Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

Gene editing for invasive species control: conservation considerations and knowledge gaps

Margaret Byrne, Dorian Moro, Malcolm Kennedy, Susan Campbell Mark Tizard

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Department of **Agriculture and Food**

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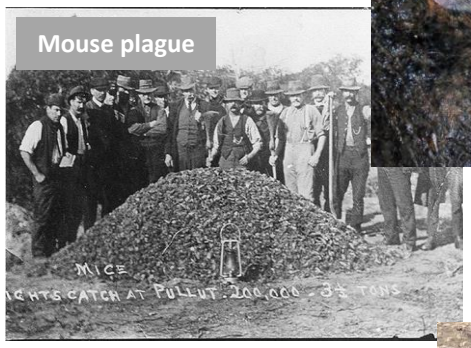
CSIRO

Introduced and invasive mammals

- House mouse
- European rabbits
- Feral foxes
- Feral cats
- Black rats

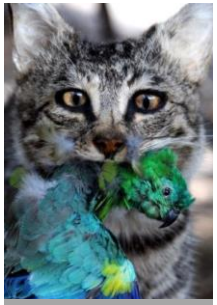



Black rats


Rabbit damage


Rabbit plague


Feral foxes


Feral cats

Biodiversity impacts of introduced invasive mammals

PNAS PNAS PNAS

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Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement

John C. Z. Woinarski[a,b,1], Andrew A. Burbidge[c], and Peter L. Harrison[d]

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This Feature Article is part of a series identified by the Editorial Board as reporting

Edited by William J. Bond, University of Cape Town, Cape Town, South Africa, and

The highly distinctive and mostly endemic Australian land mammal fauna has suffered an extraordinary rate of extinction (>10% of the 273 endemic terrestrial species) over the last ~200 y: in comparison, only one native land mammal from continental North America became extinct since European settlement. A further 21% of Australian endemic land mammal species are now assessed to be threatened, indicating that the rate of loss (of one to two extinctions per decade) is likely to continue. Australia's marine mammals have fared better overall, but status assessment for them is seriously impeded by lack of information. Much of the loss of Australian land mammal fauna (particularly in the vast deserts and tropical savannas) has been in areas that are remote from human population centers and recognized as relatively unmodified at global scale. In contrast to general patterns of extinction on other continents where the main cause is habitat loss, hunting, and impacts of human development, particularly in areas of high and increasing human population pressures, the loss of Australian land mammals is most likely due primarily to predation by introduced species, particularly the feral cat, *Felis catus*, and European red fox, *Vulpes vulpes*, and changed fire regimes.

conservation | biodiversity | marsupial | predation | feral animal

The world's biodiversity is in decline as humans increasingly use our planet's natural resources and modify its environments (1). Much of the current biodiversity decline is occurring in areas subject to the most rapid human population growth and highest rate of habitat loss and transformation, and in countries

'…the loss of Australian land mammals is … due primarily to predation by introduced species, particularly the feral cat, Felis catus, and European red fox, Vulpes vulpes …'

Earlier Losses

European settlement at 1788 marks a particularly profound historical landmark for the Australian environment, the opening up of the continent to a diverse array of new factors, and an appropriate baseline for measuring biodiversity change (9). However, the continent was not then paradisiacal: its mammal fauna had undergone profound changes before that date. The fossil record attests to appreciable change in the Australian mammal fauna over the previous hundred thousand years, most notably the loss of the continent's megafauna (10). The principal cause of these losses remains sharply contested but most likely involved a combination of rapid climate changes, environmental changes associated with the establishment of Aboriginal fire management, and hunting by Aboriginal people (who arrived on the continent about 50,000 y ago) (10–12). The arrival of the dingo, *Canis lupus dingo*, about 3,500 y ago (13) most likely caused further decline and change in the abundance of many species, although its role in broadscale extirpations at and since that time remains debated (14–16).

Taking Stock: The Current Conservation Status of and Outlook for the Australian Land Mammal Fauna

Our comprehensive review (7) concluded that 28 Australian endemic land mammal species have become extinct since 1788.

