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An introduction to ecological challenges concerning the use of genetically-modified mosquitoes for disease control

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Vector-borne diseases such as malaria and dengue constitute a major obstacle to socio-economic development in much of the tropics and remain high on the list of priorities for the improvement of public health. Sadly, financial as well as logistical constraints prevent a rapid amelioration of this situation (*Reducing risks, promoting healthy life* 2002). Unlike other infectious diseases, vector-borne diseases stand out because of their complex way of transmission, requiring passage from man to man or animal to man through an arthropod vector. This method of transmission implies the simple principle that removal of the vector will lead to the elimination of the disease. Indeed, the control of malaria, dengue, and other vector-borne diseases has relied – to a large extent – on vector control and, in certain areas (malaria in Western Europe, the Soviet Union and India, and dengue in the Americas), has been extremely successful. The introduction of synthetic insecticides (DDT, dieldrin a.o.) in the twentieth century created great optimism that vector-borne diseases could be controlled or even eradicated (Najera 1989). It is now realized that this optimism was unjustified. The main reasons were the development of insecticide resistance in the arthropod vectors, the sociological resistance to recurrent house spraying and a lack of political will to consolidate eradication efforts sufficiently well-funded for an adequate coverage of all disease-endemic regions (Greenwood and Mutabingwa 2002). Today the control of vector-borne diseases depends on a variety of methods including indoor spraying with insecticides, insecticide-treated bed nets, drainage and other means to eliminate mosquito-larval habitats, bioinsecticides, insect-growth regulators for larval control, and biological control (Curtis 1991). All such methods are combined with other health-control interventions such as drug treatment and vaccination. Although these methods help to reduce the disease burden by interruption of transmission, they do not remove or eradicate the pathogen and they leave the vectors to thrive in their natural habitats. It has proven increasingly difficult to eradicate an arthropod vector, and where successful control has been reported, this was often the result of temporary interruption of transmission to clear the human reservoir of the pathogen as has been the case for malaria. Hence we know of 'anophelism without malaria' in many countries around the Mediterranean and the continental USA (Bruce-Chwatt and De Zulueta 1980). In other areas, such temporary successes have led to a rebound effect, a.o. in India and Sri Lanka.

A different approach to vector-borne disease control is the proposition of using genetic methods to either reduce the density of the vector population or replace competent vectors with genetically modified counterparts that have been made

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refractory to parasite infection or development and no longer can transmit target pathogens or parasites. In the nineteen-seventies genetic control was studied using hybridization or sterile-insect technology. The development of molecular-genetic technology, allowing identification of genes and manipulation of such genes within or among species, opened unprecedented possibilities for genetic control of arthropod vectors. The World Health Organization organized a meeting in Tucson, Arizona, USA in 1991 to discuss the potential for the control of vector-borne diseases by genetic manipulation of vectors (*Report of the meeting 'Prospects for malaria control by genetic manipulation of its vectors'* 1991). Participants in the meeting concluded that there was ample opportunity for the application of such technology and research began to explore a) the identification of refractory genes, b) whether such genes could be introduced in vectors of major diseases such as malaria, dengue, South-American trypanosomiasis, and others and c) how desirable genetic traits could be driven into natural vector populations. An important aspect of this work would be the availability of a genomic map of the target vector, in order to identify the location of specific genes to be manipulated. The latter has been recently accomplished for the malaria vector *Anopheles gambiae* Giles *sensu stricto* (Holt et al. 2002). In addition, proof must be provided that insect vectors could be stably transformed to carry (a) refractory gene(s). Recently, this was achieved in the mosquito *Anopheles stephensi* Liston, which was transformed so that binding of the malaria parasite *Plasmodium berghei* to the mosquito's midgut membrane and sporozoite passage across the epithelium of the salivary glands were significantly reduced (Ito et al. 2002). Thus, several important goals for the development of transgenic mosquitoes have been met. This raises the question, however, whether enough attention has been paid to the issue how transgenic insects will fare in a natural environment. Surprisingly, relatively little attention has been paid to the important questions regarding fitness of released transgenic mosquitoes compared to wild siblings that they are meant to replace and how parasites will respond to barriers to infection in their arthropod hosts (Clarke 2002; Boëte and Koella 2003). Recently, however, Catteruccia, Godfray and Crisanti (2003) reported a study revealing that fitness of genetically transformed *An. stephensi* was significantly reduced compared to non-transformed *An. Stephensi* of the same laboratory stock. This report serves as a reminder that, although in the last few years several studies have reported successful transformation of mosquitoes (Jasinskiene et al. 1998; Catteruccia et al. 2000; Ito et al. 2002), controlling disease with transgenic mosquitoes is in the very early phase of development. All such studies must incorporate research on behavioural and physiological traits to ascertain that the transformed insects can compete with the wild populations which they are meant to replace. The study by Catteruccia, Godfray and Crisanti (2003) made this clear, reinforcing the fact that manipulated phenotypes must be rigorously examined when genetic manipulation of field populations is being considered. One possibility to study these effects has been suggested by Knols et al. (see elsewhere in this volume 2003), where a large contained semi-field system is proposed to create an arena for ecological vector studies that mimic a natural, undisturbed, habitat for mosquitoes. Current technology suggests that genetic manipulation of insect vectors is now available for wide use, but in contrast, the required reciprocal studies required to investigate the behaviour and survival of such transgenes in natural settings have apparently been largely overlooked.

