Engineered Gene Drives and their Value in the Control of Vector-Borne Diseases, Weeds, Pests, and Invasive Species

Kathleen Hefferon and Ronald Herring

Abstract Genetic engineering has created potential for moving medical and agricultural research and application frontiers forward in unprecedented ways. Despite its accepted use as a powerful tool in medical research, genetic modification and genome editing technologies remain controversial in large-scale ecological intervention and open-field agriculture. Gene drive is a technology based on genome editing that enables a trait to be pushed through a given population at a greater than expected rate. While gene drives show enormous promise as a way to address a number of challenges, such as the reduction of populations of disease-spreading pests and invasive species, they also incite great social unease because of unknown risks. The following chapter describes the mechanics of gene drives and how they could be utilized to control vector-borne diseases, weeds, and crop pests and even protect populations of endangered species. Limitations and risks associated with gene drive technologies, such as containment strategies and potential resistance, are discussed. Finally, the social impacts of gene drives with respect to international governance and public acceptance are considered.

Keywords Gene drive · Genome editing · Informed consent · Unwanted spread · Containment strategies · Regulation · Biosafety · Ethical considerations · Hypothetical risk · Uncertainty

Introduction

Biotechnology is among the most scientifically promising, yet socially controversial, issues today (Lewontin 2001; Doudna and Steinberg 2017). While genetically engineered crops and livestock are steadily entering the marketplace, adoption is limited due to biosafety and risk grounds throughout much of the world. The

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introduction of genome editing has rekindled this debate by stimulating divergent world views concerning the place of humans in the natural world (Čartolovni 2017). More recently, the concept of generating synthetic gene drives to reduce or possibly even eliminate some human disease vectors and crop pests is beginning to capture the world's imagination (Wimmer 2013). Natural gene drives are selfish genetic elements that use a variety of mechanisms to ensure that they are transmitted to subsequent generations at greater than expected rates (Cutter and Jovelin 2015). Synthetic gene drives based on the CRISPR/Cas9 genome editing system could potentially alter the genetic characteristics of natural populations of organisms in ways relevant to the goals of public health, conservation, and agriculture (Zentner and Wade 2017).

Social concerns of ecology and environmental safety have been heightened because the result of such gene drives may impact wild ecosystems. (Courtier-Orgogozo et al. 2017).

It has now become possible, for example, to envision the release of a gene drive that could vastly reduce the size of mosquito populations that transmit human pathogens. The end result could be virtual elimination of disease burdens (e.g., malaria, dengue) that humans have endured for millennia. While potentially conferring such significant benefits, such alterations raise concerns that species could be pushed to extinction or that gene drive traits could be transferred to nontarget species. The social dimensions of gene drive must be explored, including the dimension of both risks and benefits to humans and, consequently, how gene drives will be governed, particularly across neighboring countries and jurisdictions.

As one recognition of this pressing issue, the US National Academies of Sciences, Engineering, and Medicine (NASEM) established the Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct. The committee summarized their analysis in the report, "Gene drives on the horizon: Advancing science, navigating uncertainty, and aligning research with public values" (NAS 2016). The report concluded that while attractive and not without great potential, gene drives required further study to ensure their responsible release. It was recognized that gene drive technologies could provide solutions to world problems that are difficult to address, such as vector-borne diseases, increases in pesticide and herbicide resistance of agricultural pests, and infiltration of invasive species on fragile ecosystems. However, a gene drive that is implemented and then runs out of control could eliminate certain species and change the environment as we know it permanently. This chapter concentrates on the use of gene drives to control populations of insects, weeds, and invasive or endangered species.

Principles of Gene Drive

Gene drives are systems of inheritance that are biased, so that the likelihood of a sequence of DNA being passed between generations and throughout an entire population is greatly increased (Sinkins 2011). The pattern of inheritance in the presence of a gene drive becomes altered so that most or all offspring from the cross between a gene drive and a wild-type individual will inherit a particular genetic trait (Esvelt et al. 2014). The development of CRISPR/Cas9 and other synthetic genome editing tools has now greatly facilitated this process.

