environment has an inverse relationship with larval density. More competition can lead to smaller adults, which has a relationship with increased vector competence and adult longevity (Alto, Reiskind and Lounibos 2008, Hawley 1985, Nasci 1986). It is also possible that *Ae. albopictus* employed a strategy that allows them to develop more slowly than the competitor in the presence of the competition to reap the benefit of larval nutrient build up (Tsurim et al. 2013).

Either way, the differences in developmental time exhibited by these geographically separated populations seem to imply genetic differences between

groups. The ability of the *Ae. albopictus* to adapt to differing climates and for these adaptations to occur in relatively few generations could have serious implications for mosquito control efforts. If the response to changing environmental temperature is unique to each latitude, then female fitness measurements may be more regionally dependent than previously thought when considering the role it plays in longevity and disease transmission. This study reinforces the importance of adding to the body of knowledge of mosquito ecology, and more investigation into phenotypic plasticity among mosquito populations.

Table 21 Results from significance testing on transformed data for *Aedes aegypti* (Linnaeus) from inter and intraspecific competition. Data were log $_{10}$ transformed to ensure normal distribution before statistical analysis. Treatments showed significant effects on female and male development time. Significant *p* values in bold type.

Dependent Variable	Means	Source of Variation	DF	F Value	Pr>F
Aedes aegypti Female					
Wing Length	2.92 mm	trt	4	0.36	0.8368
Developmental time from larval hatch to pupation	7.08 days	trt	4	6.3	**0.0013**
Aedes aegypti Male					
Wing Length	2.57 mm	trt	4	0.66	0.6229
Developmental time from hatch to pupation	6.92 days	trt	4	9.12	**<.0001**

Table 22 Tukey-Kramer HSD treatment means separation of *Aedes aegypti* developmental times. Data were log_{10} transformation to ensure normal distribution. Means separation was carried out to determine which treatments were significantly different from each other. Results are for the *Aedes aegypti* (Linnaeus) only present in the multispecies treatments. Key for treatments in data table: A1= one *Ae. aegypti* larva, A10= ten *Ae. aegypti* larvae, G5A5= mixed species treatment of 5 Georgia *Ae. albopictus* and 5 *Ae. aegypti*, O5A5= mixed species treatment of 5 Ohio *Ae. albopictus* larvae and 5 *Ae. aegypti* larvae, T5A5= mixed species treatment of 5 Tennessee *Ae. albopictus larvae* and 5 *Ae. aegypti larvae*

(A) *Aedes aegypti* Female

N	Mean Developmental Time in Days	Treatment	A1	A10	G5A5*	O5A5*	T5A5*
1	7	A1		0.4928	1	0.9996	0.9348
6	9.33	A10			0.1979	**0.0056**	**0.0005**
2	7	G5A5*				0.9997	0.8711
11	6.82	O5A5*					0.6702
9	6.22	T5A5*					

(B) *Aedes aegypti* Male

N	Mean Developmental Time in Days	Treatment	A1	A10	G5A5*	O5A5*	T5A5*
3	7	A1		0.282	0.9999	0.8871	**0.4511**
12	8.08	A10			**0.0127**	**0.011**	**<.0001**
12	7	G5A5*				0.8296	0.1269
4	6.5	O5A5*					0.961
12	6.25	T5A5*					

Table 23 Results from significance testing on transformed data for *Aedes albopictus* (Skuse). Data were log_{10} transformed for normal distribution before statistical analysis. Treatments had significant effects on female developmental time. Significant p values in bold type; none of the treatments significantly affected the males in any of the measured traits.

