<table>
<thead>
<tr>
<th>Section</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Lack of kdr mutations in a population of Asian tiger mosquitoes from Costa Rica</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Chaves, Luis Fernando; Kawashima, Emiko; Futami, Kyoko; Minakawa, Noboru; Marin Rodriguez, Rodrigo</td>
</tr>
<tr>
<td>Citation</td>
<td>Bulletin of Insectology, 68(1), pp.61-63; 2015</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2015-06</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/35440">http://hdl.handle.net/10069/35440</a></td>
</tr>
</tbody>
</table>

© Bulletin of Insectology
Lack of kdr mutations in a population of Asian tiger mosquitoes from Costa Rica

Luis Fernando Chaves$^{1,2}$, Emiko Kawashima$^{1}$, Kyoko Futami$^{1}$, Noboru Minakawa$^{1}$, Rodrigo Marín Rodríguez$^{2}$

$^{1}$Institute of Tropical Medicine (NEKKEN), Nagasaki University, Japan
$^{2}$Programa de Investigación en Enfermedades Tropicales (PIET), Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

$^1$Departamento de Control de Vectores, Ministerio de Salud, San José, Costa Rica

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 (Diptera Culicidae) is an important vector of two major

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 Aedes 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 (AGACAAATGTGGATCGCTTCC) and AaSCR4 (GGACGCAATCTGGCTTGTTA) for loci 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 A. 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 Aedes albopictus infestations.
previously observed in USA (Marcombe et al., 2014), we found an individual mos-
quito has been established over a longer time period (Marcombe et al., 2014). 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 synonym-
ous DNA substitution that does not lead to an amino acid substitution) in 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 mos-
quito 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.

Acknowledgements

Junko Sakemoto (NEKKEN, Japan) offered valuable administrative support. Yukiko Higa, and Hitoshi Kawada (NEKKEN, Japan) and the reviewers provided insightful comments on the analysis methods and ms contents. Kogomi Minagawa (NEKKEN, Japan) helped with laboratory procedures and data processing and Mario Baldi (UNA, Costa Rica) helped with the mos-
quito sampling. This study was funded by the Japan Ini-
tiative for Global Research Network on Infectious Dis-
eases (J-GRID), MEXT, Japan and Nagasaki University (Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases).

References

BONIZZONI M., GASPERI G., CHEN X., JAMES A. A., 2013.- The invasive mosquito species Aedes albopictus: current knowl-
CALDERÓN ARGUEDAS O., TROYO A., AVENDANO A., GUTIER-
REZ M., 2012.- Aedes albopictus (Skuse) en la Región Hu-
DONNELLY M. J., CORBEL V., WETMAN D., WILDEING C. S., WILLIAMSON M. S., BLACK IV W. C., 2009.- Does kdr geno-
type predict insecticide-resistance phenotype in mosqui-
FUTAMI K., VALDERRAMA A., BALDI M., MINAKAWA N., MARÍN RODRIGUEZ R., CHAves L. F., 2015.- New and co-
mplex haplotypes shape genetic diversity in Asian tiger mos-
KASAI S., NG L. C., LAM-PHUA S. G., TANG C. S., ITOKAWA K., KOMAGATA O., KOBAYASHI M., TOMITA T., 2011.- First de-
tection of a putative knockdown resistance gene in major mosquito vector, Aedes albopictus.- Japanese Journal of In-
f ectious Diseases, 64: 217-221.
ocurrence of point mutations in the voltage-gated sodium channel of pyrethroid-resistant Aedes aegypti populations in Myanmar.- PLoS Neglected Tropical Diseases, 8: e2632.
KLIOT A., GHANIM M., 2012.- Fitness costs associated with insecticide resis-
LEWONTIN R. C., 1965.- Selection of colonizing ability, pp. 79-

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 synonym-
ous 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 other-
wise 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 envi-
ronment 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 mos-
quito 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 mos-
quito 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.


Authors’ addresses: Luis Fernando Chaves (corresponding author: lchaves@nagasaki-u.ac.jp), Emiko Kawashima, Kyoko Futami, Noboru Minakawa, Institute of Tropical Medicine (NEKKEN), Nagasaki University, 852-8523, Sakamoto 1-12-4, Nagasaki, Japan; Rodrigo Marín Rodríguez, Departamento de Control de Vectores, Ministerio de Salud, San José, Costa Rica.

Received October 5, 2014. Accepted March 12, 2015.