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The use of gene editing to create gene drives for pest control in New Zealand

Royal Society Te Apārangi Gene Editing Panel

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Background

The revolution in gene editing technologies is making it easier to change genetic material with huge potential benefits in many sectors including healthcare, agriculture and conservation. However, the technology to carry out gene editing and the ideas about how it might be applied are, in many cases, moving well ahead of the knowledge about how to safely effect the desired changes. For example, in conservation applications, gene editing could be used to make a native species resistant to disease, but this might accidentally make it more susceptible to drought.

As a technology, gene editing is rapidly moving ahead of any consensus on the rights and wrongs of how it should be used. So to explore the implications of gene editing technology for New Zealand, the Royal Society Te Apārangi has convened a multidisciplinary panel of some of New Zealand's leading experts to consider the social, cultural, legal and economic implications of revolutionary gene-editing technologies for New Zealand to:

- Raise awareness of the scientific possibilities and associated public issues of new gene-editing technologies to inform debate
- Provide information and guidance for policy makers to address current and new issues needing to be clarified or resolved
- Show where gene-editing applications are covered by established policies and regulations and where changes are needed
- Provide a New Zealand perspective to the global discussion on this technology and identify where global consensus is important

This paper is one of a series[1] considering the implications of the technology in health, pest control, and agricultural situations, and is accompanied by a companion discussion paper inviting public feedback, and a fact sheet on how these technologies work and are being used and applied [1].

To help consider the implications for pest control in New Zealand, this paper[2] highlights three scenarios which raise specific considerations for three different types of pest. In particular, these case studies consider:

- The range of scientific complexities of developing a gene drive for different organisms
- The implications for the spread of animals with the gene drive to different countries.

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Introduction

The last two decades have seen a substantial increase in our knowledge and ability in genetics. Researchers have now developed tools, chief among them being CRISPR[3], to enable the manipulation of specific genes within an organism's genetic material with greater and greater precision in the modification process, and fewer and fewer unintended changes elsewhere in the genome (see box 1). With their wide availability and simplicity, these gene-editing technologies are now being used to significantly accelerate research, and offer new treatments for a range of genetic diseases, while new agricultural products are beginning to be commercialized. However, alongside the development of the technology, the concept of genetic engineering, or genetic modification, has raised ethical and values-based questions in many societies.

> **Box 1: Gene editing with CRISPR**
> Bacteria possess an immune system that recognises invading viral DNA and cuts it up, making the invading virus DNA inactive. This type of immune system is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) [3]. In 2012, it was discovered that by modifying this mechanism, it was possible to target and cut any DNA sequence and edit genomes [4]. Cells which have their DNA cut by CRISPR, will repair these cuts as 'instructed' if specific DNA repair information is provided. By altering this repair information, it is possible to change a gene of interest, for example, from one that causes disease to one that does not [5, 6].

Modern advances in gene-editing technologies now provide potential novel solutions for the challenges of pest control through the development of gene drives [7-10]. Much of the research on gene editing of pests published to date has concentrated on species that cause human diseases [11-15]. However as researchers begin to understand and consider the use of gene-editing techniques in pest control, more and more species are being considered as potential targets, from agricultural pests [16] to unwanted predators.

New Zealand has unique requirements when it comes to pest control [17]. New Zealand's natural and agricultural environments are beset with pest species, imported deliberately or accidentally. Pests range from mammalian omnivores such as the Brushtail Possum [18-21], that impact our native birds and their food sources, through to a wide assortment of predators such as rats, cats, stoats and ferrets, and insect predators such as *vespulid* wasps [22]. Weeds increasingly impact our ecosystem structure and integrity [23] and the recent discovery of the fungal disease myrtle rust threatens many native and valued plant species. Our marine and freshwater ecosystems are also threatened by pests such as sea squirts [24], koi carp [25] and invasive algae [26]. Our agricultural production ecosystems are threatened by crop and pasture pests such as leafroller moths and Argentine stem weevil [17], and weeds such as ragwort and dock. New Zealand also actively maintains a biosecurity cordon to inhibit the colonisation of our islands from new pest threats. Major biosecurity threats from pests include fruit flies (e.g. Queensland fruit fly and the Mediterranean fruitfly), the brown marmorated stink bug and lymantrid moths such as the gypsy moth.

Within our native ecosystems, intensive poisoning and trapping has been undertaken for many mammalian pests. As a result of their control, it is now known that these ecosystems rebound well after key pest suppression and removal [27-30]. In many places in New Zealand, including offshore islands [28, 29], isolatable peninsulas and predator-proofed ecosanctuaries, predators have been eradicated. The benefits of control to native wildlife have been immense, even extending outside

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[3] CRISPR in this paper is being used to refer to the CRSIPR-Cas9 gene editing technique.

such sanctuaries. The Zealandia ecosanctuary in Wellington has increased native bird life in the surrounding city to the point that a rare native parrot, the kākā, is considered by some to be becoming a local pest species itself [31]. New Zealand agencies have cleared many offshore islands of pests, including the removal of Norway rats from the 11,000 hectares of Campbell Island. New Zealand's expertise in this area is well recognised internationally [32].

New Zealand has led the way internationally in pest management, incorporating significant biological control. However, ongoing improvement in existing pest management methodologies and novel approaches are required as various classes of pesticides are being withdrawn for ecological and public health reasons [17]. While classical biocontrol has had success in pastoral ecosystems, there now appear to be emerging issues around possible pest resistance and limitation of further opportunities based on regulatory and social requirements [17].

New Zealanders understand the risks they face from invasive species, both economically and environmentally. To achieve significantly reduced impacts, greater diversity will be needed in available management tools. This has been accentuated by the recently announced goal to make New Zealand predator-free by 2050, with a focus on mammalian pests in natural ecosystems where the challenge is to achieve landscape level eradication. New Zealand is already at the forefront of developing new pesticides, trapping technologies, and biological control technologies, as well as using Trojan females and sterile insect techniques [33-35] (described below). A gene edited gene drive may offer a further opportunity to expand our arsenal for pest control in New Zealand, although the development of gene drives is still very much in its infancy, and possible implementation of a gene drive approach in New Zealand is still a long way off.

What are gene drives?

CRISPR gene editing can be used to create a 'gene drive' to spread a gene rapidly through generations. In sexual reproduction, one set of chromosomes is provided from each parent and combined in their offspring. If one set of chromosomes contains a 'gene drive' it will cut the partner chromosome that lacks the gene drive and copy itself onto this chromosome. In this way gene drives are a genetic system with the ability to 'drive' themselves and nearby genes through populations of organisms over many generations [1]. For example, in normal sexual reproduction, offspring inherit two versions of every gene, one from each parent. Each parent carries two versions of the gene as well, so chance (50:50) normally governs which particular variant of the gene will be passed on. But 'gene drives' ensure that a certain gene will almost always be passed on, allowing that variant to spread rapidly through a population (see Figure 1). In this way it would be possible, for example, to spread a gene that suppresses fertility in females in a pest species population.

