

## Transgenic Mosquitoes and the Fight Against Malaria: Managing Technology Push in a Turbulent GMO World

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**Abstract.** Genetic modification (GM) of mosquitoes (which renders them genetically modified organisms, GMOs) offers opportunities for controlling malaria. Transgenic strains of mosquitoes have been developed and evaluation of these to 1) replace or suppress wild vector populations and 2) reduce transmission and deliver public health gains are an imminent prospect. The transition of this approach from confined laboratory settings to open field trials in disease-endemic countries (DECs) is a staged process that aims to maximize the likelihood of epidemiologic benefits while minimizing potential pitfalls during implementation. Unlike conventional approaches to vector control, application of GM mosquitoes will face contrasting expectations of multiple stakeholders, the management of which will prove critical to safeguard support and avoid antagonism, so that potential public health benefits can be fully evaluated. Inclusion of key stakeholders in decision-making processes, transfer of problem-ownership to DECs, and increased support from the wider malaria research community are important prerequisites for this. It is argued that the many developments in this field require coordination by an international entity to serve as a guiding coalition to stimulate collaborative research and facilitate stakeholder involvement. Contemporary developments in the field of modern biotechnology, and in particular GM, requires competencies beyond the field of biology, and the future of transgenic mosquitoes will hinge on the ability to govern the process of their introduction in societies in which perceived risks may outweigh rational and responsible involvement.

### INTRODUCTION

The history of malaria vector control is typified by the search for compounds that interfere with vital physiologic processes in the mosquito.<sup>1</sup> Ever since Ross' first transmission models,<sup>2</sup> it became clear that adult mosquito survival represents an Achilles heel in the epidemiology of malaria.<sup>3</sup> This fundamental understanding led to the search for compounds with insecticidal activity that would persist in the environment in which these were applied. Most, if not all, new developments in the control of anophelines presented incremental improvements of this concept. Resistance was, and is still today, viewed as an unavoidable consequence of widespread insecticide use that can either be managed or overcome by the discovery of new compounds with desirable (i.e., long-term mosquitocidal) characteristics.<sup>4,5</sup> Based on phenomenal historical successes around the globe, the huge efforts underway to scale-up use of insecticide-treated bednets (ITNs)<sup>6,7</sup> and the renewed acceptability of DDT for vector control following the agreement to the Stockholm Convention on Persistent Organic Pollutants,<sup>8–11</sup> it is hardly surprising that the mainstay of malaria vector control remains focused on chemical control. Disturbingly, all of these methods depend on the ability to reach target communities, acquisition of consent or willingness to use them, and cost and correct use. Both the use of ITNs and indoor residual spraying (IRS) target mosquito vectors in the domestic environment, and interest in peridomestic control strategies (e.g., larval control) is slowly reviving.<sup>12,13</sup>

The above contrasts sharply with the more radical concept to render mosquito populations refractory to infection with *Plasmodium* parasites.<sup>14</sup> The focus here is on the ability to modify the genome of *Anopheles* in such a manner that the acquired phenotypical traits are inherited in a non-Mendelian

fashion and spread through a population with the aim to eventually replace a susceptible with a refractory population. Unlike the aim to shorten the daily survival of the vector, it is now the competence to transmit disease that serves as the intervention target. Alternatively, genetic engineering may convey conditional lethality,<sup>15</sup> but focus here will be on engineered refractoriness. In contrast with insecticide-based strategies, however, there are no precedents (yet) of successful application, leading to the classification of this approach as linear technology-push.<sup>16</sup> In the absence of market pull mechanisms, present in some circumstances for ITNs, furtherance of this approach depends on basic scientific developments and proof-of-principle experimentation, followed by stepwise introduction in suitable disease-endemic country (DEC) settings. Using the same process, genetic control strategies such as the sterile insect technique (SIT),<sup>17</sup> in which large numbers of radiation-sterilized males confer sterility to the pest population on release, have created new market opportunities for the control of insect pests, which are now expanding.<sup>18</sup> Although the concept here is population suppression and/or local elimination, it is probably the only benchmark available at present for vector control strategies based on modern biotechnological tools.<sup>19</sup> The novelty of the approach thus poses challenges on several fronts. First, there are various technical hurdles affecting progress, posing challenges to the scientific community. Second, the transition from the laboratory to the field requires careful planning to manage the risk of project failure or premature termination. Third (and unknown at this stage), it remains to be seen under what ethical, legal, and societal frameworks (ELSI) adoption in DECs can be secured. In this article, we aim to shed light on all three challenges, with emphasis on the latter two, which inevitably will prove vital in the medium to long term.

### TECHNOLOGICAL CHALLENGES

Fifteen years ago, a report was published by the World Health Organization that focused on prospects of using ge-

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netic modification of anopheline mosquitoes for malaria control.<sup>20</sup> Building on concepts posed in the late 1960s<sup>21</sup> and genetic modification of *Drosophila*,<sup>22</sup> a strategic roadmap was developed, with the following targets: a transgenic mosquito by 2000, a refractory mosquito by 2005, and field trials by 2010.<sup>23</sup> Dramatic advances in modern biotechnology resulted in stable germ line transformation of *Anopheles stephensi*,<sup>24</sup> *An. gambiae*,<sup>25</sup> and *An. albimanus*<sup>26</sup> as planned, with the first refractoriness to *Plasmodium berghei* engineered in *An. stephensi* ahead of schedule.<sup>27</sup> However, by 2006, engineering of refractoriness to *P. falciparum* has not yet been reported. Current availability of the *An. gambiae* genome sequence enables functional studies on human malarias and anophelines in the search for novel control strategies.<sup>28</sup> These successes heightened enthusiasm beyond those directly involved<sup>29,30</sup> and resulted in the formation of new perspectives within related disciplines. Ecologic aspects of the approach have been reviewed,<sup>31–33</sup> besides issues related to the transition of the approach from the bench to the field.<sup>34</sup> Reviews and books on the subject continue to be published as it matures.<sup>35–37</sup>

