

Title: Molecular Approaches to Examining the Population Biology of *Aedes albopictus* in North America.

Abstract:

Aedes albopictus is an invasive mosquito species to the United States that has rapidly increased its geographic range since its initial introduction into Texas in the mid-1980s. Genetic variation at two previously developed nuclear restriction fragment length polymorphism (nRFLP) probes will be examined to investigate the dynamics of the establishment and range expansion of *Ae. albopictus* in North America. Individuals will be genotyped at these highly variable loci in order to quantify levels of within- and between- region genetic variation. These data will be fundamental in understanding the dynamics of dispersal behavior through: 1) identification of genetic drift during population establishment, and 2) identification of migration between different regions throughout the current range of *Ae. albopictus* in North America.

Description of Project:

The Asian tiger mosquito, *Aedes albopictus*, is a species of mosquito originally from Southeast Asia that has recently expanded its geographic distribution to include most of Asia, Europe, Madagascar, Hawaii, Brazil, Africa, and portions of the continental United States (Kambhampati and Rai 1991). *Aedes albopictus* was initially introduced to the United States in the vicinity of Houston, Texas through a used-tire shipment from temperate Japan in the mid-1980s (Hawley et al. 1987). Since the time of its initial introduction into North America, *Ae. albopictus* has spread as far south as Florida and as far north as New Jersey and Ohio (across approximately 14° of latitude). This geographic range expansion has been accompanied by the rapid adaptation of *Ae. albopictus* to the substantial climactic variation that occurs across its current range in the United States. There is also evidence that *Ae. albopictus* is displacing other non-native species of mosquitoes in some areas. For example, in Florida it is in the process of displacing its close relative *Aedes aegypti* (O'Meara et al. 1995). In Texas, *Ae. albopictus* has been determined to be the most abundant artificial container breeding mosquito (Hawley et al. 1987).

In general the spread of exotic species is known to affect human health, agriculture, and the natural environment. *Aedes albopictus* is capable of transmitting dengue fever (Shroyer 1986), yellow fever (Miller 1989), West Nile virus (Hollick et al. 2002), and a variety of other North American arthropod-borne viruses (Shroyer 1986). Several cases of dengue fever have been introduced to the United States from Africa, South America, the Caribbean, and Southeast Asia since 1970 (Knudsen 1986). These considerations suggest that the possibility for future endemic transmission of dengue in this country by *Ae. albopictus* is possible (DeFoliart et al. 1986), especially because of the aggressive man-biting habit of this species. *Aedes albopictus* is also capable of inhabiting different environmental niches including urban, tire-dump habitats as well as more rural, tree-hole environments (Livdahl and Walley 1991). Consequently, the invasion of North America by *Ae. albopictus* is a major public health concern, and understanding the range expansion and adaptation that this species has undergone since the time of its initial introduction is fundamental to controlling this potential vector of human disease.

Previous Work:

Aedes albopictus mosquitoes have been shown to have very low levels of mitochondrial DNA (mtDNA) variability (Kambhampati and Rai 1991, Birungi and Munstermann 2002). Mitochondrial DNA is a very important tool in studies examining population structure due to the fact that it has a high rate of evolution relative to nuclear loci. Previous studies have suggested that the low mtDNA variability in *Ae. albopictus* may be due to a small founding population during the initial colonization of North America. This hypothesis also implies a single introduction into North America.

However, in previous research that I have conducted, I have hypothesized that this low mtDNA variability in *Ae. albopictus* could also, at least in part be the result of the infection of *Ae. albopictus* by the bacterial endosymbiont *Wolbachia* spp. (Armbruster et al. 2003). *Wolbachia* spp. is a rickettsiae-like maternally inherited bacterial parasite that modifies host reproduction in order to quickly propagate itself through the host population. Due to its maternal inheritance, *Wolbachia* spp. have the ability to drive one mitochondrial haplotype to fixation in a host population during the course of its spread through that population (Turelli and Hoffman 1995). My new hypothesis also suggests the possibility for multiple introductions of *Ae. albopictus* into North America, which would have important implications for our understanding of the invasion and adaptation of this species. For example, if multiple introductions of *Ae. albopictus* from genetically disparate source populations have occurred; such increased genetic diversity could allow the species to adapt more rapidly to the many diverse environments across its range in the United States.

