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Discoveries

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A Comparative Assessment of Self-limiting Genetic Control Strategies for Population Suppression

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Abstract

Genetic control strategies are promising solutions for control of pest populations and invasive species. Methods utilizing repeated releases of males such as sterile insect technique (SIT), release of insects carrying a dominant lethal (RIDL), self-limiting gene drives, and gene disruptors are highly controllable methods, ensuring biosafety. Although models of these strategies have been built, detailed comparisons are lacking, particularly for some of the newer strategies. Here, we conducted a thorough comparative assessment of self-limiting genetic control strategies by individual-based simulation models. Specifically, we find that repeated releases greatly enhance suppression power of weak and self-limiting gene drives, enabling population elimination with even low efficiency and high fitness costs. Moreover, dominant female sterility further strengthens self-limiting systems that can either use gene drive or disruptors that target genes without a mechanism to bias their own inheritance. Some of these strategies are highly persistent, resulting in relatively low release ratios even when released males suffer high fitness costs. To quantitatively evaluate different strategies independent from ecological impact, we proposed constant-population genetic load, which achieves over 95% accuracy in predicting simulation outcomes for most strategies, though it is not as precise in a few frequency-dependent systems. Our results suggest that many new self-limiting strategies are safe, flexible, and more cost-effective than traditional SIT and RIDL, and thus have great potential for population suppression of insects and other pests.

Keywords: genetic pest control, self-limiting gene drive, SIT, RIDL, modeling, genetic load

Introduction

Insect pests such as fruit flies and mosquitoes post significant threats to agriculture and human health (Lounibos 2002 Papadopoulos et al. 2024). Traditional pesticides can lead to environmental contamination (Tang et al. 2021), nontarget species impact (Silva et al. 2023), and challenges such as rapidly developing resistance (Ma et al. 2021). In recent years, alternative pest control strategies have shown great potential. Sterile insect technique (SIT) successfully reduces the populations of targeted species by releasing sterile males, which mate with females and produce no viable offspring (Galvin and Wyss 1996; Vreysen et al. 2000; Koyama et al. 2004; Enkerlin et al. 2015; Gato et al. 2021). Another strategy involves the use of *Wolbachia*, a symbiotic bacterium that causes cytoplasmic incompatibility (LePage et al. 2017), which has seen promising effects in controlling pest population and reducing diseases in multiple regions (Hoffmann et al. 2011; Zheng et al. 2019; Pinto et al. 2021; Lim et al. 2024). These methods avoid side effects of chemical pesticides, but also require substantial release sizes to suppress the population, resulting in considerable resource demands.

Genetic engineering approaches such as release of insects carrying a dominant lethal (RIDL) and female-specific RIDL (fsRIDL) have also been developed. These systems contain a dominant, conditional lethal gene element, which can be repressed in the lab environment. When modified males are released into wild populations, this element will cause dominant lethality in their offspring (just female offspring for fsRIDL) (Phuc et al. 2007). RIDL has been developed in *Drosophila melanogaster* (Thomas et al. 2000) and proved its feasibility in field tests of *Aedes aegypti* populations (Harris et al. 2011, 2012; Carvalho et al. 2015). fsRIDL allows easy sex sorting and has been built in *Drosophila suzukii* (Li et al. 2021a), *Ceratitis capitata* (Fu et al. 2007; Leftwich et al. 2014), *Aedes albopictus* (Labbe et al. 2012), and seen success in *Ae. aegypti* field tests (Spinner et al. 2022). RIDL, fsRIDL, and genetic SIT strategies (Kandul et al. 2019; Maselko et al. 2020; Li et al. 2021b; Upadhyay et al. 2022) avoid harm caused by radiation or chemosterilization commonly seen in older SIT methods, increasing the chance of successful mating for released males. However, massive release sizes are still required to eliminate local populations.

Gene drive systems represent a more recent innovation in genetic pest control (McFarlane et al. 2018). They are selfish genetic elements that bias Mendelian inheritance to increase their frequency in the population, including suppression drives, which reduce or eliminate the population, and modification drives, which prevent transmission of diseases (Wang et al. 2022). Various types of gene drives have been built in *D. melanogaster* (Chen et al. 2007; Champer et al. 2017; Carrami et al. 2018; Buchman et al. 2018b; Guichard et al. 2019; Champer et al. 2020b; Kaduskar et al. 2022; Auradkar et al. 2024; Lawler et al. 2024), *D. suzukii* (Buchman et al. 2018a; Yadav et al. 2023), *Anopheles gambiae* (Hammond et al. 2016; Kyrou et al. 2018;

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Carballar-Lejarazú et al. 2020, 2023; Simoni et al. 2020; Ellis et al. 2022; Hoermann et al. 2022; Green et al. 2023), *Anopheles stephensi* (Gantz et al. 2015; Adolfi et al. 2020; Xu et al. 2025), *Ae. aegypti* (Li et al. 2020; Anderson et al. 2024), and *Plutella xylostella* (Xu et al. 2022). Super-Mendelian inheritance was also achieved in mammals (Grunwald et al. 2019) and plants (Liu et al. 2024; Oberhofer et al. 2024) (see also recent review articles for additional examples; Hay et al. 2021; Bier 2022; Verkuijl et al. 2022; Wang et al. 2022, 2025).

Powerful, self-sustaining suppression drives can spread through a population rapidly with even a small release size. Their frequency increases until reaching a stable equilibrium or completely suppressing the population. However, they also raise ecological and ethical concerns due to their uncontrolled spread (Collins 2018; Long et al. 2020; Annas et al. 2021). Additionally, achieving high drive efficiency in some systems remains challenging (Oberhofer et al. 2018; Reid et al. 2022; Yang et al. 2022; Anderson et al. 2023). Confined suppression drives are more controllable and require an introduction frequency above a certain threshold to increase their frequency and avoid elimination (Dhole et al. 2019; Champer et al. 2020a; Champer et al. 2021a; Metzloff et al. 2022; Chen et al. 2023; Zhu and Champer 2023). However, they can still persist in the environment if population elimination is not achieved (in cases where they lack suppressive power). In contrast, self-limiting drives, even in their ideal forms in some cases, will decrease in frequency (perhaps after an initial increase) and be eliminated from the population over time (Gould et al. 2008; Edgington and Alphey 2018; Champer et al. 2019; Noble et al. 2019; Webster et al. 2020; Oberhofer et al. 2021). These drives are thus temporary if releases are stopped, but a single release often fails to provide adequate power for successful suppression.

Gene disruptors are genetic systems that target specific sites and disrupt gene functions. Unlike gene drives, a gene disruptor does not increase its frequency in a population (with a few exceptions such as Y-linked X-shredders that can also be classified as gene drives). Instead, they rely on large or repeated releases to affect the population. These gene disruptors are naturally self-limiting and confined and thus less likely to provoke public opposition. Suppression gene disruptors with different targets have been modeled and built in *D. melanogaster* and *A. gambiae* (Galizi et al. 2014, 2016; Burt and Deredec 2018; Malik et al. 2020; Geci et al. 2022; Haber et al. 2024; Johnson et al. 2024; Tolosana et al. 2025). Although gene disruptors never replicate themselves, they may act for multiple generations before being lost from the population. Therefore, gene disruptor strategies can be substantially more efficient than directly releasing individuals with disrupted target alleles.

Aside from the type of genetic control system, ecological context can also have a profound impact on the efficiency of suppression. For example, overcompensation may happen when some larvae die at an early stage and leave resources to other offspring, leading to even more adults in the next generation due to reduced competition (Evans et al. 2022). Such resilience varies in different species and regions, influencing several pest control programs (Bouyer 2023). It is therefore important to learn how different strategies respond to varying density dependence.

The suppressive power of a system is usually best measured by genetic load, which refers to the fitness reduction of the whole population compared to a similarly sized wild-type population. Reduced fitness can be caused by mutations or introduction of deleterious genes (Bertorelle et al. 2022). It is often defined as the loss of reproductivity or growth rate of the population (Deredec et al. 2008; Beaghton et al. 2019; Dhole et al. 2020), which may result from disruption of fertility or viability genes, or a bias in the sex ratio. Here, we use a specific definition of genetic load in gene drive that specifically refers to total effects of transgenic constructs on whole-population reproductivity, which is the fractional reduction in viable offspring compared with a wild-type population of the same size. Though this term has reached common usage in gene drive, it somewhat differs from the definition of genetic load in classical population genetics, which refers to fitness reductions caused by all suboptimal mutations in a genome.

For self-sustaining gene drives, genetic load for a drive is usually recorded when it reaches its equilibrium frequency. It serves as a good measure of the suppressive power of a drive because it is not affected by species-specific and ecological parameters such as density-dependent growth rates. Together with the low-density growth rate, it is an accurate predictor of population elimination outcomes in most panmictic models (Deredec et al. 2008, 2011; Alphey and Bonsall 2014). However, for systems that involve repeated releases, the dynamics of genetic load are more complex due to increasing relative release ratio as the wild-type population declines. Equilibrium could be reached at a population size that varies based on ecological parameters, which can change the genetic load, even for the same drive and release size.

While a substantial body of previous work has focused on assessing self-limiting genetic control strategies with simulations or mathematical models (Phuc et al. 2007; Gentile et al. 2015; Natiello and Solari 2020; Geci et al. 2022; Connolly et al. 2024; Haber et al. 2024; Johnson et al. 2024; Zhu et al. 2024; Tolosana et al. 2025; Willis and Burt 2025), most of them modeled ideal systems or had specific ecologies, which creates difficulties when comparing different strategies. In this study, we compared various types of genetic control strategies for population suppression involving repeated releases, focusing mostly on self-limiting strategies and also including some new designs. We also systematically explored the impact of ecological parameters on some of these systems. Finally, we introduce "constant-population genetic load" to evaluate the power of different systems, potentially predicting suppression results and allowing for easier comparisons of different systems and release strategies independent of ecology.

Materials and Methods

Genetic Control Strategies

Our genetic control strategies fall into 3 classes: modified alleles, gene drives, and gene disruptors (Fig. 1).

Modified Allele Strategies

We assess several types of genetic population suppression strategies. For SIT, sterile males (irradiated (Galvin and Wyss 1996; Vreysen et al. 2000; Koyama et al. 2004; Enkerlin et al. 2015; Gato et al. 2021) or genetic engineered (Kandul et al. 2019; Maselko et al. 2020; Li et al. 2021b; Upadhyay et al. 2022)) are released into the population. When females mate with these males, no viable offspring will be generated.

