JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

Center for Health Security

GENE DRIVES: PURSUING OPPORTUNITIES, MINIMIZING RISK

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EXECUTIVE SUMMARY

In the future, it may be possible for humans to manipulate entire ecosystems with little continuous input through the use of emerging biotechnologies. Gene drives are one such technology, themselves derived from nature, with the potential to make directed and highly specific modifications to the genetics of entire populations, with repercussions for whole ecosystems. While there has been extensive public analysis of the risks and benefits of gene drives for the control of malaria, which will likely be their first practical application, this report anticipates the world after that initial application. Here we make recommendations for the responsible governance of gene drives as a used and normalized tool.

GENE DRIVES: PURSUING OPPORTUNITIES, MINIMIZING RISK

EXECUTIVE SUMMARY

What is a gene drive?

Gene drives are a type of genetic element capable of biasing inheritance patterns of a targeted host species, which results in the gene drive's improving the chances of its own inheritance in subsequent generations. While gene drives occur naturally, they have more recently been cultivated as a powerful biotechnology that can be used to facilitate the manipulation of host population genetics. Gene drive systems, though varied in their molecular approaches, can be designed for 2 ultimate goals: (1) suppression of the target population, or (2) genetic alteration of most of the host population to express a desired trait.

Driven mostly by biotechnological advances harnessing the CRISPR-Cas9 system, it has become possible to create engineered examples of the naturally occurring gene drive phenomenon. These new engineered gene drives have shown potential to spread targeted genetic changes through wild populations. This capability is radically different from previous genetically modified organisms (GMOs) released into the environment, which would normally be expected to live alongside the unmodified wild organism, or even to die out.

What are the risks associated with gene drives?

With such a sweeping ability to alter the genetics of entire populations, the use of gene drives carries risks. Some of these risks have an extremely low probability of occurring, but they dominate the conversation surrounding gene drive use because of potential catastrophic consequences. For example, concerns about agricultural applications of gene drives appear to be the foundation of many arguments against gene drive research because of worry about potential adverse large-scale impact of gene drives for some species. Such an outcome would damage global food security and create severe economic damage. However, given the success of GMO crops and the reliability of traditional selective strain farming techniques, gene drives are a suboptimal choice to modify agricultural products.
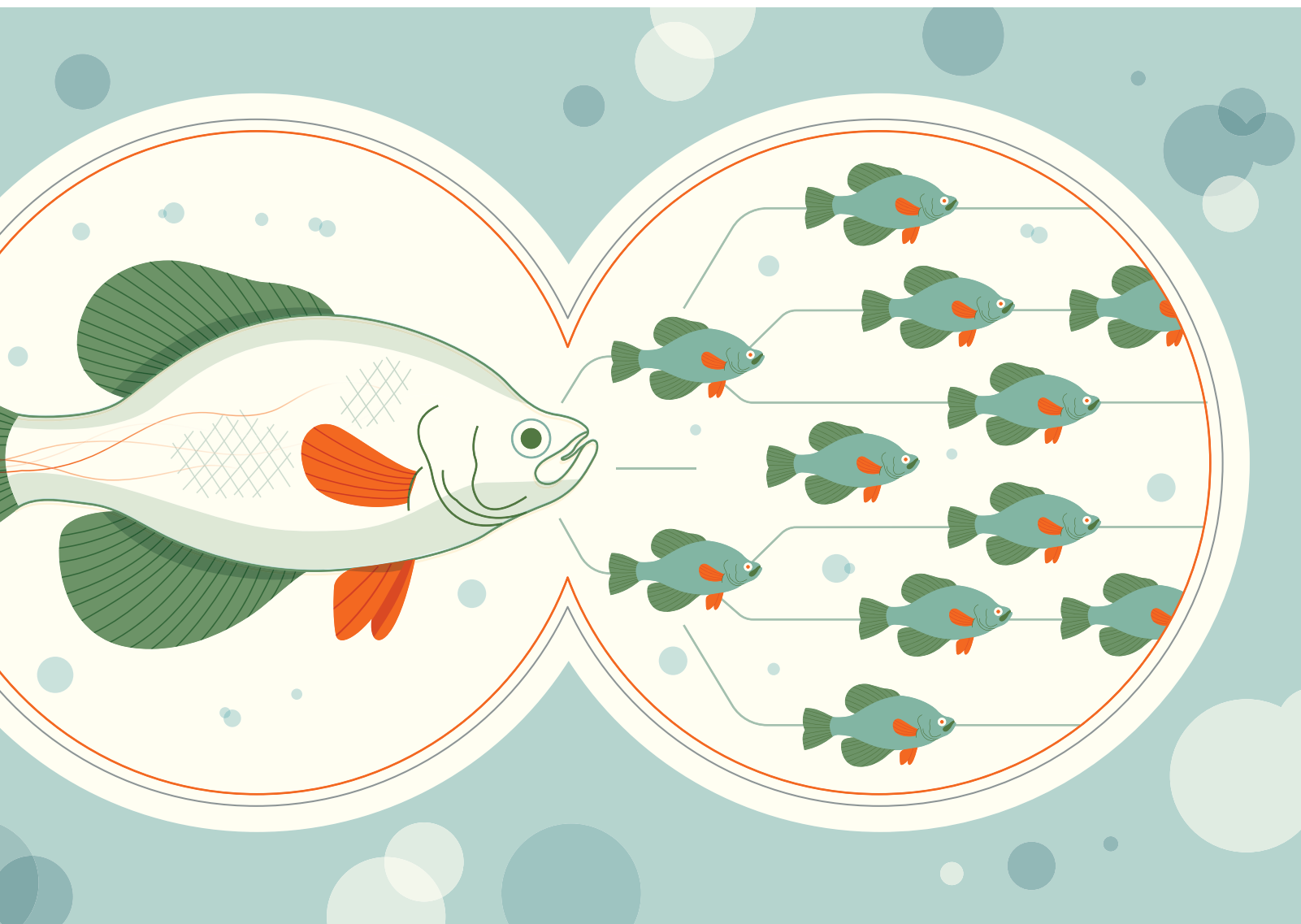
While it is unlikely that gene drives will be used by the agricultural industry, strong and explicit legislation is needed to ensure that potentially damaging applications of gene drives are carefully assessed and regulated if they are to be used. Since this technology has yet to be successfully validated in the field, it also carries with it a fear of the unknown. The complexity of the underlying science of gene drives further complicates efforts to communicate about them. Until the legitimate risks and benefits of gene drives can be better communicated to the wider public, this technology will face challenges around acceptance.

Some of the fear surrounding gene drive use is misplaced, but there are several legitimate risks associated with gene drive use. These risks can involve complex, long-range consequences in the evolution and ecology of the altered host species. A deployed gene drive may have off-target effects, could cross between closely related species, or could simply not work as it was intended. While these risks can never be fully characterized or reduced to zero, they can be deeply understood through further research and managed with appropriate regulations.

EXECUTIVE SUMMARY

How might gene drives be used?

This report anticipates that the first gene drive release will be an outgrowth of current research by the nongovernment organization Target Malaria to control malaria spread by introducing a gene drive into mosquitoes. This outcome seems highly probable due to the immense burden of malaria to human health, malaria's resistance to conventional control efforts in the developing world, and the extensive work by Target Malaria to achieve transparency, stakeholder acceptance, and local governance approval. The World Health Organization recently stated that it may not be possible to eliminate malaria given current challenges, making gene drives all the more appealing as a control mechanism in the near future. Given the work done by Target Malaria, it is fair to anticipate that if such a gene drive is to be used in this setting, it will be both safe and effective at controlling malaria in sub-Saharan Africa, with minimal off-target or unintended impacts on the environment.

Rather than further consider the malaria use case, this report focuses on the anticipated challenges that will emerge after the malaria use case has provided a clear proof of principle of the technology and its benefits. With this proof of concept, dozens of advocacy groups will feel encouraged to move forward with their own gene drive research and deployment goals, including those for whom mosquitoes are not the target species, and beyond malaria management as the purpose of the gene drive. At this time, challenges surrounding biological interaction between 2 or more gene drives, cross-border spread, and use in agriculture should be considered, and policy and regulation will be needed to safely develop and deploy these technologies.

Recommendations

This study analyzed the current state of gene drive technologies, the ways in which they might be deployed in the field, and the state of regulatory policy governing their development. Elements of legislation should include monitoring, risk assessment, tiered registration, and the requirement for a reversal drive to be developed and ready to deploy if needed at the same time the original drive is deployed.

This report makes the following policy recommendations:

1. Governments should create national tiered registry systems for gene drive research and development. The closer a group is to the release of a gene drive, the more information would be required. An international tiered registry system should be a long-term goal for the field.
2. Regulatory bodies tasked with overseeing gene drives should evaluate each drive system on a case-by-case basis, because of the wide range of intended impacts, means of delivery, and potential benefits.
3. Governments should avoid full moratoriums on gene drive research but still mandate appropriate biosafety, risk assessment processes, and regulatory controls.
4. Governments and international organizations should create special international arrangements for the use of gene drives in species of agricultural importance or in human-influenced species.
5. Governments should require that no gene drive be released in the field without a tested reversal drive.
6. Gene drive research regulatory protocols should mandate the incorporation of multiple containment strategies to mitigate the risk of spread in the event of an accidental release or laboratory escape.
7. Governments should require coordination and communication between researchers and local and international stakeholders as a prerequisite to gene drives deployment.

INTRODUCTION

Gene drives are a biotechnology capable of manipulating nearly an entire population's genetics in the wild without human intervention after release. While still in the research and development stages, there are many proposed beneficial applications of gene drives, including decreasing the burden of malaria, controlling invasive species, and mitigating the risks of other diseases spread by vectors.[1] However, there are also legitimate risks associated with gene drives that have created significant controversy surrounding research and development.[2-4] These risks include uncontrolled spread of the gene drive or unintended ecological consequences that affect either the target species or another species through direct or secondary effects. For example, an alteration in a mosquito population could have unintended consequences for species that rely on mosquitoes as a food source or even increase the incidence of other diseases in the absence of the eliminated one.

Gene drive: A genetic system that biases toward its own inheritance. These genes spread faster than expected by natural selection or Mendelian inheritance through a population as a result of this biasing. There are natural gene drive systems as well as man-made systems.

Because of their ability to have large effects for a relatively modest investment of resources, gene drives could cause widespread harmful effects, such as large-scale population collapse, either deliberately or accidentally. Governments in countries where work on gene drives is being done should have a regulatory framework in place to govern and oversee this technology, but most countries have not established those frameworks. Similarly, there are no established international approaches or coordination efforts around gene drive research or deployment, with the critical exception of Target Malaria, a highly collaborative and carefully planned effort to use gene drives to eliminate malaria.

Gene drives are a tool that allows genes to spread through a population despite fitness costs to the host organism. Fitness, or the ability to survive and reproduce, is an innate attribute of all organisms and depends on the genetics of the individual organism and its environment. Individual organisms can be more or less fit than other individuals in their population; individuals who are more fit have a better chance of surviving and reproducing, thereby passing on their genetics to the next generation at a higher rate than individuals who are less fit.

Gene drives are genetic tools that humans can use to engineer wild populations. The term *gene drive* does not refer to any specific technology or genetic mechanism; it is a functionally defined term. An analogy would be that "automobile" is defined as a land vehicle that transports people, independent of how it is designed and what its component parts include. The defining characteristic of gene drives is the increased rate at which they cause themselves to be inherited.

As such, gene drives are a type of selfish genetic element. Selfish genetic elements are genes or segments of DNA that spread through a population, despite a negative impact on the host.[5] An example of this in nature is the P element transposon in Drosophila fruit flies, which acts as a naturally occurring gene drive. It has spread through global wild populations of the species *Drosophila melanogaster* (fruit flies) in the past few decades and has been highly studied by genetics researchers.[6]

Used as a biotechnology, gene drives allow genes that would normally not be passed on because of an associated fitness penalty to be passed on anyway. A gene that does not have a negative impact on an organism's fitness will not require a gene drive, because the gene is likely to propagate through the population on its own. Potential uses for this capability include altering or diminishing a wild species, such as an insect that carries human diseases or an invasive species that causes ecological harm, or altering a species with economic value or cost.

The first genome editing of the human germ line resulted in hasty efforts by regulators to catch up with developments that had moved past them.[6] To avoid this situation for gene drives, policy should be put in place now to create norms and regulations to manage the risks and opportunities posed by gene drives. Artificial biological agents that have the capability to self-propagate have special ethical, safety, and security concerns and require special approaches to governance. Scientists working in the field have already established important containment and safety guidelines for responsible gene drive research, but a gap still remains in national and international governance specific to gene drives.[7-12]

Gene: *A sequence of nucleotides that forms the basic unit of heredity. Can be used by a cell as instructions to guide the creation of proteins.[165]*

Fitness cost: *The degree to which a gene reduces the ability of an organism to survive and bear offspring. Under the right circumstances, certain genes will increase the ability of an organism to survive and then are passed on to the organism's offspring; this is the process of natural selection, and these genes can be said to have a negative fitness cost. Human-altered genes often exert a fitness cost on their hosts.[165]*

Fitness: *The genetic contribution that an organism makes to future generations.[165]*

Efforts to regulate gene drives will be complicated by numerous technical features of gene drives and their mechanisms of action, including their ability to spread widely beyond the location where they are introduced, such as across national borders, and the inherent difficulty in accurately modeling their effects and dynamics. This report is intended for policymakers interested in creating and implementing policies for gene drives.

Benefits and Risks of This Technology

In the current age of biotechnological innovation, gene drives as a technology are uniquely positioned to address multiple high-priority and high-level challenges in ways that would otherwise require expensive and ongoing interventions. Foremost among the possible benefits of gene drives is the potential to significantly decrease the burden of vector-borne diseases on human populations. Control of vector-borne diseases currently requires coordinated efforts from public health, medicine, and vector control fields, among many others. Gene drives designed to address this problem propose to simultaneously decrease the probability of contracting the disease by decreasing the number of disease-carrying vectors as well as eliminate the need for continuous input of pesticides or prophylactic treatments.

In the same manner, gene drives could be employed to decrease the burden of disease on animals or plants of economic importance.

Increasing the productivity and reliability of the world's food supply would be an invaluable contribution. This goal could be accomplished in several ways, including by increasing the resilience of an economically important species against disease or by decreasing a pest's ability to infect an economically important species.

Another envisioned beneficial application of gene drives is the management of invasive species. The specificity of gene drives could allow environmental management of invasive species safely, without the need for toxic pesticides, and over large geographic areas.

Finally, climate change is one of the most pressing global concerns in which gene drives could have an impact. Animals or plants with heightened susceptibility to changing environmental conditions brought on by climate change could receive course corrections through targeted gene drive applications.

In spite of all of these valuable benefits, there must be careful consideration of risks associated with gene drives before any are deployed. Important among these risks is the potential for cross-border spread of gene drive–containing organisms, particularly considering that this technology is highly controversial and that most countries still lack an appropriate and explicit regulatory strategy. The confluence of biocontainment strategies, ecological movement, national governance structures, and international politics make for a uniquely complicated discussion on how such research and possible deployment should be allowed to move forward.

Additionally, there is the risk of the gene drive inadvertently causing off-target impacts in the target species. Off-target effects could range from the drive simply not creating the desired effect to creating harmful or unforeseen biological changes in the target species. Even if the drive did work as intended in the target species, ecological modeling is notoriously complicated, and therefore it is difficult to predict how an alteration or suppression of the target species will affect other components of its ecosystem. There could be cascading and self-amplifying effects once the target species is modified; these could include a drop in associated predator or prey species or result in another equally undesirable species filling the niche once occupied by the target species.

Another risk is of interbreeding and subsequent hybridization between the target species and a closely related species. Hybridization could result in the drive being unintentionally transferred and spreading across species for which it was not intended.

Finally, it remains unclear how 2 gene drives deployed in the same target species might interact on a molecular level. The drives might cancel each other out, compound each other's effects, or, more likely, not interact at all. However, as more groups enter the gene drives field, this question will become particularly important to answer.

What Are Gene Drives?

Gene drives are different from other genetically engineered organisms, because they are designed to spread themselves and any genes that they carry through the population at super-Mendelian rates—meaning they are transmitted to progeny more often than expected with typical inheritance of sexually reproducing species.[13]

In traditional Mendelian inheritance patterns, the offspring of 2 parents will receive 1 set of chromosomes from the father and 1 set from the mother.[14] In super-Mendelian inheritance, the offspring still receives 2 different sets of chromosomes from each parent, but all or most of the surviving progeny will carry and express the gene drive.[15] In this way, gene drives are self-propagating through successive reproductive cycles.

Figure 1A. Mendelian inheritance in ticks: Offspring have a 50% chance of receiving the altered gene. The altered gene does not significantly penetrate the population after 4 generations.
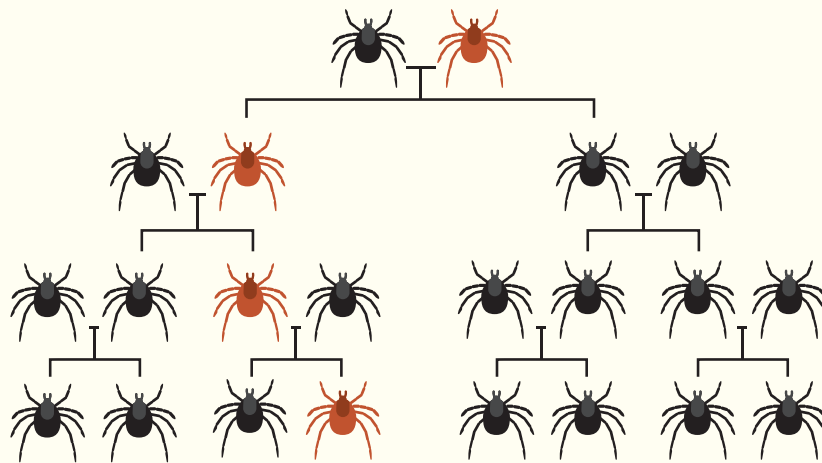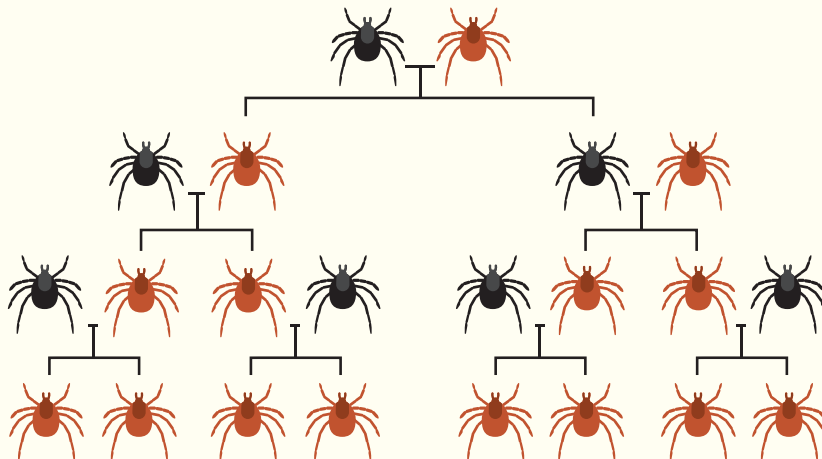

Figure 1B. Super-Mendelian inheritance in ticks: Offspring have a 100% chance of receiving the altered gene. The altered gene significantly dominates the population after 4 generations. (not pictured: non-altered individuals may still exist in the population, but at very low levels)

Mendelian inheritance:
The dominant natural process of genetic inheritance as discovered by Gregor Mendel. Most animals have 2 copies of each chromosome and inherit 1 of each chromosome from each set of parents. Ordinarily, which chromosome, and thus which gene, the offspring inherits is random, but gene drives are an exception to Mendelian inheritance.[14]

There are a wide variety of mechanisms to achieve this effect, some that have been discovered in nature and some that have been developed in laboratories. Many gene drives created through bioengineering have primarily relied on modified clustered regularly interspaced short palindromic repeats (CRISPR) systems. CRISPR is a natural defense mechanism of bacteria and other single-celled microorganisms against viruses. In these organisms, the CRISPR system will recognize and remove viral DNA or RNA that has invaded the bacterial genome through a cutting mechanism. CRISPR systems allow researchers to easily make specific edits to a gene or genome using programmable nucleases that cleave DNA or RNA at only a defined sequence.[16,17] These CRISPR-based gene drives use reprogrammed nucleases, or proteins that cut DNA or RNA, to cause targeted damage in order to manipulate the natural genetic repair systems within cells to copy a desired genetic sequence from 1 chromosome to the same genetic site on other copies of that chromosome.[18] The gene drive is not designed to be inserted at random onto any chromosome or at any site; however, CRISPR systems do have some off-target activity.

