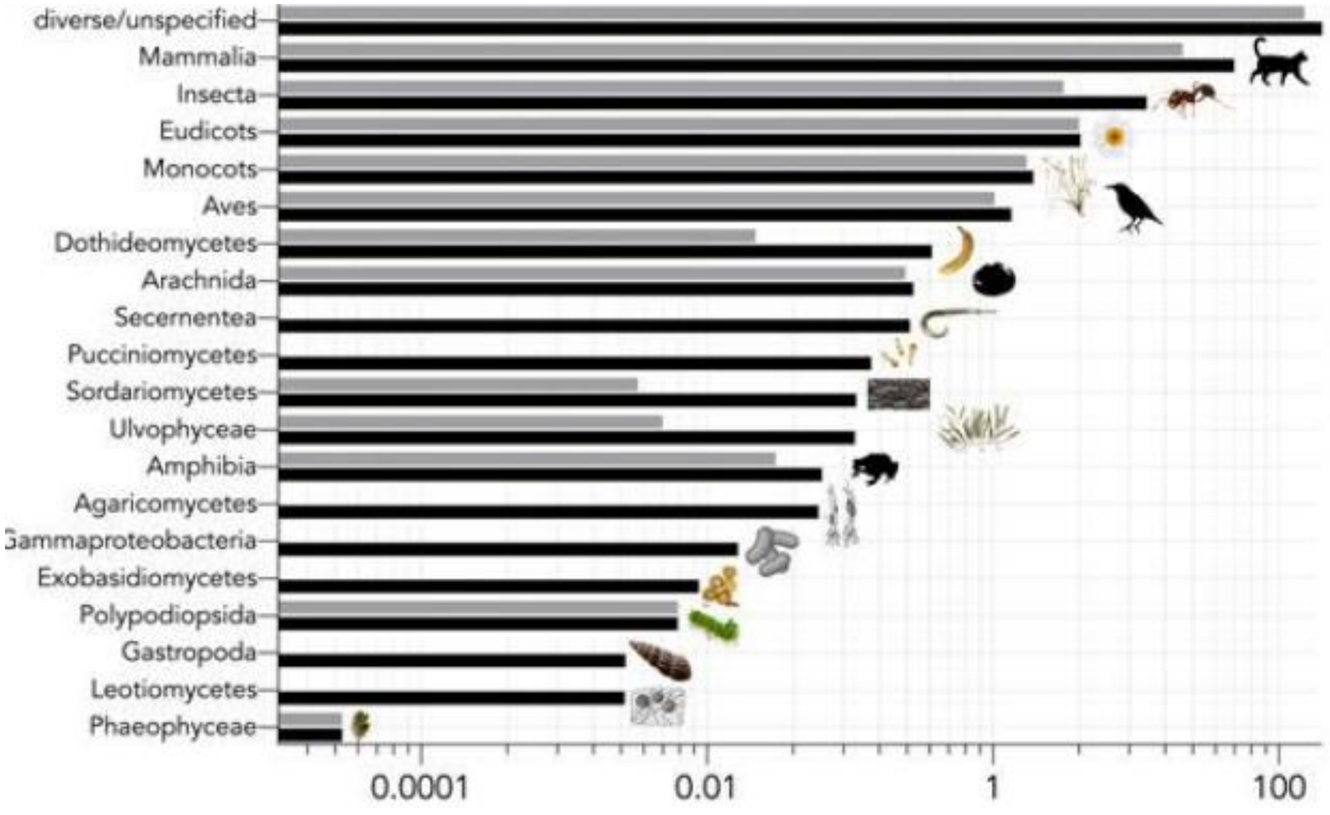
Prospects for genetic biocontrol for vertebrate pest control in Australia

Outline:

- Established pests in Australia
- Why we love self-disseminating biocontrols
- A very brief introduction on genetic biocontrol (GBC)
- Australian perspective: Stakeholder engagement
  - Prioritisation framework for stakeholders and potential funders of GBC work
  - Public attitudes
- Australian perspective: Technical developments & future plans
  - Mice
  - Rabbits

Established pests in Australia



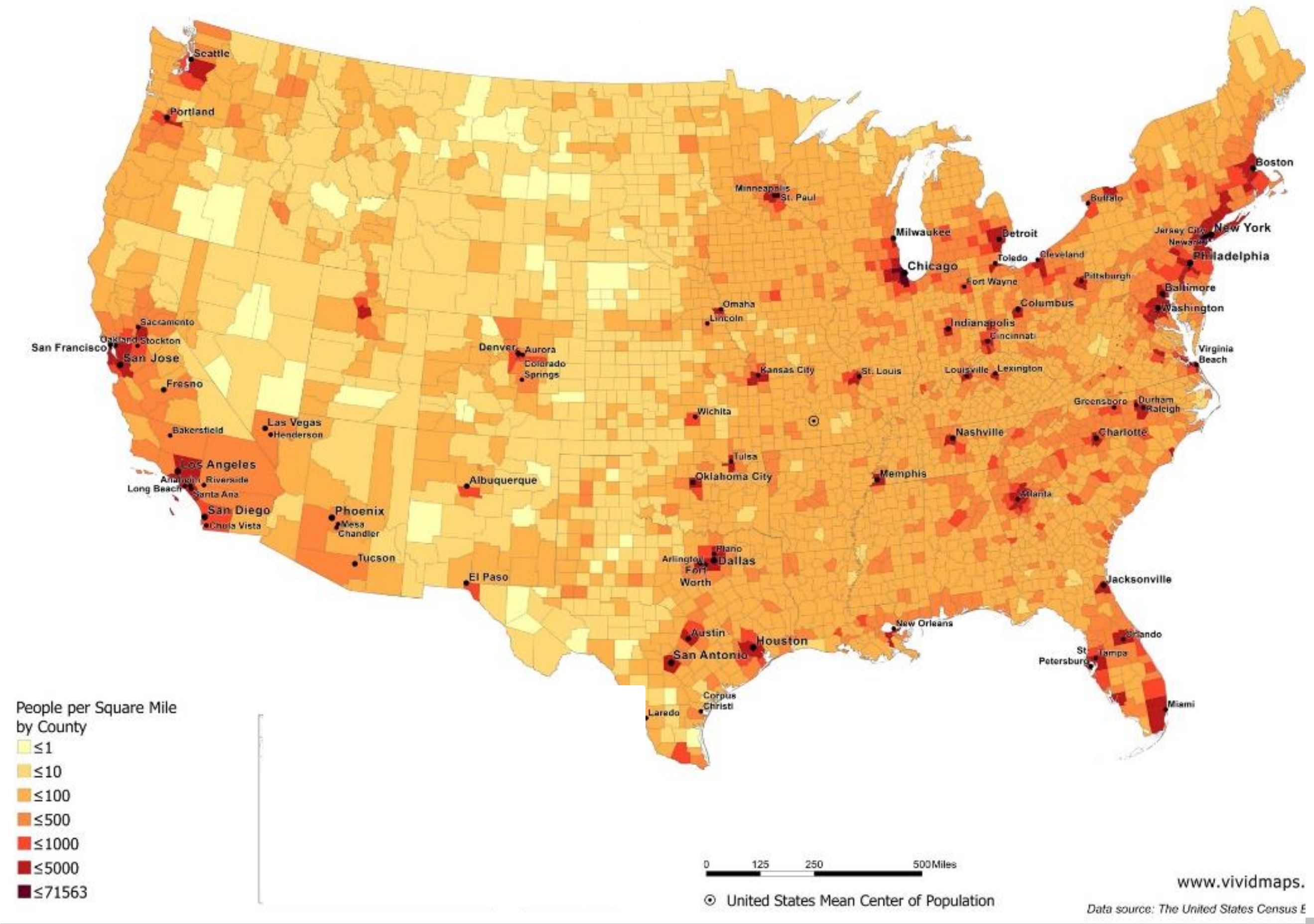
Since the 1960s, Australia has spent or incurred losses of a total of at least **US$298.58 billion** from invasive species.

Bradshaw et al., 2021, NeoBiota

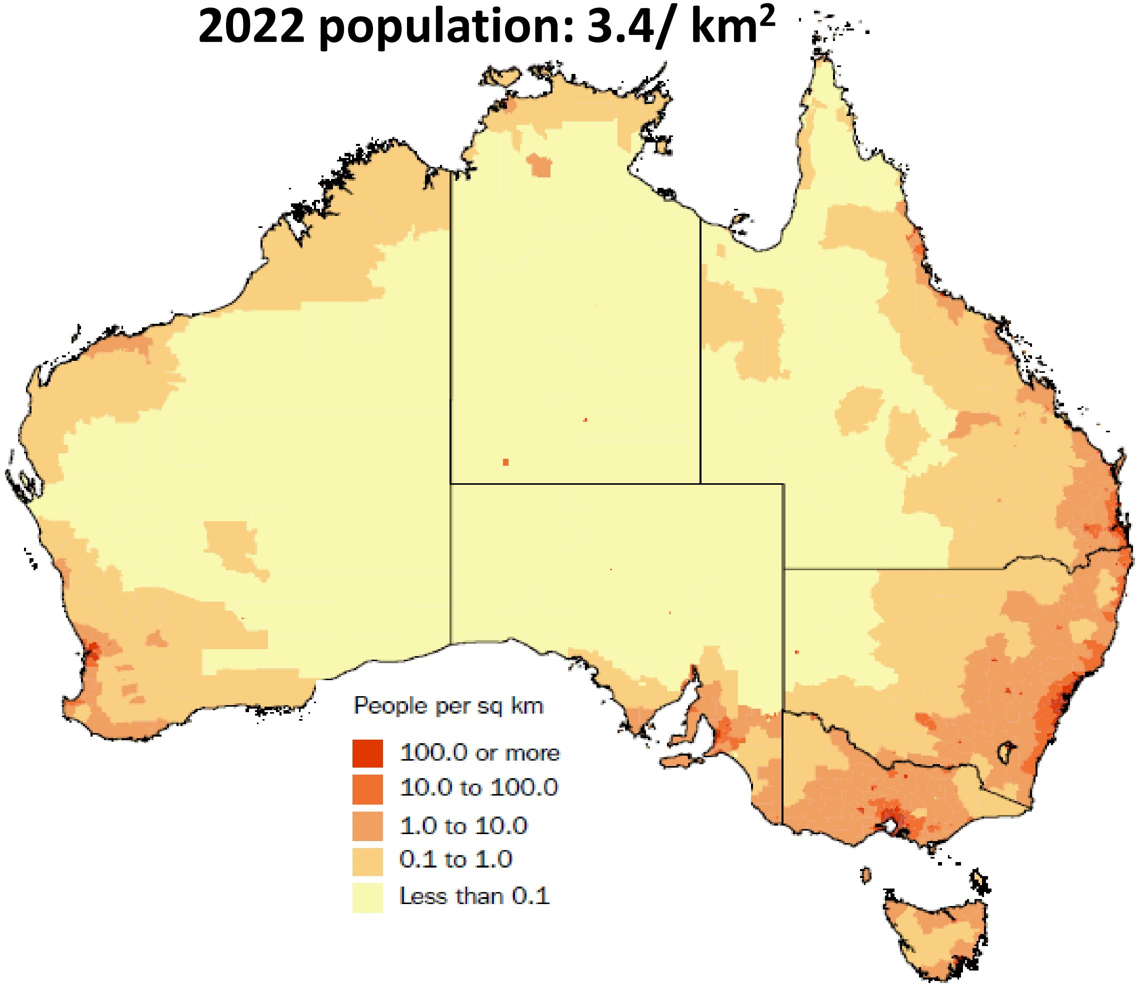
Managing established pests & weeds……..

Why Australia loves biological controls….

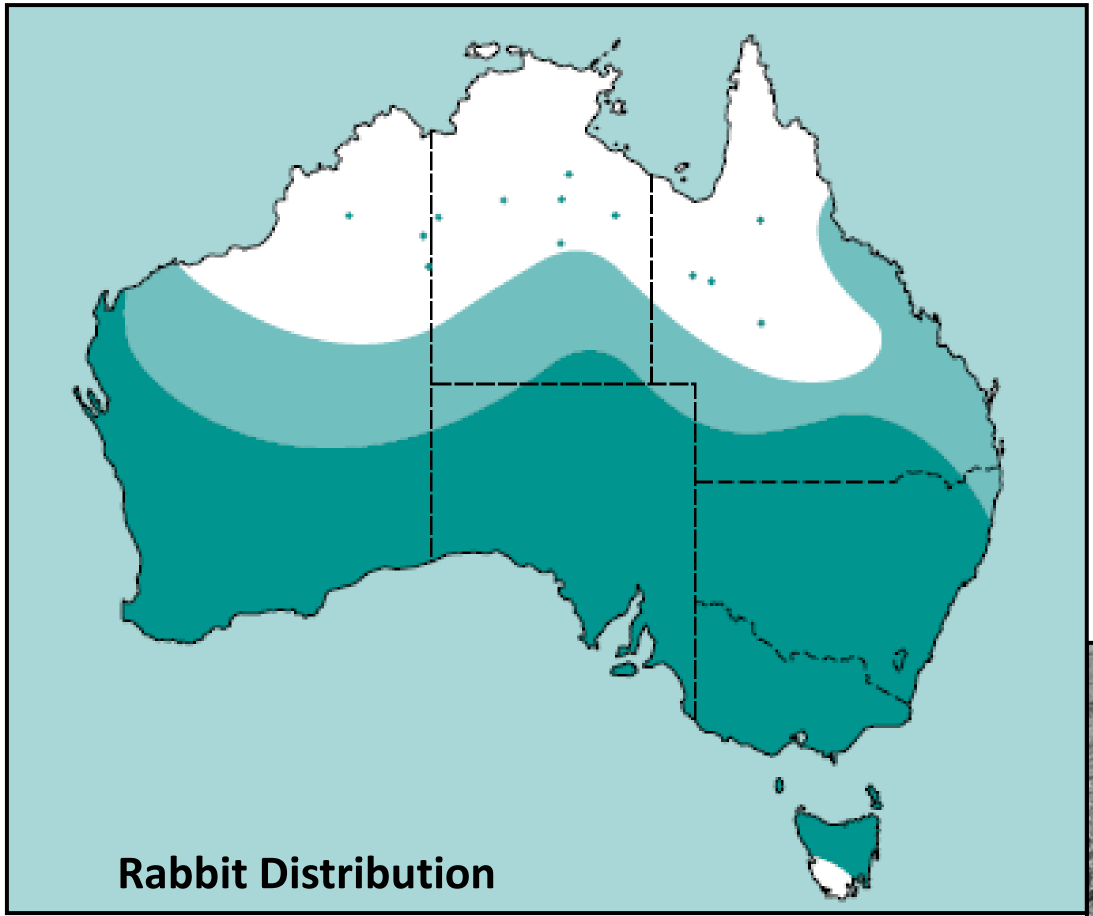
Contiguous US: 7.66 million km^2
2022 population: 42.9/ km^2