These encouraging developments were presented to the broader malaria research and control community during a plenary debate at the 4th MIM Pan-African Malaria Conference, held in Yaoundé, Cameroon, in November 2005. After explanatory remarks on contemporary issues in the field of genetically modified (GM) mosquitoes,<sup>38</sup> 196 participants expressed their views on 10 statements (Figure 1). More than one half of the respondents viewed the above successes as indicative for future successful implementation of the approach (Figure 1, Q1) and were confident that systems to target human malarias will be developed (Figure 1, Q2). Three of four respondents believed that the lessons learned from previous genetic control trials, coupled with a vast increase in knowledge of the target organisms, will increase the likelihood of success in future trials (Figure 1, Q3). However, a variety of technical hurdles, related to biologic constraints remain, the most salient of which are listed below.

**Mosquito fitness.** The introduction of novel phenotypic traits in transgenic mosquitoes may induce a fitness cost that could impede the effectiveness of genetic drive mechanisms and thus the spread of transgenes in target field populations.<sup>39</sup> Expression of marker proteins and effector molecules, particularly when expressed using ubiquitous promoters, may be detrimental to the mosquito.<sup>40</sup> In addition, transgene insertion may interfere with gene expression of the mosquito strain because of the essentially random nature of genomic insertion. This so-called insertional mutagenesis may be naturally selected against or be overcome through selection of lines with higher fitness.<sup>41</sup> However, the inability to control insertion site location using conventional vectors has led to the development of vectors that can provide a stable docking site for site-targeted transgene insertion. This, coupled with the use of suicide vectors, will increase safety and make transgene expression more predictable.<sup>42</sup> However, it will still be necessary to select, from a series of insertions with docking sites, the most fit. Finally, the genetic make-up of transgenic lines and inbreeding have been posed as additional factors affecting the fitness of transgenic lines.<sup>39,43</sup> This latter factor is probably not a serious concern and can probably be dealt with using a well-designed breeding scheme. These shortcomings may be overcome by circumventing the mosquito genome altogether and instead focus on genetic

modification of symbiotic (midgut) bacteria as a means to deliver anti-pathogen products.<sup>44</sup> Whatever approach is adopted in the coming years, compensation for fitness loss, preferably by conferring fitness advantages to transgenics that outweigh cost-benefit equilibria in susceptible wild-type mosquitoes,<sup>45</sup> will remain a critical challenge. The measurement of fitness in the laboratory with laboratory strains is at best inadequate and at worst misleading. The only meaningful measure of fitness will be when the GM mosquitoes are competed against wild mosquitoes in the field. As a concept to address this, Scott and others<sup>46</sup> called for the development of consensus methodology in fitness studies based on stepwise progression from laboratory to semi-field settings (see below), with competitiveness of GM mosquitoes as a key criterion.

**Genetic drive.** On the premise that transgenic lines with acceptable fitness can be developed, the next challenge will be to drive the desirable attributes to fixation in field populations. In contrast with SIT campaigns, in which massive repeated releases of sterile males are practiced, the use of males able to drive a transgene into a target population may require the production and release of much smaller numbers, thereby gradually altering its susceptibility in favor of refractoriness. Reviews on genetic drive mechanisms<sup>47</sup> and desirable attributes thereof<sup>48,49</sup> focus on the possible fitness costs associated with the drive mechanism itself (as described above), the rate at which introgression of transgenes in populations occurs, and the ability of the mechanism to drive large genetic constructs. Models have been developed to study these phenomena.<sup>50,51</sup> These indicate that the complete linkage between genes delivering anti-pathogen traits, and the genetic drive mechanism deployed, besides stable and reliable levels of phenotypic expression of transgenes, is mandatory to accomplish adequate penetration of the target population and impact on disease transmission. If there is a fitness cost associated with a refractoriness effector gene, recombination between it and the driver will eventually lead to fixation of the driver alone without the effector gene, with no long-term impact on vector competence.

A second major issue to take into account is the response of the target organism (i.e., the *Plasmodium*), to the anti-pathogen trait. This “trait” will be a protein that either prevents development of the parasite or kills it. The pressure on the parasite population will undoubtedly elicit a response given the time that the population replacement process may take. A mutational event that enables the parasite to negate the effects of the anti-pathogen trait will rapidly be selected for despite the ongoing gene driving process. Unfortunately, in contrast to the other two factors mentioned above, parasite response to selection pressure in the field is not a “researchable topic” and may only be studied under artificial laboratory conditions. Hopefully, targeting the *Plasmodium* population with parasitocidal compounds will not result in the development of resistance as occurred when the vector population was targeted with insecticidal compounds.

