

First molecular genotyping of insensitive acetylcholinesterase associated with malathion resistance in *Culex quinquefasciatus* Say populations in Malaysia

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Abstract

BACKGROUND: Given that there is limited available information on the insensitive acetylcholinesterase in insect species in Malaysia, the present study aims to detect the presence of G119S mutation in the acetylcholinesterase gene of *Culex quinquefasciatus* from 14 residential areas across 13 states and a federal territory in Malaysia.

RESULTS: The *ace-1* sequence and PCR-RFLP test revealed the presence of glycine–serine *ace-1* mutation in the wild populations of *Cx. quinquefasciatus*. Both direct sequencing and PCR-RFLP methods demonstrated similar results and revealed the presence of a heterozygous genotype at a very low frequency (18 out of 140 individuals), while a homozygous resistant genotype was not detected across any study site in Malaysia. In addition, statistical analysis also revealed that malathion resistance is associated with the frequency of *ace-1^R* in *Cx. quinquefasciatus* populations.

CONCLUSION: This study has demonstrated the first field-evolved instance of G119S mutation in Malaysian populations. Molecular identification of insensitive acetylcholinesterase provides significant insights into the evolution and adaptation of the Malaysian *Cx. quinquefasciatus* populations.

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Keywords: G119S mutation; *ace-1^R*; propoxur; malathion; *Culex quinquefasciatus*; Malaysia

1 INTRODUCTION

Extensive use and overreliance on insecticides for vector-borne disease control have contributed to insecticide resistance development in the target species.¹ In Malaysia, susceptibility of mosquitoes against various insecticides has been studied extensively and described by various approaches such as WHO larval and adult bioassays,^{2–8} enzyme microassays^{9–12} and protein electrophoresis.¹² However, so far nothing has been reported pertaining to insecticide resistance gene detection at the molecular level. There is a dearth of evidence of insecticide resistance in Malaysian mosquitoes on a molecular basis.

Culex quinquefasciatus is one of the most common mosquitoes in residential areas in Malaysia.¹³ Its significance as a vector of urban bancroftian filariasis has been documented in this region.¹⁴ Specifically, insecticide resistance in the Malaysian *Cx. quinquefasciatus* has been well observed. Over the years, insecticide resistance towards carbamates and organophosphates in Malaysian *Cx. quinquefasciatus* has been reported.^{4,6–9,11} Indeed, an elevated level of esterase activity has been identified to play a key role in organophosphate and carbamate resistance development in the mosquito.^{9,11} Conversely, numerous studies have also reported that mutation at the acetylcholinesterase target site (G119S) is the main factor conferring resistance

in organophosphates and carbamates.¹⁵ However, in Malaysia, previous published studies have focused mainly on biochemical characterisation of the metabolic-based mechanism,^{9–11} and there is a lack of evidence on insecticide resistance conferred by target-site insensitivity in this mosquito species.

Based on a previous report by the present authors, Malaysian *Cx. quinquefasciatus* populations have developed a wide spectrum of insecticide resistance towards propoxur and malathion, as demonstrated by WHO larval and adult bioassays. In addition,

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