Figure 1. Example of a gene drive in a mosquito population [1]
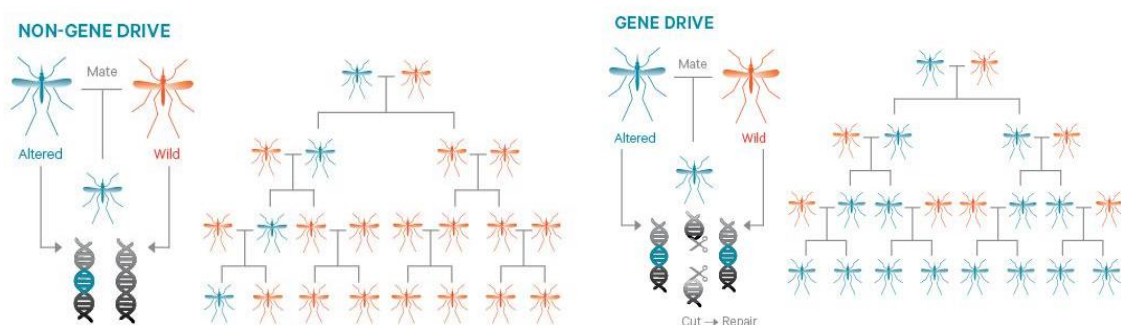
The science behind gene drives

In 2003, Austin Burt described how gene drive systems could be an efficient means for population suppression of pest insects [36]. A gene drive is a gene which creates an enzyme which cuts both strands of DNA within a targeted area of the genome and is copied across because of a DNA repair system. This DNA repair system is a 'rescue process', whereby an organism with a double-stranded break in its DNA will try and repair that break by copying any similar sequence it can find in the cell [37]. In the case of the gene drive cut, this leads to the gene drive being copied into the gap made by the gene drive itself. This then leads to guaranteed inheritance of the gene drive to all offspring and is the basis for the gene drive mechanism [36]. To be useful for population suppression, the targeted area for the gene drive should be within a gene essential for viability or fertility of the pest insect. Modelling has shown that suppression is particularly efficient if the gene drive is targeted to a gene essential for females but not males, or a gene required for germ-cell development or reproduction in one sex [36, 38].

The implementation of this system in the past has been hampered by the difficulty in modifying the gene drive to recognize a specified site within a specific genome [39] using previous genetic modification technologies. However, the advent of CRISPR technologies [40] has given new life to the gene drive idea. CRISPR makes use of a bacterial system that allows cells to cut invasive DNA that has been encountered previously [41]. The system consists of a cutting enzyme that can be targeted to any sequence using a small RNA sequence, called a guide RNA [41]. The combination of the DNA cutting enzyme and specific guide RNA that guides the enzyme to a particular sequence, provides the technology to cut and target the sequence required [42, 43]. In bacteria, the guide sequence is derived from an invading virus or other organism. However, the guide sequence can be almost any sequence at all. Using a guide RNA to target a specific sequence in a pest genome, a gene drive mechanism using CRISPR is easily able to target and modify recognition sites [44].

To illustrate a gene drive system, consider the situation of a release of a few genetically modified insects that carry a dominant fluorescent protein marker gene. All the offspring from mating between the fluorescent genetically modified insects and wild type (non-fluorescent) insects will be fluorescent as the fluorescence gene is a dominant one. Most likely these insects will mate with the numerous wildtype insects in the environment. From these matings, in the absence of a gene-drive, only half of the offspring will show fluorescence because of normal patterns of inheritance. In the following generation, even fewer of the population will show fluorescence because crossing with non-fluorescent wild type insects again only result in half the offspring carrying the fluorescence gene (represented in Figure 1). Now consider a release of a few insects carrying the fluorescent protein marker gene linked to a gene drive. As for the original non-gene-drive release, all the offspring from matings with wild type insects will be fluorescent as they will carry the dominant fluorescence gene. In the genome of this first generation, the gene drive will cause a cut in the chromosome that does <u>not</u> contain the fluorescence gene and the insertion of a copy of the gene drive with the fluorescence gene. This repair process is likely to be near 100% efficient; all the gametes will contain a chromosome with the gene drive and the linked fluorescence gene. Thus, when the first-generation insects mate with wild type insects, all the offspring in this second generation will also be fluorescent. Further generations will continue to lead to the marker gene being driven into all offspring (see Figure 1).

Assuming that carrying the gene drive and marker gene have no negative effects on the animal's reproductive fitness in being able to pass its genes to the next generation, a 1% release could theoretically lead to 99% of the local population carrying the marker gene after just 9 generations [36, 38]. For population suppression, the gene drive would alter an essential gene, perhaps a gene essential for, for example, female development or fertility [36].

Scenarios for the use of gene drives for pest control in New Zealand

In view of the challenges around economically sustainable, effective nationwide pest eradication, the potential of genetic technologies, such as gene drive systems could be evaluated. In this review, a series of scenarios is used to examine the potential from such approaches for the control of three key pests in New Zealand. All three scenarios, outlined in Table 1, are discussed in terms of the pest control opportunities they present, along with technical, social and legal ramifications.

Table 1. Three gene edited gene drive scenarios for pest control in New Zealand

	Scenario 1: Insect	*Scenario 2: Possums*	*Scenario 3: Stoats & Rats*
Species	*Vespine wasps, Argentine stem weevil, Australian sheep blowfly*	*Brushtail possums*	*Stoats & rats*
Aim	*Eradication*	*Eradication*	*Eradication*
Justification: Conservation, Agriculture or other	*Conservation & Agriculture. Wasps attack native birds and insects and deplete critical food resources.*	*Conservation & Agriculture: Predator on native birds & invertebrates, eats native plants, carrier for bovine TB*	*Conservation & Agriculture: Predator on native birds & invertebrates, eats native plants, carrier for diseases*
Genetic target	*Fertility or sex ratio*	*Fertility*	*Fertility or sex ratio*
Nature of gene editing	Inactivation of gene	Inactivation of gene? (not yet known)	Insertion of new gene? (not yet known)
Affects target individuals or passed on to future generations	Passed on to future generations	Passed on to future generations	Passed on to future generations
Method of transmission of CRISPR gene edit: Virus, bacteria, compound, other.	Direct injection into embryo	Direct injection into egg cell	Direct injection into egg cell
Are non-naturally arising genes introduced into the genome?	No	No	No

Scenario 1: Insect pests in New Zealand

Invasive wasps

Environmental rationale for control

Two colony-living social wasp species in the genus *Vespula* were accidentally imported into New Zealand and became established here. These colony-living wasps are different from the many solitary species of wasps native to New Zealand, which have evolved here with other insects and plants over thousands of years, and have never been considered a nuisance. The common wasp (*V. vulgaris* (L.)), however, was first recorded from New Zealand in 1921 and became abundant in the 1970s [22]. The German wasp, *V. germanica* (F.), became widespread and abundant in New Zealand after an incursion in 1945 [45]. These Vespine wasps are both now distributed throughout New Zealand, with the common wasp as the dominant social wasp in beech forests [46]. They are especially abundant wherever there are large quantities of honeydew produced by scale insects. This honeydew provides considerable carbohydrate food resources and is plentiful in approximately a million hectares of native beech forest [47]. The world's highest recorded *Vepsula* densities are observed in New Zealand, with up to 40 nests per hectare [48] and numbers exceeding 370 wasps per square metre of tree trunk [49]. The biomass of *Vespula* in honeydew beech forests has been estimated as similar to, or greater than, the combined biomasses of birds, rodents and stoats [50].

