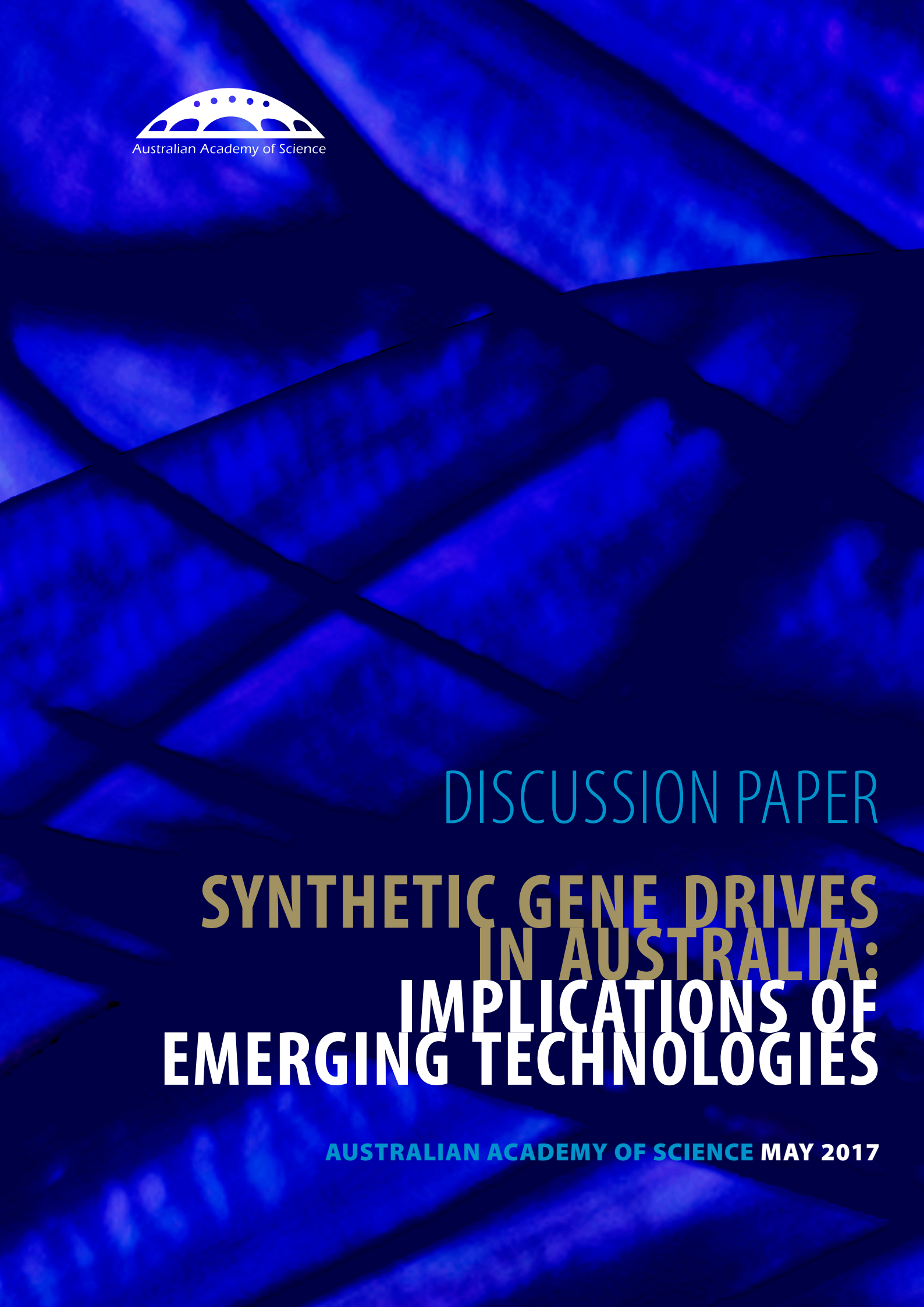


DISCUSSION PAPER

# SYNTHETIC GENE DRIVES IN AUSTRALIA: IMPLICATIONS OF EMERGING TECHNOLOGIES

AUSTRALIAN ACADEMY OF SCIENCE MAY 2017


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# INTRODUCTION

Gene drive mechanisms (or gene drives) cause a gene to spread throughout a population at a rate higher than would normally occur. Scientists have been observing examples of biased inheritance generated by natural gene drive mechanisms for many years. However, significant advances in genetic and molecular tools for genome editing have brought synthetic gene drive technology within the reach of many more researchers, and research has accelerated greatly in recent years. Since 2015, scientists have published four proof of concept studies in yeast, mosquitoes and the fruit fly *Drosophila* to demonstrate the feasibility of using synthetic gene drives for purposes such as combating vector-borne disease, suppressing pest populations, or for introducing desired characteristics into target organisms. As with many new technologies, the potential applications and benefits are far reaching, as are the potential impacts—both intended and unintended—on public health, conservation and ecology. This rapidly developing area represents an additional method of manipulating populations alongside traditional and other methods (Table 1).

The pace at which the gene drive research is moving has triggered international discussion (for example, Nuffield, 2016; NAS, 2016a). The scientific community has raised concerns as to when organisms modified with synthetic gene drives should be released, and there is significant discussion amongst scientists regarding best practice and strategies to manage and mitigate any hazards involved (Akbari et al., 2015; Oye et al., 2014).

To inform government and community consideration of these issues, this discussion paper by the Australian Academy of Science considers synthetic gene drives in a specifically Australian context and highlights the potential benefits and hazards of possible applications, emphasising the need to eventually consider these within a risk assessment framework. The paper discusses environmental hazards, social and economic issues (including trade implications) and how the technology can be managed within Australia's governance arrangements. Our unique Australian environment generates a number of issues specific to our country; the Academy intends this discussion paper to complement the international discussion underway and to inform Australian governments and our community about gene drives in Australia.