Fitness of GM mosquitoes, the search for appropriate genetic drive mechanisms, and the response of the parasite will remain challenges for the foreseeable future. However, in line with the confidence expressed by the majority of the respondents during the debate in Yaoundé, we are of the opinion that improved understanding of insect transformation and mosquito genetics, behavior, and ecology will enable the de-

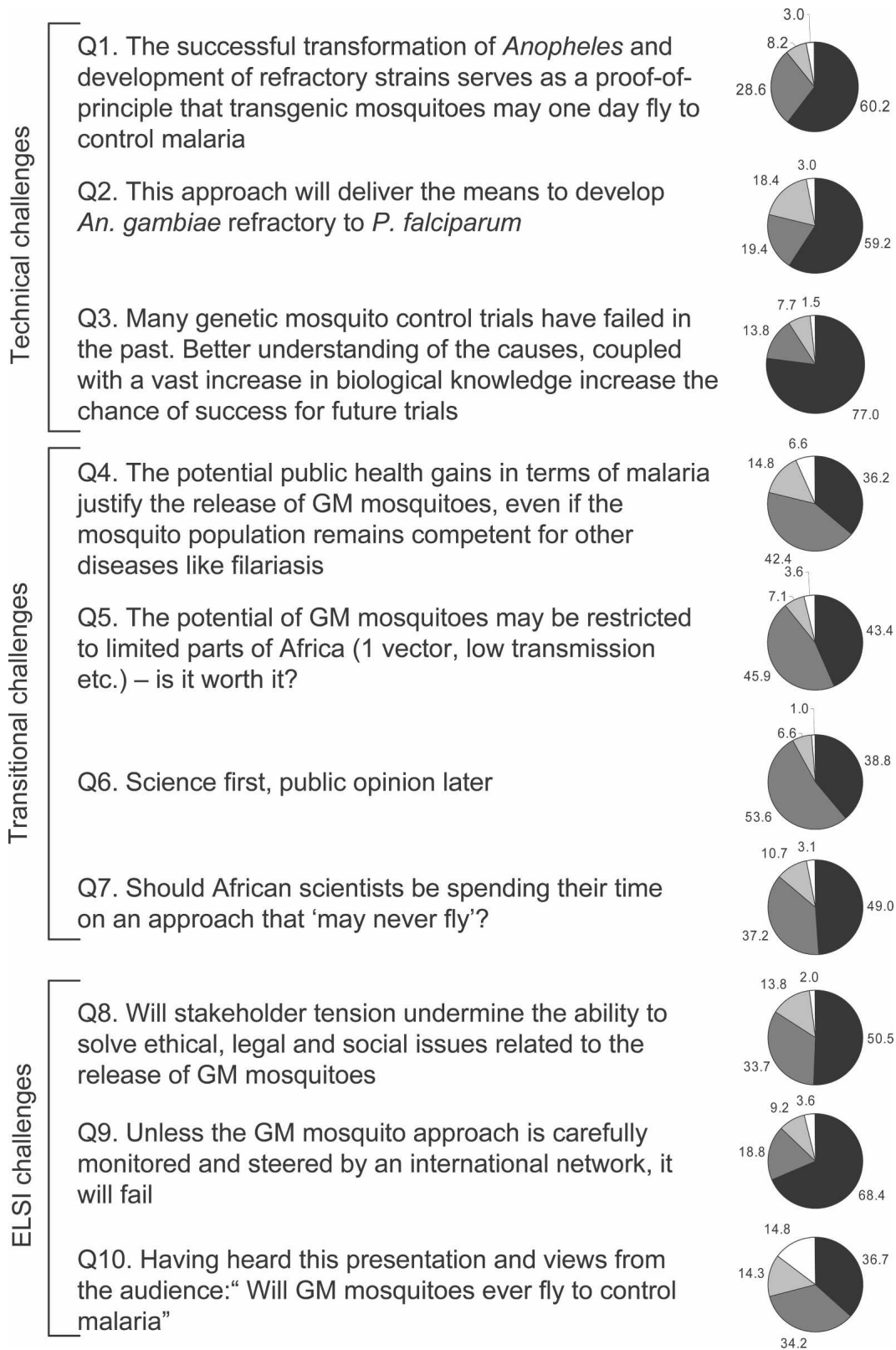


FIGURE 1. Views from the malaria research and control community ( $N = 196$ ) in response to statements presented during a plenary debate at the 4th MIM conference held in Yaoundé, Cameroon, in November 2005. The debate was titled: “Is the transgenic mosquito as a weapon against malaria ever going to fly?” Black, yes; dark gray, no; light gray, no opinion; white, no answer.

velopment of GM mosquito strains worthy of testing beyond the confines of the laboratory.

### TRANSITIONAL CHALLENGES

Given such accomplishments, a new array of challenges emerges.<sup>52</sup> The complexity of these form the interface between the technological and the ELSI described below.<sup>53</sup> A stepwise approach has been proposed for fitness evaluations,<sup>46</sup> and this model can be expanded to include components of the transitional change to bridge laboratory and field research (Figure 2).<sup>34</sup> In furtherance to previous studies on transgenic insects, such as the pink bollworm,<sup>54</sup> which were limited by tethering the insects and cutting off their wings, the endpoint of studies on GM mosquitoes will be their full evaluation in contained semi-field environments in a DEC setting. At least two of the four Grand Challenges in Global Health projects on genetic control of disease vectors (although these focus on *Aedes* rather than anopheline vectors)<sup>55,56</sup> aim for this goal before 2010. Although this target is clear and restrictive in the sense that concerns related to open-field releases can be controlled, it does pose additional issues, particularly the choice of DEC setting.