The extreme abundance and effects of both these wasps has resulted in them being listed amongst '100 of the World's Worst Invasive Alien Species' [51]and as a 'critical issue' for New Zealand entomology [52]. Their large densities exert intense predation pressure on native invertebrates. For example, vulnerable species of native caterpillars were observed to have almost no chance of surviving to become adults during times of peak wasp population densities [53]. Similarly, the probability of an orb web spider surviving until the end of a wasp season is

effectively nil [54]. They are strong competitors with native predators [55], and these competitive effects over a short evolutionary period may have even altered the morphology of native species [56].

Economically, a recent analysis suggested these wasps annually cost approximately $133 million to the New Zealand economy [57]. The direct economic impacts of wasps are largely associated with their predation on bees, with flow-on effects associated with impacts on pollination (in 2015 approximately 20% of beehive losses in the North Island were due to wasp attack [58]). This economic review also suggested wasps have substantial impacts on animal health, forestry, arable farming, horticulture, tourism, human health and even traffic crashes [57]. Wasps are one of the most dangerous and lethal animals for humans, and they periodically kill New Zealanders; approximately 1,300 people per year are estimated to seek medical attention as a result of wasp stings throughout New Zealand [59, 60].

Current control options
Effective wasp control options are currently limited to small-scale operations involving pesticides or other chemicals (e.g. petrol). These pesticides may be effective on relatively small scales but the use of toxins over large areas such as the 1 million hectares of beech forest currently overwhelmed with high wasp numbers is impractical. Prior attempts at self-sustaining options that would be suitable for such large area, such as biological control, have been unsuccessful [46, 61].

Potential future approaches
A variety of additional and 'next-generation' pest control approaches have been proposed and are being developed for wasps, funded through New Zealand's Biological Heritage National Science Challenge. These approaches include the use of the Trojan female technique, which utilizes the release of females with naturally occurring mitochondrial DNA defects that cause male infertility, and is seen as a novel and humane approach for pest population control [33]. Other approaches in the National Science Challenge include gene silencing[4] technologies, the use of pheromones for mating disruption, which require annual replacement and use at each site, or biological control options [59]. These can all form part of a 'toolbox' approach that can be used in combination. The individual limitations of each approach highlight the need to expand the 'toolbox' to discover and refine new technologies based on a good biological understanding [17].

Another potential approach is the sterile insect technique, which involves the release of large numbers of sterile insects that mate with an established insect population, leading to an effective reduction in that population. In these techniques, some of which use genetic modification to create the sterile insects, a huge number of insects must be released to ensure that matings with sterile insects are more common than those between unmodified fertile insects. The sterile insect technique has been an effective approach for eliminating screw-worm, medfly and the Mexican fruit fly [62], and has recently been used to control mosquito populations in Brazil [63]. This technology has not been used broadly in New Zealand [64], perhaps because of the large number of insects needed for release, and the large cost associated with their production. In addition, social insects have only one reproductive individual per colony and so the impact for wasps of introducing a large number of sterile males in the region is uncertain.

Technical/scientific considerations of gene drives
The development of a gene drive system in wasps using CRISPR faces a number of challenges. Current gene drive methods would require genetic modification of the Common or German wasp genome, a technology not previously developed. Genetic modification of honeybees [65, 66] using CRISPR based approaches has been carried out, and given the similarities of social wasps and bees, it seems likely that this technical barrier will be able to be overcome. In both cases, microinjection of honeybee eggs or larvae was required to achieve transformation [65, 66]. Some understanding of the basic biology of wasp embryos will also be required for transformation to be achieved.

Another set of barriers to the development of gene drives in wasps is the nature of wasp genetics and their social organisation. Vespine wasps genetically are quite unlike other pest species already targeted by gene drive systems. These wasps, like many wasp species, have haplodiploid sex determining systems, meaning males are haploid (have one copy of their genome) and females are diploid (have two copies). Males develop, like clones, from unfertilised eggs laid by the queen. The alternative haploid and diploid generations may have significant, unknown consequences for the inheritance of a gene drive system.

The social organisation of the wasp hive, with a single queen and non-reproductive workers is also a critical factor in the development of gene-drive for these species. Rather than the approach used in mosquitoes of trying to spread a

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[4]A gene silencing pesticide uses double stranded RNA to prevent the operation of targeted genes, and is applied as a pesticide.

gene drive that damages reproductive fitness in a population [11, 14], a gene drive system might fail if queens made defective by a gene drive system do not spread their genes, ensuring the gene drive will be rapidly removed from the population with little pest-control benefit.

Containing complex eusocial insect species (i.e. those with different worker castes, overlapping generations, and cooperative care for their young) is challenging and so it seems likely that computer modelling will be required to assess the potential impact of a gene drive system in a vespine wasp species, and to determine the optimum efficiency of a gene-drive approach in achieving wasp extinction. Computer modelling will also be required to understand how many modified wasps might need to be released, and where, to have the most significant effect.

International considerations
While *Vespula* wasps in New Zealand are a critical pest, in their native European range they are valued and important components of the ecosystem. Social wasps were not introduced deliberately to New Zealand, but have hitchhiked here [45], presumably in import cargo. Given this route of introduction, the use of any gene drive system must take into account the possibility that modified wasps might be transported to regions where these wasps are valued. While New Zealand would greatly benefit from eradication of these pests, their extinction here must not mean global extinction of the entire species.

Regulatory considerations
Genetically modified animals are defined as new organisms under the HSNO Act, and therefore wasps containing gene drive systems would be classified as 'new organisms'. Risk assessments of organisms produced through gene drive systems would be carried out under the provisions of the HSNO Act on a case-by-case basis by the Environmental Protection Authority. Importation of wasps with gene drives would also be regulated under the Biosecurity Act 1993.

Other possible insects of focus

Argentine Stem Weevil
Arthropod pests include such species as the Argentine Stem Weevil (*Listronotus bonariensis*). The Argentine Stem Weevil is native to Brazil, Uruguay, Argentina, Bolivia and Chile, and is a pernicious pest of pasture grasses that costs NZ up to $250 million p.a. [67]. Biocontrol combined with endophyte-based plant resistance[5] has kept the pest in check [68], but the effectiveness of the biocontrol agent (the parasitoid wasp *Mictroctonus hyperodae)* is decreasing, probably though genetic resistance arising from continual selection pressure [69, 70]. This is a critical problem, as it is possible that the full cost of the Argentine Stem Weevil may fall on New Zealand's pastoral industries. Thus, there is good reason to consider the use of genetic technologies.

Australian sheep blowfly
Despite its name the Australian sheep blowfly is native to Africa and North America. The blowfly causes large lesions on sheep and, left untreated, can prove fatal to the animal. It has huge animal welfare implications in NZ and Australia. The Australian blowfly is expected to have an increasing impact, both in incidence and in geographical spread, as a result of climate change. In contrast to wasps and weevils, development of a gene drive for genetic control of the Australian sheep blowfly *Lucilia cuprina* should be relatively straightforward. This is because the technology for germline (or hereditary) modification has already been developed [71, 72]. The technology, first developed in New Zealand, has since been adapted to the New World Screwworm, a blowfly that is a major pest of livestock in the Americas [73]. Further, the *transformer* gene has been shown to be essential for female but not male development [74] and thus would be a good target for a gene drive. Genetically modified strains of *L. cuprina* have been developed that produce only males, which could be used for a genetic control program [75, 76]. However, these strains have not been adopted by the sheep industry in New Zealand or Australia because of the rearing and distribution costs of their use in an eradication campaign, and the perceived difficulty in obtaining regulatory approval. A gene drive for population suppression would be much more economical as at least 100 fold fewer flies would need to be released [77].

