The promise and peril of CRISPR gene drives

Genetic variation and inbreeding may impede the propagation of gene drives based on the CRISPR genome editing technology

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Gene drives are selfish genetic elements that use a variety of mechanisms to ensure they are transmitted to subsequent generations at greater than expected frequencies. Synthetic gene drives based on the clustered regularly interspersed palindromic repeats (CRISPR) genome editing system have been proposed as a way to alter the genetic characteristics of natural populations of organisms relevant to the goals of public health, conservation, and agriculture. Here, we review the principles and potential applications of CRISPR drives, as well as means proposed to prevent their uncontrolled spread. We also focus on recent work suggesting that factors such as natural genetic variation and inbreeding may represent substantial impediments to the propagation of CRISPR drives.

Keywords:
Cas9; CRISPR; daisy drive; gene drive; homing endonuclease; segregation distortion

Introduction

The ability to genetically alter wild populations could allow us to address key issues in public health, conservation, and agriculture. However, spreading defined genetic alterations through wild populations is difficult. First, targeted genetic modification of any metazoan has until recently been a labor-intensive and difficult endeavor. Second, artificial genetic alterations often carry a fitness cost, reducing viability, fecundity, or mating prowess, and are thus selected against. Third, modifications must be efficient, gene-specific, and species-specific to act quickly (on the time scale of local pest invasions or disease epidemics) but exclusively on target species. Engineered genetic alterations must ensure their propagation in order to meaningfully impact the genetic makeup of a target population. Drawing inspiration from naturally occurring gene drives, selfish genetic elements that favor their own transmission to offspring, researchers are developing synthetic gene drives based on the powerful clustered regularly interspersed palindromic repeats (CRISPR) genome editing technology.

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Abbreviations:
Cas9, CRISPR-associated protein 9; **CRISPR,** clustered regularly interspersed palindromic repeats; **gRNA,** guide RNA; **HEG,** homing endonuclease gene; **ITD,** immune-to-drive; **NHEJ,** nonhomologous end joining; **PAM,** protospacer adjacent motif.

Natural gene drives

Violations of Mendelian segregation, variously called gamete selection, segregation distortion, or meiotic drive [1], result in an offspring population with unequal representation of parental alleles, biased in favor of the distorting allele. These "gene drives" change the gene frequency of a population in the absence of natural selection. *Segregation Distorter* (*SD*) in *Drosophila melanogaster* [2], *spore killer* (*Sk*) elements in ascomycetes [3], *t* haplotypes in *Mus musculus* [4], *MEDEA* in the red flour beetle, *Tribolium castaneum* [5], and cytoplasmic-male sterility (CMS) in plants [6] and in arthropods with *Wolbachia* [7] are well-studied naturally occurring gene drive systems.

The evolutionary genetics of naturally occurring gene drives [6, 8–11] shows that they spread rapidly through randomly mating populations in spite of deleterious effects on fertility or viability. These deleterious effects, however, favor

the evolution of drive suppressors which can theoretically arise anywhere in the genome [12]. Given that naturally occurring drives can propagate through a population despite negative effects on fertility or viability, it has been suggested they might provide a way to spread a deleterious trait through a population, reducing its size and growth rate [13–16].

Nuclease-based gene drives

Homing endonuclease genes (HEGs) are a class of naturally occurring gene drives. HEG-encoded HEs recognize and cleave a specific sequence in the corresponding region of the chromosome(s) that lacks the HEG. The resulting double-strand break (DSB) is repaired via homologous recombination with the HEG-containing chromosome, thereby copying the HEG to the damaged wild-type chromosome. This highly efficient [17] process is referred to as "homing" [18].

In 2003, Austin Burt proposed gene drives based on HEGs as a means to modify natural populations [19]. The first synthetic HEG drive used the *Saccharomyces cerevisiae* I-*Sce*I HE in the malaria mosquito *Anopheles gambiae* [20]. This study showed that a synthetic HEG-based drive could be efficiently propagated in a laboratory population, but it required an engineered I-*Sce*I recognition site in the target locus. Further work with natural HEs produced a sex ratio distortion system for *A. gambiae* based on I-*Ppo*I, which selectively cleaves X-linked ribosomal DNA sequences resulting in mostly male offspring due to shredding of the male X chromosome [21], an approach also recently implemented with CRISPR [22]. Further development of HEs has proceeded slowly due to the need for time-consuming protein engineering to alter their sequence specificity [23, 24]. Gene drives based on TAL effector and zinc finger nucleases, which have more easily altered sequence specificities, contain repetitive elements, and so suffer from instability due to recombination [25]. The limitations of engineered HEG drives could be overcome through the use of CRISPR-based genome engineering.

