ADULT MOSQUITO ECOLOGY IN SOUTHWESTERN GEORGIA

by

EVA ANN WHITEHEAD

(Under the Direction of Alan Covich and Stephen Golladay)

ABSTRACT

Understanding the ecology of mosquitoes is important for implementing control measures and explaining mosquito-borne disease prevalence. I compared mosquito population dynamics to selected weather variables and land use/ cover in a longleaf pine dominated landscape on the Gulf Coastal Plain of Georgia. Important factors for determining mosquito presence/ absence were precipitation, temperature, humidity, and drought index. *Aedes albopictus* and *Culex* spp. mosquitoes were associated with sites that had the most anthropogenic influence, while *Coquillettidia perturban*s and *Psorophora ferox* were associated with natural land cover such as wetlands and forested land. Arbovirus testing yielded one isolation of West Nile virus and three isolations of Potosi virus. This low arbovirus prevalence is likely due to the diversity of the wildlife in the area or factors related to the bird community, which typically serves as a reservoir for arboviruses. Examination of mosquito host-feeding patterns showed the mosquitoes collected predominantly fed on white-tailed deer.

INDEX WORDS: Mosquito community, weather, spatial ecology, land use, arbovirus, host-feeding patterns

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INTRODUCTION

Mosquito communities can vary both spatially and temporally within an area (Schäfer et al. 2008). The variation observed can be due to habitat preferences, host preferences, and environmental factors (Zhong et al. 2003, Schäfer et al. 2008). Understanding the spatial and temporal dynamics of mosquito communities is important not only for implementation of control measures but also for explaining vector-borne disease prevalence (DeGroote et al. 2007). Previous studies have determined associations between climatic and landscape patterns with both relative mosquito abundance and transmission of mosquito-borne illnesses (Afrane et al. 2005, Leisnham et al. 2006, Pecoraro et al. 2007). The mosquito nomenclature in this thesis is based on Darsie and Ward (2005).

MOSQUITO RESEARCH IN GEORGIA

Mosquitoes were first extensively studied in Georgia as a result of malaria's prevalence in the southern portion of the state (Womack 1997c). During the mid-twentieth century, investigators at the Emory University Field Station at Newton contributed a great deal to the scientific literature on *Anopheline* vectors of malaria and the dynamics of this disease (Love and Goodwin 1959, Womack 1997c). Researchers at the field station also provided many mosquito county distribution records as well as ecological data on many mosquito species in the state (Love and Goodwin 1961).

The most extensive mosquito surveillance project in the state was conducted as a result of the introduction of the Asian tiger mosquito, *Aedes albopictus* (Skuse), to the United States (Womack 1997c). In Georgia, the Asian tiger mosquito, a nuisance species and potential disease vector, was first recorded in Atlanta and Savannah in 1986, but its presence was soon documented in other areas of the state (Womack et al. 1995). At the end of survey launched in 1991 to document the species' presence in the state, all 159 counties in Georgia were found to contain the Asian tiger mosquito (Womack et al. 1995). The spread of the mosquito across the state most likely occurred between 1986 and 1990 and was due to the commercial movement of tires (Womack et al. 1995).

Another intensive statewide mosquito surveillance occurred after Tropical Storm Alberto caused flooding in southwestern Georgia in 1994 and numerous hurricanes and tropical storms caused additional flooding throughout the southeastern U.S. the following year (Smith and Floore 2001). Due to concern about the potential increased risks of mosquito-borne diseases, federal recovery funds were appropriated for development of mosquito and arbovirus surveillance programs in Georgia (Smith and Floore 2001). Surveillance was launched in 1997 throughout the state in order to provide the Division of Public Health of the Georgia Department of Human Resources (GDHR) with a statewide compilation of potential disease vectors in Georgia (Smith and Floore 2001). One GDHR-funded surveillance team trapped mosquitoes in 13 counties in north Georgia and collected 14 species of mosquitoes (Nolan 1997). *Culex quinquefasciatus* Say was the most frequently collected species throughout the northern counties (Nolan 1997). Another surveillance team focused on sampling in seven counties in central Georgia that were within approximately a 60 mile radius of Macon State College during June 1997

(Womack 1997a). Out of the total 238 adult mosquitoes and 391 larvae collected, 20 mosquito species were identified (Womack 1997a). Crawford County produced the greatest number of mosquitoes and was the most species rich county (Womack 1997a). *Ae. albopictus* was the most abundant species collected during the central Georgia survey (Womack 1997a). In September, the same surveillance team surveyed 13 western Georgia counties (Womack 1997b). A total of 15 different mosquito species were collected and the largest number was from Pike County (Womack 1997b). Similar to the earlier survey in central Georgia, *Ae. albopictus* was the most abundant species collected in western Georgia (Womack 1997b). Twenty counties in the southern third of Georgia were also surveyed during the summer of 1997 (Smith and Floore 2001). Collections taken weekly over four weeks yielded a total of 5,994 mosquitoes and 29 species (Smith and Floore 2001). All species collected represented new county records with the exception of *Ae. albopictus*, *Culex territans* Walker, and *Cx. quinquefasciatus* that had been collected previously (Smith and Floore 2001). Counties having the greatest abundance of mosquitoes included Atkinson, Calhoun, Worth, and Ware counties (Smith and Floore 2001). The greatest species diversity (24 species) was collected from Worth County (Smith and Floore 2001). *Aedes vexans* (Meigen) was the most abundant species collected in the 20 southern Georgia counties surveyed (Smith and Floore 2001).

Besides the Asian tiger mosquito, two other recently introduced mosquito species are now known to be reproducing in Georgia: *Culex coronator* Dyar & Knab and *Ochlerotatus japonicus japonicus* (Theobald). *Cx. coronator* was originally collected from Dougherty County in 2006 and subsequently collected that year in Muscogee and Lowndes counties (Kelly et al. 2008). In 2007, the species was collected along the

Georgia coastline in Chatham County as well (Moulis et al. 2008). While West Nile virus and St. Louis encephalitis virus have been detected in *Cx. coronator* mosquitoes, no direct evidence suggests that this species is an important vector of any arbovirus (Aitken et al. 1969, Kelly et al. 2008). *Oc. japonicus*, a mosquito species endemic to Asia, was initially collected in Fulton County in 2002. In subsequent years, it was collected in additional counties outside of the metro-Atlanta region, which indicates that its range is more widespread than originally thought (Gray et al. 2005). The occurrence of *Oc. japonicus* in Georgia is a potential cause for concern, because it has been shown to transmit West Nile virus effectively in laboratory studies (Turell et al. 2005).

Since the introduction of West Nile virus in Georgia, the bird-feeding *Culex* group has become a priority among mosquitoes being monitored within the state (Gray 2008). The larvae of *Cx. quinquefasciatus*, the primary vector of WNV in Georgia, are often associated with water rich in organic matter such as sewage treatment ponds, catch basins, polluted ditches, and the combined sewer overflow systems in Atlanta (Calhoun et al. 2007). In 2005, multiple cases of West Nile virus were found near the CSOs, and a study was launched to identify the role of CSO systems in the WNV vector mosquito production in Atlanta (Calhoun et al. 2007). The study documented that urban streams and creeks in Atlanta receiving effluent from CSO facilities can be major breeding sites for *Culex* mosquitoes, especially *Cx. quinquefasciatus*, and could continue to pose a significant WNV exposure risk until a new CSO system in Atlanta is fully operational (Calhoun et al. 2007).

EMORY UNIVERSITY FIELD STATION: ICHAUWAY'S MOSQUITO HISTORY

Mosquito research at Ichauway, the property of J.W. Jones Ecological Research Center, was initiated in 1939 by Emory University through a malaria research program supported by Robert W. Woodruff, the former owner of Ichauway Plantation (Goodwin 1989). Following a break in research at the field station from May 1942 until June 1944 due to World War II, the station was operated jointly by Emory and the U.S. Public Health Service's Office of Malaria Control in War Areas (Goodwin 1989). The station was closed on September 8, 1958 due to lack of funding and also because it appeared that the resurgence of malaria was unlikely in the United States (Goodwin 1989).

The work undertaken in 1939 had two primary objectives related to malaria: continuation of a drug program for control of malaria and implementation of a program for research on the natural history of malaria (Goodwin 1989). The latter work involved studies on the ecology of malaria vectors, primarily *Anopheles quadrimaculatus* Say, both in the field and under experimental conditions. Prior to 1952, collections were concerned primarily with the occurrence and abundance of *Anopheles* species (Smith and Love 1956). In 1949, Goodwin undertook studies to determine whether *An. quadrimaculatus* dispersed quickly or remained in the vicinity of breeding sites. Data from these studies suggested that female mosquitoes dispersed over a larger area from the breeding area than males most likely because females were in search of a host blood meal (Goodwin 1949). A study undertaken to document the overwintering activities of *An. quadrimaculatus* as well as any other species that might be associated with them found that adults of *An. quadrimaculatus* and *Anopheles punctipennis* (Say) were generally distributed in small numbers in hollow trees during the winter (Zukel 1949b). Adult females of *Uranotaenia sapphirina* (Osten Sacken), *Culex erraticus* (Dyar & Knab), *Culex peccator* Dyar & Knab, and *Cx. quinquefasciatus* were also found during the fumigations of hollow trees suggesting that these species also overwinter as fertile adults (Zukel 1949b). Bellamy (1950) later documented *An. quadrimaculatus* maintaining a large and reproducing population during the winter of 1948-49 near ponds that were known breeding areas. Temperatures that winter were the warmest observed in the previous 20 years. Dissection of female mosquitoes led to the frequent discovery of undigested blood in the gut of *An. quadrimaculatus* and *An. punctipennis* collected throughout the winter, supporting the notion of these species being active during cooler weather (Zukel 1949a). While studying the effect of environmental variables such as precipitation, air temperature, water-surface temperature, and pond stages on the abundance of adult *An. quadrimaculatus* in the field, Goodwin and Love (1957) found that the temperature of water in the mosquito breeding areas was a critical limiting factor in mosquito abundance.

Along with field observations, extensive laboratory experiments were conducted to determine the effects of certain environmental variables on *An. quadrimaculatus* (Platt et al. 1957). Using adults placed in an environmental chamber, researchers found that mosquitoes were attracted to the area with relative humidity ranging between 60-70% (Platt et al. 1957). In temperature experiments within chambers, mosquitoes were found to be most active between 27-28°C (Platt et al. 1957). Upper lethal temperatures for most individuals were between 40-44°C, with consistent differences noted for both sexes and under different humidity levels (Platt et al. 1957). No consistent response was found in experiments that tested preferences towards light intensities and wavelengths, except for a higher level of agitation in the presence of red and blue light (Platt et al. 1957).

Along with studies on adults, several studies at the Emory University Field Station focused on development of the immature stages of *An. quadrimaculatus* and other disease vectors. Love (1954) documented variation in hatching of ova among successive clutches oviposited by female *An. quadrimaculatus*, confirming the hypothesis that sufficient sperm can be retained by females to fertilize ova throughout their life span. Later, an experiment was conducted to determine the relation of naturally occurring temperatures to larval development and mortality (Goodwin and Love 1957, Love and Goodwin 1959). The study found that no ova hatched at temperatures below 10°C, and 90% of first and second instar larvae were killed by temperatures of 7°C (Love and Goodwin 1959). Mean temperature above 16°C was necessary for most second instar larvae to develop to the third instar (Love and Goodwin 1959). Under natural temperatures, the developmental time from ova to adult varied from 15 to 51 days (Love and Goodwin 1959). An additional laboratory experiment examined the relationship between surface water temperatures and larval development by exposing larvae and pupae to constant and fluctuating high surface-water temperatures (Love and Whelchel 1957). The results of the experiments demonstrated that larval mortality is affected by temperature, duration of exposure, and speed with which the temperature changes (Love and Whelchel 1957). In 1952, a study was conducted to determine the effect of photoperiod on the development of *Ochlerotatus triseriatus* (Say) in southwestern Georgia (Love and Whelchel 1955). Experiments showed eggs hatched under any photoperiod length, but pupation slowed as the photoperiod shortened in winter months

(Love and Whelchel 1955). However, these larvae later readily pupated when stimulated by a longer period of light at a moderate temperature (Love and Whelchel 1955).

Beginning in 1952, mosquito collections at the field station were made at weekly intervals and were directed towards comprehensive sampling of mosquito fauna, instead of just focusing on malaria vectors (Smith and Love 1956). Platt et al. (1958) investigated multiple environmental variables potentially associated with the abundance of *Ae. vexans* mosquitoes collected during a night. The study found that abundance was directly related to relative humidity and increased with relative humidity during the night until an optimum relative humidity between 70 and 80% was reached (Platt et al. 1958). In nearby Thomasville, Georgia, a study on the effect of low temperatures on the winter survival of the invasive *Aedes aegypti* (Linnaeus) mosquito in various naturally occurring containers indicated that average weekly air temperatures below 15°C killed most larvae (Smith and Love 1958). However, larvae found in containers in sheltered positions were able to survive during periods when the average weekly temperature was as low as 9°C (Smith and Love 1958).

Smith and Love (1956) compared the mosquito abundance and community composition during a drought year to that of a year of normal precipitation which had preceded, demonstrating that mosquito abundance and potentially disease prevalence can be impacted by patterns of rainfall. During the year of normal rainfall, *An. quadrimaculatus*, *Anopheles crucians* Wiedemann, and *Ur. sapphirina* made up almost 98% of the mosquito catch (Smith and Love 1956). During the drought year, *Aedes*, *Ochlerotatus*, and *Psorophora* species composed almost 98% of captures from light traps (Smith and Love 1956). The study also succeeded in refuting the hypothesis of mosquito abundance being reduced in a drought year by showing that approximately 80% more mosquitoes per night were collected during the drought year compared to the normal year (Smith and Love 1956). Later, in an effort to determine the extent of natural vertical stratification among mosquito species, collections were made using mechanical sweep nets on a tower at six elevations from three feet to 50 feet (Love and Smith 1958). *Anopheles* and *Uranotaenia* mosquitoes rarely attained an altitude of 15 feet (Love and Smith 1958). *Aedes* and *Psorophora* mosquitoes were more active at higher elevations, and *Culex* mosquitoes occurred most frequently at intermediate elevations (Love and Smith 1958). *Culiseta* mosquitoes were evenly distributed from six to 50 feet. Love and Smith (1958) suggested that the pattern of vertical distribution of mosquitoes observed in the study was correlated with their host feeding preferences.

In 1961, Love and Goodwin published a report that summarized general seasonal abundance patterns of mosquito species in southwestern Georgia based on unpublished observations made at the Emory Field station between 1939 and 1957. Of the 51 species of mosquitoes reported from Georgia at the time, 42 were collected at Ichauway (Love and Goodwin 1961). The most species collected (13) came from the genera *Aedes* and *Ochlerotatus* (Love and Goodwin 1961). *Ochlerotatus mitchellae* (Dyar) and *Ochlerotatus canadensis canadensis* (Theobald) were collected primarily during cooler months, while *Ae. vexans* was found from early spring until late fall (Love and Goodwin 1961). Of the six species of *Anopheles* found, the two most abundant were *An. crucians* and *An. quadrimaculatus* (Love and Goodwin 1961). *An. quadrimaculatus* was the predominant species from June through October, while *An. crucians* was most abundant early in the season (Love and Goodwin 1961). During the study, the genus *Culex* was represented primarily by *Cx. territans* and *Culex restuans* Theobald in the winter and by *Culex nigripalpus* Theobald, *Culex pilosus* Dyar & Knab, *Cx. quinquefasciatus*, *Cx. erraticus*, and *Cx. peccator* during the summer (Love and Goodwin 1961). The genus *Psorophora* was represented by eight species. *Psorophora ciliata* (Fabricius), *Psorophora columbiae* (Dyar & Knab), and *Psorophora ferox* (von Humboldt) were the most common *Psorophora* species collected, and their average seasonal distribution was from May through October (Love and Goodwin 1961). The smaller genera such as *Uranotaenia* were represented by only one or two species at the field station. *Ur. sapphirina* was abundant, while few *Uranotaenia lowii* Theobald were collected (Love and Goodwin 1961). Two species of *Culiseta* were collected: *Culiseta inornata* (Williston) and *Culiseta melanura* (Coquillett) (Love and Goodwin 1961). *Orthopodomyia* was represented by one species, *Orthopodomyia signifera* (Coquillett), and *Toxorhynchites* was represented by *Toxorhynchites rutilus septentrionalis* Dyar & Knab (Love and Goodwin 1961). *Coquellitidia perturbans* (Walker) was the only species collected from that genus (Love and Goodwin 1961).

The last paper published on the mosquito research conducted at the field station focused on comparing the vertical distribution of mosquitoes in a wooded area to an open area (Love et al. 1963). Over a 12-month period, mosquitoes were collected in traps at 6-, 25-, and 40-foot elevations in an oak hammock and at 6- and 25-foot levels in an adjacent open field (Love et al. 1963). In general, *Ochlerotatus, Aedes,* and *Culex* were more abundant at the higher elevations, while *Anopheles* and *Uranotaenia* were more abundant at lower elevations at both locations (Love et al. 1963). Mosquito abundance was greater in the wooded area than the open field (Love et al. 1963).

MOSQUITO-BORNE ARBOVIRUSES IN GEORGIA

While mosquito arbovirus surveillance conducted in Baker County, Georgia has been limited, it is known that several mosquito-borne viruses circulate in the state each year and are capable of causing disease in humans and other animals. The most well-documented arboviruses known to infect humans in the state are St. Louis encephalitis virus (SLEV), LaCrosse encephalitis virus (LACV), Eastern Equine encephalitis virus (EEEV), and West Nile virus (WNV) (Lance-Parker et al. 2002). Although small numbers of cases of these arboviruses excluding West Nile, have been reported since 1990, more cases may have been undiagnosed or unreported (Lance-Parker et al. 2002). St. Louis encephalitis virus is a flavivirus that occurs throughout Georgia but is rarely diagnosed (Lance-Parker et al. 2002). The primary mosquito vectors of SLEV are *Culex tarsalis* Coquillett, *Cx. quinquefasciatus* complex, and *Cx. nigripalpus* (Lance-Parker et al. 2002). LaCrosse encephalitis virus is a bunyavirus of the California encephalitis group and is seen primarily in northern Georgia (Lance-Parker et al. 2002). The vectors of LACV are *Ochlerotatus* mosquitoes, primarily *Oc. triseriatus*, and the virus reservoir is thought to be small mammals like squirrels and chipmunks (Alatoom and Payne 2009).

