Classical biological control research @ CSIRO – New directions for vertebrates, invertebrates and weeds

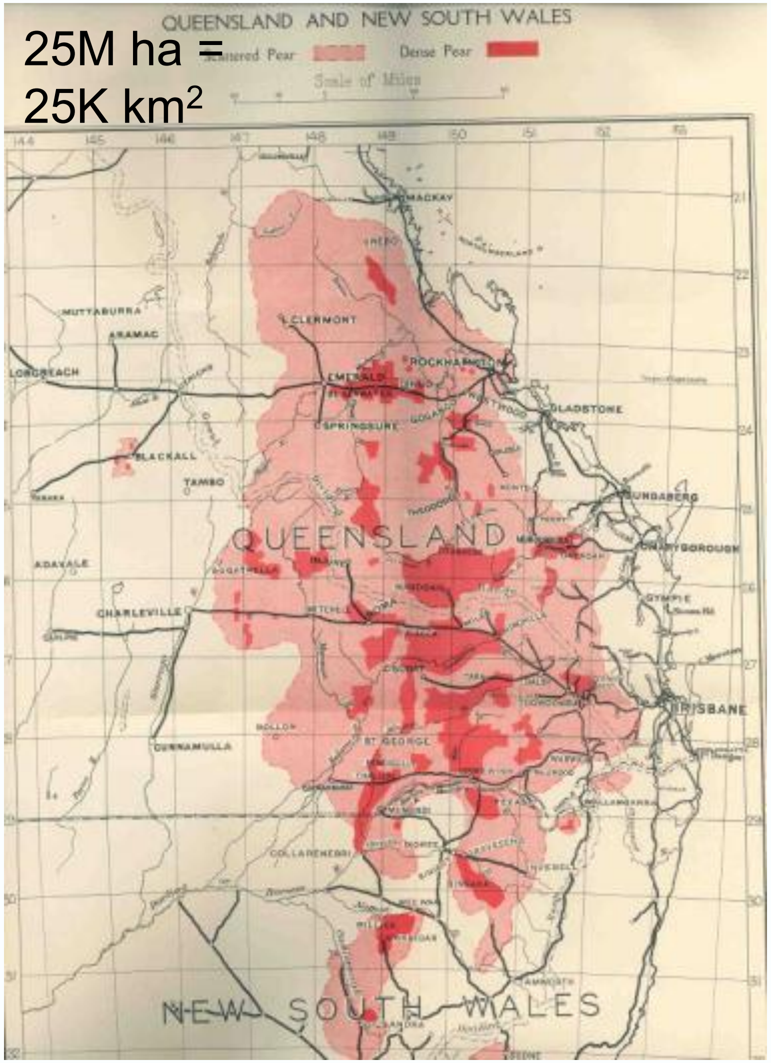
CSIRO Health & Biosecurity
- CSIRO European Laboratory

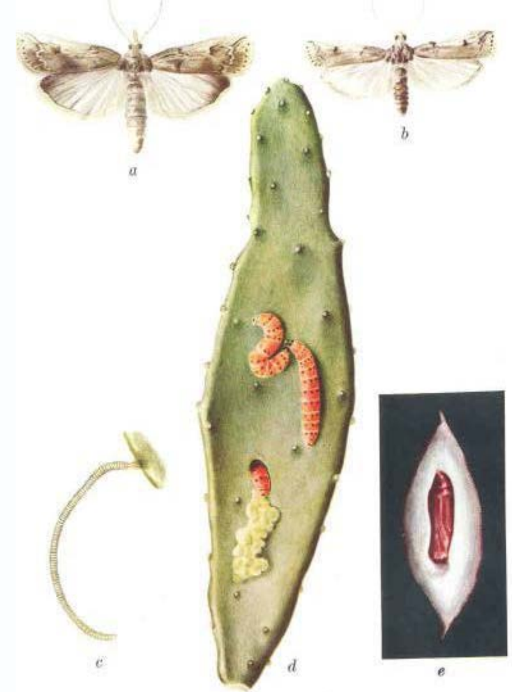
Andy Sheppard, Research Program Director

Outline

- Classical biological control in Australia - 110 years of success
- Next generation gene-tech based biocontrol opportunities
- CSIRO European laboratory activities

Opuntia & Cactoblastis 1921-1940





Cactoblastis cactorum.

AU weed biocontrol milestones ...