Dependent Variable	Means	Source of Variation	DF	F Value	Pr >F
Aedes albopictus Female					
Wing length	2.95 mm	trt	6	1.93	0.0992
Developmental time from hatch to pupation	7.59 days	trt	6	5.5	**0.0003**
Aedes albopictus Male					
Wing length	2.45 mm	trt	8	1.09	0.38
Developmental time from hatch to pupation	6.46 days	trt	8	1.27	0.2766

Table 24 Tukey-Kramer HSD treatment means separation for female *Aedes albopictus* (Skuse) developmental times. Data log_{10} transformed to ensure normal distribution. Mean developmental time in days not transformed. * Result is for *Aedes albopictus* (Skuse) only in mixed species treatments. Key for treatments in data table: G1= one Georgia *Ae. albopictus* larva, G10= ten Georgia *Ae. albopictus* larvae, G5A5= mixed species treatment of 5 Georgia *Ae. albopictus* and 5 *A. aegypti*, O10= ten Ohio *Ae. albopictus* larvae, O5A5= mixed species treatment of 5 Ohio *Ae. albopictus* larvae and 5 *A. aegypti* larvae, T10= 10 Tennessee *Ae. albopictus* larvae

N	Mean Developmental Time in Days	Treatment	G1	G10	G5A5*	O10	O5A5*	T10	T5A5*
2	10	G1		0.8317	0.943	0.2101	0.4046	0.337	0.7665
11	8	G10			0.018	0.4479	0.8634	0.7464	0.9991
5	13.4	G5A5*				**0.0002**	**0.0026**	**0.0006**	**0.0622**
10	6.5	O10					0.9995	0.9985	0.9787
6	6.83	O5A5*						1	0.999
11	6.82	T10							0.9986
3	7.33	T5A5*							

LITERATURE CITED

Agnew P., C. Haussy, and Y. Michalakis. 2000. Effects of density and larval competition on selected life history traits of *Culex pipiens quinquefaciatus* (Diptera:Culicidae). Journal of Medical Entomology 37: 732-35

Adebote D.A., J.S. Oniye, S.I. Ndams and K.M. Nache. 2006. The breeding of mosquitoes (Diptera: Culicidae) in peridomestic containers and implication in Yellow Fever transmission in villages around Zaria, northern Nigeria. Journal of Entomology 3: 180-88

Agnew P., M. Hide, C. Sidobre and Y. Michalakis. 2002. A minimalist approach to the effects of density-dependent competition on insect life-history traits. Ecological Entomology 27: 396-402

Alto B.W., L.P. Lounibos, S. Higgs, S.A. Juliano. 2005. Larval competition differentially affects arbovirus infection in Aedes mosquitoes. Ecology 86: 3279-88

Alto B.W., M.H. Reiskind, L.P. Lounibos. 2008. Size alters susceptibility of vectors to Dengue virus infection and dissemination. American Journal of Tropical Medicine and Hygiene 79: 688-95

Anderson J.F., T.G. Andreadis, A.J. Main, D.L. Kline. 2004. Prevalence of West Nile virus in tree canopy-inhabiting *Culex pipiens* and associated mosquitoes. American Journal of Tropical Medicine and Hygiene 21: 112-19

Andreson J.F., T.G. Anderadis, C.R. Vossbrinck, S. Tirrell, E.M. Wakem, R.A. French, A.E. Garmendia, H.J. Van Kruiningin. 1999. Isolation of West Nile virus from mosquitoes, crows, and a Cooper's hawk in Connecticut. Science 286: 2331-3

Apostol B.L., W.C. Black IV, P. Reiter, B.R. Miller. 1996. Population genetics with RAPD-PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. Heredity 76: 325-34

Armistead J.S., J.R. Arias, N. Nishimura, L.P. Lounibos. 2008. Interspecific larval competition between *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae) in Northern Virginia. Journal of Medical Entomology 45: 629-37

Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms. Advances in Ecological Research vol.25 Academic Press Ltd., London, pp.1-58.

Badvaev A. 2005. Stress-induced variation in evolution: from behavioral plasticity to genetic assimilation. Proceedings of the Royal Society B 272: 877-86

Bedhomme S., P. Agnew, Y. Vital, C. Sidobre, Y. Michalakis. 2005. Prevalence-dependent costs of parasite virulence. PLoS Biology 3: e262

Benedict M.Q., R.S. Levine, W.A. Hawley and L.P. Lounibos. 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonotic Disease 7: 76-85

Belk, M.C., D.D Houston. 2002. Bergmann's rule in ectotherms: a test using freshwater fishes. The American Naturalist. 160: 803 – 808.

