

# AGENDA

## NE-1043: Biology, Ecology & Management of Emerging Disease Vectors

Wednesday 27 February 2013

Trump Taj Mahal  
Bronze Room  
10:30 – 1:30

- I. 10:30 – 10:45: Preliminary Business Meeting
  - A. Introductions and round table
  - B. Distribution of meeting packet and reports
  - C. Review of meeting agenda
  - D. Modifications and additions to the agenda
  
- II. 10:45 am – 1:00 pm: Cooperator reports (15 min each, with ppt and discussion)
  - A. Ned Walker, Michigan State University
  - B. Paul Leisnham, University of Maryland
  - C. Ted Andreadis et al., Connecticut Agricultural Experiment Station
  - D. Stephen Dobson, University of Kentucky
  - E. Dina Fonseca et al., Rutgers University
  - F. Brian Federici and Margaret Wirth, University of California Riverside
  - G. Others (Dan Strickman, Dan Kline)
  
- III. 12:00 pm: Buffet style lunch during cooperator reports
  
- IV. 1:00 pm: Final Business Meeting
  - A. Future of the research committee: research or coordination?
  - B. Guidance from Dr. Kirby Stafford
  - C. Election of officers: any volunteers?
  - D. 2014 meeting date and location
  - E. Registration payment to Ned Walker. \$65/attendant.
  - F. Adjourn

## Guidance from committee administrative adviser Dr. Kirby Stafford

Ideally, a request to rewrite would be submitted at this March meeting, but it won't be accepted without a mid-term review and there is little time between February 27 and March 11, especially with me gone for three days the first week in March. A request can be submitted at the summer meeting and still keep on track, but in this case it might help to start work on a proposal ahead of time. The group needs to seriously think about whether to try and renew as a multi-state research project or as a coordinating committee. From what I can tell, the group is really functioning as a coordinating committee as your research activities are not jointly planned and multistate. However, while a CC will cover travel and getting together as a group, I believe it won't cover research expenses. I have to double check that. We haven't used CCs much in the northeast region.

**Multistate Research Coordinating Committees (CC) and Education/Extension and Research Activity (ERA):** The membership of a CC or an ERA is made up of an AA, CSREES representative, scientists, and as applicable, extension specialists and/or extension agents. A CC or ERA provides opportunity for scientists, specialists, and others to work cooperatively to solve problems that concern more than one state, share research data, and coordinate research and other types of activities. This is presently one of the most common mechanisms for functionally integrated activities such as the regional IPM programs.

**Multistate Research Projects:** The membership of a Multistate Research Project is called the technical committee, and is made up of SAES scientists, an AA, CSREES representative, other public and private sector scientists, and as applicable, extension specialists and/or extension agents. This type of activity involves cooperative, jointly planned research employing multidisciplinary approaches in which a SAES, working with other SAESs, the Agricultural Research Service (ARS), or a college or university, cooperates to solve problems that concern more than one state and usually more than one region. In addition, the following must be demonstrated in the project proposal:

1. The objectives are clearly focused.
2. Each participant listed has direct involvement in the accomplishment of the stated objectives.
3. The project is multistate and multidisciplinary.
4. The project proposal has been peer-reviewed.
5. The proposed project is oriented toward accomplishment of specific outcomes and impacts and based on priorities developed from stakeholder input.
6. The project is responsive to CSREES goals.

## REPORTS BY COOPERATOR

### Andreadis, Theodore et al. NE-1043 Annual Report 2012 Connecticut Agricultural Experiment Station

#### **Objective 1. Strengthen basic and applied research on the pathogen, hosts, and environmental factors that influence mosquito-borne disease emergence**

The overwintering ecology of *Culiseta melanura* (Coquillett) was studied in a seasonally flooded evergreen forest swamp in south central Connecticut in an effort to clarify which larval stages successfully overwinter in the northeastern United States, and to determine the degree to which larval development and/or mortality occur during the winter months. A total of 8,626 immature *Cs. melanura* were collected weekly for analysis from subterranean crypts and cavities located under the roots of trees from December 13, 2011 to May 31, 2012. Despite the formation of ice on the surface water at the entrance holes to the crypts, water temperatures within the cavities remained above freezing (ave. = 1.8 °C) throughout coldest winter months of January and February. A heterogeneous population of 2nd, 3rd and 4th instar larvae was recovered throughout the winter and early spring in the same relative proportions (30%, 30%, 40%, respectively) with no significant change in their comparative abundance during this period providing unequivocal evidence that all three instars successfully overwinter in the region. Findings further demonstrate that larvae undergo no development during the winter and do not appear to be impacted by any measurable mortality. The cessation of larval diapause and a resumption of development were observed in mid-April and were coincident with a gradual increase in water temperature within the crypts to 9°C, in agreement with a previously calculated developmental thermal minimum of 8.5°C for *Cs. melanura*. This resulted in a protracted period of pupation that encompassed a minimum of five weeks, followed by a staggered emergence of adults and an overlap of the residual overwintering population with larvae of the first summer generation (CT).

#### **Objective 2. Use knowledge of mosquito, pathogen, vertebrate reservoir, and environment interactions to enhance ability to predict and prevent conditions leading to disease.**

Mosquitoes within the *Culex pipiens* complex have been implicated as major vectors of West Nile virus (WNV) in North America due to their seasonal abundance, vector competence and high field infection rates. However, the role of *Cx. p. pipiens* complex mosquitoes in enzootic amplification of WNV among avian hosts and epidemic transmission to humans varies throughout its geographical distribution and was accordingly reviewed. In the northeastern United States, *Cx. p. pipiens* is recognized as the primary enzootic vector responsible for amplification of virus among wild bird populations. However, because this mosquito is strongly ornithophilic, its role in transmission to humans appears to be more limited in this region. In the north central and Mid-Atlantic States by contrast, *Cx. p. pipiens* shows an increased affinity for human hosts and has been incriminated as a key bridge vector. In southern regions of the United States, *Culex p. quinquefasciatus* are more opportunistic feeders, and are thought to be principal enzootic and epidemic vectors. In western regions of the United States where *Culex tarsalis* predominates, especially in rural areas, *Cx. p. pipiens* and *Cx. p. quinquefasciatus* play roles that are more limited and are recognized as secondary vectors. In the southwestern United States *Cx. p. quinquefasciatus* also appears to be the predominant vector in urban habitats, but only a secondary vector in more rural environs. The direct involvement of *Cx. p. pipiens* form molestus in WNV transmission is largely unknown, but human-biting *Cx. p. pipiens* are more likely to

have a probability of genetic ancestry with *Cx. p. pipiens* form *molestus*. The detection of WNV from overwintering populations of diapausing *Cx. p. pipiens* and non-diapausing *Cx. p. quinquefasciatus* and their role in local overwintering of WNV were also examined (CT).