**Field site selection.** It seems logical to select field sites on the assumption that, given successful evaluation of novel strains in semi-field environments, GM mosquitoes will be

released in the country and the environment in which such evaluations took place. Beyond the stipulated criteria that should govern site selection,<sup>57,58</sup> such as geographic isolation, genetic make-up of the vector population,<sup>59</sup> disease transmission intensity, etc., which in essence match those of other genetic control approaches such as SIT, broader concerns related to future open-field releases emerge. First, it must be considered that population replacement strategies leave a population capable of vectoring other pathogens (e.g., filarial worms). More than 40% of the respondents considered this scenario unjustifiable (Figure 1, Q4). Second, these prerequisites limit the scale and scope of opportunities. There are very few sites in Africa with sufficient ecological or physical (e.g., islands) isolation and hypoendemic malaria transmission by a single vector species where evaluation may take place. Our effort to develop SIT against *An. arabiensis* has (thus far) led to the identification of just two sites with appropriate conditions (Northern Sudan and the island of La Réunion). However, despite the high levels of investment in genetic control strategies (still a fraction of the global investment in drugs and vaccines R&D)<sup>60</sup> and the (possible) limited applicability of these in sub-Saharan Africa, 45% of the respondents consider the endeavor worthwhile (Figure 1, Q5). We share this view, because experiences in proof-of-principle settings may serve as the basis for expansion of the approach to more intricate settings, as has been experienced with SIT against tsetse flies (with the initial elimination of *Glossina austeni*

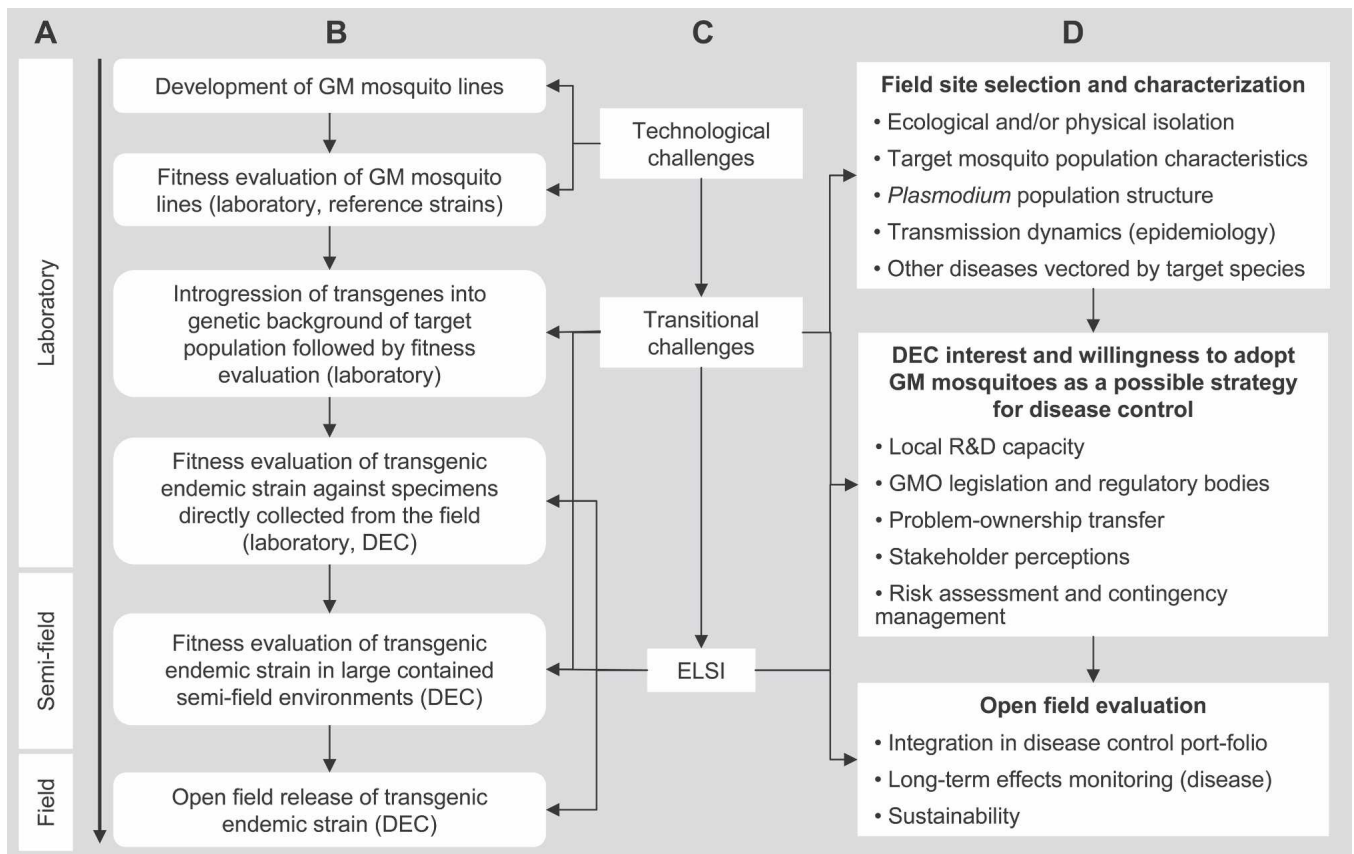


FIGURE 2. Staged progression of research toward field implementation of GM mosquitoes from laboratory to open field settings (A), the model proposed for fitness evaluation of transgenic mosquito lines (B; after Scott and others),<sup>46</sup> and transitional and ELSI issues (C) affecting field site selection and open field releases (D).



from the island of Unguja, Zanzibar,<sup>61</sup> followed by current expansion of activities in more complex settings on mainland Africa).

The key point addressed here is that the selection of field sites, even with the intermediate aim of semi-field evaluation of GM strains, requires commitment planning based on wider issues (e.g., biotechnology policies, public opinion) that may be present in the DEC setting of choice (Figure 2). One half the respondents shared this view (Figure 1, Q6).

