VECTOR-BORNE DISEASES

Beyond insecticides: new thinking on an ancient problem

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Abstract | Vector-borne disease is one of the greatest contributors to human mortality and morbidity throughout the tropics. Mosquito-transmitted diseases such as malaria, dengue, yellow fever and filariasis are the main contributors to this burden. Although insecticides have historically been used to try to control vector populations, over the past 15 years, substantial progress has been made in developing alternative vector control strategies ranging from biocontrol methods through to genetic modification of wild insect populations. Here, we review recent advances concerning these strategies and consider the potential impediments to their deployment, including the challenges of obtaining regulatory approval and community acceptance.

DALY
(Disability-adjusted life year). The number of years lost owing to morbidity or mortality of a disease. This measure is preferable to simple mortality measures, as it better captures the disease burden for debilitating but often self-limiting diseases like dengue and malaria.

Insect-transmitted diseases are present in more than 100 countries worldwide, predominantly in developing countries in the tropics (FIG. 1a). Although progress is currently being made in combatting some of these diseases, including malaria, Chagas disease and filariasis, case burdens are still high, and for some diseases (for example, dengue), the problem is worsening globally. One-sixth of the world's infection-associated DALY (disability-adjusted life year) estimate is attributed to vector-borne disease, and more than 90% of this fraction is due to mosquito-transmitted agents; in fact, malaria parasites contribute more to the burden than any other pathogen[1] (FIG. 1b). Recent WHO estimates predict that there are 50–100 million cases of dengue per year — second only in the vector-borne diseases to malaria (for which there are 216 million cases annually). But measures such as DALY, incidence or annual mortality rate for a disease greatly underestimate the importance of the disease to communities. When the social and economic impacts of diseases like dengue are also considered, then the enormity of their effect on communities can be fully appreciated[2,3].

For many years, much of the medical research community has been focused on the development of vaccines or drugs for mosquito-borne diseases. As yet, there is no effective vaccine for malaria, although Phase III trials of the most advanced vaccine, RTS S/AS01 (which is being developed by GlaxoSmithKline, PATH and the Bill and Melinda Gates Foundation), are showing some promise, with up to a 50% reduction in disease rates in African children[4]. The development of vaccines for malaria has been slow owing to the complexity of the different life stages of the parasite and our poor understanding of the human immune response correlates. Ultimately, multiple vaccines might be required to target different life stages as well as different parasite species[5]. The current antimalarial drugs of choice include a range of artemisinin-based combination therapies[6]. These drugs function well to limit mortality and are fairly low risk for the development of resistance[7]. However, there is a need for drugs that can kill all stages of the parasite in a single dose if this approach is to be effective in the push for malaria eradication[8]. By contrast, there are few, if any, drugs available for treatment of the major arbovirus diseases[9]. Instead, greater progress has been made with the preventative, vaccine-based approach, from the yellow fever vaccine developed in the 1930s[10] through to the more recently developed vaccines for Japanese encephalitis (reviewed in REF. 11). Several vaccines are in development for dengue, the most advanced of which has just recently completed Phase IIb field trials in Thailand, with mixed results[12]. Vaccine design for dengue has been far more challenging than for other arbovirus diseases owing to the existence of multiple serotypes, the complexity of the human immune response to dengue virus and the propensity for sequential infections to result in more severe forms of the disease[13]. Great strides have also been made in targeting lymphatic filariasis with mass drug administration of anthelmintics, chiefly ivermectin. However, effective, long-term treatment of populations with anthelmintics has its challenges with respect to sustained delivery and coverage as well as potential resistance in the nematode[14]. For all these diseases, some of

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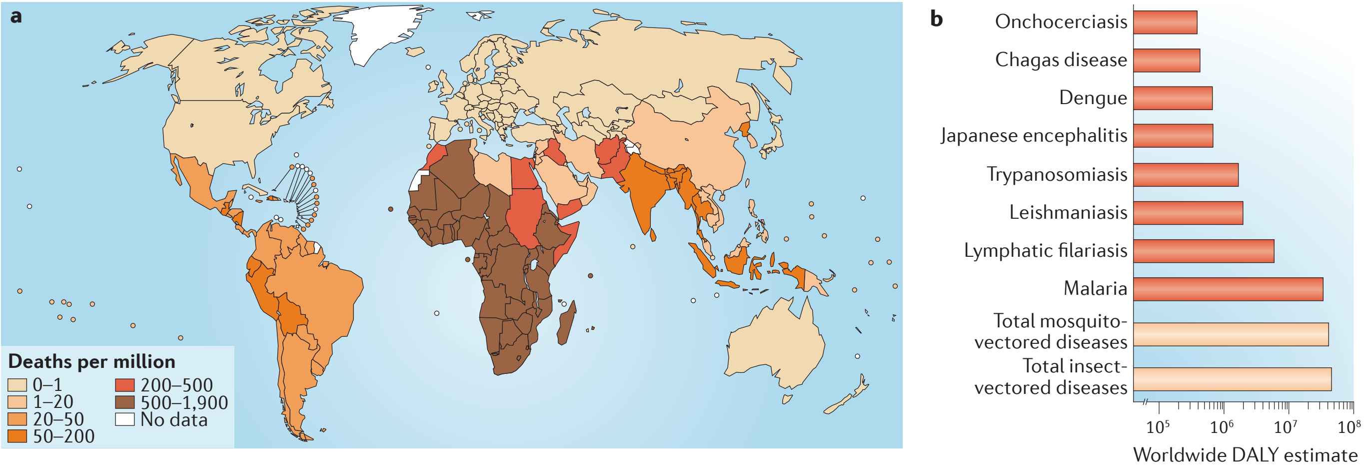

Figure 1 | **Vector-borne diseases are a global problem.** **a** | Heat map showing the worldwide incidence of deaths caused by vector-borne disease. **b** | Worldwide DALY (disability-adjusted life year) estimates for a range of reportable vector-borne diseases. Data for parts **a** and **b** are taken from REF. 1.

the most effective interventions have targeted the mosquito instead of the pathogen through the use of insecticides (see extensive reviews[15,16]). Although insecticides have been shown to be effective in many contexts, the financial cost of their application can be prohibitively high, their widespread application logistically difficult in both very urban and remote areas, and their efficacy unstable owing to the evolution of resistance in their target insects. Despite the successes, the ongoing case burden demonstrates that insecticides, as they are currently being deployed, are not sufficient to bring these diseases under control.