**Table 1: Description of various methods of biological manipulation of populations.**

| Method of manipulation | Description |
|---|---|
| Biological control | A method of controlling invasive weeds and pests using their own natural predators or parasites against them. Successful Australian examples include the control of prickly pear and skeleton weed. This approach is itself not without risk, as demonstrated by the well-known case of the cane toad in northern Australia. |
| Plant breeding | A systematic method of selecting plants with desirable characteristics for further breeding. It may include crossing closely related plant species to produce new crop varieties, or the use of chemicals or radiation to randomly generate mutants that happen to display desirable traits. |
| Animal breeding | As for plant breeding, this method aims to establish a line of animals with specific traits based on selective breeding, although related species are less commonly crossed and animals are less commonly exposed to radiation and mutagenic chemicals for this purpose. |
| Gene technology | This is a broad term that includes a variety of genetic manipulation techniques that are used to alter an organism's DNA. |
| Gene therapy | An application of gene technology involving the introduction of corrective genes to replace defective or missing genes to treat genetic disorders, usually in humans. |
| Synthetic gene drive | An application of gene technology that increases the prevalence of a genetic variant within a population. Natural gene drive mechanisms are also known; these are sometimes harnessed for manipulating populations without the use of gene technology. |

The Australian Academy of Science recommends that:

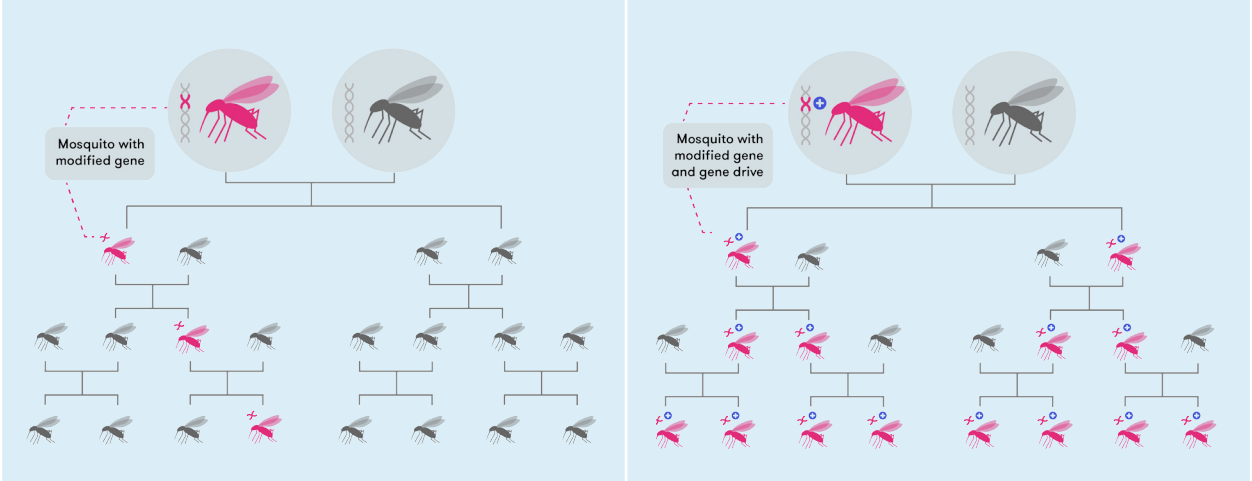
1. There continues to be clear and transparent communication of governance arrangements regarding regulation of synthetic gene drives.
2. Resources be provided to study synthetic gene drives in isolated laboratory populations with sample sizes and time frames that are large enough and/or long enough to observe processes such as selection, resistance evolution, population structuring and transmission distortion, together with the intended and potentially unintended consequences that these process may lead to.
3. Stringent, multiple containment measures be taken when researching synthetic gene drives.
4. Any decision to release a synthetic gene drive continues to be made on a case-by-case basis following a comprehensive environmental risk assessment which includes ecological and evolutionary modelling.
5. There be clear communication and consultation with the public through appropriate channels from the earliest stages of gene drive research, particularly with affected communities.
6. The wider implications of synthetic gene drives (e.g. trade implications) be considered.

# BACKGROUND

Gene drives produce a biased form of inheritance. They overcome normal Mendelian inheritance, where one copy of a gene is inherited from each parent, and greatly increase the chances of an allele passing from a parent to its offspring (Figure 1). This results in the preferential increase in the frequency of a specific genotype over many generations and the entire population may eventually come to possess only that genotype.

Synthetic gene drives are being developed to influence a target population via two primary methods: population suppression or population alteration. A synthetic gene drive that is designed to suppress a population would, over many generations, reduce the number of individuals within a population following its introduction—possibly to zero. A synthetic gene drive designed to alter some characteristic of a population would involve a modified genetic element

**Figure 1: An idealised illustration of Mendelian versus gene drive inheritance rates. Through standard Mendelian inheritance (left), offspring have a 50% chance of inheriting a modified gene carried by one of their parents. With a gene drive mechanism (right) the modified genes are eventually inherited by 100% of the offspring, allowing the gene to spread rapidly through the population. Images from Nova: Science for curious minds, modified from 'CRISPR, the disruptor', www.nature.com**



that is then spread throughout the population, for example to confer resistance or immunity to a certain parasite or disease.

A number of basic criteria are required for a synthetic gene drive to work. Firstly, the organism must reproduce sexually. This means that viruses, bacteria, many plants and some animals which use other means to reproduce cannot be altered in this way. Secondly, to be practical, the organism must reproduce rapidly. Elephants and trees with long generation times are therefore not ideal targets whereas insects, some plants and small vertebrates such as rodents and fish could be successful candidates. In addition, the organism must also be able to be transformed, and the trait of interest must have a simple genetic basis.

Whilst synthetic gene drives could technically be used in humans, we are unlikely candidates due to the combination of the complex ethical issues this would raise and the lack of efficacy from a practical perspective. Our long generation times would mean a gene drive-mediated change would take hundreds of years to spread throughout a human population. In most jurisdictions any research in this area would also be heavily regulated by existing legislation; in Australia extensive coverage would be provided by the *Research Involving Human Embryos Act 2000* and the *Prohibition of Human Cloning Act 2002*.