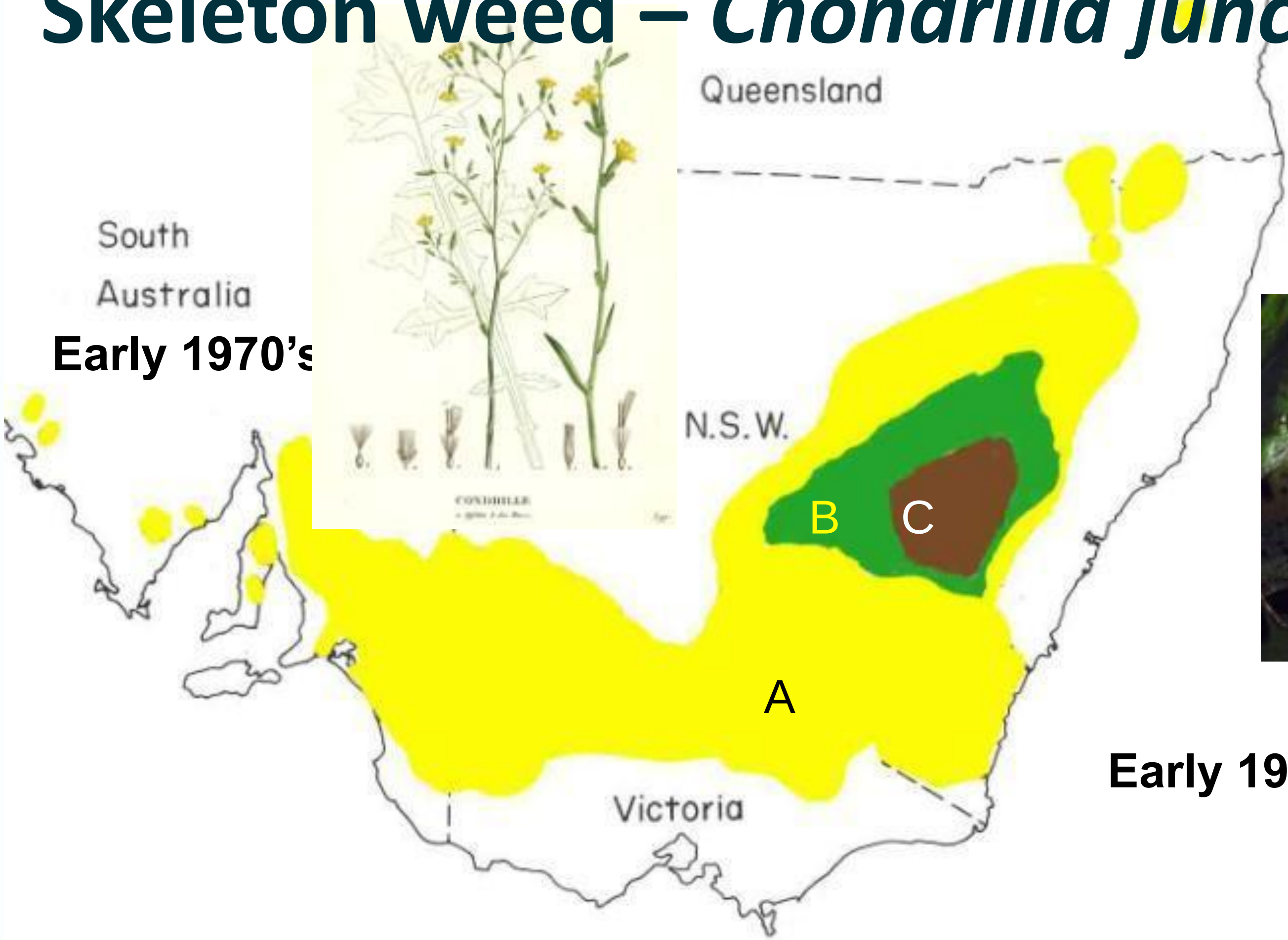
CSIRO European laboratory

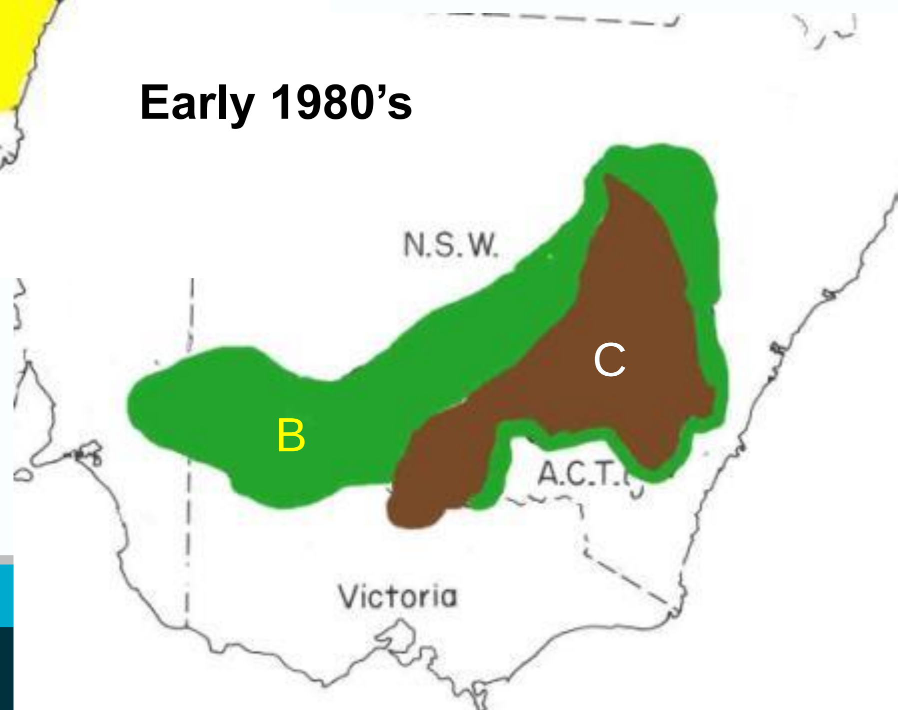
- 1971 first acceptable release of a plant pathogen – *Puccinia chondrillina* against skeleton weed
- 1974 Wapshere's – "*centrifugal phylogenetic testing*" revolutionised biocontrol risk assessment

Policy consequences

- 1986 Biological control Act and other regulatory mechanisms developed to regulate contentious releases

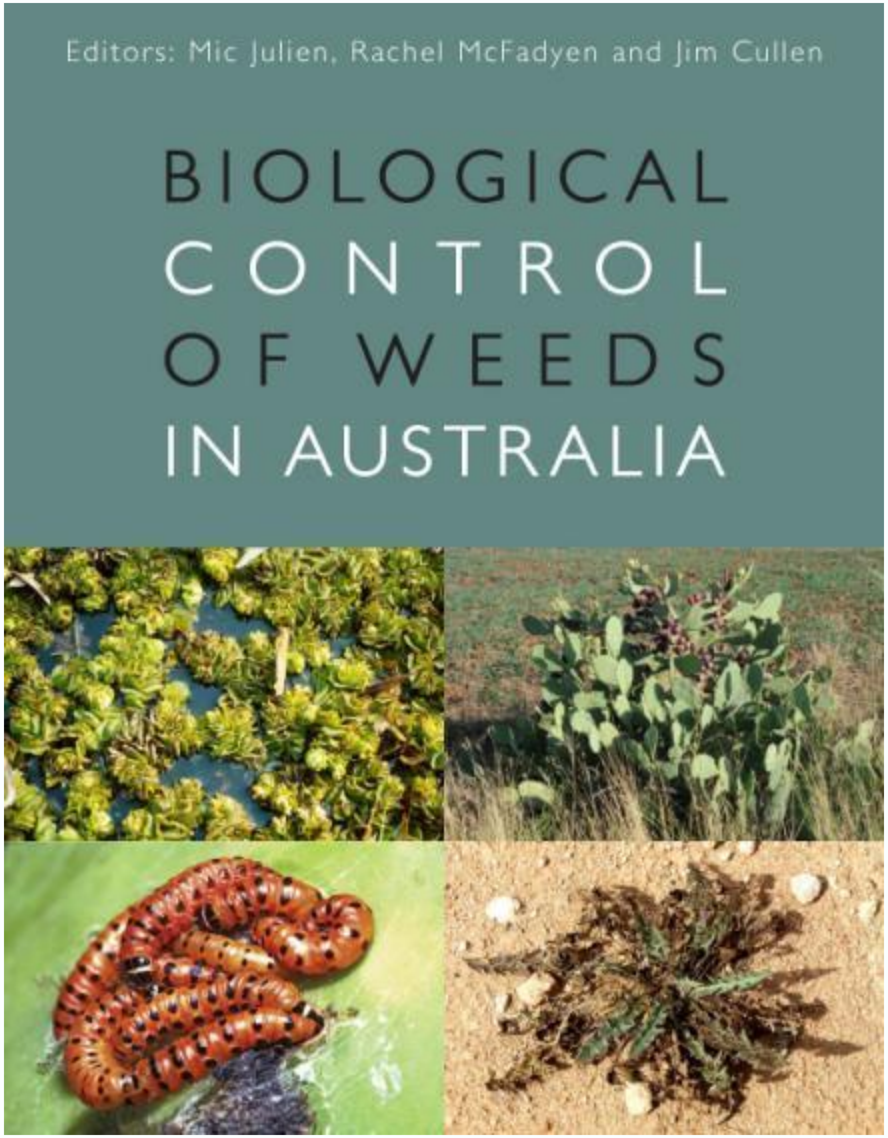
Skeleton weed – *Chondrilla juncea*





Changes in weed genotype frequency after release of *Puccinia chondrillina*

First weed biocontrol revival in 1970's



1st 110 years

69 biocontrol programs

<u>58 with releases complete</u>
14 completely successful
11 seasonal/regional success
11 unsuccessful
[22 too early]

69% program success rate against all plant forms

Overall BCR of 23:1 ($11B benefit for $0.5B cost)

Environmental, social & scientific benefits

Negligible non-target issues

Arthropod biological control since 1910

- Since 1910, 98 pests or groups of pests have been involved, totalling some 150 species

- Collembola (1), Hemiptera (56), Thysanoptera (1), Orthoptera (2), Coleoptera (9), Diptera (7), Lepidoptera (13), Hymenoptera (4), Acari (4) and Diplopoda (1)

- Natural enemies: predators, parasitoids, nematodes, fungi

- 30 of the target pests are very well controlled
  20 more are no longer important pests
  = 66% target success rate

- Most recent success – *Bemisa tabaci* (Silverleaf whitefly) - 2005

Biological control based war on rabbits

(Agricultural losses $206 million ANNUALLY)