Agricultural impacts of introduced invasive mammals

Pest	$m
Birds	313.1
Rabbits	206.0
Wild dogs	48.5
Mice	22.8
Foxes	21.2
Feral Pigs	9.2
Total	**620.8**

Gong et al (2009) The economic impacts of vertebrate pests in Australia. Invasive Animals Cooperative Research Centre

Current control

Integrated chemical / physical management practices

- Poison baiting combined with habitat removal, fencing (rabbits, mice, rats)
- Poison baiting, shooting (fox)
- Poison baiting, trapping (feral cat)
- Biological control: rabbits: myxoma, RHDV1, RHDV1 (K5)

Western Shield
fox and feral cat baiting over 3.8 m ha

- Varying levels of efficacy
  - Short-term results in population control
- Unintended ecological consequences (mesopredator release)

Alternative practices

- Sterile males (invertebrates, carp)
- Virally vectored immuno-contraception
  - – mice, rabbits, foxes
- Gene drives (eg. CRISPR-Cas9)
  - ➢species-specific
  - ➢non-lethal
  - ➢Multiple applications
    - ➢susceptibility to an impactor from the environment, a disease
    - ➢intolerance for a key nutrient
    - ➢an inducible internal toxin
    - ➢disruption of a sex-determining gene to skew sex ratios to drive populations suppression or extirpation

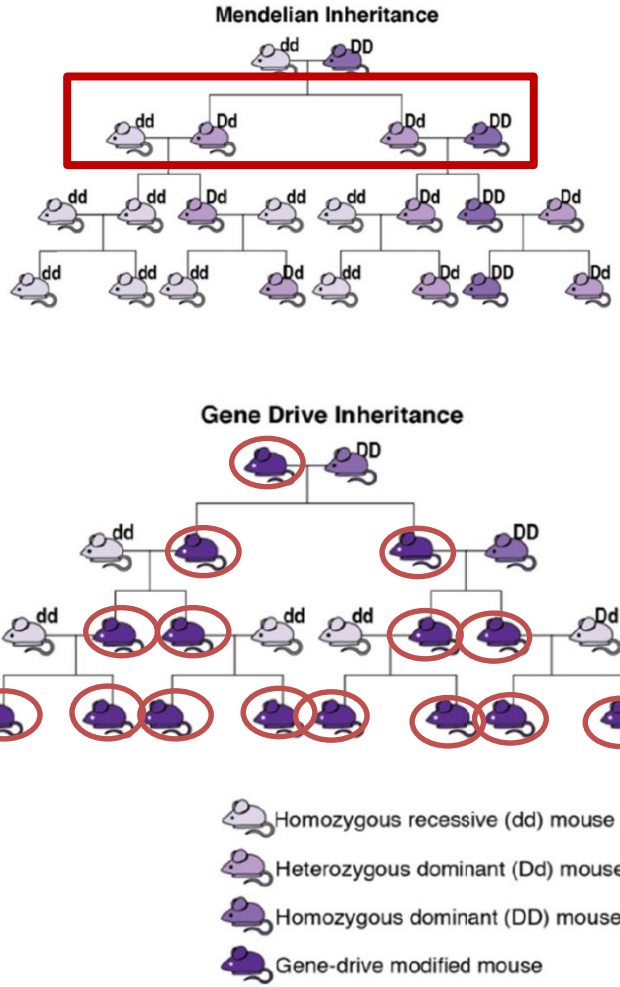
Gene editing

- Normal rules of inheritance
  - one allele from female and one allele from male
  - pass on only one of these to each offspring

- *Meiotic drives* are unusual naturally occurring genetic elements that distort the normal inheritance and gene segregation, leading to (theoretically) full transmission of the character

- Adapted for use in cells for medical research and possible treatments

- **Precise** editing of a **single gene** in the tens of thousands that make up an animal's DNA code



Disrupt sex determining gene – skew sex ratio – population extirpation

Considerations

OECD

Working Party on Biotechnology, Nanotechnology and Converging Technologies (BNCT)

Directorate for Science, Technology and Innovation

This workshop aims to stimulate a wide-ranging discussion that involves scientists, business leaders, policy makers and opinion leaders. It will be a cross-disciplinary event to exchange information and approaches across countries on the science, governance, and economics of gene editing innovations in:

- Applications in Agriculture & Aquaculture
- Environmental Applications, and
- Applications in Human Medicine.