**Problem-ownership transfer.** Given the novelty of the approach, commitment planning becomes essential. Commitment here refers to the willingness of DEC to first evaluate and ultimately adopt the approach as a possible tool for malaria control to augment their established strategic portfolio of disease control methods (e.g., ITNs, IRS). Clearly this will depend on the effectiveness of the proposed strategy and outcome of laboratory evaluations. The likelihood of adoption, in turn, will increase with investment in knowledge and skills acquisition (or capacity and capability building) in the DEC with regard to the GM mosquito approach.<sup>34,62</sup> The ultimate aim is to transfer problem-ownership to the DEC. This model is now being used by the IAEA when developing area-wide integrated SIT programs against tsetse flies in collaboration with its Member States (MSs). Beyond the technical support provided in the form of expert advice, training (through fellowships), and provision of equipment, MSs bear full responsibility for the development and implementation of the programs. The inherent implication here is that DECs, *ad in-*

*terim*, will engage their often limited human and financial resources towards research rather than control efforts. Interestingly, one half the respondents favor this (Figure 1, Q7) and apparently see maturation of equality in terms of competence and partnership as beneficial.

Beyond problem-ownership transfer and, given the controversial nature of genetically modified organisms (GMOs), the establishment of an international and independent coordinating entity has been called for repeatedly.<sup>53,57,63,64</sup> This body could coordinate research efforts, focus on the broader dissemination of scientific progress to multiple stakeholders, and facilitate collaborative efforts and partnership strengthening within and beyond the scientific community. The establishment of a guiding coalition may safeguard against potential antagonism and ensure integrity of all those involved during the transitional and implementation phases of the GM mosquito endeavor. The proposed constituency of this coordinating entity is shown in Figure 3. Executive responsibility, in accordance with problem-ownership transfer, lies with experts, regulatory and executive authorities, and WHO officials in DECs, with additional (international) expertise serving in an advisory capacity. As transition from the laboratory to the field proceeds, the committee evaluates scientific progress and gives recommendations for subsequent research. Although this committee is not yet in existence, nearly 70% of the respondents agreed that its absence in this or any other suitable format and constituency could lead to failure of the GM mosquito endeavor (Figure 1, Q9).

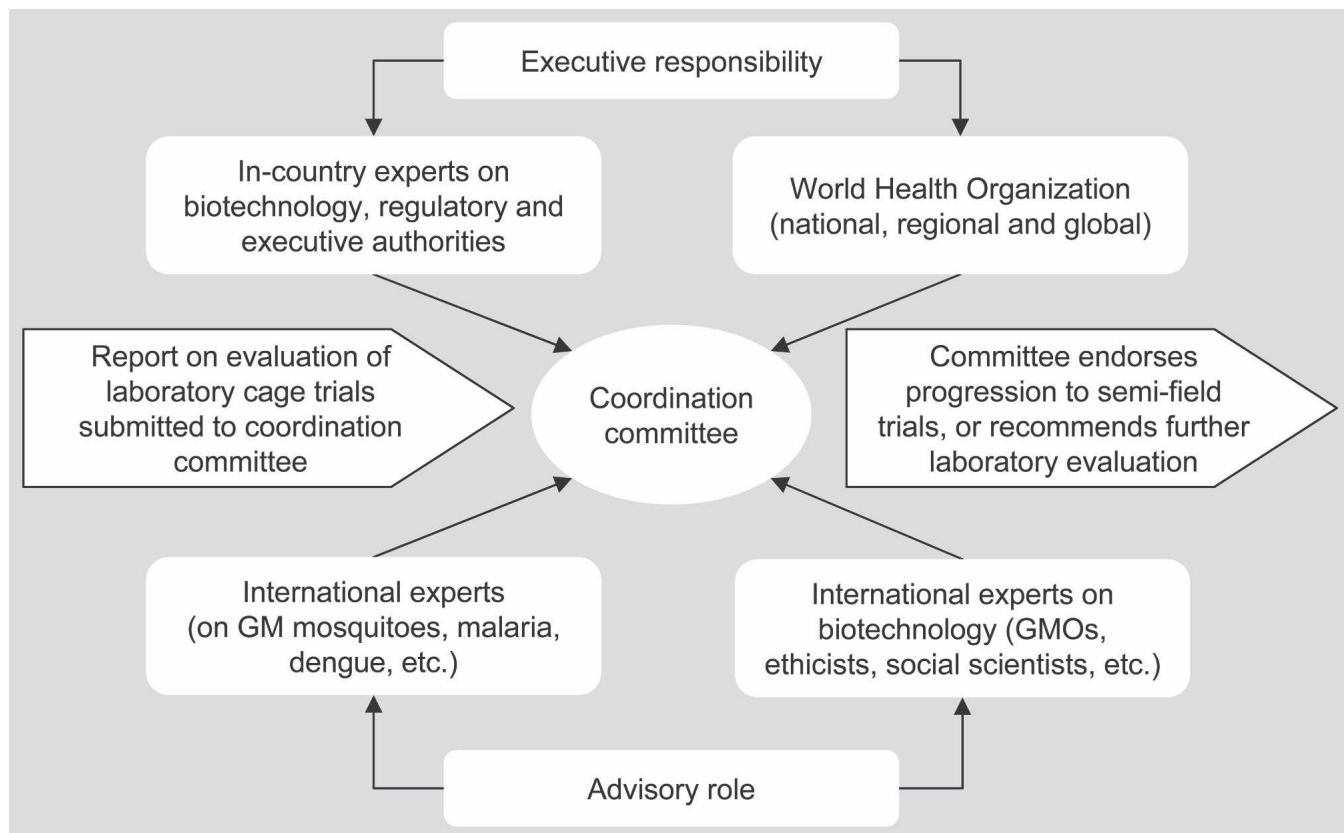


FIGURE 3. A proposed mechanism for coordinating research on GM mosquitoes (modified after Knols and Bossin<sup>57</sup>). A coordination committee consisting of expert representatives from DECs where trials are planned, supported by in-country, regional, and global World Health Organization experts takes executive responsibility for phased progress evaluation and decision making is augmented by outside expert views in an advisory capacity.

