

## Review

# Advances in CRISPR gene drives for mosquito population control

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CRISPR-based gene drive (GD) systems bias allele inheritance during meiosis, enabling transgenes to spread at rates exceeding Mendel's law of segregation. This capability underlies their potential as powerful tools for controlling mosquito-borne diseases. GDs can be engineered either to suppress mosquito populations or to modify them by introducing traits that block pathogen transmission. Recent advances have focused on improving evolutionary stability, with modeling studies providing insights into expected population dynamics. With a focus on the most current population modification GDs, we discuss advances in GD architectures — including integral and allelic drives, combined modification–suppression systems, and both homing and non-homing toxin–antidote designs — that expand the range of possible strategies and address limitations of early homing drives. Numerous antipathogen effectors with strong pathogen-blocking activity can now be coupled to these systems, with current efforts assessing their durability against genetically diverse pathogens. Key challenges remain, including resistance evolution, ecological impacts, and long-term stability. Nonetheless, GDs offer a promising approach for reducing disease transmission, especially in regions where conventional interventions are difficult to sustain.

## Addresses

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## Introduction

Gene drives (GDs) bias the inheritance of genetic elements, enabling them to spread rapidly through target populations. In conventional Mendelian inheritance, each allele has a 50% chance of being passed on to progeny, but GDs can exceed this frequency, allowing them to increase and persist in populations despite their fitness costs. There are many examples of naturally occurring GDs, such as transposable elements, meiotic drives, and *Medea* elements, with unique mechanisms to bias their inheritance. Early genetically-engineered GDs, including *Medea* [1,2], maternal-effect lethal underdominance [3], engineered translocations [4], and synthetic sex distorters [5] were inspired by these natural systems. The development of nuclease-based GDs, such as transcription activator-like effector nucleases and zinc-finger nucleases [6], and non-Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) homing endonuclease drives [7,8] further expanded GD capabilities. These natural GDs and pioneering engineering efforts set the stage for later breakthroughs in programmable genome engineering in the CRISPR age.