Eastern equine encephalitis virus, an alphavirus, is found throughout the state but is most prevalent in southern Georgia (Lance-Parker et al. 2002). The virus is transferred between birds by *Cs. melanura*, but dead-end hosts such as humans and horses are typically infected through *Aedes*, *Ochlerotatus*, *Coquillettidia*, and *Culex* bridge vector species (Harvala et al. 2009). The first isolation of EEEV from wild-caught *Cq. perturbans* mosquitoes in the United States occurred in Georgia in 1948 (Howitt et al. 1949). The virus was also later isolated from pools of three species (*Oc. mitchellae*, *An.*

crucians, and an unknown species of *Culicoides*) in Georgia in 1956 (Karstad et al. 1957). At the time, the isolation of EEEV from *Oc. mitchellae* in the wild was considered significant, because species of that genus had proven to be successful vectors of the virus in the laboratory (Karstad et al. 1957).

The summer of 1963 was a period of major EEEV activity and as a result, an investigation was undertaken in the Waycross, Georgia area to determine which species of mosquitoes were infected with the virus (Chamberlain et al. 1969). The 60,198 mosquitoes collected and tested for virus yielded a total of 167 isolations of five different arboviruses, comprised of 35 isolations of EEEV, 15 of Western Equine encephalitis virus (WEEV), 42 of Tensaw virus, 31 of Flanders virus, and 44 of Keystone virus (Chamberlain et al. 1969). *Cs. melanura* was the main species infected with EEEV, WEEV, and Flanders viruses (Chamberlain et al. 1969). The majority of the Tensaw virus isolates came from *An. crucians* and *Ps. columbiae* mosquitoes, while all of Keystone isolates were from *Ochlerotatus atlanticus* (Dyar & Knab). Little is known about Flanders virus except that it is often isolated from *Cs. melanura* and other bird-feeding mosquitoes and thought to be nonpathogenic to vertebrates (Chamberlain et al. 1969, Nasci et al. 2001). Tensaw virus is a bunyavirus that is widespread in the southeast, and is closely related to Cache Valley virus found throughout the United States (Chamberlain et al. 1969). The complex that Keystone virus belongs to is known to produce an encephalitic disease in humans, and its natural hosts are most likely wild rodents, rabbits, and hares (Chamberlain et al. 1969).

Although the clinical signs of EEE infection in horses and humans had been previously described, EEE in pigs was not described until pigs on a farm in Berrien

County, Georgia began dying from an unknown disease (Pursell et al. 1972). EEE virus was isolated from a four week-old pig that came from the farm and inoculated into uninfected pigs (Pursell et al. 1972). Following the inoculation, the symptoms of the disease's progression were documented, proving that EEE was in fact a clinical disease and providing information for the diagnosis of future porcine encephalitis of unknown origin (Pursell et al. 1972). Later, after substantial cases of EEE in pigs were reported from Georgia in 1991 and Florida in 1994, a study was undertaken to determine the prevalence of exposure to EEEV in selected populations of domestic swine in Georgia and feral swine on Ossabaw Island, Georgia (Elvinger et al. 1996). The study found 32 out of 1247 samples from domestic swine and 62 out of 376 samples from feral swine were positive for antibodies to EEEV (Elvinger et al. 1996). Results from this survey indicated that domestic and feral swine are exposed to EEEV and that EEEV infection is probably under-diagnosed in swine, even though it causes significant financial losses in the affected swine herds (Elvinger et al. 1996).

Although antibodies to EEEV in white-tailed deer (*Odocoileus virginianus*) had been reported from Florida and North Dakota, U.S.A., clinical disease due to EEEV was first documented in deer in Houston County, Georgia (Tate et al. 2005). Further serologic testing was later performed on 99 serum samples collected from hunter-killed white-tailed deer throughout the state during October and November 2001 (Tate et al. 2005). Neutralizing antibodies to EEEV were detected in 14% of the deer tested with the majority of the EEEV antibody-positive deer coming from two locations within the Coastal Plain physiographic region, an area known to be enzootic for EEEV (Pursell et al. 1972, Tate et al. 2005). Although that study was the first report of a clinical case of

EEEV infection in deer, it is now assumed that deer are commonly infected with this virus in Georgia but are relatively resistant to clinical disease (Tate et al. 2005). The first fatality of a white-tailed deer to WNV was documented in Decatur County, Georgia (Miller et al. 2005). While the prevalence of WNV infection in white-tailed deer is unknown, deer are most likely dead-end hosts like horses (Miller et al. 2005).

West Nile virus encephalitis is caused by a flavivirus closely related to SLEV (Lance-Parker et al. 2002). WNV was introduced to Georgia in 2001 by birds migrating from the northeastern and middle Atlantic states and is now found throughout the state (Lance-Parker et al. 2002). In 2002-2003, a serologic study was conducted on WNV exposure rates of rock pigeons from a single location near metropolitan Atlanta, Georgia (Allison et al. 2004). The study found that overall seroprevalence rates per collection for 2002 were 16-45% and 11%-50% in 2003, and 4.1% of the birds were viremic (Allison et al. 2004). The seroprevalence and viremia levels observed in this study indicated that rock pigeons could be involved in amplifying WNV in urban settings (Allison et al. 2004).

In order to study the role of *Culex* mosquitoes and certain species of birds in the transmission cycle of WNV in the southeastern United States, Godsey et al. (2005) sampled mosquitoes and birds during a one-week period in southern Georgia and northern Florida near sites where WNV had been detected in dead birds. The researchers also evaluated the infection rate in captive birds compared with free-ranging birds to see if captive birds, such as chickens, could serve as sentinels for enzootic transmission in the southeast (Godsey et al. 2005). Virus testing of mosquito pools collected during the study yielded 15 isolates of WNV from northern Florida and one from southern Georgia

(Godsey et al. 2005). While the Florida isolates were from pools of *Culex salinarius* Coquillett, *Cx. nigripalpus*, *Cx. quinquefasciatus*, and *Cs. melanura*, the only Georgia isolate was from *Cx. salinarius* (Godsey et al. 2005). Virus testing also yielded three isolates of Keystone virus from pools of *An. crucians* complex, *Oc. canadensis*, and *Oc.* species mosquitoes collected Lowndes County, GA (Godsey et al. 2005). In Florida, the northern cardinal, northern mockingbird, common ground-dove, common grackle, and house sparrow were the most frequently infected wild, free-ranging birds (Godsey et al. 2005). Out of the 76 captive and free-ranging birds tested from Georgia, one chicken was positive for WNV-neutralizing antibodies (Godsey et al. 2005). The results from this study indicated that the primary transmission cycle of WNV in the southeastern United States involves passerine birds and *Culex* mosquitoes, particularly *Cx. quinquefasciatus*, *Cx. salinarius*, and *Cx. nigripalpus* (Godsey et al. 2005). Furthermore, chickens may serve as effective sentinels in this region (Godsey et al. 2005).

Out of the 83 species of birds tested between 2000 and 2004 in Georgia, antibodies to WNV were detected in 37 species (Gibbs et al. 2006a). Rock pigeons, northern cardinals, common ground doves, gray catbirds, and northern mockingbirds had the highest seroprevalence rates for WNV during those three years (Gibbs et al. 2006a). Northern cardinals, specifically, were widespread throughout the state and represented 29.8%, 50.0%, and 83.2% of the seropositive birds collected in 2002, 2003, 2004 making them potentially excellent avian indicators of WNV transmission in the state (Gibbs et al. 2006a).

A subsequent study built upon Gibbs et al. (2006a) using GIS and logistic regression analyses to predict the distribution of WNV in the state of Georgia and attempt to identify human and environmental predictor variables important in the determination of WNV distribution (Gibbs et al. 2006). The study found that for the 2002 data collected, the probability of WNV being present in an area increased with observed urban-suburban land use and low January temperatures (Gibbs et al. 2006). The 2003 WNV cases were only positively associated with urban-suburban land use (Gibbs et al. 2006). In 2004, the probability of WNV positive sites was lower in the mountains than in other physiographic regions and was positively associated with the intermediate level of housing density (Gibbs et al. 2006). The models that ended up being the best fit for the 2002-2004 data indicated that human activities in the urban/suburban landscape may provide reservoir host and vector habitats suitable for efficient WNV transmission (Gibbs et al. 2006). To evaluate how West Nile virus antibody prevalence in natural avian communities co-varied with land use, songbird populations were sampled along an urban-rural gradient in Atlanta, Georgia (Bradley et al. 2008). Out of the 499 birds tested over three years, antibody prevalence increased from rural to urban sites, especially among Northern Cardinals (Bradley et al. 2008). Findings from this study demonstrated that ecological factors associated with urbanization can influence WNV infection patterns (Bradley et al. 2008).

MOSQUITO HOST-FEEDING PATTERNS

Knowledge of the blood-feeding behavior of mosquito populations is necessary for evaluating their role as vectors and for assessing the role of vertebrates as potential reservoir hosts involved in the maintenance and amplification of zoonotic pathogens (Molaei et al. 2008). Current patterns of host species choice by mosquitoes are the product of millions of generations of natural selection and are governed by a number of factors such as host availability and abundance, host characteristics such as defensive behavior and body mass, as well as flight behavior and feeding periodicity of mosquitoes (Clements 1999, Molaei et al. 2008, Hamer et al. 2009, Lyimo and Ferguson 2009). Host availability is a function of ecological and behavioral factors that influence the probability of a mosquito coming in contact with a host (Hamer et al. 2009). Host choice may also be influenced by previous feeding experience that causes mosquitoes to learn which hosts are suitable for feeding (Lyimo and Ferguson 2009).

The latter theory was tested by an experimental study in which mosquitoes known to have fed on either cows or pigs for their first blood meal were allowed to choose between the same hosts for their second blood meal (Mwandawiro et al. 2000, Lyimo and Ferguson 2009). While *Aedes* species showed no host fidelity, three *Culex* species used within the study were most likely to choose the host species that was first fed upon for their second blood meal (Mwandawiro et al. 2000, Lyimo and Ferguson 2009).

In addition, the variation in physical and chemical composition of a host's blood may influence the reproductive success of mosquitoes and create selection for preferential feeding on optimal host species (Lyimo and Ferguson 2009). Multiple laboratory studies in which mosquitoes fed on blood from different species indicated that mosquito reproduction and survival is often highest after feeding on hosts commonly fed upon in nature (Lyimo and Ferguson 2009). However, these studies may have generalized the mosquito's host species in nature and/or only looked at one component of mosquito fitness, so they are not entirely conclusive (Lyimo and Ferguson 2009). Although many mosquito species exhibit characteristic trends in host species choice, this behavior can vary spatially and temporally (Lyimo and Ferguson 2009).

Investigations of host-feeding preferences of mosquitoes in Georgia are limited to studies that took place at the Emory University Field Station at Newton and in the city of Waycross during the mid-to-late twentieth century. After documenting that *Ae. vexans* flies at heights that would make it likely to feed on avian hosts and suspecting its role in encephalitis transmission, Love and Smith (1958) examined 35 freshly engorged *Aedes vexans* only to find that none of them had fed on avian hosts. During an outbreak of eastern equine encephalitis in Waycross, Georgia in 1963, Chamberlain et al. (1969) collected blood-engorged *Cs. melanura* and *Oc. atlanticus* mosquitoes to examine the role of these mosquitoes in the transmission of EEEV. They found that the majority of the *Cs. melanura* mosquitoes collected fed predominantly on avian hosts but did take 4% of their blood meals from mammals whereas, *Oc. atlanticus* fed almost solely on mammalian hosts (Chamberlain et al. 1969). The feeding patterns of *Cs. melanura* observed in this study supported the theory of EEEV transmission between birds and mammals by this mosquito species (Chamberlain et al. 1969).

While published host-feeding studies from Georgia are limited, many studies on the subject have been published from surrounding states. In the first study to investigate the host feeding patterns of mosquitoes in the coastal plain habitat of North Carolina, Irby and Apperson (1988) found relatively large numbers of four mosquito species (*Oc. canadensis*, *Oc. triseriatus*, *Cx. peccator*, and *Cx. territans*) that fed on amphibian or reptile hosts. *Cs. melanura*, *Cx. quinquefasciatus,* and *Cx. restuans* were the only species collected in large numbers that had fed predominantly on birds. In general, the *Culex* mosquitoes fed primarily on passerine birds; however, a relatively large proportion (39%) of *Cx. quinquefasciatus* fed on either turkeys or chickens (Irby and Apperson 1988).

Relatively large numbers of two species (*Oc. atlanticus* and *Ps. ferox*) fed predominantly on mammals, and the majority of their blood meals were deer (50%), canine (23%), or rabbit (17%) (Irby and Apperson 1988). A 1993 study conducted in Lake Falls basin, North Carolina found that *An. quadrimaculatus* fed on 11 mammalian species, with the largest percentage of blood meals taken from white-tailed deer (Robertson et al. 1993). This species was also found to rarely feed on birds (Robertson et al. 1993). *Cx. erraticus* exhibited a more diverse feeding pattern with blood meals identified from 17 species of mammals, birds, and ectotherms with white-tailed deer, turtles, and columbiform birds being the most common hosts (Robertson et al. 1993). After the introduction of *Ae. albopictus*, Richards et al. (2006) described the feeding behavior of that species as well as other sympatric species in a suburban area of North Carolina. The blood meals from the three most commonly collected species, *Ae. albopictus*, *Ae. vexans*, and *Oc. triseriatus*, were taken mainly from mammals such as humans, deer, squirrels, dogs, cats, and raccoons (Richards et al. 2006). *Ae. albopictus*, specifically, fed mainly on humans, cats, and dogs (Richards et al. 2006). Only 26% of its blood meals were identified as avian, with the majority of those avian blood meals being from domestic chickens, and a similar pattern was observed for *Ae. vexans* (Richards et al. 2006).

In a long-term investigation of the feeding patterns of mosquitoes in Florida, Edman (1971) used antisera to identify the blood meal source of thousands of mosquitoes. The study found that over 90% of the blood meals from the *Aedes*, *Ochlerotatus*, *Coquillettidia*, *Mansonia*, and *Psorophora* mosquitoes came from mammalian hosts, with the majority being cows, rabbits, and armadillos (Edman 1971). As a part of the same long-term investigation, Edman (1979) also studied the feeding patterns of *Culex* mosquitoes of the subgenus *Melanoconion*. Out of the species studied, *Cx. erraticus* and *Culex iolambdis* Dyar fed primarily on birds, while *Cx. pilosis* and *Cx. peccator* fed predominantly on reptiles (Edman 1979). In contrast *Culex cedecei* Stone & Hair fed on a variety of mammals, primarily rodents. Documenting these feeding patterns provided insight into *Cx. opisthosus* being the species within the member of the *Melanoconion* subgenus responsible for vectoring Venezuelan equine encephalitis virus among rodents in southern Florida (Edman 1979).

In a more recent study from Florida intended to identify mosquito species that were feeding on alligators at a central Florida alligator farm during and after an epidemic of suspected WNV in 2003, the investigators were only able to identify the blood meal source for 13 mosquitoes (Rodrigues and Maruniak 2006). Seven of those mosquitoes, which represented three species (*Cx. erraticus*, *Mansonia dyaria* Belkin, Heinemann & Page, and *Mansonia titillans* (Walker)) had fed on American alligators (Rodrigues and Maruniak 2006). Because mosquitoes of the genus *Culex* are known to be the most important vectors of WNV, finding *Cx. erraticus* mosquitoes that fed on alligators could implicate *Cx. erraticus* in the transmission of WNV in the epidemic at the alligator farm (Rodrigues and Maruniak 2006). However, additional collecting and vector competence studies are necessary to identify the most likely vectors of WNV on alligator farms (Rodrigues and Maruniak 2006).

In order to gain more information on the transmission of EEEV and the birds that serve as the virus reservoir in Alabama, researchers identified avian-derived blood meals from four species of mosquitoes (*Ae. vexans*, *Cq. perturbans*, *Cx. erraticus*, and *Cs. melanura*) at a site in the Tuskegee National Forest (Hassan et al. 2003). Out of 198 samples that produced detectable blood meal derived PCR products, a total of 83 mosquitoes with avian-derived blood meals were analyzed (Hassan et al. 2003). The two most common avian hosts identified were the Carolina chickadee and the yellow-crowned night-heron, and these birds were consistently over-represented in mosquito blood meals when compared with their abundance estimated by point counts (Hassan et al. 2003). These findings suggested that the mosquitoes collected were selectively targeting particular avian species, which could affect the transmission of the virus if those avian species are superior reservoirs for EEEV (Hassan et al. 2003). At the same study location in central Alabama area, known to be a focus of EEEV, a later study was conducted to determine the arbovirus prevalence and host feeding patterns of three vectors of EEEV: *Ur. sapphirina*, *Cx. erraticus*, and *Cx. peccator* (Cupp et al. 2004). EEEV was detected in each species during the study's three-year sampling period, except for one year when pools of *Cx. peccator* were negative. While only two blood meals were identified from *Ur. sapphirina*, 130 blood meals were identified from *Cx peccator* with 81% of those blood meals being from *Agkistrodon piscivorus*, the cottonmouth (Cupp et al. 2004). *Cx. erraticus* was found to feed upon various birds and mammals but primarily on white-tailed deer in the study (Cupp et al. 2004).

