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# Current thoughts about the integration of field and laboratory sciences in genetic control of disease vectors

*Thomas W. Scott*[#]

## Abstract

Realizing the full potential of genetic control of vectors for disease prevention will require development of a research agenda that captures the willingness of people with diverse expertise to work together toward constructive and substantive goals. Below I review the five ecological and population biology topics that are central to contemporary genetic vector-control programmes and present opportunities of collaboration between people engaged in primarily laboratory- versus field-based research activities: (1) spread and stability of introduced genes; (2) evolutionary consequences of mosquito transformation; (3) entomological risk, pathogen transmission and disease severity; (4) quantitative analyses of mosquito biology, disease and genetically modified mosquito (GMM) control; and (5) procedural issues. I point out opportunities for greater, mutually beneficial interaction between laboratory- and field-based scientists. I draw four general conclusions from this analysis. First, an improved understanding of ecological topics associated with GMMs will provide the conceptual and factual foundation for application of genetic-control technology. Second, four topics that should be considered research priorities are male biology, mating behaviour, colonization and mass-production effects, and population biology. Third, in addition to greater collaboration between ecologists and molecular geneticists, genetic-control programmes will require recruitment of expertise from outside the vector-borne disease arena, greater involvement by scientists from disease-endemic countries (DECs), training for young scientists, adequate funding, and a sustained effort. Fourth, collaboration will be a central component of the legacy and success of genetic control for vector-borne disease prevention.
**Keywords:** genetically modified mosquitoes; genetic control; vector ecology

## Introduction

Much of the enthusiasm during the past 15 years for the strategy of reducing mosquito-borne disease with genetic control of vectors was, and continues to be today, based on potential. Evidence of the excitement with which the promise for genetic control has been blessed is the articles that have been published on the topic, both supporting and challenging the concept. Even an incomplete list of published reports restricted to malaria and dengue vectors is impressive in terms of the number in print, the profile of the forums in which these were published, and the reputations of the participants involved (Alphey 2002; Alphey and Andreasen 2002; Aultman et al. 2000; Aultman, Beaty and Walker 2001; Beaty 2000; Benedict and Robinson

[#] Dept. of Entomology, Univ. of California, Davis, CA 95616 USA. E-mail: twscott@ucdavis.edu

2003; Bradbury 2002; 2003; Braig and Yan 2002; Catteruccia, Godfray and Crisanti 2003; Christophides, Vlachou and Kafatos 2004; Coleman and Alphey 2004; Collins 1994; Collins and Paskewitz 1995; Collins et al. 2000; Curtis 1994; 2000; 2002; 2003; Gould and Schliekelman 2004; Irvin et al. 2004; Ito et al. 2002; James 2000; 2003; James et al. 2001; Kiszewski and Spielman 1998; Moreira et al. 2004; Olson et al. 1996; 2002; Reisen 2003; Scott et al. 2002; Scott and Morrison 2003; Spielman 1994; 2003; Spielman, Beier and Kiszewski 2002; Tabachnick 2003; Takken and Scott 2003). In order to successfully complete the next critical steps – i.e., evaluation and application – I submit that the espoused potential will not be realized without more integrated efforts among people engaged in molecular research that is primarily laboratory-based, ecological investigations that are primarily field-based, and population genetics studies that offer a conduit of constructive exchange between the laboratory and field. For this to happen, it is essential that a research agenda be developed that captures the willingness of people with diverse expertise to work together to suppress mosquito vectors, while maintaining their focus on the primary goal of reducing human morbidity and mortality.

Success of genetic-control programmes during the 1960s and 1970s was at least in part due to mutually beneficial interactions among scientists with complementary, but different expertise. Gould and Schliekelman (2004) noted that, "During the era of classical genetic control research there appears to have been incredibly good communication and cooperation between theoretical and empirical researchers. Indeed much of the empirical work was inspired by results of population genetics studies. There has been a tendency for the sophistication of modern science to isolate researchers involved in molecular work from those doing ecological and population genetics studies. We think that more interaction between these scientists at the very early stages of genetic control projects could increase the chances of […] ushering in a new and long-lived, golden era of genetic control". What can be done to address this challenge of elevating cross-disciplinary, collaborative research?