Economic and environmental impacts

Crop damage, competition with livestock

Soil erosion

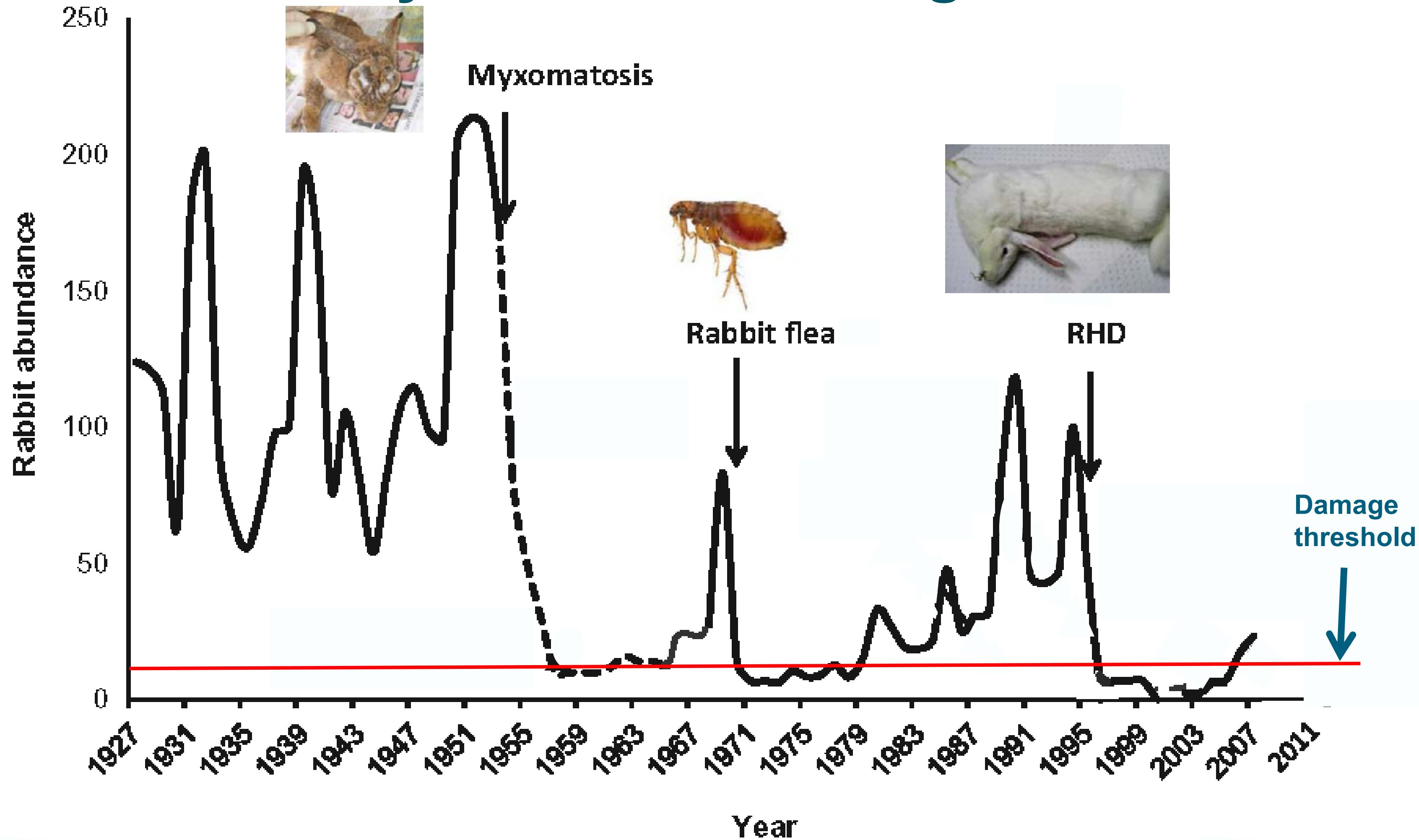
35 animal and 121 plant species threatened

Prevents native regeneration even at <1rabbit/ha

Benefit - A$70 B for agricultural industries over 60 yrs

Images: Brian Cooke, Wheeler et al (2002), Alf Manciagli

60 yrs of rabbit biological control



Virulence

Interactions between pathogens

Virus attenuation

Host resistance

Immunity

Rabbit haemorrhagic disease RHD strains in Australia

1996 - virus (RHDV1) – deliberately released

2009 - Benign RCV – detected endemic to AU rabbits RHDV1 immunity

2013 - China RHDVa – appeared

2015 - RHDV2 – appeared

2017 – RHDV-K5 (Korean RHDVa) - proposed

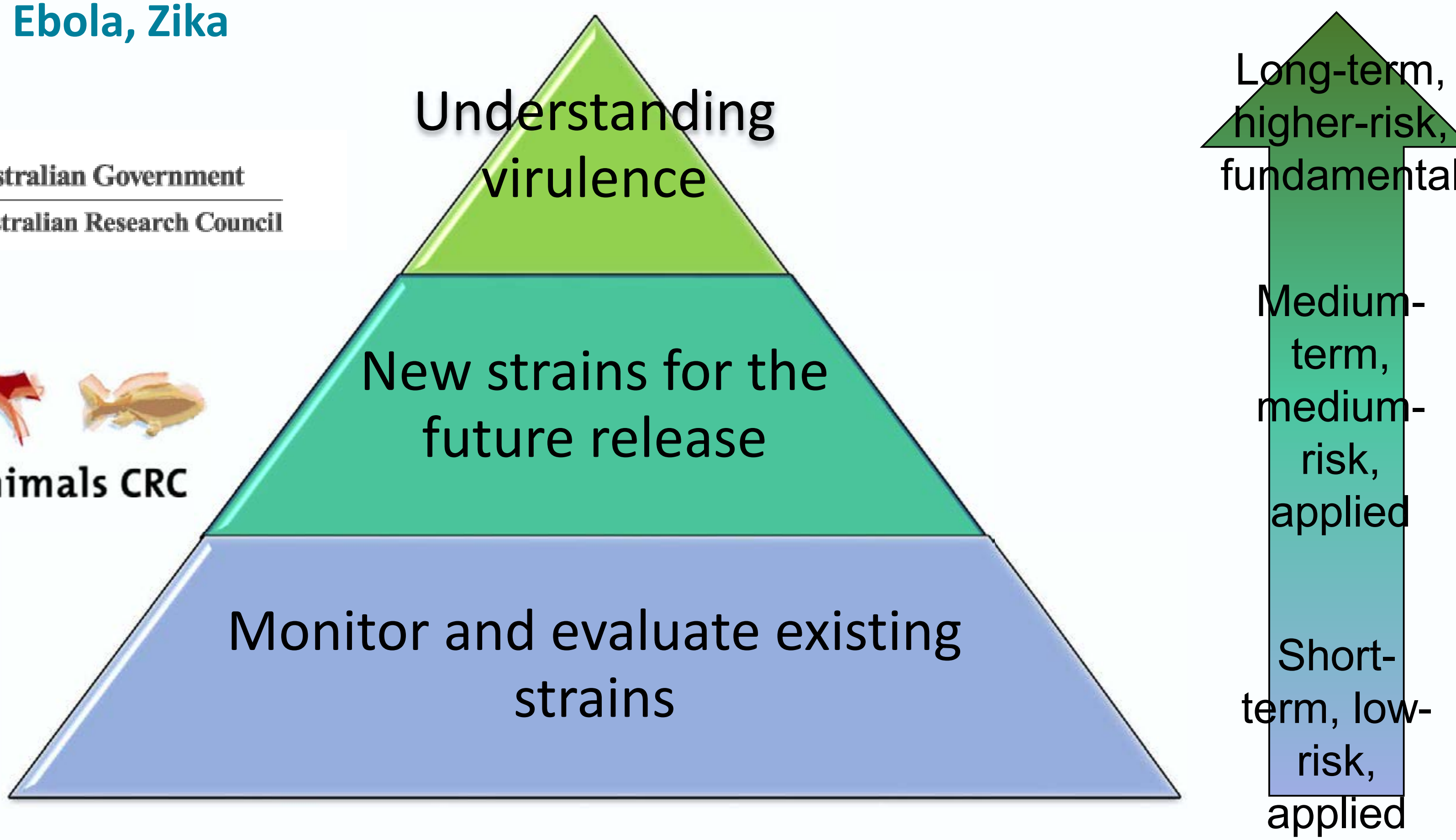
RHDV virology is important!