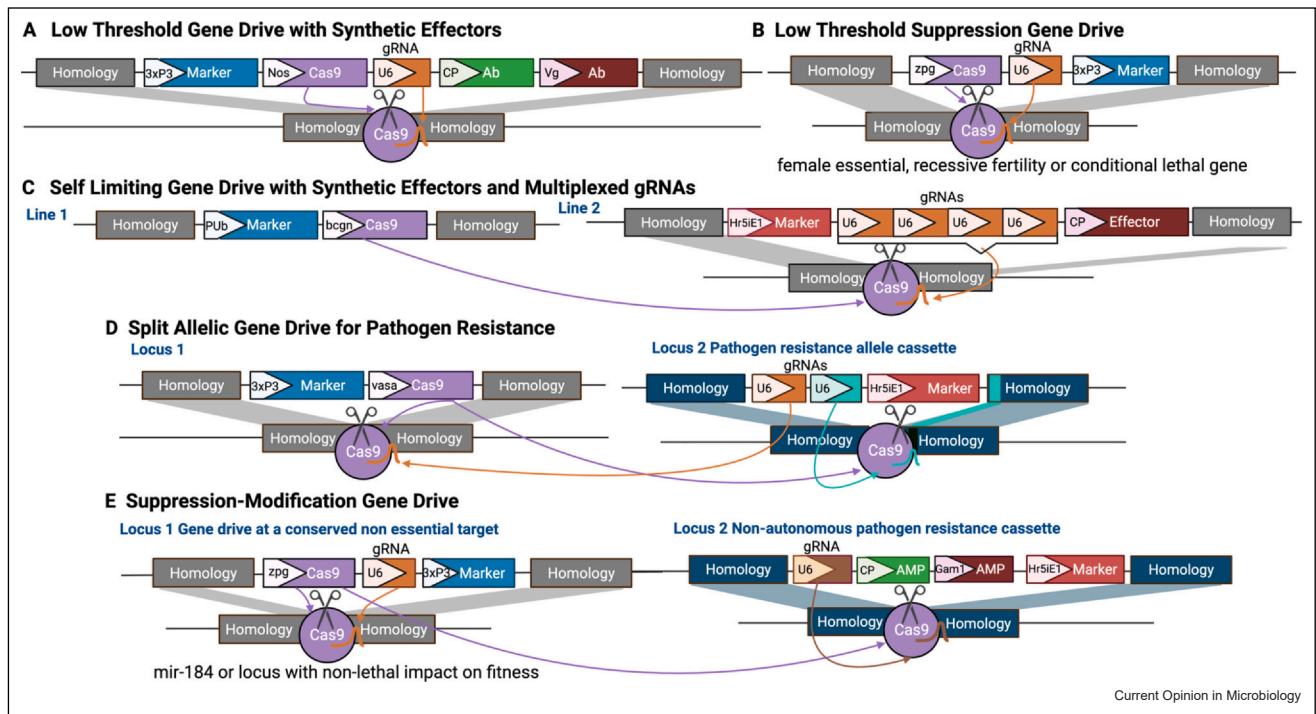
Most contemporary homing-based GDs (HGDs) leverage CRISPR biology in which an endonuclease encoded by the Cas9 gene works in concert with a guide RNA (gRNA) to introduce a double-stranded break at a specific target site and copy itself and often a linked cargo onto a homologous chromosome via homology-directed repair (HDR). This drive-biased inheritance results in progeny inheriting two copies of the GD rather than one, as expected under Mendel's law of random segregation, thereby increasing the frequency of the GD in the population over time. GDs have been proposed for population suppression, aiming to reduce or eliminate target populations. Suppression drives are particularly valuable for species where local population elimination is ideal, including disease vectors, agricultural pests, and invasive species that directly damage agricultural commodities, endangered species, or sensitive habitats. However, populations of species that can vector disease-causing pathogens can also be engineered to be incapable of pathogen transmission. This approach, termed population modification (or replacement), increases the frequency of beneficial cargo genes or alleles that are inherited with the drive, such as antipathogen genes that render the target organism incapable of pathogen transmission. As the population modification drive spreads, the population shifts to a non-

vector phenotype, while population size remains largely unchanged. Population modification has been a long-standing focus of insect management and has been achieved in multiple mosquito species using CRISPR HGDs. The first reported CRISPR-based HGD in mosquitoes was constructed in the Asian malaria mosquito, *An. stephensi* [9], a prominent vector in India and Pakistan that has recently been established in parts of Africa. Since then, GDs for population modification have been developed with diverse designs and functions in three genera, including *Anopheles*, *Aedes*, and *Culex* mosquito species. This review provides an overview of recent population modification GDs in mosquitoes and discusses concerns about their long-term impact on pathogen transmission.

### General gene drive designs and design considerations

Early iterations of CRISPR HGDs were predicted to have wide dispersal capabilities, even possibly on a global scale. However, the spread and persistence of GDs in a population are strongly influenced by its design, the target species, and factors such as selection pressure for drive resistance, population size, drive-induced loads that impact fitness, and gene flow in the release environment [10,11]. Most CRISPR-based HGDs developed to date in mosquitoes are linked systems ('autonomous') with the Cas9- and gRNA-encoding DNA sequences inherited together at the same locus (Figure 1a). These are typically low-threshold,