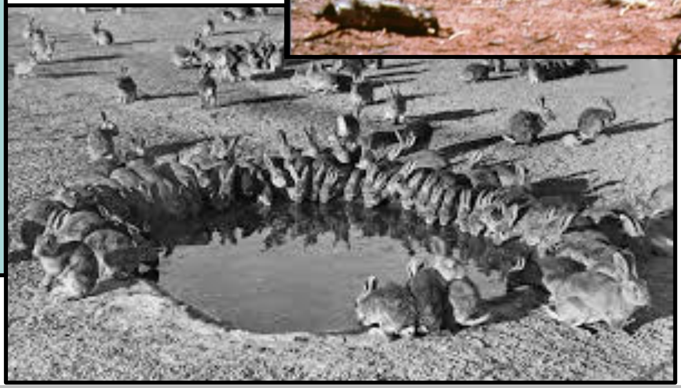
Australia: 7.66 million km^2
2022 population: 3.4/ km^2



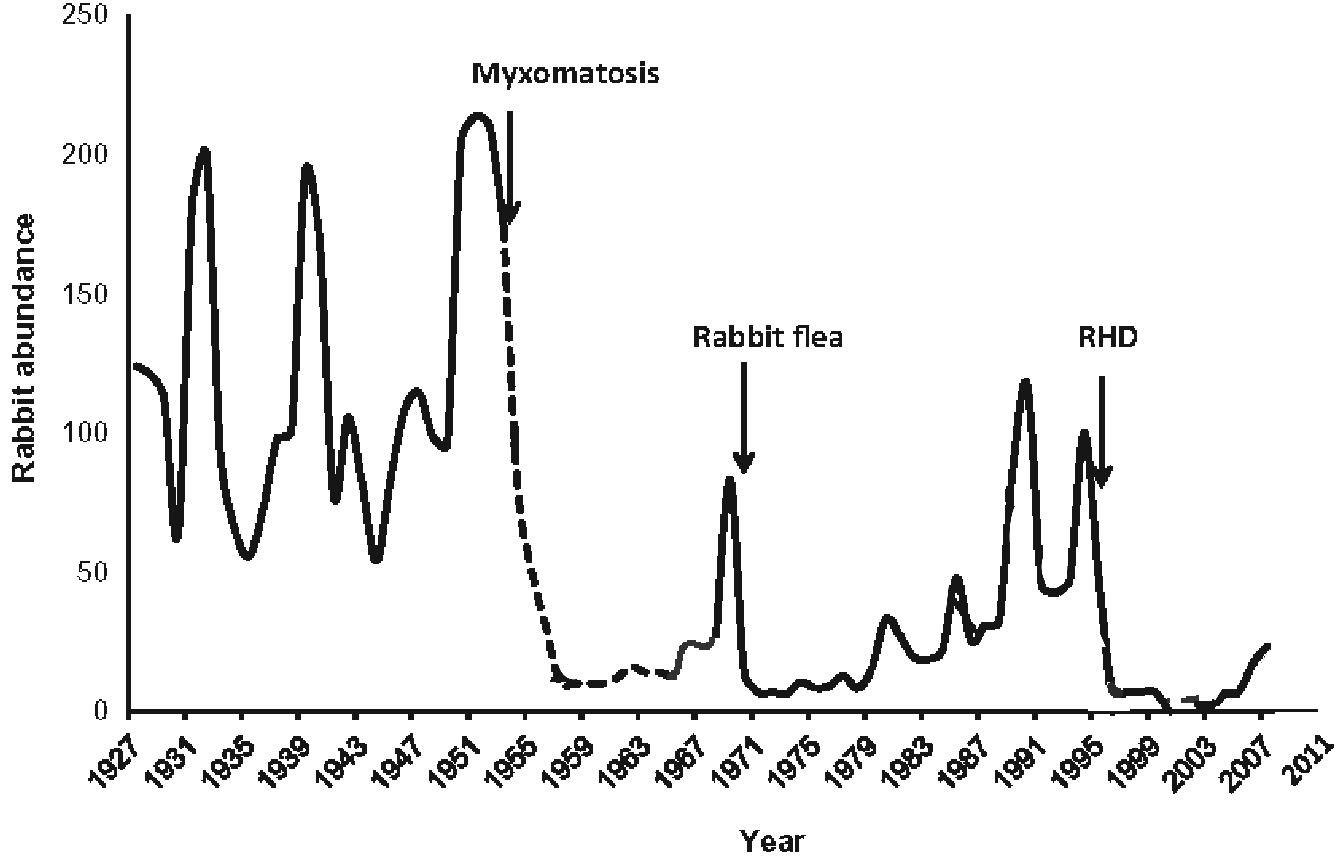
Example for successful landscape-scale biocontrol: rabbits



- Myxoma virus (1950)
- Rabbit calicivirus(es) 1996 onwards



Example for successful landscape-scale biocontrol: rabbits



- Rabbit biological control delivered >$70 billion AUD economic benefits over 60 years
- Self-disseminating, incl. insect transmission over distances
- Once established causes repeated outbreaks
- NOT a silver bullet: Virus will not eradicate the host
- ongoing host-pathogen co-evolution
- Repeated adjustments required to maintain gains

Conservation Biology

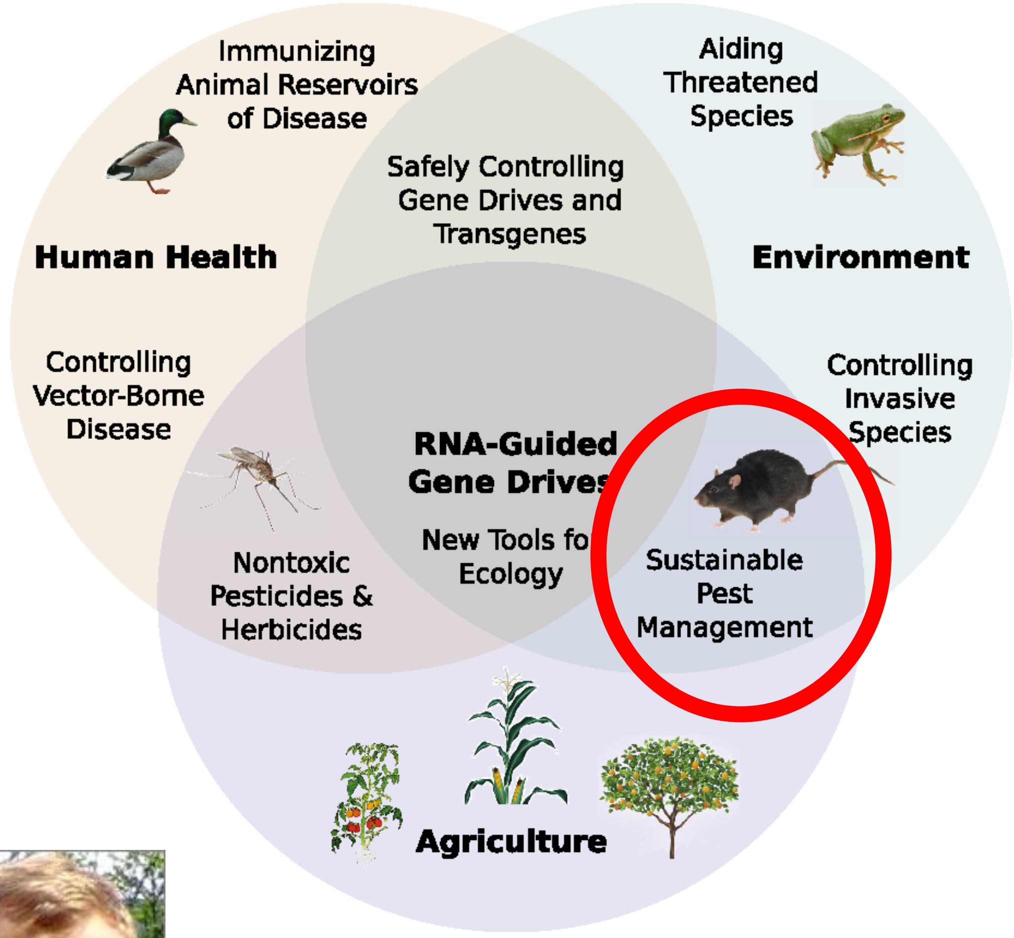
Contributed Paper

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[†‡]

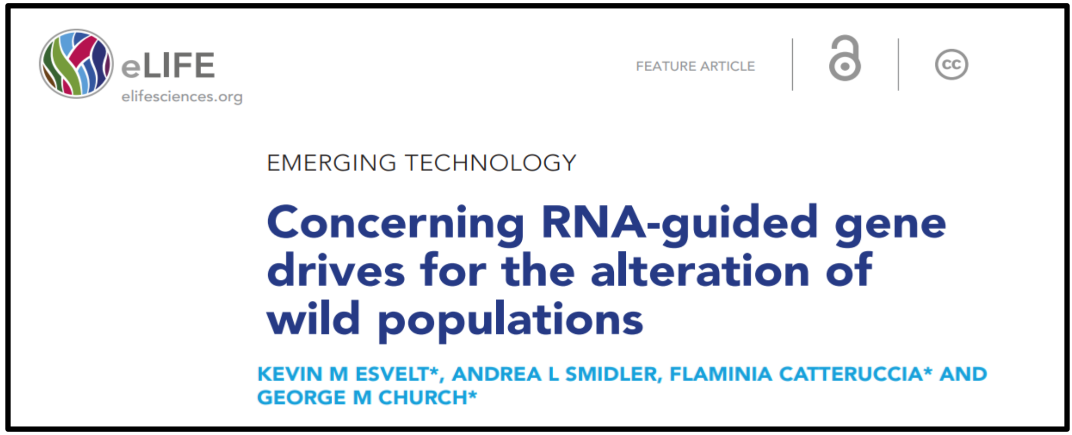
- ➢ Suppression of rabbits -> suppression of foxes and cats!
- ➢ Recovery of 3 mammalian species through 20 years of sustained rabbit suppression from RHDV
- ➢ Removal from the IUCN red list

Genetic biocontrol for pest animal population control – new hope?



- Combining precision engineering using CRISPT/Cas9 and cellular repair mechanisms to create a selfish gene from scratch

Esvelt et al 2014



eLIFE elifesciences.org

FEATURE ARTICLE

EMERGING TECHNOLOGY

Concerning RNA-guided gene drives for the alteration of wild populations

KEVIN M ESVELT*, ANDREA L SMIDLER, FLAMINIA CATTERUCCIA* AND GEORGE M CHURCH*

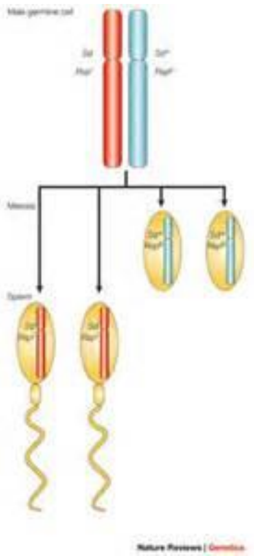
What are “Gene Drives” ?

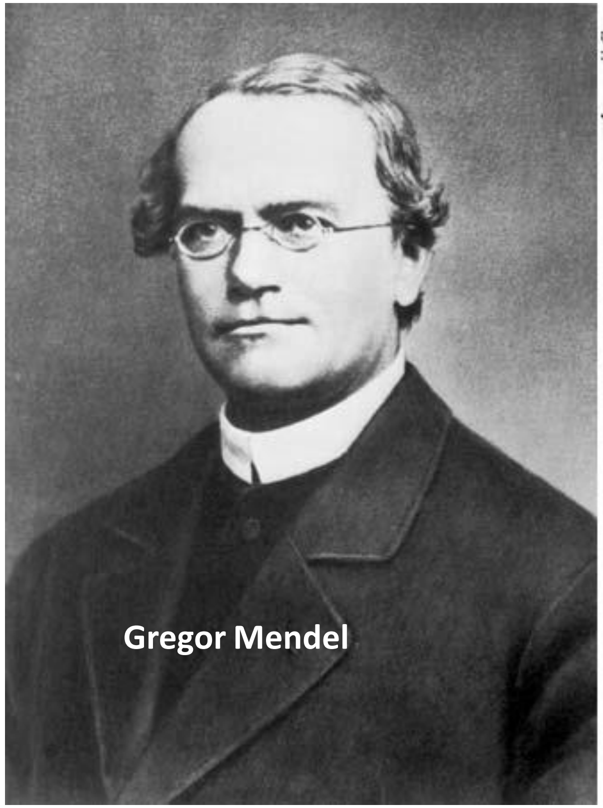
Also: meiotic drives, ‘selfish genes’

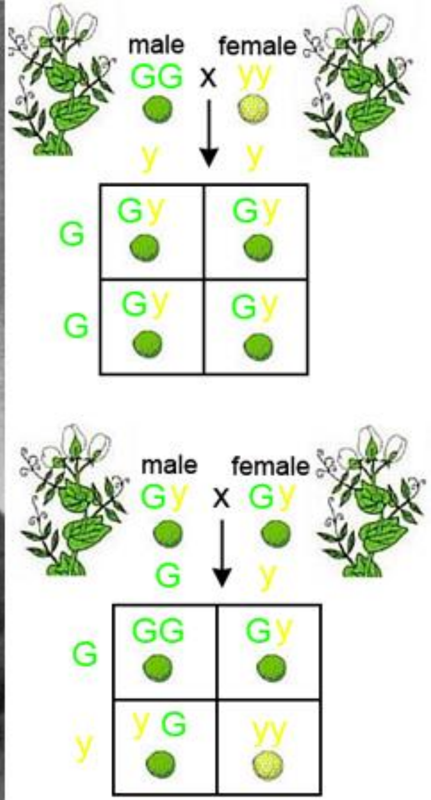
=> break the laws of Mendelian inheritance (>50% inheritance of certain alleles)

Natural drives:

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







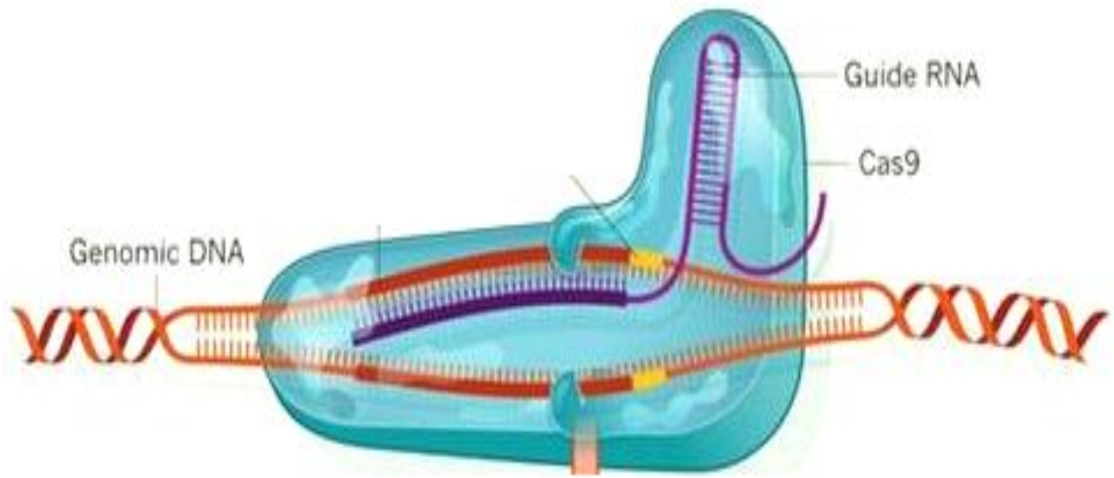
Combining 'precision engineering' component: CRISPR-Cas9 with natural repair mechanisms to create a new form of gene drive