"Grand experiment in disease emergence and evolution"

E.g. Ebola, Zika

Australian Government
Australian Research Council

Invasive Animals CRC



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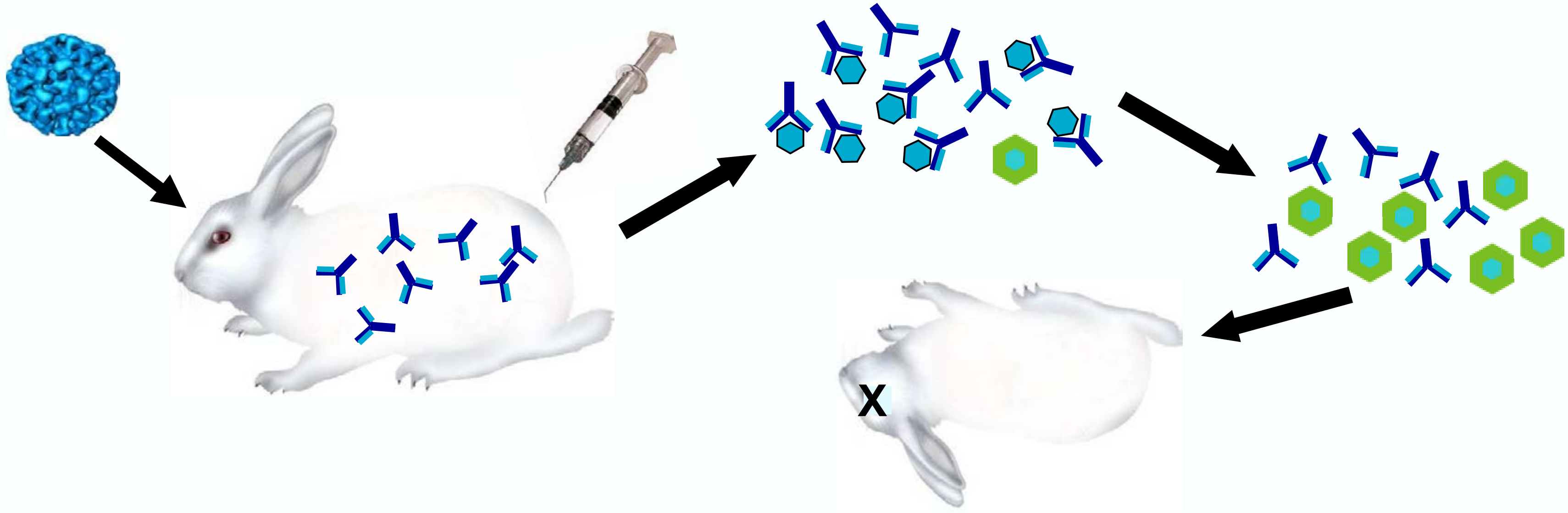
RHD-Accelerator

Platform technology for natural selection of novel RHDV strains *in vivo*

Continuous supply of suitable calicivirus serotypes for future releases → sustainable management

Passaging of virus in rabbits under controlled selection pressure



End product: virus that can overcome pre-existing immunity


DANGER

Carp as an invasive species in the Murray Darling Basin

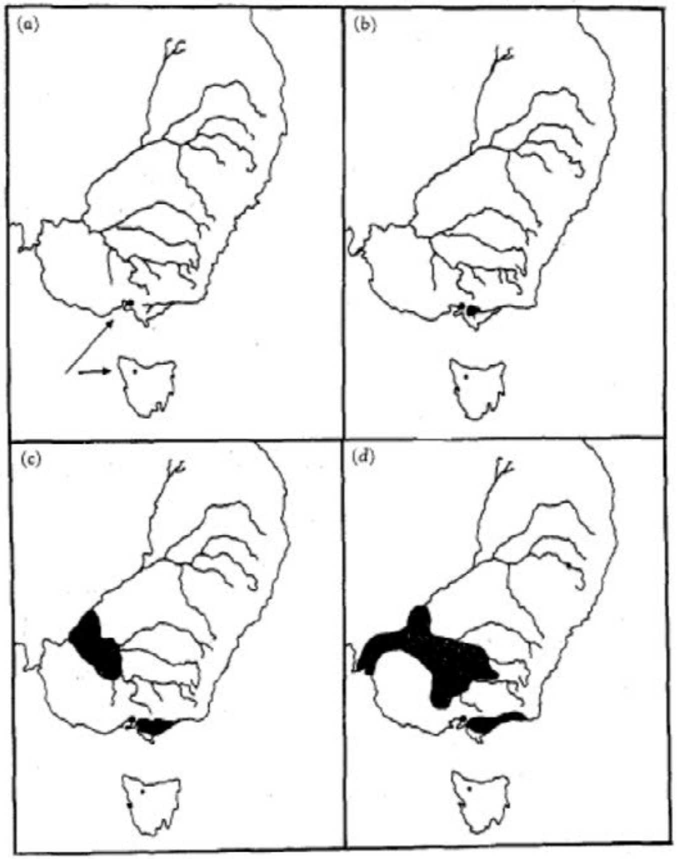
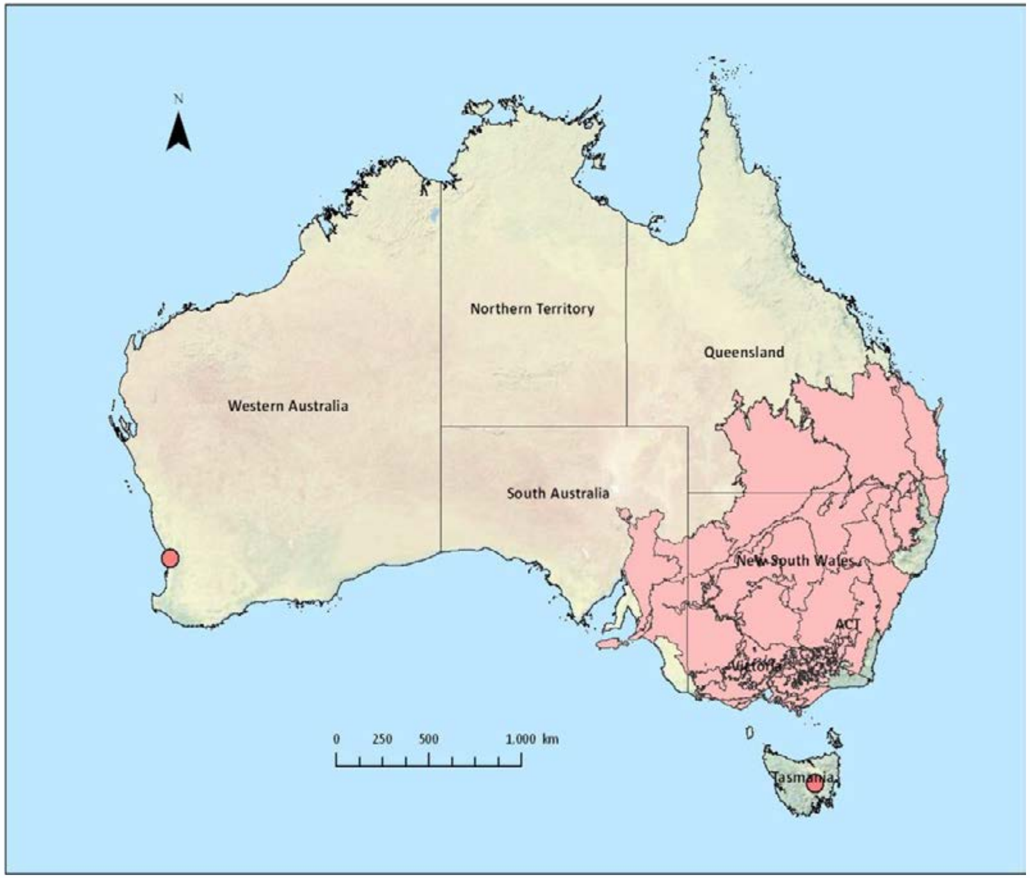