Alternative vector control strategies

During the past 15 years, researchers have been developing a range of alternative vector control strategies that do not rely on the use of insecticides or the creation of new vaccines or drugs. These approaches are typically focused on either reducing mosquito abundance or preventing the transmission of pathogens by the mosquito (see TABLE 1 for a summary of the vector species and the diseases that they transmit). Together with the more traditional approaches for vector control, there are now four major classes of interventions that have had demonstrated success (TABLE 2). None of these methods is a panacea, and often a combination of approaches provides the best outcome[17].

The first class of intervention, environmental management, includes both modification of the natural environment to reduce the breeding habitat of mosquitoes and modification of human habitats or behaviours to reduce biting incidence (TABLE 2). As mosquitoes vary in their larval habitats of choice (man-made water sources, natural brackish or fresh water, and so on) and in their biting behaviour (time of day, indoors or outdoors, and so on), some of the interventions are better suited to

Table 1 | **Vector species and the diseases that they spread**

Vector	Geographical spread	Primary vectors for	Natural *Wolbachia* infection?	Genetically tractable?
Aedes aegypti	Tropics worldwide	Chikungunya disease, dengue and yellow fever	No	Yes[128]
Aedes albopictus	Tropics and subtropics worldwide	Dengue, West Nile virus disease and various types of encephalitis	Yes	Yes[129]
Anopheles gambiae	Sub-Saharan Africa	Malaria	No	Yes[130]
Other *Anopheles* spp. (>28)	The Middle East, North Africa, the Mediterranean, the Far East, Australasia, South America and Central America	Malaria	No	Yes for *Anopheles stephensi*[131], *Anopheles albimanus*[132] and *Anopheles arabiensis*[133]; theoretically possible for others
Culex quinquefasciatus	Tropics and subtropics worldwide	Lymphatic filariasis	Yes	Yes[134]

The distribution of mosquito species around the world is variable, and so is the ability of particular species to serve as pathogen vectors. The table summarizes some of the major vectors of diseases across different world regions[135,136]. In each of the genera listed, other species exist that also serve as vectors.

Brackish
Slightly salty; pertaining to water such as that present in estuaries.

Table 2 | **Past approaches that have demonstrated effectiveness for mosquito vector control**

Approach	Disease targeted	Effectiveness
Environmental modification		
Draining wetlands and ditches	Malaria[137–139]	Field trials showed reductions in both vector numbers and malaria transmission rates
Community clean-up campaigns for mosquito breeding habitats	Filariasis[140] and dengue[141]	Field trials showed reductions in numbers of adult mosquitoes
Screening windows	Dengue[142], filariasis and malaria[143]	Epidemiological studies indicated a lack of window screens is a risk factor for dengue transmission, and field trials and commercial application of window screening reduced vector abundance
Biological control		
Larvivorous fish	Dengue[144] and malaria[145]	Field trials in water storage and natural habitats showed reductions in numbers of larvae
Larvivorous copepods	Dengue[21]	Field trials showed elimination of vector and dengue from some communities, and reductions in others
Bacterial pathogens (*Bacillus thuringiensis*)	Dengue[146] and malaria[147]	Field trials showed reductions in larval survival and adult biting rates
Fungal pathogens (*Beauvaria* spp.)	Dengue[148] and malaria[149]	Laboratory trials for dengue and field trials for malaria both showed reductions in vector survival
Endosymbionts (*Wolbachia*)	Filariasis[24]	Field trials led to local elimination of vector
Chemical treatment		
Indoor residual spraying	Malaria (reviewed in REF. 25)	Commercial application led to reductions in disease transmission
Insecticide-treated bed nets	Dengue[150], Japanese encephalitis[151] and malaria[30,152]	Field trials led to reductions in vector populations and transmission for dengue and reductions in disease incidence for Japanese encephalitis; commercial application showed decreases in disease incidence and death for malaria
Personal protection	Malaria[153]	Commercial application showed decreases in disease incidence
Mosquito traps	Dengue[154], malaria[155] and filariasis[156]	Field trials showed traps were successful in capturing mosquitoes
Genetic modification		
Sterile insect technique	Malaria[37] and West Nile virus disease[36]	Field trials showed population reduction or elimination of the vectors

Anthropophilic
Preferring humans over other animals as a blood meal source.

Copepods
Small freshwater crustaceans (in the context of this Review, of the genus *Mesocyclops*) that prey on mosquito larvae.

DDT
(Dichlorodiphenyl-trichloroethane). An organo-chlorine-based insecticide that has been used since the Second World War to control insects. The insecticide is banned in some countries because of its potential ill effects on human health and non-target species, but it is still used intensively in Africa in regions of high malaria transmission.

particular vector species than to others[18,19]. For example, for anthropophilic mosquitoes like *Aedes aegypti*, a species that breeds in and around houses, draining of wetlands would not be effective. Similarly, bed nets will not be effective against the mosquitoes that bite during the day and transmit dengue.

Biological control represents a second class of intervention and includes the use of natural predators or pathogens against mosquitoes. Recently, copepods have been successfully deployed to control *A. aegypti* larvae in water storage containers in small communities in Vietnam, leading to local elimination of adult mosquitoes and a reduction in dengue incidence[20,21]. A different strategy uses *Wolbachia pipientis* (referred to simply as *Wolbachia*), which is an obligate intracellular bacterium that lives inside insects and is transmitted vertically from mother to offspring (BOX 2). The infection affects insect sperm in a manner that prevents successful reproduction between infected males and uninfected females, and between infected males and females that harbour different strains of *Wolbachia*[22,23]. This strategy was first deployed in 1967 in Burma as a measure against filariasis vectors, when large numbers of *Wolbachia*-infected male *Culex quinquefasciatus* mosquitoes were released into wild populations, demonstrating the ability of these infected insects to eliminate local mosquito populations[24]. More recently, *Wolbachia*-based strategies have expanded both in terms of their mode of action and their vector targets (see below).