Nuclease: A protein enzyme that breaks a strand of DNA or RNA.[14]

The homing drive is one of the simplest and most commonly referred to examples of CRISPR-based drive systems. Typically, a homing drive uses CRISPR/Cas9 technology to cause itself to be copied from a chromosome that carries it at a specific site to the same site on another copy of the same chromosome in the same cell. In this way, regardless of which copy of the chromosome is inherited by an offspring, the gene drive will be on it (see Appendix 2: Technical Review on homing drives for more detail).

CRISPR (clustered regularly interspaced short palindromic repeats):
A genetic system originally discovered in bacteria, which use it as an adaptive immune system against viral DNA. CRISPR consists of a series of stored pieces of collected genetic material, separated by repeating sequences of DNA, and is paired with an endonuclease like Cas9. When the stored DNA is expressed as a guide RNA, it binds with any matching sequence of DNA present. The endonuclease recognizes the guide RNA and cuts the corresponding piece of DNA in half. This system can be used for flexible gene editing and as a component of gene drives.[18]

Homing drive (also "mutagenic chain reaction"):
A drive that uses guide RNAs to "home in" on a specific gene sequence, cut it, and replace it with the drive system via homology-directed repair, thus converting an allele pair that is heterozygous into one that is homozygous within the cell.[17,166]

While CRISPR drives are the most frequently discussed drive type for population alteration or population suppression, there are other drive systems being studied with a range of desired outcomes and methods to control a population (see Appendix 2: Technical Review). Some gene drives take advantage of toxin-producing genes to suppress population sizes or sex-biasing systems to decrease the number of individuals who are able to reproduce. These other gene drive systems reduce the survival rate of offspring that do not have a chromosome carrying a copy of the drive, rather than copying the drive from chromosome to chromosome. Although CRISPR-based gene drives are commonly discussed, they are by no means the only type of gene drive system (see Figure 2).

Population alteration (also "modification" or "replacement"):
Spreading or "replacing" a specific gene or genes throughout a population or species.[17]

Population suppression:
Reducing the size of a population—for example, by reducing the number of viable embryos, or by causing new embryos to be exclusively male. This effect can in principle drive a population or species to extinction.[61]

While the point of a gene drive is to spread through an entire population, there are a number of variant gene drive systems that are designed to spread through the population only for a certain period of time or a certain number of generations (see Appendix 2 sections on Split Drives and Multi-Part Drives). Also, there are variant gene drive systems that are designed to spread only in a specific geographic range (see Appendix 2 sections on Underdominance Drives/Threshold Drives). Broadly, these gene drives are referred to as "self-limiting drives" because of this intrinsic biocontainment ability.

Drive System	Drive Type	Also known as	What does the drive do to the target popuation?		Is the drive system self-limiting?		Does the drive rely on CRISPR-Cas?	
			Suppression	Alteration	Yes	No	Yes	No
Global drive systems	Homing drive	Homing endonuclease gene (HEG) drive	🟡	🟡		🔴	🟢	
	Precision drive	Locally fixed allele (LFA drive)	🟡	🟡		🔴	🟢	
Toxin-antitoxin systems	Medea drive			🔴		🔴		🔴
	Inverse Medea drive			🔴		🔴		🔴
	Killer-rescue drive		🟢			🔴		🔴
Underdominance systems	One-locus-one-toxin	1L1T	🟡	🟡	🟢			🔴
	Two-loci-two-toxin	2L2T	🟡	🟡	🟢			🔴
	One-locus-two-toxin	1L2T	🟡	🟡	🟢			🔴
Split drive systems	Li confinement drive			🔴	🟢		🟢	
	Standard split drive			🔴	🟡	🟡	🟢	
Multi-part systems	Daisy drive	Daisy-chain		🔴	🟢		🟢	
	Daisy quorum drive			🔴	🟢		🟢	
	Daisyfield drive			🔴	🟢		🟢	
Sex-biasing systems	Female fertility drive		🟢			🔴		🔴
	X-shredder drive	Driving Y chromosome drive	🟢			🔴		🔴
	Medusa		🟢			🔴		🔴

🟢 Always yes　🟡 Variable/design-dependent　🔴 Always no

Figure 2. Overview of notable gene drive systems that are most frequently researched in the field. Filled circles represent what methods or characteristics each drive system and sub-type possesses.

"Intrinsic biocontainment" is a mechanism incorporated into an organism's genetics to render the organism's growth, reproduction, or spread contained within boundaries or conditions in which it would not otherwise be contained. While the homing drive is the most discussed form of a gene drive, a self-limiting drive is a more likely candidate for deployment, because initial tests suggest self-limited drives will be safer than a homing drive.

In this way, the gene drive replicates itself inside the genome of the host organism and increases the chance that the organism will pass the altered genetic sequence on to its offspring. Because these gene drives depend on creating directed alterations to a host genome using genetic engineering, they are considered a type of genetically modified organism

(GMO). In contrast to traditional GMO crops, where the farmer has control over the geographic distribution and proportion of the GMO population to non-GMO population, gene drives can disperse over wide geographic areas without human intervention and rapidly become the dominant genetic makeup of the overall population.

There are 2 possible goals of a gene drive: either to alter or to suppress the population of its host organism. An alteration drive, also called a modification or replacement drive, is designed to introduce, replace, or delete a specific gene or multiple genes in the majority of the target population without significantly affecting the overall population size.[17,18] Depending on the type and design of the drive, the proportion of the population that is ultimately altered can vary.[13,19]

One use of an alteration drive would be to spread genes that make a mosquito species resistant to carrying a human pathogen, such as malaria.[20,21] Another use of this type of drive might be to disrupt and disable a gene that causes herbicide resistance in weeds. A suppression drive, conversely, introduces genes that make organisms either sterile or less fit to reproduce, with the intention of decreasing the birth rate of viable offspring.[18,22] This reduces the total number of organisms in a population and potentially reduces the targeted population to extremely low levels. Proponents of this type of drive suggest that they could be designed to control invasive species or kill off all malaria-carrying mosquitoes.[23-25]

Why "Beyond Malaria"?

This report looks beyond malaria, in recognition that the first gene drive likely to be deployed in the wild will be a gene drive released in *Anopheles gambiae* in sub-Saharan Africa for the control of malaria. There are a number of considerations surrounding that anticipated first use of gene drives.

Malaria is a problem of exceptional scale and devastation. The World Health Organization reported that in 2016 there were 216 million cases of malaria worldwide, causing 445,000 to 731,000 deaths, 90% of which were in Africa, and most of those in children.[26] Because of the exceptional human cost of endemic malaria, exceptional methods of control are seen to be justified.

In the developed world, controlling the mosquito vector of malaria has succeeded in eliminating it from whole regions, and malaria can be cured or prevented with a number of well-established interventions.[27] However, the disease has proven resistant to control with conventional methods in the developing world, often for cultural and economic reasons.[28] Control of malaria can be further complicated by the fact that it can be maintained in reservoirs both in the mosquito population and in asymptomatic humans.[29-32] New methods are needed to bypass the factors that impede more traditional methods of malaria control in the developing world.

Significant resources have been committed to an organization, Target Malaria, that is focused on the gene drive solution to malaria, with contributions from the Gates Foundation, the Open Philanthropy Project, and other contributors.[33,34] Target Malaria is putting significant effort into engaging with African stakeholders and the public and is also considering ecological risks associated with their intended gene drive usage.[34,35]

Complete buy-in for the deployment of an antimalarial gene drive is not present and probably not possible. Numerous groups concerned with biological diversity, indigenous peoples' rights, and agricultural precedent have written public letters opposing the release of gene drives, or any gene drive research that might enable their release.[4,36] For this report, extensive efforts were made to contact these groups for inclusion in the semi-structured interview process. All such efforts were met with no response at all or an unwillingness to speak with the project team.

Because of the huge disparity in funding among those groups exploring the technology and those opposing it, the massive human scale of the malaria problem, and extensive efforts to engage with and involve affected populations and governments, it is highly likely that a gene drive in *Anopheles gambiae* to control malaria will eventually be deployed. Burkina Faso has reportedly approved the use of a gene drive to be released in their country, and in 2019 it allowed a small trial release of mosquitoes modified with a homing endonuclease.[37,38] Technical and regulatory aspects of the anti-malaria usage case of gene drive technology have already been published.[39-44]

This report describes the potential benefits and risks of gene drives beyond the malaria use case, and it provides recommendations for governments regarding a regulatory approach after an anti-malaria gene drive has been put in place and when gene drives are being considered for many other applications. Some of the important issues that will need to be addressed as the technology is developed and considered for deployment include: management of uncertainty and risk, the need for coordination between parties deploying gene drives, the different value and risk profiles of distinct gene drive technologies, the proper identification and engagement of

stakeholders, countermeasures, and attribution by third parties. There are several goals for which gene drives may be an ideal tool, including pest management, invasive species control, and reduction of diseases other than malaria carried by mosquitoes and other vectors.

There are a number of previously published and ongoing studies that fully elaborate on the ethical considerations surrounding the basis of gene drive technology.[45-47] While the ethical status of gene drives should inform the foundational decision-making process, it is beyond the scope of this report to further assess ethical considerations. The focus of this report is to provide recommendations for government regulation and oversight of this technology.

Countermeasure: A product that can be used after a gene drive has been released into a population to stop it or render it inert. Certain countermeasures can even restore the wild phenotype to the affected population.[164]

METHODS

Literature Review

In order to identify potential experts and stakeholders for interviews, an initial literature review of gene drives was conducted, including popular press articles, legal commentaries, regulatory documents, and scholarly articles discussing gene drives or a related topic. Searches were conducted through PubMed, Web of Science, Google, and Google Scholar, looking back 10 years. This literature review was not exhaustive of all aspects of gene drives but instead was designed to inform our analysis and recommendations, as well as to find the experts and stakeholders most active in the area and identify appropriate experts for interviews. Technical aspects of gene drive mechanisms and effects were reviewed in papers identified through searches on bioRxiv and Google Scholar, as well as from papers and reports recommended by interviewees.

To review the governance of gene drives, a literature review was performed of relevant legal documents, press releases, and scholarly reviews of governance. US and international legislation on gene drives was collected by searching scholarly articles, government websites, and law libraries for documents relating to gene drives, genetic modification, and biodiversity. Translations of Russian documents were performed using Google Translate, and Chinese legislation and documents were researched using US law libraries that provided analyses and translations. Brazilian legislation and documents were translated by in-house staff.

Interviews

Semi-structured qualitative interviews were conducted with experts from a broad variety of backgrounds, including molecular biology, evolutionary biology, ecology, bioethics, law, global health, and biocontainment. Interviewees were drawn from academia, government, and nongovernmental organizations (NGOs) from 4 countries that are active in gene drive research or activism. In total, we spoke with 24 experts between June and August 2019.

In order to consider multiple stakeholder viewpoints, extensive efforts were made to reach several individuals named on both the November 2018 Outreach Network for Gene Drive Research open letter (supporting further gene drive research) and the October 2018 Global Food and Agriculture Movement open letter (supporting a moratorium on gene drive releases)

for interviews. In total, 5 people named on the pro-gene drive letter were interviewed, and 13 people named on the anti-gene drive letter were contacted. None of those in public opposition to gene drive use or research agreed to be interviewed; however, some responded by referring to their previously published statements on these issues.

The semi-structured interviews were conducted using an initial set of questions developed for all interviewees as well as questions designed for those with certain areas of expertise. Interviews were conducted on a not-for-attribution basis. All professional opinions were those of the interviewees and not of their organizations. The findings and recommendations in this report do not necessarily reflect those of the people who were interviewed for this project, but they do reflect the judgments of the authors of this report.
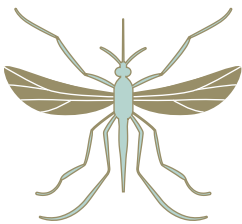
FINDINGS

In species where humans already have full genetic control, gene drives offer no major genetic innovation or advantage. In wild species, gene drives may provide the means for genetic control not possible by other means.

When considering whether or how to use gene drives in a host species, one of the most important factors is the level of control that humans have over the genetics of the host species' population. Host species can be classified broadly into 3 levels of control: human farmed, human-influenced, and wild.

1. **Human-farmed species** are species that humans have nearly complete genetic control over as a consequence of traditional farming practices. This category includes animals like cows and chickens and plants like corn and soybeans. Selective breeding enables humans to control the genetics of such species by selecting for specific traits and excluding others. For example, selective breeding alone was used approximately 14,000 years ago to transform grey wolves (*Canis lupus*) into domesticated dogs (*Canis familiaris*).[48]

2. **Human-influenced species** are plants or animals that are harvested by humans but are not maintained in a greenhouse, plantation, pen, or other location dedicated to the propagation of that species. This includes species that are regularly hunted or captured in the wild, such as deer or codfish. Species in this category often have an economic value to humans and are commonly used as a food source. While humans may influence the genetics of these populations—for example, through selective hunting[49]—human-influenced species' genetics are not directly controlled through the selective breeding and propagation of individuals with desired traits.

3. **Wild species** are organisms that humans have little or no genetic control over, such as hawks or mosquitoes. Neither are they frequently hunted, harvested, nor otherwise selected for by human activities. Humans do have an impact on these species, but primarily through broad and indirect influences on the ecosystem.

Human-influenced species: A species that is not domesticated but whose populations are nonetheless subject to significant human control—for example, fish that are heavily harvested by humans, or deer whose populations are controlled by hunting.

The distinctions among these 3 groups are important, because gene drive use in each of these categories has different risks and goals. Gene drives are a tool for controlling a population through its genetics. As such, populations in which we already have strong genetic control, such as human-farmed species, do not require gene drives to accomplish most goals. For example, a farmer could plant a genetically modified version of soybeans that are more resistant to drought without relying on a gene drive for the same purpose. The farmer already has complete control over the genetics of his crop species through selective seed planting and therefore has no need for a self-propagating genetic alteration system. A normal GMO or traditional selective breeding will, in almost all cases, be an easier and more controlled method for applications in farmed species because of the inherent genetic control that farming entails.

Unlike farmed species, wild and human-influenced species are not easy to control genetically. Therefore, gene drives could in principle be used to make genetic changes across wild or human-influenced populations.

Natural selection: The process by which variations in the forms and functions of an organism give rise to nonrandom survival of those organisms and thus over generations to the nonrandom inheritance of forms conducive to life.[167]

Gene drives would be a more effective method for altering a wild population than a non–gene drive GMO, because gene drives would not require the labor- and cost-intensive process of deploying the GMO species in an ongoing, sustained manner. Gene drives in wild species could be used to control ecological disruption from a wild invasive species, control agricultural disruption from a wild pest species, or control the spread of pathogens carried by wild vector species.[50]

Gene drives might be most economically effective in human-influenced populations, because these species often have economic value and humans more regularly interact with these species. For example, oceanic codfish live in the wild and are free to move about their range as a wild species, but humans regularly affect the population, including overall numbers, fish size, and migratory patterns, through fishing endeavors.[51] Similarly, deer are an important game animal and are thus affected by human hunting. Human behaviors alter the selection pressures on these populations by preferentially selecting certain phenotypes over others, which alters the population's genetics over time.[52]

Phenotype: Specific characteristics or organisms that can be identified or distinguished by direct inspection or only by finer methods of measuring or description.[169] Phenotype, a measure of the traits of the organism as observed, is distinguished from genotype, which is a representation of the genes contained in that organism regardless of whether they produce an observable trait or not.

Gene drives in either of these species could be designed to improve the economic opportunities associated with hunting and fishing, such as increasing individual animal mass or increasing the birth rate. However, genetic manipulation of such species could be controversial, because multiple stakeholders have competing interests. For example, one group may want to improve the climate resilience of oceanic codfish. This benefit, however, would effectively render all oceanic codfish GMOs, destroying their value in some markets. In addition, unlike farmed species, human-influenced species are not intentionally confined to a specific area such as a farm or range and are free to roam across jurisdictions or borders. This could make regulation concerning the deployment of gene drives in such species difficult.[53,54]

Currently, most gene drive work is conducted in mosquitoes, which are a wild population. The goal of that work is to protect humans from diseases carried by mosquitoes. As gene drive technology develops and is more widely explored as an option for population alteration, the goals of gene drive–based interventions may change from protecting human health to promoting economic interests.

There is an intrinsic limit to how accurately computer models will predict the efficacy and dynamics of a gene drive's spread and behavior. This limitation is due to a number of factors, such as the need to make simplifying assumptions to make the problem computationally tractable, the presence of localized and nonrandom mating of the host species, and the impracticality of perfectly complete characterization of all relevant data needed to predict the behavior of a gene drive. Examples of where such complete data would be hard to gather and incorporate into a model include the rates of rare events, such as interspecies cross-breeding, or rare genetic variants at the target insertion site of a homing drive.[55,56]

There is a need for gene drive development in model organisms, such as *C. elegans* or yeast, that can be grown to exceptionally large numbers and for many generations to experimentally validate computer models (see Appendix 2: The Limits of "Intrinsic Biological Control"). Despite the limitations of models, they can still be a helpful tool in mitigating post-deployment risks while designing gene drives. A firm understanding of the population dynamics and diversity, host organism behavior and biology, and population range will help to mitigate the risk of an unanticipated consequence by improving modeling parameters and aiding researchers in designing a more appropriate gene drive.[57]

Gene drives have distinct risks, depending on the type of gene drive**, the gene** of interest **being modified** through the population, **the goal of the gene drive, the intended environment** in which the gene drive will be released, **and the host organism**. The potential negative consequences of a gene drive release and the likelihood of those consequences should always be considered as part of a risk assessment. Risk assessment cannot provide definitive answers, but rather can help elucidate potential harm to the environment, humans, or animals from a gene drive. This information enables researchers and responsible parties to create more focused and thoughtful strategies to mitigate the chance of an unintended harmful outcome.

Uncertainty surrounding gene drives is substantial, because there are many possible variations and applications of this technology. However, this uncertainty is not irreducible: Potential consequences can be categorized, assessed, and addressed to some extent. Each of these steps in the risk assessment help reduce uncertainty. Risk assessment approaches like constructing representative scenarios, red teaming potential consequences of gene drive use, or identifying and categorizing the concerning characteristics of a specific gene drive help build protections to mitigate gene drive risks. In particular, identifying

potential consequences of highest concern, the technical likelihood of those consequences, and the resources and training that would be necessary to avoid those outcomes help scientists and policymakers prioritize attention and resources toward well-designed interventions.

Several governments have taken the approach of regulating genetic engineering, and by extension gene drives, with standards surrounding the scientific *process* used to create the GMO. Others have approached regulation with an explicit focus on the final *product*. These different approaches to regulation will shape the structure of risk assessments and will affect the methods used by researchers and companies in creating their products. **A checklist or one-size-fits-all framework for risk assessment** based on the process used to create the gene drive **will not be appropriate.** Not only can the drive mechanisms be vastly different between different drives using the same process, which creates different risk profiles, but the genes of interest and host species will also change and have their unique needs and considerations. A gene drive in a fish to improve its resilience to algal blooms will have different risks, characteristics, and safety needs than a gene drive to reduce the ability of mosquitoes to be infected with dengue virus, even if they use a similar method.

Gene drives created with the intent to change a product that might be consumed by humans will need a toxicology assessment to determine if the gene drive could have adverse effects for humans that eat the product, even though the gene drive would not theoretically alter the human population directly. Another consideration is that a gene drive released in a particular species in one environment might have different risks and benefits than the same drive released in a different environment. The potential for interbreeding between a species or strain of a species to be modified with other species or strains should be addressed.[55] There are several variables to consider for potential failure modes or unintended consequences for each individual gene drive, so each gene drive should be considered independently as a unique product.