Working through breakout sessions and case studies, the meeting will highlight policy issues of applications in each sector. It will also explore the important issues shared across these sectors that are critical to economies, including issues of science policy, innovation and governance.

In addition, the workshop aims to address certain economic questions raised by gene editing, notably IP issues and business and financing models, with a view to highlighting important aspects of responsible commercialisation.

Workshop on

Gene Editing in an International Context: Scientific, Economic and Social Issues across Sectors

29 – 30 September 2016

Sir Frederick G. Banting Building at Tunney's Pasture*

*251 Sir Frederick Banting Driveway, Ottawa, Ontario, Canada K1A 0K9

GENE DRIVES IN AUSTRALIA

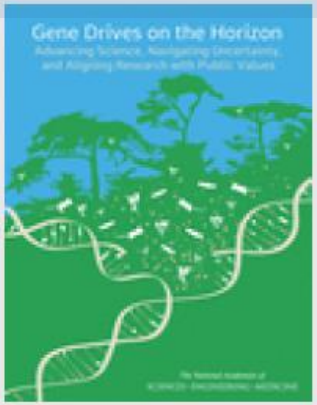
DISCUSSION PAPER. NOVEMBER, 2016

INTRODUCTION

Gene drive mechanisms cause a gene to spread throughout a population at a rate higher than would be predicted by Mendelian inheritance. Research on synthetic gene drives has accelerated recently due to significant advances in genome editing tools. Since 2015 scientists have published four proof of concept studies in yeast, mosquitoes and the fruit fly Drosophila to demonstrate the feasibility of using synthetic gene drives for purposes such as combating vector-borne disease, suppressing pest populations, or for introducing desired characteristics into target organisms. The potential applications are far reaching, as are the potential impacts– both intended and unintended–on public health, conservation and ecology. This rapidly developing area represents an additional method of manipulating populations alongside traditional and other methods as listed in Table 1.

The pace at which the science and technology field is moving has triggered international discussion on gene drives (Nuffield, 2016; NAS, 2016a). There is a need for governments and communities around the world to consider if, when and how it will be permissible to release organisms with synthetic gene drive mechanisms into the environment. Concerns have been raised in the scientific community as to whether organisms modified with synthetic gene drives should be released, and there is significant discussion amongst scientists on best practice and mitigation strategies.

This discussion paper is a contribution from the Australian Academy of Science, in which the (i) the benefits and risks of synthetic gene drives; (ii) ways to minimise the potential risks release of a gene drive modified organism; and, (iii) ways to limit the duration of th modification in the environment. This report discusses ecological and environmental economic issues (including trade implications) and governance issues from an Australi perspective. Our unique Australian environment generates a number of issues specific to report reflects such benefits and problems. The Academy intends that this discussio government and community thinking and decisions about gene drive technology in Austral



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]**

Sciencexpress

Policy Forum

Regulating gene drives

Kenneth A. Oye,[1,2*†] Kevin Esvelt,[3*] Evan Appleton,[4] Flaminia Catteruccia,[5,6] George

nome engineering that uses the CRISPR nuclease Cas9 to cut sequences specified by guide RNA molecules (5, 6). This technique is in widespread use and has already engineered the genomes of more than a dozen species. Cas9 may nable "RNA-guided gene drives" to dit nearly any gene in sexually reproducing populations (1).

