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Genetic control strategies for population suppression in the *Anopheles gambiae* complex: a review of current technologies

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Malaria continues to pose a critical public health threat, with mosquitoes from the *Anopheles gambiae* complex acting as the main vectors of the disease in sub-Saharan Africa, where approximately 95% of malaria-related deaths occur. Despite significant advancements in vector control, such as insecticide-treated bed nets and indoor spraying, the effectiveness of these interventions is increasingly compromised by various challenges, including rising levels of insecticide and pathogen resistance, mosquito behavioural adaptations, and persistent funding gaps. In this context, genetic vector control strategies have shown considerable promise, primarily based on findings from controlled laboratory studies. This review explores the development of these genetic approaches within the *Anopheles gambiae* complex and outlines future directions for their advancement and potential integration into malaria control efforts.

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Introduction

Malaria remains a major global health challenge, causing nearly 600 000 deaths in 2023, with approximately 95% in sub-Saharan Africa. This marks an increase from earlier years, partly attributed to COVID-19 disruptions, rising insecticide resistance, and climate-driven changes in transmission dynamics [1]. Although conventional vector control methods, such as insecticide-treated nets and indoor residual spraying, remain key to reducing disease transmission, innovative solutions are urgently needed.

Genetic control is a promising complementary tool in the fight against malaria, involving the release of genetically modified (GM) mosquitoes to either suppress or modify target populations to block malaria transmission [2]. These technologies include self-limiting methods requiring repeated releases for localised impact and low-threshold self-sustaining systems that persist and spread through wild populations. The latter are expected to provide long-term and more cost-effective population control than the former [3].

CRISPR-based genome editing has driven progress in this field. This dual system comprises CRISPR-associated (Cas) nucleases and guide RNAs (gRNAs), which can be programmed to direct the nuclease to a specific 20-base-pair sequence in the genome, enabling precise and efficient genetic modifications [4,5].

Although genetic technologies offer great potential for malaria control, their large-scale deployment in the field has yet to occur. The release of GM mosquitoes in African countries would commonly be regulated under national legislation derived from the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD) and, in some cases, additionally under environmental legislation, but typically requires the approval of national competent authorities in each country of intended release.

Self-sustaining genetic vector control strategies, such as gene drive technologies, designed to spread beyond initial release sites and potentially across borders, may pose additional regulatory considerations that will benefit from ongoing regional harmonisation of decision-making. International and Africa-centred bodies, including the World Health Organization (WHO), the institutions of the CBD, and the African Union Development Agency (AUDA-NEPAD), are working to strengthen regulatory capacities and promote harmonised approaches for the responsible implementation of these technologies [6–8].

In parallel, experts in the field are developing evidence-based recommendations to guide environmental risk assessment methodologies tailored to gene drive applications for malaria vector control [9] while also establishing best practices for conducting open-field trials [10••].

This review explores advances in self-limiting and self-sustaining strategies for suppressing *Anopheles gambiae* populations (Figure 1), focusing on recently developed technologies, their potential impact on malaria control, and their current implementation status.

Self-limiting strategies for population suppression

Sterile insect technique

The sterile insect technique (SIT) is a population suppression strategy that relies on the mass release of sterilised male mosquitoes, typically through radiation. These sterile males compete with wild males for mates but produce no viable offspring, leading to a gradual decline in the target population over time [11,12].

The first application of SIT in *Anopheles albimanus* in the 1970s established the technical feasibility of mass-rearing and irradiation-induced sterilisation of males capable of competing with wild males under semifield conditions, despite notable operational constraints [13,14]. This work paved the way for subsequent field trials in Sudan targeting *An. arabiensis*, which further assessed SIT efficacy and identified critical limitations such as reduced mating competitiveness of sterile males, incomplete sterilisation, and inefficient, non-scalable sex separation methods [15].

To address these challenges, strategies include mechanical sorting by pupal size dimorphism, endectocides in blood meals, and genetic sexing strains that link selectable markers, such as insecticide resistance or temperature-sensitive lethal mutations, to sex loci for female removal [16]. However, when targeting predominantly rural vectors such as *An. gambiae*, which require continuous, high-density male releases, the cost-effectiveness of these methods remains limited, and their impact has yet to be proven.