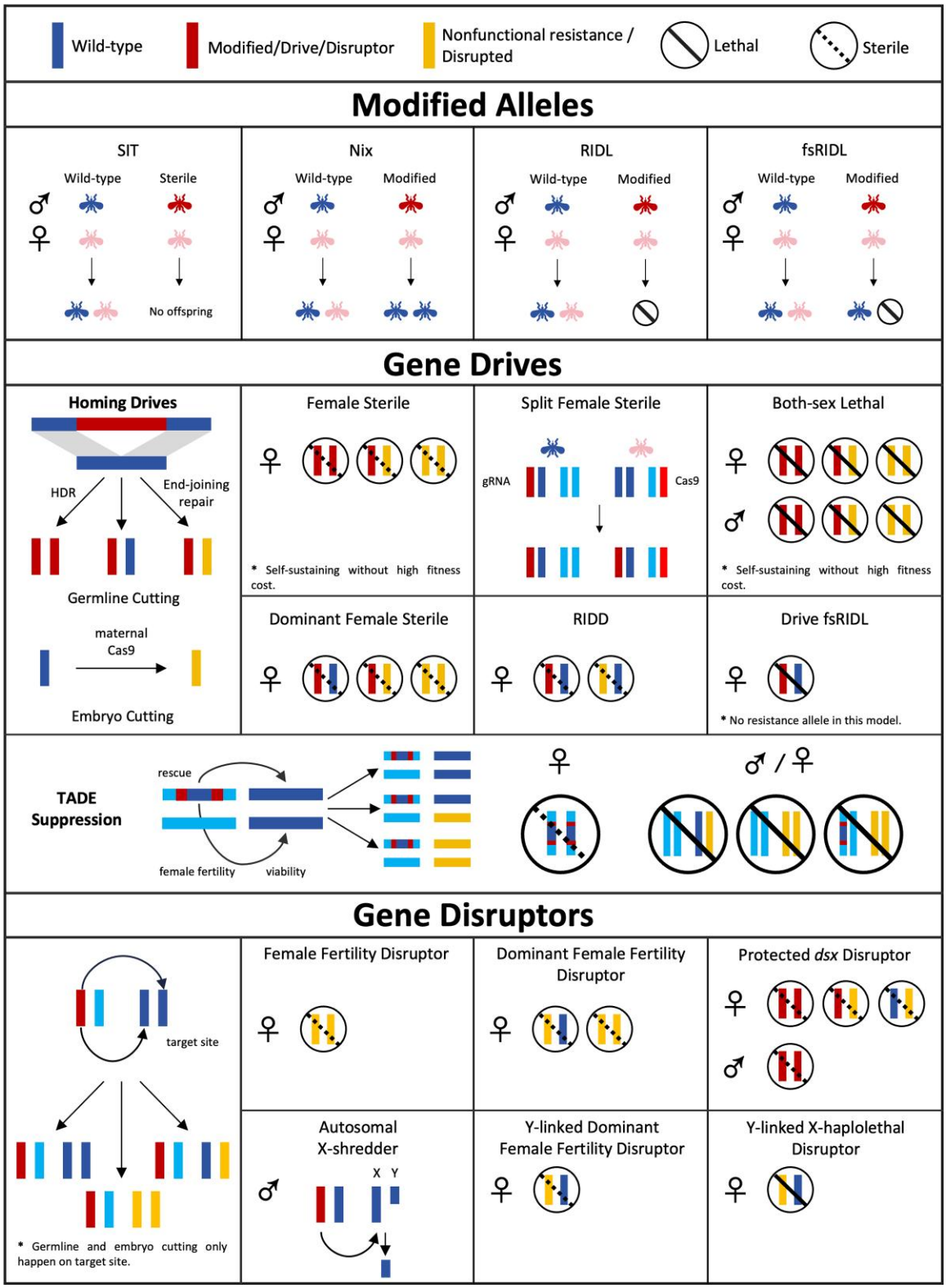

Fig. 1. Overview of systems and mechanisms. Each system's mechanism for population suppression. RIDL can be early or late acting, affecting larval competition. For gene drive heterozygotes, Cas9 causes cleavage at target sites in germline cells, leading to drive conversion or nonfunctional resistance allele (also called disrupted allele) formation (drive conversion can only happen in homing drives). Maternal deposition of Cas9 and gRNA may also form nonfunctional resistance alleles in the early embryo. Genetic control systems may result in sterile, nonviable, or sex-biased offspring, depending on their mechanisms.

RIDL elements contain a dominant lethal gene that can be suppressed in lab environment (e.g. with food additives such as tetracycline). Male late-RIDL homozygotes are released into the population, and all of their offspring (or just female offspring for late-fsRIDL) will die before reaching adulthood but still participate in larval competition (Fu et al. 2007; Phuc et al. 2007; Li et al. 2021a; Spinner et al. 2022). For early RIDL and early fsRIDL (Thomas et al. 2000), offspring are

nonviable before the larval stage and do not contribute to the competition. Therefore, SIT and early RIDL have the same performance in our simulations since released males will not have any viable offspring in both situations. In real-world conditions, SIT could potentially have worse performance, but this would depend on the type of sterilization and mating system.

Nix is a male-determination factor in *Aedes* mosquitoes, and stable expression of *Nix* will convert females into fertile males (Hall et al. 2015; Aryan et al. 2020). Wild-type males have 1 copy of *Nix*. Released males have 2 *Nix* copies on another pair of autosomes, but only half of them have a natural *Nix* (we also considered releases where all males have a natural copy of *Nix*). This line needs to be maintained by using repressible *nix*, similar to RIDL systems. Offspring with at least 1 copy of *Nix* will become fertile males. Note that *Ae. aegypti* males converted from females through expression of *Nix* alone are flightless, because they lack expression of the *myo-sex* gene responsible for male flight, which is critical for mating in the wild (Aryan et al. 2020). It may be possible to co-express of *Nix* and *myo-sex* to restore flight in males. However, in *Ae. Albopictus*, *Nix* masculinizes females while keeping their full flight capability (Lutrat et al. 2022). Thus, we model *Nix* males as fully fertile and competitive. The system works to suppress pest populations by biasing the sex ratio.

Gene Drive Strategies

CRISPR-based drives represent a well-studied and promising class of gene drives. Cas9 and gRNA elements are usually combined with a germline-specific promoter, and ideally, Cas9 only cuts the target site in germline cells. However, somatic expression has been a challenge for suppression drives because they usually target a necessary gene related to fertility or viability without rescue. Disruption of these genes in somatic cells leads to fitness costs (30% for drive heterozygotes by default), which can affect females or both sexes, depending on the type of gene drive (Hammond et al. 2017; Edwards et al. 2021).

Here, we modeled mainly 3 categories of CRISPR drives: (i) self-sustaining homing suppression drives, (ii) self-limiting homing suppression drives, and (iii) confined toxin-antidote dominant embryo (TADE) suppression drive.

For CRISPR homing drives, homology-directed repair after germline cleavage converts the wild-type allele into a drive allele in germline cells of drive heterozygotes at a rate equal to the drive conversion efficiency, forming a super-Mendelian inheritance pattern. In some cases, end-joining repair takes place and forms resistance alleles, which prevent future drive conversion due to sequence changes. Functional resistance alleles will easily outcompete drive alleles but can be avoided by use of multiple gRNAs and conserved target sites (Champer et al. 2018; Kyrou et al. 2018; Oberhofer et al. 2018; Champer et al. 2020c; Yang et al. 2022). In our simulations, only nonfunctional resistance alleles were included. The total germline cut rate was set to 1 by default, which means all wild-type alleles in germline cells of drive heterozygotes are converted into drive or nonfunctional resistance alleles. If the mother is a drive carrier, half of wild-type alleles in the embryos (which, in this case, are all passed from the father) would also be converted into nonfunctional resistance alleles due to maternal deposition of Cas9 and gRNA (Gantz et al. 2015; Hammond et al. 2017; Champer et al. 2018; Edwards et al. 2021).

The first self-sustaining drive we considered is the female sterile homing drive (Burt 2003; Kyrou et al. 2018). It targets a haplosufficient female fertility gene, so females without any wild-type alleles will be sterile. A well-studied target for such a drive is the *doublesex* (*dsx*) gene, which regulates sex differentiation in insects. Males and females exhibit distinct splicing mechanisms for *dsx* transcripts (Burtis and Baker 1989), and targeting the female-specific exon can block functional protein formation and cause recessive female sterility. Another self-sustaining drive is the both-sex lethal drive (Burt 2003; Deredec et al. 2011; Oberhofer et al. 2018; Malik et al. 2021; Champer et al. 2021b), which targets a haplosufficient viability gene. Offspring without wild-type alleles are nonviable and do not contribute to larval competition in our model, but late-acting lethality is also possible (Bender et al. 1997; Jia et al. 2024).

Female sterile homing drive can be converted to a self-limiting drive by separating gRNA and Cas9 elements onto different chromosomes. The gRNAs sit in the female fertility gene and causes recessive female sterility, while the Cas9 is at a different genomic location (the drive will function similarly if these elements are reversed; Champer et al. 2019). Drive conversion only occurs when both gRNA and Cas9 are present in the same cell, and this will only increase the frequency of the gRNAs in the female fertility gene. The Cas9, however, is not able to increase its frequency and will be lost when in sterile females, which makes this drive self-limiting. Another example of self-limiting drives is the dominant female sterile homing drive (Hammond et al. 2016; Yadav et al. 2023). It also targets a female fertility gene, but also has somatic expression that disrupts wild-type alleles, thus causing dominant female sterility in individuals that are initially drive/wild-type heterozygotes. More recently, RIDD (release of insects carrying a dominant-sterile drive) was developed (Chen et al. 2024), in which both drive and nonfunctional resistance alleles cause dominant sterility in females by using multiple gRNAs targeting to the female exon of *dsx*. The last kind of self-limiting drive we explored is drive late-fsRIDL (Burt and Deredec 2018; Zhu et al. 2024; Willis and Burt 2025). It is similar to late-fsRIDL, but linked with a CRISPR/Cas9 element, allowing it to spread rapidly while still being self-limiting due to dominant female lethality.

All drives described above are homing drives and rely on homology-directed repair to increase their frequencies. TADE suppression drive, however, has a distinct mechanism. The drive element sits in a female fertility gene and causes recessive sterility. Meanwhile, the gRNA targets a distant haplolethal gene, which results in dominant lethal disrupted alleles, which can be rescued by a recoded copy within the drive. If an offspring bears more disrupted haplolethal alleles than drive alleles, it becomes nonviable, and females with 2 drive alleles are sterile. TADE increases its frequency by eliminating wild-type alleles and does not have an introduction threshold in its ideal form. However, a threshold appears if there are any fitness costs or disrupted allele formation in the early embryo due to maternal deposition. Therefore, it is confined and will not invade neighbor populations unless migration is above a critical frequency.

Gene Disruptor Strategies

Gene disruptors are less powerful than gene drive systems because they cannot bias their inheritance, so homozygous release are often required to achieve suppression. These systems are alleles with CRISPR/Cas9 or other nucleases, which are inherited in a Mendelian pattern themselves, that

target and disrupt specific genes required for fertility or viability (such disrupted alleles are equivalent to nonfunctional resistance alleles in homing drives). No rescue is carried by the disruptor. To maintain a stable homozygous line, conditional Cas9 expression is needed. Small molecules, heat shock and optical strategies have been reported to generate conditionally controlled Cas9 cutting (Zhou and Deiters 2016). These allow consideration of homozygous releases because homozygotes can be generated prior to any Cas9 cleavage, which would only take place in released organisms.

We started with gene disruptor strategies similar to previously described drives, including both recessive and dominant female fertility disruptors. Next, we analyzed a disruptor from a recently proposed "protected dominant negative editor" system (Willis and Burt 2025), where the authors designed an editor sitting in a recessive viability locus and targeting a haplolethal site, with a recoded copy of the haplolethal gene to protect itself. This study also proposed several variants. We considered a practical variant, the protected *dsx* disruptor, which sits in an early both-sex exon of *dsx* gene and targets its female-specific exon as in the RIDD system above. The disruptor itself is recessive both-sex sterile, and the disrupted target site is dominant female sterile. These disruptors protect themselves from disrupted target site in females because they stop *dsx* expression in the early exon and thus avoid dominant sterility.

Another disruptor we considered is the autosomal X-shredder. In this case, any repeated sequence can be targeted even if not within a gene, and the goal for males carrying this shredder to have fewer or no female offspring. The sex ratio of the population is thus biased toward males and facilitates suppression. X-shredding systems have been developed in *A. gambiae* (Galizi et al. 2014, 2016), *D. melanogaster* (Malik et al. 2020), and *C. capitata* (Meccariello et al. 2021).

Transformer (*tra*) gene and its paralogs are key switches of sex determination in multiple insects. Knockdown of *tra* causes masculinization and converts XX individuals into intersex individuals or fertile males in *Lucilia cuprina* (Williamson et al. 2021), *Bactrocera dorsalis* (Liu et al. 2015), *C. capitata* (Pane et al. 2002), and *Musca domestica* (Hediger et al. 2010). Homing drives targeting *tra* has also been developed in *C. capitata* (Meccariello et al. 2024). We modeled a population suppression system targeting *tra*, where individuals with no functional *tra* copy became fertile males.

Finally, we modeled 2 Y-linked disruptors that only disrupt target genes in the male germline. One is the Y-linked dominant female fertility disruptor (Tolosana et al. 2025), forming dominant female sterile alleles, and the other targets a haplolethal gene on the X chromosome, which leads to dominant lethality in female offspring (males will never receive the dominant lethal allele). Similar systems targeting haplolethal genes on the X chromosome have been developed in *D. melanogaster* (Lawler et al. 2024) and *A. gambiae* (Haber et al. 2024).