New pests
Important arthropod incursion threats exist overseas that are still not present in New Zealand, but which could arrive. Species such as the Queensland fruit fly (*Bactrocera tryoni*), the brown marmorated stink bug (*Halyomorpha halys*),

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[5] An endophyte is a bacterium or fungus that lives with in a plant without causing disease. These endophytes can enhance resistance of host plant against insect herbivores by production of defensive compounds in the plant

and the glassy winged sharp shooter (*Homalodisca vitripennis*), would have major impacts on our predominantly agricultural economy if they became established here, attacking grapes, kiwifruit, apples, citrus and stone fruit, corn and many other valuable crops. Gene drives, because of the research needed to develop them, are unlikely to be useful as first-responses to a biosecurity incursion, but given that many pest species present biosecurity risks overseas, it may be possible in the future to utilise a gene drive developed for control elsewhere. For example, gene drive systems are being developed for spotted wing *Drosophila*, a fruit fly that is a major invasive pest of soft skinned fruits such as blueberries[6].

Scenario 2: The brushtail possum

Environmental rationale for control
Perhaps New Zealand's most significant mammalian pest is the brushtail possum (*Trichosurus vulpecula*). This marsupial was first brought to New Zealand from Australia with the aim of establishing a fur industry in 1837 [78]. The possum, as it is known in New Zealand, found an environment with few of the challenges of Australia and grew to plague proportions in New Zealand forests. Along with eating native trees [20] and predating native birds [79] and invertebrates [80], the possum is also a carrier for bovine tuberculosis [81], and thus possum control is carried out for conservation and agricultural purposes. It is indeed this latter problem that has driven most of the current program of possum control in New Zealand. The ecology of possums in New Zealand is also well known, and has fed into computer models for exploring possum population dynamics under different control scenarios [82]. Consequently, it is possible to model the impacts of a gene drive in controlling possum populations in New Zealand.

Current control options
Possum control costs the New Zealand government approximately $110 million /year [83], much of which is spent on aerial distribution of poison baits. Other approaches, such as traps and bait stations, are also used. These technologies are effective when animals are at high densities but become less effective as densities drop [84]. Gene drives and other genetic solutions may provide an opportunity to add to the 'toolbox' of approaches to achieve national eradication.

Technical/scientific considerations of gene drives
Although valued in their native range in Australia, possums are a pest unique to New Zealand and, as such, little work has gone into the development of novel methods of possum control beyond our shores. Over about twenty years, major projects were run in New Zealand, focused on establishing immunocontraception as a tool for possum control, which uses an animal's immune system to prevent it from fertilizing offspring [85]. While these projects were ultimately wound up, they did provide knowledge of possum reproduction and genetics [21] that may be useful in the era of gene editing and gene drives.

One key barrier that needs to be solved in possums, and is necessary for a gene drive, is the ability to genetically modify the organism, a feat never achieved in a marsupial. To do so would require the generation of reasonable quantities (100-1000s) of oocytes. Techniques for superovulation, and implanting embryos [86, 87] into possums have been developed as part of a reproductive control approach to possums [88], and could be used to generate oocytes for manipulation.

If genetic modification of possums is possible, there will be a need to identify what genes or processes should be targeted for a gene drive system. In comparison to the mouse, little is known about functional genetics in marsupials, mainly due to the lack of a well-established model system. Several marsupial genomes have been sequenced [89], providing a resource for further genetic work, but understanding the function of marsupial genes is only making slow progress. Some potential vulnerabilities are known, particularly around reproduction, milk production, and water balance, but there is still a lot of work to do to determine the viability of such targets.

With no well-established marsupial model system, the best option may be to adapt gene drives developed in mice that target genes or processes that are similar in possums. To this end, sequencing the possum genome, now underway as part of the Biological Heritage National Science challenge, is an important and necessary first step in developing a potential gene drive.

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[6] https://swdmanagement.org/

The use of possums with gene drives to control wild possum populations would require very large numbers of altered animals to be bred and released (1-10% of the wild population). Taking an average density of around one possum per hectare [90, 91], it would require a quarter of a million altered possums to be distributed throughout the country for a 1% release. This would involve successfully putting one altered possum into every 100 ha, including rugged back country.

International considerations
One area of concern is around the control and containment of a possum gene drive. As envisaged the gene drive would be specific to possums, likely targeted to a specific vulnerability such as fertility, with the only organisms affected being the offspring of those possums that mate with a possum possessing the gene drive. The spread of the gene drive would occur through the possum population as large numbers of gene drive possums were distributed throughout the country, and the possums disperse. This would be effective for the goal of widespread control and eradication in New Zealand. However, there would likely be an issue for Australia if a gene drive possum were to find its way or be deliberately released there, because in Australia brushtail possums are a protected species and an important part of many Australian ecosystems. While the likelihood of release may be extremely small, even the prospect of such an incident suggests the need for a means to turn off a gene drive. Currently, the only mechanism available to deliberately switch off a gene drive is to use another gene drive. However, recent work suggests that, over time, evolution will work to thwart gene drives (see below), so the issue of rare escapees may be less of an issue than anticipated. Nonetheless mechanisms to switch off a gene drive need to be thoroughly explored.

Regulatory considerations
As for wasps, genetically modified possums are defined as new organisms under the HSNO Act, and therefore possums containing gene drive systems would be classified as 'new organisms'. As with wasps, risk assessments of organisms produced through gene drive systems are carried out under the provisions of the HSNO Act on a case-by-case basis by the Environmental Protection Authority. In addition, the Animal Welfare Amendment Act (No 2) 2015 has amended the meaning of *manipulation* and includes reference to *genetic modification* and *killing*. The implications of this Act for this scenario are unclear for its use in pest management/control/ eradication, as 'genetic modification' and 'biological product' are not defined in the Animal Welfare Act.

Scenario 3: Rodents and stoats

Environmental rationale for control
Environmental rationale for control like possums, stoats are a predominantly New Zealand problem, with the Orkney and Shetland Islands the only other place on the globe that shares the problem of invasive stoats [92]. Stoats (*Mustela erminea)* are ferocious predators that do significant damage to many of our native bird populations and have contributed to the extinction of five native species [93]. Rats are also a very serious pest problem. In New Zealand there are three rat species: the ship or common rat (*Rattus rattus*), the Norway or brown rat (*Rattus norvegicus*) and the Polynesian rat or kiore (*Rattus exulans*). Of the three, the ship rat is of greatest conservation concern, but all prey on native species [94].

Current control options
These pests in New Zealand are currently controlled in many different ways depending on the target species, including the widespread use of biodegradable 1080 poison (sodium fluoroacetate), a naturally occurring metabolic poison most effective against mammalian pests [95]. 1080 is a cost-effective and safe pest control tool [96], especially when distributed by air in rugged, heavily forested terrain where trapping is not viable. However, its use remains controversial in some sections of the community [97]. Other pest control measures include innovative new approaches to trapping, including the development of self-resetting traps [98]. Technologies for identification of pests, and targeted removal have also improved [99] and many of these technologies are now available to the general public.