Advances in genetic engineering for SIT

Genome engineering can advance vector control by overcoming key limitations of SIT. One approach to improving sex separation is the generation of an *An. gambiae* strain with a fluorescently marked Y chromosome, unique to males [17]. Another exploits the sex-specific splicing of the *doublesex* (*dsx*) gene, a crucial regulator of sexual differentiation, for early, sex-specific fluorescent marking [18].

These genetic systems should facilitate high-throughput, automated male sorting using flow cytometry platforms such as COPAS, supporting the scalability of SIT programs when coupled with male-sterilisation technologies.

In parallel, the availability of highly precise endonucleases and the advancement of molecular tools for temporal and spatial gene expression [5] have unlocked a broad spectrum of possibilities for inducing male sterility, circumventing the limitations and fitness costs associated with irradiation.

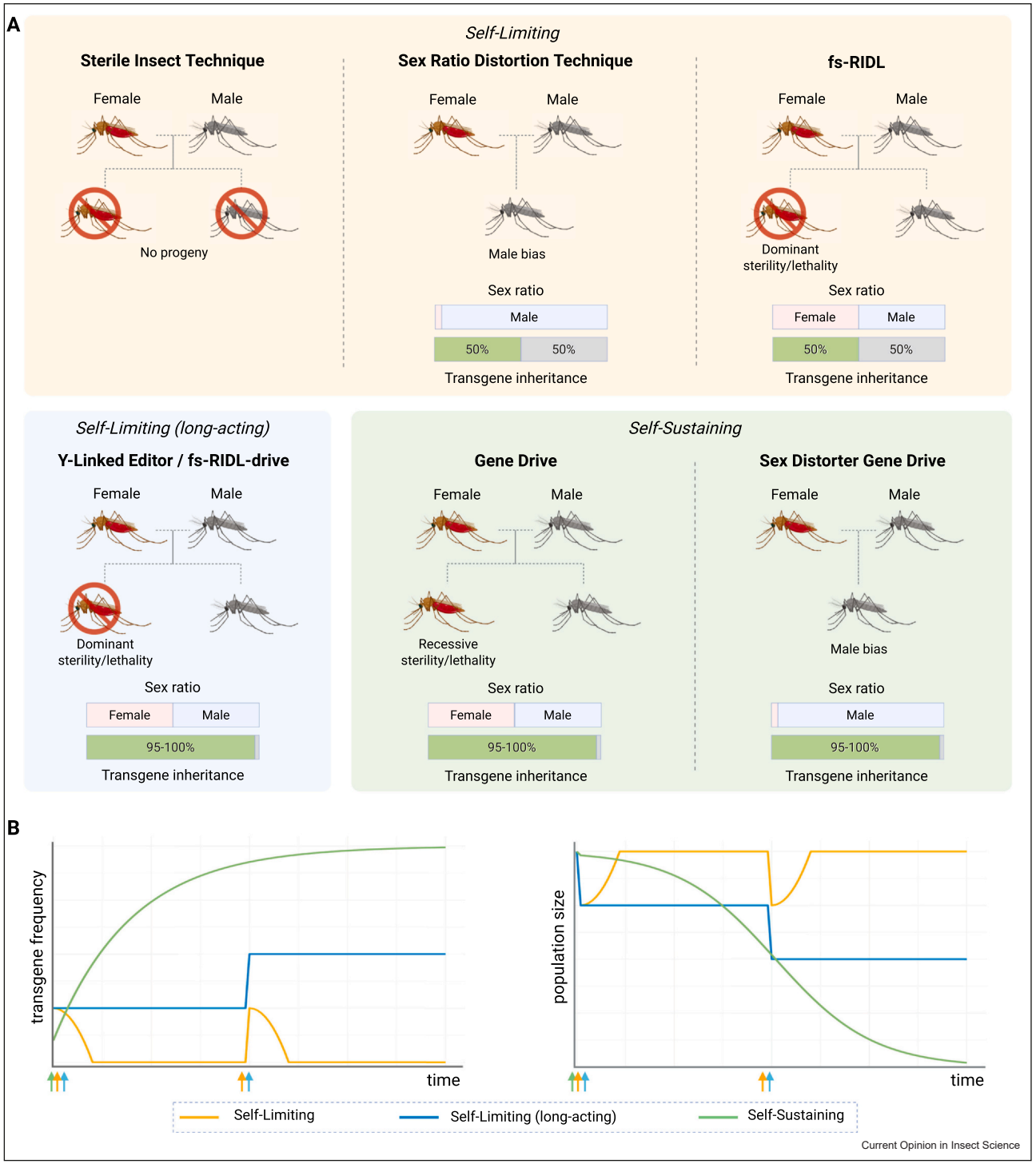
Windbichler et al. [19] engineered *An. gambiae* males to express I-PpoI endonuclease, which cleaves X chromosome-linked ribosomal repeats during spermatogenesis. This biases sperm toward Y-bearing and causes dominant embryo lethality due to the persistence of the I-PpoI in the embryo, where it cleaves the maternally inherited X chromosome (Figure 2). In laboratory settings, these transgenic males showed mating ability comparable to wild-type males, reducing the fitness costs of radiation-based sterilisation [19]. However, rearing this strain requires continuous crossing of transgenic females, in which the nuclease is not expressed, with wild-type males. Additionally, before release, both transgene screening and sex sorting would be necessary to ensure that only sterile transgenic males are deployed.

