Gene drive systems for insect disease vectors

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Abstract | The elegant mechanisms by which naturally occurring selfish genetic elements, such as transposable elements, meiotic drive genes, homing endonuclease genes and *Wolbachia*, spread at the expense of their hosts provide some of the most fascinating and remarkable subjects in evolutionary genetics. These elements also have enormous untapped potential to be used in the control of some of the world's most devastating diseases. Effective gene drive systems for spreading genes that can block the transmission of insect-borne pathogens are much needed. Here we explore the potential of natural gene drive systems and discuss the artificial constructs that could be envisaged for this purpose.

Insect-borne diseases impose an enormous burden on global health and agricultural output; malaria alone kills over a million people every year out of half a billion cases[1]. Insecticides have largely failed to deliver long-term solutions, and new control strategies are desperately needed. Replacement of insect vectors with genetically modified counterparts is a long-established control concept[2–4], but it is only recently that some key advances have brought this goal within sight. The best characterized examples come from mosquitoes and include stable germline transformation[5–7] and transformation or transfection with artificial constructs, which encode peptides or RNAs that reduce their ability to transmit malaria or dengue virus[8–11]. Naturally occurring alleles of immune-system genes with strong effects on *Plasmodium* transmission have also been identified[12,13].

Creating a transgenic strain that does not transmit disease is not in itself of practical value because these strains would need to be released on a scale that would be unfeasible given the wide areas that are inhabited by vectors of human tropical diseases. Effective large-scale population replacement strategies would require the development of reliable mechanisms for biologically spreading the crucial genes. Several categories of naturally occurring 'selfish' genetic elements that show non-Mendelian inheritance are known to spread within populations even when they provide no benefit to the host organism[14]. Efforts are now underway to exploit these mechanisms to drive anti-pathogen effector genes into mosquito populations. Surprisingly, given their importance, far less research effort is being expended on the development of such gene drive systems compared with studies of vector–pathogen interactions, transmission dynamics or population genetics studies[15]. The potential for the successful development of a gene drive system from the various genetic mechanisms that have been proposed will be evaluated here.

Criteria for gene drive systems

Candidate gene drive systems must be evaluated relative to criteria that affect their potential for success[16,17]. The most crucial criterion is that the drive mechanism must be powerful enough to spread effector genes to fixation (or close to fixation[18]) on a timescale that is appropriate for a disease-control programme. The system must also be as resistant as possible to the potential loss of linkage between the drive mechanism and the effector gene(s) to be driven. The ability to spread new or modified effector genes over time would be a valuable or even essential characteristic to counteract loss of linkage, mutational inactivation of the effector, or the development of resistance or evasion by the pathogen. The chances of a pathogen evolving resistance to a genetic intervention would be reduced substantially if multiple, independently acting effector genes could be spread at the same time, so the ability to spread large multi-gene constructs would be the ideal. Because many tropical diseases have multiple vectors, it would also be an advantage if a particular drive system functioned in several vector species.

Finally, the drive mechanism should be as safe as possible, without a significant risk of causing undesirable side effects in the target vector or of causing ecological damage in non-target species[19]. Ideally, mechanisms would be available to allow the removal of the effector gene(s) from populations in the case of unanticipated

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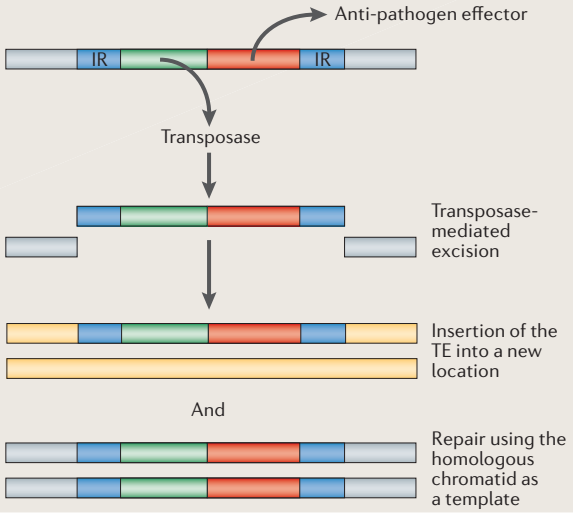
Box 1 | **Types of transposable element**

There are various classes of transposable element (TE) that differ in their mode of transposition (reviewed in REF. 26). Type 1 elements, including long-terminal repeat (LTR) retrotransposons, retroposons and long-interspersed repetitive elements (LINEs), use an RNA-mediated mechanism of transposition that involves a polymerase-like protein with a reverse transcriptase domain. Some LINE-like elements are site specific[40]. Short-interspersed repetitive elements (SINEs) also transpose using RNA but do not themselves encode reverse transcriptase, so probably make use of the machinery of autonomous retroposons that share similar terminal sequences for *trans*-mobilization. Retrotransposons are unsuitable for use as a binary transformation tool with donor and helper plasmids, but could be considered as an autonomous system in the context of gene drive, although LTRs can have enhancer or silencer effects, which could be problematic.

