cialization and the avoidance of intraspecific competition. This selective force is presumably driving the speciation process. It has been proposed that co-adapted chromosomal inversions are crucial for establishing populations in marginal habitats that could lead to the formation of new species, although the inversions per se are not the cause of the evolution of subsequent barriers to gene flow (*25*).

The taxonomic and genetic complexity of *A. gambiae s.s.* has serious consequences for malaria transmission. The ongoing speciation process leading to the M form has extended the transmission potential of this vector in space and time (*23, 24*). In dry areas of West Africa where malaria is hyper- to holoendemic (*26*), this taxon is able to exploit breeding opportunities due to human activities that would otherwise be available only to *A. arabiensis*; such is the case in areas of Eastern Africa with a similar climate (like northern Sudan) where *A. gambiae s.s.* is absent and malaria is hypo- to mesoendemic (*27*). Moreover, in dry savannas, the ability of the M form to breed year-round in permanent human-dependent larval habitats extends the malaria transmission period well beyond the rainy season, when the S form apparently disappears (*28*). Analogous situations are seen with other Afrotropical malaria mosquito vectors such as *A. funestus*, which has two West African chromosomal forms (Folonzo and Kiribina) that clearly differ in their degree of contact with humans and therefore have quite different vectorial potentials (*29*). It is likely that in both *A. gambiae* and *A. funestus*, chromosomal inversions allow more specialized and therefore more efficient exploitation of both spatial and temporal environmental heterogeneity. This is expected to have implications for such traits as the survival probability of individual mosquitoes and the stability of vector populations, both important features of malaria epidemiology (*30*).

The complete genome sequence of *A. gambiae* will deepen our understanding of the process of adaptation and speciation of this insect vector. One immediate application of this information, already in progress, is the cloning of inversion breakpoints on chromosome 2. Comparative analysis of the sequences across and surrounding each breakpoint will allow us to identify and study the gene clusters protected by recombination and may yield clues about the origin of inversions and their importance.

The concentration within four closely related species of the *A. gambiae* complex (*A. gambiae*, *A. arabiensis*, *A. melas*, and *A. merus*) of several inversions along the central and subtelomeric sections of the 2R chromosomal arm is unlikely to be coincidental. These inversions may be associated with genome regions that encode traits of ecological and behavioral importance. The availability of the entire *A. gambiae* genome will facilitate polymerase chain reaction–based assays that will complement laborious karyotyping of semigravid adult females, providing new opportunities for field studies on mosquito ecology and behavior. A long-term goal is gene discovery using a complete genome chip. The very recent divergence of the *A. gambiae s.s.* molecular forms and the likelihood that only a few genes are involved in reproductive isolation and ecological diversification means that the entire *A. gambiae* genome will have to be screened in order to identify differences in gene sequence and coordinated gene expression between incipient species.

A. gambiae provides us with an exceptional opportunity to observe evolution in action, potentially operating over the time frame of the thousands of years since humans began to modify the Afrotropical ecosystem (*1, 6, 24*). The buildup of barriers to gene flow during the speciation process resulting in separation of the molecular forms of *A. gambiae* can be compared to a glass half full. Now, we must fully elucidate the mechanisms and dynamics of evolutionary change in *A. gambiae* populations—information that will be essential if we are ever to control this nefarious insect vector.

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VIEWPOINT

The Ecology of Genetically Modified Mosquitoes

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Ecological and population biology issues constitute serious challenges to the application of genetically modified mosquitos (GMM) for disease control.

Optimism that mosquito-borne diseases such as malaria, dengue, and filariasis can be effectively controlled or even eradicated with inexpensive drugs, vaccines, or insecticides has been sorely tested (*1*). The impact of drugs is debatable, vaccine development is slow, and mosquitoes are becoming resistant to insecticides, including those used to treat bed nets (*2*). Such shortfalls have been used to justify research on mosquito population replacement—that is, the release into natural mosquito populations of genetically modified mosquitoes (GMM) rendered refractory to pathogen infection—to reduce or eliminate disease transmission (*3*).

A big hurdle to battling vector-borne diseases is our incomplete understanding of parasite transmission ecology, which is hampering GMM efforts in particular and public health initiatives in general. The GMM strategy should serve as a case study for ways to improve overall disease prevention, because the

importance of ecological information to improving human health is not limited to GMM, malaria, or dengue. An international meeting of vector ecologists (Wageningen, Netherlands, June 2002) (*4*) sought to define the key aspects of mosquito ecology and population biology necessary to evaluate GMM as a disease control strategy. The list of recommendations serves as an inventory of challenges that the GMM strategy must meet in order to be safely and effectively deployed. Discussion was limited to anopheline vectors of malaria and to *Aedes aegypti*, the vector of dengue virus, because the genetic manipulation of these mosquito species is the most advanced.

Spread and Stability of Introduced Transgenes

Effective mechanisms to drive antiparasite transgenes from released laboratory strains into natural mosquito populations are still being developed. Ecologists need to study gene flow, with emphasis on mosquito mating patterns and reproductive behavior, mosquito population size and structure, mechanisms of population regulation, genetic exchange between neighboring populations, and fitness and phenotypic effects of colonization and mass rearing.