Studies on the vector-host interactions of *Culex pipiens* complex mosquitoes by sequencing portions of mitochondrial cytochrome b gene indicate that *Cx. p. pipiens* f. *pipiens* predominantly feed on avian hosts (93.1%), and focus feeding activity on several key bird species, in particular the American robin, the gray catbird, and the house sparrow in Connecticut. However, *Cx. p. quinquefasciatus* indiscriminately feed on both birds and mammals. *Culex p. quinquefasciatus* in Harris County - Texas and southern California acquired 39.1% and 88.2% of bloodmeals from birds, respectively. Mammalian-derived bloodmeals constituted 52.5% and 9.6% in the two regions, respectively. The most frequent avian hosts for this mosquito species in the southwestern U.S. were the mourning dove, the white-winged dove, the house sparrow and the house finch. Humans infrequently served as the source of bloodmeals for *Cx. p. pipiens* and *Cx. p. quinquefasciatus*. Microsatellite analysis of mosquitoes from Chicago, Illinois showed that *Cx. p. pipiens* f. *pipiens* with mammalian- derived bloodmeals had significantly higher ancestry and proportion of hybrids from *Cx. p. pipiens* f. *molestus* than did those with avian-derived bloodmeals (CT)

#### **Objective 4. Enhance surveillance technologies for mosquitoes and mosquito-borne pathogens.**

Disease outbreaks caused by eastern equine encephalitis virus (EEEV; Togaviridae, Alphavirus) may be prevented by implementing effective surveillance and intervention strategies directed against the mosquito vector. Methods for EEEV detection in mosquitoes include a real-time reverse transcriptase PCR technique (TaqMan assay), but we report its failure to detect variants isolated in Connecticut in 2011, due to a single base-pair mismatch in the probe-binding site. To improve the molecular detection of EEEV, we developed a multi-target TaqMan assay by adding a second primer/probe set to provide redundant targets for EEEV detection. The multi-target TaqMan assay had similar performance characteristics to the conventional assay but also detected newly evolving strains of EEEV. The approach described here increases the reliability of TaqMan assay by creating backup targets for virus detection without sacrificing sensitivity or specificity (CT).

Twelve reported mosquito attractants, alone or in combination, and 3 different types of traps were evaluated under field conditions for their attractiveness to host-seeking and oviposition-seeking female *Aedes japonicus japonicus* and associated woodland species in Windsor, CT, in 2010 and 2011. This study highlights the effectiveness of combining CO<sub>2</sub> with the TrapTech® Mosquito Lure in a Centers for Disease Control and Prevention (CDC) miniature light trap for collection of *Ae. j. japonicus* and associated woodland mammalian-feeding mosquitoes. The TrapTech Mosquito Lure is a proprietary blend of Bedoukian Research, Inc. It contained 250 mg of R-1-octen-3-ol and 1900 mg of ammonium bicarbonate, which were slowly released from a plastic disperser. On average, 567 *Ae. j. japonicus* individuals were collected per trap per night in the CDC miniature light traps baited with CO<sub>2</sub> plus TrapTech Mosquito Lure. The numbers collected in this trap were 28 times and 100 times greater than the numbers of *Ae. j. japonicus* collected in the CDC miniature light trap baited only with CO<sub>2</sub> and the gravid trap baited with hay infusion, 2 commonly used traps to assess abundance of *Ae. j. japonicus*. The average catches of other mammalian-biting species, *Ae. cinereus*, *Ae. triseriatus*, *Ae. trivittatus*, *Ae. vexans*, *Anopheles punctipennis*, *An. quadrimaculatus*, *Coquillettidia perturbans*, and *Culex salinarius*, were all significantly greater in the CDC miniature light trap baited with CO<sub>2</sub> plus TrapTech Mosquito Lure than in traps with CO<sub>2</sub> alone, but their average numbers were not as

large as were those of *Ae. j. japonicus*. These data demonstrate that the TrapTech Mosquito Lure used in combination with CO<sub>2</sub> in a CDC miniature light trap has potential to be a versatile and simple surveillance method for *Ae. j. japonicus* and other species (CT).

## **Publications**

1. Anderson JF, McKnight S, Ferrandino FJ. 2012. *Aedes japonicus japonicus* and associated woodland species attracted to Centers for Disease Control and Prevention miniature light traps baited with carbon dioxide and the TrapTech Mosquito Lure. *J Amer Mosq Control Assoc* 28:184-191.
2. Andreadis TG. 2012. The contribution of *Culex pipiens* complex mosquitoes to transmission and persistence of West Nile virus in North America. *J Amer Mosq Control Assoc* 28s:137-151.
3. Andreadis TG, Armstrong PM. 2012. 2012: a record year for West Nile virus activity in Connecticut. *CT Weekly Agric Report* 92:1-4.
4. Andreadis TG, Shepard JJ, Thomas MC. 2012. Field observations on the overwintering ecology of *Culiseta melanura* in the northeastern United States. *J Amer Mosq Control Assoc* 28:286-291.
5. Andreadis TG, Simakova AV, Vossbrinck CR, Shepard JJ, Yurchenko YA. 2012. Ultrastructural characterization and comparative phylogenetic analysis of new Microsporidia from Siberian mosquitoes: evidence for coevolution and host switching. *J Invertebr Pathol* 109:59-75.
6. Armstrong PM, Prince N, Andreadis TG. 2012. Development of a multi-target TaqMan assay to detect eastern equine encephalitis virus variants in mosquitoes. *Vector-Borne Zoonotic Dis* 12:872-876.
7. Hardstone MC, Andreadis TG. 2012. Weak larval competition between the invasive mosquito, *Aedes japonicus japonicus* (Diptera: Culicidae) and three resident container-inhabiting mosquitoes under standard laboratory conditions. *J Med Entomol* 49:277-285.
8. Molaei, G, Huang S, Andreadis TG. 2012. Vector-host interactions of *Culex pipiens* complex mosquitoes in northeastern and southern USA. *J Amer Mosq Control Assoc* 28:127-136.
9. Molaei G, Andreadis TG, Armstrong PM, Thomas MC, Deschamps T, Cuebas-Incle, E, Montgomery W, Osborne M, Smole S, Matton P, Andrews W, Best C, Cornine III F, Bidlack E, Texeira T. 2012. Vector-host interactions and epizootiology of eastern equine encephalitis virus in Massachusetts, USA. *Vector-Borne Zoonotic Dis* (in press).
10. Morningstar RJ, Hamer, GL, Goldberg TL, Huang S, Andreadis TG, Walker ED. 2012. Diversity of *Wolbachia pipientis* strain wPip in a genetically admixed, above-ground *Culex pipiens* (Diptera: Culicidae) population: association with form molestus ancestry and host selection patterns. *J Med Entomol* 49:474-481.
11. Simpson JE, Hurtado PJ, Medlock J, Molaei G, Andreadis, TG, Galvani AP, Diuk-Wasser MA. 2012. Vector host-feeding preferences drive transmission of multi-host pathogens: West Nile virus as a model system. *Proc R Soc B* 279:925-933.