The Target Malaria consortium introgressed this genetic approach into *An. coluzzii* and, in 2019, conducted the first open-field release of GM mosquitoes in Africa. Although not aimed at entomological or epidemiological impact, the study provided crucial data on male survival and dispersal, proving the technical feasibility of GM mosquito deployment in natural settings [20]. The sterile *An. coluzzii* strain remains the only GM strain released within the *Anopheles gambiae* complex, marking a key step for using GM mosquitoes in malaria-endemic regions.

CRISPR-based approaches have expanded the versatility of genetic vector control by enabling the precise disruption of genes essential for mosquito fertility. For instance, a split system consisting of independent Cas9- and gRNA-expressing lines was designed to target the *zero population growth* (*zpg*) gene, essential for germ cell development. When these lines are crossed, the resulting progeny exhibit reduced fertility in females and complete sterility in males. Nonetheless, it would still be necessary to sort by sex before any potential release to ensure that only sterile males are deployed (Figure 2) [21].

Building on this strategy, Apte et al. [22••] developed precision-guided SIT (pgSIT) for *An. gambiae*. By crossing strains expressing Cas9 and gRNAs, the system simultaneously targets genes essential for male fertility (*zpg* and *β2-tubulin*) and female viability (*femaleless* [*fle*] and the female-specific isoform of *dsx* [*dsxF*]), resulting in progeny with near-complete male sterility and female lethality (Figure 2). Although this approach still requires the maintenance of two separate parental strains, the female lethality in the progeny produced by their cross eliminates the need for sex sorting before release. Laboratory trials showed pgSIT males exhibit high survival

Figure 1


A
Self-Limiting
Sterile Insect Technique
Female
Male
No progeny
Sex Ratio Distortion Technique
Female
Male
Male bias
Sex ratio
Male
50%
50%
Transgene inheritance
fs-RIDL
Female
Male
Dominant sterility/lethality
Sex ratio
Female
Male
50%
50%
Transgene inheritance
Self-Limiting (long-acting)
Y-Linked Editor / fs-RIDL-drive
Female
Male
Dominant sterility/lethality
Sex ratio
Female
Male
95-100%
Transgene inheritance
Self-Sustaining
Gene Drive
Female
Male
Recessive sterility/lethality
Sex ratio
Female
Male
95-100%
Transgene inheritance
Sex Distorter Gene Drive
Female
Male
Male bias
Sex ratio
Male
95-100%
Transgene inheritance
B
transgene frequency
time
population size
time
Self-Limiting
Self-Limiting (long-acting)
Self-Sustaining
Current Opinion in Insect Science

The figure illustrates the outcomes of self-limiting (yellow shading), self-limiting long-acting (blue shading), and self-sustaining (green shading) strategies developed for population suppression within the *Anopheles gambiae* complex. **(a)** In all cases, transgenic males are released to mate with wild females. Mosquitoes marked with a red circle indicate dominant lethality or sterility effects occurring in that generation. The expected sex ratio and transgene inheritance rates are provided for each technology as coloured bars at the bottom. **(b)** Idealised representation of transgene frequency (left-hand plot) and effect on population size (right-hand plot) after single or multiple releases (arrows) for the three classes of strategies: self-limiting transgenes are expected to decline over time after release and provide a transient suppression after each release event (yellow line); self-limiting long-acting transgenes persist in the population at release frequency and lead to limited yet sustained population suppression (blue line); additional releases (arrows) increase overall frequency and population suppression; self-sustaining low-threshold approaches progressively increase in frequency and provide long-lasting suppression, even after a single and smaller release (green line). The representation only shows general trends for the different vector control categories. Detailed dynamics are determined by specific transgene designs and vary between and within strategies.

and effectively suppress mosquito populations, supporting its potential for scalable SIT programs [22,23].

