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Transgenic Mosquitoes for Malaria Control: From the Bench to the Public Opinion Survey

Christophe Boëte and Uli Beisel

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1. Introduction

The recent field releases of genetically modified mosquitoes in inter alia The Cayman Islands, Malaysia and Brazil have been the source of intense debate in the specialized press [1, 2] as well as in the non-specialized mass media. For the first time in history (to our knowledge), transgenic *Aedes aegypti* were released in the Cayman Islands in 2010 by a private company, Oxitec, in collaboration with the local Mosquito Research and Control Unit (MRCU) [3]. The releases were followed by other releases in Malaysia in 2010/11 and then in Brazil in 2011 [4]. While the releases in Malaysia and Brazil were publicised beforehand, the releases in The Cayman Islands were only announced publicly one year after the fact [1, 5]. This lack of transparency, not to say the secrecy, in the way the first trial was conducted is without much doubt the major reason for the controversy that emerged. Brushing aside years of discussion in the scientific world and a shared recognition of the importance to consider ethical, legal and social issues this first trial could be read as a fait-accompli: the cage of transgenic mosquitoes has now been opened [6]. Oxitec faced harsh criticism for these releases, both within the scientific community, as well as from non-governmental organisations, such as GeneWatch that accused the company of acting like "a last bastion of colonialism". A vector-borne diseases method for control has rarely been the subject of such discussion not even concerning its potential efficacy at reducing the burden associated with a vector-borne disease.

Focusing on malaria control, this chapter reviews the major technological milestones associated with this technique from its roots to its most recent development. Key-points in the understanding of mosquito ecology are going to be presented, as well as their use in models whose major aim is to determine the validity of the transgenic approach and to help designing successful strategies for disease control.

Furthermore, the ethical and social points related to both field trials and wide-scale releases aiming at modifying mosquito populations (and thus controlling vector-borne diseases) are going to be discussed as well as the question of public engagement and the role scientists might play in fostering debate and public deliberation. While large part of the laboratory research is done in the Global North, most of the vector-borne diseases are endemic in the Global South. We suggest that the geopolitics related to the genetically modified (GM) mosquitoes as well as the specificity of Southern contexts needs to be considered when discussing the application of this technology.

2. Why acting on the vector population: How efficient are transgenic methods for malaria control?

When discussing the epidemiology of malaria the gold standard is the description of the R_0 [7-9]. Focusing on the vector compartment suggests that the spread of malaria can be curved either by reducing the mosquito population or by decreasing their vectorial capacity. In other words, one either aims to decrease the number of mosquitoes or to make them less efficient in transmitting the parasites. These two strategies can both be addressed by vector control including through a transgenic approach: population reduction or population replacement. However, when looking closely at R_0 one can notice that the parameters that are affected by those strategies are not the most likely ones to curve transmission efficiently. The mortality of mosquitoes (μ) and their biting rate (a) are indeed affecting R_0 in an exponential and in a quadratic manner respectively. In this respect, they are the parameters whose modifications affect R_0 and consequently the human prevalence mostly (see Box 1). This means that modifying a linear parameter is less likely to lead to a drastic change in malaria epidemiology. For example halving the vector population density (m) is going to reduce R_0 by two but because of the non-linear relationship between R_0 and the human prevalence (y) the decrease of the latter one is not going to be affected in such a manner especially in a context of high transmission.

3. Technology: What has lead to GM mosquitoes for malaria control?

The roots of the technology can be traced back to the early 80's/90's when the knowledge gained in genetics in *Drosophila* research sparked the development of new tools in the fight of vector-borne diseases. The plan was straightforward with three milestones to be achieved in a decade: i) the stable transformation of *Anopheles* mosquitoes by 2000 ii) the engineering of a mosquito unable to carry malaria parasites by 2005 and iii) the development of controlled experiments to understand how to drive this genotype of interest into wild populations by 2010 [10].