Current pest control measures, as demonstrated by the removal of pests from large offshore islands, are effective; but they are relatively expensive and take a lot of planning [17, 100]. Given the alternatives of a broad-range poison dropped from the air, and expensive and intensive trapping campaigns, gene-drive solutions could provide another avenue for pest control [44].

Technical/scientific considerations of gene drives
While New Zealand researchers have spent decades understanding the ecology, reproduction and, more recently, the genetics of possums, researchers are less well informed about many of these key issues for stoats [101]. One

potentially promising avenue to explore is to harness the significant efforts made in understanding the reproduction and genetics of mink, a related species valued for its fur that is farmed in parts of the Northern hemisphere [102, 103].

Unlike possums and stoats, rats are global pests that are implicated in food spoilage, the spread of diseases of global concern e.g. bubonic plague, and are a key conservation threat around the globe [104]. Thus, New Zealand might not have to solve the problem alone and active efforts are underway to tap into international initiatives now aimed at establishing gene drives for the control of invasive rodents [105]. Rats are also among the best-studied mammals, so there is no shortage of knowledge on reproduction or genomics, although most of this knowledge comes from the Norway rat, a well-established lab model that was among the first mammal to have its complete genome sequenced [106]. Less is known about the ship rat, although it has just had its genome sequenced by a New Zealand team, as a legacy project from the Allan Wilson Centre, which should provide an important stepping stone towards the challenge of establishing a gene drive for rats [107].

While establishing gene drives in rats will be less challenging than for stoats and possums, there are still significant practical barriers to establishing such a system. One of these is that rats are surprisingly hard to genetically manipulate [108]. Huge efforts have gone into solving this issue, with some progress made in recent years [109, 110]. However, this may be a major challenge to the use of gene drives for controlling rodents in New Zealand, and that mice (also a significant pest) might be the easiest species to target in the first instance.

Several international groups are looking to develop gene drive solutions for mice. One of the most advanced is a project that aims to link a sex determining factor to a naturally occurring gene drive to produce mice that produce predominantly male offspring [111]. While feasible in theory, there are multiple questions, as yet unanswered, that may thwart the efforts to use these in the wild to achieve population control [112]. For example, researchers do not yet know if the health, survival and reproductive success of mammalian species carrying such modifications might be impaired and how frequently mutations might arise in the gene drive or its cargo gene that could disable them. Robust modelling to explore the possibilities by which gene drives may fail, need to be undertaken in a similar way to those for insect systems [8].

As with possums, the use of gene drives to control wild populations of rodents and stoats would likely require the breeding and repeated release of very large numbers of altered animals over large areas.

International considerations
Globally, while rats are pests in many contexts, they are also important providers of ecosystem services e.g. pollination or critical elements of ecosystem food webs. Eradicating rats in New Zealand, where our ecosystems were free of rodents up until human arrival around 800 years ago, may have few knock-on effects. However, in other parts of the globe the effects on natural systems might be very different. Rats are very good invaders, disperse well, and hybridise with closely related species, making the accidental release and spread of gene drive modified rats a serious consideration. Stoats are less likely to be inadvertently spread, but they are an important animal in northern European ecosystems, so even the prospect of dispersal from New Zealand will mean the need for a means to turn off the gene drive.

Regulatory considerations
As for wasps and possums, genetically modified animals are defined as new organisms under the HSNO Act, and therefore stoats and rats containing gene drive systems would be classified as 'new organisms'. Risk assessment of organisms produced through gene drive systems are carried out under the provisions of the HSNO Act on a case-by-case basis by the Environmental Protection Authority. As with the use of a gene drive in possums, the implications of Animal Welfare Amendment Act (No 2) 2015 for this scenario are unclear for its use in pest management/control/eradication, as 'genetic modification', 'biological compound' and 'management' are not defined in the Animal Welfare Act.

Social considerations

Social license to operate
Relational trust and communication between the public, government, and scientists will need to be healthy for new genetic technologies to be accepted. The idea of releasing a genetically modified organism that leads to the extinction of a species speaks to the darkest fears expressed about GM technology. That leading conservationists have expressed

similar fears[7] only reinforces such concerns. The need to control invasive predators and pests is known, what is problematic is the way it is done and the unknown consequences on an ecosystem. While trapping and shooting are seen as acceptable by some, the use of poisons is more controversial, with protests about the use of 1080, in particular, occurring. In this environment, gene drive technologies might have a place because of their species specificity. Alongside this, there is jurisdiction under the Resource Management Act for local councils to control the use of genetically modified organisms via regional policy instruments[8] and there may be implications of this on the use of gene drive pest control techniques.

Māori cultural considerations

From a Māori perspective, there are concerns that genetic modification, including gene editing, is at odds with Māori tikanga, in that it may interfere with natural processes pertaining to whakapapa and violate the tapu of different species. Māori communities will need to be well informed about the implications, benefits and risks associated with gene editing in pest control. Education and consultation will be central to empowering whānau, communities, hapū and iwi to assess the social, moral, ethical and health considerations of gene editing within different contexts and scenarios.

For the three scenarios, in Māori terms, the ethical considerations relate to whakapapa (of the organism, as well as the relationship/kinship between humans and other species), tika (what is right or correct), manaakitanga (cultural and social responsibility/accountability, e.g. to other nations who value wasps), and mana (justice and equity) [113]. Other relevant Māori values include tapu (restrictions), tiakitanga (guardianship), and whānaungatanga (support of relatives). Implicit in those considerations would be the question of who stands to benefit from the introduction of a gene drive in this scenario; what are the risks to the ecosystems of other nations; and where do Māori accountabilities lie in terms of the outcomes [114]. In addition, broader impacts on Māori also need consideration, including any negative financial impacts on whānau that may arise, and the assurance of Māori participation in decision making regarding use of these technologies.

As part of this project, Māori perspectives and broader cultural contexts are being sought by the Panel in a parallel process.

New Zealand regulation of the use of genetic modification for pest control

Genetic modification in New Zealand, such as using gene editing on a pest to include a gene drive, is regulated primarily by central government through the Hazardous Substances and New Organisms Act (1996) (HSNO Act). Gene drives will be regulated by the HSNO Act if they come within the definition of an 'organism' and 'new organism' in this Act. 'Organism' is defined in the HSNO Act and includes a genetic structure (other than a human cell) that is capable of replicating itself, whether that structure comprises all or part of the entity[9]. The definition of 'new organism' includes genetically modified organisms (GMOs) and organisms belonging to species that were not present in New Zealand prior to July 1998[10]. The definition of a GMO is expressly defined in supporting regulations[11], but otherwise the HSNO Act defines GMOs as 'any organism in which any of the genes or other genetic material have been modified by in vitro techniques; or are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by in vitro techniques' (see Figure 2). The EPA can make a rapid assessment for 'low risk genetic modification'[12].

