and effectively transmit dengue virus even at very low population densities because they preferentially and frequently bite humans (*23*). A successful GMM dengue control program that falls short of vector eradication will result in a reduction in human herd immunity and a corresponding decrease in already low transmission threshold levels. Because there is no commercially available vaccine or clinical cure for dengue, predicting and testing transmission thresholds is among the most important unanswered questions in dengue epidemiology and GMM-based control approaches.

Quantitative Analyses of Mosquito Biology, Disease, and Control by GMM

A goal of future quantitative analyses should be to accurately predict outcomes of proposed interventions instead of simulating events retrospectively. For example, continental-scale predictions of malaria disease burden are currently being made on the basis of remotely sensed environmental data that influence mosquito population dynamics and, in turn, patterns of pathogen transmission (*24*). Simulation models have been used to predict entomological thresholds for dengue transmission (*25*). Mathematical models have been developed to identify parameters required to predict the dynamics of transgene drive mechanisms in vector populations (*5, 6, 13, 26*). Different drive strategies have been examined and predictions made for the likely success of each (*5*). An analysis of population genetics and epidemiology has concluded that in areas of intense malaria transmission, GMM control programs will have little if any effect unless mosquito refractoriness is very close to 100% (*13*).

Conclusions

The meeting participants reached consensus on four procedural issues. First, there is an urgent need to develop uniform processes for dealing with the ethical, legal, and social issues related to GMM technology (*27*). It would be most helpful if an international body like the World Health Organization established guidelines, regulatory mechanisms, and safety, containment, and conservation protocols. Second, for the GMM approach to be initially successful and ultimately sustainable, its proponents must identify and develop the capacity for human resources and research infrastructure at sites earmarked for technology evaluation and long-term application. Third, continued evaluation of GMM technology will require semi-field facilities (such as large outdoor cages), followed by release of GMM on isolated oceanic or ecological islands that have been thoroughly characterized with respect to the genetic and ecological makeup of local mosquito vector populations and site-specific patterns of pathogen transmission and disease. Fourth, in addition to population replacement, genetic strategies for mosquito population reduction [such as RIDL (release of insects carrying a dominant lethal) and negative heterosis] in isolated urban areas merit consideration (*28*).

Addressing these goals will require coordinated interaction among scientists from diverse disciplines. Only by studying the system in total will we gain greater insight into the complexity of interactions that are essential for the design, implementation, and evaluation of progressively more successful disease management strategies. Such an ambitious agenda will require adequate funding, collaboration between ecologists and molecular geneticists, recruitment of expertise from outside the vector-borne disease arena, training for young scientists, and the expectation of a sustained effort. The longitudinal field studies required to address some of the ecological issues identified will last a decade or more. In all these actions, people from the countries where GMM technology is most likely to be applied need to be more fully involved.

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29. We thank the following Wageningin meeting participants for helpful comments: K. Aultman, P. Billingsley, D. Charlwood, C. Curtis, J. Edman, J. Koella, G. Lanzaro, S. Lindsay, P. Lounibos, D. O'Brochta, S. Randolph, W. Reisen, D. Rogers, M. Sabelis, A. Spielman, C. Taylor, and Y. Touré.

VIEWPOINT

Malaria Control with Genetically Manipulated Insect Vectors

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At a recent workshop, experts discussed the benefits, risks, and research priorities associated with using genetically manipulated insects in the control of vector-borne diseases.

This is a partial report of a workshop—Genetically Engineered Arthropod Vectors of Human Infectious Diseases—jointly sponsored by the World Health Organization, the MacArthur Foundation, the National Institute of Allergy and Infectious Diseases, and London's Imperial College—originally planned for 12 September 2001 in London (but reconvened in successive sessions later in London and Atlanta). These workshops sought to encourage communication between the laboratory-oriented molecular biologists, whose work had suggested the potential of genetic control strategies, and the population geneticists, ecologists, and public health specialists, whose involvement would be crucial in moving the work beyond the laboratory. The meeting participants were charged with considering the benefits and risks of using genetically engineered arthropod vectors as public

health tools and mapping out a research agenda for their development. The task of engineering different vector species and the risks associated with various methods of genetic engineering are vastly different and could not be addressed in a single report. What follows is the consensus of the working group on germline-transformed organisms developed for control of malaria transmission (authors listed above) and other participants. The reports of the working groups on paratransgenesis (transformation of obligate symbionts in insects) and on other vector-borne diseases will be presented in the near future.