With the newfound ease of genome editing, the potential for gene drive technology has taken on new implications. Gene drives enabled by genome editing offer the potential to stop the spread of mosquito-borne diseases such as malaria, dengue, and Zika. Gene drives could also block the spread of weeds or even bring some species back from the endangered list through removal of invasive predators/competitors. In this case, mosquitoes containing chromosomal translocations could be mated with wild-type mosquitoes and produce heterogeneous progeny that are sterile. As a result, release of mosquitoes harboring this male-producing factor could impact the sex ratio of the mosquito population so that females reached a number below the level required for efficient disease transmission (Wieczorek 2016). This initial approach encouraged work on the use of CRISPR-Cas9 as a new tool to reduce mosquito populations (Hammond et al. 2016). For example, genes that confer a recessive female sterility phenotype can be disrupted. CRISPR-Cas9 gene drive constructs designed to target and edit each gene involved in reproduction can be inserted into the female sterility gene locus, resulting in a massive increase of sterile females. Population modelling has demonstrated that this type of gene drive could be used to effectively target female reproduction in a mosquito population. The technology could also be extended to edit mosquitoes so that they are no longer able to transmit infectious diseases (Singer and Frischknecht 2016).

Self-Limiting Gene Drives

Along with gene drive technology comes significant perceived risks such as ecological damage or other unintended consequences. As a result, efforts have been made to develop gene drive systems that can spread through or be recalled from a given population (Marshall and Akbari 2018). One potential option is the use of self-limiting drives such as the Daisy drive, which would involve the development of gene drive systems that are temporally and spatially limited so that uncontrolled consequences can be easily curbed (Dhole et al. 2018). In a Daisy drive system, components of the CRISPR machinery are scattered throughout the genome in fragments so that none could "drive" on their own (Fig. 1). In spite of their spatial separation in the genome, they are functionally arranged in a Daisy chain fashion so that one element is needed to get the other one started. Local populations under drive technology can be better controlled by providing a means to limit spread based on the timing of release. Alternatively, in underdominant gene drive systems, where heterozygotes for the drive allele have a lower fitness than their homozygotes and wild-type counterparts, the rate of spread is dependent on the invasion frequency threshold, therefore requiring large release of the transgenic organism. On the other hand, this provides underdominant gene drive systems the property of improved

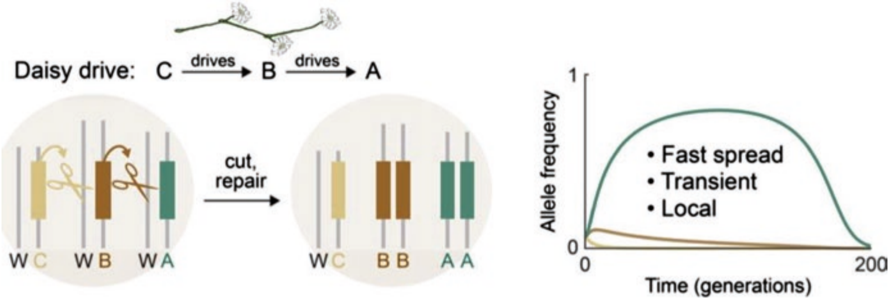

Fig. 1 Mechanism and population-level effect of endonuclease gene drives. (Figure derived from DiCarlo et al. Nature Biotechnology volume 33, pages 1250–1255, 2015)

local confinement to a given region (Champer et al. 2018). Since the changes made do not render the organism more fit in its environment, the first gene drive unit will eventually disappear by natural selection since it does not promote its own transmission. This will create a chain reaction, and the other units will also disappear in turn. It is expected that these sorts of gene drives can be used to serve functions for local populations and either be transient or made stable by incorporating a more permanent gene drive.

One possible challenge to Daisy drives is the fact that large differences can exist with respect to the minimum release size that may be required to drive a given trait into a particular population. The release size required to make an impact in certain instances will be much greater than in others. This may result in a failure to retain Daisy drives in a geographically localized state, particularly if the cost of fitness by incorporating the gene drive trait is low (Marshall and Akbari 2018). On the other hand, drive mechanisms that require the release of individuals at higher frequencies have the potential to be highly localized and also reversible, making them attractive systems for mitigating vector-borne diseases and other pest problems (Leftwich et al. 2018).

General Limitations of Gene Drive Technologies

There are several limitations to the use of gene drives. For example, since gene drives require multiple generations to induce change throughout a population, generation time and ability to mate with its wild-type relatives over multiple generations become integral to success of this strategy. Only organisms with a relatively short generation time and the ability to sexually reproduce, such as mosquitoes, could be managed using a gene drive strategy. Organisms which reproduce asexually, either through clonal division (banana) or through the ability to self-fertilize (dandelion), would exhibit different dynamics with a gene drive. Similarly, long-living organisms such as many tree species would not be suitable for gene drive technology. The evolution of resistance is another limitation of gene drives and will be discussed later in the chapter. A final limitation is that many of the traits spread through gene drives are in fact harmful or present reduced fitness to the organism. Eventually another gene drive will be needed to ensure that the intended driven trait is not replaced, depending on the circumstances. This suggests that gene drives may not be permanent solutions; rather they may only be transient fixtures that must be elicited periodically (Godfray et al. 2017)

In spite of these limitations, gene drives offer potential solutions to some of humanity's most pressing problems. The following section details several potential applications.