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



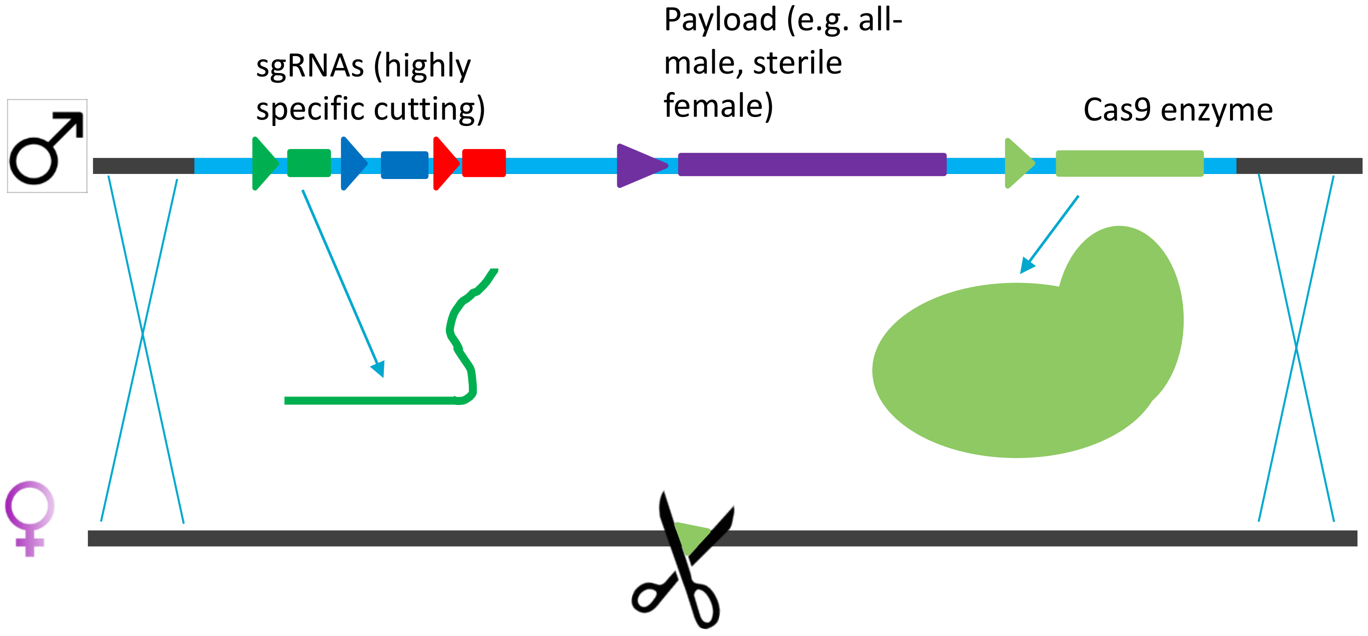
How often will this cut a piece of DNA (at random)

Frequency of a **20 nt sequence =**

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

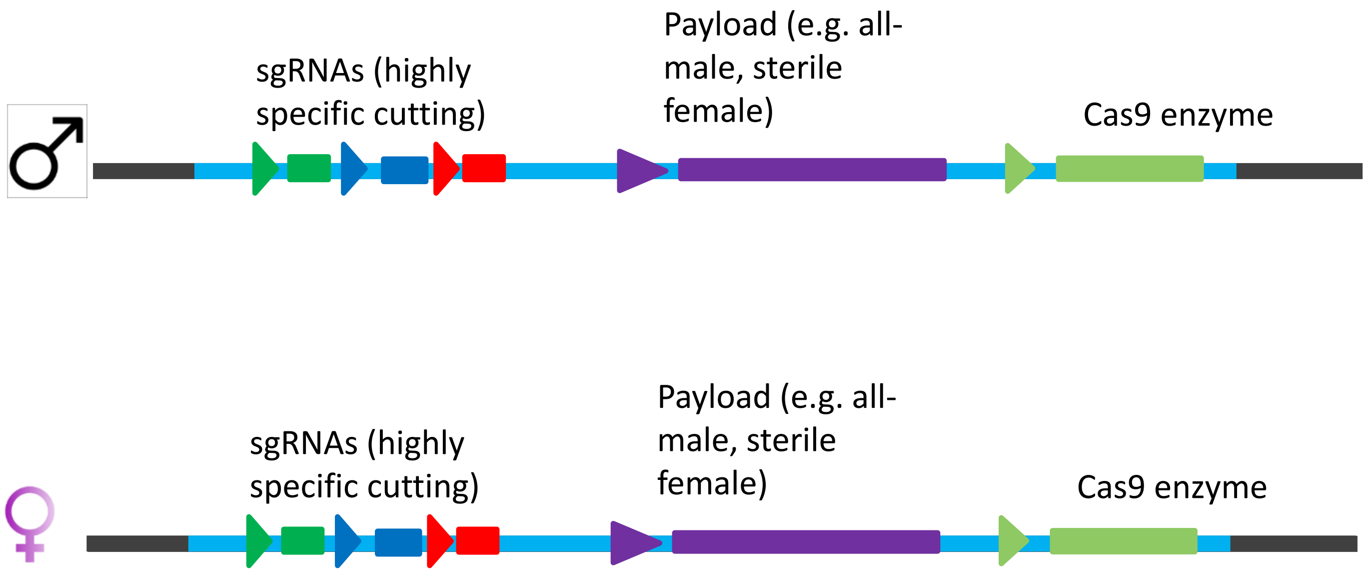
(human genome has approx. 6 billion nt)

The components of a gene drive system:



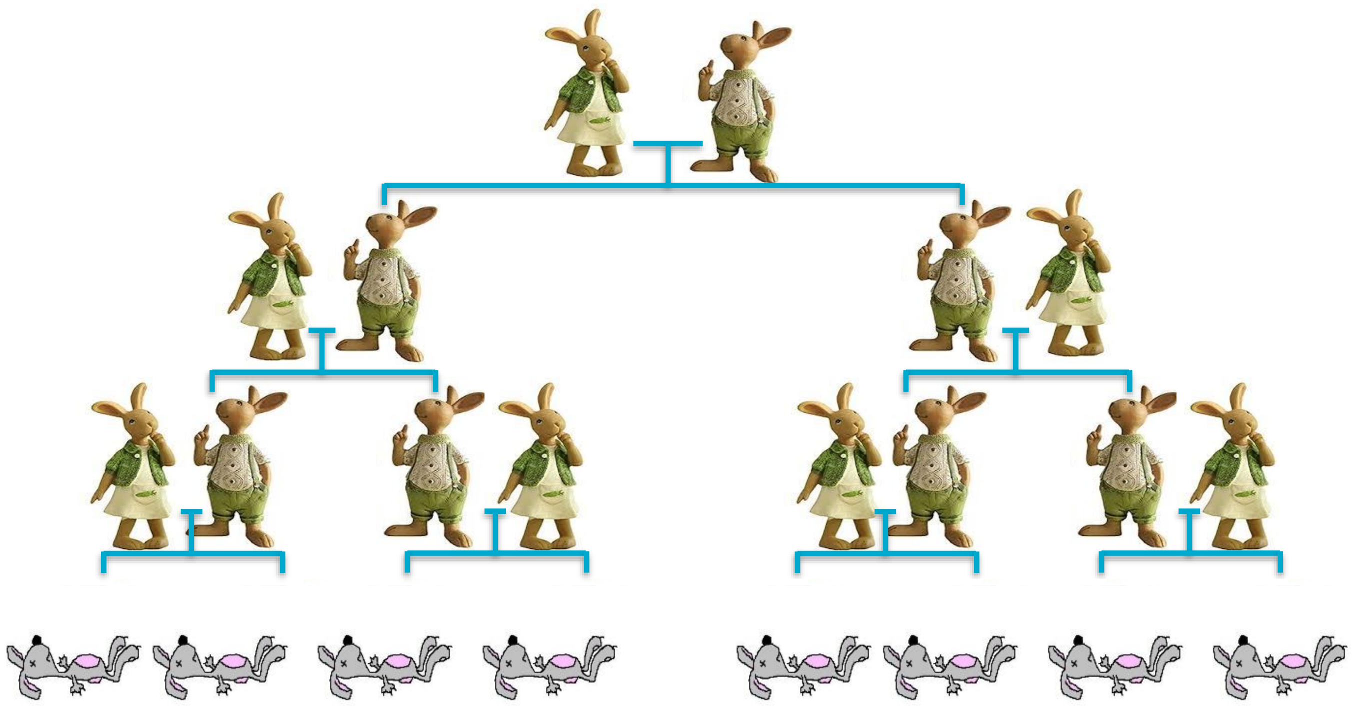
Mark Tizard CSIRO

The components of a gene drive system:



100% of offspring inherit desired trait (s)

Cas9-based gene drives – biasing sex ratios?



Excitement

- Humane
- Species specific
- Self-disseminating

- NOT CONTAGIOUS (spreads by sexual reproduction only)
- Does not require the repeated release of large numbers of animals
- Hope ?
- Dare we use the “E”- word again?

Panic

- Uncontrollable
- Irresponsible
- GM
- Won’t work anyway
- Regulatory nightmare
- International implications
- Ecological and trade risk?
- Humans playing god

BIOSAFETY

Safeguarding gene drive experiments in the laboratory

Multiple stringent confinement strategies should be used whenever possible

By **Omar S. Akbari[1,2], Hugo J. Bellen[3,4], Ethan Bier[5,*], Simon L. Bullock[6], Austin Burt[7], George M. Church[8,9], Kevin R. Cook[10], Peter Duchek[11], Owain R. Edwards[12], Kevin M. Esvelt[8,*], Valentino M. Gantz[5], Kent G. Golic[13], Scott J. Gratz[14], Melissa M. Harrison[15], Keith R. Hayes[16], Anthony A. James[17], Thomas C. Kaufman[10], Juergen Knoblich[11], Harmit S. Malik[18,19], Kathy A. Matthews[10], Kate M. O'Connor-Giles[14,20], Annette L. Parks[10], Norbert Perrimon[9,21], Fillip Port[6], Steven Russell[22], Ryu Ueda[23,24], Jill Wildonger[25]**

PNAS PNAS

Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat?

Bruce L. Webber[a,b], S. Raghu[c], and Owain R. Edwards[a,1]

[a]*Land & Water, Health & Biosecurity, Commonwealth Scientific and Industrial Research Organisation, Floreat, WA 6014, Australia;* [b]*School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia; and* [c]*Health & Biosecurity, Commonwealth Scientific and Industrial Research Organisation, Brisbane, QLD 4001, Australia*

Scientists have recognized the potential for applying gene drive technologies to the con- but whether we should. Here we explore the implications of recent developments in

Driven to Extinction

Gene drive technologies provide the ability to disperse engineered genes throughout target populations much more quickly than would be possible via simple genetic inheritance (5). In nature, selfish genetic elements use a similar strategy, generating multiple copies across the genome to improve the chances that they

NEWS · 29 NOVEMBER 2018 · CORRECTION 30 NOVEMBER 2018

UN treaty agrees to limit gene drives but rejects a moratorium

Treaty's vague language on how researchers can release engineered organisms has both opponents and supporters of the technology claiming victory.

Ewen Callaway

NATURE | NEWS

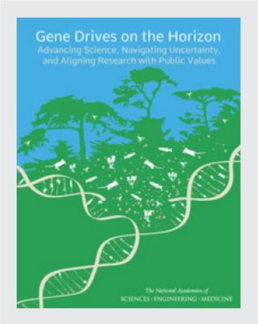
'Gene drive' moratorium shot down at UN biodiversity meeting

Freeze on genetic technology would have been a disaster, say scientists, but activists plan to renew the fight.

Ewen Callaway

The National Academies of SCIENCES ENGINEERING MEDICINE THE NATIONAL ACADEMIES PRESS

This PDF is available at http://nap.edu/23405 SHARE



Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values

DETAILS

230 pages | 7 x 10 | PAPERBACK
ISBN 978-0-309-43787-5 | DOI 10.17226/23405

www.science.org.au/gene-drives

Transparency beats a moratorium anytime…

Australian Academy of Science

Guiding Principles for the Sponsors of Gene Drive Research

- **Advance quality science to promote the public good**
- **Promote stewardship and good governance**
- **Demonstrate transparency and accountability**
- **Engage thoughtfully with communities, stakeholders and publics**
- **Foster opportunities to strengthen capacity and education**

CSIRO

Exploring Natural and Engineered Gene Drives for Eradications of Invasive Rodent Populations

GBIRd
Genetic Biocontrol of Invasive Rodents

1. Proceed cautiously, with deliberate stepwise methods and measurable outcomes.
2. Engage early and often with the research community, regulators, communities, and other stakeholders.
3. Maintain an uncompromising commitment to biosafety, existing regulations, and protocols as minimum standards (e.g. NASEM, 2016; AAS, 2017).
4. Use, and participate in developing, best practices.
5. Operate only in countries with appropriate regulatory capacity.
6. Be transparent with research, assessments, findings, and conclusions.

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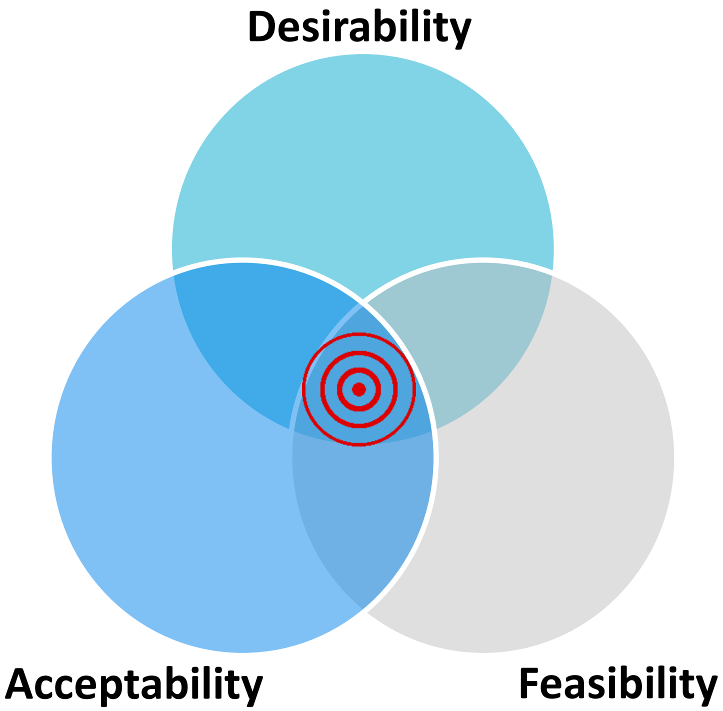
  - Public attitudes
- Australian perspective: Technical developments & future plans
  - Mice
  - Weeds
  - Rabbits

Developing a prioritisation and investment decision framework for vertebrate Genetic Biocontrol

Centre for Invasive Species Solutions (CISS) project 2018-2021



- Can we do it?
- Should we do it?
- Who decides
- Who pays?