**Containment and risk management.** With robust and suitable transgenic strains available, appropriate DEC settings and problem-ownership transfer, and a guiding coalition in place, trials in contained semi-field systems pose the next set of challenges. Although guidelines for the handling, transportation, and laboratory confinement for transgenic mosquitoes have been developed,<sup>65</sup> no such guidelines are currently available for similar activities (and semi-field research) in DECs. It is to be expected that research on GM mosquitoes in their endemic environment will require heightened levels of containment, and a task force is currently drafting such guidelines (A James, personal communication). Clearly, such guidelines will have to amalgamate international, national, and perhaps local guidelines.<sup>66</sup>

Semi-field research (i.e., strain evaluation in large outdoor enclosures) has been undertaken since the early days of genetic mosquito control<sup>67</sup> but has only recently been used for research on African anophelines.<sup>68</sup> A clear challenge, required for the evaluation of mosquito fitness, will be the establishment of self-perpetuating populations with overlapping generations in such enclosures. The concomitant exposure of such populations to *Plasmodium* infections to evaluate their refractoriness will pose additional hurdles and will have to use an artificial bloodfeeding system for this purpose.<sup>69</sup> Experience with this type of research is still limited, as are issues related to the physical (i.e., structural) and biologic containment (e.g., release of sterilized or otherwise reproductively impaired specimens) or inadvertent release of GM mosquitoes (i.e., the response to accidents).

Risk, which is the product of an identified hazard (i.e., the degree of severity of the adverse consequence) and the likelihood of that hazard actually occurring,<sup>70</sup> and risk management (reducing the likelihood of hazards or mitigating the impact of adverse consequences) form a crucial part of research on genetically engineered organisms. Such risks can be probabilistic, hypothetical, or speculative. Only the former two classes are amenable to study, because at least the hazard is known or can be assumed to exist on scientific grounds. Only in such cases can risk assessment progress through scientific research. We have recently developed a risk classification matrix for research on transgenic mosquitoes in semi-field systems based on 1) the reproductive ability of the strain tested, 2) the genetic drive system used, and 3) the nature of the effector molecule (M Benedict, H Bossin, and B Knols, unpublished data). This indicates that fertile insects carrying genetically engineered transposable elements to drive novel anti-pathogen traits will require the highest containment levels. The nature of the vector used for gene transfer and its mobility properties both intra-genomic and inter-genomic (i.e., lateral or horizontal transfer) are thus of primary importance in risk assessment.<sup>71</sup>

**Stakeholder involvement.** Most publications that focus on ELSI (see below) advocate the involvement of the broader stakeholder community at a time when open-field releases are foreseen.<sup>72–74</sup> Here we also underscore the importance of initiating this process earlier, during the transitional stages, when planning semi-field systems research. Even if this research will be undertaken in isolated areas in DECs, it will not go unnoticed in the community, press, etc., and requires careful management. Research on (non-transgenic) mosquitoes in large outdoor enclosures in Kenya and Tanzania<sup>56</sup> has raised considerable concern (if not anxiety) in nearby communities

(H Ferguson and G Killeen, personal communication), and this is likely to be more so in research involving GM mosquitoes (Figure 1, Q8 and Q9). Potential risks involved, even when considered relatively small (such as the ability of transgenic mosquitoes to vector other diseases), may be perceived and viewed in a different light outside of the scientific community.

## ETHICAL, LEGAL, AND SOCIAL ISSUES

It is noteworthy that the ethical, legal, and social issues surrounding the application of GM mosquitoes are nearly always featured last in publications on the topic (including this one).<sup>32–37</sup> Given the controversies on GMOs, this is hardly surprising, and it is often argued that basic research should provide the answers needed to dampen the debate and the risk of polarization.<sup>75</sup> Research is the only route through which risk assessment and future evaluation of the public health benefits of the approach can progress. The view, held by 34% of the malaria research and control community, that the approach “will never fly” (Figure 1, Q10) is therefore both surprising and worrying at the same time. It indicates that, even in an informed group of malariologists, skepticism remains strong over acceptance and tolerance of ambiguity and uncertainty that accompanies (any) scientific endeavor. It has taken four decades of research to develop promising malaria vaccines and 20 years from the first application of insecticide on a bednet to the currently available long-lasting impregnated nets that are being introduced in Africa on a large scale. What matters here is that views on GM mosquitoes are not likely to be viewed in isolation, but as part of a wider debate over developments in biotechnology (i.e., GM plants,<sup>76</sup> stem cell research, animal cloning, etc.) and the perception of these, fueling skepticism and/or antagonism. Seeking coherence and agreement within the malaria community to avoid further polarization is thus called for. The coordination committee described above (Figure 3) could well undertake this task.