**Walker, Edward et al. NE-1043 Annual Report 2012 Michigan State University**

**Objective 1. Strengthen basic and applied research on the pathogen, hosts, and environmental factors that influence mosquito-borne disease emergence**

The research conducted under this umbrella multistate project is designed to address critical issues and weak elements in this emergence and perpetuation process, to facilitate disease surveillance and exploit control opportunities. A fundamental question relates to the range of host choice of mosquitoes and the genetic and environmental contributions to this choice. We focused studies on genetics of host selection through controlled rearing, laboratory host choice, and comparative analyses within the *Culex pipiens* species complex of closely related taxa. The form *Culex pipiens pipiens* was found to prefer birds in both two-choice and no-choice tests whereas *Culex pipiens molestus* preferred mammals in identical test conditions. The typological designation of these strains is supported by presence of autogeny in form *molestus* females and absence of autogeny on form *pipiens* females. Results support suppositions that genes from the relatively cryptic *Culex pipiens molestus* could introgress into the more free flying and West Nile virus transmitting form *Culex pipiens pipiens*, explaining virus transmission amongst birds and to mammals by this single species. Experimental crosses in F1 and F2 stages are underway, and genetic background analyses using microsatellite allele markers are on going. Diversity of *Wolbachia pipiensis* in the *Culex pipiens* under study was shown to be high, and divergent between the *molestus* and *pipiens* forms. Crossing experiments suggest one way cytoplasmic incompatibility, forming a mating barrier which is probably based in *Wolbachia* strain diversification. The spatial and temporal distribution of *Culex pipiens* remained a focus of research due to its importance as a vector of West Nile virus, which resurged in 2012 in the United States in epidemic form. Precipitation and temperature regimes profoundly influenced production of adults of this species from anthropogenic larval habitats (water filled, street catchbasins); water regime stability was a key factor as these systems experience flush-out events with heavy rain. However, adult populations were less influenced by such events as revealed by cross-correlation analyses of long term light trap data. A system for quantifying dispersal of adult *Culex pipiens* was developed using amendment of catch basin habitats with nonradioactive, stable isotopes into the water. Results showed permanent marking of adults and dispersal to ranges of 1.5 km from original larval habitats. Sentinel birds were established in roost and no-roost zones of bird habitats and seroconversion rates to WNV were tested in these birds. Results showed lower seroconversion rates for birds set in roosts, suggesting that per capita risk of WNV exposure of wild birds in roosts is lower, supporting the concept of the herd effect. However, roosts could still function as amplification sites because the aggregate number of birds forming viremia in roosts could be higher than birds in solitary resting locations.

**Objective 2. Use knowledge of mosquito, pathogen, vertebrate reservoir, and environment interactions to enhance ability to predict and prevent conditions leading to disease.**

We expanded upon our model of the effects of environmental factors on West Nile virus disease risk have to include forecasts of transmission intensification in 2012, an abnormally hot and dry year. The model incorporates temperature as degree week above 22 degrees C threshold and precipitation in temporal lags. Data came from the Chicago region, the main study area. High ambient temperature was the strongest temporal predictor of increased infection in *Culex* mosquitoes, with cumulative high temperature differences being a key factor distinguishing years with higher mosquito infection and higher human illness rates from those with lower rates. The spring and summer of 2012 were unusually hot and degree week accumulation was the highest since the 2005 period when our data collection began. Forecasts predicted early and intense virus amplification in the vector population. Sampling of *Culex* spp. populations followed by analysis of WNV infection rates in pooled mosquitoes provided qualitative confirmation of these forecasts.

## **Publications**

1. Hamer GL, Donovan DJ, Hood-Nowotny R, Kaufman MG, Goldberg TL, Walker ED. Evaluation of a stable isotope method to mark naturally-breeding larval mosquitoes for adult dispersal studies. *J Med Entomol* 2012; 49: 61-70.
2. Gardner A, Hamer G, Hines A, Newman C, Walker ED, Ruiz M. Temporal change in precipitation and temperature affects habitat for larval *Culex* (Diptera: Culicidae) in storm water catch basins in suburban Chicago, USA, 2010. *J Med Entomol* 2012; 49: 270-276.
3. Morningstar RJ, Hamer GL, Goldberg TL, Huang S, Andreadis TG, Walker ED. Diversity of *Wolbachia pipientis* strain wPip in a genetically admixed, above-ground *Culex pipiens* (Diptera: Culicidae) population: association with form molestus ancestry and host selection patterns. *J Med Entomol* 2012; 49: 474-81.
4. Chuang T-W, Knepper RG, Stanuszek WW, Walker ED, Wilson ML. Cross-correlation map analyses show weather variation influences on mosquito abundance patterns in Saginaw County, Michigan, 1989 -2005. *J Med Entomol* 2012; 49: 851-58.
5. Kaufman MG, Stanuszek WW, Brouhard EA, Knepper RG, Walker ED. Establishment of *Aedes japonicus japonicus* and its colonization of container habitats in Michigan. *J Med Entomol* 2012; 49: 1307-17.

## **NE-1043: Biology, Ecology & Management of Emerging Disease Vectors**

**Dina M. Fonseca and Nina Fefferman, NJ**

□ Period the Report Covers: 10/2011 to 09/2012

### **NE1043 SAES-422: Accomplishments**

#### **Objective 1. Strengthen basic and applied research on the mosquito, pathogen, hosts, and environmental factors that influence disease emergence.**

A graduate student (Ashley DeNegre) worked with Fefferman to mentor an undergraduate student (Yi Ming Yu) for a summer research project to implement a computational simulation of the theoretical model developed during a previous project year, investigating the impact of relative biting rates among infected and uninfected hosts on the threshold between endemic and epidemic rates of emerging disease. Two student posters were given at national meetings (MAA and JMM), and 3 student seminar talks have been given (2 at Rutgers and one at CUNY) to student research groups.

We have obtained the necessary families of *Aedes albopictus* mosquitoes (known single male and female and respective offspring) to simultaneously identify and examine the inheritance of thousands of Single Nucleotide Polymorphisms (SNPs). We currently have 10 families started from virgin females (separated as pupae and allowed access to a single male). The number of offspring per family is  $26.5 \pm 3.3$  (Mean $\pm$ SE), which considering SNPs rarely have more than 2 alleles (max is 4: A, C, G, T) will provide more than adequate statistical power to examine their inheritance. We will test if SNPs are inherited following standard Mendelian patterns. Only those that do will be useful to perform population genetic analysis. SNPs that are sex-linked or exhibit non-Mendelian inheritance will be explored for other objectives.