Figure 1



Schematic representations of conventional and more advanced GDs in mosquitoes. (a) Representative of a linked (autonomous) GD construct expressing dual anti-malarial antibodies, similar to the design in [76]. The drive has a *nanos* gene promoter (Nos) driven Cas9 (lavender) and U6 gene promoter driven gRNA (orange) for the direct integration of the drive to a locus in the *cardinal* gene. These components are inherited along with genes encoding two anti-plasmodium antibodies (green and burgundy) and are marked with an eye-specific promoter (3xP3) driven fluorescent marker (blue). (b) A low threshold suppression drive with a *zero population growth* (zpg) gene promoter-driven Cas9 (lavender) and U6 driven gRNA (orange) targeting a conserved female-essential gene, and is marked with an eye-specific promoter (3xP3) driven fluorescent marker (blue). (c) Split GD with multiplexed gRNAs similar to a recent *Ae. aegypti* drive [45]. At locus 1, there is a *vasa* gene promoter driven Cas9 (lavender) and an eye-specific promoter (3xP3) driven fluorescent marker (blue). Locus 2 has multiple U6-driven gRNAs (orange) targeting different sites in the same gene, and a ubiquitous Hr5iE1 gene promoter-driven marker (red). Both constructs need to be co-inherited to direct GD. (d) Gene construct representative of an allelic drive where locus 1 has *vasa* gene promoter driven Cas9 (lavender) and an eye-specific promoter (3xP3) driven fluorescent marker (blue). The second locus encodes two U6-gRNAs and a marker (red). One gRNA (orange) facilitates copying of the drive, and the other gRNA (teal) targets a specific site adjacent to the 5'-end of the coding sequence of a disease-susceptible allele. This allele is then replaced with a disease-resistant allele encoded into the homology arm of the GD. (e) A suppression-modification GD [44] has one locus with a linked GD that produces Cas9 (lavender) and gRNAs (orange) that target a non-essential gene with a fitness cost, such as *mir-184* [44]. The GD at locus 1 also provides Cas9 *in trans* to locus 2, which encodes anti-plasmodium antibodies (green and burgundy), and a gRNA (brown). Both loci also encode unique fluorescent markers. For each design, the homology arms, which match the sequences immediately to the 5'-end (left arm) and 3'-end (right arm) of the cut site, guide HDR and allow the entire drive cassette (Cas9, gRNAs, effectors, marker genes, etc.) to be copied precisely into the chromosome. Created in BioRender (2026).

capable of population invasion from low initial frequencies, and are the drives initially that showed the potential via modeling to spread far beyond the release sites into other populations as a result of gene flow. Low threshold systems are attractive for vector control due to their minimal release requirements, but they pose significant challenges in terms of reversibility and spatial containment [12,13]. In contrast, high-threshold, frequency-dependent drives often require multiple high release ratio frequencies and are eliminated from a population if their frequency drops below a critical threshold [12,13]. GDs can also be self-limiting, whereby the biased drive inheritance is only temporary, limiting their population persistence and spread in both space and time. For example, split or modular drive systems are HGDs in which the Cas9 and gRNA transgenes are inherited separately, thereby limiting their spread as the non-driving Cas9 transgene is gradually lost (Figure 1c) [14–17]. Therefore, the specific design features in the linkage of key HGD components can lead to greater spatial and temporal confinement of drives and are key to understanding more advanced HGD systems.

Cas9-gRNA linkage is not the only major design consideration for CRISPR HGDs. For genetic technologies with applications to pest or disease control, GDs must overcome the load-induced fitness costs associated with the drive and disruption of their target site. A GD and cargo that reduce pathogen transmission but cause low mosquito fitness is unlikely to persist in the population, even when paired with a high-efficiency drive [18]. Early laboratory studies of CRISPR HGDs also revealed that error-prone end-joining (EJ) DNA repair mechanisms at the drive cleavage site can rapidly generate functional and non-functional cleavage-resistant alleles. These EJ-mediated resistance alleles negatively affect drive efficiency and persistence, and as they accumulate in populations, the drive is removed [19,20]. Therefore, many of the early CRISPR HGD designs would likely go extinct in wild populations before spreading widely. For example, an *An. stephensi* HGD system linked to anti-malarial effector genes failed to reach fixation in laboratory populations due to a significant fitness load (reduced female fertility and fecundity) in mosquitoes either homozygous for the drive or heteroallelic for the drive and a non-functional mutant allele in the kynurenine hydroxylase-encoding (*kh*) target gene. The rapid accumulation of drive-resistant alleles in the laboratory population led to drive extinction in some populations within a few generations, due to the fitness costs of these nonfunctional alleles [21,22]. Later iterations of this drive reduced the generation and accumulation of resistance alleles by incorporating a recoded *kh* target sequence to remove loss-of-function *kh* alleles and rescue *kh* function [23]. Notwithstanding, functional resistance alleles still emerged. This example underscores that despite high homing efficiencies, the fitness of CRISPR

HGDs and their propensity to develop EJ-mediated drive-resistant alleles are important factors in drive performance.

