VECTOR-BORNE DISEASES

Controlling vector-borne diseases by releasing modified mosquitoes

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Abstract | *Aedes* mosquito-transmitted diseases, such as dengue, Zika and chikungunya, are becoming major global health emergencies while old threats, such as yellow fever, are re-emerging. Traditional control methods, which have focused on reducing mosquito populations through the application of insecticides or preventing breeding through removal of larval habitat, are largely ineffective, as evidenced by the increasing global disease burden. Here, we review novel mosquito population reduction and population modification approaches with a focus on control methods based on the release of mosquitoes, including the release of *Wolbachia*-infected mosquitoes and strategies to genetically modify the vector, that are currently under development and have the potential to contribute to a reversal of the current alarming disease trends.

Aedes aegypti
The primary mosquito vector of epidemic transmission for viruses, such as dengue, zika and chikungunya. *A. aegypti* is prevalent primarily in tropical and subtropical regions of the world and is particularly adapted to urban habitats.

Sterile insect technique
(SIT). The radiation or chemical treatment of male mosquitoes, which renders them sterile. When they are released in the field and they mate with wild-type females, they cannot produce offspring.

Incompatible insect technique
(ITT). The release of *Wolbachia*-infected males, which, when mated with wild-type females that contain no *Wolbachia* or a different, incompatible strain of *Wolbachia*, produce no offspring owing to cytoplasmic incompatibility.

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Aedes aegypti is exquisitely adapted to tropical and subtropical cities as its preferred habitat, living and breeding within people's dwellings and the waste that accumulates around them. As tropical cities continue to grow, often outstripping the delivery of adequate infrastructure to manage either water delivery or waste removal, this mosquito has flourished. This rapid urban growth together with widespread global air travel, which enables human pathogens to travel as easily as their hosts, creates the perfect conditions for human disease to rapidly spread, which we are currently experiencing globally.

The burden of *Aedes*-transmitted disease has considerably increased over the past 50 years[1,2]. The incidence of dengue, now the world's most common mosquito-borne viral disease, grew more than 30-fold during this period[3]. Dengue viruses are estimated to infect around 400 million people per year, and over half of the world's population is at risk of the disease[4] (FIG. 1).

More recently, chikungunya virus emerged from Africa in the mid-2000s, spreading first across India and Asia and then into the Americas in 2013 (REF.[5]). Zika virus outbreaks occurred in the South Pacific in 2013 and in the Americas in 2015 (REF.[5]). Infection with Zika virus in the Americas coincided with a surge in cases of microcephaly and other congenital abnormalities. Even yellow fever, for which an effective vaccine exists, is re-emerging. Recent outbreaks started in Angola in late 2015, and the virus quickly spread to the Democratic Republic of Congo, Kenya and China[6]. In late 2016, hundreds of cases of yellow fever have been reported in Brazil[7].

This unprecedented global emergence of viruses that are transmitted by arthropod vectors (arboviruses) is thought to be caused by a combination of human population growth, increasing globalization, a rapid rise in population-dense cities in tropical areas and major expansion of the geographical range of *A. aegypti*[1,5,8]. Existing methods that aim to reduce disease by suppressing mosquito populations through the physical removal of breeding sites or the application of insecticides targeting either larvae or adults are unable to cope in this new global context. To effectively limit or prevent future outbreaks, novel public health interventions are desperately needed[3].

New vector control approaches that involve the release of mosquitoes currently fall into two broad classes: they either aim to reduce the vector population or modify the vector to make it refractory to pathogen transmission. Reducing mosquito populations through suppression approaches is based on the intuitive assumption that as virus transmission is dependent on a bite from an infectious mosquito, reducing mosquitoes will lower transmission and disease. However, although this is clearly true if the mosquito population can be completely eliminated, the impact on disease if population suppression is only partial is much less clear. Currently, there is little experimental evidence (for example, from randomized controlled trials with epidemiological end points) that indicates the effectiveness of imperfect mosquito-suppression strategies[9,10].

Novel population reduction approaches involve rearing and releasing large numbers of male mosquitoes that cannot produce viable offspring when they mate with wild females. Over the course of many generations of continual release of these males, the size of the vector population should be substantially reduced, which in turn should reduce disease transmission. These methods include the sterile insect technique (SIT), the incompatible insect technique (IIT) and various genetic modification strategies (FIG. 2).