Beyond internal stakeholders (i.e., the malaria research and control community), a much broader framework of stakeholder groups, affected by or able to affect the GM mosquito endeavor, can or might exert their influence in the foreseeable future. Their importance and necessary inclusion in various capacities cannot be overemphasized and yet has received only marginal attention to date. Stakeholder influence has increased dramatically over the last few decades, enforced by the internet and other modern communication tools, enabling criticism to emerge from all corners of society and not just through the press. With beliefs and values already stretched by the global GMO debate, the pace with which government-imposed, novel, yet controversial, initiatives in several instances is taking place in DECs is likely to be affected. Failure to involve key stakeholders at a sufficiently early stage may lead to the disruption, or worse, to the termination of contained and open-field trials of GM mosquitoes. The history of genetic control trials against culicine mosquitoes in India in the mid-1970s<sup>77,78</sup> shows how opposition can have far-reaching consequences. After several years of work on field testing of the mating competitiveness of sterile male mosquitoes,<sup>79</sup> accusations that the project was meant to obtain data for biologic warfare using yellow fever were launched in the

press and taken up by opposition politicians. Shortly afterward, a well-prepared attempt to eradicate an urban *Ae. aegypti* population by sterile male releases was banned by the government of India 2 days before its launch.<sup>80</sup> Given that only (non-biting) males were to be released, no data relevant to biologic warfare could have been collected, yet such rumors about this project still persist.<sup>81</sup> Reluctance of WHO at the time to intervene in what they viewed as an internal Indian political matter has been listed as a reason why the situation escalated,<sup>82</sup> hinting once more at the need for an impartial global coordinating entity for genetic control trials. A clear task would be to develop stakeholder power matrices<sup>83</sup> to estimate the power and legitimacy each stakeholder has in terms of influence and claims, from which the potential impact on the purpose of research trials can be deducted. Figure 4 uses this approach to understand the opposition Monsanto faced when intending to enter the European market with GM crops.<sup>84</sup> Similarly, it addresses the position of key stakeholders in a GM mosquito project whereby the likely position of non-governmental organizations (NGOs), the general public, etc., may change over time in search for increased power. The role of DEC partner institutions and their governments will become critical in managing the position of these groups, highlighting the need for problem-ownership transfer and empowerment of DEC partners.

The role of (currently disempowered) target communities inhabiting areas earmarked for possible future releases of GM mosquitoes requires special attention. Several challenges emerge here. First, some societies or parts thereof may be more willing to accept evaluation of the approach in their surroundings than others. This results in a paradox: should biologic criteria (see above) outweigh social acceptance or *vice versa*? Congruence between the governing variables of these criteria is preferred but may not be assumed to be in existence at present. Second, the nature of genetic control interventions (per definition area-wide) will affect all citizens in release areas, requiring informed community consent.<sup>85</sup> The widely advocated involvement of target communities in field evaluation of the approach is likely to be influenced by a debate on the uncertainties and ambiguities related to it in view of existing alternative vector control tools with proven efficacy. If malaria eradication in isolated settings (e.g., islands) can be accomplished through integrated disease management (e.g., mass chemotherapy combined with ITNs),<sup>86</sup> application of alternative approaches with unknown efficacy will require careful evaluation from an ethical perspective. The boundaries for this consent, moreover, are blurred. The ability of vectors to disperse actively and/or passively (e.g., on cars, boats, aircraft) places any trial beyond the confines of a given spatial dimension and must be fully recognized. It is likely that acquisition of consent for open-field releases will be initiated centrally and follow international and country-specific guidelines. Subsequently, community consent may be obtained through community consultation of inhabitants of ear-marked areas and take the form of intense dialog with elected representatives of those communities, based on local governance frameworks.

Of particular concern is the epidemiologic consequences of reductions in transmission intensity and how this may affect the development of immunity to malaria and the age at which infections are acquired.<sup>87</sup> Discussions on this issue have been widespread with regard to currently used vector

control interventions (such as ITNs)<sup>88</sup> and center around pre-intervention levels of transmission (and related levels of immunity), incomplete efficiency of refractoriness,<sup>89</sup> and/or collapse of the transgene system. In the latter case, or with the introduction of competent vectors into areas freed of malaria for extended periods of time, the likelihood of epidemics is real. It is clear, therefore, that the introduction of GM mosquitoes will necessitate long-term monitoring of the impact on malaria epidemiology.

With only few surveys having been undertaken to assess the viewpoints from communities regarding the use of GM mosquitoes,<sup>90</sup> the current and future attitudes toward the approach may be influenced by global developments in the field of biotechnology and require careful analysis to avoid repetition of negative past experiences.

## FUTURE PERSPECTIVES

In the above sections, we described multiple challenges that the GM mosquito endeavor will face in the coming years. Open-field releases are not anticipated in the next 5–10 years, during which time the arena of malaria and its control will undergo changes that will affect the likelihood of implementation. Although forecasting is often inaccurate, some salient points may be emphasized that can either positively or negatively affect GM mosquito strategies. For instance, by 2025, 90% of the world's population will live in developing countries,<sup>91</sup> and 52% of the African population will live in urban environments, of which 300 million will be in slums.<sup>92</sup> Environmental modification and/or degradation may lead to reduction of malaria, and high population densities may result in a shift of interest toward case management, personal protection measures, or larval control.<sup>93</sup> On the other hand, genetic control strategies may become particularly attractive as operations over relatively small areas can achieve maximum impact in number of people protected.<sup>94</sup> Moreover, it has been observed that urban areas harbor island populations of one species (e.g., *An. stephensi* and *An. arabiensis*) surrounded by another species in rural settings (*An. culicifacies* and *An. gambiae* s.s., respectively), essentially rendering the urban species an isolated population.<sup>95</sup>