Type 2 TEs transpose by a direct DNA cut-and-paste mechanism rather than using an RNA intermediate; in this case the increase in copy number is brought about by repair mechanisms that use the homologous chromatid as a template (see figure). DNA transposons from several families contain terminal inverted repeats (IRs) that flank a transposase-encoding region. Most of the elements used so far for germline transformation are in this class, including *P*, *Mos1*, *piggyBac*, *Hermes* and *Minos*.



negative effects. Extensive risk assessment and laboratory testing would in any case be necessary before field releases could be contemplated; effector genes could be brought to high frequency and tested for efficacy in small field-trial populations by simple mass release, with release of individuals carrying the effector that is linked to the drive mechanism only at the final stage of a project.

The type of gene to be spread is also an important consideration. Transgenes that interfere with pathogen transmission but substantially decrease the fitness of the engineered mosquito would be extremely difficult to spread through populations, even with a powerful drive system. If there is a fitness cost that is due to metabolic costs of transgene expression or toxic effects of the anti-pathogen effector or marker genes, then constructs with mutationally inactivated or deleted effector genes would be advantageous and could become fixed in the population. Fitness costs that are caused by insertions disrupting other genes are also of concern, but fitness costs that are due to inbreeding could be controlled for by outcrossing before assessment[20,21]. Mathematical modelling can help to predict the utility of different drive systems, as long as realistic values for the fitness costs of the effector transgene and for the pest's population structure are used. Fitness effects can be measured by carefully examining parameters such as female fecundity, mating competitiveness and behaviour, or more easily in cage experiments that monitor frequency over several generations, although these approaches might still miss fitness effects that would be incurred only in the natural environment. Estimating realistic rates of mutational inactivation or recombinational loss of inserts is also important, but this is difficult to do in the laboratory given the very low frequency of such events.

A wide variety of selfish genetic elements exist, but the classes of drive mechanism that are best understood or seem most likely to yield an effective drive system are those that involve transposable elements (TEs), meiotic drive, homing endonucleases, engineered underdominance and *Wolbachia* endosymbionts; these systems will be considered in more detail.

Transposable elements

TEs of several classes (BOX 1) are able to move to new locations in the genome and increase their copy number; therefore their frequency of inheritance from a heterozygous individual will be greater than the Mendelian ratio of 0.5 (REF. 22). TEs will increase in population frequency, as long as the rate of super-Mendelian inheritance outweighs any fitness costs that result from insertional gene inactivation or chromosomal rearrangements through recombination between elements in different genomic locations. A well-documented natural example of this spread is the invasion of *Drosophila melanogaster* by the *P* element during the last century, following acquisition from *Drosophila willistoni*[23,24].

TEs are common and widespread; for example, they constitute at least 15% of the genome of *Anopheles gambiae*[25]. Their extensive use as laboratory transformation tools in *Drosophila* and several pest insect species means that their molecular biology is comparatively well characterized[26], although much less is known about their population dynamics. Autonomous TEs encode enzymes such as transposases and are therefore able to effect their own transposition, whereas non-autonomous elements lack this ability but might achieve mobility through cross-mobilization. Most TEs in insect genomes have been inactivated over time through the accumulation of mutations. A TE-based gene drive system would require the use of autonomous elements, for example with an effector gene tightly linked to the transposase locus[27–30].

The first major challenge is to identify active elements that have rates of transposition in the target species that are high enough to be useful in a drive system. The *Hermes*, *Minos*, *Mos1* and *piggyBac* elements have all proved to be useful integration tools in mosquitoes, but their rates of germline remobilization are very low[31]. Regulatory mechanisms, whether host-generated or autoregulated[32–34], that inhibit transposition might be