The Potential of Gene Drives to Reduce Vector-Borne Diseases (VBD)

Vector-borne diseases, primarily spread by mosquitoes and ticks, account for 17% of all known infectious diseases (de la Fuente et al. 2017). Most vector-borne diseases are found in tropical and subtropical regions, where populations tend to be poorer and have fewer resources for surveillance and response. More recently however, infectious diseases such as Lyme disease, Zika and dengue, previously found exclusively in warmer regions, have been making their way to more temperate regions of the world (Tjaden et al. 2018). This spread stems from a complex mixture of climate change, globalization, and international trade, thus creating the emergence of new threats.

More than half of the world's populace resides in urban areas; this proportion continues to climb (United Nations 2018). This population shift may well result in the growth of concentrated populations of the poor, typically lacking in safe drinking water, waste management, and basic healthcare services. Disease burden can then be disproportionately high in poor communities. Moreover, malnourished populations typically lack essential vitamins and minerals and thus lack robust immune systems. The death rate due to vector-borne diseases is already high, and the World Health Organization (WHO) has issued a call for vector control strategies that are both unified and easy to mobilize throughout urban and rural communities alike. These strategies for vector control include a strengthened surveillance system and rapid in-field diagnostic tests so that infectious diseases such as Zika, dengue, and Chikungunya can be quickly identified and epidemics prevented. An enhanced, consistent, and unified surveillance and response infrastructure will require education, extensive communication, urban planning, availability of health services, government commitment, and strengthened policies between countries at risk of impact by vector-borne disease (Schorderet-Weber et al. 2017).

Current strategies to block disease transmission by mosquitoes and ticks include insecticides, bed nets, protective clothing, improved sanitation, and water management. While some of these approaches have proven to be effective, they are clearly not sufficient. Insecticides can be high in cost, and insects eventually may develop resistance to them. Bed nets and various forms of protective clothing are not distributed thoroughly enough to fully block mosquito and tick bites (Rakotoson et al. 2017). Urban planning to improve sanitation and still water containment strategies require social infrastructure that may not be readily available. While vaccines do exist for some infectious diseases, many do not and thus cannot detour the spread of infection. Projects such as the world Mosquito Program incorporate the use of endosymbionts such as *Wolbachia*, a bacterium that can infect mosquitoes and disrupt disease transmission (Curtis and Sinkins 1998). Ticks may also be infected with *Wolbachia* species, which can decrease their motility and thus reduce the occurrence of disease transmission (Indriani et al. 2018; Calvitti et al. 2010). Improved knowledge of microbial communities in mosquito and tick populations could potentially be controlled using endosymbionts based natural gene drives (Bull 2015).

The general concept that genetics can be used to control mosquito populations is not new; however, the use of gene drive technology has offered the possibility to generate a way to limit the spread of diseases such as Zika, dengue, and malaria through the control of insect vectors (Macias et al. 2017). In Brazil, the biotech company Oxitec is using a small laboratory, mobile production approach to generate genetically engineered mosquitoes that carry a gene that causes their offspring to die before reaching maturity. By releasing these mosquitoes into the area surrounding towns and small cities, the company hopes to reduce vector-borne diseases prevalent in the area, such as Chikungunya and dengue (Paes de Andrade et al. 2016). In this case, Oxitec generates male mosquitoes with a self-limiting gene that kills them before they are mature enough to reproduce, as well as a reporter gene whose promoter is regulated by sensitivity to tetracycline. By including the antibiotic tetracycline in the insects' water, the self-limiting gene becomes inactivated so long as they remain under caged conditions. The mosquitoes are able to reach maturity and the males are released into the environment to mate with wild females. The offspring lack access to tetracycline outside of the lab, and as a result, the self-limiting gene becomes activated, causing an early death. Using this approach, mosquito populations plummeted by over 60% in 2016 when Oxitec males were released in the Cayman Islands. It is important to note that not all of the mosquitoes released were sterile (Evans et al. 2019).

