70 years of rabbit biocontrol in Australia

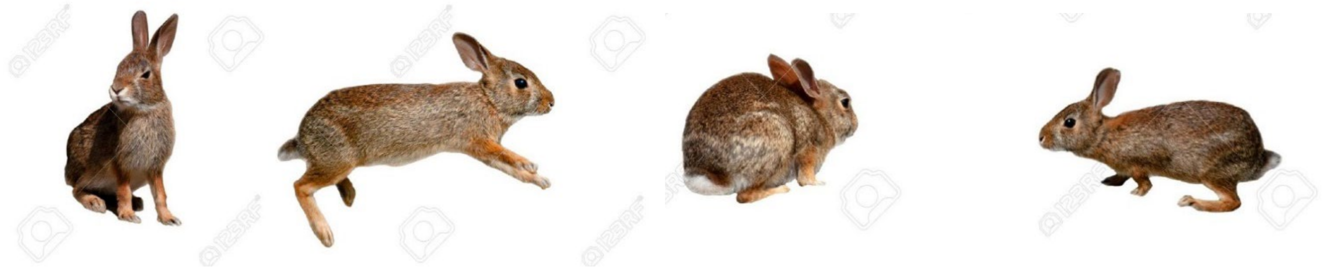
An ongoing co-evolutionary arms race

Tanja Strive| Montpellier, July 2022

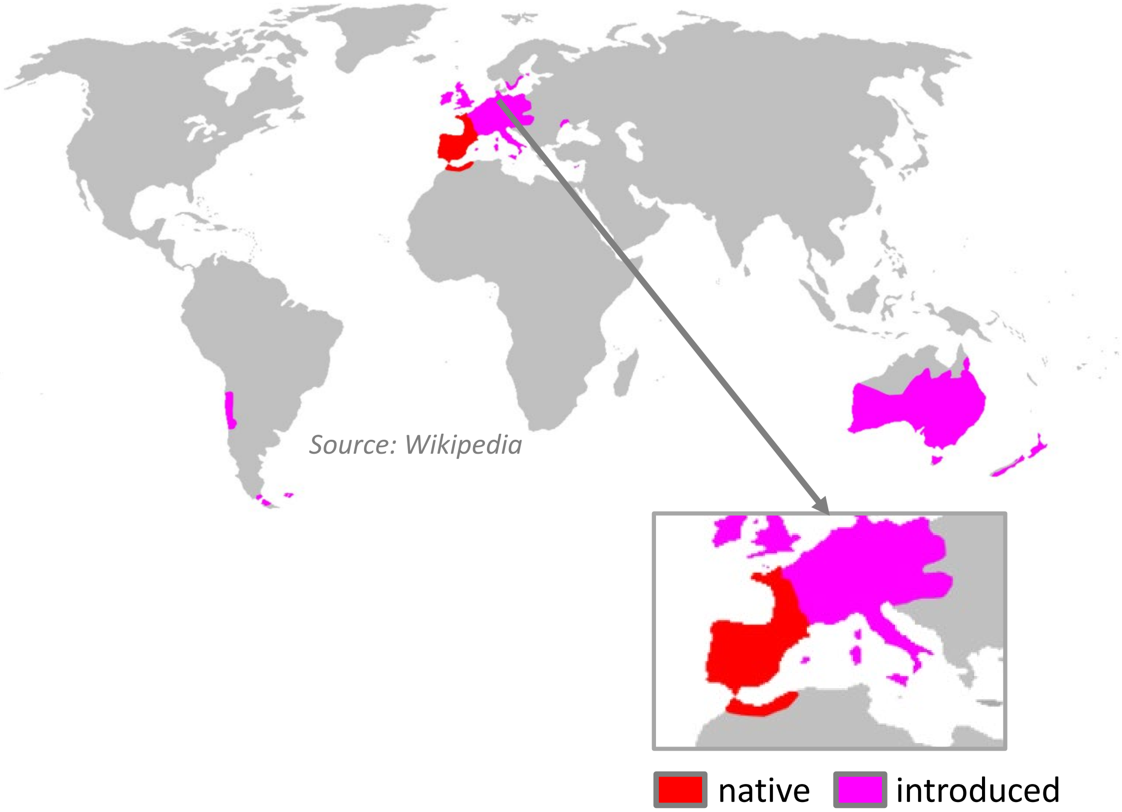
Australia's National Science Agency

Outline
70 years of rabbit biocontrol

- Rabbits and rabbit biocontrol in Australia
    - Myxoma virus & RHDV
    - Host-pathogen co-evolution
- Impacts of rabbit biocontrol
- Current & future biocontrol initiatives in Australia



European rabbits *(Oryctolagus cuniculus)*



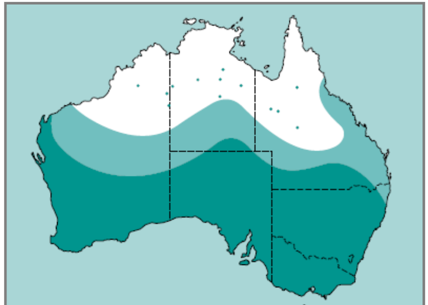
Iberian lynx
(Lynx pardinus)

Spanish Imperial eagle
(Aquila adalberti)

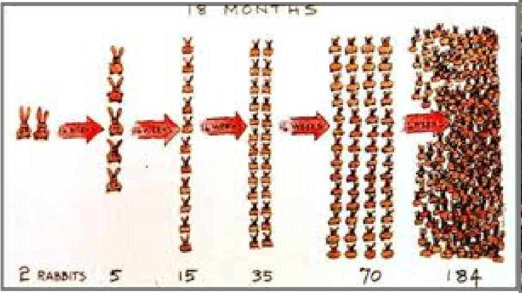
Sacramento-Moreno et al., 2007
Delibes-Mateos et al., 2009

The European rabbit (*Oryctolagus cuniculus*): A perfect invader

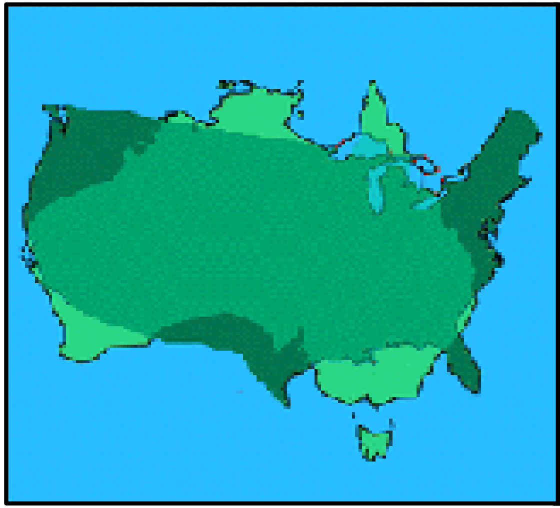
- 26 rabbits (released in 1859 for hunting)
- Colonised continent in 70 years
- Ideal environment, few diseases
- Gestation period 28 days, mate same day of giving birth
- Large litters (5-7), early sexual maturity
- Peak estimate: 0.5 – 10 billion rabbits
- Massive economic and ecological impacts



Saunders et al., 1995

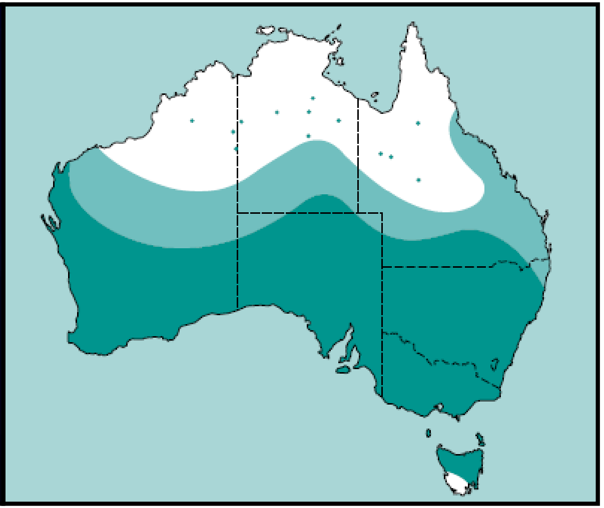


Australia in 1930:



6.7 million km^2

6.5 million people
(in 1930)



Estimated
0.5 – 10 billion rabbits

⇒ Conventional rabbit control (shorting, trapping, etc) ineffective

⇒ Self-disseminating (=biological) controls were needed for continental scale control

Biological control of the European rabbit (*Oryctolagus cuniculus*) - *early days*

1887: NSW-Government promised £24,000 rewards for eradication of rabbits

Pasteurella multocida (Chicken cholera)
Not species specific, did not transmit

Louis Pasteur
1822-1895

Adrien Loir
1862-1941

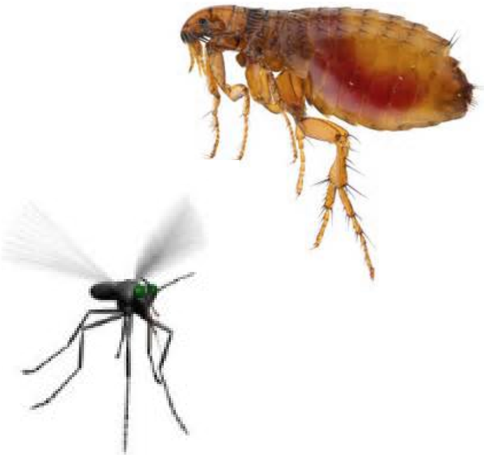
Myxoma virus (MYXV): an emerging disease of rabbits

Family: *Poxviridae*, Genus: *Leporipoxvirus , ds-DNA, 160 kb*

Natural host: Tapeti
(Sylvilagus brasiliensis)
Mild infection (cutaneous fibromas)

Jumped species to European rabbit 1896
(*Oryctolagus cuniculus)*
Highly virulent generalised disease

Transmission (mechanical) by biting insects
Fleas & mosquitoes



MYXV – release and impacts

- 1950 release: >90% mortality rates in some areas
- Within 2-3 years attenuated MYXV strains appeared (and dominated)
- Rabbits developed genetic resistance
- Rabbit numbers increased again!

- Introduction of rabbit fleas to boost transmission
  - *Spilopsyllus cuniculi (1966)*
  - *Xenopsylla cunicularis (1995)*

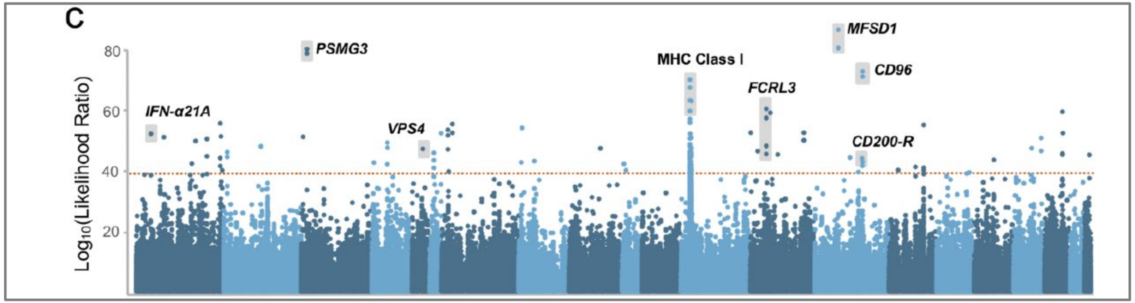
Dame Jean Macnamara 1914-2010

MYXV – text book example for *'Trade-off Theory "* of Virulence Evolution of an emerging disease

Frank Fenner
1914-2010

- Selection of moderately virulent phenotype for maximum transmission by biting insects:
  - Increased survival rates and survival times
  - Longer disease period
  - Increased opportunity for mosquitoes/fleas to pick up virus from skin lesions
  - Much more severe disease & high welfare impact

- Rapid (2 year) selection for host resistance acting on standing genetic variation: Immune modulating genes



UNIVERSITY OF CAMBRIDGE

Science RESEARCH ARTICLES

Cite as: J. M. Alves *et al.*, *Science* 10.1126/science.aau7285 (2019).

Parallel adaptation of rabbit populations to myxoma virus

Joel M. Alves[1,2,3*], Miguel Carneiro[2,4*], Jade Y. Cheng[5,6], Ana Lemos de Matos[7], Masmudur M. Rahman[7], Liisa Loog[3,8], Paula F. Campos[6,9], Nathan Wales[6,10,24], Anders Eriksson[11], Andrea Manica[12], Tanja Strive[13,14], Stephen C. Graham[15], Sandra Afonso[2], Diana J. Bell[16], Laura Belmont[7], Jonathan P. Day[1], Susan J. Fuller[17], Stéphane Marchandeau[18], William J. Palmer[19], Guillaume Queney[20], Alison K. Surridge[16], Filipe G. Vieira[6], Grant McFadden[7], Rasmus Nielsen[5,6], M. Thomas P. Gilbert[6,21], Pedro J. Esteves[2,22], Nuno Ferrand[2,4,23], Francis M. Jiggins[1*]

The race continues............