Bergmann, C.1847. "Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse". *Göttinger Studien* **3** (1): 595–708

Bevins S. 2008. Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). Biological Invasions 10: 1109-17

Black W.C., K.S. Rai, B.J. Turco, D.C. Arroyo. 1989. Laboratory study of competition between United States strains of *Aedes albopictus* and *Aedes aegypti* (Diptera, Culicidae). Journal of Medical Entomology 26: 260-71

Bonizzoni M., G. Gasperi, X. Chen, A.A. James. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends in Parasitology 29: 460-68

Borah J., P. Dutta, S.A. Khan, J. Mahanta. 2011. A comparison of clinical features of Japanese encephalitis virus infection in the adult and pediatric age group with Acute Encephalitis Syndrome. Journal of Clinical Virology 52: 45-49

Bosak P.J., W.J. Crans. 2002. The structure and function of the larval siphon and spiracular apparatus of *Coquillettidia perturbans*. Journal of the American Mosquito Control Association, 18 (4):280-283

Bradshaw W.E., C.M. Holzapfel. 2006. Evolutionary response to rapid climate change. Science 312: 1477-78

Bradshaw W.E., C.M. Holzapfel. 2001. Genetic shift in photoperiodic response correlated with global warming. Proceedings of the National Academy of Sciences 98: 14509-11

Brown J.E., V. Obas, V. Morley, J.R. Powell. 2013. Phylogeography and spatio-temporal genetic variation of *Aedes aegypti* (Diptera: Culicidae) populations in the Florida Keys. Journal of Medical Entomology 50: 294-99

Calisher C.H., N. Karabasos. 1988. Arbovirus serogroups: definition and geographic distribution. In: Monath, T.P. (ed) The arboviruses: epidemiology and ecology. Vol I. Boca Raton, Florida: CRC Press. 19-57 pp.

Caminade C., J.M. Medlock, E. Ducheyne, K.M. McIntyre, S. Leach, M. Baylis, A.P. Morse. 2012. Suitability of European climate for the Asian tiger mosquito *Aedes albopictus*: Recent trends and future scenarios. The Journal of the Royal Society Interface 9: 2708-17

Cerný O., J.Votýpka, M. Svobodová. 2011. Spatial feeding preferences of ornithophilic mosquitoes, blackflies and biting midges. Medical and Veterinary Entomology 25: 104-08

Chandler J.A., J. Parsons, P.F.L. Boreham, G.S. Gill. 1977. Seasonal variations in the proportions of mosquitoes feeding on mammals and birds at a heronry in western Kenya. Journal of Medical Entomology 14: 233-40

Chaves-Carballo E. 2005. Carlos Finlay and Yellow Fever: triumph over adversity. Military Medicine 170: 881-85

Clarke K.R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117-43

Cleaveland S., M.K. Laurenson, L.H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society London B 356(1411):991-99

Clements A.N. 1992. The biology of mosquitoes, development, nutrition and reproduction. Chapman and Hall, London.

Clements A.N. 1999. The biology of mosquitoes volume II: sensory, reception and behaviour. CABI Publishing, Wallingford.

Conover W.J., R.L. Iman. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. The American Statistician 35: 124-29

Crans W.J. 1963. Continued host preference studies with New Jersey mosquitoes, 1963. Proceedings of the Fifty-first Annual Meeting New Jersey Mosquito Extermination Association

Darbro J.M., L.C. Harrington. 2006. Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. Journal of Medical Entomology 43: 83-92

Darsie R.F., R. A. Ward. 2004. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville.