Several strategies have been developed to improve the evolutionary stability of HGDs. To address the production and accumulation of EJ-mediated resistance alleles in populations, researchers have evaluated more restrictive germline-specific promoters to drive Cas9 expression [24–26], multiplexing gRNAs to reduce resistance [12,27–30], targeting highly-conserved or functionally-constrained sites [30,31], and designing drives that eliminate progeny with resistance alleles. The latter can be achieved by disrupting essential genes while providing a rescue element that regains target function, ensuring that only drive carriers survive [23,32–35]. However, whether these strategies can delay resistance formation and, ultimately, the extinction of the drive in a population is not necessarily straightforward. A recent study in *An. stephensi* showed that the classic strategy for gRNA multiplexing, whereby gRNA target sites are designed adjacent to each other, can slightly reduce homing rates compared to additive gRNA multiplexing, which entails more distant spacing between gRNA target sites, although this difference was not significant [36]. Mathematical modeling suggests that both classical and additive gRNA multiplexing in GDs can persist in populations and reach high population frequencies, but additive multiplexing is more sensitive to the cumulative fitness costs of each drive element. [36]. Resistance poses a greater challenge to population suppression drives, which impose higher fitness costs, than to population modification GDs [37,38].

### Recent advancements in mosquito population modification drives

A number of next-generation population modification drives are in development in mosquitoes. For example, integral GD (IGD) may address potential regulatory concerns surrounding the development and release of synthetic transgenes into populations by using native promoters and regulatory elements [39–41]. They insert DNA sequences at loci that will drive endogenous effector genes along with Cas9 expression. IGDs have been integrated into the *An. gambiae zero population growth* (*zpg*) and *nanos* genes, enabling Cas9 expression from their respective endogenous regulatory elements and frameworks for their assessment in the field have been devised [42]. These drives are predicted to have many advantages, including being smaller than other CRISPR HGDs, which may make them more stable and the ability to be modularized across multiple genes, potentially incurring a lower fitness cost and lending flexibility in their design. The selection of functionally relevant and highly conserved target sites for IGDs can also reduce the formation and accumulation of resistance alleles.

Allelic drives, which bias the inheritance of specific alleles, are efficient at spreading protective alleles into populations (Figure 1c). A recent proof of concept for an allelic drive in mosquitoes was built in *An. stephensi* and biased the inheritance of a *Plasmodium* refractory allele of the *FREPI* gene, reducing infection prevalence by 50% and sporozoite numbers in the salivary glands by five-fold [43]. Although conversion rates were moderate (<86%) in small laboratory cage studies, the allele reached 94% frequency within eight generations at a 1:3 release ratio. This approach also may have a lower fitness cost, since drive-strain fitness is not burdened by the load associated with antipathogen transgenes. However, this proof-of-concept was developed only in an engineered congenic strain, with no diversity at or near the allele-conversion site. The Cas9 encoding gene was also unlinked to the cassette containing the FREPI donor allele and the gRNA. Therefore, whether this approach would work as a standard allelic drive, resulting in the biased inheritance of both the drive and the allele-conversion cassette (Figure 1c), or in a more genetically diverse wild-type population, remains uncertain. Moreover, it will also not be trivial to identify and validate multiple alleles that confer more complete disease resistance, or to determine whether these alleles will perform similarly in natural populations of mosquitoes with native pathogens.

