



### Introduction

**Background:**

- The mosquito, *Aedes aegypti*, vectors yellow fever, dengue, zika, and chikungunya<sup>1</sup>.
- Dengue fever affects >300 million people per year<sup>1</sup>.
- Pyrethroids are one of the most common insecticide classes used to control *Aedes aegypti*<sup>2</sup>.
- Pyrethroids target the voltage-gated sodium channel gene (VGSC)<sup>2</sup>.
- Genetic resistance to pyrethroids develops with continued use of the insecticides<sup>2</sup>.
- Many mutations exist, but two are important in the Americas: V1016I and F1534C<sup>3,4</sup>.
- Mutations are physically linked in the genome, but typically do not rise in frequency together.
- Previous studies observe this pattern, but samples are limited<sup>3,4</sup>.
- One study indicates that heterogeneity exists in insecticide resistance frequency at the scale of a city block, but sampling efforts are not dense enough to understand the prevalence<sup>4</sup>.

**Iquitos, Peru:**

- Pyrethroids were used in Iquitos to control dengue epidemics from 2002 - 2014.
- During this time, phenotypic resistance to pyrethroids increased until they were replaced in 2014 for malathion, an insecticide with a different mode of action.
- In Iquitos, mosquitoes have been collected continually since the year 2000, providing a sample set that includes periods before, during, and after the use of pyrethroids.
- Each archived mosquito is associated with collection site GPS coordinates.
- The city is an ideal place to perform large scale population suppression experiments.
- We examine temporal and spatial patterns of insecticide resistance in *Aedes aegypti* to better understand the dynamics of insecticide resistance evolution.

Figure 1. This figure is intended to clarify the complicated nomenclature found in the literature and used within this poster. The mutations are single nucleotide polymorphisms (SNPs) that cause amino acid substitutions in the voltage gated sodium channel gene (VGSC). Multiple SNPs within the gene form haplotypes. \*Amino acid positions are determined based on the homologous mutation in the house fly VGSC.

Causative SNP	Amino Acid Substitutions in <i>kdr</i> Protein		Haplotype
	Position 1016*	Position 1534*	
Wild Type (wt)	Val	Phe	wt1016/wt1534
V1016I	Ile	Phe	Ile1016/wt1534
F1534C	Val	Cys	wt1016/Cys1534
V1016I & F1534C	Ile	Cys	Ile1016/Cys1534

### Objectives

- Determine if V1016I and F1534C mutations are found in *Aedes aegypti* from Iquitos, Peru.
- Determine when these mutations arose in the population.
- Calculate the selection coefficient for each mutation while under selection.
- Analyze spatial patterns of emergence to observe heterogeneity.
- Determine if genetic heterogeneity can be induced following an intense suppression experiment.

### Materials & Methods

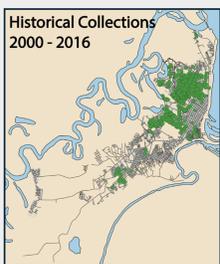


Figure 2. Map of Iquitos, Peru. All samples collected from 2000 - 2016 are plotted.

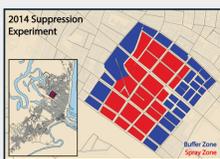


Figure 3. Map of 2014 suppression experiment. The buffer zone is shaded in blue. The spray zone is shaded in red.

**Collections:**

- All mosquitoes collected have GPS coordinates and stored at -80°C.
- Historical: 2000 - 2016
  - Locations - throughout city of Iquitos (Fig. 2).
- Suppression Experiment: 2014
  - Location - ~500 m x ~500 m area of Iquitos (Fig. 3).
  - Mosquitoes collected from January - October.
  - 6-week intensive spray regime completed: 4/28/14 - 6/2/14

**Laboratory Methods:**

- gDNA Isolations with Blood and Tissue protocol for Qiagen DNeasy Kit or Canadian Center for DNA Barcoding protocol.
- kdr Genotyping based off modified versions of protocols<sup>4,5</sup>.
  - All samples genotyped twice to ensure accuracy.
- 4,756 mosquitoes processed: gDNA isolated and genotyped.

**Analytical Methods:**

- All graphs analyzed and produced in R v. 3.3.2, package ggplot2.
- Maps produced in QGIS v. 2.18.13.
- Repeated G-test performed in R v. 3.3.2 package RVAideMemoire.

### Results

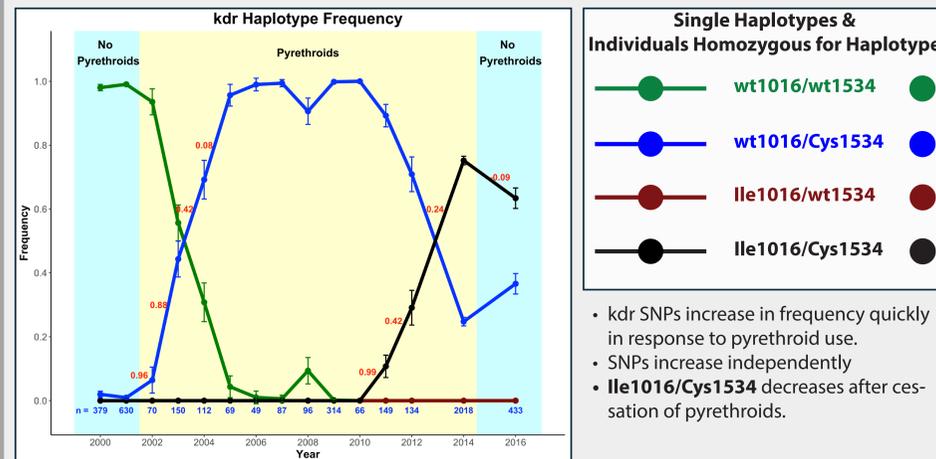
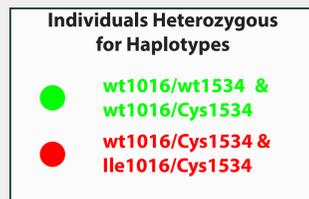


Figure 4. *kdr* haplotype frequencies from 2000 - 2016 in Iquitos, Peru. Shaded areas represent periods pyrethroids were used. Selection coefficients for certain years are printed in red near the haplotype line. Number of individuals genotyped per year in blue below y=0.0. Error Bars = 95% CI.