Significance:

My current project focuses on examining the population structure of *Ae. albopictus* in North America. Population structure refers to levels of within- and between-population genetic variation. The data obtained from such a population structure study will be significant because they will provide an estimate of the potential for independent evolutionary change among populations throughout the geographic range *Ae. albopictus* in North America. These data will also estimate the amount of migration between populations of *Ae. albopictus* mosquitoes in North America, a parameter of potential importance in the prediction of the spread of disease by this mosquito species. Additionally, the data provided by these analyses could also have potentially important implications for the control of this medically important mosquito species. For example the selection of control methods depends greatly on the degree of genetic variation in a species because the variation is a good indication of the organism's ability to develop pesticide resistance or overcome other methods of control (Downie 2002). Previous attempts to investigate the population structure of *Ae. albopictus* have been met with little success due to this low variability in the mtDNA genome of *Ae. albopictus*. Consequently, it is now necessary to examine the population structure of this species by studying nuclear loci.

Proposed Methodology:

In order to examine the population structure of *Ae. albopictus* in North America and examine the effects of the range expansion of this species on its population structure, I propose to test the following hypotheses:

- H₀₁: The geographic range expansion of *Aedes albopictus* in the United States has not affected the WITHIN-region genetic variation in derived populations (Florida and New Jersey) at the edge of the species range relative to the ancestral Texas populations.
- H₀₂: The geographic range expansion of *Aedes albopictus* in the United States has not affected the BETWEEN-region genetic variation in derived populations (Florida and New Jersey) at the edge of the species range relative to the ancestral Texas populations.

I will use two previously developed nuclear Restriction Length Fragment Polymorphism (nRFLP) probes to examine the nuclear genetic variation in *Ae. albopictus*. In the past these markers have been used extensively in creating genetic linkage maps in *Aedes* mosquitoes (Severson et al. 1995), but have not yet been applied to population structure studies.

These probes were used to genotype 54 individuals from Texas (four collection sites), 101 from Florida (three collection sites), and 47 from New Jersey (two collection sites) at each of these two loci. Samples of *Ae. albopictus* from these three geographic locations were previously collected and selected because they represent the extent of the current geographic range of *Ae. albopictus* in the United States.

The actual procedure first involves the extraction and purification of the genomic DNA from the individual mosquitoes by a phenol-chloroform extraction protocol. After the DNA has been extracted and purified it can be cut by a restriction enzyme. In this case the restriction enzyme *EcoR*I was used, which recognizes the base-pair sequence of 5'-GAATTC-3'.

Once the genomic DNA has been digested by *EcoR*I, it can then be loaded into an agarose gel, where the DNA fragments will be separated according to their size by standard electrophoresis. The DNA is then transferred from the gel to a nylon membrane by a process called a Southern blot. The membrane can then be exposed to ultraviolet light, causing the DNA fragments to covalently bond to the nylon membrane. The membrane is then probed with the previously developed probes (see above). These probes bind to homologous DNA on the membrane identifying fragment length polymorphisms of individual mosquitoes, and allowing me to determine if different homologous chromosomes have the same sequence in a particular portion of the DNA strand.

Over the course of the previous academic year (as well as the summer preceding it), I have performed the procedures described above. The bands resulting from the probing of the membranes will now be scored (assigned a length in base pairs based on the ladder DNA on the membrane), and the resulting data will then be analyzed to examine differences between the three different regions.

The data analysis will consist of the calculation of the average within-region genetic diversity (Π_n) and the average between-region genetic variation (G_{ST} ; Nei 1975). I will then compare estimates of the mean (+/- standard error) within- and between-region genetic diversity in order to evaluate the consequences of the geographic range expansion on the population structure of *Ae. albopictus* in North America.