During the 2001 workshop on Genetically Engineered Arthropod Vectors of Human Infectious Diseases at London's Imperial College there appeared to me to be a lack of balance in the contributions of people with laboratory versus field expertise. The geneticists had, in general, given more thought and spent more time engaged in research directed at a contemporary strategy for genetic control than had most vector ecologists. A week later, at the International Congress of the Society for Vector Ecology in Barcelona, Willem Takken and I decided to convene a meeting of vector ecologists for the purpose of defining key ecological and population-biology issues necessary for responsible evaluation and application of genetically modified mosquitoes (GMM) for disease control. The meeting would be largely limited to vector ecologists because we wanted to develop as much as possible a consensus on the topic prior to engaging our laboratory colleagues. This would be the first meeting of vector ecologists to discuss genetic control of mosquitoes to prevent malaria and dengue. That meeting resulted in two publications. One summarized the meeting (Scott et al. 2002) and the other provided detailed thoughts by meeting participants on a list of key challenges that the GMM strategy needs to address in order to be safely and effectively deployed (Takken and Scott 2003). Because at the time the most progress in genetic modification had been made with anopheline vectors of malaria and *Aedes aegypti*, discussion was limited to those taxa. Below I review five topics that are central for a genetic-control programme to prevent malaria and dengue transmission. In the spirit of bridging the gap between laboratory and field research in

disease vector control, when appropriate, I will note opportunities for greater, mutually beneficial interaction between laboratory- and field-based scientists.

## Spread and stability of introduced genes

Fundamental to the success for GMMs for disease prevention will be that gene constructs spread and persist in target populations. To achieve this it will be important to understand the genetic structure of mosquito populations, patterns of mosquito reproduction, the size of target populations, how populations are regulated, and requirements for colonization of wild-type mosquitoes and mass rearing of GMMs. Knowledge of target population structure will likely be more important for anophelines than for *Ae. aegypti* because many anopheline populations exhibit restricted gene flow among sympatric sibling species and/or different chromosomal forms (Lanzaro and Tripet 2003). Reproductive barriers between or among different populations could undermine a population replacement strategy if not all competent vectors are eliminated or rendered refractory to parasite transmission.

Because successful mating is a basic component of any genetic-based control strategy and our understanding of mosquito mating is underdeveloped, there is an urgent need for increased research on this topic (see Takken et al., elsewhere in this volume). The application of contemporary molecular tools to dissect the details of mosquito mating offers an opportunity for productive collaboration between laboratory- and field-based scientists. Similarly, an opportunity exists for significant contribution in understanding the ecology of male mosquitoes, which has been largely ignored in preference of females. Females have been the focus of attention because they are responsible for pathogen transmission by bite. But in a genetic-control programme it is likely that releases of GMMs will be limited to males in order not to increase the bites per night suffered by humans living at release sites (Alphey et al. 2002). Therefore, fitness of males – which rarely has been examined for mosquitoes – assumes high status as a critical component of a successful genetic-control programme.

The number of GMMs released will be determined at least in part by the size of the target population. Estimation of effective population size can be complicated by seasonal fluctuations that could, depending on the circumstances, aid or hamper genetic-control efforts. Consequently, there is a need for greater effort with more sophisticated analyses to characterize the size and structure of mosquito populations (Taylor and Manoukis 2003). Likewise, population biologists and ecologists can contribute to the GMM strategy by explaining the processes by which the size of mosquito populations is regulated. Differences in population regulation among genetic subdivisions could lead to an unpredicted advantage for one population over another (Rasgon and Scott 2004).

During the development of GMMs it will be essential that protocols be worked out for colonization of wild-type mosquitoes that will be used for genetic modification and for mass rearing GMMs. Because adaptation to a laboratory setting can reduce fitness compared to the wild-type mosquitoes with which GMMs will be expected to compete (Munstermann 1994; Mukhopadhyay et al. 1997), there is a need to develop requirements or guidelines for minimizing the loss of fitness and altered phenotypic expression due to colonization and mass rearing. This is another area in which people with field- and laboratory-based expertise can work together and make a significant contribution to a GMM control programme.

