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## **Discussion – Ecological challenges concerning the use of genetically modified mosquitoes for disease control: synthesis and future perspectives**

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The preceding chapters of this book focus on ecological aspects for the application of genetically modified mosquitoes for disease control. Although the novelty of the approach to render disease vectors incapable of transmitting some of the world's most debilitating diseases is underscored and unanimously recognized in these contributions, it is clear at the same time that the authors' views on the topic of feasibility are controversial and deserve continued debate. Being the first of its nature, the 'Wageningen Meeting' as it has become known, marked the beginning of a renewed impetus towards enhancing the still trifling knowledge on the ecology and behaviour of major *Anopheles* and *Aedes* disease vectors. The recognition of this knowledge gap by the wider scientific community and funding agencies alike will hopefully follow, and in itself presents ample justification for the publication of this volume.

A second important outcome of the meeting was the recognition that scientific expertise in countries earmarked for future application of novel vector-control interventions is sorely lacking. Not only do those countries often have limited scientific capability overall, their capacity in terms of research on the basic ecology and population biology of disease vectors is even less advanced. In spite of various ongoing international initiatives to improve this situation (Killeen et al. 2002), it will require additional efforts to enthuse young scientists in disease-endemic countries to engage in the study of vector biology and infectious-disease control. Failure to establish a competent cadre of scientists and absence of their involvement, through full partnership in decision-making processes and research alike, will be an impediment to improving public health and merely result in the repetition of past mistakes (Desowitz 1993). Fortunately, it is widely agreed that one should proceed with genetically modified mosquito field trials only if the likelihood of public-health benefits can be maximized, potential adverse effects for humans and the environment can be minimized, and the advance of this endeavour includes full collaboration with scientists in endemic countries (Alphay et al. 2002; Scott et al. 2002).

The third common denominator in the preceding contributions is the fact that advances in the understanding of ecological processes that affect mosquito populations and transmission of disease will be of value to *any* intervention methodology, be it the ones that are currently advocated (for example, insecticide-treated bed nets) or those that are being developed to augment existing methodology,

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like genetically modified mosquitoes. Critical in this regard, particularly in the case of malaria in sub-Saharan Africa, will be the divergence from the intradomiciliary to the peridomestic domain. The 'ease' of targeting host-seeking female vectors indoors, by using residual insecticides on the walls and roofs of houses or on bed-net material, curtains etc. will not apply to interventions that aim to reduce mosquito populations in open-field settings. This historical focus (on the indoor environment) is probably the leading cause for a still marginal understanding of vector population biology, the underlying causes for observed dynamics in populations, and the forces that drive such processes as dispersal, mating patterns, and speciation. Some of the intricacies of peridomestic anopheline life history remain a mystery such as dry-season survival, the nature of precopulatory mating barriers and male feeding behaviour, to name but a few. Similar knowledge gaps exist for *Aedes aegypti* ecology, population biology, and its role in transmission of dengue and yellow-fever viruses. A myriad of questions that have surfaced in this volume relate to these critical understudied topics. Consequently, an important research need is to understand these processes in order to maximize the likelihood of success for any intervention that targets vectors in the peridomestic domain, including genetic-control strategies.

Another major issue for concern when moving away from the intradomiciliary domain will be the shift from individual-based intervention technology to community-wide applications. The release of genetically modified or sterile insects will require consent at the community level, which is in stark contrast to existing methodologies whereby individuals can simply decide whether or not to use such tools as an insecticidal mosquito coil or bed net. What will happen if the old man under the tree will not tolerate the release of genetically modified insects? Although such social and ethical issues were not discussed at length (but see Touré et al. in this volume) either during the Wageningen meeting (Scott et al. 2002) or the London meeting that preceded it (Alphay et al. 2002), such controversies will ultimately be a significant challenge for the application of peridomestic intervention technologies (including genetic as well as non-genetic approaches, like larval control). Although it has been repeatedly stated in this book that ecological knowledge lags behind advances in molecular entomology, it is perhaps the sociological perspective that needs as much, if not more attention to ensure support and goodwill at all levels. It will be essential in areas earmarked for application to have support ranging from the affected communities to their country's public-health authorities (Aultman, Beaty and Walker 2001; Touré et al. in this volume).