Regarding malaria most recent research has concentrated on the development of an *Anopheles* strain that has the ability to interrupt transmission through the synthesis and production of molecules able to block the development of the parasite. A few years ago, the SM1 peptide was shown to reduce malaria oocysts number by about 80% [11]. More recently, it was

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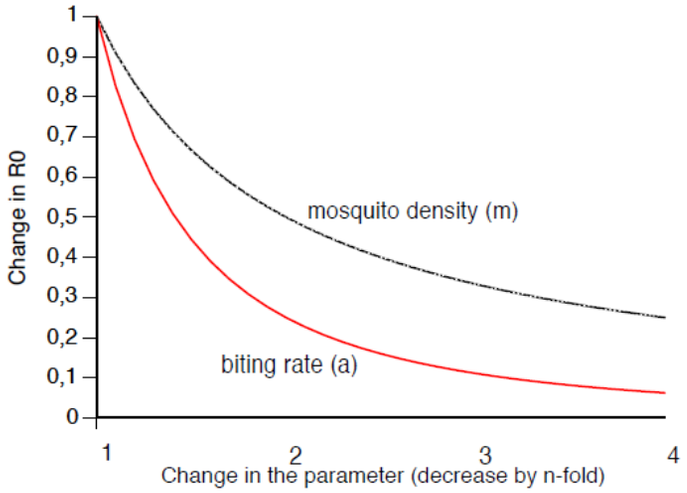
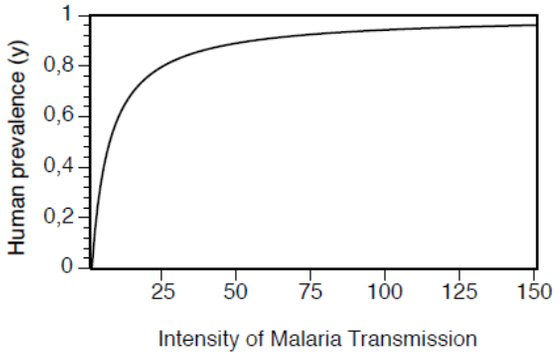
$$R_0 = \frac{ma^2 b_1 b_2 e^{-\mu T}}{r\mu} \quad \text{(equation 1)}$$

m : Number of mosquitoes per host.
a : Biting rate of mosquitoes on their host.
μ : Mortality of adult mosquitoes
T : Incubation time of the the parasite in the mosquito vector
r : Recovery rate of humans
b1 : Infectiousness of hosts to mosquitoes.
b2 : Susceptibility of humans



Evaluating the impact of several parameters on R0 permits to determine that a decrease in the biting rate is affecting R0 in a greater manner than a change of the same magnitude in mosquito density. The biting rate (a) appears indeed quadratically whereas the mosquito density (m) is a linear factor in equation 1.

$$y = \frac{R_0 - 1}{R_0 + \frac{a}{\mu}} \quad \text{(equation 2)}$$



Box 1. The Ross-MacDonald model permits to describe R0 which is the number of secondary case arising from a single one in an otherwise uninfected population (Macdonald 1957; Koella, 1991). It permits to determine the relative importance of the different parameters implicated in the transmission of malaria (equation 1). From the R0 value, a simple expression permits to determine the prevalence in the human population (equation 2). As seen on the graph above, only a large decrease in the intensity of transmission (estimated by R0) can affect significantly the human prevalence (y).

synthesised from a transgenic entomopathogenic fungi [12], this later one is by-itself (in its natural version) already considered as a potentially interesting method to develop [13-15]. Other potential solutions currently developed rely on single-chain antibodies [16-18]. Using the $\varphi C31$ integration system for the first time in *An. stephensi* it is now possible to insert the transgene of interest in a permanent manner at chromosomal 'docking' site using site-specific recombination and to have a tissue- and sex-specific expression. The authors have then shown that the prevalence and number of oocysts decreased when the transgenic mosquitoes were

challenged with *Plasmodium falciparum* [17]. If technology has been able to determine how the insertion of a transgene can be made to change a vector to a quasi non-vector, the next question to answer concerns the spread of this construction in natural populations of mosquitoes.