Day J.F., J.D. Edman. 1984. Mosquito engorgement on normally defensive hosts depends on host activity patterns. Journal of Medical Entomology 21: 732-40

Deitz K.C., G. Athrey, M.R. Reddy, H.J. Overgaard, A. Matias, M. Jawara, A. Della Torre, V. Petrarca, J. Pinto, A.E. Kiszewski, P. Kengne, C. Constanini, A. Caccone, M.A. Slotman. 2012. Genetic isolation within the malaria mosquito *Anopheles melas*. Molecular Ecology 21: 4498-513

De Jong R., B.G.J. Knols. 1995. Selection of biting sites on man by two malaria mosquito species. Cellular and Molecular Life Sciences 51:80–84

Dia I., H. Ba, S. Mohamed, D. Diallo, B. Lo, M. Diallo. 2009. Distribution, host preference and infection rates of malaria vectors in Mauritania. Parasites and Vectors 2: 61

Dufrene M., P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67: 345-66

Edman J.D., H.W. Kale. 1971. Host Behavior: its influence on the feeding success of mosquitoes. Annals of the Entomological Society of America 64: 513-16

Edman J.D., L.A. Webber. 1974. Effect of vertebrate size and density on host-selection by caged *Culex nigripalpus*. Mosquito News 35: 508-12

Edmund J.D., L.A. Webber, H.W. Kale. 1972. Host- feeding patterns of Florida mosquitoes. II. Culiseta. Journal of Medical Entomology 9: 429-34

Edwards S., R. Massey. 2011. The impact of livestock production on local economies: summary of literature. MP752. University of Missouri Extension

Ejiri H., Y. Sato, K-S. Kim, T. Hara, Y. Tsuda, K. Murata, K. Saito, Y. Watanabe,Y. Shimura, M. Yukawa. 2011. Entomological study on transmission of avian malaria parasites in a zoological garden in Japan: blood meal identification and detection of avian malaria parasite DNA from blood-fed mosquitoes. Journal of Medical Entomology 48: 600-07

Fish D., S.R. Carpenter. 1982. Leaf litter and larval mosquito dynamics in tree-hole ecosystems. Ecology 63: 283-88

Gillies, M.T. 1964. Selection for host preference in *Anopheles gambiae*. Nature 203:852-854

Gouagna L.-C., R.S. Poueme,K. R, Dabiré, J.-B. Ouédraogo, D. Fontenille, F. Simard. 2010. Patterns of sugar feeding and host plant preferences in adult males of *Anopheles gambiae* (Diptera: Culicidae). Journal of Vector Ecology 35: 267-76

Grimstad P.R., E.D. Walker. 1991. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. Journal of Medical Entomology 28: 378-86

Grimstad P.R., L.D. Haramis. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus III. Enhanced oral transmission by nutrition-deprived mosquitoes. Journal of Medical Entomology 21: 249-56

Hankison S.J., M.B. Ptacek. 2008. Geographical variation of genetic and phenotypic traits in the Mexican sailfin mollies, *Poecilia velifera* and *P. petenensis*. Molecular Ecology 17: 2219-33

Haramis L.D. 1985. Larval nutrition, adult body size, and the biology of *Aedes triseriatus*. Ecology of mosquitoes; proceedings of a workshop: 431-37

Hawley W.A. 1985. The effect of larval density on adult longevity of a mosquito, *Aedes sierrensis*: epidemiological consequences. Journal of Animal Ecology 54: 955-64

Hawley W.A., P. Reiter, R.S. Copeland, C.B. Pumpuni, G.B. Craig. 1987. *Aedes albopictus* in North America: probable introduction in used tires from Northern Asia. Science 236: 1114-16

Hii, J.L.K., M. Chew, V.Y Sang, L.E Munstermann, S.G. Tan, S. Panyim, S. Yasothornsrikul.1991. Population genetic analysis of host seeking and resting behaviors in the malaria vector, *Anopheles balabacensis* (Diptera: Culicidae). Journal of Medical Entomology 28: 675-684.