- 1990s MYXV field strains are hypervirulent in unselected domestic rabbits, causing a completely new disease phenotype

PNAS

Next step in the ongoing arms race between myxoma virus and wild rabbits in Australia is a novel disease phenotype

Peter J. Kerr[a,b], Isabella M. Cattadori[c,d], June Liu[b], Derek G. Sim[c,d], Jeff W. Dodds[e], Jason W. Brooks[e], Mary J. Kennett[e], Edward C. Holmes[a,f], and Andrew F. Read[c,d,g,1]

[a]Marie Bashir Institute for Infectious Diseases and Biosecurity, Charles Perkins Centre, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW 2006, Australia; [b]CSIRO Health and Biosecurity, Black Mountain Laboratories, Acton, ACT 2601, Australia; [c]Center for Infectious Disease Dynamics, The Pennsylvania State University, University Park, PA 16802; [d]Department of Biology, The Pennsylvania State University, University Park, PA 16802; [e]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802; [f]Sydney Medical School, University of Sydney, Sydney, NSW 2006, Australia; and [g]Department of Entomology, The Pennsylvania State University, University Park, PA 16802

Edited by Simon A. Levin, Princeton University, Princeton, NJ, and approved July 14, 2017 (received for review June 23, 2017)

Peter Kerr

THE UNIVERSITY OF SYDNEY

PennState College of Agricultural Sciences

CSIRO

“Now, *here*, you see, it takes all the running you can do, to keep in the same place”

(Lewis Caroll, Through the looking glass)

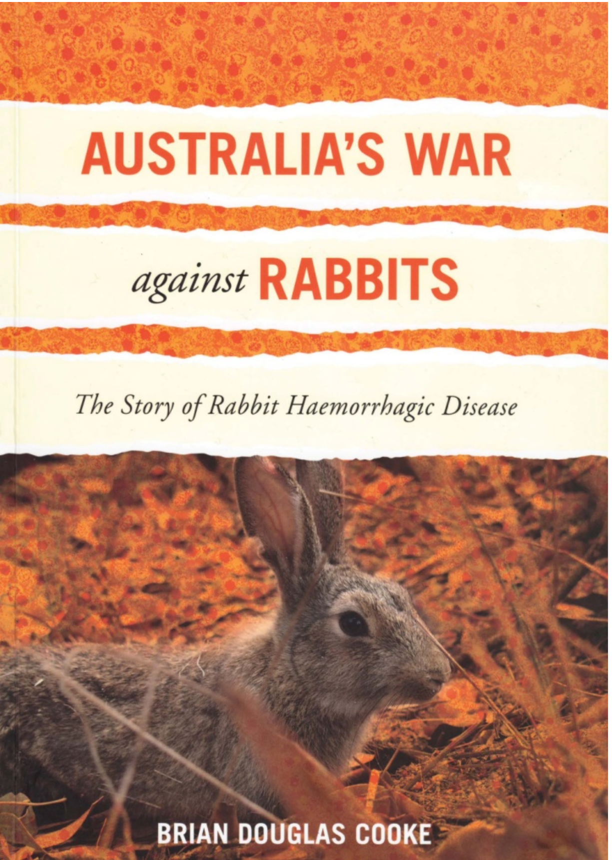
New hope for Australia’s feral rabbit problem

Rabbit Haemorrhagic Disease Virus (RHDV)

Family: *Caliciviridae*, Genus: *Lagovirus* , *+ss-RNA, 7.5 kb*

- Emerging disease of rabbits (China 1984)
- Infectious hepatitis with >95% case fatality rates
- Death 36-72 h.p.i.
- Short period of clinical signs (~12h)
- Young rabbits innately resistant to lethal disease
- Does not grow in conventional cell culture
- Species specific (infects only European rabbits)

Bringing RHDV to Australia



- Quarantine tests (CSIRO AAHL) commenced in 1991
- Later: field transmission trials on Wardang Island
- Escaped in 1995 (Fly transmission!)
- Highly successful in reducing rabbit populations (> 95% in parts of Australia)
- In 1997 illegally introduced into New Zealand

> some evidence of genetic resistance
> No significant reduction in virulence observed


Brian Cooke

Virulence evolution of RHDV is the opposite of MYXV

=> More recent field strains kill a higher proportion of rabbits faster


Virology 464-465 (2014) 415–423

Contents lists available at ScienceDirect

Virology

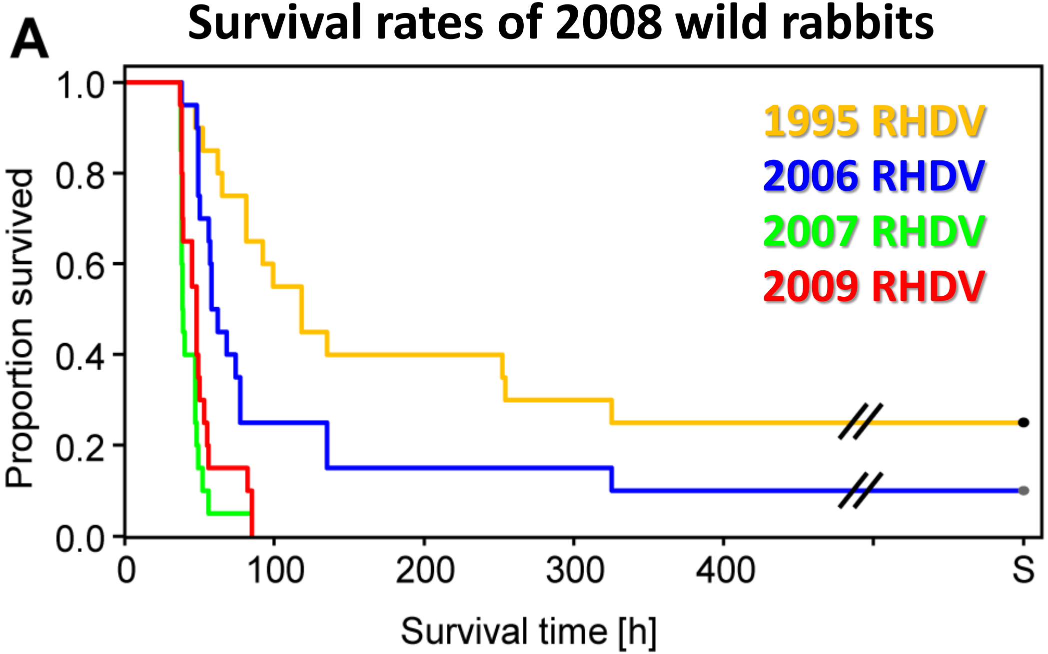
journal homepage: www.elsevier.com/locate/yviro

ELSEVIER

Increased virulence of rabbit haemorrhagic disease virus associated with genetic resistance in wild Australian rabbits (*Oryctolagus cuniculus*)

Peter Elsworth [a,b], Brian D. Cooke [b,c], John Kovaliski [b,d], Ronald Sinclair [b,d], Edward C. Holmes [e], Tanja Strive [b,f,g,*]

Survival rates of 2008 wild rabbits

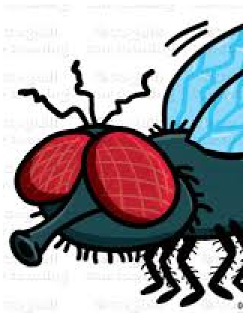


WHY?

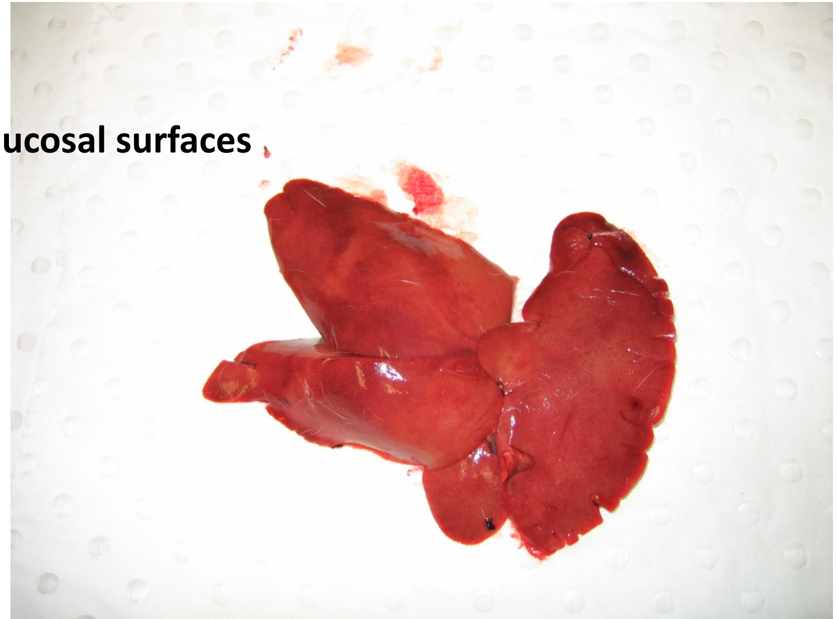
Transmission of RHDV:

Direct contact, fomites AND flies

Asgari et al., 1998
McColl et al., 2002
Elsworth et al., 2014
Schwensow et al., 2014
Hall et al., 2018



- Rabbit dies
- VERY high concentration of virus in dead body (10e9 copies/mg, ~10e15/carcass)
- Body decays or is opened up by scavengers
- Fly feeds on carcass
- Flies to next rabbit warren
- Lands on grass and/or rabbit's mucosal surfaces



Plays a key role in distance transmission between non-connected rabbit populations

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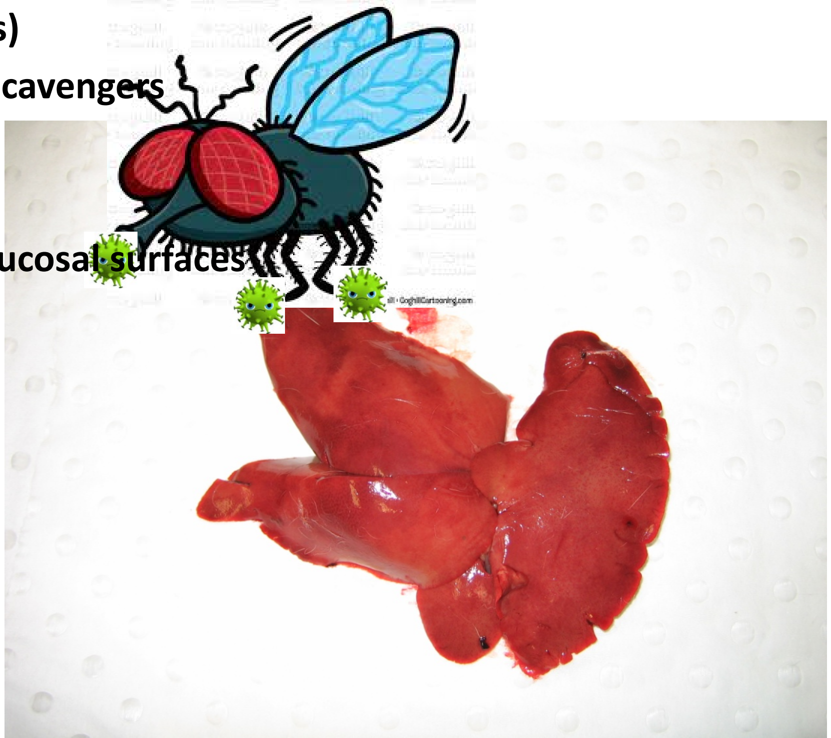
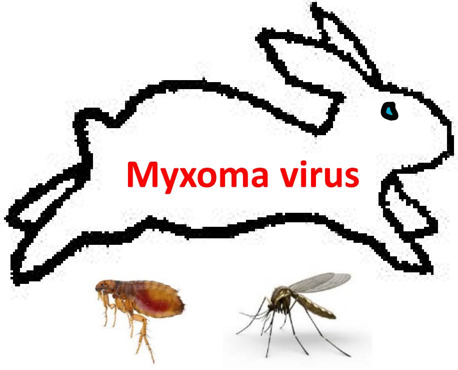
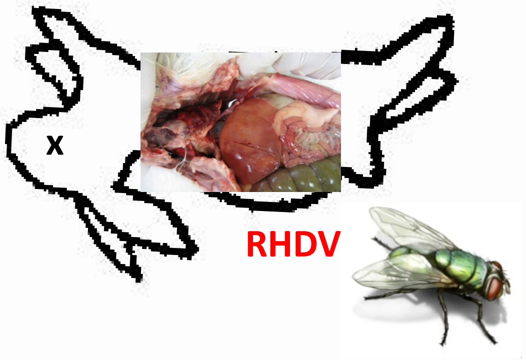


Plays a key role in distance transmission between non-connected rabbit populations

⇒Both evolve towards maintain virulence levels optimal for mechanical insect transmission