4. Mosquito ecology: First hurdle at the door of the Lab

When the ecological and evolutionary issues related to the potential use and impact of *Plasmodium*-resistant transgenic mosquitoes started to be discussed about a decade ago [19, 20], most studies aimed at providing information on the fitness of genetically-modified mosquitoes were based on the use of natural mosquito immune responses as a model system. This was mainly driven by the fact that using the natural immune system of mosquitoes in a transgenic approach was considered of some potential interest [21], and also because the only fully effective system against malaria parasite was the melanization response (also known as melanotic encapsulation) in selected lines of mosquitoes [22]. The mechanism leading to the death of the parasite because of melanization remains unclear. It seems that death can occur because of starvation (by isolation from the hemolymph) as well as because of the cytotoxic function of melanin [21, 23]. The melanization response was then considered as a model of what could happen with an artificial peptide mimicking an immune response and thus aiming at reducing the number of parasites in the mosquito.

Before considering the cost associated with resistance that could impair the spread of resistance in mosquito populations, it is important to notice that the sole insertion of an exogenous gene (not even conferring any anti-parasitic advantage) leads to a drastic decrease in *Anopheles stephensi* fitness [24]. However, recent work with site-specific insertion seems to bring a less negative outcome in term of fitness [18]. This even seems to be the case when all different groups including the control group (called wild) derive from a lab colony and the fitness reduction due to the colonisation process is probably significant. Concerning the cost of resistance, mosquitoes are no exception and reduced fitness associated with the absence of parasite can be observed. Thus, several studies have measured the associated cost in *Anopheles stephensi* carrying a transgene conferring resistance again the rodent malaria parasite *P. gallinaceum*. Regardless if resistance was provided by the expression of SM1 (termed for salivary gland- and midgut binding peptide 1) [25] or the phospholipase A2 gene (PLA2) [26], a fitness cost was associated with it. Even in conditions where harbouring an allele conferred an advantage i.e. when mosquitoes were fed on *Plasmodium*-infected blood, the SM1 transgene could not reach fixation revealing that the benefit of resistance was counterbalanced by the cost of resistance in the transgenic homozygotes [27]. In any case the construction needs to follow a couple of requirements for the promoter and the gene of interest for the method to have some chances of success [28]. The gene of interest needs to express in a temporal manner i.e. after a blood-meal is taken, but also only in the tissues where it could efficiently impact the parasite life cycle, such as the midgut epithelium and the salivary glands.

Recent work on GM mosquitoes have also been done with *Aedes* that are not resistant towards a pathogen but that are carrying a gene that makes nearly all their offspring non-viable in a

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natural environment [29-31]. To date such a strategy has not been developed for the *Anopheles* genus.

For the strategy considering the replacement of malaria vector by their modified non-vector version, this question of a cost associated with resistance leads necessarily to the idea of the need to use a driving system in order to favour the spread of resistance in natural populations of mosquitoes.

5. Driving an allele of interest in natural populations of mosquitoes

The idea of using a gene drive to affect the epidemiology of vector-borne diseases is not a recent idea as the use of chromosomal translocation to reduce mosquito populations was already proposed in 1940 by Serebrovskii [32]. It was revived later with the idea to use those translocations to drive alleles conferring refractoriness in mosquito populations [33].

Thus the spread of refractoriness in mosquito populations could be facilitated if the allele, conferring resistance but also associated with a cost, was linked with an element whose spread is not Mendelian. One of the techniques for which various models provide information is the use of transposable elements. A tandem made of a transposon and an allele of interest can spread easily and fixation can be reached [34, 35], even if the cost of resistance is particularly high [36].

Using intracellular bacteria associated with cytoplasmic incompatibility, such as *Wolbachia*, is also an idea that has been explored. Modifying them so that they could harbour the allele of interest would permit, at least in theory, to favour the spread of the allele of interest [37, 38]. There is no natural infection of *Anopheles* by *Wolbachia* but work is in progress trialling infections of *Anopheles gambiae* cells by *Wolbachia pipientis* (strains wRi and wAlbB) in the lab [39]. However, up to now no such sustainable transformation has been done [40].