Dual population suppression and modification drive strategies are also under development. A recent GD in *An. gambiae* combines population modification with suppression of HGD strategies by targeting a highly-conserved microRNA [44]. Knockout of miR-184 produced multiple negative fitness effects, including shortened lifespan, impaired flight, and bloodmeal-associated mortality, but fertility remained intact. These phenotypes reduced vectorial capacity, allowing the drive to spread and fix in caged populations. Modeling indicated that even partial fitness costs, in particular, reduced lifespan and post-bloodmeal mortality, could lower malaria transmission, with some advantages over fully lethal suppression strategies due to reduced resistance selection.

Most of the advances in mosquito GD technology have focused on malaria vectors in the *Anopheles* genus. GDs in non-*Anopheles* mosquito species have lower homing efficiencies, reducing their drive fixation rates. For example, improvements have been made in the initial homing rates in *Ae. aegypti*, however, this species still has homing rates much lower (~84–94%) [25,45] than those of *Anopheles* mosquitoes. Recent *Ae. aegypti* homing CRISPR GD systems were linked drives targeting a single locus [46] or split drives targeting multiple loci (Figure 1b) [45]. The GDs increased in frequency across generations in small cage populations, but the single-target drive accumulated substantial GD-blocking

resistance indels [46]. The multiplexed-target drive developed resistance alleles, but not at all target loci [45]. Despite this, both achieved consistent super-Mendelian inheritance, although further evaluation is needed in diverse populations. These homing rates may make population modification drives feasible for *Aedes* mosquitoes, but not suppression drives, which demand very high efficiencies to offset their heavy fitness costs. Until homing improves, alternative non-GD genetic suppression technologies, such as precision-guided sterile insect technique (pgSIT) [47–49], Release of Insects carrying a Dominant Lethal (RIDL) [50], traditional sterile insect techniques [51] or *Wolbachia* Incompatible Insect Technique (IIT) [52] should continue to be used and developed.

Additional GD systems developed in model organisms may be adapted for mosquitoes in the future, particularly to counter resistance. Toxin-antidote homing-dependent GDs bias inheritance by cleaving an essential haplosufficient gene (toxin) and inserting a cleavage-resistant rescue version (antidote) at the same site [23]. Individuals that do not inherit the drive are eliminated due to the loss of gene function. Cleave-and-rescue (ClvR) [53], or CRISPR toxin antidote drives [35], which are homing-independent, achieve biased inheritance by targeting essential genes and rescuing only drive carriers. These GDs are less prone to resistance, as individuals that do not inherit the drive or inherit indels at the drive site are killed, which may prove more effective in mosquito species with low or variable homing rates.

### Engineering disease resistance for population modification strategies

Population modification strategies are predicated on antipathogen effector genes that are linked to the drive and driven into the population to block pathogen transmission. If either the drive or the linked antipathogen effector fails, then this approach may not work. A wide range of strategies have been employed to develop anti-pathogen effectors including targeting the pathogen with RNA interference (RNAi) [54–60], single-chain antibodies [61–64], antimicrobial peptides/proteins (AMPs) [39,65–69], Cas13-mediated viral RNA targeting systems [70], overexpressing immune factors [71–74], or converting alleles to variants that impair transmission [43]. Therefore, a wide range of different anti-pathogen effectors can be employed to make mosquitoes resistant to pathogen transmission, but their effectiveness against the genetically diverse pathogen populations found in the wild remains to be demonstrated. Resistance management for effector genes needs to prevent or delay emergence, and monitoring for continued efficacy, while developing new effectors to replace those that are no longer effective [75]. Prevention requires raising the selection barrier by using

multiple effectors targeting different viral sites or parasite stages, and reducing pathogen population size [76]. Effectors must robustly block transmission across diverse hosts to avoid escape mutants and ensure durability at large, even continental, scales [75].