Sex ratio distortion technique

Compared to SIT, genetic control strategies are more effective when effector elements are heritable and persist in the population, reducing the need for frequent and dense releases.

An example is the sex distortion technique, which suppresses mosquito populations by biasing progeny toward males, the non-biting, non-disease-transmitting sex.

Galizi et al. [24] modified the I-PpoI nuclease, previously used by Windbichler et al. [19], by introducing a single amino acid substitution that reduced the enzyme's half-life. When expressed in male gametogenesis, this destabilised variant produced highly male-biased progeny without causing embryonic lethality (the maternally inherited X chromosome remained unaffected) (Figure 2). Notably, introgression experiments confirmed its applicability across species within the *Anopheles gambiae* complex [25,26] and replacing I-PpoI with CRISPR-Cas9 enabled more flexible genome targeting while preserving sex distortion and mating ability [27].

A recent study further showed that targeting either of two single-copy ribosomal protein genes on the X chromosome, rather than repetitive sequences, induces prezygotic sex distortion in *An. gambiae* (Figure 2), suggesting even single DNA breaks can enable Y chromosome drive (i.e. early elimination of X-bearing gametes). Additionally, the study provided evidence that early expression of autosomal sex ratio distorters, before meiotic sex chromosome inactivation, can strongly bias the male ratio [28•]. This supports the development of Y-linked sex distorters, powerful vector control strategies that bias progeny toward males, all inheriting the transgene due to its Y chromosome linkage, which has been difficult to engineer.

fs-RIDL and fs-RIDL-drive

The Release of Insects carrying a Dominant Lethal gene (RIDL) is a vector control strategy that employs mosquitoes expressing a dominant lethal gene, repressed during rearing before release. This approach can be refined to achieve female-specific lethality (fsRIDL), allowing males to survive and transmit the modification in a Mendelian fashion, enhancing suppression efficacy and sustainability under certain release scenarios [29,30].

Spinner et al. [31] engineered a tetracycline-repressible system in *Aedes aegypti*, where female-specific splicing drives overexpression of tTAV (tetracycline-controlled transactivator), causing female lethality by sequestering essential transcription factors. In 2024, this fsRIDL technology was applied in a pilot field release in Djibouti targeting the invasive *An. stephensi*, a species outside the *Anopheles gambiae* complex, serving as a proof of concept for larger control efforts [32].

