Review

Genetic control of *Aedes* mosquitoes

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Aedes mosquitoes include important vector species such as *Aedes aegypti*, the major vector of dengue. Genetic control methods are being developed for several of these species, stimulated by an urgent need owing to the poor effectiveness of current methods combined with an increase in chemical pesticide resistance. In this review we discuss the various genetic strategies that have been proposed, their present status, and future prospects. We focus particularly on those methods that are already being tested in the field, including RIDL and *Wolbachia*-based approaches.

Keywords: *Aedes*, Genetic engineering, RIDL, SIT, IIT, *Wolbachia*, Dengue

Introduction

Aedes mosquitoes transmit a range of pathogens that cause substantial human morbidity, mortality, and suffering. Dengue, the most important mosquito-borne viral disease with 50–400 million infections per year worldwide,[1,2] is transmitted primarily by *Ae. aegypti*. Several other *Aedes* species are competent vectors for dengue in the laboratory and *Ae. albopictus* in particular has been responsible for some transmission in the field, though it appears much less epidemiologically significant than *Ae. aegypti*.[3] The common name of *Ae. aegypti* is the yellow fever mosquito, indicating another major arbovirus transmitted by mosquitoes of this genus, and there are many more; chikungunya has come to prominence more recently with a major outbreak in the Indian Ocean in 2005–6[4,5] and some transmission in Italy in 2006.[6] Pathogen transmission is not confined to viruses – lymphatic filariasis in the South Pacific is vectored by *Ae. polynesiensis*; specific characteristics of this vector may have contributed to the failure of drug-based control programmes in the region.[7,8]

A vaccine has long been available for yellow fever, but remains some way off for dengue, following disappointing results from a recent large trial of the leading candidate.[9,10] With no licensed vaccine or specific drug (whether prophylactic or therapeutic), dengue control focuses on the major mosquito vector, *Ae. aegypti* – and vector control is expected to remain essential even when drugs or vaccines eventually become available. However, current mosquito control methods have limited effectiveness against some key species which breed in small dispersed bodies of water. For *Ae. aegypti*, these might be water storage containers or rain-water filled artificial containers such as buckets, vases, general refuse, or blocked rainwater gutters. Both private properties and public spaces will have large numbers of such potential breeding sites. Each one may be treated easily by tipping out the water or treating with a chemical or biological toxin, however finding and treating a high enough proportion for effective control is extremely difficult and impractical in most settings. Adulticides are also of limited effectiveness, compounded by increased resistance and the relative ineffectiveness of bednets against day-biting mosquitoes. The inadequacy of current technology is clear: for example, the efficient and well-resourced programme in Singapore, working with a cooperative citizenry, has not been able to prevent epidemic dengue.[11–13] This, combined with recent enabling technical advances in mosquito genetics, provides the underlying motivation for the development of new genetics-based approaches.

Genetics-based approaches have several features in common[a]. Since they depend on vertical (mating-based) transmission of heritable elements[b], they are

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[a] Genetic control may be defined as ''Dissemination, by mating or inheritance, of factors that reduce pest damage" and area-wide control as "Reducing pest damage using measures whose effectiveness depends on application over large expanses'' (Mark Benedict, *pers. comm.*). All proposed genetic strategies are intended for area-wide use, though the minimum useful area varies by species and strategy.

[b] One exception might be 'paratransgenesis', the use of modified microbes to change the phenotype of insects with which the microbes associate. Depending on the microbe, horizontal transfer of the modified microbe between insects might be possible. Paratransgenesis is not discussed further in this review.

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extremely species-specific. Populations can only be affected by the genetic system if they can interbreed with carriers of that genetic system; other populations will not be directly affected. This species-specific aspect is very attractive from an environmental perspective, as it means that these approaches are exquisitely targeted to the pest or vector species of interest. On the other hand, this feature may be a limitation where multiple pest species are transmitting the same pathogen, in which case a more broad-spectrum approach may be preferred. An additional advantage of genetic control methods is that the control agent, modified insects, will actively disperse and seek mates, so the methods are 'homing' or actively target-seeking, as well as specific.

