

Research

Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by deep targeted sequencing

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The capacity of mosquitoes to resist insecticides threatens the control of diseases such as dengue and malaria. Until alternative control tools are implemented, characterizing resistance mechanisms is crucial for managing resistance in natural populations. Insecticide biodegradation by detoxification enzymes is a common resistance mechanism; however, the genomic changes underlying this mechanism have rarely been identified, precluding individual resistance genotyping. In particular, the role of copy number variations (CNVs) and polymorphisms of detoxification enzymes have never been investigated at the genome level, although they can represent robust markers of metabolic resistance. In this context, we combined target enrichment with high-throughput sequencing for conducting the first comprehensive screening of gene amplifications and polymorphisms associated with insecticide resistance in mosquitoes. More than 760 candidate genes were captured and deep sequenced in several populations of the dengue mosquito *Ae. aegypti* displaying distinct genetic backgrounds and contrasted resistance levels to the insecticide deltamethrin. CNV analysis identified 41 gene amplifications associated with resistance, most affecting cytochrome P450s overtranscribed in resistant populations. Polymorphism analysis detected more than 30,000 variants and strong selection footprints in specific genomic regions. Combining Bayesian and allele frequency filtering approaches identified 55 nonsynonymous variants strongly associated with resistance. Both CNVs and polymorphisms were conserved within regions but differed across continents, confirming that genomic changes underlying metabolic resistance to insecticides are not universal. By identifying novel DNA markers of insecticide resistance, this study opens the way for tracking down metabolic changes developed by mosquitoes to resist insecticides within and among populations.

[Supplemental material is available for this article.]

Mosquitoes are vectors of numerous human diseases, representing a major threat for public health worldwide (Lounibos 2002). Dengue and Chikungunya viruses are both transmitted by the mosquito *Aedes aegypti* and represent a burden in more than 100 countries putting more than 2.5 billion people at risk (WHO 2009, 2014). Since the Second World War, chemical insecticides have been massively used for controlling vector populations and reducing disease transmission, but their efficacy is now threatened

by resistance mechanisms developed by mosquitoes. Insecticide resistance is widespread in *Ae. aegypti* and affects most insecticides used for vector control (Ranson et al. 2010). Resistance to pyrethroid insecticides, the primary insecticide family used against adult mosquitoes, is particularly worrying in the context of the re-emergence of dengue and other arboviruses worldwide (Bhatt et al. 2013). Although attempts are made to develop new insecticides or alternative mosquito control strategies (Scholte et al. 2004; Lacey 2007; Hoffmann et al. 2011; Walker et al. 2011; Harris et al. 2012), their large-scale implementation in tropical

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