TWO NOVEL SINGLE NUCLEOTIDE POLYMORPHISMS IN THE VOLTAGE-GATED SODIUM CHANNEL GENE IDENTIFIED IN Aedes aegypti MOSQUITOES FROM FLORIDA

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ABSTRACT
Aedes aegypti, the primary vector of dengue, Zika, chikungunya, and yellow fever viruses, is known to be resistant to pyrethroid-based insecticides in Florida. To improve our knowledge on the mechanism(s) responsible for this resistance, we sequenced 106 Ae. aegypti individuals collected from throughout Florida and examined mutations in a known insecticide resistance gene, voltage-gated sodium channel (VGSC; AAEL023266), also commonly known as the knockdown resistance (kdr) gene. Through whole genome sequencing, we identified 2 novel nonsynonymous single nucleotide polymorphisms (SNPs), F174I and E478K, and 5 known SNPs, V410L, S723T, V1016I, D1763Y, and Q1853R, of which 4 were reported in Floridian Ae. aegypti for the first time. These SNPs provide a basis for further studies examining their contribution to pyrethroid resistant phenotypes, such as increased time of survival after insecticide exposure. This sequence data can be used to develop a multiplex genotyping assay to investigate the SNP frequencies in a larger number of samples and to examine their phenotypic contribution to pyrethroid resistance in Ae. aegypti.

Key Words: Aedes aegypti, SNP, Florida, resistance, kdr, pyrethroids

INTRODUCTION
Aedes aegypti (L) is found in peninsular Florida with no known established populations currently in the Panhandle (Parker et al. 2019). Where distributed in Florida, Ae. aegypti can be found in urban and suburban areas due to greater availability of artificial containers within these landscapes compared to rural areas (Braks et al. 2003). Urban landscaping additionally provides highly suitable larval habitats for production of several mosquito species. Ornamental bromeliads, which are commonly used for landscaping in tropical and subtropical areas, can also be utilized as a larval habitat by Ae. aegypti (Wilke et al. 2018, Brown et al. 2019). As the predominant mosquito species responsible for transmitting dengue, Zika, chikungunya, and yellow fever viruses, Ae. aegypti is an important public health vector. Currently, no vaccines are available for most Aedes-borne viruses, increasing the necessity to control mosquito populations to prevent local disease outbreaks.

While the best method for controlling mosquitoes is an integrated mosquito management plan that utilizes multiple techniques such as larviciding, biological control, and source reduction, adulticides are most frequently used by the 60+ mosquito control programs spread throughout Florida to reduce mosquito populations, especially during mosquito-borne virus outbreaks (Lloyd et al. 2018). Pyrethroids are among the most common insecticides utilized globally and within the state of Florida (Lloyd et al. 2018). These chemicals are synthetically derived versions of pyrethrins (Bond et al. 2014), naturally occurring insecticidal compounds found in the chrysanthemum flower, and are commonly utilized for mosquito control due to their characteristically low mammalian toxicity and broad-spectrum application (EPA 2009). Similar to DDT, pyrethroids bind to voltage-gated sodium channels (VGSC) affecting depolarization activity and leading to neuronal failure (Coats 1990). This class of chemicals is differentiated into two types (I and II) by the presence or absence of a α-cyano-3-phenoxybenzyl group (Coats 1990). Overuse of pyrethroids for controlling mosquitoes in addition to exposure to chemicals in urban runoff, used in agriculture and pest control, as well as from other sources can result in strong selection pressure towards resistant individuals. Pyrethroid resistance in Florida Ae. aegypti populations has been well documented (Estep et al. 2018, Parker et al. 2020, Schluep and Buckner 2021, Scott et al. 2021). In particular, Parker et al. 2020 recently tested 37 Ae. aegypti populations from across Florida and reported that 95% of these populations were resistant to at least one pyrethroid.
Genetic point mutations within the VGSC can confer pyrethroid resistance in mosquitoes. For example, some nonsynonymous point mutations, which cause changes in amino acid sequences, within the VGSC can adversely affect the ability of a pyrethroid to bind effectively to its protein channel, resulting in knockdown resistance (kdrr) (Soderlund and Bloomquist 1990). Knockdown resistance has been documented in multiple mosquito species including Ae. aegypti (Brengues et al. 2003, Saavedra-Rodriguez et al. 2007, Reimer et al. 2008, Martins et al. 2009, Babu et al. 2015, Li et al. 2015, Mack et al. 2021). In Ae. aegypti, the 1016 and 1534 amino acid positions in the VGSC have become a focal point for determining pyrethroid resistance (Brengues et al. 2003, Smith et al. 2016). Adult Ae. aegypti mosquitoes with 1016I and/or 1534I mutation(s) have been shown to display increased insecticide resistance to pyrethroids (Ishak et al. 2015, Estep et al. 2018, Hayd et al. 2020). A recently studied VGSC nonsynonymous point mutation that results in knock down resistance is V1016I, an amino acid substitution at the 1016 position from a valine (V) to an isoleucine (I). Aedes aegypti from Florida exhibiting heterozygotic (V/I) and homozygotic (I/I) kdrr genotypes at the 1016 position have been detailed in prior studies (Estep et al. 2018, Scott et al. 2021).