Though some genetic strategies have been developed using classical genetics, such as the Sterile Insect Technique (SIT) (see section below: Population Suppression Strategies – Sterile Males Section), recombinant DNA methods provide a step change in our ability to design and build highly specified, versatile, powerful genetic systems. Several key *Aedes* species have now been transformed, either by recombinant DNA methods using transposon vectors,[14–18] or by artificial infection with various *Wolbachia*, a diverse group of intracellular bacteria.[7,19,20] This opens the door to the development of powerful new genetics-based tools with which to control major vector-borne diseases.

Classifying Genetic Control Strategies

A bewildering variety of genetic control strategies have been proposed; these can be categorised according to the intended outcome, or according to the expected dynamics of the genetic element in the target population. Regarding intended outcome, this may be to reduce the number of individual vector mosquitoes – population suppression – or to reduce the ability of individual mosquitoes within the population to transmit the pathogen. This latter approach is known as 'population replacement' or, because the mosquitoes are made refractory to transmission of the pathogen, 'refractory insect strategy'.[21–24] However, the target population is not really replaced; rather a genetic element is introduced into it through breeding of released modified mosquitoes with wild individuals, thereby changing the phenotype of some or all individuals in that population – those that carry the new genetic element.

Regarding the expected dynamics of the genetic element in the target population, the element may be intended to persist indefinitely in the target population, potentially also increasing in frequency within the target population and spreading to invade additional populations. These are termed 'self-sustaining' genetic systems. The alternative is systems which will not spread or persist, rather they will decrease in prevalence over time and can be maintained in the target population only by periodic release of additional carriers. These are known as 'self-limiting' genetic systems.

Population Suppression Strategies – Sterile Males

The most familiar genetics-based population suppression strategy is SIT. This relies on the release of large numbers of sterile males to seek, court, and mate wild females, thereby reducing the reproductive potential of the target wild population. If enough of the wild females mate sterile males then the target population will decline and collapse. SIT has been used successfully for more than 50 years against several major agricultural pests, using radiation-sterilised insects.[25,26] However, the use of radiation imposes several undesirable limitations, including logistical issues, and the somatic damage unavoidably caused by the sterilising dose of radiation used.[27–30] Several field trials using radiation- or chemo-sterilised mosquitoes have been conducted, with some success, but there are also problems including poor performance of irradiated mosquitoes.[31] Though classical methods have recently been revisited for *Ae. albopictus*[32,33] and *An. arabiensis*,[34] several alternatives have been explored to avoid the need for irradiation, and to provide additional enhancements, while retaining the many attractive aspects of classical SIT.[35] Though 'sterile' may strictly indicate agametic sterility, meaning that no gametes are produced, for SIT agametic sterility is not intended or used as it is important that spermatozoa are in fact produced. If aspermic males were used, sperm competition in remating females would likely lead to fertile sperm winning over (non-existent) sperm from sterile males. This would lead to most or all of the eggs from females that mate more than once being fertilised by unmodified sperm and therefore being viable, unless all of their mates are sterile. Increased remating might therefore represent a form of selectable behavioural resistance. However, the barriers to remating vary; where physical barriers such as mating plugs occur selection for increased remating may be less likely. Instead of 'agametic', in the context of SIT and this review 'sterile' simply means that some or all of the offspring die. For instance, *Wolbachia* can induce a form of sterility known as Cytoplasmic Incompatibility (CI), in which embryos from uninfected females fertilised by sperm from infected males fail to develop. Infected males are therefore sterile when mated with uninfected females, though fertile when mating with infected females. This can potentially be used as a sterilising principle for SIT, this variant being called the Incompatible Insect Technique, IIT.[36] In classical SIT,

the radiation doses used induce dominant lethal mutations in the irradiated sperm such that most eggs die after being fertilised by such sperm. About 95–99% sterility is typical for Mediterranean fruit fly SIT programmes;[37,38] higher sterility can be achieved with more radiation, but at the cost of further damaging the insects. *Wolbachia* achieve a similar effect – death of offspring of incompatible crosses – in IIT, though the biochemical and genetic mechanism is unknown.