#### **Objective 2. Use knowledge of mosquito, pathogen, vertebrate reservoir, and environment interactions to enhance ability to predict conditions leading to disease.**

One graduate student (Karen Wylie) continued her work with Fefferman to analyze the outcomes from a model developed in the previous project year to predict the differences in density- versus frequency-driven disease transmission due to differences in host-vector interactions, ecology,

and environmental conditions. This model focuses on predicting which vectors will play the most important role in disease transmission under which conditions when multiple species (including mosquitoes) can vector a disease.

Further, Fefferman and Wylie co-mentored an undergraduate student (Colleen Thiersch) for a summer research project in which she worked to create a computational simulation based on the analytic system of equations from the theoretical model.

Invasive mosquitoes have caused inordinate human suffering because they can drive local or exotic disease epidemics. To understand factors that deter or promote invasiveness we studied a new exotic mosquito in North America and Europe and in its native East Asia. We traced the source of all exotic populations to central Japan, where we found homogeneity across populations. Spatiotemporal analyses of exotic populations revealed that (1) genetic diversity declined precipitously outward from introduction points limiting expansion; (2) populations across the exotic range had very different genetic signatures; (3) all broadly expanding populations (=invasive) had genetic signatures resulting from admixture of separate introductions. We found that human-assisted rapid movement of specimens across the exotic range “rescued” self-limiting introductions by mixing locally differentiated genotypes. We propose that invasiveness is not a required pre-existing trait in mosquitoes, but instead can evolve in the exotic range leading inexorably to dangerous human disease vectors.

### **Objective 3. Develop strategies to control mosquito vectors.**

Funded by the Area-wide management of the Asian tiger mosquito (USDA-ARS) we have tested, optimized, and shared with the mosquito control community multiple methodologies to control *Aedes albopictus*. We have made multiple presentations at AMCA, ESA, NJMCA, ASTMH, made available detailed Standard Operating Procedures (SOPs) and published multiple peer-reviewed publications.

### **Objective 4. Enhance surveillance technologies for mosquitoes and mosquito-borne pathogens.**

The models (both analytic and computational) of Fefferman, DeNegre, and Yu will, once completed, provide not only prediction about the conditions under which endemic disease in non-human hosts will be likely to emerge in human populations, but will be able to predict levels of prevalence in suites of potentially infected species which will constitute greater risk for emergence into human outbreaks than absolute counts within single species or total numbers of identified cases without regard for the distribution across species.

Blood meal analysis (BMA) is a useful tool for epidemiologists and vector ecologists to assess which vector species are critical to disease transmission. In most current BMA assays vertebrate primers amplify DNA from a blood meal, commonly an abundant mitochondrial (mtDNA) locus, which is then sequenced and compared to known sequences in GenBank to identify its source. This technique, however, is time consuming and costly as each individual sample must be sequenced for species identification and mixed blood meals cloned prior to sequencing. Further, we found that several standard BMA vertebrate primers match sequences of the mtDNA of the Asian tiger mosquito, *Aedes albopictus*, making their use for blood meal identification in this species impossible. Because of the importance of *Ae. albopictus* as a vector of dengue and chikungunya viruses to humans, we designed a rapid assay that allows easy identification of human blood meals as well as mixed meals between human and nonhuman mammals. The assay consists of a nested PCR targeting the cytochrome b (cytb) mtDNA locus with a blocking primer in the internal PCR. The blocking primer has a 3' inverted dT modification that when used with the Stoffel Taq fragment prevents amplification of nuclear cytochrome b pseudogenes in humans



and allows for the continued use of *cytb* in BMA studies, as it is one of the most species-rich loci in GenBank. We used our assay to examine 164 blooded specimens of *Ae. albopictus* from suburban coastal New Jersey and found 62% had obtained blood from humans with 7.6% mixes between human and another mammal species. We also confirmed the efficiency of our assay by comparing it with standard BMA primers on a subset of 62 blooded *Ae. albopictus*. While this assay was designed for use in *Ae. albopictus*, it will have broader application in other anthropophilic mosquitoes

### **Objective 5. Develop strategies for sustainable mosquito control by including training at all levels.**

Brian Johnson, a PhD student through DENR has joined my laboratory. He is working on the effect of urban wetlands on WNV transmission. Rhiannon Andre-Tucker is an undergraduate working on local adaptation in *Aedes albopictus*". We are preparing a manuscript together. Rebekah Heiry has left to join Hartz Company has a full time researcher but will be making a presentation at the American Mosquito Control Association meeting in Feb 2013 on her work in the lab on mosquito behavior and control. Talks by Dina Fonseca outside of the scientific meeting circuit aimed at enhancing understanding of vector-borne diseases and mosquito control. May 17 and May 25, 2012: two 3-hour presentations entitled "Enlightened mosquito control" to the Rutgers Environmental Stewards, at the Atlantic County Utility Authority, NJ and the Duke Farms, Hillsborough, NJ. 2012, December 5 – Oral presentation on West Nile virus at the Emerging Infectious Diseases Conference organized by the Rutgers College of Nursing. 2012, March 28-30, Atlantic City, NJ – Annual Meeting of the NJ Mosquito Control Association "Current update on *Aedes albopictus* and the Asian tiger mosquito project". 2012, March 16-19, Hartford, CT – 83<sup>rd</sup> Annual Meeting of the Entomological Society of America Eastern Branch. Xu J, Fonseca DM. "Invasiveness Associated with multiple introductions of a temperate mosquito. 2012, Feb 26-Mar 1, Austin, TX – 78<sup>th</sup> Annual Meeting of the American Mosquito Control Association "The pros and cons of egg counts".

### **Publications (2012)**

1. Versteirt V, De Clercq EM, Fonseca DM, Pecor J, Schaffner F, Coosemans M, Van Bortel W. 2012. Bionomics of the established exotic mosquito species *Aedes koreicus* in Belgium, Europe. **Journal of Medical Entomology**. 49(6): 1226-1232.
2. Mogi M, Armbruster P, Fonseca DM. 2012. Analyses of the northern distribution limit of *Aedes albopictus* (Diptera: Culicidae) with a simple thermal index. **Journal of Medical Entomology**. 49(6): 1233-1243.
3. Strickman D, Fonseca DM. 2012. Autogeny in *Culex pipiens* complex mosquitoes from the San Francisco Bay Area. **American Journal of Tropical Medicine and Hygiene**. 87(4):719-26.
4. Versteirt V, Pecor J, Fonseca DM, Coosemans M, Bortel WV. 2012. Confirmation of *Aedes koreicus* (Diptera: Culicidae) in Belgium and description of morphological differences between Korean and Belgian specimens validated by molecular identification. **Zootaxa**. 3191:21-32.

