Novel technologies for the prevention, surveillance and control of IAS

Andy Sheppard | Dec 2019
Introduced and invasive pest animal species

- Mouse plague
- Dengue fever
- Rabbit plague
- Feral foxes
- Cane toad plague
- Carp infestation
- Aphid infestation
- Fruit fly crop damage
Pests, weeds and diseases – What do they cost us?

Australian damage costs:

Weeds - $6B p.a  
= $146/ha

Feral animals - $1B p.a.

Pests & diseases $2B p.a.
Feral animals (59 sp.)

Top threat to:
• native mammals (22 extinctions)
• island bird biodiversity

Top 3 threat to:
• threatened ecosystems,
• wetlands and riparian zones

(IUCN & National Land & Water Resources Audit 2002)

Damage costs $1 Billion per annum
Australia’s history

- c. All Australia’s pests weeds and diseases are IAS now 120 yrs managing the problem
- AU: 1 tax payer per 2 km\(^2\) (1990) or 3500 m\(^2\) (2020) vs EU: 1 tax payer per 100 m\(^2\)
- IAS the top threat to biodiversity


- AU has to be bold – adaptive management approach from start
- Few early mistakes e.g. Cane toad
- New Biosecurity ACT 2015 – Chief Environmental Biosecurity Officer
- Dept of Ag just merged with Dept of Env
Rabbit biological control

(Cooke et al., 2013)

$70B benefit over 60 years
Paterson’s curse Biological Control: $1B benefit
New technology options

• Citizen crowd sourcing
• ICT technologies
  • Airborne imaging
  • Automated vehicles
  • Sensor networks
• Detection technologies
• Gene technology – next gen biocontrol?

Data analytics the only delivery limitation
Crowd sourcing

• Direct – citizen science portals
• Indirect – social media analytics
  – twitter (WHO, AU)
  – Artificial intelligence & machine learning
UAV detection

Complex “3D” imaging of large inaccessible areas
Includes visible, multi- and hyper-spectral; LiDAR (Light Detection And Ranging); and FLIR (thermal infrared) imaging technology

**Context:**
Hyperpsectral/LIDAR – weeds
Thermal infrared – vertebrate pests/RIFA

**Regulations:** CASA line of sight limitation

**Satellites:** imagery increasingly sophisticated and public access a government priority due to key agricultural benefits.
Low orbital satellites coming with 5G
Robotics for the widespread pest and weed management

- Detection
- Mapping
- Treatment

Development & annual detection and treatment costs of high density infestations less than using current human practice, savings increase with land value.
Sensor Networks

Visual, movement, audio & physiology data of monitored targets
Wireless radio connected sensor networks
Attachable/implantable
Machine learning AI capability

Application:
• Small scale continuous mapping/monitoring
• Livestock movement and health
• Monitoring/mapping bees and pest animals
• Audio detection e.g. invasive frogs and fish

Regulatory Context: Acceptable
New technology options

• Crowd sourcing
• ICT technologies
• Detection technologies
  • Smart trapping
  • Biosensors
• Gene technology – next gen biocontrol?
Smart trap surveillance
- supporting the protection and growth of global fruit and vegetable exports.
Unique Sensors – Behavioral “fingerprints”
• Automated wireless pest specific trapping from visual/behavioural cues

• Technology-based real-time detection service and image notification of pest detection

• Growers and biosecurity agencies can respond immediately to pest detection,

• Protection of existing markets and supporting the development of new overseas markets.

Customers make 30% profit and half their labour
Volatile biomarkers = chemical or group where detection level allows diagnosis
Developing sensors for target biomarkers

**Screenable** library of 300 nematode olfactory receptors that can be developed into CYBERNOSE® sensors

Method for discovering new biosensors for volatile analytes. PCT lodged April 2014.
New technology options

- Crowd sourcing
- ICT technologies
- Detection technologies
- Gene technology – next gen biocontrol?
  - SWOT
  - Gene drives - global concerns, effective? risky?
3 x Gene-tech pest/weed management interventions

• **RNA interference** - Specific biopesticide applied/consumed
  breaks protein synthesis

• **Self-limiting genetic solutions** (Mendelian inheritance) – mass rearing and release strategies