Other constructions that would favour the spread of resistance have also been considered [41, 42]. Among them the use of HEG (Homing Endonuclease Genes) has been the centre of a lot of attention in the last years [43-45]. Apart from those systems another approach relies on the use of pairs of unlinked lethal genes. In this case, each gene is associated with the repressor of the lethality of the other one and this system is called engineered underdominance [46]. With respect to those methods a number of recent papers have been focusing on theoretical work aiming at spreading an allele conferring resistance as well as containing it. If the aim of a GM approach is to favour the spread of an allele conferring resistance it is also important to consider that self-limitation could be a real advantage to avoid the establishment of the transgene in non-target populations. Such an approach has been studied in theoretical analysis with the *Inverse Medea* gene drive system [47] and with the *Semele* one [48].

If the speed at which the construction of interest can spread in mosquito populations is a major issue, authors have also shown that in the case of the use of transposable elements one of the problems is the stability of the system with the probability of disruption [49].

However, if the spread of an allele conferring resistance is a target that can be reached, the real aim should be a strong decrease in the prevalence of the disease or even its elimination. Two models merging population genetics and epidemiology have pointed out the major importance of the efficacy of resistance [36, 50]. They have shown that a significant reduction in malaria prevalence can only be obtained if the efficacy is close to 1 especially when a release of resistant mosquitoes is done in high transmission areas.

If recent work claims that the engineered-mosquito do not suffer too much from carrying a resistant allele [17], this remain only valid under lab conditions where environmental conditions remain fairly stable and usually favourable. It is interesting to note that the survival of the mosquitoes in Isaacs et al. study reaches about 35 to 40 days which is probably far more that what happens under natural conditions.

As shown with natural immune responses, environmental conditions experienced at the larval or at the adult stage can greatly affect the host-parasite interactions and thus the outcome of an infection [51]. A reduction of 75% on food availability at the larval stage in lines selected for refractoriness [22] leads to a decrease in the proportion of the mosquitoes able to melanize half of the surface of a foreign body (a Sephadex bead) of more than 50% of it [52]. Even more worryingly, a recent paper [53] revealed the complex effects of temperature on both the cellular and humoral immune responses on the malaria vector *Anopheles stephensi*. What is highly interesting in this study is that not only temperature can affect immune responses but also that different immune responses are affected in different manners by temperature. The authors have studied the melanization response, the phagocytosis (a cellular immune response that lead to the destruction of small organisms or apoptotic cells) and the defensin (an antimicrobial peptide) expression. The three of them are higher at 18°C while the expression of Nitric Oxide Synthase (active against a large number of pathogens [54]) peaks at 30°C and the one of cecropin (an antimicrobial peptide) seems to be temperature-independent. Concerning melanization it is important to note that if the melanization rate is higher at 18°C, the percentage of melanised beads -introduced inside the mosquito to measure its immunocompetence- (at least partly) was higher when the temperature increased (fig. 1).

This result highlights the difficulties to define what is an optimal temperature for the melanization response especially as it is also involved in developmental processes. The complexity of the immune function appears also with cecropin expression that despite being independent from temperature was affected by the administration of an injury or the injection of heat-killed *E. coli*. Other works have also revealed that the immune function is affected in a complex manner by a variety of environmental parameters such as the density of conspecifics or the quality of food resources [55]. Apart from showing the need to better understand the impact of the complex interactions between temperature and other variables on the vector competence, this work also highlights the crucial importance to take them into account when determining the potential outcome of the interactions between the natural immune function, the allele conferring resistance in a GM mosquito and finally the resulting vectorial competence under a large variety of ecological conditions.