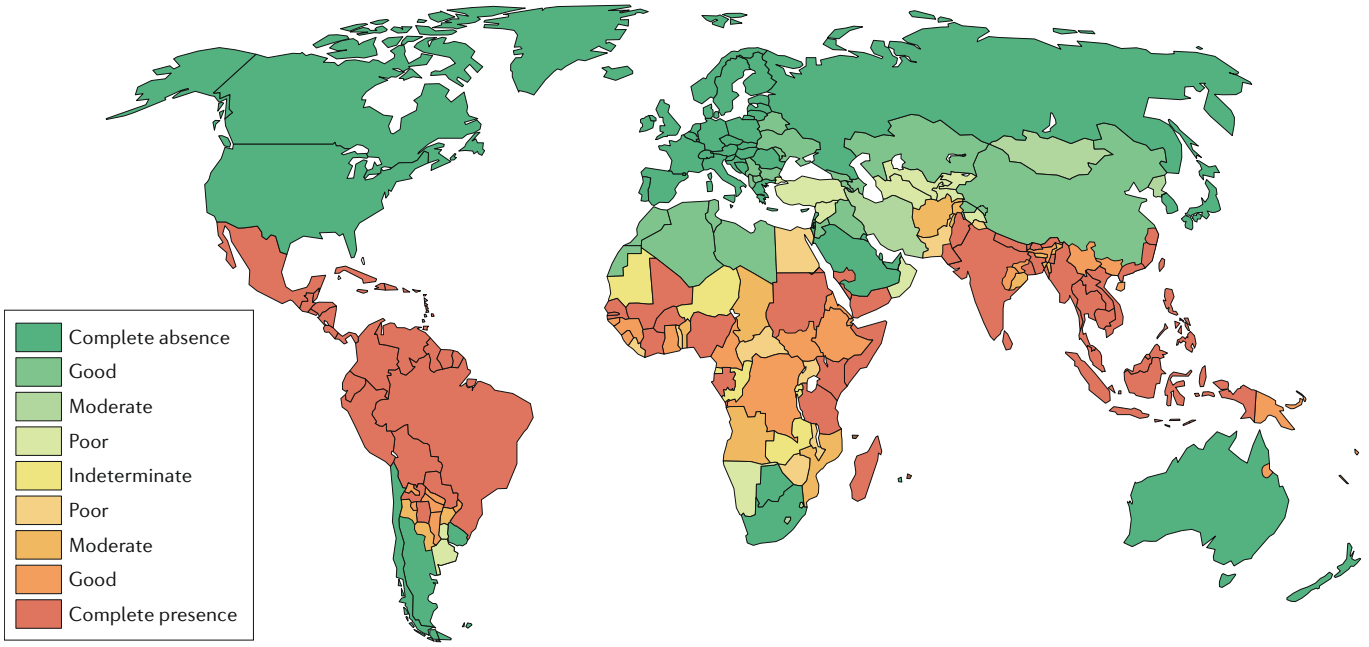

Fig. 1 | **The global distribution and burden of dengue.** Evidence consensus map showing the complete absence to complete presence of dengue. Green colours indicate evidence consensus towards absence of dengue, and orange and red colours indicate consensus towards presence of dengue. Darker colouring indicates more data supporting a conclusion about the presence or absence of dengue in a country. Figure adapted from REF.[4], Macmillan Publishers Limited.

By contrast, population modification approaches involve the release of both male and female mosquitoes that carry a heritable factor that reduces or blocks their ability to transmit viruses, such as dengue or Zika. As these modified mosquitoes mate with wild mosquitoes, the factor will spread through the population, effectively rendering the mosquitoes incapable of transmitting the pathogen without the need for population suppression. These approaches include the deployment of pathogen-blocking endosymbiotic bacteria *Wolbachia pipientis* (FIG. 3) and gene-drive mechanisms, such as the CRISPR–Cas9 system, coupled with transmission-blocking gene constructs (FIG. 4).

Before any new vector control approach can be deployed at scale, it should progress from laboratory-based proof-of-concept experiments to semi-field and then open field releases[11]. To achieve this, researchers must adapt the vector control methodologies for large-scale releases, satisfy regulatory requirements and commit to investing great effort in community engagement. Strong public support is critical for adoption of any new technology, as these stakeholders have the power to help 'pull' a technology to the field — or, alternatively, prevent its implementation. Finally, if field releases are successful, the task of demonstrating epidemiological impact for a particular technology still remains. Effective epidemiological studies require the support and involvement of the community at the trial site, approval by the government, collaboration with the existing health system and robust financial support.

In this Review, we describe, evaluate and compare novel vector control methodologies that are based on either the modification of the mosquito population or mosquito population suppression approaches and that require the active release of modified mosquitoes. We highlight knowledge gaps and discuss lessons learned from field releases that may aid in the success of these or other approaches going forward.

Population modification approaches

Wolbachia *to target virus transmission*. The endosymbiotic bacterium *Wolbachia pipientis*, referred to as *Wolbachia* from here on, naturally infects an estimated 40–60% of all insect species[12,13]. It is vertically transmitted via the host egg, and many *Wolbachia* strains manipulate host reproduction to provide an advantage to infected females — most commonly by inducing cytoplasmic incompatibility (CI). Infected females can mate successfully with both infected and uninfected males, which enables the rapid spread of *Wolbachia* throughout a population (FIG. 3b). The expression of CI also provides a method to suppress insect populations by releasing *Wolbachia*-infected males into a population of naturally uninfected female insects, thus effectively sterilizing those females (see below, also FIG. 3a).

Recently, it was discovered that, in addition to inducing CI in insects, *Wolbachia* can protect its natural host *Drosophila melanogaster* from pathogenic viruses, such as Drosophila C virus[14,15]. Since that initial observation, a number of different *Wolbachia* strains were shown to prevent the transmission of a range of viruses and parasites in laboratory studies[16–23] by preventing pathogen replication within the insect[16,24].

The properties of CI coupled with the inhibition of virus replication provide the basis for a novel intervention strategy against mosquito-transmitted diseases. By releasing both male and female mosquitoes that are infected with *Wolbachia* into a wild population, it should be possible for *Wolbachia* to invade that population.

Wolbachia pipientis
A naturally occurring bacterial endosymbiont that is estimated to be present in 40–60% of all insect species. Commonly referred to as just *Wolbachia*.

CRISPR–Cas9
A genome-editing tool that was developed from adaptive immune systems found in bacteria and archaea. The system is composed of a nuclease, Cas9 and a guide RNA that targets the nuclease to a specific DNA sequence for cleavage.

Cytoplasmic incompatibility
(CI). When *Wolbachia*-infected male mosquitoes mate with uninfected females, the resulting progeny die during early embryogenesis. If the female is also infected with the same *Wolbachia* strain, that infection can rescue the embryonic lethality, resulting in viable progeny.