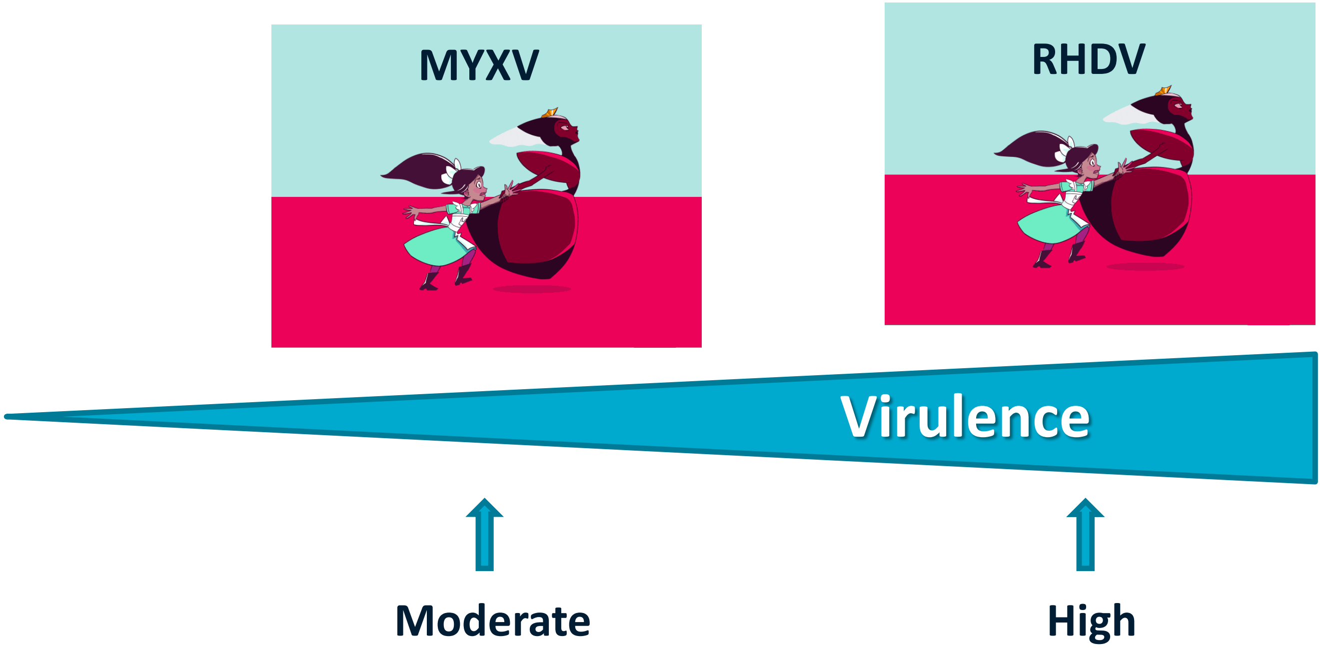
Biting insects
Moderate virulence



Carrion feeding flies
High virulence

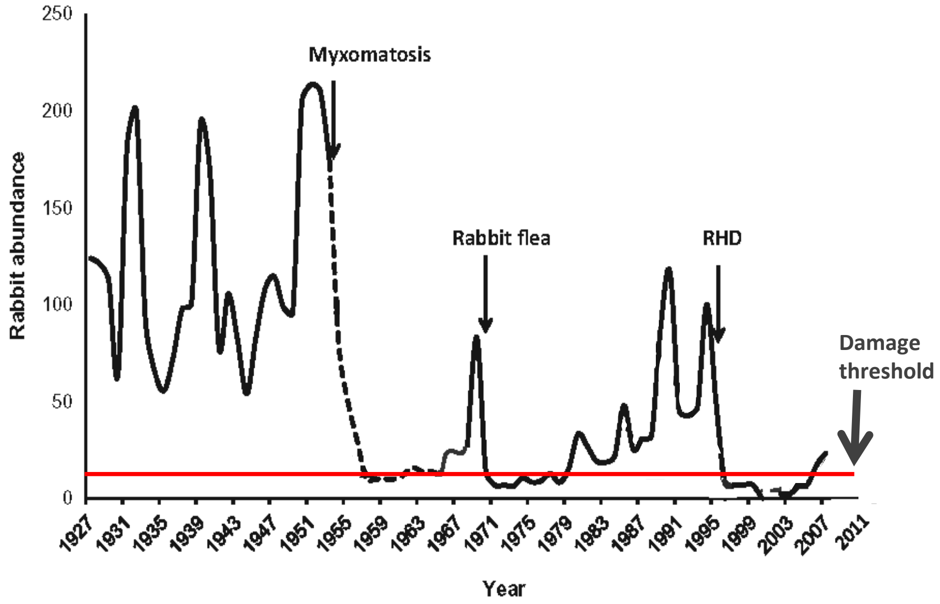
For RHDV the <u>dead</u> animal is (not the sick animal) is an important source of distance transmission via insects

⇒Both evolve towards maintain virulence levels optimal for mechanical insect transmission



Has biocontrol worked?

VERY effective, but NOT a silver bullet !



(Cooke et al., 2013)

- Must be followed up with conventional control tools
- Virus will not eradicate the host
- ongoing host-pathogen co-evolution
- Repeated adjustments required to maintain gains

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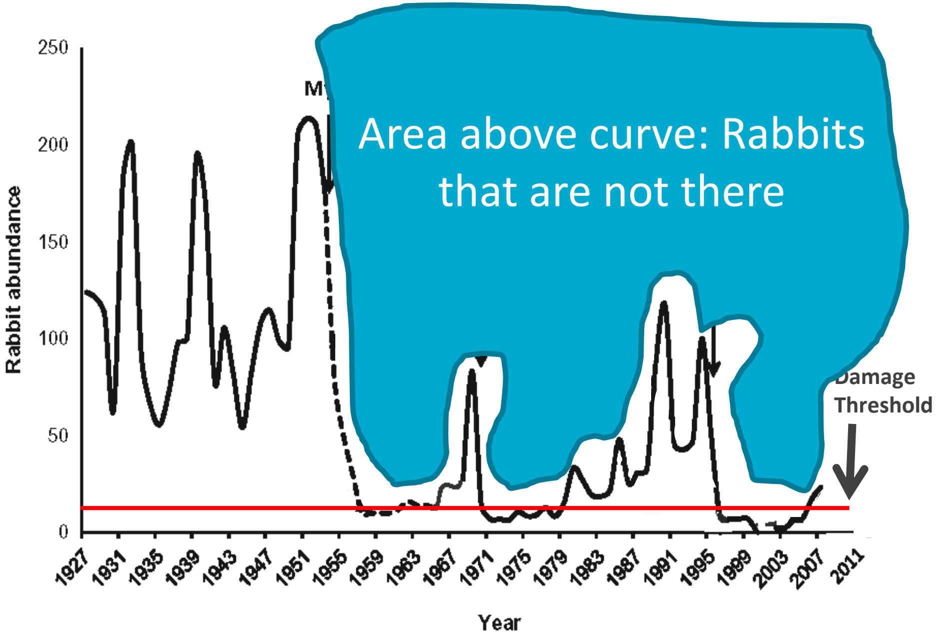


(Cooke et al., 2013)

- Must be followed up with conventional control tools
- Virus will not eradicate the host
- ongoing host-pathogen co-evolution
- Repeated adjustments required to maintain gains

- NOT about killing lots of viruses
- BUT about preventing rabbit impacts

Economic benefits of rabbit biocontrol

Myxoma virus
1950

Rabbit fleas
1968 and 1993

Calicivirus
(RHDV)
1996

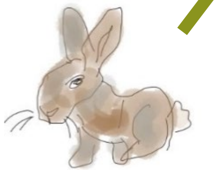
1950-2010 (60 years): Combined cumulative benefits estimated at $70 billion to the Australian agricultural industries
(…..and counting….)

(Cooke et al., 2013)

Ecological benefits
=> Top down and bottom up trophic effects

Sustains high population numbers of feral cats and foxes

Key drivers for mammalian extinction in Australia



Competition and land degradation

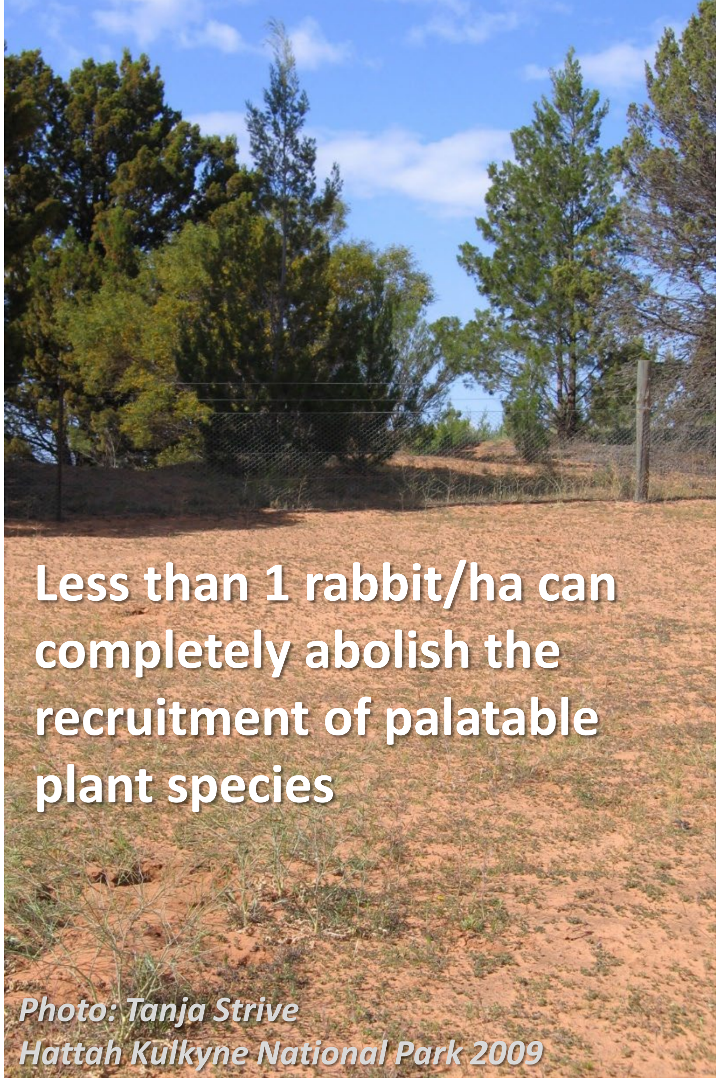
Conservation Biology

Contributed Paper

Rabbit biocontrol and landscape-scale recovery of threatened desert mammals

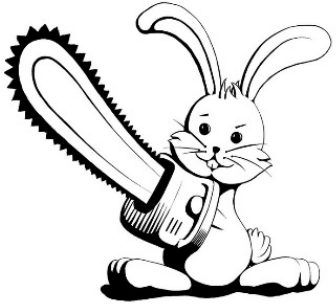
Reece D. Pedler,* ¶ Robert Brandle,* John L. Read,†‡ Richard Southgate,§ Peter Bird,** and Katherine E. Moseby†‡

- Recovery of 3 mammalian species through 20 years of sustained rabbit suppression from RHDV
- Removal from the IUCN red list



- **Long term suppression of rabbits has led to the recovery of many native plants, e.g. Sheoak & Cypress pine *(Allocasuarina sp., callitris sp.)***

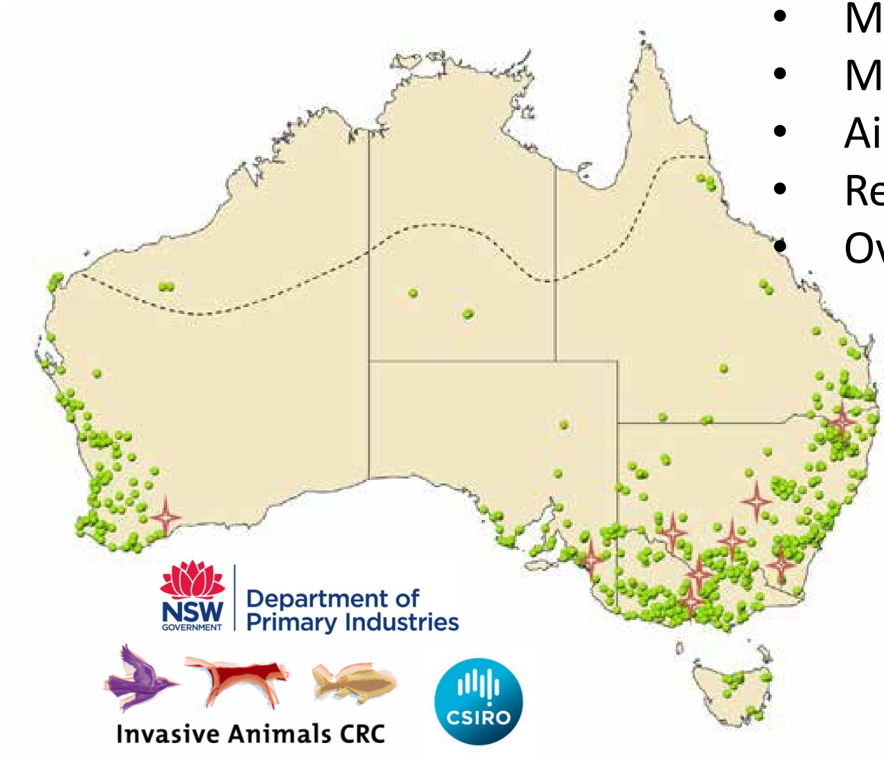
Sandell 2002




Today in Australia >300 threatened species and ecological communities are impacted by competition and land degradation caused by rabbits.
Rabbit exclosure on Macquarie Island
Image: Brian Cooke

Recent rabbit biocontrol developments in Australia

March 2017: Nationwide release of a new RHDVa variant from Korea (K5, GI.1a):



- More effective in genetically resistant rabbits
- More effective against endemic benign caliciviruses
- Aimed at slowing down rate of rabbit increase
- Released at >300 sites across Australia
- Overshadowed by the arrival of another RHDV.....