If there is not a statistically significant difference in the mean values of Π_n between the different regions (Texas, New Jersey, and Florida), then H₀ will be accepted. Consequently, it will be concluded that the range expansion of *Ae. albopictus* in the United States has not affected the within-region gene diversity. The first inference of such a result would be that there has been little to no genetic drift in *Ae. albopictus* populations

during this range expansion. Secondly, there will be no conclusive evidence that New Jersey, Florida, or Texas populations have received multiple introductions from genetically disparate host populations. This result would support the hypothesis that there was a single, initial introduction in Texas in the mid-1980s (Hawley et al. 1987), with no subsequent introductions in other geographic regions of the United States.

However, if there is a statistically significant difference in the mean values of Π_n , then H_{01} will be rejected. In this case, I will conclude that the geographic range expansion of *Ae. albopictus* in the United States has led to differences in levels of within-region gene diversity. The pattern of these observed differences will allow me to make inferences about the dynamics of the invasion of *Ae. albopictus* and the subsequent spread of the mosquito in North America. For example, if there is increased within-region gene diversity in the ancestral Texas population relative to the New Jersey and Florida populations, I will conclude that a population bottleneck has occurred during the range expansion of *Ae. albopictus* throughout the United States. Such a conclusion would also imply that mosquitoes in these derived regions would be less likely to develop pesticide resistance due to this low within-population gene diversity (Downie 2002). Additionally, these mosquitoes might be slower to adapt to the differing environmental conditions that occur throughout their range.

Alternatively, if there is increased within-region gene diversity in New Jersey relative to Texas and Florida, then it can be inferred that there was a small founding population in Texas that spread to Florida. This would also support previous suggestions that Texas was invaded by a small founding population of *Ae. albopictus*. However, the high level of gene diversity in New Jersey (relative to Texas and Florida) would suggest that there have been multiple introductions into New Jersey. These are only two possible scenarios, but conclusions from other scenarios would be assessed in a similar manner.

If there are no statistically significant differences between replicate G_{ST} values from the different geographic regions of the United States, H_{02} will be accepted. If this hypothesis is accepted, it can be concluded that the geographic range expansion of *Ae. albopictus* in the United States has not affected levels of between-region genetic variation. I would then conclude that the gene flow (i.e. migration) between different regions is approximately equivalent. This result would suggest similar control strategies may be appropriate for *Ae. albopictus* across its entire range in the United States because the movement of individuals between regions is similar. Additionally, this result would imply that *Ae. albopictus* mosquitoes from all regions of the study would have a similar rate at which they are capable of spreading disease.

Lastly, if I find statistically significant differences in G_{ST} values between geographic regions, I will reject H_{02} . Such a conclusion would indicate that there are genetic differences between regions, and several types of conclusions may be possible depending upon the specific patterns of differences. For example, a pattern of low (or no) difference in G_{ST} values between Florida and Texas, but high differences in G_{ST} values between Florida and New Jersey as well as between Texas and New Jersey, would suggest two possibilities. First, these differences in observed G_{ST} values could be due to an independent introduction of *Ae. albopictus* into New Jersey. Alternatively, this result might suggest high levels of gene flow between Texas and Florida populations, and relative genetic isolation of New Jersey populations due to low levels of gene flow with Texas and Florida populations. Such high levels of gene flow between Texas and Florida might be explained by considering domestic used-tire shipments through which *Ae. albopictus* eggs and larvae could be rapidly dispersed over vast geographic distances. Additionally, differences in G_{ST} values between regions would suggest that different control methods might have to be used in geographically disparate regions because of the implied isolation between regions. I might also conclude that *Ae. albopictus* mosquitoes from these different regions could potentially have different rates at which they were capable of spreading disease.

In conclusion, my study will provide fundamental insights into the basic population biology of *Ae. albopictus* in North America. My results will have important implications in the understating of the life history, possible control methods, and potential for disease transmission and further range expansion of this medically important mosquito species in North America.

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