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Report of the working-group meeting

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Introduction

The continued threat of vector-borne diseases calls for both reactive and proactive efforts to mitigate the significant morbidity and mortality they cause. To anticipate future demands for novel vector control strategies, the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) formalized a work plan to evaluate genetic-control strategies for prevention of the transmission of selected vector-borne diseases. The original plan focused only on malaria, but was later expanded to include dengue fever. The plan calls for research to determine if genetics, and specifically genetically modified mosquito vectors, could be used to supplement established control methods that rely on chemicals and environmental management. Buoyed by support provided by TDR, the John D. and Catherine T. MacArthur Foundation, the Wellcome Trust, the Burroughs Wellcome Fund, National Institutes of Health (USA) and other funding agencies, research on genetically modified vectors (GMVs) has resulted in the proof-of-principle demonstration that malaria-resistant and dengue-resistant mosquitoes can be produced. It remains to be demonstrated that laboratory-derived engineered mosquito strains can be deployed effectively and safely in the field.

Following the laboratory achievements in genetically modifying mosquitoes, three workshops (London 2001, Atlanta 2001, Wageningen 2002) began a process to discuss issues of benefits and risks in the use of GMVs. A number of recommendations were proposed at the different workshops and are summarized here:

Laboratory-based research

There is a need for continued research in the laboratory-based development of GMVs for disease control. This research includes improvement in transgenesis technology for *Anopheles gambiae* and other relevant anopheline species, as well as for *Aedes aegypti*.

Research on the validation of anti-pathogen effector molecules must be continued. Specifically, the predictive value of animal model systems for what might be expected with human pathogens needs to be determined. Research is needed to determine the potential for development of pathogen resistance to effector molecules. The genetic load imposed on vectors as a result of carrying effector molecules must be assessed using rigorous standards for fitness. The long-term stability of effector gene constructs in transgenic mosquitoes must be evaluated.

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Long-term preservation of laboratory strains will become increasingly important. These will include reference and transformed strains of specific insects.

There is a need to develop additional targets of genetic manipulation that may result in non-biting vectors, those with altered host preferences and other mechanisms that may not target specifically the pathogens.

Translational research

There is a need for increased interplay between laboratory and field-based research, so that advances from the laboratory are moved to the field and insights from the field help shape increasingly rigorous laboratory-based investigations. Basic research must be done on target populations, which could include topics like behaviour (reproduction, host location and host preference); population size, structure and dynamics; and physiology (reproductive and feeding). Research must be undertaken that measures the impact of field conditions on GMVs. Examples include, but are not limited to, the effects of colonization on fitness, behaviour, physiology and pathogen transmission. An increased effort must be directed toward identifying useful mechanisms to manipulate gene flow (gene drive) in target populations. This should include Mendelian systems, non-Mendelian mechanisms, and symbionts, including paratransgenesis. To meet these goals field sites for long-term study must be identified, characterized, staffed by personnel with appropriate expertise and resources, and designed to facilitate training as well as integrative research.

Safety and efficacy research

There is an urgent need to identify and define biological factors that will be important for risk assessment. These include horizontal transfer of genetic material, modified vector competence and vectorial capacity, and issues associated with the release of nuisance vectors.

Communication and education research

There is a fundamental need for collaborative work with other committees and public policy groups to evaluate decision-making processes associated with the social, ethical and legal aspects of using genetics to control vector-borne diseases.

In order to substantially elevate the prospects for successfully applying genetic tools to control vector-borne diseases, there is a need to develop consensus opinions regarding key areas that integrate field and laboratory research. The following sections describe the outcome of deliberations held in an interactive workshop format. It was structured to respond specifically to previous recommendations and prioritize laboratory and field research areas.

Objectives and expected outcomes

The SWG was to set a research agenda to address issues and challenges about genetic control of disease vectors and develop a strategic plan to bridge laboratory and field research. This would provide a basis for TDR to define its own vector research programme, taking into account its comparative advantages. The objectives of the meeting were to:

- identify research areas that will integrate molecular and non-molecular research orientated towards the use of genetics in the control of vector-borne diseases;

- identify key areas of research that take into consideration the ecological, behavioural and other field-related parameters that will affect the strategies for using genetics to control vector-borne diseases;
- establish priorities for TDR and partners-funded research to achieve a meaningful incorporation of ecological factors in genetic control;
- identify training opportunities that will result in researchers who are able to consider the laboratory-based advances in relation to the relevant ecological context.

A dialogue between the various disciplines involved in vector-borne-disease control and GMV development (e.g. molecular biologists, geneticists, physiologists, population biologists and ecologists) was expected to result in identification of key research areas for the application and evaluation of genetics to control vector-borne diseases. The production of this workshop report would subsequently provide guidelines for the TDR Molecular Entomology Committee work plan and research agendas of its partners. A strengthened research portfolio should then stimulate research in key areas related to GMV development through solicitation for research proposals.

The widening gap between laboratory and field-based research on disease vectors has been noted with concern. This meeting was the first to bridge this gap and facilitate future collaboration and training of scientists with multidisciplinary expertise in genetic control of vector-borne diseases. Successful development, evaluation and refinement of genetic strategies to control vector-borne diseases can only be expected if both laboratory and field-based scientists merge their efforts and strategically focus their impetus in this endeavour.

Genetic disease-vector control: past, present and future

Historical perspective of application of genetics to vector control

The concept of genetic control of insect pests and vectors was formulated independently three times in the 1930s-40s, obtaining encouraging results with releases of radiation-sterilized male tsetse. This was followed in the 1970s by work on mosquitoes using sterile males and systems, which could potentially be developed further to be used for driving genes into populations. Chemosterilized male *Anopheles* separated from females by a genetic system were successfully released in El Salvador. In India chemosterilization, cytoplasmic incompatibility, translocations and meiotic drive were tested with culicine mosquitoes in field cages, for mating competitiveness in the field and in some cases in village-wide release trials. The field tests in India of *Culex quinquefasciatus* and *Aedes aegypti* with various genetic-control systems indicated adequate mating competitiveness for females of wild origin. However, corresponding tests with *Cx. tarsalis* in the USA gave evidence for assortative mating. Corresponding tests with promising GM strains would be important. During the earlier stages of testing these could be radiation-sterilized to ensure that any unexpected adverse effects of the GM constructs would not be propagated in the wild population. In India, a town-wide eradication attempt planned in 1975 with *Ae. aegypti* was stopped due to spurious claims about biological warfare.

The historical examples mentioned above have in common a reduction of the mosquito populations through the use of either sterilized males or strains that lead to an unbalanced male-female ratio. In other words, the sterile-insect technique (SIT) in a wider sense of the term, an approach that has proven successful in the control of other insect pests such as, for example, the Mediterranean fruit fly and the New World

Screwworm. Although approaches of this kind are not to be excluded from consideration in the control of disease vectors, the focus of attention today is different: it entails the use of GMVs that will, ideally, not be able to transmit particular disease agents. Naturally, genetic modification can also be considered for the production of so-called sexing strains, i.e. strains that produce males only and that can, therefore, be used in SIT approaches. This strategy, though, was not discussed further at the meeting and, thus, emphasis will be put on the development of genetically modified, refractory vector strains that, after release in the environment, will eventually replace the resident populations leading to an end of disease transmission.