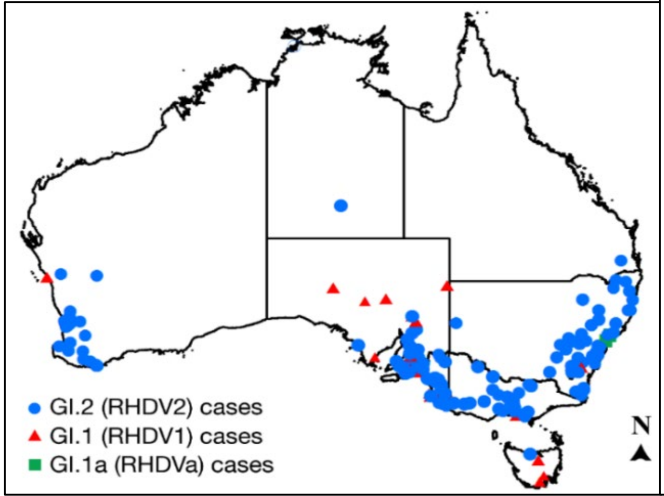
RHDV2 (GI.2) incursion (NOT released)

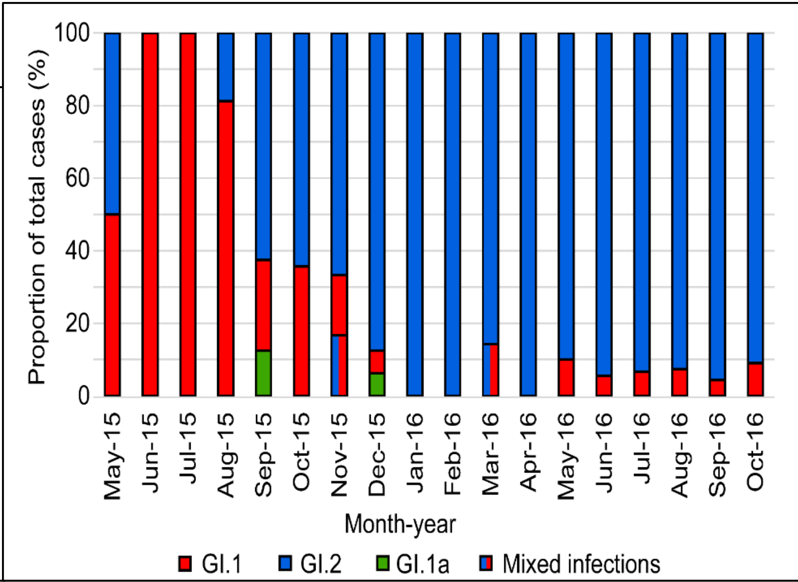
RHDV2: A new rabbit calicivirus

- Emerged in Europe in 2010
- Can overcome immunity to other strains (to some degree)
- Can kill young rabbits and other lagomorphs (incl. European hares)
- First reported in Australia in May 2015
- Has become dominant strain in Australia

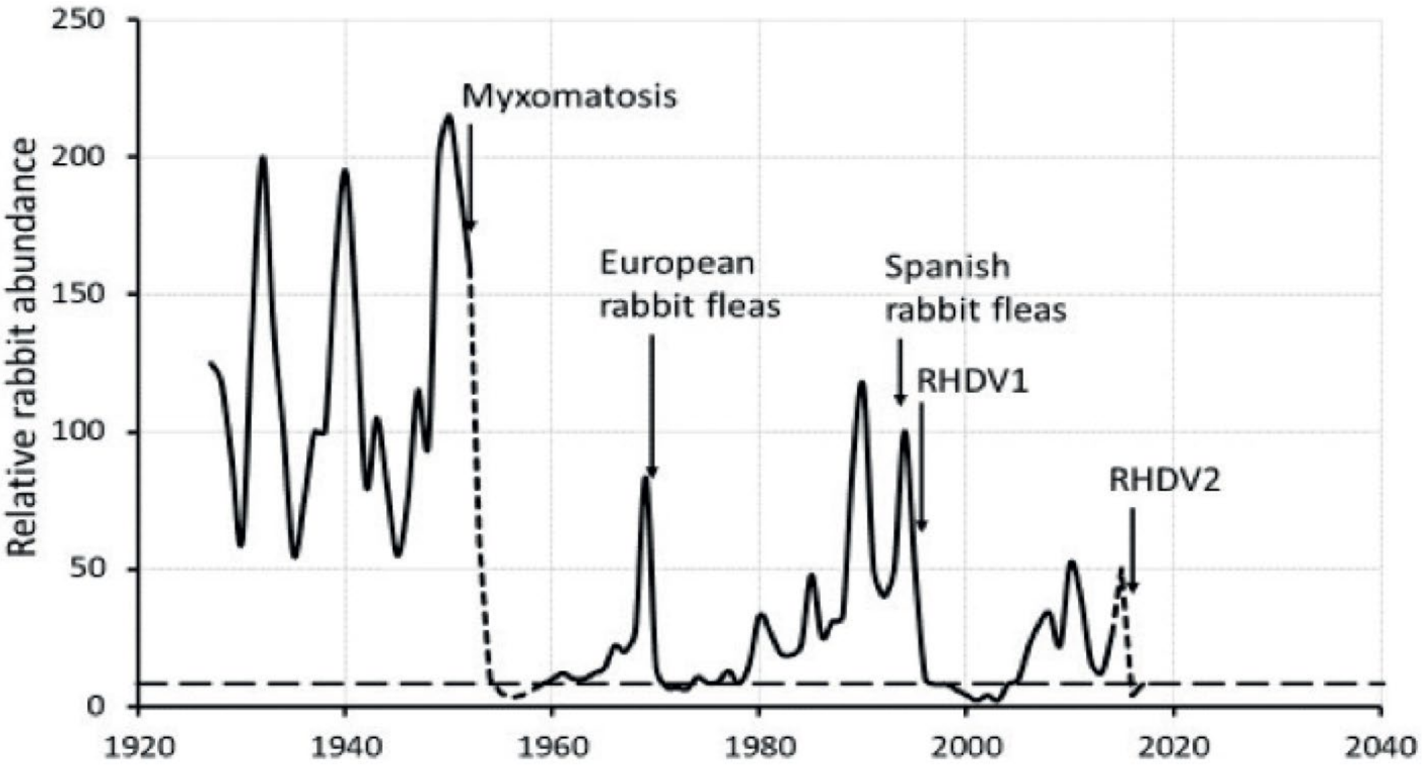
Global spread
(all continents)
'lagomorph covid'

Mahar, Hall et al 2018





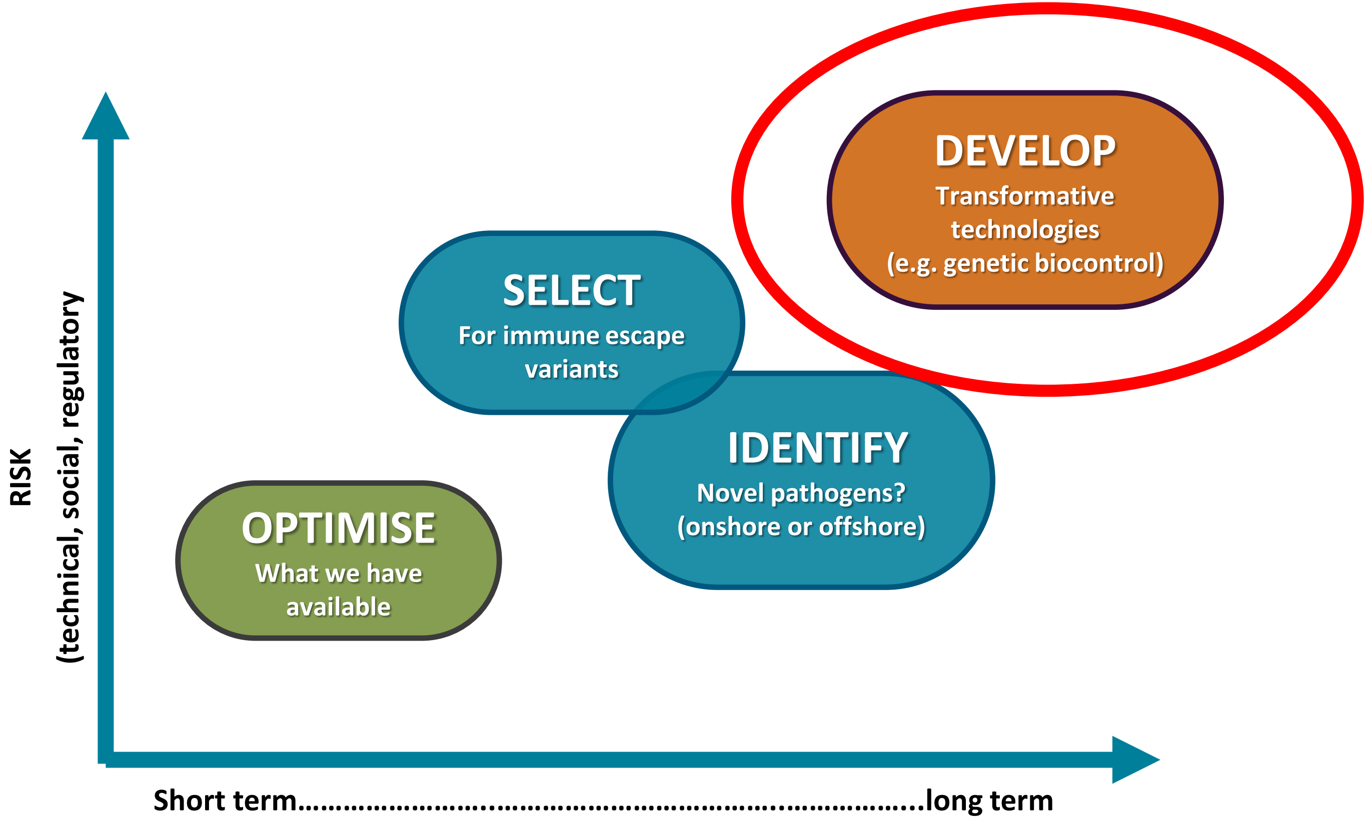
RHDV2 reduced Australian rabbit populations by ~60% (average)



Ramsey et al., 2020
Cooke et al., 2018

What's next in the Biocontrol pipeline?

AUSTRALIA'S RABBIT BIOCONTROL PIPELINE STRATEGY FOR 2022-27



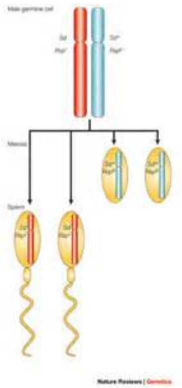
Genetic biocontrol of invasive animals:

1. Precision genetic engineering of the pest species

+

2. ‘driving’ the engineered trait (genes) into populations to control them

Example "Gene Drive"

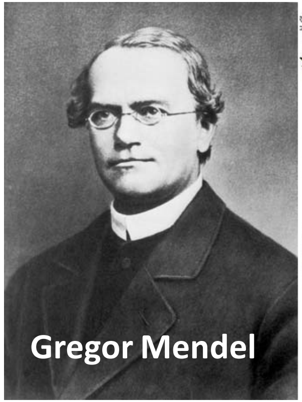


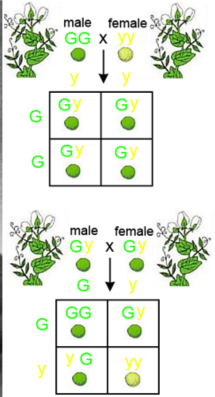
During sexual reproduction of diploid organisms, the alleles segregate and the embryo gets one from each parent (50:50)

Gene drives break the laws of Mendelian inheritance (>50% inheritance of certain alleles)

Natural gene drive:
meiotic drives, 'selfish genes', >50% inheritance

- *sd* in drosophila
- *t complex* in mice
- Homing endonucleases (HEGs)





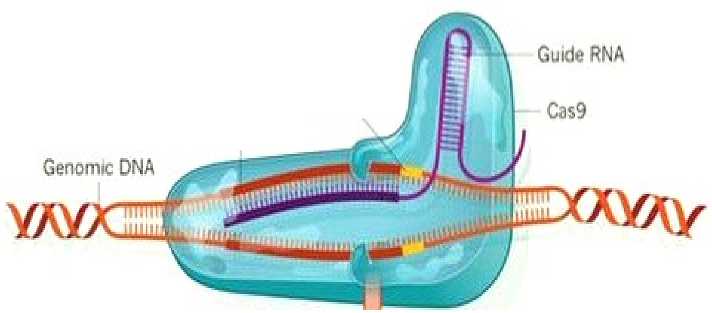
The ‘Precision engineering’ component: CRISPR-Cas9

Genome engineering tool derived from bacterial “immunity” system

CRISPR – clustered regularly interspaced short palindromic repeat

Cas9 - CRISPR associated protein 9 nuclease - cuts DNA

Guide RNA – tells Cas9 where to cut



How often will this cut a piece of DNA (at random)

Frequency of a **20 nt sequence =**

$4^{20} = 10^{12}$ = **1 in 1000 billion**

(human genome has approx. 6 billion nt)

Nobel Prize:

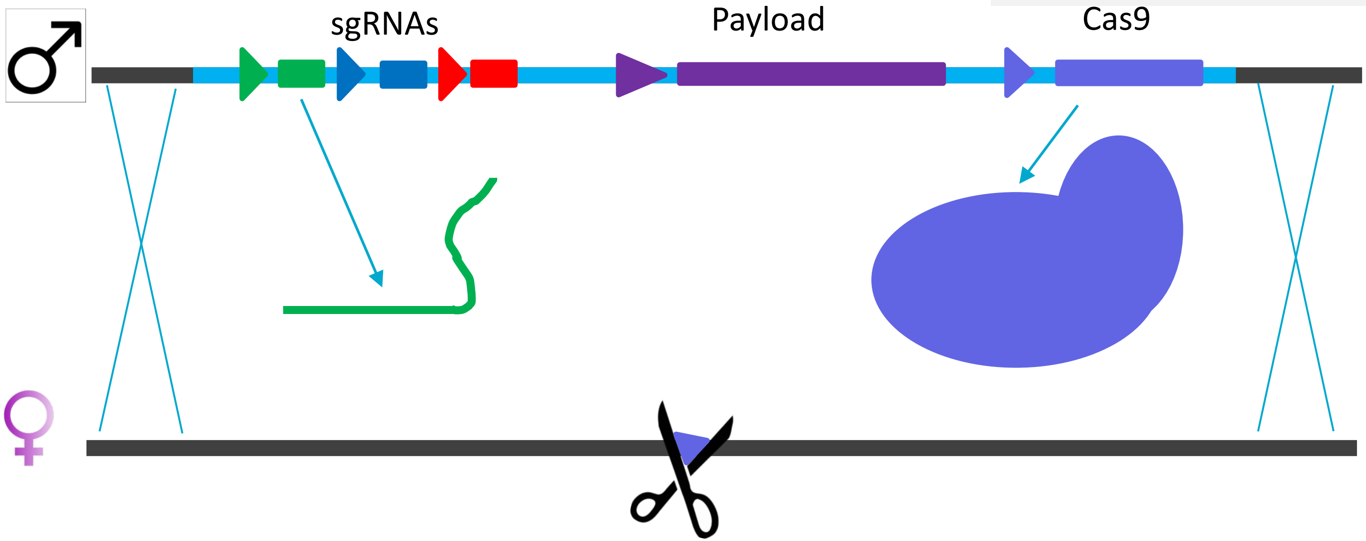
Jennifer Doudner, Emanuelle Charpentier 2012:

The components of a gene drive system:

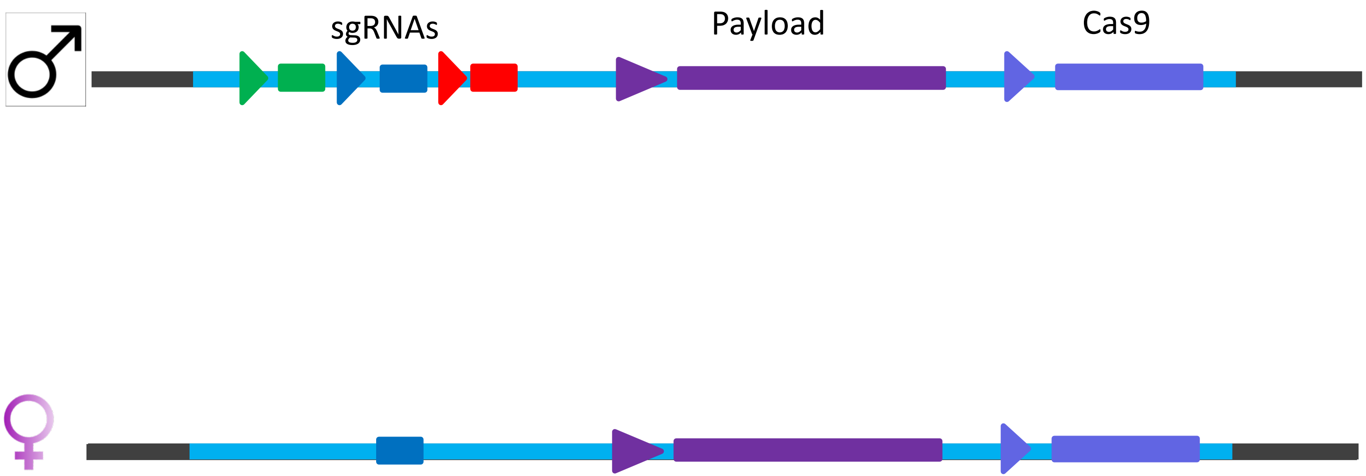
Genes encoding the Guide RNA(s)

Payload gene with biological impact e.g. **SRY (sex bias)**

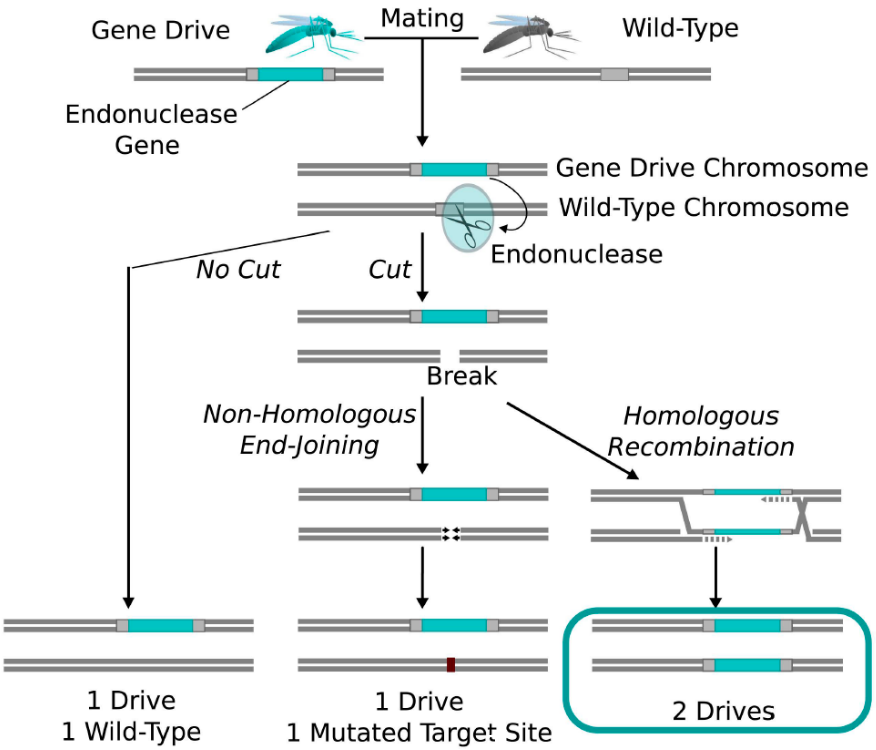
Gene for Cas9
Timing of Cas9 expression can be regulated
- Always 'on'
- Only in germline



The components of a gene drive system

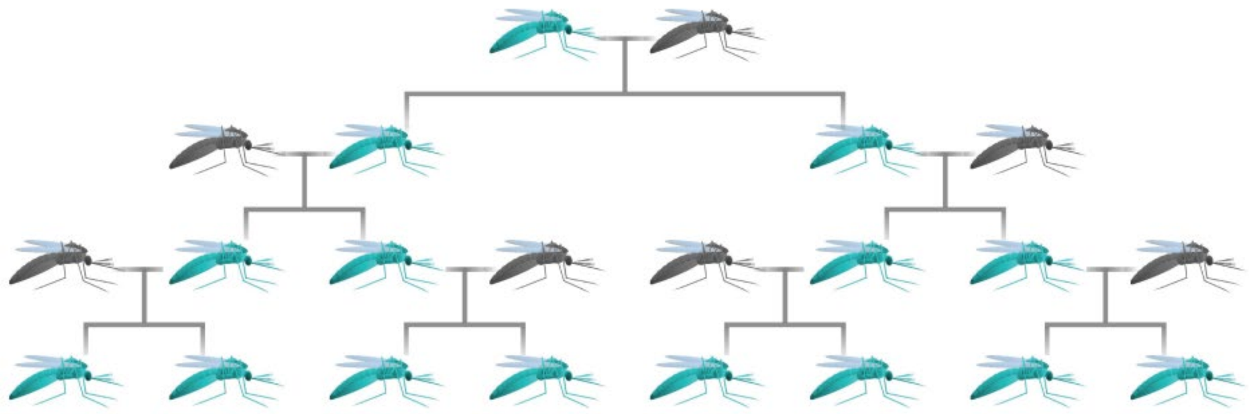


Gene drive mode of action (3 possible outcomes)



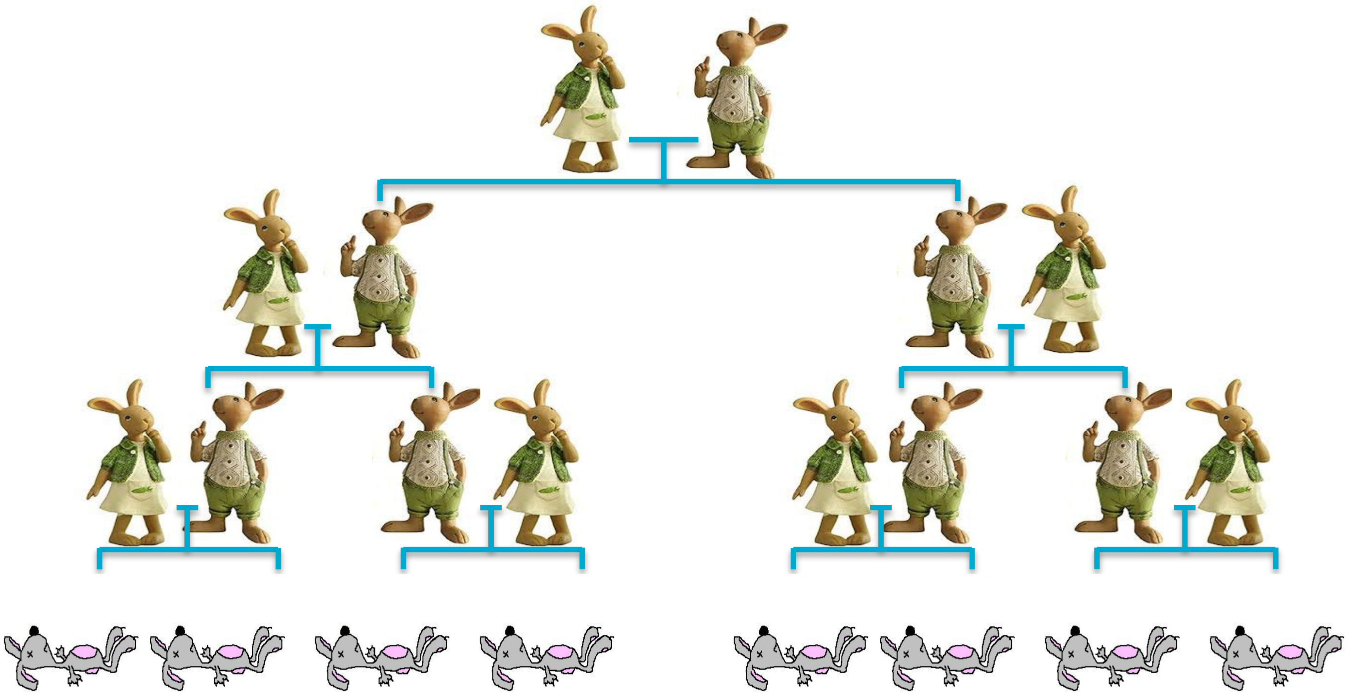
Esvelt et al., 2014 elife

Cas9-based gene drives



- 100% homing efficiency would lead to all offspring inheriting the trait (data from **insects and nematodes** show the real efficiency is between 60%-95%)
- In theory enables the drive to spread until it is present in all members of the population – even if it is mildly deleterious to the organism
- If trait was gene that turned all offspring into phenotypic males – population collapse

Cas9-based gene drives – biasing sex



Recent break through in mice !

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results

Follow this preprint

Leveraging a natural murine meiotic drive to suppress invasive populations

Luke Gierus, Aysegul Birand, Mark D. Bunting, Gelshan I. Godahewa, Sandra G. Piltz, Kevin P. Oh, Antoinette J. Piaggio, David W. Threadgill, John Godwin, Owain Edwards, Phillip Cassey, Joshua V. Ross, Thomas A.A. Prowse, Paul Q. Thomas

doi: https://doi.org/10.1101/2022.05.31.494104

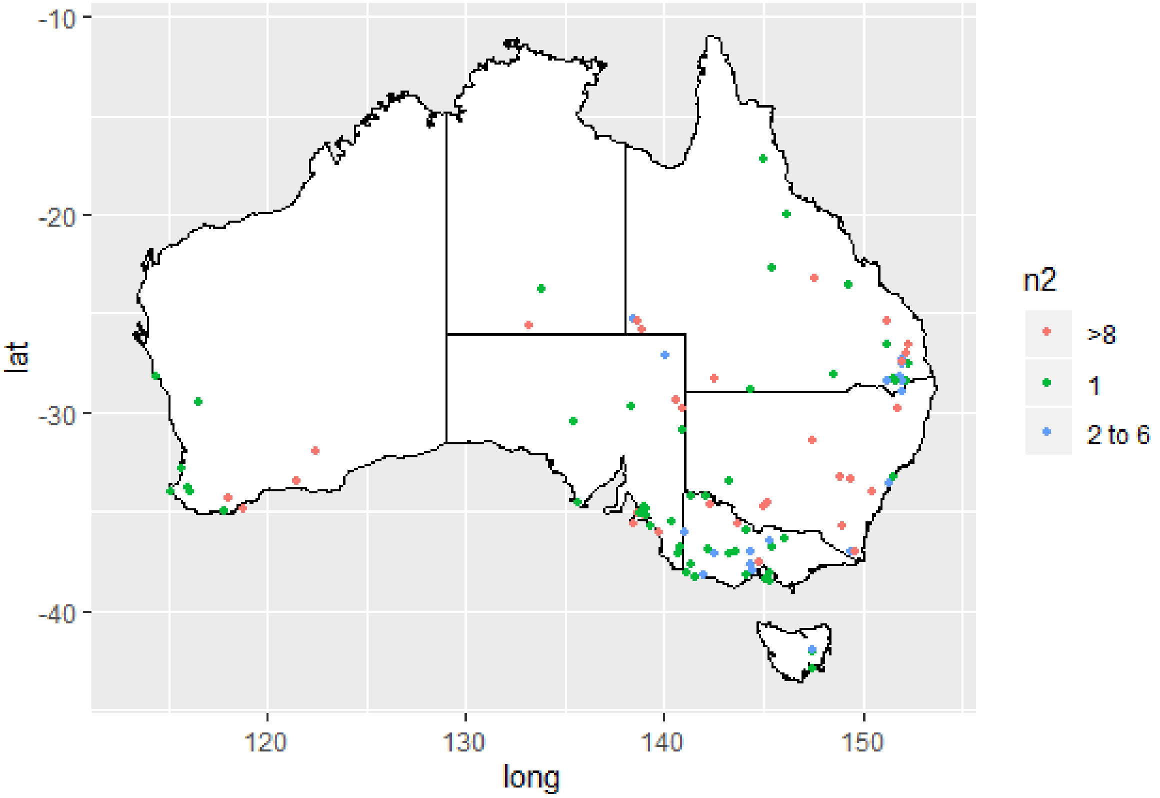
=> Transfer to rabbits?