What appears to be clear is that the expression of genes involved in the anti-parasitic response are not only influenced by the sole host-parasite interactions but that the environment is a

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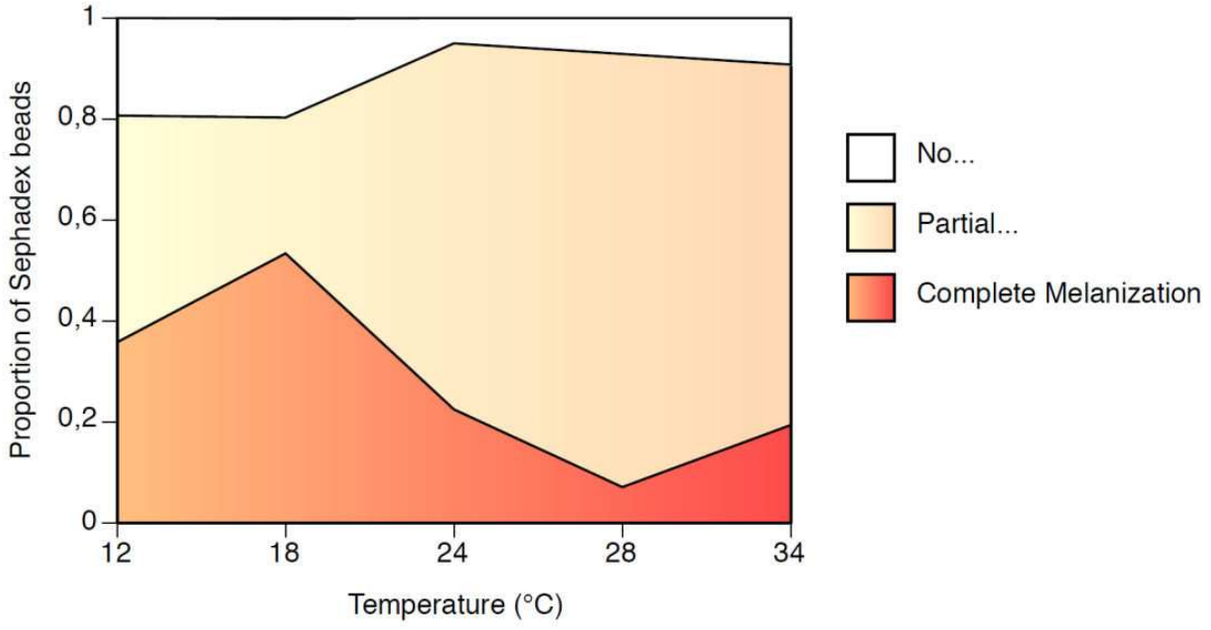

Figure 1. Influence of the temperature on the melanization response of Sephadex beads in the malaria vector Anopheles stephensi. The melanization of beads was measured 24h after the injection. The proportion of completely melanized beads was the highest at 18°C whereas the higher proportion of beads being at least partially melanized occurs at higher temperatures (modified after Murdock et al. 2012)[53].

crucial factor be it the abiotic conditions, such as temperature and its daily variations, or biotic factors, such as parasites encountered at the larval or adult stage [56, 57].

On the side of the parasite it would be naïve not to consider an evolutionary response in the face of selective pressure represented by any (natural or artificial) resistance. The quick selection of resistance against artemisinin in South-East Asia in the last years [58] and the evidence of its genetic basis [59] suggests that it is reasonable to envision the selection of parasite strains able to overcome any engineered resistance mechanism. Using transgenic *Plasmodium*-resistant mosquitoes can be considered equivalent to artificially increasing the investment of the mosquito in an immune response. Referring to some theoretical work [60] this is assumed to be followed by an increase in the parasite investment to avoid resistance. In the long term this would lead to a decrease in the effectiveness of the programme aiming at decreasing malaria prevalence or the need to 'play evolution' by monitoring the parasite population and releasing transgenic mosquitoes for which resistance could be modified as in an arm race with parasite evasion.

What is then important is to determine the longer-term of such a strategy regarding parasite virulence. Some answers have already been provided by theoretical work concerning the impact on parasite virulence to humans and mosquitoes in the case of dengue [61]. The authors examined four distinct situations: blocking transmission, decreasing mosquito biting rate, increasing mosquito background mortality or increasing the mortality due to infection; if all of them are associated with a benefit in terms of disease incidence, only the ones affecting mosquito mortality seem to pose the smallest risk in term of virulence to humans. It is important to note the scarcity of studies aiming at providing empirical data on this topic even

if experimental evolution with mosquitoes and parasite can provide interesting results in a reasonable number of generations [62]. This lack of data not only concerns dengue but also malaria as has already been discussed in a paper on possible outcomes of the use of transgenic *Plasmodium*-resistant mosquitoes [63].