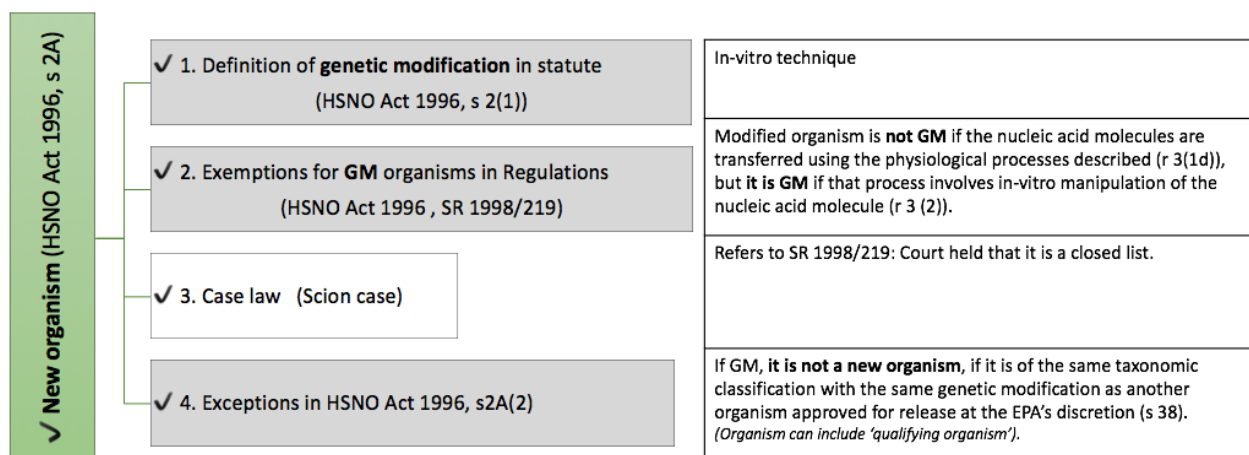[7] http://www.etcgroup.org/files/files/final_gene_drive_letter.pdf
[8] http://www.mfe.govt.nz/more/hazards/risks-new-organisms/what-are-new-organisms
[9] HSNO Act, s2(1)
[10] HSNO Act, s2A
[11] HNSO Act, SR 1998/219
[12] HSNO Act s 41(c) and SR 2003/152 r 4

Figure 2. Summary of the process determining a new organism according to the HSNO Act

New organism (HSNO Act 1996, s 2A)		
✔ 1. Definition of **genetic modification** in statute (HSNO Act 1996, s 2(1))	In-vitro technique
✔ 2. Exemptions for **GM** organisms in Regulations (HSNO Act 1996 , SR 1998/219)	Modified organism is **not GM** if the nucleic acid molecules are transferred using the physiological processes described (r 3(1d)), but **it is GM** if that process involves in-vitro manipulation of the nucleic acid molecule (r 3 (2)).
✔ 3. Case law (Scion case)	Refers to SR 1998/219: Court held that it is a closed list.
✔ 4. Exceptions in HSNO Act 1996, s2A(2)	If GM, **it is not a new organism**, if it is of the same taxonomic classification with the same genetic modification as another organism approved for release at the EPA's discretion (s 38). *(Organism can include 'qualifying organism').*

It is unlawful to import, develop, field-test and release any 'new organism' without approval from the Environmental Protection Authority. If there is uncertainty about whether an entity is a GMO (or even an 'organism' or 'new organism'), there is a formal determination the Environmental Protection Authority can undertake pursuant to the HSNO Act (s 26). The HSNO Act is enforced at the New Zealand border under section 28 of the Biosecurity Act 1993.

The case studies evaluated in this paper highlight a complicated regulatory framework with many 'grey' areas. The current regulatory framework may permit gene editing for pest control in containment and for release, as each application is assessed on a case by case basis. An application would need to be made to the Environmental Protection Authority (EPA) for approval under the HSNO Act for development and field testing in containment. Further applications would be required for release from containment, and controls may be imposed by the EPA. The HSNO Act further prescribes the mandatory assessment and decision-making process for applications, including a risk assessment of the new organism's effect on native species, biodiversity, and natural habitats[13]. The EPA will decline the application if the minimum standards cannot be met.

The following legislation and associated amendments require evaluation alongside the Hazardous Substances and New Organisms Act 1996, for pest control using gene editing technologies (See Figure 3):

● Animal Welfare Act 1999 and the Animal Welfare Amendment Act (No 2) 2015 (Animal Welfare Act)
● Agricultural Compounds and Veterinary Medicines Act 1997(ACVM Act)
● Biosecurity Act 1993 (Biosecurity Act) Conservation Act 1987 (Conservation Act)

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[13] HSNO Act, section 36. Minimum standards:
  The Authority shall decline the application, if the new organism is likely to—
  (a) cause any significant displacement of any native species within its natural habitat; or
  (b) cause any significant deterioration of natural habitats; or
  (c) cause any significant adverse effects on human health and safety; or
  (d) cause any significant adverse effect to New Zealand's inherent genetic diversity; or
  (e) cause disease, be parasitic, or become a vector for human, animal, or plant disease, unless the purpose of that importation or release is to import or release an organism to cause disease, be a parasite, or a vector for disease.
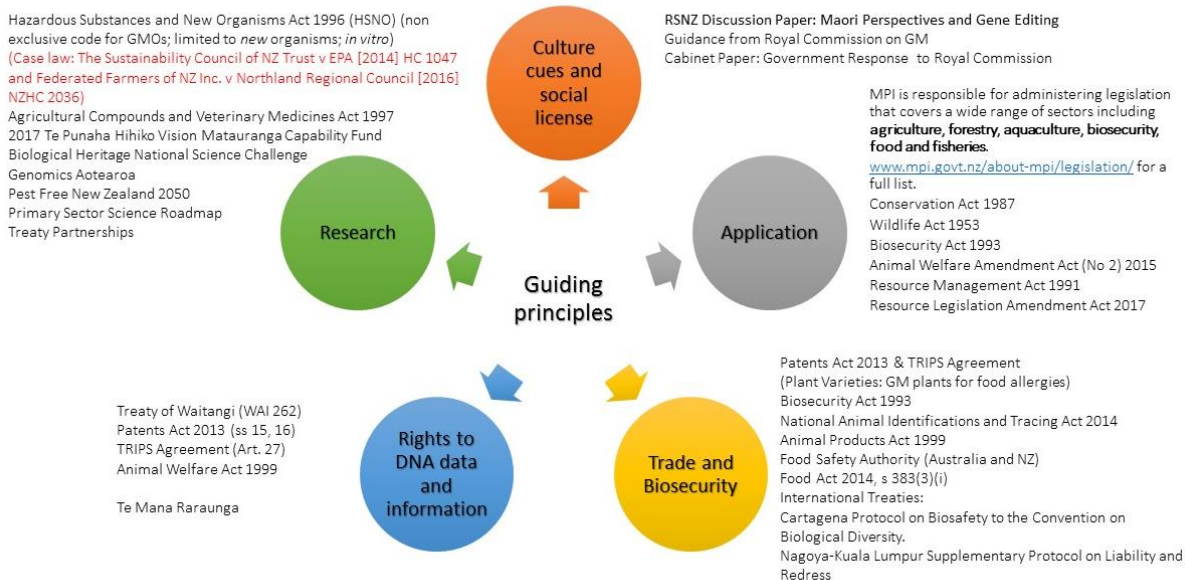HSNO Act, section 37. Additional matters to be considered:
  The Authority, when making a decision under section 38, shall have regard to—
  (a) the ability of the organism to establish an undesirable self-sustaining population; and
  (b) the ease with which the organism could be eradicated if it established an undesirable self-sustaining population.