CENTRE FOR INVASIVE SPECIES SOLUTIONS
WWW.INVASIVES.COM.AU

CSIRO

GOVERNMENT OF WESTERN AUSTRALIA
Department of Primary Industries and Regional Development
Department of Biodiversity, Conservation and Attractions

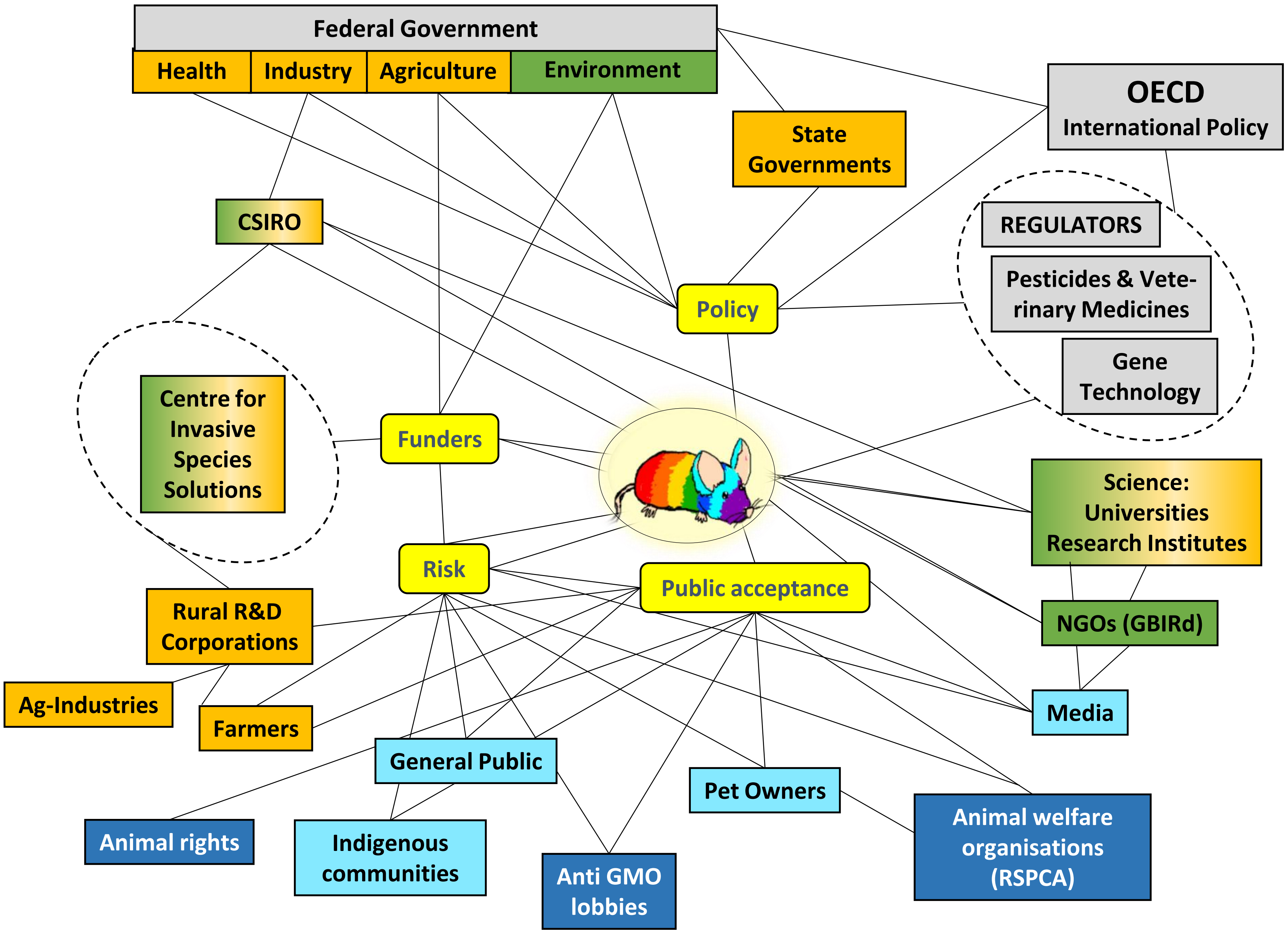
Path forward for a coordinated development of genetic biocontrol technologies for vertebrates, considering:

- Elicit Stakeholder ‘push and pull-factors’: differences in priorities and investment appetite from various key private and public organisations involved in pest species management.

- Develop framework to aid decisions if, when and how to invest

Proof of concept in mammals not yet achieved at the time

A very messy and incomplete stakeholder map



Input:

- Relevant published literature
- Team's expert knowledge
- Interdisciplinary Team (genetics/ecology/biocontrol), social science, government representatives

- Two stakeholder workshops
- Offline survey (subset of participants)

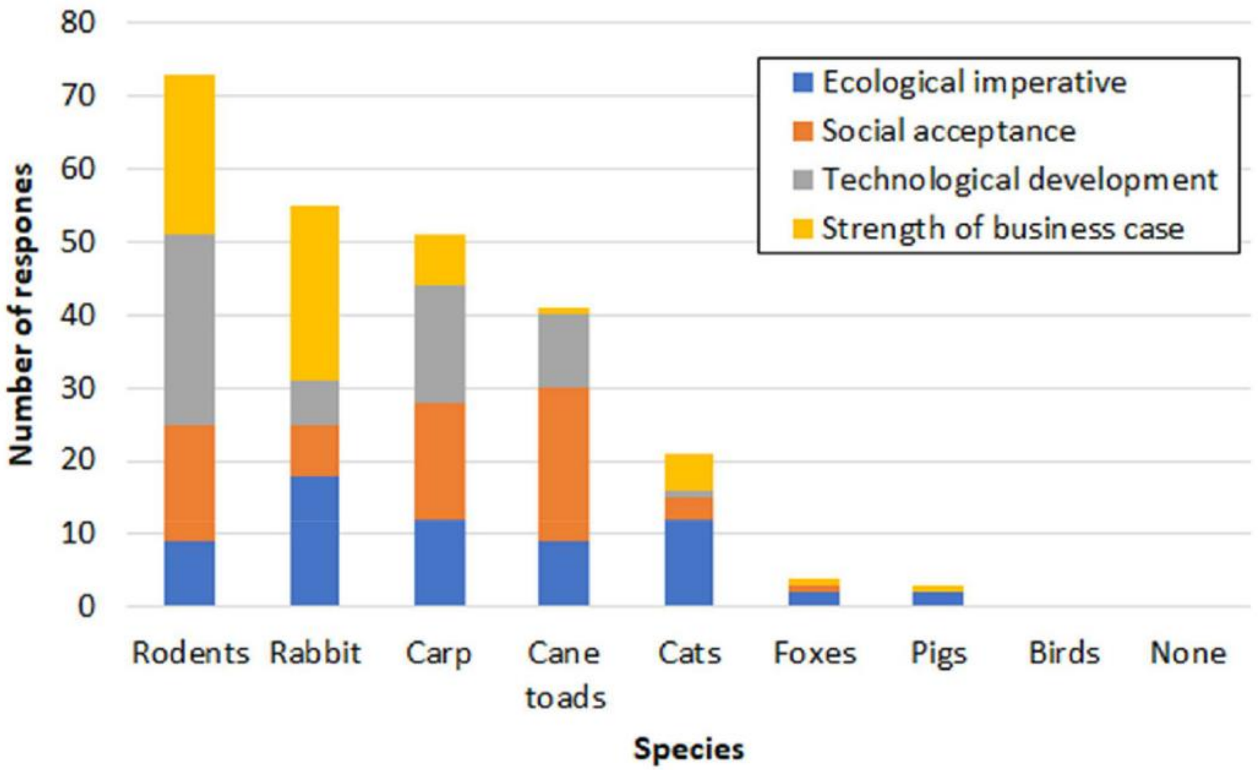
Workshop 1 (in person)

- 36 participants
- Broader pest and invasive species research community, state and federal agencies, NGOs incl. RSPCA
- Share perspectives, highlight risks and species-specific discussions

Workshop 2 (virtual)

- 18 participants
- Potential investors and industry reps, government decision makers, NGOs
- Present knowledge and ideas on broader decision-making environment, appetite for investment and enabling factors

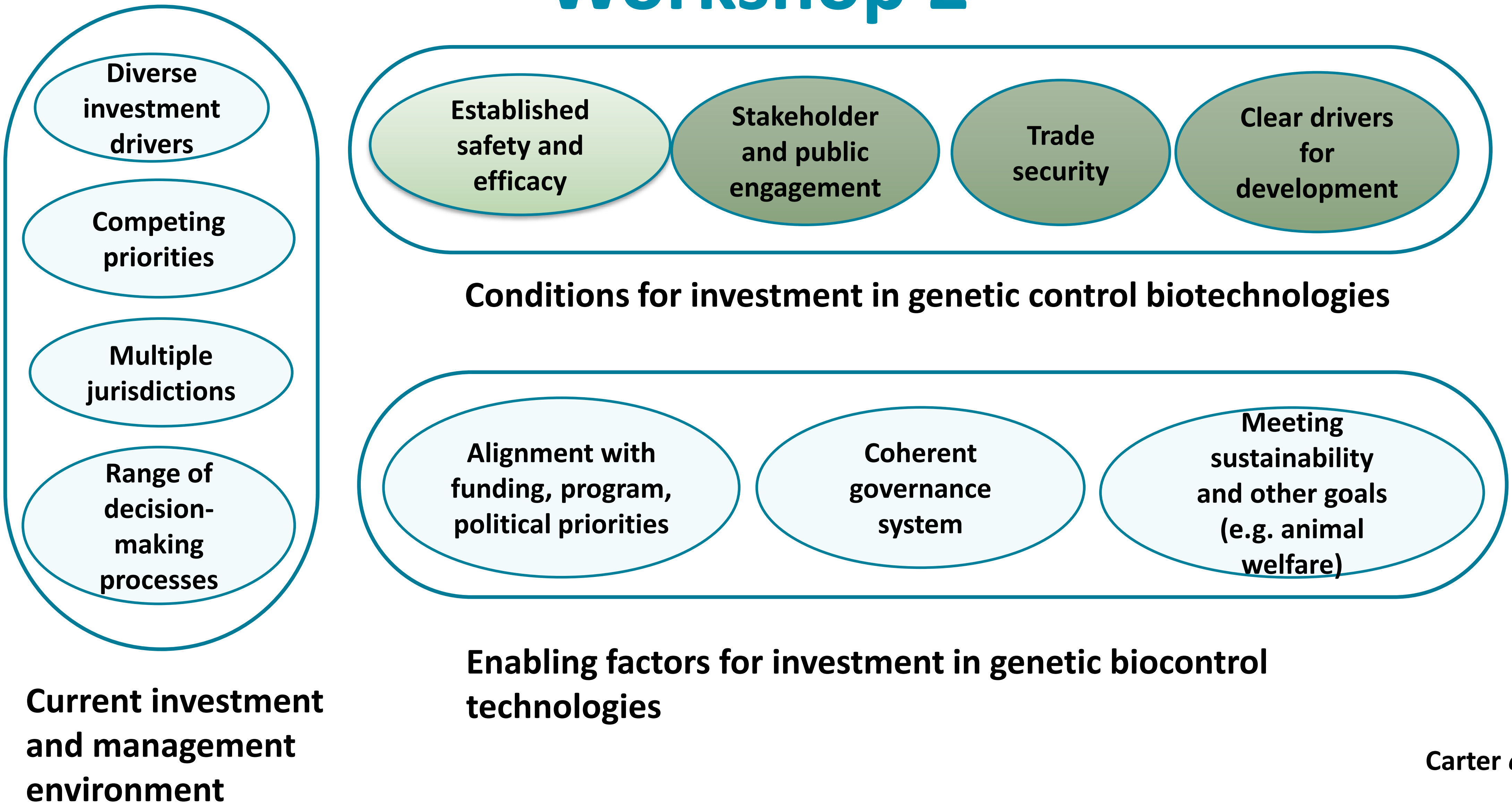
Workshop 1



=> Proof of concept needed in mammals

Carter et al., 2022

Workshop 2



Carter *et al.* 2021

Recommendations

- Early comprehensive stakeholder research and engagement strategy:
  - Seek multiple-stakeholder support, address social risks, cultural and ethical factors
- Governance structure with clear oversight and national coordination
- Trade risk analysis and post-deployment monitoring
- **Pipeline from animal laboratory studies to field implementation (staged proof of concept needed!)**

Pipeline

1. Proof of concept in a model animal species
2. Acquisition of essential background data/closing of knowledge gaps for target species
3. Transfer of the technology from model organism to the target species, and
4. Implementation and rollout in target species.

Essential background data:

- Population structure/dynamics
- Gene flow/migration
- Social structure
- Behaviour
- Genetics/genomics: identify target sequences unique to target population
- Reproductive biology/genetic engineering

- -> useful in non-gene drive contexts too

Technology

Effective management

Get started

Tools

Multisector perspectives

Effective humane

Transformational

Welfare

Potential, acceptability

Enthusiastic

Cautious optimism

Enthusiasm openness

Watching brief

Transformative

Working tool

Wildlife matters

Enthusiastic, driven

New potential

Community's ready

Future focussed

Ability to make a difference

Regulation

Great change

Curiosity; transformational

Useful

The future

Transformative; futuring

Game-changer


GENETIC BIOCONTROL TECHNOLOGY FOR VERTEBRATE PESTS: DECISION FRAMEWORK SUMMARY

A REPORT BY WENDY RUSCOE, SUSAN CAMPBELL, LUCY CARTER, ADITI MANKAD, PETER BROWN, MARGARET BYRNE, KEVIN OH, MARK TIZARD & TANJA STRIVE

COLLABORATION INNOVATION IMPACT

CENTRE FOR INVASIVE SPECIES SOLUTIONS

CSIRO

GOVERNMENT OF WESTERN AUSTRALIA

Department of Primary Industries and Regional Development

Department of Biodiversity, Conservation and Attractions

frontiers in Agronomy

ORIGINAL RESEARCH
published: 31 January 2022
doi: 10.3389/fagro.2021.806569

Conditions for Investment in Genetic Biocontrol of Pest Vertebrates in Australia

Lucy Carter [1,2], Aditi Mankad [1,2], Susan Campbell [3], Wendy Ruscoe [2,4], Kevin P. Oh [4,5], Peter R. Brown [2,4], Margaret Byrne [6], Mark Tizard [2,4,7] and Tanja Strive [2,4]*

CSIRO Synthetic Biology Future Science Platform

Aditi Mankad & Team, CSIRO Synthetic Biology Future Science PLatform

- Perception of pest animals as a major problem in Australia
- Basic education on genetic control technology (incl. YouTube Video)
- Attitudes towards genetic control technology

Do Australians think pest species are a problem?