The Bill & Melinda Gates Foundation has now joined forces with a consortium known as Target Malaria to focus on removing malaria-carrying mosquitoes in West Africa. Target Malaria incorporates the CRISPR-Cas9 self-sustaining gene drive strategy into male mosquitoes of specific species that carry malaria, which, upon release, will mate with wild females. It is estimated to take multiple generations until all the modified mosquitoes will be eliminated from the population. Although the population of this specific species of mosquito may thus collapse, no impact on other mosquitoes or other insect species within that ecosystem is generally expected, although some evidence exists that this may not always be the case (McFarling 2017; Collins 2018; Fontaine et al. 2015).

Research concerning the gene editing of mosquitoes is accelerating. For example, Li et al. (2018) have successfully used the CRISPR/Cas9 system for highly efficient, site-specific mutagenesis in a diversity of malaria vectors including *Anopheles albimanus*, *A. coluzzii*, and *A. funestus* (Hammond et al. 2016). Similarly, Kyrou et al. (2018) have managed to negatively impact female *Anopheles gambiae* mosquito populations by focusing on alternatively spliced transcripts that are responsible for sex differentiation. Recently a cargo gene comprised of small RNAs which target Zika virus has been generated in *Aedes aegypti* mosquitoes that could significantly reduce Zika infection (Buchman et al. 2018).

Using Gene Drive to Combat Lyme Disease

Lyme disease, transmitted by ticks, is caused by *Borrelia* bacteria and is responsible for symptoms ranging from neurological problems to arthritis. Lyme is found in both the USA and Europe, with hundreds of thousands of people diagnosed every year. Infecting humans, dogs, horses, and deer, the main reservoir of the *Borrelia* species of bacteria are mice. *Borrelia* has a highly complex genome, compared to many other bacteria, and carries multiple plasmids that provide unique pathologies, tropisms, and manifestations of disease (Casjens et al. 2017, 2018). A project that focuses on creating transgenic mice that harbor immunity to the bacteria has gained momentum, and the future possibility of employing a gene drive to carry the trait through wild populations is now under consideration (Hammond et al. 2016). Mice would either express an antibody to render them resistant to Lyme disease or else would be immunized against a protein found in tick saliva, which in turn would protect the mice against *Borrelia* and other forms of disease carried by ticks. This approach differs from a conventional vaccination as the acquired immunity would be passed on from one generation to the next. The modifications can be made using CRISPR-Cas9 genome editing technologies (Enzmann 2018). The plan is to release these edited mice into the wild, beginning with unpopulated islands and then moving on to island communities such as Nantucket and Martha's Island, where mouse populations are contained and Lyme disease is highly prevalent. The short reproductive cycle of mice enables them to be assessed for the presence of infected ticks over a relatively short time period (Bouchard 2017).

Gene drive technology in mammals such as mice, however, is far behind that of mosquitoes. Only last year has a gene drive been partially successfully implemented in mice (Grunwald et al. 2019). Researchers were able to use a CRISPR-based gene drive to change the coat color of mice from black to gray over the course of one generation (Grunwald et al. 2019). Since this initial attempt resulted in only female mice inheriting the gene drive approximately 86% of the time, more effort will be required to improve transmission efficiency before the drive could be successfully applied to a wild population. Improvements in gene drive technologies implemented on rodents such as mice could eventually be applied to reduce or eradicate rodent-borne deadly infectious diseases such as Lassa fever virus and hantavirus.

Gene Drive to Reduce Persistence of Weeds and Pests

Resistance of weeds to herbicides remains a significant agricultural challenge (Godfray et al. 2017). The problem could feasibly be addressed through the use of a synthetic gene drive which could replace resistant alleles with their original, herbicide-sensitive counterparts. Similarly, a gene drive could revert insect pests who have developed resistance to commonly used pesticides such as Bt. While attractive, the technology would work only on organisms which sexually reproduce and would require that fields be kept pesticide/herbicide-free until the drive fixes. This would require cooperation from farmers who own neighboring fields that drives may spread to and thus could be difficult to implement.

Alternatively, a sensitizing gene drive could be utilized so that pest or weed populations were made vulnerable to molecules that did not affect them previously. If, for example, a gene drive provides a novel sensitivity to a chemical compound or small molecule, then an insect pest or weed species could be made vulnerable upon exposure to that small molecule. The concept is attractive as it would enable the presence of a specific species to be under strict control. It would also permit farmers to use chemicals or small molecules which are more benign to human health and the environment.