Anticipated impact of genetic tools in vector control and expected benefits from genomics

Presently, research efforts are concentrated on developing all the required tools for such an endeavour and providing proof of principle. Although the idea of replacing wild populations with refractory ones was already suggested in the 1980s, the compulsory germ-line transformation techniques were only developed, initially for *Aedes*, in 1998. More disease vector insects followed in the next few years, most prominently *Anopheles gambiae* in 2001. Even if the molecular toolbox has not yet been filled with all the required devices the availability of germ-line transformation made it possible to start working towards answering the crucial question of whether vector strains can be engineered to produce insects that are not able to transmit pathogens. Based on a series of experiments that used either a *Plasmodium gallinaceum/Aedes aegypti* or a *Plasmodium berghei/ Anopheles stephensi* system, it is clear today that, such an approach is, at least in principle, possible. Therefore, the task that is now faced by the research community is to expand the required techniques so that the strategies can be tested under conditions that come closer to the actual interventions in the future.

The expansion of the toolbox will have to encompass a variety of entomological and general molecular methods and approaches, such as the effector gene construct(s) to be used (including the actual gene and its entire control machinery) and the drive system that will be employed to spread the GMV population and replace the existent ones. Care will have to be taken to ensure ‘fool-proof’ mechanisms: an early failure may not only have negative effects on the future of the approach, one should also be aware of the fact that a malfunction of the system may lead to a complete permanent breakdown of the method depending on the individual components chosen. Therefore, genetic stability of such constructs is something that demands extreme attention.

To help prevent a complete failure, it will also be necessary to provide answers to basic questions that deal both with the GMV disease-refractory strains to be used (e.g. fitness, long-term stability of the engineered constructs, etc.) and the populations that these strains will be replacing. It becomes obvious that a shift in research is emerging. This shift does not reflect a major change in the long-term objectives but, rather, it encompasses the spread of new methodologies that allow for a much faster acquisition of data from the field. For example, the lack of easily scored markers (n.b.: the term ‘easy’ reflects speed, availability of resources, cost, etc.) made the study of mosquito populations somewhat tedious. The advent of nucleotide sequence markers and RFLPs, and especially the first generation genomic tools such as microsatellites, RAPDs and other ‘anonymous’ markers, provided new approaches to analyse mosquitoes and other vector insects at the population level. It should be stressed here that the use of microsatellites in population studies makes it easier to move a larger

part of the respective research to laboratories located in disease-endemic countries (DECs). Only a few years later, whole genome sequence (WGS)-derived genetic markers, such as SNPs are now bound to speed up the collection of data from field research, although the use of these very powerful markers relies on the availability of a WGS for the organism of interest, and is still relatively costly. Finally, it should be stated that an ever-growing pool of sophisticated software for population analysis accompanies the modern molecular techniques.

A major thrust in knowledge acquirement is expected from the availability of additional WGS of disease vectors. The new era was initiated by *An. gambiae* but genome projects are under way for different vector arthropods, including *Ae. aegypti* and tsetse. Considering the fact that the *An. gambiae* WGS has only been available for less than two years, it is highly encouraging to see the wealth of information that has emerged from its use. This wealth relates to an increased knowledge on gene systems that are, directly or indirectly, linked to diseases (e.g. immune system), on comparative genomics and, thus, on specific evolutionary aspects, and last but not least, the aforementioned development of specific tools. It is clear that with more genomes being analysed, the benefit for the research community will also increase, given all potential applications that will materialize. These applications will have to include tools for monitoring genetic structure and stability of vector populations, also aiding studies on dispersal (gene flow) of vector populations. Moreover, on the level of actual genomic research, emphasis will have to be given to postgenomic research on the level of general and specific RNA profiling, but also on fields that are underrepresented in vector biology today, such as proteomics.

What is needed?

The shift mentioned earlier can also be described as a move from descriptive population genetics to experimental and applied population genetics. This is a development that is a prerequisite for the successful use of GMV in attempts to control disease. Although at a purely scientific level the chances of success for this are becoming better through the availability of improved techniques, a few more items are required. These include a push to improve partnerships and create or expand existing networks between DEC scientists and laboratories in the North. This has been recognized as a *conditio sine qua non*, given the particular interest of DECs in solving the problem, combined with the fact that the problem is, in reality, international.

Current state and future needs of laboratory and field sciences

Laboratory science: progress and bottlenecks in GMV development

Although proof of principle has been achieved and vectors that are refractory to parasites already exist in the laboratory, releases of GMVs, even at a small-scale experimental level, are not anticipated in the near future. Critical laboratory-based research must be carried out to solve problems that have already been identified. This needs to include the following:

1) Identification of new promoters. A number of vector promoters (tissue-, sex-, stage-specific promoters) from *Anopheles gambiae* and *Aedes aegypti* have now been characterized and are ready for use in GMVs. However, further research is needed to identify and characterize new promoters. For example, the identification of *Anopheles gambiae* salivary-gland promoters that function in adult female salivary glands is urgently needed.

2) Development of new effector genes. The SM1 peptide does not interfere with *Plasmodium falciparum* entry into midguts or salivary glands of *Anopheles gambiae* and new effectors must be designed specifically for this parasite–vector system. Therefore, additional genes will have to be tested for their ability to suppress the transfer of aetiological agents by the insect vectors. It will probably be necessary to design multiple effector-gene strategies to target the parasite and prevent development of pathogen resistance to a given GMV approach through their very close linkage. This will also be true for ongoing endeavours to develop GMVs resistant to dengue viruses. For instance, homology-dependent RNAi approaches are now being developed that express virus-derived double-stranded RNAs that target and destroy multiple regions of a dengue-virus RNA genome.
3) Characterization of genetic drive systems. A major area of research that urgently needs to be addressed is the development of methods to drive effector genes into mosquito field populations. While several approaches are being considered, such as transposable elements, *Wolbachia*, meiotic drive and paratransgenesis, their feasibility remains to be demonstrated. Each approach has both potential benefits and problems as drive mechanisms. Transposable elements have made genetic transformation of vectors possible and have for a long time been considered good gene-drive candidates, but to date, little has been done to test whether current transposons can act as drive mechanisms. Natural transposons carrying 'foreign' genes do exist but their spread is extremely slow, if detectable at all. In addition, fully loaded transposons, which will have to carry effector genes and transformation markers, have neither been tested for their ability to move nor for stability. These types of experiments are urgently needed to assess the feasibility of GMV approaches, in addition to a thorough molecular investigation of the non-transposon-based spread mechanisms mentioned above.

Many other laboratory-based issues remain to be solved for the successful population replacement of competent vectors using GMV approaches, but major milestones have been reached. It should be stated that there exists no universal consensus that the GMV strategy will provide the long-sought solution to the problem of disease-vector control. For example, many meeting participants expressed confidence that major advances have taken place in the laboratory-based GMV research and remain very optimistic about the role of GMVs in arthropod-borne-disease mitigation. In contrast, some felt that GMVs may not present the ultimate, but they recognize that they may also have an important role in vector-population reduction strategies, particularly in producing large quantities of males (e.g. *Aedes aegypti*) for SIT strategies. Indeed, GMV lines with SIT applications have apparently been generated that may lead to field trials much sooner than GMVs developed for population replacement approaches. If so, this could provide important information on release strategies and ethical, legal and social issues (ELSI) for all GMV approaches.

Field science: current thoughts about genetic applications and integration of field and laboratory science

Laboratory-based research for developing a GMV approach will have to be complemented with field-based research to understand the ecology of vectors and arthropod-borne-disease transmission in DECs. Research areas, in which field-based research is necessary to complement ongoing laboratory-based research, have to be identified; investigations need to start now to provide critical insights into GMV-based control of both malaria and dengue. It should be noted that some of these field

studies are currently proceeding in selected DECs, but much more needs to be done and new relationships between DECs and non-DECs need to be formalized. These field-based studies include:

1) The determination of spread and stability of a transgene in vector populations by developing appropriate models of mosquito mating behaviour.
2) The definition of the genetic structure of mosquito populations to determine gene flow and probability of transgene spread.
3) The definition of vector population size with seasonal fluctuations.
4) The definition of factors controlling population regulation, and a number of other parameters for eventually designing GMV releases.