Sterility – death of most or all offspring – can also be achieved by using dominant lethal alleles introduced into the genome by recombinant DNA methods, rather than by irradiation. In the most direct analogous system, so far described only in *Anopheles*, a nuclease is expressed in the male germline.[39] This gives a sterilising effect much like radiation – and presumably by a similar mechanism, induction of double-stranded breaks in the insect's chromosomes. Interestingly, the system was designed to cut the X chromosome exclusively, and thereby selectively kill female offspring, though this was not achieved and would in any case be difficult in *Aedes* mosquitoes that lack a Y chromosome. The underlying molecular system, using sequence-specific nucleases called homing endonucleases (HEGs), is remarkably flexible depending on the precise design. In theory, both self-limiting systems like this SIT example and invasive, self-sustaining genetic systems can be developed with these tools.[40,41] Furthermore, although the SIT-like systems described here are clearly self-limiting, self-sustaining population suppression strategies using HEGs have been described, in which reduced-fitness traits are driven into the target population using the super-Mendelian inheritance property of HEGs; in principle this could drive a population or even a species to extinction.[40,41]

We have developed a SIT-like system called RIDL (Release of Insects carrying a Dominant Lethal).[42] Here, rather than inducing dominant lethals when required, as with radiation, a dominant lethal transgene is inserted, but its expression is artificially repressed to allow the insects to be reared. One advantage of this approach over the use of DNA damage or CI is the ability to select the time of death of the offspring. Radiation and CI kill affected individuals as embryos, but where there is significant larval density-dependence, a later lethal period can be considerably preferable.[43–49]

All control interventions place pressures on the target population that may select for various forms of resistance, and genetic control methods are no exception. As mating-based systems, one obvious potential mode of resistance is assortative mating, whereby females are selected to avoid the engineered males. In practice, in decades of use of radiation-based SIT there have been few examples of this, a melon fly control programme in Okinawa being perhaps the only well-documented example.[50] Even then, control was successfully achieved simply by releasing more sterile males. Other genetic strategies may have additional potential resistance modes. The use of zygotically active lethal genes in RIDL provides flexibility in terms of engineering the time – and/or sex, see in the following section – of death. In principle, it also allows the possibility of resistance to the zygotic killing mechanism,[51] though this has not yet been observed. Given the large number of effector molecules available, one might expect that new strains could be developed faster than such resistance would emerge; other approaches such as stacking traits may also be useful should this type of resistance prove an issue in practice.

Large-scale Separation of Males and Females – Genetic Sexing Strains

A further issue is that of sex separation. This is not essential for efficacy – the New World screw-worm was eliminated from a continent by a classical SIT programme releasing both males and females – but it is highly desirable.[25] Female mosquitoes will bite and potentially transmit disease even if sterilised. The lifespan of released mosquitoes will likely be reduced by laboratory rearing and handling, significantly reducing their capacity to transmit disease in addition to any effect of the modification itself, nonetheless the possibility of deliberate or accidental release of females may adversely affect public acceptance. Sterile-male methods (e.g. SIT, IIT, RIDL) do not require the release of females, however self-sustaining releases of *Wolbachia* do require the release of some females because *Wolbachia* is maternally inherited. Therefore it has been proposed that special strategies using male-biased release should be used to minimise the number of females released,[52] though in fact no sex separation was used for the first such release trial.[53]

For some strategies there are additional reasons to remove females beyond their potential to bite. For SIT, large-scale field experiments with Mediterranean fruit flies showed that male-only releases were 3–5 times more effective per male than mixed-sex releases; the sterile females are thought to 'distract' the sterile males from seeking out wild females.[54] For the *Wolbachia*-based IIT specifically there is an additional requirement for sex separation – the infected females are fully fertile with both infected and uninfected males, furthermore all their progeny inherit the infection. This means that release of even a single infected female could potentially lead to the alien *Wolbachia* spreading in the target population. Where the target species is naturally infected with a different, incompatible, strain of *Wolbachia*, the

resulting bidirectional incompatibility will likely limit the spread of the new infection beyond the target area, at least for small target areas. However, if the target species is naturally uninfected, this could lead to the spread of the infection throughout the species. The natural history of *Wolbachia*, which indicates many independent invasion events, shows this is possible, but not the likelihood, which may be very low per female. This is likely to be seen as an undesirable outcome and therefore a significant risk, unless species-wide invasion is the intent of the release.