# GENE DRIVE MECHANISMS

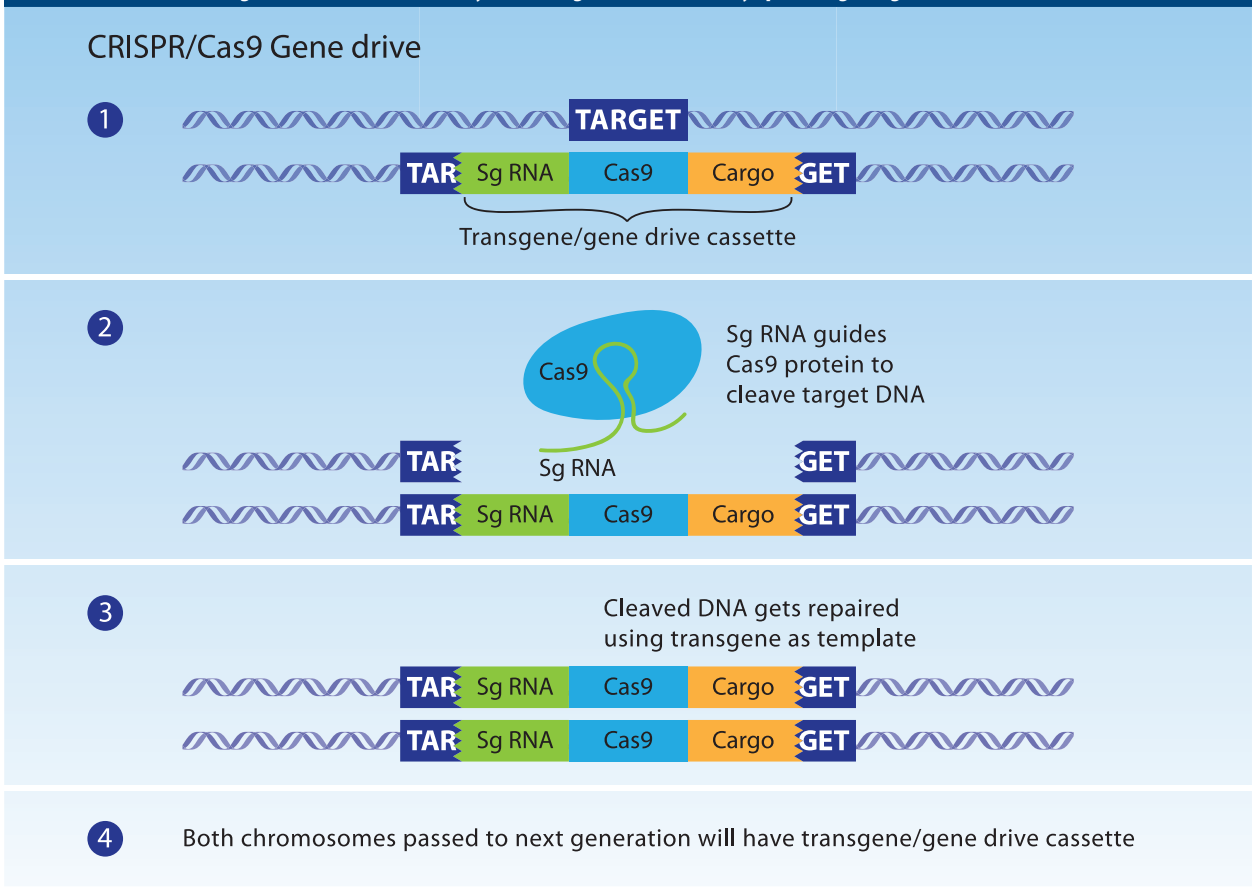
Scientists have been observing examples of biased inheritance generated by natural gene drive mechanisms for many years. The concept of a 'synthetic gene drive' was devised almost 50 years ago by Christopher Curtis who proposed using translocations (rearrangements of genetic material) to drive anti-pathogenic genes into wild species (Curtis, 1968). This idea was further developed by Austin Burt (2003; 2014), an evolutionary geneticist, who discussed how a synthetic gene drive could be used to prevent insects spreading diseases such as malaria.

There are many different types of natural gene drive mechanisms (Appendix 1). These can be characterised by attributes such as the rate of spread, species specificity, fitness cost, susceptibility to resistance, removability and reversibility (Champer et al., 2016). The rate of spread is an important consideration. So called 'high threshold' gene drives would only spread if the number of individuals with the drive genotype reaches a high level. These types of drive systems could be confined to local areas and breeding populations by controlling the number of individuals with and without the drive. In contrast, 'low threshold' gene drives, which are considered invasive, would spread with a low initial release, requiring only a small number of gene drive-carrying organisms to be released to spread. Natural *Wolbachia* infections provide examples of drives with high and low thresholds (Nguyen et al., 2014; Hoffmann et al., 2011). It is worth noting that no synthetic gene drives have yet been released into wild populations so the concepts discussed here are untested to date on such systems.

Recent advances in gene editing tools allow organisms to be edited much more efficiently and more accurately than previously possible. Scientists can now harness gene drive mechanisms which were previously merely theoretical to control or alter natural populations. While not a gene drive tool in its own right, clustered regularly interspaced short palindromic repeats of base sequences (CRISPR), can be used as part of a system to produce a synthetic gene drive. When CRISPR is paired with a guide RNA and with specific proteins, such as Cas9 (CRISPR associated protein 9) that cuts DNA, it can be used to efficiently edit genetic material. In natural prokaryotic systems, CRISPR/Cas9 is produced by host bacteria to remove viral DNA by targeting repeats associated with viral insertions, as a kind of immune system to combat infections. For gene editing purposes, the Cas9 protein and guide RNA are injected into the cell to cut the DNA at a sequence complementary to the RNA guide. For synthetic gene drives, the target organism is transformed with a construct that includes the gene for the Cas9 protein, a guide RNA that is complementary to the sequence at the insertion site, and the 'cargo' gene controlling the desired trait (Figure 2). The guide RNA directs Cas9 to produce a double stranded cut in the DNA at the target site in the other chromosome. This triggers the cell's repair mechanism, which copies the entire construct (Figure 2). If germ cells are targeted, the new sequence can then be passed on to offspring ensuring the editing changes can occur in each generation. A CRISPR-based gene editing technique was used in all four synthetic gene drive proof-of-concept studies in 2015. These studies generated laboratory-

based gene drives in yeast *Saccharomyces cerevisiae* (DiCarlo et al., 2015), fruit fly *Drosophila melanogaster* (Gantz & Bier, 2015) and two mosquito species *Anopheles stephensi* (Gantz et al., 2015) and *Anopheles gambiae* (Hammond et al., 2016).

**Figure 2: A synthetic CRISPR/Cas9 gene drive. Sg RNA is the guide RNA, Cas9 is an endonuclease which cuts the DNA and cargo is the desired genetic material added. When all three elements are present in a gene drive cassette this ensures that each chromosome will have the desired cargo and will be inherited by the next generation thereby spreading the gene drive.**




# POTENTIAL USES IN AUSTRALIA

Australia has a unique environment with highly diverse flora and fauna that have evolved in relative physical isolation over a long time period. A number of pests, diseases and invasive species that Australia has acquired from other parts of the world do not have close relatives in this country. This genetic differentiation and our well-established governance frameworks may make Australia an attractive setting in which to test synthetic gene drives that target pest species.

Any release of an organism containing a synthetic gene drive would be required to comply with our governance arrangements which include the requirement for a comprehensive risk assessment.

Australia has had mixed success in using deliberate biological introductions to reduce invasive and feral species populations. One success story is the control of prickly pear, a cactus which was introduced to Australia in 1788 and quickly became an invasive species spreading rapidly throughout eastern Australia. A South American insect, *Cactoblastis cactorum*, was introduced as a biological control and successfully reduced the prickly pear population. Other introductions, particularly that of cane toads to suppress cane beetles, have had far greater negative consequences than their modest positive contribution in the sugar cane fields. Mechanisms used for screening and testing biological control agents have prevented a repeat of such destructive introductions in the last few

decades, highlighting the efficacy of Australia's strong governance framework.