Ho B.C., A. Ewert, L.M. Chew. 1989. Interspecific competition among *Aedes aegypti, Aedes albopictus*, and *Aedes triseritatus* (Diptera:Culicidae) larval development in mixed cultures. Journal of Medical Entomology 26: 615-23

Hoffmann A.A., M.J. Hercus. 2000. Environmental stress as an evolutionary force. BioScience 50: 217-26

Jost L. 2010. The relation between evenness and diversity. Diversity 2: 207-32

Juliano S.A., L.P. Lounibos, G.F. O'Meara. 2004. A field test for competitive effects of *Aedes albopictus* on *Aedes aegypti* in south Florida: differences between sites of coexistence and exclusion? Oecologia 139: 583-93

Kamgang B., C. Brengues, D. Fontenille, F. Njiokou, F. Simard, C. Paupy. 2011. Genetic structure of the tiger mosquito, *Aedes albopictus*, in Cameroon (Central Africa). PLoS ONE 6: e20257

Kawka M., J.O. Horbańczuk, M. Sacharczuk , G. Zieba, M. Lukaszewicz, K. Jaszczak , R Parada.2007. Genetic characteristics of the ostrich population using molecular methods. Poultry Science 86: 277-81

Kilpatrick A.M., L.D. Kramer, M.J. Jones, P.P. Marra and P. Daszak. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biology 4 (4): 82

Kingsolver, J.G. and R. B. Huey. 2008. Size, temperature and fitness: three rules. Evolutionary Ecology Research 10: 251-268.

Kirby M.J., S.W. Lindsay. 2009. Effect of temperature and inter-specific competition on the development and survival of *Anopheles gambiae* sensu stricto and *An. arabiensis* larvae. Acta Tropica 109: 118-23

Koella J.C., E.O. Lyimo. 1996. Variability in the relationship between weight and winglength of *Anopheles gambiae* (Diptera: Culicidae). Journal of Medical Entomology 33(2): 261-264

Koenraadt C., M. Kormaksson, L. Harrington. 2010. Effects of inbreeding and genetic modification on *Aedes aegypti* larval competition and adult energy reserves. Parasites and Vectors 3: 92

Kothera L., E.M. Zimmerman, C.M. Richards, H.M. Savage. 2009. Microsatellite characterization of subspecies and their hybrids in *Culex pipiens* complex (Diptera:Culicidae) mosquitoes along a north-south transect in the central united states. Journal of Medical Entomology 46: 236-48

Kruskal J.B. 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29: 1-26

Kruskal J. B., M. Wish 1978. Multidimensional Scaling. Sage Publications, Beverly Hills, California.

Laarman J.J. 1958. The host-seeking behavior of Anopheline mosquitoes. Tropical and Geographical Medicine 10: 293-305

Lefevre T., L.-C. Gouagna, K.R. Dabire, E. Elguero, D. Fontenille, F. Renaud, C. Costantini, F. Thomas. 2009. Beyond nature and nurture: phenotypic plasticity in blood-feeding behavior of *Anopheles gambiae* s.s. when humans are not readily accessible. American Journal of Tropical Medicine and Hygiene 81: 1023-29

Lounibos L.P., G.F. O'Meara, R.L. Escher, N. Nishimura, M. Cutwa, T. Nelson, R.E. Campos, S.A. Juliano. 2001. Testing predictions of displacement of native *Aedes* by the invasive asian tiger mosquito *Aedes albopictus* in Florida, USA. Biological Invasions 3: 151-66

Lucas E.A., W. S. Romoser. 2001. The energetic costs of diving in *Aedes aegypti* and *Aedes albopictus* pupae. Journal of American Mosquito Control 17: 56-60

Maciel-De-Freitas R., Codeço CT, Lourenço-De-Oliveira R . 2007. Body size-associated survival and dispersal rates of *Aedes aegypti* in Rio de Janeiro. Medical and Veterinary Entomology 21: 284-92

Manda H., L.C. Gouagna, E. Nyandat, E.W. Kabiru, R.R. Jackson, W.A. Foster, J.I. Githure, J.C. Beier, A.Hassanali.2007. Discriminative feeding behaviour of *Anopheles gambiae* s.s. on endemic plants in western Kenya. Medical and Veterinary Entomology 21: 103-11

Mather P.M. 1976.Computational methods of multivariate analysis in physical geography. Wiley, London.