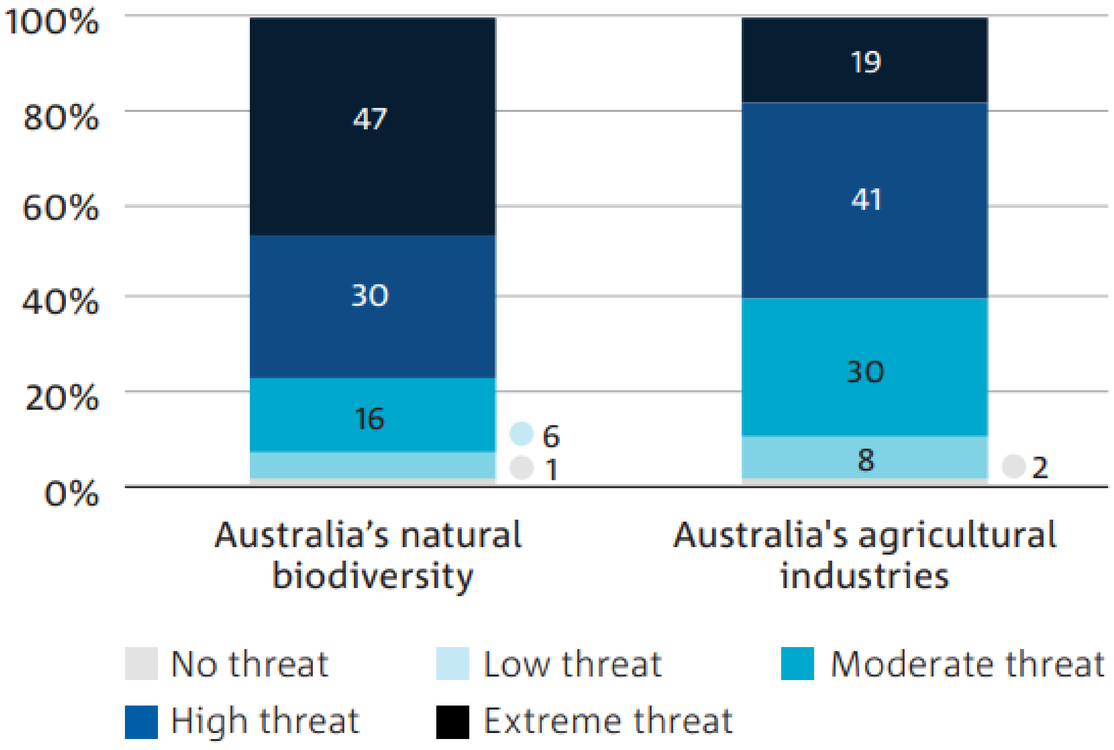


Figure 2. Public views on the level of threat that feral animals (e.g., cats, rabbits, foxes, pigs) pose to Australia's natural biodiversity and Australia's agriculture industries, as a percentage (%) of total responses.

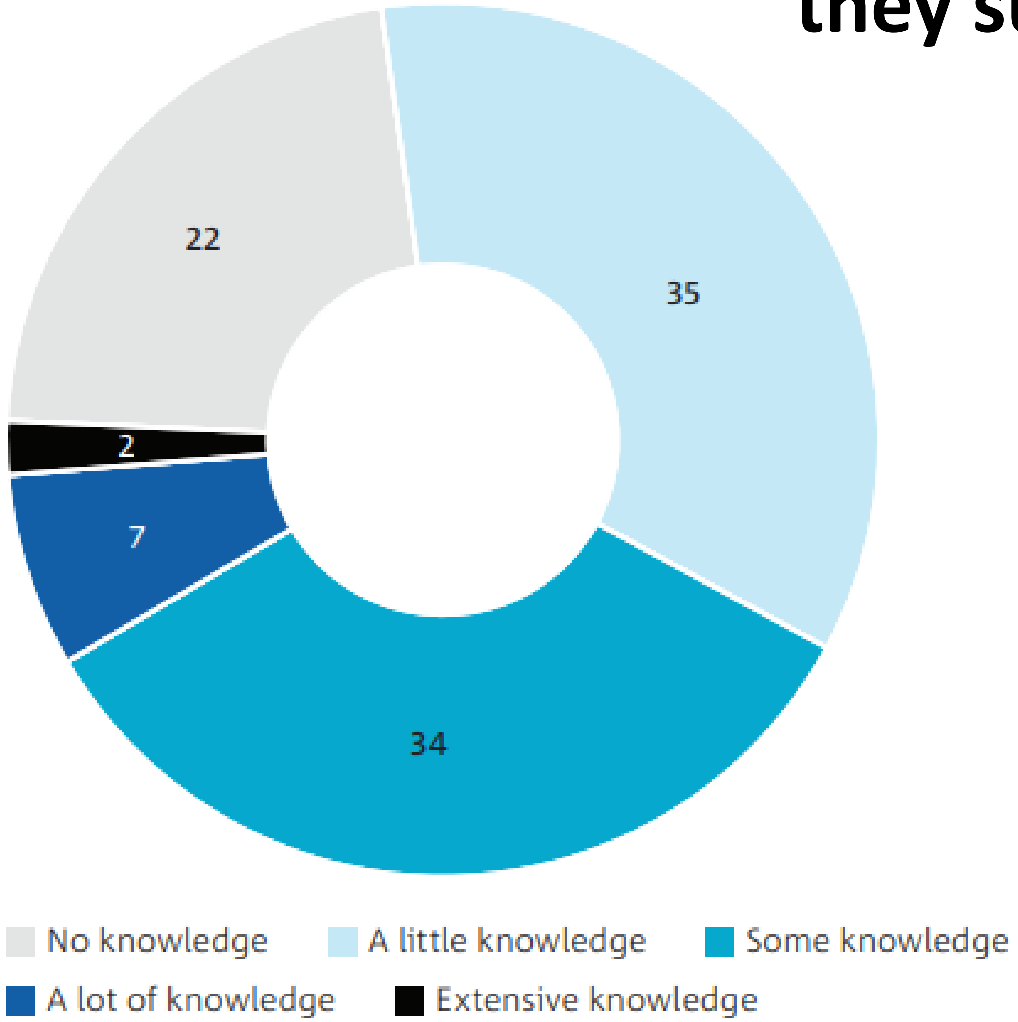
Feral Cats

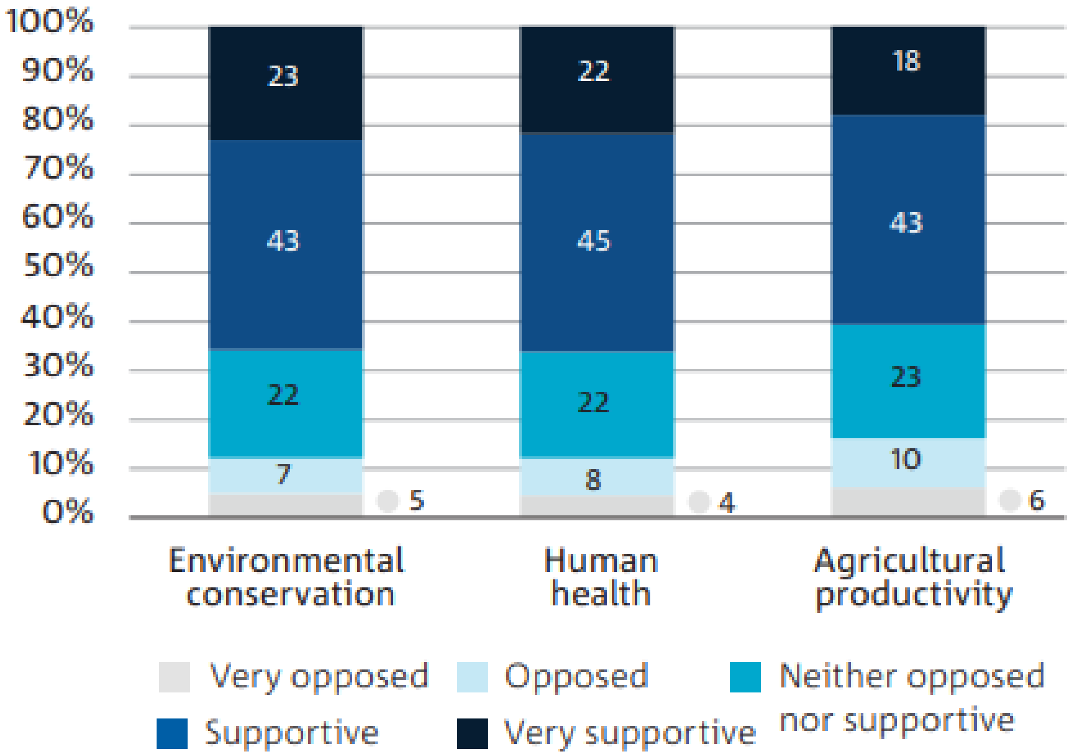
- Kill an estimated
  - 3.2 million mammals, 1.2 million birds, 1.9 million reptiles and 250,000 frogs *per day*, the majority are native species.
  - 1.8 billion native animals/year

- Inhabit >90% of continent

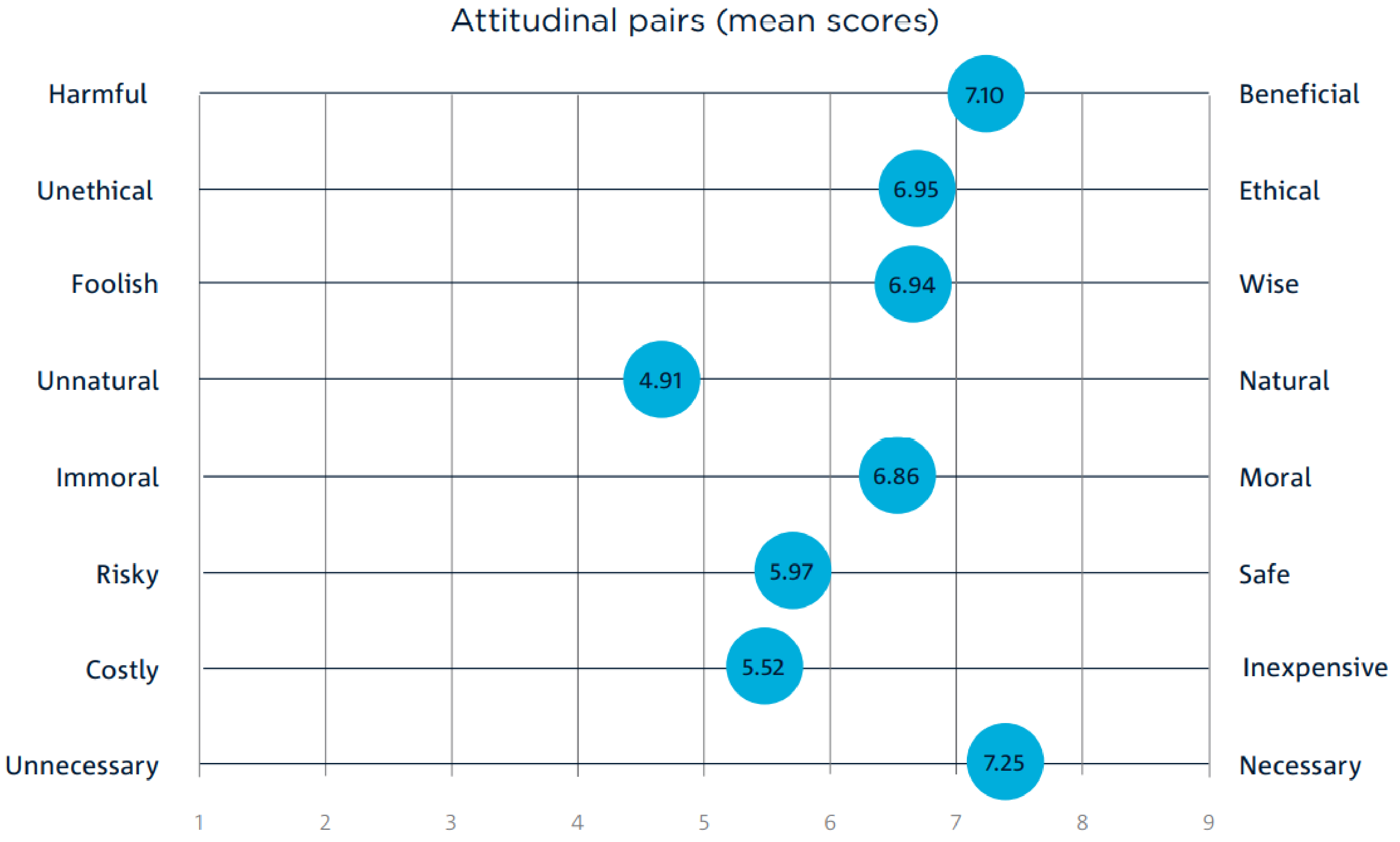
- Broadscale controls can be ineffective

What do Australians know about synthetic biology and would they support its use?