Notably, consideration should be given to how reversal drives are developed in relation to their counterpart gene drive. A reversal drive is a gene drive that spreads through a population that has already been modified by a specific target gene drive and over-writes or reverses the modification that the first drive introduced, such that the organism is genetically in its original state and cannot be further modified by the original gene drive. An immunization drive achieves the same end effect as the reversal drive but spreads through the portion of the population that has not yet been modified by the target gene drive. While the reversal drive will be a unique drive compared to the original drive, they should be developed in parallel and assessed together as 2 parts of 1 product.

A lack of coordination between groups intending to release a gene drive in the same region could pose risks.
There is currently a lack of coordination between groups working to develop new gene drives. With uncertainty surrounding gene drive behavior following release, it will be important to address potential interactions between gene drive technologies of 2 or more projects in the event of concurrent release or a new release in the same population as a previous release. It is unclear how 2 gene drives designed to modify the same species will interact in vivo. Although it is highly unlikely that 2 groups would target the same genetic sequence in the same species with the same type of drive, the effects of 1 drive could be detrimental to the desired goals of another.

For example, an alteration drive that intends to make the target mosquito population more resistant to malaria infection would be a wasted effort if another group simultaneously released a suppression drive that would rapidly decrease the reproductive population. Just as the intended outcomes of different independent gene drive releases might interfere with one another, the molecular-level mechanisms of some types of gene drives are able to interfere with one another, such as 2 or more gene drives using the same toxin/antitoxin system (see Appendix 2: Gene Drive Interactions Are Possible).

These **interactions between 2 gene drives could create an unexpected and unintended consequence**. Such interactions will need to be considered before gene drives are released, and provisions should be implemented to monitor how the gene drive behaves independently, and with previously released gene drives, following deployment. Since gene drives are designed to persist in the environment, the potential for interactions between independently deployed drives will accumulate with time.

Stakeholder engagement will be critical before deployment. Organizations must make substantive efforts to communicate the benefits and risks of gene drive applications throughout each stage of development.

A single gene drive deployment could affect thousands, if not millions, of people and have an extreme ecological impact. Despite their potential for such wide-ranging effects, it is conceivable that a release could occur without sufficient efforts to inform key stakeholders of all risks and benefits. It is universally accepted within the field that gene drive research must incorporate stakeholder feedback throughout the development of a gene drive, but questions remain about the best methods for implementation.

During expert interviews and the review of technical and policy-based literature, several themes for future stakeholder engagement were frequently repeated. **Gene drive deployments in natural populations may disregard national borders**, complicating coordination, and the degree of technical literacy needed to grasp the nuances of this technology may burden communication and education efforts.

Target Malaria, the most prominent ongoing gene drive initiative, has received praise for their continuous communication and education efforts, their efforts to meet ethical standards, and their regional approach to stakeholder engagement. Future gene drive research has the opportunity to model its engagement plans similarly, with strategies, like a tier-based implementation, providing a natural structure for their integration. Overall, stakeholder engagement should play an integral role in the development of future gene drive technologies.

There is little explicit mention of gene drives in existing legislation at national and international levels.

While some nations have explicit mention of genetically modified organisms in their legislation, few mention the term "gene drives." Our case studies on GMO legislation (see Appendix 3) include many of the major national participants in gene drive research, such as the United States and the European Union (EU). Gene drives are a relatively new technology that, in some cases, may fall under the legal term of "genetically modified organism." This will depend on the country's definition of a GMO.

But the lack of clear and specific language regarding gene drive technology is a major gap in national and international legislation. Because of this, it is often unclear under which regulation or legislation a gene

drive effort would fall and which government agency would be responsible for its oversight. This ambiguity is evident in the US system, despite having a Coordinating Framework of Biotechnology. Gene drives may be subject to regulation by the Food and Drug Administration (FDA), the US Department of Agriculture (USDA), or the Environmental Protection Agency (EPA), but it depends on what organism is being targeted and for what purpose, creating an uncertain regulatory system for academics, nonprofits, or companies wanting to research or deploy this technology.

Our case study identified only 3 legislative bodies that have specific language on gene drive research: the EU, Brazil, and Uganda. The EU, in a recent court decision, explicitly stated that gene drive–modified organisms would fall under their definition of a GMO, and so they are subject to all relevant GMO legislation.

Brazil created a resolution in 2018 to clarify the process of gaining approval of gene drive research, with clear language that informs researchers of their duty to seek approval from the designated regulatory authority, CTNBios (Comissão Técnica Nacional de Biossegurança), even if the gene drive technology is not clearly a GMO under law.

Uganda's recent Genetic Engineering Regulatory Bill has clear language on gene drive technology, with details on how to maintain safety in research, and has explicit language regarding liability in the case of negative impacts of a gene drive technology. Such definitive language in legislation regarding gene drives helps prevent misunderstanding and gives a clear process for regulating risks.

The specific mention of gene drives in these 3 examples is commendable, because gene drives are distinguished from other forms of GMOs. More national governments should follow this example and make explicit regulations regarding gene drives.

Gene drives are a type of invasive species.
An often-cited concern surrounding gene drives is that, once a gene drive is released, it might be very difficult or even impossible to recall. Further, it is extremely difficult to model or predict what a drive might do when interacting with the larger ecology in which it is deployed. This uncertainty is not without precedent in the release of biological agents, both intentional and accidental; it is directly parallel to the case of invasive species, which also can rapidly outcompete native species and be nearly impossible to recall once released.

Further, in a biological sense, **a gene drive is, in fact, an invasive species**; it is a genomic parasite that invades an ecological niche in the environment in which it lives—in this case, the host organism's genome is that niche. As such, in countries with thorough invasive species legislation, these regulations could be updated to incorporate gene drives.
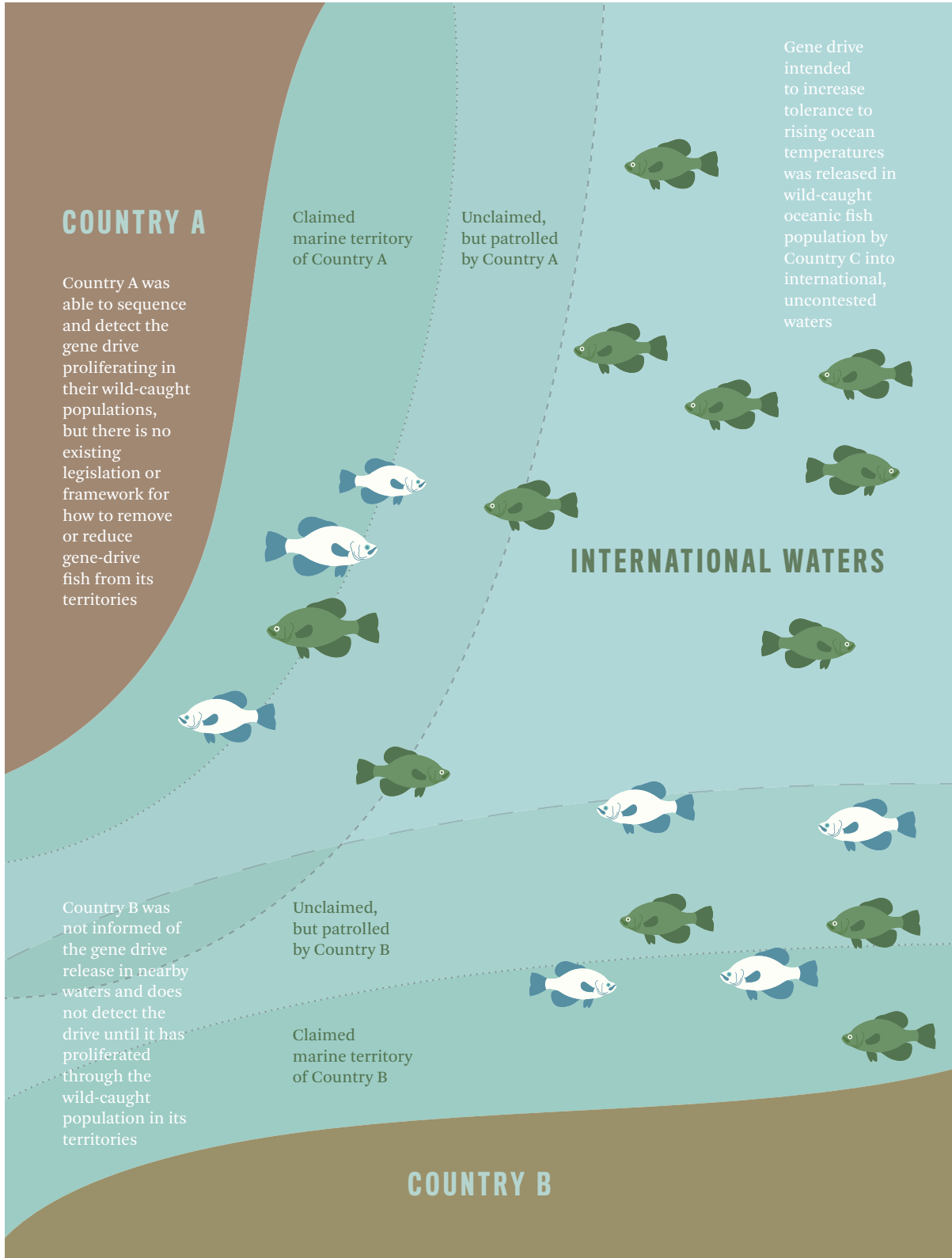
There are no proposed countermeasures to gene drives that are not themselves gene drives.
A countermeasure would be an intervention designed and deployed, possibly by a third party, after detecting an unwelcome or misbehaving gene drive spreading through a host species. The only viable countermeasure to a gene drive, in almost all cases and with our current biotechnological capabilities, would be another gene drive.

Beyond other gene drives, the closest concept to a countermeasure identified by participants was a genetic containment or control element designed to keep the drive regionally confined to its area of initial release. However, this would not be a true countermeasure, as it would have to be designed into the gene drive and exist before the drive is released. If a drive were released without a genetic containment element, a countermeasure drive could be introduced into the population that would be intended to immunize the host population to the first drive or reverse its effects.

As discussed in Appendix 2, no individual gene drive can be expected to reach 100% of the target wild population—meaning that any countermeasure in the form of a gene drive cannot be expected to be 100% effective either. There is also a possibility that the countermeasure could cause its own unique negative impacts in addition to the impacts caused by the original gene drive.

One participant noted that while it is impossible to recall a gene drive, it may be possible to stop a gene drive if measures are taken nearly immediately after its release. For example, a gene drive released in fish in a large river system could potentially be halted by immediately collecting all original gene drive fish and monitoring fish born soon after the initial release for evidence that they are carrying the gene drive. This would be a costly and labor-intensive process and would require sequencing the genomes of nearly all fish to ensure all members carrying the gene drive were removed from the population. It is unlikely that this approach could be successfully implemented, and it should not be relied on as a countermeasure.

COUNTRY A

Country A was able to sequence and detect the gene drive proliferating in their wild-caught populations, but there is no existing legislation or framework for how to remove or reduce gene-drive fish from its territories

Claimed marine territory of Country A

Unclaimed, but patrolled by Country A

Gene drive intended to increase tolerance to rising ocean temperatures was released in wild-caught oceanic fish population by Country C into international, uncontested waters

INTERNATIONAL WATERS

Country B was not informed of the gene drive release in nearby waters and does not detect the drive until it has proliferated through the wild-caught population in its territories

Unclaimed, but patrolled by Country B

Claimed marine territory of Country B

COUNTRY B

Cross-boundary movement of gene drives in wild-caught, human-influenced populations
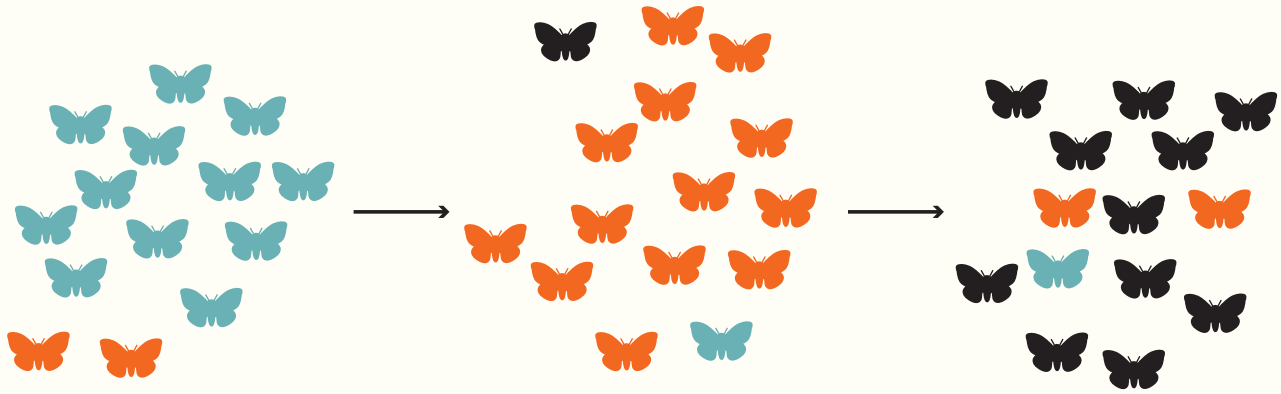
Gene drive is released:

At the time of release, the majority of the population is wild-type. It will take several generations until the gene drive individuals saturate the population through mating.

Many generations later:

The majority of the population is gene drive individuals with very few wild-type individuals still remaining. Escape mutants, progeny of gene drive parents with resistance to the gene drive mechanism, are beginning to evolve away due to selective pressure.

Many more generations later:

The majority of the population is escape mutants with very few gene drive individuals and even fewer wild-type individuals. The escape mutants did not revert back to wild-type, and any further mating with gene drive individuals will not result in the gene drive dominating the population makeup.

Gene drive

Wild-type

Escape mutant

Represents a homing drive system and assuming no migration into the population

While there is no foolproof mechanism to recall a gene drive, it may be possible to counteract the effects of a gene drive by reducing the gene drive's saturation in the total population. If the drive carried a fitness consequence to the organism, which is a common outcome of genetic modification, then the non–drive population would gradually be selected over the drive population until the percentage of the drive population was negligible and unable to rebound as the dominant group. This strategy would not be effective in certain types of gene drives, such as an underdominance system (see Appendix 2).

Gene drives are likely to gradually lose their potency over time as genetic resistance evolves. Genetic modifications often carry fitness costs that compromise the ability of individual organisms to reproduce. Over time, random, undirected modifications result in adaptive changes that make the organism more fit overall but with diluted potential to pass on the gene drive. This natural fail-safe, which is also a barrier to efficiency and long-term success of gene drives, will arise as selection pressures favor not having the drive cassette in the genome.[58]

Cassette: A mobile region of genetic material (typically DNA) that contains a gene and recombination site allowing for it to be integrated into a larger genetic construct (eg, a chromosome).[163]

Some researchers have published methods they believe will slow or inhibit the evolution of resistance mutants.[59-62] Unless a drive is carefully designed to specifically combat the evolution of genetic resistance mutants, a gene drive is likely to peter out on its own over time.[58]

There are many different attitudes among experts and stakeholders toward the use of countermeasures. Some believed that gene drive countermeasure development was important and could imagine it being deployed effectively. Other participants placed a higher value on pre-release risk assessment and modeling, with the reasoning that if these assessments identify the possibility of off-target effects, then the gene drive should not be released at all. However, these approaches are not zero-sum. A pre-release risk assessment is unlikely to be able to identify and quantify all possible off-target effects in situ, and even if there were a small chance of off-target effects, knowledge of such effects may not be enough to prevent the eventual authorized release of that drive.

A moratorium on gene drive research would mean that research into reversal drives would be stopped.

The Biological and Toxin Weapons Convention should be interpreted as prohibiting harmful gene drives.
A weaponized gene drive should be subject to the terms of the Biological Weapons Convention (BWC). Article I of the convention, in addition to serving as a prohibition of biological weapons, also functions as a definition:

> Article I
> Each State Party to this Convention undertakes never in any circumstances to develop, produce, stockpile or otherwise acquire or retain:
> (1) Microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes;
> (2) Weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict.[63]

This prohibition is not limited to just organisms or toxins but "agents." Because of the ambiguous nature of that term, the fact that a gene drive is not "microbial" is irrelevant; it is still a biological agent. This is in keeping with the 1986 Biological Weapons Convention Review Conference report, which stated:

The Conference, conscious of apprehensions arising from relevant scientific and technological developments, *inter alia*, in the fields of microbiology, genetic engineering and biotechnology, and the possibilities of their use for purposes inconsistent with the objectives and the provisions of the Convention, reaffirms that the undertaking given by the States Parties in Article I applies to all such developments.[64]

A host organism that carries a gene drive could therefore be considered a biological agent. Any gene drive that is created and has "no justification for prophylactic, protective or other peaceful purposes" is considered under the purview of the BWC. Further, because a gene drive is effectively a mechanism that pushes any gene, including a potentially harmful gene or its products, through a population, it would also fall under the purview of the United Nations Security Council (UNSC) Resolution 1540.[65] Therefore, a gene drive that purposefully pushes harmful genes into a population should be prohibited by the BWC and other disarmament treaties and agreements including UNSCR 1540.

The attribution of harmful gene drives to specific laboratories should be possible.

Gene drives could be used by malicious actors to do harm, released by irresponsible or anonymous actors, or accidentally released by legitimate researchers. In any of these cases, attribution will be an important component for identifying who should be held liable for the harm that was caused. Attribution of a released gene drive could be accomplished by analyzing the technology and deducing the mechanism(s) used to create the drive. In addition, the DNA sequence–based approach to the attribution of engineered plasmids and organisms—assisted by machine learning—is an active area of research.[66]

The relatively small number of scientists currently doing gene drive research regularly publish their research and the DNA sequences involved. In the case of release, organisms with a gene drive could be sequenced, and these sequences could be compared to the sequences in the published literature. Because of the small size of the current field, attribution should be a relatively simple task. However, as the field expands and the technology becomes more accessible, attribution will become increasingly difficult.

Once a gene drive is released into the environment, it can be sequenced by any interested party, so the genetic structure of a gene drive is not something that can be obscured. It is unlikely that technical capabilities in the design and deployment of gene drives could be kept secret from other scientists or the public.

Technical attribution of genetic elements has been demonstrated.[66] This is possible because engineered DNA, as with any engineered construct, requires that the engineer make design decisions. These decisions are signatures of the engineer's skills, background, and training. This should also be true for the design of gene drives, including the design of the payload genes and the drive mechanisms themselves.

Payload gene (also "cargo gene"): A gene added to a gene drive in order to be driven (alongside the drive) throughout a population in order to give organisms a desired property.[168]

RECOMMENDATIONS

Because gene drives are capable of cross-boundary spread, the international community should recognize their shared responsibility for gene drive governance and oversight. These recommendations are intended for national governments and international agencies to promote collective responsibility of this technology.

1. *National governments should require gene drives at an advanced level of development to undergo individualized risk/benefit assessments before deployment. Understanding the specific risks and benefits of 1 gene drive will not necessarily translate to other gene drives.*

Because of the significant variation among gene drive technologies, host species, proposed use cases, and use environments, each deployment-ready gene drive will be unique. Therefore, the benefits and risks should be judged on a case-by-case basis for each gene drive as a product, rather than blanket assessments on gene drives as a technology. Risk assessments are already performed by scientists and their institutional review boards (IRBs), but given the potential for gene drives to spread in the environment, gene drives should also undergo national level review and risk assessment before field deployment. Such a risk assessment should consider the following:

- Genomic components of the gene drive, including the drive component and the gene of interest
- Expected dynamics of the gene drive in the intended population, according to computational models and experimental data
- Host species' genetic diversity at the genomic site(s) of the gene drive
- Potential for off-target effects
- Interbreeding potential between the host species and related species
- Primary and secondary ecological effects, including a food web analysis
- Efficacy of the reversal gene drive or other mitigation strategies

While assessing the gene drive, the gene of interest should be assessed with the same rigor as any other GMO gene would be assessed. In addition to the gene of interest, the drive components (such as the CRISPR and Cas9 genes) should also be assessed both as a GMO and as a construct with the ability to self-propagate. Assessments should consider the potential benefits of the gene drive, including both the benefits of the gene of interest as well as the usage of a gene drive mechanism, and determine if the same result could be reached with a non–gene drive GMO or other intervention.