Investigating the theoretical feasibility of genetic biocontrol for Australian wild rabbits

Know your genetics!

- Whole genome sequencing of ~300 individual Australian rabbits

- + ~180 from European collaborators



Key questions the dataset can answer:

Safety:

- Understand genetic diversity of Australia's wild rabbits

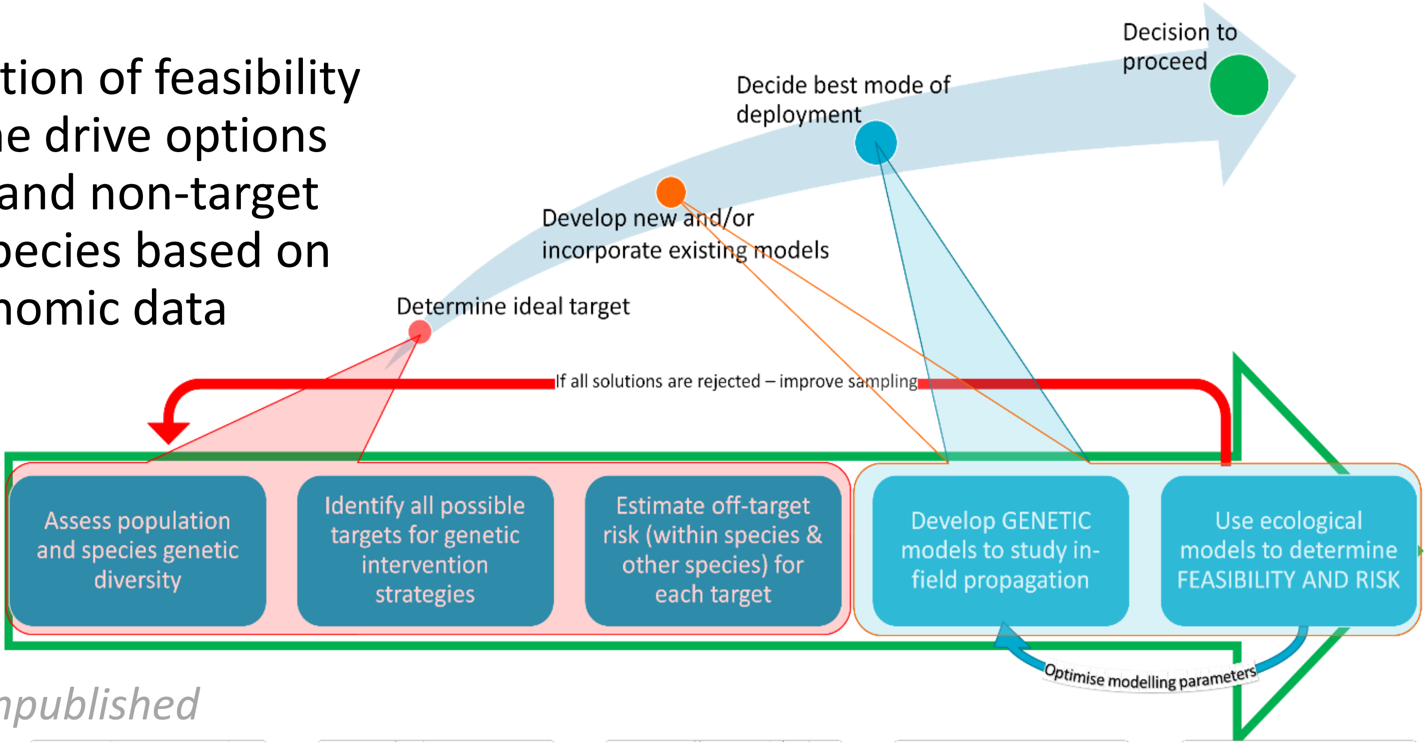
- Look for unique target sites ("Private alleles") that an Australian rabbit specific
  - shared amongst all Australian populations or just sub-populations?
  - absent (or at low frequency only) in Rabbits from the European continent?

Potential efficacy:

- Based on the genetic structure of the population, the spread of a theoretical genetic biocontrol can be modelled

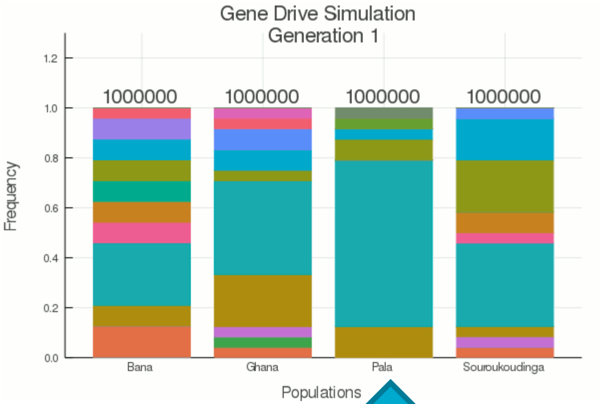
Gene drive Utility And Risk Determination pipeline (GUARD)

- *In silico* evaluation of feasibility and risk of gene drive options against target and non-target populations/species based on population genomic data

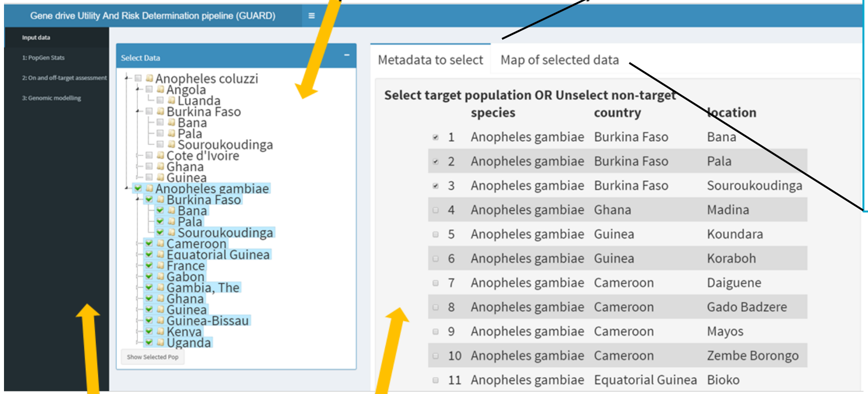


Yeap, Rane et al, unpublished

Example: Population sensitive gene drives for mosquitoes

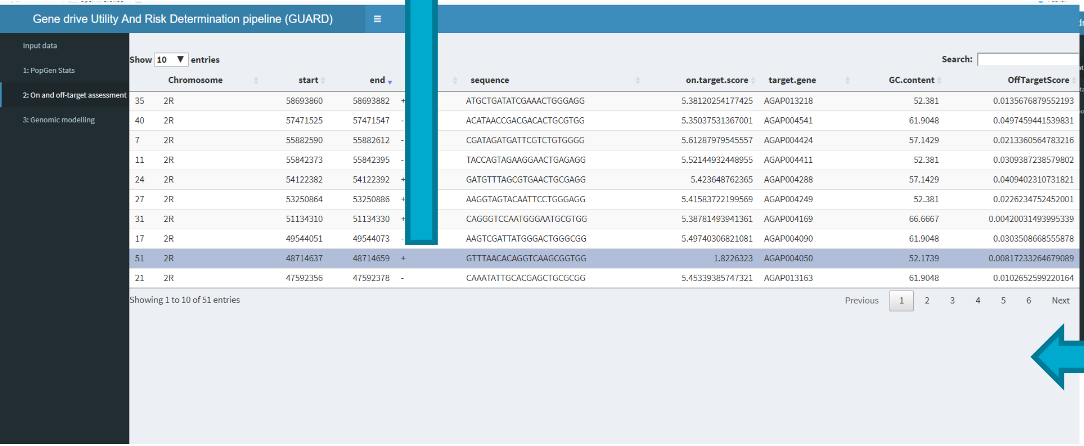


Population data to analyse

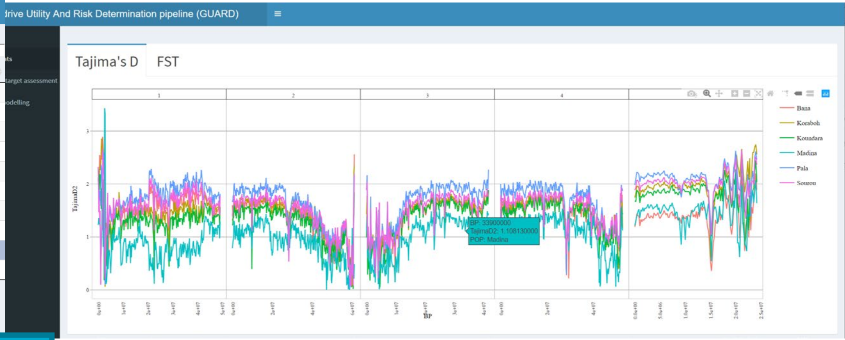


Modules in tabs

Selecting target population



	Chromosome	start	end		sequence	on.target.score	target.gene	GC.content	OffTargetScore
35	2R	58693860	58693882	+	ATGCTGATATCGAAACTGGGAGG	5.38120254177425	AGAP013218	52.381	0.0135676879552193
40	2R	57471525	57471547	-	ACATAACCGACGACACTGCGTGG	5.35037531367001	AGAP004541	61.9048	0.0497459441539831
7	2R	55882590	55882612	-	CGATAGATGATTCGTCTGTGGGG	5.61287979545557	AGAP004424	57.1429	0.0213360564783216
11	2R	55842373	55842395	-	TACCAGTAGAAGGAACTGAGAGG	5.52144932448955	AGAP004411	52.381	0.0309387238579802
24	2R	54122382	54122392	+	GATGTTTAGCGTGAACTGCGAGG	5.423648762365	AGAP004288	57.1429	0.0409402310731821
27	2R	53250864	53250886	+	AAGGTAGTACAATTCCTGGGAGG	5.41583722199569	AGAP004249	52.381	0.0226234752452001
31	2R	51134310	51134330	+	CAGGGTCCAATGGAATGCGTGG	5.38781493941361	AGAP004169	66.6667	0.00420031493995339
17	2R	49544051	49544073	-	AAGTCGATTATGGACTGGGCGG	5.49740306821081	AGAP004090	61.9048	0.0303508668555878
51	2R	48714637	48714659	+	GTTTAACACAGGTCAAGCGGTGG	1.8226323	AGAP004050	52.1739	0.00817233264679089
21	2R	47592356	47592378	-	CAAATATTGCACGAGCTGCGCGG	5.45339385747321	AGAP013163	61.9048	0.0102652599220164



Future work:

Can we transfer the functionality of the mouse proof of concept into rabbits?

=> Internal CSIRO investment into GBC (3 year)…..

watch this space !

Proceed – but with caution!

- Science
- Social License, Liaison
- Risk assessment
- Gene technology regulators

Australia's National Science Agency

Public perspectives towards using gene drive for invasive species management in Australia

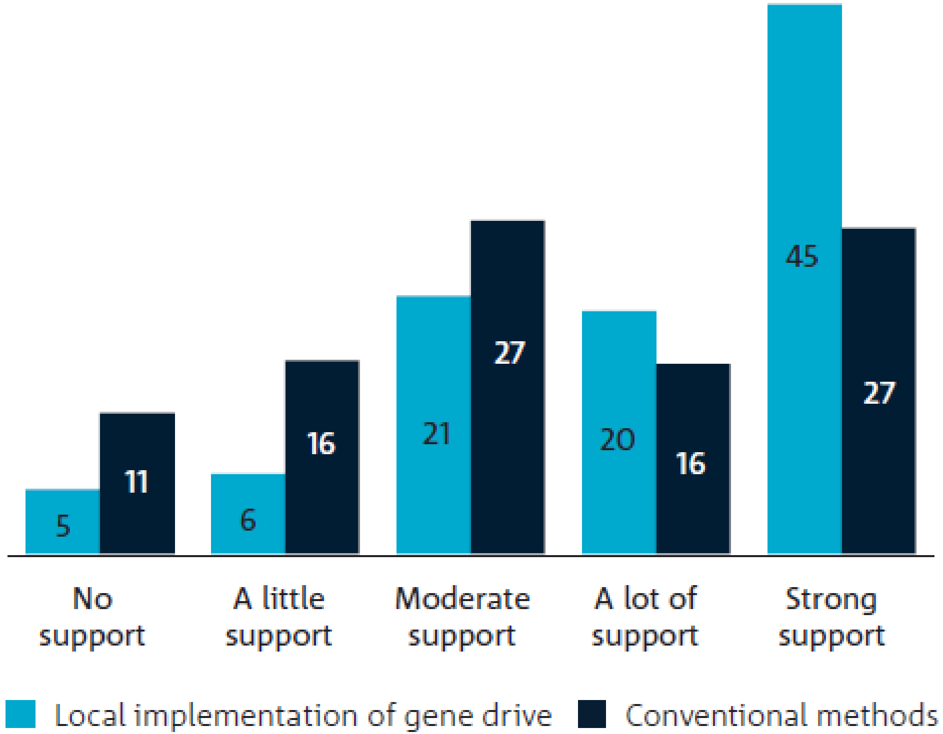


Figure 8. Levels of support for the local implementation of gene drive technology and conventional cat control methods, as a percentage (%) of total responses.