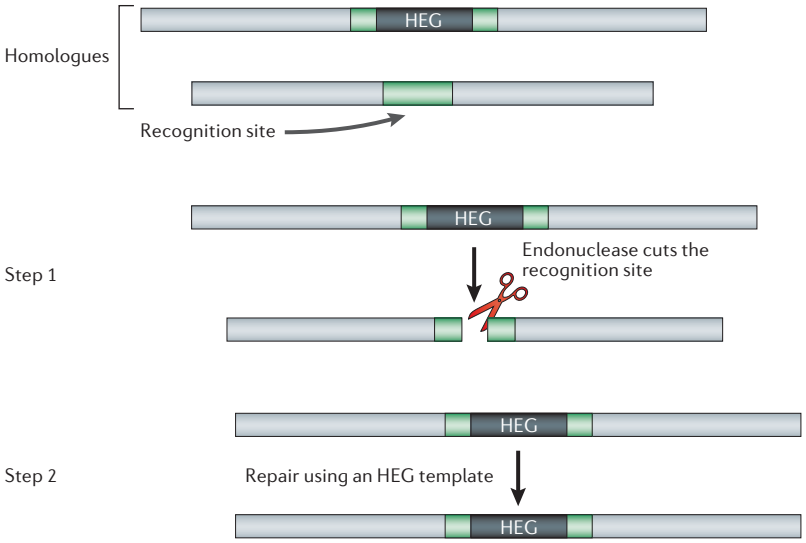


Figure 1 | **The mechanism by which homing endonuclease genes increase in frequency within a population.** A specific homing endonuclease gene (HEG) is typically found inserted between two specific sequences of DNA within the genome (light green). The HEG (dark green) codes for the production of an enzyme that recognizes these two specific coding sequences when they are not interrupted by the presence of an HEG. In individuals that carry the HEG on only one of two homologous chromosomes, the enzyme catalyses a break within the DNA sequence of the chromosome that lacks the HEG (step 1), which is then naturally repaired using the HEG within the homologue as a template (step 2).

present. This applies particularly if the active TE has been identified from the genome of the target species itself, such as *Herves*, which has recently been identified in *A. gambiae*[35]. Frustratingly, our knowledge of how transposition is regulated is still far from satisfactory. It is likely that only one gene-spreading opportunity would be possible with any particular TE because regulatory mechanisms would prevent further transposition, but again more data need to be collected.

The rate of transposition can vary with element size. *Mos1* elements show an exponential reduction in rates of transposition with increasing size[36]. A 'loaded' *Mos1* would probably take too long to reach population fixation to be useful for disease-control programmes. A strong selective pressure for mutational loss of the transgene to be spread would result in the spread of a TE without its load, as has been observed experimentally for *P* elements[37]. *Mos1* elements are small and it remains to be seen if larger TEs are more readily able to tolerate inserts; there are currently insufficient data for the other important TEs that are used in disease vectors. This issue might represent the most serious obstacle to the successful implementation of TE-based drive. The inherent tendency of TEs to recombine with other copies elsewhere in the genome and the imprecise nature of some transposition events are expected to make them particularly prone to loss of inserts (although copies that carry deletions would need to maintain both inverted repeats and the transposase to remain autonomous).

In the case of TEs that do not show site-specific integration, disruptions of gene function through insertional mutations and genomic rearrangements introduce a low-probability risk of unexpected adaptive changes in the vector. For example, natural TE-generated inversions in *A. gambiae* are associated with aridity tolerance[38]. The wide range of hosts for TEs also brings with it a risk of movement into non-target species. Natural TE spreading events do occur, albeit rarely. Nevertheless, the risks of actually increasing disease transmission by the vector through TE-generated adaptations must be rated as extremely slim. A possible reduction in the expression of the anti-pathogen effector gene as a result of position effects or silencing means that the relationship between phenotype and construct presence and its copy number would be complex for TEs that do not insert into specific sites, and insertional fitness effects would also be difficult to predict.

If TEs are to be used as gene drive systems, those elements that have high rates of replication and mobilization in target species must be identified. Moreover, endogenous elements in target species and the effects of transgene inserts on rates of replication and mobilization must be characterized, and mechanisms of regulating transposition must be better understood[30]. The identification of germline-specific promoters might allow the design of modified TEs that have improved transposition rates and species specificity[39]. These are difficult aims that will require considerable research effort. An exploration of the utility of elements other than type 2 TEs as drive systems, particularly site-specific TEs[40] (BOX 1), should also be encouraged, as these would have more predictable effects.