There are many potential local and international applications of gene drives in areas such as public health (specifically looking at interactions with pathogens), environmental conservation and agriculture, targeting both animals and plants. Gene drives can provide significant positive benefits to certain problems, especially where alternative methods are ineffective, damaging to the environment and/or costly. Australian-specific examples are described below; more detail is provided in Appendix 2.

## DISEASE APPLICATIONS

Insect-borne infectious diseases are a serious and significant global public health issue, and Australia is not immune. Malaria, dengue, Ross River fever (named after its place of discovery in Queensland) and Zika are all spread by mosquitoes and despite research efforts vaccines are still many years away from being widely available. Other methods to control mosquito populations are in jeopardy due to an increase in insecticide resistance. Current research in Australia is investigating how to suppress the transmission of dengue: a disease estimated to infect 390 million people each year worldwide (Bhatt et al., 2013) and which occurs in parts of northern Australia. Using a natural or synthetic gene drive to reduce mosquito populations, or make the mosquitoes less susceptible to becoming carriers, would help reduce the spread of this disease.

Other potential disease control applications include gene drives in vector insects to prevent the spread of livestock diseases such as blue tongue virus and systems to reduce wildlife diseases such as avian malaria that threaten endangered species.

## INVASIVE SPECIES AND THE ENVIRONMENT

Introduced invasive species can devastate native flora and fauna through predation, competition or parasitism. Gene drives may have the potential to restore native biodiversity through a number of routes, either by controlling specific invasive species or conferring competitive advantages on native animals. In Australia, suggestions to date include a synthetic gene drive to reduce the population of black rats on Lord Howe Island, cane toads in the tropics, European carp in the Murray Darling Basin and rabbits across the continent.

## AGRICULTURAL APPLICATIONS

Australian agriculture is a promising area for gene drive applications. Controlling organisms that damage important crops or carry crop diseases would provide a major boost to agricultural productivity and competitiveness. Introducing

**Disease applications**

| Problem | Examples of current solutions | Potential problems with current solutions | Potential beneficial consequences of gene drive |
|---|---|---|---|
| **Insect-borne diseases** | Spraying of chemicals, vaccination, wear long sleeve clothing, mosquito nets. | Several hundred thousand humans die every year from mosquito-borne diseases. Spraying of non-selective chemicals damages the environment and kills beneficial insects. Current non-chemical solutions rely on changes in human behaviour. Many solutions are costly to implement in remote regions. | A gene drive designed to prevent a mosquito from transmitting a pathogen would have positive consequences by reducing the spread of disease. The mosquito would still be present to retain its ecological function. Suppression of populations of exotic mosquitoes and midges will likely have few detrimental effects. |

**Invasive species and the environment**

| Problem | Examples of current solutions | Potential problems with current solutions | Potential beneficial consequences of gene drive |
|---|---|---|---|
| **Invasive species** | Traps and poisons, and other vector control strategies (e.g. ballast water exchange). | Invasive plants and animals predate and out-compete native Australia flora and fauna. Inaction could result in the extinction of native species. Some traps and poisons are non-selective and vector control strategies can be costly to implement. | A gene drive to control an invasive species could restore native species populations and ecosystem function. |

**Agricultural applications**

| Problem | Examples of current solutions | Potential problems with current solutions | Potential beneficial consequences of gene drive |
|---|---|---|---|
| **Agricultural pests** | Spraying of pesticides. | Spraying of chemicals damages biodiversity and decreases beneficial invertebrates due to non-selective nature of many chemicals. Pesticides become ineffective when resistance evolves. | A gene drive to eliminate a weed or pest could reduce chemical spraying and potentially increase farmer's crop yields. |

genes that reverse pesticide or herbicide resistance would help farmers to continue to control insects and weeds by chemical methods.

Suppressing or modifying invertebrate pests would be valuable for farmers and land managers. Targets for suppression include fruit fly pests, which attack soft fruits and cause significant crop loss, as well as various moths, mites, thrips and other pest invertebrates which attack vegetables and broad acre crops. Pests like diamondback moths, *Lucilia* blowflies and redlegged earth mites that have developed resistance to chemical pesticides are particularly important targets for control. Synthetic gene drives might also be developed to modify insect and mite vectors to reduce their ability to transmit plant viruses.

# POTENTIAL HAZARDS AND CHALLENGES

Despite the significant benefits synthetic gene drives may provide, an unplanned or poorly managed release of a gene drive modified organism could potentially change the environmental landscape well beyond the site of its introduction.

The introduction of foreign species and their genes into a new environment is not new. With human exploration and travel we have introduced new species into different environments either inadvertently (e.g. within ships' ballast water) or consciously (e.g. new crops, garden flowers or even animals for sport hunting) for many decades. Many invasive and feral species have become established in Australia, some of which have caused ecological and environmental damage. The introduction of new genes occurs both through new mutations arising in existing populations and though the movement of genes from one population to another. For instance, insecticide resistance genes in Australian insect pests have likely arisen both locally following mutation and been introduced from overseas populations (Umina et al., 2014).

Significant technical and knowledge challenges remain which must be overcome to engineer a successful synthetic gene drive, and these challenges should not be underestimated. The four proof of concept studies published over 2015 have all been in laboratory organisms which are highly uniform and unlike wild populations. The genetic constructs produced in controlled laboratory conditions are unlikely to perform in the same way in natural environments where conditions are much more variable and unpredictable. Additionally in a wild population, a trait which reduces the biological fitness of an organism—for instance a gene drive containing a construct designed to suppress reproduction—will slow down the spread of the gene drive.

The release of a low threshold synthetic gene drive designed to spread genes throughout an entire population demands additional care. The consequences of such releases are potentially widespread, and hence international consideration and consultation may be required. The spread of genes between populations—gene flow—must be understood prior to the release of any synthetic gene drive, but this is particularly important with low threshold drives. The possible transfer of genes between distinct species must also be considered. Gene drives shouldn't be implemented in species where there is potential for introgression with non-pest native species.

There is the possibility that releases of gene drive modified organisms will lead to unpredicted and undesirable side effects. Past eradication of pest species by conventional means such as baits or sprays have in some instances allowed another problematic pest to flourish as a result of a vacated niche or the withdrawal of predation (Dutcher, 2007). We must consider equivalent problems that might arise from possible future use of gene drive modified organisms.