CRISPR gene drives

CRISPR is an RNA-guided genome editing tool based on a bacterial system for defense against phage infection [26]. Simple CRISPR editing requires two components: a nuclease and a guide RNA (gRNA). The nuclease, commonly Cas9 from *Streptococcus pyogenes* (SpCas9), is targeted to a specific region of the genome by the gRNA, a small RNA containing a region called the spacer, consisting of 17–20 nt [27] homologous to a region of interest in the target genome termed the protospacer. To be recognized as a target, the protospacer must lie directly upstream of a short DNA sequence called the protospacer adjacent motif (PAM, NGG for SpCas9). Cas9 recognizes the PAM, denatures DNA adjacent to the PAM, and attempts to form a gRNA:DNA hybrid. If a stable hybrid is formed, Cas9 remains associated with the site long enough to generate a DSB. If a suitably stable gRNA:DNA hybrid is not formed, Cas9 dissociates and resumes its target search [28].

In a CRISPR gene drive, a transgenic organism carries a drive cassette, encoding Cas9, and a gRNA with or without additional cargo flanked by regions of homology to the locus of interest. In most cases, one allele of the targeted locus is converted to a drive allele through homologous recombination with the vector bearing the drive cassette. The second wild-type allele is then converted to a drive allele by Cas9 and gRNA expressed from the initial drive allele (Fig. 1). This process is identical to the phenomenon of homing.

The first CRISPR drive, the mutagenic chain reaction (MCR), was implemented in *D. melanogaster* [29]. Gantz and Bier used a drive consisting of Cas9 and a gRNA flanked by regions of homology to the X-linked recessive *yellow* (*y*) gene, enabling phenotypic screening via body color. In accordance with the expected super-Mendelian inheritance of the drive, y^- females were recovered in the F_1. Crossing of y^{MCR} females to y^+ males yielded a y^- phenotype in 95–100% of the F2 progeny as opposed to the expected Mendelian proportion of 50%. These data indicate that copying of a drive via HDR is highly efficient in *D. melanogaster*, though drives targeting *y* are less efficient than initially reported (59–62% vs. 95–100%) [30, 31]. A similar CRISPR-based drive in *S. cerevisiae* disrupted the *ADE2* locus via HDR-mediated integration >99% of the time [32]. CRISPR-based drives have also been tested in *Anopheles stephensi* [33] and *gambiae* [34], where transmission efficiency is >91%. Together, these studies provide proof-of-concept that CRISPR gene drives can be efficiently propagated through laboratory populations.

Impediments to CRISPR gene drive propagation

Laboratory populations differ from wild populations, which display features that could slow or halt the propagation of a CRISPR drive. In natural gene drives, any genetic alteration that suppresses the drive is favored by selection, leading Charlesworth and Charlesworth to posit that "a newly evolved distorter system will be inefficient" [12]. Here, we discuss features of natural populations that pose obstacles to efficient propagation of CRISPR drives and how they might be surmounted.

Variation in target sequences

Cas9 and other CRISPR nucleases are sequence-specific by virtue of association with a gRNA and so natural variation, such as single nucleotide polymorphisms (SNPs), in a wild population could impair or eliminate recognition and subsequent cleavage. Variation could also be introduced by nonhomologous end joining (NHEJ)-mediated repair of Cas9-generated DSBs and subsequent modification of the target sequence via indel. As a deleterious allele drives through a target population, preexisting drive-resistant variants enjoy a fitness gain proportional to the fitness cost of the deleterious allele being driven. SNPs can occur in three distinct regions of the target site: the PAM, seed, or outer protospacer. The PAM