Homing endonuclease genes

Homing endonuclease genes (HEGs) encode endonucleases that recognize a specific sequence that flanks the HEG, but only when this sequence is not interrupted by the HEG itself (FIG. 1). Therefore, if an HEG is introduced into the target sequence on one of a pair of homologous chromosomes of a diploid organism, the endonuclease will cut only the copy of the chromosome that lacks the HEG. If the two homologous chromosomes are close to each other — for example, during meiosis — DNA repair mechanisms might use the chromosome that carries the HEG as a template for repairing the cut homologue. If this process occurs in the germ line, the proportion of gametes that contain the HEG will be greater than 0.5 and in some cases has been shown to be over 0.9 (REFS 41,42). Because HEGs are generally spliced out when the gene into which they are inserted is expressed, the gene function is maintained.

In principle, if an HEG that carries an anti-pathogen gene is able to (or is engineered to) cleave a particular highly conserved target gene in the insect's genome, it should be capable of population invasion from a very low starting frequency if the insertion does not significantly decrease the insect's fitness. Because HEGs are expected to remain in one genomic location they are predicted to be more genetically stable than TEs. Unlike with TEs, gene silencing is not expected to be a problem because fixation of an HEG results in no more than two gene copies. Unfortunately, HEGs have only been reported in fungi, plants, bacteria and bacteriophages[43–47]. Although

research has begun to investigate whether HEGs can operate in *D. melanogaster*, the potential for developing an HEG-based functional system in insects is unknown. Data are also lacking on the potential effects of inserts on the efficiency of cutting and repair, and the potential for loss of the insert owing to incomplete repair[48,49]. It seems likely that any HEG would need to be substantially modified before it could be used in insects, unless the gene targeted by the endonuclease was highly conserved. Creating an artificial HEG might be possible[41] using zinc-finger endonucleases that are engineered to target a sequence in the pest species[50], expressed during meiosis and introduced into the correct locus by homologous recombination.

HEGs have also been proposed as a way of inducing a population crash by targeting essential genes[41]. In this case the HEG would be engineered so that it would not be spliced out during gene expression, thereby producing a homozygous lethal mutation. Theoretically, this is an attractive alternative to driving anti-pathogen transgenes for population replacement, although it could prove difficult to achieve and would impose strong selective pressures for the evolution of suppressors.

Meiotic drive mechanisms

Meiotic drive occurs (usually in males) when a particular heterozygous locus segregates at a frequency greater than the expected Mendelian 0.5 ratio, through destruction or disabling of the homologous chromosome[51]. The driver locus (or loci) targets a particular responder region, but is itself linked to an insensitive allele of the responder so that it is not suicidal (FIG. 2). There are various mechanisms of meiotic drive and some result in a reduction in functional sperm, although they do not necessarily lead to reduced fertility. The driver will spread in a population as long as the increase in transmission is greater than any deleterious effects on fertility. Meiotic drive systems are often associated with small inversions that suppress recombination between their breakpoints.

The best characterized example of autosomal drive is *SD* (*Segregation distorter*) in *D. melanogaster*, a complex of 3 loci on chromosome 2. The components of the complex are an allele of the RanGAP gene *Sd*; an enhancer of distortion *E(SD)*; and a repeated array responder *Rsp*. Sensitivity to drive rises with increased copy number of *Rsp*[52–55]. The mechanism by which only sperm that contain sensitive chromosomes are affected by the RanGAP protein that is encoded by *Sd* is unknown, but one possibility is that this protein is preferentially sequestered in sensitive nuclei, leading to mislocalization, failure of chromatin condensation and therefore sperm dysfunction[56].

In the dengue fever vector mosquito *Aedes aegypti*, a meiotic drive allele M^D is closely linked to the dominant male-determining gene *M* on chromosome 1 (Y) and causes breakage of the homologous chromosome 1 (X) that carries the *m* allele[57–60]. There is a similar system in the *Culex pipiens* group[61], which are vectors of filariasis and arboviruses. Males are heterozygous (*Mm*) at the sex locus, whereas females are homozygous (*mm*), which results in a highly male-biased sex ratio. Meiotic-drive genes on the Y or male-determining chromosome are expected to produce a strongly male-biased sex ratio; such genes could be used to induce a population crash[62,63] as long as the local population has no insensitive alleles on the X chromosomes. In practice, many natural populations are insensitive to M^D (REFS 64,65). An insensitivity allele could itself be linked to an anti-pathogen gene and would be expected to spread if released into a sensitive population together with M^D. Moving natural drive systems such as M^D or *SD* into distantly related pest species would almost certainly be impossible because the responder would not be conserved in the target species. Similar examples could be sought in target species, but autosomal drive genes are likely to have gone to fixation, eliminated the original responder region and therefore become undetectable. Sex-chromosome drive would either have resulted in a local population crash or the selection of suppressor alleles, and therefore might be difficult to use. Creating an artificial version of meiotic drive using zinc-finger endonucleases that are engineered to be expressed during meiosis might prove a more promising alternative, as would also