After Cupp et al. (2004) revealed *Cx. peccator* feeding on cottonmouths, a study was conducted to determine if ectotherm-feeding mosquitoes were generalists or if they fed disproportionately on some hosts (Burkett-Cadena et al. 2008). The researchers found four species of mosquitoes (*Cx. territans*, *Cx. peccator*, *Cx. erraticus,* and *Cs. melanura*) that fed on ectotherm vertebrate hosts, with *Cx. peccator* and *Cx. territans* feeding primarily upon vertebrate ectotherms (Burkett-Cadena et al. 2008). *Cx. peccator* fed largely upon reptiles but tended to not be host specific, while *Cx. territans* fed primarily upon amphibians and was found to be quite host specific (Burkett-Cadena et al. 2008). In contrast to Cupp et al. (2004), ectotherm abundance data combined with host-feeding patterns from this study showed that although cottonmouths were commonly targeted hosts, they did not appear to be a preferred host (Burkett-Cadena et al. 2008). However, bullfrogs appeared to be a preferred host for both *Cx. peccator* and *Cx. territans* (Burkett-Cadena et al. 2008). The findings of this study illustrated that while some species of mosquitoes have host preferences for mammals and birds, some species such as *Cx. peccator* and *Cx. territans* have host preferences among vertebrate ectotherms (Burkett-Cadena et al. 2008). These findings are potentially important related to the transmission of EEEV by mosquitoes that target vertebrate ectotherms that may serve as competent reservoirs for the virus (Burkett-Cadena et al. 2008).

A study with the objective of characterizing the host-feeding patterns of potential vector mosquitoes for WNV, with particular emphasis on indentifying avian hosts of *Culex*, mosquitoes, examined the feeding patterns of 21 mosquito species from New York, New Jersey, and Tennessee (Apperson et al. 2004). At the study sites in Tennessee, mammophilic mosquito species, such as *An. quadrimaculatus* and *Cx. erraticus*, fed mainly on domestic dogs (Apperson et al. 2004). *Cx. pipiens* complex proved to be primarily ornithophilic, but still had some mammal-derived blood meals suggesting its potential involvement in the transmission of WNV in the U.S. (Apperson et al. 2004). In a later study that focused on Tennessee, approximately 2,000 blood-fed mosquitoes were collected in 2002 and 2003 at sites located in Memphis and surrounding areas of Shelby County, Tennessee. *Cx. pipiens* complex and *Cx. restuans* were found to be primarily ornithophilic; however, it was clear that neither species were entirely ornithophilic (Savage et al. 2007). *An. quadrimaculatus* and *Cx. erraticus* took the majority of their blood meals from mammals but did occasionally take meals from birds (Savage et al. 2007). The domestic dog was the most frequently utilized host for *An. quadrimaculatus* and *Cx. erraticus*, while the raccoon was the preferred host for those *Cx. pipiens* complex and *Cx. restuans* mosquitoes that fed on mammals (Savage et al. 2007). This demonstrated that host feeding choice may vary widely within a single species from location to location, which may in turn affect the role that each species plays both as an enzootic and as a bridge vector in different regions (Savage et al. 2007).

OBJECTIVES

The purpose of this project was to examine the ecology of the mosquito community at Ichauway, the property of the J. W. Jones Ecological Research Center in southwestern Georgia, which has been largely unstudied since the closing of the Emory University Field Station in 1958. While recent mosquito surveys have taken place in southwestern Georgia, the data published are based only on surveys that lasted a few days to four weeks due to the goal of gaining information at a statewide level (Womack et al. 1995, Smith and Floore 2001). Moreover, the survey of mosquitoes in southern Georgia did not include Baker County, the site of this project (Smith and Floore 2001). Smith and Floore (2001) could not detect natural fluctuations in species composition and abundance associated with changes in weather and climate in southern Georgia due to the short length of their study. However, my study took place over an entire field season to not only gain information on the community composition but also the changes in composition associated with land cover/ use, weather, and time of year.

Arbovirus prevalence studies have been conducted in the southeastern U.S. including Georgia, but those studies have been conducted during times of known arbovirus epidemics (Chamberlain et al. 1969, Godsey et al. 2005, Gibbs et al. 2006b). This study aimed to provide more information on the transmission of arboviruses in the environment under baseline, non-epidemic levels, specifically in a rural area. Finally, while host-feeding patterns studies are prominent throughout the states surrounding Georgia, the host-feeding patterns research conducted in Georgia has been very limited (Love and Smith 1958, Chamberlain et al. 1969, Apperson et al. 2004, Richards et al. 2006, Rodrigues and Maruniak 2006, Savage et al. 2007, Burkett-Cadena et al. 2008). In an effort to provide more complete information on the ecology of mosquitoes in southwest Georgia, data on host-feeding patterns was also collected. The information on community composition, arbovirus prevalence, and host-feeding patterns gained from this study will be built upon in future projects that will use small, isolated wetlands as ideal model systems to examine linkages between environmental change, complex food webs, and ecology of mosquito-borne disease in both natural areas and transformed areas (Kirkman et al. in revision).

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CHAPTER 2

SPATIAL AND TEMPORAL VARIATION OF ADULT MOSQUITOES AT ICHAUWAY IN SOUTHWESTERN GEORGIA

ABSTRACT

To determine if mosquito population dynamics were related to weather patterns or land use/ cover at Ichauway, the property of the J. W. Jones Ecological Research Center in southwestern Georgia, mosquito community composition data were compared to weather variables and land use/ cover data during the 2008 mosquito season. Models relating mosquito abundance or presence/absence to weather variables and time of year were compared using Akaike's Information Criterion (AIC) model selection. Precipitation, temperature, humidity, and Keetch-Byram Drought Index were important factors correlated with mosquito abundance or presence/absence for the species considered. A cluster analysis, which grouped sites based on the percentages of land use/ cover and hydric soils in the 1-km radius surrounding collection sites, and an indicator species analysis were used to investigate the associations between mosquito species collected and groups of sites with similar land use/ cover. *Aedes albopictus* (Skuse), *Culex coronator* Dyar & Knab, *Culex quinquefasciatus* Say, and *Culex salinarius* Coquillett were associated with sites that had the most anthropogenic influence, while *Coquillettidia perturbans* (Walker) and *Psorophora ferox* (von Humboldt) were associated natural land cover such as wetlands and forested land.

INTRODUCTION

Understanding the spatial and temporal dynamics of mosquito communities is important not only for implementation of control measures but also for explaining vector-borne disease prevalence within an area (Godsey et al. 2005, DeGroote et al. 2007). Previous studies have determined associations between both climatic and landscape patterns with relative mosquito abundance and transmission of mosquito-borne diseases (Afrane et al. 2005, Leisnham et al. 2006, Pecoraro et al. 2007). Several mosquito-borne viruses circulate in Georgia each year, which are capable of causing disease in humans and other animals (Lance-Parker et al. 2002). The most well-documented arboviruses known to infect humans in the state are St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV), LaCrosse encephalitis virus (family *Bunyaviridae*, genus *Orthobunyavirus*, LACV), Eastern Equine encephalitis virus (family *Togoviridae*, genus *Alphavirus,* EEEV), and West Nile virus (family *Flaviviridae,* genus *Flavivirus,* WNV) (Lance-Parker et al. 2002).

The spatial variation observed in mosquito communities can be influenced by competition, host preferences, and habitat preferences (Zhong et al. 2003, Schäfer et al. 2008). The relationship between habitat preferences and mosquito communities can be examined in depth by relating landscape spatial data to local mosquito abundances through GIS techniques (DeGroote et al. 2007). For example, Diuk-Wasser et al. (2006) modeled the relationship between the distribution of WNV mosquito vectors, land use/cover data, and vegetation indices in Connecticut. Reiter and Lapointe (2007) used GIS to examine the relationship between landscape factors and the spatial distribution and abundance of *Cx. quinquefasciatus*, the primary vector of avian malaria in Hawaii.

Zhou et al. (2007) examined the impact of environmental heterogeneity and larval habitats on the spatial distribution of *Anopheles gambiae* s. s. and *An. funestus* adult mosquitoes, the most important malaria vectors in the highlands of western Kenya, in order to determine malaria transmission foci where control efforts should be directed.

While the relationship between land use/cover and mosquitoes is important, weather variables also influence the population dynamics of mosquitoes by regulating the timing of mosquito larval development and emergence of adult mosquitoes (DeGroote et al. 2007). Relationships between seasonal and daily activity of human-biting mosquitoes and temperature, photoperiod, and precipitation were studied in the lower delta of the Paraná River in Argentina (Loetti et al. 2007). Reisen et al. (2008) modeled the risk of encephalitis virus infection by comparing the relationships between *Culex tarsalis* Coquillett abundance, temperature, precipitation and snowpack in California over a 50-year period. Pecoraro et al. (2007) examined the population dynamics of WNV vectors in Seattle, Washington using a landscape factor, degree of urbanization, as well as the climatic factors of temperature and precipitation.

Several studies have reported occurrence and distribution of specific mosquito species in Georgia (Smith and Floore 2001, Gray et al. 2005, Kelly et al. 2008, Moulis et al. 2008). Other recent studies have examined the relationship between land use/ cover data, human demographic data, climate ecological correlates, and WNV infection in birds (Gibbs et al. 2006, Bradley et al. 2008). However, none of these studies examined the relationship between potential mosquito vectors and ecological factors in the Gulf Coastal Plain of southwestern Georgia. To determine if mosquito population dynamics were related to weather patterns or landscape, I compared mosquito community composition data to selected weather variables and land use/ cover data during the 2008 mosquito season.

MATERIALS AND METHODS

Study area

The study occurred at Ichauway, the property of the J. W. Jones Ecological Research Center in southwestern Georgia. Ichauway is a remnant 11, 736 hectare longleaf pine-wiregrass ecosystem, and the landscape immediately surrounding Ichauway is dominated by center-pivot irrigated row crop agriculture (Michener et al. 1998, Smith et al. 2006). Elevation at Ichauway ranges from 27 m to 61 m above mean sea level. The climate is humid sub-tropical with annual average rainfall of 1310 mm and average daily temperatures ranging between 21-34 °C in summer and 5-17 °C in winter (Martin and Kirkman 2009).

Mosquitoes were collected from eight sites at Ichauway: Big Slough, Collins Pond, Flint Riparian, Horse Hammock Slough, Mim’s Store Drain, Parmalee Pond, Richardson Flat, and Hall Corner Pond (Figure 2.1). Big Slough and Horse Hammock are riparian sloughs along Ichawaynochaway Creek with their canopies dominated by bald cypress (*Taxodium distichum*). Collins Pond is a cypress gum swamp with the dominant tree species being swamp black gum (*Nyssa sylvatica* var. *biflora*) and bald cypress (*Taxodium ascendens*), sparse ground-flora, and dark-stained waters that have low dissolved oxygen levels (Watt and Golladay 1999, Kirkman et al. 2000, Battle and Golladay 2001). The Flint riparian site is located in the southern portion of Ichauway property, north of the Flint River’s confluence with the Ichawaynochaway Creek. The land use on the side of the Flint River opposite from Ichauway property is dominated by pine plantations and center-pivot agriculture. The Mim's Store site drains wetlands nearby. Its over story canopy is dominated by hardwood trees such as oaks (*Quercus* spp.). Parmalee Pond is a cypress savanna characterized by clay soils, an interspersed ground-flora composed mainly of panic grasses *Pancium* spp. and broomsedge (*Andropogon virginicus* L.), and an over story canopy of pond cypress (Kirkman et al. 2000). Richardson Flat is a grass-sedge marsh with dense ground-flora dominated by panic grasses and cutgrass (*Leersia hexandra* Sw.) (Kirkman et al. 2000). Hall Corner Pond is a hardwood depression on the edge of Ichauway property that receives runoff from an adjacent agricultural area (Figure 2.1).

Sampling methods

The locations for collection sites were selected by initially creating two transects across the property and picking eight points off those transects. Trap placement at each site was determined by selecting an area that had the potential for producing mosquitoes. Population abundance data were obtained through weekly trapping at each site from mid-May through early November 2008. The order in which sites were sampled each week was doubly randomized by randomly assigning a number to each of the sites at the beginning of the field season and by using a random number table to determine the sequence each week. Typically, trapping occurred at two sites per night each week.

Adult female mosquitoes were collected using Centers for Disease Control (CDC) miniature light traps and gravid traps (John W. Hock, Gainesville, FL) (Silver 2008). Light traps were baited with dry ice, powered with 6-V batteries, and suspended 1.5 m above the ground. Gravid traps were baited with hay-infused water and powered with 6-V batteries. Traps were operated from late afternoon until early the next morning. Collected mosquitoes were transported live to the laboratory and anaesthetized using triethylamine. Adults were identified to species using standard keys (Darsie and Morris (2003) and Darsie and Ward (2005)). Nomenclature used here is based on Darsie and Ward (2005). In August 2008, Tropical Storm Fay resulted in 33 cm of rainfall over a 96 hr period. For collections that resulted in large quantities of mosquitoes after the tropical storm, the total number of individuals collected and species composition were estimated by weight to the nearest 0.01 g. For these estimations, three sets of 100 randomly chosen individuals of mixed species were weighed, identified, and the mean was used to estimate the total catch, cf. Schäfer et al. (2008).

Weather data

For the entire field season, data on relative humidity, precipitation, temperature, and Keetch-Byram Drought Index, which estimates the amount of soil moisture by tracking daily maximum temperatures and rainfall (Dolling 2009), were obtained from the Georgia Automated Environmental Monitoring Network weather station on Ichauway property (listed as the Newton station at www.georgiaweather.net). Average temperature and relative humidity were calculated for the following time periods preceding each mosquito sampling date: 1 d, 1 wk, and 2 wk. Cumulative precipitation was calculated for the following time periods preceding each mosquito sampling date: 2 wk, 4 wk, and 6 wk. These data were used to compare weather conditions with mosquito abundance or presence/absence.

Spatial analysis

To quantify land use/ cover around sites, a data layer that was mapped at 1:12,000 scale from 1992 color infrared aerial photography and updated frequently to reflect changes in land cover from more recent photography and field observation. The land use/ cover surrounding sites was validated using 2006 color infrared aerial photography, including areas outside of the Ichauway property. Using ArcGIS (ESRI, Redlands, CA, v. 9.1), the percent area of each of the following nine land use/ cover categories were determined within a 1-km radius of each study site (total area = 3.14 km^2): open water, residential/highways, agriculture, scrub, hardwood, bottomland hardwood, pine, forested wetland, and non-forested wetland. In order to quantify the percentage of hydric and non-hydric soil within the 1-km radius of each site, I used a data layer created from the Natural Resources Conservation Service Baker County Soil Survey. For areas within the 1 km radius around sites but outside of the Ichauway property, I used the U.S. Department of Agriculture Soil Conservation Service State Soil Geographic (STATSGO) database for Georgia. The presence of hydric and non-hydric soil was further validated using 2006 color infrared aerial photography.

Data analysis

I developed 52 *a priori* models to explain daily abundance or presence/absence of selected mosquito species using combinations of the following 11 descriptor variables based on the importance of the variables in previous studies and field observation: two week cumulative precipitation (2wkprecip), four week cumulative precipitation (4wkprecip), six week cumulative precipitation (6wkprecip), one day average relative humidity (1drh), one week average relative humidity (1wkrh), two week relative humidity (2wkrh), one day average temperature (d1temp), one week average temperature (wk1temp), and two week average temperature (2wktemp), Keetch-Byram Drought Index (DI), and time (Table 2.1). I analyzed daily log-transformed *Aedes vexans* (Meigen) abundance as a function of all 52 *a priori* models using PROC GENMOD (SAS Institute Inc., Cary, NC). Due to presence of multiple zero values in abundance data for all other mosquito species considered besides *Ae. vexans*, I analyzed daily presence/absence of other species as a function of all 52 *a priori* models using PROC LOGISTIC (SAS Institute Inc., Cary, NC). I used the second-order Akaike's Information Criteria (AIC_c) for small sample sizes to identify the best model for each mosquito species and to predict variable importance (Burnham and Anderson 2002, Conner et al. in press). I used a Hosmer and Lemeshow (2000) test to evaluate goodness-of-fit for the global model for all species considered (Burnham and Anderson 2002, Conner et al. in press). I considered the model with the lowest AIC_c to be the best model, but included all models with $\Delta AIC_c < 2.0$ from the best model as the best set of approximating models (Burnham and Anderson 2002, Conner et al. in press). I calculated Akaike weights for each model as an estimate of the probability of the model being the best model of the *a priori* set. I then calculated variable weights by summing model weights for all models containing the variable. Once all model weights and variable weights were calculated, I used model averaging to calculate parameter estimates and unconditional standard errors for all variables (Burnham and Anderson 2002, Conner et al. in press). Predictor variables were considered useful if the 95% confidence interval surrounding the parameter estimate did not contain zero and the variable weight was high (i.e., > 0.80) (Miles et al. 2006, Miller and Conner 2007, Conner et al. in press).

A cluster analysis was used to group sites based on percentages of land use/ cover and hydric soils in the 1-km radius surrounding sites using PC-ORD (MjM Software Design, v.5.10) with the Euclidean distance measure and Ward's group linkage method.

Once sites were clustered, the associations between groups of sites and a subset of mosquito species collected, selected based upon their abundance in sampling collection and/or their status as a potential arbovirus vector, were assessed using the indicator species analysis (ISA) in PC-ORD (Dufrene and Legendre 1997). The following mosquitoes species were used in statistical analyses: *Ae. albopictus, Ae. vexans, Cq. perturbans, Cx. coronator, Culex erraticus* (Dyar & Knab), *Culex nigripalpus* Theobald, *Cx. quinquefasciatus, Culex restuans* Theobald, *Cx. salinarius, Ochlerotatus triseriatus* (Say), and *Ps. ferox* (Turell et al. 2005, Cupp et al. 2007, Kelly et al. 2008). The ISA was performed on log-transformed weekly abundance data collected from each site grouped according to the cluster analysis results. The indicator species analysis produces indicator values (IV) for each species based on the concentration of species abundance and faithfulness of occurrence in a particular group (McCune et al. 2002). If the P-value for the indicator value is statistically significant, then the species is considered to be an indicator of that group (McCune et al. 2002). An IV closer to 100 indicates a stronger species association with that group (McCune et al. 2002). Indicator values are tested for statistical significance using the Monte Carlo randomization method (McCune et al. 2002).