In 1991, a scientific workshop in Arizona assessed the prospect for malaria control by genetic manipulation of vector populations (see the Viewpoint by Morel *et al.* on page 79) (*1*). The basic concept of genetic control of vector-borne diseases was proposed by Curtis in 1968 (*2*), but major advances in the molecular manipulation of *Drosophila melanogaster* during the 1980s encouraged reevaluation of this idea. The WHO/TDR summary document of the meeting laid out a clear list of research aims that would have to be met before a genetic control strategy could be field tested (*3*). These aims fell into three categories: (i) the development of genetic engineering tools that could be used with malaria vectors; (ii) the identification of effector genes that could block parasite transmission; and (iii) the development of effective methods for driving these effector genes to fixation in natural vector populations.

The first two aims have been largely achieved. Several different but effective methods of germline transformation have been developed and used in at least three species of malaria mosquito vector (*4*–6); two different laboratories have developed genetic constructs that significantly reduce vector competence in experimental malaria models (*7*, *8*). A large set of molecular markers has been developed and is being used in studies of gene flow and population structure in anopheline malaria vectors (*9*–*13*). But there has been no significant progress in developing methods for driving desirable genes into wild populations and especially for ensuring the necessary unbreakable linkage between the drive system and the gene to be driven (see the Viewpoint by Scott *et al.* on page 117) (*14*).

Consideration of the potential use of genetically modified organisms (GMOs) is driven by the realization of the enormous human cost of diseases like malaria, and of the inadequacy of present control measures. Perhaps the most important theme emerging from the workshop was the recognition that control strategies involving GMOs could potentially provoke serious public mistrust and resistance to their implementation. Therefore it was strongly recommended that all work leading to the development of specific genetic control strategies targeted at malaria vectors should involve both public health specialists and scientists from disease-endemic countries and (where possible) the general public in areas where field trials could be implemented. Because field trials of genetically modified mosquitoes would have to be preceded by long-term, longitudinal studies of potential field-trial sites, the local community and its own scientists and health experts can easily be involved.

The goal of producing GMOs intended to benefit human health has been perceived more favorably by the public than that of producing GMOs for agricultural or domestic animal research. However, meeting participants strongly argued that this positive public perception could be rapidly undermined by an actual field trial of a transgenic arthropod that failed to provide a significant and tangible health benefit to the resident human community. It was therefore recommended that all preliminary research designed to lead to field trials of the efficacy of a transgenic arthropod-based disease control strategy should involve fully contained laboratory or cage environments. Release should be permitted only when all relevant parameters had been investigated in either contained environments or in open field studies that did not involve transgenic arthropods. Furthermore, field trials involving release of transgenic arthropods should take place only when all members of both scientific and local community review groups were assured that such trials had a very high probability of producing a significant and measurable public health benefit for the local community.

Many important ecological and population genetic issues must be understood before any release program can be contemplated, and such issues will be specific not only to individual vector species but also to local populations (see the Viewpoint by Scott *et al.* on page 117) (*14*). Understanding the dynamics of a natural population will require years of study, with the time frame dependent on the stability and repeatability of yearly cycles. Thus, given progress in the laboratory, it is important to start the ecological and population genetic study of potential target populations soon, as this will be the biggest scientific limitation to implementing genetic control field trials. A large number of technical problems will have to be addressed, ranging from the feasibility of producing an effective release strain to the design and assessment of release strategies with specifically predicted goals. To address such problems will require the involvement of ecologists and population geneticists. Most participants recommended that study of potential field-trial sites should be initiated immediately at multiple different locations, recognizing that the initial phase of fieldwork might show one or more of the selected sites to be unsuitable. Because the biology of vector populations at any such site would have to be studied for many years before field trials could be designed, the community cannot investigate different sites sequentially.

GMOs could be used in either of two ways for malaria control. The initial concept (expressed in the 1991 meeting) was to engineer mosquitoes with an altered phenotype that would be introduced into the population in such a way that the new trait would spread and become dominant. These strategies target the malaria parasite, rather than the mosquito itself, for reduction. There is an immediate research need for the study of drive systems in *Anopheles* species. These drive systems also present a potential hazard because they may generate unintended phenotypes and have unforeseen, potentially harmful ecological effects. Autonomous transposons, for example, could increase the mutation rate through multiple genomic insertions, leading to unanticipated alterations in the biology of the target species. Tight linkage of the drive system and the engineered gene is also an important issue in that its loss in the progeny of released mosquitoes could lead to loss of public health efficacy and loss of the molecular tool for future engineering efforts. Although transposon and symbiont systems have garnered the most attention to date, participants recognized the need to explore any possible drive system that could continue to propagate a released genetic construct through the target population after initial release.

An alternative use of genetic engineering for malaria control takes a more traditional approach. This involves targeting the mosquito population per se for reduction. Proposed improvements in sterile insect techniques, including release of insects carrying dominant lethals (RIDL) (*15*), and other mechanisms of genetic sexing may alter the prognosis for these strategies. In these situations the release of large numbers of insects presents other specific challenges: for example, the need to release only male mosquitoes so as not to increase the number or nature of mosquito bites per person per night. In the absence of an existing drive

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system, participants considered the use of inundative release of refractory mosquitoes as a strategy for limited field-testing of the performance of specific genetically engineered vector strains. Although considered suitable only for a small vector population with limited interpopulation gene flow (such as a real or ecological island setting), the ability to limit or quickly control unforeseen risks in the genetic manipulation of an island population will be important in early-stage trials designed to demonstrate the efficacy of particular genetic modifications of the vector population.