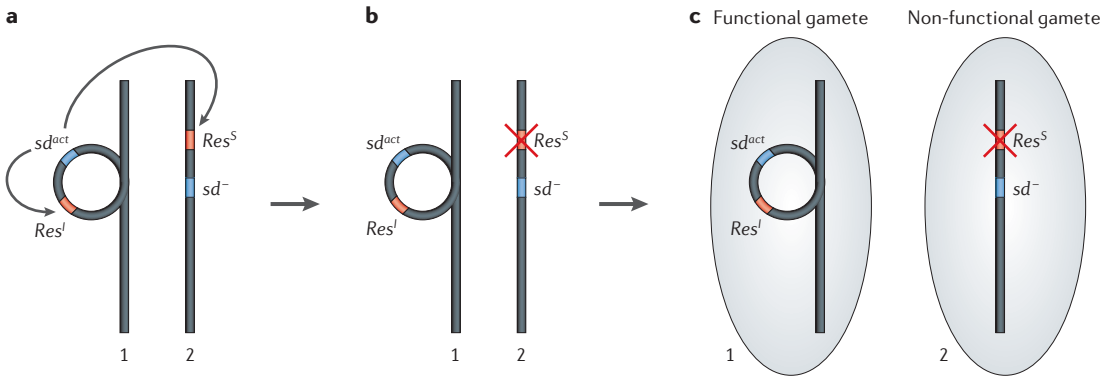


Figure 2 | **Segregation distortion.** In part **a** homologous chromosome 1 carries the active form of the segregation distortion allele (sd^{act}) and the insensitive allele (Res^I) for response to the product of sd^{act} within an inversion. Homologous chromosome 2 lacks the *sd* gene or has an inactive allele of the gene (sd^-), and has a responder allele (Res^S) that is targeted by the product of the sd^{act} allele. During meiosis, homologous chromosome 2 either does not form a gamete (**b**) or forms a non-functional gamete (**c**).

be the case for HEGs, because an endonuclease that targets a repeated region might overwhelm the usual mechanisms of chromosome repair.

There is another category of meiotic drive mechanism in *Tribolium* beetles. Here a region of nuclear DNA called *Medea*, or maternal-effect-dominant embryonic arrest, causes the death of all offspring of heterozygous females that do not inherit a copy of the *Medea* gene(s)[67–69]. When the frequency of the *Medea* sequence is rare it is expected to increase very slowly (proportional to the square of its frequency, if selection is not density dependent) even if there are no effects on fitness beyond the death of offspring that do not have the Medea gene(s). However, if insects that contain an engineered construct with *Medea*-like properties were released at higher frequencies, the construct could increase rapidly even if there were some negative effects on fitness. The *Medea* gene(s) has not yet been identified and might not operate in other species, but a molecular understanding of its function could lead to the development of artificial *Medea*-like constructs.

Underdominance

A trait such as fitness is considered to be underdominant if the average value for the trait in offspring is lower than it is in either of the parents[70]. If the definition of underdominance is extended to include the fitness of the second generation of offspring, then mating of insects with normal and translocated chromosomes can be considered to result in underdominance because a substantial portion of the second generation will lack a full genome and will die. A characteristic of underdominant traits that are controlled by two alleles at a single locus, or two translocation chromosome types, is that even when both types confer exactly equal fitness when homozygous, one of the two will be lost in a large, randomly interbreeding population. The initial frequency of the two alleles or chromosome types is the major determinant for which one is lost. This outcome is explained by the fact that individuals who are homozygous for the less common allele or chromosome are more likely to mate with an individual of the opposite type, resulting in unfit offspring. If the introduced chromosome includes closely linked anti-pathogen effector genes, and substantial numbers of carriers are released, the trait should increase in frequency and ultimately become fixed.

It has proved difficult to use naturally occurring underdominance systems such as translocations to drive useful genes into populations[2,71–73]. Nevertheless, a method of engineering an underdominant system that would require lower release levels and would involve two constructs inserted in separate linkage groups has recently been proposed[74–76]. Each construct has four components: a *trans*-acting suppressor, a *cis*-acting promoter, a toxin-coding sequence and an anti-pathogen gene with its own promoter. The suppressor on the first construct represses the promoter for the toxin gene on the second construct; the suppressor on the second construct represses the promoter on the first construct (FIG. 3). When an insect carries at least one copy of each construct it will survive.

