

**Phylogeographic and Population Genetic
Structure, and '*kdr*' type gene mutations
of *Aedes aegypti* in Sri Lanka**

Hettiyakandage Sachini Dinusha Fernando

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Phylogeographic and Population Genetic Structure, and 'kdr' type
gene mutations of *Aedes aegypti* in Sri Lanka

By

Hettiyakandage Sachini Dinusha Fernando



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Declaration

The work described in this thesis was carried out by me under the supervision of Prof. B. G. D. N. K. De Silva and Dr. Menaka Hapugoda and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma.



H. S. D. Fernando

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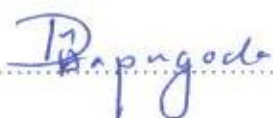
Prof. B. G. D. N. K. De Silva

Date

Department of Zoology

Faculty of Applied Sciences

University of Sri Jayewardenepura



07/06/2019

Dr. Menaka Hapugoda

Date

Molecular Medicine Unit

Faculty of Medicine

University of Kelaniya

Declaration

We certify that the candidate has incorporated all corrections, additions and amendments recommended by the examiners to this version of the Ph.D thesis.



Prof. B. G. D. N. K. De Silva
Department of Zoology
Faculty of Applied Sciences
University of Sri Jayewardenepura

07/06/2019

Date



Dr. Menaka Hapugoda
Molecular Medicine Unit
Faculty of Medicine
University of Kelaniya

07/06/2019

Date

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LIST OF ABBREVIATIONS

A	Adenine
AGE	Agarose Gel Electrophoresis
AD	<i>Amno Domini</i>
AS-PCR	Allele-Specific Polymerase Chain Reaction
ATP	Adenosine Triphosphate
BG	BioGents
bp	base pair
C	Cytosine
CO ₂	Carbon Dioxide
COI	Cytochrome C Oxidase subunit I
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DNA	Deoxyribonucleic Acid
dNTP	Deoxy Nucleotide Triphosphate
DSS	Dengue Shock Syndrome
E	Envelop protein
EDTA	Ethylenediaminetetraacetic Acid
F1534C	Phenylalanine1534Cysteine
g	gram

G	Guanine
GABA	Gamma-Aminobutyric acid
GIS	Geographical Information System
Gly923Val	Glycine923Valine
GPS	Global Positioning System
h	haplotype
Hd	Haplotype diversity
H _E	Expected Heterozygosities
H _O	Observed Heterozygosities
HWE	Hardy- Weinberg Equilibrium
IDT	Integrated DNA Technologies
Ile1011Met	Isoleucine1011Methionine
Ile1011Val	Isoleucine1011Valine
k	Average number of nucleotide differences
Kb	Kilo bases
kdr	knockdown resistance
km	kilometre
L	Litre
LD	Linkage Disequilibrium
Leu982Trp	Leucine982Trptophan
M	Molar

m	metre
µg	Microgram
µl	Microlitre
mA	milli Ampere
ml	millilitre
mM	millimolar
mtDNA	mitochondrial DNA
NADH	Nicotinamide Adenine Dinucleotide
NaV	Sodium Voltage Channel
ND4	NADH dehydrogenase subunit 4
ng	nanogram
nm	nanometre
no.	number
NS	Non Structural
NUMTs	nuclear mtDNA
°C	Centigrade
PCR	Polymerase Chain Reaction
pmol	picomole
RNA	Ribonucleic Acid
rpm	revolutions per minute

R_s	Allelic Richness
RT	Room Temperature
SDS	Sodium Dodecyl Sulfate
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
STR	Short Tandem Repeats
T	Thymine
TBE	Tris-borate-EDTA
Tris	Tris(hydroxymethyl)aminomethane
TE	Tris EDTA
U.S.A	United States of America
UV	Ultra Violet
V	Volt
Val1016Gly	Valine1016Glycine
Val1016Ile	Valine1016Isoleucine
WHO	World Health Organization
yr	years
π	Nucleotide diversity
π_a	placement substitution site
π_s	synonymous substitution site

