VIEWPOINT

An Overview of Insecticide Resistance

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Insecticide resistance poses a serious threat to current malaria control efforts. The *Anopheles gambiae* genome will enable identification of new resistance genes and will provide new molecular targets for the design of more effective insecticides.

The introduction of DDT for control of the mosquito vector of malaria in the late 1940s, and the early eradication of malaria from the periphery of its transmission range by residual house spraying with this insecticide, led directly to the malaria eradication campaign of the 1960s backed by the World Health Organisation. At the end of the 1960s, the concept of eradication was formally dropped in favor of sustainable control, largely because insecticide resistance was being selected for among the mosquito species that transmit malaria. There has recently been a resurgence in antimalarial activities with the Roll Back Malaria initiative and Global Fund for Health, which support extensive use of pyrethroid-impregnated bed nets for mosquitocontrol campaigns in Africa and other malaria-endemic regions. It is not clear how much the current large-scale pyrethroid resistance of mosquitoes in West Africa will affect these efforts, and what will replace the pyrethroid-treated nets if selection of multiresistance mechanisms results in widespread failure of this strategy (*1*). Genomics will play an increasingly important part in the development of new malaria control tools (Fig. 1). Comparing the genomes of the malaria vector *Anopheles gambiae* and of the fruit fly *Drosophila melanogaster* will not only yield new hormonal, neuronal, and regulatory molecular targets for the development of new classes of insecticides, but will also allow us to attack existing insecticide resistance and to boost the life-span of currently available insecticides.

The resistant phenotype—an insect that survives a dose of insecticide that would normally have killed it—is relatively easy to monitor with direct insecticide bioassays. However, in many cases the actual molecular mechanisms responsible for the resistant phenotypes are still unknown. The availability of the *A. gambiae* genome will allow us to determine the exact molecular changes that have resulted in resistant phenotypes. For example, up-regulation of one or more members of the cytochrome P450 gene families can produce broad-spectrum insecticide resistance. Currently, the mechanisms that regulate insecticide resistance are poorly understood. *Drosophila* may not be a good model in this respect because it is not an insect pest, and so is not subjected directly to large-scale insecticide control programs. In contrast, *A. gambiae* has multiple resistance mechanisms that have been field-selected in both East and West Africa through exposure to DDT and pyrethroids (*2*, *3*).

Research into insecticide resistance is obviously ripe for the move from the static genome map to the functional genomics approach, which will allow us to understand the evolution of resistance in these complex organisms through modulation of gene expression. Material from East Africa has already been subjected to standard genetic quantitative trait loci (QTL) mapping, which has defined a polytene chromosome region within which the regulator of P450 gene expression must be encoded (*4*). The availability of the *A. gambiae* genome sequence now allows us to use new molecular microsatellite markers from the sequence to narrow down this control region to a few kilobases of DNA. Open reading frames can then be identified and candidate genes analyzed for function with recently developed anopheline transformation technology. Regulatory genes controlling the expression of glutathione transferases (enzyme families that are important for protecting insect cells from insecticides) will be similarly defined from QTLs that are already mapped to the *A. gambiae* polytene chromosomes.

In *Culex* mosquitoes and aphids, elevated esterases confer organophosphate resistance through gene amplification, with multiple copies of DNA amplicons of about 30 kb being integrated stably into the insect genome, either contiguously or, in the case of aphids, sometimes disparately (*5*, *6*). The resistant phenotype results from a complex tissue-specific interplay of differential up-regulation of these amplified genes and in the case of aphids involves changes in DNA methylation (*7*). The sequenced *A. gambiae* genome is from an insecticide-susceptible strain, and there are obvious orthologs for the amplified *Culex* esterases. To date, there is no evidence of esterase gene amplification–based resistance in any *Anopheles* species. However, as new resistant strains of *A. gambiae* are investigated, amplifications may well be found, and analysis of the genome sequence surrounding the amplicons will allow us to elucidate the size of the units and may shed light on the amplification mechanisms.