In addition to V1016I and F1534C, recent studies have identified three other single nucleotide polymorphisms (SNPs), V410L, S723T, and D1763Y within the VGSC that seem to be associated with pyrethroid resistance in Ae. aegypti (Haddi et al. 2017, Chung et al. 2019, Saavedra-Rodriguez et al. 2019). The V410L mutation was first identified in Brazilian Ae. aegypti populations (Haddi et al. 2017) but has also been recorded in Ae. aegypti populations in Africa and Mexico (Villanueva-Segura et al. 2020, Ayres et al. 2020). Recently, the D1763Y mutation was discovered in Ae. aegypti populations from Taiwan (Chung et al. 2019). In the continental United States, California experienced significant increased allele frequencies for the V410L, S723T, and V1016I mutations in two major cities between 2013 and 2018 and the detection of a new kdr mutation, the Q1853R (Kelly et al. 2021). However, no studies have documented the occurrence of the V410L, S723T, D1763Y, and Q1853R mutations within Ae. aegypti from Florida.

Combinations of kdr mutations can also act synergistically with each other to increase pyrethroid insensitivity (Al Nazawi et al. 2017). Several studies have reported heightened levels of pyrethroid resistance in mosquitoes that express two kdr mutations compared to one (Du et al. 2013, Hirata et al. 2014, Al Nazawi et al. 2017, Haddi et al. 2017). For example, Haddi et al. (2017) witnessed higher deltamethrin and permethrin dose-response curves for Ae. aegypti with a V410L and F1534C profile compared to adults with the F1534C mutation alone. Additionally, Al Nazawi et al. (2017) reported increased time to mortality against deltamethrin in Ae. aegypti with a V1016G and S989P haplotype. In Florida, Estep et al. (2018) not only detected the V1016I mutation in Ae. aegypti, but also documented it co-occurring with the F1534C mutation. The F1534C mutation has the ability to confer pyrethroid resistance solitarily, however a stronger positive correlation between higher permethrin resistance ratios and higher homozygote resistant genotype, ICC, frequencies were observed for Floridian Ae. aegypti containing the mutations V1016I and F1534C (Estep et al. 2018). These three studies are important examples of the additive effect of kdr mutation combinations in increasing pyrethroid sensitivity.

As part of a larger project aimed at understanding dispersal of Floridian Ae. aegypti with respect to environmental conditions and/or landscape, we obtained 106 Ae. aegypti specimens from 15 counties in Florida between 2016-2021. Using whole genome sequence data, we screened for nonsynonymous SNPs occurring within the VGSC. Five previously documented point mutations (V410L, S723T, V1016I, D1763Y, and Q1853R) associated with pyrethroid resistance and two novel point mutations (F174I and E478K) were identified. Here, we provide the geographic distribution, alternate allele frequency, and depth coverage of these nonsynonymous mutations.

**MATERIALS AND METHODS**

**Mosquito Sample Collection**

Between 2016-2021, interested Florida mosquito control programs were provided with an Aedes egg collection kit. The kit contained seed germination paper (Anchor Paper Express, Plymouth, MN) as an oviposition substrate, binder clips, 480 mL black plastic cups (Gary Austin Advertising, Jackson, TN), a microcentrifuge tube with 1:1 lactalbumin/yeast mixture as an oviposition attractant, and collection instructions (Parker et al. 2019). Oviposition cups containing seed germination paper were placed in the field by participants, and the seed germination paper was replaced once a week. Egg papers were collected and sent to the University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory (UF/IFAS FMEL). Field-collected egg papers were air-dried, if necessary, then hatched in 1 L rearing trays. Larvae were fed 1:1 lactalbumin/yeast ad libitum. Pupae were transferred into water-filled cups and placed in a 30.5 x 30.5 x 30.5 cm Bug Dorm adult rearing cage (Bioquip®, Rancho Dominguez, CA). A cotton ball soaked in 10% sucrose solution was provided as a carbohydrate.
source for emerged adults. All life stages of the mosquitoes are reared in a walk-in bioroom set at 83°F ± 2°F and 70% humidity ± 5% with a 12:12 LD photoperiod. Adults were identified to species by morphology (Burkett-Cadena 2013), and female Ae. aegypti mosquitoes were transferred to individual microcentrifuge tubes containing 70% ethanol solution for future DNA extraction.