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Phylogeographic and Population Genetic Structure of *Aedes aegypti* in Sri Lanka

H.S.D. Fernando

ABSTRACT

Mosquitoes as disease causing vectors, are of major relevance as insect invaders, due to their ability to transmit pathogens to humans and their ability to adapt to human built environment. *Aedes aegypti* (Linneaus), major vector for Dengue Viruses (DENV), originated as a canopy dwelling species in Africa, feeding on non-human primates, invaded the tropics and subtropics of the world through globalization and trade. In Sri Lanka, dengue is the most serious arboviral infection accounting for severe number of deaths annually. The present study was conducted to investigate the evolutionary history of *Ae. aegypti* in Sri Lanka, and to study the present genetic structure of *Ae. aegypti* mosquito populations within the country.

Ae. aegypti mosquitoes were sampled from eight districts during the period of 2013-2015 in Sri Lanka. The phylogeographic relationships of Sri Lankan *Ae. aegypti* was studied in an macro-evolutionary time frame using two mitochondrial Deoxy Ribonucleic Acid (DNA) markers named Cytochrome C Oxidase 1 (*COI*) and NADH subunit 4 (*ND4*) in PART 1 of the study. The population genetic structure of *Ae. aegypti* in the island was studied using eleven microsatellite loci in PART 2 of the study and the results were used to interpret gene flow patterns and population structuring of the

species in the island. The presence and distribution of knockdown resistant (*kdr*) alleles were studied, hypothesizing a resistant mosquito population for the most commonly used insecticide in the country, pyrethroids, in PART 3 of the study. Here three most commonly occurring point mutations, Phenylalanine 1534 Cysteine (F1534C), Valine 1016 Isoleucine (Val1016Ile) and Valine 1016 Glycine (Val1016Gly) in the sodium voltage gated channel was genotyped through Allele Specific Polymerase Chain Reaction (AS-PCR) and real time PCR assays.

The phylogeographic study in PART 1 of the study recorded 14 *COI* and 40 *ND4* haplotypes for the first time in Sri Lanka, with the highest genetic diversity recording for *ND4* gene. Presence of two mitochondrial lineages of *Ae. aegypti* was recorded among Sri Lankan mosquitoes. These two Sri Lankan lineages were related respectively to East and West African specimens and the analysis of gene flow patterns revealed abundant gene flow between South-East Asian countries and Sri Lanka. The population structure analysis in the PART 2 of the study revealed high genetic diversity in Sri Lankan *Ae. aegypti* with all the studied loci being polymorphic in all populations. The software STRUCTURE estimated three genetic clusters with the presence of weak isolation by distance pattern. A non-equilibrium analysis of gene flow rates and patterns indicated abundant bidirectional gene flow among all samples collected. The study of *kdr* allele mutations in the PART 3 of the study revealed the presence of F1534C mutation in Sri Lanka. The mutant allele was found to be wide spread in the island and it can be hypothesized that there is a resistant mosquito population for the insecticide. However V1016I and V1016G mutations were not recorded in the present study.

The efficiency of transmitting DENV and the ability to resist insecticides is dependent upon the genetic composition of the *Ae. aegypti* population. The identification of the presence of *Ae. aegypti* belonging to the two clades, representing the origins of East and West Africa suggests differences in flavivirus susceptibility and resistance to insecticides. Population genetic structure of the Sri Lankan *Ae. aegypti* revealed highly genetically differentiated populations due to genetic drift. Vector control through source reduction and insecticide treatment of breeding sites along with the seasonal changes may create periodic population reductions leading to genetic drift. Genetic drift may result in an increase of selectable genetic variation and could lead to the selection of insecticide resistance genes thus making the newly found population resistance to commonly used insecticides. Thus the results of the present study are of utmost importance to in understanding the corridors and barriers for mosquito movement and in turn, develop the strategies for controlling DENV vector mosquitoes more effectively.

Key words: *Aedes aegypti*, phylogeographic structure, mitochondrial DNA, population genetic structure, microsatellites, *kdr*