To reduce potential negative effects a advance of construction and testing, svelt et al. have proposed several novl types of drives (1). Precision drives ould exclusively affect particular spees or subpopulations by targeting equences unique to those groups. Imunizing drives could block the spread f unwanted gene drives by preemptvely altering target sequences. Revertted changes introduced by an initial ngineering, even restoring the original effects would not necessarily be reded gene drives have yet to be demon-

certainly be allowed, but only under the strictest conditions and with appropriate safeguards.

In less than three years, CRISPR has become a key tool for biologists. 'Should they stop before it is too late?' is therefore an immaterial question.

pressed by propagating traits that reduce reproductive capacity (1, 2). Potential beneficial uses of such "gene drives" include reprogramming

Environmental and security aspects

A recent workshop examined key questions concerning effects of devel-

WORLD VIEW *A personal take on events*

Regulate gene editing in wild animals

The use of genome-modification tools in wild species must be properly governed to avoid irreversible damage to ecosystems, says **Jeantine Lunshof.**

Gene editing is a hot topic following a flurry of interest in the use of CRISPR tools to modify human embryos. As an ethicist in a genome-engineering lab, I am an eyewitness to these recent scientific developments and I do have concerns about the way gene

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*

Driven to Extinction

Gene drive technologies prov disperse engineered genes th

Potential for pest management

Investigate gene editing as a sustainable and economic landscape-wide alternative to population control of invasive species

while understanding and addressing the ecological risks

- Identify requirements of target species in risk framework
  - Knowledge
  - Desirable characteristics/features
- Identify knowledge gaps to aid future research priorities

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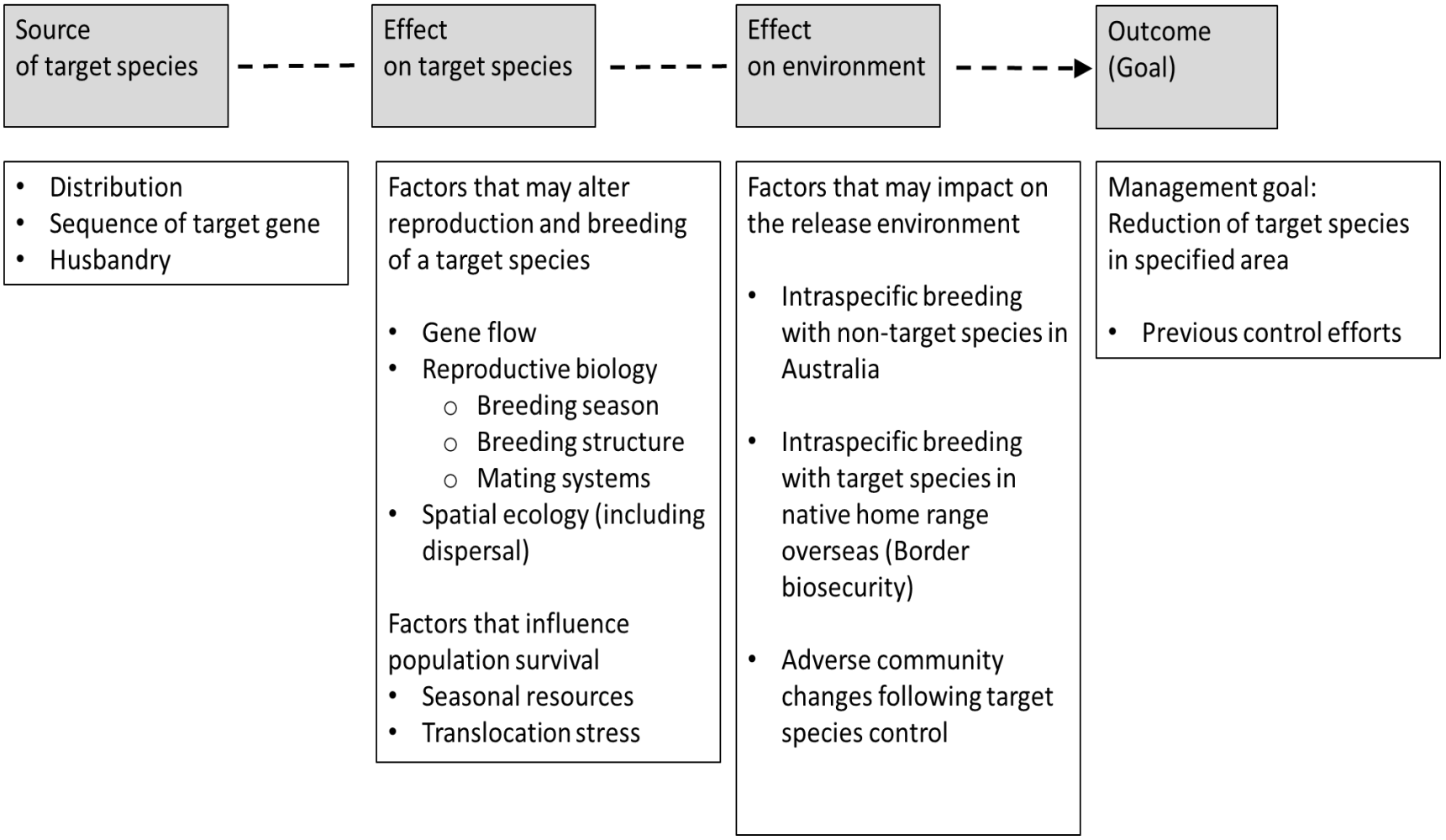
Review Paper