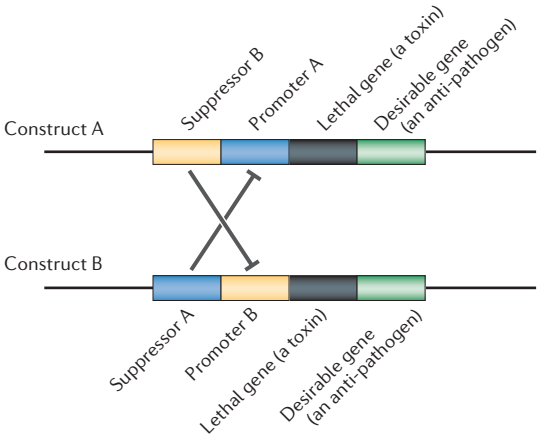


Figure 3 | **An example of an engineered underdominant system that is based on mutual suppression of lethal constructs.** Underdominance is a genetic condition in which the offspring (and/or grandoffspring) from a cross of two different genotypes are less fit than either parental genotype. When there is underdominance, one of the two genotypes becomes fixed in the population. Engineered underdominance can be achieved by developing two unique constructs (A and B) that are inserted on non-homologous chromosomes. Each of these constructs has a suppressor DNA sequence that shuts down the promoter on the alternative construct that controls expression of a toxin-coding gene. Each construct also includes an anti-pathogen gene with its own promoter. When an engineered strain with these two constructs breeds with the native population a substantial proportion of the F2 generation inherits only one construct and is killed by the action of the toxin-coding gene[75].

If insects with both constructs are released into the wild, a proportion of the second generation will die because they contain only one of the constructs and the promoter for the toxin-coding sequence will therefore be active. If the released engineered insects exceed a threshold frequency (of around 27% under ideal conditions) then the two constructs will become fixed in the population, assuming there is no fitness cost associated with the constructs.

The system's advantage lies in the expectation that the constructs are stably inserted and the anti-pathogen gene could be expressed in a tissue-specific and time-specific manner. In addition, because an anti-pathogen gene can be inserted into each of the constructs there is some insurance against failure that might occur due to a mutation in, or deletion of, one of the copies of the anti-pathogen gene. Furthermore, engineering different anti-pathogen genes into both of the constructs would ensure that the parasite would have to overcome two evolutionary hurdles, as long as both genes continue to function.

Several challenges face this approach to gene drive. Engineering a system with these properties that does not have major fitness costs will not be easy. It has been proposed that two specific RNAi genes, or a modified tetracycline-inhibited expression system, could function as *trans*-acting suppressors, but some leakage in suppression

of the lethal gene seems likely. Technical difficulties aside, this system, under realistic conditions, requires the insects carrying the constructs to be released at a much higher frequency than the previously discussed systems. The frequency threshold could be reduced by insertion of two copies of each construct as long as fitness costs were low[76]. If there were significant fitness costs that were due to one or both constructs then the release threshold that must be exceeded would be higher and a small fraction of wild-type insects would be expected to persist indefinitely in some populations.

Underdominant systems would not be appropriate for large-scale replacement of disease vectors on continental scales because the levels of release needed would not be economically viable. However, they could be well suited to replacement of defined target populations where unregulated further spread is not desirable.

Wolbachia

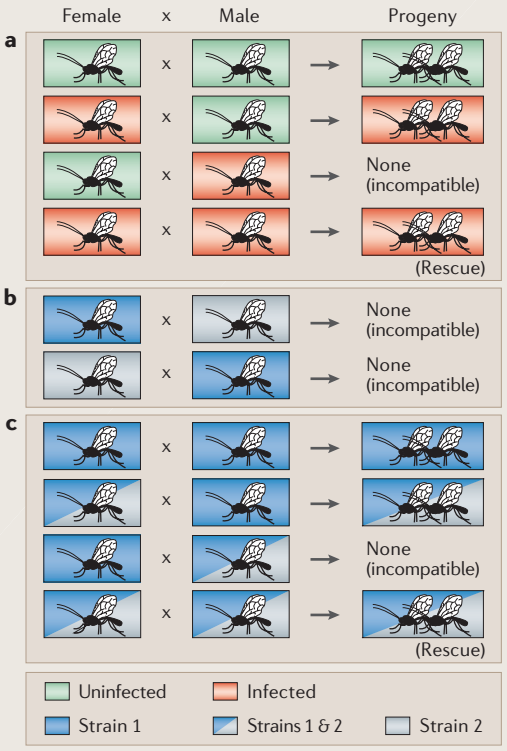
Wolbachia are maternally inherited intracellular bacteria that manipulate the reproduction of a diverse range of arthropod hosts to their own advantage[77,78]. One form of such manipulation, seen in many disease-vector species, is cytoplasmic incompatibility (CI). Unidirectional CI is seen in crosses between *Wolbachia*-infected and uninfected individuals; modified sperm from *Wolbachia*-infected males are unable to complete fertilization of uninfected eggs. By contrast, a rescue function allows eggs from infected females to develop normally (BOX 2). This provides a frequency-dependent reproductive advantage to infected females.

