# Hypothetical gene drive / environmental release case study 1

National Gene Technology Scheme
An Australian, State and Territory Governments Collaboration

## SCENARIO 1: **Gene Drive Fruit Fly**

These scenarios have been developed to provide guidance to proponents and are hypothetical only. These scenarios are intended to demonstrate the regulatory requirements and risk considerations which may apply, depending on the gene drive organism that is proposed to be deployed.

## Introduction

Australia has two economically significant fruit fly species – the Queensland fruit fly (*Bacterocera tryonil* 'Qfly') and the Mediterranean fruit fly (*Ceratitis capitata* 'Medfly'). According to the Department of Agriculture, Fisheries and Forestry (DAFF), fruit flies cost Australia hundreds of millions of dollars per year in control measures and lost access to international markets.[1]

Fruit fly management is carried out by the Commonwealth and jurisdictional governments under the guidance of the Australian National Fruit Fly Management Protocols. This includes the maintenance of Pest Free Areas (PFAs) in Tasmania and the Riverland region of South Australia.[2]

The aims of the National Fruit Fly Strategy 2020-2025, developed by the National Fruit Fly Council, are "reducing the risk of exotic fruit fly incursion, effective and efficient management of established fruit fly species, and a robust national system to manage risk and underpin market access."[3]

Fruit fly management usually involves the use of agrichemicals and/or netting. This includes cover spraying,[4] bait spraying, and other methods.

The Sterile Insect Technique (SIT) has also been very effective for managing fruit fly populations. It involves rearing sterile male flies by irradiating the pupae with x-rays and releasing them into the wild to mate with wild type females, leading to a reduction in fruit fly populations. SIT have been used to eradicate Qfly and Medfly from NSW, VIC, SA and WA, and to control fruit fly outbreaks, including in PFAs.[5] Whilst irradiation is not classed as genetic modification under the Act, the use of SIT establishes a precedent for the release of modified fruit fly into the environment to control fruit fly numbers.

## Gene Drive

In 2021, Meccariello et al.[6] published the first demonstration of a gene drive in Medfly. This gene drive works using the 'X-shredding' system, which interferes with the transmission of the X-chromosome to offspring by introducing multiple DNA double-stranded breaks during male meiosis. This results in predominantly male progeny.

The authors noted however that the male to female ratio achieved by this gene drive would not be sufficient for use in the field, necessitating further work to improve the gene drive system.

Other gene drives systems have been developed in fruit fly not found in Australia, such as in the model organism *Drosophila melanogaster*[7], and the crop pest *Drosophila suzukii*.[8]

The OGTR issued a licence in 2021 authorising contained research to explore split gene drive designs in Australia using *Drosophila melanogaster* as a model organism.

1. https://www.agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/fruit-flies-australia/the-measure/economic-studies
2. https://www.agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/fruit-flies-australia/management
3. National-Fruit-Fly-Strategy-2020-25.pdf (preventfruitfly.com.au)
4. Control methods | National Fruit Fly Council (preventfruitfly.com.au)
5. https://www.agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/fruit-flies-australia/management/sterile-insect-technique
6. https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-021-01010-7
7. A nickase Cas9 gene-drive system promotes super-Mendelian inheritance in Drosophila - PMC (nih.gov)
8. https://www.pnas.org/doi/10.1073/pnas.1713139115

# Hypothetical gene drive / environmental release case study 2

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## SCENARIO 2: **Gene Drive Mouse**

These scenarios have been developed to provide guidance to proponents and are hypothetical only. These scenarios are intended to demonstrate the regulatory requirements and risk considerations which may apply, depending on the gene drive organism that is proposed to be deployed.

## Introduction

High mouse populations can cause significant crop damage, with Australia's worst ever mouse plague, in 1993, causing an estimated $96 million worth of damage. Higher populations of mice are becoming a persistent problem, rather than the previous boom and bust population trends.[9]

Baiting is the primary strategy used to manage mice in Australia.[10] Zinc phosphide is commonly used for mouse baits and is highly toxic if acute exposures occur to humans through preparation and handling of baits. Baiting can be expensive, with baiting costs exceeding $150,000 for some farmers during the 2021 eastern Australia mouse plague.[11] Whilst financial support is available for farmers affected by mice infestation,[12] there is no centralised management strategy for mouse management in Australia.

## Gene Drive

Researchers at the University of Adelaide have developed the first gene drive in mammals, which introduces female infertility in the common house mouse (*Mus musculus*). This gene drive modifies a naturally occurring gene drive element, known as the *t* haplotype, to spread faulty copies of a female fertility gene.

This means that females are progressively reduced from the population, leading to the suppression of mouse numbers. However, the authors noted that many other factors, such as possible resistance, still require thorough exploration before deployment.[13] The authors also used computer modelling to determine the effectiveness of the system.