**DNA extraction/library prep**

DNA was extracted from 106 individual mosquito specimens utilizing a magnetic bead-based DNA extraction protocol described by Chen et al. (2021). DNA concentrations were measured using the Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA) and a Qubit instrument (Life Technologies, Carlsbad, CA) for each sample. A genomic DNA library was constructed with the QIAseq FX DNA Library UDI kit (Qiagen, Valencia, CA) using 20 ng input DNA for each mosquito. Enzymatic fragmentation was carried out at 32°C for 11 minutes followed by 65°C for 30 minutes. Ligation of adapters was performed at 20°C for 2 hours. PCR amplification of constructed libraries was conducted for 8 cycles of denaturation at 98°C for 20 seconds, annealing at 60°C for 30 seconds, and DNA extension at 72°C for 30 seconds. Library cleanup was conducted using PCRClean DX (Aline Biosciences, Woburn, MA). Library concentrations were measured with Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA) and a Qubit instrument (Life Technologies, Carlsbad, CA).

**Sequencing and data analysis**

Construct libraries were sequenced as 150bp paired-end reads using a NovaSeq instrument (Illumina) at the University of Florida Interdisciplinary Center for Biotechnology Research (ICBR) Nextgen DNA Sequencing Core. Fastp version 0.20.1 was used to trim raw reads (Chen et al. 2018). Trimmed reads were mapped to the Ae13CLOV028MT (Genbank ID: MH431876) using BWA-MEM (Li 2013) version 0.7.15 recommended by Schmidt et al. (2018) to minimize the impact of mitochondrial reads mapping to the nuclear genome due to presence of pseudogenes (Hlaing et al. 2009). After mapping to mitogenome, unmapped and mate-is-unmapped reads were filtered utilizing Sambamba (Artem et al. 2015), converted to fastq files using Samtools version 1.12 (Li et al. 2009) and mapped to the AaegL5 reference genome (Matthews et al. 2018) using BWA-MEM (Li 2013) version 0.7.15. Qualimap version 2.2 was used to calculate mapping statistics (Okonechnikov et al. 2016). Freebayes (Garrison and Marth 2012) version 1.0.1 with standard filters and population priors disabled was used for joint variant calling of all samples. Repeat regions were soft-masked in the AaegL5 reference genome and SNPs in these regions were removed from analysis. Further analysis only focused on biallelic SNPs with a minimum of 6X coverage. A 10% missing data threshold was used to filter SNPs.

Distribution maps of the SNPs were constructed using mapchart.net. A VGSC image was constructed using Protter version 1 (Omasits et al. 2014). Locations of SNPs were determined by alignment with Ae. aegypti (ACB37021.1) (Aedes aegypti genome working group 2017) and Musca domestica (ANW06229) (Scott et al. 2014) reference sequences transferring SNP annotations from Aedes to Musca to identify Musca protein position. Structural annotations were identified by alignment with Drosophila melanogaster (SCNA_DROME) reference sequence (Matthews et al. 2015).

We calculated the alternate allele frequency for each SNP identified by dividing the observed alleles for each genotype by the total number of copies of all the alleles at that particular genomic coordinate. Then the SNPs were classified as being common or rare in the Ae. aegypti sampled based on their calculated alternate allele frequencies. We considered any SNP identified in ≥ 25% of Ae. aegypti as common and any SNP found in < 25% of Ae. aegypti as rare.