RESULTS

Mosquito species

Approximately 58,000 mosquitoes were collected over 355 trap nights from 78 dates. The mosquitoes collected belonged to 30 species within the following nine genera: *Aedes, Anopheles, Coquillettidia, Culiseta, Culex, Ochlerotatus, Orthopodomyia, Psorophora*, and *Uranotaenia* (Table 2.2). Four species (*Ae. vexans* (50 %), *Ps. ferox*

(25%), *Oc. sticticus* (4%), and *An. crucians* (3%)) comprised over 80% of the total mosquitoes collected. *Ae. vexans*, the most abundant species collected, was present from mid-May through the beginning of November (Figure 2.2). *Ps. ferox* reached its highest population density during week 37 of the field season, approximately two weeks after the largest rainfall event of the season (Tropical Storm Fay). *Oc. sticticus* was collected in small numbers until it also exhibited a peak in population density during week 37 of the field season. *An. crucians* was most abundant early in the season and its population fluctuated greatly.

Weather patterns

Over the sampling period, temperatures ranged from -0.4°C to 37.4 °C. Average daily humidity ranged from 45.1% to 98.2%. The total precipitation for the study period was 50.62 cm. Weather patterns had a strong influence on the mosquito species modeled, but predictive variables varied by species. The best model ($w = 0.4632$) for predicting daily abundance of *Ae. vexans* over time contained all precipitation variables considered, one day average relative humidity and temperature, one and two week average temperature, and time (Table 2.3). The best model for predicting the presence/absence of all other mosquito species considered varied by species (Table 2.3). The results of Hosmer and Lemeshow (2000) tests suggested that all global models satisfactorily fit the data.

Focusing on the importance of individual variables through the use of model averaged parameter estimates suggested that daily abundance of *Ae. vexans* was best predicted by the following variables: total precipitation for two and six weeks, average temperature for one day and two weeks, and average relative humidity for one day (Table

2.4). Of the variables examined, the daily presence/ absence of *Ae. albopictus* was best predicted by average relative humidity for one and two weeks. *Cx. coronator* was best described by total precipitation for four weeks and time of year, while *Cx. erraticus* was best described by these variables as well as average relative humidity for one day and one week. Six week total precipitation, one day average temperature, and drought index were most useful in describing *Cx. nigripalpus* daily presence/ absence. The pattern of *Cx. restuans* presence/ absence was best described by one day average temperature. Six week total precipitation best described presence/absence of *Oc. triseriatus*.

Spatial distribution

The cluster analysis grouped sites based on percentages of land use/ cover and hydric soils into the following three assemblages: Group 1: Big Slough, Collins Pond, and Horse Hammock, Group 2: Mim's Store Drain and Richardson Flat, Group 3: Flint Riparian, Hall Corner Pond, and Parmalee Pond (Figure 2.3). Of the 11 species considered, the ISA identified seven species with significant indicator values (5% level of significance) for one of the three groups of sites (Table 2.5). *Ps. ferox* (IV = 27.5, P = 0.002) was the only species associated with the sites Big Slough, Collins Pond, and Horse Hammock. *Ae. albopictus* (IV = 17.7, P = 0.0270) and *Cq. perturbans* (IV = 12.8, P = 0.0062) were both associated with Mim's Store Drain and Richardson Flat. *Cx. coronator* (IV = 17.1, P = 0.0484), *Cx. quinquefasciatus* (IV = 34.7, P = .0056), and *Cx. salinarius* (IV = 28.8, P = 0.0002) were all associated with Flint Riparian, Hall Corner Pond, and Parmalee Pond.

DISCUSSION

Results of the AIC analysis indicated that total precipitation over two weeks and six weeks, average temperature over one day and two weeks, and daily average relative humidity were the most influential weather variables determining *Ae. vexans* daily abundance. The positive correlation with six weeks total precipitation is likely due to this mosquito being a floodwater species, and immature stages are often found in temporary rain-filled pools (Carpenter and LaCasse 1955). However, its negative correlation with two week precipitation may be related to *Ae. vexans* preferring to lay its eggs on moist soil rather than directly on the surface of standing water. The importance of average temperature over two weeks prior to the collection date is probably related to the effect of temperature on larval development time and emergence (Shone et al. 2006). The positive correlation between *Ae. vexans* daily abundance and average temperature on the day of collection is potentially a reflection of warm temperatures encouraging host-seeking (Shone et al. 2006). Daily average relative humidity influences the survival of mosquitoes and could certainly also contribute to the abundance pattern observed for *Ae. vexans.*

Ae. albopictus is susceptible to body fluid loss at low relative humidity (Hylton 1967), which may explain the importance of average relative humidity on the daily presence/absence of this species. Alto and Juliano (2001) found that while increased temperature alone resulted in greater production of adults, increased temperature coupled with decreased precipitation led to higher adult mortality. The results of my study agree with Alto and Juliano (2001), who predicted that *Ae. albopictus* will not be able to establish itself successfully in arid regions even if warmer temperatures might allow for expansion of its range in North America.

Cx. coronator and *Cx. erraticus* are late season species that lay their eggs on the surface of standing water. This life-history characteristic potentially explains the importance of four week total precipitation and time of year in modeling their presence/absence. Daily average relative humidity influences the survival of mosquitoes and could certainly also contribute to the daily presence/absence pattern observed for *Cx. erraticus*. Boiling et al. (2005) found the most important variable of interest affecting *Cx. erraticus* abundance to be temperature. Robertson et al. (1993) found peaks in *Cx. erraticus* abundance in North Carolina in summer and early fall which is consistent with the patterns documented in this study.

The negative correlations between daily presence/absence of *Cx. nigripalpus*, six weeks total precipitation, and Keetch-Byram Drought Index documented in my study are consistent with the observations of Shaman et al. (2003), which found that antecedent drought, coincident wetting, and the emergence of *Cx. nigripalpus* were all associated with increased prevalence of SLEV in wild birds in southern Florida. The negative correlation with average temperature on the day of collection observed for the presence/absence of both *Cx. nigripalpus* and *Cx. restuans* is most likely because while warm temperatures can encourage host-seeking and oviposition (Shone et al. 2006), temperatures too high can lead to the desiccation of mosquitoes. Using ovitrapping, Jackson and Paulson (2006) documented peaks in *Cx. restuans* abundance in late June, mid-July, and August in southwestern Virginia.

Oc. triseriatus is a tree hole/container breeder and its negative correlation with six weeks total precipitation is likely related to the effect of rainfall on the volume and persistence of water within tree holes and other potential breeding sites. Excessive rainfall may cause some tree holes to overflow, which could lead to a loss of eggs, larvae, and pupae. In a survey of tree holes in Pennsylvania, Paradise (2004) reported that higher densities of *Oc. triseriatus* larvae were always found in tree holes that maintained high water volumes. *Cq. perturbans, Cx. quinquefasciatus, Cx. salinarius*, and *Ps. ferox* presence/absence were not predicted using weather variables in this study. However, associations between these species and certain sites revealed through the ISA suggested a land use/ cover link for these species. My population data were not collected independently of sites, so microclimatic factors cannot be eliminated as potentially influencing their presence/absence.

The associations between the selected mosquito species and groups of sites revealed in the ISA seem logical based on the ecology of the species and similarity of the land use/ cover surrounding sites within each group. The sites within group one (Big Slough, Horse Hammock, Collins Pond) are forested wetlands that have surrounding land use/ cover comprised of primarily coniferous forest with little cultivated land (Figure 2.4; Table 2.6). *Ps. ferox*'s association with these sites reflects its preference for breeding in woodland pools (King 1960).

The ISA-based association between *Cq. perturbans* and group two sites is likely because it was collected in greatest abundance from Richardson Flat, the site with the largest percentage of wetland within its 1-km radius (Figure 2.4). Rochlin et al. (2008b) found that abundance of *Cq. perturbans* in Suffolk County, New York increased with wetland land use/ cover area surrounding a site.

Ae. albopictus is associated with group two due to its abundance at Mim's Store Drain (Table 2.2). Its low IV for the group, 17.7, is because it was also trapped in comparable abundance at Parmalee Pond, a site delegated into group three by the cluster analysis (Table 2.1). Although natural breeding sites for *Ae. albopictus* (i.e., tree holes) could be found at all sites, the abundance of this species collected from Mim's Store Drain and Parmalee Pond is probably due to human influence. Mim’s Store Drain is located near a residential area of Ichauway, and Parmalee Pond is located near a large farming operation adjacent to Ichauway property. Both sites have artificial containers nearby that would allow for increased breeding of *Ae. albopictus*. In a recent north central Florida study, Obenauer et al. (2009) found that 98% of *Ae. albopictus* captures occurred at suburban sites compared to forested sites, and the site with the greatest abundance of *Ae. albopictus* was closest to a major highway and residential areas.

Cx. coronator’s association with group three sites (approximately 25-40% of their 1-km radius in cultivated land) is potentially important. *Cx. coronator*, a species common to the American tropics, has recently expanded its range in the southeastern United States (Gray et al. 2008). Although observations on the occurrence and distribution of *Cx. coronator* larvae and adults in the southeastern United States exist (Goddard et al. 2006, Gray et al. 2008, Kelly et al. 2008, Moulis et al. 2008), this report is the first to attempt to associate *Cx. coronator* adult distribution with land use/ cover. WNV and SLEV have been detected in *Cx. coronator* mosquitoes. The impact this species will have on arbovirus transmission in areas where it has been recently found is unknown (Aitken et al. 1969, Kelly et al. 2008). Studying the link between the distribution of this species, its life history, and land use/ cover in the southeastern United States is important to understanding its potential as a disease vector.

Cx. salinarius' relatively high IV coefficient of 28.8 with group three is most likely due to 95% of its total abundance trapped at Hall Corner Pond (Table 2.2). *Cx. salinarius* breeds in fresh and saltwater marshes, lakes, ponds, but can also be found in water polluted with organic matter (Rochlin et al. 2008a). Hall Corner Pond receives runoff from an adjacent agricultural area and has higher nutrient and suspended solids concentrations compared to undisturbed wetlands (S. Golladay, J.W. Jones Research Center, unpublished data), which makes it a suitable breeding site for *Cx. salinarius*. *Cx. quinquefasciatus*, the primary vector of WNV in Georgia, is also known to breed in water rich in organic matter (Calhoun et al. 2007). Its IV coefficient of 34.7 was the highest of all species examined for the group of sites with the least natural land cover. In a study of the spatial distribution and abundance of *Cx. quinquefasciatus* in Hawaii, Reiter and Lapointe (2007) found agricultural lands and forest fragmentation significantly increased the abundance of this species, similar to the observations of its distribution in my study.

Ae. vexans' lack of association with any group of sites is potentially due to weather conditions during the field season that allowed this species to successfully breed at all sites. Substrate moisture is probably the most important factor acting as an oviposition attractant for *Ae. vexans* (Horsfall et al. 1975). Given the large amount of rainfall received during the field season due to a tropical storm and the presence of hydric soils at sites (Table 2.6), substrate moisture was high throughout the study area. *Ae. vexans* was also widespread and the most abundant species collected in a previous survey of 20 southern Georgia counties (Smith and Floore 2001). *Oc. triseriatus* was most likely not significantly associated with any groups because of the species' propensity for breeding in tree holes and artificial containers (Paradise 2004). The presence of deciduous trees where tree holes are common is not limited to one group of sites (Figure 2.4). *Cx. restuans*, *Cx. nigripalpus*, and *Cx. erraticus* were not significantly associated with any groups of sites possibly because these species were trapped in relatively equal numbers from each of the groups (Table 2.2).

This study showed that weather and land use/ cover affect the temporal and spatial distribution of mosquitoes in southwestern Georgia. However, it is important to note that the seasonal distribution and abundance of mosquitoes documented here reflect a year that had extreme drought conditions followed by a tropical storm event. This may not apply to years when different climatic conditions prevail. This study was as an initial exploratory analysis of mosquito ecology in southwestern Georgia, but it demonstrated that the population dynamics of certain mosquitoes can be predicted using regional climate and land use/ cover information. In order to further understand the ecology of mosquitoes, and arbovirus vectors in particular, larval and adult sampling is needed at replicated sites across a wider range of disturbed conditions in southwestern Georgia for a longer time period in the future.

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Table 2.1. Models used in the second-order Akaike's Information Criteria (AIC$_c$) analysis

wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh wk_2_rh d1_temp wk_1_temp wk_2_temp DI time
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_2_rh d1_temp wk_1_temp wk_2_temp DI time
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh d1_temp wk_1_temp wk_2_temp DI time
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp DI time
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp DI
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh wk_2_rh d1_temp wk_1_temp wk_2_temp time
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh d1_temp wk_1_temp wk_2_temp time
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp time
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh wk_2_rh d1_temp wk_1_temp wk_2_temp
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh d1_temp wk_1_temp wk_2_temp
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp
wk_2_precip wk_4_precip wk_6_precip d_1_rh DI time
wk_2_precip wk_4_precip wk_6_precip d_1_rh DI
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh wk_2_rh time
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_2_rh time
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh time
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wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh wk_2_rh
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_2_rh
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh
wk_2_precip wk_4_precip wk_6_precip d_1_rh
wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp DI
wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp DI
wk_6_precip d_1_rh d1_temp DI
wk_6_precip wk_2_temp DI
wk_6_precip d1_temp DI
wk_6_precip wk_2_temp
wk_6_precip d_1_rh
wk_4_precip wk_6_precip d_1_rh
wk_4_precip wk_6_precip time
wk_2_precip wk_4_precip wk_6_precip time
wk_2_precip wk_4_precip wk_6_precip
wk_2_precip wk_6_precip wk_2_temp time
wk_2_precip wk_6_precip wk_2_temp
wk_2_precip wk_4_precip wk_2_temp
wk_2_precip wk_4_precip d_1_rh
wk_2_precip wk_6_precip d_1_rh
wk_2_precip d_1_rh wk_2_temp
wk_2_precip DI wk_2_temp
wk_2_precip d_1_rh DI
wk_2_precip
wk_4_precip
wk_6_precip
wk_2_rh
wk_1_rh
d_1_rh
DI
d1_temp
wk_1_temp
wk_2_temp
time

Table 2.2. Number of mosquitoes per species collected during May- November 2008 at eight sites in Ichauway, the site of the J. W. Jones Ecological Research Center in southwestern Georgia. Location of sites can be found in Figure 2.1.

Species	Big Slough	Collins Pond	Flint Riparian	Hall Pond	Horse Hammock	Mim's Drain	Parmalee Pond	Richardson Flat
Ae. albopictus (Skuse)	2	1	11	7	3	29	30	5
Ae. vexans (Meigen)	2730	5887	3679	3384	3007	3080	5008	2428
Oc. atlanticus (Dyar & Knab)	0	3	0	0	0	0	1	2
Oc. canadensis canadensis (Theobald)	71	209	0	0	8	61	531	34
Oc. fulvus pallens (Ross)	66	9	6	3	137	6	7	5
Oc. infirmatus (Dyar & Knab)	188	297	2	21	202	79	86	15
Oc. mitchellae (Dyar)	3	50	0	8	28	5	418	21
Oc. sticticus (Meigen)	1364	136	6	10	641	239	40	34
Oc. thibaulti (Dyar & Knab)	10	9	0	0	6	0	6	0
Oc. triseriatus (Say)	23	27	13	5	36	104	55	4
An. crucians Weidemann	105	870	14	654	39	14	102	82
An. punctipennis (Say)	75	25	25	9	56	51	1	5
An. quadrimaculatus Say	14	51	38	94	7	8	18	8
Cq. perturbans (Walker)	0	2	1	1	3	2	7	12
Cs. melanura (Coquillett)	0	1	0	0	0	1	0	0
Cx. coronator Dyar & Knab	3	3	10	85	7	22	58	69
Cx. erraticus (Dyar & Knab)	71	6	24	88	22	25	5	48
Cx. nigripalpus Theobald	83	45	42	150	28	39	9	112
Cx. quinquefasciatus Say	59	28	169	359	51	146	169	279
Cx. restuans Theobald	13	18	80	68	31	11	3	31
Cx. salinarius Coquillett	40	5	21	1544	9	3	0	9
Cx. territans Walker	35	37	12	7	40	9	10	4
Or. signifera (Coquillett)	4	2	2	2	1	5	3	0
Ps. ciliata (Fabricius)	1	292	2	307	34	0	135	27
Ps. columbiae (Dyar & Knab)	1	32	37	75	2	1	45	97
Ps. cyanescens (Coquillett)	9	10	170	384	1	5	160	20
Ps. ferox (von Humboldt)	7203	426	17	76	6094	271	341	5
Ps. howardii Coquillett	17	36	13	41	34	0	64	0
Ps. mathesoni Belkin & Heinemann	4	8	0	0	0	0	0	0
Ur. sapphirina (Osten Sacken)	11	18	0	7	12	2	2	1
Total	12205	8543	4394	7389	10539	4218	7314	3357

Table 2.3. Best fitting models explaining the effects of weather variables and time on mosquito abundance or presence/absence for all species considered. The number of predictor variables (*K*) in each model included the intercept term. Models with lower second order Akaike's information criterion difference (Δ AIC$_c$) and greater Akaike weight (w_i) were more supported by data.