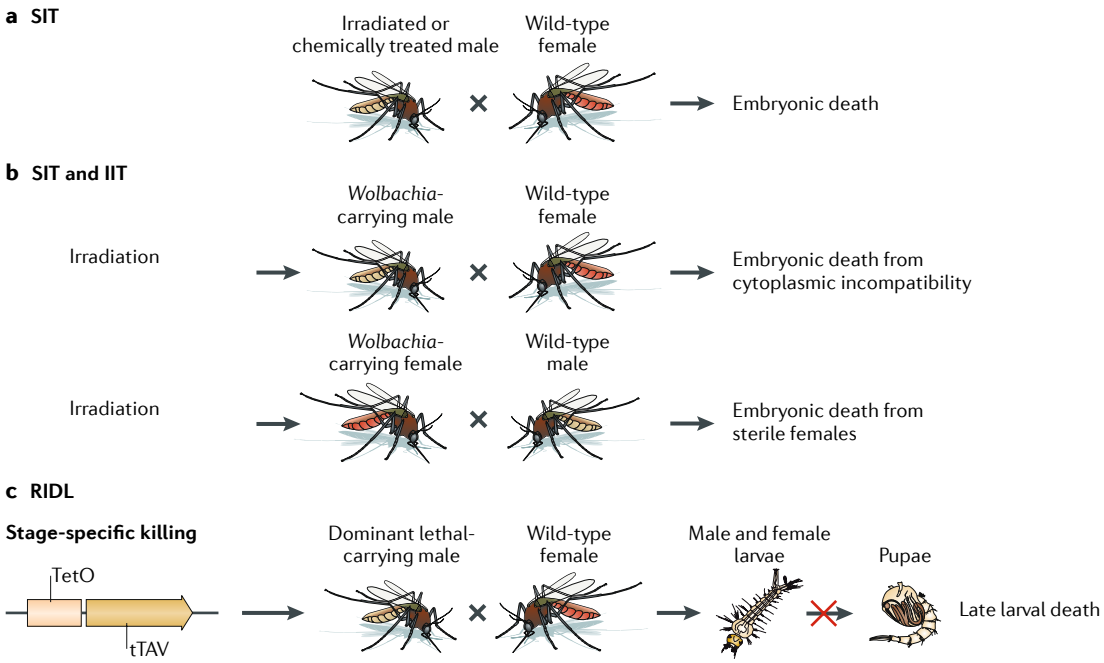

Fig. 2 | **Modification of vectors for population reduction.** New vector control approaches that involve the release of mosquitoes aim to reduce the vector population. **a** | In the sterile insect technique (SIT) approach, male insects are exposed to either irradiation or sterilizing chemicals, causing large-scale random damage to the insect chromosomes or dominant lethal mutations in the sperm. These males are then released into the wild population, and when they mate with wild females, viable offspring are rarely produced, eventually leading to a substantial decrease in vector population size. **b** | In the incompatible insect technique (IIT) approach, a *Wolbachia* strain is stably introduced into a colony of a mosquito species. Only *Wolbachia*-infected males are released, which, when mated to females that do not harbour the same *Wolbachia* strain or that do not carry *Wolbachia*, results in the death of their offspring owing to cytoplasmic incompatibility. A combination of IIT and SIT could be used to suppress mosquito populations. During this approach, *Wolbachia*-infected mosquitoes are treated with low-level irradiation. As in IIT alone, mating between *Wolbachia* males and wild females will not produce offspring. In the case of accidental female releases, these irradiated females are sterile and cannot reproduce with wild or *Wolbachia*-infected males. **c** | Release of insects carrying a dominant lethal (RIDL) is a suppression strategy whereby males that carry a transgene that causes late-acting lethality are released in the open field. These males mate with wild-type females, and the resulting offspring die before reaching the pupal stage. TetO, tetracycline operator; tTAV, tetracycline-repressible transcriptional activator.

Wolbachia-infected females would have a reproductive advantage compared with wild-type females owing to the induction of CI, and *Wolbachia* would naturally spread throughout the population until nearly all mosquitoes carry it. The *Wolbachia*-infected females would then have greatly reduced ability to transmit a virus to humans, and disease should decline and potentially be eliminated from communities[25,26].

In contrast to many insects, including many mosquito species, *A. aegypti* is not a natural host for *Wolbachia*, and therefore to use *Wolbachia* to modify a mosquito population, the bacteria must be introduced into the mosquito through microinjection and a stable colony needs to be established[24,27]. Subsequently, it needs to be determined whether *Wolbachia* can reduce the vector competence of the mosquito. To date, eight different *Wolbachia* strains have been transinfected into *A. aegypti*: *w*Mel, *w*MelPop-CLA, *w*MelCS, *w*Ri, *w*Au, *w*AlbA, *w*AlbB and *w*Pip[24,27–30]. Importantly, it was shown that *Wolbachia* can limit the transmission of a range of human pathogens by *A. aegypti*, including dengue, Zika and chikungunya viruses[16,17,19,24], which suggests that this intervention simultaneously targets multiple diseases.

Vector competence
A measure of the ability of arthropod vectors to acquire and transmit viruses in their saliva.

Using *Wolbachia* to reduce the ability of the mosquito population to transmit disease has a number of desirable attributes. The method requires the release of far fewer mosquitoes than population reduction methods such as SIT or IIT (TABLE 1). Moreover, once *Wolbachia* is established in a mosquito population, it is expected to be maintained at a high frequency indefinitely[31]. In Australia, initial releases of *Wolbachia*-infected male and female insects were undertaken for 10 weeks, and *Wolbachia* infection has persisted in wild mosquito populations at frequencies above 90%[32]. Therefore, mosquitoes infected with *Wolbachia* need to be deployed only once, which is in contrast to population suppression strategies (which requires the repeated deployments of modified mosquitoes as the natural vector population recovers). As a result, *Wolbachia*-based replacement strategies are cost-effective, and, moreover, as costs occur only for the initial deployment, donor fatigue might be less of a problem for this approach, as no ongoing recurrent funding is needed to sustain the intervention. Finally, as this method involves the release of both female and male mosquitoes, there is no need for the laborious and error-prone process of sex sorting before release.