**RESULTS**

One hundred and six adult female Ae. aegypti mosquitoes were obtained from 15 counties in central and south Florida (Table 1). The genome of all 106 individual samples were successfully sequenced and filtered for nonsynonymous SNP mutations. Depth coverage for samples ranged between 7-10x approximately. In total, 7 SNPs within the VGSC were identified, 2 of which were novel (F174I and E478K) and 5 previously known (V410L, S723T, V1016I, D1763Y, and Q1853R). Three of the four novel SNPs (V410L, S723T, V1016I, D1763Y, and Q1853R) were observed in Ae. aegypti from all 15 sampled counties (Figure 1A), displayed alternate allele frequencies of approximately 70 to 73%, and were considered common (Table 2). The known SNP Q1853R was documented in Ae. aegypti from II of the 15 counties (over 70%; Figure 1B) sampled and was also classified as common due to its 26.7% alternate allele frequency. Interestingly, every individual that contained the Q1853R SNP also contained the V1016I mutation. We did not see a consistent co-occurrence between any other mutations. The final known SNP, D1763Y, was only found in two counties (Figure 1A) and displayed a minute alternate allele frequency of 1.0% (Table 2), which led us to classify this mutation as rare. The two novel SNPs identified, F174I
and E478K, were found in *Ae. aegypti* isolated to single counties and displayed alternate allele frequencies of approximately 1.0%, which also led to their classification as rare (Table 2). Structurally, the novel SNPs were located in the intercellular (E478K) and transmembrane (F174I), regions of the VGSC channel, respectively, based on constructed protein structure (Figure 2).

**Figure 1.** The geographic distribution of single nucleotide polymorphisms (SNPs) identified in *Ae. aegypti* mosquitoes collected from 15 counties in Florida. (A) Previously known SNPs, V1016I, S723T, and V410L, (blue), D1763, V1016I, S723T, and V410L (yellow); (B) Q1853R (red); and (C) the rare point mutations identified, E478K (purple), and F174I (orange).

**Figure 2.** Topology of the mosquito sodium channel highlighting novel Florida and known SNP locations. Known mutation positions based on Mack et al. (2021). The VGSC is comprised of four homologous repeat domains (I-IV), each containing six helical transmembrane segments (1-6, 7-12, 13-28, 19-24 in Figure 2). Blue-filled circles indicate the two novel SNPs identified in this study. The green circles represent previously documented SNPs (Haddi et al. 2017, Mack et al. 2021).
Table 1. Collection sites of *Aedes aegypti* samples used in this study.

<table>
<thead>
<tr>
<th>County</th>
<th># of samples</th>
<th>City</th>
<th>Latitude</th>
<th>Longitude</th>
<th>CollectionYear</th>
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</thead>
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<tr>
<td>Broward</td>
<td>3</td>
<td>Davie</td>
<td>26.0693</td>
<td>-80.2082</td>
<td>2020</td>
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<tr>
<td>Broward</td>
<td>3</td>
<td>Hollywood</td>
<td>26.0350</td>
<td>-80.1768</td>
<td>2020</td>
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<tr>
<td>Broward</td>
<td>3</td>
<td>Miramar</td>
<td>25.9863</td>
<td>-80.2462</td>
<td>2020</td>
</tr>
<tr>
<td>Collier</td>
<td>9</td>
<td>Naples</td>
<td>26.1392</td>
<td>-81.7582</td>
<td>2020</td>
</tr>
<tr>
<td>Hillsborough</td>
<td>18</td>
<td>Tampa</td>
<td>27.9873</td>
<td>-82.4782</td>
<td>2020</td>
</tr>
<tr>
<td>Lee</td>
<td>2</td>
<td>Fort Myers</td>
<td>26.6528</td>
<td>-81.8118</td>
<td>2020</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>3</td>
<td>Miami</td>
<td>25.7546</td>
<td>-80.2235</td>
<td>2020</td>
</tr>
<tr>
<td>Monroe</td>
<td>4</td>
<td>Key Largo</td>
<td>25.0872</td>
<td>-80.4477</td>
<td>2020</td>
</tr>
<tr>
<td>Palm Beach</td>
<td>3</td>
<td>Haverhill</td>
<td>26.6886</td>
<td>-81.1135</td>
<td>2020</td>
</tr>
<tr>
<td>Pasco</td>
<td>3</td>
<td>Holiday</td>
<td>28.1863</td>
<td>-82.7452</td>
<td>2020</td>
</tr>
<tr>
<td>Pasco</td>
<td>2</td>
<td>New PortRichey</td>
<td>28.2619</td>
<td>-82.7055</td>
<td>2020</td>
</tr>
<tr>
<td>Polk</td>
<td>2</td>
<td>Auburndale</td>
<td>28.0497</td>
<td>-81.7767</td>
<td>2020</td>
</tr>
<tr>
<td>Polk</td>
<td>4</td>
<td>Haines City</td>
<td>28.1147</td>
<td>-81.6136</td>
<td>2020</td>
</tr>
<tr>
<td>Polk</td>
<td>3</td>
<td>Lakeland</td>
<td>28.1145</td>
<td>-82.0033</td>
<td>2020</td>
</tr>
<tr>
<td>Sarasota</td>
<td>6</td>
<td>Sarasota</td>
<td>27.3700</td>
<td>-82.4841</td>
<td>2020</td>
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<tr>
<td>Seminole</td>
<td>5</td>
<td>Sanford</td>
<td>28.8264</td>
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<td>2020</td>
</tr>
<tr>
<td>St. Lucie</td>
<td>4</td>
<td>Port St. Lucie</td>
<td>27.3093</td>
<td>-80.3405</td>
<td>2020</td>
</tr>
<tr>
<td>St. Johns</td>
<td>4</td>
<td>St. Augustine</td>
<td>29.9012</td>
<td>-81.3126</td>
<td>2020</td>
</tr>
<tr>
<td>Indian River</td>
<td>3</td>
<td>Vero Beach</td>
<td>27.5872</td>
<td>-80.3734</td>
<td>2016</td>
</tr>
<tr>
<td>Manatee</td>
<td>24</td>
<td>Palmetto</td>
<td>27.5485</td>
<td>-82.5995</td>
<td>2018</td>
</tr>
</tbody>
</table>

