Molecular Entomology

I RATIONALE

Malaria, dengue and dengue haemorrhagic fever, and human African trypanosomiasis (HAT) are on the increase. Although vector control methods are available to interrupt transmission of these diseases, their effectiveness has been limited by logistics problems, development of resistance to insecticides, and high cost. Novel, sustainable approaches to control are urgently needed.

Recent molecular advances in the understanding of vector genetics and vector-parasite/virus relationships have provided novel tools for the study of disease transmission. Among these are germ-line transformation of mosquitoes, detailed genetic and physical maps, molecular genetic markers for the identification of cryptic species, detection of pathogens in vectors, gene flow studies, detection of insecticide resistance, and the complete genome of *Anopheles gambiae* as well as collections of expressed sequence tags (ESTs) from a variety of disease vectors. However, much work remains to be done to identify, at the molecular level, the role of insect vectors in disease transmission, and the mechanisms for interfering with vector competence. The ultimate goal is to use knowledge acquired by this programme to develop new strategies for the control of disease transmission.

At present, the Molecular Entomology Committee will support relevant proposals on all vectors of the malaria parasite and dengue virus (*Anopheles* and *Aedes* mosquitoes), and preparatory work useful in promoting genomics activities of HAT vectors (*Glossina* flies).

II OBJECTIVES

The objectives of the Molecular Entomology Committee are to:

- Understand the molecular basis of vectorial resistance. This will involve genetic and molecular biology studies of vector-parasite/virus interactions, and of midgut, haemolymph and salivary gland targets for disruption of pathogen transmission. Special attention should be given to the search for antiplasmodial/antiviral molecules and processes.
- Further develop genetic and molecular tools for engineering insect vectors resistant to pathogen transmission and improve germ-line transformation methods.
- Understand mosquito population biology, genetics and dynamics in order to be able to apply these new methods effectively in the field (gene flow, mating barriers between wild mosquito populations, and adaptive mechanisms to environmental conditions).
- Evaluate (in the field and laboratory, and by computer modelling) factors affecting the competitive fitness and vectorial capacity of *Anopheles* and *Aedes* (mating, host-seeking behaviour, oviposition, gene driver systems, and genomic stability).
- Facilitate insect vector genome sequencing, mapping, and post genomic analysis. Identify novel insecticide resistance mechanisms and develop tools for monitoring them in field populations.
- Assess the requirements to be considered before deploying transgenic vectors. This will include biosafety studies; risk/benefit evaluation; development of guidelines and principles; site preparation; baseline data collection on vector biology, ecology and genetics; and issues about ethical, legal and social implications (ELSI), so as to develop an evidence base for policy and minimize the risk to humans and the environment from use of biotechnologies for disease vector control.
- Build capacity in disease endemic countries (DECs) through training and participation in molecular biology, genomics, and postgenomics (e.g. bioinformatics, gene discovery, functional analysis) activities on disease vectors as well as biosafety assessment. Provide support to disease endemic country investigators for the use of insect vector genome data.

### III INDICATORS AND PROGRESS

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<th>INDICATORS</th>
<th>PROGRESS since 2002</th>
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| Genomics and post genomics advances            | - Integrated genetic and physical maps compiled through *in situ* hybridization of thousands of bacterial artificial chromosomes (BACs) to polytene chromosomes  
- RNAi technique exploited for gene function analysis  
- *Aedes aegypti* genome project in progress  
- *Anopheles*-specific bioinformatics resources (*AnoBase* web site and *Anopheles* CD) developed  
- Tsetse genome project activities initiated                                                                                                                        |
| Genetic transformation advances                | - Stable, reproducible germline transformation of *Ae. aegypti*, *Culex quinquefasciatus*, *An. stephensi* and *An. gambiae* achieved  
- Progress achieved on *Wolbachia* transformation  
- Improved transposable elements constructs developed and used for mosquito transformation  
- Tissue-specific (midgut, salivary glands, hemocytes, fat body) promoters shown to function in transgenic mosquitoes  
- Fluorescent dominant protein markers shown to function in transformed mosquitoes                                                                                           |
| Molecular targets in mosquito-pathogen interactions identified | - Several antiplasmodial effector gene products shown to inhibit parasite development in mosquitoes  
- A stable *P. berghei* green fluorescent protein (GFP) parasite constructed to study transmission in mosquitoes  
- RNAi-induced inhibition of dengue virus replication accomplished                                                                                                    |
| Olfaction in mosquito-host interactions advances | - Odorant binding proteins from *An. gambiae* and *Ae. aegypti* cloned and characterized  
- Genes encoding candidate odorant receptors that are selectively expressed in olfaction organs identified from *An. gambiae*                                                                                      |
| Population genetics advances                   | - Distribution of M and S molecular forms of *Anopheles gambiae* established in Africa  
- Insecticide resistance genes (*Kdr, Ace1, Cyt P450*) frequency distribution among vector populations established  
- *Ae. aegypti*, *An. funestus* and *An. culicifacies* population structure studied with molecular markers                                                                                           |
| Capacity building for molecular entomology     | - Yearly high level specialized course in molecular entomology (Biology of Disease Vectors) co-sponsored (about 10-15 participants from DECs trained per course)  
- Two training centres in functional genomics and bioinformatics for insect vectors established (in Thailand and Mali)  
- About 20 new research projects funded for 1-2 years duration                                                                                                                                 |
| Leadership in agenda setting in molecular entomology | - An International *Glossina* Genome Initiative (IGGI) established to facilitate *Glossina* genome activities  
- A meeting to bridge laboratory and field research on genetic control of disease vectors organized and coordinated  
- A scientific working group (SWG) meeting on Insect Disease Vectors and Human Health suggested directions to update the TDR vector research agenda                                                                 |
IV HOW THE STEERING COMMITTEE WORKS