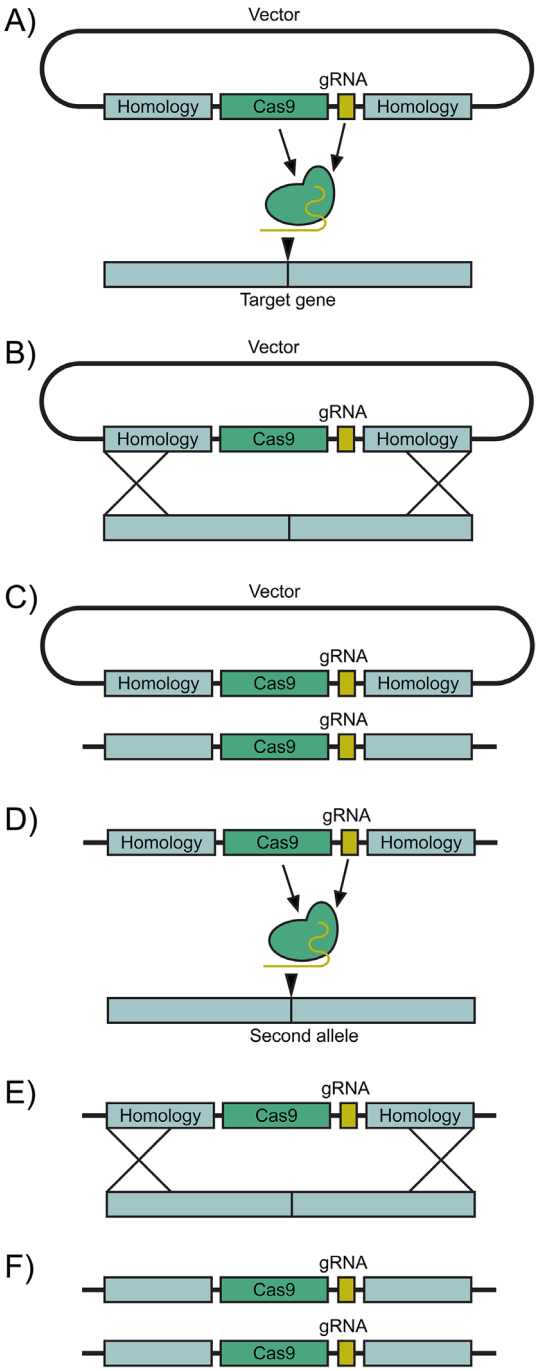

Figure 1. Schematic representation of a basic CRISPR gene drive**. A:** A vector encoding Cas9 and a gRNA flanked by homology arms is introduced into the organism of interest via transgenesis. The gRNA targets Cas9 to DNA, where it generates a DSB in the gene of interest. **B:** Homologous recombination between the homology arms of the vector and the DNA flanking the Cas9-induced DSB occurs, resulting in **C:** copying of Cas9 and the gRNA into the disrupted locus. **D:** Cas9 and gRNA produced from the drive allele cleave the remaining wild-type allele, generating a DSB. **E:** The DSB in the second wild-type allele is repaired via homologous recombination between the drive and wild-type alleles, resulting in **F:** homozygosity for the drive allele.

is required for target site recognition and SNPs that alter the PAM are likely to eliminate Cas9 recognition and cleavage, though SpCas9 tolerates additional PAMs, such as NAG, in vitro [35, 36]. The seed region, generally thought to consist of the five bases immediately upstream of the PAM, is the nucleation point for the formation of the gRNA:DNA hybrid [37] and critical for stable Cas9 binding.

The outer region of the protospacer, beyond the seed, is more flexible, so a single SNP would be unlikely to strongly impact Cas9 recognition and cleavage, though this may depend on the identity and position of the changed base [35, 38–41]. For instance, Hsu et al. [35] performed a comprehensive in vitro analysis of the effects of single gRNA:DNA (rN:dN) mismatches on Cas9 cleavage with gRNAs targeting the human *EMX1* gene. Positions 8–15 of the spacer were, on average, moderately sensitive to mismatches, though the severity of the negative impact on cleavage was dependent on the gRNA and DNA base mismatch identity. For example, an rA:dC mismatch at position 15 severely impaired cleavage, while an rA:dG mismatch at the same position had no effect. Positions 5 and 6, outside of the canonical seed region, were highly sensitive to mismatches. Moreover, some mismatches in the outer protospacer region increased cleavage efficiency, suggesting that some variants might increase the prevalence of off-target effects, a result confirmed by an independent study [41]. Work with Cpf1, another CRISPR nuclease, has found similarly variable effects of gRNA/DNA mismatches on cleavage [42]. Can the tolerance of Cas9 or other CRISPR nucleases to gRNA:DNA mismatches be predicted? It has been suggested that mismatches that generate wobble gRNA:DNA base pairs are well tolerated by Cas9 and Cpf1 [42, 43]. However, such wobble pair-generating mismatches are not uniformly innocuous: introduction of an rU:dG wobble pair at position 15 of various *EMX1* gRNAs resulted in substantial decreases in cleavage [35]. We propose that, if data on genetic variation for the target population is available, gRNAs should be tested for function against variant targets in vitro prior to use in a drive construct.