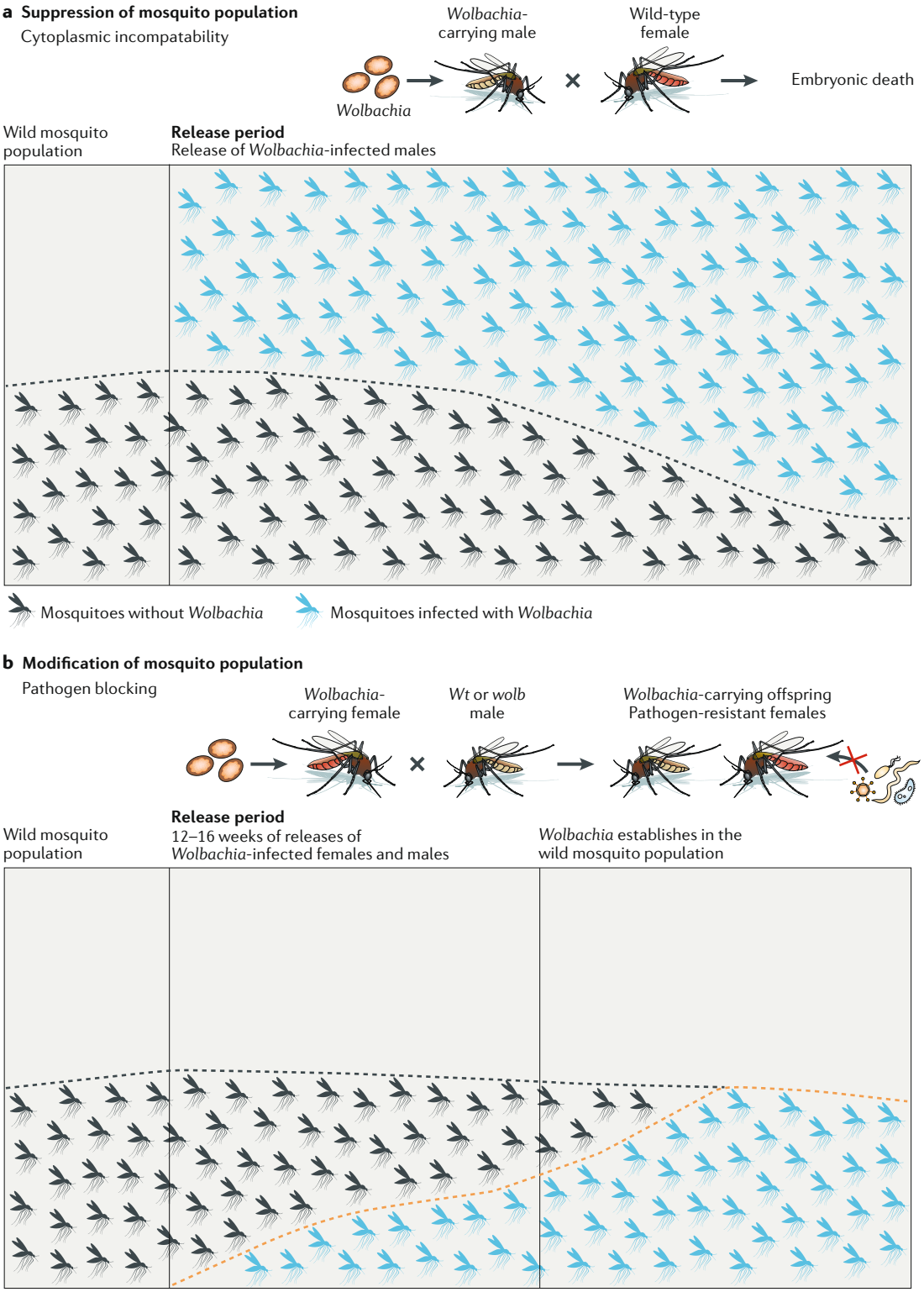

Fig. 3 | **Using *Wolbachia* to reduce or modify populations. a** | The use of *Wolbachia* in the incompatible insect technique (IIT) approach results in population reduction. In IIT, a *Wolbachia* strain is stably introduced into a colony of a mosquito species. Only *Wolbachia*-infected males are released, which, when mated to females that do not harbour the same *Wolbachia* strain or that do not carry *Wolbachia*, results in the death of their offspring owing to cytoplasmic incompatibility (CI). Large numbers of males are released to increase the number of incompatible matings that are occurring. Over time, the population of disease-competent mosquitoes will decrease. **b** | *Wolbachia* can also be used to modify a mosquito population. Both *Wolbachia*-infected (*wolb*) male and female mosquitoes are generally released over a 12–16-week period. CI provides a reproductive advantage to *Wolbachia*-infected females, resulting in the spread and establishment of *Wolbachia* in the population. These *Wolbachia*-infected females are resistant to arboviruses such as dengue, Zika and chikungunya. *Wt*, wild type.

