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Lack of *kdr* mutations in a population of Asian tiger mosquitoes from Costa Rica

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Abstract

Use of insecticides has led to the fixation of alleles for single amino acid changes in the voltage gated sodium channel (VGSC) associated with knockdown resistance (*kdr*) in insects of agricultural or medical importance. In this short note, we study allele frequencies of 5 *kdr* loci, S989, I1011, L1014, V1016 and F1534 in a population of the Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera Culicidae), collected in the only identified location with persistent infestations in Costa Rica. We found no mutant alleles associated with *kdr* resistance, and a synonymous point mutation was observed at loci V1016. Our results likely reflect the recent invasion of the Asian tiger mosquito in Costa Rica, and also provide a baseline for future studies looking at the global distribution of *kdr* mutations in this important vector of dengue and chikungunya viruses.

Key words: insecticide resistance, invasive species, *Aedes albopictus*, dengue, chikungunya.

Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera Culicidae) is an important vector of two major human arboviruses: chikungunya and dengue (Paupy *et al*., 2009), as well as, a major invasive species, which has expanded its range out of its native S.E. Asia over the last 150 years (Bonizzoni *et al*., 2013). *Ae. albopictus* was first recorded in Costa Rica during the late 2000s (Marín *et al*., 2009) and a phylogeographic analysis found that some globally widespread mitochondrial COI haplotypes are common in the area (Futami *et al*., 2015).

Monitoring mosquitoes for their insecticide resistance provides valuable information to optimize insecticide based vector control and manage insecticide resistance (Donnelly *et al*., 2009). Several mutations in the gene for voltage gated sodium channel (VGSC) have been described in vectors of disease (Donnelly *et al*., 2009; Kasai *et al*., 2011). These mutations give raise to knockdown resistance (*kdr*) alleles, given that mosquitoes with the mutant alleles are more likely to survive lethal insecticide doses, and have a fitness advantage via increased survival when DDT or pyrethroid insecticides are applied (Donnelly *et al*., 2009). Mutations of *kdr* have been observed in domains II (where wild type alleles code the following amino acids at specific sites: S989, I1011, L1014, and V1016), and III (where F1534 is a wild type allele) of the VGSC (Kasai *et al*., 2011). To the best of our knowledge no study has asked whether *kdr* mutant alleles are present in *Ae. albopictus* from Costa Rica, and here we investigate the presence of *kdr* mutant alleles in samples from the only identified location in Costa Rica with persistent *Ae. albopictus* infestations.

Materials and methods

Mosquito collection

We collected mosquitoes from an organic pineapple farm in Sarapiquí (10°26'03.80"N 84°07'14.75"W), the only place where a persistent *Ae. albopictus* infestation has been identified in Costa Rica (Calderón Arguedas *et al*., 2012). We collected a total of 58 mosquitoes using 3 CDC backpack aspirators operated during a total of 6 h over a 1.6 ha surface in December 2012. Samples were killed by flash freezing them at −5 °C before their morphological identification with a taxonomic key (Rueda, 2004) and kept in ethanol at 99%. Voucher specimens are available at the Entomological Collection in the Institute of Tropical Medicine of Nagasaki University.

Molecular *kdr* detection

DNA was then extracted from 3 homogenized legs from each mosquito in a mixture of extraction solution (20 µl) + tissue preparation solution (5 µl) REDExtract-N-Amp Tissue PCR Kit (Sigma) for DNA extraction. The solution was heated at 95 °C for 3 min and neutralized (Futami *et al*., 2015). The extraction was followed with an initial DNA amplification using the primers, and a modified version of the protocol, presented by Kawada *et al.* (2014). We specifically used AaSCF1 (AGACAATGTGGATCGCTTCC) and AaSCR4 (GGACGCAATCTGGCTTGTTA) for loci S989, I1011, L1014, and V1016 in the domain II of the VGSP. We also used AaSCF7 (GAGAACTCGCCGATGAACTT) and AaSCR7 (GACGACGAAATCGAACAGGT) for F1534 in the domain III of the VGSP. The PCR solution contained 1µL of 10X Ex Taq buffer, 0.8 µL of dNTP mix, 0.05 µL of Ex Taq HS, 0.4 µM of each primer, and 1 µL of the DNA template, completing 10 µL of solution with MILIQ® water. The PCR was executed as fol-