Table 2. The positions of the single nucleotide polymorphisms with accompanied allele and amino acid information.

<table>
<thead>
<tr>
<th>SNP Group</th>
<th>Musca aa&lt;sup&gt;2&lt;/sup&gt; position</th>
<th>Chromosome</th>
<th>Genomic coordinate</th>
<th>Reference allele</th>
<th>Alternate allele</th>
<th>Alternate allele frequency</th>
<th>Aaeg&lt;sup&gt;2&lt;/sup&gt; aa&lt;sup&gt;1&lt;/sup&gt; position</th>
<th>Reference aa</th>
<th>Alternate aa</th>
<th>Mean Depth and STDV</th>
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</thead>
<tbody>
<tr>
<td>Known</td>
<td>410</td>
<td>3</td>
<td>316080722</td>
<td>C</td>
<td>A</td>
<td>73.3%</td>
<td>108</td>
<td>V</td>
<td>L</td>
<td>7.63 +/- 4.54</td>
</tr>
<tr>
<td>Known</td>
<td>723</td>
<td>3</td>
<td>316014588</td>
<td>A</td>
<td>T</td>
<td>73.0%</td>
<td>711</td>
<td>S</td>
<td>T</td>
<td>8.65 +/- 6.40</td>
</tr>
<tr>
<td>Known</td>
<td>1046</td>
<td>3</td>
<td>315983763</td>
<td>C</td>
<td>T</td>
<td>70.4%</td>
<td>1012</td>
<td>V</td>
<td>I</td>
<td>7.47 +/- 3.61</td>
</tr>
<tr>
<td>Known</td>
<td>1763</td>
<td>3</td>
<td>315932009</td>
<td>C</td>
<td>A</td>
<td>1.0%</td>
<td>1794</td>
<td>D</td>
<td>Y</td>
<td>7.65 +/- 4.58</td>
</tr>
<tr>
<td>Known</td>
<td>1853</td>
<td>3</td>
<td>315931672</td>
<td>T</td>
<td>C</td>
<td>26.7%</td>
<td>1884</td>
<td>Q</td>
<td>R</td>
<td>9.67 +/- 7.92</td>
</tr>
<tr>
<td>Novel</td>
<td>478</td>
<td>3</td>
<td>316067895</td>
<td>C</td>
<td>T</td>
<td>1.1%</td>
<td>476</td>
<td>E</td>
<td>K</td>
<td>7.79 +/- 5.59</td>
</tr>
<tr>
<td>Novel</td>
<td>174</td>
<td>3</td>
<td>31601951</td>
<td>A</td>
<td>T</td>
<td>1.0%</td>
<td>189</td>
<td>F</td>
<td>I</td>
<td>8.29 +/- 4.69</td>
</tr>
</tbody>
</table>

<sup>2</sup>aa = amino acid
<sup>1</sup>Aaeg = *Aedes aegypti*
DISCUSSION

*Aedes aegypti* is an important vector species of dengue, Zika, and chikungunya viruses in Florida. The lack of developed vaccines for many of *Aedes*-borne diseases creates heavy reliance on mosquito population control for preventing disease transmission. Insecticidal use is an integral part of adult mosquito control in Florida (Lloyd et al. 2018). The presence and development of insecticide resistance among mosquito populations can potentially undermine the effectiveness of mosquito-borne disease control tools currently utilized.

