

Using micro-arrays technology to investigate insecticide resistance in malaria vectors.

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Insecticide resistance is a major threat to the success of all vector control programs. This is particularly true for malaria, where most national control programs rely extensively on residual house spraying with insecticide or the use of insecticide treated nets. Insecticide resistance is primarily caused by alterations in the target sites, reducing insecticide binding, or increased rates of insecticide detoxification. The recent release of the full genome sequence of the African malaria vector *Anopheles gambiae* and the development of new molecular techniques have opened up the possibility of conducting **a full analysis of the enzymes families involved in insecticide detoxification in malaria vectors**. Recently, the Liverpool School of Tropical Medicine in collaboration with the Institute of Molecular Biology and Biochemistry in Crete have developed a **'detoxification chip'** to study the expression profile of all genes putatively implicated in insecticide resistance in this mosquito and perhaps in other *Anopheles* species.

The first step of this project was the construction of an array containing fragments of all genes involved in insecticide resistance in mosquitoes. This array, now operational is composed of more than 200 different elements. It includes all genes coding for **cytochrome P450 monooxygenases, glutathione S-transferases and carboxylesterases**. In addition, several other candidate resistance genes have been incorporated. These include genes involved in **oxidative stress response** and those located within the boundaries of pre-determined quantitative trait loci associated with insecticide resistance. One of the major difficulties in micro-array studies is the specificity of the probes printed onto the slides because cross hybridizations between different closely related mRNAs could induce mistakes in the interpretation of the results. This is a particular problem when studying the expression of closely related members of a gene family. Considering this, the software 'PrimeGene' has been used to design unique probes to each gene. Then, a combination of cDNAs and 70-mers oligos were used to construct the array.

The next step of this project is the hybridization of the chip with various mRNA species. The first hybridization studies will focus on the **comparison of gene expression between insecticide-susceptible and insecticide-resistant strains**. Various insecticides will be studied, but with a first focus on pyrethroids because of their widespread use in malaria control. These studies will initially use *An. gambiae* laboratory strains but we will also extract RNA from *Anopheles* specimens collected from field populations showing reduced susceptibility to insecticides. In addition, we are hopeful that the array will also be utilizable for other *Anopheles* species.

A related aim is to study the effect of sub-lethal insecticide exposure on **induction of the genes involved in insecticide detoxification** and the relationship of this process with insecticide resistance. In addition to studying insecticide resistance and gene induction, the array will be used to address a wide range of important questions related to vector control. For example: expression of detoxifying genes during mosquito development, between different tissues, between different transcripts of alternatively spliced genes, between males and females, after blood feeding, after parasite infection, between parasite-resistant and parasite-susceptible strains etc...

By focusing on the study of detoxifying genes and using a high probe specificity design, the 'detoxifying chip' will be **a valuable and updatable tool to study the expression of genes potentially related to insecticide resistance**. Members of these gene families have a large number of different functions besides detoxification, and the array will provide an ideal tool for testing hypothesis of the *in vivo* function of individual members of these complex families. The results from these experiments will complement future experiments using whole genome arrays and help provide a global picture of factors governing gene expression in malaria vectors.