Fitness Costs

Fitness costs refer to usually unintended reproductivity loss caused by the suppression systems. When targeting essential genes, we assume that heterozygotes have a lower fitness (f and m for females and males respectively, 0.7 by default for one or both sexes in all models unless otherwise indicated, see below—this value was chosen to represent highly imperfect gene drives that are still far from dominant-female sterile systems) due to somatic expression and disruption of target genes. Effects of maternal carryover are modeled separately by embryo cutting. Fitness costs may apply to both sexes or just females, depending on the target gene. Fitness costs lead to less mating success for males and fewer offspring for females, respectively. Specifically, when fitness of heterozygotes is set to 0, they become infertile due to severe somatic expression and cutting. Total sterility of heterozygotes has been reported in systems originally designed to cause recessive female sterility (Hammond et al. 2016; Du et al. 2024).

For female fertility drives, female drive heterozygotes have a fitness value equal to f. For both-sex viability drive, drive heterozygotes of both sexes are influenced. For TADE suppression drive, if a drive carrier has the same number of drive alleles and disrupted alleles, its fitness equals f or m. If it has more drive alleles, it endures a lower fitness of the square of f or m. If it has more functional gene copies, it has a fitness of the square root of f or m. Similar equations are used for fitness costs in gene disruptors.

No fitness cost was imposed on SIT, late-RIDL, late-fsRIDL, and drive late-fsRIDL to form a better base line. *Nix*, autosomal X-shredder, *Tra* disruptor, and Y-linked dominant female fertility disruptors had no fitness cost in our models due to their special mechanisms. All these are likely to have small fitness costs realistically, but in our default parameters, we only model the moderate fitness costs from somatic nuclease expression and other aspects of essential gene targeting with high expression that are particularly difficult to avoid.

Panmictic Model

We used SLiM software (version 4.2.2) (Haller and Messer 2023) to develop individual-based panmictic models with overlapping generations that approximates the lifecycle of relatively short-lived insects that undergo larval competition (See default parameters in supplementary table S1, Supplementary Material online). Each time-step in this model corresponds to 1 week (essentially 1 reproductive cycle). All code is available on GitHub (https://github.com/Hanyue22/Assessment-of-Self-limiting-Systems).

We start the simulation with a wild-type population with $K = 100{,}000$ by default. Newly hatched larvae (age = 0) undergo a 1-week larval stage before reaching adulthood (age ≥ 1). The age structure of adults is linearly decreasing from hatching to maximum age. The proportion of adults of age i is thus $P_i = \frac{\omega - i + 1}{\sum_{j=1}^{\omega} j}$, where i is the age of individual and ω is the maximum lifespan, which is 6 week in our models. Therefore, the age distribution from age 1 to 6 is usually 6/21, 5/21, 4/21, 3/21, 2/21, and 1/21. The survival rate of adults (age > 0) is age related: $S_{\text{Adult}} = \frac{\omega - i}{\omega - i + 1}$ (5/6, 4/5, 3/4, 2/3, 1/2, and 0 from age 1 to 6).

After equilibrating for 10 week, we release $n = \frac{K \cdot r}{2 \cdot \rho}$ transgenic age 1 males (heterozygotes for drives, homozygotes for modified alleles and disruptors except where noted) into the population each week. Here, ρ is the average lifespan for individuals surviving the larval stages (individual that reach reproductive age), which is the average generation time ($\rho = \sum_{i=1}^{\omega} i \cdot P_i = 2.67$). r is the release ratio of transgenic males to wild-type males in the original population per generation (fewer males are released each week/time step because the generation time is 2.67 week). We also used single releases

with $r = 1$ for the self-sustaining female sterile homing drive, $r = 3$ for both-sex lethal homing drive and $r = 10$ for TADE suppression drive. Female fecundity and male mating attractiveness are decided by the fitness value (f and m respectively), which is determined from an individual's genotype. Within a 6-week maximum lifespan, each fertile adult female randomly selects a male every week and will then mate with the male with a success rate equal to the male's fitness. If this check is failed, the female will select another potential candidate. Each female has a maximum of 10 chances to mate every week (but will reproduce at most once per week). If none of them succeed or if the selected male is infertile, she will not produce offspring that week. Males are allowed to mate with multiple females. The number of offspring produced is calculated based on the female's fitness and is drawn from a Poisson distribution with an average of $\frac{f \cdot \theta}{\rho}$ each week, where θ is the average offspring number from a single wild-type female per generation, which is 100 by default.

Based on their genotype, nonviable offspring are then removed from the population. All offspring then undergo larval competition. The competition ratio of larvae (λ) is defined as the ratio of the number of actual offspring this week (α) to the expected offspring number at carrying capacity ($1/2 \cdot \mathrm{K} \cdot \theta / \rho$). This gives: $\lambda = \frac{2\alpha \cdot \rho}{\theta \cdot \mathrm{K}}$. Competition ratio is the relative competition compared to the population at normal equilibrium without any transgenic individuals. The actual survival rate of larva is affected by the competition ratio, shape of the density growth curve, and the low-density growth rate (β). Our models use a linear density growth curve unless otherwise indicated: $Survival_{\mathrm{Offspring}} = P_1 \cdot 2 \cdot \rho \cdot \frac{((1-\beta)\cdot\lambda)+\beta}{\theta}$. P_1 stands for the proportion of age 1 individuals, which is 6/21 in our models.

We also explored the influence of a concave curve ($\mathrm{Survival}_{\mathrm{Offspring}} = P_1 \cdot 2 \cdot \beta \cdot \frac{\rho}{\theta \cdot ((\beta-1)\cdot\lambda+1)}$) and a convex curve ($\mathrm{Survival}_{\mathrm{Offspring}} = P_1 \cdot 2 \cdot \rho \cdot \frac{((1-\beta)\cdot(\lambda+1)\cdot(\lambda-1))+1}{\theta}$) with varying low-density growth rate. To avoid excessive fluctuation as low-density growth rate increased (which can eliminate the population even without any releases of a suppression system and are thus unlikely to be realistic), we modified the linear and convex curves for some simulations where this could be an issue. They were applied only when competition was below the normal level, and if offspring number was boosted above that threshold due to overcompensation, the concave curve would be used to stabilize the population. To simulate possible ecological dynamics in many scenarios, a combination of concave and linear curves was used in some simulations ($\mathrm{Survival}_{\mathrm{Offspring}} = (1-\delta) \cdot P_1 \cdot 2 \cdot \rho \cdot \frac{((1-\beta)\cdot\lambda)+\beta}{\theta} + \cdot P_1 \cdot 2 \cdot \rho \cdot \frac{((1-\beta)\cdot\lambda)+\beta}{\theta}$). A density character δ, ranging from 0 to 1, was employed to control the shape of the mixed curve and to represent the proportion contributed by the linear component (supplementary fig. S1, Supplementary Material online). These mixed curves were only used in simulations in Fig. 7.

Numbers of adult fertile females were recorded each week. If there is no fertile females in the population before week 267 (within 100 generations), the simulation ends in successful population elimination. If suppression fails, the average number of fertile females from the latter half of simulation (week 133 to 267) is used to show the remaining population size.

The minimum release ratio for elimination is determined by binary search. Initially, the search is conducted within a range of release ratio from 0 to 50 starting at 25 (adjusted based on the specific system). If elimination occurs in at least 3 out of 5 simulations, the search moves downward; otherwise, it moves upward. During this phase, a smaller capacity ($K = 10{,}000$) is used to expedite the process. Once the precision of the binary search (step size) decreases to a predefined threshold (varying by systems), a more refined search begins. The default capacity ($K = 100{,}000$) is applied. Starting from the lower bound of the identified range, the release ratio is incremented in steps of 0.01 until elimination occurs in 5 out of 10 simulations. The release ratio is then considered to be the critical release threshold.

Constant-population Genetic Load

If the population size is artificially kept constant during repeated releases, the relative release ratio remains the same, and the genotype frequencies will eventually reach an equilibrium. We achieve this by calculating the number of viable offspring based on the drive mechanism, number of fertile females, and proportion of different genotypes.

The average offspring number was then adjusted to keep viable juvenile production at the natural level. For most systems (though not late-RIDL and several that cause sex-biases), it also means that the native female population is kept around the original level. We then measure the average constant-population genetic load after the system reaches equilibrium from week 100 to 150. One exception is the Y-linked X-haplolethal disruptor which reaches equilibrium slower at release ratio of 0.1, and its constant-population genetic load was recorded from week 200 to 250. Equations and explanations for each strategy can be found in the Supplementary Information and the reproduction callback of corresponding models.

Data Collection

All simulations were carried out on the High-Performance Computing Platform of the Center of Life Science at Peking University. Data were analyzed and visualized using Python. All models, data, and scripts are available on GitHub (https://github.com/Hanyue22/Assessment-of-Self-limiting-Systems).

Results

SIT, RIDL, *Nix,* and Self-Limiting Gene Drives

To broadly compare different types of population suppression systems with repeated releases, we first evaluated the performance of several self-limiting systems (Fig. 2a). SIT eliminated the population when the release ratio was above 3.69, representing the number of transgenic males that are released per generation for each male in the wild-type population at its normal carrying capacity. Note that if SIT releases were insufficient to eliminate the population, the final equilibrium size was boosted above normal carrying due to reduced larval competition and subsequent higher survival of offspring. This was due to our choice of a linear density growth curve and does not occur with a concave Beverton-Holt curve. Density growth curves likely differ between species, but these effects are consistent with previous reports using linear density growth curves (Phuc et al. 2007) and some (though not all) field observations of mosquitoes (Bouyer 2023). Note also that we applied no fitness costs for SIT, which means the transgenic male's mating success rate was equal to wild-type males. This may be consistent with laboratory performance of

genetically engineered sterile males, but SIT males generated with methods such as irradiation will likely have a higher fitness cost (Pérez-Staples et al. 2012) and requires a larger release size to achieve suppression (Shelly et al. 2007; Flores et al. 2017).

Early RIDL is equivalent to SIT, but early fsRIDL is more efficient. Not only are insects easier to rear (the system itself will remove females prior to release), but male offspring, which do not directly contribute to reproduction, will still contribute to larval competition and will still pass on female-lethal alleles to half of their offspring. Thus, the critical release ratio falls from 3.69 to 3.0 (Fig. 2b). Late-RIDL allows larvae carrying a lethal allele to contribute to competition, enhancing the suppressive effect and substantially reducing the critical release ratio to 0.96. Both-sex late-RIDL performed better than late-fsRIDL (with a critical release ratio of 1.86—see "Drive late-fsRIDL" in Fig. 2d with zero drive conversion) under default settings because wild-type alleles were eliminated from all offspring of released males in late-RIDL but not late-fsRIDL. For late-fsRIDL, released males produce surviving male heterozygotes, which can later mate with females and have half of their daughters survive. In contrast, offspring of RIDL homozygotes all die before reaching adulthood, sacrificing some RIDL alleles in male heterozygotes to more quickly remove wild-type alleles. Thus, the relative release ratio increased. This advantage, however, was not consistent under all ecological parameters (see section "Impact of density growth curve on population suppression").

The suppression power of late-fsRIDL was greatly enhanced by combination with homing gene drive (Fig. 2) (Burt and Deredec 2018; Zhu et al. 2024; Willis and Burt 2025). Here, we assumed no resistance alleles. The critical release ratio linearly declined as drive conversion rate increased, becoming equivalent to late-RIDL at 60% drive conversion while retaining the advantage of more efficient rearing. At 100% drive conversion (and no fitness costs), the system remains at the release frequency, so any level of repeated release can eliminate the population. Resistance alleles would decrease drive late-fsRIDL's efficiency, but could be largely avoided with an essential target and rescue system in the drive, together with other precautions against functional resistance (Zhu et al. 2024).

Nix is a gene responsible for sex determination in several species and has been proposed to be used for genetic control (Hall et al. 2015; Aryan et al. 2020; Lutrat et al. 2022). A self-sustaining suppression drive could be developed by linking it to a homing drive, further biasing the sex-ratio toward males and potentially eliminating the population. However, this system would be self-sustaining, so we do not model it here. Instead we focus on a self-limiting system without homing. To develop a self-limiting system, we could repress *Nix* expression in a rearing facility, so individuals that are homozygous for a *nix* transgene could be generated, half of which would also contain a native copy of *nix*. All progeny of these released males would be males with 1 or 2 copies of *nix* on autosomes. Though this strategy allowed for maximum larval competition, it needed a relatively higher release ratio (5.58) for population elimination (Fig. 2e). This is because it does not directly affect female fertility, only converting some to males, which themselves will not have all male offspring if they mate (thus competing with subsequent released males). If combined with genetic or mechanical sex-sorting in a rearing facility, males with 3 copies of *Nix* could be obtained, reducing the release ratio to 4.69 (supplementary fig. S2a, Supplementary Material online). The efficiency of this system could be further improved with additional repressible *Nix* transgene loci (Schliekelman and Gould 2000a,b; Schliekelman et al. 2005), unless expression of even more *Nix* copies caused significant fitness costs or if repression is not sufficient.