Wolbachia population invasion has been directly observed in nature in *Drosophila simulans*[79]. Many *Wolbachia* infections show wide tissue distribution

Box 2 | ***Wolbachia* and cytoplasmic incompatibility**

Wolbachia infections induce various patterns of cytoplasmic incompatibility (CI).

- Unidirectional CI is typically seen between infected and uninfected populations. By providing a frequency-dependent reproductive advantage to infected females, which unlike their uninfected counterparts can mate successfully with any male in a mixed population, *Wolbachia* can rapidly increase in frequency (panel **a**).
- Bidirectional CI is seen between populations that are infected with different *Wolbachia* strains. If such populations are in contact, an unstable equilibrium results, and whichever strain forms the local majority is expected to reach local fixation (panel **b**).
- If bidirectionally incompatible *Wolbachia* strains are combined to form a superinfection, superinfected males will be incompatible with females that are only infected with one strain, owing to the absence of the rescue factor for the strain that the female lacks. Because the reciprocal cross is fully compatible, these superinfections are expected to spread through populations that only harbour one of the strains[88] (panel **c**).



The published sequence of the *w*Mel strain from *Drosophila melanogaster*[92] and several other *Wolbachia* genomes that are in various stages of completion provide a wealth of information that will aid the development of *Wolbachia*-based drive systems. *Wolbachia*'s numerous transposable elements and phages provide promising tools for transformation[92–96] and for introducing variant CI genes. The presence of many repetitive and mobile elements in *Wolbachia* genomes also demonstrates that it is relatively tolerant of the presence and expression of non-essential genes, with inefficient selection[92], so there should be no strong selective pressures for the loss of inserted effector genes.

The ability to monitor gene expression will allow the identification of suitable promoter sequences, and the study of secretion systems will provide an ability to engineer the export of effector proteins to the host cytoplasm where they can come into contact with pathogens (although in *Wolbachia* strains in which there is high phage lytic activity the use of specific secretion mechanisms might be unnecessary). An understanding of the molecular mechanism of CI will have a major effect on the ability to most effectively use *Wolbachia* in transgenic control strategies. Genome sequencing has revealed a large number of ankyrin repeat-encoding genes that are excellent candidates for involvement in CI[92,97,98]; their function is under investigation in several laboratories.

Successful interspecific transfer of *Wolbachia* by microinjection of early embryos is now well established and has recently been used to introduce *Wolbachia* into the naturally uninfected pest species *Ceratitis capitata* (Mediterranean fruitfly)[99] and the important dengue vector mosquito *Aedes aegypti*[100], both resulting in high frequency of maternal transmission and high penetrance of CI. These results are cause for optimism about whether other important uninfected target species, such as the malaria vector *Anopheles gambiae*, will also be able to support *Wolbachia* and CI.

within the host[80], and therefore could be used to express transgenes that target pathogens in vector species[81–84].

The most important parameters that affect the spread of *Wolbachia* are the relative hatch rates from incompatible versus compatible crosses (penetrance of CI), the relative fecundity of infected females, and the maternal transmission efficiency. If *Wolbachia* reduce fecundity or show imperfect maternal inheritance, the frequency of infected individuals must first exceed a threshold value before spread can begin[85]. *Drosophila simulans* has been used as a model species for understanding *Wolbachia* population invasion, although the infected *Aedes albopictus* and *Culex quinquefasciatus* mosquito species show more favourable spread dynamics — very high frequencies of maternal transmission and penetrance of CI, and lack of observable fitness costs[86,87]. The threshold frequency that would have to be exceeded before *Wolbachia* becomes positively selected would be far lower than in *D. simulans*.

There are several advantages of using *Wolbachia* as a drive system. Its wide host range[77] means that *Wolbachia* that are transformed to express a particular anti-pathogen product would probably also be applicable to a range of secondary vectors, although this also means there is a small risk of movement into non-target species (although such events do happen, they are ecologically very rare). Outcrossing to maximize fitness is facilitated by its maternal inheritance, because no selection would be necessary after crossing infected females with field-caught males. Opportunities for recombination with wild-type *Wolbachia* strains would be limited owing to its strict intracellular location and maternal inheritance, and it is not expected to be unduly affected by the presence of inserts (BOX 2). Repeated spread and invasion of naturally *Wolbachia*-infected populations are possible using superinfections of different *Wolbachia* strains that can be created by microinjection[88].

