# Genetic biocontrol for invasive vertebrate pests

**Dr Stephen Frankenberg**

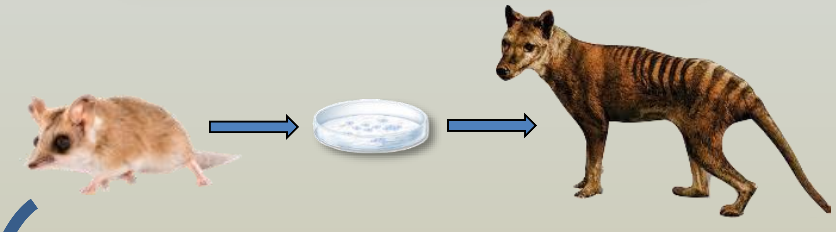
THE UNIVERSITY OF MELBOURNE

# Frankenberg Lab

## Prof. Andrew Pask

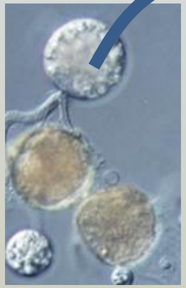
TIGRR LAB
Thylacine Integrated Genetic Restoration Research

### Thylacine de-extinction



### Engineering chytrid fungus immunity in frogs

chytrid fungus



antibodies produced by alpaca

transgenes expressing antibodies in frog skin

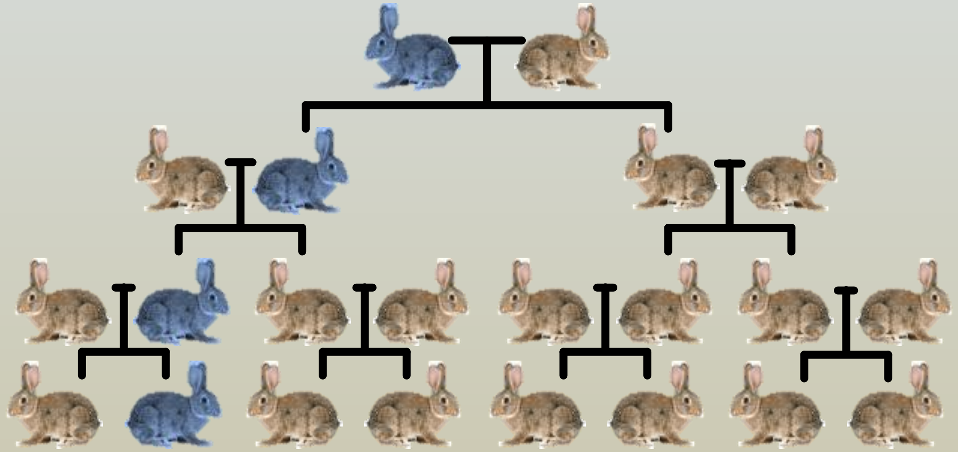
### Genetic biocontrol of invasive pests

### Engineering cane toad toxin resistance in the northern quoll

A single edit in a known gene is predicted to confer toxin resistance

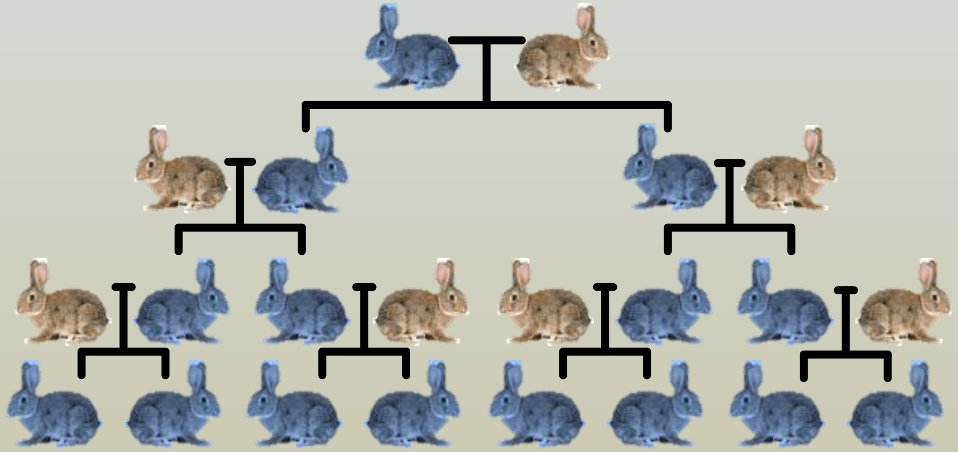
# Gene drives targeting female fertility genes for suppression/eradication of invasive pest populations