The likelihood that the intended result will be reached, the potential consequences of failures, and the extent to which the potential consequences could be mitigated should be identified. Experimental data, expert opinion, and computational models should be used for these assessments. However, the limitations of computer models should be recognized and accounted for during the risk assessment.

An important indirect consequence of case-by-case risk assessment of gene drives—as a unique product rather than as a blanket assessment of the technology—is that gene drive releases in farmed species are less likely to pass a cost-benefit analysis. In human-farmed species, there are other genetic modification methods that are less complicated and less likely to result in uncontrolled spread or off-target impacts than

gene drives. Concern about gene drives in agriculture informs much of the opposition to gene drive research and development, so adopting a policy that reduces the likelihood of such applications may help diminish those concerns. Gene drive use for human-farmed species should be considered to have lower benefits and higher risk than uses in other species and should have a unique regulatory approach that properly weighs the risks and benefits in it.

2. *An international tiered registry system should be created. The closer a group is to the release of a gene drive, the more information would be required. This kind of registry would facilitate transparent assessments, coordination of different gene drive efforts, and stakeholder communication. It would also be a step toward ensuring that proper regulation was in place to allow safe usage in the field.*

Given the potential for uncertain or harmful impacts on the environment, an international registry for gene drives should be created. All countries in which gene drive research is being performed or a gene drive could be deployed should participate. This would provide international transparency and increase knowledge about the emerging field. It would reduce the chances that multiple gene drives are released inadvertently into the same ecosystem.

Such a registry could be managed by an NGO or an international organization, as is the case in which the World Health Organization (WHO) is creating a registry for germline editing. Individual governments should have a similar registry including all gene drive research and plans for deployment occurring within that country. The individual government registry should be a first step, given that this would be under the purview of a single government and it could be accomplished more quickly. We recommend creating a tiered system that protects the intellectual property of researchers before they have the opportunity to patent or publish their work, but also provides regulators and the public with necessary information before a gene drive is released in their community.

Tier 0—No Registration Requirement:
Research that consists entirely of computer simulations, or involves isolated testing or development of genetic components that might eventually be incorporated into a functioning gene drive, such as CRISPR-Cas9, or that might eventually inform such an effort such as sequencing the genetic diversity of a potential host organism would not require registration.

Tier 1—Academic, Industry, or Organization Research Stage:
All researchers working on creating any gene drive in a laboratory would be required to register their work. This would include drives in *C. elegans* and other model organisms. The researchers would be required to identify all individuals on the research team and in their organization, the biosafety officer(s) and IRB(s) involved, the host species of the drive, the type of drive being created, and the containment mechanisms being used. Specific genetic sequences or other proprietary information would not be required for reporting in this tier.

Tier 2—Field Trial Stage:
Before approval could be given for a field release of a gene drive, researchers would have to provide all the information required in Tier 1 with appropriate updates, demonstrate they have engaged with the local community around the test site, provide modeling work demonstrating the predicted spread of the drive in the future, show all biosafety and containment systems that are in place, and provide a detailed report on the intended outcomes of the gene drive. At this stage, a national authority should conduct an independent risk assessment, as described in the first recommendation.

Tier 3—Imminent Release Stage:
Before any gene drive is released into the environment, the responsible party would need to provide all the information required in Tiers 1 and 2, plus the specific genetic sequences of the drive, and signed documents demonstrating national approval of the release (see below for more detail on community approval and engagement), and the responsible party would need to demonstrate that a reversal drive has been created and successfully tested. Like Tier 2, this stage would require submitting evidence that an independent national authority had conducted a risk assessment for the gene drive.

3. *Governments should not place a blanket moratorium on gene drive research. There are valuable potential gene drive applications (eg, vector control, invasive species control) that are worth studying with the appropriate biosafety, risk assessment process, and regulatory controls in place.*

There have been calls to implement a moratorium on gene drive research and development.[67] Many supporters of a moratorium are concerned about the possibility that gene drives in plants might have catastrophic consequences, putting the world's food security in peril. While the world's food security is critical, threatening the global supply of an entire crop is unlikely because of several safety standards and other barriers to long distance travel of a plant across hemispheres or continents. Additionally, there are a limited number of plants susceptible to gene drives. Gene drives provide several potential opportunities that might improve human health and even strengthen the food supply, such as decreasing the incidence of vector-borne diseases or controlling invasive species that threaten the food supply.

Because gene drives have the potential to significantly improve human health by decreasing disease burden, there should not be a blanket moratorium on gene drive research and deployment. Such a moratorium would limit the potential to solve problems so far unsolved using other strategies. Additionally, a blanket moratorium on gene drive research would have a negative impact on research into countermeasures to gene drives, such as reversal drives.

While a blanket moratorium or ban on all gene drive research and deployment would be inappropriate, there could be situations in which specific types of gene drives or gene drives in certain host species could appropriately be restricted or banned. For example, it may be appropriate to limit research on gene drives in specific crop species that are susceptible to gene drives, perhaps restricting them to government laboratories verified to have the highest safety and security controls.

There are several types of gene drives, some of which pose more risk than others. For example, self-limiting drives are considered safer options that non-self-limiting drives because there is less risk for uncontrollable spread of the gene drive.[1] The type of gene drive mechanism, the gene of interest, and intended host species are key factors that could be the basis of categorical bans or moratoriums on some gene drive research; it may be appropriate to limit the riskiest

gene drive research while allowing research for safer and potentially more beneficial research to continue. It may also be appropriate to implement a moratorium or permanent ban on *deployment* of specific types of gene drives in certain circumstances following an in-depth risk assessment.

4. *National governments should create specific regulations for the use of gene drives, with particular attention to regulating and protecting human-influenced species.*

Unlike most GMO organisms, which mostly remain in the area in which they were cultivated, the potential for gene drive organisms to cross national borders makes international agreement on regulations and communication about gene drive research a high priority. Governments should be creating regulatory approaches to this technology in advance of its deployment, rather than attempting to manage an emerging problem only after a gene drive has been released. National governments should create legislation that explicitly addresses gene drive research and development. This legislation should require: the establishment of a single national authority responsible for the oversight of gene drives in the

country; performance of an independent risk assessment before any gene drive is released in the environment or a field release; required participation in a national tiered registry; development of reversal drives in tandem with primary drives; and the creation of a system for periodic monitoring of a gene drive following its release into the environment.

While national governments begin to address these issues individually, there is a need for international cooperation and the development of harmonized approaches to deployment, given the potential for cross-border spread. Large discrepancies in gene drive legislation between nations, particularly those that share borders, could create points of contention, especially if there is gene drive spread to a country with stricter gene drive regulation or to one with lower capacity to respond to such spread. Therefore, international harmonization of national approaches should be pursued. To this end, export control, patent law, and invasive species management regulations could be used as a method of controlling gene drives.

National governments should ensure human-influenced species are considered and protected in regulations. Human-influenced species are valued by human populations as food sources while also filling important environmental niches. Because these species are important for both human interests and for maintaining ecosystems, special efforts should be made to protect these species from extraneous and potentially harmful gene drives. As many such species are sexually reproducing with relatively short generation times, they could be suitable for a gene drive intervention. Gene drives could be deployed to help protect these species from effects of climate change, invasive species, or other threats.

However, gene drives could also be used to try to place undue control by one stakeholder on these species at the expense of other stakeholders for economic gain. For this reason, governments should pay special attention to protecting this class of species through specific regulations regarding human-influenced species. There is a need for international attention to this issue because, unlike farmed species, which are geographically limited to their location of cultivation, human-influenced species often have international ranges, as is the case with migratory birds or ocean-caught fish.

Elements of legislation should include monitoring, risk assessment, tiered registration, and the requirement for a reversal drive to be developed and ready to deploy if needed at the same time the original drive is deployed.

5. *Governments should require that gene drives cannot be used without reversal drives having been already developed, tested, and ready to be deployed if there were the need for emergency reversal. Systematic monitoring of gene drive impacts on the environment should be in place and required before release is approved.*

Because the only effective gene drive countermeasure in most situations would be another gene drive, reversal drives or immunization drives should be designed and created in parallel with the primary drive. While no gene drive should be released without extensive risk assessments and studies to demonstrate its safety to the highest extent possible, gene drives will always pose some risk.

Therefore, mitigation measures that can be implemented after release should be explored and developed before the primary drive is released. Reversal and immunization drives, being the only such measures available with current technology, should be developed in tandem with the primary gene drive. Regulatory efforts for gene drives should require reversal measures as part of the overall gene drive application, not a separate product requiring separate approval. There should be criteria

established before a gene drive is released about the circumstances under which the reversal drive will be released and who will be responsible.

Part of any gene drive release should be monitoring the population in which the genetic elements have been introduced to assess whether the gene drive is behaving as anticipated. This monitoring should include sampling the target population and sequencing its members' genomes. Related species in the area should also be systematically monitored to ensure the drive has not moved into these species. Ecological assessments should be conducted regularly to assess the impact the drive has had on the environment and its ecology.

6. *Governments should require gene drive researchers to design their drive technologies with intrinsic and extrinsic containment strategies to mitigate the risk of spread in the event of an accidental release or laboratory escape.*

There are a number of approaches that scientists are already taking to manage identified gene drive risks. Scientists have organized to create guidelines for safe gene drive research, and there are multiple governance structures that affect the use of gene drives.[12]

One of the key concerns for gene drive research is an accidental release of a gene drive into the environment. Because of this concern, several safety measures have been published to encourage responsible research surrounding gene drives, including physical barriers, geographical limitations, and genetic containment mechanisms.[12,25] Physical barriers include laboratory infrastructure, organism containment strategies, and protocols designed to mitigate the likelihood of test organisms escaping the lab into the environment. These strategies are similar to other physical containment procedures for any laboratory working with animals or pathogens.

Geographical containment refers to moving the lab work outside of the range of the organism, such as conducting the research on a continent that does not house a natural population of the animal or plant. In this way, if the organism were to escape the lab, it would not have a natural population to mate with and spread the drive through. The limitation to this type of system is that animals can move across geographical regions.

In addition to physical and geographic controls, genetic control mechanisms are elements built into the gene drive system to inhibit the drive from functioning if the organisms were to escape the lab. For instance, kill-switch systems and drive systems in which the nuclease and target sequence are located at different points in the genome are types of genetic containment (see Appendix 2 on split drives for more on this

method). However, these systems could fail as the drive system evolves in response to selection pressures. Scientists working on gene drives have recommended that at least 2 of these methods—physical, geographic, or genetic—be put into place before developing a gene drive, and these recommendations seem sensible and warranted.[12]

7. *Governments should require coordination between researchers and local and international stakeholders before gene drives are deployed.*

Even if one country approves release of a gene drive, neighboring countries may not have developed gene drive legislation to appropriately assess or respond to gene drive proposals, or they may have total bans on all gene drive technology. Under the Cartagena Protocol, signatory nations have certain responsibilities to perform appropriate risk assessments and acquire specific approvals from bordering countries for potential transboundary movement, whether intended or unintended, of living modified organisms resulting from modern biotechnology.[53] Currently, 171 signatory parties adhere to these levels of international coordination. While gene drives have not been explicitly mentioned in the protocol, they pose the same kind of concerns for international spread.

RECOMMENDATIONS

Governments should require coordination and communication among researchers, stakeholders, governments, and NGOs. This could help decrease the risk of failure or redundancy once gene drives arrive at the deployment stage after years of risk assessment and development. Groups working on gene drives and in the humanitarian sector should coordinate to ensure their efforts are not deleterious to each other and in order to maximize each intervention's impact and safety. For example, traditional vector-control groups should be involved in gene drive coordination efforts for gene drives in mosquitoes. There would not be much use in releasing a population suppression drive into mosquitoes immediately before a mass insecticide spraying campaign. Both traditional vector-control and gene drive methods have the potential to make a meaningful combined impact on human disease burden when used in tandem.

Gene drives could augment traditional vector control interventions, since gene drives do not rely on changing human behavior and would require little input of resources after release. This is in contrast to insecticide-treated bednets, which are often limited by whether people are trained to use nets, the level of communication that has encouraged a population to use nets, and the capacity to replenish old nets.[68,69] Researchers must also coordinate with non–gene drive groups working in the field. If a gene drive were released for mosquitoes and then vector control officials had a mass spraying campaign, both groups' efforts would ultimately be minimized.

The scope of gene drive technology would vary widely depending on the specific application. During the conceptualization of a novel gene drive application, there should be a dialogue in the scientific community to discuss ethical considerations and to develop a methodological road map to build meaningful stakeholder engagement in the targeted area. Part of this methodology should focus on identifying and approaching regional groups, local community organizations, and government entities as a way to build a network of stakeholders that accurately represent the best interests of the community. Transparency and open lines of communication with stakeholders should be ongoing throughout the production of a gene drive application, and researchers should work to communicate steps taken to mitigate concerns that may arise during stakeholder conversations.

National governments should establish a protocol that empowers local stakeholders to have a constructive input in the decision to deploy a gene drive technology. There is an opportunity for gene drive technologies to solve stakeholder concerns. It is imperative that research in deployment efforts recognize the ethical implications of a decision and make dedicated efforts to create a product that reflects the concerns and best interests of an affected public.

There are limited means through which local communities can stay informed on gene drive research, including through news media penetration or education campaigns initiated by the research teams themselves. It should be mentioned that many groups in this space already perform admirable community outreach,[69] but it is less clear how communities can initiate outreach from their side with the scientific community regarding these issues. Local stakeholders should be encouraged to communicate not only with researchers, but also with their governments to facilitate active civic engagement and government accountability.

Interviewed experts frequently commented on the importance of communicating and coordinating with local and regional authorities during each stage of gene drive development. Civic engagement and a sense of government accountability will lay important groundwork for continued involvement with stakeholders and give the community a sense of partnership over alterations in their environment.

CONCLUSION

Gene drives could be a powerful solution to solving health and commercial problems that are difficult or impossible to solve otherwise, such as malaria, other vector-borne diseases, and invasive species. However, there is also uncertainty surrounding how a gene drive will behave once it is actually deployed, and gene drives therefore pose potential serious risks.

It is also clear that there are some applications for which gene drives will likely never represent a favored method for legitimate action. For example, gene drives will likely never be the most useful way to address challenges in farmed agricultural species. The case-by-case regulatory framework that we recommend is anticipated to disfavor gene drive releases in farmed species.

This outcome is notable, as agricultural GMO concerns heavily inform much of the opposition to the use of gene drives. If it becomes apparent to those who are opposed to the use of gene drives in agriculture that this use case is unlikely to occur, then perhaps there can be more productive conversations between those for and those opposed to gene drives moving forward.

In that vein, activities with human-influenced species such as wild-caught oceanic fishing, which already represent a challenge for international regulation, could be a point where gene drive regulation may benefit strongly from coordination with an international organization such as WHO or the Food and Agriculture Organization (FAO) of the United Nations. International organizations could help in this endeavor to provide unbiased judgment over the regulation of certain species that have high potential to cross borders or, in the case of oceanic species, to provide more oversight on the release of gene drives into international waters.

However, as the uneven acceptance of the Cartagena Protocol demonstrates, acceptance of international regulations is often quite slow, and there is an imperative for regulation of gene drives to be developed in a timely manner. Thus, while regulation and guidance from international organizations will be of great assistance as the state of the gene drives field moves forward, the regulatory burden must ultimately be taken up by national authorities to ensure up-to-date guidance for scientists and organizations pursuing these means. One international organization where acceptance is already near universally present is the Biological Weapons Convention; while it is sincerely hoped that gene drives will never be used as weapons of war, it is clear that such a use would be prohibited by the Biological Weapons Convention as currently written.

While there are many challenges to regulating these complex technologies, there are some concrete governance steps that could be taken to minimize risks while also allowing potential beneficial applications to be fully explored. These steps most notably include updating national laws and regulations to specifically address and assign responsibility for gene drive regulation, as part of existing genetically modified organism statutes, or creating new ones. Further, coordination among gene drive–releasing groups is essential for technical reasons, particularly with regard to multiple gene drives being released in the same host organism. Gene drives are likely to spread across borders and thus involve multiple national regulatory frameworks; thus, there is great need for a single unified international registry of gene drive projects, both active and past. As an interim step toward such a unified international registry, this report recommends the creation of national tiered registries of gene drive projects for any countries currently involved in or looking to become involved in such research.
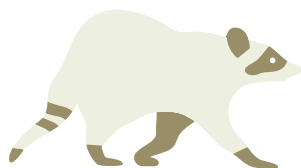
APPENDIX 1

LIST OF INTERVIEWEES

All interviewees' opinions were their own and not those of their organizations.

Aggrey Ambali, PhD, New Partnership for Africa's Development

Felix Beck, MSc, University of Freiburg

Jackson Champer, PhD, Cornell University

Genya Dana, PhD, World Economic Forum

Jason Delborne, PhD, North Carolina State University

George Dimopoulos, PhD, Johns Hopkins University

Rebecca Efroymson, PhD, Oak Ridge National Laboratory

Laura Epstein, CVM, US Food and Drug Administration

Kevin Esvelt, PhD, Massachusetts Institute of Technology

Jacqueline Fletcher, PhD, Oklahoma State University

Stephen Higgs, PhD, Kansas State University

Betty Lee, PhD

Philipp Messer, PhD, Cornell University

Paul Ndebele, PhD, George Washington University

Samantha O'Loughlin, PhD, Imperial College London

Kimberly Orr, DVM, PhD, US Department of Commerce

Larisa Rudenko, PhD, Massachusetts Institute of Technology

Jacob Sherkow, JD, New York Law School

Philip Thuma, MD, Johns Hopkins University

Renee Wegrzyn, PhD, Defense Advanced Research Projects Agency

Nikolai Windbichler, PhD, Imperial College London

Laurie Zoloth, PhD, University of Chicago

APPENDIX 2

TECHNICAL REVIEW

Introduction

This appendix is intended to provide a broad overview of the biotechnology of gene drives. The descriptions of each drive type are not intended to be an exhaustive review, but rather are intended to help orient those who have a scientific background but who are unfamiliar with the key underlying biological mechanisms.

While CRISPR-based gene drives are among the most commonly discussed gene drive systems, this review encapsulates other kinds of systems currently being discussed in the literature. Some of these systems are further along in the development process than others. Additionally, the underlying biotechnologies that enable gene drives are also under development. Advances that enable the effectiveness and feasibility of gene drive technologies must be considered on a case-by-case basis. For example, the recent development of CRISPR Prime is unlikely to further enable CRISPR-based gene drives, because it does not support insertion of gene-length fragments.[70]

This review also discusses several considerations about how, when, and why certain drive systems would be preferable to others and in which situations gene drive use might be ineffective for altering a population.

Gene drives can be naturally occurring or man-made. There are many possible gene drive mechanisms with different properties, but all of them share a single defining quality: They are inherited from generation to generation at a rate greater than classic Mendelian inheritance would predict. Gene drives based on powerful biotechnology tools like CRISPR can achieve rates of inheritance close to 100%—that is, nearly all offspring of a mating pair with only 1 gene drive parent will inherit the gene drive.[13] Because these drives are passed to offspring of sexually reproducing organisms, each successive generation of offspring carry this inheritance-biasing property. On a population level, this means that gene drives can quickly spread altered genes though a sexually reproducing population after starting with a small number of organisms engineered to carry the drive.