Gene Drives to Control Crop Pathogens

Gene drive is under consideration as a means to address environmental problems that have not been solved by traditional conservation practices. For example, in Florida, the spread of citrus greening disease, a bacterial disease transmitted by the insect *Diaphorina citri* (psyllid species) that is destroying the citrus industry, has been particularly newsworthy. Currently, citrus growers have resorted to the spraying of antibiotics throughout their orchards to protect against the disease (McKenna 2019). While a GM-resistant citrus alternative exists, the possible use of a gene drive to control insect vectors has been explored. In this case, a self-sustaining gene drive that would spread a strain of the insect that would be incapable of transmitting the disease could replace the existing insect population (Baltzegar et al. 2018). A gene drive solution for the invasive East Asian fruit fly, *Drosophila suzukii*, which damages berries and soft-skinned fruits across the globe has also been under consideration (Li and Scott 2016). Oxitec plans to focus next on diamondback moths, a well-known crop pest and invasive species responsible for approximately $5 billion worth of damages in the USA every year. While gene drive moths are preferable to pesticides for many, others have raised concerns about the ecological impacts of their release, as well as the possibility of acquired resistance (Scharping 2017).

Gene Drives to Protect Invasive/Endangered Species Populations

Invasive animal or plant species, such as cane toads, brown rats, and purple loosestrife, have caused great ecological damage (Webber et al. 2015). The top ten invasive species found in the United States are responsible for approximately $42 billion in damage every year. Alternatively, the plight of endangered species, such as amphibians sensitive to fungal diseases, is also of great concern. A synthetic gene drive could be a valuable tool to address both population types. A self-limiting drive could be implemented to target invasive organisms who sexually reproduce, such as the enzyme responsible for the production of the toxin in the saliva of the cane toad, to reduce their populations and restore natural ecosystems (Webber et al. 2015). Similarly, a gene drive, aimed at population modification, that is tailored to an endangered frog and salamander species could offer resistance to fungal pathogens and enable them to thrive once more in their natural environments. Kohl et al. (2019) conducted a survey to determine moral acceptability of gene drive technologies for the purpose of either eliminating an invasive species or protecting an endangered species. Their results suggested that the general public was more accepting of a gene drive to improve the survival of an endangered species rather than eliminate environmentally problematic wildlife populations.

Limitations and Risks Associated with Gene Drive Organisms

Concerns Regarding Appropriate Containment Strategies

Gene drives potentially promise eradication of some of humanities' worst pests. However, they also elicit concerns ranging from practical difficulties with regard to conducting field trials, unforeseen ecological changes or other complexities, and their long-term efficacy in the field (Moro et al. 2018), to the development of target site resistant populations that could prove to be unstoppable (Callaway 2017). Besides the general biology of gene drive, other challenges include governance, development, and adherence to legal structures and public acceptance (Nash et al. 2019). How the potential for ecological impacts can be addressed is discussed in the following section.

Containment measures can be administered and implemented in several ways. For example, Adelman et al. (2017) described the need for standard operating procedures (SOPs) concerning gene drive mosquitoes. While a series of SOPs will be essential for any release of gene drive organisms, implementation across multiple countries may be essential for success. Containment strategies to be set in place include an examination of land use, facilities constructed to house the target species (including labs and cages), biosafety protocols, removal of wastes, and shipping and transport precautions. International laws will be necessary to ensure that proper regulation. Staff can conduct initial trials regarding containment management by starting with non-transgenic mosquitoes, followed by transgenic varieties once competence has been demonstrated (Quinlan et al. 2018). While physical containment of gene drive organisms may be achievable in this fashion, additional safety procedures are required to ensure that gene drive strains can be identified and are trackable (Benedict et al. 2018). This will require creation of biosafety committees with methodologies to track and control gene drive organisms that are released.

Concerns about uncontrolled spread of gene drives can be addressed in other ways. For example, self-limiting drives such as the Daisy drive could generate short-lived gene drives within a given population. Alternatively, since a successful gene drive requires a particular threshold number of genome edited organisms to be released into the wild, having in reserve a large population of wild-type organisms available to be released if necessary could overcome this threshold and mitigate the impact of a gene drive within a given population.

Possible Resistance Developed Toward Gene Drives

Another means by which a gene drive could overcome containment strategies and undergo uncontrolled spread is through the natural genetic variation within a given population (Zentner and Wade 2017). Sequence polymorphisms found within a given population can prevent the endonuclease from cleaving a target gene. Eventually, these naturally resistant variants will increase in abundance to the extent that the gene drive is eliminated. For example, if enough variation existed within a malaria-carrying mosquito species, a gene drive may not completely exterminate all members of a local population. Genetic variants that possessed resistance could indeed escape the effects of a gene drive and reseed a new “resistant” population of mosquitoes. This new malaria-bearing gene drive resistant mosquito population would be more difficult to control than the original population. Hammond et al. (2017) assessed the potential of emergence of resistance to gene drives in a caged mosquito population by running a female infertility-based gene drive for 25 generations. The authors observed a gradual decrease in frequency of gene drive, followed by a slow spread of mutations within the target gene that rendered it resistant to cleavage by the endonuclease. These mutations were endonuclease induced and increased at rates which were consistent with positive selection.