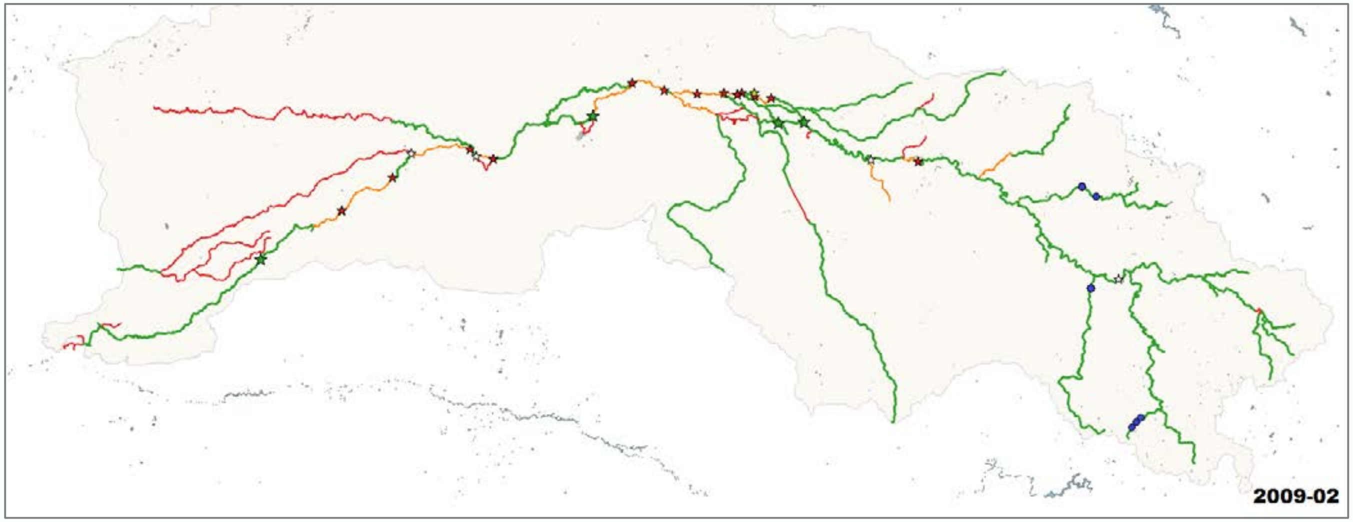
Fig. 4. Distribution of *C. carpio* 'Boolara' in (*a*) 1960, (*b*) 1964, (*c*) 1970, and (*d*) 1974.

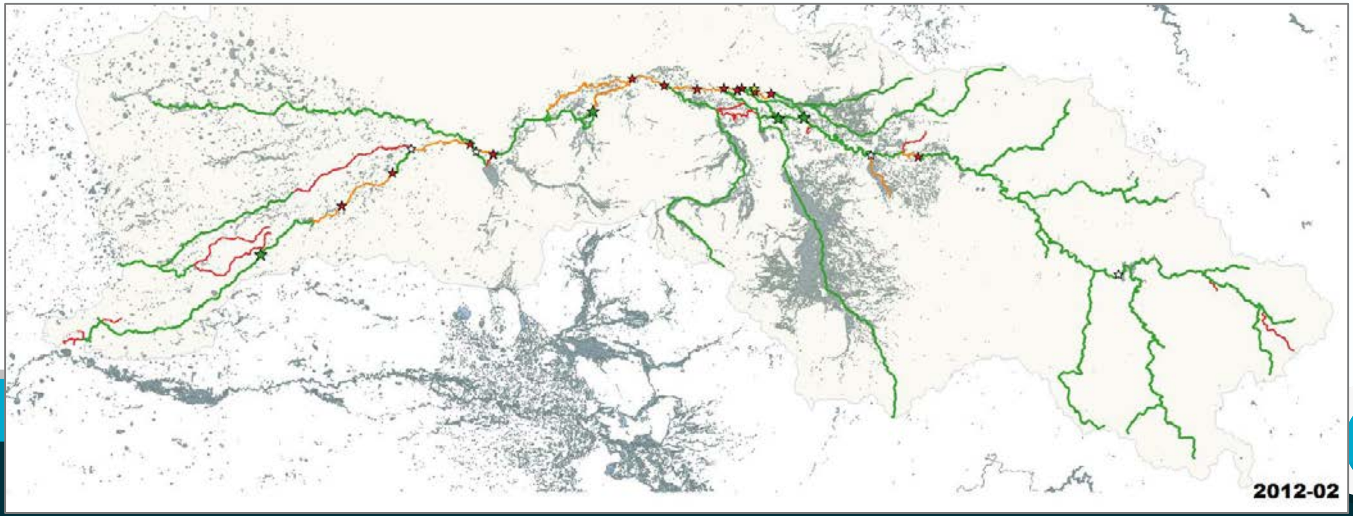


2016

Lachlan river systems modelling

Main river channel connectivity

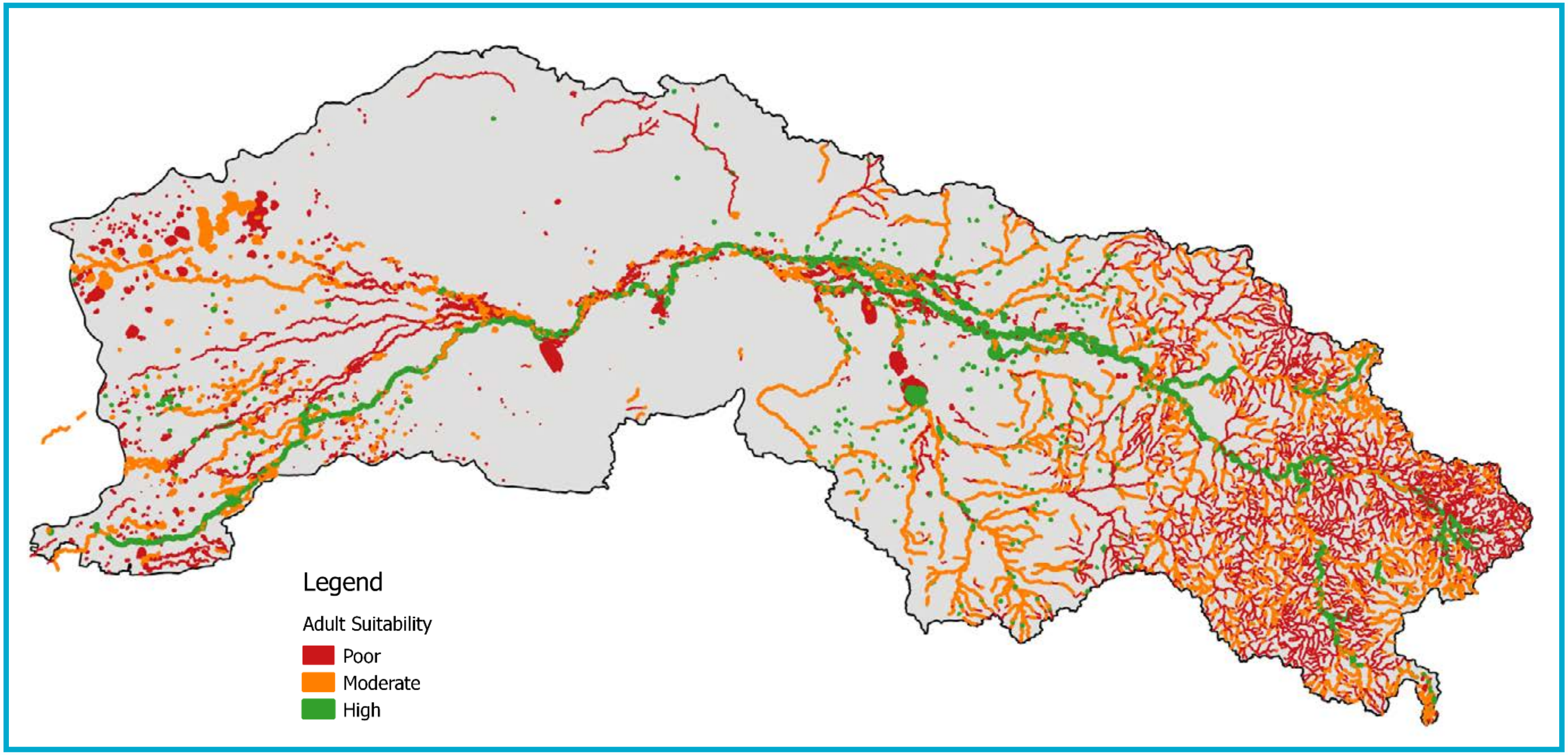




Legend

- Two way fish movements
- D/S from weir only
- NO flow
- Two way over weir
- Nil movements over weir
- Carp trapping