There are several characteristics shared by all gene drives that lead to some common risks. For example, specific gene drive mechanisms may fail to function as intended, either by gradually ceasing to spread at super-Mendelian rates or from unintended spread, and gene drives have varying risk profiles depending on their mechanisms. There are risks based on unpredictable factors, such as an escaped organism, a mutation, or a series of mutations, leading the drive system to have unexpected ramifications. Another risk is inefficiency, where unpredictable factors merely cause the system not to work effectively. Other risks include the evolution of resistance to the gene drive in the target species, analogous to the spread of antibiotic resistance in bacteria.[56,71] Different molecular systems have different likelihoods of resistance evolution, and the dynamics of suppression and resistance in populations are understudied.[57] Of the types of gene drives listed in this section, only some have been put into animals for experiments and others remain solely theoretical.

Different Types of Gene Drives

In this section, we review several types of gene drives, summarizing the basic technical details for each type of gene drive, including the functionality, limitations, and benefits.

Homing drives

Subtype	Why use this type of drive?
Homing drive	The purpose of deploying a homing drive is to spread the drive without built-in limitations.
Precision drive	The purpose of deploying a precision drive is to spread the drive within a naturally occurring subpopulation of the host species.

The most well-known and commonly discussed type of gene drive is the CRISPR-Cas9 homing drive. These drives are known for their ability to spread altered genes quickly through populations and even to suppress populations. To create such a gene drive, 1 chromosome of the host organism is altered to include 3 crucial elements in the drive cassette: an endonuclease (Cas9), an engineered guide RNA that directs the endonuclease protein to the correct genetic locus, and the payload gene or "gene of interest." Unlike other GMOs, this process creates a gene drive because the engineered guide RNA targets the same site in the genome in which the 3 elements are inserted— that is, the homing gene drive has a biological activity specific to the insertion site of the drive itself. The result will be a system that will repeatedly insert itself at that same site onto other copies of the same chromosome.

When expressed in an embryo heterozygously alongside the wild-type version of the chromosome, the endonuclease and guide RNA combination cleave the wild-type site that the guide RNA targets. The cell's repair mechanisms will then "repair" the broken chromosome by copying from the homologous region of its pair chromosome carrying the gene drive cassette. The drive system is thus specifically engineered to take advantage of this "copy and paste" style repair mechanism. The result is that the cell becomes homozygous for the gene drive carrying 1 or more genes of interest, even if there was only

1 copy in the cell initially. Offspring that inherit the gene drive subsequently also become homozygous at this site because of the cut, copy, repair process.[17,18] More recent technologies may be able to use transposon elements, trading off independence from the host's homology-directed repair machinery with a longer inserted sequence.[72]

This type of drive is essentially unconfinable in the wild; there is no way to confidently restrict it to a limited population.[53] However, if mutated versions of the target site are circulating in the wild population, then the drive could potentially spread to the entire population. These mutations to the target site, or resistance mutations, would actually be driven by the action of the gene drive.

These mutations arise when the homology-directed DNA repair mechanism, the crux of how the drive spreads through a population, is not used to repair the break in the DNA. If the cell repairs the break in DNA with another type of DNA repair mechanism, such as nonhomologous end-joining, then the target site could be drastically altered such that the guide RNA is unable to direct the endonuclease to the correct target site. Because the gene drive itself is likely detrimental to the fitness of the organism (see The Limits of "Intrinsic Biological Control"), such resistance mutations are likely to be under positive selection, causing the gene drive to eventually die off once it has saturated the population with either itself or resistance mutants.[58]

There are a number of strategies to reduce or bypass the formation of such resistance mutations, like multiplexing gRNA (creating multiple sites at which the gene drive can insert) and expression control over when the CRISPR-Cas9 is active in the organism's life cycle.[73]

The wild-type genetic target must be carefully chosen. The precision drive, a subtype of the homing drive, specifically targets a genetic sequence that is not found in most of the host organism individuals, but rather is unique to a local subpopulation of the species. The homing drive then suppresses or alters the local population but is restrained from altering the broader species population, which does not have the unique target site. This technique is best suited for relatively genetically isolated populations, such as populations on an island.[17,74]

Split drives

Why use this type of drive?
The purpose of deploying a split drive is to limit the spread of the drive only to hosts with a pre-engineered genetic context.

A split drive is a type of homing drive in which the gene drive cassette components are not all located in the same chromosomal region but rather spread out over different sites or even different chromosomes. This strategy may be pursued for several reasons, like making the drive more fragile to easily render it nonfunctional to prevent unintended spread.[12]

Depending on the drive's design, certain drive components may not be driven to super-Mendelian inheritance themselves, but rather only drive other components to super-Mendelian inheritance when in their presence. In this way, the success of each component relies on the success and presence of the other components. The result is that the whole system does not infinitely propagate through future generations. As such, split drives can be self-propagating for a short time, like a basic homing drive, or they can be self-limiting. Self-limiting split drives have been used in laboratory experiments to study gene drive dynamics, while reducing the risk of a self-propagating system escaping into the environment.[12,15,75]

The *Aedes aegypti* confinement drive designed by Ming Li and colleagues is an excellent case study for split drive systems.[75] In this system, 1 genetic locus is altered to contain a fluorescent indicator protein gene and a corresponding guide RNA. The gene for fluorescence was chosen because it is easy to track, but other payload genes could replace it. A separate genetic element encodes the Cas9 protein and is inserted at a different site. When all cassette components are present in a cell, the components combine to drive the fluorescent indicator gene and guide RNA onto its paired chromosome, thus making the cell homozygous even if only 1 copy of the altered locus were present initially. As a containment mechanism, the Cas9 gene is not over-propagated and is maintained in the population by the scientists repeatedly adding genetically altered Cas9-carrying males back into the population. Several variations of the drive apparatus were tested before the final version was found to operate efficiently.[75]

Daisy drives: Multipart split drives

Why use this type of drive?
The purpose of deploying a daisy drive is to limit the spread of the drive to a specific time or number of generations after the initial release.

A daisy drive is a gene drive that incorporates several split drives in a stepwise fashion. Daisy drives are basically a homing drive that lasts only for a certain number of generations.[76] While there are numerous alternative genetic architectures to achieve the effect of a daisy chain drive, here we will summarize the most commonly discussed version.

In the daisy drive system, there are N separated loci in a genome. Locus A, which contains an endonuclease (eg, Cas9) and a guide RNA for driving Locus B. Locus B encodes an endonuclease and matching guide RNA for driving Locus C. This continues until reaching Locus N, which contains the gene of interest. Locus A is not driven—that is, it will be inherited only at normal Mendelian rates. Beginning after the first generation of breeding from the release of the daisy drive into a wild-type population, Locus A's concentration is dispersed via Mendelian inheritance. Locus B is inherited only at super-Mendelian rates in the presence of Locus A. So, once a breeding individual is missing Locus A, Locus B becomes undriven and also disperses via Mendelian inheritance. This goes on until the gene of interest at Locus N is no longer driven at all, and all genes are dispersed via Mendelian inheritance.[77]

Daisy drives, unlike standard homing drives, are designed to be self-limiting as they spread through their host species population. Theoretically, there are scenarios in which random and uncontrollable genetic recombinations could reconfigure a daisy drive, or even a split drive, such that lower elements in the daisy-chain drive higher elements and thus render all of the reconfigured daisy drive no longer self-limiting in its spread through a host species population.[76] Such a broken system is more likely to emerge if all of the elements in the daisy chain use the same promoters and proteins, since genetic material is more likely to recombine on chromosomes where there are homologies. Design of daisy drives that do not use repeat promoter or endonuclease sequences is necessary to reduce this risk, although this also constrains genetic design space, as there is not an indefinite pool of promoter and endonuclease sequences appropriate for all tasks.[76]

A variant on the daisy drive is the daisyfield drive, which also spreads for a finite number of generations and then reverts to standard Mendelian inheritance. The system consists of a nuclease and gene of interest at 1 locus, and then, scattered throughout the genome, numerous guide RNAs targeting that locus. The gene of interest is driven through each generation, as long as there are guide RNAs present. However, the guide RNAs are themselves spread between different chromosomes and are dispersed in each generation, as altered organisms breed with wild-type populations. Once an organism no longer has guide RNAs, the gene of interest and nuclease are no longer driven, and the drive cassette disperses via Mendelian inheritance. This can be combined with ordinary daisy chain drives in different ways. Because only 1 cutting "step" takes place, a pure daisyfield drive is predicted to be more efficient than a daisy drive.[78] Another variant of the daisy drive is the daisy quorum drive, which is discussed further in the section on under-dominance drives.

Toxin-antitoxin drives

> **Why use this type of drive?**
>
> The purpose of a toxin-antitoxin drive is to spread the drive without self-limitation.

Before the discovery of CRISPR, toxin-antitoxin systems were, and still are, an engineerable method to bias which genes an organism's offspring inherits. Toxin-antitoxin systems consist of a toxin gene, a gene for a corresponding antitoxin, and a gene of interest. Any offspring that inherit the toxin gene must also inherit the antitoxin gene in order to survive. In many such systems, the offspring requires the antitoxin gene to survive, even if the offspring itself does not possess the toxin gene. In such cases, the anti-toxin gene is necessary to counter residual toxicity from parental cells. Unlike other drive types that copy the drive fragment onto another copy of the chromosome, these drive systems work by killing off progeny that do not have all the components of the drive.

One archetypal version is the killer-rescue drive. In this system, altered individuals have a toxin or other lethal gene on 1 chromosome and a matching antidote gene on another. The payload gene is then added to the antidote locus. Any progeny that inherit the toxin-containing chromosome will perish without the antitoxin-containing chromosome with the payload gene.[79]

Another class of toxin-antitoxin system involves sex-linked toxin expression to kill embryos or individuals that do not carry the drive. Sex-linked traits related to the expression of genes reside on the sex-determining chromosome; in mammals, these are the X and Y chromosomes. The most well-known of these is the *Medea* system, a naturally occurring gene drive in the flour beetle, which has been adapted for use in other insects. Females with the *Medea* gene express the inhibitor of an essential gene in their eggs (whether or not the eggs carry *Medea* themselves). *Medea* also contains an "antidote" version of the essential gene that is immune to the inhibitor, expressed in the egg cell itself. When an egg from a *Medea*-carrying mother is fertilized, it will survive if it has inherited a *Medea* gene from either parent, and it will die if it has not.[80,81] Conversely, in the inverse *Medea* drive system, the fertilized egg expresses the toxin, and the antidote is expressed by the mother. The fertilized eggs perish unless their mother has the antidote.[80]

In the *Semele* system, selection happens among adults, rather than zygotes. *Semele* drives contains a sex-linked toxin and antitoxin. Males with the *Semele* drive express a toxin in their semen. The toxin can either be lethal or cause infertility in any wild-type females who mate with the gene drive males. *Semele* females express the antitoxin, and thus they and their offspring are immune to the toxin's effects. This drive can act as a suppression system if only drive males are released, or as an alteration system if both drive males and drive females are released. In the case of alteration, the wild population will eventually be replaced by surviving *Semele* offspring.[82] Multiple releases of altered individuals are needed to saturate the toxin-antitoxin genes through an entire population.[82]

Underdominance drives (or Threshold drives)

> **Why use this type of drive?**
>
> The purpose of an underdominance drive is to limit the gene drive's spread to certain geographic areas.

Underdominance drives, also known as threshold drives, are non-CRISPR-based and are intrinsically self-limiting (**Figure 3**). These drives select exclusively for offspring with 2 copies of the gene drive, because individuals with only 1 copy of the gene drive are much less fit than either individuals with 2 gene drive copies or individuals with 2 wild-type alleles.

The behavior of this type of drive is dependent on the ratio of the number of individuals in each group to the number of individuals in the population as a whole (gene drive–containing individuals, and non–gene drive–containing individuals).[83] This creates a threshold effect; if enough homozygous individuals carrying the gene drive are present in the population to reach the required threshold, then the drive will likely persist in that environment. Conversely, if that threshold is not reached, then the gene drive will be eliminated from the population over time.[84]

Therefore, the success of this drive system depends on releasing enough altered individuals into the target population, such that breeding between wild-type and altered individuals overwhelms wild-type/wild-type breeding.[85] The drive is likely to stay relatively confined to 1 area, since mixing between wild-type and altered populations leads to less viable offspring, resulting in selection against the drive.[86] This is useful because it grants a form of control in effect size and geographic area to those releasing a gene drive.

A mechanism to counteract this type of drive would be to release more wild-type organisms into the population to disrupt the required mixing threshold.[87] These systems often share molecular mechanisms with toxin-antitoxin systems and tend to be labeled by the number of loci and toxins involved.

One underdominance variant is "1 locus, 1 toxin" (1L1T). The 1L1T system uses haplo-insufficient genes, where 2 copies are required for each cell to be viable (**Figure** 4). Once an appropriate haplo-insufficient gene is chosen, an altered version of the haplo-insufficient gene is designed with identical function but different sequence. An inhibitor to the original wild-type version of the haplo-insufficient gene is also designed (typically an interfering RNA). Because the gene that it inhibits is haplo-insufficient, the inhibitor functions as a toxin by reducing the total expression of the haplo-insufficient gene to levels equivalent to only 1 copy being present. Organisms that have 2 copies of the altered gene are immune to the inhibitor. The inhibitor's gene, the altered haplo-insufficient gene, and any payload genes are then all linked on the same chromosome of the host organism. When this chromosome enters the population, 3 results are possible: (1) offspring homozygous for the wild-type gene overcome haplo-insufficiency and are viable; (2) offspring that are heterozygous will have their wild-type gene expression inhibited, and the only copy of the altered gene is not sufficient to produce viable offspring; or (3) offspring homozygous for the altered gene overcome haplo-insufficiency and are viable.

The "2 loci, 2 toxin" (2L2T) system, also known as the "2 locus engineered underdominance" system, is a variant similar to a killer rescue drive. In this case, however, both loci contain a toxin, as well as an antitoxin for the opposing locus's toxin. Offspring must inherit neither or both loci in order to survive the effects of both toxins. In this way, heterozygotes are rapidly eliminated from the population. As time progresses, the number of homozygous wild-type individuals will decrease, and the number of homozygous gene drive individuals will increase.

The "1 locus, 2 toxin" (1L2T) system is a variant on the 2L2T system, in which each pair of toxin and antitoxins exist on different versions of the same chromosome. This system selects for offspring that are either homozygous for the wild-type gene or for offspring that are heterozygous for both wild-type genes.[83]

The daisy quorum drive is another theoretical self-limiting drive system and is a combination of a daisy

Both chromosomes contain allele for critical protein

Critical threshold of mRNA is transcribed and translated into functional product

Normal phenotype

Only one chromosome contains allele for critical protein

Critical threshold of mRNA is not reached— resulting product is not sufficient to produce a normal phenotype
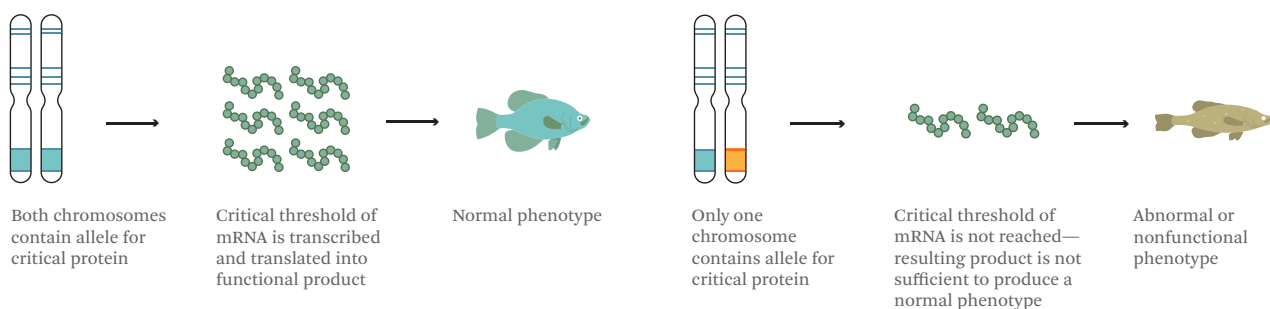
Abnormal or nonfunctional phenotype

Figure 3. Offspring who are homozygous for an underdominance gene, possessing a normal phenotype, are more fit and able to reproduce. Offspring who are heterozygous for an underdominance gene display an abnormal or nonfunctional phenotype and are less fit to reproduce.

drive and an underdominance drive. The underdominance component consists of 2 separate drive mechanisms, each based around a different haplo-insufficient gene. Each of these drive mechanisms includes a Cas9 protein and an intentionally altered haplo-insufficient gene. When targeted with the appropriate guide RNAs in a heterozygous cell, the drives activate and replace the wild-type gene on their chromosome with the other haplo-insufficient gene through homologous repair. To be viable, a cell must have neither or both of the haplo-insufficient gene drive copies. Therefore, the drive tends to spread only where there is a large concentration of pre-altered individuals, which increases the chances that offspring will inherit both altered chromosomes. This is the "quorum" part of the drive.[88]

The guide RNAs for both drive mechanisms, meanwhile, are propagated by a daisy drive. Each drive element consists of guide RNAs for each "quorum" drive, as well as a guide RNA for the next daisy drive element. The first daisy drive element is undriven, and the final consists only of guide RNAs for the quorum drives. In each new generation, the chain's previous element begins to disperse via Mendelian inheritance. Because the daisy drive spreads the quorum elements, all self-spreading capability for the payload gene is lost once the daisy chain is expended.[88] There are many moving parts in this drive system, making it increasingly likely that mutations will render the drive nonfunctional over time;

however, it remains a potentially powerful tool for creating gene drives with limited temporal and geographic spread as long as such resistance mutations are rare evolutionary events.

Sex-biasing drives

Why use this type of drive?
The purpose of sex-biasing drives is to suppress the population of the host species by causing an unbalanced ratio of sexes to be born.

Sex-biasing drives are gene drives that affect the sex ratio of offspring and, like all gene drives, are inherited by offspring. Over time, as only males or only females are born and reproduce with remaining wild-type members of the other sex, the population will shrink.[24] Note that some toxin-antitoxin drives also differentially affect males or females, but they do so on the basis of the individual containing the gene drive, regardless of its sex, and do not result in driving the entire population to 1 sex.

These drives are, like homing drives, potentially capable of unlimited global spread. There is, however, strong selection pressure against a genetic trait that drastically reduces its host's fitness, such as drastically changing the ratio of males to females, suggesting that mutation may readily emerge in populations with these drives.

In population A: both chromosomes contain allele for critical protein

Critical threshold of mRNA is transcribed and translated into functional product

Normal phenotype: can continue to reproduce in population A

Population AB heterozygous hybrids: Differences between alleles of the non mixing populations create fitness costs

Critical threshold of mRNA for neither population requirement is reached—resulting product is not sufficient to produce a normal phenotype

Abnormal or nonfunctional phenotype: reduced reproduction

In population B: both chromosomes contain allele for critical protein

Critical threshold of mRNA is transcribed and translated into functional product

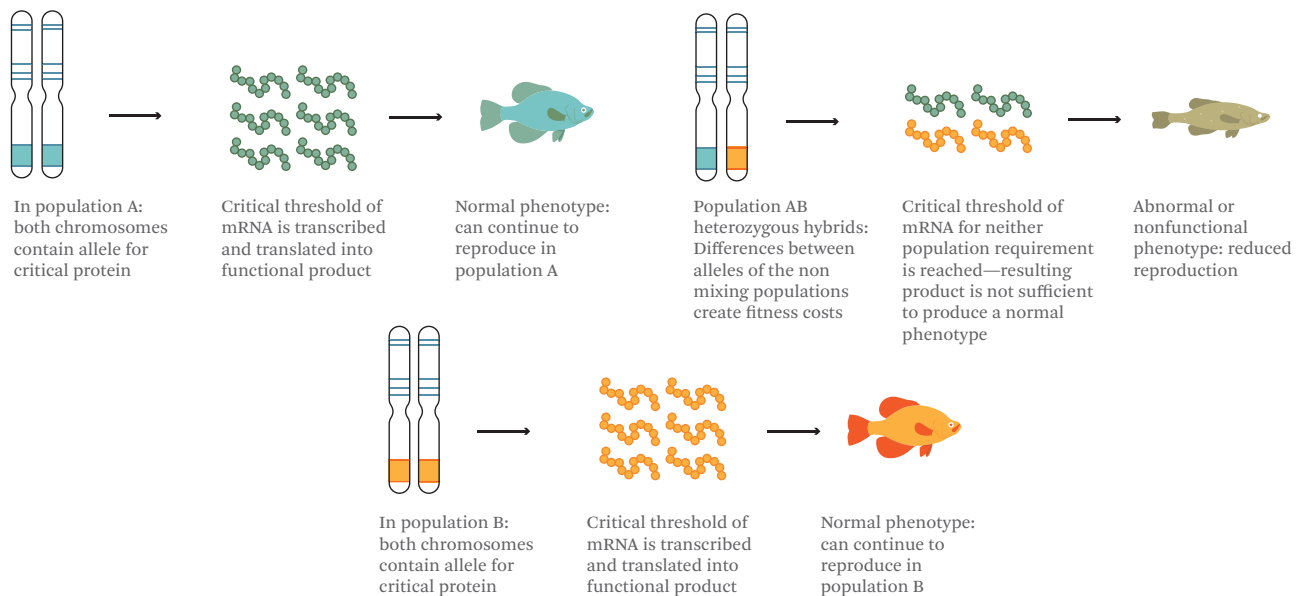Normal phenotype: can continue to reproduce in population B

Figure 4. Haplo-insufficiency, relies on the decreased fitness of heterozygous offspring for an underdominance gene. Resulting hybrids have reduced reproductive ability compared to the homozygous offspring of either parental population.