A strategy proposed to address this development would be the creation of a gene drive targeting multiple sites within the target gene, so that it would be statistically next to impossible to find a natural variant that harbored mutations at all sites (Godfray et al. 2017). This strategy may, however, be difficult to implement in a very large population, as it would also require a large number of guide RNAs to ensure that resistance was not selected for. Oberhofer et al. (2018) created multiple cleavage sites within a specific gene to prevent spurious mutations that conferred resistance from occurring, although homing rates were modest. Another strategy to avoid resistance would be to create several temporally successive gene drives, each targeting a select array of multiple sites. Incorporation of both temporal drives and multiple target sites might prevent resistance from emerging.

Other Containment Strategies

One way to mitigate unknown ecological consequences of a gene drive could be through the simultaneous development of a reversal drive that restores the original phenotype and is ready to install at a given moment. This reversal drive could be used to overwrite any nucleotide changes that were spread by the first gene drive (Khamis et al. 2018). Alternatively, the production of sensitizing drives that are able to render the target organism vulnerable to a particular chemical could also be implemented to shut off the effect of the gene drive (Godfray et al. 2017). The presence of the chemical would have either a toxic or an inhibitory effect to the gene drive, resulting in refined control of the gene drive and greatly reduced ecological risk, although not restoring the original phenotype. Finally, the use of computational modeling to predict the outcome of a gene drive on a population of given size is a great way to identify their potential ecological impact (Edgington and Alphey 2018). This can be performed in conjunction with field trials using genetically engineered organisms that lack the gene drive function necessary to spread the trait in question and provide insight regarding properties including dispersal patterns of released insects, mating success, gene flow, and persistence. Such a plan exists for Target Malaria and is being implemented now. Currently, mathematical modeling of populations is used to determine the possibility of natural resistance taking place and taking over (Baltzegar et al. 2018). Another concern that may not be adequately captured by computational modeling is unknown behavior of the target organism in terms of mating and movement over their lifespans. For example, some mosquito species mate in swarms; others do not. Furthermore, mosquitoes raised in the lab or in cages may not serve as accurate models for their wild counterparts (Olena 2017). This can be investigated using RIDL field trials before implementing a gene drive approach (e.g., the Target Malaria phased project).

Social Impacts: The Risk/Benefit/Uncertainty Calculus

Gene drive technology will enter an entirely new space of social acceptance and regulatory treatment: genuinely unknown terrain in which social impacts will prove decisive for progress. Might insights gained from regulation and acceptance of recombinant DNA (rDNA) pharmaceuticals and agricultural plants provide clues for coming social dynamics?

It is of great importance that a continuous assessment of how the general public perceives gene drive technologies is performed well before any gene drives are released. Jones et al. (2019) analyzed the attitudes of the US public and identified strong support for the use of gene drives to control invasive species when no other options are available. Moreover, the authors found that people who do not support GMOs within their food supply nonetheless supported the use of engineered gene drives to control invasive agricultural pests. This interesting finding suggests that concerns regarding GMO food are not necessarily transferred to other technologies that require genetic engineering. Large portions of the population remain undecided regarding their judgment of gene drives. Public attitudes are known to undergo rapid changes, as has been shown with public perception of GM crops in Europe, for example. It is interesting that the authors also found an unclear response to natural gene drives such as *Wolbachia*-based control of insect pests. While attractive to some for their "natural" component, such a gene drive would be next to impossible to control in terms of spread, in comparison to some of the synthetic gene drive implementation strategies.