## Evolutionary consequences of mosquito transformation

Understanding and minimizing fitness costs associated with genetic modification, which can be conditional and/or correlated with other life history traits, will be critical in a population replacement scheme for spreading and maintaining stable resistance to pathogen transmission. This is an area that offers multiple opportunities for collaboration between population biologists and molecular biologists who are engineering GMMs. For example, we can ask what are the evolutionary costs of genetic modification to mosquitoes, and how will they shape plans for interfering with pathogen transmission? What effect will imperfect interference have on the evolution of pathogen resistance and how will it be managed in a disease prevention programme? Boëte and Koella (2003) predict that even if a gene for refractoriness was driven to fixation in an anopheline population, if malaria transmission is intense, prevalence of infection in humans will decrease only if transmission interference is close to 100%. If this is true, success of population replacement strategies will depend in large part on the efficacy of the effector gene(s). Thus, Boëte and Koella (2003) provide guidance to the people who are engineering GMMs by predicting the extent to which vector competence will need to be reduced.

What effect will natural environmental conditions and genetic background have on phenotypic expression of resistance? Variation in phenotypic expression can result in some mosquitoes carrying a ‘refractory transgene’ but not expressing a ‘refractory phenotype’ (Tabachnick 2003). Even if the gene is driven to fixation, this could lead to less than perfect replacement of competent with refractory mosquitoes and the opportunity for parasites to evolve resistance to GMMs.

What effect will imperfect interference have on the evolution of pathogen resistance and how will it be managed in a disease prevention programme? Although parasite resistance has been discussed in a variety of platforms, there has been relatively little empirical work done to determine to what extent this might be a problem for GMMs. If resistance does evolve, can we predict the virulence characteristics of resistance phenotypes? Every effort should be made to avoid selection of parasites that are more virulent than the ones that preceded release of the GMM.

A question that is frequently asked and will certainly need to be addressed is whether GMMs have enhanced capacity to transmit pathogens other than the one that they are intended to block. Vector competence studies will need to be carried out with co-occurring pathogens demonstrating that GMMs will not transmit unintended pathogens.

Will changes in parasite populations in response to a GMM affect the efficacy of other disease prevention programmes? For example, will vaccines or anti-parasite drugs be compromised? The time is right to begin to provide details for how GMMs will be incorporated into an integrated disease prevention programme. This kind of analysis will require collaboration among people with a diversity of expertise.

## Entomological risk, pathogen transmission and disease severity

The conceptual foundation of genetic mosquito control is that reduction in the density of competent vectors, whether directly – population reduction – or indirectly – population replacement – will decrease human infection and disease. For this strategy to be successful we need to know the degree to which mosquito populations must be reduced in order to produce the desired public-health outcome. In other words, we

need to understand the quantitative relationships between density of competent vectors, human infection, and disease (Scott and Morrison 2003). This will include avoiding the so-called 'rebound effect'. That is, if transmission becomes unstable, primary adult infections could result in an unexpected increase in epidemic disease. Short-term reduction in malaria or dengue transmission could, but does not necessarily (Maxwell et al. 2002), create such a situation by increasing the number of people surviving childhood without an infection and the benefit of a protective immunological response. It may also be necessary to partition the relative contributions to parasite transmission by different mosquito species or chromosomal forms if several sympatric mosquito populations sustain transmission. If only one population of mosquitoes is removed from transmission, what will the impact be on the number of new human infections?

The entomological inoculation rate (EIR, i.e. the number of mosquitoes with sporozoites biting a person per unit of time) is a powerful measure of entomological risk for malaria transmission (Smith, Leuenberger and Lengeler 2001). For example, Charlwood et al. (1998) reported that when infections were low, risk of human malaria infection increases with the EIR. When infections were high, an increase in EIR did not raise parasitaemia in infants. Despite its advantages, there are at least two unresolved difficulties associated with application of the EIR for assessing the risk of malaria transmission and disease. First, for ethical reasons it is increasingly difficult to use human bait to collect anophelines that may be infected with parasites. This has resulted in efforts to correlate collections in traps to those from people. In some cases the relationship is good, in others it is not (C. Constantini and D. Fontenille, pers. comm. Mathenge et al. 2004). There is a need to develop a standardized methodology for capturing human-host-seeking anophelines that does not require direct exposure to humans (Mathenge et al. 2002; 2004). Second, establishing the relationship between EIR and malaria-specific mortality centres on the difficulties associated with the quality of cause-of-death data. Smith, Leuenberger and Lengeler (2001) explain the details of this dilemma and highlight the need for continued research efforts at the interface between medical entomology and epidemiology.