One sex-biasing drive is a female fertility drive, which is a homing drive that targets a haplo-insufficient female fertility gene and inactivates it. The outcome is that females who are homozygous for the altered gene are infertile. In this scenario, the initial release population contains males and/or females that are heterozygous for the altered gene and thus are able to reproduce and pass on the altered gene.[89] The drive is activated only during gamete production, so the offspring of the released mosquitoes produce only homozygous offspring (and thus infertile females). As the number of infertile females increases, the total population drops.[22,24]

An X-shredder drive, a type of sex-biasing drive, is a genetic package located on the Y chromosome in an XX/XY-sexed species. An XX/XY-sexed species is one in which the sex of an individual is determined by the presence or absence of sex-determining chromosomes. In other species, the anatomical sex of an individual might be determined by other factors, such as temperature at a certain stage of embryo development.

An X-shredder encodes endonucleases that specifically target regions of the X chromosome. The endonucleases cut the X chromosome at these sites and render it nonfunctional. The drive's endonuclease is expressed during meiosis in males, so that males with at least 1 copy of the drive produce sperm with only the Y chromosome.[85,90] Because this drive targets multiple loci found on nearly all X chromosomes, it is less likely to result in resistance development among subsequent generations than other types of gene drives.[24] Similar W-shredder systems, which target the W chromosome of ZW sex determinant species, such as cane toads, have also been proposed.[91]

A non-CRISPR-containing drive system has been proposed that is a combination of a sex-biasing and a toxin-antitoxin drive. This combinatorial system is called the Medusa drive, and it is a population suppression drive system similar to a Medea or a 2L2T drive. Both the X and Y chromosomes (in an XX/XY-sexed species) contain toxin and antitoxin, corresponding to those on the opposing chromosome. Thus, only offspring with both the X and Y chromosomes survive and pass along the drive as well. The population quickly becomes male-biased and crashes.[80] This type of drive requires a lower release threshold than other sex-biasing drives, but multiple releases of the drive carrying individuals are probably needed to avoid population grow-back.[81]

Non-CRISPR endonuclease drives

Why use this type of drive?
A non-CRISPR-based drive, such as a transposon, could be used in non-sexually reproducing species where other gene drives would not be effective.

Prior to the discovery of Cas9, there was more interest in other classes of enzymes used to recognize and cleave specific DNA sequences. These endonucleases include transcription activator–like effector nucleases (TALENs) and zinc finger nucleases (ZFNs). These enzymes do not rely on a corresponding guide RNA to recognize proteins, but rather on the amino acid sequence and structure of the protein. "Alphabets" of amino acid sequences have been assembled that allow the creation of enzymes for essentially arbitrary sequences. Creation of these methods is more laborious and less flexible than working with CRISPR-Cas9, but they can theoretically be used in a homing or other drive mechanism in much the same way.[92]

Other Biasing Genetic Elements

In addition to the gene drives described above, there are a handful of other genetic or parasitic elements that are both inherited and biased toward their own inheritance. The genetic elements discussed in this section are naturally occurring examples of gene drives that can be used to address some of the same problems as other types of gene drives but are not as popular as options anymore because of their limitations. These elements tend to be less understood or less modifiable than CRISPR drives but can theoretically propagate genes through a population, similar to the gene drives described above.[18]

The B chromosome is a "parasitic" or "selfish" chromosome found in some species. It tends to be small, noncoding, and nonessential and to occur singly rather than in pairs like most chromosomes. Many B chromosomes use strategies to "accumulate" or inherit at super-Mendelian rates—for instance, by causing embryonic cells containing many B chromosomes to be more likely to mature into gamete-producing cells.[93] In theory, promoters and genes could be placed in B chromosomes and thus spread through a population at super-Mendelian rates, although B chromosome biology is not well understood.[18]

A much more common element is a transposable element, or transposon, like the P transposons in *Drosophila melanogaster* described in the introduction.

Transposons are mobile pieces of DNA that promote their own copying and reinsertion into different points in the genome and thus are capable of super-Mendelian inheritance.[94] Intentionally adding genes into these elements could potentially allow them to be used as a gene drive.[95] Typically, naturally occurring transposons have a much slower integration rate than the homing drives discussed earlier and would be expected to spread through a population more slowly than other engineered drive systems. Unlike homing drives, they do not need to insert at a specific site and therefore are not subject to resistance mutations at the insertion site. Further, the copy number of the transposable element can rise far beyond the limited number of target sites that would exist for a homing drive with numerous deleterious biological implications.

Finally, there is an insect parasite that behaves similarly to a gene drive. *Wolbachia* is an intracellular bacterium in some insects that is inherited maternally via egg cytoplasm. Different *Wolbachia* strains have different effects on the reproduction of the infected insect, which can include killing insect embryos of parents that are not infected with compatible *Wolbachia* strains, thus selecting for their own inheritance in embryos.[18] Through such effects, it is possible to conceptualize *Wolbachia* as a population suppression drive.

Technical Considerations for All Gene Drives

The Limits of "Intrinsic Biological Control"

The principle of "intrinsic biological control" (also referred to as "intrinsic biocontainment") is central to several aspects of the function of gene drives. An intrinsic biological control is a mechanism designed into the genetics of an organism with the intent of containing that organism's growth, reproduction, or spread within predetermined boundaries or conditions. By contrast, an extrinsic control would be something outside the organism itself, such as a cage or containment vessel. Gene drives can be considered an example of an intrinsic biological control on the genetics and viability of their host species.

An example of an intrinsic biological containment system would be a genetic "kill switch" that rendered the organism carrying it unable to survive unless an artificially supplied chemical is present. Such an organism would be able to survive only in a laboratory setting where that chemical would also be supplied, but it would die if it were to escape the laboratory into an environment without the chemical.

Intrinsic biological controls have certain known dynamics as a result of natural selection. The organism carrying an intrinsic biological control, while perhaps designed to be more useful to humans, is at a disadvantage relative to uncontrolled versions of the same organism. This is a direct consequence of the control itself and is independent of the exact mechanism of control. The intrinsic control forces the organism to live in a smaller ecological niche than it could otherwise exploit.

The wild version of the organism, which has not been modified with an intrinsic biological control, has the comparative advantage that it can still exploit the full ecological niche. That comparative advantage is also conferred to individuals of the modified organism that have acquired mutations that render the intrinsic biological control no longer effective. This is referred to as an "escape mutant."[96,97] Once such a mutation exists, because it has an advantage over its controlled brethren, it will come to dominate the population as a consequence of natural selection.

Escape mutations are rare, but "rare" is a relative term; specifically, rare is relative to the number of opportunities for the mutation to occur and to be selected for. In the kill switch example described above, if only 20 organisms escaped the laboratory, and the chances of 1 of them being an escape mutant (that is, the intrinsic biological control was broken and this specific individual *could* survive without the artificially supplied chemical) were 1 in a million, then the chances that the intrinsic biological control would fail are quite low: 20 in a million. This is not because 1 in a million is a low rate of escape mutation in any absolute sense, but because it is low *compared* to the anticipated number of opportunities for the organism to escape control. If 20 billion organisms escaped the laboratory instead of 20, and the rate of escape mutation was still 1 in a million, some 20,000 organisms that escaped the laboratory would also be able to live without the chemical, and the intrinsic biological containment would have failed.

This has direct implications for the effectiveness of gene drives, as many of them generate "resistance mutations" that prevent their own spread. These resistance mutations are escape mutants to the intrinsic biological control that is the gene drive. The number generated is very high, because the gene drive's own activity causes the rate of such mutations to scale with the gene drive's own spread.[58] Further, in any practical

release of gene drives in the wild, the number of wild organisms eventually carrying it will be very high relative to even large field trials, so even a very small absolute rate of resistance or escape mutation may be high when compared to the number of opportunities for escape mutation formation afforded over such a large wild population.

This principle that escape mutation rate and population size are numbers that describe the probability of success or failure of an intrinsic biological control only in relation to one another applies to some gene drives in another capacity. Many gene drive systems being proposed in the literature are so-called "self-limiting drives." These are drives that are designed to spread for only a certain number of generations inside their host species, or only within certain geographic regions, or under certain circumstances. In these cases, the gene drive is still an intrinsic biological control on its host species, but the genetic mechanisms that are designed to limit its spread are also intrinsic biological controls *on it*. Just as escape mutants will occur in the host organism at some rate to let it escape from the control of the gene drive, so escape mutations will form in the gene drive at some rate to let it escape from the self-limiting mechanism that prevents its uninhibited spread. In both cases, the absolute rate of mutation is not meaningful without comparing it to the number of opportunities for it to happen and be selected for.

A risk assessment of gene drive applications is essential for proper regulation of gene drive technology; this is, in part, because of the wide variety of gene drive architectures and types already being considered today. In the future, it is likely that still more gene drive variations with different intrinsic controls will be proposed and developed. Yet, the basic principle that intrinsic biological controls always are at risk of escape mutations will be just as true of them as of any other intrinsic biological control.[98] For this reason, any risk assessment of gene drives must consider these questions: How often will escape mutants form? How many opportunities will those mutants have to overtake the controlled gene drive or organism? And how strong will the selection favoring escape mutants be?

Host Species Considerations

In order for a gene drive to be a viable intervention outside of the lab, the host species must reproduce sexually. Also, because gene drives work by skewing inheritance ratios across generations, they work fastest in species with short generation times. Some sexually reproducing species, such as trees, can have extremely long generation times relative to human lives and economic cycles, making gene drive interventions in such species less attractive, because they would not come to fruition on human time scales.[23,57]

Other issues that can complicate the viability of some gene drive systems in certain hosts are the ploidy of the organism; ploidy refers to the number of copies of a chromosome that each organism of a cell contains. In many animals, that is 2 copies of most chromosomes, but in some species, particularly some plants, it can be much higher. Also, some gene drives such as sex-biasing drives may not work in all species. This is because sex in many species is controlled by environmental factors, such as the temperature of the embryo at a certain time in development, rather than inheritance of a sex-determining chromosome. Finally, the propensity for the host organism to interbreed with other species and produce viable offspring directly affects the safety and risks associated with releasing a gene drive into such hosts.

Because the concerns raised over gene drives are focused on an agricultural model and informed by the predominantly plant-based GMOs currently used in agriculture, it is worth briefly considering a plant-based gene drive from a technical perspective. While wholly theoretical at this point, and needing to overcome certain technical challenges, gene drives in plants are conceivable.[57]

Even so, not all plant species are reasonable hosts for a gene drive. Broadly, cultivated species of plants fall into 3 categories: asexually cultivated, monoecious, and dioecious. An asexually cultivated species is propagated by cuttings and grafts. A monoecious species is propagated by seeds derived from flowers. However, each individual monoecious plant has flowers with both male organs (stamens) and female organs (pistils). The term *monoecious* in plants is analogous to *hermaphrodite* in animals. Dioecious plants are of either the male or female sex, but not both. Because gene drives spread through the population of a host species by altering hereditary inheritance ratios during sexual reproduction, they would

not be able to spread through a population of asexually reproducing plants. Similarly, monoecious plants, which most often fertilize themselves, would be highly inefficient at spreading a gene drive through a population. Therefore, a gene drive will be efficient only in dioecious species.

In agricultural crops, gene drives are likely to be a useful or effective technology only in dioecious annually cultivated plants. Many cultivated plant species, particularly major staple crops, are asexually propagated or monoecious, but there are exceptions, such as soybeans. Because plants often require the intervention of a pollinating insect, and hives of pollinating bees are routinely shipped from farm to farm, there is potential for widespread and rapid dissemination of a gene drive by a pollinator vector. Similarly, wind-borne pollen is often a major factor in the reproduction of some plants, suggesting a delivery vector for a plant-based gene drive similar to crop dusting might be achievable. Finally, annually planted species have a generation time that is more amenable to gene drive use than perennials.

In Findings, we pointed out that there are material differences among species that are human farmed, human influenced, and wild with regard to their suitability for host species of gene drives. There are few opportunities for gene drive use directly in human-farmed species because of the de facto genetic control that human farmers exercise over the species that they cultivate. However, there are still potentially valuable applications of gene drives for agriculture, but most of them will involve the control of pest species. The most obvious examples would be to reverse herbicide resistance in weed species or to suppress weed species populations directly. Also, many insects such as leafhoppers, aphids, squash bugs, and whiteflies transmit diseases to plants.[99-102] Controlling these vectors of agriculturally important diseases could allow gene drives to have a positive impact on cultivated crops without ever being deployed in plants.

Gene Drive Interactions Are Possible
It is possible for 2 or more gene drives to interact at the genetic level. In fact, some gene drives are designed to do so; split drives and daisy drives are specifically designed to limit the spread of the payload gene by making the presence of 1 or more drive elements dependent on the presence of other elements. Similarly, the 2L2T underdominance system is designed to allow the spread of 1 drive element only when correlated with another element at a second locus. It is also possible for 1 drive to inhibit or reverse another, as is the mechanism of reversal drives and immunizing drives, discussed briefly in the Findings section.

Mechanistically, some of these drive-drive interactions would occur by competition for the genomic site that they both are designed to occupy. For example, an immunizing drive might disrupt the site that another homing drive was targeted against. Other gene drive interactions occur because the presence of 1 genetic component of the gene drive mechanism is split from the other components, making its presence a condition for the operation of the whole system. That condition is meant to be satisfied by a specific chain reaction of events as it spreads in the population of the host species.

Gene drive interactions become relevant to the question of policy and regulation when one considers that not all parties releasing a gene drive in a host organism may be cooperating with or even aware of each other's activities.

Consider the case of a split drive: Split drives are sometimes used as a form of intrinsic biocontainment. The payload gene and guide RNA will spread only through a special laboratory strain of the host population that also produces CRISPR/Cas9 compatible with that guide RNA. Wild versions of the host population will not produce CRISPR/Cas9, and thus, even if a drive-carrying individual escapes and breeds with wild populations, the drive is crippled and will not spread without its missing CRISPR/Cas9 component. However, if a second group had released a CRISPR/Cas9-based homing drive into the wild population, the split drive escapee would be able to introduce its gene drive into the population due to the previously engineered presence of the compatible CRISPR/Cas9. As a result, when 1 group altered the genetics of a host organism, the intrinsic biocontainment safety precaution of the other group was disabled. Similarly, 2 gene drives released into the same host organism and based on the same toxin-antitoxin system would alter the pattern of one another's spread. The possibility of gene drives interacting, and the resulting unintended consequences of such interactions, underscores the need for careful regulation of gene drive technologies.

APPENDIX 3

SELECTED NATIONAL APPROACHES TO GENE DRIVE REGULATION AND GOVERNANCE

Gene drive technology is rapidly expanding and evolving throughout the world, and its application in organisms may have international impact as it spreads across borders. While substantial literature has suggested guidelines and frameworks for the use of gene drives, the regulations driven by legislation vary among countries.[1,10,68,87,103,104] WHO has published guidance specifically for release of genetically modified mosquitoes, including mosquitoes with gene drives, which has detailed methods on each step of the process, from development to field trials to release.[41] There is no other WHO guidance on gene drives.

It is important to consider regulations in countries that are developing the technologies and to assess whether and how these differ from the countries that potentially host semi-field and field trials of gene drive organisms. Consequently, we conducted a review of the current legislation in several countries that are leading gene drive research. While this effort attempted to encompass several countries where gene drive research is relevant, this appendix serves to provide a representative sample of legislation. This report is not intended to be comprehensive of all legislation existing worldwide today.

The United States
The United States currently divides responsibility for and regulation of genetically modified organisms across its major departments and agencies. The guiding principle behind US regulation is that it is product based, in that regulations focus heavily on the end product that may include a gene drive, but they are not focused on regulating the product because it has a gene drive.

The Food and Drug Administration (FDA), the US Department of Agriculture (USDA), and the Environmental Protection Agency (EPA) are the regulatory agencies that usually oversee genetically modified organisms (GMOs). Within the USDA, there is the Animal and Plant Health Inspection Service (APHIS), which would likely be involved in many gene drive efforts in plants or animals. APHIS is responsible for maintaining permits for approved regulated organisms, which includes monitoring interstate movement.[105]

There is no specific legislation or regulation regarding gene drives, but because of the nature of modifying the genome of the organism, gene drives theoretically fall under GMO legislation. Further, the specific agency managing the regulation of genetic modification of an organism depends on the class of organism being modified. For instance, genetic modification or a gene drive of animals, including mosquitoes and mice, would fall under the primary governance of the USDA. Their Animal Health Protection Act (AHPA) of 2000 could include gene drives under the category of veterinary countermeasures (7 U.S.C. §109).[106] A gene drive in animals may also be subject to FDA regulation, through their guidance on intentionally altered genomic DNA in animals. This document specifically mentions the rise of CRISPR, although it does not cite gene drives specifically. These animals would require pre-market approval and subsequent monitoring by the FDA.[107] However, a suppression drive in mosquitoes may be classified as an insecticide, in which case it would fall under the EPA's jurisdiction—specifically under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) or the Toxic Substances Control Act (TSCA).[108,109] The EPA also has the National Environmental Policy Act (NEPA), which stipulates that agencies "prepare detailed statements assessing the environmental impact of and alternatives to major federal actions significantly affecting the environment."[110] If a technology such as gene drives were to be implemented, the responsible agency would need to complete an Environmental Assessment (EA) or an Environmental Impact Statement (EIS). This would be applicable, however, only if a federal agency were to enact a policy that involved gene drive technology.

While the United States has extensive regulations regarding GMOs, it is clear that the current mechanisms are complex and decentralized. Gene drives may target several different types of organisms, from mosquitoes to mice to crops, which will complicate the question of which regulatory agency is responsible for the drive. In addition, there is a lack of specific language and definitions of gene drive technologies in the current legislation. This could further confuse which regulations and agencies are the appropriate ones for a new gene drive. Having clear, specific language and regulatory hierarchies would improve the ability of the US government to monitor and regulate new gene drive technology.

Regarding gene drives in agriculture and livestock, there are 3 current regulations that could be interpreted to apply to gene drives. These are the Federal Meat Inspection Act (FMIA), the Poultry Products Inspection Act (PPIA), and the Egg Products Inspection Act (EPIA) of the USDA.[111-113] Some of these have not been updated in years, including the FMIA, which was initially adopted in 1906. If gene drives are to be used in agriculture, then specific language relating to gene drives may need to be added to these acts so that the regulation is explicit and clear to any farmers or biologists interested in implementing gene drives.