A major conclusion of literature on existing rDNA organisms and products is that aggregate cost-benefit analysis under conditions of low information has driven social acceptability and regulatory response to the genomics revolution (Herring and Paarlberg 2016). Where benefits are high and demonstrable, and alternatives inferior, risks are largely accepted: rDNA pharmaceuticals illustrate this logic. These were decisive questions differentiating biotech medicine from biotech agriculture. Regulation and acceptance of rDNA pharmaceutical products was not problematic: subjected to existing regulation, such products were accepted without any exceptional stigma. There was demonstrable utility, and safety was vetted by trusted authorities – personal physicians and official science, as in the Food and Drug Administration in the USA. Risks were explicitly detailed and known to consumers; these risks were accepted for demonstrable benefits, absence of alternatives, and the extreme risks of doing nothing. Agricultural rDNA products – in marked contrast – were politically encoded as "GMOs" and restricted or blocked in much of the world. For rDNA agricultural plants there is to date no documented incremental hazard in comparison with other means of inducing new traits in plants and yet "risk" is the dominant theme in restricting spread of the technology globally (Lewontin 2001). On the benefit side of the equation, consumers of GMOs typically derived little or no benefit but are confronted with a powerful risk narrative built around the unnatural nature of "Frankenfoods."

In these existing implementations of biotechnology, the difficulty has been in coming to an appropriate *aggregate* risk/benefit analysis for society as a whole: whose risk, whose benefit? In pharmaceuticals and foods, risks are divisible and individual choices are feasible. Environmental risk management is fundamentally different: individual risk perceptions are subordinated to ecological scale dynamics, over which unanimous consent is unlikely.

For new risk/utility regulatory regimes governing gene drives to become accepted, and effective, social consensus around aggregate risk and benefit must be achieved. The history of biotechnology to date raises large cautions: such agreement assumes polities that do not exist in any meaningful sense and hard-to-conjure enforcement tools.

In a technical sense, risk is hazard multiplied by exposure or probability of hazard. Determining hazard definitively is problematic: how many "unknown unknowns" escape conventional science, how many black swans? Moreover, at the frontiers, there is no way to predict unknown *future* hazards; "risk" in this sphere can be socially constructed only in hypothetical or "anticipatory" terms, generating a distinctive politics of precaution (Gupta 2011). Even if there is no new hazard at all, proving the absence of risk is impossible for science (Giddens 1999). In these circumstances, different interpretations of risk will proliferate and parties will engage in strategic behavior to secure support for the "framing" of regulatory positions they prefer (Benford and Snow 2000). In the current environment of populist rejection of authoritative knowledge in general, and science in particular, the probability of successful risk politics increases; the plausibility of deploying gene drives widely declines.

Despite the power of risk politics evident in the global rift over GMOs, the benefit side of the equation of technology at the frontier is qualitatively greater than in previous genetic engineering episodes. Risks of uncontrollable diseases of humans, animals, and crops constitute a crisis that may produce social and political openings. Cancer patients accept the risks of powerful chemicals on the grounds that alternatives are worse. Thus gene editing technologies may resemble more the path of rDNA pharmaceutical, risk but high benefits, more than rDNA crops, perceived risk but few consumer benefits. Genome editing and gene drives promise aggregate benefits unimaginable a few decades ago, in human health, agriculture, and environmental integrity. With the dreadful risks of climate change before us, every possible tool in the toolkit for adaptation assumes even greater importance. Yet the potential for unknown hazards increases with greater potential for utility.

The second lesson from existing biotechnology in society is that rigorous monitoring and regulation are assumed for legitimacy and effectiveness, but both remain egregiously elusive. Failure of regulation in turn increases the risk side of the equation. Gene drives are especially difficult in this regard because ecological systems do not stop at artificial boundaries on a map, either locally or globally. A social compact on a scale adequate to satisfy precautionary logic is difficult to conjure; there are essentially no exemplars. More important, agreement in paper treaties has proved as often as not toothless. Scientists and officials consider precise guidelines for gene editing deployment whereas the technology itself almost uniquely permits operation outside regulation of any kind. What means of surveillance and enforcement powers can even be conjured on any meaningful scale? Climate science offers cautions. Despite wide global scientific consensus, national and local interests outweigh species interest politically and the absence of any real power to implement agreements is obvious.

We can illustrate these issues with regard to gene drives with one example from the United States, where biotechnology is widely accepted. Gene-drive mosquito technology offers great potential benefits, but democratic politics does not always accord with independent scientific assessment (Meghani and Kuzma 2018). Some groups will inevitably contest the transparency and thoroughness of the science; some organizations exist to do specifically this. Many local people in the Florida Keys remain opposed to the release of Oxitec's GM mosquitoes within their neighborhood despite extensive analysis by the USDA, FDA, and the CDC. Classified as a pesticide with potential environmental impact, the EPA is exerting regulatory oversight of the organisms. The EPA has also approved the release of *Wolbachia*-infected mosquitoes in both California and the Florida Keys.