Figure 3. New Zealand legislation influencing gene editing technologies in animals and organisms

NZ Regulation: Gene editing & gene drives in pest control & primary industries

Hazardous Substances and New Organisms Act 1996 (HSNO) (non exclusive code for GMOs; limited to *new* organisms; *in vitro*)
(Case law: The Sustainability Council of NZ Trust v EPA [2014] HC 1047 and Federated Farmers of NZ Inc. v Northland Regional Council [2016] NZHC 2036)
Agricultural Compounds and Veterinary Medicines Act 1997
2017 Te Punaha Hihiko Vision Matauranga Capability Fund
Biological Heritage National Science Challenge
Genomics Aotearoa
Pest Free New Zealand 2050
Primary Sector Science Roadmap
Treaty Partnerships

RSNZ Discussion Paper: Maori Perspectives and Gene Editing
Guidance from Royal Commission on GM
Cabinet Paper: Government Response to Royal Commission

MPI is responsible for administering legislation that covers a wide range of sectors including **agriculture, forestry, aquaculture, biosecurity, food and fisheries.**
www.mpi.govt.nz/about-mpi/legislation/ for a full list.
Conservation Act 1987
Wildlife Act 1953
Biosecurity Act 1993
Animal Welfare Amendment Act (No 2) 2015
Resource Management Act 1991
Resource Legislation Amendment Act 2017

Culture cues and social license

Research

Application

Guiding principles

Treaty of Waitangi (WAI 262)
Patents Act 2013 (ss 15, 16)
TRIPS Agreement (Art. 27)
Animal Welfare Act 1999

Te Mana Raraunga

Rights to DNA data and information

Trade and Biosecurity

Patents Act 2013 & TRIPS Agreement
(Plant Varieties: GM plants for food allergies)
Biosecurity Act 1993
National Animal Identifications and Tracing Act 2014
Animal Products Act 1999
Food Safety Authority (Australia and NZ)
Food Act 2014, s 383(3)(i)
International Treaties:
Cartagena Protocol on Biosafety to the Convention on Biological Diversity.
Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress

Regulatory process

There is, however, no clear regulatory framework for specifically evaluating gene drive technologies as a method for controlling pests

HSNO Act

The HSNO Act has been described as a comprehensive, strict and rigorous code [115] and additional amendments sought to increase restrictions following release of the organism, including reassessment (section 63), conditional release (section 38) and clarifying the meaning of genetically modified organism (Statutory Regulation 1998/219, r 3(ba)).

Regulation of genetically modified organism under the HSNO and Resource Management Acts have been challenged in the New Zealand courts. Most notable was the Scion case[14], which clarified the classification of gene edited organisms as 'genetically modified organisms' for the purposes of the HSNO Act[15]. The Northland Regional Council case clarified that Regional Councils control the use of genetic modification through their regional policies and district plans under the Resource Management Act[16]. Both of these cases have wide ranging implications for New Zealand and are not limited to genetically modified crops. Central government consequently amended regulations to clarify the exemptions to the HSNO Act (EPA, HSNO Act SR 1998/219). Central government has also amended legislation (Resource Legislation Amendment Act 2017) introducing a new regulation making power to prohibit or remove specified rules or types of rules by Territorial Authorities that would duplicate, overlap, or deal with the same subject matter that is included in other legislation. Rules that regulate the growing of GM crops do not apply[17].

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[14] *The Sustainability Council of New Zealand Trust v The Environmental Protection Authority* [2014] NZHC 1067.

[15] The High Court Judge ruled that the exemption list in the Regulations is a closed list. The conclusion was based on an interpretation of the language of the Regulation and that the regulations did not prescribe factors for the EPA to add other techniques to the list. The Judge interpreted the HSNO Act and the regulations as not implicitly giving the EPA discretionary power to add to the exemption list and ruled that the EPA could not expand the exemption list to include techniques similar to chemical mutagenesis and adding to the exemption list was a political decision, not an administrative decision**.**

[16] *Federated Farmers of New Zealand v Northland Regional Council* [2015] NZEnvC 89.

[17] Resource Legislation Amendment Act 2017, s 360D.

New Zealand has a network of legal instruments and treaties that require consideration alongside review of the HSNO Act, when introducing new biotechnologies. These include the Treaty of Waitangi[18] (the Waitangi Tribunal Report recommending that Māori have a greater interest in genetic modification[19]) and the Resource Management Act 1991 (the ability of regional councils to control the use of genetically modified organisms through regional policy statements or district plans). A recent amendment to the Resource Management Act has introduced a new provision that allows the prohibition or removal of certain rules that would duplicate, overlap with, or deal with the same subject matter that is included in other legislation[20].

Agricultural Compounds and Veterinary Medicines Act 1997 (ACVM Act)

In addition to the HSNO Act, the Agricultural Compounds and Veterinary Medicines Act 1997 (ACVM Act) has possibly the greatest effect on this technology. Depending on the interpretation of 'veterinary medicine', a gene drive intervention could be regulated under the ACVM Act and thereby assessed under the Conditional Release and Release statutory provisions in the HSNO Act (potentially bypassing the Containment provision).

A veterinary medicine, according to the ACVM Act (s 2(1)), means any substance, mixture of substances, or biological compound used or intended for use in the direct management of an animal.
- Note that direct management is not defined in the Act.

The HSNO Act defines a 'qualifying veterinary medicine' as a veterinary medicine that is, or contains, a new organism and meets the criteria set out in section 38I(3) of the HSNO Act.
- A new organism has the same meaning in the ACVM Act and in section 2A of the HSNO Act.
- A qualifying organism means a new organism that is or is contained in a qualifying veterinary medicine (HSNO Act, s 2(1)).

Royal Commission on Genetic Modification

The 2001 Royal Commission on Genetic Modification report concluded that, 'New Zealand should preserve its opportunities by allowing the development of genetic modification whilst minimising and managing the risks involved.' The Royal Commission's overall strategy was supported by the Government. However, the Government required that research practices adhere to strict safety guidelines, including secure containment, thereby limiting discretion when determining the conditions of the research. Government also required a precautionary approach to be exercised in the operation of the HSNO Act (s 7): '*All persons exercising functions, powers and duties under this Act including, but not limited to, functions, powers, duties under sections 28A, 29, 32, 38, 45, and 48, shall take into account the need for caution in managing adverse effects where there is scientific and technical uncertainty about those effects*'.