It is also important, however, to put the hazards presented by gene drive modified organisms into perspective. A 100% effective gene drive can only ever double in frequency with each generation inheriting the drive mechanism. Mosquitoes have an average generation time of three weeks and it would take multiple generations to spread a gene drive to a portion of a local population. By comparison, a viral pandemic would affect national and international populations in a matter of weeks. While there should be caution in regard to the use of synthetic gene drives, there would be time to react if an unintended release or unexpected effect were detected.

The potential of evolution to modify gene drives and the constructs being driven also needs to be carefully considered. Resistance to the gene drive is likely to evolve unless other DNA repair systems that organisms possess can be turned off or multiple, independently acting, drive systems are developed. Before release into the environment, likely evolutionary changes in each genetic construct and their consequences

will need to be carefully modelled and evaluated. In addition, untargeted changes in the genome associated with the creation of drives may need to be evaluated.

Hazards pertinent to the applications of synthetic gene drives relating to pathogens, invasive organisms and agricultural applications are discussed in more detail below.

## HAZARDS RELATED TO PATHOGEN CONTROL

There are several hazards associated with releasing an organism containing a gene drive which results in the extinction of an insect-borne disease. Removing one vector could allow another potentially dangerous species to take its place by competitive- or predator-release processes. Releasing a gene drive modified organism that was only partially successful could also cause a loss of herd immunity in previously exposed populations. Public health would benefit in the short term but possibly not in the longer term, because individuals within the population may become more susceptible to the disease as the vector recovers from the initial suppression.

## HAZARDS RELATED TO INVASIVE SPECIES CONTROL

Ecosystems are highly interlinked systems within which the abundance of each species is governed by the balance of births, deaths, immigration and emigration. Their dynamics are controlled by positive and negative feedback cycles that respond to external forces in ways that are often difficult to predict. Introduced non-native species, if they are successful and flourish, can alter these processes and cause significant changes to the abundance of native species, and the feedback cycles they operate within. Gene drive modified organisms offer the potential to restore impacted ecosystems by suppressing invasive species, potentially to extinction. Modified ecosystems, however, may not return to a previous (desired) state even if the drive is successful. Furthermore, species that have become reliant on the invasive species could suffer as its abundance was reduced, and other harmful species could be released from predation pressure or competitive exclusion, and thereby flourish. Regardless of the cause—be it through a gene drive, attack by an invasive species or habitat loss—extinction of species requires careful and serious consideration.

Gene drive modified organisms may also spread naturally, or through human-mediated dispersal mechanisms, to other regions and other parts of the worlds. A possible consequence of creating a synthetic gene drive aimed at eradicating European carp or rabbits in Australia could be that the drive spreads overseas where these animals have important food, cultural and/or ecological values.

## HAZARDS RELATED TO CONTROL OF AGRICULTURAL PESTS

The spread of gene drive modified organisms also poses hazards in agriculture domains. Efforts to improve agriculture in Australia using synthetic gene drives may target problem weeds such as *Echinochloa colona*, or barnyard grass. This is a damaging weed for Australian farmers but in India the seeds of this grass are used to prepare a dish consumed on festival fasting days. Consequently, if a gene drive modified organism was released to suppress the weed population in Australia it could also affect a food source in other parts of the world. Elimination of a pest species might also create an empty niche that could be filled by other pests, as in the case of redlegged earth mites that show competitive interactions with other species of earth mites.

Significant technical limitations currently exist for gene drives in weeds. Gene drives can only function if double strand DNA breaks are repaired by homologous recombination, but some plants use non-homologous end joining pathways which prevents the use of the current generation of synthetic gene drive constructs.

Another challenge for agriculturally related gene drives is to avoid the development of resistance (Fukuoka et al., 2015). Resistance alleles can prevent a gene drive from spreading in pests and weeds (Champer et al., 2016). Efforts to avoid the development of resistance include stacking traits so that there are multiple defences to target the same pests and weeds. This strategy is already used in GM crop plants with resistance to insects where multiple insecticide genes are stacked together to reduce the likelihood of insects evolving resistance.

# SOCIAL AND ECONOMIC DIMENSIONS

Based on available information, which is currently limited, there is very little public awareness of the term 'gene drives' or of the science and technology associated with this term. Negative attitudes to all genetic modification persist despite almost 30 years of GMOs being globally available, and many scientific studies providing strong evidence that there are no adverse effects to human health due to consumption of GMOs (Nicolia et al., 2014; NAS 2016b). Within Australia, there are relatively few GM products on the market compared for instance to the United States, although GM-derived vegetable oil and soy flour have been in widespread use for the past two decades.

Public opinion regarding GMOs appears to vary widely within the Australian community, although there are few scholarly studies on attitudes towards GM foods (as noted by Lea, 2005). Community attitudes to biotechnology have been monitored in Australia by the Commonwealth Government, under the auspices of Biotechnology Australia (from 1999–2007), the Department of Industry (in 2010 and 2012) and the Office of the Gene Technology regulator (in 2015).[1] These surveys show some volatility in Australian public opinion regarding GM and biotechnology. Australians are generally viewed to be less cautious than Europeans and more sceptical than residents of the USA about GM. Anti-GM activism (in the form of direct action) in Australia has been far more limited than in Europe and the United States (Hindmarsh, 2008). There continues to be popular concern about the potential for drift between GM and non-GM crops (particularly organics, for example the recent court case in Western Australia (Paull, 2015)), the use of GM in crops destined for the food supply (even when no GM material remains in the final product) and the role of multinationals in GM particularly in the developing world. In short, the key issue underlying public attitudes to GM is that competing arguments are grounded in extremely diverse understandings and assumptions, particularly about what counts as evidence (predominantly of risk or lack thereof), and how to balance risks and benefits, especially with regard to new innovations. These arguments are likely to recur in the case of synthetic gene drives.

As in the case of GMOs, the concerns of potentially affected communities need to be carefully considered in regard to gene drives. Community engagement will be important from the earliest stages of gene drive research. Community engagement around control of carp involving genetically-based approaches (Thresher, 2008) and *Wolbachia* releases (Hoffmann et al., 2011; Kolopack et al., 2015) provide case studies. Any unintentional release—even without harmful consequences—could cause widespread public distrust of scientists, transgenics and transgenic products, and the field of gene drive research more generally. Transparent information provision and policy, cultural respect and engagement with social and ethical implications of this type of research will be imperative for the possible benefits of synthetic gene drives to be realised, in alignment with best practice strategies in science engagement (see for example Department of Industry, Innovation, Science and Research, 2010) and to avoid community backlash such as occurred in the case of GM policy and regulation (Schibeci & Harwood, 2007). The potential benefits of gene drives and the consequences of inaction are also important to convey to the public. There is a risk that lack of action or continued ineffective action could cause damage to the environment and be unnecessarily costly.