**Normal (Mendelian) inheritance**



Altered gene does not spread

**Gene drive inheritance**



Altered gene is always inherited

Success will depend on:

- **efficient gene drive design**
- **the ability to produce animals carrying gene drives**

# For good gene drive design, we need:

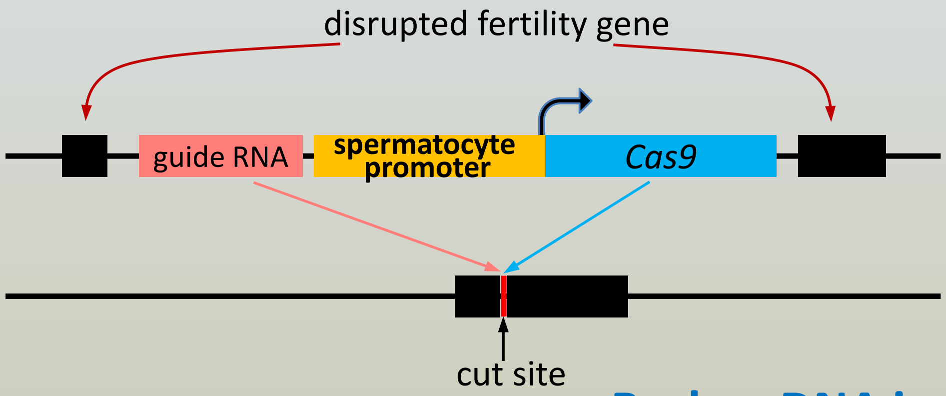
- suitable target genes essential for female development or fertility

e.g. **zona pellucida** (coat surrounding egg)

- efficient copying of the gene drive in the cells ("spermatocytes") that develop into sperm

# Improving CRISPR-based gene drive copying efficiency

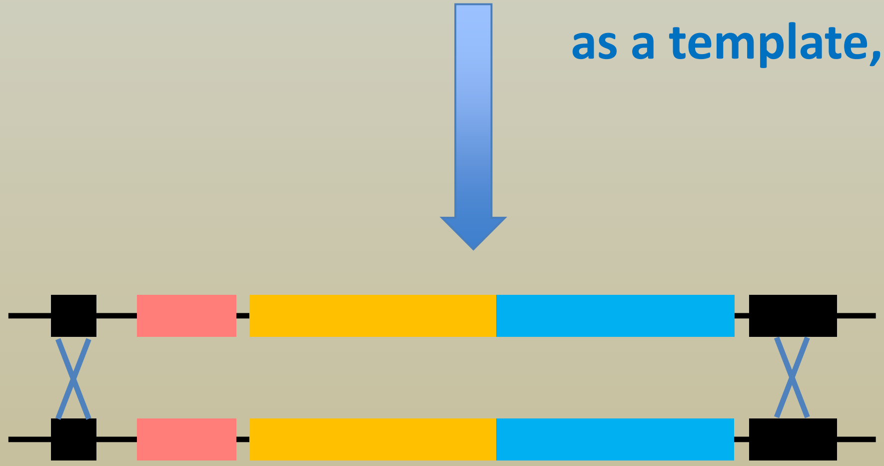
- **Cas9**: enzyme that cuts DNA in target gene at precise location (determined by **guide RNA**)
- **Amount** and **timing** of Cas9 is determined by the **promoter** in spermatocytes