6. Vector control: To be or not to be transgenic-based

As mentioned earlier one of the major points to consider with transgenic mosquitoes used for malaria control are the ethical and societal issues and public acceptance of this high-tech method. Even though the importance of societal acceptance of GM mosquitoes has been recognised for a decade [64], studies on acceptability remain scarce. One first study conducted in Mali mapped out several crucial aspects of potential acceptance or rejection of GM mosquitoes [65]. While Marshall reports that his interviewees were generally "pragmatic" about the technology, acceptance was dependent on several conditions.

If people were supportive of a release of transgenic mosquitoes for malaria control, they first wanted to see evidence of safety for human health and the environment prior to releases. In addition, proof of efficacy of the technology in reducing malaria prevalence was requested. Lastly people declared that they would prefer the trial to be done outside of their village and when comparing GM crops and GM mosquitoes, people were more sceptical of the latter. Even if this not a rejection of the idea of using a GM technology for health purpose, it is important to note that a population, even if at risk of contracting malaria, remains cautious about the idea of using such a technology. This should remind us how, in the 70's, a decade-long programme conducted by the WHO in India utilising the sterile insect technique (SIT) ended in a chaotic way after the publication of inaccurate information in the Indian press [66].

Secondly, the question of regulation has recently been highlighted as crucial [5, 67]. Because the social and environmental implications of GM mosquitoes are significant and potentially irreversible, and as the regulatory attention that GMOs have received in Europe suggests broad-based trials and releases require robust legislation and international agreements. These regulations are still under development, and it is important to note that at the time of the first releases in The Cayman Islands international guidance on open field releases of GM mosquitoes was still in preparation [67, 68]. While the existing Cartagena Protocol on Biosafety is considered to be applicable to GM crops, it is in need of specific amendments in order to work for GM mosquitoes [69].

Furthermore, in terms of regulation one has to distinguish between two different types of GM mosquitoes. While regulation and tracking might be possible for genetically sterilised mosquitoes as they are self-limiting in their spread, tracking and containment of GM mosquitoes with self-spreading genetics, i.e. fertile mosquitoes that block disease transmission, is considered almost impossible, or at the very least extremely difficult [70, 71]. This distinguishes GM mosquitoes from earlier GM technologies, such as for the modification of crops. GM and non-GM crops can be separated from each other and marked by labels on GM products, it can thus be seen as a technology of choice. However, the accuracy of this argument is only limited. As

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for instance Lezaun has shown, bees have proven to be effective agents of cross-pollination between GM and non-GM crops, thus subverting regulations that aim to keep GM and non-GM crops separate [72]. GM insects, however, are markedly different. The elusiveness of mosquitoes will likely be a major impediment to tracking, containment and comprehensive regulation, as for instance the spread of *Aedes albopictus* and herewith the increased risk of arboviral transmission in new locations across the world has shown, mosquitoes are hard to contain. This renders GM mosquitoes as a no-choice technology – once released, GM mosquitoes will stay in our environments.

A second major issue in terms of the social and ethical implications of GM mosquitoes is the question by whom and how they are produced and implemented. GM modification of insects is an expensive high-tech intervention and research so far has mainly been located in resource rich laboratories in the Global North, rather than in disease-endemic developing countries [73]. This enrols the technology thoroughly into discussions about technology transfer and development initiatives from North to South, and sits uncomfortably with the West's history in colonial exploitation and tropical medicine. Aside from this imbalance in bio-capital and agenda setting, GM mosquitoes are as much a product of the biotech industry as they are tools for public or global health. Are GM mosquitoes currently seen as a public good or a commercial product? While most of the research and development of GM mosquitoes has so far been funded by public institutions –both national research foundations -such as the US National Science Foundation- and philanthropic organisations -such as the Bill and Melinda Gates Foundation and the Wellcome Trust, the mosquitoes that have been released were part of a commercial project. The emerging GM mosquito industry has caught the interest of private biotech firms. The first company to produce and market GM mosquitoes is Oxford Insect Technologies (Oxitec), founded by a group of entomologists as a spin-off company of Oxford University. The company is a for-profit-enterprise, so far has mainly been funded by public entities and venture capitalists, and is one of the main drivers of high-end developments in the field. As discussed in the introduction, Oxitec was the first to release sterile GM mosquitoes into the wild in the field trials in The Cayman Islands. A fundamental issue that is raised through the dominance of Oxitec in the field is the tension between GM mosquitoes as a public health tool and a commercial product [74-76]. While GM mosquitoes in malaria control would be used as a tool of disease control and to foster public health, companies like Oxitec follow different aims – they have to become profitable and eventually make profits with their GM entities. This tension brings another social issue of GM mosquitoes to the forefront, namely the question of how one conducts field trials with GM mosquitoes in an ethical way?