Modeling the impact of genetic variation on CRISPR drives

Using four geographically distinct populations of the flour beetle *T. castaneum*, we modeled the effects of standing genetic variation on CRISPR gene drive spread [44]. We found SNPs in all three of the gRNA regions discussed above. As mutations in the PAM and seed regions of gRNAs would be predicted to abrogate Cas9 binding and cleavage, we considered such alleles immune-to-drive (ITD). We determined that an ITD at a frequency as low as 1% rapidly removed a CRISPR drive ($\leq$10 generations) carrying a highly deleterious genetic modification designed to suppress a population. Our modeling also showed that low-frequency ITDs also removed a drive with a moderate fitness cost, albeit more slowly. Notably, the prevalence of the ITD increased until the drive was effectively eliminated from the population in all cases, consistent with selection favoring drive suppressors in natural systems. This moderate fitness cost scenario is akin to a drive bearing a desirable modification of a nonessential locus, such as the insertion of antimalarial genes

into the *Kh* gene, which causes white eyes when homozygously disrupted, in *A. stephensi* [33]. The relationship between fitness cost, drive elimination, and ITD prevalence in our model is illustrated in Fig. 2. Similar conclusions regarding the evolution of resistance were reached by Unckless et al. [45] and Noble et al. [46]. Interestingly, recent experimental work has also shown variable drive efficiency in different genetic backgrounds despite identical target sequences. Using *D. melanogaster* as a model, Champer et al. [31] tested the efficiency of two drives in diverse populations from around the world. The efficiency of a single drive varied from $40 \pm 4\%$ to $63 \pm 3\%$, indicating that background genetic variation also has a substantial impact on drive propagation.

ITDs may also be generated when NHEJ-mediated repair of CRISPR-induced DSBs generates indels, rendering the target refractory to further editing. Such induced ITDs are frequently generated in *A. stephensi* [33] and *D. melanogaster* [31] drive systems, both in the germline and embryonic soma, through the action of maternal Cas9. Induced ITDs are thus likely to represent a substantial impediment to efficient drive propagation that is additive with standing ITDs.

Overcoming genetic variation

How might a CRISPR drive overcome genetic variation, either natural or induced? One strategy involves the use of a drive carrying multiple gRNAs, targeting a conserved region of an essential gene [47]. The concept behind this approach is that the gene would need to accumulate and/or already possess a large number of SNPs to be resistant to all gRNAs, making the evolution of genetic resistance less likely. Furthermore, variants in essential genes are more likely to be harmful than neutral, and therefore be rare or absent owing to purifying selection. Lastly, CRISPR-induced indels would disrupt an essential gene and therefore be quickly eliminated from the population. However, if NHEJ repairs DSBs in a way that does not impose a significant fitness cost, resistance may still be induced. Indeed, Unckless et al. concluded that if 0.1–1% of all NHEJ-generated ITDs were able to persist in a population, resistance would still develop [45]. It is thus important to reduce the incidence of such ITDs. Recent work has suggested that the choice of promoter driving Cas9 expression influences the rate of induced ITD formation [30]. Using the promoter of the germline-restricted gene *nanos*, instead of that of *vasa*, markedly reduced the rate of NHEJ-induced ITD formation in a drive targeting nonessential eye color genes due to reduced leaky somatic Cas9 expression. The efficiency of *nanos-Cas9* drives was also increased by the addition of a second gRNA.

We note that ITDs could perhaps provide a "brake" on CRISPR drives, allowing them to persist in a population for many generations, depending on their fitness cost, before they are eventually removed by natural selection. Models show that there is a lag period during which the prevalence of the drive is high and the prevalence of the ITD is low [44–46]; thus, populations can still be modified for several generations before the frequency of the drive plummets. This braking approach may be safer than the use of multiple gRNAs, which increases the potential for off-target effects and could be more difficult to control in a wild population.

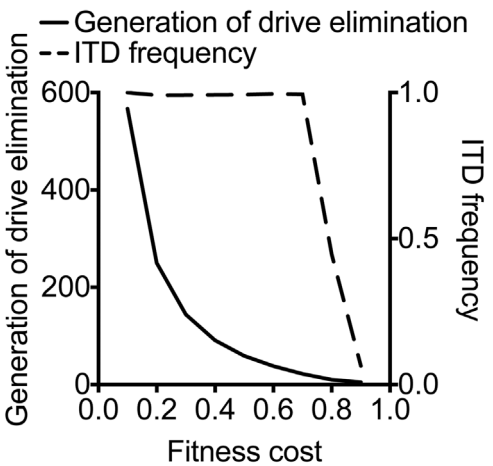

Figure 2. The relationship between fitness cost, generation of drive elimination, and ITD frequency. We modeled the generation of drive elimination and ITD frequency with varying fitness costs. The drive was assumed to be highly efficient ($c = 0.95$) and near-dominant ($h = 0.98$), and inbreeding was not considered.