Field-based studies must also be designed and implemented to determine the evolutionary consequences of transformation, including fitness costs, phenotypic variation in effector-gene expression, and whether GMVs will effect transmission of other pathogens or ongoing vaccination, drug or vector control programmes. Obviously, the latter will have to be initiated in the laboratory. Field-based studies are also needed to develop mathematical models that can identify knowledge gaps in our understanding of disease transmission, thereby allowing researchers to better predict outcomes of potential release scenarios. A number of these studies can (and must) be initiated now in selected DECs. There have been ongoing efforts to formalize interactions of DEC scientists with laboratory- and field-based scientists of non-DECs in the context of the Gates Grand Challenges in Global Health initiative. Funding of some of these proposals should help in cementing these relationships, but it is clear that success of GMV approaches depends heavily on developing close working relationships with DEC scientists and other DEC partners.

The challenges

While approaches based on GMVs represent potentially useful solutions for mitigating malaria and dengue, there remain gaps in our knowledge that have slowed or prevented the development of genetic-control methods. These gaps exist between the state-of-the-art laboratory development of novel transmission-blocking tools and knowledge of field properties of mosquitoes that will affect their use, and between scientists in the developed world and the DEC scientists who would be responsible for implementing the technology. Further gaps exist among scientists and the agencies that would be responsible for the deployment of any genetic-control strategy, and in policies and procedures for evaluating how genetic-control methods fit into the overall strategy of existing or planned control programmes. Finally, gaps exist between the enthusiasm of scientists for these genetic methods and the level of awareness of potential end-users for the risks and benefits of using them for controlling malaria/dengue transmission. The challenge is to close these gaps in knowledge sooner rather than later.

Regional situation reports

As malaria and dengue/yellow-fever vectors (various anopheline species, *Aedes aegypti* and *Ae. albopictus*) are currently at the forefront of studies concerning genetically modified mosquitoes, the situation reports presented below focus mainly on these species. It is realized, however, that in due course vectors of other human pathogens will be included as well. These concern vectors of African trypanosomiasis, Chagas disease, onchocerciasis, leishmaniasis, filariasis and arbo-viral diseases other

than dengue and yellow fever. Several species of vectors of these diseases are already being considered for genetic modification and GMV strategies.

Africa

In recent years, work on African malaria vectors has concentrated much on investigations of species and population genetics associated with malaria transmission. In addition, studies have focused on the emergence of insecticide resistance and molecular tools for rapid assessment of such resistance. In much of tropical Africa, *Plasmodium falciparum* is the dominant malaria parasite, with *P. malariae* occurring at an incidence of 10%.

Studies on species complexes and population genetics

Important malaria vectors in Africa are members of the *Anopheles gambiae*, *An. funestus*, *An. nili* and *An. moucheti* complexes. These complexes consist of closely related sibling species with often distinctly different behaviours and ecological adaptations. Whereas *An. gambiae s.l.* and *An. funestus* are widely distributed across the continent, the latter two species complexes are mostly found in forest areas of Central and West Africa. All of these species express high heterogeneity in genetic make-up as well as in environmental adaptation, and this affects their significance as malaria vectors. Notably, not all sibling species within these complexes are vectors because of different feeding preferences.

Anopheles gambiae complex

Currently seven members of the complex have been described, of which *An. gambiae s.s.*, *An. arabiensis*, *An. melas*, *An. merus* and *An. bwambae* are malaria vectors, in this order of significance. *An. gambiae s.s.* and *An. arabiensis* are mostly panmictic across tropical Africa while *An. melas*, *An. merus* and *An. bwambae* have restricted distributions. In West Africa *An. gambiae s.s.* exhibits great genetic variability, appearing in five chromosomal forms (Savana, Bamako, Mopti, Bissau, Forest). In East and Southern Africa, only one chromosomal form (Savana) of *An. gambiae s.s.* can be found. Chromosomal forms Bissau and Forest are more often associated with humid zones, whereas the Mopti form appears to have adapted to dry zones. Recently it was shown that *An. gambiae s.s.* consists of at least two distinct molecular forms, S and M, which are characterized by their rDNA haplotype. Ongoing research shows that both molecular forms are widely distributed across the continent, with the exception of East and Northeast Africa, where so far only the S form has been found. Cytotaxonomic and molecular-genetic studies suggest that *An. gambiae s.s.* may be a sibling group expressing incipient speciation. This phenomenon has been associated with environmental changes such as irrigated agriculture and deforestation.

Anopheles funestus complex

Currently 11 species of this complex have been described, of which 10 are present in Africa. Of these only *An. funestus s.s.* is considered a malaria vector. This species is found across tropical Africa and an important malaria vector due to its high degree of anthropophily and endophilic character. Two chromosomal forms of *An. funestus s.s.* have been found: Kiribina and Folonzo. These forms are present at least from Senegal to Cameroon. From separate studies in Southeast and West Africa it was shown that genetic heterogeneity of *An. funestus* increases with geographical distance.

Anopheles nili complex

This group of malaria mosquitoes currently exists of 4 different taxonomic groups, *An. nili s.s.*, *An. carnevalei*, *An. ovengensis* and *An. somalicus*. The first three species are efficient malaria vectors, with a high degree of anthropophily, whereas *An. somalicus* is zoophilic and not a vector.

Anopheles moucheti complex

Two members have been recognized, *An. moucheti s.s.* and *An. bervoetsi*. Both are sympatric in distribution, mainly restricted to central Africa. The species are morphologically identical. Locally, *An. moucheti s.l.* can be an important malaria vector.

From recent investigations it appears that members of these 4 complexes can now all be distinguished by PCR. Microsatellites are considered useful genetic markers to establish heterogeneities within a species as well as for genetic fingerprinting. Some studies using SNPs have been initiated with *An. gambiae s.s.*, but they are still rare and costly.

Studies on insecticide resistance

Indoor residual spraying for malaria control has been widely applied in Africa, but was abandoned in many countries in the nineteen sixties, often for non-scientific reasons. However, in many countries resistance against DDT and dieldrin was reported. Locally, resistance against carbamates and malathion (an organophosphate) has also been reported. Studies with insecticide-treated nets (ITNs) began as of 1980 and were rapidly expanded on an experimental scale in several countries. The nets were impregnated with synthetic pyrethroids, mostly permethrin and deltamethrin. Because of the great success of these studies in reducing child morbidity and mortality by factors varying from 20 to 60% (depending on local epidemiological conditions), ITN technology was officially adopted by WHO as one of the main strategies for malaria control, through the Roll Back Malaria programme. Resistance of *An. gambiae s.s.* against pyrethroids was first observed in the Ivory Coast in 1993; since then resistance frequencies as high as 80% have been reported in this country. In 1994 resistance was also detected in *An. gambiae s.s.* from Kenya. The resistance reported from Ivory Coast has not been associated with the use of ITNs or indoor spraying, but with agricultural use of insecticides, notably for cotton production.

A special case of insecticide resistance in malaria vectors was reported from South Africa. In 1996 this country, having used DDT for malaria control since 1950, switched to pyrethroids for indoor spraying. Since then malaria incidence rapidly increased. The principal malaria vector, *An. funestus s.s.*, was found to be highly resistant against the pyrethroids. The resistance was based on a metabolic mechanism of mixed-function oxidase. For these reasons South Africa resumed DDT spraying in 2001, and malaria cases have dropped since then. However, *An. arabiensis* developed resistance against DDT (dieldrin resistance of *An. arabiensis* had been reported earlier). The regional situation is complicated as Mozambique does not allow DDT spraying and continues to use pyrethroids.