The trade implications of gene drive modified organisms released in Australia must also be considered. Australian exports to an importing country with different gene technology legislation to our own could be detrimental to trade relationships and generate other economic issues. Unintended consequences of a gene drive modified organism may include increased import requirements such as increased testing and documentation. A gene drive targeting pest fruit flies may be a problem for countries such as Japan which have highly specific regulations on fruit imports. These potential trade impacts should be discussed with Australian industries prior to release to ensure they are comfortable with the risks. In addition early engagement with key importing countries for trade is highly recommended.

A significant ethical concern is commercialisation and ownership of intellectual property. A patent for the technology of RNA guided gene drives was filed by Esvelt and Smidler in 2014 (WO 2015105928 A1). There are currently two competing patents (Zhang versus Doudna) over the CRISPR gene editing technology (Egelie et al., 2016). For a synthetic gene drive with applications in public health and conservation, there may be very little scope for commercialisation. As in other areas of biotechnology, the patenting of gene editing and gene drive technologies may raise ethical and economic issues and thus present impediments to ongoing research. Conversely, intellectual property can reward innovation and allow time for new products to be developed.

**1** See www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/reports-other for the OGTR 2015 survey and https://industry.gov.au/industry/IndustrySectors/nanotechnology/Publications/Pages/Public-Attitude-Research.aspx for earlier surveys.

# MITIGATION STRATEGIES

Gene drives have the potential to solve intractable problems in diverse areas of public health, agriculture and conservation but also present a range of social and environmental hazards. It is vital that the use of technology is open and peer reviewed, with research intentions made clearly transparent to the public. The Academy recommends scientists adhere to best scientific practices and follow the responsible conduct of research when investigating gene drive modified organisms[2]. Ethical consideration of both social and environmental consequences should be considered prior to commencing any research. The *National Framework of Ethical Principles in Gene Technology 2012* provides guidance on values and ethical principles in relation to gene technologies.

Such considerations should include a thorough and quantitative investigation of alternative methods to address the experimental problem. Not all problems that can be addressed by a gene drive modified organism should be: if there is an alternative available that will achieve the same outcome while presenting fewer hazards then it should be prioritised over new technologies. On the other hand, if a synthetic gene drive is the best solution it should be considered to prevent the consequences of inaction or ineffective action.

Multiple stringent confinement strategies should also be used to avoid the unintentional release of a gene drive modified organism while in development (Akbari et al., 2015; Oye et al., 2014). Molecular and physical confinement measures are described below in addition to possible safeguards that may be prepared in advance of a gene drive release.

## MOLECULAR CONFINEMENT

There are a number of options which can be considered during the design of a gene drive construct that can act as a molecular confinement measure. These include:

- using synthetic target sequences that are not in natural populations and therefore could not spread to wild organisms
- targeting unique sequences which are very specific to the target organism to avoid a gene drive spreading to closely related species. For example targeting the toxin genes of cane toads which are not found in other amphibians
- choosing a gene drive mechanism which has a low ability to spread, known colloquially as high threshold drives—these help confine the spread of a gene drive to a local breeding population. If the threshold is not exceeded, the drive system is lost from a population. This concept is illustrated by the loss of *Wolbachia* from natural populations (Nguyen et al., 2015)
- designing a gene drive which is not self-sufficient by physically separating the elements. In the case of CRISPR/Cas9 drive technology the Cas9 and guide RNA would be separated, known as a split gene drive system. This has been tested in yeast (DiCarlo et al., 2015)
- designing a gene drive that would stop after a few generations. This would limit the capacity of the gene drive to spread. Figure 3 demonstrates this 'daisy chain' gene drive where each genetic element drives the next (Noble et al., 2016).

**Figure 3: Example of a 'daisy chain' gene drive. A daisy chain system consists of serially dependent, unlinked drive elements which are on separate chromosomes. These genetic elements drive the next element and are lost over time which limits the time and location of the gene drive spread.**


**2** www.science.org.au/supporting-science/science-policy/position-statements/ethics-and-integrity

## PHYSICAL CONFINEMENT

Appropriate training of researchers in best practice and using precautions to limit human errors are very important. Other physical measures which can be implemented include:

- following the specific guidelines for work on mosquitoes as outlined within *The guidance framework for testing genetically modified organisms* (WHO, 2014)
- avoid transferring gene drive modified organisms between laboratories. Instead DNA constructs or information sufficient to reconstruct the gene drive should be sent, if required
- ensuring that all work takes place in suitably confined premises as currently defined by Physical Containment levels PC2[3] or PC3[4] (Office of the Gene Technology Regulator) or Biosecurity Insectary Containment levels BIC2[5] or BIC3[6] (Department of Agriculture and Water Resources).

## REPRODUCTIVE AND ECOLOGICAL CONTAINMENT

Options for reproductive and ecological containment include using:

- reproductive barriers, such as using a laboratory strain which cannot reproduce with wild organisms.
- ecological confinements, such as developing a gene drive in an area where there are no viable mates or an area which is only temporarily habitable for that organism.

## SAFEGUARD MEASURES

In addition to the containment measures described above, a strategy to mitigate potential ecological and environmental consequences from the accidental release of a gene drive or from unanticipated impacts of an intentional release is highly recommended. Options include:

- an immunisation gene drive to block the spread of unwanted gene drives by pre-emptively altering the target sequence thereby preventing the gene drive from spreading (Esvelt et al., 2014)
- a reversal gene drive designed in parallel with any gene drive experiment to overwrite any unwanted changes of a gene drive (DiCarlo et al., 2015)
- trialling a gene drive using a benign change to enable the effectiveness of a gene drive spread to be studied prior to a release
- ecological modelling to help predict the potential consequences resulting from a gene drive release (for example, see Unckless et al., 2017).

Wherever possible, the likely effectiveness of safeguards should be assessed in a quantitative way based on current knowledge.

# CURRENT REGULATORY STATUS

The rapid advances in gene editing and gene drive technologies present substantial challenges to current regulatory systems that are under active consideration in numerous jurisdictions (Nuffield, 2016; NAS, 2016a; Secretariat CBD, 2015). There are important differences between gene editing and gene drives. As organisms with a gene drive may spread beyond geographical borders, this raises many questions including who should, ultimately, make the final decision on a gene drive release? And who bears responsibility for any negative consequences?

The ability of gene drives to intentionally spread a trait through a population carries important implications for the governance of gene drive research, not only for the regulatory framework but also the informal processes of implementing a gene drive. The informal processes include public engagement, addressing societal expectations, communication, and mitigation strategies which have been discussed in the previous sections.