The third class of intervention, chemical treatment, represents the most highly used approach to date. Indoor residual surface spraying of DDT in houses, for example, is one of the most effective means of controlling malaria transmission, despite environmental concerns over the toxicity of the insecticide to non-target organisms[25]. Indoor residual spraying is also effective against *A. aegypti*, the main vector of dengue virus[26,27]. Insecticide-treated bed nets have also been highly

Box 1 | ***Wolbachia***

Wolbachia pipientis (referred to simply as *Wolbachia*) is an endosymbiotic bacterium that is present in up to 65% of all insects and some arachnids, freshwater crustaceans and filarial nematodes[63]. The closest relatives of *Wolbachia* are members of the genera *Rickettsia*, *Anaplasma* and *Ehrlichia*[113]. Members of these three genera naturally infect or are vectored by arachnids and cause disease in humans. Like these relatives, *Wolbachia* has a reduced genome, and there is substantial evidence of dependence on the host cell for a range of nutritional resources[114]. As yet, tools have not been developed for genetic transformation of the *Wolbachia* genome, despite decades of effort. Living inside vesicles of host origin[115], *Wolbachia* infects the gonads, where it ensures transmission to the next host generation (from mother to egg) and orchestrates a range of reproductive manipulations of the host. Although cytoplasmic incompatibility is the most common form of reproductive manipulation in insects, the symbiont can also cause feminization of genetic males, parthenogenesis and male killing, depending on the host species[116]. Each one of these effects directly or indirectly benefits the infected females and hence assists with the spread of *Wolbachia* through host populations[117]. Estimates indicate that *Wolbachia* infections can spread in wild populations at rates of up to 100 km per year[118]. *Wolbachia* also infects the somatic tissues of hosts, with distributions and densities varying between the different host–*Wolbachia* strain associations[119]. Infections in somatic tissues might help to explain some of the other phenotypes that have been associated with *Wolbachia* infections, such as a shortened lifespan[65,71], altered locomotor activity[120,121] and poor blood feeding in mosquitoes[77]. Although there are some rare examples of fitness effects, for the most part curing insects of their *Wolbachia* infections has little effect on the insect[122]. This is in contrast with the *Wolbachia* present in filarial nematodes: in this case, the host is dependent on the microorganism for its reproduction[123]. *Wolbachia* has occasionally, over large evolutionary timescales, jumped hosts, although horizontal transmission events seem to be rare with respect to geological timescales[124]. The creation of new host–*Wolbachia* associations has involved the often painstaking process of transinfection (the movement of *Wolbachia*-infected embryonic material to a recipient egg from a donor egg[125] or directly from *Wolbachia*-infected insect cells reared in culture[64,65]). The phenotypes induced by *Wolbachia* are often more extreme in these new hosts[97], a pattern that may result from a lack of co-adaptation[126,127]. In parallel, there are often increases in *Wolbachia* densities and tissue distributions that may explain these shifts[82,125].

effective against the night-biting anopheline species that transmit malaria. The use of bed nets by children has led to decreases in mortality as a result of malaria and in malaria transmission[28]. In pregnant women, the use of bed nets has lead to greater survival and health of their offspring following birth[29]. However, there are challenges relating to the distribution of insecticide-treated bed nets and the maintenance of their effectiveness[30], and there is some evidence that mosquito behaviour is shifting from indoor to outdoor biting or from night to dawn biting[31] in areas where these nets are used[32]. Furthermore, all approaches based on insecticides are threatened by the evolution of resistance in mosquito populations[25].

The fourth and final class of intervention strategy involves genetic modification of the vectors (TABLE 2). The sterile insect technique (SIT) is the oldest and most tested example of such a strategy. In a SIT approach, male insects are exposed to either γ-irradiation or sterilizing chemicals, causing large-scale random damage to the insect chromosomes or dominant-lethal mutations in the sperm[33]. These males are then released in far larger numbers than occur in the wild male population, and when they mate with wild females, viable offspring are rarely produced. With ongoing releases of these males, the population reduces to low levels or is completely eliminated. Conventional SIT requires the production of large numbers of insects and the ability to separate males from females before release. Releasing females would add to the vector population and also introduce mutations from the sterilization treatment into wild populations. There is some level of female leakiness associated with most systems of male production for SIT. A second and potentially larger issue is that the released males often exhibit reduced mating competitiveness in the field, requiring the release of large numbers to compensate. These males might also exhibit low-level fertility and, hence, might pass on some of their mutations into wild populations[34]. Finally, without complete eradication of a vector across the landscape, migration from outside the release area means that ongoing releases can be required.

SIT has a mixed history of success for mosquitoes, as some trials have demonstrated reductions in target populations, whereas other trials have not[33,35]. The most successful initiatives include the eradication of *C. quinquefasciatus*, a local vector of West Nile virus, on an island off Florida, USA[36], and the elimination of *Anopheles albimanus*, a local malaria vector, in El Salvador[37], both of which were achieved by the release of chemosterilized males. The development of SIT approaches is underway for other mosquito vectors, with the aim of controlling malaria[38,39], Chikungunya disease and dengue[40]. With regard to the history for non-mosquito vectors, a SIT campaign effectively eliminated a species of tsetse fly, the vector for African sleeping sickness, on the island of Zanzibar[41]. Perhaps the best examples of effective SIT, however, come from the control of agricultural pests. The New World screw-worm, which is a pest of livestock primarily, was eradicated from Southern USA, Mexico and Central America[42], and more recently, Northern Africa was protected from infestation by the release of sterile insects[42]. The pink bollworm, a lepidopteron pest of cotton, was targeted by SIT approaches beginning in 1968 in the USA. SIT against this invasive insect has been most useful in preventing colonization of new areas (reviewed in REF. 43). SIT programmes for both these pests are still ongoing, and their methods are being continually improved, which is a testament to their success[44–46].

In this Review, we highlight alternative vector control strategies from two of the classes described above — namely, the genetic modification of vector species and the use of a particular biological control agent, *Wolbachia*. We describe the rationale for the various approaches, the stage of development that each has reached and the likely scalability of the technologies. We also discuss the issue of obtaining approval for such approaches, both from the relevant regulatory bodies and from the wider public.