Model	K	Δ AIC$_c$	w_i
Ae. albopictus			
wk_2_rh wk_1_rh wk_2_temp	4	0.0000	0.4409
wk_2_temp wk_1_rh wk_2_rh d_1_rh	5	1.7474	0.1840
wk_2_rh wk_1_rh wk_2_temp wk_1_temp	5	1.7534	0.1835
Ae. vexans			
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp time	9	0.0000	0.4632
Cq. perturbans			
wk_1_temp d1_temp wk_1_rh	4	0.0000	0.1311
wk_1_rh d_1_rh	3	0.2444	0.1160
wk_1_temp wk_1_rh	3	0.2564	0.1153
wk_2_temp wk_1_rh	3	0.5254	0.1008
wk_1_rh	2	1.3651	0.0662
wk_1_temp d1_temp wk_1_rh wk_2_rh	5	1.5734	0.0597
wk_1_temp d1_temp d_1_rh wk_1_rh wk_2_rh	6	1.6302	0.0580
wk_2_precip wk_1_temp d1_temp wk_1_rh	5	1.7264	0.0553
Cx. coronator			
wk_2_precip wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp time	9	0.0000	0.3016
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh d1_temp wk_1_temp wk_2_temp time	10	1.2245	0.1635
wk_2_precip wk_4_precip wk_6_precip d_1_rh DI time	7	1.6749	0.1306
Cx. erraticus			
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh time	7	0.0000	0.4803
wk_2_precip wk_4_precip wk_6_precip d_1_rh wk_1_rh wk_2_rh time	8	1.0710	0.2812
Cx. nigripalpus			
wk_4_precip wk_6_precip wk_2_rh d1_temp wk_2_temp DI	7	0.0000	0.1887
wk_4_precip wk_6_precip wk_2_rh d1_temp wk_1_temp wk_2_temp DI	8	0.2620	0.1655
wk_4_precip wk_6_precip wk_1_rh d1_temp wk_1_temp wk_2_temp DI	8	0.3060	0.1619
wk_4_precip wk_6_precip wk_2_rh d1_temp wk_1_temp DI	7	0.5600	0.1426
wk_4_precip wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp DI	8	1.2600	0.1005
wk_6_precip d_1_rh d1_temp wk_1_temp wk_2_temp DI	7	1.9430	0.0714

Table 2.3 continued. Best fitting models explaining the effects of weather variables and time on mosquito abundance or presence/absence for all species considered. The number of predictor variables (*K*) in each model included the intercept term. Models with lower second order Akaike's information criterion difference (Δ AIC$_c$) and greater Akaike weight (w_i) were more supported by data.

Model	K	Δ AIC$_c$	w_i
<u>*Cx. quinquefasciatus*</u>			
wk_6_precip d1_temp wk_1_temp wk_1_rh	5	0.0000	0.2072
wk_6_precip wk_2_precip wk_1_temp d1_temp wk_1_rh	6	1.4628	0.0997
wk_6_precip wk_1_temp d1_temp wk_2_rh	5	1.5810	0.0940
wk_6_precip wk_4_precip wk_1_temp d1_temp wk_1_rh	6	1.9758	0.0771
<u>*Cx. restuans*</u>			
wk_2_precip wk_6_precip wk_2_rh d1_temp wk_1_temp	6	0.0000	0.2824
wk_2_precip wk_6_precip wk_1_rh d1_temp wk_1_temp	6	0.7540	0.1937
wk_2_precip wk_4_precip wk_6_precip wk_2_rh d1_temp wk_1_temp	7	1.9639	0.1058
wk_2_precip wk_6_precip wk_1_rh wk_2_rh d1_temp wk_1_temp	7	1.9969	0.1041
<u>*Cx. salinarius*</u>			
wk_4_precip	2	0.0000	0.0822
wk_1_rh	2	0.0460	0.0803
wk_1_temp	2	0.2930	0.0710
wk_2_temp	2	0.3440	0.0692
Time	2	0.3790	0.0680
wk_4_precip wk_2_temp	3	0.5073	0.0637
wk_4_precip wk_1_rh d_1_rh	4	0.5959	0.0610
wk_2_rh	2	1.2510	0.0439
wk_2_precip	2	1.8850	0.0320
d1_temp	2	1.9290	0.0313
<u>*Oc. triseriatus*</u>			
wk_4_precip wk_6_precip	3	0.0000	0.2322
wk_4_precip wk_6_precip d_1_rh	4	0.5726	0.1744
wk_6_precip d_1_rh	3	1.3860	0.1161
<u>*Ps. ferox*</u>			
wk_6_precip wk_1_temp DI time	5	0.0000	0.1650
wk_6_precip wk_2_temp DI time	5	0.6480	0.1193
wk_6_precip d1_temp DI	4	0.8996	0.1052
wk_6_precip wk_2_temp DI	4	0.9596	0.1021
wk_6_precip d1_temp DI time	5	1.1510	0.0928
wk_6_precip wk_2_rh wk_1_temp DI time	6	1.5478	0.0761
wk_2_precip wk_6_precip wk_1_temp DI time	6	1.7068	0.0703

Table 2.4 Model-averaged parameter estimates, their unconditional standard errors (SE), 95% confidence intervals (CI), and variable weights for parameters used to predict daily abundance or presence/absence of selected mosquito species during the 2008 mosquito season in southwestern Georgia, USA. Data are only presented for those parameters with 95% confidence intervals that did not contain zero and with a high variable weight.

Variable	Coefficient	SE	95% CI	Variable Weight
Ae. albopictus				
wk_1_rh	0.2382	0.0907	0.0604-0.4161	0.9698
wk_2_rh	-0.3507	0.1149	-0.5759- -0.1254	0.9862
Ae. vexans				
week2precip	-0.0086	0.0032	-0.0148- -0.0023	0.9999
week6precip	0.0041	0.0021	-0.1665- -0.0254	0.9999
d1_temp	0.1827	0.0794	0.0271-0.3384	0.9887
wk_2_temp	-0.6476	0.2654	-1.1678- -0.1274	0.9987
d_1_rh	-0.0630	0.0225	-0.1070- -0.0189	0.9900
Cx. coronator				
week4precip	0.0845	0.0392	0.0077-0.1613	0.9774
time	2.9715	1.5119	0.0081-5.9349	0.9996
Cx. erraticus				
week4precip	0.0734	0.0342	0.0063-0.1405	0.9968
d_1_rh	-0.1076	0.0481	-0.2018- -0.0134	0.9994
wk_1_rh	-0.3143	0.1315	-0.5721- -0.0566	0.9489
time	1.5879	0.7509	0.1162-3.0597	0.8666
Cx. nigripalpus				
week6precip	-0.0754	0.0360	-0.1458- -0.0049	0.9999
d1_temp	-0.9726	0.3813	-1.7200- -0.2252	0.9900
DI	-0.0256	0.0091	-0.0434- -0.0077	0.9724
Cx. restuans				
d1_temp	-0.4468	0.2269	-0.8916- -0.0021	0.9929
Oc. triseriatus				
week6precip	-0.0083	0.0038	-0.0157- -0.0010	0.9999

Table 2.5. Indicator species analysis after Dufrene and Legendre (1997) computing indicator value (IV) coefficient of selected mosquito species across sites grouped by landscape features in southwestern Georgia, USA.

Species	Group	IV	Mean	S. Dev	P*
Ae. albopictus	2	17.7	12.0	2.36	0.0270*
Ae. vexans	1	35.8	34.2	1.44	0.1270
Cx. coronator	3	17.1	12.2	2.45	0.0484*
Cx. erraticus	3	20.1	16.9	2.67	0.1206
Cx. nigripalpus	2	12.5	12.4	2.43	0.3963
Cx. quinquefasciatus	3	34.7	26.0	2.51	0.0056*
Cx. restuans	2	8.8	10.9	2.47	0.8222
Cx. salinarius	3	28.8	11.4	2.46	0.0002*
Cq. perturbans	2	12.8	5.9	1.88	0.0062*
Oc. triseriatus	2	15.7	13.6	2.54	0.1764
Ps. ferox	1	27.5	12.5	2.59	0.0002*

Group = identifier for group with maximum observed IV
* Indicates P is significant at 95% level

Table 2.6. The percent area of nine land use/ cover categories and hydric soils within a 1-km radius of eight study sites (total area = 3.14 km^2) at Ichauway assessed using ArcGIS (ESRI, Redlands, CA, v. 9.1).

Percentage of Land use/ cover and Hydric soils	Big Slough	Collins Pond	Horse Hammock	Mim's Drain	Richardson Flat	Flint Riparian	Parmalee Pond	Hall Pond
Coniferous forest	76.9	79.2	70.8	86.6	64.4	43.0	59.8	54.4
Deciduous forest	1.6	5.1	2.4	3.6	11.8	3.9	1.8	1.8
Bottomland deciduous	3.6	0.0	12.9	2.6	0.0	13.1	0.0	0.5
Forested wetland	0.6	5.1	1.5	0.0	0.1	0.0	5.6	0.2
Non-forested wetland	0.0	0.2	0.0	0.0	22.6	1.3	2.3	3.6
Scrub	4.8	1.2	0.9	0.8	0.0	5.9	1.0	0.0
Agriculture	8.8	8.3	3.4	5.9	1.0	25.8	26.3	39.5
Residential	0.1	0.9	5.2	0.2	0.0	0.1	3.2	0.1
Open water	3.5	0.0	2.9	0.5	0.0	6.9	0.0	0.0
Hydric soils	10.5	19.3	9.5	0.0	24.8	10.3	13.1	8.8

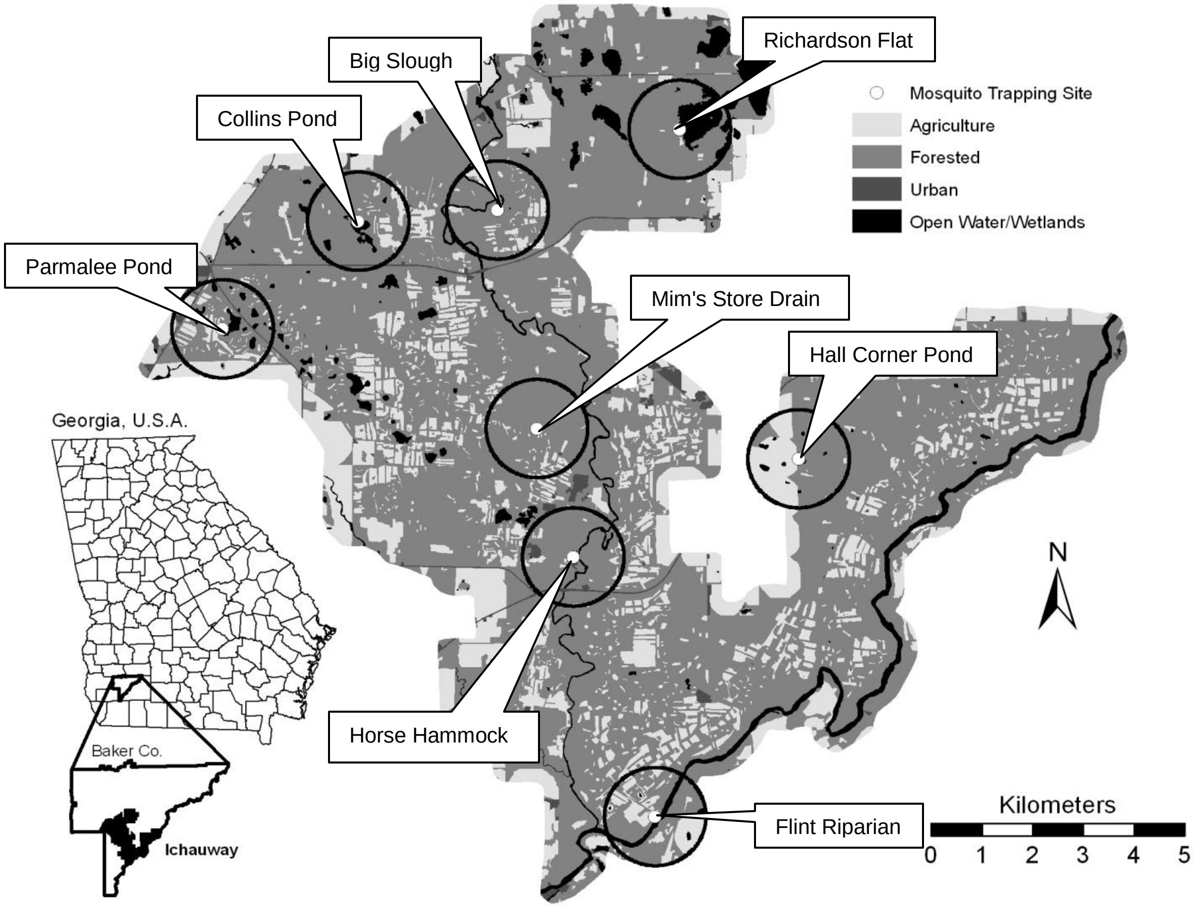

Figure 2.1. The eight sites at Ichauway in southwestern Georgia, USA, where adult mosquitoes were collected from May to November 2008. The 1-km radius surrounding each site that was used in spatial analysis is outlined in black.

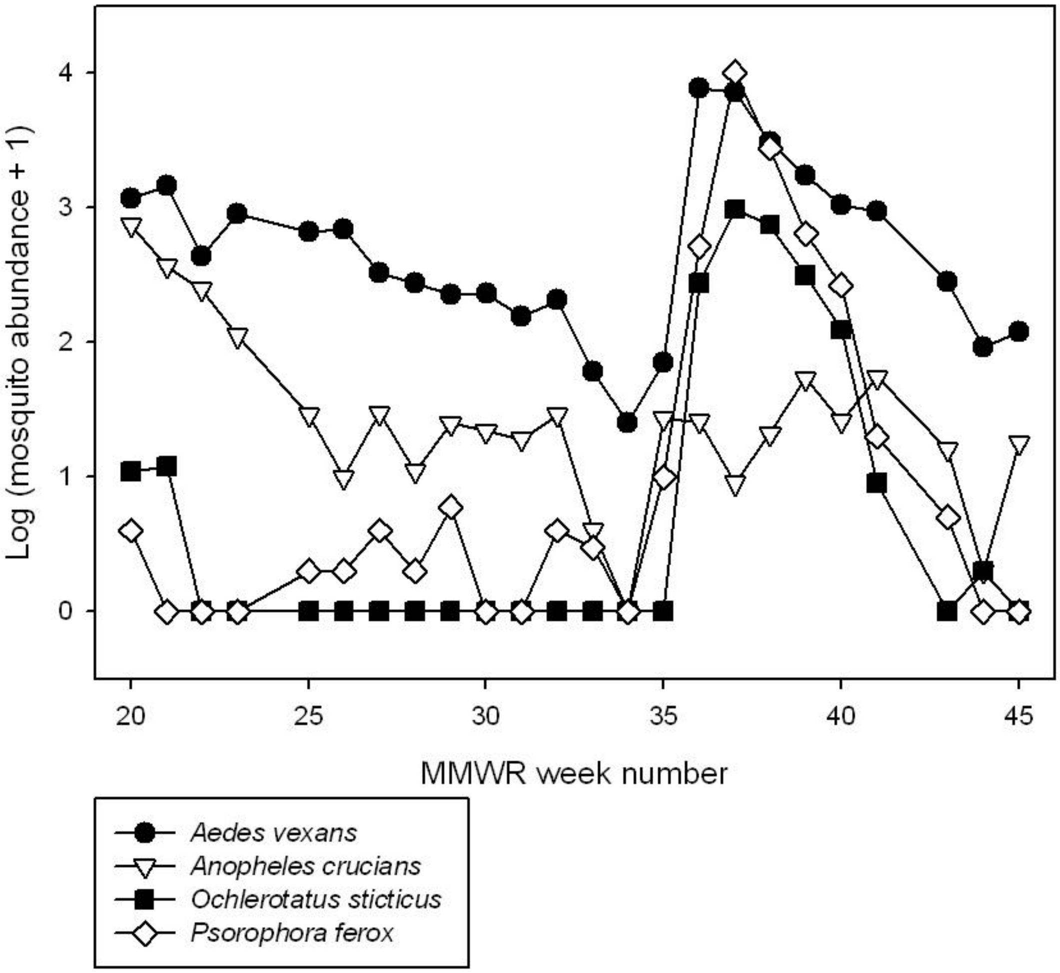

Figure 2.2. Seasonal distribution of the four most abundant mosquito species collected at Ichauway in southwestern Georgia, USA from mid-May to early November 2008. MMWR week number, located on the x-axis, is the method that the Centers for Disease Control (CDC) uses to number weeks during the year in which week one of the year contains the first of January.

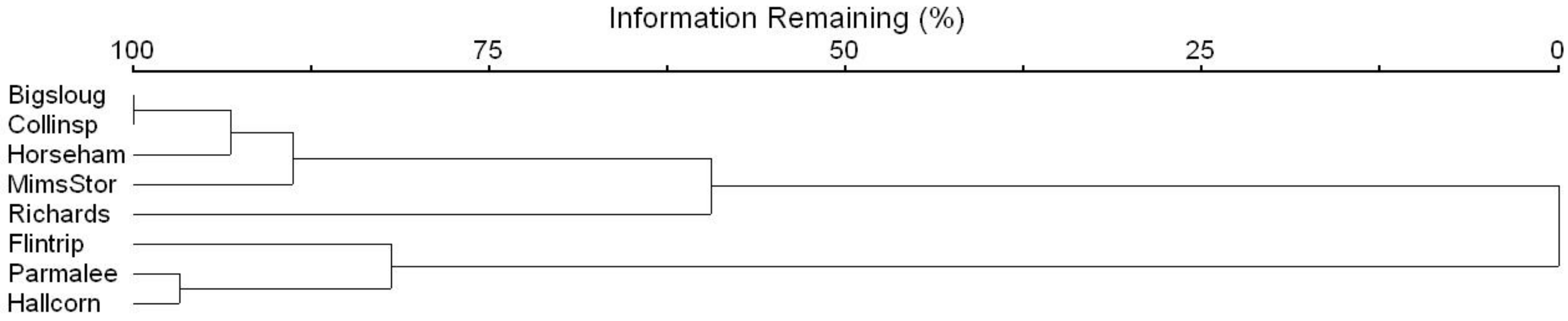

Figure 2.3. A cluster analysis of the percent area of nine land use/ cover categories and hydric soils within a 1-km radius of eight study sites, based on percentage of similarity and with Euclidean distance measure and Ward’s group linkage method.