Nevertheless, historical attempts to apply mosquito genetic-control strategies to reduce vector populations and curb disease transmission show that communities and governments of countries like Myanmar, Pakistan, El Salvador and Kenya, and European and US donor agencies have been supportive of such programmes. For years Zanzibar supported mass releases of sterile tsetse flies (*Glossina austeni*), which led to eventual eradication of this species from that island (Msangi et al. 2000). Whether similar support can be generated for the release of genetically modified mosquitoes remains to be seen. More important at this stage is that appropriate lessons have been learned from past experiences. Although some failures could be attributed to factors other than those related to the technology applied, like the outbreak of civil war in El Salvador, which led to the abandonment of the SIT programme with *Anopheles albimanus*, most failures were attributed to insufficient understanding of the ecology of wild mosquito populations and the fate of released material. Although such programmes were staged over two decades ago, these same hurdles still need to be overcome today, and specifically centre on the lack of knowledge in four areas:

1. *Male biology*. Historically, control efforts have focused on female mosquitoes, because they transmit pathogens that cause disease. A consequence of this is that knowledge of male life history is limited. Dependence of males on nutrients from plants, and how availability thereof affects survival and fitness, is a key element in genetic-control strategies yet thorough understanding of that kind of information is lacking. The factors that govern swarm formation, sustenance, and mate choice are similarly relatively unknown. Considering the fact that releases of female insects will likely be regarded as unethical (due to their blood-feeding habit), it will be parameters like those mentioned above that directly affect the efficient transmission of transgenes or sterile sperm from released males to wild females.
2. *Mating behaviour*. Although it is generally assumed that anophelines mate at swarm sites, apparent absence thereof in several areas where researchers have spent considerable time searching for them, suggests that other mating strategies may exist. It will be critical to ascertain whether or not this is the case. Similarly, do male *Ae. aegypti* locate and mate with females when they are not engaged in flight around human hosts? Do anopheline and *Aedes* mosquitoes mate assortatively? If so, based on what information are those choices made? Segregation mechanisms that keep hybridization between sibling species to a minimum, and even yield substantial reproductive isolation between chromosomal and molecular forms of *An. gambiae sensu stricto* have been recorded (e.g. Wondji, Simard and Fontenille 2002; Della Torre et al. 2002). Although the merits of molecular medical entomology may at times be questioned (Curtis 2002), molecular taxonomy has played a key role in defining reproductive isolation between sympatric populations of what was thought to be one taxon just years earlier. This information is vital for any genetic-control strategy because even a small population not targeted by the intervention may, due to reproductive isolation, undermine the benefits of releases by maintaining disease incidence and parasite prevalence at or close to pre-intervention levels.
3. *Colonization and mass-production effects*. The transition of wild mosquitoes to insectary conditions, more so for anophelines than for *Ae. aegypti*, is difficult and frequently fails. Mating, particularly in small-cage conditions (stenogamy) rather than through swarm formation outdoors, exerts a strong selection pressure for genotypes that thrive in such artificial conditions and hence causes a reduction in genome diversity. The build-up of subsequent generations and mass production for release was reported to result in reduced mating competitiveness (see Reisen in this volume). Non-compromised mating ability and adult survival are two critical elements for the success of genetic-control strategies. The impact of colonization and mass rearing deserves more thorough investigation. Similarly, behavioural determinants of gene flow in mosquito populations and the influence thereon of laboratory maintenance require intensive investigation in order to establish quality-control protocols for mosquitoes earmarked for release.
4. *Population biology*. Population biology issues will strongly impact genetic-control strategies. Investigators involved in several previous attempts to apply genetic-control techniques for disease control attributed failure to limited understanding of the 'background' population into which altered insects were introduced. Classical approaches, such as mark-release-recapture were utilized to determine ratios at which released insects should be introduced into wild

populations. Yet, some of the more critical components that will affect transgene technology, such as the factors that determine the spatial and temporal variations of allele frequencies and affect population structure, have only been studied to a limited extent. For example, dynamics of seemingly village-bound subpopulations of highly anthropophilic mosquitoes across fragmented landscapes support the need for a more thorough understanding of mosquito metapopulations. This will, however, constitute a significant scientific challenge because of complications associated with fluctuations in gene flow among 'patches' of mosquitoes and variation within (seasonal) and among effective population sizes (see Taylor and Manoukis in this volume).