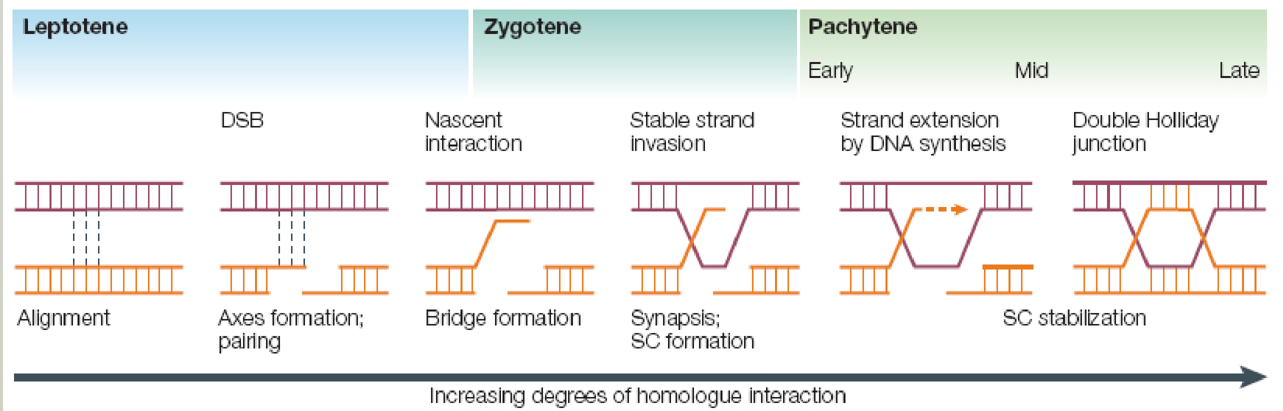

**fertility gene containing gene drive insertion**

**wild-type version (allele) of fertility gene**

**Broken DNA is repaired using the gene drive as a template, creating a second copy**



**MEIOSIS**: natural crossing over between chromosomes to repair DNA breaks



zebrafish

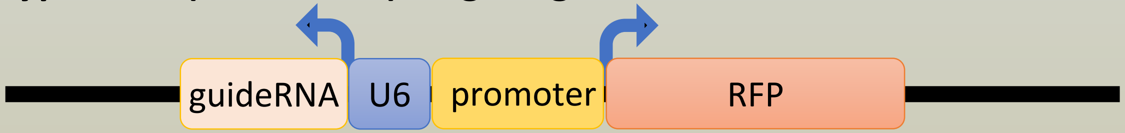
# Optimising gene drive copying efficiency

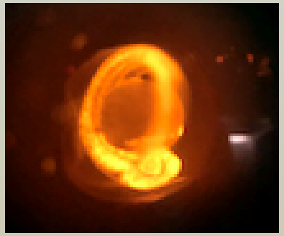
Clancy Lawler

Dr Patricia Jusuf

Proof-of-principle ”split gene drive” targeting ***cyp19a1a* (aromatase)** gene, which is essential for female development

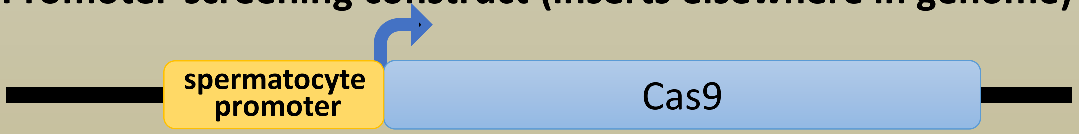
***cyp19a1a* (aromatase) targeting construct**



zebrafish embryo expressing red fluorescent protein (RFP)