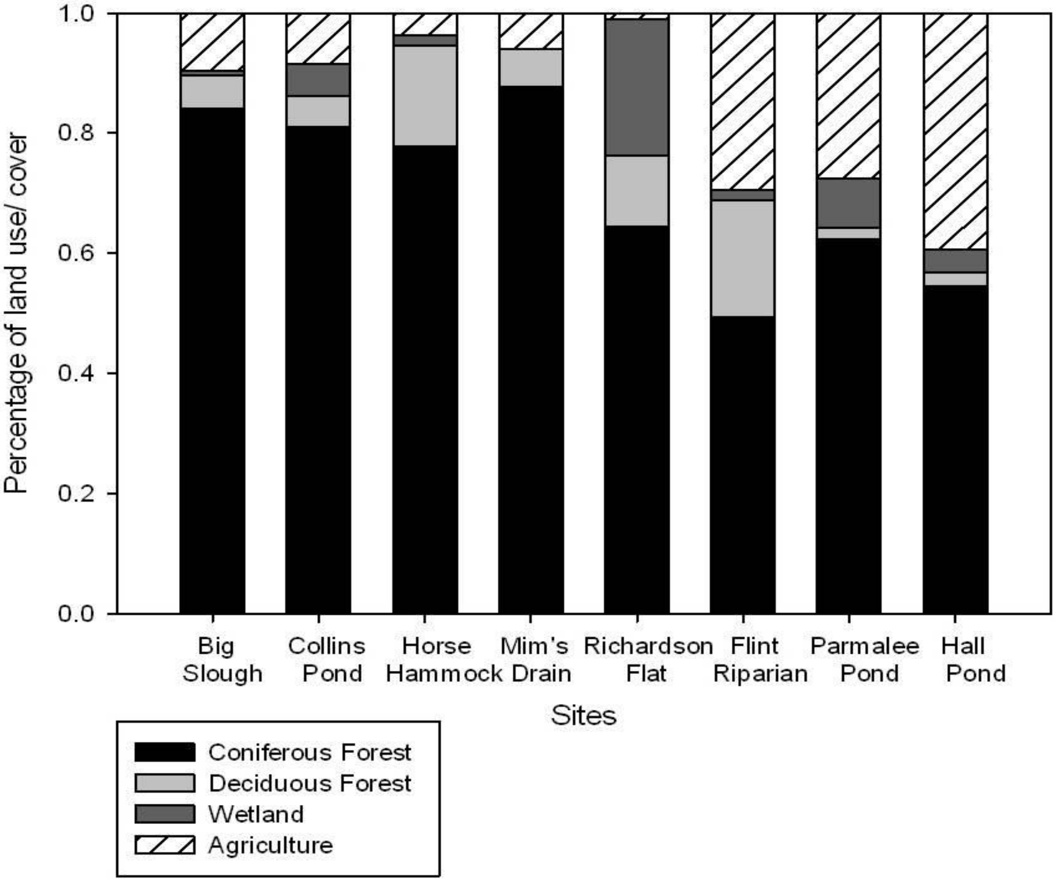

Figure 2.4. Percentage of four dominant land use/ cover categories within 1-km radius of eight collection sites at Ichauway in southwestern Georgia, USA, where adult mosquitoes were collected from mid-May to early November 2008.

CHAPTER 3

ARBOVIRUS PREVALENCE AND HOST-FEEDING PATTERNS OF MOSQUITOES AT ICHAUWAY IN SOUTHWESTERN GEORGIA

ABSTRACT

Data on mosquito community composition, arbovirus prevalence, and mosquito host-feeding patterns were collected over one field season in southwestern Georgia in 2008. Approximately 58,000 mosquitoes were collected over 355 trap nights on 78 dates. Four species (*Aedes vexans* (Meigen) (50%), *Psorophora ferox* (von Humboldt) (25%), *Ochlerotatus sticticus* (Meigen) (4%), and *Anopheles crucians* Wiedemann (3%)) comprised over 80% of the total mosquitoes collected. 9603 mosquitoes (987 pools) were tested for arboviruses. West Nile virus (WNV) was detected in one pool of *Culex quinquefasciatus* Say mosquitoes, and Potosi virus (POTV) was detected in two pools of *Ae. vexans* mosquitoes and one pool of *Anopheles punctipennis* (Say) mosquitoes. The most abundant blood-fed species collected were *Anopheles quadrimaculatus* Say and *Ae. vexans*, which fed predominantly on white-tailed deer. While the host was identifiable for only two *Cx. quinquefasciatus* blood meals, both were birds. No association could be made between WNV occurrence and land cover, while POTV occurrence could be associated with coniferous and deciduous forest. The WNV prevalence documented in this study is potentially related to the diversity of the wildlife in the area and/or low *Culex* mosquito abundance during the amplification phase of the virus.

INTRODUCTION

Many infectious diseases that affect humans and wildlife are caused by pathogens that reside within wildlife hosts. Several of these pathogens are transmitted through arthropod vectors such as mosquitoes (Allan et al. 2009). Because these multi-species interactions are potentially complex and variable, ecological factors can strongly affect epidemiological patterns (Allan et al. 2009). The interaction between susceptible host reservoirs and competent vectors can be influenced by land use/ cover, elevation, human population density, physiographic region, and temperature (Gibbs et al. 2006).

Numerous studies have examined factors that influence the prevalence of arboviruses and their vectors (Kilpatrick et al. 2007, Pecoraro et al. 2007, Bradley et al. 2008, Allan et al. 2009). In Louisiana, WNV infection rates among *Culex* mosquitoes declined with increasing wetland land area surrounding sites (Ezenwa et al. 2007). Mosquito arbovirus prevalence in Houston, Texas was higher in collections made near waste materials and containers that held rainwater (Rios et al. 2006). Lower socioeconomic status of the local community was also related to higher arbovirus prevalence (Rios et al. 2006). Reiter and Lapointe (2007) found agricultural lands and forest fragmentation significantly increased the probability of capturing *Culex quinquefasciatus* Say, the primary vector of avian malaria and avian pox in Hawaii.

Host species choice by mosquitoes is governed by a number of factors such as host availability and abundance, host characteristics such as defensive behavior (e.g. tail swatting) and body mass, as well as flight behavior and feeding periodicity (Clements 1999, Molaei et al. 2008, Hamer et al. 2009, Lyimo and Ferguson 2009). Knowledge of the blood-feeding behavior of mosquito populations is necessary for evaluating their status as vectors and for assessing the role of vertebrates as potential reservoir hosts in the maintenance and amplification of zoonotic pathogens (Molaei et al. 2008). Many studies have examined host feeding patterns of mosquitoes within the United States (Apperson et al. 2004, Gingrich and Williams 2005, Molaei et al. 2008, Hamer et al. 2009). For example, in a long-term investigation in Florida, Edman (1971) found that over 90% of the blood meals from *Aedes*, *Ochlerotatus*, *Coquillettidia*, *Mansonia*, and *Psorophora* species came from mammalian hosts, with the majority being cows, rabbits, and armadillos. In the first study of mosquito host-feeding patterns in the coastal plain of North Carolina, Irby and Apperson (1988) documented that *Cx. quinquefasciatus* and *Culex restuans* Theobald fed primarily on passerine birds. Burkett-Cadena et al. (2008) found that *Culex territans* Walker, *Culex peccator* Dyar & Knab, *Culex erraticus* (Dyar & Knab), and *Culiseta melanura* (Coquillett) fed on ectothermic vertebrate hosts in Alabama's Tuskegee National Forest, with *Cx. peccator* and *Cx. territans* feeding primarily upon cottonmouths and bullfrogs.

Limited research has been conducted on the arbovirus prevalence and mosquito host-feeding patterns in Georgia (Love and Smith 1958, Chamberlain et al. 1969, Godsey et al. 2005, Gibbs et al. 2006, Bradley et al. 2008). Most studies have dealt with information collected over short periods of time, primarily during outbreaks of Eastern Equine encephalitis virus (EEEV) and West Nile virus (WNV) (Chamberlain et al. 1969, Godsey et al. 2005). During one season in 2008, I collected data on the ecology of mosquitoes with a focus on mosquito community composition, arbovirus prevalence, and host-feeding patterns in southwestern Georgia.

MATERIALS AND METHODS

Study area

The study occurred at Ichauway, the property of the J. W. Jones Ecological Research Center in southwestern Georgia. Ichauway is a remnant 11, 736 hectare longleaf pine-wiregrass ecosystem, and the landscape immediately surrounding Ichauway is dominated by center-pivot irrigated row crop agriculture (Michener et al. 1998, Smith et al. 2006). Elevation at Ichauway ranges from 27 meters to 61 meters above mean sea level. The climate is humid sub-tropical with annual average rainfall of 1310 mm and average daily temperatures ranging between 21-34 °C in summer and 5-17 °C in winter (Martin and Kirkman 2009). In 2008, the study area experienced moderate drought conditions, which occur when multiple drought indicators are at levels expected once in 10 years (U.S. Drought Monitor 2008). Then in August 2008, 33 cm of rainfall over a 96 hr period from Tropical Storm Fay resulted in flooding throughout the area.

Mosquitoes were collected from eight sites at Ichauway: Big Slough, Collins Pond, Flint Riparian, Horse Hammock Slough, Mim's Store Road Drain, Parmalee Pond, Richardson Flat, and Hall Corner Pond (Figure 3.1). Big Slough and Horse Hammock are riparian sloughs along the Ichawaynochaway Creek with their canopies dominated by bald cypress (*Taxodium distichum*). Collins Pond is a cypress gum swamp with the dominant tree species being swamp black gum (*Nyssa sylvatica* var. *biflora*) and pond cypress (*Taxodium ascendens*), sparse ground-flora, and dark-stained waters that have low dissolved oxygen levels (Watt and Golladay 1999, Kirkman et al. 2000, Battle and Golladay 2001). The Flint riparian site is located in the southern portion of the Ichauway property, north of the Flint River's confluence with the Ichawaynochaway Creek. The land use on the side of the Flint River opposite from Ichauway property is dominated by pine plantations and center-pivot agriculture. The Mim's Store site drains nearby wetlands. Its over story canopy is dominated by hardwood trees such as oaks (*Quercus* spp.). Parmalee Pond is a cypress savanna characterized by clay soils, an interspersed ground-flora composed mainly of panic grasses *Pancium* spp. and broomsedge (*Andropogon virginicus* L.), and an over story canopy of pond cypress (Kirkman et al. 2000). Richardson Flat is a grass-sedge marsh with dense ground-flora dominated by panic grasses and cutgrass (*Leersia hexandra* Sw.) (Kirkman et al. 2000). Hall Corner Pond is a hardwood depression on the edge of Ichauway property that receives runoff from an adjacent agricultural area (Figure 3.1).

Sampling methods

The locations for collection sites were selected by initially creating two transects across the property and picking eight points off those transects. Trap placement at each site was determined by selecting an area that had the potential for producing mosquitoes. Population abundance data were obtained through weekly trapping at each site from mid-May through early November 2008. The order in which sites were sampled each week was doubly randomized by randomly assigning a number to each of the sites at the beginning of the field season and by using a random number table to determine the sequence each week. Typically, trapping occurred at two sites per night each week.

Adult female mosquitoes were collected using Centers for Disease Control (CDC) miniature light traps and gravid traps (John W. Hock, Gainesville, FL) (Silver 2008). Light traps were baited with dry ice, powered with 6-V batteries, and suspended 1.5 m above the ground. Gravid traps were baited with hay-infused water and powered with 6-V batteries. Traps were operated from late afternoon until early the next morning. Collected mosquitoes were transported live to the laboratory and anaesthetized using triethylamine. Adults were identified to species using standard keys (Darsie and Morris (2003) and Darsie and Ward (2005). Nomenclature used here is based on Darsie and Ward (2005). For collections that resulted in large quantities of mosquitoes after Tropical Storm Fay, the total number of individuals collected and species composition were estimated by weight to the nearest 0.01 g. For these estimations, three sets of 100 randomly chosen individuals of mixed species were weighed, identified, and the mean was used to estimate the total catch, cf. Schäfer et al. (2008).

A subset of the mosquitoes collected in light and gravid traps were sorted into pools of up to 25 individuals based on species, collection date, and collection site and stored at -80˚C for later arbovirus testing. In addition to blood-engorged mosquitoes collected in light and gravid traps, blooded mosquitoes were also collected from two four-cubic-feet resting boxes and tree holes at sites using a backpack aspirator. The two walk-in (4 x 4 x 4 ft.) resting boxes were placed at Mim's Store Drain and Hall Corner Pond, because these sites allowed for the easiest transport of the resting boxes into the field. Mosquitoes resting in vegetation and on the surface of water at sites were also collected using the backpack aspirator. The blood-fed mosquitoes collected were stored at -80˚C for future blood meal analysis.

Virus isolation and identification

For virus testing, mosquito pools of up to 25 individuals were homogenized in 1 ml virus isolation media (Minimum Essential Medium supplemented with 1,000 U penicillin G, 1 mg streptomycin, 0.25 mg gentamicin sulfate, 0.5 kanamycin monosulfate, 2.5 mg/ml amphotericin B, and 1% bovine serum albumin) using a Qiagen® Mixer Mill 300 (Valencia, CA) then clarified by centrifugation (10 min @ 10,000 rpm). A 100 µl aliquot of the resulting supernatant fluid from each sample was inoculated onto a separate well of a 12-well plate with confluent two-day-old Vero Middle America Research Unit (MARU, [Vero M]) cell culture monolayer and incubated at 37°C in a 5% CO_2 atmosphere. Cell cultures were examined daily for seven days for signs of virus replication, which was evidenced by cytopathic effects (CPE) on the Vero cells (Beaty et al. 1989, Schmidt 1989, Karabatsos 1994). Samples not displaying CPE within the first seven days were subpassaged onto two-day-old Vero M cells and observed for an additional seven days. Samples were discarded and reported as negative if CPE were not evident within 14 days. If CPE were observed within a cell plate well, cultures were initially tested for the presence of WNV using West Nile Virus VecTest strips (Medical Analysis Systems, Inc., Camarillo, CA). Reverse transcription-polymerase chain reaction was performed to confirm WNV or identify other viruses in cultures displaying CPE. In summary, total RNA was extracted from the supernatant using a QIAamp® Viral RNA Mini Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. Reverse transcription of RNA to cDNA and subsequent primary amplification were carried out in a single-tube reaction using WNV specific primers (Gibbs et al. 2005) or a combination of virus specific and group-wide primers. Amplification products were visualized by electrophoresis using a 2% agarose gel containing 0.5 µl of ethidium bromide.

The estimated minimum infection rate (MIR), which uses the assumption that a positive pool contains only one infected mosquito, was calculated for mosquito species positive for any arbovirus using the standard formula cited in Condotta et al. (2004). A binomial confidence interval was calculated for arbovirus prevalence using Statistical Analysis Software (SAS Institute, Inc., Cary, NC).

Blood meal identification

Total nucleic acids were extracted from blood-fed mosquitoes using a QIAamp® Viral RNA Mini Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer’s instructions. Segments of host mitochondrial DNA were amplified using mammalian (Mam 16s-F and Mam16s-R), avian (Avi16s-R and Avi16s-F), and ectotherm (Rept-sp-F and Ecto-univ-R) group-wide primers in separate reactions. Amplification products were visualized by electrophoresis using a 2% agarose gel containing 0.5 µl of ethidium bromide. Bands of the correct size, based on comparison to positive DNA controls, were excised then purified with the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Purified PCR products were sequenced in forward and reverse directions at the Molecular Genetics Instrumentation Facility, The University of Georgia, with an ABI 3100 automated sequencer (Applied Biosystems, Perkin Elmer Corp., Foster City, CA). The sequences were assembled and edited using the Sequencher software package, version 4.1.4 (Gene Codes Corp., Ann Arbor, MI). A nucleotide-nucleotide BLAST (blastn) search was performed to determine the most similar sequences of the target genes published in GenBank (http://www.ncbi.nlm.nih.gov/).

Spatial analysis

To quantify land use/ cover around sites, I used a data layer that was mapped at 1:12,000 scale from 1992 color infrared aerial photography and updated frequently to reflect changes in land cover from more recent photography and field observation. The land use/ cover surrounding sites was validated using 2006 color infrared aerial photography, including areas outside of the Ichauway property. Using ArcGIS (ESRI, Redlands, CA, v. 9.1), the percent area of each of the following nine land use/ cover categories were determined within a 1-km radius of each study site (total area = 3.14 km^2): open water, residential/highways, agriculture, scrub, hardwood, bottomland hardwood, pine, forested wetland, and non-forested wetland.

RESULTS

Approximately 58,000 mosquitoes were collected over 355 trap nights from 78 dates during this study. The mosquitoes collected belonged to 30 species and the following nine genera: *Aedes*, *Anopheles*, *Coquillettidia*, *Culiseta*, *Culex*, *Ochlerotatus*, *Orthopodomyia*, *Psorophora*, and *Uranotaenia* (Table 3.1). Four species (*Ae. vexans* (50%), *Ps. ferox* (25%), *Oc. sticticus* (4%), and *An. crucians* (3%)) comprised over 80% of the total mosquitoes collected. The most abundant species, *Ae. vexans*, was present from mid-May through the beginning of November (Figure 3.2). *Ps. ferox* reached its highest population density during week 37 of the field season, approximately two weeks after the largest rainfall event of the season (T. S. Fay). *Oc. sticticus* was collected in small numbers until it also exhibited a peak in population density during week 37 of the field season. *An. crucians* was most abundant early in season and its population fluctuated greatly.

9603 mosquitoes (987 pools) were tested for arboviruses. West Nile virus (WNV) was detected in one pool of *Cx. quinquefasciatus* mosquitoes collected from the Flint Riparian site on October 7, 2008. The WNV seasonal minimum infection rate (MIR) of *Cx. quinquefasciatus* was 0.9 infected mosquitoes per 1,000, while the WNV MIR of all mosquitoes tested was 0.1 infected mosquitoes per 1,000 (Table 3.2). Potosi virus

(POTV) was detected in two pools of *Aedes vexans* Meigen mosquitoes collected from Collins Pond on June 5, 2008. Potosi virus was also detected in one pool of *Anopheles punctipennis* (Say) mosquitoes collected from Mim's Store Drain on June 4, 2008. The POTV seasonal minimum infection rate of *Ae. vexans* and *An. punctipennis* were 0.4 and 11.5 infected per 1,000 respectively, while the POTV MIR of all mosquitoes tested was 0.3 infected per 1,000.