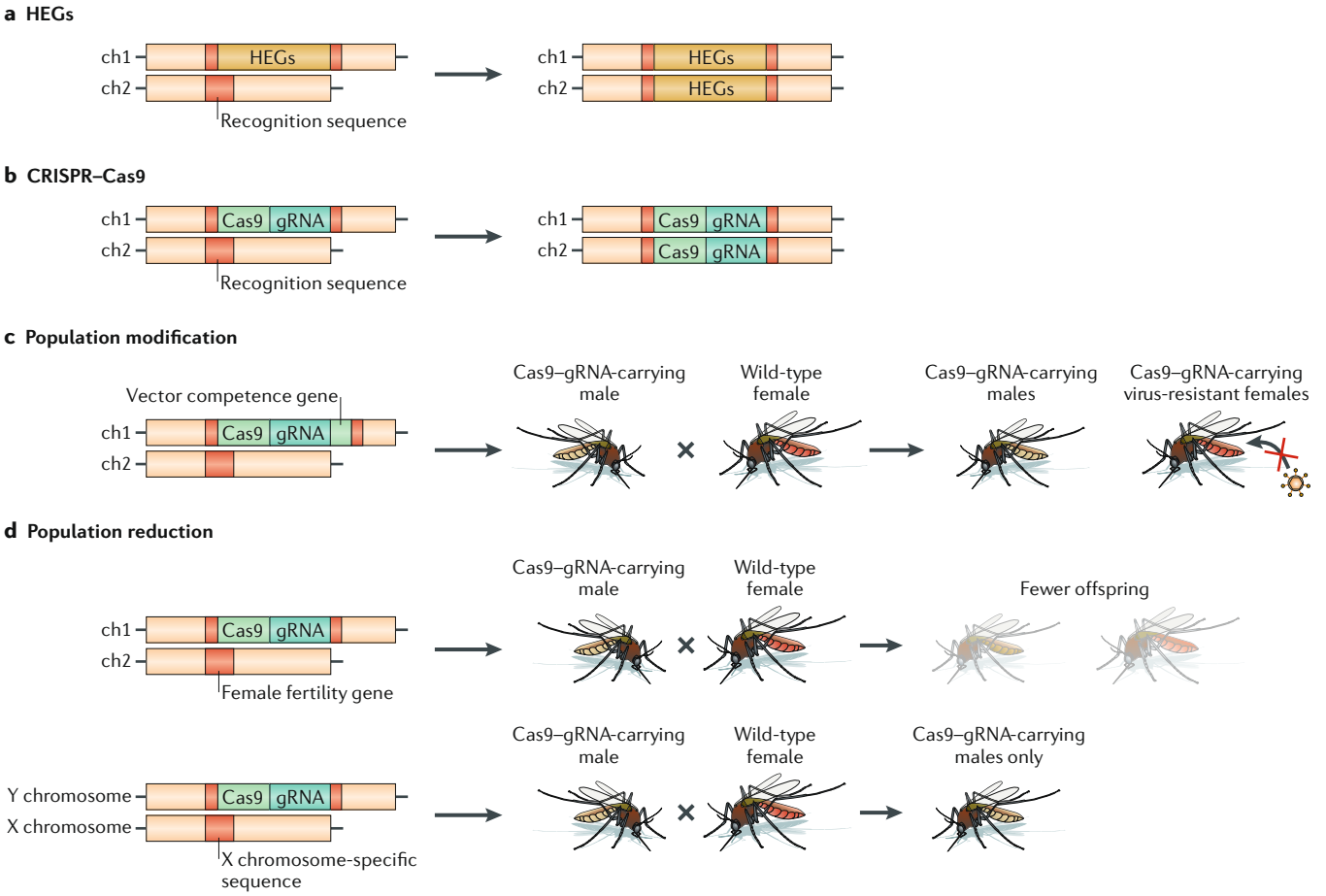

Fig. 4 | **Gene drive approaches to modify or reduce populations.** Homing endonuclease genes (HEGs) encode endonuclease genes that recognize a specific DNA sequence and catalyse a break, which is then naturally repaired through homology-directed repair resulting in non-Mendelian inheritance (part **a**). The CRISPR–Cas9 system is analogous to HEGs (part **b**); however, a guide RNA (gRNA) provides sequence specificity for DNA cleavage by the Cas9 nuclease, which is then repaired through homology-directed repair. Examples of CRISPR–Cas9 used in population modification and reduction approaches include the addition of vector competence genes with the Cas9–gRNA construct, resulting in virus-resistant offspring[99] (part **c**); the creation of a gRNA to target female fertility genes, resulting in sterile females[100]; and the creation of a gRNA to target X chromosome-specific sequences, resulting in a reduction in female offspring[101] (part **d**). The Cas9–gRNA constructs inherited by any surviving offspring result in its continual spread.

Ongoing field trials. Currently, the World Mosquito Program (WMP; previously known as the Eliminate Dengue Program)[33] is undertaking deployments of *A. aegypti* infected with *Wolbachia* in five countries with strong community support. These studies have shown that the *w*Mel strain of *Wolbachia* can quickly spread to near fixation in the wild mosquito population, and in the field sites in Australia, where this approach has been studied the longest, the frequency of the *w*Mel strain in the mosquito population has remained stable since the initial deployment in 2011 at rates of around 90% or greater[32,34]. Large-scale releases are now underway in Brazil (Niteroi and Rio de Janeiro), Colombia (Bello and Medellin) and Indonesia (Yogyakarta)[35] in the form of randomized cluster trials or large non-experimental deployments that cover more than two million inhabitants each.

Work in Australia has shown that the method can be deployed successfully at low cost across small cities. Moreover, early time series observational data from these sites indicate no observations of local dengue transmission once *Wolbachia* is established in the local mosquito population[36]. A randomized cluster trial of the method is currently in progress in Yogyakarta[33] (ClinicalTrials.gov, NCT03055585). This trial, which is estimated to finish in 2019, is expected to provide high quality epidemiological evidence on the degree of disease reduction through the use of *Wolbachia*-infected mosquitoes.

Releasing female mosquitoes is not without issue. As female mosquitoes bite, their release can be a source of discomfort for individuals. During the period of active releases, the number of female mosquitoes present in the mosquito population will temporarily increase, and the community may experience higher biting pressure. The released *Wolbachia*-carrying female mosquitoes are expected to decrease, rather than increase, the transmission of arboviruses, such as dengue, Zika and chikungunya[16,19,24]; however, communities need to accept an approach that superficially seems to counter years of health promotion messages advising to

Table 1 | **Comparison of different vector control technologies that are currently being developed**

Technology	Laboratory proof-of-concept	Field release	Scaled deployment beyond 50 km²	Re-application required	Approximate release rate (mosquitoes per ha weekly)
Population modification					
Wolbachia	+	+	+	No	10–100[75,109]
CRISPR–Cas9	+	–	–	No	<1
Population suppression					
SIT	+	+	–	Yes	1,000[a67]
IIT (*Wolbachia*)	+	+	–	Yes	1,000–10,000[a72,73,114]
RIDL	+	+	–	Yes	25,000–50,000[a35,90]

IIT, incompatible insect technique; RIDL, release of insects carrying a dominant lethal; SIT, sterile insect technique. [a]Males per ha weekly.

kill mosquitoes. This issue needs to be addressed with strong community engagement[37].

Publicly available risk analyses provide confidence about the safety of the method[33,38,39], but some concern has been raised that *Wolbachia* infection might enhance the transmission of other pathogens. For example, it has been suggested that transient *Wolbachia* infections (that is, temporary infections of *Wolbachia* injected into the body of the mosquito followed by pathogen challenge) may increase infection rates, although not dissemination or transmission rates, of West Nile virus in the mosquito *Culex tarsalis*[40]. However, in stably transmitted *Wolbachia* infections (where the *Wolbachia* infection is stable, infects germline tissues and is maternally transmitted by the mosquito), no enhancement of transmission of any virus, including West Nile virus, has been shown[16–18,21,24,41–43]. Similarly, transient *Wolbachia* infections in anopheline mosquitoes have suggested that, in certain contexts, vector competence for infections with *Plasmodium* spp. might increase[44–46], whereas in naturally infected anophelines, vector competence is reduced[47,48]. By contrast, natural *Wolbachia* infections of *Culex pipiens* mosquitoes have been shown to increase susceptibility to infections with *Plasmodium relictum*[49]. Clearly, extensive testing is needed before release to ensure that the control measure does not inadvertently exacerbate disease.

Although *Wolbachia* is a vertically transmitted endosymbiont, comparisons of host and bacterial phylogenies suggest that horizontal transmission occurs[50,51], leading to the concern that the introduction of *Wolbachia* to a novel host such as *A. aegypti* could result in the horizontal transfer of the bacterium to predators or other insects. Laboratory-based and field-based experimental testing for horizontal transfer of *Wolbachia* from *A. aegypti* has found no evidence of transfer[52,53]. Moreover, 40–60% of all insect species are naturally infected with *Wolbachia*[13,54], and it is unlikely that introducing *Wolbachia* into one more species will increase the frequency of horizontal transmission, especially when closely related *Wolbachia*-infected mosquitoes, such as *Aedes albopictus* and *Aedes notoscriptus*, already inhabit the same larval habitats as *A. aegypti*. Furthermore, the fact that *A. aegypti* is not a natural host for *Wolbachia* despite shared habitats with the aforementioned species provides further support that horizontal transmission is unlikely to occur.