Sex separation can be efficiently achieved for some species of mosquitoes, including *Ae. aegypti*, using physical methods based on the size difference between male and female pupae.[55–58] Strains that allow genetics-based automated separation of males and females are known as 'genetic sexing strains'. Several have been developed using classical genetics, notably the 'MACHO' strain which contributed greatly to the success of an SIT programme against *An. arabiensis* in El Salvador.[31,59] However, modern genetics provides more options and also allows such systems to be transferred more readily from one species to another. Several have been developed.[60–65] In principle, any selectable induced sexual dimorphism could be used, but in practice two approaches have been followed, either sex-specific expression of a fluorescent marker allowing automated sorting,[61,66] or sex-specific conditional lethality allowing facile elimination of one sex from a cohort during rearing.[62] It is possible to use a repressible female-killing system both for sex separation and also for field control.[42,67–69] Insects are reared with the lethal system repressed to provide a colony. Cohorts for release are then reared without the repressor, so that females are eliminated. The resulting males, homozygous for a dominant female-specific lethal gene are released to mate with wild females. All offspring from such a mating inherit one copy of the female-lethal transgene, so daughters die. These are both the vectors and the reproductive potential of the population. Heterozygous sons will pass the transgene on to half of their offspring, resulting in some additional control, though the high fitness cost of a female-lethal trait means that the transgene will be rapidly eliminated from the target population unless maintained by periodic release of additional homozygous males. This is female-specific RIDL, fsRIDL, which has some similarities to the classical field female-killing (FK) systems developed in *Lucilia cuprina*[70] and is in principle more efficient than SIT.[71] Furthermore, the use of female-lethal systems may provide additional benefits in terms of resistance management for other approaches used in an integrated vector management programme.[72,73] fsRIDL strains have been developed for *Ae. aegypti*[63,74] and *Ae. albopictus*,[75] using flightlessness as a lethal trait.

Refractory Insects

Several approaches have been described for making mosquitoes refractory to malaria, including the expression of specific antibodies,[76] peptides,[77] or manipulating cell signalling.[78] For the arboviruses transmitted by *Aedes* mosquitoes, RNAi seems an attractive mechanism for suppressing virus replication. Transgene-based expression of a hairpin RNA corresponding to part of the DEN2 virus in either the midgut[79] or salivary glands[80] has been shown to provide a strong block to virus transmission. However, for the midgut-expressing line, expression of the anti-DEN2 hairpin and the associated refractory phenotype were lost after about 13 generations,[81] suggesting that expression may impose a significant fitness cost, and also perhaps that the unusual inverted repeat structure involved may be subject to some form of epigenetic silencing.

Gene Drive Systems

A refractory gene will only have an epidemiologically useful effect if it is present in a significant fraction of the target population. It will probably also have to keep both prevalence and effectiveness high for many vector generations. How can this be achieved? Getting to a high prevalence by simple introgression is difficult in a numerically large population, though not necessarily impossible.[82] However, since the refractory gene is likely to impose a fitness cost on the mosquitoes, it is likely that both be selected against in terms of prevalence, and also perhaps in terms of loss of function.[83] A system is therefore required which will increase the prevalence within the population over time, despite a selective disadvantage. Such systems are termed 'gene drive systems'. Selfish DNA systems,[84] which have this property of spreading despite not providing an individual fitness benefit, are the main source of inspiration for the design of gene drive systems. Several systems have been proposed,[85] but none developed even to proof-of-principle stage in a mosquito. However, a *Medea*-like system has been demonstrated in *Drosophila melanogaster*,[86] using a design which should in principle be transferable to mosquitoes.[87]

One interesting proposal is the 'killer–rescue' system.[88] By using a lethal transgene and an unlinked repressor, this provides an initial increase in allele frequency of the repressor, but over time both the lethal transgene and the repressor decline in frequency. Though having some gene drive properties, this is therefore still a self-limiting system, which helps to illustrate that there is a spectrum of invasiveness or persistence in genetic systems. At one extreme we have high-penetrance dominant

