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Biosafety and risk assessment in the use of genetically modified mosquitoes for disease control

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Abstract

The development and implementation of the release of genetically modified mosquitoes (GMM) for interrupting pathogen transmission represent a major challenge, despite the fact that several achievements have been made about *Anopheles* and *Aedes* mosquitoes. There are major biotechnology challenges remaining about the improvement of the stability of a gene construct and its expression for a robust and complete interruption of pathogen transmission and the devise of safe means of spreading foreign antipathogen genes through mosquito populations in the wild.
The implementation obstacles to overcome include proper risk assessment and management, conduct of studies to ensure safety for humans and the environment, devise of appropriate control strategies based on sound gene-driving systems, address properly ethical, legal and social implications of the release of GMM and public concerns. Although the development of GMM as disease-control tool is technically feasible, for proper implementation no field release must be undertaken until clear scientific proof of safety for humans and the environment and efficacy is provided and ELSI concerns and public acceptance are properly addressed.

Keywords: genetically modified mosquito; biosafety; risk assessment; disease control; malaria; dengue

Introduction

Effective disease-vector control has been very difficult to achieve, particularly in developing countries, due to various factors, such as development of insecticide resistance by the vectors, poor knowledge of the biology of the vectors, inappropriate implementation strategies (technical and operational) and also limited human capacity.
To overcome these difficulties, it becomes necessary to apply selective, targeted and site- specific control strategies based on increased knowledge of the biology of vector-pathogen-human interactions, on the improvement of existing control tools and on the development of new and innovative vector-control tools and strategies.

The limited understanding of vector biology, emergence of insecticide resistance and failure of other malaria-control measures, served as an impetus for a historic

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meeting convened by TDR* and the MacArthur Foundation in Tucson, Arizona in 1991. On that occasion a small group of scientists, stimulated by the advances of molecular biology, proposed an unorthodox approach: the use of the modern tools of genetics to overcome the role of *Anopheles gambiae* in malaria transmission, making it unable to host and/or transmit *Plasmodium* (*Report of the meeting 'Prospects for malaria control by genetic manipulation of its vectors'* 1991). This revolutionary idea was accepted by the Joint Coordinating Board of TDR, and a new area of work – Molecular Entomology – was launched with a long 20-year work plan, focusing on three major milestones: (1) by 2000, develop the basic tools for the stable transformation of anopheline mosquitoes; (2) by 2005, engineer a mosquito unable to carry the malaria parasite(s); (3) by 2010, carry out controlled experiments for understanding how to drive this genotype into wild populations. Milestone 1 was reached exactly on target (Kokoza et al. 2000; Catteruccia et al. 2000); milestone 2 was achieved three years ahead of schedule (Ito et al. 2002). Next to *Plasmodium*-incompetent mosquitoes, it was also considered that *Aedes aegypti* (L.) should be transformed to become refractory to the dengue viruses.

In this paper, the challenges for the development and implementation of new and innovative control measures such as the release of genetically modified mosquitoes (GMM) are analysed in light of the necessity of addressing safety assessment and management, ethical, legal and social implications (ELSI) and public acceptance and decision making.

Challenges for development and implementation of the control strategy

Several achievements have been made towards the genetic transformation of vectors unable to transmit disease pathogens.

- *Anopheles stephensi* Patton transformation was achieved using Minos transposable element and the marker gene of the Enhanced Green Fluorescent Protein (EGFP) (Catteruccia et al. 2000).
- *An. stephensi* expressing a 12-amino-acid peptide (termed SM1) that binds specifically to mosquito midgut and salivary-gland tissues (Ghosh, Ribolla and Jacobs-Lorena 2001), was also engineered using piggyBac transposable element, the EGFP marker gene and the synthetic gene corresponding to SM1, and made unable to sustain *Plasmodium berghei* development and transmission (80% reduction in transmission) (Ito et al. 2002).• *An. gambiae*, the major vector of malaria in Africa was transformed using piggyBac marked with EGFP (Grossman et al. 2001) and SM1 was proved to be able to bind to its midgut and salivary-gland tissues.
- A white-eyed strain of *Ae. aegypti* was transformed (coloured eyes) in 1998, with 50% transformation achieved with *Hermes-Cinnabar* (Jasinskiene et al. 1998), and 4% with *Mariner-Cinnabar* (Coates et al. 1998).
- A transgenic *Ae. aegypti* refractory to dengue virus (and other pathogens) was engineered (Olson et al. 1996) with a viral transducing system using a double subgenomic Sindbis virus (dsSIN) containing a sequence from DEN-2 virus, to induce resistance in *Ae. aegypti* to DEN-2 virus replication and transmission.

- A stable transgenic *Ae. aegypti* mosquito (*Hermes-Vg-DefA*) produced (a blood-meal induced) defensin with antibacterial activity in the fat body (Kokoza et al. 2000).

Despite these achievements, biotechnology challenges remain about the improvement of the stability of a gene construct and its expression for a robust and complete interruption of pathogen transmission, the devise of safe means of spreading foreign antipathogen genes through mosquito populations in the wild and the devise of an appropriate genetic-control strategy based on this tool.

The solution to these technical challenges will be greatly speeded up (Morel et al. 2002) by the newly released *Anopheles gambiae* genome (Holt et al. 2002) and the forecoming *Ae. aegypti* genome sequence.