**Promoter-screening construct (inserts elsewhere in genome)**



# Inserting heritable gene drives into non-model species

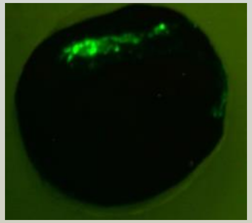
**European carp**

**cane toad**

**fox**

**rabbit**

direct microinjection
of CRISPR reagents
into thousands of eggs



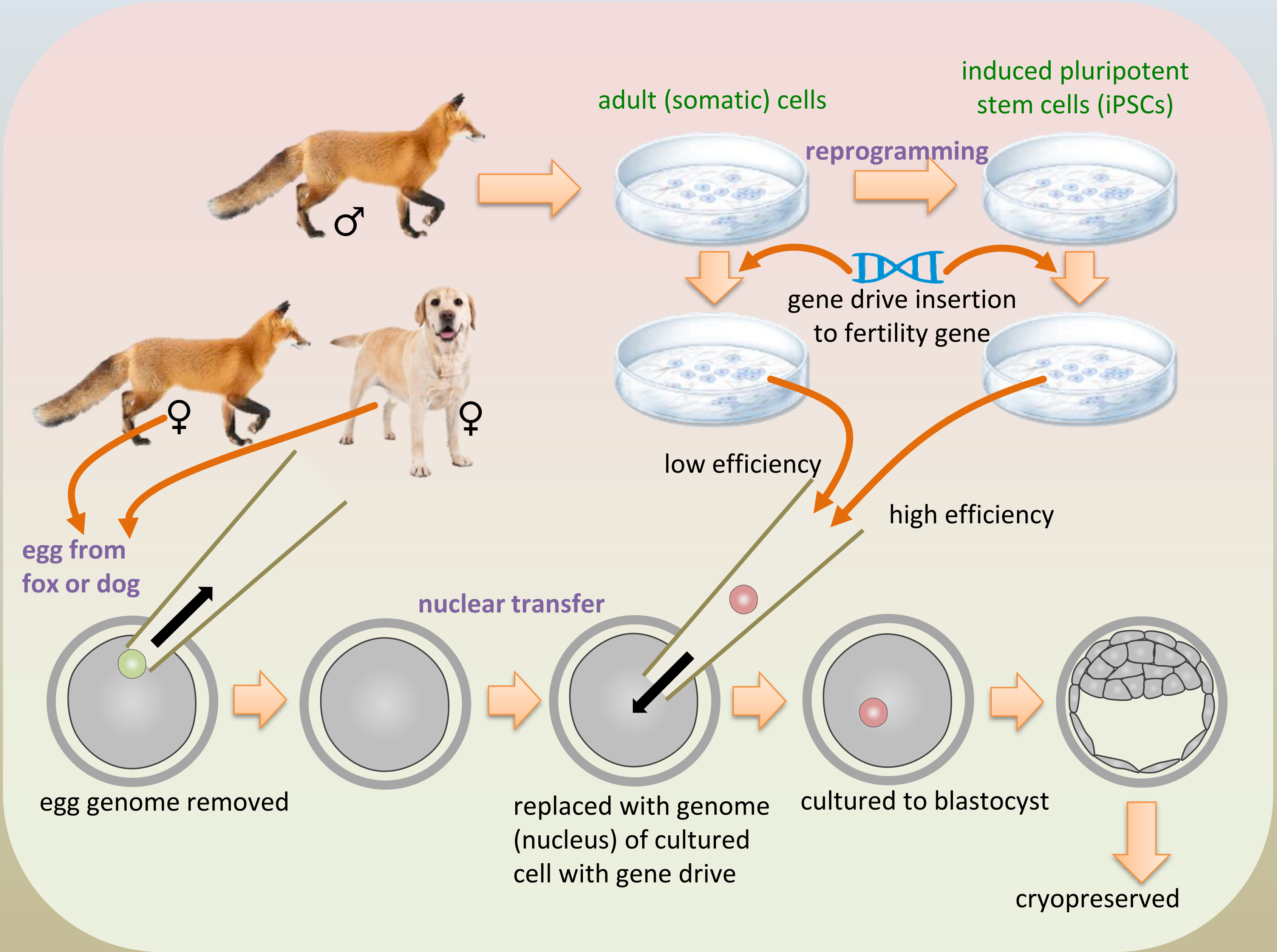
toad embryo
expressing green
fluorescence
transgene