Dominant female sterile homing drives have been generated when somatic Cas9 expression or maternal carryover eliminates wild-type alleles in drive heterozygous females (Hammond et al. 2016; Oberhofer et al. 2018). Released males are drive heterozygous, and performance is heavily dependent on the drive conversion rate. We found that this drive could successfully eliminate the population only when the drive conversion rate was above 0.4, though in this case, the required release ratio could reach much lower than late-fsRIDL or SIT (Fig. 2f). Though it would be difficult to achieve, we also modeled homozygote releases (supplementary fig. S2b, Supplementary Material online), which was similar to drive late-fsRIDL, but with the addition of nonfunctional resistance allele formation. This significantly increased efficiency when drive conversion was below 0.9.

If resistance alleles are dominant sterile along with the drive (the RIDD system (Chen et al. 2024)), then suppressive power is substantially improved (Fig. 2g). This allows male progeny to spread sterility to all daughters even if the daughters do not receive a drive allele. This efficiency is particularly valuable when the drive itself has a low conversion rate. Unlike normal recessive sterile resistance alleles, which can persist longer and also block drive conversion in males (reducing their benefit), the dominant sterile alleles more heavily contribute to population elimination and are eliminated before blocking drive conversion in males.

Another way to make a self-limiting homing suppression drive is to use a split drive system. With this, we released males that were homozygous for Cas9 and heterozygous for the gRNA allele (the drive) that sat inside and targeted a female fertility gene. Only females carrying both Cas9 and gRNA suffered the default 30% fitness cost from somatic Cas9 expression. Despite being a self-limiting system, it required only half the release size for suppression compared with late-fsRIDL when drive conversion rate reached 0.5 (Fig. 2h), likely because both male and female heterozygotes could continue spreading the drive. Below this threshold, its efficiency declined sharply but still performed better than ideal SIT with a drive conversion rate of 0.4. Time to population elimination shortened as the drive conversion rate rose from 0.6 to 1, but the required release ratio remained unchanged because a certain minimum amount of Cas9 was needed in the population for effective spread of split drive and formation of sufficient numbers of sterile drive homozygotes.

Low Efficiency Self-Sustaining Gene Drives

We next explored how self-sustaining drive performance could be improved with repeated releases. Though these unconfined drives are powerful, they often will reach a high equilibrium frequency without generating enough suppressive power (genetic load, representing the reduction in reproductive capacity of the population) to eliminate the target population. For these systems, the drive conversion rate is the main factor affecting drive efficiency, and female sterile homing drive required a conversion rate above 0.9 to succeed with a

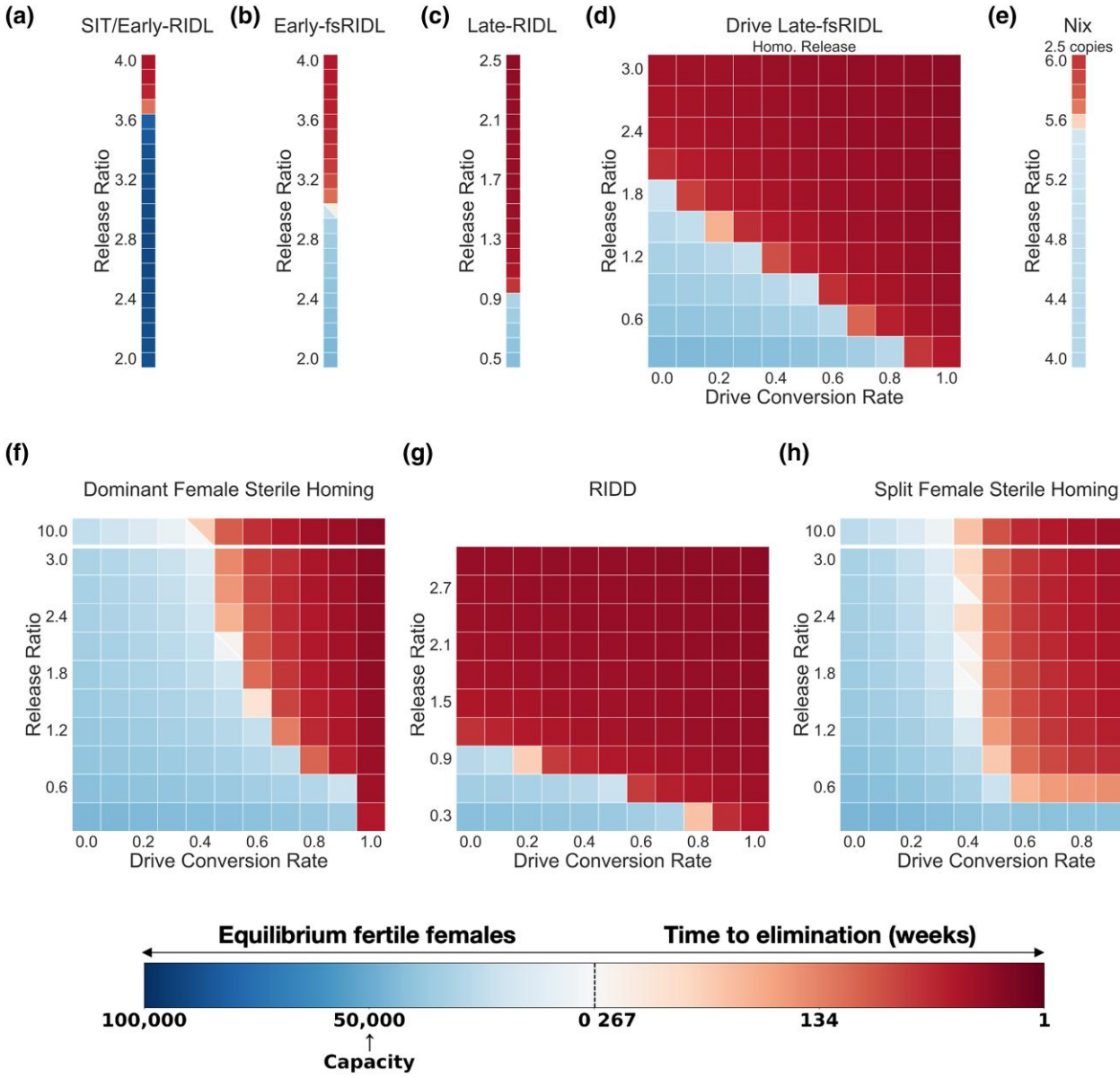

Fig. 2. Performance of self-limiting systems. Transgenic males with varying suppression systems were released each week at the per-generation release ratios shown into a wild-type population with a carrying capacity of 100,000 (50 k fertile females). With default performance parameters and varying drive conversion and release ratios, we recorded the time to population elimination (267 week at maximum) or the average number of fertile females after the system reached equilibrium. Squares with 2 colors indicate that both success and failure happened in simulations. Each point had 10 replicates.

single release with our default parameters (Fig. 3a, single release ratio = 1.0). This could be challenging for many organisms. However, with repeated releases, population elimination could be achieved with only a 40% drive conversion rate. The required release ratio decreased sharply from 40% to 60% drive conversion, reducing the required release sizes to a small level.

Another important factor was the drive fitness cost, which is common for this type of drive in heterozygous females if there is any somatic Cas9 expression or requirement for the target gene in the germline. Here, we fixed the drive conversion rate at 0.5 and varied the fitness of female drive heterozygotes (Fig. 3b, single release ratio = 1.0). Low fitness significantly harmed drive efficiency, especially when it was below 0.4, but success was still possible with higher release ratios. With sterile heterozygote females, required release ratio was 4-fold higher compared with a drive causing no fitness cost. Note that when female somatic fitness of the drive dropped to 0, female drive heterozygotes were sterile, meaning that there would be fewer fertile females than drives with higher fitness costs. If reduction in fertile females is a primary objective (such as in females in situations where this prevents biting), then dominant drive sterility could be favorable if population elimination is not possible.

We also noticed that population elimination with most homing drives (RIDD excepted) becomes impossible with even high release ratios if drive conversion was insufficient. To further investigate these aspects, we conducted an "infinite release" (in which all females were assumed to mate with drive males) while varying both the drive conversion rate and the germline resistance allele formation rate (Fig. 3c). As expected, population elimination could be readily achieved when the drive had high drive conversion, but this was also true with high total germline cut rate (drive conversion and germline

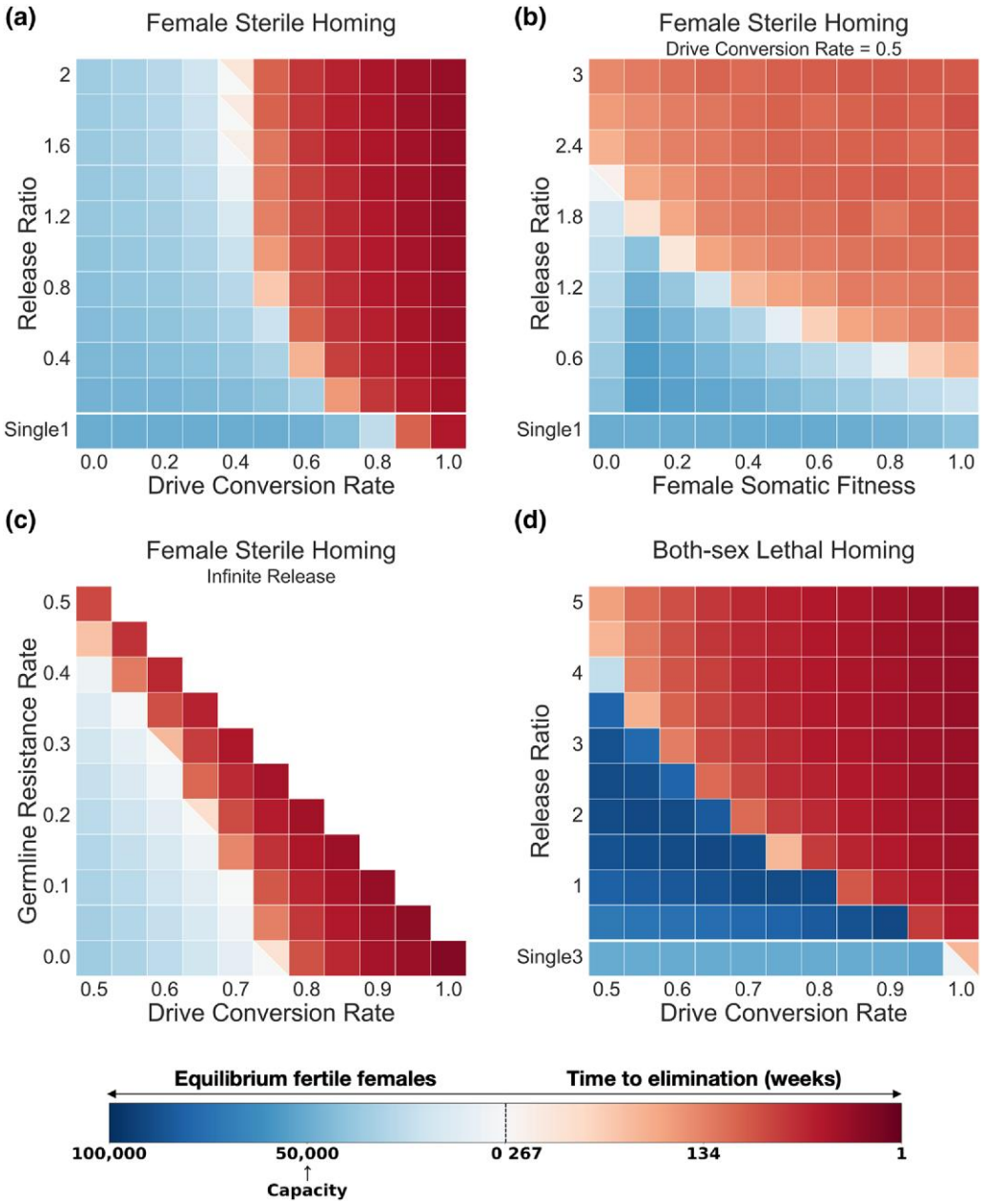

Fig. 3. Performance of self-sustaining systems. Transgenic males with varying suppression drives were released each week at the per-generation release ratios shown into a wild-type population with a carrying capacity of 100,000 (50 k fertile females). With default performance except as shown, we recorded the time to population elimination (267 week at maximum) or the average number of fertile females after the system reached equilibrium. Squares with 2 colors indicate that both success and failure happened in simulations. The single release ratio is 1.0 for (a and b), and 3.0 for (d). Each point had 10 replicates for.

resistance allele formation added together), even when drive conversion was moderate.