• **Sustaining “Meiotic” gene-drive systems** (non Mendelian) - Genetic suppression of pest/weed populations
  – eradication theoretically possible
RNAi

- Specific biocide targeting specific target genes
- Disrupts intracellular translation of DNA to Protein
- Requires delivery to and into target cells – big challenge!
- Delivery types
  a) **Endogenous** - generated within cells using GM or
  b) **Exogenous** - constructed and physically delivered to cells
RNA interference (RNAi): proven technology for trait development in plants

- Allergen free peanuts
- Wheat with healthier starch
- Healthier cottonseed oil
- Lysine rich soy beans
- Virus resistant cereals
- Blue rose
- Improved photosynthesis in canola
- Supercharged safflower
RNAi challenges

1. Formulation for effective delivery 
i.e. miRNA stability in the environment  
– nanosheets? bioclay?
2. Delivery to all target cells through plant  
epidermis or gut walls
3. Costs compared to herbicides & scalability?

Proof of concept already for suppression  
of plant viral and fungal diseases

RNAi proposed: common reed *Phragmites australis* control (USGS Great Lakes Science Center 2012).

Gene silencing targeting *Phragmites*:
- growth,
- seed production,
- vegetative reproduction,
- photosynthesis,
- flower organ development,
- pollen production

US Patent Application 14/503,675, 2014 “Treatment of one or more populations of invasive plant species with a composition comprising an effective amount of nucleic acids, wherein the nucleic acids have been produced using gene silencing techniques to target specific plant species”

Mendelian Genetics

Gene of Interest

Lacking Gene of Interest
Self limiting genetic constructs – to reduce population fitness using *Mendelian* inheritance


2. Release Insect Dominant Lethality gene (RIDL) - ♂ carrier x WT ♀ - lethal (Oxitech) – fruit flies & mosquitos

3. Incompatible Insect Technique
   - *Wolbachia*-infected ♂ x WT ♀ - lethal – mosquitos

4. Next-gen Sterile Insect Techniques (SIT) “Synthetic species” – which at F1 produce ♂ only lines incompatible to WT (no offspring) - fruit flies
Daughterless carp – Thresher et al. 2010

Gambusia

European carp

WT ♀

M13

“NEOMALE”

F1 carriers
Synthetic Speciation?
(Mike Smanski/Maciej Maselko, Univ. Minnesota, Macquarie Uni & CSIRO)

- Engineered 2-way mating incompatibility
- Engineered strain carries a lethal transcriptional activator that only binds to wild type genome.
**Meiotic Gene-drive**

Gene Drive $\rightarrow$ Wildtype

T-Sry $\leftarrow$ Genetic Approaches $\rightarrow$ CRISPR/Cas9
Self-sustaining meiotic gene-drive systems

Genetic mechanisms (natural or synthetic) that can propagate modified gene(s) through a target population via *super-Mendelian* inheritance
Natural selfish genetic elements (gene-drives)?

- **Wolbachia** in *Aedes & Culex* spp. mosquitos
  - Cytoplasmic incompatibility; infected ♂ x WT ♀ - lethal
  - Population replacement with infected lines with reduced competence

- **Y-drive** in insects e.g. *Aedes* leads to breakage of X chromosome distorting sex ratio – 80-90% heritable in wild populations

- **Medea gene element** (maternal toxin & antidote traits) in beetles, fungi & plants – all offspring without antidote gene die

- **Pre-gametic (biased meiosis) & post-gametic (gamete/pollen killers) drives** in plants

- **T-Sry mice** – male sex determining mutation in 30% of wild mice

Fitness costs often lead to reduced titres of selfish genotypes in wild populations
CRISPR – clustered regularly interspaced short palindromic repeat
Synthetic Gene-Drive system in pest management – drive deleterious genes into the genome of every pest individual in the population = eradication?

- 2002 an Idea
- 2009 discovery of CRISPR-Cas9 gene shears
- 2014 gene-drives a GM reality
- 2016 – public acceptability?
  - ethics questions
  - regulations for use?
Gene drives for pest animal population control?