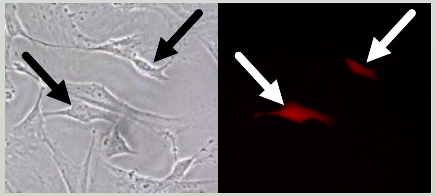
CRISPR-edit
cultured cells

nuclear transfer

**Dolly**

# Nuclear transfer pipeline for non-model species



fox cells with red fluorescence gene inserted on Y chromosome

# Risk of introduction to non-target populations

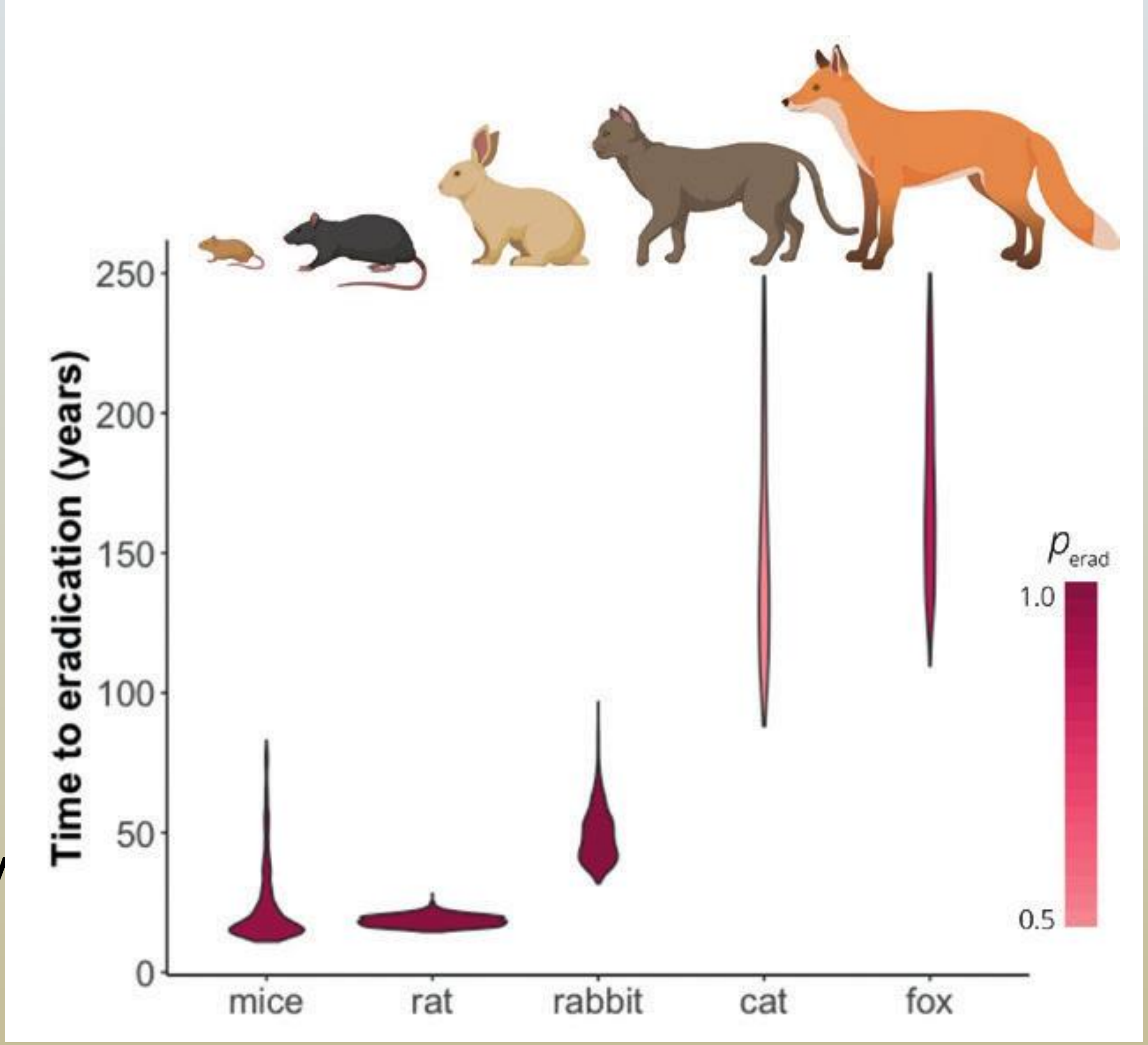

**Solution:**

Easier to engineer a gene drive-resistant allele (while maintaining normal functionality of the gene) than to engineer the gene drive in the first place

# Deployment of suppression gene drives for long-lived species

- Slow – many decades
- Will require monitoring and strategic management – e.g. regular releases of captive-bred gene drive males

"Best time to plant a tree is 30 years ago"