The Committee holds one meeting per year during which it updates the workplan and reviews research proposals addressing any of the objectives mentioned above. Grants are typically funded for one to two years with an annual budget of up to US $40 000. Progress is evaluated each year and grants may be renewed depending on the results.

The Committee welcomes the involvement of young researchers, especially those from DECs, in the project, to broaden their experience and to reinforce research skills as appropriate to the aim of the project. Training is supported by Research Capability Strengthening (RCS) grants.

Interested investigators may consult with the committee manager before applying.

V HOW TO APPLY

Researchers interested in collaborating in the above activities may download the application forms (Collaborative Research Grant form) from the TDR website at http://www.who.int/tdr/grants/forms.htm or request them from the TDR communications unit.

Proposals for 2005 should be submitted before 28 February 2005. All specific correspondence related to research covered by the Committee on Molecular Entomology should be sent to:

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<table>
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<tr>
<th>PRODUCT AND INTERMEDIATE PRODUCT</th>
<th>ACTIVITIES</th>
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<tr>
<td><strong>Product A.02.01: Identification of genes responsible for disruption of parasite/virus growth</strong></td>
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| 1.1. Genetic and molecular studies of vector-pathogen interactions | - Midgut targets for disruption of pathogen growth and development  
- Haemolymph targets  
- Salivary gland targets | In progress  
In progress  
In progress |
| 1.2. Methods for the identification of chemical signals involved in host-finding by mosquitoes | - Identify human specific volatiles, attractants and repellents  
- Chemical analysis of effective compounds and electrophysiological and behavioural tests | In progress  
In progress |
| 1.3. Understanding of determinants of mating success and of finding oviposition, resting and feeding sites | - Field and laboratory investigation of these behaviours | In progress |
| **Product A.02.02: Methods for the spread of selected genes in wild mosquito vector populations** |  |  |
| 2.1. Methods for discovery of population structure | - Analysis of genetic polymorphism and population size  
- Studies of gene flow and mating barriers between wild mosquito populations | In progress  
In progress |
| 2.2. Knowledge about mechanisms and evolution of insecticide resistance | - Identification of novel insecticide resistance mechanisms  
- Development of molecular genetic tools for monitoring resistance mechanisms in field populations | In progress  
In progress |
| **Product A.02.03 : Development of molecular tools for genetic transformation of mosquito vectors** |  |  |
| 3.1. Knowledge of genomics of *Anopheles* and *Aedes* mosquitoes | - Integration of genetic, cytogenetic and molecular maps using BAC libraries  
- Sequencing (including annotation) of *An. gambiae* genome  
- Functional analysis of the genome | Achieved for *An.gambiae*,  
Achieved  
In progress  
In progress |
| 3.2. Stable germ-line transformation | - Improvement of methods for introducing DNA into embryos  
- Improvement of efficiency and yield of germ-line transformation | Achieved  
In progress |
| 3.3. Gene driver systems | - Efficiency evaluation of transposable elements  
- Evaluation of cytoplasmic incompatibility systems | In progress  
In progress |
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| **Product A.02.05:** Requirements to be considered before deploying refractory transgenic insect vectors | - Develop guidelines and principles on the design and performance of minimum risk field research  
- Develop criteria to identify and prepare the sites  
- Conduct studies on biosafety and risk/benefit evaluation  
- Develop criteria and test methods for environmental monitoring  
- Provide the basis for collection of data on vector biology, ecology, behaviour and genetics addressing safety in the field  
- Provide basis for policy-making of relevant scientific, legal, ethical and social issues (ELSI) | To be initiated |
| **Product A.02.07:** Support and coordination of international insect genome sequencing and mapping and post genomics research activities | - *Aedes* genome project activities  
- Tsetse fly genome project preparatory activities  
- Improving *Anopheles* genome annotation | In progress  
Initiated  
In progress |
| **Build capacity for molecular entomology research activities** | - Provide advanced specialized training in molecular biology, genomics and bioinformatics of disease vectors  
- Provide training in biosafety assessment  
- Develop mechanisms for disseminating information to researchers, decision-makers and communities  
- Promote South-South and South-North research and development | In progress  
To be initiated  
In progress  
In progress |
| **Support to DEC investigators for the use of insect vector genome data (RCS Plus: E.11.21.)** | - Establish training centres for training of trainers in bioinformatics in Africa, South-east Asia and Latin America  
- Undertake training in molecular entomology and functional genomics applied to insect disease vectors  
- Fund trained investigators from DECs for conducting insect vector post genomic research | Initiated in Africa and Asia  
To be initiated  
To be initiated |