Emerging technologies

Genetic modification of the vector. There are three main approaches for genetic modification of the vector (FIG. 2). The approach known as release of insects carrying a dominant lethal (RIDL) operates similarly to traditional SIT but offers several improvements, most notably with a focus on female-killing effects (FIG. 2a; TABLE 3). Instead of random mutations, males carry and deliver female-acting transgenes into the population. One approach uses a construct that reduces the expression of a gene which is active in the flight muscle in female pupae. The result is that daughters of the released males are unable to fly to find mates or human hosts[47]. The second approach is based on transgenes that induce mortality later in life, either in pupae[48] or in adults[49]. In the laboratory, rearing of these lines is accomplished by placing the transgene under the control of a repressor that inhibits expression in the presence of tetracycline, which can be added to the diet. Because transgene transcription is driven either by female- or stage-specific promoters, the fitness of males or non-target stages carrying the constructs is much less compromised[34]. Indoor cage experiments initially demonstrated the mating success of males carrying the flightless-female construct, as evidenced by extinction of the mosquito population over time[34]. More recently, open-field releases of these same mosquitoes on Grand Cayman, in the Cayman Islands, have suggested that the released males show some reductions in mating competitiveness relative to wild males, but that this can be compensated for by releasing greater numbers[50]. Two benefits of the RIDL method that might improve community support include the short-lived presence of the genetically modified organism in the population (compared to homing endonuclease genes (HEGS); see below) and a focus on the release of males that will not increase nuisance biting (compared to all other methods, depending on how they are deployed). Of the genetic modification-based approaches, RIDL is the most advanced with respect to implementation, as the technology is currently being trialled by Oxitec in Brazil and Malaysia[51]. The approach is promising, and the scientific community is awaiting the publication of further studies that demonstrate both the capacity of transgenic males to reduce or eliminate populations and the long-term stability of the suppression in response to migrant mosquitoes from outside the release areas.

A second genetic modification strategy, one that is still in the early stages of development (TABLE 3), is aimed at improving the natural defence system of the mosquito. RNAi is an insect immune response that recognizes and degrades invading viral RNA. In one approach, a genetic construct was developed that expresses copies of an inverted repeat from a dengue virus 2 (DENV-2) genomic RNA (FIG. 2b). The resulting double-stranded RNA that forms then triggers the RNAi response and protects the mosquito from colonization of its tissues by the dengue virus encountered in blood meals[52]. After long-term laboratory rearing, however, the effectiveness of the transgene is diminished by genetic changes occurring outside the targeted region[53]. In another approach, insect densoviruses were engineered to deliver RNA copies of genes required for vector competence in the mosquito[54]. This approach exploits a second function of RNAi, which is to suppress transcription of a gene in the presence of double-stranded RNA copies of that gene. Because RNAi-based approaches target a fundamental process, similar constructs could potentially be engineered against a diverse range of arboviruses[55–57] as well as against malaria parasites[58]. As is the case for RIDL, the targeted nature of the RNAi constructs should mean fewer negative fitness consequences for released mosquitoes carrying the transgene.

A third genetic modification approach makes use of HEGS, which are selfish genetic elements that were discovered in bacteria but have since been experimentally engineered and introduced into mosquitoes for future use in disease control (FIG. 2c). HEGs encode endonucleases that recognize and cut specific DNA sequences (of ~30 bp). As HEGs insert into these specific recognition sequences, they are protected from their own activity. In an organism that is heterozygous for the HEG, the endonuclease will cut the intact copy of the recognition sequence in the chromosome that does not contain the HEG. Recombinational repair processes then use the HEG-containing strand as a template, converting the heterozyogote to a homozygote. In this way, HEGs increase their copy number in populations. Because HEGs can be engineered to recognize specific sequences, they can be developed to target mosquito genes required for vector competence[59]. Alternatively, HEGs can be used as a form of population suppression by targeting genes to induce sterility, reductions in survival or sex ratio distortions[60,61]. Thus far, HEGs have been successfully introduced into *A. aegypti*[62] and *Anopheles gambiae*[59]. In simple simulation modelling, HEGs have been predicted to be able to eliminate populations of *A. gambiae* in as little as a few years after their introduction[61].

Biological control from within. Since 1967, the potential use of *Wolbachia* in insect control has continued to be explored. One of the benefits of *Wolbachia* as a control tool is that the reproductive modifications that this organism induces in insects, known as cytoplasmic incompatibility[22,23], provide an indirect benefit to *Wolbachia*-infected females, by decreasing the reproductive output of uninfected females. Given the maternal transmission of *Wolbachia*, this provides a self-driving mechanism for population invasion, as occurs with HEGs (TABLE 3). *Wolbachia* is estimated to occur naturally in approximately 65% of all insect species[63].

Although present in many mosquito species, including *Culex pipiens* and *Aedes albopictus*, *Wolbachia* is not naturally present in any anopheline species that transmit malaria parasites or in *A. aegypti*, the primary vector of dengue viruses. In the past few years, three different *Wolbachia* strains have been successfully transinfected into *A. aegypti*, in which they have formed stable, inherited infections[64–66]. To date, only transient somatic-tissue infections have been achieved for anopheline species[67]. *Wolbachia* infections are currently being developed for a range of different control strategies ranging from population suppression approaches similar to SIT, in which *Wolbachia*-infected males effectively reduce the reproduction of wild females, to the use of *Wolbachia* to invade mosquito populations and reduce pathogen

Transgenes
Genes or genetic material that has been introduced into another organism using genetic engineering techniques.

RNAi
The process by which animals cleave double-stranded RNAs into small fragments, the presence of which directs transcriptional silencing of the corresponding gene. RNAi also has a role in immunity, as it is responsible for cutting and degrading the RNA of invading viruses.

Cytoplasmic incompatibility
The failure of embryo development in the early stages, as the result of a *Wolbachia*-infected male mating with an uninfected female. This leads to poor or no survival of the offspring. By contrast, when two *Wolbachia*-infected adults mate, the egg of the infected female 'rescues' *Wolbachia*-mediated changes to the sperm and allows the offspring to develop normally.

Transinfect
To transfer a bacterial or viral infection from one host to another by microinjection.