For the *Plasmodium* parasites causing human malaria, mosquitoes may act population-wide as reservoirs of pathogen genetic diversity, challenging the assumption that bottlenecks in the mosquito limit variation, which was a presumed benefit of targeting parasites in the mosquito [77]. Additional studies are necessary to understand the probability that parasite resistance to the effectors arises *de novo* and the rate at which resistant parasites spread within a population. As these technologies progress into field trials, these studies should discern whether they are efficient enough to disrupt transmission over an epidemiologically relevant time-scale. Evaluations as to whether there is a risk of intensifying transmission or infection are also essential. In studies of human *Plasmodium* infections, which are known to exhibit high parasite diversity, preventative and therapeutic drugs have selected drug-resistant parasites that have a fitness advantage during treatment that leads to higher intensities of infection [78,79]. Combinations of effector molecules targeting distinct parasite stages, such as m1C3 (midgut ookinetes) and m2A10 (salivary gland sporozoites) [76] will likely slow the evolution of resistance [80,81]. Testing effectors against multiple field-derived strains and over longer time scales is also critical to ensure that these effectors do not inadvertently intensify transmission. A recent study was the first to evaluate the effectiveness of genetically modified mosquitoes against field-derived malaria parasites and remarkably demonstrated impaired development of midgut parasites and a complete elimination of transmissible parasites [40]. This promising result is an important step toward building a more comprehensive GD risk assessment and a framework for GD field trials, but additional studies would strengthen our understanding of their longevity at scale.

Antimicrobial peptides and upregulation of endogenous immunity strategies have broader-spectrum activity. A recent dual-AMP GD had a significant impact on malaria sporozoite size and prevalence, while retaining high homing efficiency [27]. Expression of these AMPs was driven by insertion into endogenous genes expressed in response to mosquito blood feeding or in tissues vital to parasite development. Significant fitness deficits were attributed to these effectors, which contributed to model predictions that malaria could be interrupted, but not eliminated, by a dual AMP drive in higher transmission areas. Immune pathways produce AMPs, so the overexpression of positive regulators of these pathways is an alternative to engineering synthetic AMPs. Recent studies on the overexpression of a member of the Imd

pathway that activates AMPs that modulate bacterial and parasitic infections showed that this approach had a limited impact on *Plasmodium* parasite prevalence in *An. gambiae* [73]. Given the complexity of immune pathway regulation, a multifaceted approach that modulates both positive and negative regulators of one or more anti-parasite pathways will likely be needed to advance this approach. Further study is also needed to understand how AMP production and immune gene overexpression impact mosquito physiology, microbiome composition, and overall fitness. Both of these approaches are likely to affect mosquito immunity against non-target pathogens and microorganisms, or other processes that may alter mosquito fitness.

Resistance and potential selection for strains with increased transmissibility are also a concern for GD-linked effector genes that target viruses [75]. Mosquito-borne viral pathogens are RNA viruses with high mutation rates and genomic plasticity, and often exist as genetically diverse quasi-species. Targeting numerous sites in the viral genome, or functionally constrained or conserved sites, may delay or prevent the emergence and spread of viral resistance, despite this diversity. Many antiviral effectors rely on RNAi mechanisms [54–60], but high viral diversity has been associated with rapid RNAi resistance [82], and RNAi may also promote further viral diversification [83]. At high viral titers, RNAi responses can be circumvented, supporting the conclusion that effectors also could select for more intense infections [84]. Whether RNAi-based effectors similarly influence virus evolution remains unknown, but this underscores the need for long-term testing with diverse, low-passage strains. Targeting multiple viral loci [54], antibody-based effectors [61], or combinations of RNAi-mediated effectors [64] may overcome some of these issues, but resistance is still likely, and further studies are required. Viral sensor technologies that trigger mosquito death upon infection can complement existing antiviral effectors. For example, systems using Cas13 have been engineered to reduce viral titer, but the off-target activity of Cas13 also kills infected mosquitoes [70]. A tethered viral sensor based on the Phage P1 *cre-loxP* system has also been engineered with viral protease-specific linkers flanking the *cre* gene, which are cleaved by viral proteins, leading to viral-specific activation of an insect-specific neurotoxin at a *loxP* site [68].