- Sex bias
- Inducible toxin?
- Susceptibility
- Shorter life span

Esvelt et al., e-life, 2014
CRISPR /Cas9

• Bacterial immunity mechanism transferred to eukaryotic cells

• Directly cuts and inserts desired deleterious gene sequence at any desired point in the genome

• Works synchronously on both chromosomes circumventing normal inheritance
Artificial Gene-drive “cassette” components
Activated each time it encounters guide sequence on genomes after each mating event

GUIDE sgRNAs
• Maintain drive activity
• Target host gene function

PAYLOAD: deleterious gene construct

SCISSORS: CRISPR-Cas9
• Inducible in germ line
• Active post fertilization
Gene drive mode of action

Gene Drive

Mating

Wild-Type

Endonuclease

Gene

Gene Drive Chromosome

Wild-Type Chromosome

Endonuclease

Cut

Break

Non-Homologous
End-Joining

Homologous
Recombination

1 Drive
1 Wild-Type

1 Drive
1 Mutated Target Site

2 Drives

NHEJ can create a resistant allele!!
Gene-drive - advantages

1. **Monospecific** – “control” contained within target population by gene-flow
2. **Potential landscape-scale control** – spread rate defined by gene-flow
3. **Control disseminated by the target itself** – no application costs
4. **No environmental residues** – GM construct naturally broken down on death/population collapse
5. **Humane** – no targets killed through its use
Gene-drive requirements

1. **Genes to disrupt** – sequence & annotate target genomes to identify targets.
2. **Mutation pathway** - either through the zygote (one parent) or via germlines (directly to sperm/oocyte development of both parents)
3. **Sexual reproduction** – asexual species out of scope e.g. some weeds/pests
4. **Short life cycle** – to have sensible times to population saturation
5. **Low impact on individual fitness** under normal field conditions
Gene-drive constraints

1. **Gene flow** – selfing weeds and low seed dispersal limitations
2. **Scale of releases** – how many modified individuals to release to optimize management?
3. **Payload gene transfer success frequency** to uninfected chromosomes (variability in some targets e.g. weeds?)
4. **Mechanisms of resistance evolution** to gene-drive machinery?
Gene drive target mechanism?????

- Remove resistance to existing pesticides
- Daughterless gene-drive for pest animals?
- Remove self-compatibility in weeds?
- Induce ♀ sterility and ♂ fertility?
- Increase susceptibility to a benign chemical = new specific but eco-harmless pesticide?
- Inducible disease susceptibility gene = new biopesticide?
- Others?
Global concerns
Everything an ideal biocontrol should be:

• Humane
• Species specific
• Self-disseminating
• NOT CONTAGIOUS (spreads by sexual reproduction)
• Not repeated release of many animals
• Hope?

Should be banned

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

Excitement

Panic
Sciencexpress

POLICY FORUM

TECHNOLOGY GOVERNANCE

Editing nature: of global governance

Environmental gene editing debate

By Natalie Koñler, James P. Collins, Jennifer Kyllop, Michael Paul Nelson, Andrew Newhouse, Lynne Semenov, Rowan Jacobsen, James E. DeFries, Aladisa Caccone, Timothy Brown, Oswald J. Blanc

Scientists have recognized the potential for applying gene drive technologies to the control of pests but whether we should do so and the implications of re-engineering the genome are already hotly debated.

A new genetic technology bankrolled by the United States military has the potential to wipe out feral mice and malaria—but scientists are treading carefully, warning it could have unintended consequences.

Risks raised:

• Uncontrollable? Once released cannot be recalled
• Synthetic gene-drive carriers are GMO
• Spread to target native populations – extinction risk!
• Can we de-risk an untried technology?
• Need tough regulations!
• Moratoria (IUCN/CBD)!
NGOs organised R&D moratoria through IUCN (2016) and CBD (2018) – thwarted

Genetic frontiers for conservation
An assessment of synthetic biology and biodiversity conservation
Edited by: Kent H. Redford, Thomas M. Brooks, Nicholas B.W. MacFarlane, Jonathan S. Adams

UN treaty agrees to limit moratorium
Treaty’s vague language on how researchers will fund opponents and supporters of the technology

Ewen Callaway
Principles for gene drive research
Sponsors and supporters of gene drive research respond to a National Academies report

By Claudia Emerson,1 Stephanie James,1 Katherine Litterle,2 Filippo (Fil) Randazzo2

The recent outbreak of Zika virus in the Americas renewed attention on the importance of vector-control strategies to fight the many vector-borne diseases that continue to inflict suffering around the world. In 2015, there were ~212 million infections and a death every minute from malaria alone (1). Gene drive technology is being explored as a potentially durable and cost-effective strategy for controlling the transmission of deadly and debilitating vector-borne diseases that affect millions of people worldwide, such as Zika virus and malaria. Additionally, its suitability is being evaluated for various potential applications in conservation biology, including a highly specific and humane method for eliminating invasive species from sensitive ecosystems (2, 3).