**Harrington, Laura NE-1043 Report 2012. Cornell University**

### **Objectives:**

- 1) Strengthen basic and applied research on the pathogen, hosts, and environmental factors that influence mosquito-borne disease emergence.
- 2) Use knowledge of mosquito, pathogen, vertebrate reservoir, and environment interactions to enhance ability to predict and prevent conditions leading to disease.

During this reporting period we investigated several aspects of emerging disease vectors. We: 1) determined the capacity of select North American Strains of the Asian tiger mosquito (*Aedes albopictus*) to transmit the epidemic genotype of Chikungunya virus; 2) developed a model for the introduction of CHIKV into New York, Atlanta and New Orleans and its subsequent spread based on introduction by one individual. We developed a climate based model and combined it with an epidemiological model that simulates the introduction, replication and dissemination of the virus by the Asian tiger mosquito; 3) provided information for the public and health workers through media outlets and presentations on the risk of Chikungunya virus introduction in the United States; and 4) we developed methods to test dog heartworm vectors for ability to transmit the *D. immitis* parasite. We also conducted a review of the literature and current status of research on dog heartworm in the United States given increasing incidence and problems with control.

In consideration for the risk of Chikungunya introduction to the US, we developed a model for disease introduction based on virus introduction by one individual. Our study combined a climate-based mosquito population dynamics stochastic model with an epidemiological model to identify temporal windows that have epidemic risk. Our laboratory results demonstrated vector competence of all North American strains tested for the epidemic Chikungunya virus genotype. We ran this model with temperature data from different locations to study the geographic sensitivity of epidemic potential. We found that in locations with marked seasonal variation in temperature there also was a season of epidemic risk matching the period of the year in which mosquito populations survive and grow. In these locations controlling mosquito population sizes might be an efficient strategy. But, in other locations where the temperature supports mosquito development all year the epidemic risk is high and (practically) constant. In these locations, mosquito population control alone might not be an efficient disease control strategy and other approaches should be implemented to complement it. Our results strongly suggest that, in the event of an introduction and establishment of Chikungunya in the US, endemic and epidemic regions would emerge initially, primarily defined by environmental factors controlling annual mosquito population cycles. These regions should be identified to plan different intervention measures. In addition, reducing vector: human ratios can lower the probability and magnitude of outbreaks for regions with strong seasonal temperature patterns. This is the first model to consider Chikungunya risk in the US and can be applied to other vector borne diseases. We published our model in the journal *PloS Neglected Tropical Diseases*.

In addition, we optimized dose and other methods to infect and track dog heartworm development in mosquitoes. We followed up on our review publication in 2011 with an additional paper outlining a research agenda for moving forward.

- 3) Develop new strategies to control mosquito disease vectors.

- 4) Conduct surveillance for mosquitoes and mosquito-borne pathogens.
- 5) Develop training programs that maintain the national scientific expertise to respond to future health threats.

### **Recent Harrington publications for these projects:**

Ledemsa, N and Harrington, L.C. 2011. Topical Review: Vectors of Dog Heartworm in the United States: Vector status and factors effecting transmission efficiency. *Topics in Companion Animal Medicine*. Volume 26 (4): 178-185.

Ruiz-Moreno D, Vargas IS, Olson KE, Harrington LC. 2012. Modeling Dynamic Introduction of Chikungunya Virus in the United States. *PLoS Negl. Trop. Dis.* 6(11): e1918.

doi:10.1371/journal.pntd.0001918

Brown HE, Harrington LC, Kaufman PE, McKay T, Bowman DD, Nelson CT, Wang D and R. Lund 2012. Key Factors Influencing Canine Heartworm, *Dirofilaria immitis* in the United States. *Paras. Vect.* 5(1):245.

Menda G, Uhr JH, Wyttenbach RA, Vermeylen FM, Smith DM, Harrington LC, and RR Hoy. 2012. Associative learning in the dengue vector mosquito, *Aedes aegypti*: Avoidance of a previously attractive odor or surface color that is paired with an aversive stimulus. *J. Exper Biol* doi:10.1242/jeb.074898

Helinski MEH, Deewatthanawong P, Sirot LK, Wolfner MF and LC Harrington. 2012. Duration and dose-dependency of female sexual receptivity responses to seminal fluid proteins in *Aedes albopictus* and *Ae. aegypti* mosquitoes. *J Insect Phys.* 58(10):1307–1313

Helinski MEH and LC Harrington. 2012. The role of male harassment on female fitness for the dengue vector, *Ae. aegypti*. *Behavioral Ecology and Sociobiology*. DOI: 10.1007/s00265-012-1365-9

### **Fallon, Anne. University of Minnesota**

The obligate intracellular bacterium, *Wolbachia pipientis* (Rickettsiales: Anaplasmataceae) distorts reproduction of its arthropod hosts to facilitate invasion of naïve populations. This property makes *Wolbachia* an attractive “gene drive” agent with potential applications in control of insect vector populations. Genetic manipulation of *Wolbachia* will require in vitro systems for its propagation, genetic modification, amplification, and introduction into target insects. *Wolbachia* from the planthopper, *Laodelphax striatellus*, establishes a robust infection in clonal C7-10 *Aedes albopictus* mosquito cells. Infected cells, designated C/wStr, expressed radiolabeled proteins that were enriched in cells grown in the absence of antibiotics that inhibit *Wolbachia*, relative to cultures grown in medium containing tetracycline and rifampicin. Using mass spectrometry, we verified that tryptic peptides from an up-regulated 24 kDa band predominantly represented proteins encoded by the *Wolbachia* genome, including the outer surface protein, Wsp. We further showed that resistance of *Wolbachia* to streptomycin is associated with a K42R mutation in *Wolbachia* ribosomal protein S12, and that the pattern of amino acid substitutions in ribosomal protein S12 shows distinct differences in the closely related genera, *Wolbachia* and *Rickettsia*. We proceeded to use C/wStr cells to test whether *Wolbachia*-associated changes in antioxidant activities could provide a tool to select for infected cells using the common oxidizing agent, paraquat (PQ). Like mammalian cells, mosquito cells