Mc Cune,B., M.J. Mefford. 2011. PC-ORD. Multivariate analysis fo ecological data. Version 6. MjM Software, Gleneden Beach, Oregon, U.S.A.

Mc Iver S.B. 1982. Sensilla of Mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 19: 489-535

Meegan, J.M. 1979. The Rift Valley epizootic in Egypt. Transactions of the Royal Society of Tropical Medicine and Hygiene. 73(6): 618-23

Merritt R.W., R.H. Dadd, E.D. Walker. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annual Review of Entomology 37: 349-74

Miller B.R., M.S. Godsey, M. B. Crabtree, H.M. Savage, Y. Al-Mazrao, M.H. Al-Jeffri, A.M. Abdoon, S.M. Al-Seghayer, A.M. Al-Shahrani, T.G. Ksiazek. 2002. Isolation and genetic characterization of Rift Valley Fever virus from *Aedes vexans arabiensis*, Kingdom of Saudi Arabia. Emerging Infectious Diseases 8 (12):1492-4.

Missouri Department of Conservation. *Missouri's Fish, Forests and Wildlife*. Missouri Department of Conservation, Web. 26 Nov. 2013.

Missouri Department of Economic Development. 2008. Missouri Economic Impact Brief Agricultural Industries. Missouri Department of Economic Development. Web. 26 Nov. 2013

Muturi E. J., C.-H. Kim, B. W. Alto, M. R. Berenbaum, M. A. Schuler. 2011. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. Tropical Medicine and International Health 16: 955-64

Muturi E.J., C.-H. Kim, B.W. Alto, M.R. Berenbaum, M.A. Schuler. 2011. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. Tropical Medicine and International Health. 2011. 16(8):955-64

Mwandawiro C., M. Boots, N. Tuno, W. Suwonkerd, Y. Tsuda, M.Takagi. 2000. Heterogeneity in the host preference of Japanese encephalitis vectors in Chiang Mai, northern Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene. 94: 238-42

Nasci R.S. 1986. The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. Journal American Mosquito Control Association 2: 61-62

Nasci R.S., C.J. Mitchell. 1994. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. Journal of Medical Entomology 31: 123-26

Nasci R.S., J.D. Edman.1981. Blood-feeding patterns of *Culiseta melanura* (Diptera:Culicidae) and associated sylvan mosquitoes in southeastern Massachusetts eastern equine encephalitis enzootic foci. Journal of Medical Entomology 18: 493-500

Navarrete-Tindall N. 2010. Native Cool-Season Grasses in Missouri. Missouri Prairie Journal 31(2):20

Nelms B.M., E. Fechter-Leggett, B.D. Carroll, P. Macedo, S. Kluh, W.K. Reisen. 2013. Experimental and natural vertical transmission of West Nile virus by California *Culex* (Diptera: Culicidae) mosquitoes. Journal of Medical Entomology 50: 371-78

Newman R.A. 1992. Adaptive plasticity in amphibian metamorphosis. BioScience 42: 671-78

Novak M.G., L.G. Higley, C.A. Christianssen, W.A. Rowley. 1993. Evaluating larval competition between *Aedes albopictus* and *Aedes triseriatus* (Diptera Culicidae) through replacement series experiments. Environmental Entomology 22: 311-18

Nylin S., K. Gotthard. 1998. Plasticity in life-history traits. Annual Review of Entomology 43: 63-83

Olanga E.A., M.N. Okal, P.A. Mbadi, E.D. Kokwaro, W.R. Mukabana. 2010. Attraction of *Anopheles gambiae* to odour baits augmented with heat and moisture. Malaria Journal 9:6