Inbreeding

Another potential limitation of natural and CRISPR drives, which is more difficult to address, is inbreeding. Wild populations of mosquitoes [48–50] and mice [51, 52], proposed targets of CRISPR gene drives, display substantial levels of inbreeding. Practically, this means that there is a reduced likelihood that organisms carrying the drive will mate with the target wild population. We found that moderate inbreeding removed a drive with a high fitness cost from a population more rapidly than a rare ITD and that the effects of a rare ITD and mild inbreeding were synergistic. Together, they greatly accelerated the loss of a drive with a moderate fitness cost, with drive elimination occurring at generation 39 rather than 91. The effect of inbreeding on time to drive elimination and ITD frequency in our model is presented in Fig. 3. An independent modeling study also indicated that increased inbreeding is favored when a drive with a high fitness cost is introduced into a population [53]. Of note, inbreeding may be reduced by swarm mating [54], observed in some mosquitoes [55].

An additional mating-related concern is the mating fitness of the drive-bearing organisms. A study measuring the efficacy of a dominant-lethal allele in suppressing a wild population of *Aedes aegypti* indicated that the mating competitiveness of the released males was ~3% of that of wild males [56], a severe disadvantage. Mitigating such a mating disadvantage requires release of an enormous number of drive-carrying individuals. For instance, in the above-mentioned work, in a study area of 54 hectares, it was estimated that ~25,000 males would need to be released into each hectare on a weekly basis to ensure that 50% of the matings occurring are between released males and wild-type females, for a weekly release of ~1.35 million individuals. However, a 50% mating fraction may be an overestimate, as a previous study reported suppression at a 12% mating fraction [57]. A 12% mating fraction for population

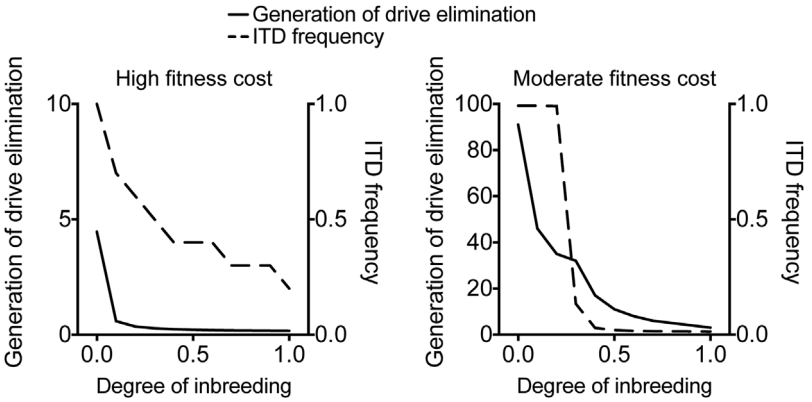

Figure 3. The impact of inbreeding on drive spread. We modeled the generation of drive elimination and ITD frequency with high ($s = 0.8$) and moderate ($s = 0.4$) fitness costs and varying degrees of inbreeding. Other parameters were as in Figure 2.

suppression still requires release of ~6,000 individuals per hectare (~324,000 total).

Safeguarding CRISPR drives

A major concern regarding the use of gene drives is that they may spread unchecked due to accidental release of a small number of drive-bearing organisms from the lab or as a consequence of planned large-scale releases. It is therefore of great importance to design and implement strategies to prevent uncontrolled drive spread. Here, we discuss drive safeguards that have been implemented or proposed and put forth an additional mechanism for controlling drive spread.

Molecular confinement

A major concern with CRISPR drive research is that accidental release of even a few individuals carrying a complete drive (i.e., containing cargo, Cas9, and a gRNA) could alter a wild population. Physical containment of such organisms is subject to human error and it has been recommended that studies of drives integrate molecular fail-safes [58]. To this end, DiCarlo et al. implemented a molecular confinement approach in *S. cerevisiae* [32]. Cas9 was expressed from a plasmid maintained via metabolic selection while the drive cassette, encoding a gRNA, was integrated at the target chromosomal locus. Because Cas9 and the cargo, including the gRNA, were not linked and the cargo requires Cas9 to be driven through a population, the drive would be rapidly eliminated from a wild population in which Cas9 is not present due to loss of the Cas9-bearing plasmid in the absence of appropriate selection. Molecular confinement may be particularly important in insect species where escape via flight is a significant risk. To this end, it has been suggested that the non-driving Cas9 gene and the drive, containing cargo and a gRNA, be inserted at distinct genomic loci. In the laboratory, homozygosity for both components of the drive could be maintained via inbreeding. However, in the event of an accidental release of a small number of drive organisms into the wild, the drive components would rapidly segregate away from one another by mating with wild-type individuals, rapidly collapsing the drive. This type of safeguard changes the drive dynamic: the drive-bearing, lab-reared organisms would need to be released in large numbers for efficient drive propagation.