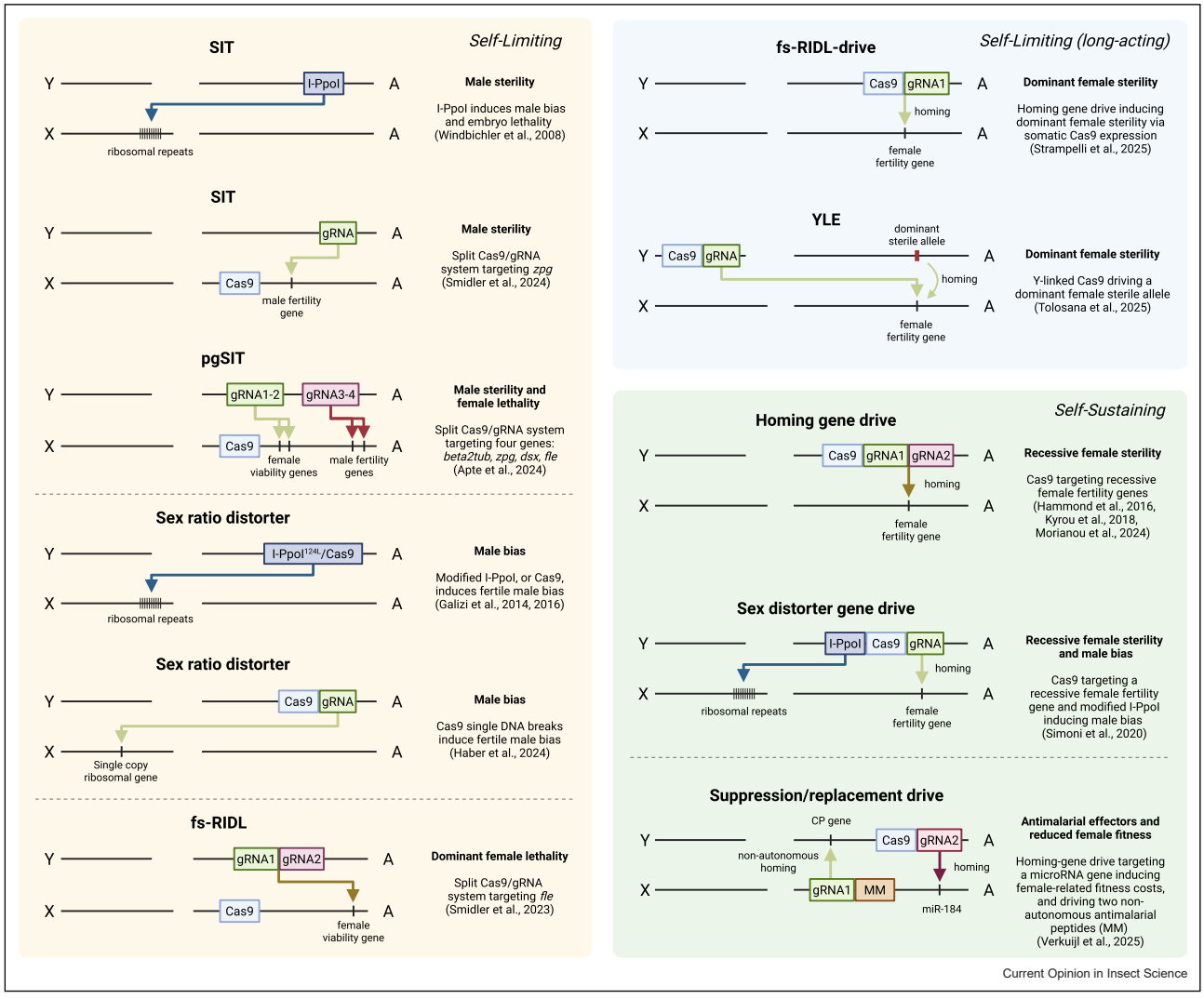
Smidler et al. [33] developed a split CRISPR-based fsRIDL system in *An. gambiae* targeting the *fle* gene (Figure 2). This system induces female-specific lethality, eliminating the need for prerelease sex sorting. Fertile males transmit the *fle* mutations and CRISPR components to their offspring, enabling prolonged, yet localised, population suppression [33].

fsRIDL's effectiveness can increase when coupled with CRISPR-based gene drive systems that promote super-Mendelian inheritance (fsRIDL-drive) [29,30], leading to a long-acting, yet self-limiting, effect. Strampelli et al. [34•] demonstrated this in *An. gambiae* by targeting *dsxF*. Their CRISPR-based system disrupted *dsxF*, biased its inheritance, and induced complete dominant sterility and inability to bite in female progeny due to somatic and early germline Cas9 expression (Figure 2), highlighting its strong potential for mosquito population suppression under laboratory cage conditions [34].

Y-linked editor

In *Anopheles* mosquitoes, sex is determined by the inheritance of the Y chromosome. Genetic constructs inducing female-specific lethality or sterility are ideal for Y chromosome linkage because the Y is absent in females, so no negative selection acts against the construct [29]. Consequently, a Y-linked construct could persist indefinitely at the released frequency without male-associated fitness costs. Unlike population suppression strategies such as SIT or fsRIDL, where populations rapidly rebound after releases