tolerate PQ over a wide range of concentrations, and for considerable lengths of time, depending on cell density at the time of treatment. When mosquito cells were plated at low density and allowed to grow in the presence of PQ, we measured an LC<sub>50</sub> of approximately 1-2  $\mu$ M. Unexpectedly, cells persistently infected with Wolbachia strain wStr, from the planthopper *Laodelphax striatellus*, grew to higher densities in the presence of 1.5  $\mu$ M PQ than in its absence. This effect of PQ was reminiscent of the improved growth of host cells that occurs in the presence of antibiotics that suppress the Wolbachia infection. A more detailed examination of growth and metabolic sensitivity indicated that wStr is about 10-fold more sensitive to PQ than the mosquito host cells. Microscopic examination confirmed that Wolbachia levels were reduced in PQ-treated cells, and DNA estimates based on the polymerase chain reaction (PCR) indicated that Wolbachia abundance decreased by approximately 100-fold over a 10 day period. Although Wolbachia genomes encode superoxide dismutase (SOD), inspection of annotated genomes indicates that they lack several genes encoding products that ameliorate oxidative damage, including catalase, which converts the PQ byproduct, hydrogen peroxide, to molecular oxygen and water. We suggest that loss of multiple genes that participate in repair of oxidative damage accounts for increased sensitivity of Wolbachia to PQ, relative to its host cells. In mosquitoes, Wolbachia causes the reproductive distortion known as cytoplasmic incompatibility, or CI. In an incompatible cross, eggs of uninfected females fail to hatch when fertilized by sperm from infected males. We used polyacrylamide gel electrophoresis and tandem mass spectrometry to identify Wolbachia proteins in infected mosquito gonads. These included surface proteins with masses of 25 and 18 kDa and the DNA binding protein, HU, which potentially plays a role in cytoplasmic incompatibility. Using reverse transcriptase polymerase chain reaction, we showed that the HU gene is transcribed in Wolbachia-infected *Culex pipiens* and *Aedes albopictus* mosquitoes. We sequenced HU genes from four Wolbachia strains and compared deduced protein sequences with additional homologs from the databases. Among the Rickettsiales, Wolbachia HU has distinct N- and C-terminal basic/acidic amino acid motifs as well as a pair of conserved, cysteine residues. Similarities to eukaryotic architectural chromatin proteins underscore a potential role for HU in cytoplasmic incompatibility. A manuscript has been submitted to "Insect Biochemistry and Molecular Biology" documenting the following: 1) Wolbachia express a DNA binding protein, HU, in mosquito testes and ovaries; 2) HU abundance is comparable to that of Wolbachia surface proteins; 3) Wolbachia HU has unique amino acid motifs absent in *E. coli* homologs; 4) HU is the first detected Wolbachia-encoded protein that potentially binds sperm DNA.

Beckmann, J. F. and Fallon, A. M. Decapitation improves detection of *Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) in *Culex pipiens* Linnaeus (*Diptera: Culicidae*) mosquitoes by the polymerase chain reaction. *J. Med. Entomol.*, 49, 1103-1108, 2012. NIHMS432423

Fallon, AM, Baldrige, GD, Higgins, LA, Witthuhn, BA. *Wolbachia* from the planthopper *Laodelphax striatellus* establishes a robust, persistent, streptomycin resistant infection in clonal mosquito cells. *In vitro cell develop biol--Animal*, 49, 66-73, 2013; chosen for feature article, *In Vitro Reports Society for In Vitro Biology Newsletter*.

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**Becnel, Jimmy. ARS-Gainesville.**

**Multistate Project Biology, Ecology and Management of Emerging Disease Vectors**

### **Objective 3. Develop new strategies to control mosquito disease vectors.**

A broad based approach to new toxicant discovery includes screening of conventional chemical libraries and structure activity relationship analysis, evaluation of registered compounds for mosquitocidal activity and bioassays of compounds derived from natural products. In addition, we are exploring gene silencing using RNAi technology as a method to knock down critical proteins in adult and larval mosquitoes with possible applications for control (Becnel, ARS-Gainesville, FL).

Studies on mosquito pathogenic viruses and microsporidia are focused on pathogen/host interactions. Studies investigating the immune response in mosquitoes challenged with a baculovirus (CuniNPV) are ongoing and sequencing of the genome of AesoNPV is underway. Complete genomic sequences for two mosquito microsporidia have been completed and RNA-seq studies are underway. *Vavraia culicis floridensis* has a genome size of approximately 6.1Mb while *Edhazardia aedis* is significantly larger at approximately 51Mb. *Anncaliia algerae* sequencing is to be completed early 2013. (Becnel, ARS-Gainesville, FL).

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- Sims, K., Becnel, J. J. and Funderburk, J. 2012. The morphology and biology of the entomophilic *Thripinema fuscum* (Tylenchida: Allantonematidae), and the histopathological effects of parasitism on the host *Frankliniella fusca* (Thysanoptera: Thripidae), Journal of Natural History, 46:17-18, 1111-1128.

Sterling, K. M., Okech, B. A., Xiang, M. A., Linser, P. J., Price, D. A., VanEkeris, L., Becnel, J. J. and Harvey, W. H. 2012. High affinity 3H-Phe uptake by brush border membrane vesicles from whole larvae of *Aedes aegypti* (AaBMVw). *Journal of Insect Physiology* 58, 580-589.

**Cortinas, Roberto. Nebraska**

**NE-1043 - Biology, Ecology & Management of Emerging Disease Vectors  
2012 Annual Report**

Prepared by Alister Bryson, Julianne Matczyszyn, & Roberto Cortinas

**Objective 1.** Strengthen basic and applied research on the mosquito, pathogen, hosts, and environmental factors that influence disease emergence.

**Objective 2.** Use knowledge of mosquito, pathogen, vertebrate reservoir, and environment interactions to enhance ability to predict conditions leading to disease.

**Objective 5.** Develop strategies for sustainable mosquito control by including training at all levels.

The highest incidence of West Nile virus infection on a per capita basis is in the western Great Plains region, although the region is sparsely populated. In addition, farming and ranching are the most important economic sectors in this semiarid portion of the US. Irrigation practices allow for various crops to be cultivated in the region. Irrigation has been associated with increased risk of various mosquito-borne pathogens in other agroecosystems, and at the regional level, irrigation appears to be associated with increased WNV risk. However, at the landscape and farm level, distinct irrigation systems may differentially contribute to WNV risk. Our goal for the present study is to assess WNV risk in relation to irrigation practices and cultivated crops. Our objectives are to describe adult and immature mosquito diversity and density and to assess WNV prevalence in host-seeking female mosquitoes associated with three types of irrigation (subsurface drip, center pivot, and gravity-fed surface irrigation) and two crop types (corn and soybean),

The two-year study is being conducted at the University of Nebraska South Central Agriculture Laboratory (SCAL) and at farms located around Clay Center, NE. During the summer of 2012, a total of 321 larval dips were taken. Over 8,800 adult mosquitoes were collected at SCAL, and 21,376 mosquitoes were collected in surrounding fields. Sixteen species have been identified including *Culex tarsalis*, *Cx. restuans*, *Cx. erraticus*, *Cx. salinarius*, *Coquillettidia perturbans*, *Aedes vexans*, *Ochlerotatus canadensis canadensis*, *Oc. nigromaculis*, *Oc. sollicitans*, *Oc. triseriatus*, *Psorophora ciliata*, *Ps. columbiae*, *Ps. cyanscens*, *Ps. discolor*, *Anopheles quadrimaculatus* sensu lato, and *An. barberi*. *Culex tarsalis* (48% of SCAL-collected, 16% of field-collected mosquitoes) and *Aedes vexans* (27% SCAL, 67% field) were the most collected species. We anticipate that severe drought coupled with very low humidity during much of the summer will impact our findings.

