

Resistance associated novel amplified esterase *esta3* gene in *Culex quinquefasciatus*, the vector for Filariasis

The mosquito *Culex quinquefasciatus* is the major vector of filariasis in tropical regions of the world. Resistance to organophosphate insecticides in these mosquitoes is associated with increasing esterase activity due to overproduction of one or more esterases. The most common resistance associated phenotype is co-elevation of the enzyme esterase Est 2 and Est β 2. Est β 1 is the second commonest elevated esterase. Here we report the amplification of a novel functional esterase *est 3* gene, in two strains of

Cx. quinquefasciatus, Colombian (COL) and Cuban (MRES) strains, which have different amplified est β 1.

On native polyacrylamide gels it had an elevated β naphthyl acetate specific esterase with the same R_f as that for the Est β 1 is involved in insecticide resistance. After five generations of temephos insecticide selection, the esterase specific activity with nitrophenyl acetate and the LC values were increased, suggesting the elevation of esterase activity. Western blot with antisera raised to Est 21 and Est β 21, indicated that the COL strain had an elevated Est 3 enzyme, which co-migrated on native gels with Est β 1. Southern and dot blot analysis revealed that est β 1 and est 3 genes, were amplified, therefore the molecular basis of the insecticide resistance in these strains were, gene amplification. However restriction digest pattern of the est β 1 gene in these two strains are different.

The non-amplified est and est genes are still present present in the Colombian strain. These are in a head to head arrangement 1.7kb apart, as found in the insecticide susceptible Pel SS strain. The screening of a genomic library of the Colombian strain indicated that two esterase genes (est 3 & est 1) are amplified at two different amplification levels. There is no evidence for any further genes amplified with the est 3 or est 1 genes. Therefore the genomic organization, of esterase genes are different in est /est 1 and est 21 /est 21 amplicons.