Identifying knowledge gaps for gene drive research to control invasive animal species: The next CRISPR step

Dorian Moro [a, *], Margaret Byrne [a], Malcolm Kennedy [b], Susan Campbell [c], Mark Tizard [d]

- Target mammal species
  - House mouse
  - European red fox
  - European rabbit
  - Feral cat
  - Black rat

Risk framework



Modified from 'Gene Drives on the Horizon.' National Academies of Sciences, Engineering, and Medicine 2016

Available knowledge

Species	Movement/ containment	Genome	Breeding Colonies	Gene flow	Reproductive biology	Spatial biology	Population regulation	Translocation stress	Species specificity	Biosecurity	Community dynamics	Prior control
Rabbit	++	+++	+++	+++	+++	+++	+++	++	+++	++	+++	+++
House mouse	+++	+++	+++	+++	+++	+++	+++	++	+++		+++	+++
Fox	+++	+++	+++	++	+++	+++	+++		+++	++	+++	+++
Feral cat	+++	+++	++	+++	++	+++	+++		++	++	+++	++
Black rat	+++	++	+++	++	+++				++		++	

Desirable characters/features

Species	Movement/ containment	Genome	Breeding Colonies	Gene flow	Reproductive biology	Spatial biology	Population regulation	Translocation stress	Species specificity	Biosecurity	Community dynamics	Prior control
House mouse	+++	+++	+++	+++	+++	+++	+++	++	+++		+++	+++
Rabbit	+++	+++	+++	+++	++	+++	+++	+++	++	+++	++	+++
Feral cat	+++	+++	++	+++	++	+++	+++		++	+++	++	++
Fox	+++	+++	++	+++	++	+++	+++		+++	++	++	++
Black rat	+++		+++	++	++		++		+++			

Knowledge gaps

Species	Life history and fecundity data (age-specific and sex-specific)	Gene flow	Clarify sex-determining genes	Density dependant reproduction and mate selection	Border transport pathways	Community interactions	Invasion biology	Fertility control
House mouse	✓	Mate selection	✓	✓	X	✓	X	✓
Rabbit	✓	Mate selection	✓	✓	X	✓	X	✓
Feral cat	X	X	✓	X	X	✓	X	X
Fox	✓	X	✓	✓	X	✓	X	✓
Black rat	X	X	partial	X	X	minimal	X	X

Considerations

- New control tools for controlling invasive mammals in Australia are urgently needed
- Gene editing is worth investigating
- Open discussion and evaluation, Community acceptance
- Acknowledge risks of gene editing technologies and options for mitigation
- Baseline information on the biology and ecology of target species is needed
- Risk assessment – framework for evaluation