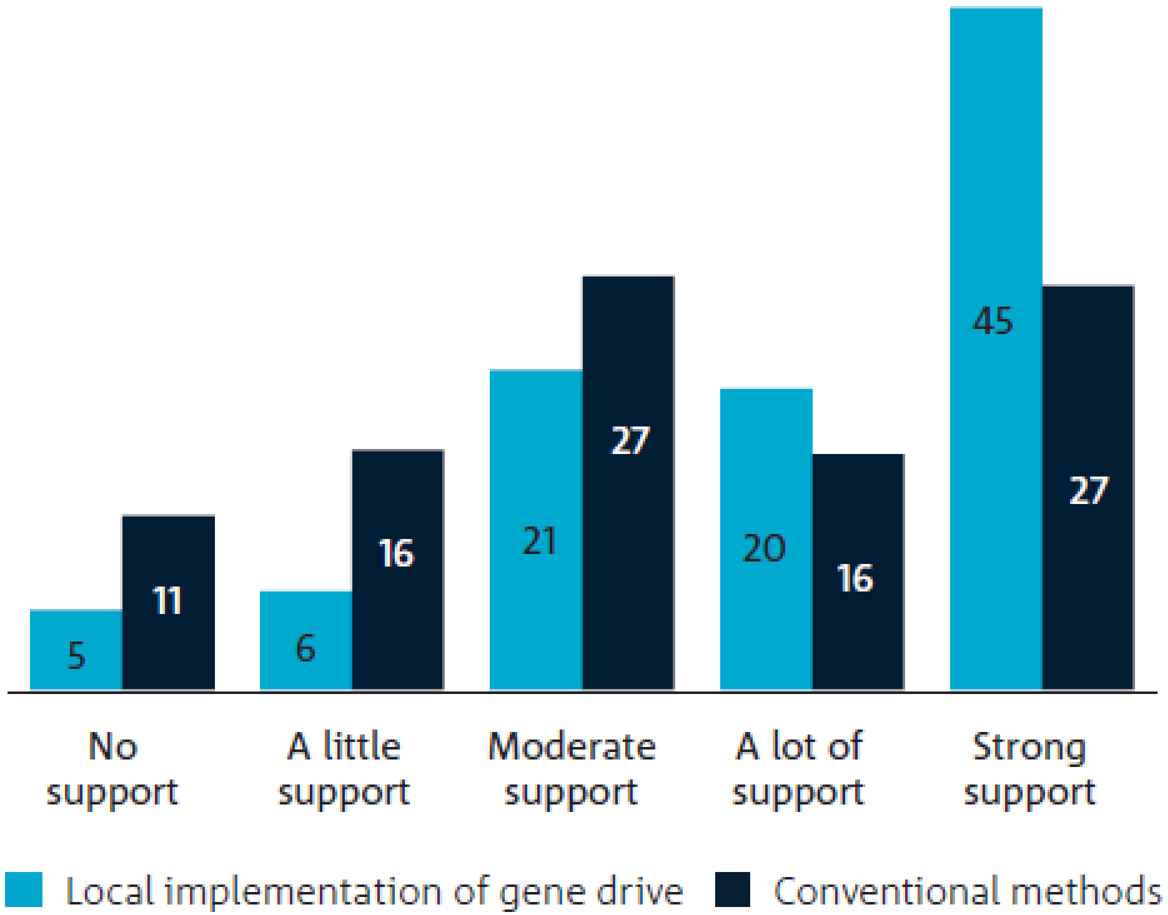




Attitudes towards using gene drive technology for feral animal control



Levels of support for the local implementation of gene drive technology and conventional control methods, % of total responses




Australia's National
Science Agency
Public perspectives
towards using gene
drive for invasive species
management in Australia

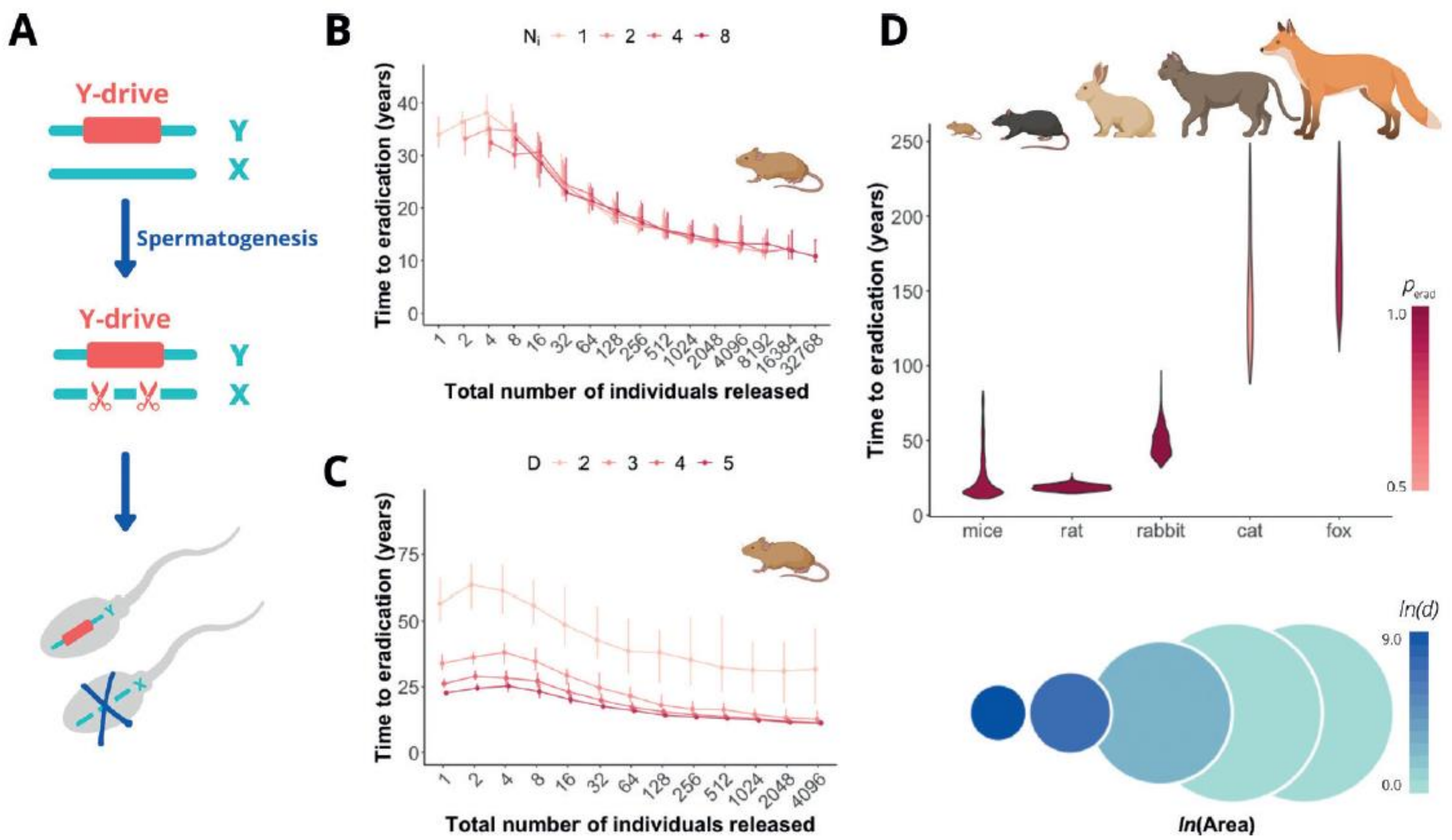
Scalability of genetic biocontrols for eradicating invasive alien mammals

NeoBiota 74: 93–103 (2022)
doi: 10.3897/neobiota.74.82394
https://neobiota.pensoft.net

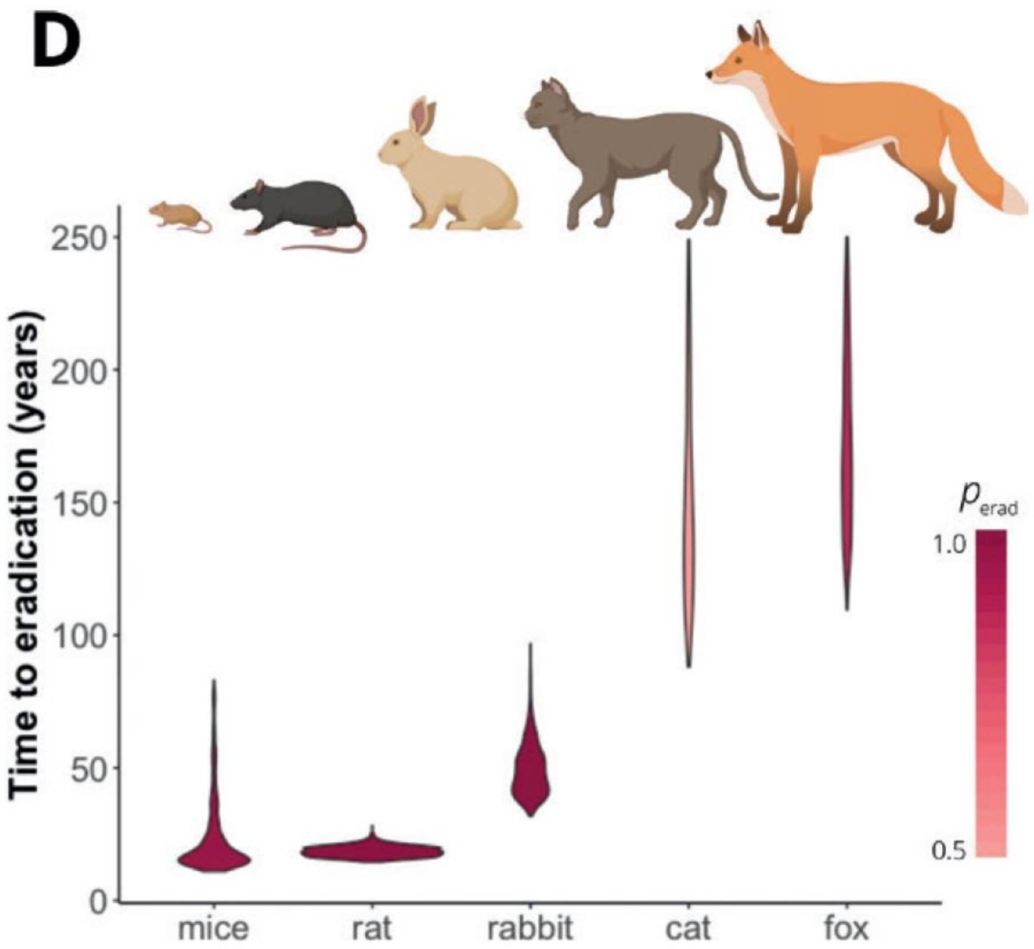
SHORT COMMUNICATION

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Aysegul Birand[1], Phillip Cassey[1], Joshua V. Ross[2], Paul Q. Thomas[3,4], Thomas A. A. Prowse[1]



CSIRO



D
Time to eradication (years)
250
200
150
100
50
0
mice
rat
rabbit
cat
fox
p_{erad}
1.0
0.5

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- **Australian perspective: Technical developments & future plans**
  - **Mice**
  - **Rabbits**

Technical Developments in genetic biocontrol– What else is going on in Australia?

….may not be a complete list…. Grey = planned/about to start

Improved gene editing methods

CSIRO: zebra fish, toads, rabbits
UoM: carp, foxes, cats, rabbits?

Target gene validation (all male, female infertile, non-toxic etc)

UoA: mice
MU: zebra fish
UoM: zebra fish, carp, toads, cats, foxes, rabbits
UTas: Gambusia
CSIRO: rabbits

Drive mechanism

UoA: mice
MU: zebra fish
UoM: zebra fish
UTas: Gambusia
CSIRO: rabbits

THE UNIVERSITY OF MELBOURNE

THE UNIVERSITY of ADELAIDE

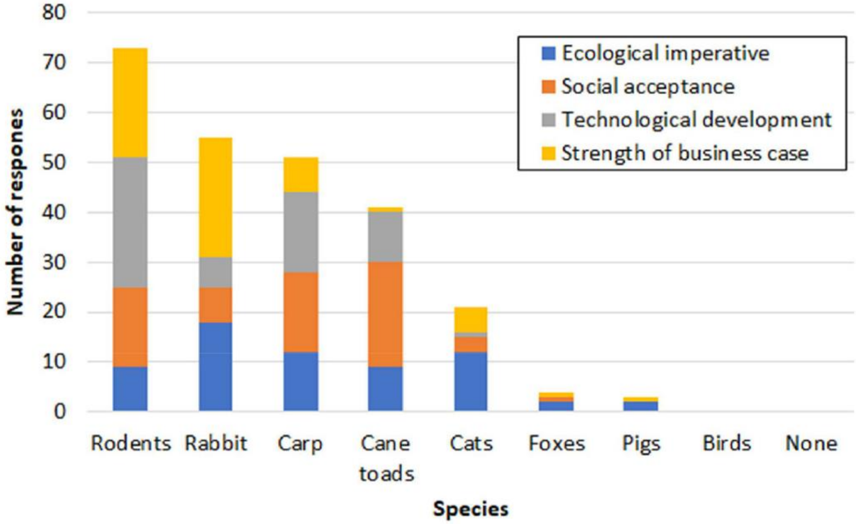
UNIVERSITY of TASMANIA

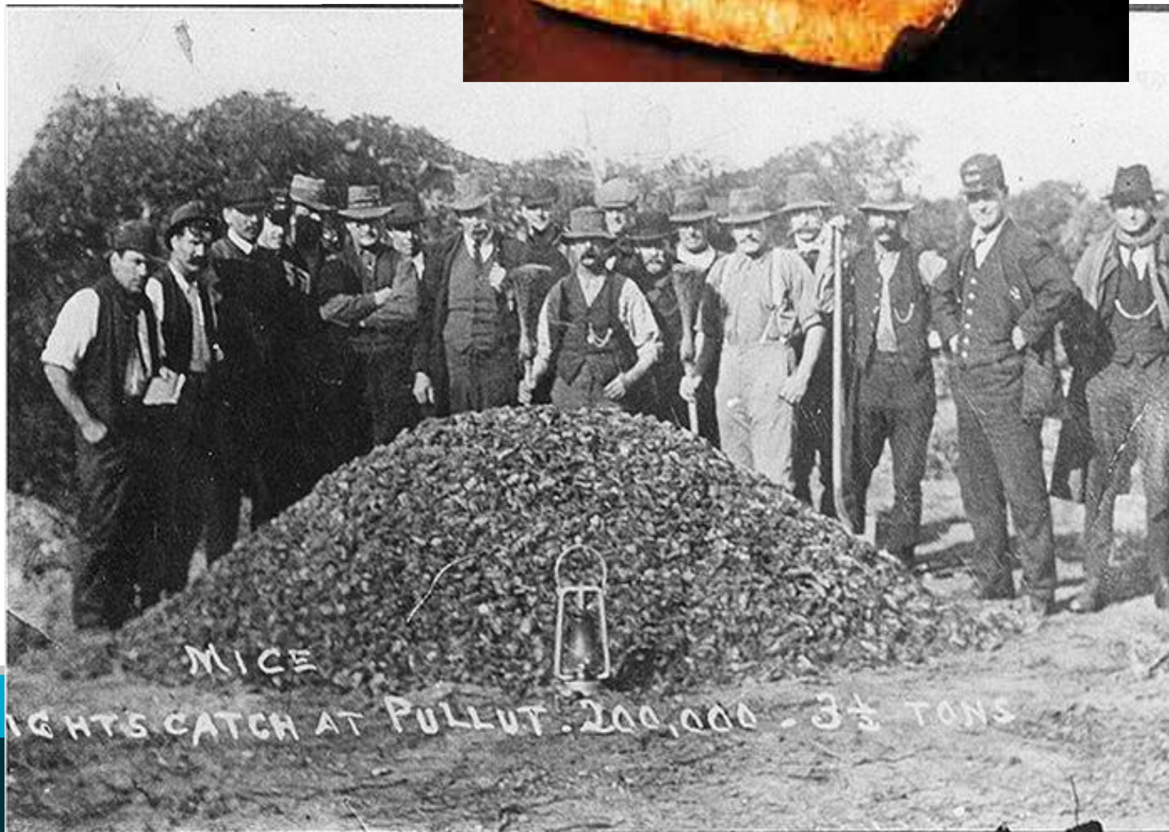
MACQUARIE University

CSIRO

Mice!

Vertebrate model systems *AND* significant pest (agricultural and environmental)





Homing not particularly effective in mice!

MENU nature International journal of science

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NEWS · 06 JULY 2018

Controversial CRISPR 'gene drives' tested in mammals for the first time

Experiments in mice suggest that the technology has a long way to go before being used for pest control in the wild.

Ewen Callaway

Homing inefficient in mice!