### Open questions and future directions

Gene-drive technologies have advanced rapidly and have the potential to have a significant impact on diseases that disproportionately affect some of the most vulnerable humans, including children under five years old, who still account for the most malaria deaths. Substantial uncertainties still remain regarding their performance in natural populations, including the interactions between

drive constructs and the extensive genetic variation present in wild populations (reviewed in [17]). Standing polymorphisms, *de novo* mutations, or drive-induced EJ-mediated resistance alleles may impair target recognition and facilitate the rapid emergence of drive-resistant individuals. Multiple strategies to delay this resistance have been tested, but, as with many current mosquito-borne disease interventions, resistance is likely inevitable, making it necessary to maintain a pipeline of GD and other technologies to replace functionally-exhausted GD systems and manage resistance, ensuring longer-term disease prevention. The ecological consequences of the drive spread are also poorly resolved, including potential impacts on population structure, vector-parasite dynamics, and interspecies interactions within complex ecosystems. The long-term evolutionary stability of drive elements and the extent to which drive efficacy also may be shaped by environmental variables such as temperature, density dependence, and population mating structure require further investigation (reviewed in [10]). The trajectory of resistance evolution under field conditions, particularly the relative fitness of resistant alleles and their capacity to spread, also remains difficult to predict. Addressing these gaps will require integrated approaches combining population genetic modeling, laboratory and semi-field experimentation, and rigorous ecological and epidemiological modeling prior to field trials. Some GDs are advancing on pre-release goals and trial frameworks (reviewed in [42]), but as they potentially move into field trials, population modification drives will require monitoring of both the GD and the anti-pathogen effectors to ensure their efficacy and safety (reviewed in [75]).

Progress toward small-scale field trials of some GD technologies is affected by regulatory challenges that so far have prevented even small-scale, country-regulated field trials in spatially or ecologically confined areas [85]. Recently, some groups have been forced to suspend their activities when their regulatory approval was rescinded. Therefore, the regulatory landscape for HGDs remains very uncertain and vulnerable to the vagaries of some political systems. The concurrent development and expansion of self-limiting genetic technologies, such as RIDL [50], *Wolbachia* IIT [52], or pgSIT in *Ae. aegypti* [48,49] and *Anopheles* [47] mosquito vectors can provide alternatives while these uncertainties are being resolved. These technologies are akin to long-established SIT, or female-killing population suppression strategies, and it may be prudent to ensure genetic technologies are available now for immediate mosquito suppression and human life-saving. In addition, these technologies may serve as a strategy to reduce the mosquito population prior to the release of population modification drives (*reduce* and *replace*). Smaller populations tend to have less diversity, making it feasible to design drives without naturally occurring resistance alleles, but they are also subject to stronger

genetic drift, which may facilitate more rapid fixation of the drive or expedite its extinction. Other factors, such as gene flow, will also play a role in small populations.

Despite these uncertainties, mosquito-borne diseases require immediate, effective prevention tools. Unlike conventional interventions, GDs may have the potential for self-sustaining and wide-scale impact, particularly in many resource-limited regions where repeated vector control measures are most needed but difficult to implement and sustain. The modularity of GDs also allows for the incorporation of diverse designs and effector strategies, enabling flexible responses to emerging pathogen threats.

### CRedit authorship contribution statement

**Robyn Raban:** Writing – original draft preparation, Writing – review & editing. **Anthony A James:** Writing – original draft preparation, Writing – review & editing, Funding acquisition. **Omar S Akbari:** Writing – original draft preparation, Writing – review & editing, Funding acquisition.

### Data Availability

No data were used for the research described in the article.

### Declaration of Competing Interest

OSA is a founder of Agragene, Inc. and Synvect, Inc., holding an equity interest. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict-of-interest policies. All other authors declare no competing interests.

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Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
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