It was predicted that introducing 256 gene drive mice into a population of 200,000 mice on an island would lead to eradication of all mice from the island in approximately 25 years.[14]

The OGTR issued a licence in 2020 authorising contained research into genetic methods to control invasive pest mice by spreading mutations that cause infertility, embryonic death or bias the sex of offspring.

9. https://www.csiro.au/en/research/animals/pests/mouse-control
10. https://www.agriculture.gov.au/agriculture-land/farm-food-drought/mouse-infestation/managing-mice
11. https://www.theguardian.com/australia-news/2021/may/11/emergency-permit-allows-farmers-battling-australian-mouse-plague-to-use-double-strength-bait
12. https://www.agriculture.gov.au/agriculture-land/farm-food-drought/mouse-infestation/financial-support
13. https://www.pnas.org/doi/10.1073/pnas.2213308119
14. https://www.abc.net.au/news/2022-11-10/university-of-adelaide-gene-drive-technology-mice-control/101639638

# Hypothetical gene drive / environmental release case study 3

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## SCENARIO 3: **Gene Drive Mosquito**

These scenarios have been developed to provide guidance to proponents and are hypothetical only. These scenarios are intended to demonstrate the regulatory requirements and risk considerations which may apply, depending on the gene drive organism that is proposed to be deployed.

## Introduction

Ross River virus (RRV) infections are the most common mosquito-borne infection in Australia, with the largest recorded epidemic occurring in 2014–2015, with 9544 cases reported in 2015.[15] At least 40 mosquito species are thought to carry RRV, including several *Aedes* mosquitoes.[16]

Another mosquito-borne illness is dengue virus infection (also called dengue fever). It is commonly more severe than RRV, with symptoms that are similar to a serious case of the flu.[17] It is carried by either the *Aedes aegypti* or the *Aedes albopictus* mosquito.[18] The number of cases of dengue fever per year in Australia is quite low due since vector mosquitoes are only found limited areas in Queensland,[19] with the World Health Organisation reporting 188 cases in 2022 as of 6 October.[20]

Clinical trials are being conducted using live vaccines containing attenuated dengue virus. However, currently mosquito-borne diseases in Australia, RRV and dengue fever are controlled by preventing bites and managing mosquito numbers.

[21]Protective measures include wearing protective clothing and mosquito repellent, avoiding mosquito ridden areas especially at dawn and dusk, and using insecticides.

There has also been a trial in Northern Queensland to minimise mosquito numbers by releasing male mosquitoes infected with *Wolbachia*, bacteria that is harmless in humans but affects reproduction in mosquitoes.[22] After 10 years this project has had a significant impact on dengue transmission, with no evidence of transmission in areas with high *Wolbachia* levels.[23]

## Gene Drive

Gene drive mosquitoes are probably the most extensively researched gene drive system, with CRISPR gene drives investigated for both population suppression and to reduce pathogen transmission.[24] Whilst much of the research to date relates to the *Anopheles* mosquito, the vector for malaria, there has been some research into the use of gene drives for dengue fever suppression.

In 2020, Ming *et al.*[25] reported a gene drive system in *Aedes aegypti* mosquitoes, the principal vector for dengue virus. This gene drive uses CRISPR to spread anti-pathogen effector genes through the mosquito population and has transmission rates of up to 94%. In addition, it is a "split gene drive", meaning two critical components of the gene drive are split into different organisms. This reduces the chance the gene drive will spread into neighbouring mosquito populations and means the gene drive can be eliminated from the population over time. The authors suggested that these features could enable safe testing in the field prior to release of a non-split gene drive.

Whilst Ming *et al.* focused on the ability for the gene drive to prevent dengue virus infections, it may also be applicable to RRV, which can be spread by *Aedes aegypti* mosquitoes.[26]

15. https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-020-05411-x
16. https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-020-05411-x
17. https://www.health.gov.au/diseases/dengue-virus-infection
18. https://www.cdc.gov/dengue/index.html
19. https://www.health.qld.gov.au/clinical-practice/guidelines-procedures/diseases-infection/diseases/mosquito-borne/dengue/virus-fever
20. https://www.who.int/docs/default-source/wpro---documents/emergency/surveillance/dengue/dengue-20221006.pdf?sfvrsn=fc80101d_124
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22. https://ecos.csiro.au/preventing-mosquito-borne-diseases/
23. https://www.worldmosquitoprogram.org/en/global-progress/australia
24. https://www.nature.com/articles/s41467-021-24654-z
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# Hypothetical gene drive / environmental release case study 4

## SCENARIO 4: **Rescue drive for frogs**

These scenarios have been developed to provide guidance to proponents and are hypothetical only. These scenarios are intended to demonstrate the regulatory requirements and risk considerations which may apply, depending on the gene drive organism that is proposed to be deployed.