The *An. gambiae* genome provides new opportunities for generating new genetic markers for characterizing the vector populations, for studying their biology, ecology (including gene flow, dispersal, behaviour) and for identifying targets to inhibit parasite growth and interruption of transmission.

The main challenges would be implementation-related issues of the control strategy to answer the questions:

- How can we successfully and safely implement genetically modified mosquitoes to interrupt malaria and dengue pathogen transmission in the field?
- How can we successfully convince the public that this goal is desirable, feasible and can be accomplished safely?

Requirements to be considered before GMM can be deployed

The obstacles to overcome include proper risk assessment and management, conduct of environmental studies to ensure safety for humans and the environment, devise control strategies based on sound gene-driving systems, address properly ethical, legal and social implications of the release of GMM and public concerns.

Safety assessment needs a strong scientific base such as the identification of scientific principles and practices for conducting safe laboratory experiments and field trials with GMM following Good Developmental Practices (GDP).

A step-wise approach needs to be used during the research and development process to minimize the potential risks of the use of the GMM to humans and the environment. This can be achieved by the provision of guidance on the design and performance of minimum-risk field research, the development of criteria and test methods for environmental monitoring, providing the basis for collection of data addressing safety in the field, the development of guidelines for dispersal, contingency measures and site rehabilitation.

Proper safety assessment and management is an important basis for policy decision. It needs to set up a procedure to minimize the potential adverse human and environmental consequences by anticipating detrimental effects that might follow the release of GMM during experimentation, by designing monitoring systems for the early detection and evaluation of adverse outcomes and by planning intervention strategies, so that new information can be gathered and interpreted to avert and if necessary, remedy adverse health or environmental effects (Wheelis et al. 1998).

Biosafety assessment for humans and the environment needs to provide public information about the biosafety issues and ensure that the information reaches the communities and decision-making bodies. A proof of efficacy and safety to be

approved by authorized biosafety and regulatory bodies before any experimental release should be properly established (Hoy 2000).

There should also be prior environmental and health studies for site selection, and based on this data the most appropriate sites should be chosen.

Ecological studies are needed to improve understanding of gene flow in mosquito populations (mating patterns, behaviour, male biology, population size and structure, mechanisms of population regulation, fitness and phenotypic effects of colonization and mass production) (see also Knols et al. elsewhere in this volume). They will help identifying suitable isolated field sites and characterize populations in terms of genetic and ecological make-up; epidemiological characterization (transmission, disease), develop appropriate contained semi-field systems to improve understanding of the biology of (transgenic) mosquitoes (Scott et al. 2002).

Moreover, models can be used to enhance understanding of biological processes, spatial and temporal variations, selection of 'suitable' areas, prediction of effects of transgene introduction and public-health outcome.

Ethical, legal and social implications (ELSI) of the potential use of GMM will also need to be properly addressed, by integrating with the scientific studies those ELSI factors that are relevant to the use of GMM, and by ensuring that all parties with legitimate concerns have mechanisms for including their input into the proposed control programs. There is also the necessity of translating risk-assessment procedures into language(s) that are easily understood by the communities concerned, and of involving the end-users in the choice of sites and plans for deployment, in clear and legally appropriate concepts of informed consent, and in promoting an understanding of the real measures of success for the programs (Macer 2002).

Information should be openly provided as broadly as possible in a two-way process, and consent should be obtained from the communities involved. The mechanisms to obtain individual and group consent need to be specifically developed for public-health interventions. The data should be made open to all so that they can benefit from global expertise and develop an international consensus. There is a need for an ongoing and active process of ethical analysis, through a variety of fora. There is also the need for the elaboration of ethical and scientific standards for research in this area (Macer 2002).

The building of public awareness and confidence is essential to develop implementation strategies that involve the end-user communities and decision-making bodies such as to raise their awareness and build confidence about the benefits and risks, to provide means to the public to be sufficiently knowledgeable to make informed decisions about the merits of deploying these programmes in their communities, to provide adequate means for information dissemination and communication, to promote South-North research and development and build capacity in Disease-endemic countries (DECs) for the understanding and the potential use of the tool.

Bearing in mind these necessities, TDR and partners (NIH, MacArthur Foundation) initiated several meetings in order to bring together the different parties (molecular biologists, population geneticists, ecologists, field biologists involved in these activities (Scott et al. 2002; Alphey et al. 2002). Further plans include more involvement of DECs in terms of North-South research and training activities, building capabilities for the use of genome data as well as developing public awareness.

Conclusion

The development of genetically modified mosquitoes as disease-control tool is technically feasible and the recently released *An. gambiae* genome provides opportunities to address the remaining scientific challenges. For the implementation-related challenges, biological, ecological and genetic information needs to be gathered on the tool and the environment in which it may be used such as to give guidance about how to devise control strategies. Public concerns and ELSI considerations need to be properly addressed and a proof of efficacy and safety as basis for policy decision needs also to be demonstrated.

For proper implementation, no field release must be undertaken until clear scientific proof of efficacy is provided and safety issues for humans and the environment, ELSI considerations and public concerns are properly addressed.

Regardless of the quality of the science, the public confidence and acceptance will be the key factors to drive the tool in use.

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