It has also been suggested that viruses may develop mutations over time that render them less susceptible or resistant to *Wolbachia*[55]. The mechanistic basis of *Wolbachia*-mediated pathogen blocking remains to be fully elucidated, but current data suggest that multiple pathways underlie this effect, which suggests that resistance will not evolve easily[56–58]. Moreover, assessments of field-released mosquitoes suggest that if resistance does develop, it will not happen quickly[34]. Furthermore, even if resistance were to develop in the future, a great reduction in disease burden may have been afforded to communities in the intervening period.

Population reduction approaches

Sterile insect technique. Reducing mosquito populations has long been a focus of disease control programmes, with the underlying assumption that reducing the number of mosquitoes present in a population will limit the probability of transmission for viruses such as dengue or Zika. Population reduction approaches have been widely used, despite limited experimental evidence supporting the epidemiological effectiveness of these approaches[10]. One such method of population reduction, the SIT, involves irradiating or chemically treating male mosquitoes to sterilize them. When these males are released and mate with wild females, no offspring are produced, eventually leading to a substantial decrease in population size[59] (FIG. 2a). SIT has been used to reduce mosquito populations with some success, although it has generally been more successful in other agricultural pest species[60,61].

Work to explore the use of SIT in *Aedes* species has begun[62–66], and the results of a pilot field trial have been published. One study, trialling SIT in *A. albopictus* at four different sites, found that eggs collected in ovitraps from treated areas had induced egg sterility rates of 18–68% compared with eggs from untreated areas, with two sites showing a significant reduction (50–72%)[67].

The use of SIT has a number of advantages. As females will not be released, communities should experience no increase in biting rates, and therefore the intervention may be more acceptable than the population modification approaches described above. It is also aligned with existing health promotion messaging of reducing mosquito population size. Finally, if the population can be suppressed, then reductions in disease

Ovitraps
Traps designed for the collection of mosquito eggs.

transmission are expected with little chance of pathogen resistance developing.

However, the temporary nature of vector population suppression has some disadvantages. Complete elimination of a vector population in an area would require large numbers of males to be released over a long time period (TABLE 1) — especially considering the biology of *A. aegypti* (for example, mosquito eggs can withstand drying for many months) — and unless the population is completely eliminated, it is expected to recover quickly if no further control measures are in place. Similarly, migration of mosquitoes from regions other than the treatment area would spark a population resurgence. This means that SIT releases need to be repeated regularly to maintain community protection from disease. It also requires mosquitoes to be sorted by sex before release, which is not straightforward, and any females that escape sorting may be a competent disease vector. Another difficulty in using SIT is the generation of sterile males that have high fitness and are reproductively competitive with wild-type males[65,68]. Finally, as for all suppression methods, a likely scenario is that the mosquito population is only reduced and not eliminated. Unfortunately, there are no experimental studies available that robustly measure the impact of incomplete suppression on epidemiological end points, and therefore the effects on disease are currently unclear.

Incompatible insect technique. A modified version of SIT, termed the IIT, can overcome the fitness costs that are associated with irradiation or chemical treatment of males by using *Wolbachia* to effectively sterilize males[69]. To implement IIT, a *Wolbachia* strain is stably introduced into a colony of a mosquito species. In contrast to population modification approaches, only male mosquitoes carrying *Wolbachia* are released into a wild population to mate with wild-type females; owing to the CI induced by *Wolbachia*, no offspring can be produced. If males are released in high enough numbers, more incompatible matings will occur, and ultimately, the mosquito population collapses (FIG. 3a).

IIT has a long history of field trials. In 1967, *Wolbachia* was first used as a population reduction strategy to control *Culex quinquefasciatus* in Burma[70]. Subsequently, semi-field and pilot field studies of IIT have been performed for *A. albopictus* and *Aedes polynesiensis*[71–73]. *Wolbachia*-infected males of the lymphatic filariasis vector *A. polynesiensis* were released over a 30-week span in French Polynesia, leading to a significant decrease (17%) in egg brood-hatch success in the treated area relative to an untreated area[72]. In Kentucky (USA), *A. albopictus* males transinfected with the *w*Pip strain of *Wolbachia* were released over a 17-week period, causing a significant decrease in the mean number of females collected, as well as a reduction in egg hatch in treated compared with untreated areas[73].

IIT has many advantages as a method of vector population reduction. The use of *Wolbachia* to 'sterilize' males is not associated with the fitness costs that can reduce male mating competitiveness in SIT approaches[68]. Depending on the strain of *Wolbachia* used, females that escape sorting and are released may have greatly reduced ability to transmit pathogens. Finally, as *Wolbachia* is naturally occurring and already ubiquitous, the public may accept this technique more easily than genetic modification or irradiation.

By contrast, the IIT still shares many of the limitations of SIT. It requires the continual release of large numbers of males to suppress the mosquito population (TABLE 1), and migration of mosquitoes from surrounding (untreated) areas will limit the long-term effectiveness of this method. As only males are introduced into the environment, an effective sex-sorting system is still required. If non-negligible numbers of females are also released, *Wolbachia* could spread through a mosquito population as in a replacement approach rather than suppress it — although the probability of this is dependent on the overall fitness effects that the *Wolbachia* strain has on the vector[31,74,75]. Although the IIT approach was tested on a small scale in pilot studies more than 50 years ago, it has yet to be shown that this approach can be scaled-up sufficiently to be an effective operational tool for disease control.

Using a combination of IIT and SIT could further reduce the need to carefully sort females from males before release. In this method, *Wolbachia*-infected mosquitoes are treated with low-level irradiation, which sterilizes females whereas males are unaffected. Females that escape sex sorting and are released into the wild cannot produce offspring and therefore would not interfere with the induced CI in the mosquito population[76,77] (FIG. 2b). The low dose of irradiation has minimal effects on male fitness in a laboratory setting, which suggests that this combined method could be effective in the field[76–79].