The use of gene drives is an emerging technology that promotes the preferential inheritance of a gene of interest, thereby increasing its prevalence in a population. A gene drive is dis-
Gene Drives on the Horizon
Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values

Australian Academy of Science

DISCUSSION PAPER
SYNTHETIC GENE DRIVES IN AUSTRALIA:
IMPLICATIONS OF EMERGING TECHNOLOGIES
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
### 2017

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<th>1. Communication/Governance</th>
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<td>Communication of governance arrangements regarding regulation of synthetic gene drives.</td>
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<th>2. Quality &amp; duration of science</th>
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<td>Resources be provided to study synthetic gene drives in isolated laboratory populations with sample sizes and time frames that are large enough and/or long enough to ensure transmission distortion, together with the intended and potentially unintended consequences that these processes may lead to.</td>
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<th>3. Stringent containment</th>
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<td>Consideration of wider implications</td>
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<th>4. Risk assessment</th>
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<td>Any decision to release a synthetic gene drive should be based on a risk assessment which includes ecological and evolutionary modelling.</td>
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<th>5. Communication/Consultation</th>
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<td>Involvement of relevant stakeholders from the outset and ongoing involvement throughout the process.</td>
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<th>6. Consideration of wider implications</th>
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<td>The wider implications of synthetic gene drives (e.g., environmental, social, economic).</td>
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Proceed – but with caution!

Science

Risk assessment

Social License, Liaison

Frameworks, underpinning science
Does the U.S. public support using gene drives in agriculture? And what do they want to know?

Michael S. Jones¹², Jason A. Delborne²³, Johanna Elsensohn²⁴, Paul D. Mitchell⁵, Zachary S. Brown¹²*,

Gene drive development is progressing more rapidly than our understanding of public values toward these technologies. We analyze a statistically representative survey \( n = 1018 \) of U.S. adult attitudes toward agricultural gene drives. When informed about potential risks, benefits, and two previously researched applications, respondents' support/opposition depends heavily \((+22\%/-19\%)\) on whether spread of drives can be limited, non-native versus native species are targeted \((+12\%/-9\%)\), or the drive replaces versus suppresses target species \((\pm 2\%)\). The one-fifth of respondents seeking out non-GMO-labeled food are more likely to oppose drives, although their support exceeds opposition for limited applications. Over 62\% trust U.S. universities and the Department of Agriculture to research gene drives, with the private sector and Department of Defense viewed as more untrustworthy. Uncertain human health and ecological effects are the public’s most important concerns to resolve. These findings can inform responsible innovation in gene drive development and risk assessment.
Do gene-drives work? & can they be made low risk?
1st target malaria mosquito

A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae

Andrew Hammond1, Roberto Gallizi1, Kyros Kyrou1, Alekos Simoni3, Carla Siniscalchi2, Dimitris Katsanos1, Matthew Griffibi1, Dean Baker2, Eric Mares2, Steven Russell3, Austin Burt1, Nikolai Windbichler2, Andrea Crisanti2 & Tony Nolan1

Gene drive systems that enable super-Mendelian inheritance of a sequence have the potential to modify homogamete in a process known as ‘homing’. Through this mechanism, the female offspring of HOMO can viably transmit to the next generation. Moreover...

Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi

Valentino M. Gantz1,2,3, Nijole Jasinskaiene3,4, Olga Tatarenkova3, Aniko Fazekas5, Vanessa M. Mancias6, Ethan Bier2,4, and Anthony A. James1,2

Genetic engineering technologies can be used both to create transgenic mosquitoes carrying antipathogen effector genes targeting human malaria parasites and to generate gene-drive systems capable of introgressing the genes throughout wild vector populations. We developed a highly effective autonomous Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (Cas9)-mediated gene-drive system in the Asian malaria vector Anopheles stephensi, adapted from the mutagenic chain-replicated to new sites while providing confidence that treated areas will remain malaria-free (5, 7).