Overview of adult suitability in the Lachlan catchment



Option 1 – 1995-2010

"Daughterless" gene technology – genetically forcing fish to be only male

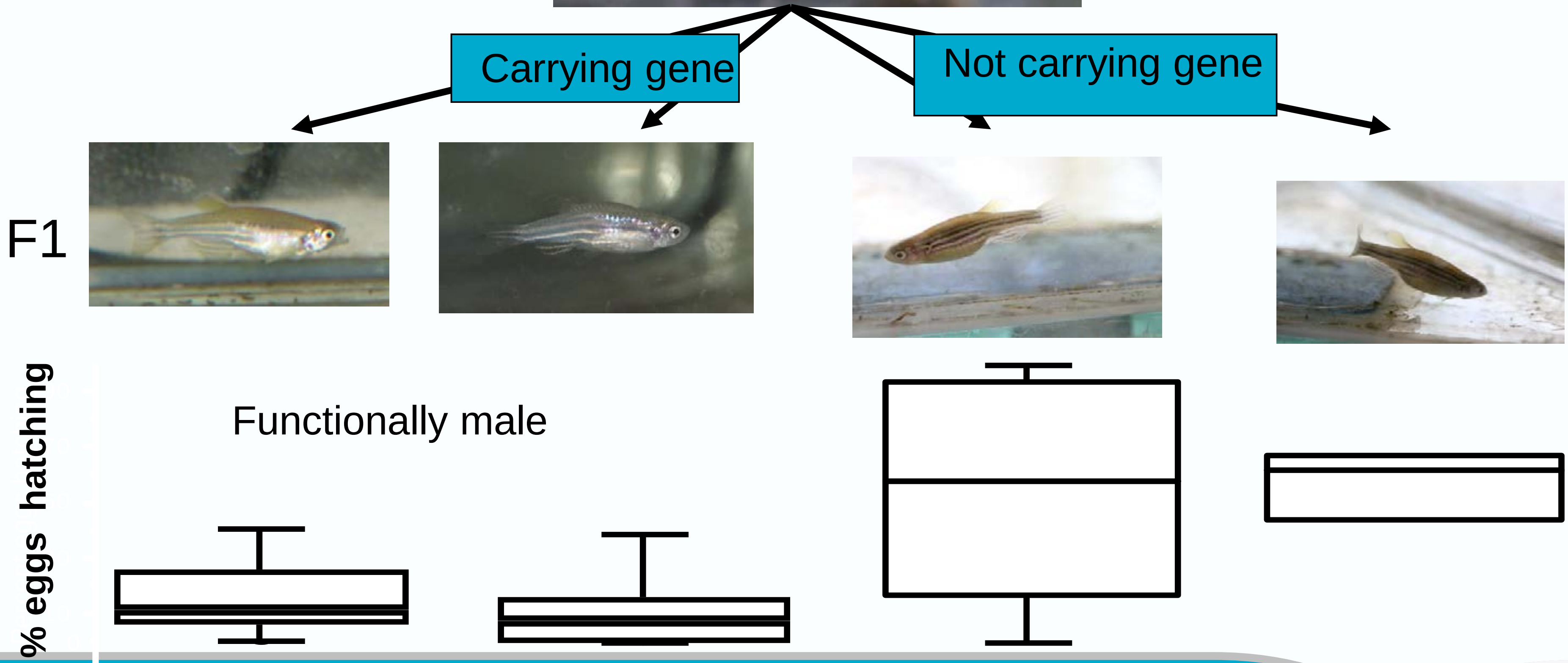
Heritable GM construct added to the fish genome

- prevents fish offspring becoming female
- Mendelian inheritance
- population male biased sex ratio

Dr Ron Thresher (Ron.Thresher@csiro.au)
CSIRO Marine & Atmospheric Research

Zebra fish prototype sterile ♀ gene

Reduced female fecundity




4th generation
Daughterless zebrafish
100% Male

Trials on common carp underway at Auburn Univ, USA

Potential daughterless carriers

Daughterless carp – need investment

Option 2

Classical biological control:
Koi herpesvirus as biocontrol agent for carp

Dr Ken McColl (Ken.McColl@csiro.au)
CSIRO Livestock Industries, AAHL Geelong

CyHV-3 in carp

First outbreak - Israel 1998

High mortality (70-100%)

- Common carp (*Cyprinus carpio carpio*)
- Koi carp (*C carpio koi*)
- Max. losses
  - water temp 17–26 °C