The principle of molecular confinement has recently been expanded in a proposal for daisy-chain gene drives, or "daisy drives" [59], wherein the drive components are separated into a linear chain, each component of which must be present for the drive propagation. The simplest daisy drive is a two-component system. The drive cassette (component "A") only propagates through a population when an unlinked, non-driving component "B" is also present. This strategy has been implemented in *S. cerevisiae* [32] as described above, wherein component A is the drive cassette bearing homology arms flanking a gRNA and component B is the unlinked, plasmid-borne Cas9. While this is an effective safeguard for preventing drive spread in the case of accidental release, it is unlikely to be an effective way to alter a population unless released in large numbers. To overcome this, Noble et al. proposed adding additional components to the daisy chain [59]. In a three-component daisy drive, non-driving component C drives component B, which in turn drives component A, the drive cassette (Fig. 4A). This addition of a single additional component ensures that the drive persists for a longer period in the population but is still eliminated after many generations. While the daisy drive theoretically provides a means by which to ensure that a drive is removed from a population after it has had its intended effect, a potential hazard exists. Any recombination event that puts a gRNA or gRNAs from an upstream component of the drive (for instance, C) into linkage with a downstream component (for instance, B) would create a self-sustaining drive that would in principle be capable of uncontrolled spread in the absence of resistance. This might be avoided by using different promoters to express gRNAs in each element of the daisy drive.

A proposed further iteration of the daisy drive is the daisyfield drive [60]. In contrast to the daisy drive, where a linear chain of components is required for drive (for instance, C drives B drives A), the daisyfield drive uses many copies of a single non-driving component B to induce propagation of a drive cassette (component A) through a population (Fig. 4B). In this case, component A consists of Cas9 along with a cargo of interest, while component B would be a gRNA targeting the locus to be disrupted. The key to the daisyfield drive is that many copies of component B are scattered throughout the genome. This is achieved by insertion of gRNAs into high-copy number repetitive elements. Progeny from a mating between a daisyfield and a wild-type organism would result in a halving of gRNA dosage due to inheritance of wild-type repeats and homozygosity for the drive (Fig. 4B). Subsequent matings would halve the number of gRNA-containing loci until none remain, and the drive cassette would then be inherited in a Mendelian fashion. The daisyfield drive circumvents the problem of recombination between components of a standard daisy drive and provides, in essence, a drive self-destruct system. However, simultaneous insertion of a sequence of