Oliver J., J.J. Howard. 2011. Fecundity of wild-caught gravid *Culiseta morsitans* (Diptera: Culicidae). Journal of Medical Entomology 48: 196-201

Padmanabha, H., C.C. Lord, L.P. Lounibos. 2011. Temperature induces trade-offs between development and starvation resistance in *Aedes aegypti* (L.) larvae. Medical and Veterinary Entomology 25: 445-453

Paulson S.L., W.A. Hawley. 1991. Effect of body size on the vector competence of field and laboratory populations of *Aedes triseriatus* for La Crosse virus. Journal of American Mosquito Control Association. 7: 170-75

Paupy C., H. Delatte, C. Bagny, V. Corbel, D. Fontenille. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microbes and Infection 11: 1177-85

Porretta D., M. Gargani, R. Bellini, M. Calvitti, S. Urbanelli. 2006. Isolation of microsatellite markers in the tiger mosquito *Aedes albopictus* (Skuse). Molecular Ecology Notes 6: 880-81

Rasheed S.B., M. Boots, A.C. Frantz, R.K. Butlin. 2013. Population structure of the mosquito *Aedes aegypti* (*Stegomyia aegypti*) in Pakistan. Medical and Veterinary Entomology 27: 430-40

Raymond M., F. Rousset. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenism. Journal of Heredity 86: 248 - 49

Reisen W.K., Y. Fang, H.D. Lothrop, V.M. Martinez, J. Wilson, P. Oconnor, R .Carney, B. Cahoon-Young, M. Shafii, A.C. Brault. 2006. Overwintering of West Nile virus in Southern California. Journal of Medical Entomology 43: 344-55

Rezza G. 2012. *Aedes albopictus* and the reemergence of Dengue. BMC Public Health 12:72

Rodriguez-Prieto I., E. Fernandez-Juricic, J. Martin. 2006. Anti-predator behavioral responses of mosquito pupae to aerial predation risk. Journal of Insect Behavior 19: 373-81

Ross R. 2002. The role of the mosquito in the evolution of the malarial parasite: the recent researches of Surgeon-Major Ronald Ross, I.M.S. 1898. Yale Journal of Biology and Medicine 75: 103-05

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8: 103-06

SAS Institute Inc. 2008. SAS/STAT 9.2 User's Guide. Cary, NC

Scheiner S.M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics 24: 35-68

Schneider J.R., D.D. Chadee, A. Mori, J. Romero-Severson, D.W. Severson. 2010. Heritability and adaptive phenotypic plasticity of adult body size in the mosquito *Aedes aegypti* with implications for Dengue vector competence. Infection, Genetics and Evolution 11: 11-16

Scott T.W., P.H. Amerasinghe, A.C. Morrison, L.H. Lorenz, G.G. Clark, D. Strickman , P. Kittayapong, J.D. Edman. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. Journal of Medical Entomology 37: 89-101

Siegel, J. P., R. J. Novak, R. L. Lampman, B. A. Steinly. 1992. Statistical appraisal of the weight-wing length relationship of mosquitoes. Journal of medical entomology 29: 711-714.

Severson D.W., A. Mori, V.A. Kassner,B. M. Christensen. 1995. Comparative linkage maps for the mosquitoes, *Aedes albopictus* and *Aedes aegypti* based on common RFLP loci. Insect Molecular Biology 4: 41-45

Shannon C.E., W. Weaver. 1949. The mathematical theory of communication. University of Illinois Press. Urbana, Illinois

Sibly R.M., D. Atkinson. 1994. How rearing temperature affects optimal adult size in ectotherms. Functional Ecology 8: 486-93

Smithburn K.C., A.J. Haddow, J.D. Gillet. 1948. Rift Valley fever: isolation of virus from wild mosquitoes. British Journal of Experimental Pathology 29: 107-21