Malaria-vector control in Africa

Currently, most countries have adopted ITNs as the main strategy for malaria intervention. Nine countries (in East and Southern Africa) use indoor residual spraying. In spite of the ITN strategy and implementation of the Roll Back Malaria strategy, on average only 2% of all children are covered effectively by an ITN, with

notable better exceptions in The Gambia and Sao Tomé (20% coverage). These strategies are sporadically and locally augmented by source reduction and larval control. Therefore, vector control for prevention of malaria is in urgent need of expansion.

Aedes aegypti in Africa

Aedes aegypti was the vector of yellow fever, particularly in urban centres. Efficient vaccination campaigns in the last century made yellow fever almost disappear. Such wide-scale vaccinations have been largely abandoned, and many people are non-immune against the virus.

Ae. aegypti in Africa can be distinguished into two different forms: *Ae. aegypti aegypti* (East Africa) and *Ae. formosus* (West and Central Africa). The species express different vectorial competence for dengue virus. *Ae. albopictus* has been recorded from Cameroon and South Africa. Recent studies in Cameroon show that *Ae. albopictus* is replacing *Ae. aegypti*, as the species is spreading eastwards from the coast. This species replacement is very similar to that observed in southern USA. There is no reported control of *Ae. aegypti* or *Ae. albopictus* in Africa.

Southeast Asia

This report on vectors and vector control of prevention of malaria and dengue in Southeast Asia is limited to Vietnam, Cambodia, Democratic Republic of Laos, Thailand, Malaysia and Singapore.

Malaria vectors and control

Malaria vectors in Southeast Asia belong to four species complexes, *Anopheles dirus*, *An. minimus*, *An. maculatus* and *An. balabacencis*, all made up of variable numbers of sibling species. The species occur in a wide diversity of habitats, from lowland rainforest to upland sunlit farmland (rice cultivation). The most important malaria species are *Plasmodium falciparum* and *P. vivax*. Overall, the malaria risk is relatively low, and is experienced by all age groups. However, forest workers in the age group of 30-39 years are most at risk.

Insecticide resistance is widespread in all vector species, presumably as a result of extensive application in agriculture, and in some areas as a result of decades of indoor residual spraying. There are good results with ITNs and this is, in most countries, the main tool for malaria prevention (notably in Vietnam, where some 11 million people are sleeping under ITNs).

Recently the first studies on population genetics of malaria vectors in Southeast Asia have been published, and it is expected that other such studies will appear soon, providing more information on the genetic make-up of Southeast-Asian malaria vectors.

Dengue vectors and control

In Southeast Asia dengue is highly prevalent and an important vector-borne disease. The main vectors are *Aedes aegypti* (urban centres) and *Ae. albopictus* (rural areas). Long-term studies on the biology, vector competence and ecology of *Ae. aegypti* are being conducted in Thailand. Recent studies on *Ae. albopictus* demonstrated high infection rates with *Wolbachia*. However, such infections were absent from *Ae. aegypti*. Therefore, translocation of *Wolbachia* from *Ae. albopictus* to *Ae. aegypti* using microinjection appears promising. Obviously, the use of *Wolbachia*

in control strategies has not yet been developed to a level where it could be considered for immediate use.

The incidence of dengue is increasing throughout the region, presumably due to deforestation (and other environmental changes) and rapid urban growth. Vector control occurs locally and mostly ad hoc, using fumigation of residential areas and larval control.

Latin America

In this region malaria is less prevalent compared to Africa and even lower than in Southeast Asia. Dengue outbreaks have been reported from the Caribbean since 1634, but in the last three decades serious outbreaks of dengue have been reported from Cuba, Peru and Brazil.

Malaria vectors and control

Malaria is present in the entire zone, with the exception of Chile. Both *Plasmodium falciparum* and *P. vivax* occur, in a ratio of 1:2. In 2003 more than 800,000 cases of malaria were reported, 40% of these from Brazil. The entire Amazon region is endemic with malaria. Vectors are *Anopheles albimanus*, *An. darlingi*, *An. pseudopunctipennis* and *An. nuñetzovari*. The former two species are most abundant and widespread, *An. albimanus* in Central America and along the west coast of South America. *An. darlingi* is a forest species, occurring from southern Mexico till southern Brazil. Unlike the Southeast-Asian and African anophelines, the American anophelines appear to be relatively homogeneous, showing far less genetic heterogeneity compared to the anophelines from the other continents.

Malaria control in Latin America is predominantly based on indoor residual spraying (notably Mexico), use of ITNs and focal larval control. The control of *An. darlingi* appears problematic, as this species exhibits high and varying degrees of exophilic and exophagic behaviour, and larval habitats can be huge and unsuitable for larval control. Malaria transmission by *An. albimanus* can be successfully prevented with ITN use.

Dengue vectors and control

In 2003, PAHO reported 380,000 cases of dengue in Latin America. To-date dengue transmission in Latin America is considered to be vectored by *Aedes aegypti*. This species was virtually eradicated from South America in the nineteen fifties, but has reinvaded all its previous habitats. Large epidemics of dengue have recently been recorded from Rio de Janeiro (Brazil) and Iquitos (Peru). Brazil has established a special dengue control programme, based on larval vector control. This is done with the insecticide abate and with biological agents such as *Bacillus thuringiensis var. israeliensis* (Bti). During epidemics, focal fogging of infected zones is also being performed. Other countries in the region are establishing dengue control programmes, with emphasis on larval control.

Key areas for research involving both laboratory and field sciences

Transition from the laboratory to the field

Current state of the art

It is essential that groups of researchers separated by virtue of differing interests, scientific disciplines or geographical separation interact with one another if GMV

development and application is to proceed satisfactorily. By sharing the same general goal of trying to reduce morbidity and mortality caused by vector-borne diseases, gaps relate to reciprocal lack of scientific skills and experience and the difficulties to communicate effectively and learn from one another.

Issues and challenges

The challenge is to develop an open, interactive research community that exploits the expertise of all scientists, empowers those in DECs and results in application of genetic-control methods that have a real and measurable impact on disease transmission. Communication, funding, clear strategic planning and development of genuine partnerships among all scientists are important ingredients for eliminating the barriers between DEC priorities and global research initiatives.

Opportunities and future directions for research and capacity/partnership building

The GMV endeavour is clearly dependent on the development of a scientific culture in which sharing of funds, building of intellectual partnerships, collaborative infrastructure development and sharing of technological achievements is commonplace. Another key aspect is the bridging of the 'language gap', requiring reciprocal training between field and laboratory biologists so that they can understand and appreciate one another's contribution towards GMV developments. Existing courses (like the BDV course) should grasp the opportunity to extend its training portfolio to include aspects not or only marginally covered previously (e.g. ecology and behaviour of disease vectors).

Drive mechanisms

Current state of the art

The hypothesis behind the development of drive mechanisms is that increasing the frequency of a gene (or allele) in a population of mosquitoes that interferes with the development or propagation of a pathogen will result in the reduction or elimination of transmission of that pathogen to humans. The expectation from a successful demonstration of this hypothesis is that a reduction in transmission will result in less disease. Research is needed in three areas, laboratory-based work to demonstrate that it is possible to use molecular tools to transform mosquitoes that would normally transmit a pathogen into ones that do not, laboratory and field-based work to develop mechanisms for moving genes made in the laboratory into wild mosquito populations, and the systematic assembly of information about target vector populations and disease transmission dynamics that is needed to model and predict how anti-pathogen genes will affect the epidemiology of a disease in a specific endemic region, to test this hypothesis.