Figure 2



Overview of genetic control strategies and associated genetic constructs for population suppression in *An. gambiae*. Self-limiting systems (yellow panel) include the sterile insect technique (SIT) approaches, which employ the endonuclease I-PpoI targeting ribosomal repeats on the X chromosome to induce dominant male sterility [19] or a split Cas9/gRNA system disrupting male fertility genes such as *zpg* [21]. Precision-guided SIT (pgSIT) uses split Cas9/gRNA to target essential female viability genes (*dsxF*, *fle*) and male fertility genes (*β2-tubulin*, *zpg*) [22]. Sex ratio distorters leverage modified I-PpoI or Cas9-based systems to bias progeny sex ratios toward males through X-chromosome shredding [24,27] or cleavage of a single X-linked locus [28]. Female-specific RIDL (fs-RIDL) employs split Cas9/gRNA targeting female fertility genes such as *fle* [33]. Autosomal targets are shown on only one chromosome of each pair for clarity. Self-limiting long-acting systems (blue panel) include the fs-RIDL-drive, a homing gene drive targeting *dsxF* that induces dominant female sterility via somatic and early germline Cas9 expression [34], and the Y-linked editor (YLE), where Y-linked Cas9 drives a dominant female-sterile allele at the *dsxF* locus [35]. Self-sustaining systems (green panel) comprise homing gene drives with Cas9/gRNA targeting single or multiple sites in recessive female fertility genes such as *dsxF* [36,39,43], sex distorter gene drives (SDGD) combining I-PpoI-mediated sex ratio distortion with Cas9 targeting female fertility genes [48], and suppression/replacement drives targeting the microRNA gene miR-184, conferring female fitness costs and encoding antipathogen peptides (magainin 2 and melittin) inserted as a non-autonomous drive into the *carboxypeptidase A1* (*CP*) gene [53]. Sex chromosomes (X and Y) and autosomes (A) are indicated. Cas9, gRNAs, and homing elements are colour-coded by their target specificity.

cease, Y-linked editor (YLE) constructs maintain sustained suppression over time. Notably, the efficacy of YLEs depends critically on the dominant penetrance of the induced mutations, editing efficiency, and the generation of recessive mutations, which can significantly diminish their suppressive impact [29].

This strategy was developed in *An. gambiae* by Tolosana et al. [35••] who integrated a CRISPR-based construct into the Y chromosome to drive a dominant female-sterilising allele at the autosomal *dsxF* locus (Figure 2). Like fs-RIDL-drives [34], cage experiments validated model predictions, achieving sustained population

suppression. Modelling based on empirical data indicated that YLEs and fs-RIDL-drives could achieve local population suppression with release rates 2- to 14-fold lower than those required for optimal SIT, RIDL, or fs-RIDL strategies. Importantly, both YLEs and fs-RIDL-drives are predicted to remain geographically confined, offering promising long-acting tools for spatially restricted vector control [29,34,35].

Rearing and deployment of the described YLE and fs-RIDL systems require continuous crossing of transgenic males with wild-type females and sex sorting before release. However, the Y-linked transgene in the YLE facilitates automated male sorting through flow cytometry, which should significantly enhance operational efficiency.

Low-threshold self-sustaining strategies for population suppression

Gene drive strategies for population suppression

CRISPR-based self-sustaining gene drive technologies represent a promising approach for the long-term suppression of *Anopheles* mosquito populations. By biasing the inheritance of genetic traits, such as disrupting recessive genes essential for female fertility or viability, these drives are designed to reduce population sizes across generations progressively.

For a gene drive to be effective at low release thresholds, it must impose minimal fitness costs on heterozygotes, target recessive genes, and include mechanisms to mitigate resistance at the target site. Early gene drive designs faced significant challenges, including high fitness costs in heterozygous females [36] and rapid development of resistance alleles [37,38]. However, drives targeting the constrained *dsxF* gene (Figure 2) have shown exceptional efficacy, leading to complete population collapse in both small- and large-cage trials [39,40].

Hancock et al. [41••] modelled releasing this drive in multiple *Anopheles* species (*An. gambiae*, *An. coluzzii*, *An. arabiensis*, and *An. funestus*) across ecologically heterogeneous regions of West Africa. The study suggested vector population reductions ranging from 71.6% to 98.4%, depending on local ecological and species-specific parameters. When integrated with existing interventions, including insecticide-treated nets and vaccination, gene drive deployment was predicted to avert from 60 to 300% more clinical malaria cases compared to conventional interventions alone, highlighting synergistic effects of combining gene drive systems with current vector control strategies [41].

Despite promising results, the emergence of resistant alleles, arising from natural genetic variation or CRISPR-induced mutations, poses a significant challenge to sustained gene drive effectiveness [42,43••]. To mitigate this, multiplexed gRNAs that target multiple sites within genes of interest have been used, significantly reducing resistance likelihood [43–47]. However, fully preventing resistance in the field may be impossible. Like other vector controls, gene drives may need periodic updates or redesigns to maintain efficacy, especially if complete population elimination is not achieved.