Advances in the above four areas of research will be of tremendous value in assessing the likelihood of success when applying genetic-control strategies. Additional, yet salient issues that need to be taken into account relate to geographical differences in the vectorial systems that influence transmission dynamics of disease. Some countries, like Cameroon, have incriminated five endemic anopheline species as malaria vectors, whereas others, like the islands of São Tomé and Príncipe and Réunion report just one (potential) vector species. *Anopheles funestus* Giles sustains intense (seasonal) transmission in many African countries alongside its *An. gambiae s.l.* counterpart, yet its inclusion in genetic-control programmes remains exceedingly difficult due to difficulties of maintaining this species in the laboratory. It is imperative, therefore, that the biology and life history of this epidemiologically important species receives increased attention, if genetic-control strategies are to be scaled-up in the future to go beyond the highly specific ecological or physical island settings now under consideration. Although not equal contributors to transmission of dengue and other arboviruses, *Aedes aegypti* and *Ae. albopictus* also can transmit viruses sympatrically, which raises similar concerns to the malaria situation for the application of genetic-control tools when multiple species of mosquito vectors are present in the same geographic location.

Some recently developed novel transgenic approaches for insect control were discussed briefly in this volume (see Curtis 2003), like application of dominant lethality or engineered underdominance. Such approaches may ultimately be employed to control mosquito vectors. However, the bulk of research to date, and the main focus of discussion in this book, relates to the ecological consequences of attachment of an antipathogenic genetic construct to an efficient genetic-drive mechanism, introduced through mosquito germline transformation, followed by the release of such genetically modified mosquitoes into the environment. Although this concept is not new (Curtis 1968), its full development has only come to flourish over the last decade, when appropriate advances in molecular biology facilitated its full application. Proof of principle, with impaired transmission capability of mosquitoes for both malaria (Ito et al. 2002) and dengue transmission (Olson et al. 1996), has been established, and has generated considerable excitement that this approach may come to fruition for use as a public-health tool. Before such advances can be expected, however, there are several important issues that need to be addressed.

Considering the fact that progress to date has been confined to laboratory settings, a myriad of unanswered questions relate to the fate of engineered mosquitoes in the natural environment. In view of the potential for detrimental effects of parasite infection on mosquito fitness (Hurd 2003) one would expect refractoriness to be a trait of high adaptive value, yet it is rare in field populations (Schwartz and Koella 2002). The staging of an effective immune response may, therefore, bear an evolutionary

cost too high for it to become naturally fixed, which explains the need for a highly effective drive mechanism to increase introduced gene frequency in wild populations. Concern has risen over the physiological consequences and altered mosquito resource allocation in response to introduction of a refractory trait, and the possible unfavourable selection pressure against such genotypes. If not carefully designed and implemented, inclusion of transgenes in the mosquito genome may at best yield complete blockage of pathogen development in the individuals possessing the trait, but may compromise mosquito fitness to the extent that such traits rapidly disappear from the target population (Billingsley; Koella in this volume; Catteruccia, Godfray and Crisanti 2003; Boëte and Koella 2003). Another pertinent issue with regard to the above concerns relates to the use of a genetic-drive mechanism (e.g. a transposon) to overcome the selective disadvantage of possessing transgenes. If a transgene that reduces fitness disassociates from its genetic driver, the transgene and its beneficial effect on pathogen transmission will be lost.

It is generally accepted that if wild mosquito populations are to be replaced with refractory ones, drive mechanisms are required to spread transgenes at rates that exceed the spread of genes by normal Mendelian inheritance. If such a system can be identified, then small 'seeding' releases of, for example, transposon-carrying individuals may be adequate to accomplish population replacement. Alternatively inundative releases could be repeated over time, but this would add logistical complexity that may render it economically unviable in comparison to established vector-control tools. The lessons learned from sterile-insect-technique programmes will certainly be of value in this regard (Benedict and Robinson in press).

