Temporal and spatial patterns of knockdown resistance in Aedes aegypti from Iquitos, Peru

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Conclusions

- The mosquito, Aedes aegypti, vectors yellow fever, dengue, zika, and chikungunya2.
- Dengue fever affects >300 million people per year.
- Pyrethroids are one of the most common insecticide classes used to control Aedes aegypti.
- Pyrethroids target the voltage-gated sodium channel gene (VGSC)2.
- Genetic resistance to pyrethroids develops with continued use of the insecticides2.
- Many mutations exist, but two are important in the Americas: V1016I and F1534C4.
- Mutations are physically linked in the genome, but typically do not rise in frequency together2.
- 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 prevalence4.

- 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 action4.
  - 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.

Objectives

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

Materials & Methods

- 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:
  - Genomic DNA isolation with Blood and Tissue protocol for Qiagen DNeasy Kit or Canadian Center for DNA Barcoding protocol.
  - kdr Genotyping based off modified versions of protocols4.

- Analytical Methods:
  - All graphs analyzed and printed in R v. 3.3.2, package ggplot2.
  - Maps produced in QGIS v. 2.18.15.
  - Repeat G test performed in R v. 3.3.2 package RVideomemo.

- 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 period, 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.

Future Directions

- Analyze suppression experiment replicate conducted in 2013.
- Will we analyze these results in the same way as the 2014 suppression experiment.
- Analyze sequencing for VSC gene.
- Because the origin of the resistant haplotypes is unknown, we will analyze the flanking sequences surrounding the VSC 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.

Sources