This study is being conducted by Alister Bryson, a Masters degree candidate. He is being assisted by Renee Berger, an undergraduate student with an interest in vector-borne disease ecology.

**Objective 1.** Strengthen basic and applied research on the mosquito, pathogen, hosts, and environmental factors that influence disease emergence.

**Objective 4.** Enhance surveillance technologies for mosquitoes and mosquito-borne pathogens.

**Objective 5.** Develop strategies for sustainable mosquito control by including training at all levels.

In rural areas of Nebraska, dry ice availability can be a problem. Octenol has a stable shelf life (if sealed), does not need to be refrigerated and is commercially available. We were interested in finding out if different formulations and concentrations of octenol could be used in lieu of dry ice in surveillance programs.

The study was conducted at ten different sites within Lancaster County, Nebraska during the summer of 2011. Liquid octenol in high and low volumes was compared with gel packets of octenol in high and low volumes to indicate if the response in total mosquito and *Culex* species mosquito attractant was comparable to traditionally used carbon dioxide in tandem with CDC light traps. CDC light traps were randomly baited with a chemoattractant treatment using a complete block design. Collections were separated out by date, location, and attractant, counted, and stored in a -80 freezer for further analysis. Overall, carbon dioxide collected significantly more total mosquitoes ( $F = 11.73$ ;  $df = 2, 18.05$ ;  $P < 0.0005$ ) and *Culex* mosquitoes ( $F = 13.11$ ;  $df = 2, 18.06$ ;  $P < 0.0003$ ) than any form of octenol. Mosquitoes have been identified and analyses are complete.

*Culex* mosquitoes that were collected during the chemoattractant studies and had the presence of a blood meal in the midgut were separated out, tested using PCR, sequenced, and analyzed on the online database system, GeneBank for blood meal content. Of the 502 *Culex* species (*Cx. erraticus*, *Cx. pipiens*, *Cx. salinarius*, *Cx. restuans*, *Cx. tarsalis*, *Cx. territans*), 205 were successfully matched with a blood meal source. Majority of the blood meals were indicated as human (74%) and the next highest blood meal source were avian species (8%). Statistical results are pending. This is the first study assessing mosquito hosts via blood meal analysis in Nebraska.

This study is being conducted by Julianne Matczynszyn, a Masters degree candidate graduating Spring 2013. She is interested in pursuing a doctoral degree with an interest in molecular approaches in entomology and nematology.

Federici, Brian A. NE-1043 2012 Report. University of California, Riverside.

### **Objective 3. Develop strategies to control mosquito vectors**

Strains of *Bacillus thuringiensis* such as *B. thuringiensis* subsp. *israelensis* (ONR-60A) and *B. thuringiensis* subsp. *morrisoni* (PG-14), which highly toxic for mosquito larvae, produce a complex parasporal body consisting of several protein endotoxins synthesized during sporulation that form an aggregate of crystalline inclusions bound together by a multilamellar fibrous matrix. Most studies of these strains focus on the endotoxins. Although it is known that parasporal body structural integrity is important to achieving high mosquitocidal toxicity, virtually nothing is known about the matrix that binds the toxin inclusions together. A proteomic analysis of this matrix identified proteins that mediate assembly and stability of the parasporal body. In addition to fragments of their known major toxins, namely Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa, peptides with 100% identity to regions of Bt152, a protein coded for by pBtoxis of *B. thuringiensis*

subsp. *israelensis*, the plasmid that encodes all endotoxins of this subspecies were obtained. Mutation of this protein using recombinant DNA technology destabilized the parasporal body matrix, and concomitantly, inclusion aggregation. Fluorescence microscopy further demonstrated that Bt152 localized to the parasporal body in both strains, but was absent in other structural or soluble components of the cell, including the endospore and cytoplasm. Ligand blots showed this Bt152 bound to the purified multilamellar fibrous matrix. Together the data show that Bt152 is essential for stability and parasporal larvicidal toxicity of these strains (CA).

The *cry19A* operon of *B. thuringiensis* subsp. *jegathesan* encodes two proteins, mosquitocidal Cry19A (ORF1; 75 kDa) and an ORF2 (60 kDa) of unknown function. Expression of the *cry19A* operon in an acrySTALLIFEROUS strain of *B. thuringiensis* (4Q7) yielded one small crystal per cell, whereas no crystals were produced when *cry19A* or *orf2* was expressed alone. To determine the function of the ORF2 protein, different combinations of Cry19A, ORF2, and the N- or C-terminal half of Cry1C were synthesized in strain 4Q7. Stable crystalline inclusions of these fusion proteins similar in shape to those in the strain harboring the wild-type operon were observed in sporulating cells. Comparative analysis showed that ORF2 shares considerable amino acid sequence identity with the C-terminal region of large Cry proteins. Together, these results suggested that ORF2 assisted in synthesis and crystallization of Cry19A by functioning like the C-terminal domain characteristic of Cry protein in the 130-kDa mass range. To determine whether overexpression of the *cry19A* operon stabilized its shape and increased Cry19A yield, it was expressed under the control of the strong chimeric *cyt1A*-p/STAB-SD promoter. In contrast expression achieved with the native promoter, overexpression yielded uniform bipyramidal crystals that were 4-fold larger on average than the wild-type crystal. In bioassays using the 4th instars of *Cx quinquefasciatus*, the strain producing the larger Cry19A crystal showed moderate larvicidal activity that was 4-fold (95% lethal concentration [LC95] = 1.9 µg/ml) more toxic than the activity produced in the strain harboring the wild-type operon (LC95 = 8.2 µg/ml) (CA).