lethal systems killing both males and females, where the transgene is not expected to persist beyond the immediate progeny of the released individuals. Then there are female-lethal systems, where the sons survive but the transgene will still disappear rapidly due to its high fitness cost. Refractory genes that are designed to be neutral will also decline in frequency, but much more slowly due to their much lower fitness cost (some fitness penalty seems inevitable). A transient gene drive system like killer–rescue can provide some boost beyond the initial allele frequency, but still eventually declines. Then on the other side of the self-sustaining/self-limiting divide – which is a very real and significant divide, notwithstanding the shades of persistence and invasiveness on either side of it – we have frequency-dependent systems like underdominance.[89–91] This has a high invasion threshold making it relatively unlikely to invade non-target populations well isolated from any target populations. Medea-like systems have a much lower invasion threshold and so are much more likely to spread aggressively into distant populations,[86,87,92] though modifications can in principle be made to reduce this.[92] Transposons, long proposed as the basis for gene drive systems though not yet demonstrated, are also potentially highly invasive.[22]

While the relationship of IIT and RIDL with the well-known SIT is clear, there are not such obvious analogies with current methods to guide the testing, deployment, and use of gene drive systems. Some affinity may be found with classical biological control, where the intention is to introduce a parasitoid or predator to control a pest population, expecting that the biocontrol agent will establish and provide long-lasting control, albeit usually incomplete, for the indefinite future. As with classical biological control, there are concerns regarding the lack of control over the gene drive system once released, its unknown evolutionary trajectory post-release, and the essentially irreversible nature of a release, at least in the case of large-scale releases. For these reasons, self-sustaining systems are seen as higher-risk.[93–98] On the other hand, while sterile-male control looks economically attractive[44,47] self-sustaining systems in principle have an even lower cost to deploy as fewer mosquitoes are required, at least after the initial introduction. This theoretical cost advantage depends on being able to use the gene drive system as a 'fire-and-forget' weapon; the more expensive the post-release monitoring required, for example to assure the ongoing prevalence, stability, and effectiveness of the modification, the lower the cost differential is likely to be.

A further issue is the possibility that success may lead to decreased vigilance or the loss of capacity to implement previously effective measures if such existed. While this applies to all control methods, whether genetic or not, it may be a significant concern in respect of the use of long-term self-sustaining systems. The 'forget' part of 'fire-and-forget' should therefore not be taken literally – such methods would still require careful ongoing monitoring for field efficacy, and the development of replacement strains prior to breakdown. This is likely to require significant ongoing resource expenditure.

Can *Wolbachia* Provide both Refractoriness and a Gene Drive System?

One striking exception to the slow progress with refractoriness and gene drive systems has come from work on *Wolbachia* in *Ae. aegypti*. Though originally developed for IIT and life-shortening strategies, it was observed that infection with certain strains of *Wolbachia* dramatically reduced susceptibility to a range of pathogens,[99–101] though potentially increasing susceptibility to others.[102] *Wolbachia* are capable of spreading through insect populations as a heritable modification by manipulating the host's reproductive biology[84,103] – in other words, *Wolbachia* has the properties of a gene drive system. This raised the possibility that certain strains of *Wolbachia* might provide a complete gene-drive-plus-refractory-gene package. Attention has focused on *w*Mel, a strain of *Wolbachia* from *Drosophila melanogaster* and a laboratory-isolated pathogenic derivative *w*MelPop. Interestingly – and highlighting the diversity of *Wolbachia* – *w*Mel infection has a similar dengue-blocking effect in *Ae. albopictus*, even though *Ae. albopictus* is naturally infected with two further strains of *Wolbachia* that do not have this effect.[104] As with cytoplasmic incompatibility, the molecular basis of this pathogen-blocking phenotype is not known, though various studies have implicated upregulation of immune genes or production of reactive oxygen species, or competition for a limited resource such as cholesterol.[100,101,105,106]

In principle, therefore, a suitable strain of *Wolbachia* could provide an invasive refractoriness phenotype. Though such invasive genetic systems are seen as relatively risky for reasons outlined above, *Wolbachia* is not especially invasive, particularly for a strain that has a significant fitness cost, as appears to be the case for *w*MelPop.[103] Introduction of a single infected female can still lead to *Wolbachia* invading that population, especially if the effective population size is low.[107]