Dengue researchers do not have a simple and reliable entomological measure for assessing risk of disease, like the EIR. The rate of *Ae. aegypti* infection with dengue virus is too low and varies too much through time and space to create a dengue risk measure analogous to the EIR. Current measures of entomological risk for dengue transmission are at best weakly correlated with human dengue infection and their relationship to disease is poorly defined. Consequently, predicting and testing the relationships among mosquito density, dengue transmission and disease are among the most important unresolved issues in dengue epidemiology and assessing the application of GMM for dengue prevention.

## Quantitative analyses of mosquito biology, disease, and GMM control

Models have made and will continue to make two vital contributions to the development and application of GMM technology (Ribeiro and Kidwell 1994; Kiszewski and Spielman 1998; Turelli and Hoffmann 1999; Focks et al. 2000; Boëte and Koella 2003; Rogers et al. 2002; Rasgon, Styer and Scott 2003; Gould and Schliekelman 2004; Rasgon and Scott 2004). First, they identify knowledge gaps and thus direct new research activities toward high-priority topics. Second, they predict outcomes of scenarios of interest. It is important that in the future modelling efforts in

the GMM arena transcend simulating events retrospectively and predict outcomes of proposed interventions.

This is clearly an area of opportunity for increased interaction among people with different and complementary expertise. Those who create models will seek the most germane and accurate data available from vector ecologists, molecular biologists and epidemiologists. The accuracy and utility of models will need to be refined by interaction among all parties involved. Model output will be invaluable for designing genetic constructs and predicting strategies for deployment and evaluation of GMMs.

## Procedural issues

Addressing three procedural issues will provide additional opportunity for interaction between field- and laboratory-based scientists. First, there is a pressing need for development of standardized processes for dealing with the ethical, legal and social issues related to GMM technology (see Touré and Manga elsewhere in this volume). It has been suggested that these kinds of guidelines would be most effective if developed by an international body like the World Health Organization (Scott et al. 2002). During 20-21 September 2004 the Pew Initiative sponsored a conference in Washington, D.C. on science and policy surrounding the release of genetically modified insects. The meeting provided a forum for interaction among people with different backgrounds and the basis for development of guidelines for research and application of GMMs. Second, it is essential that scientists, public-health officials and regulatory personnel in DECs are fully enfranchised in the development and application of GMM programmes. For that to happen, there will need to be greater participation by people and infrastructure development at GMM research field sites. Third, thorough evaluation of GMM technology will require transitional research from laboratories to semi-field facilities – large outdoor cages like those described by (Knols et al. 2003) – followed by release at geographically isolated sites. Challenge 7 in the Grand Challenges in Global Health – i.e., develop a genetic strategy to deplete or incapacitate a disease-transmitting insect population – has already, in the proposal development stage, constituted a unique opportunity for laboratory- and field-based scientists to work side-by-side toward a common and well defined goal. Let us hope grants for this challenge are awarded and exciting crosscutting science will continue.

## Conclusions

Four broad conclusions can be drawn regarding the application of genetic control of vectors and the integration of field and laboratory sciences.

First, without an improved understanding of the ecological topics discussed above, application of GMM technology will lack an appropriate conceptual and factual foundation. Understanding and applying ecological processes of a mosquito's role in pathogen transmission will be essential to achieve reduction in disease. When assessing research accomplishment on genetic control of mosquitoes in India during the 1970s, Rao (1974) explained that "Ecology of mosquitoes is the bedrock on which management of genetic control methods have been founded. The impact of the methods applied is largely determined by the behaviour of mosquitoes in nature, their numbers and their resting, feeding, mating, egg laying and dispersal habits which vary from season to season and place to place".

Second, although failures of past efforts have been attributed to factors other than the technology applied, four topics have been problematic in the past and should be

considered research priorities in contemporary programmes. They are male biology, mating behaviour, colonization and mass-production effects, and population biology.

Third, genetic control will require greater collaboration between ecologists and molecular geneticists, recruitment of expertise from outside the vector-borne disease arena, greater involvement by scientists from DECs, training for young scientists, adequate funding and a sustained effort. Genetic control will require a long-term commitment. In this regard it will be important to watch the impact of the Grand Challenges in Global Health on the development of genetic control of vectors.

Fourth, we stand on the threshold of a unique opportunity for all participants in the genetic-control paradigm. Let us hope that in the spirit of constructive and substantive interaction, regardless of participants' expertise or background, we will continue to work together to integrate genetic control into a robust strategy for disease prevention. Collaboration will be a central component of the legacy and success of genetic control for vector-borne disease.

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