Another mechanism for self-sustaining gene drive is to target a haplosufficient but essential gene for both sexes, so individuals with 2 drive or nonfunctional resistance alleles are nonviable (Burt 2003; Deredec et al. 2011; Oberhofer et al. 2018). Though such a drive with early acting lethality, would have lower genetic load that one targeting female fertility, it could still be a powerful option for suppression with many possible gene targets in different species. We thus explored the performance of a both-sex lethal drive and found that it required a much higher release ratio (Fig. 3d, single release ratio = 3.0) compared to the female sterile drive. This is partly because male drive heterozygotes would also suffer fitness costs from somatic Cas9 expression, which we modeled as a reduction in mating competitiveness (supplementary fig. S2c, Supplementary Material online). Additionally, male homozygotes could not maintain drive alleles, and yet, removal of these males did not reduce population reproductive capacity. This drive also induced overcompensation due to a reduction in viable larva. When we fixed the drive conversion rate at 0.5 and fitness to 0.7, it needed a release ratio of 4.5 to eliminate the population. Only very high drive conversion rates allowed for small release sizes.

TADE Suppression Drive

TADE suppression drive does not rely on homing to spread, making it slower, but giving the advantage of confinement.

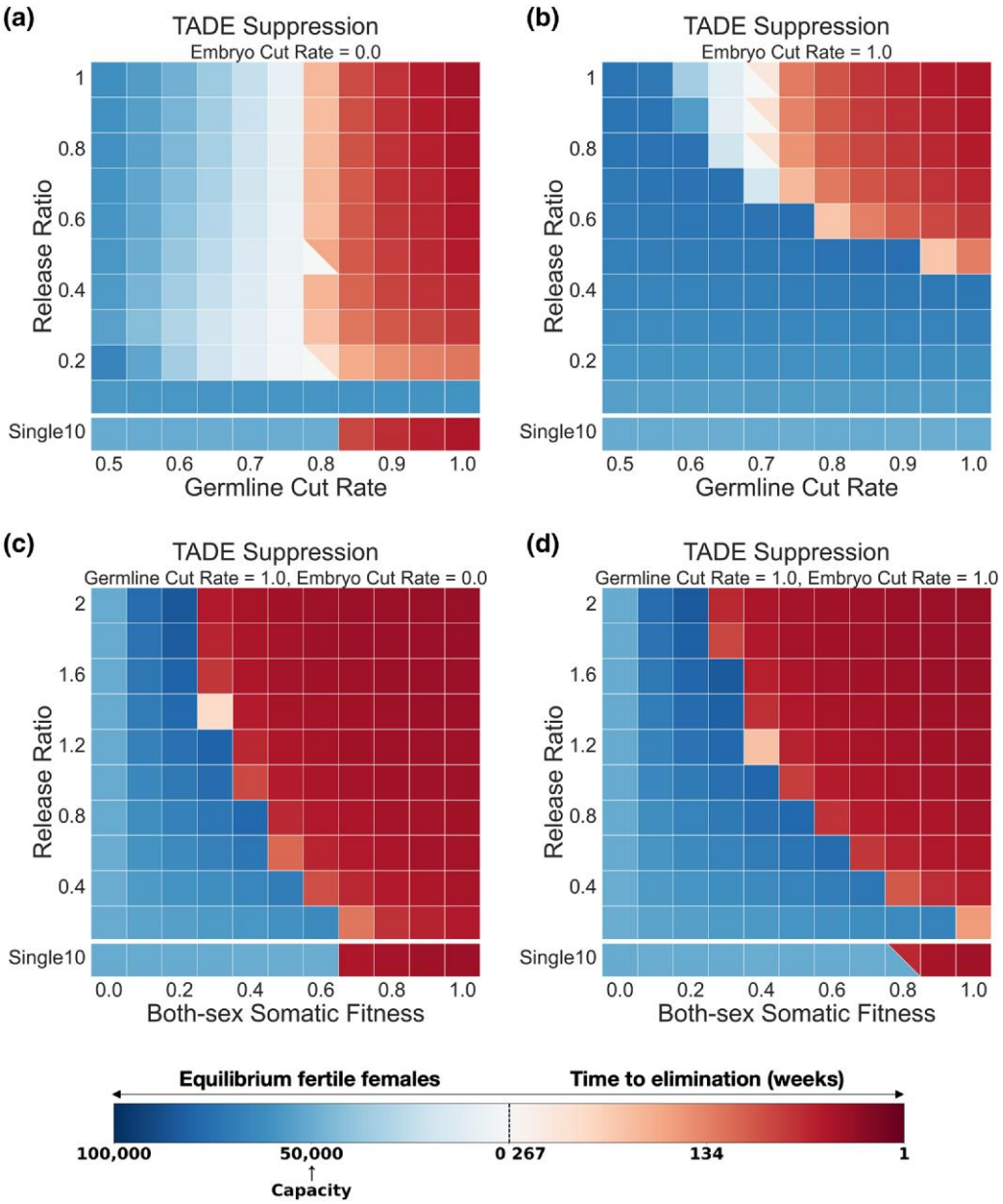

Fig. 4. Performance of TADE suppression drive. Transgenic males with TADE suppression drives were released each week at the per-generation release ratios shown into a wild-type population with a carrying capacity of 100,000 (50 k fertile females). With default performance parameters except where indicated, we recorded the time to population elimination (267 week at maximum) or the average number of fertile females after the system reached equilibrium. Squares with 2 colors indicate that both success and failure happened in simulations. The single release ratio here is 10.0. Each point had 10 replicates.

When TADE suppression drive has embryo cleavage from maternal Cas9/gRNA and fitness costs, its introduction threshold increases. Additionally, the main factor influencing its genetic load is the germline cut rate (Champer et al. 2020a), which is easier to improve than drive conversion rate in homing drives. In our model, TADE suppression drive with default fitness costs required at least 80% germline cut rate to succeed (Fig. 4a, single release ratio = 10.0), either with a single large release above its introduction threshold or with multiple releases. However, larger release sizes did not expand the parameter range for population elimination like in homing suppression drives. However, when the embryo cut rate was high, even a large single release size still resulted in failure due to the extremely high introduction threshold with these parameters (Fig. 4b, single release ratio = 10.0). In this case, efficiency could be restored using multiple releases. This required larger release sizes but could tolerate somewhat smaller germline cut rates. TADE suppression drive also suffered from overcompensation due to its embryo lethal mechanism, but the effect was much milder because female sterility accounts for most of its suppressive power.

Interestingly, for TADE without embryo cutting, we noticed that a single release performed better than repeated releases, often requiring a smaller total release size (the total number of released males from release start to elimination) for more rapid population suppression. At a release ratio of 0.1, success was not possible because the drive was removed too rapidly from the population due to fitness cost, which would not happen for an ideal TADE suppression drive, which has no introduction threshold. Although the total release amounts were

large, the introduction ratio in each generation provided a frequency that was far below the drive's introduction threshold when the population was at its capacity.

We next looked into the effect of fitness cost in TADE suppression drive. Because somatic activity from the drive allele may disrupt the wild-type target gene in somatic cells without offering sufficient rescue, both sexes could face high fitness cost. The efficiency of TADE suppression drive was susceptible to this fitness cost (Fig. 4c and d, single release ratio = 10.0, germline cut rate = 1.0). Even with full germline cut rate, the drive quickly lost its power when somatic fitness dropped below 0.7, or 0.8 when embryo cutting was high. Despite the impact of embryo cleavage and fitness costs, TADE suppression drive was relatively powerful compared to SIT and late-fsRIDL. As long as the germline cut rate was high, a repeated release strategy could cover a deficit in drive fitness, expanding its potential application compared to single release strategies.

Gene Disruptors

We define gene disruptors as any construct that targets a certain gene or chromosome without directly providing complete rescue for the disrupted target and without directly increasing their own frequency. We explored the performance of several different self-limiting gene disruptor variants.

First was a simple allele targeting a female fertility gene from a distant site. Such a system, if released in heterozygous form, could not generate sufficient power to eliminate reasonably robust populations. We thus considered homozygous releases, which could be achieved by repressing expression of the construct in the rearing facility. When the germline cutting rate was sufficiently high, the population could be eliminated with a low release ratio of 0.62 (Fig. 5a). Unlike homing drives, where embryo resistance often harmed drive efficiency, cutting in the early embryo reinforced female fertility disruptors. An embryo cut rate of 100% allowed female fertility disruptors to eliminate the population with only 50% germline cutting and an even lower release ratio (Fig. 5b). When we fixed the germline cut rate at 100%, increasing embryo cut rate somewhat decreased the release ratio threshold and duration required for suppression (Fig. 5c).

We also modeled severe expression and cutting in the somatic cells from a female fertility disruptor, which led to dominant female sterility by itself. Female offspring that inherited a disruptor would be sterile, regardless of whether they also inherited disrupted target alleles. We found that it rendered disruptors with a germline cut rate below 0.75 powerful enough to achieve population elimination (supplementary fig. S2d, Supplementary Material online), albeit with a higher release size than the version without high somatic activity. In this case, fitness costs from somatic expression reduced population fecundity and favored system efficiency, although it also reduced the persistence of the disruptor.

Dominant female fertility disruptors formed dominant female-sterile alleles (disrupted target site) in the same way as the RIDD system (Fig. 5d), while the disruptor itself was not dominant sterile (unless the female somatic fitness was specifically set to 0, rather than the default of 0.7). It was much more powerful than the recessive form, requiring only 55% germline cutting and no embryo cutting to achieve suppression with homozygous releases. It remained effective even when the construct was also dominant female-sterile due to severe somatic expression (Fig. 5e). More efficiency was lost when the release was heterozygous, but it still performed effectively with a high germline cut rate, similar to the RIDD system without any drive conversion (Fig. 5f).

The protected *dsx* disruptor was a distinct system, also based on RIDD in *doublesex*. It targeted a different site on the same chromosome (such as the female exon of *doublesex*), forming dominant female-sterile alleles. These alleles would not take effect if on the same chromosome as the disruptor, because the disruptor is placed earlier in the gene, stopping expression and thus preventing dominant sterility (Willis and Burt 2025). However, drive homozygous females and males would be sterile, lacking any functional copies of *doublesex*. The protected *dsx* disruptor was powerful enough to suppress the population with heterozygous releases (Fig. 5g). When germline cut rate was above 0.8, it allowed population elimination, with a required release ratio of 0.22 when the germline cut rate was 100%. Eliminating fitness costs significantly enhanced its efficiency, lowering the threshold to 0.04 with full germline cut rate (supplementary fig. S2e, Supplementary Material online, germline cut rate = 1.0 without fitness cost). Unlike most other disruptors, embryo cutting harmed its efficiency, greatly increasing the required release ratio to 0.18 (supplementary fig. S2e, Supplementary Material online).

X-shredders have been constructed in *A. gambiae* using both Cas9 and other nucleases (Galizi et al. 2014, 2016). With a repressible system and homozygous release, we found that the system performed fairly well (Fig. 5h). However, heterozygous releases required a much higher release ratio (a release ratio of 2.84 in ideal form) to achieve suppression (Fig. 5i).