As we alluded to in the introduction, the first releases in The Cayman Islands were conducted in a rather secretive fashion. Oxitec only published the news about the release with a one-year delay [1], leading to accusations that the releases were deliberately done in secret [75, 76]. Oxitec stated the trials were prepared and conducted in close cooperation with local Mosquito Control and Research Unit, had conformed to the British Overseas Territory's biosafety rules, and that information had been sent to local newspapers preceding the trials. However, many locals claimed they were not informed and no risk assessment documents were made available to the public on the internet. The only risk assessment document that can be found was

published by the UK parliament in 2011, over one year after the releases started [5]. The Cayman Island releases have triggered fears for entomologists working on GM mosquitoes that such secretive trials might lead to a public backlash and undermine their own extensive efforts at public engagement, some scientists for instance claimed they have spent years preparing a study site through "extensive dialogues with citizen groups, regulators, academics and farmers"[1].

GeneWatch argued that Oxitec purposefully bypassed existing international GM regulations (developed mainly for GM crops), because Cayman Islands does not have biosafety laws and is not a signatory to the Cartagena Protocol on Biosafety or the Aarhus Convention (even though since the UK is a signatory to the protocol, Oxitec had a duty to report the export of GM eggs to UK government). As a result GeneWatch reads Oxitec's actions as colonialist tactics: "the British scientific establishment is acting like the last bastion of colonialism, using an Overseas Territory as a private lab" [76].

All in all, this raises the question what ethically and socially responsible research on GM mosquitoes means? Here, the ability of researchers and stakeholders to communicate with each other is key for meaningful public engagement. In this respect, a recent survey has focused on the willingness of scientists to have interactions with a non-scientific audience [77]. One of the main findings of the survey indicates that more than 90% of scientists working on GM mosquitoes are agreeable to interactions with the public on their research. However, communication might not be enough and real discussion might not be easy between researchers and a non-scientific audience. This has been underlined by the reluctance of a fraction of the research community to have their research project evaluated by a non-scientific public [77]. Thus, while a significant proportion of researchers are ready to interact with a non-scientific audience, they seem to be less likely to accept an evaluation and a prior-agreement of a research proposal by the general public, interestingly especially researchers from the Global North are hesitant. On the other hand, many scientists in malarious countries do welcome exchanges with publics and are more willing to negotiate their research project with members of the disease-endemic communities.

In summary, the GM mosquito technology in malaria control raises a set of challenging questions. Challenges from a biological and ecological perspective are interlinked with questions about democratic decision-making, local acceptance and international regulation of these emerging entities. Such a potentially controversial technology cannot afford to skip these debates and time is ripe to focus on the ethical and sociological aspects governing the potential use of GM mosquitoes. Furthermore, it is crucial that the development of transgenic methods does not lead to a decrease in funding of classical, accepted and efficient vector control methods – indeed, they should be favoured and enhanced to continue curbing the malaria burden today.

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Author details

Christophe Boëte[1*] and Uli Beisel[2]

*Address all correspondence to: cboete@gmail.com

1 UMR 190 "Emergence des Pathologies Virales", Aix Marseille Université IRD (Institut de Recherche pour le Développement) EHESP (Ecole des Hautes Etudes en Santé Publique), France

2 Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

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