In population replacement, refractory transgenes are predicted to spread according to patterns of mosquito reproduction. A transgene drive mechanism must ensure that the spread of transgenes through a natural vector population is more rapid than the spread of genes with normal Mendelian inheritance (*3*). Success will depend on understanding patterns of effective mosquito reproduction that are relevant to transgene spread. Most population models assume random mating (*5, 6*), which almost certainly is not the case (*7*). The most plausible natural circumstance is assortative mating—the tendency for certain phenotypes to mate with one another—yet this phenomenon has been little studied in mosquitoes.

The genetic structure of target mosquito populations needs to be elucidated in order to predict the spread and maintenance of transgenes in natural populations (*8*). If reproductive barriers between the different chromosomal forms of *Anopheles gambiae* go unnoticed (*9*), the beneficial effects of an introduced refractory transgene could be negated because the mosquitoes that are not transformed will continue to transmit parasites. Transgene spread will also be influenced by the size of target mosquito populations. Population size will figure heavily in calculations before GMM release, but it is difficult to estimate (*10, 11*). We need more sophisticated analytical techniques and a better understanding of effective population size (an estimate of the number of individuals that contribute genes to subsequent generations) of the target vector species. Estimating population size is particularly difficult for species whose abundance fluctuates seasonally, as is the case for the most important malaria vectors (*11*). Regulation of population size could be beneficial or detrimental to the spread and stability of transgenes, depending on the circumstances. If mosquitoes mate assortatively and their populations are structured into reproductively separate subdivisions, different mechanisms of population regulation could lead to an unpredicted advantage for one population over another.

Before a GMM release, the numbers of genetically manipulated mosquitoes must be amplified. Adaptation to a laboratory setting can reduce mosquito fitness, and the geographic origin of mosquitoes can affect their adaptation to new habitats (*12*). Thus, the likelihood that a transgene will be driven from GMM into wild mosquito populations will depend on the balance between strength and fidelity of the drive mechanism and the reduction in fitness of the laboratory mosquito population (*5, 13*).

Evolutionary Consequences of Mosquito Transformation

Now that mosquito transformation is well established in the laboratory (*14*), we need to study the effects of genetic modification on the ability of these mosquitoes (relative to wild-type mosquitoes) to survive, reproduce, and carry out critical physiological processes. Fitness of GMM populations may affect the spread of parasite refractory transgenes into wild mosquito populations (*15*). Consequently, transgenes that reduce host fitness must be inextricably linked to the drive mechanism in order to avoid elimination by selection. What are the evolutionary costs of genetic modification to mosquitoes, and how will these costs shape plans for interfering with pathogen transmission? What effects will natural environmental conditions have on the expression of refractoriness of GMM? Will GMM have an enhanced capacity to transmit pathogens other than the one that they are intended to block? There has been little research on the plasticity of vector-borne parasites to circumvent the barriers placed before them by GMM. The *Plasmodium* malaria parasite has repeatedly demonstrated its capacity to evolve resistance to antimalarial drugs (*16*). RNA viruses such as dengue are among the most mutable of all organisms (*17*). To what extent will parasites evolve resistance to GMM, and can we predict the virulence characteristics of resistance phenotypes? Will, for example, an increase in a mosquito's immune response result in an increase in parasite-induced immunosuppression (*18*)? Will changes in parasite populations in response to GMM affect the efficacy of vaccines or antiparasitic drugs? A most undesirable outcome would be to select parasites that are more virulent than their predecessors.

Entomological Risk and Pathogen Transmission

The premise of the GMM approach is to reduce the number of competent mosquito vectors, thereby decreasing human infection. But we need to know the extent to which vector populations must be reduced in order to elicit required public health outcomes. This will be difficult because the relationship between vector density and human infection will vary with time and the particular geographic location.

Malariologists (*19*) have been more successful in relating vector density to human infection and disease than have dengue researchers. The entomological inoculation rate (EIR, the number of mosquitoes with malaria sporozoites biting a person per unit time) is a powerful measure of entomological risk for malaria transmission. Recent prospective epidemiological studies produced surprising information about the relationship between EIR and malaria morbidity and mortality (*20*). In southern Tanzania, the risk of human infection increased with EIR when human infections were low. But when infections were high, transmission became saturated and an increase in EIR did not raise parasitemias in infants. When transmission is low (the predicted situation after GMM release), the "rebound effect" must be avoided. Because primary malaria infections in adults cause more severe disease than in children, longer term vector density reduction could result in unstable malaria transmission and an unanticipated epidemic increase in disease from primary adult infections. Encouragingly, recent results from bed net studies indicate that reduction in contact between children and infected mosquitoes continues to cause reductions in malaria fever and anemia even after 3 to 4 years of bed net use (*21*). Because malaria in a given location is often transmitted by several mosquito species, if one species/form is rendered refractory, others may still transmit parasites. Also, to protect beneficial mosquitoes and promote transgene spread, we need to analyze the possible effects on human health if insecticide application is terminated after GMM release. Further investigations into the relationships among various measures of entomological risk, human infection, and disease prevalence and incidence will strengthen predictions of the success of a GMM strategy for controlling malaria transmission.

Dengue researchers do not have a simple and reliable entomological measure for assessing disease risk. Virus infection rates in *Ae. aegypti* are too low for surveillance to be based on an EIR. The currently proposed indices for *Ae. aegypti* density at best weakly correlate with human dengue infection, and their relationship to disease is understudied (*22*). *Ae. aegypti* mosquitoes persist

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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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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