- Heterogeneity in insecticide resistance exists within Iquitos.
- Mosquitoes collected in 2008 from the southern part of the city had a higher prevalence of wt1016/wt1534 haplotypes, while the northern part of the city had a high frequency of the wt1016/Cys1534 haplotype (Fig. 4 datapoint 2008 & Fig. 5 box c).

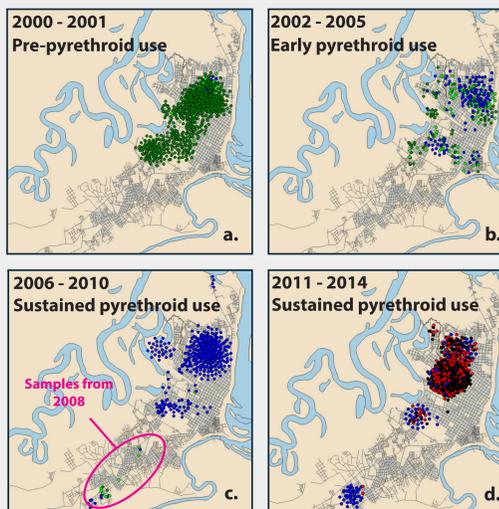


Figure 5. Maps of individual mosquitoes color coded by haplotype. a. 2000 - 2001 pre-pyrethroid use in Iquitos. b. 2002 - 2005 early pyrethroid use. c. Years 2006 - 2010 sustained pyrethroid use before the Ile1016/Cys1534 haplotype emerges. d. Years 2011 - 2014, sustained pyrethroid use following emergence of Ile1016/Cys1534 haplotype. Some points displaced in concentric circles for easier viewing.

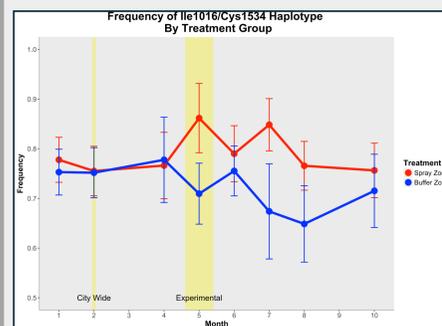


Figure 6. Frequency of the Ile1016/Cys1534 haplotype by treatment group across the year 2014. A 6-week experimental spray period occurred in the spray zone from 4/28/14 to 6/2/14 and is highlighted by a yellow block. A city-wide spray in response to a dengue outbreak occurred in February and is also highlighted in yellow. Error bars = 95% CI.

- Prior to the experimental spray period (highlighted in yellow) the frequency of the Ile1016/Cys1534 haplotype was not significantly different between treatment groups.
- After the experimental spray, the frequency of the Ile1016/Cys1534 haplotype increased in the spray zone and decreased in the buffer zone.
- A repeated G-test of heterogeneity found a significant difference (p = 0.004, df = 15) between month by treatment groups, suggesting that genetic structure exists in the population.

### Conclusions

**Objective 1:**

- Both *kdr* SNPs shown to be important in the Americas are present in Iquitos, Peru.

**Objective 2:**

- The wt1016/Cys1534 haplotype emerged in response to pyrethroid sprays in 2002 and rose to fixation by 2010.
- The Ile1016/Cys1534 haplotype first appeared in the city in 2011.
- The Ile1016/wt1534 haplotype is never observed.

**Objective 3:**

- Selection coefficients for wt1016/Cys1534 and Ile1016/Cys1534 haplotypes indicate that there was very strong selection pressure acting to increase the frequency of the *kdr* mutations.

**Objective 4:**

- Results from 2008 indicate that heterogeneity of insecticide resistance exists within the city.
- GIS mapping of individuals color coded by haplotype shows that high levels of resistance began in the northern part of the city.
- It is unknown if these SNPs arose independently within Iquitos or were introduced via migration. Future work will address this question.

**Objective 5:**

- Results from the 2014 suppression experiment indicate that significant (p = 0.004) amounts of heterogeneity can be induced with small scale focused suppression efforts.
- Future mosquito control and insecticide resistance management strategies may want to consider fine-scale levels of insecticide resistance when developing management plans.

### Future Directions

- Analyze suppression experiment replicate conducted in 2013.
  - We will analyze these results in the same way as the 2014 suppression experiment.
- Analyze flanking sequences for VGSC gene.
  - Because the origin of the resistant haplotypes is unknown, we will analyze the flanking sequences surrounding the VGSC gene. Flanking sequences are important for determining from where the wt1016/Cys1534 and Ile1016/Cys1534 haplotypes arose in Iquitos.
- Some possibilities:
  - New and independent mutations within the city.
  - Selection acting on existing low-levels of variation in the population.
  - Immigrants moving into the city from elsewhere.
- Each possibility will present a different pattern of flanking sequences in the population.



Figure 7. Flanking sequences on either side of the VGSC gene can be informative for determining the demographic history that leads to the current level and pattern of insecticide resistance in Iquitos, Peru.

### Sources

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