The availability of the complete *A. gambiae* genome sequence should stimulate a rapid shift in research aimed at improving the management of insecticide resistance. If the problem of resistance is subdivided into three stages—detection, monitoring, and manage-

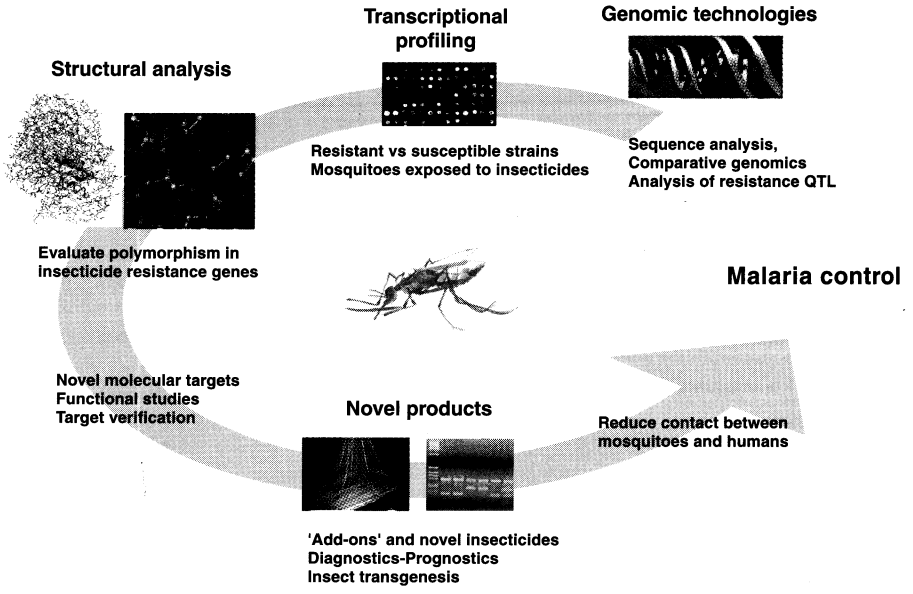

Fig. 1. Potential steps in moving from *A. gambiae* genomics to improved, insecticide-based strategies for controlling transmission of malaria.

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ment—then the benefits of this genome sequence become obvious. The genome sequence will enable access to the major regulatory genes involved in resistance, particularly if orthologous regulators control metabolically based resistance in insects generally. For example, management of resistance in practice currently involves basic rotations and mixtures or mosaics of different insecticides. Access to insect-specific metabolic enzyme regulators will provide a target for "add-ons" to current insecticides, which should expand their natural life-span by blocking common resistance pathways while leaving mammalian toxicity unaffected.

Insecticide resistance can result from direct changes to the proteins that normally bind to the insecticides. For example, mutations in sodium channels (the target of DDT and pyrethroids) and in acetylcholinesterase (AChE; the target of organophosphates and carbamates) have been well documented in many insect species including, in the case of sodium channels, mosquitoes (*3*, *8*). The *Anopheles* genome will provide information on the target site genes, facilitating cloning and mutagenesis studies to determine the precise nature of the mutations and to aid in predicting interactions between insect proteins and insecticides. In the longer term, this could lead to new insecticidal molecular targets. This approach may be especially important for AChE as there is increasing evidence for multiple *AChE* genes from the *Anopheles* and *Drosophila* genome databases (*9*). Two *AChE* genes are apparent in the *A. gambiae* genome as detailed by Ranson *et al.* in this issue (*10*), and to date no resistance-linked mutations have been identified in mosquitoes predominantly in studies on the sex-linked *AChE* gene. The *Anopheles* genome in conjunction with that of *Drosophila* also provides sequences of nicotinic acetylcholine receptor subunits, which will facilitate their cloning from other insect species. This receptor is the target of an important new group of agrochemicals, but until now studies of insect receptors have relied on coexpression of insect genes with a vertebrate subunit (*11*).

Many instances of resistance, resulting from a change in a single regulator gene, may trigger complex cascades of expression of unrelated genes. The presence of the full genome sequence will prompt a move away from the reductionist approach that has dominated the last two decades of resistance research. Such an approach has tended to result in a lack of appreciation for how the large physiological changes that often accompany resistance can influence other characteristics of the insect vector. For example, many of the large scale-up regulations of enzyme families that accompany insecticide resistance result in profound changes in oxidative stress levels in the cells where these enzymes are expressed. These are often the identical tissues in which parasites or viruses reside during transmission by insect vectors from one human to another.

Microarray and cell biology approaches are being used to define mechanisms in *A. gambiae* that enable this insect vector to be refractory to infection by malaria and other parasites. Available data already strongly indicate that an enhanced ability of the refractory insects to tolerate oxidative stress is integral to their ability to resist parasite infection (*12*). The convergence of these two lines of resistance research will be greatly facilitated by our ability to look at regulation patterns in different phenotype combinations across the whole genome. There are already indications that these are not mutually exclusive systems; for example, filarial parasites fail to develop in highly insecticide-resistant *Culex* mosquitoes (*13*).

Overall, the *A. gambiae* genome provides us with exciting opportunities to move from knowledge and understanding of how resistance genes work to the practical application of that knowledge in the field. This will fundamentally improve the control of malaria and other important vector-borne diseases and will contribute to the wider studies of resistance in agricultural pests.

References

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