Since *Wolbachia* are naturally occurring, albeit not in *Ae. aegypti* and the relevant strains are from rather distantly related insects, this use of *Wolbachia* escapes the regulatory structures and oversight put in place for recombinant DNA technology.[108] This may seem rather odd if one considers that addition of

any single gene, or less, of DNA from *Wolbachia* would trigger such an oversight, but the addition of the whole genome does not. However, it is clear that here, as for conventional genetic engineering of mosquitoes, the relevant research groups have worked hard to clarify and then to comply with all applicable regulations.[8,57,108–111]

For any self-sustaining genetic system, key questions relate to the initial ability to spread and confer the desired phenotype, and the possibility that evolutionary responses will compromise this, or have some other undesirable effect. Though in principle the large-scale use of such systems may be reversible by further genetic intervention, restoring the *status quo ante* is at best uncertain; this irreversibility has been a major discussion point in respect of gene drive systems. In the case of *Wolbachia*, one may predict that the introduced strain will co-adapt with *Ae. aegypti*, reducing the fitness cost of infection but perhaps correspondingly reducing the extent of refractoriness, as both may have the same underlying cause of overproliferation in somatic cells.[112] However, while the direction seems clear, the rate of decay is very hard to predict, and many generations of protection may be provided. Lack of permanent effect is hardly a reason not to act, but might this tapering protection have some negative aspect? Consequences might include selection for resistant strains of virus. Though initial experiments suggested that *w*Mel infection gave strong refractoriness,[113] subsequent data using blood from human patients indicated titre-dependent breakthrough.[114] This suggests that a *Wolbachia* strain with refractoriness that is incomplete – either as its initial phenotype or arising through co-adaptation with the mosquito – could select for virus strains with higher titre in humans, an undesirable trait. It is also striking that, unlike normal uninfected mosquitoes, *Ae. aegypti* infected with *w*MelPop require human blood to produce viable eggs.[115] This would appear to provide strong selection for increased human biting preference, a trait which is central to the transmission of human-specific pathogens, as well as to biting nuisance. Unlike the more catholic *Ae. albopictus*, *Ae. aegypti* has a strong preference for anthropophagy, but this is far from absolute and could presumably be increased by such selection.[116–119]

These issues illustrate the difficulty of predicting the consequences of releasing a self-sustaining genetic system relating to future evolutionary responses. The use of a 'black box' system such as *Wolbachia* has advantages and disadvantages relative to genetic engineering using well-characterised components. On the one hand *Wolbachia* is arguably natural – though this may also be true of the elements of an engineered system; in both cases the association with *Aedes aegypti* is artificially induced, a product of modern biotechnology. To further blur the lines, gene transfer from *Wolbachia* to insect nuclear genomes is well known, and this can lead to stable transfer of expressed genes.[120] Nonetheless, this 'natural' aspect is somewhat reassuring, in that *Wolbachia* strains are already widespread in the environment without known negative effects – though that many strains are harmless does not imply that all are; one could not sustain such an argument for *E. coli*, for example. On the other hand, a complex uncharacterised system is by definition less well understood and correspondingly more likely to throw up surprises. *w*Mel has an estimated 1,270 protein-coding genes in 1.3 Mb of DNA[121] – vastly more complex than the 1–4 genes in about 10–20 kb typical for current transgenic insertions. The refractoriness phenotype was a major, beneficial surprise; the human blood requirement was also entirely unexpected, and less welcome. The future evolutionary trajectory of such a complex system may reveal additional surprises – positive or negative.

However, it is a fallacy, sometimes called the nirvana fallacy, to compare actual things with idealised alternatives, for example the risks of future action with a hypothetical risk-free world. Both inaction and alternative actions have risks of their own. Nonetheless, it may be difficult both for regulatory authorities and the general public to compare the relatively well-known risks and hazards of inaction with the unknown aspects of a new technology, even when – as for genetic control – the technology seems likely to offer potentially large net benefits.

Not a 'Magic Bullet'

The above discussion has focused on genetic control methods alone. However, current control methods have some strengths as well as weaknesses; an optimal programme is therefore likely to integrate the best of current methods with new technology to achieve the goal of improved control. For example, short-term suppression by conventional methods is likely to be a desirable prelude to either sterile-male or refractory-insect methods as it will reduce the number of modified insects required to achieve a given effect. As further tools become available, such as drugs and vaccines, this integrated vector management approach will naturally expand to integrated disease management – again using an optimal mix of available tools. While there may be a certain inclination simply to 'wait for the vaccine', in practice both vaccine and vector control experts anticipate an ongoing requirement for vector control even when a cheap, effective vaccine is generally available[2] – a hoped-for but perhaps rather distant prospect.

Progress to the Field

In fact, after due consideration, national regulators in several countries have approved small-scale field trials as the next step in an incremental testing and scale-up process. Several self-limiting and one self-sustaining genetic system have been tested in the field to date.[53,57,58,122,123] Public perception has generally been positive, though these are early days. The use of *Wolbachia*, presented as 'natural'[c], has largely avoided public concerns relating to the use of recombinant DNA methods. Public response to genetic control, either in general or relating to specific applications, may vary considerably depending on a wide range of social, political, epidemiological, presentational, and cultural factors, of which the genetic element is only one; furthermore, this response may vary over time. Even for a well-established approach such as vaccination, participation rates are rarely as high as programme managers would wish, and scare stories such as that regarding MMR vaccine in the UK can still shake public confidence. However, regulatory and social factors, while crucial to the adoption of any new technology, are not the main focus of this review.

Field trials of genetic control methods known to the authors are:

1. 2009–2010 Cayman Islands: males of a RIDL strain of *Ae. aegypti*, OX513A,[43] were shown to be able to compete successfully for mates with wild mosquitoes;[58] sustained release of these 'sterile' males led to strong suppression of the target wild population.[57]
2. 2010 Malaysia: OX513A males were shown to have similar longevity and maximum dispersal to an unmodified comparator.[122]
3. 2010 French Polynesia: sustained release of *Ae. polynesiensis* males infected with a *Wolbachia* strain from *Ae. riversi*[7] for IIT trial.[8,123]
4. 2011–present: Brazil: sustained release of OX513A males led to strong suppression of a target wild population.[d]
5. 2011–present Australia: release of *w*Mel-infected male and female *Ae. aegypti* led to the invasion and establishment of *w*Mel *Wolbachia* in two target wild populations;[53] releases underway in three further areas.
6. Australia: release of *w*MelPop-infected male and female *Ae. aegypti* undertaken in two target areas; present status unknown[e]
7. 2013–present Vietnam: release of *w*MelPop-infected male and female *Ae. aegypti* on an island.[5]

To our knowledge, each of these trials has been successful in accomplishing its experimental objectives, and in no case have any negative consequences to human health or the environment been identified.

[c] "http://www.eliminatedengue.com/", accessed 18 Oct 2012 and 17 April 2013.

[d] "http://www.moscamed.org.br/2012/index.php", "http://www.oxitec.com", accessed 18 Oct 2012.

[e] "http://www.eliminatedengue.com", accessed 17 April 2013.

Prospects for the Future

One may anticipate that each of the programmes described above will develop further over the coming years, though there will doubtless be numerous technical, legal, and social challenges. In addition, one may anticipate that some of the many approaches at earlier stages of development will progress towards field trials and use. In this regard one may particularly look to synthetic biology approaches to engineered refractoriness and gene drive systems – an approach that has been long heralded and where the daunting technical obstacles are slowly being overcome.

A specific technical question relating to both genetic and conventional vector control is 'how low do you have to go'? What is the relationship between the number and competence of vectors and disease transmission? Current dengue control methods rely on population suppression. Genetics-based population suppression has the same aim, so can reasonably be evaluated on the same terms, looking for mosquito suppression, i.e. entomological endpoints. But what about refractory-insect methods, or indeed novel non-genetic methods such as spatial repellents? One would need to show an ability to reduce dengue, i.e. an epidemiological endpoint. However, this is extremely difficult for an area-wide intervention, as dengue is highly variable in time and space. Consequently, a trial to show disease suppression would likely need to have many separate treatment and control sites, each of a significant size and with many inhabitants. This is problematic in terms of scale but also in terms of funding – despite the potential, and outstanding early results, funding for genetic control has been extremely low relative to the resources devoted to drugs, vaccines, and insecticides.

Given adequate resources, the future for genetic control looks bright. Numerous research groups are developing exciting approaches; the first of these have successfully completed their first field trials. Genetic control may soon be deployed on a large scale, delivering clean, affordable, sustainable, scalable solutions to major human vector-borne diseases.

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