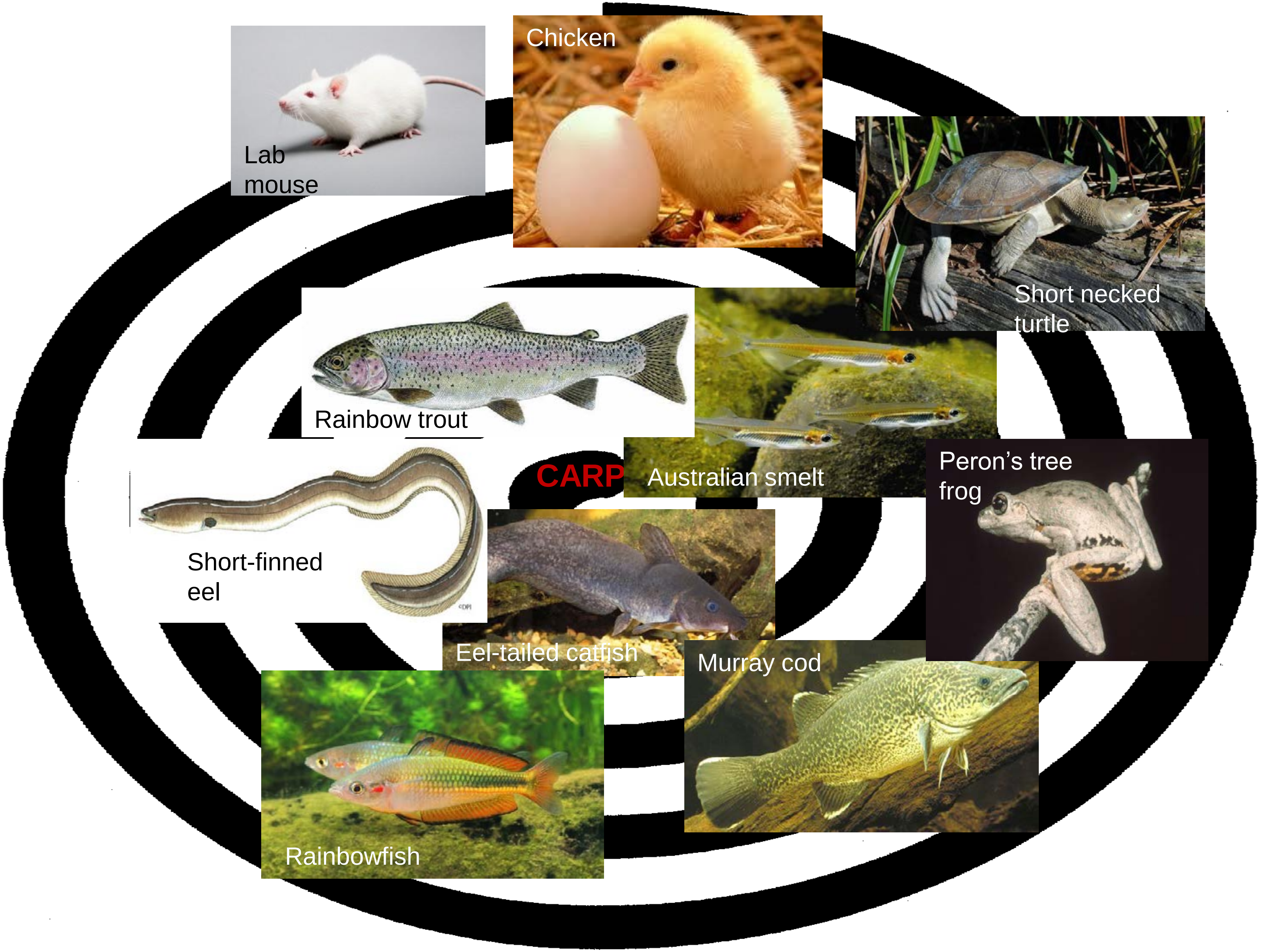
Age-related susceptibility

Larvae < juveniles > adults

Resistance

Gill necrosis

Skin necrosis



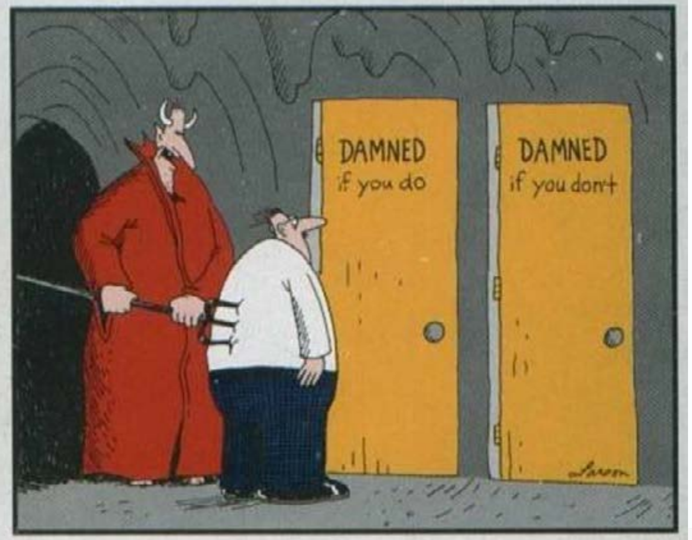
Lab mouse
Chicken
Short necked turtle
Rainbow trout
Australian smelt
CARP
Peron's tree frog
Short-finned eel
Eel-tailed catfish
Murray cod
Rainbowfish

Release 2018 strategy?

Where and when to release the virus to achieve maximum sustained ecological impact?

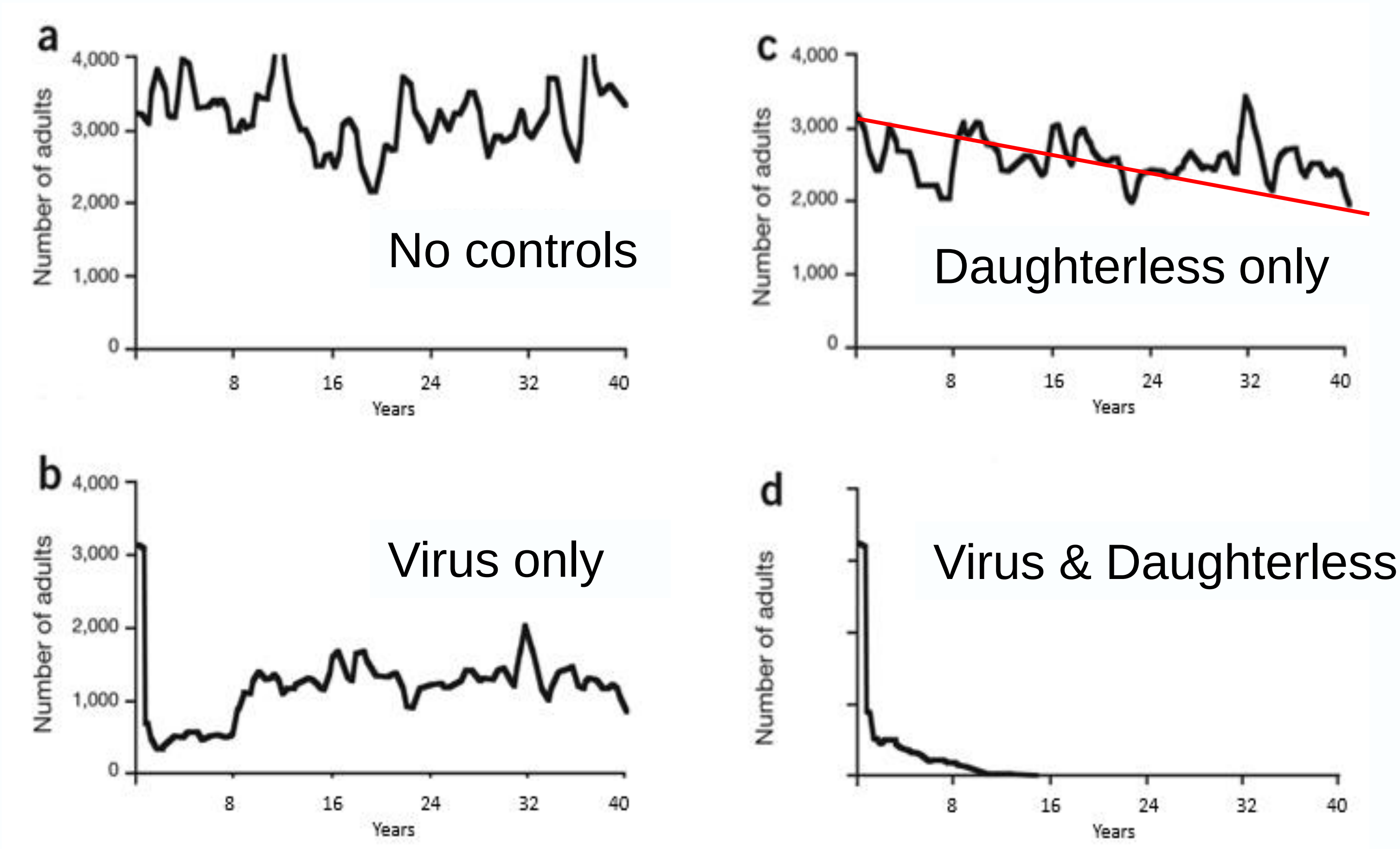
Social-economic-political issues

– Carp sympathysers - carp fertiliser industry
– Impact on water quality of dead & dying fish
– Political pressure of the "silver bullet"



"C'mon, c'mon — it's either one or the other."

Modelling for long-term carp control



Thresher et al (2014) *Nature Biotechnology* 32:424-427

Future carp control strategy?

- More virulent (vaccine-induced) strains of CyHV-3?
- Alternative/additional genetic technologies
  - Triploidy driven sterility?
  - Gene-drive?