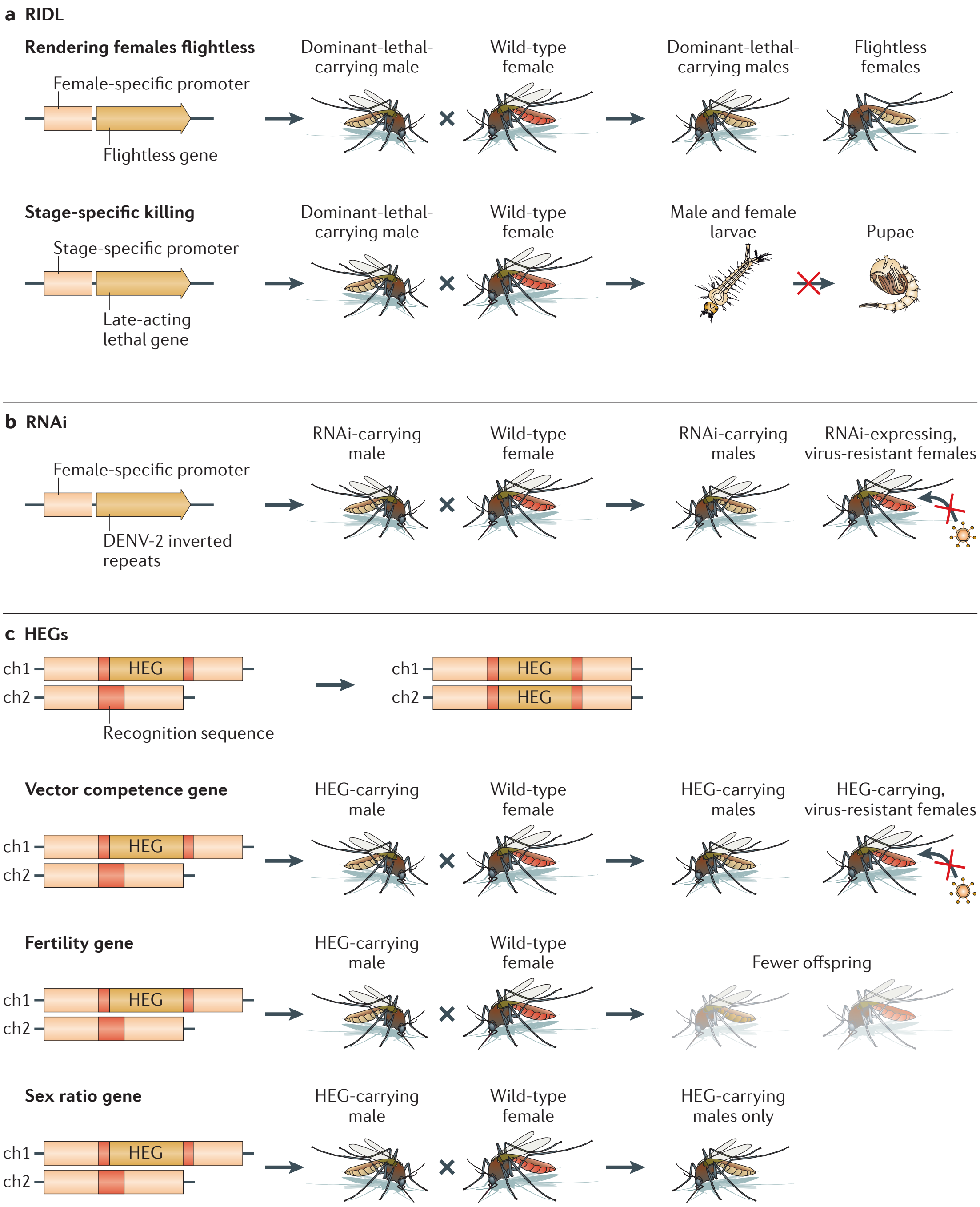

Figure 2 | **Genetic modification approaches for vectors.** **a** | Release of insects carrying a dominant-lethal allele (RIDL). In the first scenario, males carrying a female-acting transgene that results in a loss of flying ability are released in the open field. These males mate with wild-type females, and the resulting female offspring are flightless and, hence, unable to mate or find human hosts[34,47]. In the second scenario, males carrying 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 as pupae[48] (shown) or adults[49]. **b** | RNAi. In the example shown, males carry a female-acting transgene that contains an inverted repeat from dengue virus 2 (DENV-2), and are released in the open field. These males mate with wild-type females, and the resulting females express the DENV-2 repeat RNA, resulting in reduced dengue vector competence owing to the activation of RNAi[54]. Both males and females continue to pass on the transgene. **c** | Homing endonuclease genes (HEGs) encode endonuclease enzymes and recombine into the genome at sites that are homologous to the recognition sites of the encoded endonucelase, and are thus protected from self-degradation. In a heterozygote, the endonuclease that is inserted in one gene copy will cut and insert itself into the second gene copy, resulting in an individual that is homozygous. Released males carrying HEGs mate with wild-type females and produce offspring that contain the HEG. HEGs can be designed to target vector competence genes, leading to pathogen-resistant females[59]; fertility genes, leading to reduced reproductive output and lifespan; or sex-determining genes, leading to sex ratio skews[60,61]. The HEG is passed on through any surviving mosquitoes to their offspring and, hence, continues to spread.

Table 3 | **Summary of emerging technologies**

Method	Mode of action	Intended outcome	Spreading capacity	Release numbers required	Technology*	Stage of development
RIDL	Removal of flying ability through expression of female-acting transgenes	Population elimination	No	Large	GM	Field testing
	Late-acting lethality	Population elimination	No	Large	GM	In development
RNAi	Vector immunity to pathogens	Reduced vector competence	No	Large	GM	In development
HEG	Distortion of the mosquito sex ratio	Population suppression	Yes	Very small	GM	In development
	Reduction in the ability of pathogens to infect mosquitoes	Reduced vector competence	Yes	Very small	GM	In development
	Poor mosquito survival or reproduction	Species elimination	Yes	Very small	GM	In development
Wolbachia	Prevention of reproduction for wild-type mosquitoes	Population suppression	No	Large	Non-GM	Field testing
	Reduction in the lifespan of mosquitoes	Reduced vectorial capacity	Yes	Small	Non-GM	Field testing
	Inhibition of pathogen replication in mosquitoes	Reduced vector competence	Yes	Small	Non-GM	Field testing

HEG, homing endonuclease gene; RIDL, release of insects carrying a dominant lethal. *GM (genetically modified) indicates that genetic constructs were introduced into the insect genome. Non-GM indicates that neither the *Wolbachia* genome nor the host genome was modified.

transmission by shortening the adult mosquito lifespan and/or preventing pathogen replication inside the mosquito (FIG. 3).