Gene drives in plants, which could include agricultural crops, could in principle be covered by existing regulations, but there are no regulations that specifically mention gene drives. The Plant Protection Act (PPA) is under the USDA and APHIS, and includes the Biotechnology Regulatory Service.[114] In part 340 article 7, the regulation addresses genetically modified plants and the USDA's right to regulate them.[114] This would theoretically include gene drive plants. APHIS also currently has guidance regarding the proper containment of "nonindigenous" arthropods, which could include gene drive insects, in order to prevent excessive harm to plant populations.[115] Nevertheless, there is no specific language regarding gene drive use. Although gene drives in plants would fall under the definition of a GMO, current regulations are based on older genetic modification technology that does not include gene drive systems.

Consequently, a gene drive organism could be regulated by the PPA, but the PPA may not effectively address the reagents, protocols, and potential consequences of the drive. Section 3 of the PPA stipulates that the genetic material must be stably integrated, which, in their definition, is "the cloned genetic material is contiguous with elements of the recipient genome and is replicated exclusively by mechanisms used by recipient genomic DNA." Because of the nature of CRISPR, often used in homing and split gene drives, it is unclear if a gene drive would be considered stably integrated. Other drives that do not use CRISPR systems might, in this case, be included

in this regulation, whereas the CRISPR-dependent drives might not be interpreted as being included in this regulation. Although these details may seem like highly technical minutiae, these discrepancies could have legal consequences if a gene drive had negative effects on the target organism or off-target organism effects. Ambiguities such as these should be addressed now before a gene drive is deployed.

A recent proposal is currently under public comment to update the PPA to reduce the regulatory burden around genetically engineered plants. Currently, any vector that could damage a plant species in some way is referred to as a "plant pest." However, the USDA acknowledges that, with advances in genetic engineering, as with gene drives, not all genetically engineered plants are plant pests and not all genetic engineering requires a plant pest (such as a virus) to modify the genome, as with CRISPR. Updating this element of the act would reduce the number of permits needed when using plants that have been genetically engineered using modern technology (under 84 FR 26514).[115]

However, this proposed rule differs from traditional US policy in that it focuses on the product rather than the process. The product would be evaluated for "plant pest risk," and if there is sufficient risk, then the genetically engineered plant would be regulated as a plant pest. This proposed rule also offers developers and farmers the option of self-determining plant pest risk, though this would have to be verified by APHIS. It also accounts for plants that are genetically engineered to produce pharmaceutical compounds, allowing those created without plant pest vectors to have fewer regulatory obstacles. This update to the PPA may prove to encourage genetic engineering in plants, while still allowing for monitoring of safety and movement of the genetically engineered plants themselves. This genetic engineering may be accomplished by using gene drive technology. Having a case-by-case evaluation of a gene drive product would likely reduce the number of permits required, while having clearly defined, tailored regulation of a single product that would increase safety. This could encourage farmers to use gene drive–modified organisms while maintaining public safety.

Beyond animals and plants, the Public Health Service Act (PHS) of 2018 includes the creation of a center for biotechnology that could include techniques such as gene drives.[116] Much of this act is meant to encourage research to identify genetic determinants of disease, such as ALS and autism. Many of the diseases highlighted in this act have genetic factors that seem to influence the onset of disease or are suspected to have genetic determinants. The act calls for research into these genetic factors. In section 4091, subpart 3, the act calls for the creation of the National Center for Biotechnology Information, which may include gene drive technology that could have an impact on public health.[116] This section does not specifically name gene drives in the language, but such a technology would theoretically be included. It is important to note that no gene drives in humans have been proposed to date, nor, ethical objections aside, would a gene drive in humans be particularly effective for population modification because of the long human generation time. Gene drives in pests, parasites, and rodents, however, could have public health impacts and may make gene drive technology relevant for the National Center for Biotechnology Information.

Clearly, gene drive technology may fall under multiple regulations of different US agencies and departments. This conflict in proper regulation and jurisdiction may prove to complicate gene drive regulation moving forward, or it would at least require clear interagency cooperation. The United States has been forward thinking in creating the Coordinated Framework for the Regulation of Biotechnology in 1986, updated last in 2017.[117] Essentially, this framework divides the acts by responsible agency. EPA controls FIFRA, the Federal Food, Drug, and Cosmetic Act (FDCA), and TSCA. The FDA controls the FDCA (when applicable) and PHS. The USDA is responsible for the AHPA, PPA, FMIA, PPIA, and EPIA.

The FDA also released a draft for Guidance for Industry Regulation of Intentionally Altered Genomic DNA in Animals in 2017.[107] This document states that gene drives in animals do count as veterinary science and therefore could be regulated by the FDA as well as the USDA. The document does not include specific language on insects, and so the use of gene drives in pests such as mosquitoes may not be covered by this document.

Although these frameworks are excellent starts to coordination among these agencies, the advent of technology such as gene drives warrants specific

legislation that clarifies responsible agencies so that responses to issues are swift and effective. US regulatory agencies such as the FDA, USDA, and EPA have extensive legislation on GMOs. Each agency has specific jurisdiction over different classes of organisms, with the FDA monitoring animal genetic modification, the USDA monitoring plant modification, and the EPA monitoring insecticides and methods that may affect ecosystems throughout the country. These agencies have well described technologies such as CRISPR, but the application of these technologies in a gene drive system has been a more recent development that has not been adequately addressed in legislation. Gene drives have evolved rapidly in recent years, and their current refinement and applicability warrant legislation that specifically addresses them.

A unique and powerful element of gene drives is their ability to spread rapidly and effectively. This may affect an animal, such as a mosquito, in a way that may also be considered an insecticide, which would make it subject to both FDA and EPA regulations. It is currently unclear which organization would bear responsibility. The extensive impacts of gene drives, then, can subject them to regulations from several different agencies at once. Because of this overlap, specific language and guidance on gene drive technologies is warranted in the United States. The United States is a leader in science and biotechnology and so should have clear language on responsible research and application of new tools such as gene drives that can have an impact on populations of animals and plants. Past work to address GMOs has been successful and would be bolstered by further guidance on gene drive technologies and their role in GMOs.

It should be noted that much of the international effort to regulate biotechnology, especially gene drives, relies on the Cartagena Protocol on Biosafety to the Convention on Biological Diversity.[118] The United States has not signed this protocol, though hundreds of countries are signatories to this agreement on proper biosafety considerations of research on living organisms and how it may affect existing environments. It has been active for approximately 15 years and in 2018, COP (Conference of the Parties) 14 was introduced to the Convention on Biological Diversity to propose a moratorium on gene drive research.[119] This should be considered when moving forward with gene drive research in the United States and also with its scientific and political allies.
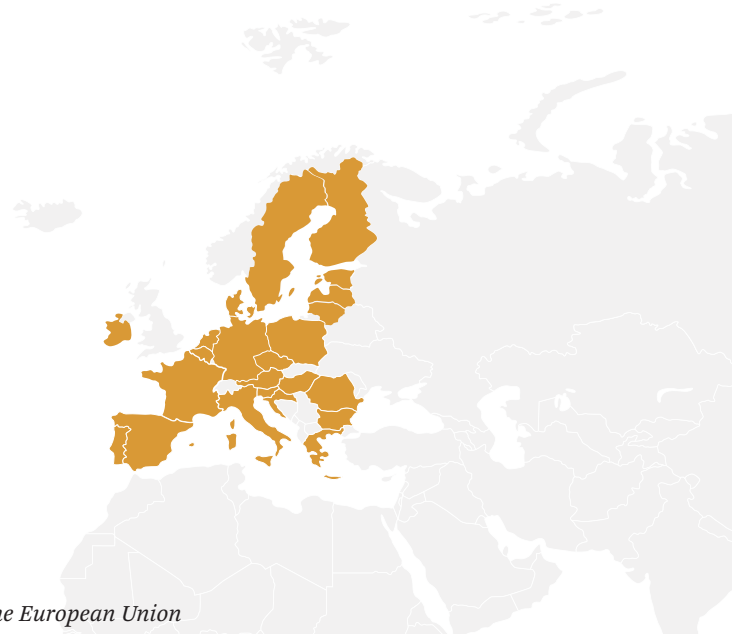
The European Union

The European Union's regulation strategy focuses on the process of creating and releasing GMOs, rather than focusing on the end product, as in the United States. Their primary GMO legislation is Directive 2001/18/EC, which is also known as the GMO Directive.[120] This directive concerns the release of any GMO into the environment, and a recent court decision in 2018 clearly established that organisms genetically modified by gene drives do fall under the term "GMO" and are subject to any GMO regulation of the EU.[121]

This is one of the best examples of a state preemptively addressing gene drive research, in specific language, and as it relates to existing legislation. This directive has prompted release of guidance from organizations such as the European Food Safety Authority on risk assessment of GM animals, though it primarily focuses on commercial applications.[122] The European Union also amended their GMO Directive, using Directive (EU) 2015/412, which clarifies that any member state can prohibit cultivation of GMOs in its territory.[123] However, gene drives will not likely obey borders, and such GMOs could potentially violate this directive.

Regulation (EC) 1946/2003 attempts to address transboundary movements of GMOs, but it relies on the responsible party's notifying another member state of an export of a GMO.[124] This process may not be possible in the case of a gene drive, unless extensive surveillance is completed to monitor movement of all gene drive organisms. If the EU facilitated a consortium of active gene drive researchers, so that they could have open communication and easily notify member states of ongoing research, this might be in line with Regulation 1946/2003.

The EU does provide legislation that attempts to track the creation of GMOs, using Regulation (EC) 1830/2003.[125] This regulation is primarily focused on food products, but it attempts to improve the traceability and labeling of GMOs. Regarding gene drive organisms, this capability would be incredibly important for liability purposes to have traceability and accountability. Though most gene drives would likely be implemented in larger organisms, such as crops or pests, the EU also provides regulation for genetically modified microorganisms and their containment through Directive 2009/41/EC, which could include any microorganisms that contain gene drives or gene drive elements.[126]

The European Union provides specific language on gene drives in legislation, which countries may seek to emulate. Clearly and preemptively addressing the specific term *gene drive* in legislation as a GMO allows clear understanding of consequences of violations of existing regulations. Gene drive technology evaluation, such as risk assessment, is currently performed according to recommendations based on their GMO legislation. The EU GMO Directive has extensive, clear language on the development, release, and monitoring of GMOs. Although recent court decisions have defined gene drive–modified organisms as GMOs, guidance should be updated to reflect this new technology. As scholar Martin Wasmer has asserted, the identification and separation of GMOs in the marketplace is a requirement of the GMO directive. However, identifying a gene drive organism may be difficult without extensive and repetitive sequencing.

Importation of gene drive organisms in the EU may also be an issue if the exporting country does not properly label the GMO as a gene drive organism.[127] Clearly identifying gene drive organisms as GMOs is a significant step for the EU to properly address this new technology, but legislation and guidance should be updated further to address some of the nuances of gene drives, such as their potential for rapid spread and how they might be identified. In the future, guidance could address risk assessments, containment strategies, and countermeasures that are tailored specifically to gene drive technology. The EU demonstrates a path forward for countries to properly address gene drive organisms, but it should continue its efforts to improve legislation and guidance.

Australia
Australia has proven to be a forward-thinking and careful actor in the regulation of biological research that may be dual-use research of concern.[128,129] While gene drives are not mentioned specifically by name in any Australian legislation to date, their governance surrounding genetic technology and biodefense is fairly extensive. The Biological Control Act of 1984 regulates using biological organisms to control potentially harmful organisms. In other words, this act relates to the release of one organism to control another potentially harmful organism.[130]

In the case of gene drives, this act would be relevant legislation for a gene drive meant to target vectors or pests, such as mosquitoes. But it could also be relevant if a reversal drive was released. If a second gene drive had to be released to counter the effects of a first gene drive, then it theoretically would qualify as biological control and could be regulated by the Australian government.

This act details the process of identifying and approving target organisms and controlling organisms and then implementing biological controls. An important element of the act is in subsection 34, which asserts that any existing agent organisms that have been released must be declared to the government. In the case of gene drives, this statement could require any released gene drives to be officially declared, and this may prevent the re-release of the same drive, or the release of a competing drive that may reduce efficacy. While the Australia Biological Control Act does not explicitly regulate gene drives, the Biological Control Act of 1984 is one of the most applicable pieces of existing legislation regarding gene drives and their potential control of harmful organisms.

The Gene Technology Act of 2000 would also likely cover gene drive technology and its implementation.[131] This act not only details the regulation of GMOs but also specifies the licensing and accreditation of users and institutions that are working with new gene technology. Because of this act, the Office of the Gene Technology Regulator has been established to process all GMO-related technology and products, and it has created a GMO register to manage GMOs that are currently released.

The act also calls for the creation of the Gene Technology Technical Advisory Committee and the Gene Technology Ethics and Community Consultative Committee, which both function to provide scientific context to novel technology that may require new policies to maintain public safety and environmental protection. These committees are composed of members of the scientific community, the public, the ethics community, and other relevant actors such as lawyers. Engaging the public regarding release of insects has been important in the success of previous trials, such as Eliminate Dengue. This release of Wolbachia-infected mosquitoes was not a gene drive, but it demonstrated that transparency and community engagement were essential to the success of the program.[132] Gene drives, though not specifically mentioned in this act, are the exact type of technology that this act concerns. These mandates may have widespread public health and environmental impacts, and having committees to constantly evaluate and explain these advances to legislators would likely help ensure the public's safety.

Australia has other legislation that may be relevant to specific types of gene drives, especially those meant to preserve biodiversity or eliminate an invasive species. The 1999 Environmental Protection and Biodiversity Conservation Act primarily focuses on conservation efforts, but if a gene drive were released to protect an endangered species, then this act might be applicable.[133] This act also concerns the international movement of plants and animals, which may be relevant in the case of imported gene drive organisms or for drive organisms able to travel across borders. Crossing national borders may be more difficult in Australia, as it is far from most other countries, but insect vectors or birds may be able to successfully migrate and would therefore be covered under this act.

A more specific piece of legislation concerning the import of organisms that may have biosecurity concerns is the 2015 Biosecurity Act, which details Australia's efforts to prevent the importation of pathogens, plants, or animals that may affect Australia's public and environmental health.[134] This mandate could apply to gene drive organisms that are imported to Australia. A suppression gene drive, for instance, would have an impact on organisms in Australia. Consequently, the import of such gene drive organisms would be highly regulated by the Australian government, which could aid in the overall security and management of the technology in Australia.

This act has incredibly detailed subsections regarding human health impacts and methods of controlling movement of goods, humans, flora, and fauna. It also details the processes of risk assessments and what to do in the case of a biosecurity emergency. The 2015 Biosecurity Act, coupled with the Biological Control Act of 1984, may be the legislation most relevant to gene drives in Australia overall. Monitoring their implementation, while also constantly assessing biosecurity risks, would help the Australian government control any gene drives released in the country.

Australia presents an excellent example for countries that seek to develop gene drive technologies in their expansive legislation surrounding safety and development of GMOs. The term *gene drive* should be defined in future legislation so that there is clear language surrounding use of the technology. Their legislation provides clear guidance on registration of novel applications, risk assessments, requirements for containment strategies, and engagement with the community. These efforts would only be enhanced by clear language defining gene drives and applicable regulations. In the future, legislation could be updated to include gene drive–specific language, such as gene drive countermeasures as a method of reversal of release. Nevertheless, their existing legislation provides excellent guidance on risk assessment, evaluation of technologies, and reporting mechanisms for regulation of new GMOs.

Brazil

Brazil is a leader in biotechnology and infectious disease research and is also burdened by many vector-borne infectious diseases. Previous releases of GM mosquitoes to combat *Aedes aegypti*, the vector of diseases such as dengue fever, have demonstrated the complexity of approving and safely completing open field releases.[135] Many currently proposed gene drives target vectors of diseases such as dengue fever, which can be found in Brazil.[136] Brazil's primary gene technology legislation is Law No. 11.105 (The Biosafety Law), which establishes the National Biosecurity Council (CNBS) and the CTNBios.[137] The CTNBios, or Comissão Técnica Nacional de Biossegurança, are committees that govern gene drive technology guidelines. They have well-defined and stratified institutional and state responsibilities, creating clear reporting hierarchies, and they are implemented across Brazil in several research institutions. The CTNBios are similar to institutional biosafety committees (IRBs) and are composed of a range of experts in biology and engineering as well as farming and defense. The Biosafety Law also covers the release of GMOs and their derivatives, stating that any release must be approved by the relevant CTNBio and have proper licensing. This law would cover the release of gene drives and make regulation of gene drives subject to CTNBio approval. The National Biosecurity Council would analyze the requests from the CTNBios for GMO release. This 2-step approval process would be an excellent measure when considering gene drive release, as it would improve security and require stringent analyses and research of any gene drive proposal.

The law further describes the Internal Biosecurity Commissions, which are required at any institution that participates in genetic engineering research. These processes essentially bridge the gap between researchers and the public and inform the public of any ongoing work that may affect them. Internal Biosecurity Commissions could be an excellent resource during gene drive research; they could keep the public informed of any trials, while also detailing public concerns to the research teams to facilitate communication.

A recent amendment to the CTNBio structure is Normative Resolution Number 16, of 2018, which articulates the technical requirements of a request to a CTNBio on "Innovative Techniques for Precision Breeding Innovation."[138] This is one of the few pieces of legislation worldwide that specifically mentions "gene drives" and creates the possibility that they will not be considered a GMO under the Biosafety Law. This resolution states that if the researcher is unsure if the gene drive technology is considered a GMO, he or she must still request approval by the CTNBio. In other words, regardless of whether or not the gene drive is considered a GMO, it must be approved by the CTNBio. This type of exact language is essential to ensure biosecurity of gene drives and their research. The specificity of their regulations further improves the ability of the Brazilian government to monitor gene drive research and enact consequences for those who violate the current regulations.

Current legislation and regulatory structures in Brazil present an excellent example of a country that is not only leading gene drive research but is also a potential host country for gene drives. Their legislation specifically mentions gene drives and defines them, and their CTNBio system provides an organized framework for developing, approving, and monitoring such technology.

It is not clear how extensive their community involvement is in discussions of gene drive deployment, but community members in the CTNBios provide a valuable perspective. In addition, legislation should address the types of reversal techniques that are applicable to gene drives—namely, that the countermeasure will likely be another gene drive. Having a clear reporting hierarchy with diverse members of advisory boards, coupled with specific language on gene drive technology, well prepares Brazil for novel gene drive applications. Legislation should be continually updated as gene drives are developed in the future.

Uganda

Uganda recently passed the Genetic Engineering Regulatory Bill of 2018, which addresses GMOs and the implementation of gene drives in that country.[139] The Ugandan Genetic Engineering Regulatory Bill primarily addresses genetically modified plants and animals, which could include insects. The bill stipulates that genetic modification on plants and animals should be contained, so that it does not immediately spread to native species with no methods of recall. This stipulation for containment during early phases of testing has been recommended by several scientific groups as essential for gene drive safety.[68] Further, "a person who owns a patent to GEM [genetically engineered material] is strictly liable for any harm it may cause and must be tasked to explain whether the harm caused was intentional or not."[140] This mandate could prove to be key in the release of gene drive organisms, if the organism or gene drive is patented. While it is not clear if gene drives could be patented in Uganda, this law strictly defines liability. This type of legislation will prove to be essential moving forward in many African countries, such as Mali, where foreign institutions may be attempting to release semi-field or field trials of gene drive mosquitoes.

These efforts by Uganda to clearly define and regulate the use of gene drives are commendable. Uganda could benefit by defining regulatory structures surrounding gene drives, so that researchers, legislators, and community members are all aware of the necessary steps to approve gene drive technologies. This will ensure that the development of gene drives is monitored and transparent from the beginning. Their stipulation of containment of gene drive organisms is essential to maintaining safety throughout the development of this novel technology, especially if it is applicable to native species of the country.

Uganda is one of the few countries to clearly define liability in gene drive release, though patenting of gene drives currently is a complex field. Uganda's efforts are exemplary steps forward in regulating gene drives, and future efforts should center on bolstering regulatory agencies and prioritizing transparency in research and government.