## Introduction

The chytrid fungus *Batrachochytrium dendrobatidis* causes the amphibian disease chytridiomycosis, which has resulted in huge population losses and local extinctions, having a 100% mortality rate in some populations.[27] Chytridiomycosis was first identified in 1993 in Queensland, but has since been shown to be found in cool and wet areas throughout Australia, with exception of the NT. The chytrid fungus has been directly linked to the extinction of 4 frog species in Australia and has been implicated in the decline of at least 10 others, including the Southern corroboree frog.[28]

The strategies to mitigate the impacts of the chytrid fungus are incredibly limited,[29] and in Australia largely focus on preventing the spread of the disease to unaffected areas.[30] This leaves populations that are currently affected vulnerable. The chytrid fungus is listed as a key threatening process under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. A national Threat Abatement Plan was developed in 2016, which included four main objectives, two of which pertained to building scientific capacity and developing research around chytrid fungus management.[31]

## Gene Drive

Whilst the gene drives discussed in the other scenarios involve eliminating a pest species or removing an undesirable trait, gene drives may also have a possible function to conserve threatened species by introducing beneficial alleles.[32] These gene drives are known as 'rescue drives'.

The chytrid fungus reproduces mainly asexually so cannot be targeted with a gene drive. However, it may be possible to use a gene drive to introduce resistance to the fungus into affected frog species.[33] It has been shown that introducing changes to the Major Histocompatibility Complex (MHC) increases the likelihood that infected frogs will survive fungal infection.[34] If changes to the MHC could be made into a gene drive, it would be possible to rapidly spread resistance to the chytrid fungus throughout frog populations.

However, these changes have yet to be converted into a gene drive system. And even if a gene drive could be generated, there are several other factors that may influence the use of such a gene drive. Firstly, reducing diversity of the MHC gene within a frog species may have the unintended consequence of making the frogs more susceptible to other pathogens.[35] In addition, it is possible that making genetic changes to native species, rather than pest organisms, could be viewed more negatively by the general public.

27. https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000550#:~:text=One%20of%20the%20most%20dramatic,and%20local%20extinctions%20%5B3%5D
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32. https://royalsocietypublishing.org/doi/10.1098/rspb.2019.2709
33. https://link.springer.com/article/10.1007/s10592-019-01165-5
34. https://www.pnas.org/doi/10.1073/pnas.1106893108
35. https://link.springer.com/article/10.1007/s10592-019-01165-5

## Potential regulatory touchpoints

The table below outlines the potential regulatory touchpoints of the various scenarios.

| Regulatory Touchpoints | Explanation | SCENARIOS<br>Gene Drive Fruit Fly | Gene Drive Mouse | Gene Drive Mosquitoes | Rescue Drive for frogs |
|---|---|---|---|---|---|
| **Gene Technology Regulation** | The Gene Technology Regulator will consider all gene drive applications in accordance with the Act. However, jurisdictional gene technology regulation may also be triggered or enacted by the environmental release of gene drives, including under the Designated Areas Principle, which is the ability for the jurisdictions to designate 'GM areas' and 'non-GM areas. | ✔ | ✔ | ✔ | ✔ |
| **Agricultural Implications** | Gene drive pest control, and the control of agricultural pathogens, will have a considerable impact on the agricultural sector. As such, agricultural legislation, on both a Commonwealth and jurisdictional level, may be enacted or triggered by the environmental release of gene drives. This may include relevant Animal Ethics Committee approvals. | ✔ | ✔ | | ✔ |
| **Environmental Implications** | The environmental release of gene drives is likely to impact environmental legislation and regulation, both on a Commonwealth and on a jurisdictional level. For example, gene drive organisms may fall under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).<br>Examples of the environmental impacts of gene drives include:<br>– If a gene drive causes the elimination of a particular species from an ecosystem, this could have serious implications for the broader biodiversity of that ecosystem, especially if the gene drive organism is a species.<br>– There is a remote potential for gene drives to jump to other species, including native species | ✔ | ✔ | ✔ | ✔ |
| **Food implications** | Whilst at this point it is unlikely gene drives will be used in food crops/animals, there is the possibility that gene drive organisms could interact with food products, potentially triggering food legislation or regulation, such as by Food Standards Australia New Zealand. | ✔ | ✔ | | |
| **Trade implications** | Gene drives may also impact on trade, as regulations in other countries may prohibit the purchase of products that could be affected by gene drives (e.g., fruit that could potentially contain gene drive fruit fly larvae). | ✔ | ✔ | | |
| **Human health implications** | Gene drives have the potential to impact on human health if the gene drive organism is a vector for human disease, or if the gene drive organism can cause human disease. | | | ✔ | |