Interestingly, for both dominant and recessive female fertility disruptors, as the germline cut rate rose above 0.85, the release ratio threshold for population elimination slightly increased. This unusual pattern was likely caused by disrupted alleles hindering the spread of disruptor alleles by sterilizing females (and removing disruptor alleles) that would otherwise have been able to further disrupt target alleles in their offspring. For example, in the dominant female fertility disruptor system, when the germline cut rate reached 100%, any female offspring inheriting the disruptor would also inherit a dominant-sterile allele and thus could not pass disruptor or disrupted alleles to the next generation. Compared to a system with only 55% germline cut rate (supplementary fig. S3, Supplementary Material online), it generated more disrupted alleles at first, but the carrier frequency of the disruptor was eventually limited, reaching a lower equilibrium frequency. A similar pattern emerged in the autosomal X-shredder system with homozygous releases, since few females would be able to inherit the disruptor under high X-shredding rate, and females would tend to enjoy greater reproductive success than males because of the sex bias created by the shredder.

The *tra* is a female fertility gene, but in some species, genetic females with *tra* knockout become fertile males. A disruptor targeting *tra* in such a species with an ideal germline cut rate and no embryo cleavage could eliminate the population with a homozygous (implying repressibility) release ratio of 1.27 (supplementary fig. S2f, Supplementary Material online). Such system might not be applicable to all pest species, but the sex transforming effect allowed disrupted alleles to be passed to the next generation, rather than being lost in a female sterile mechanism.

All gene disruptors considered above were located on autosomal chromosomes. Recently, the development of a Y-linked

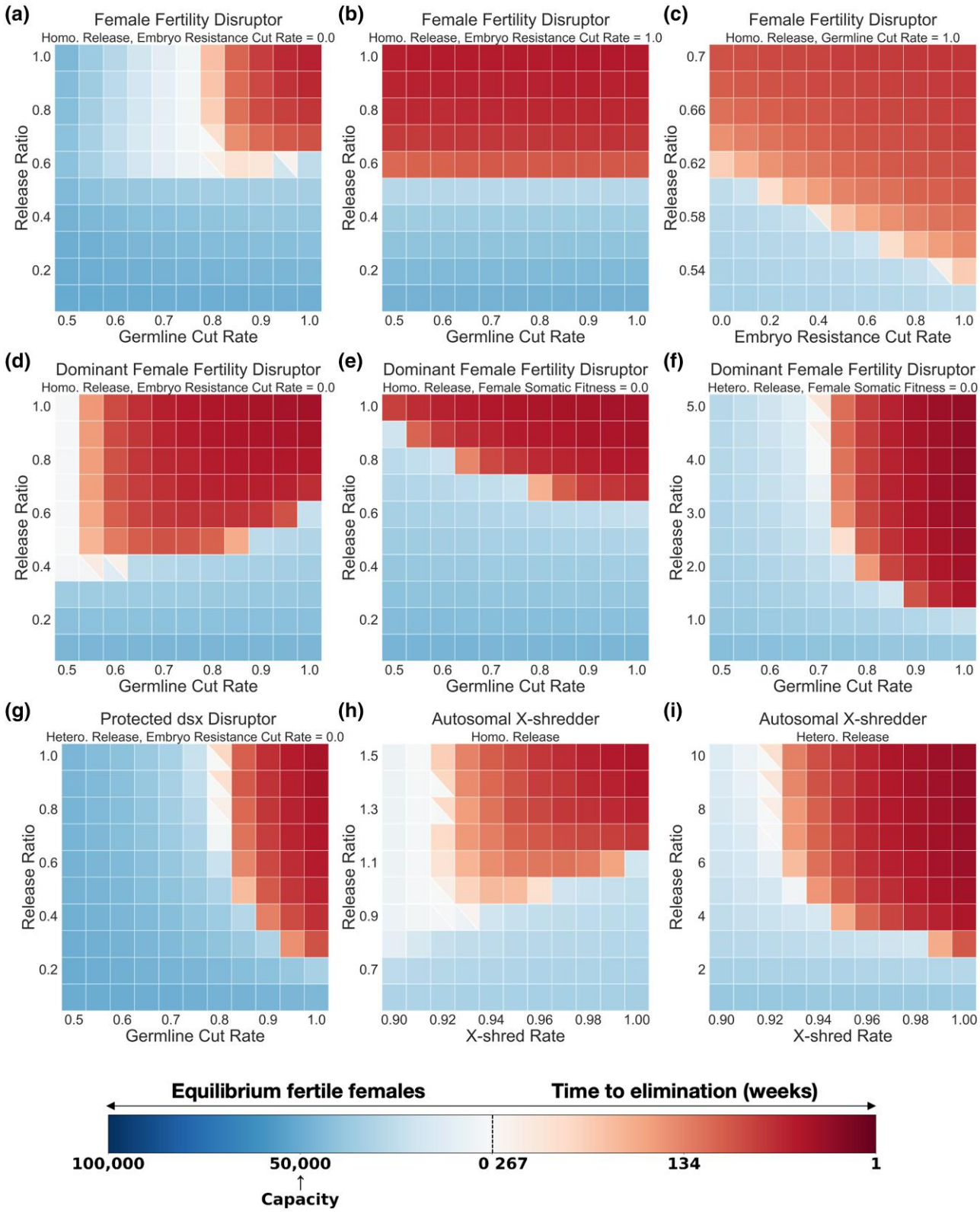

Fig. 5. Performance of gene disruptors. Transgenic males with gene disruptors were released each week at the per-generation release ratios shown into a wild-type population with a carrying capacity of 100,000 (50 k fertile females). With default performance parameters except where indicated, we recorded the time to population elimination (267 week at maximum) or the average number of fertile females after the system reached equilibrium. Squares with 2 colors indicate that both success and failure happened in simulations. Each point had 10 replicates.

gene editor (YLE) showcases the viability of Y-linked disruptor systems (Tolosana et al. 2025). We explored the performance of a Y-linked dominant female fertility disruptor (supplementary fig. S2g, Supplementary Material online), which forms dominant female-sterile alleles at an autosomal site such as *doublesex*. No fitness cost was considered in this

model because the disruptor would only be expressed in males. The system was very powerful, with germline cut rates over 0.9 eliminating the population with a release ratio of 0.1. A Y-linked disruptor targeting an X-haplolethal gene was generally as effective without fitness cost (supplementary fig. S2i, Supplementary Material online), but took longer because larval competition was reduced (female progeny would be nonviable rather than sterile). However, this system would suffer from somatic expression because the haplolethal target would usually be needed in males. With a 30% fitness cost for male carriers, efficiency dropped sharply (supplementary fig. S2h, Supplementary Material online) because the drive itself would no longer be fitness neutral. Y-linked X-haplolethal disruptors only exist in males, who never inherit a disrupted X chromosome. Thus, if the disruptor has no fitness cost itself, it will remain at constant frequency in males even without continuous releases. However, if the disruptors cause a negative impact on male carriers by decreasing their mating competitiveness (or survival), a higher release ratio is needed to compensate for the fitness cost and achieve suppression.

Assessment of Suppressive Power Using Constant-Population Genetic Load

Genetic load represents the reduction in reproductive capacity of the population (suppressive power on a scale from 0 to 1). For self-sustaining suppression drives when the drive reaches equilibrium, it is a good measure of long-term system performance. It depends only on drive characteristics and not ecology, at least in commonly used models. To determine if the drive is powerful enough to eliminate the population, genetic load can be compared to the low-density growth rate. However, it is not possible to easily use genetic load at system equilibrium for assessment suppression strategies involving repeated releases. Performance of genetic control systems with repeated releases are more heavily affected by features of different species and ecological parameters such as the full shape of the density-dependent growth curve. Specifically, genetic load sometimes fails to consistently assess system power due to changes in the relative release ratio. The relative ratio of released males to native-born males will affect the genetic load, but the number of native-born males will change as the population is suppressed, and it will do so in a manner determined by species ecology. Thus, by releasing a constant number of males per generation based on the initial male number (as in our simulations) the genetic load will change as the population size is changed. To provide an unchanging genetic load measurement for repeated release systems, we introduce the concept of "constant-population genetic load" to quantify system power. We measure this by artificially adjusting reproduction for all fertile individuals by a particular constant, calculated to maintain the native-born population size. We then allow the system to reach equilibrium during continuous releases and measure the genetic load at this point. Here, we assess the accuracy and consistency of this measurement across multiple suppression strategies.

Constant-population genetic load correlated closely with the performance of different systems (Fig. 6a, supplementary fig. S4, Supplementary Material online). Specifically, higher values tended to result in more successful population elimination (compare to other figures) and good correlation with system performance. For example, as the release ratio went up, overcompensation first led to higher average population sizes for both-sex viability homing suppression drive. However, constant-population genetic load results suggested that system power was stably increasing. Constant-population genetic load results for the dominant female sterile disruptor had similar pattern with its actual performance. It suggested that the relatively inferior outcomes when the germline cut rate rose above 0.9 were due to system deficiency rather than environmental impacts.

Additionally, both Y-linked disruptors show a similar pattern: with a fixed germline cut rate, the genetic loads across release ratios (0.1 to 1.0) are nearly identical (supplementary fig. S4, Supplementary Material online, Fig. 6b). This is because Y-linked disruptors affect only females, allowing them to accumulate in males through repeated releases, leading to the rapid replacement of wild-type Y chromosomes. For the Y-linked X-haplolethal disruptor, the population stabilizes with XX females and XY^D males. For the Y-linked dominant female fertility disruptor (at high germline cut rates), all males carry 1 disrupted allele, and fertile females carry 2 wild-type alleles, resulting in a stable genotype distribution of WW XX females and WR XY^D males. In this scenario, the equilibrium genetic load depends entirely on the germline cut rate, explaining the identical genetic loads when the cut rate is fixed, as long as the release ratio is sufficient to achieve system fixation by the end of the simulation.

We next explored the relationship between constant-population genetic load and successful elimination. For regular genetic load in self-sustaining systems, the relationship between genetic load and deterministic population elimination is straightforward. The genetic load must be equal or greater than [1 - 1/(low-density growth rate)]. Constant-population genetic load does not provide as simple a relationship because the full shape of the density growth curve (not just the low-density growth rate) and other ecological factors relating to competition will all influence the result. Nevertheless, we still uncovered a strong correlation between elimination outcomes and the constant-population genetic load for many different systems (Fig. 6b). We determined the minimum release ratio for population elimination in different systems with drive conversion rate of 0.6 or germline cut rate of 0.8 (except for the 2 Y-linked disruptors which required a germline cut rate above 0.86 to eliminate the population), and we measured the constant-population genetic load at this critical release ratio. We then compared this to our previous simulations and their associated values for constant-population genetic load. This served as an accurate predictor for population elimination outcomes for SIT, early fsRIDL and most homing drive systems, all at a similar level when holding ecological parameters constant. However, some systems with more exotic mechanisms (Y-linked disruptors, female fertility disruptors) had their critical constant-population genetic load threshold at a different level, showing that while internally mostly consistent for different drives, this value has less utility comparing certain specific system types than genetic load for self-sustaining gene drives.