Burkina Faso

Burkina Faso is a leading country in the release of genetically modified mosquitoes to reduce the burden of malaria.[141] Involved with the African Biosafety Network of Expertise (ABNE), they passed their Biosafety Law in 2012 (Law 064-2012).[142] The Biosafety Law addresses the regulation of GMOs from development to release and import. Although the legislation does not specifically mention gene drive technology, this type of modification would fall under their definition of modern biotechnology. This legislation is also applicable to genetically modified crops, including Bt cotton, which has been used in Burkina Faso since 2007.[143]

This law also created 2 organizations, the National Biosecurity Observatory (NBO) and the National Biosecurity Scientific Committee (NBSC), to manage regulations surrounding modern biotechnology and GMOs. Their responsibilities include managing the biosecurity of cross-border movement of GMOs, and to "create and make available to the public a database on the genetically modified organisms intended for human or animal consumption or for transformation."[142] This would be highly relevant in the case of multiple released gene drives, and it may make possible a registry so that multiple gene drives that might interfere with one another are documented and transparent to the public and scientists.

The law also details necessary safety measures, approval and release processes, and risk evaluations for the approved use of GMOs, which line up well with academic recommendations on safety with gene drives.[87,144] Importantly for gene drives, the law stipulates that prior to any release, there must be established countermeasures in the case of negative impacts of the organism. This leads to the articles on liability, which explicitly state that "any damage caused by this genetically modified organism is the express liability of the designer of said organism."[142]

Few countries other than Uganda have established this clear liability in the release of GMOs, and in the case of a gene drive organism, this would encourage any group releasing a gene drive to have clear testing and safety constraints in place from the beginning. While Burkina Faso's law does not specifically address gene drives, it does create a framework of biosecurity for GMOs that is an excellent step toward maintaining safety while modernizing biotechnology.

Future legislation and guidance should center on incorporating specific language about gene drive technology and providing clear frameworks for researchers to perform risk assessments, evaluations, and development of countermeasures. Their stipulation that there must be countermeasures present before release is forward-thinking and significant, especially considering that many gene drive targets may be native to Burkina Faso and could be greatly affected by any novel trials.

Russia

There is limited public transparency regarding Russia's current gene drive research, though it has regulations that address the registration and monitoring of gene modification research. The first regulation passed was 86-FZ, On the State Regulation in Genetic Engineering.[145] The top 3 directives of this legislation are "improving the human condition and protecting its health; protection and restoration of the environment, preservation of biological diversity; and increasing the efficiency of agriculture," all of which may be addressed by gene drive research.[145]

The regulation maintains that safety of the public and the environment should be at the forefront of any scientific research efforts. To preserve that safety, the Russian government requires licensing at specific "levels" of genetic research. Levels III and IV, which include "genetic manipulations at the molecular, cellular levels involving recombinant ribonucleic and deoxyribonucleic acids to create genetically modified organisms (viruses, microorganisms, transgenic plants and transgenic animals and their cells)" require licensing by the state.[145] This legislation could, theoretically, allow the state to monitor groups that are actively participating in gene drive research.

However, Russia's stance on genetically modified crops may impede gene drive research. The more recent law, 358-FZ, essentially "prohibits cultivation of genetically engineered plants and breeding of genetically engineered animals on the territory of the Russian Federation, except for cultivation and breeding of plants and animals required for scientific expertise or research."[146]

Additionally, Russia's resolution 839 requires a registry for the release of any GE crops. This resolution was halted by another resolution, 548, which delayed the start of the registry.[147] Law 358-FZ clarifies that, due to potential harm by GMOs released into the Russian Federation, the authorized local government bodies must establish checkpoints to prevent the import of such organisms into Russia. The law also concludes that any impacts or consequences of genetically modified organisms on public health, inspections of GMOs, and impact assessments can be evaluated by the Russian government and used to decide whether an imported crop will be banned.

In addition, the containment requirements of level III and IV genetic engineering research may be too stringent for gene drive semi-field or field trials. These requirements may further inhibit the research of implementing gene drives in the environment. This conflict may be remedied by specific legislation that has clear language regarding gene drive technology and that delineates the type of research with gene drive technology that is acceptable.

India

Another hub of biotechnology can be found in India. There, GMOs are regulated in the context of public health and agriculture. The primary GMO legislation in India is the Manufacture, Use, Import, Export and Storage of Hazardous Micro-organisms Genetically Engineered Organisms or Cells Rules of 1989.[148] This set of rules details the creation of institutional and state biosafety committees, as well as regulation of genetically modified microorganisms. Though the legislation does not specifically mention gene drives, this technology would fall under the definition of a gene technology and genetic engineering.

The rules further describe lists of organisms of concern or interest and the process by which import, export, or engineering of these organisms is approved by the Genetic Engineering Approval committee. This committee is composed of lawmakers and scientific experts, including from biotechnology and the department of environment, forests, and wildlife. Further, the legislation provides a static list of classifications of organisms and their risk levels, which would be useful when considering gene drive organisms and their potential release. Gene drive technology, under this set of rules, would be the type of biotechnology that the Genetic Engineering Approval committee would have to approve before any semi-field or field trials would take place, and it would likely be monitored from the beginning in the lab.

India also regulates imports of organisms and the impact they may have on agriculture and the environment. The Biodiversity Act of 2002 establishes the National Biodiversity Authority and primarily focuses on maintaining India's biodiversity and preventing overuse for commercial purposes.[149] However, a biotechnology expert must be assigned as a part of the authority, which could mean that gene drive technology that could

preserve, or harm, biodiversity in India may have to be approved by the National Biodiversity Authority. If a gene drive were to be used to preserve an endangered species, then this would certainly fall under this act and require approval.

The Plant Quarantine Act of 2003 further details the licensing for import of any plant into India, including agricultural crops. This would be relevant if a gene drive plant were to be released.[150] Section 7 details the import of insects, which would include mosquitoes. The act specifies only that one must have a permit to import insects, but it does not mention genetically modified mosquitoes. This gap could be further clarified with an amendment but would be incredibly important if other nations wanted to implement gene drive insects in India.

The final legislation that could be relevant to gene drives is the Food Safety and Standards Act of 2006, which would be applicable only if the gene drive were in an agricultural crop. This act covers genetically modified foods and describes the responsibilities of the Food Safety and Standards Authority of India, which is responsible for quality assurance and risk assessment of any genetically modified foods.[151]

While India's current GMO legislation could be relevant for gene drive technology, further amendments with specific language that addresses gene drives would bolster their security. Specifically, in the Plant Quarantine Act, gene drive mosquitoes should be addressed as insects of concern. In addition, language and guidance related to the Manufacture, Use, Import, Export and Storage of Hazardous Micro-organisms Genetically Engineered Organisms or Cells Rules should be updated to reflect current biotechnology tools, including gene drives.

India has clearly been forward-thinking in their legislation about not only genetically modified microorganisms, but also their biodiversity and animal and plant health. Future efforts should center on creating clear risk assessment guidance for researchers, including stipulations for containment of trials and reversal of any drives to be released. In addition, the Indian government could work with communities that may be affected by gene drive releases to encourage transparency and clear communication among researchers, government, and community members.

China

China is a biotechnology giant, although it appears there are only limited regulations that are publicly available that may govern gene drive technology research. The 13th Five Year Plan is the most recently released, and while biotechnology is mentioned, the plan does not describe gene drive technology.[152] The plan describes the Four Modernizations, 2 of which are agriculture and science and technology. China is seeking to modernize these fields, and given its extensive agricultural research using CRISPR technology, it is reasonable to assume that gene drive technology may also be researched.[153]

China recently made available a draft of the legislation Safety Management Regulations for Biotechnology Research and Development, which addresses biosafety issues of new biotechnologies. The draft was open for public comment in March of 2019, but to the authors' knowledge, has not been published. The law would create a "tier" system that would address different levels of risk; encourage monitoring by the Biotech Security Commission, to be created by this legislation; and require development of emergency countermeasures to any modified organism.[154,155]

China has created biosafety, rather than biosecurity, legislation. According to a recent review, the Chinese constitution maintains that the state is responsible for diversity of bioresources, as well as public health resources. In addition, the 1979 Criminal Procedure Law of the People's Republic of China stipulates that there are legal consequences for the creation or dissemination of an agent that could pose a contagious threat; this proviso could apply to a gene drive microorganism.[156]

Most recent legislation appears to focus on infectious disease agents and monitoring of epidemics, or animal and plant protection laws.[157,158] However, these laws do not mention genetic modification or gene drive technology. To our knowledge, based on available documents, there is no specific legislation regulating GMOs. There exists a regulation titled Measures on the Administration of Gene Engineering Safety, but access to this document was unavailable at the time of writing. Chinese scholars also assert that most current guidelines are issued by "low-level" government organizations and are not nationally issued.[156] It should be noted that there may also be existing regulations that addresses biotechnology such as gene drives that were not publicly available at the time of this report.

In the future, efforts should focus on creating legislation with clear language on gene drive technology. In addition, risk assessments, containment strategies, and countermeasure development should be required of all gene drive researchers. It would be beneficial for the Chinese government to continue to develop biosafety committees to oversee any developing research and to involve the community to encourage transparent communication. It is especially important for China to have regulations concerning gene drives and other biotechnologies given China's dominant role in global trade. As with other countries, it would be important and beneficial in China for there to be public transparency concerning the state of gene drive research and any legislation regarding such research.

Non-Nation Case Studies

The Cartagena Protocol

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an important international agreement that aims to maintain the safety of the public and the environment during the transport and development of living modified organisms (LMOs). The Cartagena Protocol was enacted in 2003 and, since then, has garnered more than 100 countries' signatures and ratifications.[118] Gene drive technology, while not mentioned specifically in the original protocol, would fall under the definition of modern biotechnology in Article 3, subsection i.[115] The protocol primarily focuses on the transboundary movement of living organisms modified by modern biotechnology, and this would be pertinent to gene drive import and release in a country.

Under the Protocol, the country of origin must properly notify and approve the movement of the living modified organism. In the context of gene drives, this mandate could mean that any country that has ratified the Cartagena Protocol must follow the notification procedures when releasing (even for contained field trials) gene drive organisms in another country. The protocol should provide protection for a host country, which would be especially relevant in African nations that are hosting mosquito gene drive trials.

In addition, the Protocol mandates that unintentional transboundary movement must be reported by the responsible nation as soon as possible. The protocol also explicitly recognizes the importance of educating the public on movement of LMOs. Further, the protocol suggests the consideration of socioeconomic factors that may affect a country's participation in movement of LMOs, "especially with regard to the value of biological diversity to indigenous and local communities."[118] In order to coordinate communication among scientists, government leaders, and the public, the protocol established a Biosafety Clearing House. Countries that have signed the protocol meet semiregularly and often have presentations relating to new biotechnology that may be relevant. Today, there are almost 200 countries party to the protocol, including the United Kingdom and China.[118]

The Cartagena Protocol is a global effort to maintain biosecurity with emerging technologies, such as gene drives. It would be beneficial for the Cartagena Protocol to specifically define gene drives and assert their inclusion in the "modern biotechnology" category. Nevertheless, their efforts to create a clear reporting system to monitor transboundary movements of organisms are well suited to address potential spread of gene drive organisms. The emphasis on engaging researchers, legislators, and the public in any major biotechnology effort would also be essential to the success of a gene drive deployment. The protocol also addresses risk assessment, monitoring, and liability of release of organisms, all of which are needed in gene drive deployment.

This protocol is a laudable effort to have international guidelines for biosafety, but it is completely voluntary. Countries, including the United States, that have not signed the protocol may not have to follow the same guidelines, which may lead to conflict. However, the protocol provides an example for all nations to use for regulation of gene drive technology.

African Biosafety Network of Expertise (ABNE)

While not specific to any one African country, ABNE is the development agency of the African Union that is largely involved with much of the gene drive research in Africa. Notably, Uganda and Burkina Faso are members of ABNE, which is a biotechnology focus area of the New Partnership for Africa's Development (NEPAD). ABNE has been responsible for helping enact biosafety laws all over Africa, including in Côte d'Ivoire, Kenya, Ethiopia, Ghana, Malawi, Egypt, Sudan, and South Africa, to name those countries with the most relevant legislation.[143,159]

ABNE has made remarkable progress with many African nations in helping to plan for and enact legislation on relevant biotechnology, including gene drives. One of their central goals is to help "establish and support biosafety systems" all over Africa, to keep these countries competitive and safe in the growing biotechnology sector.[159] Approximately 49 member states of the African Union have signed the Cartagena Protocol, and because of this involvement, at the 27th Ordinary Session of the African Union Summit of Heads of State and Government, ABNE was created to help serve these countries.[160]

Organizations like ABNE can aid in the creation of legislation surrounding biosafety, such as Uganda's recent bill, which could provide a useful framework for other countries hoping to enact their own laws regarding gene drive research.[161] Scientific groups have suggested methods for selecting proper release sites and legitimate collaborations to maintain safety when working with gene drives.[162] One central recommendation is to engage with the public, to maintain transparency throughout the process. ABNE provides a community for scientists and stakeholders to work together to create clear legislation on biosafety.

Summary of International Policies and Gene Drives

Regulation of GMOs and the methods of genetic research is clearly widespread, indicating a wealth of research worldwide. In reviewing the selected case studies, it is clear that most countries have biosafety guidelines surrounding GMOs to protect the health of their people and their environment. In many cases, these laws were implemented with agricultural use of GMOs as a major consideration. Several of the guidelines include internal committees, such as the CTNBios in Brazil and institutional biosafety committees in the United States, that work to enforce these regulations and educate researchers on laws relevant to their research. Such efforts would be important when considering gene drive technologies and their implementation in the field.

Several countries, including the United States, India, and Australia, also have biodiversity protection legislation that may be relevant in regard to gene drive research. If a gene drive were released in the field, it could alter the environment. The extent to which this alteration is acceptable would likely be governed by biodiversity regulations. The central gap in almost all legislation, apart from that of Brazil, the EU, and Uganda, is that gene drive technology is not specifically defined or mentioned. Moving forward, specific language on gene drives would be essential in maintaining biosecurity and environmental health and in removing ambiguity in governance for this technology. This language must be clear and definitive, as gene drives will likely not obey borders and multiple countries may need to move ahead without regulatory oversight if they are not covered by the current definitions of GMOs.

APPENDIX 4

GLOSSARY

Allele: A collective word for the possible variants of a gene that encode for 1 feature and are found consistently at 1 spot on a chromosome (eg, eye color.) Alleles can be natural or artificial.[14]

Cassette: A mobile region of genetic material (typically DNA) that contains a gene and recombination site, allowing for it to be integrated into a larger genetic construct (eg, a chromosome).[163]

Confinement: Keeping a gene drive contained to a specific population, either wild or in a laboratory study, so that it cannot affect or alter other wild organisms. Confinement may be physical (eg, walls) or genetic (eg, a precision drive).[12]

Countermeasure: A product that can be used after a gene drive has been released into a population to stop it or render it inert. Certain countermeasures can even restore the wild phenotype to the affected population.[164]

CRISPR (clustered regularly interspaced short palindromic repeats): A genetic system originally discovered in bacteria, which use it as an adaptive immune system against viral DNA. CRISPR consists of a series of stored pieces of collected genetic material, separated by repeating sequences of DNA, and is paired with an endonuclease like Cas9. When the stored DNA is expressed as a guide RNA, it binds with any matching sequence of DNA present. The endonuclease recognizes the guide RNA and cuts the corresponding piece of DNA in half. This system can be used for flexible gene editing and as a component of gene drives.[18]

Diploid: Describes a species with 2 copies of each (non-sex) chromosome. Almost all animals are diploid.[165]

Endonuclease: A type of nuclease enzyme that cuts a stand of DNA or RNA in the middle of the strand, rather than from the end of a strand. A common CRISPR-associated endonuclease is Cas9, although there are others.[14]

Fitness: The genetic contribution that an organism makes to future generations.[165]

Fitness cost: The degree to which a gene reduces the ability of an organism to survive and bear offspring. Under the right circumstances, certain genes will increase the ability of an organism to survive and then are passed on to the organism's offspring; this is the process of natural selection, and these genes can be said to have a negative fitness cost. Human-altered genes often exert a fitness cost on their hosts.[165]

Gene: A sequence of nucleotides that forms the basic unit of heredity. Can be used by a cell as instructions to guide the creation of proteins.[165]

Gene drive: A genetic system that biases toward its own inheritance. These genes spread faster than expected by natural selection or Mendelian inheritance through a population as a result of this biasing. There are natural gene drive systems as well as man-made systems.[13]

Heterozygous: An organism that has 2 different alleles on a pair of chromosomes at a given locus. Applies only in diploid species.[165]

Homing drive (also "mutagenic chain reaction"): A drive that uses guide RNAs to "home in" on a specific gene sequence, cut it, and replace it with the drive system via homology-directed repair, thus converting an allele pair that is heterozygous into one that is homozygous within the cell.[17,166]

Homology-directed repair: Many endonuclease gene drive systems rely on the DNA repair mechanism of homology-directed repair. When 1 site on a chromosome is cut with an endonuclease, animal cell repair mechanisms generally repair that break by using the other corresponding chromosome, at the same site, as a "template." The corresponding site is recognized via both sites having identical genetic sequences surrounding the break (hence "homologies"). If the other chromosome has altered genes within the same homologous sequences as the broken site, these will be copied into the other chromosome as a "repair."[18]

Homozygous: An organism that has identical alleles on both chromosomes at a given locus. Applies only in diploid species.[165]

Human-influenced species: A species that is not domesticated but whose populations are nonetheless subject to significant human control—for example, fish that are heavily harvested by humans, or deer whose populations are controlled by hunting.

Mendelian inheritance: The dominant natural process of genetic inheritance as discovered by Gregor Mendel. Most animals have 2 copies of each chromosome and inherit 1 of each chromosome from each set of parents. Ordinarily, which chromosome, and thus which gene, the offspring inherits is random, but gene drives are an exception to Mendelian inheritance.[14]

Multiplexing: One of several nearly identical processes operating in parallel. Multiplexing in the context of a homing drive would describe the use of 1 guide RNA to target multiple sites on the target chromosome. The target is less likely to evolve resistance to a multiplexed guide RNA than to a nonmultiplexed guide RNA, because all of the multiple sites would have to mutate in order to render the drive nonfunctional.[61,73]

Natural selection: The process by which variations in the forms and functions of an organism give rise to nonrandom survival of those organisms and thus over generations to the nonrandom inheritance of forms conducive to life.[167]

Nonhomologous end joining: A natural biological process for connecting 2 cut pieces of DNA, without relying on an unbroken template as a guide like another chromosome. Cells will either use this process or homology-directed repair to repair broken strands of DNA.[14]

Nuclease: A protein enzyme that breaks a strand of DNA or RNA.[14]

Locus: A specific site on a chromosome. Genes located "on the same locus" are physically adjacent.[14]

Payload gene (also "cargo gene"): A gene added to a gene drive in order to be driven (alongside the drive) throughout a population in order to give organisms a desired property.[168]

Phenotype: Specific characteristics or organisms that can be identified or distinguished by direct inspection or only by finer methods of measuring or description.[169] Phenotype, a measure of the traits of the organism as observed, is distinguished from genotype, which is a representation of the genes contained in that organism regardless of whether they produce an observable trait or not.

Population alteration (also "modification" or "replacement"): Spreading or "replacing" a specific gene or genes throughout a population or species.[17]

Population genetics: The study of gene presence and variation within a population of organisms.[165]

Population suppression: Reducing the size of a population—for example, by reducing the number of viable embryos, or by causing new embryos to be exclusively male. This effect can in principle drive a population or species to extinction.[61]

Self-limiting drive (also "localized drive"): A gene drive, such as a daisy chain, that is designed to lose its "driving" properties over time or via dilution of the population via interbreeding with wild-type individuals. The altered gene is then reduced to spreading via natural selection, like other genes, and may be selected for or against.[77]

Self-propagating drive (also "global drive"): Contrasted with a self-limiting drive, a self-propagating drive has the potential to spread through the entirety of a population, although in practice such drives would not be expected to spread to quite 100% of the population due to resistance mutations.[53]

Target population: The group of individuals intended as the subjects of a treatment, such as a gene drive. This may range in size from 1 relatively isolated breeding population to the entire species.

Wild-type (WT): The unaltered, naturally occurring version(s) of a gene or organism.[14]

APPENDIX 5

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