lows: an initial denaturation at 94 °C for 3 min; 35 cycles at 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s; ending with an elongation step at 72 °C for 10 min. Amplified fragments of the expected size were purified with ExoSAP-IT (USB Corporation) at 37 °C for 30 min, followed by 80 °C for 15 min. DNA sequencing was done employing primers AaSCF3 (GTGGAACTTCACCGACTTCA) and AaSCR6 (CGACTTGATCCAGTTGGAGA) for the 4 loci in domain II of the VGSP, and AaSCR8 (TAGCTTTCAGCGGCTTCTTC) for F1534, the only loci in domain III of the VGSP (Kawada *et al*., 2014). We used a BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems Japan Ltd.) for DNA sequencing, following the manufacturer's instructions. Briefly, 2 µM of each primer were added in a final volume of 10 µL per reaction tube. The sequencing PCR was done under the following conditions: an initial denaturation at 96 °C for 60 s; 25 cycles each at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 120 s. DNA was precipitated with 25µL ethanol at 99.8%, 1µL of 0.125M EDTA and 1 µL of 3M Sodium Acetate at room temperature for 15 min, then centrifuged at 3000G for 30 min at 4 °C. Supernatant was subsequently discarded, and 35 µL of ethanol 70% were added to the precipitate, which was centrifuged at 3000 G, 4 °C for 15 min. Supernatant was again discarded, and samples were dry by centrifugation at 200 G for 1min, 10 µL of Hi-Di Formamide were added, and this solution was heated at 95 °C for 120 s. Direct DNA sequencing was done with a 3730 DNA Analyzer (Applied Biosystems Japan Ltd.). Electropherograms of the targeted loci for *kdr* mutant amino acid substitutions for each mosquito were made with MEGA 5.0 (Tamura *et al*., 2011).

Results and discussion

We were able to extract DNA from only 56 of the 58 samples. For loci S989, I1011, L1014, V1016 and F1534 we found no *kdr* mutations. In loci V1016 we found an individual with a silent mutation (a synonymous DNA substitution that does not lead to an amino acid substitution). The lack of *kdr* alleles in our samples is within the expectations for a recent invasive species. Lewontin (1965) and Lounibos (2002) have argued that colonizing, and by extension invasive, species, try to maximize their fitness in order to succeed in their initial establishment into a new habitat. Given that insecticide resistance has fitness costs (Kliot and Ghanim, 2012), it will be natural to expect the absence of alleles that otherwise hamper the fitness of a species in the absence of a selective pressure from insecticide use. That might also be the case of our *Ae. albopictus* samples, which came from an organic farm, an insecticide free environment by definition. Although we did not find any amino acid substitution, like the F1534C observed in Singapore (Kasai *et al*., 2011), and F1534L in USA (Marcombe *et al*., 2014), we found an individual mosquito with a silent mutation at loci V1016, where the consensus codon for V changed from GTG to GTA, like previously observed in USA (Marcombe *et al*., 2014). We expect that as the Asian tiger mosquito becomes more widespread in Costa Rica, mutant *kdr* alleles might become common in populations of this major vector, as has been observed in USA, where this mosquito has been established over a longer time period (Marcombe *et al*., 2014). The data presented here, to the best of our knowledge, is the first report of its kind for Costa Rica and Central America. Our results will be a valuable baseline for future comparisons within Costa Rica, as well as, Central America, and for understanding the global distribution of *kdr* alleles in the Asian tiger mosquito.

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