In recent years, there has been a resurgence of the basic idea originally pioneered by Laven[24] and others in the late 1960s, which was to release *Wolbachia*-infected males to reduce or eliminate mosquito populations. Recent approaches have focused on population suppression for *Aedes polynesiensis* on South Pacific islands as a means of filariasis control[68,69]. The strategy is based on bidirectional incompatibility, a complexity of the *Wolbachia*-induced reproductive phenomenon, which results in unsuccessful mating between mosquitoes carrying genetically distinct *Wolbachia* strains. On South Pacific islands, *Wolbachia* strains (from sister species to those infecting the wild populations) are being introgressed into *A. polynesiensis* mosquitoes that have had their *Wolbachia* infections removed by antibiotic treatment. These transinfected lines can then serve to block reproduction of local females on release. Similar approaches have also been suggested in the case of *C. quinquefasciatus*, which is a vector of lymphatic filariasis in some regions of the world and of arboviruses in other regions[70].

One *Wolbachia*-induced trait is shortening of the adult insect lifespan; this trait is uniquely associated with a particular *Wolbachia* strain, *w*MelPop, which was discovered in *Drosophila melanogaster*. Flies infected with *Wolbachia w*MelPop live roughly half their expected adult lifespan, probably owing to host cell lysis caused by over-replication of the bacterium throughout host tissues[71]. Shortening the lifespan of mosquito vectors could theoretically reduce the transmission of a number of viruses and parasites because of the importance of the extrinsic incubation period (EIP) to disease dynamics. EIP is the time between consumption of a pathogen-infected blood meal by an insect, and pathogen escape from the gut and colonization of the salivary glands, where it can then be secreted back into the saliva of the insect. This period is typically greater than 6 days[72], which means that older insects contribute disproportionally to pathogen transmission[73]. Vectorial capacity is a measure of transmission efficiency of the disease — that is, new infections per person per day by each mosquito. It is a function of a number of factors related to the biology of the mosquito: the propensity to bite humans, the daily survival rate, the EIP, the rate of contact with humans and the lifespan[74]. Mathematical modelling of vectorial capacity shows that even small shifts in average vector lifespan can have large impacts on the transmission dynamics of a disease[73,75]. For this reason, *Wolbachia w*MelPop was selected for transinfection into *A. aegypti* for potential use against dengue virus transmission. In this new mosquito host, the strain causes an approximately 50% reduction in adult lifespan, as well as inducing cytoplasmic incompatibility[65]. Although this would suggest the possibility of large impacts on pathogen transmission, it also causes other effects that reduce mosquito fitness, such as a reduced ability to obtain blood meals in old age[76,77], and lower egg production and viability[78,79]. These detrimental effects might make it difficult for these transinfected lines to invade natural mosquito populations, particularly in regions with harsh dry seasons, where egg fitness issues will be exacerbated. It has also been suggested that it would be possible to collapse a

Bidirectional incompatibility
A phenomenon that occurs when mating males and females are infected with different *Wolbachia* strains. Eggs from the female may not be able to rescue the *Wolbachia*-induced changes in the sperm of the male. The consequence is an incompatibility in the embryo such that few or no offspring survive, despite the fact that both parents carry *Wolbachia*.

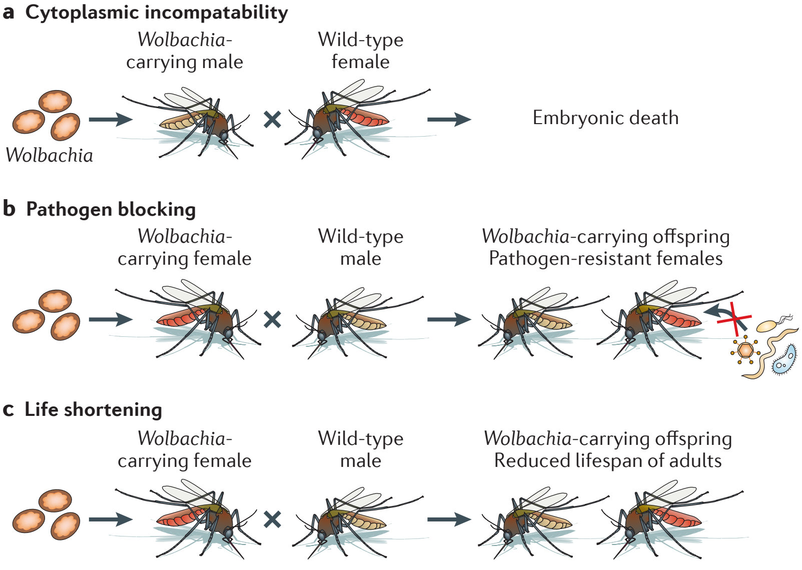

Figure 3 | **Vector control using *Wolbachia*.** **a** | The *Wolbachia* method can be used in a similar way to the sterile insect technique (SIT), with the release of an abundance of *Wolbachia*-infected males[68]. In wild populations in which *Wolbachia* is absent, there will be a reproductive incompatibility with uninfected wild females, leading to embryonic-stage death in the offspring. Alternatively, releasing males harbouring a different *Wolbachia* strain from that present in a wild population will also produce reproductive incompatibilities (not shown). Because infected females are not released, the *Wolbachia* infection does not spread. **b** | If an abundance of females harbouring a *Wolbachia* infection (which has been shown to inhibit the growth of pathogens in insects) is released, all offspring will carry the symbiont and exhibit reduced vector competence for a range of pathogens[82]. Because only females bite and transmit disease, males have not been tested for pathogen resistance. Owing to the action of cytoplasmic incompatibility, this type of *Wolbachia* infection will spread. **c** | If the strain *Wolbachia w*MelPop is released via females, it will not only provide pathogen blocking and spread via the action of cytoplasmic incompatibility, but also reduce insect lifespan. This has the potential to decrease pathogen transmission, as only older insects transmit disease[73].

mosquito population if a strain like *Wolbachia w*MelPop could invade the population before the onset of the dry season in regions that have such seasonality[78].