Genetic control technologies – unique *challenges and opportunities for Australian pest animal management*

Is it worth taking this risk? What is the risk of not trying?

Thank You

Recent RHDV and/or MYXV work

CSIRO

Robyn Hall
Egi Kardia
Maria Jenckel
Lena Smertina
Nina Huang
Ina Smith
Matt Neave
Rahul Rane
HengLin Yeap
Angel Popa
Peter Kerr
June Liu
Ros Mourant
Tegan King
Mel Piper

Hugh Mason
Megan Pavy
Madi Rutherford
Maria Jenckel
Omid Fakhri
Tom Walsh
Tek Tay
Raghu
Sathyamurthy
Mark Tizard
Agus Sunarto
Caitlin Cooper

The University of Sydney
Eddie Holmes
Jackie Mahar
JS-Eden
Mang Shi

University of Canberra — Australia's Capital University
Michael Frese
Brian Cooke
Peter Elsworth

NSW Government — Department of Primary Industries
Tarnya Cox
Emma Sawyers
Glen Saunders
Pat Taggart

Government of South Australia — Primary Industries and Regions SA
Greg Mutze
Dave Peacock
John Kovaliski
Kandarp Patel

BCM — Baylor College of Medicine
Mary K Estes

Centre for Invasive Species Solutions

Victoria State Government — Economic Development, Jobs, Transport and Resources
Dave Ramsey
Carlo Pacioni

Lorenzo Capucci

University of Cambridge
Joel Alves
Frank Jiggins

Australian Government — Department of Agriculture and Water Resources

PennState College of Agricultural Sciences
Andrew Read
Isabella Cattadori

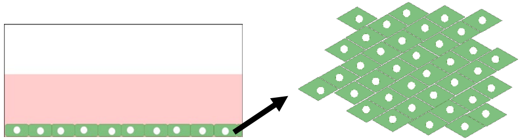
cibio
Miguel Carneiro

mla — Meat & Livestock Australia

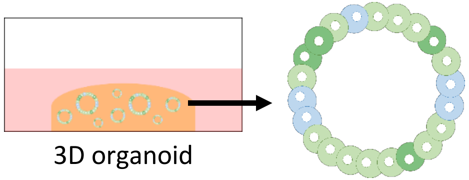
Invasive Animals CRC

awi — Australian Wool Innovation Limited

We need a robust cell culture system/reverse genetics



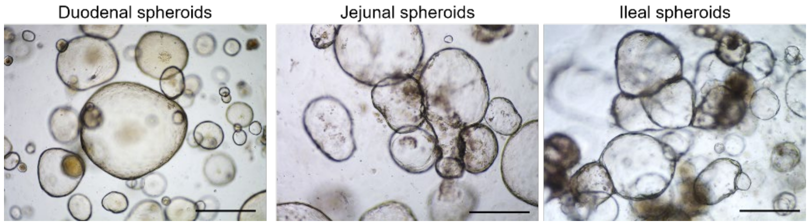
2D monolayer



3D organoid

3D culture

- Multicellular
- More relevant model
- Mimic the architecture of the parental tissue



➢ **Developing rabbit intestinal and liver organoids to study enterotropic and hepatotropic lagoviruses**

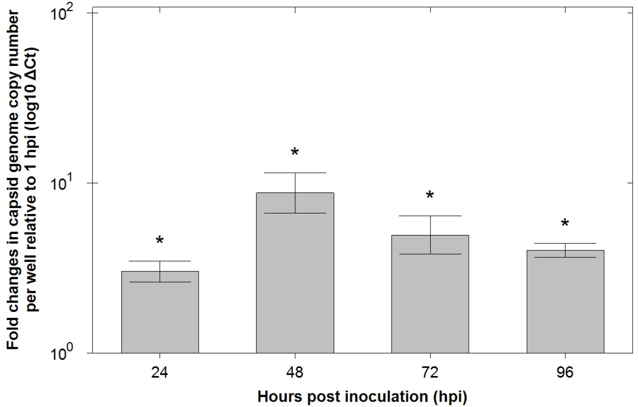
- Replication mechanisms
- Virulence mechanisms
- Tissue tropism/virulence evolution
- Accelerated natural selection of immune variants

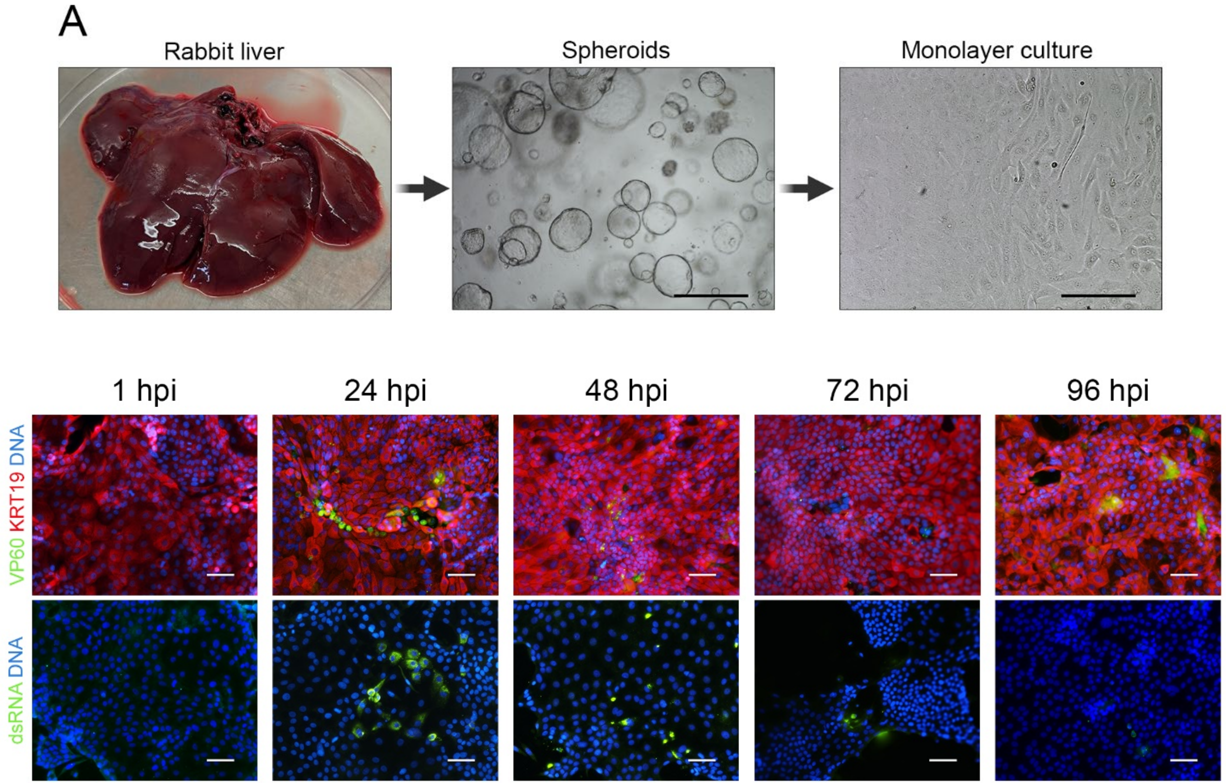
Baylor College of Medicine

Mary K Estes

Robyn N Hall, Egi Kardia, Omid Fakhri

Rabbit liver organoids: replication proof of concept





Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results

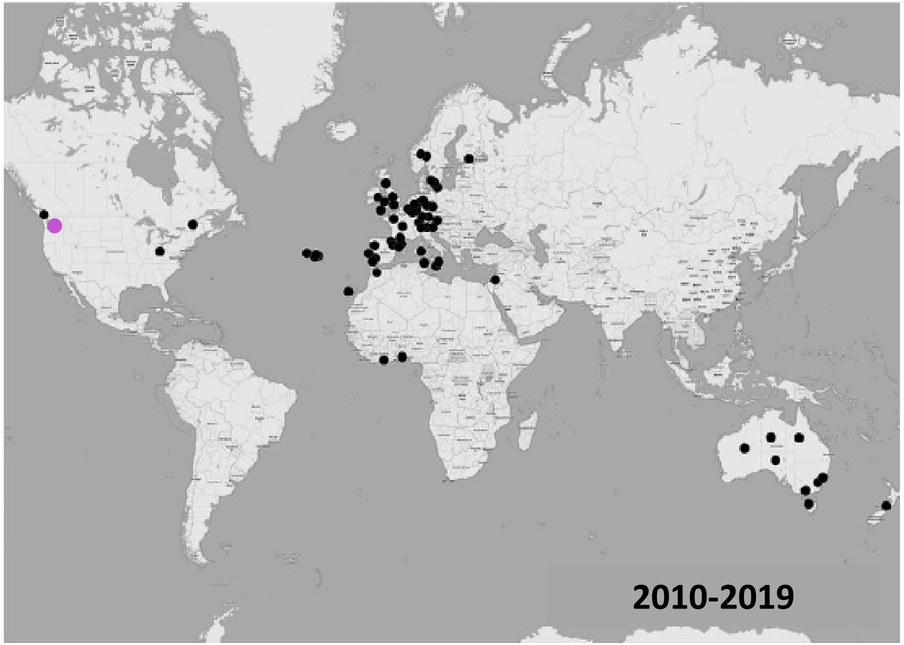
Follow this preprint

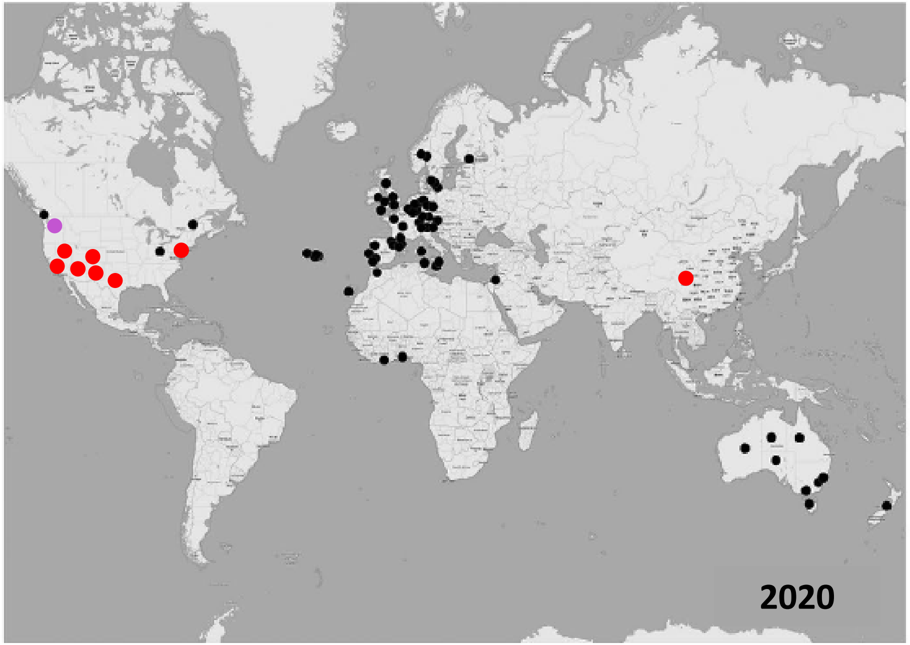
Hepatobiliary organoids derived from leporids support the replication of hepatotropic lagoviruses

Egi Kardia, Omid Fakhri, Megan Pavy, Hugh Mason, Nina Huang, Elena Smertina, Mary K. Estes, Tanja Strive, Michael Frese, Robyn N. Hall

doi: https://doi.org/10.1101/2022.04.07.487566

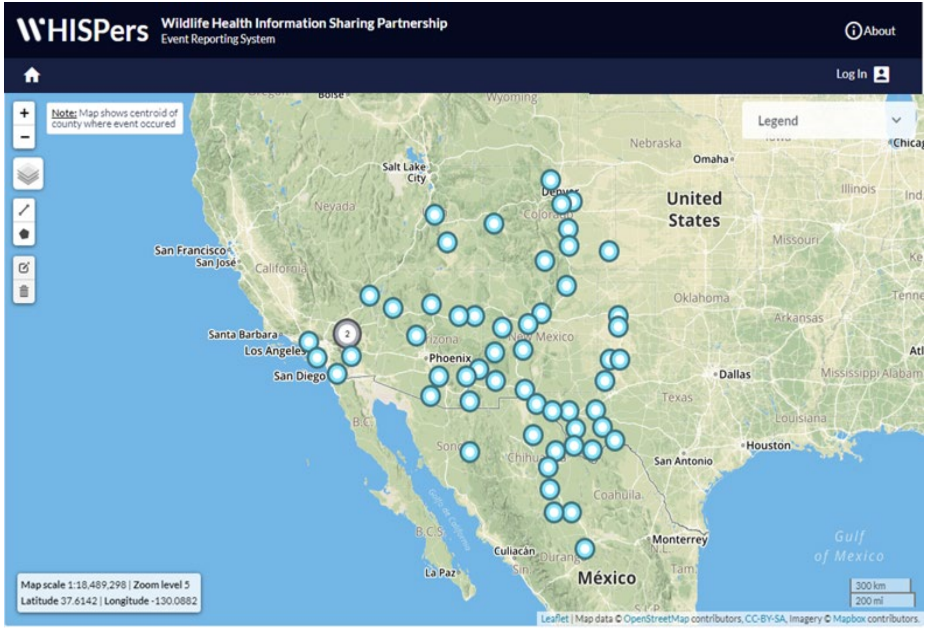
Global Spread of RHDV2: 2010 - 2020





RHDV2 in the southwestern US

- First reported in Spring 2020
- A series of native lagomorphs affected



USGS science for a changing world
National Wildlife Health Center
Wildlife Health Bulletin 2020-06