Although there was support for continued, intensive research in this area, a clear recommendation emerged that there should be no precipitous releases of transgenic arthropods. The malaria group was willing to recommend barring field trials of transgenic insects that were designed solely for research; others felt that initial field safety testing of the various individual elements of the engineered organism was crucial to development. The parallel processes of drug and vaccine development illustrate these two views. For either product, and indeed for engineered *Anopheles* mosquitoes, there is a requirement for preliminary studies of safety and efficacy in culture and in animal models before the first clinical trial is initiated. With many new drugs (other than cancer drugs), the first human trials are performed in small numbers of normal healthy volunteers, and safety is the end point examined. In these situations it would be inappropriate to endanger patients who are already sick by exposing them to a drug candidate of unknown toxicity. By contrast, when new vaccines are developed, they are most often combined with adjuvants that improve their potency or direct their effects to one or more segments of the human immune system. Under its current guidelines the U.S. Food and Drug Administration does not allow investigation of the adjuvants alone without the vaccine candidate being tested at the same time. The malaria working group requires tangible benefits at each phase of field testing. The other working groups—discussing symbionts, transducing viruses, and other mechanisms of driving traits into populations—decided to follow drug-development protocols. These differences may be appropriate given the different nature of the engineering tools and the different risks associated with each one.

Despite nearly universal recognition that enormous technical and sociological problems must be overcome before the implementation of genetic control strategies for malaria can be field tested, participants concluded that public health strategies incorporating transgenic vectors offer the potential of health benefits. Participants from disease-endemic areas, many of whom had limited prior exposure to transgenic arthropod research or policy discussions, were among the most supportive and optimistic about the public health goals such strategies hope to achieve. Participants also noted that the broad scope of biological research required for the development of genetic control strategies is likely to contribute both to the more efficient application of currently available control tools and to the development of new approaches.

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VIEWPOINT

Malaria—a Shadow over Africa

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Reduction in severe disease and death from *falciparum* malaria in Africa requires new, more effective and inexpensive public health measures. The completed genomes of *Plasmodium falciparum* and its vector *Anopheles gambiae* represent a big step toward the discovery of these needed tools.

The current focus of malaria control programs in Africa is rightly on the management of sick children through early treatment with effective antimalarial drugs. However, this cannot be the final strategy. The two first-line drugs, chloroquine and sulfadoxine/pyrimethamine (Fansidar), are no longer effective in many parts of East Africa where chloroquine resistance (introduced from Asia) is rampant. Combinations of new drugs may help to slow the emergence and spread of resistant parasites (*1*), but control strategies based on early treatment mean a never-ending struggle to develop and deploy new drugs before the *Plasmodium* malaria parasites become resistant to existing drugs. Thus, the long-term control strategy must be to interrupt the transmission of this parasite. Unfortunately, this will be extremely difficult in parts of Africa where people may be bitten as many as 1000 times a year by infected mosquitoes. Insecticide-treated bed nets—now being vigorously promoted in many parts of Africa—reduce bites from infected mosquitoes by as much as 90% (*2*). However, their effectiveness is already under threat as a result of the emergence of pyrethroid resistance in *Anopheles funestus* in Mozambique and in *A. gambiae* in agricultural areas of West Africa (*3*). Household spraying with residual insecticides is highly effective in reducing malaria in some parts of Africa, but it is logistically demanding, costly, and may have adverse environmental effects.

There are many ways to reduce malaria transmission, but none can provide a complete block in transmission, particularly in the highly endemic areas of Africa (*4*), and new approaches are desperately needed (*5*). Publication of the *Plasmodium falciparum* (*6*) and *Anopheles gambiae* genomes (*7*) represents a big step forward in our search for new tools for controlling malaria. Combined deployment of three strategies that each have the potential to reduce malaria transmission by 90%—drug treatment, vaccination, and vector control—should be sufficient to stop transmission, even in highly endemic areas of Africa. We will need to first test such strategies in areas with a low intensity of transmission before attempting the challenging task of preventing malaria transmission in the highly endemic areas of Africa.

Anyone who has thought deeply about the problem of reducing severe disease and death from malaria in Africa realizes the crucial need for a malaria vaccine. Pre-erythrocytic, blood-stage, and transmission-blocking vaccines have recently been developed by a number of groups (*8*). Each type of vaccine has a part to play in the complex, highly diverse epidemiology of malaria and the associated variety of patterns of

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