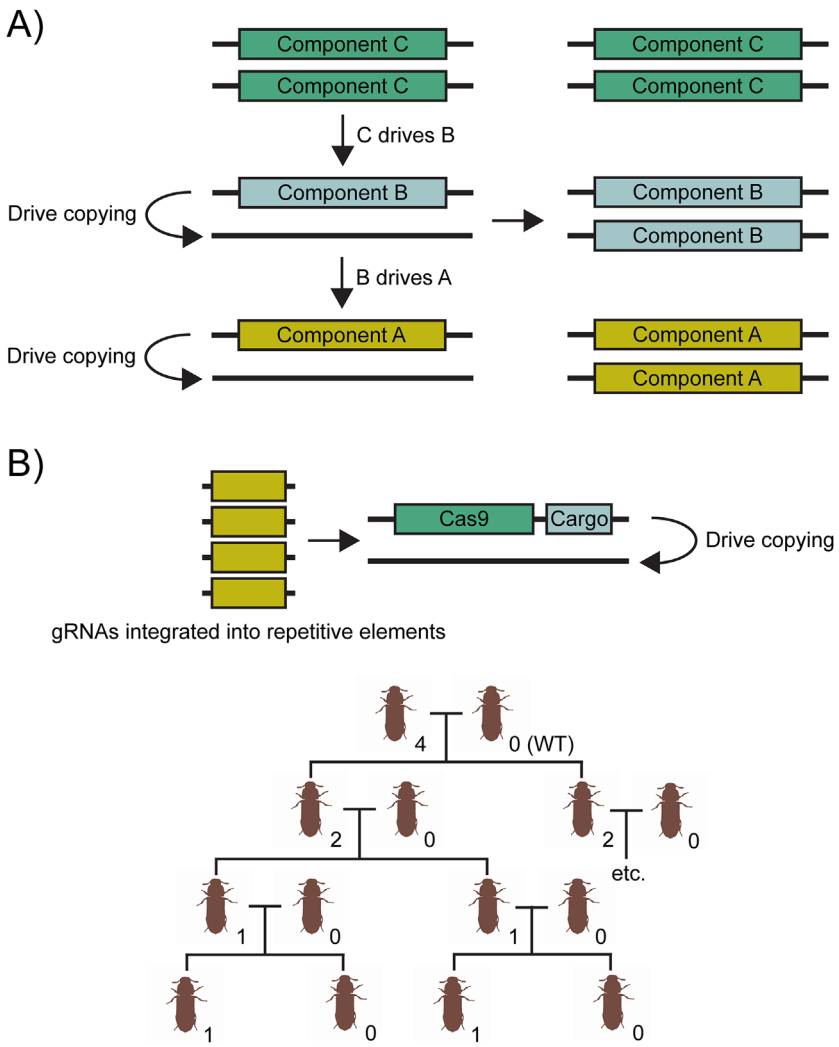

Figure 4. Various iterations of the daisy drive. **A:** A standard daisy drive with three components. A non-driving component, C, drives B, which in turn drives A, resulting in homozygosity for A and B when C is present. **B:** A simple daisyfield drive with four gRNAs, each of which drives the copying of the Cas9/cargo cassette. Also shown is a simple pedigree illustrating the inheritance of gRNA-bearing repetitive elements, assuming that each drive-bearing individual mates with a wild-type individual. The number next to each organism indicates the number of gRNAs it carries.

interest into many loci throughout the genome has not been documented and may be extremely challenging. The geographic spread of both standard daisy and daisyfield drives could be limited by combining the drive with underdominance in a daisy quorum drive [61]. It is important to note that, at this point in time, daisy-variant drives are strictly theoretical. In addition to the technical challenges of implementing such systems in the lab, their complexity might preclude them from field use due to regulatory restrictions.

Overwriting

A strategy has also been developed to limit the spread of a drive that has already been released. This strategy involves overwriting the existing drive with another drive that restores the target locus to wild-type functionality while rendering the restored allele resistant to future editing. The overwriting drive carries the standard cargo of Cas9 and a gRNA alongside a recoded version of the target gene. Recoding of the target gene via silent point mutations renders the gene resistant to future drives targeting the same region while maintaining the amino acid sequence of the encoded protein. Thus, Cas9 would be unable to recognize and cleave its initial target site and the drive can no longer be copied. This approach has been shown to be effective in *S. cerevisiae* [32] and is theoretically applicable to any species. From a regulatory perspective, it is important to note that the overwritten organisms would still be transgenic due to the presence of the overwriting drive in the population.

Removal

Strategies to remove CRISPR drives from a population have also been proposed. In one such approach, termed elements to reverse the autocatalytic chain reaction (ERACRs) [62], organisms carrying gRNAs targeting sequences flanking the drive to be removed are released. Importantly, ERACR-carrying organisms do not encode Cas9 and rely upon Cas9 protein supplied in trans by mating with drive organisms. Upon crossing, the drive-flanking gRNAs direct drive excision and replacement with the ERACR cassette. A similar strategy, termed Cas9-triggered chain ablation (CATCHA) [63], involves Cas9 disruption of its own coding sequence.

We propose another approach that would allow near-complete removal of an initial drive, though this approach would require forethought in the design of the first drive. In this approach, an initial drive cassette is constructed such that recognition sites for a site-specific recombinase, such as *loxP* for Cre or *FRT* for Flp [64], flank the cargo contained between the homology arms. If reversal is desired, a second drive carrying a cargo of the appropriate recombinase is released, in principle leading to excision of the initial drive cassette and cessation of its spread. Recombination between *loxP* or *FRT* sites leaves a scar consisting of a single copy of the recognition sequence, and so a small modification would remain in the targeted locus following drive excision. The *loxP* site is 34 bp and so its insertion may cause various alterations in gene sequence, including a frameshift that might lead to introduction of a premature stop codon. If the initial drive was contained within a gene with a high fitness cost when

disrupted, alleles disrupted by a *loxP* site following excision of the initial drive would likely be rapidly eliminated from the population. If the gene targeted by the initial drive had a more moderate fitness cost, the *loxP*-disrupted allele could remain in the population indefinitely.

We note that overwriting and reversal drives described here are subject to the same caveats in terms of genetic variation, inbreeding, and/or mating competitiveness discussed above for initial drives. Modeling of various drive control strategies has also suggested unexpected behaviors in certain situations [65] and so more work is necessary to understand if such safeguards will be effective.

Potential applications of CRISPR drives

CRISPR drives could, in theory, address numerous issues affecting human health, conservation, and agriculture. Here, we summarize potential applications for CRISPR drives and how they might be implemented.