Proof-of-principle advances in animal model systems of malaria and the epidemiological relevant dengue virus/*Aedes aegypti* combination confirm that much progress has been made to demonstrate the function of isolated promoters that can be used to drive the expression of anti-pathogen effector genes, the development of transgenesis technologies to integrate stably the effector genes in mosquito strains, and the synthesis of a variety of anti-pathogen effector genes that interferes with parasite and virus transmission. While consolidation and transfer of these efforts to human malaria parasites and their vectors is needed, it is appropriate now to engage the next major challenge and develop gene drive mechanisms.

Issues and challenges

The core features of a gene drive system are that is should be safe (minimum risk) and efficient. Safety refers to an evaluation of the risk factors associated with a particular system, and efficiency is assessed by how well it works to drive the gene through the population and the subsequent impact on transmission dynamics. Specific issues to be addressed are:

- the development of design criteria that mitigate scientific, environmental and epidemiological issues that might factor into safety and efficiency;
- development of a comprehensive approach for utilizing population replacement strategies that targets all the important vectors within a given area;
- development of procedures that introgress genes into strains of mosquitoes that are as similar as possible to the targets in the field.

Meeting participants discussed the desirable characteristics of drive systems and reviewed the potential effects of even low levels of recombination between the drive system and the effector gene(s), or back-mutation of these genes. In combination with (even) low levels of fitness cost associated with heterozygosity or homozygosity of the effector, only the drive system may become fixed in the target population. It is, therefore, essential to ensure extremely reliable linkage of the driver and effector, and genetic stability and full fitness of the effectors.

Research and control opportunities

Research on drive mechanisms will follow a path of studying natural genetic phenomena that alter segregation ratios to spread specific genes or alleles through populations. From these phenomena, specific drive systems must be designed that incorporate the features identified in the preceding section. Such drive systems must be demonstrated first to work in laboratory experiments and progress to semi-field evaluation. Eventually the systems will be introgressed into field-derived mosquito strains and evaluated for their reproductive and competitive success.

Future directions for research and capacity/partnership building

Future research must bridge gaps between the:

- state-of-the-art laboratory development of novel anti-pathogen tools and knowledge of field properties of mosquitoes that will affect their use;
- scientists in the developed world and the scientists living in DECs who would be responsible for implementing the technology;
- scientists and the agencies that would be responsible for the deployment of any genetic-control strategy;
- policies and procedures for evaluating how genetic-control methods fit into the overall strategy of existing or planned control programmes;
- enthusiasm of scientists for these genetic methods and the level of awareness of potential end-users for the risks and benefits of using them for controlling dengue and malaria transmission.

Participants emphasized that all future work should highlight the enlistment and involvement of DEC personnel to foster the level of awareness required for workable guidelines for the evaluation and application of genetic-control programmes.

A research agenda that requires a DEC component includes experiments to:

- introgress laboratory-derived systems into recent wild-derived target populations;
- evaluate drive mechanisms in semi-field settings;

- demonstrate that these drive mechanisms can spread an effector through semi-field populations;
- determine the effects on life-history traits of a drive mechanism in semi-field environments;
- determine the possibility of movement in semi-field cages of drive mechanisms between closely related species

Population genetics

Current state of the art

Over the last years, a number of excellent molecular tools have been developed that will now facilitate the characterization and monitoring of target field mosquitoes before and after releases. These tools also provide capabilities for having genetic markers that will assist the evaluation of aspects of competition between mosquitoes with and without transgenes. These tools include Multiple Displacement Amplification (MDA), which allows a 100-400-fold amplification of whole mosquito genomes, Single Nucleotide Polymorphisms (SNPs), and microarray chips.

Issues and challenges

The core features of theses tools should be that they are easily adapted for application in field research in the DECs. A major issue is that some of these tools are still highly expensive and therefore may drain limited resources rapidly. Training of personnel who can exploit these technologies deserves special consideration, given the adequacy of funds for this type of work. Specific scientific issues arise for the present compositions of microarray chips, which are based on expressed (EST) DNA sequences. Although useful, a better reagent would be a whole-genome tiling arrays.

Research and control opportunities

Participants noted the above challenges and placed emphasis on the following opportunities:

- meaningful capacity-building should be based on sharing of scientific material and biological specimens and move beyond the mere involvement of DEC partners in the collection and shipping of material to northern laboratories. It involves collaborative research, sharing of ideas and concepts, and population-genetics studies form an ideal target for this;
- some of the recently developed methods require sampling and preservation of specific life stages of vectors. The development of specific sampling protocols that provide nucleic acids (DNA and RNA) of mosquitoes that can be used to address major questions about gene flow, population structure etc., lends itself for joint involvement.

Future directions for research and capacity/partnership building

The rapid progression in identification of suitable genetic markers for mosquito population studies requires training that emphasizes hypothesis formation, data analysis and publication skills. The availability of these tools, as such, may not be sufficient to cover all aspects needed for GMV application, and it was recognized that indiscriminate application does not necessarily serve the purpose of the GMV endeavour. It was also noted that research to explore the adaptability of specific data sets for broader or different scientific questions is needed.

Effector molecule identification

Current state of the art

A number of endogenous refractory phenotypes have been recognized, and these include the melanotic encapsulation and lytic destruction of malaria parasites, and the RNAi-mediated impact on viruses. In addition, there are a number of other mechanisms, the specifics of which are unknown, which could include a lack of host factors (atreptic immunity). Synthetic refractory phenotypes include the construction of genes that have parasite anti-ligand effector molecules, host mosquito anti-receptor activities, toxins that kill the parasites, RNAi constructs that interfere with pathogen or vector genes, genes that lead to over-expression of pathogen antagonists, and inhibitors of parasite or viral gene expression. However, the paucity of molecules currently available hinders progress.

Issues and challenges

More work needs to be done in the identification/construction of effector/transgenes that target human malaria parasites, evaluations of dominance/recessiveness in endogenous mechanisms, and incomplete penetration and variable expression of transgenes. In addition, issues must be addressed of phenotypic variation in response to environmental conditions and selection by the transgene effector molecule of resistance or increased virulence of the pathogen. The prevalence (frequency) of the transgenes in the population will be important and threshold levels of refractoriness needed to interrupt transmission need to be determined. Field tests of specific genes and outcome evaluation parameters also are needed. With respect to this latter issue, assays for evaluating refractoriness to endogenous malaria pathogens are important and in need of development. Transmission-biting assays based on membrane feeding with gametocytemic blood derived from patients in transmission zones also need development.

Research and control opportunities

The issues and challenges can be met by evaluating specific genes in:

- Laboratory-based work
  - dominance/recessiveness
  - incomplete penetration and variable expression
  - selection of resistance
  - selection of virulence
  - combinations of effector mechanisms
- Field-based work
  - phenotypic variation in response to environment
  - field tests of performance and stability in cages
  - outcome evaluation
- Laboratory- and field-based
  - prevalence in mosquito population
  - threshold levels

Future directions for research and capacity/partnership building

Research is needed to identify:

- key qualitative and quantitative endpoints for the efficacy of transgene construct
- routine and reliable methods for transmission-blocking assays (membrane feeding) that do not involve feeding on children

- protocols for human subjects
- additional studies of natural refractoriness and its potential to adapt as a 'death-on-infection' effector mechanism.