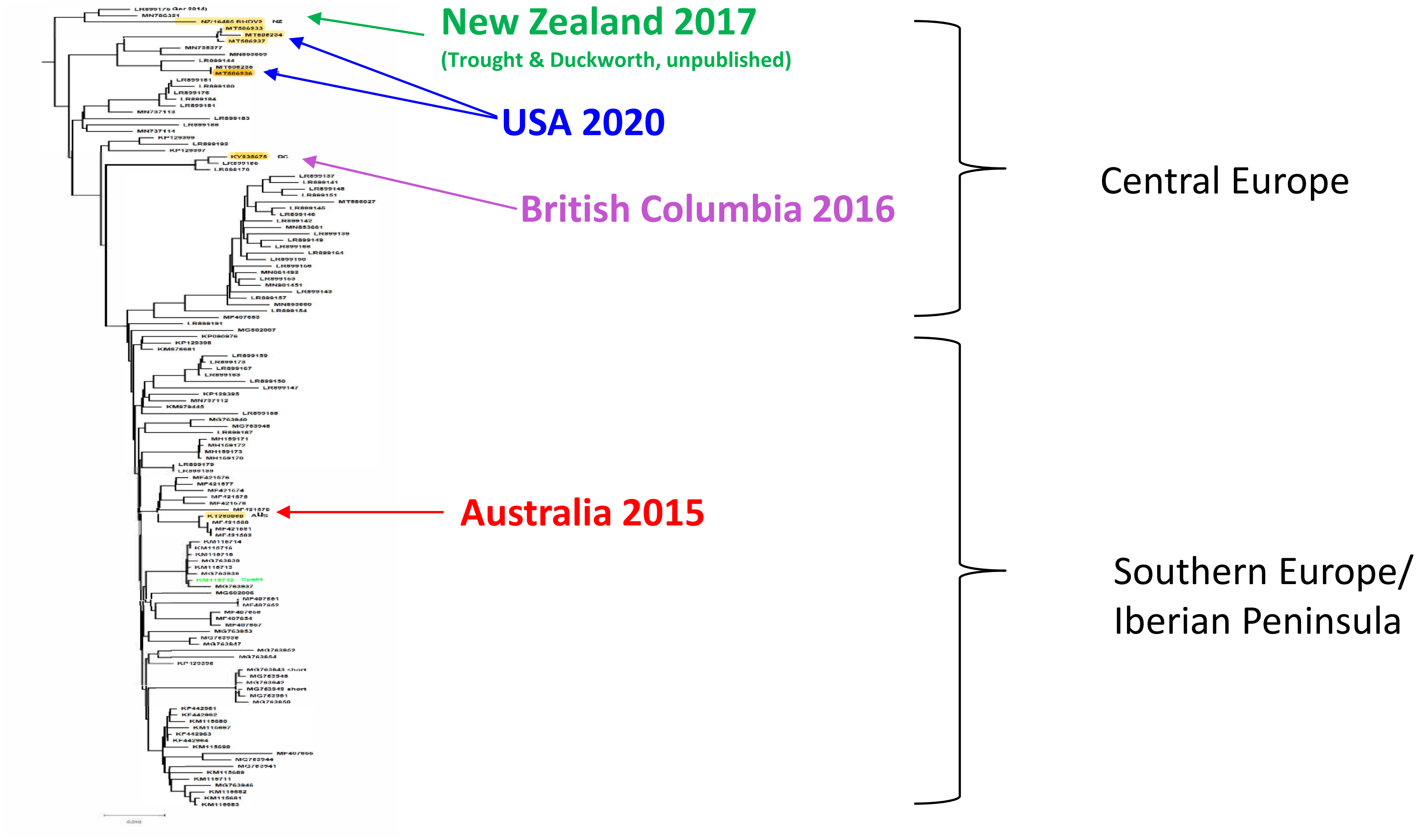
Sylvilagus floridanus

Lepus californicus

Sylvilagus audubonii

Sylvilagus nuttalii

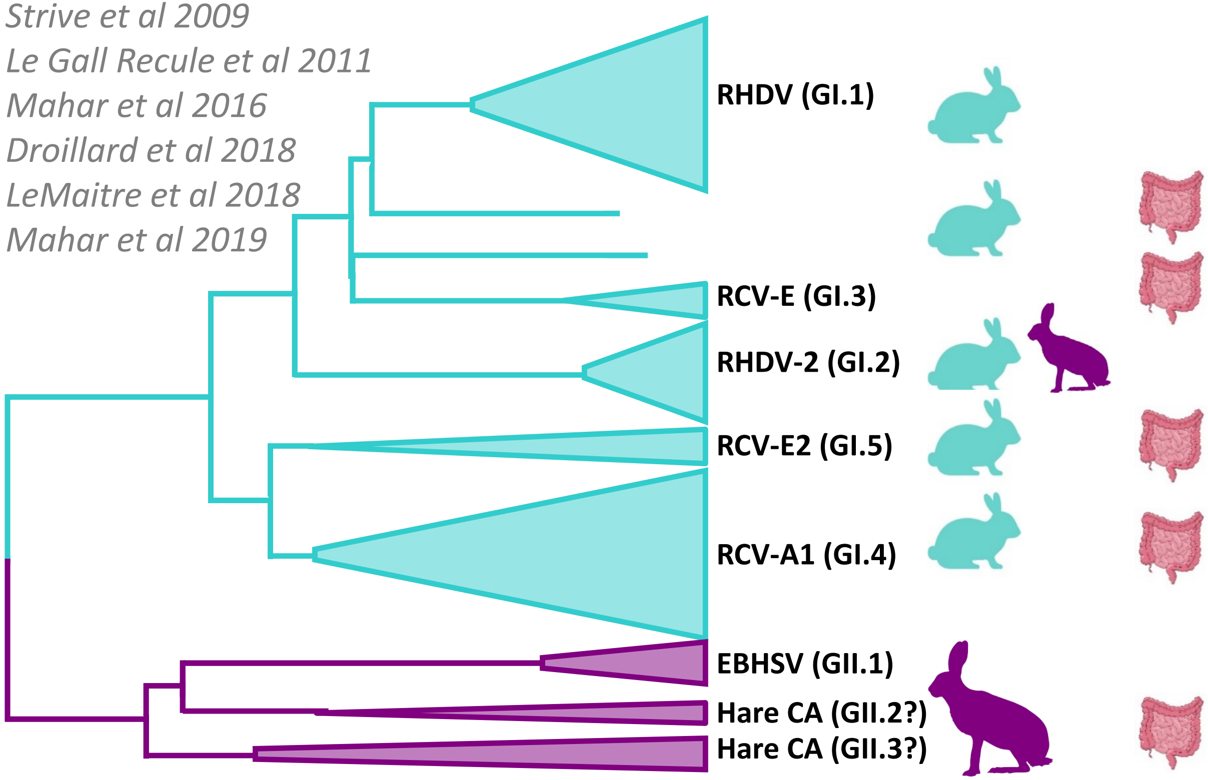
Lepus alleni


New Zealand 2017
(Trought & Duckworth, unpublished)
USA 2020
British Columbia 2016
Australia 2015
Central Europe
Southern Europe/
Iberian Peninsula

Matt Neave & Robyn Hall, CSIRO

Evolution of virulence in lagoviruses

Capucci et al 1996
Strive et al 2009
Le Gall Recule et al 2011
Mahar et al 2016
Droillard et al 2018
LeMaitre et al 2018
Mahar et al 2019



Mahar et al., 2016, Mahar et al., 2019

Evolution of virulence in lagoviruses

Capucci et al 1996
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Mahar et al 2016
Droillard et al 2018
LeMaitre et al 2018
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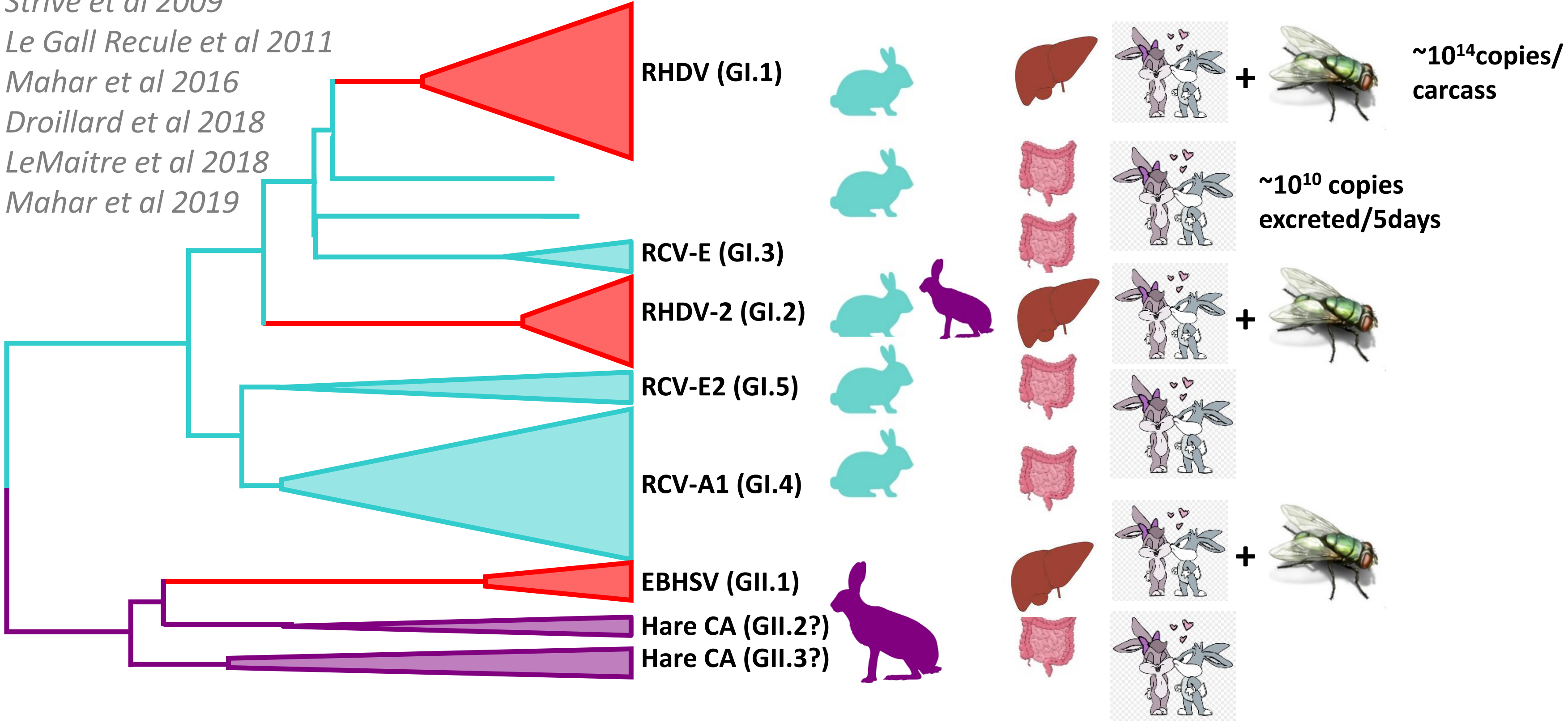


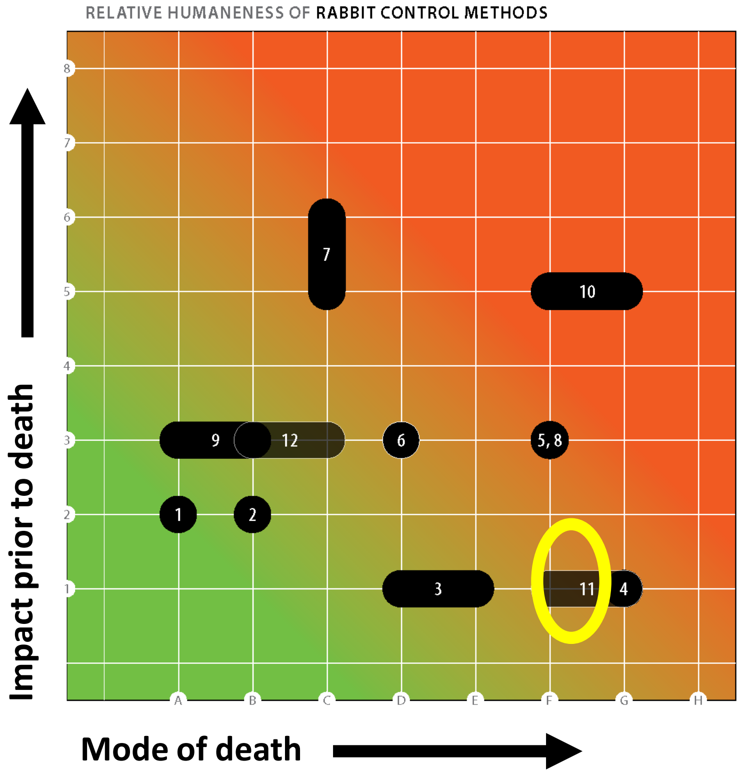
Animal Welfare

Is an integral component of pest animal control

Factor to be considered when choosing the appropriate tool for pest animal management

What is the consequence of doing nothing?

Scoring Matrix to assess the welfare impacts of rabbit control methods approved in Australia



Sharp and Saunders 2012

- Shooting (head)
- Shooting (chest)
- (Warren blasting)
- LPG Warren fumigation
- 1080 poison
- Phosphine fumigation
- Baits RHDV
- Chloropicrin fumigation
- Warren ripping
- Pindone anticoagulant
- RHDV inoculation
- Padded foot hold trap