CRISPR drives and disease control

Diseases transmitted by insects constitute an immense public health burden. In 2015, there were an estimated 212 million new cases of malaria and an estimated 429,000 malaria deaths, most of them children under 5 years of age [66]. As many as 400 million people are afflicted by dengue each year, making it the most common arthropod-transmitted disease [67]. Numerous other diseases, including zika, yellow fever, chikunguya, Lyme disease, and trypanosomiasis are transmitted by insects. In total, vector-borne diseases account for ~17% of all infectious diseases and cause upwards of a million deaths each year [68]. It has been postulated that CRISPR drives might be a means by which to control or eradicate such diseases [47].

In accordance with the tremendous toll of insect-borne diseases, in particular those transmitted by mosquitoes, on human health, much thought has been devoted to the idea of gene drives as a tool for modifying mosquito populations as a means to control the spread of diseases including malaria and dengue [16, 69–72]. In this case, the experimental side has followed the theoretical, with the first example of a HEG-based gene drive being implemented in the malaria mosquito *A. gambiae* [34]. Furthermore, two disease-vectoring mosquito species, *A. stephensi* and *gambiae*, were the targets of initial CRISPR drives targeting [33, 34]. Such drives were very effective in laboratory populations; however, their efficacy in wild populations remains to be determined.

CRISPR drives and conservation

Global travel and commerce have thoroughly broken down the biogeographic barriers that for eons isolated the flora and fauna of the continents from one another. A significant consequence of this has been the introduction of non-native species to new ecosystems. Such invasive species represent a multifaceted threat. At a biological level, invasive species reduce biodiversity, as in the case of the red imported fire ant in the United States [73], through a number of mechanisms [74] and can lead to species extinction [75]. Invasive species also impose a remarkable financial burden: in 2005, the cost of invasive species in the United States was estimated to be $120 billion as a result of environmental damage, livestock pests, human disease, and crop loss [76]. Control of invasive populations using CRISPR drives may thus be ecologically and economically beneficial; however, as discussed by Esvelt et al., there exist the problems of such a drive spreading from the invasive target population to closely related species, and the spread of the drive from the invasive target population back to its native habitat [47].

CRISPR drives and agriculture

Genetic modification is commonplace in agriculture. A well-known example is the introduction of Cry proteins from *Bacillus thuringiensis* into maize, creating bio-insecticidal *Bt* corn. However, *Bt* resistance has become remarkably common: at least 27 insect species have shown resistance in the lab [77], and some insect species, including the diamondback moth [78], and Western corn rootworm [79], have shown field-evolved resistance. Provided that the genetic basis of *Bt* resistance is known in a population of interest, a CRISPR drive could be deployed to restore sensitivity. Individuals bearing the re-sensitizing drive would be released into *Bt* cornfields to mate with resistant insects and increase the susceptibility of the population to the existing control method (*Bt*). Such a re-sensitizing drive could also be used in cases where pesticide or herbicide resistance has developed, such as with the many weeds now resistant to glyphosate [80].

CRISPR drives might also be used to reduce pest population size. For instance, various *Tribolium species*, including *T. castaneum*, which we used for modeling of resistance to CRISPR drives [44], have long been recognized as pests of stored food products, with various species feeding on over 100 different foods and frequently the most common insects found in flour mills [81]. Use of a CRISPR drive that reduced *T. castaneum* or *T. confusum* population size, perhaps by targeting reproduction or general fitness, would be of economic benefit by preventing the destruction of significant quantities of stored crops. *Tribolium*, being confined to storage facilities, represents a special case for pest elimination with drives. Most pests, such as the Western corn rootworm, are dispersed across large agricultural areas making them more challenging to target. Suppressing such a pest would likely require repeated releases of a substantial number of drive-carrying individuals over a large geographic area.

Conclusions

Alteration of wild populations with CRISPR drives is truly a new frontier in biology. Without any informative precedent as to how large-scale genetic alteration of a natural population might affect an ecosystem, it is essential that all proposed drives include robust safeguards to prevent both unintentional drive spread due to accidental release of organisms

from the lab and uncontrolled drive spread following planned release. Moreover, as advocated by Esvelt et al. [47], public discourse and societal consent are essential as gene drives mature to the point that they might be used in the field. Indeed, there is precedent for societal unease with modification of natural populations: a well-regarded and rigorous study on the use of the sterile insect technique for mosquito control in India ground to a halt when it was alleged that the project was a covert biological warfare operation by the United States [82]. The rapid pace of CRISPR drive research spurred the National Academies of Sciences, Engineering, and Medicine of the United States to conduct a project aimed at generating recommendations for responsible research on and use of such drives. We encourage all drive researchers to read the resulting report [83], which encompasses drive strategies and safeguards, issues of public trust, societal engagement, and regulation. Such a multifaceted strategy of further research, public dialogue, and regulatory formulation is necessary before any drive can be considered for release.

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