Mosquito fitness

Current state of the art

Assessing fitness of GMVs will be a critical component of genetic programmes for control of disease vectors and prevention of vector-borne disease. It is assumed that in most cases genetic modification will incur fitness costs. This could undermine a population reduction strategy by rendering the sterile released sex non-competitive for wild-type mates. In a population replacement approach, genetic drive mechanisms are used to spread desirable genes into a population, but if insects with the desirable genes are less fit than wild mosquitoes, the drive mechanism may not be strong enough to offset the impact of the fitness cost and the desirable genes may be lost from the target population. A goal for both strategies, therefore, will be to minimize fitness disadvantages associated with genetic modification. The probability of a fitness advantage resulting from modification is considered low, but if it should occur it would be expected to promote success of either intervention strategy. Consequently, for the development and deployment of GMVs it is of paramount importance that the concept of fitness be fully understood and that a consensus is reached on how best to measure the fitness of GMVs relative to the wild-type mosquitoes they will be intended to eliminate or replace.

Of the research projects that included assessment of components of GMV fitness, three took place during the 1970s and included field releases and three recently published reports concern fitness of transgenic mosquitoes. Among these studies of transgenic mosquitoes, two reported reduced fitness of transformed mosquitoes, but their experimental design made it difficult to separate inbreeding depression from transgene effects. In the third study inbreeding depression was overcome by crossing the transformed strains with a genetically diverse laboratory strain. It was concluded that detection of a fitness load depends on the effects of the expressed transgene and that transgenes will not necessarily confer a fitness cost.

Issues and challenges

Clearly defining GMV fitness and accounting for the complexity of fitness as a concept needs to be addressed, given the fact that fitness remains one of the most controversial concepts in evolutionary biology. There is a large body of literature debating precisely what fitness is, how it varies in different situations, and how best to measure it. Its etymology is believed to have been from Darwin's reference to survival of the fittest. Following the development of population genetics during the 1920s and 1930s the term evolved to its present form, which is "*success in producing offspring, irrespective of the causes of that success*".

The definition provided above is quite simple for the outcome of such a remarkably complicated and dynamic process. Definitive characterization of the causes of changes in fitness is a formidable challenge because fitness can be modified by a long list of biotic and abiotic factors, many of which are difficult to measure or dissociate empirically. Complicating issues centre on the observation that fitness can be significantly influenced by variation in environment and genetic background. Moreover, fitness is dynamic. It can change, for the same genotype, as the environment and structure of populations change.

Suggested future directions

Fitness of selected strains can be evaluated by conducting competition experiments among different parental genotypes. Relative fitness can be measured in subsequent generations as frequencies of transgene genotypes (transgene homozygotes, heterozygotes and wild-type homozygotes). The advantages of this approach are that large numbers of mosquitoes in replicate cages can be examined in a reasonable period of time, all life stages of the mosquitoes can be included in the analyses, and the performance of different genotypes can be directly compared. Such evaluations should preferably be undertaken with recently transformed lines from the target population and be adequately replicated and include appropriate controls.

A three-phase process for fitness evaluation of GMVs was proposed:

- **Phase I:** Under standard laboratory conditions, a transgenic line is paired with equal frequencies of mosquitoes from the target field population. Fitness evaluations can be used to eliminate lines with major negative impairment.
- **PhaseII:** As Phase I, but at proposed release site, with freshly collected wild-type material. GMVs will also be exposed to the ambient environmental conditions.
- **Phase III:** Strains that survive Phase II are released into large replicate outdoor semi-field systems, in equal frequencies with freshly collected wild-type material from the proposed release site.

Research should be carried out on the effects of colonization and mass rearing because adaptation to a laboratory setting can alter the genetic make-up of colonized material, modify their behaviour and reduce their fitness. It remains unknown what requirements or remedial actions will be necessary to minimize loss of fitness and altered phenotypic expression associated with colonization and mass rearing. Fitness studies will greatly benefit from the availability of life-table analyses of the species that have not already been studied. Likewise, advanced (molecular) age-grading tools are in need of further development.

Future directions for research and capacity/partnership building

By adopting a standardized, consensus methodology, results from fitness assessments in one laboratory can be compared with results from another, which will help to rapidly identify appropriate constructs and GMV strains most likely to be successfully applied in the field. The transition in fitness assessment from the laboratory (Phase I) to the field (Phases II and III) offers multiple opportunities for collaboration between laboratory- and field-based researchers. Rapid and accurate assessment of GMV fitness will be important for development, evaluation and application of novel transgenic technologies.

Mosquito mating behaviour

State of the art

Of the critical behaviours that characterize the vector life histories, mating is probably the least well understood and most understudied. Yet, as disease vectors depend on sexual reproduction for species maintenance, this aspect of vector biology should receive the highest attention when seeking new avenues for genetic control and interventions of vector-borne disease. Which behavioural steps need to be considered when mating is concerned? How do vectors use their reproductive resources (sperm

vs. eggs) and what governs those choices? How can contemporary molecular tools be applied to provide improved precision on these important questions?

Issues and challenges

Mating in most disease vectors remains poorly understood. Yet, successful mating is critical for the success of proposed strategies for vector-borne-disease control using SIT or GMVs. Results from previous studies are not conclusive and the conceptual framework for understanding vector reproductive biology (especially mosquitoes) has lagged behind what has been revealed for a variety of other organisms. For instance, little attention has been paid to the mating behaviour of *Aedes aegypti* since the 1960s and 1970s. Only recently has new progress been made with studies on anopheline mating behaviour under field conditions (in São Tomé and Mozambique). Unfortunately, such studies are few, and do not address the question how mating is accomplished and by which factors this is regulated.

Suggested future directions

Because insemination of wild female mosquitoes by released transgenic or sterile males is obviously a requirement for any genetic-control programme, it is proposed that research focuses on the following aspects of mating behaviour:

- Assortative mating and polyandry
- Factors that effect frequency of mating
- Mate location (male and female)
- Cues that control male swarming (including site selection)
- Mate choice (male and female)
- Male fitness and feeding behaviour
- Pre- and post-mating behaviour
- Sperm production and depletion by males
- Sperm utilization by females
- Frequency of multiple-species swarming
- Genes that affect mating behaviour
- Mating studies of captive mosquitoes (lab to large outdoor enclosures)
- Alternative mating strategies
- Dispersal behaviour

The above list of topics is extensive, and not all aspects may be of importance for genetic-control trials to be implemented. The overall intention of mating-behaviour studies should be to aid in the interpretation of relatively simple field studies of whether or not released male GMVs can effectively compete for females of the wild vector population.

Capacity partnership building

The above aspects appear critical for a proper understanding of mosquito population biology and genetics and offer opportunities for interaction among molecular geneticists, vector ecologists and people modelling genetic vector control strategies. For instance, in population modelling of the behaviour of gene transfer between GMV and wild populations, the frequency of wild versus GMV matings should be well understood in order to predict the number of GMV released individuals required for effective results. Also, SIT programmes require a constant monitoring of wild versus sterile matings to adjust the release rate over time. Finally, any driving mechanism of foreign DNA into wild populations requires normal mating behaviour,

and can only be evaluated once it is properly understood. Many of these aspects can *only* be addressed through field studies, offering many opportunities for collaborative research between northern and DEC scientists.

Pathogen evolution

State of the art

Research on the interaction of *Plasmodium* and dengue virus with their arthropod and human hosts, indicates that the capacity to evolve resistance to GMVs may exist. The observations that *Plasmodium* can modulate the immune response of its mosquito vectors, and that there is an absence of sterilizing immunity in human infections, besides occurrence of drug resistance, are all well-documented phenomena that are consistent with the notion that parasites may evolve resistance to barriers intended to prevent transmission by GMVs. Similarly, dengue, like other RNA viruses, lacks a proof-reading mechanism to correct errors during replication. Consequently, dengue has a high mutation rate and the capacity to change rapidly due to drift or selection in response to changes in the environment in which it reproduces.

Issues and challenges

An important issue in addressing pathogen evolution relates to consensus building of exactly what is meant when one refers to resistance and virulence. Definition of these terms will avoid confusion and aid in the development of unified conclusions across different research groups studying those topics.