It has been suggested that an ecologically appropriate and biologically safe way to address questions about the transfer of transposon-driven transgenic mosquitoes from the laboratory to the field is to use contained semi-field systems (Aultman, Beaty and Walker 2001; Scott et al. 2002). For some research topics this approach might work well, for others it may not be appropriate due to inherent limitations (Knols et al. in this volume). For instance, it may be inappropriate for studying the occurrence of transgene inactivation through mutation, phenotypic expression in the genetic background of wild populations or the development of pathogen-resistance against introduced traits. Those processes are unlikely to be expressed and identified in small-scale greenhouse studies over a short period of time. Other means will be required to obtain that kind of critical information for the use of transgene technology when applied on a large scale and over several years.

If the process of population replacement is complete and refractoriness absolute, the merits of the transgenic approach will be easily identifiable – transmission will be eliminated and disease will disappear. However, any shortfall of complete blockage of transmission or partial population replacement will require detailed insight, which does not currently exist, into the relationship between entomological risk and disease outcome. Although the relationship between vector density, transmission intensity and disease are fairly well understood for malaria (e.g. Smith, Leuenberger and Lengeler 2001), it has proven much harder to define such characteristics in the case of dengue (Scott and Morrison in this volume). Measuring the direct public-health benefits of transgene technology may likely be complex. An extension of this concern are the possible consequences that reductions in pathogen transmission and delayed acquisition of (partial) immunity may have on severity of disease. Although this so-called 'rebound effect' has not been observed in bed-net studies in Tanzania (Maxwell et al. 2002; Curtis in this volume) it remains to be seen whether these observations are universal across wider ranges of transmission intensity and over longer time periods

than have been studied to date. Overall, there is a fundamental need for improved understanding of the intricate relationships between entomological indices of risk, transmission intensity and dynamics, and disease incidence and prevalence.

Legitimate concerns have been raised regarding the various biosafety and risk factors involved in the application of transgenic tools in agriculture and human health. We, therefore, can anticipate that public concern over the inclusion of selected traits in the genome of human-blood feeding, pathogen transmitting, and free-ranging insects may be of similar if not larger magnitude compared with that observed for biotechnologically enhanced food crops. An array of ethical, legal and social issues related to the release of genetically modified mosquitoes must be addressed, in conjunction with clearly defined biosafety and risk-assessment studies. Such studies should include vector competence of target insects for diseases other than those against which they were intended to be refractory. The merits of population replacement should also be viewed with regard to sustained transmission potential of other diseases by the target population (e.g. malaria-refractory *An. gambiae* transmitting bancroftian filariasis). Above all, regulatory bodies charged with the authorization process of moving transgene technology from the laboratory to full field application, need to be established and should safeguard the two-pronged objective of this endeavour: maximizing the likelihood of public-health benefits and minimizing potential adverse effects for humans and the environment.

The Wageningen Meeting brought together a group of leading international experts in the fields of mosquito biology and ecology, infectious-disease control and epidemiology. The many stimulating discussions at the meeting were covered by news features in *Nature* (Clarke 2002) and *Science* (Enserink 2002). In highlighting the huge and intolerable burden of malaria and dengue it was once more noted that the battle staged by mankind against their mosquito vectors is far from won, and leaves many of the poorest nations in the world with insufficient capability to combat disease and halt the economic losses they incur. The expanding global efforts, both in scientific and financial terms, to turn the tide of this public-health disaster are to be applauded. Major advances, notably the completion of the *An. gambiae* and *Plasmodium falciparum* genome projects, offer prospects for expanding the arsenal of novel tools (drugs, vaccines, insecticides) to combat malaria. We hope that the ongoing *Ae. aegypti* genome project will result in similar positive public-health impacts against dengue. Further refinement of insect transgenic technology and identification of additional targets (phenotypes other than refractoriness such as altered blood-feeding behaviour or shortened life span) capable of reducing a mosquito population's capacity to transmit pathogens are to be expected over the coming decade. Moreover, novel developments in the field of molecular biology will in all likelihood deliver the means to circumvent impediments to application that exist today.

'*Ecological aspects for application of genetically modified mosquitoes*' is the first scientific contribution to draft a research agenda specifically intended to facilitate the application of novel (genetic) approaches to reduce or eliminate mosquito-borne disease. Given the appropriate financial, scientific, and human resources, we may indeed enter an era rich in successes and unprecedented in scale and magnitude, against mankind's most deadly foes.

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