In an interesting and serendipitous twist, recent discoveries have shown that *Wolbachia* can reduce the ability of certain pathogens to replicate in insects. This was first discovered in *D. melanogaster*[80,81] and then confirmed in *A. aegypti* and other mosquitoes[82–87]. In *A. aegypti*, this blocking effect extends to bacteria (*Erwinia carotovora*), filarial nematodes (*Brugia malayi*)[88], viruses (dengue virus, chikungunya virus and yellow fever virus) and the malaria parasite *Plasmodium gallinaceum*[66,82,83,89], and seems to be associated with a range of different *Wolbachia* strains, but not all strains. In only one case has *Wolbachia* been shown to enhance pathogen replication, that case being *A. gambiae* that was transiently infected with a mosquito *Wolbachia* strain and subsequently exposed to the rodent malaria parasite *Plasmodium berghei*[90]. Field experiments have commenced in Australia to test the ability of artificially introduced *Wolbachia* infections to invade and establish in wild *A. aegypti* populations. To date, *Wolbachia w*Mel has been successfully introduced into Australian mosquito populations[66,91] and, at the time of writing, has remained at fixation for more than 18 months. Additional trials examining the ability to deploy *Wolbachia w*MelPop, the life-shortening strain that also blocks dengue viruses, are currently underway. In Vietnam, regulatory approval has just been granted for trials, and there are plans for the first field release in mid 2013. In Indonesia and Brazil, community engagement has begun, regulatory approval is being sought, field sites have been identified and mosquito release strains are being produced. These new trials will begin to address issues associated with broad-scale deployment and measures of efficacy in reducing dengue.

Understanding the underlying mechanism of pathogen blocking is key to predicting its long-term stability in the field. A growing body of evidence is suggesting that the interaction of *Wolbachia* with pathogens in the mosquito is complex. The degree of pathogen blocking conferred by *Wolbachia* is positively correlated with *Wolbachia* density and/or, potentially, tissue distributions[66,82,87,92]. Initial work has shown that *Wolbachia* can boost insect innate immunity in some hosts, which may contribute to pathogen blocking, particularly in recently generated transinfections; however, there seem to be other mechanisms also acting to contribute to this effect[93]. Other hypotheses are being explored, including competition between *Wolbachia* and dengue virus for key cellular locations or subcellular molecules. Indeed, additional research currently under review from our group indicates that access to cholesterol might also have a role in the blocking effect. This complexity is potentially beneficial, as it might slow the ability of either viruses or mosquitoes to evolve resistance against the trait. In addition, if insect hosts gain a fitness advantage from *Wolbachia*-mediated pathogen blocking, particularly for naturally occurring mosquito pathogens, then we might expect co-evolution of the system to maintain the blocking trait.

Scalability of emerging technologies

Each of the mosquito control methods described above will require different numbers of mosquitoes to be introduced. HEGs, given their aggressive self-spreading nature, will require the fewest, and *Wolbachia*, with a population-driving mechanism, will require an intermediate number compared to any methods without a genetic-drive system (TABLE 3). Other methods, such as the release of sterile insects, will require inundative releases. For the RIDL test release in Grand Cayman, 465 males per hectare per week were released over a period of 4 weeks. Subsequent modelling indicated that for the technology to substantially reduce the population of wild-type mosquitoes, higher numbers would be required: on the order of 651–5,580 mosquitoes per hectare per week[50]. In Cairns, Australia, up to 275 mosquitoes per hectare per week of *Wolbachia w*Mel-infected mosquitoes were released over a period of 10 weeks. As *Wolbachia* infection frequencies rose to 80% by week 4 and to fixation by week 12, aided by the action of cytoplasmic incompatibility, it is quite possible that fewer insects could have been released to achieve a similar level of success[91]. The costs for carrying out these releases include the facilities and equipment for insect rearing, as well as well-trained staff to carry out the rearing, releases and field monitoring after the releases. The other crucial and labour-intensive aspects of these approaches are the upstream and parallel programmes of community engagement[91,94]. Although both of these methods have been successful in release over small scales, it is yet to be seen how they will scale up to cover large geographical regions.

In the shorter term, the challenge for mosquito suppression technologies, whether by *Wolbachia* or RIDL, will be the sustainability, owing to the re-establishment of local populations through incomplete suppression or migration. This, in turn, will be very context dependent in relation to local geography and ecology. For isolated populations, especially those on islands, elimination might be permanent. It is also possible that once a population has been suppressed, only small releases will be required on an ongoing basis for an area to remain mosquito free. Indeed, there is some precedent for this from SIT programmes against particular pests[43]. Theoretically, HEGs and *Wolbachia* (for pathogen blocking or life shortening) might require less ongoing effort if they are self-sustaining in populations or, even better, if they spread beyond release sites. In the case of *Wolbachia w*Mel, 1.5 years after the initial releases, the frequencies of *Wolbachia* infections remain at 100% in the communities where releases were undertaken (E.A.M and S.L.O., unpublished observations). Whether *Wolbachia w*Mel will spread beyond target release areas has not yet been tested, given that the initial field sites were deliberately selected to limit spread. Bodies of water, major highways and agricultural fields might be effective barriers to spread, as *A. aegypti* is highly anthropophilic, and adult mosquitoes are thought to disperse on the order of only hundreds of metres[95]. Planned Australian release sites for 2013 are embedded in broader tracts of human settlement and hence will allow the self-spreading capacity of these mosquitoes to be tested.

Long-term stability of emerging technologies

None of these methods have been deployed in the field long enough to empirically test their long-term stability. The main points of concern relating to stability are competitiveness of the mosquitoes and the capacity for resistance to evolve.

For both RIDL- and *Wolbachia*-based approaches, producing fit and competitive mosquitoes is key. For RIDL, ongoing attention to mosquito fitness in laboratory breeding environments is required[34,50]. Laboratory mosquitoes inbreed quickly, so repeatedly placing the genetic constructs in local wild-type backgrounds should help to minimize any potential loss of fitness through inbreeding as well as to prepare the males for competition with individuals from the same population[34,50]. A potential issue that may arise for RIDL is whether the expression or behaviour of the construct in females will be fully penetrant in different genetic backgrounds or whether it will become leaky, and if so, what the subsequent effects of that leakiness will be on suppression. For *Wolbachia* infections, there is often an associated fitness cost that might make *Wolbachia*-infected organisms less capable of surviving in the field, and might retard the establishment of the *Wolbachia* infection in the wild population. Cytoplasmic incompatibility provides a mechanism that allows *Wolbachia* to still invade host populations despite there being some fitness cost. Modelling predicts that *Wolbachia* can spread into uninfected populations even if the symbiont induces an approximate 50% reduction in host fitness, although the rate of spread declines as fitness costs increase[96]. Nevertheless, for the *Wolbachia*-based approach, selecting the ideal strain to release might need to balance any negative fitness effects with the sought-after traits of life shortening and/or pathogen blocking. Obtaining good estimates of fitness in laboratory environments is notoriously difficult, so for both RIDL and *Wolbachia*-based approaches, the empirical data coming from past and future open-field releases will provide a real understanding of competitiveness in a natural setting. These data can then be used to inform models examining optimal deployment strategies.