Out of the 190 blood-fed mosquitoes collected, the hosts of 148 mosquitoes (78%) were identified. *Anopheles quadrimaculatus* Say was the most abundant blood-fed species collected and the majority of its blood meals were of mammalian origin (n = 80, 99%), except for one blood meal which had both mammalian and avian origins (Table 3.3). *Ae. vexans* was the second-most abundant blood-fed species collected and the bulk of its blood meals were mammalian (n = 43, 98%), although one was avian in origin. While the host was identifiable for only two *Cx. quinquefasciatus* blood meals, both were birds. The hosts of the blood meals from other species collected, *Aedes albopictus* (Skuse), *An. punctipennis, Culex coronator* Dyar & Knab, *Cx. erraticus*, *Cx. restuans*, *Culex salinarius* Coquillett, *Ochlerotatus thibaulti* (Dyar & Knab), and *Ps. ferox*, were predominantly mammals (n = 23, 96%), while one was a bird. No blood meals from reptiles or amphibians were identified.

Results of BLAST searches of *cyt b* sequences showed that the *An. quadrimaculatus* mosquitoes I collected fed entirely upon one mammalian species, white-tailed deer (*Odocoileus virginianus*) (Table 3.4). The one blood meal identified from *An. quadrimaculatus* which had mixed origins was taken from a white-tailed deer and a chicken. *Ae. vexans* fed on five mammalian species, with the most common being white-tailed deer (81%) and domesticated cows (7%). The one avian blood meal belonging to *Ae. vexans* was taken from a wild turkey (Table 3.5). *Ae. albopictus*, *An. punctipennis*, *Cx. restuans*, and *Cx. salinarius* took all of their blood meals from white-tailed deer. The three blood meals identified for *Cx. erraticus* were taken from a cattle egret and two white-tailed deer. The only blood-fed *Cx. coronator* collected fed on an eastern cottontail rabbit. *Ps. ferox* exhibited mammophilic feeding patterns, and its hosts were a white-tailed deer (20%), an eastern cottontail rabbit (20%), a human (20%), a raccoon (20%), and a nine-banded armadillo (20%).

Analysis of the land use/ land cover patterns at the Big Slough, Collins Pond, Horse Hammock, and Mim's Store Drain collection sites showed relatively similar land use/ cover patterns within the 1-km radius surrounding these sites: over 70% in coniferous forest, less than 10% in cultivated land, and up 15% in deciduous forest (Table 3.6, Figure 3.3). The area within the 1-km radius around Richardson Flat was 65% coniferous forest, 12% deciduous forest, and 23% non-forested wetland. Flint Riparian, Parmalee Pond, and Hall Corner Pond sites had similar land use/ cover patterns within the 1-km radius surrounding sites: 40-60% coniferous forest and 25-40% cultivated land.

DISCUSSION

This study indicates that arbovirus prevalence at Ichauway in southwestern Georgia during the 2008 mosquito season was low (less than 1%) (Gu 2008). *Cx. quinquefasciatus* was the only species infected with WNV, with a minimum infection rate of 0.9 infected mosquitoes per 1,000. West Nile virus is a flavivirus that uses several bird species as reservoirs, specifically passerine birds (Allan et al. 2009). While the virus can be vectored by several species of mosquitoes, *Cx. quinquefasciatus* is the principal

WNV vector in Georgia (Gibbs et al. 2006, Allan et al. 2009). In contrast to this study, the WNV infection rate for *Cx. quinquefasciatus* from sites in northern Florida was 21.3 during July 2001 (Godsey et al. 2005) However, the infection rate seen in 2001 when the virus first arrived may not be indicative of current infection rate since the virus is now considered endemic in this area. Moreover, the WNV prevalence in humans in Georgia has been consistently lower than in other areas of the United States such as New York and Illinois (CDC 2009).

Documenting only one isolation of WNV in this study prevented associations being made between arbovirus incidence and land cover/ use. However, a study examining correlations between agriculture activities, climate, and incidence of human WNV cases in Colorado, Nebraska, Louisiana, and Pennsylvania found that temperature and farming activity were strongly associated with the incidence of human WNV incidence (Miramontes et al. 2006). Comparably, in an investigation of how West Nile virus antibody prevalence in natural avian communities co-varied with land use, Bradley et al. (2008) determined that antibody prevalence increased from rural to urban sites in Georgia. In addition, Gibbs et al. (2006) found that during 2002 and 2003, the probability of birds infected with WNV being present in an area increased with urban-suburban land use in Georgia.

In this study, POTV was isolated from *Ae. vexans* and *An. punctipennis*. *Ae. vexans* minimum infection rate was low (less than 1%) (Gu 2008), and *An. punctipennis* MIR was probably artificially inflated by the small number of *An. punctipennis* mosquitoes tested for arboviruses (Table 3.2). POTV was initially isolated from *Ae. albopictus* in Missouri in 1989 (Heard et al. 1991), and it was later isolated from *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Ochlerotatus*, and *Psorophora* species throughout central and eastern United States (Harrison et al. 1995, Mitchell et al. 1996, Armstrong et al. 2005). The virus has been isolated from both *Ae. vexans* and *An. punctipennis* in previous studies (Mitchell et al. 1996, Armstrong et al. 2005). Neutralizing antibodies to POTV were detected in deer from Arkansas, Missouri, Indiana, Iowa, and Colorado (McLean et al. 1996, Blackmore and Grimstad 1998). It is believed that the virus regularly infects wild deer populations, and its impact on the health of this animal appears to be minimal (McLean et al. 1996, Blackmore and Grimstad 1998). The land cover surrounding sites where POTV was detected, Collins Pond and Mim's Store Drain, is primarily coniferous and deciduous forest (Table 3.6, Figure 3.3). Forest dominated areas would allow for the interaction of competent mosquito vectors and susceptible white-tailed deer reservoirs, supporting the continued transmission of POTV. Moreover, the two species that were infected with POTV, *Ae. vexans* and *An. punctipennis*, fed primarily upon white-tailed deer (Table 3.4).

The host-feeding patterns documented here potentially suggest that the transmission cycle of WNV in southwestern Georgia is similar to pathways found in other areas of the United States (Mitchell et al. 1998, Armstrong et al. 2005, Cupp et al. 2007, Kilpatrick et al. 2007). Finding the most ornithophilic species in this study, *Cx. quinquefasciatus*, to also be the only species infected with WNV (Table 3.5) suggests that it could be serving as the most important enzootic (bird-to-bird) and epizootic (bird-to-human) vector of WNV in the area. *Cx. erraticus* has also been shown to transmit WNV in the southeastern United States (Cupp et al. 2007, Kilpatrick et al. 2007). This species fed upon both mammals and birds (Table 3.3), which potentially provides evidence of it also serving as a possible epizootic vector. Previously, WNV has been isolated from *Cx. salinarius* and *Cx. restuans* in the region, and these species are considered to be possibly important epizootic/epidemic vectors as well (Godsey et al. 2005, Savage et al. 2007). However, their role in the transmission of WNV in the study area could not be determined from the host-feeding patterns documented, because they fed exclusively on white-tailed deer. The potential role of another vector in the WNV transmission cycle in the region, *Cx. nigripalpus* (Rutledge et al. 2003, Vitek et al. 2008), is also unknown, because no blood-fed *Cx. nigripalpus* mosquitoes were collected.

The host-feeding patterns of *Ae. vexans* documented within this study are consistent with earlier findings within the same area. Love and Smith (1958) examined 35 freshly engorged *Ae. vexans* and found all had fed on mammalian hosts. My findings are also consistent with studies from other portions of the United States. In a Connecticut study on the blood-feeding behavior of the mosquitoes in the genera *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*, Molaei et al. (2008) identified white-tailed deer as the most common vertebrate host. Moreover, Molaei and Andreadis (2006) found that *Ae. vexans* obtained vertebrate blood meals most frequently from white-tailed deer and domesticated horses. A 1993 study conducted in Lake Falls basin, North Carolina documented *An. quadrimaculatus* feeding on 11 mammalian species, a much larger variety of mammalian hosts in comparison to this study (Robertson et al. 1993). But similarly, the largest percentage of blood meals was taken from white-tailed deer (Robertson et al. 1993). In the same study from North Carolina, *Cx. erraticus* also exhibited a more diverse feeding pattern, with blood meals identified from 17 species of mammals, birds, and ectotherms (Robertson et al. 1993). Cupp et al. (2004) documented

Cx. erraticus feeding upon various birds and mammals but primarily on white-tailed deer in Alabama's Tuskegee National Forest. While *Cx. salinarius* was shown to feed primarily on mammals in Gingrich and Williams (2005), its host selection was more varied than what was documented within my study. In Gingrich and Williams (2005), *Cx. salinarius* fed on dogs, horses, rabbits, deer, and birds instead of exclusively on deer. *Ps. ferox* possessed the most diverse feeding patterns within my study, and its observed feeding patterns were more varied in comparison to Molaei et al. (2008), where the species took 11 out of 12 (92%) of its blood meals from white-tailed deer.

While the number of avian hosts identified in this study was extremely low, it is potentially significant that no blood meal was taken more than once from the same bird species. This occurrence could be due to the high bird diversity present at the study area. Smith et al. (2006) documented 191 bird species present at Ichauway. High bird diversity at the study area may also be influencing the low WNV minimum infection rate through the dilution effect, in which incompetent host reservoir hosts dilute rates of disease transmission between vectors and more competent hosts (Ezenwa et al. 2006).

Ezenwa et al. (2006) found that WNV incidence in mosquitoes was negatively associated with the species richness of non-passerine birds, which are consistently found to be less competent WNV reservoirs in comparison to passerine birds. In a Saint Louis regional study, Allan et al. (2009) found that bird diversity and the overall arbovirus reservoir competence were strongly negatively correlated. In a national study, Allan et al. (2009) also found a negative relationship between human per capita incidence of WNV illness and bird diversity. However, in a Chicago study conducted by Loss et al. (2009), there was no negative correlation between avian richness and *Culex* WNV infection rate or between richness and infection status in individual birds.

The low WNV prevalence documented could also be due to the time of year when nestling and fledging birds are abundant in the area. Nestling and fledging birds may serve as effective WNV reservoirs, because they lack antibodies to the virus and while confined to their nests, they are more prone to mosquito bites (Scott and Edman 1991). Hamer et al. (2008) documented that hatch-year birds played a key role in the amplification of enzootic transmission of WNV in the Chicago, Illinois region. However, if birds in the study area fledge before *Culex* mosquitoes become abundant, the mosquitoes may not be able to take blood meals as successfully, preventing mosquitoes from playing a large role in the amplification of the virus.

The majority of blood meals identified in this study came from mammals and specifically white-tailed deer, which are present throughout the study area at approximately 3.8-5.8 deer/km^2 (Stober 2008). However, mosquitoes feeding primarily on mammals and white-tailed deer may not be a true representation of feeding patterns due to sampling techniques that were unintentionally biased towards a known mammophilic species and a white-tailed deer feeder, *An. quadrimaculatus*. This species was not prevalent in light trap collections throughout the field season (Table 3.1), yet it was consistently the most abundant blood-fed species collected from walk-in resting boxes. Furthermore, the host-feeding patterns reported are based on a small number of blood-fed mosquitoes collected. Obtaining a greater quantity of blood-fed mosquitoes may have allowed for a better representation of the actual host-feeding patterns.

The results of this study suggest that arbovirus prevalence at Ichauway in southwestern Georgia is low and this could be potentially related to the diversity of the wildlife in the area that has been previously documented (Smith 2006). An association between WNV incidence and land use/ cover could not be made because only one isolation of WNV occurred. The association between POTV and forested land seems logical, because this landscape can serve as habitat for white-tailed deer, the reservoir of the virus. However, this association is based on only three isolations of the virus, which is not very substantial. Confirming the associations between arbovirus prevalence and land use/ cover offered in this study requires future mosquito surveillance and arbovirus testing at replicated sites across a wider range of natural and disturbed conditions for a longer period of time. Also, in order to confirm the host-feedings observed in the study, much more sampling in rural southwestern Georgia is needed in a wider range of habitats. In order to gain a better understanding of the WNV transmission cycle in southwestern Georgia, it is recommended to extensively study the bird community in the area. Bird surveillance should take place throughout the entire year to understand what effect nestling, fledging, and migratory birds may have on the WNV transmission cycle in the area.

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Table 3.1. Number of mosquitoes per species collected during May- November 2008 at eight sites in Ichauway, the site of the J. W. Jones Ecological Research Center in southwestern Georgia. Location of sites can be found in Figure 3.1.

Species	Big Slough	Collins Pond	Flint Riparian	Hall Pond	Horse Hammock	Mim's Drain	Parmalee Pond	Richardson Flat
Ae. albopictus (Skuse)	2	1	11	7	3	29	30	5
Ae. vexans (Meigen)	2730	5887	3679	3384	3007	3080	5008	2428
Oc. atlanticus (Dyar & Knab)	0	3	0	0	0	0	1	2
Oc. canadensis canadensis (Theobald)	71	209	0	0	8	61	531	34
Oc. fulvus pallens (Ross)	66	9	6	3	137	6	7	5
Oc. infirmatus (Dyar & Knab)	188	297	2	21	202	79	86	15
Oc. mitchellae (Dyar)	3	50	0	8	28	5	418	21
Oc. sticticus (Meigen)	1364	136	6	10	641	239	40	34
Oc. thibaulti (Dyar & Knab)	10	9	0	0	6	0	6	0
Oc. triseriatus (Say)	23	27	13	5	36	104	55	4
An. crucians Weidemann	105	870	14	654	39	14	102	82
An. punctipennis (Say)	75	25	25	9	56	51	1	5
An. quadrimaculatus Say	14	51	38	94	7	8	18	8
Cq. perturbans (Walker)	0	2	1	1	3	2	7	12
Cs. melanura (Coquillett)	0	1	0	0	0	1	0	0
Cx. coronator Dyar & Knab	3	3	10	85	7	22	58	69
Cx. erraticus (Dyar & Knab)	71	6	24	88	22	25	5	48
Cx. nigripalpus Theobald	83	45	42	150	28	39	9	112
Cx. quinquefasciatus Say	59	28	169	359	51	146	169	279
Cx. restuans Theobald	13	18	80	68	31	11	3	31
Cx. salinarius Coquillett	40	5	21	1544	9	3	0	9
Cx. territans Walker	35	37	12	7	40	9	10	4
Or. signifera (Coquillett)	4	2	2	2	1	5	3	0
Ps. ciliata (Fabricius)	1	292	2	307	34	0	135	27
Ps. columbiae (Dyar & Knab)	1	32	37	75	2	1	45	97
Ps. cyanescens (Coquillett)	9	10	170	384	1	5	160	20
Ps. ferox (von Humboldt)	7203	426	17	76	6094	271	341	5
Ps. howardii Coquillett	17	36	13	41	34	0	64	0
Ps. mathesoni Belkin & Heinemann	4	8	0	0	0	0	0	0
Ur. sapphirina (Osten Sacken)	11	18	0	7	12	2	2	1
Total	12205	8543	4394	7389	10539	4218	7314	3357

Table 3.2 Seasonal minimum infection rates (MIR) with 95% confidence intervals (CI) among arbovirus-positive mosquitoes at Ichauway in southwestern Georgia during 2008, as determined by reverse transcriptase polymerase chain reaction (RT-PCR)

Species	Virus	No. of infected pools	No. of pools tested	No. of mosquitoes	MIR[a] (95% CI)
Ae. vexans	Potosi	2	306	4767	0.42 (0.41-0.43)
An. punctipennis	Potosi	1	12	87	11.49 (4.79-18.19)
Cx. quinquefasciatus	West Nile	1	102	1148	0.87 (0.85-0.89)

[a] Number of infected per 1,000 mosquitoes

Table 3.3. Number and percent of blood meals by host class for mosquitoes collected at Ichauway in southwestern Georgia in 2008

Species	Avian	Mammal	Mixed avian-mammal	**Total**
Ae. albopictus		1 (100)		1
Ae. vexans	1 (2)	43 (98)		44
An. punctipennis		1 (100)		1
An. quadrimaculatus		80 (99)	1 (1)	81
Cx. coronator		1 (100)		1
Cx. erraticus	1 (33)	2 (67)		3
Cx. quinquefasciatus	2 (100)			2
Cx. restuans		1 (100)		1
Cx. salinarius		8 (100)		8
Oc. thibaulti		1 (100)		1
Ps. ferox		5 (100)		5

Table 3.4. Number and percent of blood meals identified to one mammalian host from mosquitoes collected at Ichauway in southwestern Georgia in 2008

Species	Armadillo	Cat	Cow	Deer	Horse	Human	Rabbit	Raccoon	**Total**
Ae. albopictus				1 (100)					1
Ae. vexans		1 (2)	3 (7)	35 (81)	1 (2)	2 (5)	1 (2)		43
An. punctipennis				1 (100)					1
An. quadrimaculatus				81 (100)					81
Cx. coronator							1 (100)		1
Cx. erraticus				2 (100)					2
Cx. restuans				1 (100)					1
Cx. salinarius				8 (100)					8
Oc. thibaulti				1 (100)					1
Ps. ferox	1 (20)			1 (20)		1 (20)	1 (20)	1 (20)	5

Table 3.5. Number and percent of blood meals identified to one avian host from mosquitoes collected at Ichauway in southwestern Georgia in 2008

Species	Cattle egret	Chicken	Northern cardinal	Pigeon	Turkey	**Total**
Ae. vexans					1 (100)	1
An. quadrimaculatus		1 (100)				1
Cx. erraticus	1 (100)					1
Cx. quinquefasciatus			1 (50)	1 (50)		2

Table 3.6. The percent area of nine land use/ cover categories and hydric soils within a 1-km radius of eight study sites (total area = 3.14 km^2) at Ichauway assessed using ArcGIS (ESRI, Redlands, CA, v. 9.1).

