The genetic architecture of target-site resistance to pyrethroid insecticides in the African malaria vectors *Anopheles gambiae* and *Anopheles coluzzii*

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**Abstract**

Resistance to pyrethroid insecticides is a major concern for malaria vector control, as these are the only compounds currently approved for use in insecticide treated bed-nets (ITNs) and are also widely used for indoor residual spraying (IRS). Pyrethroids target the voltage-gated sodium channel (VGSC), an essential component of the mosquito nervous system, but mutations in the *Vgsc* gene can disrupt the activity of these insecticides, inducing a “knock-down resistance” (*kdr*) phenotype. Here we use Illumina sequence data from phase 1 of the *Anopheles gambiae* 1000 Genomes Project (Ag1000G) to provide a comprehensive account of genetic variation at the *Vgsc* locus in mosquito populations from 8 African countries. In addition to three known variants that alter the protein-coding sequence of the gene, we describe 19 previously unknown non-synonymous variants at appreciable frequency in one or more populations. We report a highly significant enrichment for non-synonymous variation occurring in linkage with the known L995F resistance allele, indicating the evolution of multiple secondary variants that either enhance or compensate for the L995F phenotype. We also describe a possible resistance variant I1527T, which occurs in linkage with two non-synonymous variants in codon 402. We use an analysis of haplotype sharing on the flanks of the gene to refine our understanding of the origins and geographical spread of resistance variants within the gene. We characterise 11 distinct lineages, each of which carries one or more resistance alleles and appears to be undergoing a rapid and recent expansion in one or more populations. We provide preliminary evidence that the most successful and widespread resistance lineage (F1) originates in West Africa and has subsequently spread to countries in Central and Southern Africa. We also reconstruct a putative ancestral haplotype for each lineage, and analyse patterns of recombination to show that lineages are unrelated and thus represent independent outbreaks of resistance. Our data demonstrate that the molecular basis of pyrethroid resistance in African malaria vectors is more complex than previously appreciated, and provide a foundation for the development of new genetic tools to predict resistance phenotype and track the further spread of resistance.