We further examined systems where the threshold prediction had a lower accuracy when comparing to our previous simulations (supplementary fig. S5, Supplementary Material online). The split female sterile homing drive and autosomal X-shredder were influenced mostly by parameter spaces with binary outcomes (elimination in some simulations and population persistence in others with the same simulation parameters). For the female fertility disruptors (recessive and

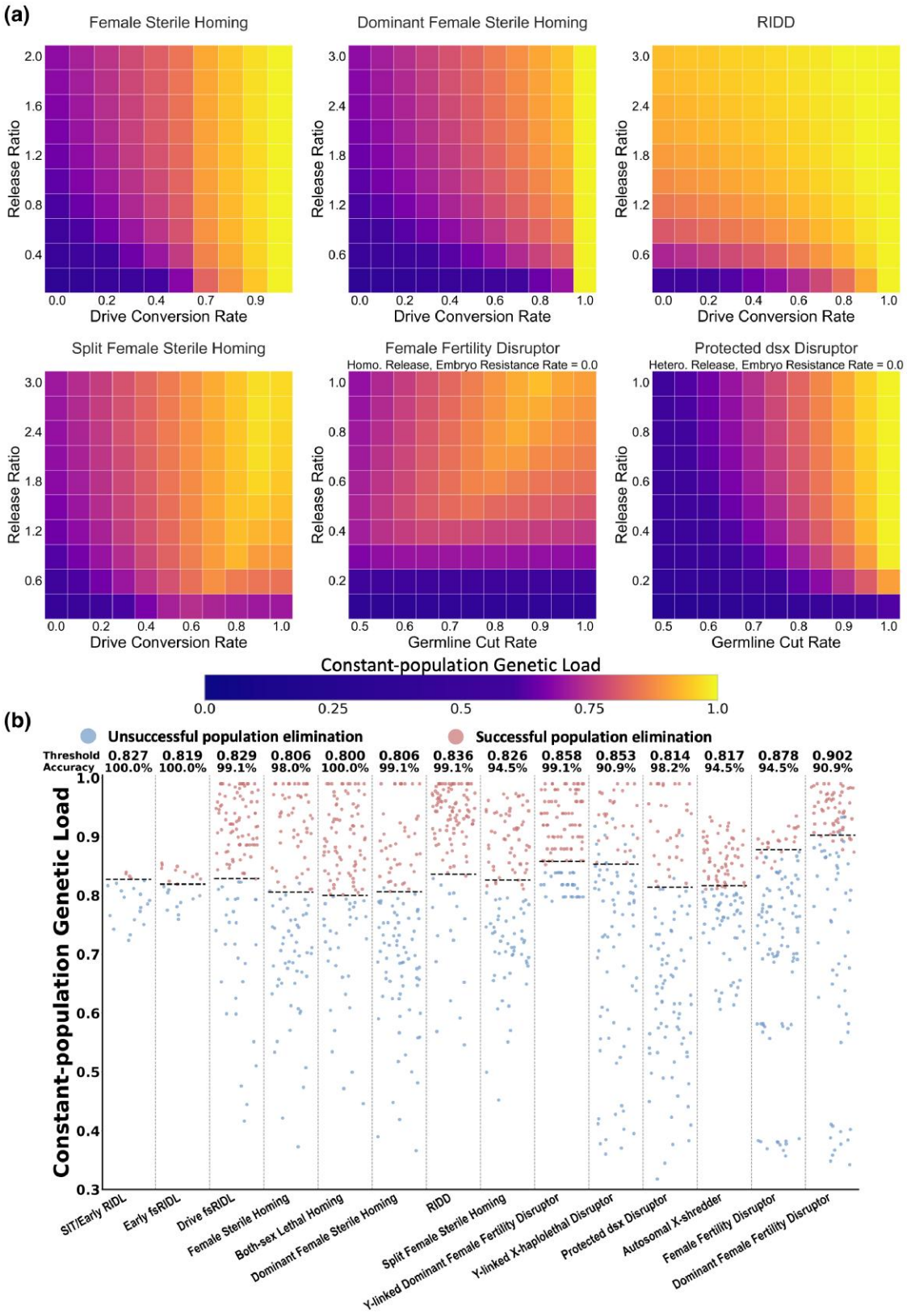

Fig. 6. Constant-population genetic load. a) Transgenic males were released each week at the per-generation release ratios shown into a wild-type population, with the population being kept around its carrying capacity of 100,000. With default performance parameters except where indicated, we recorded the average constant-population genetic load from week 100 to 150, when most systems reached equilibrium, except for the Y-linked X-haplolethal disruptors, which reached equilibrium slower at release ratio of 0.1 (week 200 to 250). Each dot represents a certain parameter set from our earlier data obtained from constant-population genetic load simulations and normal simulations. Dashed lines represent the constant-population genetic load at the critical release ratio needed for population elimination. This critical release threshold and the accuracy of using this to predict results in the other points are shown above the graph.

dominant) and Y-linked X-haplolethal disruptor, accuracy fell near the borders of population elimination. The threshold measured at different performance parameters varied, probably due to the inhibiting effect of high germline cut rate. For late-RIDL and TADE suppression, the thresholds were difficult to measure in the first place. The late-acting, both-sex lethal mechanism of late-RIDL hindered the equilibrium state near elimination. For TADE, its frequency-dependent nature and unique mechanism including both-sex lethal and female sterile effects led to rapid population elimination in the constant-population simulations, preventing accurate measurements (this system is usually self-sustaining and best assessed with conventional genetic load).

Impact of Density Growth Curve on Population Suppression

Ecological factors can greatly affect suppression systems involving repeated releases. In addition to the low-density growth rate, the shape of the density growth curve can play a more important role than for self-sustaining gene drives. We thus explored the performance of different systems under various ecological conditions. The shape of the density growth curve affected population stability and resilience. A "concave"-shaped Beverton-Holt curve resulted in stable population level but low resilience in response to a genetic load, as shown in some laboratory populations (Hassell et al. 1976). A "convex" curve allowed population size to remain high when facing moderate suppressive power, as observed in some large mammalian herbivores (Owen-Smith 2006). Our default setting in simulations was a "linear" curve, with intermediate effects. Overcompensation can occur with any curve that allows greater growth than our concave curve, which was evident in several SIT programs across the world (though these did not represent the majority of cases) (Bouyer 2023).

We first investigated the impact of low-density growth rate on the 4 classic suppression systems: RIDL, late-fsRIDL, early fsRIDL, and SIT (equivalent to early RIDL). We determined the release ratio threshold needed for population elimination with the linear density-dependent growth curve (Fig. 7a). With a low-density growth rate of 2, late-fsRIDL required the lowest release ratio for population elimination (0.15), outcompeting late-RIDL since males could still spread the female-specific lethal alleles. When low-density growth rate rose above 4, however, late-RIDL became more powerful.

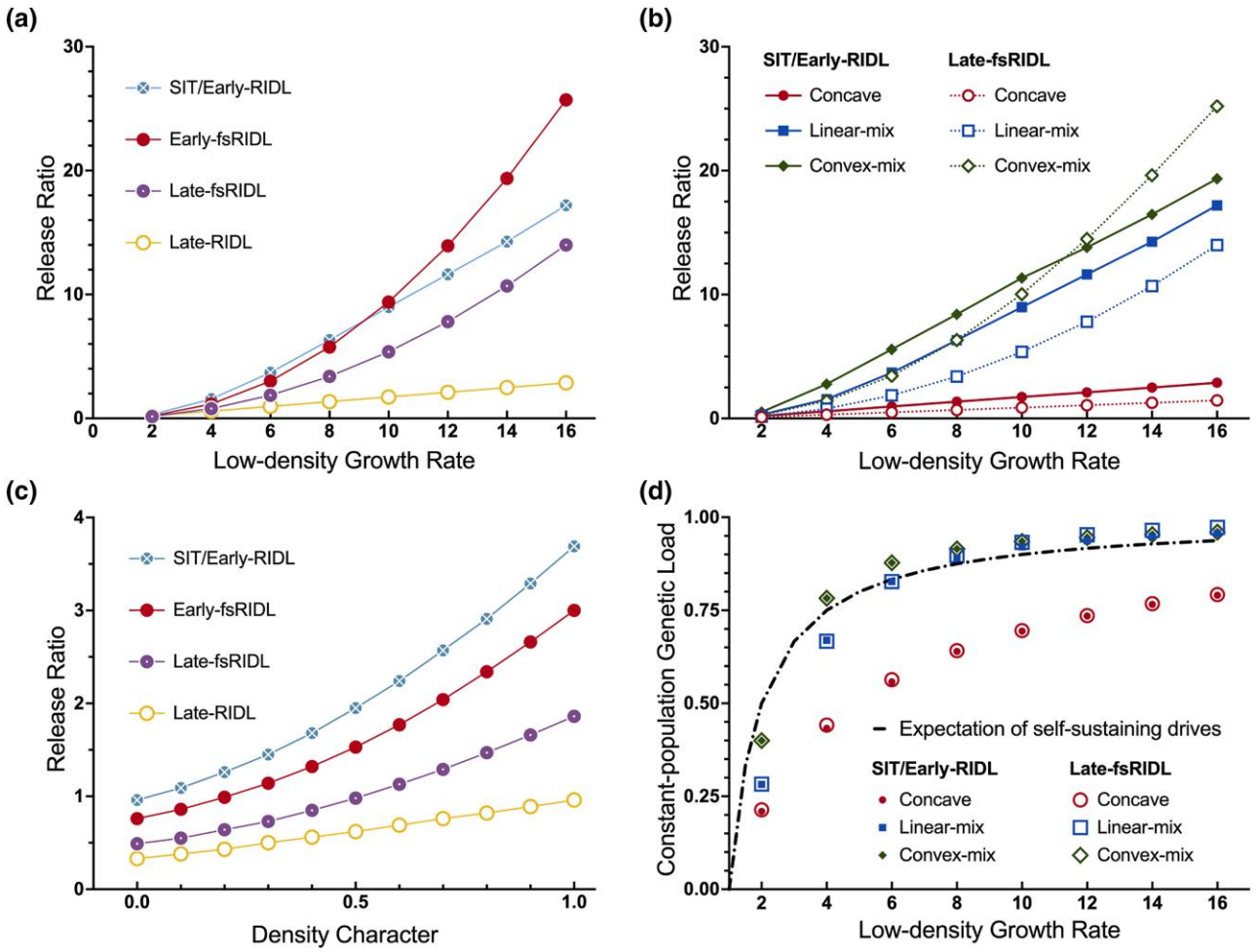

Fig. 7. Impact of ecological parameters. Critical release ratio threshold needed for population elimination using several systems are shown for a) linear density growth curve and varying low-density growth rate, b) different shaped density growth curves and varying low-density growth rate, and c) varying density character showing a transition from concave (0) to linear (1) with a low-density growth rate of 6. d) Constant-population genetic load threshold needed for population elimination with differently shaped of density growth curves with varying low-density growth rates. The dashed curve represents the genetic load threshold for elimination after a single release of a self-sustaining suppression drive, which is equal to 1-1/β. Each spot had 5 replicates.

The 2 early acting systems were less efficient throughout entire range. SIT required a higher release ratio when low-density growth rate was below 8 but outperformed early fsRIDL when it was over 10. Overall, the both-sex systems were linearly impacted by low-density growth rate, while female-specific systems were more substantially affected by higher low-density growth rate due to their reduced ability to eliminate wild-type alleles.

We next tested the release ratio threshold of late-fsRIDL and SIT/early RIDL under different low-density growth rates with all 3 kinds of curves (Fig. 7b). As expected, higher low-density growth rate increased release sizes. The convex curve required the highest release ratio to achieve elimination, and the concave curve needed the lowest. The late-fsRIDL system usually performed better, but it was more strongly affected by the low-density growth rate in linear and convex curves. It was only inferior to SIT for the convex curve when low-density growth rate was over 10. This is because the main advantage of late-fsRIDL over SIT comes from higher larval competition from offspring of released males. If this is less important because population size will remain high until near the point where population elimination is suddenly achieved, then late-fsRIDL is left with the disadvantage of surviving male progeny of released males carrying wild-type alleles and competing with released males. Because late-RIDL allows for full competition while removing wild-type alleles rapidly, its critical release ratio increases only linearly as the density curve transitions from concave to linear, while all other systems are more greatly affected (Fig. 7c).

We then measured the corresponding constant-population genetic load at the critical release ratio needed for population elimination for SIT and late-fsRIDL, comparing them to the theoretical necessary ratio based only on the low-density growth rate (Fig. 7d). Because the predictive threshold of constant-population genetic load was consistent across multiple systems, this result indicated the approximate required intrinsic power for a genetic control system to eliminate the population under different low-density growth rate. Consistent with the release ratio result, it was the easiest to suppress the population with a concave curve, where a small genetic load will already rapidly reduce the population size. This, in turn, rapidly increases the relative release size (release size compared to actual population in later time points) in our simulations, further contributing to population suppression. Of course, high growth must still be overcome in the final stages of elimination. For a linear or convex curve, a small genetic load could induce overcompensation, decreasing the relative release size, or at least preventing it from increasing nearly as quickly. Therefore, a higher constant-population genetic load is required to eliminate the population because it becomes more challenging in the initial stages. Thresholds under all circumstances tended to converge to the theoretical expectation as the low-density growth rate further increased, which makes it most difficult to eliminate the last part of the population for all 3 curves when population density is low.

Discussion

In this study, we modeled various genetic control systems (Table 1), focusing on self-limiting strategies involving repeated releases of transgenic males. Results suggested that several types of gene drive and gene disruptors could eliminate pest populations substantially more effectively than SIT and RIDL systems, offering a feasible alternative for population suppression with potentially lower resource investment. To facilitate comparison between the large number of potentially effective systems utilizing repeated releases, we proposed constant-population genetic load to evaluate system power, showing that it is often a useful way to assess these systems irrespective of ecological factors.