It will be critical to develop non-onerous means for assessing the capacity for parasites to avoid interference from GMVs and to characterize virulence of resistant parasites. A system that is operationally practical needs to be developed, should provide this essential information, and be standardized across different research groups and laboratories.

An undesirable outcome from a GMV release would be the evolution of resistant parasites that are more virulent than their predecessors. A rich body of literature on the evolution of virulence demonstrates that increased virulence is not necessarily disadvantageous to parasites. If the efficiency of transmission is linked to virulence in a way such that increasing virulence similarly increases the basic reproductive rate of the parasite, virulent phenotypes will have a selective advantage over those that are less virulent.

Suggested future directions

A practical strategy to assess and/or monitor evolution of parasite resistance in the mosquito and virulence to humans needs to be developed. Research should be carried out on the effects of genetic variation in mosquito and parasite populations on vector–parasite interactions, the evolution of resistance, and virulence characteristics of resistant parasites.

The requirements necessary for minimizing the adverse effects (e.g. mosquito colonization and parasite culturing) of carrying out vector–parasite interaction studies in the laboratory need to be defined. A deepened understanding of environmental effects on vector–parasite interactions will be beneficial in the study of GMVs.

Capacity partnership building

Expertise on parasite resistance and evolution of virulence needs strengthening and may be acquired from outside the discipline of vector biology.

Modelling

State of the art

Models have had a profound impact on understanding processes of pathogen transmission and reducing disease. In general, models make two vital contributions to the development and application of disease-prevention technology. First, they help identifying knowledge gaps and second, they enable predicting outcomes of scenarios of interest. Past contributions of modelling to genetic-control strategies for vector-borne diseases and future opportunities were recently reviewed. It was noted that "during the era of classical genetic-control research there appears to have been incredibly good communication and cooperation between theoretical and empirical researchers. Indeed much of the empirical work was inspired by results of population genetics studies. There has been a tendency for the sophistication of modern science to isolate researchers involved in molecular work from those doing ecological and population genetics studies. More interaction between these scientists at the very early stages of genetic-control projects may increase the chances of producing useful strains and ushering in a new and long-lived, golden era of genetic control".

Issues and challenges

Although it is uniformly agreed that models will be an integral part of developing, evaluating, and deploying genetic strategies for vector-borne-disease control, there is debate regarding how they can be best constructed and used. For example, the relative merits of emphasizing generality, realism or precision remain questionable. The precise role and involvement of empirical researchers in the development, validation and application of models of genetic vector control need to be clarified. Accessibility and user-friendliness for non-specialists need to be addressed, as does the adaptation of models for field application. Existing models of genetic vector control illustrate the relative merits of different approaches. Some provide estimates for spread of transposons and associated genes across all species, but are applicable only to specific species. Others account for spatial heterogeneity but do not explain movement of transposons within genomes, which could profoundly influence their spread within a population.

Suggested future directions

Models should be used as a quantitative framework that can assist researchers and guide policymakers in more accurately assessing the field-implementation costs and potential for success of a variety of transgenic approaches. Modelling efforts should transcend simulation of events retrospectively and be more productively directed at predicting outcomes of proposed interventions. Predicting outcomes will require close collaboration among modellers, laboratory-based molecular geneticists, vector ecologists, and epidemiologists. This kind of reciprocal interaction is essential to obtain the necessary insights to advance vector-based genetic control. Complexity and generality of models will depend on their intended purpose, which will need to be determined based on interaction between the people developing models and those using their output. Models should be constructed in ways that make them easily accessible to users. Finally, suggested modelling topics could include efficiency of drive mechanisms, epidemiological impact on disease, etc.

Capacity partnership building

Construction, validation and application of models constitute one of the best examples of how people with different kinds of expertise can work together in mutually beneficial ways. For example, modellers can provide specific information to people creating transgenic mosquitoes regarding the kind and extent of transposon movement that is best suited for driving transgenes through target populations. They can also make recommendations for the best GMV release strategies. Vector ecologists can provide essential information for modelling mosquito population and pathogen transmission dynamics. Molecular geneticists can provide empirical information on transformation and transposition processes.

Identification and characterization of field sites

State of the art

The ongoing development of GMVs for application in genetic-control programmes against major tropical diseases such as malaria and dengue is gradually advancing to a stage where scientists involved are planning future field trials or, more in the short term, semi-field evaluations. The transition of research efforts from the laboratory to (semi-)field environments raises a number of important issues like ELSI (ethical, legal and social issues; addressed elsewhere), and also the selection of appropriate field sites. Once identified, target pest populations need to be characterized. At present, field sites for future genetic-control interventions are being selected, based on the biological characteristics these should possess. There is, however, no coordination of these activities (see section 6).

A priori, it can be noted that previous genetic-control trials (e.g. employing cytoplasmic incompatibility (CI), chromosomal translocations, the sterile-insect technique, etc.) have addressed many analogous problems related to present-day site selection and characterization, albeit without the genetic-engineering component that adds new and unique concerns. The following sections focus on GM mosquitoes, though most concepts apply similarly to other disease vectors.

Issues and challenges

The transition of laboratory research on GM mosquitoes to full programmatic implementation in disease-endemic settings encompasses a series of steps, each with its own unique challenges. Historical genetic-control attempts focused mainly on two key aspects affecting the potential for success, i.e. knowledge of the local vector population, and partial to full isolation of the target population.

CI trials in Myanmar were conducted in a village surrounded by rice fields, where the target pest did not occur. In Kenya, genetic-control trials against *Ae. aegypti* in the 1970s focused on villages and a small area surrounding them. The necessity for applying genetic control against isolated populations remains valid today. It has been proposed to target *Anopheles arabiensis* populations in urban areas surrounded by *An. gambiae s.s.* or urban *An. stephensi* populations surrounded by *An. culicifacies*. Others have suggested going beyond ‘ecological islands’ described above, and move to physical islands. Genetic-control trials have delivered dramatic successes through eradication of target pests, such as the eradication of *Glossina austeni* from the island of Zanzibar by 1997.

With regard to the application of GM approaches for disease-vector control, further containment (in terms of selecting isolated populations) is needed, to overcome potential adverse effects of the introduction of GM insects. The choice for physical islands, far from mainland populations, seems the best option in that regard

Beyond (1) geographic isolation, there are several more key factors affecting the selection of a field site, such as (2) occurrence in a narrow geographic range and (3) appropriately sized area (small enough to be manageable, large enough to be convincing); presence of (4) panmictic populations of (5) one vector species (although another closely related non-transmitting species may be useful as a 'control'); the target species occurs in relatively (6) low density and can be suppressed with existing vector control tools, and (7) disease transmission, in order to measure a public-health impact of the intervention.

These biological pre-requisites should serve as the first criteria when selecting a field site. Secondary criteria include (8) accessibility of the site and availability of research infrastructure, and the availability of (9) detailed entomological and epidemiological information.

Suggested future directions

Specific research topics related to field site selection and characterization should follow an evaluation of the existing human resources and scientific/public-health infrastructure at the chosen sites, and include:

- Collection of basic ecological and biological data of the target species, including relative population densities, adult and larval distribution patterns and an assessment of the role of the target species in disease transmission.
- Assessing the degree to which the target population is genetically isolated from surrounding populations and a description of the genetic structure of the population.
- Creating a geographic information system that describes the ecology of each site and is fully integrated with information from the ecology and population-genetics studies.
- Baseline studies on parasite transmission and disease epidemiology.
- Developing a system for oversight of research activities at field sites.
- Semi-field research in countries with established research and scientific collaborations.