However, studies like ours that identify and screen for SNPs in pyrethroid resistant populations could allow for detection of reduced insecticide sensitivity in mosquito populations, indicating a need for changes in control strategy. Our results corroborate Estep et al. (2018)’s findings of the V1016I mutation associated with pyrethroid resistance being common in Floridian *Ae. aegypti* mosquitoes. Additionally, this study is the first to document the occurrence and distribution of the V410L, S723T, D1763Y, and Q1853R mutations in Florida. Our findings also indicate a shared geographic distribution of two recently identified mutations, V410L and S723T, with the V1016I mutation in Florida. Two locations, Hollywood in Broward County and Haines City in Polk County, had only variant homozygotes (L/L, T/T, and L/I) for three of the previously documented mutations, V410L, S723T, and V1016I, in all sampled adult *Ae. aegypti* mosquitoes. Variant homozygotes for S723T and V1016I were additionally found in Davie in Broward County. All other sampled locations exhibited mixed genotypes of homozygote wildtype (V/V, S/S, and V/V) variant individuals and heterozygote individuals (V/L, S/T, and V/I).

While our study did detect the F1534C mutation previously reported in Florida *Ae. aegypti* by Estep et al. (2018), it was excluded from analyses, because its depth coverage was less than 6X. Upon further investigation, low mapping reads in the F1534C region on chromosome 3 were most likely responsible for the low depth coverage. The low mapping reads may have been potentially due to the presence of similar sequences on a portion of chromosome 1 and the F1534C region on chromosome 3 within the reference genome, which caused amplified segments to align to chromosome 1 instead of chromosome 3. This issue was not observed with the other detected mutations. Interestingly, Fan et al. (2020) did not detect the F1534C mutation in *Ae. aegypti* collected from St. Augustine, Florida. However, the authors did detect the F1534C mutation in *Ae. aegypti* collected from other locations around the world (Fan et al. 2020). Perhaps the different methodologies utilized in our study and by Estep et al. (2018) and Fan et al. (2020) may explain the differences observed regarding F1534C. Future studies using qRT-PCR and mutation-specific primers are planned to validate the detection of F1534C in our *Ae. aegypti* samples. The presence of Q1853R in over 70% of the counties sampled and high allele frequency (26.7%) suggest that this mutation arose much earlier in time, which allowed for further dispersal throughout the state. In addition the Q1853R mutation was also found co-occurring with the V1016I mutation in *Ae. aegypti* mosquitoes. This co-occurrence pattern is similar to V1016I + F1534C observations detected in *Ae. aegypti* by Estep et al. (2018). Whether Q1853R functions additively or multiplicatively with F1534C to affect pyrethroid resistance is yet to be determined. Further studies are needed to assess the impact of the co-occurrence of these mutations as well as the novel SNPs we detected on *Ae. aegypti*’s phenotypic response to insecticides.

The occurrence in only one county and low allele frequency (approximately 1.0%) documented for both of our novel SNPs, F174H and E478K, suggest that these mutations arose recently and have had a limited chance of dispersal to other locations. Still, it is plausible that these rare mutations could be detected in additional locations if sampling is increased to include a substantial number of individuals from other parts of the state. Additionally, larger scale population genetic studies involving increased sample sizes per city and/or county are needed to accurately assess the distribution of the novel mutations that we identified. A limitation of our study is the minimal sampling of approximately 2-24 mosquitoes per city. The resolution of allele frequency for any given city or county needs to be examined with much larger samples. In future studies. Nonetheless, our genome data provides template sequences that future studies can use to develop genotyping assays to examine fine-scale abundance of SNPs identified and their contribution to phenotypic insecticide resistance.

The VGSC gene is one of hundreds of genes that have the potential to influence insecticide resistance (Saavedra-Rodriguez et al. 2008, Faucon et al. 2015, Campbell et al. 2019). As such, examining just the VGSC is only scratching the surface of potential functional changes in insecticide resistance genes. Our genome data will allow us to look for nonsynonymous mutations in other insecticide resistance genes. It is evident that Florida *Ae. aegypti* harbor many *kdr* mutations that can functionally impact insecticide resistance. In addition to the effort to characterize their functions in the laboratory, a multiplex SNP genotyping
Two novel snps in Floridian Aedes aegypti

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