Over the last 15 years, dramatic progress has been made in the development of malaria vaccines,<sup>96</sup> and availability of these will probably affect the future of vector control interventions negatively. Given the efforts to increase uptake of ITNs in much of sub-Saharan Africa, problems with insecticide resistance will presumably increase, necessitating alternative vector control strategies. At present, there seems to be little to offer beyond the ITN/IRS era, which may influence the perception and willingness to apply genetic control strategies favorably. Whether ongoing globalization will dissolve the current differences in perception of GMOs remains to be seen, yet it seems unlikely that complacency and acceptance will prevail in all corners of society. Preparedness for antagonism to avoid another incident like the India example described above is essential, yet little has been done at present in this direction. Any genetic control trial receiving bad press and opposition is likely to affect similar efforts worldwide, with the real risk of delaying implementation of strategies that potentially may save thousands of lives every year. Holistic and integrative approaches, although tedious, time-

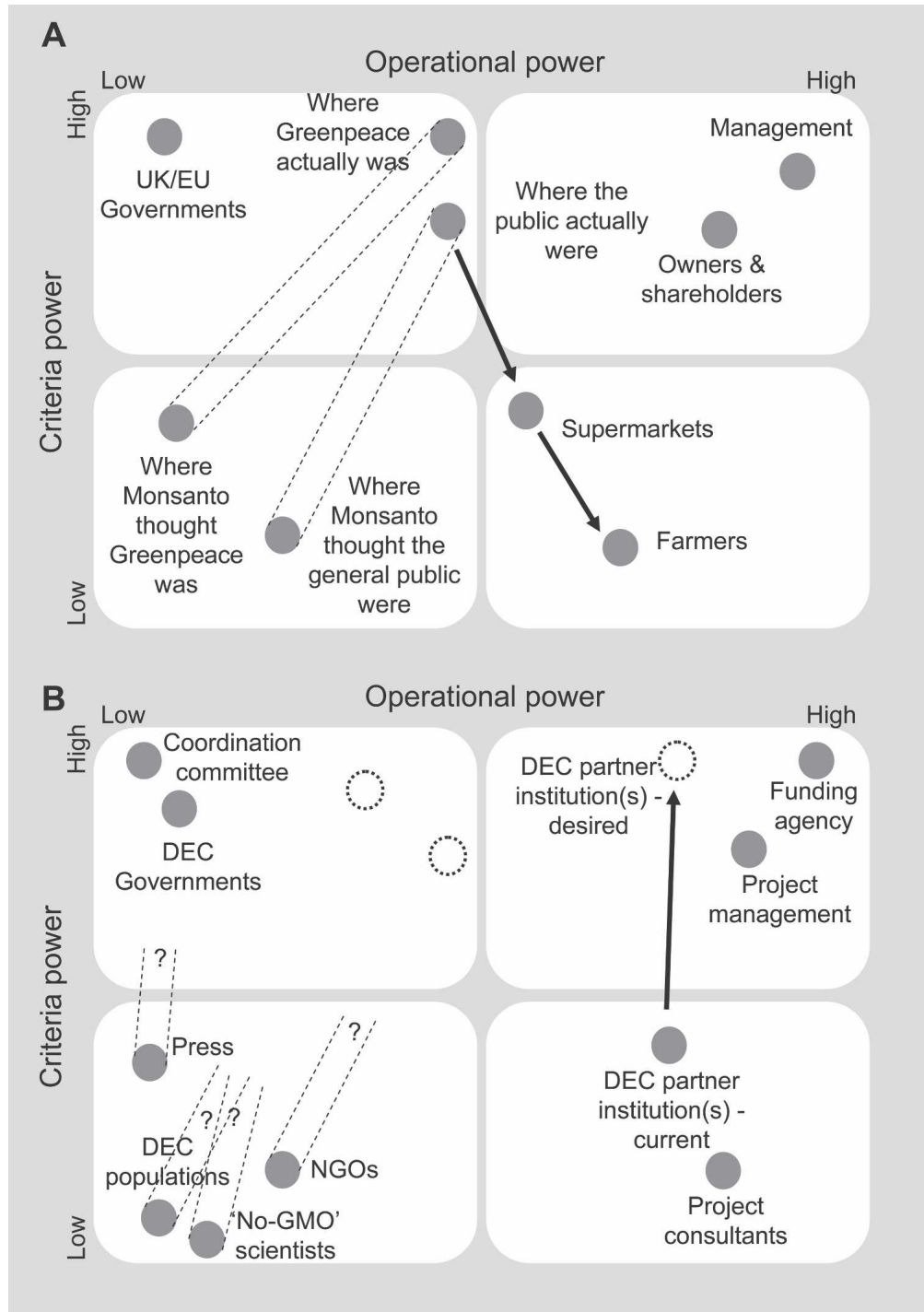


FIGURE 4. Stakeholder power matrices plotting the position of key stakeholders for the Monsanto case (A) with regard to criteria power (i.e., the power to define goals, aims, and purposes of Monsanto) and operational power (i.e., the power to influence the operations of Monsanto and its allocation of a range of resources). Arrows indicate the flux in influence and outcomes (Greenpeace affected consumer demands, in turn influencing willingness of supermarkets to sell and farmers to produce GM crops). A similar diagram for a hypothetical project working toward releases of GM mosquitoes for disease control (B) highlights the need for strengthening the position of DEC partner institutions (through problem-ownership transfer and capacity building) to avoid similar antagonism of currently disempowered stakeholder groups.

consuming, and resource-intensive, are needed to move genetic control trials forward in a manner that maximizes support of all stakeholders involved, with the greatest chance to properly assess the merits in terms of public health benefits. Current efforts focus mainly on the technical challenges listed

above, and good science and persistence are key ingredients to face these. Resolving transitional and implementation challenges may prove much more complex and time consuming.<sup>97</sup> However, these will ultimately determine whether “transgenic mosquitoes will ever fly to control malaria.”



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