Genetically modified mosquitoes. Although a number of transgenic systems have been developed to suppress mosquito populations, few have progressed to field releases[80–83]. Oxitec has developed a number of transgenic approaches that are based on their release of insects carrying a dominant lethal (RIDL) methodology[80,84]. The OX513A mosquito strain has been used most successfully to date. This mosquito strain has a tetracycline-repressible transcriptional activator (tTAV) under the control of its own binding site tetracycline operator (TetO), creating a positive-feedback loop in which the expression of tTAV results in late-larval lethality[84]. When the mosquitoes are reared on a diet supplemented with tetracycline, it binds tTAV, preventing its binding to TetO, which decreases the production of tTAV and allows the mosquitoes to thrive. When OX513A males are released into the wild and mate with wild-type females, they pass on the transgene to their offspring; owing to the lack of tetracycline in their diet, the transgene is expressed, leading to late-larval death[84–86] (FIG. 2c).

Two field studies have compared the fitness of OX513A mosquitoes with that of wild-type mosquitoes for male competitiveness[87] as well as dispersal and longevity[88]. The life expectancy and maximal dispersal distance of OX513A are similar to those of wild-type mosquitoes, but the mean distance travelled is significantly lower[88].

Field releases of these mosquitoes have been performed in the Cayman Islands and Bahia, Brazil. The release of OX513A in the Cayman Islands allowed researchers to perform real-time comparisons of the effective numbers of males required to achieve a significant decrease in mosquito populations. Under their highest release ratios, they found an 80% relative reduction in treated versus untreated areas over a 23-week period[89]. In Brazil, mosquitoes were released over the course of 1 year. A 95% reduction in the local population of *A. aegypti* was observed based on adult trapping data and an 81% reduction based on egg trapping data[90]. Currently, Oxitec is performing releases in the Cayman Islands, Panama and Brazil, with plans for substantial expansion in their Brazil release sites[35].

Oxitec's methodology has several advantages over traditional suppression methods. The radiation used in SIT generates dominant lethals in a nonspecific manner — which can also lead to strong fitness effects and lowered mating competitiveness of males. The RIDL method specifically engineers a dominant lethal, thereby limiting off-target effects. Additionally, engineering allows for control of when the lethality is induced (that is, when the mosquitoes die in their lifespan)[84]. In contrast to the SIT, which induces lethality generally at the embryonic stage, lethality of OX513A is induced at late-larval stages[59], suggesting that although OX513A larvae ultimately die before adulthood, they still compete with wild-type larvae for food, possibly enhancing population suppression[84]. Although the public may have concerns about the release of genetically modified insects, their concerns may be alleviated by the fact that the OX513A-based approach is a self-limiting technology. As the transgenic mosquitoes require tetracycline in their diet for survival, mosquitoes that carry the transgene cannot survive more than one generation in the field.

One of the largest limitations of a RIDL technology such as the OX513A-based approach is that it requires large numbers of males to be released for successful suppression (TABLE 1), and this can be technologically and financially difficult. For the release of OX513A in both the Cayman Islands and Brazil, the planned field site sizes had to be decreased owing to rearing limitations and a requirement to maintain a mating fraction of 50% for genetically modified males[89,90]. This suggests that large-scale releases could be difficult to maintain. As with SIT and IIT, accurate sex sorting is required for this RIDL method. Although sex-sorting methods have become more efficient, rates of accidental release of females were previously reported to be between 0.02 and 0.33%[88–90]. For large-scale releases, such as those planned in Brazil for which Oxitec estimates releases of 30–60 million males per week[35], this would result in the unintended daily release of thousands of females. In addition, a considerable community engagement effort to build sufficient trust for widespread deployment of genetically modified mosquitoes is required[91].

Emerging technologies

A number of developing technologies exist that have not yet progressed to field trials. Numerous laboratory-based studies have shown that the use of transgenes can be effective in limiting pathogen transmission through the expression of genes that target the pathogen or that are effective in suppressing mosquito populations by targeting genes involved in reproduction or by using sex distortion systems. Although these systems show promise, the difficulty lies in how to spread the transgenes to all mosquitoes in a population. One of the most promising methods to solve this problem is the use of transgenes to generate a gene-drive system, a strategy that was first proposed nearly 15 years ago[92]. Gene-drive systems alter normal Mendelian inheritance to greatly increase the odds that the drive system will be passed on to offspring. An effective gene-drive system could be used to establish disease inhibitors or population repressors in a population. Homing endonuclease genes (HEGs) were the initial inspiration for a gene-drive system. HEGs encode proteins that recognize and cleave a 15–30 bp DNA sequence. By placing HEGs within their target sequences, the chromosome on which it was located would be resistant to cleavage. Cleavage of chromosomes that contain only the recognition site would occur, and owing to homology-directed repair (HDR), a heterozygote would be converted into a homozygote (FIG. 4a). HEGs have been developed in *Anopheles* and *Aedes* mosquitoes in proof-of-concept experiments[93–95].

The CRISPR–Cas9 system has been used in genome editing for a number of years in diverse organisms[96,97]. A study showed that placing the genes that encode Cas9 and a guide RNA (gRNA) into the template used for HDR generated a mutagenic chain reaction capable of gene drive[98] (FIG. 4b). Subsequent work showed that in laboratory settings, CRISPR–Cas9 could be used to spread anti-*P. falciparum* effector genes into an *Anopheles stephensi* population[99], to target genes required for female fertility in *Anopheles gambiae*[100] and to create a sex distortion system that targets female *A. gambiae*[101], suggesting that this system can be used for both population suppression and population modification (FIG. 4c,d). However, optimization of this methodology is still required before commencing field trials. The first two studies discussed above[99,100] used the regulatory regions of the germline-specific gene *vasa* to induce the expression of Cas9 in the germ line, thus causing only heritable mutations. However, one study found that the expression of Cas9 was not completely restricted to the germ line, resulting in somatic mutations[100]. A second study found that maternal deposition of the Cas9 protein from the mother into the developing egg caused double-stranded DNA breaks during early embryonic development before a homologous chromosome was present as a repair template, resulting in an increase in non-homologous end joining (NHEJ) repair rather than HDR[99]. Repair via NHEJ often results in point mutations, insertions or deletions of sequences, which destroy the Cas9 recognition site and thus the generation of resistant alleles[99,100].