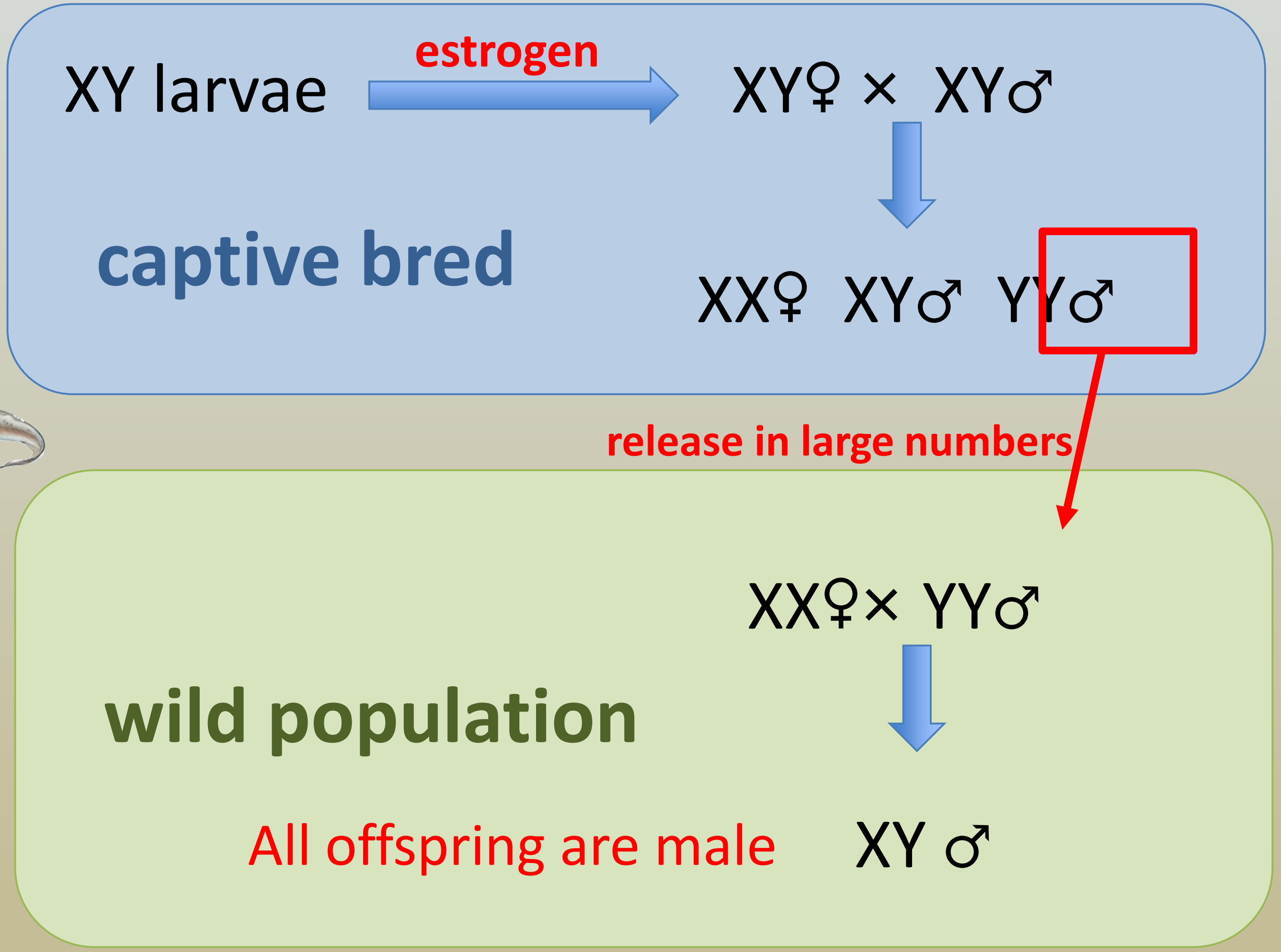

Simulation of 256 gene driv
individuals released into a
population of 200,000

source: Birand *et al.* 2022

# Smooth newt: future "cane toad of the south?"

- Invasive population detected in south-east Melbourne in 2011
- Appears to be spreading

**Trojan Y – quick, non-self-sustaining biocontrol**



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## Collaborators

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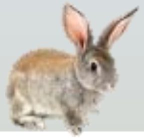
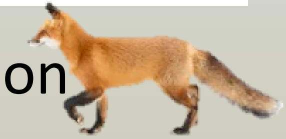
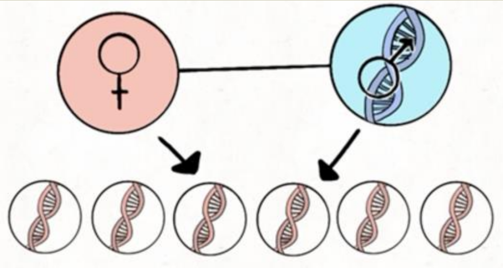


Australian Government Department of Agriculture, Water and the Environment

RABBIT-FREE AUSTRALIA Bilbies Not Bunnies

Russel & Womersley Foundation



https://frankenberg.science.unimelb.edu.au/

**Gene drive explained by my 11-year-old niece:**

https://www.youtube.com/watch?v=U3JqPYveNjk