Percentage of Land use/ cover and Hydric soils	Big Slough	Collins Pond	Horse Hammock	Mim's Drain	Richardson Flat	Flint Riparian	Parmalee Pond	Hall Pond
Coniferous forest	76.9	79.2	70.8	86.6	64.4	43.0	59.8	54.4
Deciduous forest	1.6	5.1	2.4	3.6	11.8	3.9	1.8	1.8
Bottomland deciduous	3.6	0.0	12.9	2.6	0.0	13.1	0.0	0.5
Forested wetland	0.6	5.1	1.5	0.0	0.1	0.0	5.6	0.2
Non-forested wetland	0.0	0.2	0.0	0.0	22.6	1.3	2.3	3.6
Scrub	4.8	1.2	0.9	0.8	0.0	5.9	1.0	0.0
Agriculture	8.8	8.3	3.4	5.9	1.0	25.8	26.3	39.5
Residential	0.1	0.9	5.2	0.2	0.0	0.1	3.2	0.1
Open water	3.5	0.0	2.9	0.5	0.0	6.9	0.0	0.0
Hydric soils	10.5	19.3	9.5	0.0	24.8	10.3	13.1	8.8

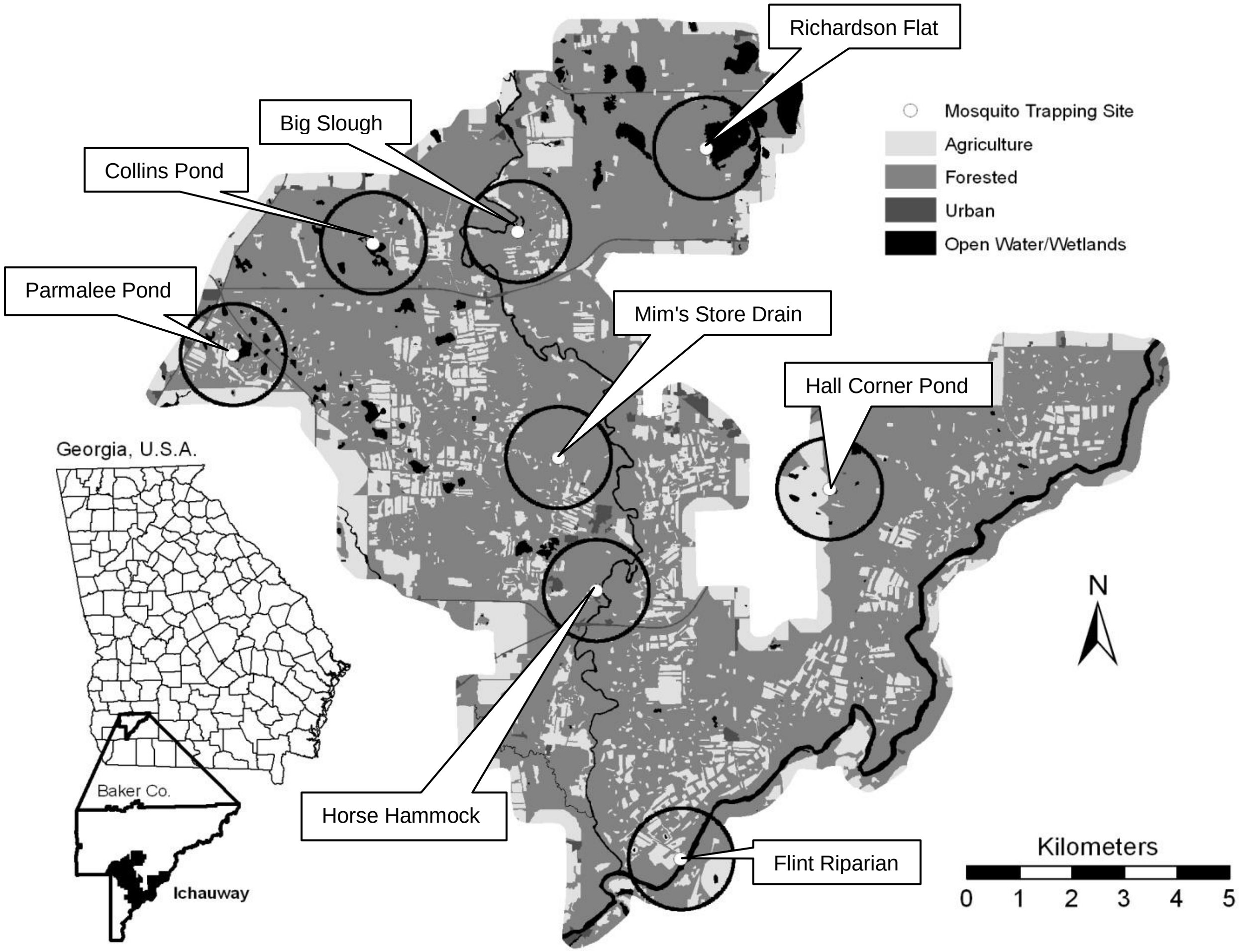

Figure 3.1. The eight sites at Ichauway in southwestern Georgia, USA, where adult mosquitoes were collected from May to November 2008. The 1-km radius surrounding each site that was used in spatial analysis is outlined in black.

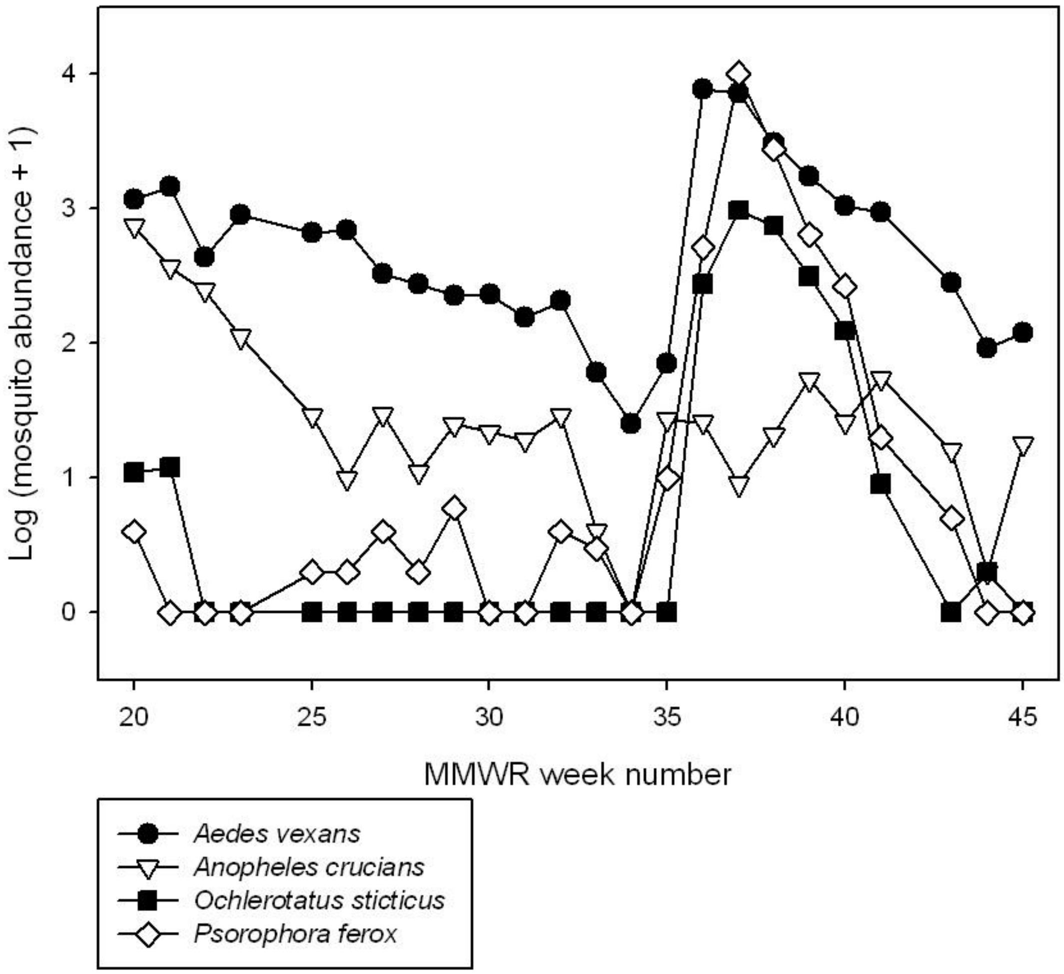

Figure 3.2. Seasonal distribution of the four most abundant mosquito species collected at Ichauway in southwestern Georgia, USA from mid-May to early November 2008. MMWR week number, located on the x-axis, is the method that the CDC uses to number weeks during the year in which week one of the year contains the first of January.

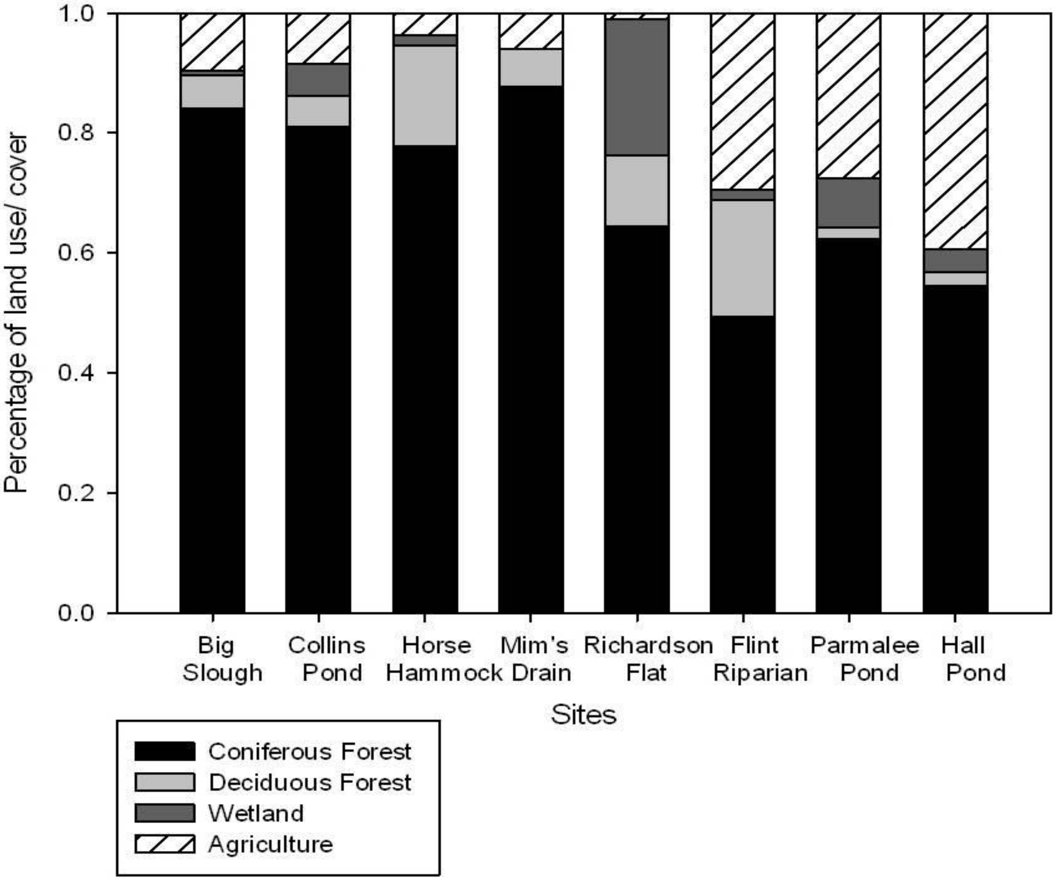

Figure 3.3. Percentage of four dominant land use/ cover categories within 1-km radius of eight study sites at Ichauway in southwestern Georgia, USA, where adult mosquitoes were collected from mid-May to early November 2008.

CHAPTER 4

CONCLUSIONS

Multiple diseases affecting humans and wildlife are caused by viruses that reside within wildlife hosts, and some of these viruses are transmitted through arthropod vectors such as mosquitoes (Allan et al. 2009). Understanding the spatial and temporal dynamics of mosquito communities is important not only for implementation of control measures but also for explaining vector-borne arbovirus prevalence within an area (Godsey et al. 2005, DeGroote et al. 2007). Recognizing arbovirus prevalence of important vectors permits infection risk prediction in animals and/or humans (Kilpatrick et al. 2005). Quantifying arbovirus prevalence, along with identifying mosquito host-feeding patterns allows for assessment of vertebrates as potential reservoirs involved in the persistence and amplification of arboviruses in an area (Molaei et al. 2008).

This study expands our knowledge of mosquito ecology in the Gulf Coastal Plain of southwestern Georgia. The objectives of this research were to (1) examine factors that potentially influence the mosquito community composition and (2) determine the arbovirus prevalence and host-feeding patterns of mosquitoes during one field season in rural southwestern Georgia. Mosquito sampling occurred from mid-May through early November 2008 on a weekly basis at eight sites on Ichauway, the property of the J.W. Jones Ecological Research Center.

Mosquito collections captured 30 mosquito species in the study area, with *Aedes vexans* (Meigen) being the most abundant species. *Anopheles* species were abundant early in the field season. *Psorophora* and *Ochlerotatus* species became abundant following flooding from Tropical Storm Fay (late August 2008). *Culex* species became abundant later in field season due to standing water left from Tropical Storm Fay.

A cluster analysis was used to group sites based on percentages of land use/ cover and hydric soils within a 1-km radius of collecting sites, and an indicator species analysis (ISA) was used to investigate the association between mosquito species and groups of sites with similar land use/ cover patterns. These analyses revealed that *Aedes albopictus* (Skuse), *Culex coronator* Dyar & Knab, *Culex quinquefasciatus* Say, and *Culex salinarius* Coquillett were associated with sites that had the most anthropogenic influence, while *Coquillettidia perturbans* (Walker) and *Psorophora ferox* (von Humboldt) were associated natural land cover such as wetlands and forested land.

Future studies should consider collecting mosquitoes at a greater number of replicated sites to allow for stronger correlations between land use/ cover and mosquito species. Also, sampling the larval population at sites would allow for stronger correlations with environmental variables and land use/ cover because larvae are confined to the area where they are collected, while adults may disperse for kilometers, depending on the species. In future research, it is also recommended to compare associations found between adults, larvae, land use/ cover and environmental conditions at sites to see if associations between mosquito species and sites change with the life history stage of the mosquito and its needs for taking nectar and blood meals.

I used an information theoretic approach to predict important weather variables influencing mosquito abundance and presence/absence. Model averaged parameter estimates suggested that daily abundance of *Ae. vexans* was best predicted by total precipitation for two and six weeks, average temperature for one day and two weeks, and average relative humidity for one day. Of the variables examined, the daily presence/ absence of *Ae. albopictus* was best predicted by average relative humidity for one and two weeks. *Cx. coronator* was best described by total precipitation for four weeks and time of year, while *Cx. erraticus* was best described by these variables as well average relative humidity for one day and one week. Six week total precipitation, one day average temperature, and drought index were most useful in describing *Cx. nigripalpus* daily presence/ absence. The pattern of *Culex restuans* presence/ absence could be best described by one day average temperature. Six week total precipitation best described presence/absence of *Oc. triseriatus*.

Of 9603 mosquitoes tested for arboviruses, West Nile virus (WNV) was detected in *Cx. quinquefasciatus*, but the WNV minimum infection rate (MIR) was low (less than 1%) (Gu 2008). Potosi virus (POTV) was detected in *Ae. vexans* mosquitoes and *Anopheles punctipennis* (Say), but at low rates. Documenting only one isolation of WNV in this study prevented associations being made between arbovirus incidence and land cover/ use. The sites of POTV incidence were surrounded primarily by coniferous and deciduous forest. Associating POTV with forested land seems logical, because this landscape can serve as habitat for white-tailed deer, a reservoir of the virus. However, this association is based on only three isolations of the virus, which is not very substantial. Confirming associations between arbovirus prevalence and land use/ cover will require additional mosquito surveillance and arbovirus testing at replicated sites across a wider range of environmental conditions and for longer periods of time.

The low WNV prevalence documented could be due to the high bird diversity present at the study area through the dilution effect, in which incompetent host reservoir hosts dilute rates of disease transmission between vectors and highly competent hosts (Ezenwa et al. 2006). The low WNV prevalence observed could also be due to nestlings and fledglings leaving nests before *Culex* mosquitoes become abundant during the year, thus minimizing the likelihood of encountering potential virus reservoirs and the role that these mosquitoes play in the amplification of the virus. In order to gain a better understanding of the WNV transmission cycle in southwestern Georgia, it is recommended to conduct future mosquito surveillance and also extensively study the bird community in the area. Bird surveillance should take place throughout the entire year to understand what effect nestling, fledging, and migratory birds have on the WNV transmission cycle in the area.

Out of the 190 blood-fed mosquitoes collected, the hosts of 148 were identified. *Anopheles quadrimaculatus* Say and *Ae. vexans* were the most abundant blood-fed species collected and the majority of their blood meals were of mammalian origin. While the host was only identifiable for two *Cx. quinquefasciatus* blood meals, it was the single species which fed entirely on birds. No blood meals from reptiles or amphibians were identified.

While the majority of all blood meals identified in this study came from mammals and specifically white-tailed deer, mosquitoes feeding primarily on mammals and white-tailed deer may not be a true representation of feeding patterns due to sampling techniques that were unintentionally biased towards a known mammophilic species and a white-tailed deer feeder, *An. quadrimaculatus*. This species was not prevalent in light trap collections throughout the field season, yet it was consistently the most abundant blood-fed species collected from walk-in resting boxes. Furthermore, the host-feeding patterns reported are based on a small number mosquitoes collected. Obtaining a greater quantity of blood-fed mosquitoes in future studies may allow for a better representation of the actual host-feeding patterns. It is recommended to have walk-in resting boxes at more than just two locations in an effort to increase the diversity of blood-fed mosquitoes collected in the future. In future collections of blood-fed mosquitoes, more effort should be put into collecting resting blood-fed females in natural resting areas such as tree holes and in vegetation to potentially provide a better representation of species in the area.

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