We and others are pursuing a population-modification approach that involves the introduction of genes that confer a parasite-resistance phenotype to mosquitoes that otherwise would be fully capable of transmitting the pathogens (8–15). The expectation is that the introduction of such an effector gene at a high enough frequency in a vector population would decrease or...
Gene drives tested in mammals for first time

Technology worked inconsistently in mice.

BY EWEN CALLWAY

A controversial technology that can alter the genomes of entire species has been applied to mammals for the first time. In a preprint published on 4 July, researchers and researchers have suggested that the technology could help to kill off rodent pests. The technique has attracted controversy — and even a failed attempt to ban its global use — because, if released in the wild, organisms carrying gene drives might be hard to contain.

Zygotic pathway gene-drives didn’t work due to resistant NHEJ
Germline sex distortion gene-drive: “Y & X -chromosome shredder”

Y- shredder (Y-CHOPE) programmable endonuclease ‘shreds’ Y chromosome - published 2019

X- shredder (potentially simpler) under development

Unique allele characterization of Island mice populations to contain gene-drives

• evaluate the efficacy of locally-fixed alleles as a means of spatially limiting gene-drives.
• creating CRISPR constructs targeting known unique alleles within a particular test mouse population.
• semi-natural experiments crossing drive-carrying mice both with susceptible (i.e. with a locally-fixed allele) and non-susceptible mice genotyping offspring.
Kevin Esvelt’s Daisy Chain

Local drive (self-exhausting)

few more

Self-Exhausting (Daisy) Drive
Local Drive Wild-Type

Daisy-Chain Drive

Noble C., Min P., Chojnacki J. et al. (2014) bioRxiv

Noble C., Min P., Chojnacki J. et al. (2014) bioRxiv

Noble C., Min P., Chojnacki J. et al. (2014) bioRxiv
Programmable Base Editors (generation counters)

- Based on CRISPR-Cas9 endonucleases modified by:
  - removing nuclease domains
  - fusing to cytidine deaminase or adenine deaminase

- Targeting base editors to genomic loci induces specific GC to AT or TA to CG mutations

- Mutations not dependent on HDR/NHEJ double-strand DNA break repair pathways
Base editors on Y-chromosome introduce dominant female-sterile/lethal mutations to other chromosomes.

All male offspring carry base-editor system and mutation. All female offspring are sterile/non-viable.
Future targets?
Cane toads?

Gene-edit a toxin free cane toad?

Knockout pigment gene 1st!

Control Toad

Cas9-Tyr-Toad

@ 6 months

1 month ago successfully generated bufotoxin hydrolase knockout cane toad tadpoles!

Caitlin Cooper & Mark Tizard
NATIONAL CARP CONTROL PLAN

Carp need to be controlled in Australia - we're working on how.

The Plan will be delivered to the Australian Government in December 2019.

Learn more
Gene drives in plants: opportunities and challenges for weed control and engineered resilience

Luke G. Barrett¹,³, Mathieu Legros¹,³, Nagalingam Kumaran², Donna Glassop¹, S. Raghu² and Donald M. Gardiner¹

¹CSIRO Agriculture and Food, Canberra, Australian Capital Territory, Australia

Cats? — strong AU NGO support

**Advantages**
- No threat to pet cats (not contagious)
- Fecund, promiscuous and nomadic target (facilitates spread)
- Humane
- Long-term solution?
- Need better tools

**Disadvantages**
- Long generation interval (5-10 months)
- Captive GM cats competitive fitness?
- Long life spans, => long time to see results?
- Logistical nightmare for public acceptance
New Project through CISS:

“Gene-drive business decision system to prioritise vertebrate pest species for the potential development of gene drive-based population control tools in Australia”

Aim:
- better understand demand, technical knowledge gaps, risk analysis and social licence
- establish framework & prioritization system
- inform an appropriate gene-drive investment strategy
Gene-tech’s a great new technology with potential we can’t ignore. Our role is to understand the possibilities, the people will decide if they want to use it!
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