The benefits and risks in altered mosquitoes are especially complex and hence uncertain for reasons elaborated in previous sections: likelihood for success in achieving intended outcomes, possible impact on nontarget species, risk of spreading uncontrollably, and unanticipated societal effects. Gene drives in mosquitoes then illustrate the range of social issues of greatest importance: tremendous potential benefits achievable in no other way and uncertainty that can be coded as unacceptable risk.

First of these hypothetical risks are unintended consequences for health and the environment. What is the target species and what are its habits, location, and distribution? How much certainty is enough certainty when putatively sufficient studies have been completed? What level of governance is appropriate, which is a potential chokepoint for preventing deployment: local, national, and international polities? Practical questions of social importance follow: What are best practices for delivering gene drive technology, as well as evaluations and assessment before and after the release of gene drives? How much are lab research and controlled field trial work necessary to authorize release of gene drives into the environment? How and for how long would necessary post-release surveillance studies be conducted? How would persistence of gene drive organisms be determined, to ensure unwanted spread is not occurring? For how long? How intrusive/transparent will the level of engagement be between researchers and the public? Does a community have the capacity to oversee the safe and controlled release of gene drive organisms? What containment strategies will be implemented?

Assuming answers to these questions are adequate for local and national acceptance, how will a neighboring country without adequate scientific capacity or policies be involved in decisions? Perhaps a resolution could be found within the Florida Keys, but globally scientific and regulatory capacity vary greatly. How would international spread of altered organisms be predicted adequately, halted, or mitigated? As the scope of intervention extends, how will the weight of social forces opposed to hacking evolution be addressed? Are there theological complications over space in the arrogance of intervening in “God’s plan”? Will transnational advocacy networks that have powerfully mobilized against GMOs stimulate sufficient risk politics to blunt scientific consensus should it surface? What political and financial forces will mobilize on behalf of new opportunities to relieve the burden of disease in some countries, increase agricultural productivity, or sustain endangered species? What is the scope of informed consent when consequences are species-wide, not merely in one’s own backyard? Who has veto power?

Unless peoples’ confidence in answers to these questions can be incorporated into final decisions, regulatory uncertainty will impede investment of time, energy, and money necessary for development, blunting the effectiveness of the technology no matter how potentially beneficial. What we have learned so far is that the greatest risk of all may well be to block new technologies on the basis of ideological fundamentalism rather than rational comparison of probable risks and benefits in democratic and scientifically legitimate ways.

Conclusions

The perception of gene drive technology presents a controversy similar to previous experiences with genetically modified organisms, yet unique in important ways. Multiple stakeholders, both pro- and anti-GMO, have created uncertainties that depress investment and complicate world trade. Specific influencers that may swing in the favor of gene drive technologies could include an increased death toll due to the rise of vector-borne diseases such as malaria in the advent of climate change, the intensity of pest pressure in a farmers' field, or the potential loss of a now endangered species to extinction in the wild. Deterrents to offset the use of gene drives could comprise of unexpected events, such as loss of control, resistance of the target species, malfeasance, or changes to an ecosystem that are detrimental to nontarget species. The costs and benefits of gene drive are distributed unevenly and at multiple levels.

Geography at all scales weighs heavily in this future. Mosquitoes do not recognize borders. Farmers who did not actively agree to the adoption of a gene drive technology to remove a crop pest may reject adamantly the presence of gene drive pest residues in their crops, even if they benefit from pest reduction generally. It is unclear how organic farmers will perceive GM insects or how they will be classified in organic certification programs. These decisions would then significantly impact international food trade between countries harboring markets that are gene drive-friendly and countries that are not. On the other hand, the removal of invasive agricultural pests will offer relief to some countries which have in the past failed phytosanitary (SPS) regulations due to the presence of an invasive insect species. Gene drive to greatly reduce or even eliminate such invasive species in produce could remove quarantines and reinstate access of these countries to the global market.

This past November, the United Nations Convention on Biological Diversity (CBD) in Sharm El-Sheikh, Egypt, rejected a proposal to temporarily ban the release of organisms carrying gene drives. While not a formal moratorium, the requirements for release of a gene drive remain vague. Part of the explanation for this result is failure to provide for community engagement, with particular emphasis on underrepresented communities that may be most affected by this technology. Community engagement is the strategy taken by the organization Target Malaria, which hopes to test gene drive mosquitoes in Africa by 2024. Although the scientific potential of gene drives as a beneficial technology continues to proceed, its progress from a social perspective will remain hindered due to regulatory uncertainty and deep-seated reservations.

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