Mendelian crosses were used to study the mode of inheritance of Cry toxin resistance in a *Culex quinquefasciatus* Say colony (CqAB11A) that evolved insecticide resistance under laboratory selection with a deletion mutant of *Bacillus thuringiensis* subsp. *israelensis* lacking the Cyt1Aa toxin component but containing its three major Cry toxins, Cry4Aa, Cry4Ba, and Cry11Aa. High levels of resistance were observed to Cry toxins. F1 offspring of reciprocal crosses to a sensitive colony showed intermediate levels of resistance with no maternal effect, indicating autosomal inheritance. Dose-response data for backcross offspring deviated significantly from the monofactorial model when tested with Cry4Aa+Cry4Ba+Cry11Aa, Cry4Aa+Cry4Ba, or Cry11Aa. However, tests with Cry11Ba from *B. thuringiensis* subsp. *jegathesan* fit the monofactorial model. Dominance of F1 offspring was calculated at different concentrations of Cry-toxin suspensions and, as reported for other Cry-resistant *Culex*, generally decreased as concentration increased. A subset of colony CqAB11A was reared without selection pressure for 18 generations with little change in susceptibility, indicating a highly homozygous population. Consistent with reports for other Cry-resistant *Culex*, the data show these mosquitoes evolved resistance to *B. thuringiensis* Cry toxins at multiple loci in response to selection pressure and that cross-resistance to Cry11Ba was conferred by one of those loci (CA).



## **Publications**

Diaz-Mendoza M, Bideshi DK, Ortego F, Farinos GF, Federici BA. 2012. The 20-kDa chaperone-like protein of *Bacillus thuringiensis* spp. *israelensis* enhances yield, crystal size and solubility of Cry3A. *Letts Appl Microbiol* 54: 88-95.

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Diaz-Mendoza M, Bideshi DK, Federici BA. 2012. A 54-kDa protein encoded by pBtoxis is required for parasporal body structural integrity in *Bacillus thuringiensis* subsp. *israelensis*. *J Bacteriol* 194:1562-1571.

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## **Paul Leisnham**

**Maryland Agricultural Experiment Station. University of Maryland College Park.**

## **Multistate Project NE-1043.**

## **2012 Annual Progress Report.**

## **Publications**

Leisnham, P.T. and S.A. Juliano. 2012. Impacts of Climate, Land Use, and Biological Invasion on the Ecology of Immature *Aedes* Mosquitoes: Implications for La Crosse Emergence. *EcoHealth* 9: 217-228.

## **Objective 1. Strengthen basic and applied research on the mosquito, pathogen, hosts, and environmental factors that influence disease emergence.**

Predictions for three hypotheses of *Aedes japonicus* invasion into North America were tested: spatial partitioning among individual water-filled containers, variable container resources, and keystone predation. We measured *A. japonicus* and *A. albopictus* abundances and *Toxorhynchites rutilus* presence among treeholes and tires in Washington, D.C., and tested competition between *A. japonicus* and *A. albopictus* with or without *T. rutilus* in laboratory microcosms. Consistent with the spatial partitioning hypothesis, *A. japonicus* was negatively correlated and independently aggregated with *A. albopictus*, and not associated with *T. rutilus*, among field containers. Consistent with the variable resource hypothesis, container resources (detritus) showed high spatial variability and *A. japonicus* was more strongly associated with them than *A. albopictus*. In laboratory microcosms, *A. albopictus* was competitively dominant over *A. japonicus*, which is consistent with the few prior studies of competition between these species. *Toxorhynchites rutilus* predation severely lowered performances of both *Aedes* species but more severely lowered *A. japonicus* performance than *A. albopictus* performance when all three species co-occurred, therefore yielding no evidence for keystone predation (MD).

Mosquito larval microhabitats (mostly water-holding containers) in six neighborhoods in the Washington, D.C. metropolitan area that varied in socioeconomic status (SES) and housing structure (row houses versus stand-alone houses) were sampled to test associations of these neighborhood characteristics with microhabitat quantity and parameters. Nested associations of neighborhood characteristics and microhabitat parameters on mosquito presence and density were also tested. Thirty-four percent (33.9%) of sampled microhabitats contained mosquito larvae, and 93.1% of larvae were *Aedes albopictus* or *Culex pipiens*. Four specific container types (drains, planters, garbage cans, and buckets) accounted for the majority of water-holding (56.0%) and mosquito-positive (50.6%) microhabitats sampled. We found no associations between SES or housing structure with total container abundance per yard or total mosquito density per container. However, container purpose varied with SES, with lower SES neighborhoods having higher numbers of disused containers than high SES neighborhoods and lower numbers of functional containers than both low and medium SES neighborhoods. *Aedes albopictus* was 83% more abundant in disused containers, while *Cx. pipiens* were more abundant in functional containers, possibly due to species-specific oviposition preferences related to habitat permanence (MD).

Mosquito communities were sampled in three historically ditched tidal salt marshes and three paired control (unditched) marshes on the Maryland Delmarva Peninsula, as part of a multi-year project whereby the ditched marshes will receive a management intervention (ditch plugging) in Winter of 2013-14 as part of a Before-After-Control-Intervention Study. Two of the three ditched marshes had a higher proportion of randomly located sample sites with mosquito larvae than their paired control marsh. The majority of larvae in all marshes were *Anopheles bradleyi*. In 2013, random study sites will again be sampled, as well as randomly selected ponds and ditches in ditched marshes and sites of high mosquito abundance among all marshes as part of targeted sampling (MD).

Dobson, S  
University of Kentucky

Relevant to strategies requiring the release of adult mosquitoes, we have devoted effort to developing additional safeguards against the unintended release of fecund females. We have evaluated for chronic effects caused by the exposure of adult *Aedes* mosquitoes to methoprene. We applied methoprene to adults, both through (1) droplet application to the abdomen and (2) as an aerosol, examining for treatment effects on ovary development, adult longevity and fecundity. The results demonstrate that relatively high doses are required to affect adult survivorship. In contrast, significant impacts on both fecundity and egg hatch were observed for females treated at the lower dosages.

Outcomes/Impacts:

Development of an integrated control strategy and specific recommendations for area-wide control of the Asian tiger mosquito. Assess the feasibility of initiating large field cage experiments on the sterile insect technique. Provide for and encourage environmentally sound, scientifically based, and professional control by mosquito control agencies.

Publications:

Andrews ES, Crain PR, Fu Y, Howe DK & Dobson SL (2012) Introduced *Wolbachia* Infection Affects Production of Reactive

Oxygen Species and *Brugia pahangi* Survival within *Aedes polynesiensis*. PLoS Pathogens. In Press.

Calvitti M, Moretti R, Skidmore AR & Dobson SL (2012) *Wolbachia* strain wPip yields a pattern of cytoplasmic incompatibility enhancing a *Wolbachia*-based suppression strategy against the disease vector *Aedes albopictus*. Parasites & vectors. In Press.

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Participants: