Genetic Control of Mosquitoes

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Abstract

Genetics can potentially provide new, species-specific, environmentally friendly methods for mosquito control. Genetic control strategies aim either to suppress target populations or to introduce a harm-reducing novel trait. Different approaches differ considerably in their properties, especially between self-limiting strategies, where the modification has limited persistence, and self-sustaining strategies, which are intended to persist indefinitely in the target population and may invade other populations. Several methods with different molecular biology are under development and the first field trials have been completed successfully.

INTRODUCTION

Genetic control: a method of biological control that depends on the dissemination of heritable factors that reduce pest damage

Vector: an organism that can mediate the transmission of a pathogen between hosts

Vectorial capacity: efficiency with which a vector population can transmit a pathogen

Sterile: an insect modified such that on mating it produces fewer viable offspring than an equivalent unmodified insect

Mosquitoes transmit various pathogens that lead to significant human morbidity and mortality. Being incapable of direct vertebrate-to-vertebrate transmission, many of these pathogens are completely dependent on mosquitoes for transmission. The mosquito therefore represents an important target for strategies aimed at reducing or eliminating transmission of such pathogens. Mosquitoes also create severe biting nuisance in many regions. Traditional methods such as the use of insecticidal chemicals have had success but may be restricted by concerns over environmental impact or the evolution of resistance to the toxin. Genetic strategies are attracting increasing interest as supplements or alternatives to such traditional methods. Genetic control may be defined as "dissemination, by mating or inheritance, of factors that reduce pest damage" (Mark Benedict, personal communication). Many approaches are being investigated and several have already reached the field.

Genetic control strategies depend on the introduction of a heritable element into the target population, so that the "modified pest becomes a biocontrol agent against its unmodified brethren" (34). This dependence on mating and vertical transmission distinguishes genetic control strategies from other biological control methods such as the use of predators, parasitoids, or infectious microbial agents. Instead, the control agent is a version of the pest itself, with a heritable modification that alters its properties in a desirable way.

CLASSIFYING GENETIC CONTROL STRATEGIES

Genetic control strategies share several key features. They are area-wide methods, in contrast to vaccines, drugs, and bednets, which are directed at individual humans (though widespread use may have additional community effects). As a result, those in the program area are equally protected irrespective of wealth, social status, or education. All genetic strategies exploit the mate-seeking behavior of the modified insect to provide a control agent that self-disperses and actively seeks wild pest insects. Being mating-based, genetic methods are also extremely species-specific. This minimizes off-target effects, but where multiple vector species are present, multiple control tools may be required. Genetic control should be seen not as a magic bullet, but as a set of powerful new methods that, integrated with current methods, should provide improved outcomes.

Genetic control strategies may be classified in several ways. Two key aspects are the intended outcome and the persistence of the genetic element.

Outcome-Based Classification: Suppression or Replacement

Population suppression strategies reduce the number of competent vectors in the target population or area, similar to the goal of insecticide-based programs. In contrast, population replacement strategies reduce the vectorial capacity of some or all of the mosquitoes in the target population; any reduction in numbers is of secondary importance.

For example, sterile-male methods aim to suppress target populations. Modified sterile males are released to mate with wild females; the modification results in the death of some or all of the offspring of such mating. If sufficient sterile males are released for a sufficient time, the target population will be suppressed and potentially even eliminated. In some cases (e.g., nonnative pests), suppression or elimination may be the desired end point. However, population replacement may reduce the potential for niche replacement, whereby removal of one pest leads to replacement by another (1).

Population replacement methods spread a novel trait, such as reduced ability to transmit a pathogen, into the target population. Whereas this novel trait will be beneficial for humans, in most cases it is likely to be deleterious to the mosquito (48, 52). Malaria and dengue infect only

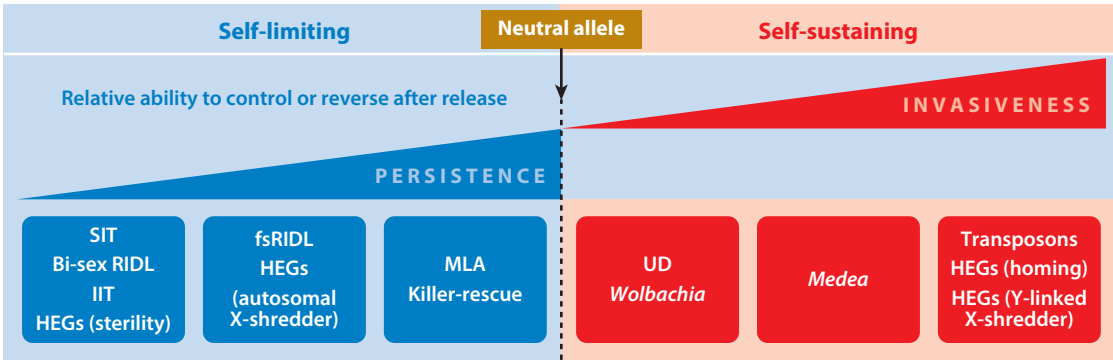


Figure 1

Self-limiting and self-sustaining genetic systems. Genetic systems with a strong fitness penalty are rapidly eliminated by natural selection. This is attractive in terms of control and potential reversibility of the intervention, but maintaining the modification in the target population requires ongoing periodic releases. In some cases this may not be necessary, for example, if a limited-duration intervention breaks the transmission cycle, leading to local elimination of the vector or the pathogen. Invasive genetic systems may require more [invasive *Wolbachia*, underdominance (UD)] or fewer (transposons, HEGs) modified insects for initial establishment (see below and **Figure 4**), but then the modification should persist indefinitely and perhaps invade other populations and species. This may reduce long-term program costs, though ongoing monitoring for prevalence and efficacy of the modification is required. However, restricting self-sustaining modifications to defined target populations is problematic, as is removing the modification from a population once present. The relative persistence or invasiveness of various genetic control systems is indicated. Although suppression strategies are generally self-limiting and replacement strategies self-sustaining, this is not necessarily the case. HEGs may drive recessive lethal or sterile alleles into a population, potentially forcing it to extinction. For initial trials of a refractory gene, or where modest prevalence and duration are adequate, refractory genes may be used with a self-limiting driver system (e.g., Killer-Rescue; 35), or no driver system (MLA; 66). Abbreviations: fsRIDL; female-specific RIDL; HEG, homing endonuclease gene; IIT, incompatible insect technique; MLA, multi-locus assortment; RIDL, release of insects carrying a dominant lethal genetic system; SIT, sterile insect technique.

a fraction of the total mosquito population and even then impose only modest fitness costs. The small average benefit from artificial resistance is therefore likely to be outweighed by the costs of the modification. The novel trait must then persist or spread in the target population despite conferring a fitness penalty, in other words, in the face of natural selection. However, many naturally occurring selfish DNA systems can do this. In principle, such a system can be co-opted as a gene drive system to drive the novel trait or cargo into the target population. However, maintaining the link between driver and cargo is potentially problematic, especially when this link needs to be maintained over many generations in a target field population (see below).

Selfish DNA: DNA sequences that can spread within a host population without providing a selective advantage to the host organism

Self-sustaining element: a genetic element expected or designed to persist indefinitely and perhaps to increase in frequency and/or invade other populations or species

Self-limiting element: a genetic element or modification that disappears from the target population over time, e.g., through natural selection

Persistence/Invasiveness

A consideration more fundamental than the intended outcome of use is the degree to which the modification will persist or even spread in the target population after release (**Figure 1**).

In self-limiting strategies the modification tends to disappear from the target population unless replenished by periodic release of additional modified insects. In contrast, self-sustaining strategies use a modification intended to persist indefinitely and perhaps even spread within the initial target population or to other populations. The sterile-male methods described above are obviously self-limiting as the lethal or sterile factor disappears rapidly from the target population, maintained only by periodic release of additional modified males. In contrast, gene drive systems are intended to spread themselves within the target population and are almost invariably self-sustaining. Self-limiting systems are readily controlled as they remove themselves from the target population if

not actively maintained by further releases; self-sustaining systems, lacking this feature, are seen as more controversial and risky (30).

Though the binary divide between self-limiting and self-sustaining systems is real and important, there is a spectrum of persistence or invasiveness within each type (**Figure 1**). Unless supplemented by further releases, a lethal system that kills all individuals inheriting it will disappear from the target population within a generation, whereas a female-killing system will persist somewhat longer but still rapidly disappears. An element with a modest fitness penalty will disappear more slowly, and some systems may even show an initial increase in frequency before eventually declining. Similarly, self-sustaining systems may be designed to spread only if present above a high initial frequency and decline otherwise; these systems are relatively noninvasive whereas others may have a low or zero threshold for spread. In several cases similar molecular designs may have a different invasiveness depending on their exact configuration. The potential use of homing endonuclease genes (HEGs) exemplifies this. Burt and colleagues (16, 28) proposed an elegant set of designs ranging from strongly self-limiting to highly invasive, all using these long-recognition-site endonucleases. Similarly, *Wolbachia* can provide either the self-limiting, sterile-male incompatible insect technique (IIT) or an invasive genetic system. Because specific modifications (e.g., HEGs, *Wolbachia*) can give different outcomes depending on how they are used, this is not a helpful basis for classification. Current proposed strategies are therefore discussed in relation to their persistence or invasiveness postrelease.

Homing endonuclease gene (HEG): HEGs are selfish DNA elements encoding proteins (endonucleases) that recognize and cleave specific DNA sequences of ~20–30 nucleotides

***Wolbachia pipientis*:** a diverse group of vertically transmitted intracellular bacteria that manipulate their host's reproductive biology to facilitate their spread

IIT: incompatible insect technique

SELF-LIMITING METHODS

Sterile-Male Systems

The most strongly self-limiting strategies involve the use of sterile insects. Although the use of spermless males has been proposed (73), most sterile-male strategies involve the use of heritable dominant lethal genes or mutations (1, 13). For fully penetrant sterile/lethal traits the modification is strongly self-limiting, as it disappears within a generation unless additional modified insects are released (**Figure 2**). Even where only a subset of the offspring is affected (e.g., only females), the

Figure 2

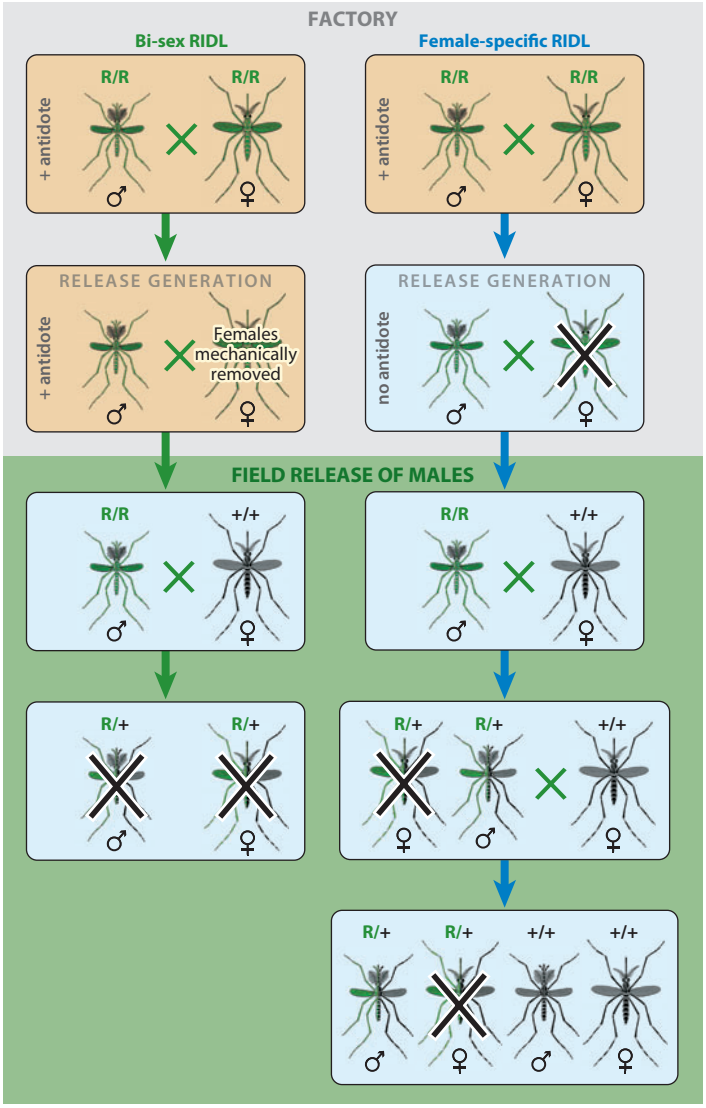
Sterile-male systems. Bi-sex lethal sterile-male systems kill both sexes of offspring, illustrated for RIDL in the left column. A strain homozygous for the RIDL transgene (R) is reared in the presence of an antidote that represses the lethal gene (33, 46, 65). Males intended for release are separated from females—for example by mechanical separation based on size (38, 39)—and released. Progeny of matings with wild females inherit one copy of the lethal gene and die. SIT-, IIT-, and HEG-based methods give similar mating outcomes, despite differences in the underlying biochemical mechanisms. If fully penetrant (100% of affected progeny die), the modification will disappear within a generation of releases ceasing. However, complete penetrance is not essential (65); radiation-based SIT programs typically use partially sterilizing doses, balancing somatic damage with sterility (59). Female-killing systems such as fsRIDL provide genetic sexing (automated elimination of females from the release population) by allowing expression of the female-killing system in the release cohorts. Offspring of released fsRIDL males inherit the lethal gene; the daughters die but the sons live. If these sons mate, then additional females are killed, though the high fitness cost of female lethality means this is still strongly self-limiting. An autosomal X-shredder has similar properties, whereas a Y-linked X-shredder is inherited by all sons and therefore persists and potentially even spreads in the target population (16, 28). Female-killing, but not bi-sex lethal, systems facilitate introgression of background genes from the mass-reared strain into the wild population, which can help spread additional desirable traits (2, 4, 6). Abbreviations: fsRIDL, female-specific RIDL; RIDL, release of insects carrying a dominant lethal genetic system; HEG, homing endonuclease gene IIT, incompatible insect technique; SIT, sterile insect technique.

severe fitness penalty ensures that the modification is rapidly and predictably lost from the target population unless periodically replenished.

Classical sterile insect technique (SIT) trials used DNA-damaging agents such as γ-radiation to induce lethal modifications (25). More recently, several different approaches have produced modified lines of mosquitoes that can generate sterile males. This avoids the need for radiation-sterilization and may thereby produce more effective sterile insects. In one example, a nuclease is expressed in the male germ line to generate chromosome breaks (82), similar to radiation but avoiding somatic cells. Release of insects carrying a dominant lethal genetic system (RIDL; 2, 65, 74) uses a lethal gene expressed in the zygote, rather than in the father, which gives much greater flexibility as the lethal gene can be designed to act at a chosen time in development (see sidebar, Genetics, Ecology, and Modeling). However, this flexibility depends on zygotic expression of the lethal gene, which could in principle make this approach more susceptible to environmental and

SIT: sterile insect technique

RIDL: release of insects carrying a dominant lethal gene or genetic system



GENETICS, ECOLOGY, AND MODELING

Genetics alone is not sufficient to predict the outcome of genetic interventions; ecology is also crucial. Population dynamic effects can directly influence control strategies. Mathematical modeling allows exploration and understanding of these issues across a wide range of parameters and scenarios.

Example 1: Late-acting lethals

Where density dependence acts at the larval stage, an early-lethal sterile-male method that kills affected offspring as embryos will lead to decreased density-dependent mortality of wild-type conspecifics. This reduces the effectiveness of the intervention (65, 84). However, if the lethal effect acts after the density-dependent phase, e.g., pupal death, the doomed larvae still compete with conspecifics, improving effectiveness and removing the possibility of inadvertently increasing the adult population (3, 8, 65, 84).

Example 2: Seasonal fluctuation

Many insect populations show temporal fluctuations in abundance. For a release of a given number of modified mosquitoes, the ratio of modified to wild mosquitoes will clearly be affected by fluctuations in the target mosquito population. More interestingly, the required release ratio can be strongly affected by seasonal variation, both for self-sustaining systems, such as invasive *Wolbachia* (37), and for sterile-male methods (80).

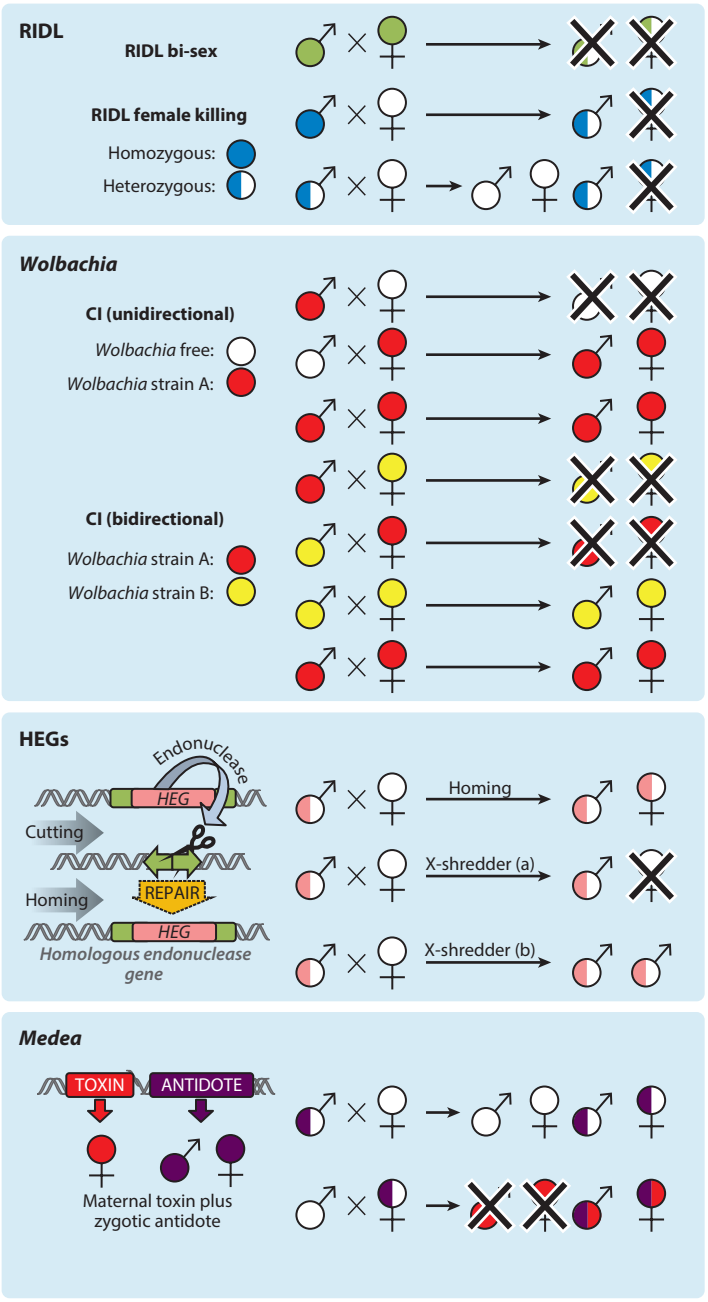
Cytoplasmic incompatibility (CI): a phenomenon that results in certain sperm being unable to form viable zygotes with certain eggs

genetic background effects on the expression of the lethal gene than is the case for paternal-effect approaches. Sterility can also be created by artificial infection with various strains of *Wolbachia*, a diverse group of intracellular bacteria (79). *Wolbachia* are not infectious between insects on normal timescales; rather they are maternally transmitted, like mitochondria, being passed from mother to her offspring. Infected males are useless to the maternally inherited *Wolbachia* for propagation; instead they produce modified sperm that produce viable zygotes only with eggs from infected females, a phenomenon known as cytoplasmic incompatibility (CI). *Wolbachia* in effect conducts a

Figure 3

Selected genetic strategies. RIDL and fsRIDL involve the release of transgenic males homozygous for a repressible dominant lethal gene to mate wild females (*open symbols*). For bi-sex RIDL, as for SIT, the heterozygous offspring are killed by the inherited lethal. In fsRIDL, the lethal gene is female-specific, so daughters die but heterozygous sons live; if these mate, half of their offspring inherit the female-lethal transgene. For *Wolbachia*, all embryos produced by an infected mother are infected. In addition, embryos are nonviable if fertilized by a male carrying a *Wolbachia* strain not carried by the mother. This is the basis of IIT. If only one strain is involved, this is unidirectional CI; if two strains are involved, this is potentially bidirectional. Invasive *Wolbachia* strategies release infected females, as well as males, spreading the *Wolbachia* strain into the target species. HEGs cut a specific nucleotide sequence. If the sequence is X-linked and the HEG is expressed during spermatogenesis, this may result in (*a*) nonviable daughters or (*b*) the elimination of X-bearing sperm. If the X-shredder HEG is Y-linked, all sons carry it, which can lead to invasion through meiotic drive. When the HEG is inserted into the potential cut site, repair of the HEG-induced cut can copy the HEG-bearing allele, a phenomenon known as homing. The occurrence of homing in the germ line causes super-Mendelian inheritance, whereby more than 50% of the offspring of a HEG heterozygote carry the HEG; this tends to allow the HEG to invade. Synthetic *Medea* uses a maternal toxin and a zygotic antidote to achieve a somewhat similar effect, as the only viable offspring of a heterozygous female are those carrying the *Medea* element. Abbreviations: CI, cytoplasmic incompatibility; HEG, homing endonuclease gene; IIT, incompatible insect technique; fsRIDL, female-specific RIDL; RIDL, release of insects carrying a dominant lethal genetic system; SIT, sterile insect technique.

sterile-male attack on uninfected females (**Figure 3**). *Wolbachia*-infected males were successfully used for sterile-male control of *Culex* mosquitoes even before *Wolbachia* was known to be the etiological agent of CI (49). This is the basis of the incompatible insect technique (IIT). However, *Wolbachia*-infected males are fertile with infected females; if any infected females were released, *Wolbachia* would tend to spread through the target population, neutralizing the sterility effect (36, 60). This risk—and the possibility of spread and establishment beyond the release area—is



much reduced where the target population is naturally infected with an incompatible strain, to give bidirectional CI.

Removal of females before release is essential for IIT and desirable for other sterile-male methods for several reasons. Female, but not male, mosquitoes bite, so sterile females might cause nuisance or even transmit disease. Field experiments with sterile Mediterranean fruit flies (*Ceratitis capitata*) found male-only releases three to five times more effective than mixed-sex releases; sterile females are thought to distract sterile males from seeking wild females (67). Separation by physical or behavioral methods may be possible (38, 39), but genetic methods should be cheaper and more accurate. Several such genetic sexing strains have been developed, including conditional female-specific lethals, allowing selective elimination of the females when an artificial condition is applied (inducible lethal) or withdrawn (repressible lethal) (18, 33, 46, 64).

fsRIDL: female-specific RIDL

Vector competence: the physiological ability of an individual vector to become susceptible to the pathogen and to become infectious

Refractory gene: a gene that reduces the ability of a vector carrying it to transmit a pathogen

Female-Killing Systems

Female-killing systems for genetic sexing can also provide "sterilization" (**Figure 2**). Offspring of released males homozygous for a female-specific lethal would inherit a copy of the female-killing gene such that daughters would die. Females represent the reproductive potential of the population, such that this provides control much like conventional SIT (14, 74). Sons carry one copy of the female-killing gene, so half of their daughters will die, providing further control. This is female-specific RIDL (fsRIDL). In principle, this approach can be considerably more effective than SIT, IIT, or other bi-sex-lethal systems, especially if the female-lethal gene is present at more than one locus (68, 74). The lethal gene still disappears rapidly from the target population if releases stop, owing to the strong fitness penalty associated with female lethality, but not quite as quickly as a bi-sex lethal trait.

For organisms with X and Y chromosomes—*Anopheles gambiae*, for example, but not *Aedes aegypti* (which does not have distinct sex chromosomes; rather it has a male-determining locus on an otherwise undifferentiated chromosome 1)—a similar effect can in principle be achieved by expressing during spermatogenesis a nuclease that specifically cuts the X chromosome (16, 28, 82). If such an X-shredder nuclease is located on an autosome, the system is much like fsRIDL. However, if the X-shredder is located on the Y chromosome, it can be a self-sustaining, invasive element, an artificial meiotic drive system (17, 28). Expression from the Y chromosome may be difficult due to heterochromatinization, but not impossible (22). In principle, release of even a modest number of insects carrying such a system can drive a target population to extinction by reducing the production of females. Furthermore, because the system can invade adjacent populations or species with incomplete mating barriers, widespread effects may be anticipated.

Refractory Genes

Population replacement strategies reduce the vectorial capacity of the modified mosquitoes. For this to significantly affect disease, a high proportion of the mosquitoes in the population must carry a modification that strongly reduces vectorial capacity. Although life-shortening (55), behavioral modification (21), and other traits have been proposed, attention has focused on modifications that make the mosquito refractory to transmission of the pathogen, i.e., reduce vector competence. Many prototype refractory genes have been demonstrated using single-chain antibodies, innate immunity peptides, artificial peptides, altered cellular signaling (78), *Wolbachia* (57), or RNA interference (31). For some of these, notably *Wolbachia*, the molecular and biochemical basis of the refractory trait is currently unclear.

Self-Limiting Replacement Methods

Artificial modification is expected to have some fitness cost, whereas mosquito-borne pathogens may impose little cost on the mosquito. Furthermore, only a small fraction of the total population is infected. It is therefore unlikely that refractoriness-inducing modifications will spread through a mosquito population by natural selection (48, 52). A gene drive system capable of spreading the refractory gene cargo will likely be required (see below). However, introduction by mass release may suffice for experimental trials, especially if insects carrying multiple copies of the refractory gene are used (66). Indeed, it would be desirable to test thoroughly with self-limiting systems before moving to self-sustaining systems, releases of which are far less readily reversed (13).

SELF-SUSTAINING METHODS

Unlike the self-limiting systems described above, self-sustaining systems are intended to persist indefinitely in the target population. They are expected to increase in prevalence within the target population and to spread to other populations under some circumstances. This invasive nature may be desirable in that it reduces the cost of deployment (although other ongoing costs such as postrelease monitoring for prevalence and efficacy may still be substantial unless a truly reliable, evolution-proof system could somehow be designed). However, this comes at the cost of greatly reduced control. For self-limiting systems trait quality can be assured through procedures in the rearing facility, whereas for self-sustaining systems production is in the field, which is harder to monitor and to adjust.

Numerous designs and a few prototypes have been developed for self-sustaining systems (70). These are all selfish DNA elements that can spread through a population without providing a fitness advantage to the individuals carrying them by imposing a relative disadvantage on nonmodified alleles or individuals. Many selfish DNA elements are known in nature (17); some gene drive systems use these directly (e.g., invasive *Wolbachia*,[1] transposons), whereas others replicate their key traits with synthetic components (e.g., synthetic *Medea*, see **Figure 3** and Reference 20).

Frequency-Dependent Gene Drive Systems

Different self-sustaining systems differ in their invasiveness. A key property is the extent to which the spreading potential depends on the frequency of the element within the population. If the system is frequency-dependent, then there may be a threshold frequency below which the element tends to decrease in frequency and above which it tends to increase (**Figure 4**). This threshold depends on the intrinsic properties of the system and the fitness costs of the specific implementation.

In general, a system with a lower invasion threshold will be more invasive because this threshold will be exceeded more readily. Fewer mosquitoes then are needed for establishment, but the modification is also more likely to spread to other populations or species with incomplete mating barriers. Underdominance-based and invasive *Wolbachia* systems have relatively high invasion thresholds; *Medea* systems have lower thresholds; and transposons and some HEG designs can have a zero threshold. However, there are three major caveats. First, these barriers are often calculated for single introductions, whereas the threshold may be dramatically lower for drip-feeding (i.e., sustained introductions over time), as is likely to occur between incompletely isolated populations (37, 54). Second, both source and target populations may fluctuate in time; this can further reduce the degree of migration necessary for invasion (37). Finally, thresholds have historically been

[1]This use referred to here as "invasive *Wolbachia*" to distinguish from the self-limiting use of *Wolbachia* in IIT.

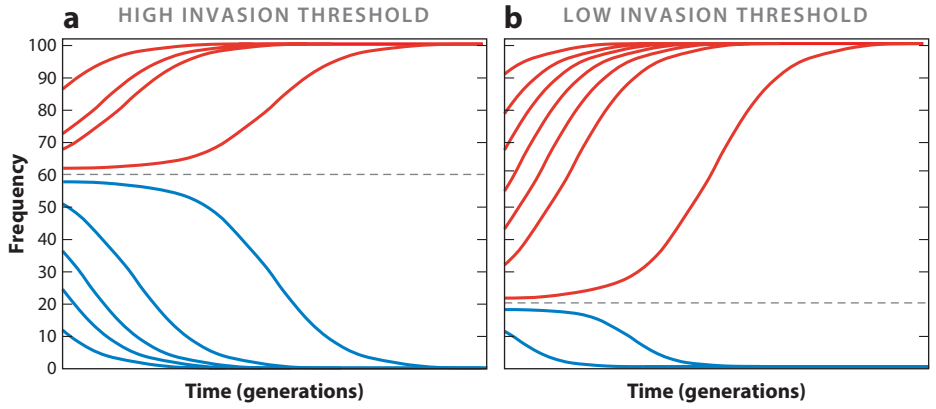


Figure 4

Frequency-dependent gene drive systems. Many invasive genetic systems have a frequency threshold for invasion. Above a certain unstable equilibrium frequency (*horizontal dashed line*) they tend to increase in frequency; below it they tend to decrease. The element may then increase to fixation (100% prevalence) or reach another equilibrium short of fixation. These outcomes depend on the properties of the genetic system and specific parameters within it, especially fitness costs. Typically, a high fitness cost imparts a drag, increasing the invasion threshold, slowing the spread, and possibly preventing the element from reaching fixation. Graphs illustrate the frequency evolution over time of a hypothetical system with a (*a*) high or (*b*) low invasion threshold.

calculated with deterministic models, which provide an illusory certitude to the unwary—it is not the case that introductions below the critical prevalence mean the modification will not invade the population, merely that it is less likely (43). Despite its high invasion threshold in deterministic models (76), the natural history of *Wolbachia* indicates that it has repeatedly swept through entire species from initial infection of a single individual.

Gene Drive Systems

Initial designs for highly invasive (zero threshold) gene drive systems focused on engineered transposons, inspired by the natural history of transposons, especially the rapid global spread of the *P* element in *Drosophila melanogaster*. However, no transposon-based gene drive system has yet been demonstrated in a mosquito, and artificial transposons are remarkably immobile in *Aedes aegypti* after integration into the genome (69).

Gene drive system (gene driver): a selfish DNA system, expected to spread a novel trait through a target population or species

Homing: HEG-induced cleavage of DNA can be repaired using a HEG-containing allele as a template, duplicating the HEG

Applications of HEGs that spread by homing (**Figure 3**) are also highly invasive (16, 28). Homing and associated gene drive have been demonstrated in *Anopheles gambiae* with an artificial target (81). A Y-linked X-shredder should also be highly invasive, at least if it has little fitness cost to males and if affected X chromosomes are excluded from mature sperm so that potential daughters are replaced by X-shredder-bearing males rather than merely killed (28).

Synthetic *Medea*-like elements, inspired by the naturally occurring selfish *Medea* elements of *Tribolium castaneum*, have been constructed (20). The system uses rationally designed synthetic elements that replicate the inheritance pattern of *Medea* without borrowing its molecular components. The invasion threshold for *Medea* is not zero but very low unless the element has a high fitness cost, such that *Medea* would likely invade across all but the strongest barriers (20, 40). However, illustrating the flexibility of synthetic approaches, related toxin-antidote systems can in principle be designed that are much less invasive (54).

Less invasive systems, i.e., ones with a higher predicted invasion threshold, include underdominance-based and *Wolbachia*-based systems. In the case of underdominance (negative heterosis) a hybrid of A and B has lower fitness than either A or B alone. In such a situation, hybrids are at a disadvantage, so the rarer of A and B is at a disadvantage—given random mating, the rarer type will mostly mate the other type and produce hybrid offspring, whereas the more abundant type will mostly mate its own kind and produce higher-average-fitness offspring. This leads to unstable dynamics where the more abundant form dominates—an invasion threshold. Underdominance can be achieved by chromosome translocations (24) or, with more flexibility, transgenic methods (26, 51).

Wolbachia strains are selfish DNA elements (though sometimes showing some symbiotic aspects) that can spread throughout target populations or species by distorting their host's reproductive biology (see above), for example, inducing CI to disadvantage uninfected females. This gene drive system may be useful for driving nuclear transgenes (76), but attention has focused on spreading specific *Wolbachia* strains that themselves reduce vector competence (77). These are *w*Mel, from *D. melanogaster*, and *w*MelPop, a pathogenic mutant of *w*Mel. Not all *Wolbachia* strains induce significant refractoriness; indeed many vector species already carry one or more *Wolbachia* strains. This refractoriness can suppress a wide range of pathogens (44, 57) but also potentially increases susceptibility to others (42). The molecular basis of the transmission-blocking property is not known; studies have variously implicated increased production of immune proteins or reactive oxygen species or competition for a limited resource such as cholesterol (15, 44, 57, 63). Nonetheless, the effect raises the possibility that these strains of *Wolbachia* might provide a complete package of gene drive system plus refractory gene.

System Decay: Resistance, Linkage, and Coevolution

All control systems are subject to evolution and the potential for resistance, but these are particular concerns for self-sustaining systems as the modification must remain stable and effective for many generations in a wild population. In essence, field use of self-sustaining systems sets up a multicomponent system of host, vector, pathogen, refractory gene, and gene driver to run for many generations—at least for many generations of the vector and pathogen—with the hope that the key novel traits of refractoriness and spreading ability are maintained long enough to have a significant effect on human morbidity and mortality. There are several predictable failure modes to consider, though the likelihood and consequences may be mitigated by suitable design.

Resistance in the mosquito population. All control methods tend to provoke an evolutionary response from their targets, and genetic methods are not immune to this. Even though refractory genes do not directly target the mosquitoes, silencing has been observed (32). Lethality/sterility systems clearly provide strong selection for resistance; assortative mating provides one obvious mechanism, though such resistance has rarely been observed during the decades of field use of SIT. Zygotic lethal systems may permit additional resistance modes (5). The lack of congruence between phylogenies of *Wolbachia* strains and their hosts implies that *Wolbachia* strains have a residence time shorter than speciation time, though the mechanism of resistance and loss is unknown. HEGs may generate resistant target sites through nonhomologous end-joining (28).

On the other hand, genetic control strategies involving inundative release and viable hybrids, notably fsRIDL, can help manage resistance to other interventions (4, 6), because susceptibility alleles will enter the wild population from the mass-reared strain. This property could provide significant additional benefit for an integrated program using several control methods.

The linkage problem. For any gene driver–cargo system, if the driver-cargo linkage is broken the driver may continue to spread without its refractory cargo. Because the cargo is expected to hinder spread, through its associated fitness penalty and possibly also physical size, runaway cargo-less drivers may spread faster and take over the population (23, 53). Though originally discussed for transposons, this applies to all driver-cargo systems. For example, *w*Mel provides a CI-based driver and a refractory trait. Both are encoded in its 1.3-Mbp genome but are presumably separable traits. In *D. melanogaster*, *w*Mel appears to protect against insect pathogens such as *Drosophila* C virus (72), so perhaps *w*Mel will be advantageous to a mosquito host in some circumstances, though increasing the fitness of the mosquito population may not be seen as desirable. However, if the refractory trait provides a net fitness cost, it is expected to fade away over time (75). The end result of such separation is that the target population is transformed with the driver but not the cargo (23, 53). A key unknown is the rate at which breakdown may occur.

Some gene driver methods lack a separate cargo and thereby largely avoid this linkage problem. For example, HEGs targeting recessive-lethal or female-sterile genes may impose a fitness load sufficient to drive a population to extinction (16, 28). Although resistance may arise at the target site, this should lead to loss of the HEG, not to spread of the HEG without the intended phenotype; that would require more unlikely events such as second-site resistance or the HEG somehow moving to another homing-compatible site.

Coevolution. Evolution of the driver-cargo system within the mosquito population will likely lead to loss over time of the intended cargo phenotype (see above). However, there are additional system components, notably the pathogen (e.g., malaria parasite, dengue virus). The pathogen will be strongly selected for resistance to the refractory trait; such resistance would reduce the effectiveness of the genetic control, which could lead to resurgence of disease after a period of apparently good control. Ongoing monitoring for efficacy of the refractory gene is therefore essential. However, similar issues apply to all control methods. The key concern is whether resistance, or coevolution of system components, could lead to more virulent strains of the pathogen. Although generally unlikely, plausible scenarios can be found, especially for some refractory insect approaches (56). Initial experiments suggested that *w*Mel infection gave strong refractoriness, but subsequent data using blood from human patients indicated titer-dependent breakthroughs (77). This finding suggests that a *Wolbachia* strain with refractoriness that is incomplete—either as its initial phenotype or arising through coadaptation with the mosquito (see above)—could select for virus strains with higher titer in humans, an undesirable trait.

System decay: mitigation. That a system will not last forever does not mean it should not be used. Even ten years of good protection could bring substantial benefits. Furthermore, it should be possible to develop replacement versions of some systems in less time than resistance can arise. For example, the list of potential effectors for a lethal system such as RIDL is essentially limitless. Similarly for HEGs, resistance at one site to one HEG should not give cross-resistance to others. The situation is less clear for *Wolbachia*-based approaches, as we do not know the molecular basis of CI and refractoriness; however, it seems likely that a new strain of *Wolbachia* could be used to remove an old one through bidirectional incompatibility.

REGULATION AND PUBLIC PERCEPTION

There is more to successful deployment of a new technology than excellent science (**Figure 5**). SIT, the archetypal self-limiting system, is well understood in operational terms and has a long history of safe use; recent variants such as IIT and RIDL may draw on this precedent. Self-sustaining

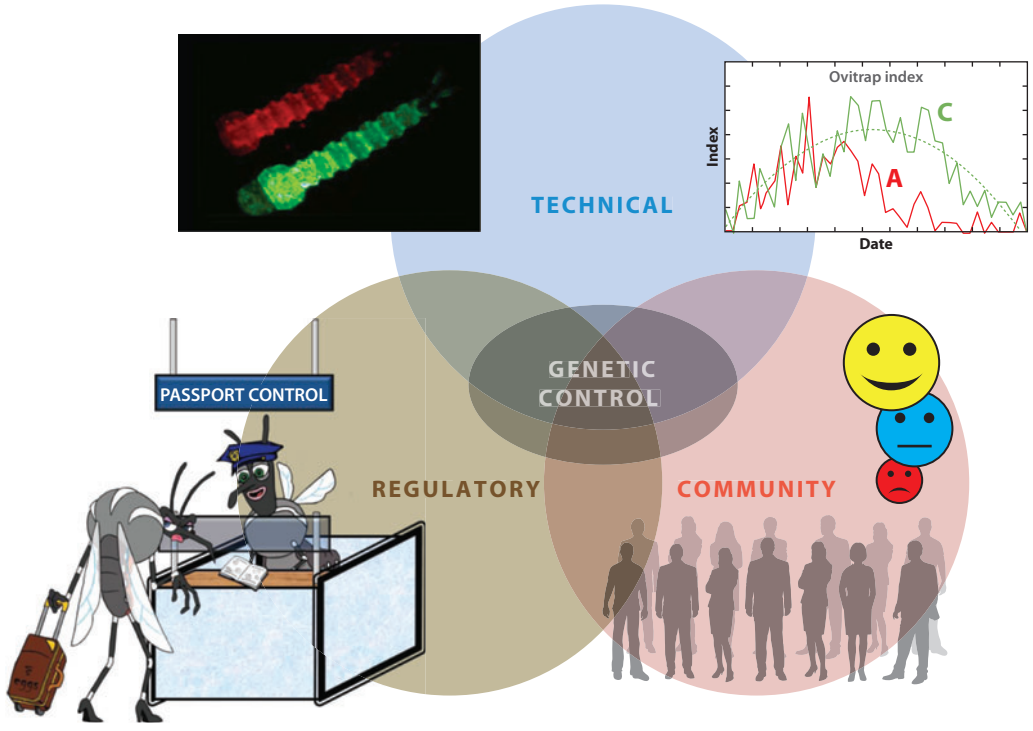


Figure 5

Bringing new technology to the field. Successful implementation of genetic control requires a technically effective strain, but this is far from sufficient. The approval of a wide range of stakeholders is required, notably the potentially affected communities and relevant regulatory authorities.

systems are more novel than, but somewhat analogous to, classical biological control, in which alien predators or parasitoids are introduced to control invasive pests. Self-sustaining genetic systems and classical biological control promise cheap, sustained control, with potential disadvantages of irreversibility of introductions and lack of control postrelease. Use of modified pests and mating-based mechanisms should limit the scope for off-target effects relative to classical biological control, but only long-term field experience will determine this. It would seem appropriate to gain such experience first with self-limiting systems, particularly where self-sustaining systems have self-limiting cousins—different configurations of the same components that give self-limiting effects, for example, sterile males and autosomal X-shredders for HEGs and bidirectional IIT for *Wolbachia*.

New technologies often fit awkwardly into nations' existing regulatory frameworks. Invasive systems may face particularly severe regulatory barriers, not least because they are unlikely to respect political borders. This has led several commentators to call for new, harmonized international regulations (7, 62), and various international agencies are developing guidelines. Existing frameworks for genetically modified organisms provide a basis for regulating those genetic strategies to which they apply, but non-genetically modified approaches are generally not included, despite their similarities. Nonetheless, as for conventional genetic engineering of mosquitoes, the relevant research groups have worked hard to clarify and comply with all applicable regulations (10, 27, 38, 58, 60, 71).

Table 1 Recent field trials of genetic control methods[a]

Date	Location	Method	Outcome	Reference(s)
2005–2009	Italy	SIT	Release of irradiated male *Aedes albopictus* induced sterility in target populations; population suppression was observed in some locations	12
2009–2010	Cayman Islands	RIDL	Males of a RIDL strain of *Aedes aegypti*, OX513A, competed successfully for mates with wild mosquitoes; sustained release of these sterile males led to strong suppression of the target wild population	38, 39
2010	Malaysia	RIDL	RIDL OX513A males have life span and maximum dispersal similar to an unmodified comparator	47
2010	French Polynesia	IIT	Sustained release of *Aedes polynesiensis* males infected with a *Wolbachia* strain from *Aedes riversi* induced sterility in a target population	60
2011–Present	Brazil	RIDL	Sustained release of RIDL OX513A males led to strong suppression of two target wild populations; larger subsequent program in progress[b]	–
2011–Present	Australia	Invasive *Wolbachia*	Release of *w*Mel-infected male and female *Aedes aegypti* led to the invasion and establishment of *w*Mel *Wolbachia* in two target populations; releases in three additional areas are in progress[c]	41
2012–2013	Australia	Invasive *Wolbachia*	Release of *w*MelPop-infected male and female *Aedes aegypti* in two target areas; does not appear to have self-sustained[c]	–
2013–Present	Vietnam	Invasive *Wolbachia*	Release of *w*MelPop-infected male and female *Aedes aegypti* on an island; in progress[c]	–

[a]The IIT and RIDL trials additionally confirmed the self-limiting, reversible nature of these methods (38, 39, 60).
[b]**www.moscamed.org.br/2012/index.php; www.oxitec.com**.
[c]**http://www.eliminatedengue.com/progress/**.
Abbreviations: IIT, incompatible insect technique; RIDL, release of insects carrying a dominant lethal gene; SIT, sterile insect technique.

After due consideration, regulators in several countries have approved limited field trials as part of an incremental testing and scale-up process. Public perception has generally been positive (38, 41), though it is too soon to determine long-term trends. Public response to new technologies, including genetic control, may vary considerably depending on social, political, technical, epidemiological, presentational, and cultural factors, of which the genetic aspect is only one, and may change over time. Even for human vaccination, a well-established technology, participation rates are rarely as high as program managers would wish, and scare tactics can still shake public confidence.

PROGRESS TO THE FIELD AND FUTURE PROSPECTS

Successful field trials have recently been conducted for several genetic control methods (summarized in **Table 1**). These trials followed extensive laboratory and cage trials (9, 11, 19, 29, 45, 50, 61, 77, 83). Each trial successfully accomplished its experimental objectives, and in no case have any negative consequences to human health or the environment been identified. With several exciting methods entering trials of varying scales, and a pipeline of even more powerful methods under development, the prospects for genetic control to help provide clean, effective, sustainable mosquito control seem bright indeed.

SUMMARY POINTS

1. Multiple genetic control strategies are under development; several have been successfully tested in initial field trials.
2. For genetic control, the modified pest becomes a biocontrol agent, acting via mating. This leads to a very high degree of species specificity. Furthermore, the modified mosquitoes actively disperse and seek out conspecific mates.
3. Current mosquito control methods generally suppress target mosquito populations. Some genetic control strategies share this goal (population suppression), but others aim to modify some or all of the mosquitoes in the target population to reduce their ability to cause harm (population replacement).
4. A defining aspect of genetic control methods is the extent to which the modification is expected to persist in the target population. In self-limiting strategies the modification disappears over time unless it is maintained in the target population by further releases of modified insects. In self-sustaining strategies the modification persists in the target population indefinitely and is expected to spread within this and other populations under at least some circumstances.

FUTURE ISSUES

1. How should different stakeholders be consulted and engaged?
2. How can regulatory frameworks that are harmonized, respected, and science led and that balance risks and benefits in a proportionate and consistent manner be adapted or developed?
3. How should potential risks relating to the long-term instability/evolution of self-sustaining genetic elements be investigated, assessed and mitigated? How will postrelease monitoring of self-sustaining elements be conducted, and who will pay?

4. How can we develop and validate rational, systematic design approaches to reduce development times while increasing the predictability of outcomes?
5. How can these genetic strategies be combined with each other and conventional methods for optimized integrated pest management, and with drugs, vaccines, etc., for integrated disease management?
6. How can large numbers of high-quality modified insects be reared economically? In this context, how can insect quality be assessed?
7. How can the epidemiological impact of complex interventions and combinations of interventions be demonstrated?
8. How can these powerful new technologies be applied in areas that need them but do not have deep enough pockets to pay high regulatory costs, e.g., conservation biology?

DISCLOSURE STATEMENT

The author is an employee and shareholder of Oxitec Ltd. Oxitec is developing genetic control methods for mosquitoes and other pest insects. Oxitec and the University of Oxford hold intellectual property relating to the subject matter of this paper.

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72. Showed that *Wolbachia* infection could confer resistance on *D. melanogaster* to RNA viruses—this led to the proposed use of such *Wolbachia* strains to make mosquitoes less susceptible to various pathogens.

74. Described potential genetic improvements to sterile-male methods (RIDL, fsRIDL) and proof-of-principle strains of *D. melanogaster*.

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