To support this effort, high-throughput mutagenesis assays can be used to identify and predict resistance mutations at target loci. This approach not only informs the refinement of gene drive designs but also underscores the importance of thorough prerelease assessments to evaluate resistance risks and guide the development of more robust and durable gene drive systems [43].

Notably, previous work combined a single-gRNA *dsxF* gene drive with the X-chromosome shredding endonuclease I-PpoI to develop a sex distorter gene drive system that biases offspring sex ratio toward males while achieving super-Mendelian inheritance (Figure 2) [48]. Mathematical models suggest that this combined approach could suppress *An. gambiae* populations more rapidly than gene drives targeting female fertility alone, as also shown in laboratory cage trials [48]. However, population dynamics are highly influenced by the fertility of released males and 'escapee' females, potentially leading to prolonged chasing dynamics [48,49].

Gene drive strategies for combined population suppression and modification

While population suppression methods aim at reducing the number of disease vectors, population modification (or replacement) strategies aim to reduce the vector populations' ability to transmit pathogens by introducing antiparasitic effector genes that block pathogen development. Although several systems for population modification have been developed in *An. gambiae*, they are beyond the scope of this review and will not be discussed [50–52].

A recent study described a gene drive in *An. gambiae* that combines suppression and modification strategies by targeting the conserved microRNA mir-184, highly expressed in the midgut, an essential tissue for *Plasmodium* development [53••]. While disruption of mir-184 did not directly affect female fertility, it reduced mosquito lifespan, impaired flight, and increased susceptibility to blood-feeding stress, collectively decreasing population fitness. Laboratory cage trials demonstrated rapid drive fixation and co-propagation of a non-autonomous antimalarial effector gene, achieving both transmission blocking and partial suppression (Figure 2). This study presents a novel framework for integrated gene drive

approaches and highlights the potential of targeting conserved noncoding RNAs.

However, combining suppression-modification drives may pose challenges due to conflicting selective pressures, particularly when high fitness costs or effector expression is suboptimal [53].

Conclusions and future directions

Recent advances in CRISPR technologies, including sex distorters, dominant genetic editors, and low-threshold gene drives, have expanded the genetic toolkit potentially available, upon regulatory approval, for open-field trials. These innovations offer transformative potential for vector-borne disease management [41]; however, challenges remain to ensure sustained effectiveness.

A central concern is the evolution of resistance. Multiplexed gene drives aim to mitigate resistance by targeting several genomic sequences simultaneously. Nevertheless, empirical data indicate that one or more gRNAs can be lost within these constructs, functionally reverting them to single-gRNA systems [43,51]. This reversion increases the likelihood of resistance allele formation at the target site, diminishing efficiency and long-term success. Elucidating the molecular mechanisms underlying transgene instability and developing strategies to mitigate it remain critical research priorities.

In addition, expanding the molecular diversity of CRISPR-based systems is essential to enhance their flexibility and robustness. Most current platforms developed for *Anopheles* mosquitoes use Cas9 nucleases, which may constrain optimisation across target sites and mosquito species. Consequently, the exploration of alternative effectors is advised. Recent applications of Cas12 in *Drosophila* [54] provide a promising foundation for extending the use of alternative Cas nucleases in mosquito genome engineering [55]. Similarly, emerging base editing technologies, capable of introducing precise nucleotide substitutions without inducing double-strand breaks, have demonstrated specificity and efficacy in flies, with potential for equivalent efficiency in mosquitoes [56–58].

Innovative designs might also involve CRISPR-based antidote-rescue systems predicted to achieve rapid, geographically restricted suppression of *Anopheles* populations, providing a favourable balance between impact and containment [59]. Other theoretical approaches to limiting spread could involve exploiting natural genetic diversity to restrict CRISPR activity to private alleles, preventing spread outside genetic islands [60].

Beyond molecular design, potential field deployment of GM mosquitoes requires careful consideration. Genetic elements from different modified strains could interact through hybridisation in overlapping release areas or after dispersal. Whether intentional or not, such interactions should be investigated to understand genetic constructs' compatibility, stability, and long-term dynamics within the same vector population.

Responsible development and deployment of genetic technologies require integrated research, regulatory oversight, and proactive community engagement.

Data Availability

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

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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