A literature review of vector-borne diseases reveals that few studies have been conducted to examine gene flow among vectors, their mating behaviour, the interactions between vectors sharing one habitat, how parasites and pathogens respond

to the introduction of new vectors etc. Moreover, even if it becomes technically possible (with the efficient genetic-drive system) to introduce an allele conferring resistance to the malaria parasite into a mosquito-vector population, the effect on human prevalence and malaria morbidity and mortality would remain the heart of the problem, and the predicted effect of transmission reduction on malaria mortality and morbidity is debatable (Smith, Leuenberger and Lengeler 2001; Trape et al. 2002). Last but not least, any disease-prevention method based on genetically modified organisms (GMO) needs to be conducted under the social, legal and ethical rules of the societies and governments where the GMO is released.

Questions on topics ranging from ecology, parasitology, evolution and epidemiology to sociology, must be answered before any release of transgenic vectors is contemplated and will, without doubt, be useful for disease-control programmes that transcend vector transgenesis.

If not, the prospects for reducing disease are not good, similar to several of the sterile-insect programmes for malaria control in the nineteen-seventies (see Reisen elsewhere in this volume 2003). Given the time and resources spent on current studies using transgenic technology, such failures cannot be afforded for several reasons. First and foremost, people of disease-endemic countries urgently require a solution for reduction of the burden of disease. One would be ill-advised if these studies provided false hopes of a lasting solution to their problems. Second, only a few decades ago scientists confidently declared that insecticides would permanently halt transmission of malaria, yellow fever and dengue. A short while later it became evident that the biological plasticity of the vectors had been underestimated, rendering many of them insecticide-resistant. A similar problem occurred with the malaria parasite, whose plasticity led to the rapid development of resistance against many anti-malarial drugs (White et al. 1999). The scientific world should not repeat such a spectacle of hope, followed by defeat because technologies that have not been tested properly in a natural environment were introduced too soon. Third, a vast amount of public spending has gone into new research and training on mosquito vectors. Scientists owe it to the public that their research will meet the promises with which the numerous research grants were accepted. This takes time, and ecological research is an integrated aspect of the – up to now – mostly laboratory-directed studies.

In the summer of 2002 a group of vector ecologists met in Wageningen, The Netherlands, to discuss these issues and to provide a list of study themes that should be addressed urgently in the advent of the release of transgenic mosquitoes for disease control. This book contains the thoughts and ideas of the participants of the workshop, as well as recommendations for future research. We hope that this book will serve as a reference for scientists, administrators and health officials with an interest in novel methods for vector-borne-disease control.

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