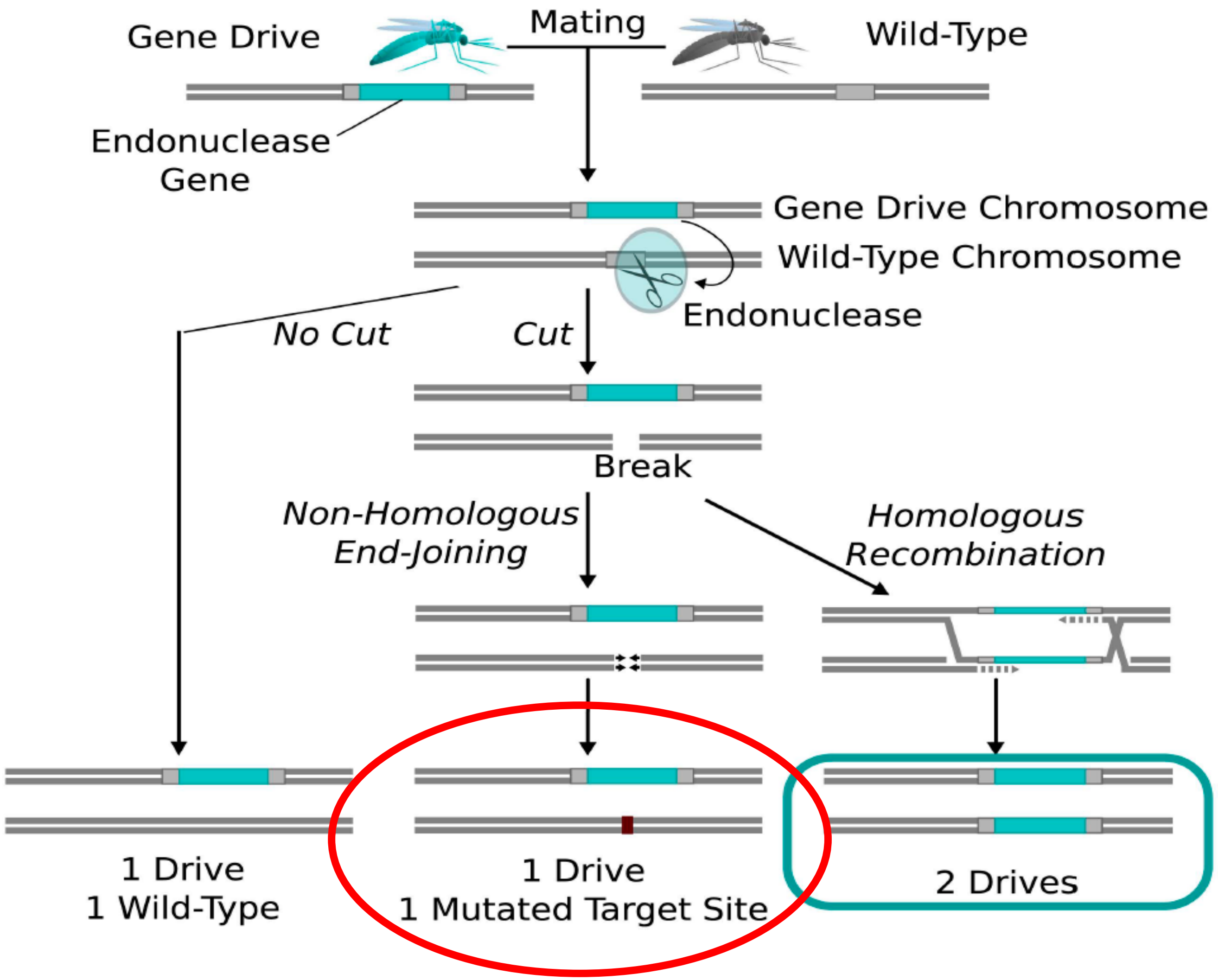
MENU nature International journal of science

Letter | Published: 23 January 2019

Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline

Hannah A. Grunwald, Valentino M. Gantz, Gunnar Poplawski, Xiang-Ru S. Xu, Ethan Bier & Kimberly L. Cooper

Nature (2019) | Download Citation



Esvelt et al., 2014

Recent break through in mice !

PNAS RESEARCH ARTICLE | GENETICS OPEN ACCESS

Leveraging a natural murine meiotic drive to suppress invasive populations

Luke Gierus[a,b,1], Aysegul Birand[c,1], Mark D. Bunting[a,b], Gelshan I. Godahewa[b,d], Sandra G. Piltz[a,b], Kevin P. Oh[e,f], Antoinette J. Piaggio[g], David W. Threadgill[h], John Godwin[i], Owain Edwards[e,j], Phillip Cassey[c], Joshua V. Ross[k], Thomas A. A. Prowse[c] and Paul Q. Thomas[a,b,2]

Edited by James Bull, University of Idaho, Moscow, ID; received August 3, 2022; accepted October 4, 2022

- Non-homing drive (natural t-complex)
- Combining T-allel (drive)+ female infertile target
- 95% of offspring inherited female infertile marker
- Split gene drive

Manaaki Whenua Landcare Research

USDA

GBIRd Genetic Biocontrol of Invasive Rodents

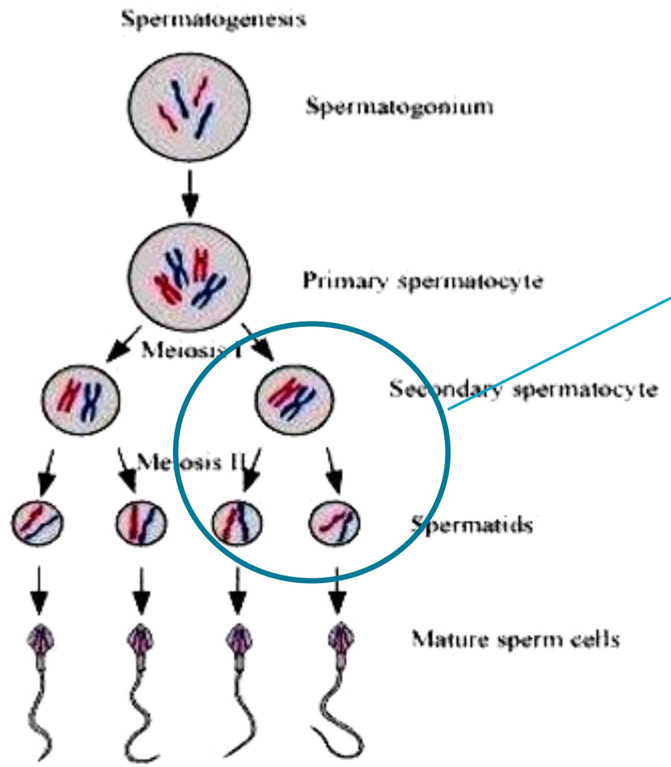
CSIRO

THE UNIVERSITY of ADELAIDE

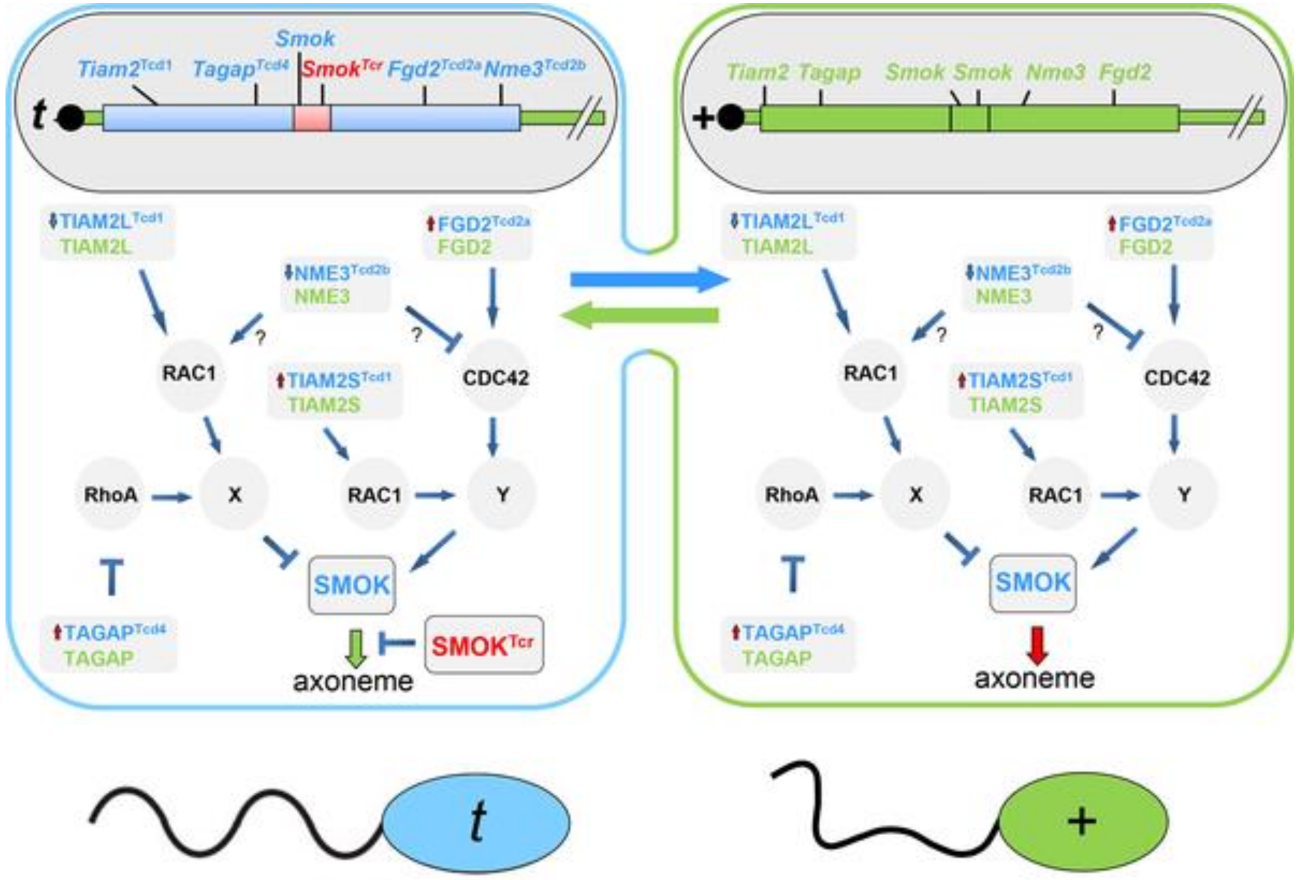
NC STATE UNIVERSITY

ISLAND CONSERVATION Preventing Extinctions

Drive: T-haplotype in mice - ~ 40 Mb arm of Chromosome 17

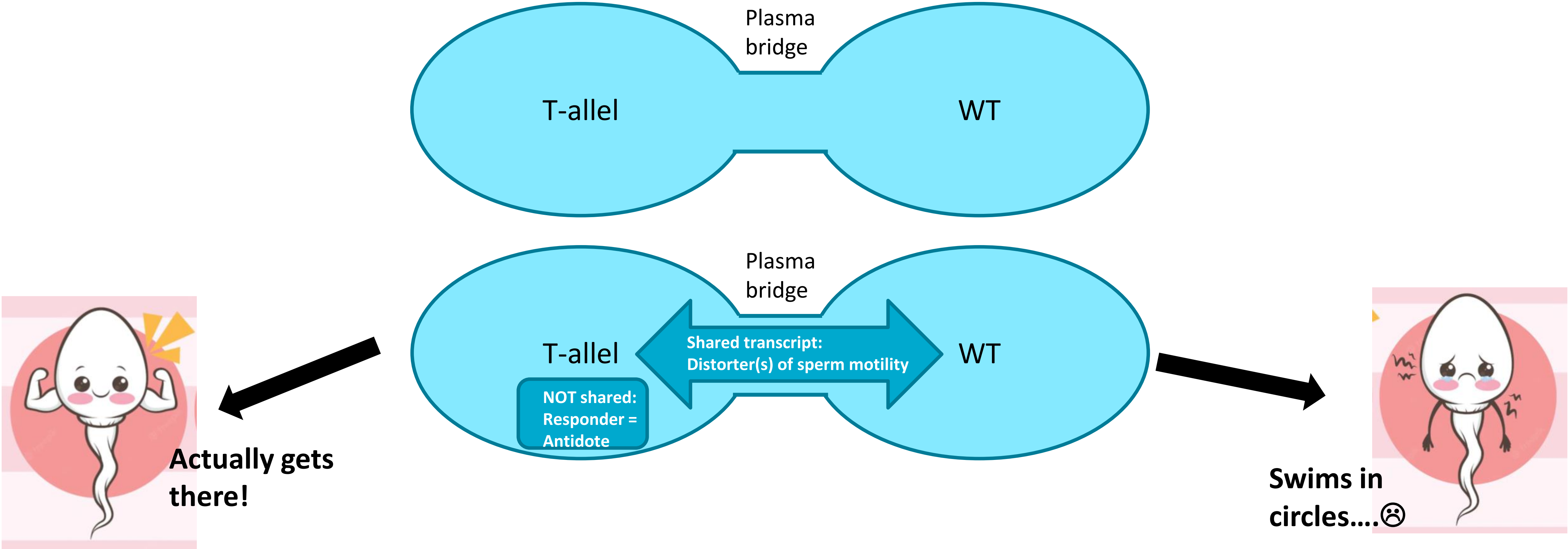


Process of spermatogenesis



Charron Y, Willert J, Lipkowitz B, Kusecek B, Herrmann BG, et al. (2019)

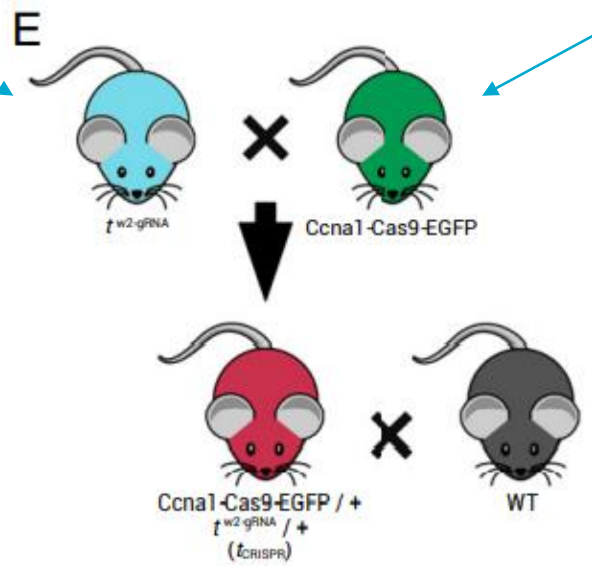
Simple version:
Shared distorters and non-shared responders selectively interfere with sperm motility



Split Gene drive design:

Adding gRNA into t-locus (Chr 17)
(targeting prolactin-> female infertility)

Adding Cas9 to different chromosome



Prolactin is cut in the male germline – transmitted to 95% of offspring of heterozygous males

Outline:

- Established pests in Australia
- Why we love self-disseminating biocontrols
- A very brief introduction on genetic biocontrol (GBC)
- Australian perspective: Stakeholder engagement
  - Prioritisation framework for stakeholders and potential funders of GBC work
  - Public attitudes
- Australian perspective: Technical developments & **future plans**
  - Mice
  - Rabbits

Mice on islands – the locally fixed allele advantage?