One disadvantage to the use of a *Wolbachia*-based effector-gene expression system is that insect tissue or stage-specific promoters to restrict transgene expression, which reduce fitness costs, cannot be used. An alternative that would allow the use of such promoters would be to identify and use the genes that control CI as the basis of a nuclear drive system, analogous to *Medea*. Modelling has shown that this approach can be efficient in *Wolbachia*-infected populations[89]; however, the feasibility of this strategy can only be properly assessed when the mechanism of CI is fully elucidated.

Alternative strategies for using *Wolbachia* and CI have also been proposed. A virulent *Wolbachia* strain called *w*MelPop shortens adult lifespan in *D. melanogaster*, in addition to inducing CI[90]. If successfully transferred into vector insects this phenotype could be used to reduce disease transmission[83,91]. Only a small percentage of the population survives long enough to transmit pathogens, owing to the pathogen extrinsic incubation period. Modelling has shown that despite reducing fitness such a strain could still spread under a range of conditions, although increased release frequencies would be required, and could markedly reduce disease by removing the sector of the mosquito population that is responsible for most pathogen transmission.

The most important research priorities for the development of a *Wolbachia*-based drive system are achieving stable *Wolbachia* transformation and developing the means for secretion of effector gene products into the

Table 1 | **Comparative characteristics of potential drive systems***

Characteristic	Classes of potential drive systems				
	Transposable elements	**Natural meiotic drive**	**Engineered meiotic drive or HEG**	**Engineered underdominance**	***Wolbachia***
Is a release threshold required before population spread begins?	No	No	No	Comparatively high	Usually low
Is efficiency of drive dependent on insert size?	Yes	No	No; unknown for HEGs	No	No
Is there a mechanism for repeated spread?	Different transposable elements might be required	No	Redesign of target sequence	Different promoters and suppressors	Incompatible strains
Can insect tissue-specific promoters be used?	Yes	Yes	Yes	Yes	No
Is there a mechanism for transgene removal from the population?	No	No	Redesign of target sequence	Large-scale release of wild-type insects	Incompatible strains
Is there a risk of spread to non-target species?	Low	Close to zero	Close to zero	Close to zero	Low
Is the system known to function in important pest species?	Yes	Yes, but insensitivity alleles occur	No	No	Yes
Is there a potential use for the same system in secondary vectors?	Yes	Unlikely	Yes	Yes	Yes

*In many cases the data that support specific characteristics of a drive system are still preliminary. In this table we make the assumption that for all drive systems, strains can be constructed with low fitness costs and appropriate levels of gene expression. HEG, homing endonuclease gene.

host cytoplasm at sufficient concentrations in the relevant tissues to disrupt parasite development. The transfer of *Wolbachia* into important target species such as malaria vectors, followed by an assessment of whether it can be transmitted at high rates and induce high levels of CI without high fitness costs, is also needed.

Conclusions

Given the overwhelming burden of insect-borne disease, the recent rapid advances that prove the principle of blocking pathogen development in the vector have given real impetus and urgency to work on gene drive mechanisms. These are exceptionally important systems that deserve intensive research effort, but remain relatively understudied. TABLE 1 summarizes some of the relative advantages and disadvantages of the potential drive mechanisms that are discussed here. Various technological barriers must be overcome in each case before field trials can even be contemplated. Although significant recent advances have been made, particularly in TE and *Wolbachia* research, the emphasis has been on evolutionary and functional aspects rather than specifically on their development for gene drive.

The various types of drive mechanism should not be viewed as competing systems. Different characteristics will be needed in different situations. For example some systems (such as underdominance) would be better suited to the early stages of an intervention where unregulated spread would be disadvantageous, whereas others could be used only when the safety of the construct has been established beyond doubt. The availability of two or more independent drive mechanisms would markedly increase the chances of success of an intervention. Multiple lines of research are to be encouraged, including those of less-studied systems that are nevertheless theoretically attractive; ultimately, the drive system that becomes most widely used might be one that is entirely novel and not described here. The creative design and engineering of drive systems that have improved characteristics compared with naturally occurring systems will be needed if this 'grand challenge' of insect population replacement is to be met.

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Competing interests statement
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DATABASES
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