International governance

The Cartagena Protocol on Biosafety (the Biosafety Protocol) is designed to address the biosafety risks presented by GMOs when these move across borders. Established under the Convention on Biodiversity, this international treaty is founded on the principle of prior informed consent with respect to the transboundary movement of living modified organisms (LMOs). It puts a duty on an exporting party to seek prior informed consent from the destination country (Article 7). However, the procedures only work for intended movements across the border of a single nation. The protocol does not define best practice guidelines, for example, for standards for assessing effects, estimating damages, or mitigating harms [75]. While these may be seen as 'gaps', it could also be argued that best practice

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[18] NZ Law Commission (2002). *Liability for loss resulting from the development, supply, or use of genetically modified organisms.* Study Paper 14.The Law Commission looked into the issue of liability for loss resulting from GMOs and described the adverse cultural effects of GM on Maori: "*Concerns have also been raised by Maori, which arise from a different belief structure, Although the basis for many of the Maori cultural objections to genetic modification vary among iwi, they are usually based around impacts on whakapapa, mauri, kaitiakitanga and rangatiratanga. The traditional Maori worldview considers all parts of the natural world to be related through whakapapa. Genetic modification risks interfering with such relationships, and threatens the sanctity of mauri (life principle) and wairua (spirit) of living things. Concluding that genetic modification may affect Maori's ability to be kaitiaki (guardians) of their taonga and particularly their ability to care for valued flora and fauna*".
[19] Anna Kingsbury (2011). Intellectual Property. WAI 262. *NZ Law Journal*, September 2011, 273.
[20] Resource Legislation Amendment Act 2017

guidelines are best left out of such rigid instruments. The related Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress identifies response measures in the event of damage to the conservation and sustainable use of biological diversity resulting from living modified organisms that result from transboundary movements. It does not define rules governing liability and redress for damage, but requires Parties to either apply their existing general law on civil liability or develop specific legislation that addresses (as appropriate): damage; standard of liability (including strict or fault-based liability); channelling of liability where appropriate; and the right to bring claims.

Concerns around the potential unintended impacts of gene drives were highlighted in a US National Academies of Science review of gene drives [116] which noted:

"Gene drives do not fit well within the existing regulatory logic of confinement and containment because they are designed to spread a genotype through a population, making confinement and containment much more difficult (or even irrelevant) and the environmental changes introduced by release potentially irreversible.… Research on gene drives is global. Responsible governance will need to be international and inclusive, with clearly-defined global regulatory frameworks, policies, and best practice standards for implementation."

This will have implications for New Zealand's international social license to develop gene drives that could potentially threaten other countries' native species.

Safety mechanisms for gene drives

In their 2014 article, Esvelt and colleagues outlined a variety of uses for CRISPR gene drives in human health, agriculture and the environment [44]. Importantly, the authors noted that the potential efficiency of CRISPR gene drive systems posed a requirement for a high certainty of laboratory containment before they are deemed safe to move out of the laboratory. They suggested parallel development of a 'reversal' gene drive that would restore the original gene, but with a slightly different sequence that would not be targeted by the original guide RNA.

Although Esvelt *et al.* [44] had highlighted the need for safeguards, the ease and efficiency of the CRISPR-mediated gene drive in the fruit fly *Drosophila melanogaster* [7] was a surprise to many. These results have led to wide discussion of the risks of gene drives. Recently, scientists working on CRISPR [117] recommended a number of safeguards, including:
1. Perform gene drive experiments outside the ecological range of the organism (e.g. *Anopheles* mosquito in Boston). Consequently, if any individuals do escape the laboratory they would likely perish and/or have no potential mates.
2. Use a laboratory strain that cannot reproduce with wild organisms.
3. Have a high level of laboratory containment, using multiple substantial physical barriers. In practice, this could be a higher level of containment than is currently recommended for transgenic strains of the species of interest (i.e. for organisms containing genetic material into which DNA from an unrelated organism has been artificially introduced). For example, using air blast fans and higher precautions to prevent escape (e.g. sealing possible escape routes).

In 2016, another safety mechanism was developed, called the 'daisy-chain' gene drives [118], which gradually vanish after 50-100 generations. To create these gene drives that don't spread indefinitely, the gene drive is split into three or more parts to create a 'daisy chain'. Each part contains a genetic element that drives the next element in the chain so that element A can only copy and paste itself if element B is present. Element B can only copy and paste itself if element C is present. And element C, crucially, cannot copy and paste itself at all – it can only spread by normal breeding, to half of offspring. When the gene drive animals are released, they carry all three elements. Then, when they mate with their wild counterparts, all the offspring will inherit element A and B, but only half will inherit element C. In the following generations, element B will spread rapidly and A will spread even more rapidly, but C will gradually die out. Once it does, B will start to disappear, and finally A will too. By adding more elements to the daisy chain, the gene drive could be made to persist longer in the wild. This could allow the use of gene drives locally without the worry about the risk of worldwide spread.

Evolutionary resistance to gene drives

The promise of gene drives lies in their inherent ability to rapidly move target alleles to fixation in a very short period of time to generate a desired effect on a population. If all individuals within a population are susceptible to the gene drive linked gene, then it is predicted that the gene drive version of the gene will rapidly spread. However,

substitutions, insertions or deletions within the DNA adjacent to the gene drive that occur through natural mutational processes or during gene-drive mediated DNA cutting are expected to lead to a resistant version of the gene [8]. Most cells also have an alternative pathway for repairing double-stranded breaks, known as non-homologous end joining (NHEJ) [119]. With NHEJ, the broken ends of DNA are fused together without regard to matching similar sequences. Errors during this repair process can lead to small deletions or insertions in the genetic code, called mutations. In many cell types, this type of repair can outnumber repairs that try to copy similar sequences in the cell. A NHEJ mutation of the gene drive recognition site would suppress its targeting accuracy [36].

Because many resistant versions of the gene will have greater Darwinian fitness than the gene drive gene, population level resistance to the gene drive is expected to appear [8]. In fact this is what was observed in the laboratory-based gene drive experiments on *Anopheles gambiae* mosquitos [14] and *Drosophila* [120].

In addition to the gene drive process generating resistant versions of the gene, it is also predicted that many pest species will harbour genetic variations resistant to the gene drive construct. While gene drive approaches have not been used in the field, many species targeted for control via gene drives harbour significant levels of genetic variation, especially insects which are likely early targets for gene drive. In these cases, under known mutation rates and with large population sizes, mutations in the DNA adjacent to the gene drive sequence are inevitable. If these variants lie within the target region of the gene drive then, as with the mutant gene versions generated though NHEJ, it is expected that these gene versions will be selected for and subsequently lead to individuals resistant to the gene drive. For example, [121] measurements of genetic variation in *Anopheles gambiae* across Africa through whole genome resequencing found that approximately half of the potential gene drive target sites had variants in the wild that would disrupt targeting by the gene drive construct.

How can resistance be overcome? Detailed population genomic surveys of the target pest species would need to be employed to assess variation across all potential gene drive target sites. Ideally, this would include whole genome resequencing to detect the presence of variants across potential target sites. These data would also yield information to guide the identification of alternative target sites in the same gene or alternative genes. This approach would also have the advantage in aiding the prediction of off-target effects. Large numbers of individuals would need to be assayed as resistant versions of genes are expected to be strongly selected for, even from very low initial frequencies [8]. Based on the population genomics results from *An. gambiae* [121], gene drives are unlikely to work unless multiple genes and multiple target sites within those genes are targeted. Increasing the number of target sites in the genome leads to a corresponding increase in the probability of off-target effects with the associated safety and ethical concerns. The use of multiple guide RNAs could also be used to target a wide range of gene variants [14]. Again, this approach requires detailed knowledge of gene variation. A further approach could be to target a conserved region of a biologically essential gene [44].

Another implication of this resistance is that intentionally releasing a resistant gene into a population could be an effective means of reversing the effects of a gene drive [122].

For further information

For more information and resources about gene editing, visit the Society's web pages: https://royalsociety.org.nz/gene-editing/, or contact info@royalsociety.org.nz.

Appendix 1: Contributors to the technical paper

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