Australia has a well established regulatory framework for gene technology. Our national, integrated regulatory scheme is a process-based system that was set up to protect people and the environment by identifying and managing the risks posed by live and viable GMOs. The *Gene Technology Act 2000* (the Act) covers work with GMOs in certified contained laboratory conditions as well as intentional releases to the environment under limited and controlled conditions (field trials), through to unrestricted releases.

**3** www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/PC2-4/$FILE/PC2LABv3-1-1.pdf

**4** ogtr.gov.au/internet/ogtr/publishing.nsf/Content/PC3-4/$FILE/PC3LABv3-May2012.pdf

**5** www.agriculture.gov.au/SiteCollectionDocuments/biosecurity/import/arrival/approved-arrangements/7.2-requirements.pdf

**6** www.agriculture.gov.au/SiteCollectionDocuments/biosecurity/import/arrival/approved-arrangements/7.3-requirements.pdf

| Table 2: Australian regulatory environment for GMOs | | |
|---|---|---|
| **Agency** | **Relevant legislation** | **Scope** |
| Office of the Gene Technology Regulator | *Gene Technology Act 2000* | Genetically modified organisms, including gene drives. |
| Department of Agriculture and Water Resources | *Biological Control Act 1984* | Assessment and authorisation of biological control activities. |
| | *Biosecurity Act 2015* | Assessment and management of biosecurity risks from diseases and pests. Includes provisions addressing importation of products presenting a biosecurity risk. |
| Department of the Environment and Energy | *Environmental Protection and Biodiversity Conservation Act 1999* | Protection and management of nationally and internationally important flora, fauna, ecological communities and heritage places. |
| Australian Pesticides and Veterinary Medicines Authority | *Agricultural and Veterinary Chemicals (Code) Act 1994* | Agricultural pesticides and veterinary medicines. |
| | *Agricultural and Veterinary Chemicals Administration Act 1994* | |
| Food Standards Australia and New Zealand | *Food Standards Australia New Zealand Act 1991* | Food and food technology (including food produced using gene technology). |
| Therapeutic Goods Administration | *Therapeutic Goods Act 1989* | Human therapeutics, including medicines and medical technologies. |

Where gene technology is used to introduce or create a gene drive in an organism, the resulting organism will be considered to be a GMO and subject to regulation under the Act.[7] Hence, the use of site-directed nucleases (SDNs) such as CRISPR/Cas9 to produce a gene drive modified organism would be regulated.

To enhance coordinated decision making and avoid duplication, the Act requires consultation between regulatory agencies that have complementary legal responsibilities and expertise in relation to the evaluation and use of GMOs and GM products (Table 2).

Where a synthetic gene drive modified organism targets invasive species, a range of legislative provisions may also apply. The *Biological Control Act 1984* (Commonwealth) assesses and authorises biological control activities. Each state and territory has their own version of this act (except the ACT, which is under the Commonwealth act). As such organisms can potentially cross state and territory borders, agreement across Australia will be needed for the release of a synthetic gene drive modified organism to control invasive organisms. In addition, the *Biosecurity Act 2015* targets biosecurity risks entering Australia from overseas relating to animal and plant pests and diseases so a gene drive modified organism imported from overseas would likely be subject to this act. The *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC), which protects and manages nationally and internationally important flora, fauna, ecological communities and heritage places, may also need to be considered.

Some work with gene editing and gene drive technologies may be subject to control as a consequence of Australia's membership of a number of international counter-proliferation regimes. The Defence Trade Controls Act was introduced in 2012 to prevent sensitive goods and technologies that could be used for offensive purposes (known as 'dual use') going to individuals, states or groups of concern.

The regulatory environment continues to evolve in response to changes in technologies. At the time of writing The Gene Technology Regulator, the independent statutory office holder responsible for administering the *Gene Technology Act 2000*, is conducting a technical review of the Gene Technology Regulations 2001, with community consultation and engagement. This review is explicitly considering gene drive technology. The Department of Health will be undertaking a scheduled review of the *Gene Technology Act 2000* in 2017, and Food Standards Australia and New Zealand has commenced a review to consider food derived using new breeding techniques, including gene editing technologies.

Australia also works with other countries to harmonise approaches in biotechnology and new technologies in agriculture. In January 2016, Australia released a joint statement with Argentina, Brazil, Canada, Paraguay and the Unites States advocating removal of global barriers to the trade of agricultural biotechnology and promotion of science-based regulatory approaches.

**7** www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/53139D205A98A3B3CA257D4F00811F97/$File/OGTR%20guidance%20on%20gene%20drives.pdf

# RECOMMENDATIONS

Synthetic gene drives have the potential to solve seemingly intractable problems in public health, environmental conservation and agriculture. However, they also have the potential to cause negative environmental and human health effects.

The Australian Academy of Science recommends that:

1. There continues to be clear and transparent communication of governance arrangements regarding regulation of synthetic gene drives.
2. Resources be provided to study synthetic gene drives in isolated laboratory populations with sample sizes and time frames that are large enough and/or long enough to observe processes such as selection, resistance evolution, population structuring and transmission distortion, together with the intended and potentially unintended consequences that these process may lead to.
3. Stringent, multiple containment measures be taken when researching synthetic gene drives.
4. Any decision to release a synthetic gene drive continues to be made on a case-by-case basis following a comprehensive environmental risk assessment which includes ecological and evolutionary modelling.
5. There be clear communication and consultation with the public through appropriate channels from the earliest stages of gene drive research, particularly with affected communities.
6. The wider implications of synthetic gene drives (e.g. trade implications) be considered.

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# APPENDIX 1: EXAMPLES OF NATURAL AND SYNTHETIC GENE DRIVE MECHANISMS

## HOMING ENDONUCLEASE GENES

Site-specific selfish genes such as homing endonuclease genes (HEGs) can spread through populations as a gene drive due to their biased inheritance (Burt, 2003). They cleave a unique stretch of genomic DNA and as the cell repairs the hydrolysed DNA the HEG is copied into the cleaved site. Consequently the frequency of HEGs increases and they spread throughout a population.

There are other current gene editing techniques such as Zinc Finger Nucleases (ZFNs), Transcription Activator-like Effector Nucleases (TALENs) and CRISPR (Clustered regularly interspaced short palindromic repeats) which also utilise nucleases to cleave at specific sites. While not a gene drive in its own right, CRISPR/Cas9 is a gene editing tool that can be used to produce synthetic gene drives that increase the inheritance of a particular trait as outlined in the main text. Note that the vast majority of gene editing applications does not involve the creation of a gene drive.

## TRANSPOSABLE ELEMENTS

Gene drives can be generated by manipulating transposable elements, also known as jumping genes. These are small DNA segments which can excise themselves and randomly insert into different parts of the genome. This results in multiple copies within the genome. The *P*-element transposon is a type of transposable element well studied in the *Drosophila melanogaster* (Rubin & Spradling, 1982). An active *P*-element can be modified and in this way can rapidly spread the modified sequence throughout a population.