Suom C., H.S. Ginsberg, A. Bernick, C. Klein, P.A. Buckley, C. Salvatore and R.A. LeBrun. 2010. Host-seeking activity and avian host preferences of mosquitoes associated with West Nile virus transmission in the northeastern U.S.A. Journal of Vector Ecology 35 (1): 69-74

Sutcliff J.F. 1987. Distance orientation of biting flies to their hosts. Insect science and its application/ sponsored by the International Centre of Insect Physiology and Ecology (ICIPE) and the African Association of Insect Scientists (AAIS) 8: 611-16

Taylor L.H., S.M. Latham, M.E. Woolhouse. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society London B 356:983-89

Tempelis C.H. 1975. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. Journal of Medical Entomology 11: 635-53

Temu E.A., R.H. Hunt, M. Coetzee. 2004. Microsatellite DNA polymorphism and heterozygosity in the malaria vector mosquito *Anopheles funestus* (Diptera: Culicidae) in east and southern Africa. Acta Tropica 90: 39-49

Tsurim I., A. Sillerbush, O. Ovadia, L. Blaustein, Y. Margalith. 2013. Inter- and intra- specific density-dependent effects on life history and development strategies of larval mosquitoes. PLOS One 8(3): e57875

Turell M.J., M.R. Sardelis, D.J. Dohm, M.L. O'Guinn. 2001. Potential North American vectors of West Nile virus. Annals of the New York Academy of Sciences 951: 317-24

Unlu I., A.J. Mackay, A. Roy, M.M.Yates, L.D. Foil. 2010. Evidence of vertical transmission of West Nile virus in field-collected mosquitoes. Journal of Vector Ecology 35: 95-99

van Uitregt V.O., T.P. Hurst, R.S. Wilson. 2012.Reduced size and starvation resistance in adult mosquitoes, *Aedes notoscriptus*, exposed to predation cues as larvae. Journal of Animal Ecology 81: 108-15

Walker E.D., D.L. Lawson, R.W. Merritt, W.T. Morgan, M.J. Klug. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. Ecology 72: 1529-46

Wang R., L. Zheng, Y. T. Toure´, T. Dandekar, F. C. Kafatos. 2001. When genetic distance matters: measuring genetic differentiation at microsatellite loci in whole- genome scans of recent and incipient mosquito species. PNAS 98: 10769-74

West-Eberhard M.J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20: 249-78

Yakubu A.A., A. Singh. 2008. Livestock: An alternative mosquito control measure. Sokoto Journal of Veterinary Sciences 7: 71-74

Yee DA, Kaufman MG, Juliano SA. 2007. The significance of ratios of detritus types and micro-organism productivity to competitive interactions between aquatic insect detritivores. Journal of Animal Ecology 76: 1105-15

Yee WL, Foster WA. 1992. Diel sugar-feeding and host-seeking rhythms in mosquitoes (Diptera: Culicidae) under laboratory conditions. Journal of Medical Entomology 29: 784-91

Zhong-Cheng Y., H. Zhong. 2005. Comparison of adult mosquito community structure on various habitats. Insect Science 12: 193-97

VITA

Margo Lynn Mire began her educational journey in east Tennesssee as a non-traditional student in 2002 after completing a Certified Nursing Assistant course and receiving CNA licensure. She enrolled in Walter State Community College in Morristown, TN with plans of becoming a nurse in 2002. After moving to Jefferson City, MO in 2003 she began taking classes at Lincoln University of Missouri with plans of finishing her nursing degree. As it turned out, she graduated magna cum laude in 2007 with her Bachelors of Science in Biology from Lincoln University of Missouri. During this time aspirations of completing a doctoral program had started. In the fall of 2008 Margo began the Plant, Insect, and Microbial Sciences Ph.D. program in the entomology program area at the University of Missouri in Columbia.

Margo has gained experience during her educational career through teaching and tutoring undergraduate students at Lincoln University. Being a non-traditional student has allowed Margo to experience her degree programs with insights and thoughtfulness that only life experiences can give. It has been a very rewarding and fulfilling journey that has included her husband and family.