Capacity partnership building

Perhaps the most significant challenge in terms of GMV development and implementation is the full participation of and genuine collaboration with partner institutions in DECs that meet the above criteria. In all likelihood, the suitability of island settings will be countered by the absence of appropriate institutional frameworks and local competence. It will be important to develop general guidelines for GMV implementation irrespective of the field site/country involved. A regulatory framework residing under a larger umbrella such as the WHO seems to be best suited for this (see Section 6).

Integrated disease management

Current state of the art

Most NMCPs focus on early diagnosis and treatment, personal protection, health education and vector control. While anticipated, vaccines and GMVs are currently not available. Current vector control measures emphasize personal protection by use of ITNs or IRS, and to a lesser extent source reduction by environmental management (drainage, filling), biological control (larvivorous fish) and larviciding (Bti, *Bacillus sphaericus* (Bs), temephos).

Issues and challenges

It was recognized that lack of sufficient knowledge on the ecology, behaviour and genetic background of vectors (in most disease-endemic areas at least two or more vectors occur sympatrically) hinders conceptualization of effective integrated approaches to disease-vector control. Given the adoption of GMVs in existing disease and vector control programmes, it remains unknown how the impact of GMVs on disease epidemiology will be gauged in a background of other control activities. Moreover, it remains unclear how GMVs will compare with other control strategies in socio-economic terms. Much effort is currently being undertaken to upscale the use of ITNs at community level and enhance sustainability of this intervention. If transgene spread occurs at similar rates in low-density or high-density populations, then both GMVs and ITNs may be applied concurrently.

Research and control opportunities

Future directions for research and capacity/partnership building relate to the integration of GMV development in ongoing vector research programmes in DECs. This requires significant expansion of both capability and capacity of DEC partner institutions. It was suggested to identify centres of excellence in DECs to tackle GMV development as a local solution rather than an adopted one from the North, advocating a transition in problem ownership. A real opportunity for DEC partners is advocacy of the GMV approach to policymakers, communities and NGOs, to promote GMVs as an integral component of IVM. A change in mind-set, from operating at household level (IRS, ITNs etc.) to community-wide and area-wide scales will require the development of approaches consistent with health-policy frameworks operating at such scales (e.g. district level).

Epidemiological impact assessment

Current state of the art

At least two frameworks exist to evaluate the entomological impact of a genetic-control approach for malaria. The vectorial capacity equation and the entomological inoculation rate (EIR) could, in principle, provide empirical measures of the effects of introduction of GMVs. For dengue, such measures are not available.

Issues and challenges

Challenges include evaluating the relevance of reproductive and physiological fitness to transgenic engineering. An important property of a GMV is its reproductive fitness compared to that of wild-type conspecifics. Transgenic mosquitoes must be as physiologically fit as the wild types. Transformation strategies must not compromise fitness. A major emphasis in genetic-control strategies is blocking the sporogenic cycle. The current genetic-modification strategies that probably have the least effects on fitness are blocking the penetration of the midgut and salivary glands by sporozoites. The result would be females that cannot support the sporogonic cycle. The possibility of reducing anthropophagy seems initially attractive. The approach would be to change the blood-feeding preference of *An. gambiae* from man to animals. Some of the sibling species of the *An. gambiae* complex such as *An. arabiensis* and *Anopheles quadriannulatus* can be highly zoophilic and it may be possible to introgress the relevant genes to reduce anthropophagy.

Research and control opportunities

The endpoint for any introduction of a GMV should be to measure a public-health benefit. To date, however, it remains unknown what entomological thresholds need to be attained before an epidemiological impact becomes measurable. These issues have been similar when measuring the impact of other interventions (e.g. ITNs) but become more complicated when introducing GMVs in areas where other interventions are practiced concurrently (see 5.10). Entomological monitoring tools that are efficient in collecting GMVs for research purposes are urgently required.

Future directions for research and capacity/partnership building

Participants agreed that GMV evaluation, if, for instance, based on *Plasmodium* sporogony interference, should have as its prime target the sporozoite rate. This can be done by collecting large numbers of females in the field and subjecting them to ELISA assays. Given that a GMV release programme yields a substantial reduction in the sporozoite rate within the targeted population, it would then be justified to proceed to measures of impact related to disease, e.g. incidence, morbidity and mortality.

Ethical, legal and social issues

Current state of the art

The technical feasibility of the development of transgenic mosquitoes unable to transmit malaria and dengue pathogens has been demonstrated in the laboratory. However biotechnological and implementation challenges remain to be addressed in order to make this approach a control method applicable as a public-health tool in disease-endemic settings.

Issues and challenges

The principle challenge is to provide a proof of efficacy and safety of the use of GMVs for disease control. This proof is required to initiate addressing the ELSI of the potential use of GMVs.

Research and control opportunities

There is a commitment from WHO/TDR as part of a wider network of research institutions and funding agencies to advance the area of genetic control of vectors. In addition it has been recognized that major initiatives (e.g. the Gates Grand Challenges in Global Health initiative) accommodate vector-research components and provide new research opportunities.

Future directions for research and capacity/partnership building

The working group noted that this area has not received sufficient attention over the last decade, but recommended specific areas for research:

- To provide proof-of-principle and biosafety assessment (risk management) of the use of GMVs for disease control by:
  - conducting studies on the efficacy, biosafety and risk/benefit evaluation through long-term efforts to clarify the scientific uncertainties under different experimental conditions and with the involvement of DEC investigators;
  - providing the basis for collection of data on vector biology, ecology, behaviour and genetics addressing efficacy and safety in the field;
  - developing guidelines and principles on the design and performance of efficacy and minimum-risk field research;

  - developing criteria and test methods for environmental monitoring;
  - developing criteria to identify and prepare field sites for approach evaluation.
- To ensure the public that this goal is desirable, feasible and can be accomplished safely, by:
  - developing a strategy to make the information available to the public and the media such as to raise their awareness and address their concerns about possible environmental and human-health risks;
  - bringing all parties together on common ground that can lead to objective, scientific, legal, ethical and social-based decisions by policymakers, bearing in mind that many people may not endorse the scientific efficacy and risk analyses.
- To develop a plan to gather all the information necessary for legal and regulatory approvals, particularly documentation related to biosafety and ethical review and national/local-authority approval.
- To enhance capacity in DECs: for biosafety assessment, risk/benefit evaluation, environmental monitoring, and data collection on the vector biology, ecology, genetics and behaviour.
- To promote South-South and North-South research collaboration based on well-defined ethical, equitable and scientific standards.
- To develop mechanisms for dissemination of information to researchers, decision-makers, affected communities, the public and the media, and develop procedures for obtaining informed community consent.

Coordination and follow-up mechanism

The development and implementation of GMVs is a multidisciplinary endeavour that requires ever-widening circles of involvement within society. Alhough it is recognized that many activities will remain laboratory-based for some time to come, involvement of field-orientated scientists is becoming increasingly important. This, in turn, raises issues related to communities living in such field settings, the political climate in the affected country, etc.

The need to establish a coordinating board to engage in the provision of guidance and steering to all levels within the molecular-science – society continuum, was noted and endorsed.

Although the constituency and mandate of this body need further deliberation, it was agreed that:

- it should take a platform function, communicate to all stakeholders in the GMV endeavour, and in particular sustain a mechanism for addressing media enquiries;
- it should engage in constant contextual analysis of the external environment in order to monitor developments and perception changes in the public and scientific community alike;
- it should play a catalytic and proactive role in establishing, securing and strengthening partnerships among stakeholders in the GMV field.

Membership of this board will be based on rotation, vested interest and adequate expertise, and should strive for an appropriate balance between members from DECs and developed nations.