Resistance may take different forms depending on the technology. With RIDL-based approaches, resistance can arise in response to the construct, especially if there is incomplete penetrance in its expression in local genetic backgrounds. In the short term, this will mean survival of these resistant individuals in the population, and in the longer term, it will mean spread of the resistance alleles through the descendants of the survivors. Resistance against HEGs could arise because there is always a proportion of the cleavage events that are not repaired by recombinational processes. Other repair mechanisms do not generate a copy of the HEG and, more importantly, often alter the target site, rendering it resistant to future HEG insertion[61]. If these repaired alleles confer greater fitness than the HEG-containing allele, then the HEG will be lost from the population. In the *Wolbachia* system, there are two mechanisms by which resistance could be generated. First, mosquitoes could evolve resistance against a particular strain of *Wolbachia*, reducing its densities or restricting its tissue distribution. There is precedence for exactly this occurring in *Drosophila simulans* in response to transinfection with *Wolbachia w*MelPop[97]. Arguably, some reduction in the effect of *Wolbachia w*MelPop on the mosquito might be welcome, improving its ability to spread by diminishing its effect on fitness[98]. Nevertheless, 4 years after the production of the *Wolbachia w*MelPop-infected mosquito, *Wolbachia w*MelPop densities still remain high enough to cause life shortening (E.A.M. and S.L.O., unpublished observations). In the future, this particular means of resistance might be countered by the subsequent release of *A. aegypti* infected with multiple strains of *Wolbachia* that, owing to bidirectional incompatibility, would sweep and replace any single infections[73]. Second, it is also possible that pathogens themselves will evolve a means to evade *Wolbachia*-based blocking. As the mechanism (or mechanisms) underpinning pathogen blocking is not known, it is difficult to predict whether resistance will evolve easily.

As with all disease interventions, including insecticides[25] and vaccines[99], the evolution of resistance is a risk. In the simplest case, 10–20 years of efficacy by one of these emerging methods alone could permanently change the face of disease transmission globally. It is also clear that pairing these new methods with more traditional interventions such as vaccines, as is done with insecticides, or with rotations of different insecticides, as is done with antibiotics, might offer better protection against disease risk as well as an extended lifetime of efficacy.

Regulatory approval and community consultation

In the 1970s, the WHO carried out SIT releases for multiple species, including *A. aegypti* and *Culex* spp., in Dehli, India[100–103]. The fate met by the project, however, is a reminder of the importance of government and community consultation. Untruths reported by the media included the idea that the United States was using the WHO-associated research project to test dangerous chemosterilization methods in India and that the unstated goal of the programme was to develop biological weapons. In the undercurrent of these accusations was the subtext of scientific imperialism. Although the project might also have been a victim of the geopolitics of the time, it would surely have benefited from an active and effective community engagement campaign. The result was that the Mosquito Control Group, which had begun to have some successes, had its programme prematurely terminated[104–107]. The lesson, of course, is that even with great scientific success, such programmes can fail if the correct relationships are not formed with the public and the government.

The emerging technologies that are discussed here will need to develop authentic methods for community and broader stakeholder engagement if they are to be successfully deployed around the globe[108]. In some cases, there might be clear guidelines for how to achieve regulatory approval, especially for genetically modified organisms, as was the case for a recent release of RIDL mosquitoes in Malaysia[51]. In preparation for the release, Oxitec carried out a 30-day public consultation process that involved newspaper advertisements, public forums and surveys. In Australia, identifying an agency to take on the *Wolbachia* project was not immediately obvious, as the insects were not genetically modified organisms, and both *Wolbachia* and mosquitoes are native to Australia. In the case of the *Wolbachia* roll out, the primary goal was to develop a plan for acquiring regulatory approval in Australia and to demonstrate the willingness of the country to accept the technology at home before exporting it to other countries[91,109].

The process of obtaining regulatory approval involved having the Australian government science agency, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), carry out an independent and comprehensive risk assessment of the technology[110,111]. The conclusion from this analysis was that the approach carried negligible risk. An international panel of experts subsequently reviewed the risk analysis. Both the risk analysis and the panel's review were then provided to the Australian government agency that is responsible for regulating biopesticides, which was given carriage of the decision. In parallel, the research team met with local communities to hear their concerns using various means to engage, including town hall meetings, dedicated focus groups, meetings with existing community groups and 'science in the pub' activities. The information flow was two-way, with researchers explaining the work and the community raising concerns. These concerns were addressed through explanation or, in some cases, additional research[111,112]. During the 2-year process, public opinion went from 69% support to 87% support, as measured by anonymous questionnaires and telephone polling. At the same time, local, state and federal politicians were consulted and briefed on the project[91,111]. With a well-documented body of public consultation and backing, a body of scientific evidence in support of the feasibility of the programme and an external risk assessment, the regulating agency was then able to evaluate the case and undertake its own risk analysis, and decided to provide its support[109–111]. During ongoing releases, communication with the community has been continuous via individual meetings, public access to a visible shopfront (where the public can walk in at any time to make enquiries of the research team), monthly newsletters showing the latest research results of the trial and regular updates provided through the media. The government regulators also required that particular data be collected during the releases as part of the permit conditions. This includes data on mosquito abundance, the establishment of *Wolbachia*-infected populations outside the intervention areas and the impacts of the releases on non-target organisms. These data are then returned to the government for review. In contrast to earlier genetic control trials carried out in the 1970s in India, the current *Wolbachia* trials in Australia have so far met with strong community support and involvement. As field trials move to other countries, the challenge will be to sustain this standard of community engagement.

Conclusions

The rapid development of these new vector-based interventions is the result of sustained investment into this research area over the past 15 years by multiple agencies. The fruits of that investment are now being realized, and if the new challenges around regulation and community authorization can be met, then we are likely to have a suite of new technologies to apply against these diseases in a fairly short period of time. These approaches each have the potential to have major impacts on disease incidence by themselves. They are also compatible and could be used in conjunction with any emerging vaccines or drugs and alongside the better application of existing tools, such as insecticides. Used in combination, they might be even more powerful, but as yet these new tools are still being developed and trialled in isolation. Pprogrammes of combined implementation should be considered when these new approaches have been sufficiently developed, to demonstrate efficacy. Either alone or in combination, the power of such new approaches might make it possible to turn the tide on these persistent human diseases.

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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