## MEIOTIC DRIVE

Meiotic drive is a gene drive mechanism interfering with meiotic processes to cause a distortion of allelic segregation compared to expected Mendelian inheritance (McDermott & Noor, 2010). This has been reported in *Drosophila melanogaster*, in the house mouse *Mus musculus* and in plants *Zea mays* and *Silene*. Within *Zea mays* the Abnormal 10 (Ab10) chromosome affects segregation of chromosome 10 and causes heterozygous chromosomal pair separation of 70% rather than the typical 50% expected with Mendelian inheritance.

## UNDERDOMINANCE

Underdominance is selection against heterozygous progeny where the homozygotes have an increased fitness and one of the homozygous forms can be driven to a high frequency. Underdominance was proposed as a method of controlling sheep blowfly in Australia several decades ago (Whitten, 1971). Current approaches for establishing underdominance have been achieved by RNA interference in *Drosophila melanogaster* to suppress an endogenous gene (Reeves et al., 2014).

## MATERNAL-EFFECT DOMINANT EMBRYONIC ARREST

Maternal-effect dominant embryonic arrest (*Medea*) can be used to suppress a population by targeting and silencing a maternal gene necessary for embryonic development. This was first discovered in a flour beetle and causes death in any offspring that lack the *Medea*-bearing chromosome (Beeman et al., 1992), allowing the *Medea* element to spread.

## CYTOPLASMIC INCOMPATIBILITY

*Wolbachia* are bacteria that manipulate the reproduction of a diverse range of arthropod hosts to their own advantage (Sinkins & Gould, 2006). They are a common intracellular microbe which can generate a gene drive in infected host individuals by triggering incompatibility between eggs and sperm or by male killing. They are maternally inherited and change the population dynamics to favour infected females. A rescue function allows eggs from infected females to develop normally when mated to infected males. Current research trials on release of mosquitoes which carry *Wolbachia* have focused on preventing the spread of viruses such as Zika and dengue whose transmission is suppressed by *Wolbachia*. However these bacteria could also be used to potentially spread genes engineered into *Wolbachia* or other maternally transmitted factors such as mitochondria.

## CYTOPLASMIC MALE STERILITY

Cytoplasmic male sterility is another form of non-Mendelian inheritance (Laughnan & Gabay-Laughnan, 1983). This condition is widespread among higher plants and results in a plant unable to produce functional pollen, i.e. male sterile, due to a sterility inducing mitochondrial gene which is maternally inherited. This is used extensively in agriculture to generate hybrid seed, these seeds usually result in larger, more vigorous plants.

# APPENDIX 2: POTENTIAL GENE DRIVE APPLICATIONS

## DISEASE

A gene drive could be used to reduce mosquito populations to help reduce the spread of diseases. Advances in gene editing techniques have led researchers to develop a CRISPR/Cas9 gene drive targeting a female sterility gene. This would lead to more male offspring than females and over multiple generations reduce *Anopheles gambiae* populations to a level where disease transmission of malaria is limited (Hammond et al., 2016). Although malaria is not an issue in Australia, we do experience other human viral diseases spread by mosquitoes, such as dengue and Ross River fever. Another approach is using *Wolbachia*, a bacterium which infects mosquitoes, to reduce transmission by *Aedes aegypti* populations in north Queensland, which is the main vector of dengue (Hoffmann et al., 2011).

## INVASIVE SPECIES AND THE ENVIRONMENT

A gene drive could be used to reduce the population of the non-indigenous mouse species *Mus musculus* on islands around the world, or specific to Australia, to reduce the population of black rats on Lord Howe Island. Introduced rodents can negatively affect an islands ecosystem by competing with native species and by destroying their habitats. Current efforts to eradicate invasive rodents have disadvantages including using toxic chemicals which can damage the environment or mechanical traps which don't discriminate between introduced or native species. A gene drive targeting a sex determining gene, *Sry*, to produce more male offspring than females could lead to a reduced population of mice after several generations (Cocquet et al., 2012).

Cane toads were first introduced to Australia in 1935 as an attempt to biologically control cane beetles which damaged sugarcane crops. Since their release in north Queensland the cane toad has spread and caused the decline of many native species. The skin of the cane toad is toxic and has poisonous glands across its back and the tadpoles are highly toxic if ingested. These toxic defences have poisoned many native Australian animals. A gene drive could detoxify the cane toad to reduce the detrimental effects of this invasive species or could control the population of cane toads directly. The cane toad is the only toad species in Australia, so a targeted gene drive could be specific to just the cane toad and not affect native frog species.

Another invasive species in Australia is the European carp. It was introduced over 100 years ago and has colonised many waterways throughout Australia causing major environmental impacts. Carp now dominate many river systems and reduce water quality, increase erosion, spread diseases and reduce native fish numbers. A gene drive to reduce the number of females and create an all-male population would be one mechanism to eradicate the European carp.

Rabbits are a classic example of an invasive, destructive species. Rabbits were introduced to Australia in 1859 for hunting but have since caused extensive damage, competing with livestock for grazing, spreading weeds, accelerating erosion and reducing biodiversity. It is estimated that rabbits cause A$200 million per year of economic damage.[8] Efforts to control rabbit populations have had mixed success in the past, namely through biocontrol programs using viruses including Myxomatosis and calicivirus. However resistance has developed in some Australian rabbits meaning the rabbit population is again on the rise. A gene drive to reduce rabbit numbers would be highly beneficial for Australian farmers and our environment.

## AGRICULTURE

Gene drive systems hold a lot of promise in controlling agricultural invertebrate pests such as fruit flies, moth pests, thrips and mites. These pests tend to have short generation times and have often become problematical to control due to the evolution of resistance to widely-used pesticides such as pyrethroids and organophosphates.

Gene drive systems may also help deal with weed issues. For instance, *Echinochloa colona*, also known as barnyard grass or jungle rice, is a damaging weed for agricultural production in Australia. It particularly affects rice, sugarcane, maize, sorghum and summer fallow crops and since 2007 several populations have developed glyphosate resistance (Thai et al., 2012). Glyphosate is a herbicide commonly used to control weeds. The production of herbicide resistant crops have dramatically changed weed control practices. However after decades of herbicide use weeds are developing resistance, reducing the efficacy of glyphosate for weed control. A gene drive to reverse herbicide resistance would be valuable especially for Australian cotton farmers.

**8** www.csiro.au/en/Research/BF/Areas/Managing-the-impacts-of-invasive-species/Biological-control/Controlling-those-pesky-rabbits