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ORIGINAL ARTICLE
Evolutionary Applications Open Access
WILEY

Population genomics of invasive rodents on islands: Genetic consequences of colonization and prospects for localized synthetic gene drive

Kevin P. Oh[1,2] | Aaron B. Shiels[1] | Laura Shiels[1] | Dimitri V. Blondel[3] | Karl J. Campbell[4,5] | J. Royden Saah[4,6] | Alun L. Lloyd[6,7] | Paul Q. Thomas[8] | Fred Gould[6,9] | Zaid Abdo[2] | John R. Godwin[3,6] | Antoinette J. Piaggio[1]

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Locally Fixed Alleles: A method to localize gene drive to island populations

Jaye Sudweeks[1], Brandon Hollingsworth[2], Dimitri V. Blondel[3], Karl J. Campbell[4], Sumit Dhole[5], John D. Eisemann[6], Owain Edwards[7], John Godwin[3,8], Gregg R. Howald[4], Kevin P. Oh[6,9], Antoinette J. Piaggio[6], Thomas A. A. Prowse[10], Joshua V. Ross[10], J. Royden Saah[4,8], Aaron B. Shiels[6], Paul Q. Thomas[11], David W. Threadgill[12], Michael R. Vella[2], Fred Gould[5,8] & Alun L. Lloyd[1,2*]

Potentially suitable locally fixed target alleles exist on Islands!

(e.g. zar1 on Thevenard Island, zygote arrest 1, female sterility, multiplexing potential)

Drive becomes ineffective pretty quickly once the frequency drops

Current proposals under development:

Next stage proof of concept: development and contained pen trials of gene drive mice with an Australian **island-specific gene drive**

Plans to progress mouse GBC work in Australia (CSIRO & Uni Adelaide)

- Clarify regulatory and policy pathways (funded, planned workshop 1st half 2023)

Phase I (current proposal development):

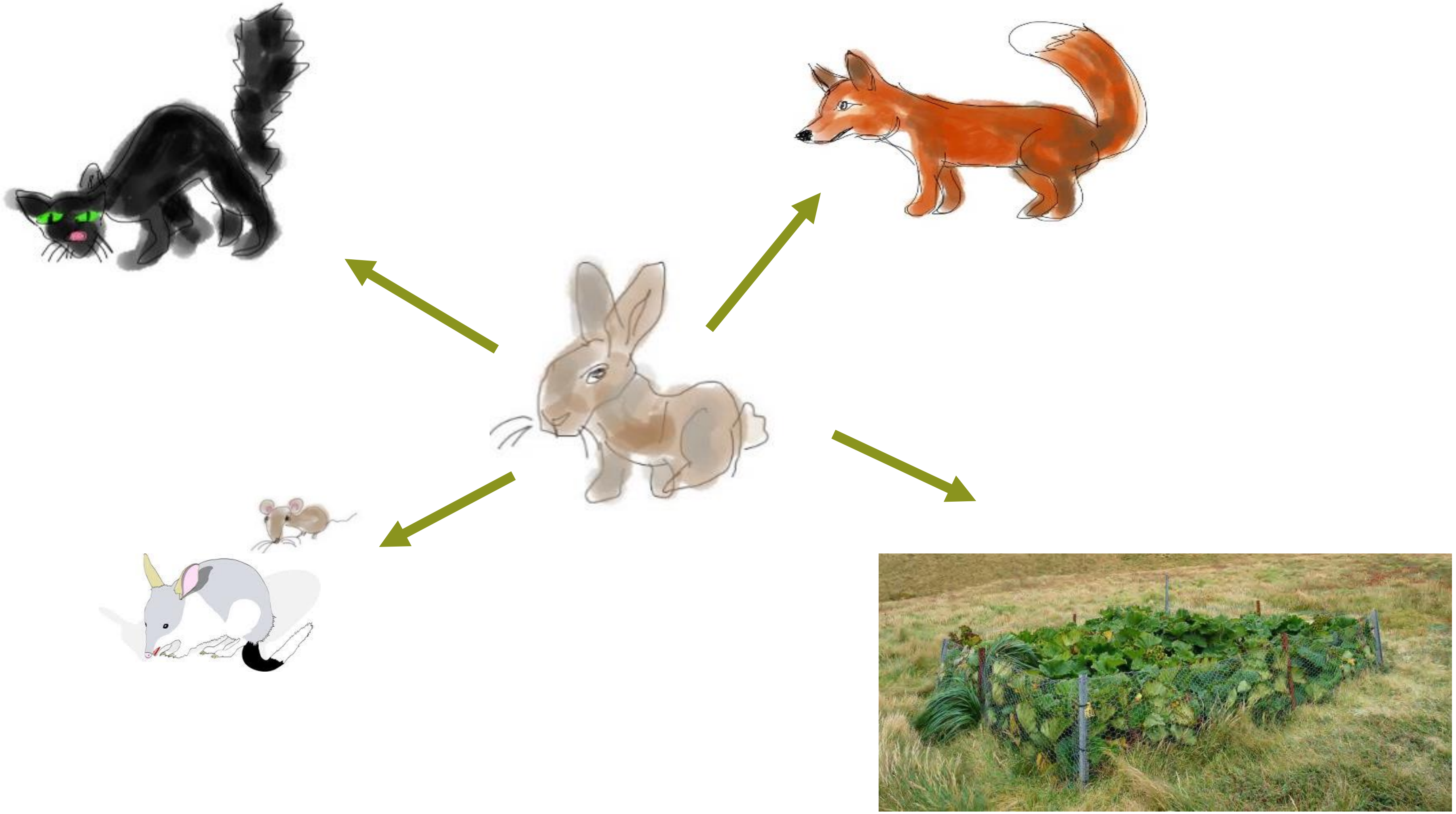
- All-in-one mouse (Paul Thomas), no split gene drive
- Guide RNA for Thevenard-specific female infertility allele
- Cage trials-> pen trials
- More island genomics, trial-site genomics (frequency of target allele)
- Island selection review (is there another more suitable Island with a private allele)
- Review of Indigenous stakeholder considerations and implications for field testing
- Social perception component (GBC for mice in environmental and agricultural contexts)

Phase II:

- finalise mouse design, more pen trials, site specific risk assessment, release plan

Rabbits

– keystone species with top-down and bottom up effects

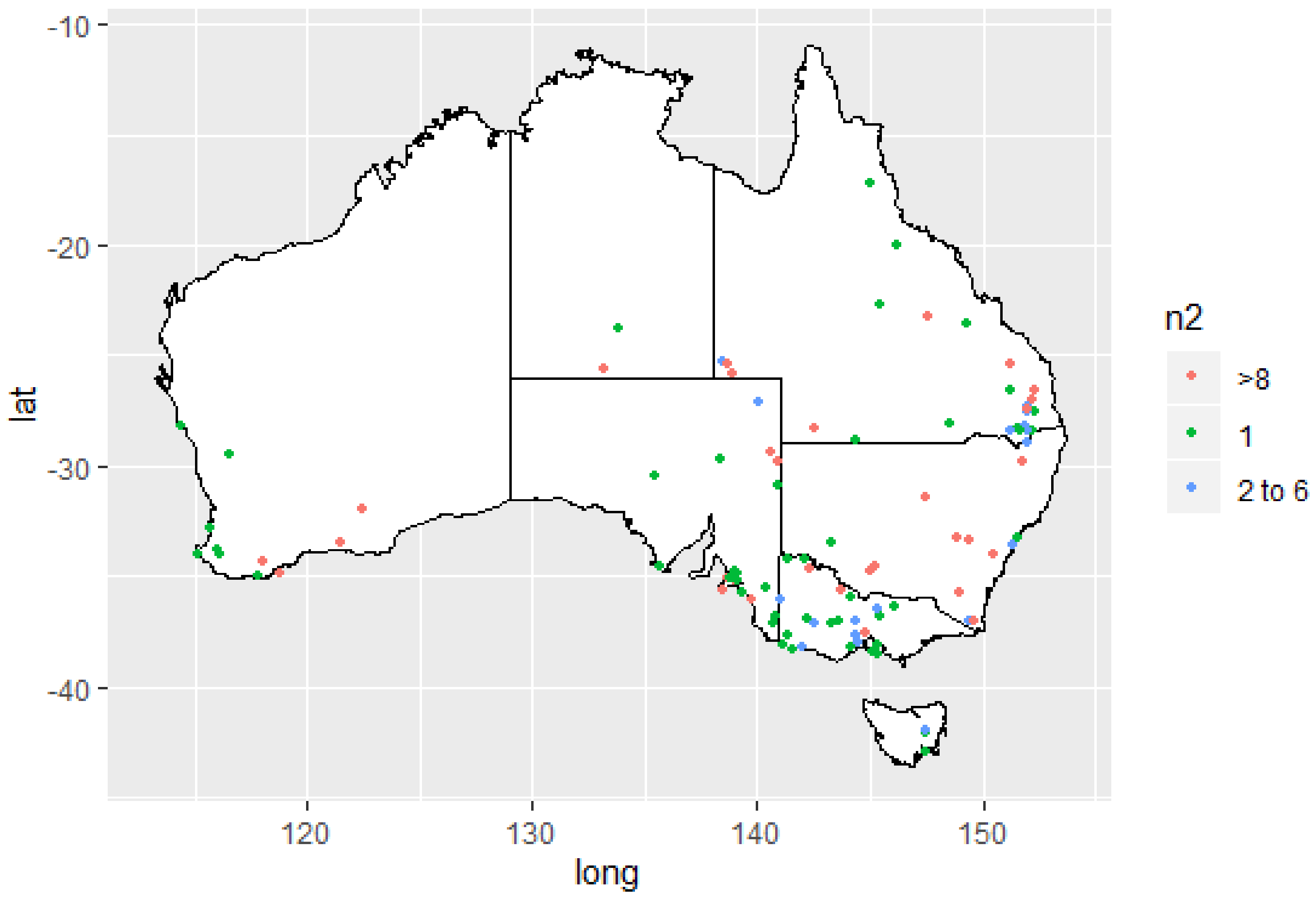


Investigating the theoretical feasibility of genetic biocontrol for Australian wild rabbits

Know your genetics!

- Whole genome sequencing of ~300 individual Australian rabbits

- + ~180 from European collaborators

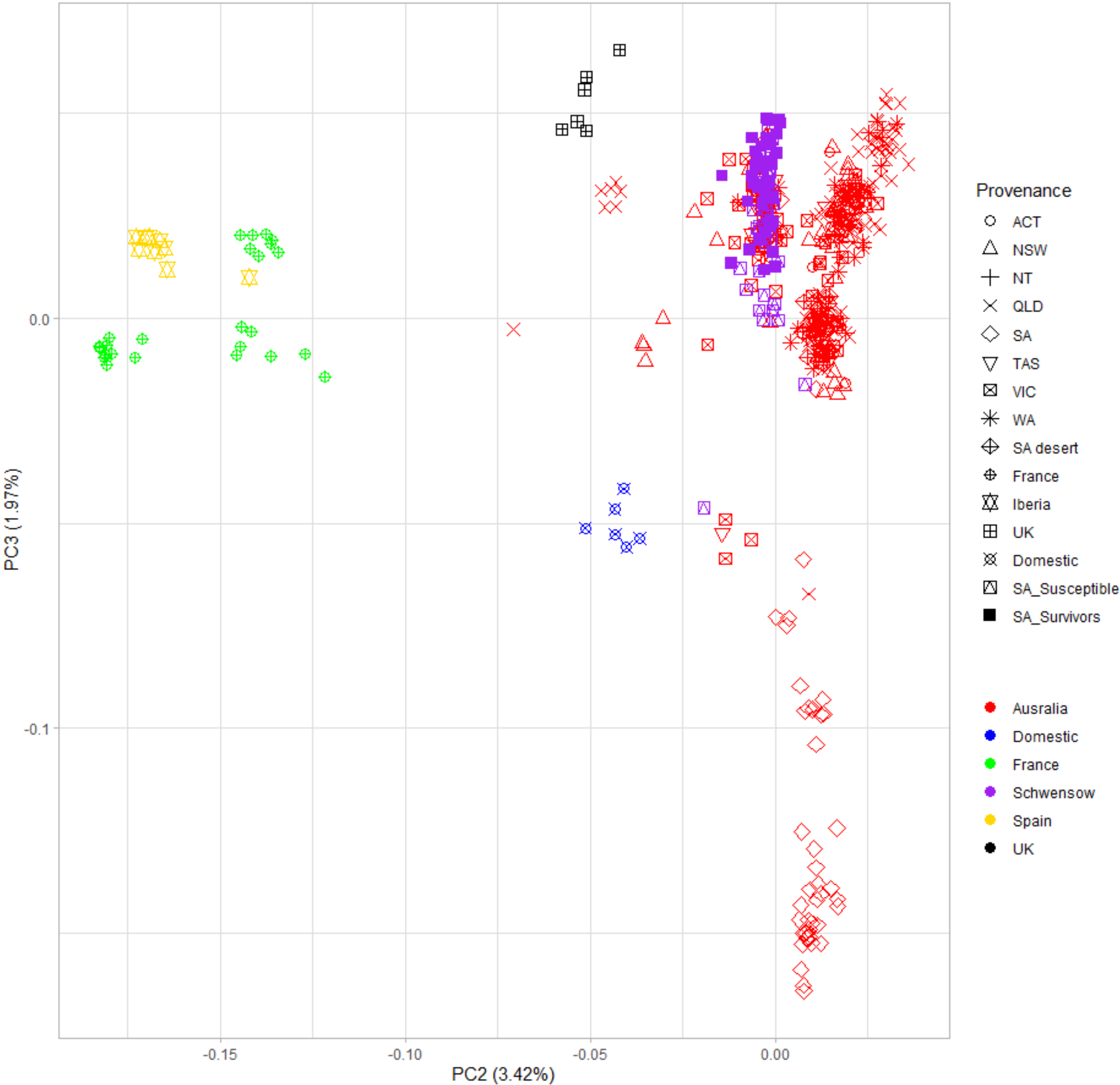


UNIVERSITY OF CAMBRIDGE
Joel Alves
Frank Jiggins
cibio
Miguel Carneiro
CSIRO

Key questions the dataset can answer:

Safety & efficacy:

- Understand genetic diversity of Australia's wild rabbits
- Look for locally fixed alleles
- Based on the genetic structure of the population, the spread of a theoretical genetic biocontrol can be modelled



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

Are there unequally shared transcripts during spermatogenesis leading to unequal inheritance of certain alleles?

RESEARCH

RESEARCH ARTICLE SUMMARY

SPERM GENOMICS

Widespread haploid-biased gene expression enables sperm-level natural selection

Kunal Bhutani*, Katherine Stansifer*, Simina Ticau*, Lazar Bojic, Alexandra-Chloé Villani, Joanna Slisz, Claudia M. Cremers, Christian Roy, Jerry Donovan, Brian Fiske, Robin C. Friedman†

- SS- transcriptomics on developing rabbit sperm
- Unequally shared alleles = selfish genes?

=> prospects for synthetic t-mechanism?

- Transfer Sperm assisted genetic modification technology to rabbits
- Validate target genes in rabbits

We’re hiring!
Transfer genetic control technologies to rabbits

- Genome engineer
- Veterinary scientist

Canberra, Australia

Thank You

Australian Government
Department of Agriculture and Water Resources

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