The CRISPR–Cas9 gene-drive method can potentially be extremely powerful. Only small numbers of the modified mosquitoes might need to be released (TABLE 1), as the modification should drive itself throughout a mosquito population[98], and nearly any sequence of interest can be targeted. However, similarly

Homing endonuclease genes
(HEGs). Selfish genetic elements encoding endonucleases that recognize a specific DNA sequence and catalyse a break, which is then naturally repaired through homologous repair.

Homology-directed repair
(HDR). A repair mechanism of a DNA double-strand break, whereby the homologous chromosome is used as a template for repair.

Non-homologous end joining
(NHEJ). A repair mechanism for DNA double-strand breaks, whereby the two DNA ends are ligated without the need for a homologous template, often resulting in small indels or the introduction of mutations.

to HEGs, the CRISPR–Cas9 system is susceptible to developing resistance owing to mutations that can occur in the recognition site. As described above, multiple laboratory-based studies using CRISPR–Cas9 for gene drive have reported the accumulation of mutations that led to CRISPR-resistant alleles[98–100], which halt the spread of any modifications throughout a population. Furthermore, based on theoretical modelling, evolution of resistance against the CRISPR–Cas9 system is inevitable[102,103]. The emergence of resistance might be avoided or at least prolonged by targeting multiple sequences, by targeting conserved sequences that cannot tolerate disruption or by being more mindful of when releases occur in relation to seasonality of the vector population[102]. Whereas the OX513A strain is self-limiting, the CRISPR–Cas9-drive is self-promoting. The potential for uncontrolled spread of genetic modifications has caused concern among the scientific community[104], resulting in the publication of guidelines not only pertaining to field releases of such modified organisms but also preventing the accidental release of the modified organisms from laboratories[104–107].

Lessons learned

Most of the different technologies described above are still in early developmental stages, with limited examples of field releases, and only *Wolbachia*-based population modification approaches, as undertaken by the WMP[33], are being utilized at operational scales in medium-sized cities. A number of lessons are being learned that generally apply to all the approaches.

Importance of field-cage studies. Advocates of phased testing approaches have stressed the importance of preliminary testing of technologies in semi-field cages before open field release[11]. The construction of these facilities is expensive and time consuming, and evidence suggests that they may not actually provide data that are more useful than data collected from small laboratory cages in regard to evaluating an approach. Even very elaborate field cages[108] do not mimic the true field situation. For example, semi-field-cage experiments demonstrated successful establishment of the *w*MelPop strain of *Wolbachia* in a mosquito population, but it was later shown that this *Wolbachia* strain could not be established following open field releases[24,109]. Those findings together with the expense of such preliminary testing strategies indicate that field-cage studies should be carefully considered and relevant to the question being addressed and not automatically recommended.

When the WMP first started to undertake field releases, there was some concern that *Wolbachia* might spread in an uncontrolled manner. There was good evidence documenting regional[110,111] and even global sweeps[112] of *Wolbachia* infections in naturally infected hosts, raising the prospect that once *Wolbachia* was released, it might spread to locations that might not have approved its release. Initial field testing was done very carefully in Australia in geographically isolated areas to evaluate the ability of *Wolbachia* to spread[75]. Over time, it was realized that in *A. aegypti*, the spreading rates of the *w*Mel strain of *Wolbachia* were very slow[113], and the initial concern was unfounded. The current controversy around the potential uncontrolled spread of CRISPR–Cas9 gene-drive technology is injecting an even greater sense of caution into this area[104,105,107]. The theoretical ability of gene-drive systems to spread from very small numbers of released individuals and to alter an entire wild population is of concern, as we do not fully understand possible adverse consequences of such a release and may not be able to assess it before release. Current gene-drive methodologies do not have reversibility built into the system, so if negative consequences are observed, it would be difficult to stop the intervention from spreading. However, the emerging issues of resistance with this technology suggest that, similar to *Wolbachia*-based approaches, the power of the gene-drive systems that are being developed might be overstated. Although there is merit in a cautious framework to evaluate and test these methods, it must be balanced against the public health need of new technologies to protect people from ongoing disease outbreaks. Testing and regulatory frameworks need to be sufficiently flexible to be able to adapt to less stringent and time-consuming testing procedures if empirical evidence shows that risks are likely to be overstated. Otherwise, technology that is urgently needed may be unnecessarily impeded in its adoption and use.

A common feature of all these new vector control tools is that they rely on the release of mosquitoes into the environment to control the diseases they transmit. This requires communities to have high levels of trust to willingly participate given that the health promotion messages used for decades have been based on the dangerous nature of mosquitoes and the need to kill them to reduce disease risk. Even the most robust and elegant technology will fail to be implemented if communities will not accept it. Recently, this was exemplified by the difficulties Oxitec has faced in applying the RIDL methodology in open releases in Florida, where deep issues of mistrust towards genetic modification technology, government and industry have led to open protests and stalling of testing plans of a potentially robust and useful technology[91]. Serious attention and resourcing are required for effective community engagement programmes associated with these technologies so that trust and acceptance can be built with the communities that will be the end recipient of the technology[37]. This engagement is costly and time consuming and needs to start early, even before a given technology is fully developed. Unfortunately, many of the scientists involved in the development of new technologies are laboratory-focused specialists with little experience in field application or the principles of effective community engagement.

Conclusion

Existing vector control methods are clearly unable to cope with the unprecedented emergence and re-emergence of arboviral diseases. A number of novel methods under development show promise in curbing the ability of *A. aegypti* mosquitoes to transmit pathogens.

Within the next few years, we expect that evidence for the effectiveness of these new interventions will accumulate. Critical to wide-scale adoption of any of these approaches will be rigorous epidemiological evidence showing the impact on disease, not just entomological indicators.

Many of these technologies are being developed by scientists who are not located in disease-endemic countries. Ultimately, collaborations between scientists and governments of affected countries are needed to test and apply the technology. This requires open and authentic partnerships to be developed very early with these collaborators so that they are active participants in the development and implementation of the technology in their countries. Without their full support and ownership, there is no pathway to adoption.

Equally as important will be the sustainability and cost-effectiveness of the different approaches for disease-endemic countries with limited resources for control programmes. Hopefully, at least some of these technologies will prove to be cost saving for health ministries, in which case adoption pathways will be more straightforward. With solid epidemiological evidence and community support, their widespread implementation might reverse the current alarming global disease trend.

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