Numerous genetic control strategies have been proposed and verified in many species over the past decades. As researchers start to contemplate field trials for more powerful strategies (Long et al. 2020; Connolly et al. 2024), a central question emerges: which system should we opt for? This can be further deconstructed into 4 aspects: (i) what is our desired outcome, (ii) where do we aim to control the pest, (iii) what systems can be built in the target species, and (iv) what is the effectiveness of the viable systems? The first question tends to be straightforward, dividing scenarios into population modification or suppression, though the level of desired suppression could vary in some cases. The second question is very important, but also potentially complex. Self-sustaining gene drives and even confined gene drives could potentially be less desired due to a variety of factors.

The third question addresses the feasibility of implementing specific strategies in the target species. Once an objective is set, possible strategies may still be limited because not every system can be successfully implemented in a target species due to a lack of genetic knowledge and the absence of universal transgenic technologies applicable to nonmodel organisms. Even when successful, efficiency can vary. For instance, gene drives with the capability to eliminate population by single release often necessitate a well-characterized target site, reliable gene-editing tools, and promoters with high germline specificity and minimal maternal deposition—all of which may be difficult to meet in some species. According to our modeling results, gene disruptors are usually less powerful than even self-limiting gene drives, relying on repeated releases of homozygous transgenic males in some cases. However, they still outcompete SIT and late-fsRIDL with high germline cut rate, which is easier to achieve than high drive conversion rate. The self-limiting nature of gene disruptors can mitigate safety concerns, and easy access to conditional/repressible systems (Table 1 "Requires repressibility") or effective insertion sites on the Y chromosome would make more of them potentially feasible solutions for pest control. With limited options, it is crucial to select a system with relatively better efficacy and feasibility based on simulation outcomes.

The final question focuses more on the practical effectiveness in real-world applications, as opposed to the intrinsic efficiency of systems considered in the third question. Genetic pest control strategies often have eco-evolutionary feedback, which can greatly influence their outcomes, and such impacts are closely correlated with features of targeted species as well as the climate and terrain of targeted areas (Combs et al. 2023; Kim et al. 2023). The density growth curve, including the low-density growth rate, is a general description of population response to suppressive power. We showed that the shape of density growth curve and low-density growth rate significantly changed the performance of different strategies, suggesting that specific control methods should be applied according to species and environmental conditions. A system outcompeting another in 1 situation may be a lot weaker under alternative conditions. Therefore, performance of systems and suppressive power required for population elimination in specific species and environments should be fully considered when

Table 1 Properties of suppression systems

	Strategy	Ideal release ratio	Default release ratio	Minimum total release	Requires repressibility	Resources used for females	Requires sex-sorting	Requires outcrosses	Sensitive to overcompensation
Modified Alleles	Genetic SIT (Maselko et al. 2020; Upadhyay et al. 2022)/early RIDL	3.69	3.69	66	No/Yes	Yes	Yes	Yes/No	Yes
	Late-RIDL (Thomas et al. 2000)	0.96	0.96	20	Yes	Yes	Yes	No	No
	Early fsRIDL (Fu et al. 2007)	3.00	3.00	79	Yes	No	No	No	No
	Late-fsRIDL (Fu et al. 2007)	1.86	1.86	48	Yes	Yes	No	No	No
	Nix (Aryan et al. 2020; Lutrat et al. 2022)[a]	4.69/5.58	4.69/5.58	114/135	Yes	No	No	No	No
Gene Drives	**Self-sustaining drives**								
	Female sterile homing (Kyrou et al. 2018; Oberhofer et al. 2018)	<0.01	0.04	5.8	No	Yes	Yes	Yes	No
	Both-sex lethal homing (Oberhofer et al. 2018)	<0.01	1.18	45	No	Yes	Yes	Yes	Yes
	Self-limiting drives								
	Split female sterile homing (Champer et al. 2019; Yadav et al. 2023)	0.54	0.56	26	No	Yes	Yes	Yes	No
	Dominant female sterile homing (Yadav et al. 2023)	0.03	0.84	31	No	Yes	Yes	Yes	No
	RIDD (Chen et al. 2024)	0.03	0.3	12	No	Yes	No	Yes	No
	Drive Late-fsRIDL (Zhu et al. 2024)	0.03	0.38	13	Yes	No	No	No	No
	TADE drive								
	TADE suppression (Champer et al. 2020a)	<0.01	0.17	8.7	No	Yes	Yes	Yes	Partially
Gene Disruptors	**Autosomal-linked disruptors**								
	Female fertility disruptor	0.45	0.62	22	Yes	Yes	Yes	No	No
	Dominant female fertility disruptor[aa]	0.62/1.07	0.62/1.07	16/29	Yes/No	Yes	No	Yes	No
	Protected *dsx* Disruptor (Willis and Burt 2025)	0.04	0.22	11	No	Yes	Yes	Yes	No
	X-shredder (Galizi et al. 2014; Galizi et al. 2016; Malik et al. 2020; Meccariello et al. 2021)[b]	1.11/2.84	1.11/2.84	27/76	Yes/No	No	No/Yes	No/Yes	No
	Tra disruptor (Meccariello et al. 2024)	1.26	1.27	42	Yes	No	No	Yes	No
	Y-linked disruptors								
	Dominant female fertility disruptor (Tolosana et al. 2025)	0.03	0.03	3.5	No	Yes	No	Yes	No
	X-haplolethal disruptor (Haber et al. 2024; Lawler et al. 2024)	0.04	0.82	22	No	No	No	Yes	No

Release ratio refers to minimum drop size in each generation for population elimination. "Ideal" indicates ideal system performance parameters, and "Default" indicates default parameters. "Minimum total release" is for elimination with default parameters assuming the same release size each generation (See supplementary table S2, Supplementary Material online). "Requires repressibility" indicates need to repress expression of a construct element for homozygous release. "Resources used for females" indicates where female larvae consume resources during rearing. "Requires sex-sorting" assumes release of sterile/intersex females but not fertile females is allowed. "Requires outcrosses" indicates the need for rearing/crossing separate strains. "Sensitive to overcompensation" indicates possible overcompensation after release.
[a]Males with 3 Nix copies (from sex-sorting)/Half males with 3 Nix copies and half with 2.
[b]Homozygous/heterozygous release.

determining the optimal system if at all possible. This will often not be the case, though due to a lack of ecological data even in many relatively well-studied species. We proposed constant-population genetic load as a measurement of intrinsic system power in repeated releases and explored the suppressive threshold for different systems and conditions. Despite a few exceptions, constant-population genetic load shows good consistency and reliable predictive power for multiple systems, making it a potentially valuable alternative method to consider different systems independently of the specific scenarios that may lack available data.

Economic and labor resources are additional considerations for efficiency that we did not consider here because they would usually vary based on location, pest species, and potentially even specific systems. Especially important for these is the complexity of rearing facilities. For many drives, resources in the final release rearing batches are spent on females, reducing efficiency at this step (Table 1 "Resources used for females"). Mechanical sorting, either by sex or with fluorescence, can also add time, cost, and complexity to rearing (Table 1 "Requires sorting"—note that the tables assumed that intersex females may be released, but not sterile females, but this may sometimes be acceptable). For some systems, it is necessary to rear separate batches of insects and then cross them together (usually requiring sex-sorting) to generate the batch required for deployment, adding an additional consideration (Table 1 "Requires outcrosses"). Some of these can be mitigated in many of these systems by adding genetic complexity to improve efficiency, such as male-killing that takes effect to release batches. All these considerations interact with the costs of deployment per insect and setup/facility maintenance costs to provide a full assessment of resources required for a release program. For example, in our simulations, the release ratio required for RIDL is lower than that for late-fsRIDL under many conditions. However, late-fsRIDL can automatically filter out females, thereby reducing costs significantly. Early fsRIDL is less effective than late-fsRIDL per released male, but up to twice as many males could be generated for the same amount of food because females will be nonviable before they consume a significant amount of food. Another consideration is when a system has a higher release ratio threshold, but a shorter duration for population elimination, resulting in a smaller total release size. We provided an approximate estimate of the minimum total release size for population elimination for each system based on our data with default parameters (Table 1 "Minimum total release", supplementary table S2, Supplementary Material online), which shows that the minimum release ratio is not the optimal ratio for minimizing the total resource investment. Optimization of release patterns with dynamically changing ratio and area covered could further reduce the total release size, though other considerations (such as the advantages of being able to do a wider release using a lower release ratio) could also affect which system may be the best in a specific scenario. The comprehensive cost of each strategy, including research and development, material preparation, release implementation, as well as long-term management and monitoring, is an important factor for its application prospects.

Achieving a balance between efficiency and controllability has been a substantial challenge in the field of genetic pest control. There are instances where the long-term persistence of genetically modified elements within a population is undesirable, or where we may need the ability to halt the genetic intervention and reverse its impact. Previously proposed ideas including elements that disrupt the drive by releasing another gene drive (Oberhofer et al. 2020), or simply releasing wild-type individuals to dilute and eliminate drive alleles (Akbari et al. 2013). Such solutions are costly, and the release of a second kind of transgenic individual may cause further safety concerns, particularly if an initial release was associated with an unexpected problem. A repeated release strategy using a self-limiting system provides an alternative. Compared with SIT and late-fsRIDL, repeated releases of weak gene drives and disruptors require a much lower release ratio, thereby conserving resources. Meanwhile, in contrast to self-sustaining homing drive that spread from a single introduction, ceasing repeated releases will result in the allele frequency gradually diminishing for self-limiting systems (supplementary fig. S6a, Supplementary Material online). This strategy also renders low-cost genetic control feasible in some species where it is difficult to develop a homing system with high drive conversion rate and low fitness costs. Moreover, by selecting appropriate size and duration of repeated releases, this flexible strategy allows complete eradication of target population, maintaining it at low level, or full recovery after a short-term period of suppression (supplementary fig. S6b, Supplementary Material online).

Our panmictic models in this study are generalized and may be widely applicable to many different systems where offspring compete. However, other modeling approaches are possible. Previous research on spatial models indicates that chasing and long-term persistence of wild-type alleles could happen for self-sustaining suppression drive and hinder the elimination of population (Champer et al. 2021b; Zhang et al. 2024). This would be less likely for self-limiting systems released in a limited area, but the nature of migration from outside the area could still drastically change outcomes and increase required release sizes. Progress has also been made in building gene drive models considering interspecies interaction (Liu et al. 2023), disease transmission (Hancock et al. 2024), and temporal dynamics (Eckhoff et al. 2017). Investigating how repeated release strategies and self-limiting systems perform in such scenarios may yield important insights. Constant-population genetic load fills a gap in evaluating the power of repeated release strategies but does not perfectly predict suppression results in some systems. A better understanding of these could result in improved predictions.

It is well understood that released males that were reared in a laboratory environment have reduced mating competitiveness due to a variety of factors. These can include but are not limited to incomplete nutrition, lack of environmental cues, and effects arising from the wait between adult maturation and actual release. Release of eggs instead of adults is possible for many systems (traditional SIT and both sex RIDL do not allow this, and it may not be possible when sorting is required, depending on the sorting system, see Table 1), but it remains unclear if this actually provides greater overall efficiency due to greater egg mortality, which could very substantially exceed mortality of naturally laid eggs. We did not model such considerations, which would theoretically increase critical release sizes by a similar factor for all systems. However, some systems may have a greater advantage for reducing reared male fitness costs in real-world conditions, potentially giving them an additional advantage.

In summary, we performed a detailed comparison of genetic control strategies for population suppression, including SIT,

RIDL, several homing drive variants, TADE suppression drive, and several classes of gene disruptors. Many self-limiting strategies are highly feasible and represent large improvements over current state-of-the art systems, which may be particularly desirable if even more cost-effective suppression gene drives cannot be developed due to technical challenges or cannot be deployed due to ecological, social, or other considerations. Moreover, constant-population genetic load effectively evaluated the power of different repeated release systems, allowing it to serve as a promising predictor of population elimination when comparing strategies. However, ecological factors and rearing economics should also be considered when determining the most promising approach in specific scenarios.

Supplementary Material

Supplementary material is available at *Molecular Biology and Evolution* online.

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Data Availability

Raw and processed data reported in this article are available on GitHub (https://github.com/Hanyue22/Assessment-of-Self-limiting-Systems), along with all SLiM models and python scripts.

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