LETTERS

nature biotechnology

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

Andrew Hammond[1], Roberto Galizi[1], Kyros Kyrou[1], Alekos Simoni[1], Carla Siniscalchi[2], Dimitris Katsanos[1], Matthew Gribble[1], Dean Baker[3], Eric Marois[4], Steven Russell[3], Austin Burt[1], Nikolai Windbichler[1], Andrea Crisanti[1] & Tony Nolan[1]

Gene drive systems that enable super-Mendelian

homozygote in a process known as 'homing'. Through this mechanism,

Outline

- Classical weed biological control - 110 years of success
- Next generation gene-tech based biocontrol opportunities
  - RNAi
  - Gene-drive
- CSIRO European laboratory activities

Modern gene technology – the new era

We can now…

- Remove or knockout specific genes – GMO?
- Regulate gene activity via exogenously delivered microRNA into cells – RNA interference – GMO?
- Add new functional genetic sequences at precise points in the genome - GMO
- Switch individual nucleotides at precise points in the genome – "precision genome engineering" – GMO?
- Swamp desired genetic constructs into sexually reproducing populations (Mendelian inheritance based sterile feral techniques) - GMO
- Genetically-drive deleterious genes into all individuals in sexually reproductively-isolated populations of single species- GMO

RNA interference (RNAi)
platform biotechnology

The Prime Minister's Prize for Science won by CSIRO in 2007

Ming-Bo Wang *Peter Waterhouse*

Small sections of RNA that can modify gene function inside plant cells

RNA interference (RNAi):

proven technology for trait development in plants

Allergen free peanuts

Wheat with healthier starch

Supercharged safflower

Healthier cottonseed oil

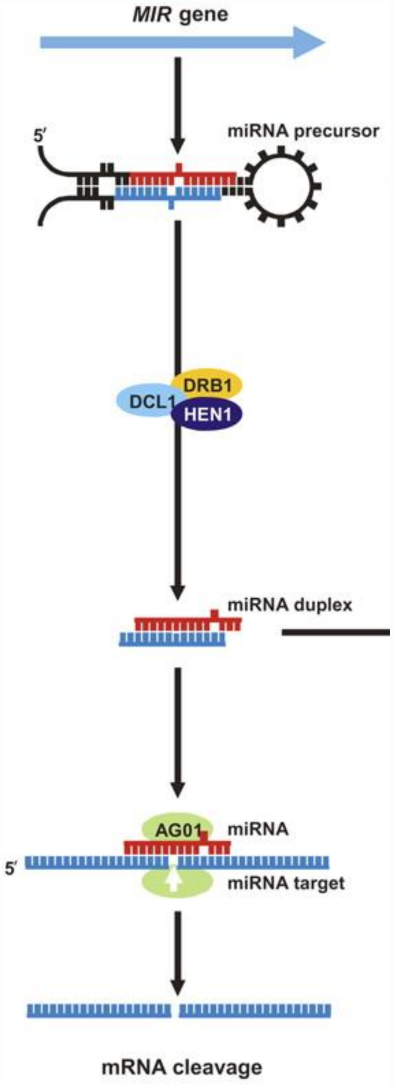
Lysine rich soy beans

Virus resistant cereals

Blue rose

Improved photosynthesis in canola

RNA interference achieved via MicroRNAs which are…



- 18-24 nt single stranded RNA of gene expression
- Part of normal physiological and metabolic development
- Filter mechanism in information transfer from genome to phenotype.
- Often involved in disease immunity and infection
- Some microRNAs can trigger an amplification response to cause silencing of a gene
- Delivery
  a) Endogenous - generated within cells using GM or
  b) Exogenous - constructed and physically delivered to cells

RNAi for weed/pest management

- **What to target?**
  - Pesticide resistance genes? – Company interest to prolong extant pesticides s
  - Disease susceptibility ? – take out disease resistance
  - Genes that facilitate pollination/reproduction?
  - Other genes vital for viable embryo production?
  - Disease vector competency – demonstrated for mosquitos and being trialled for fruitflies

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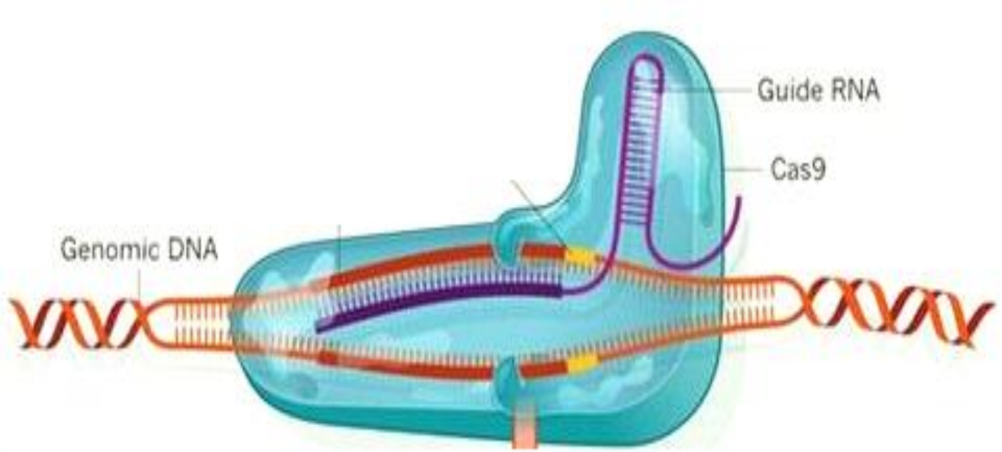
Gene-drive derived from bacterial "immunity" system

<u>CRISPR (Cas9)</u> – "*clustered regularly interspaced short palindromic repeat associated protein 9 nuclease*"

Directly cuts and insert desired gene sequence, based on presence of short existing nucleotide code sequence



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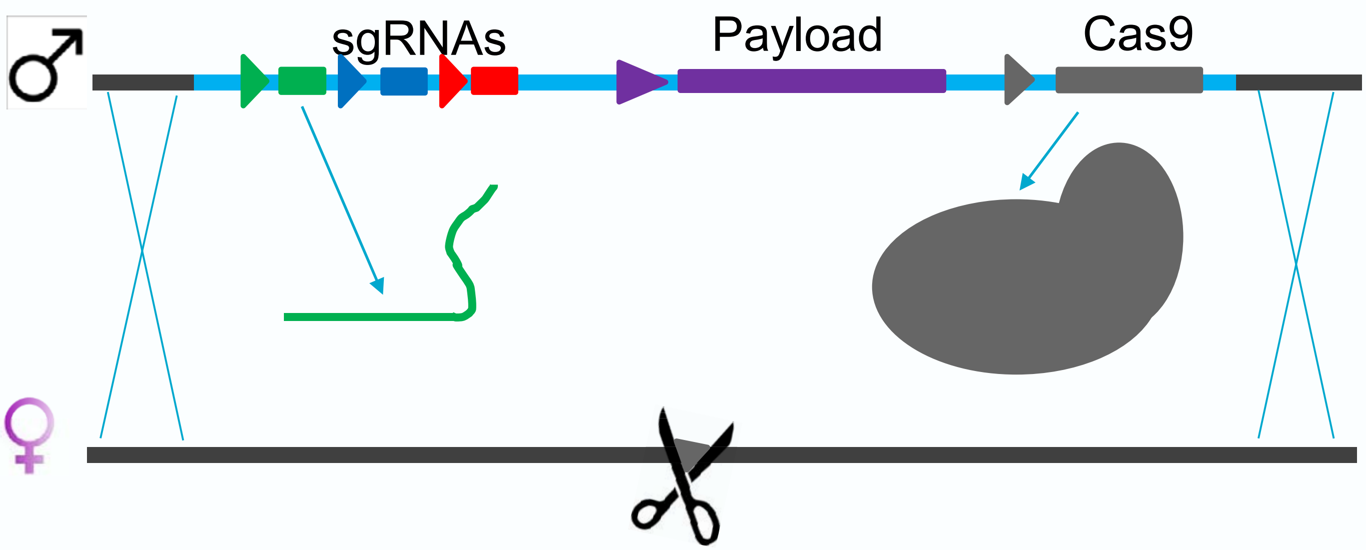
Gene-drive system components

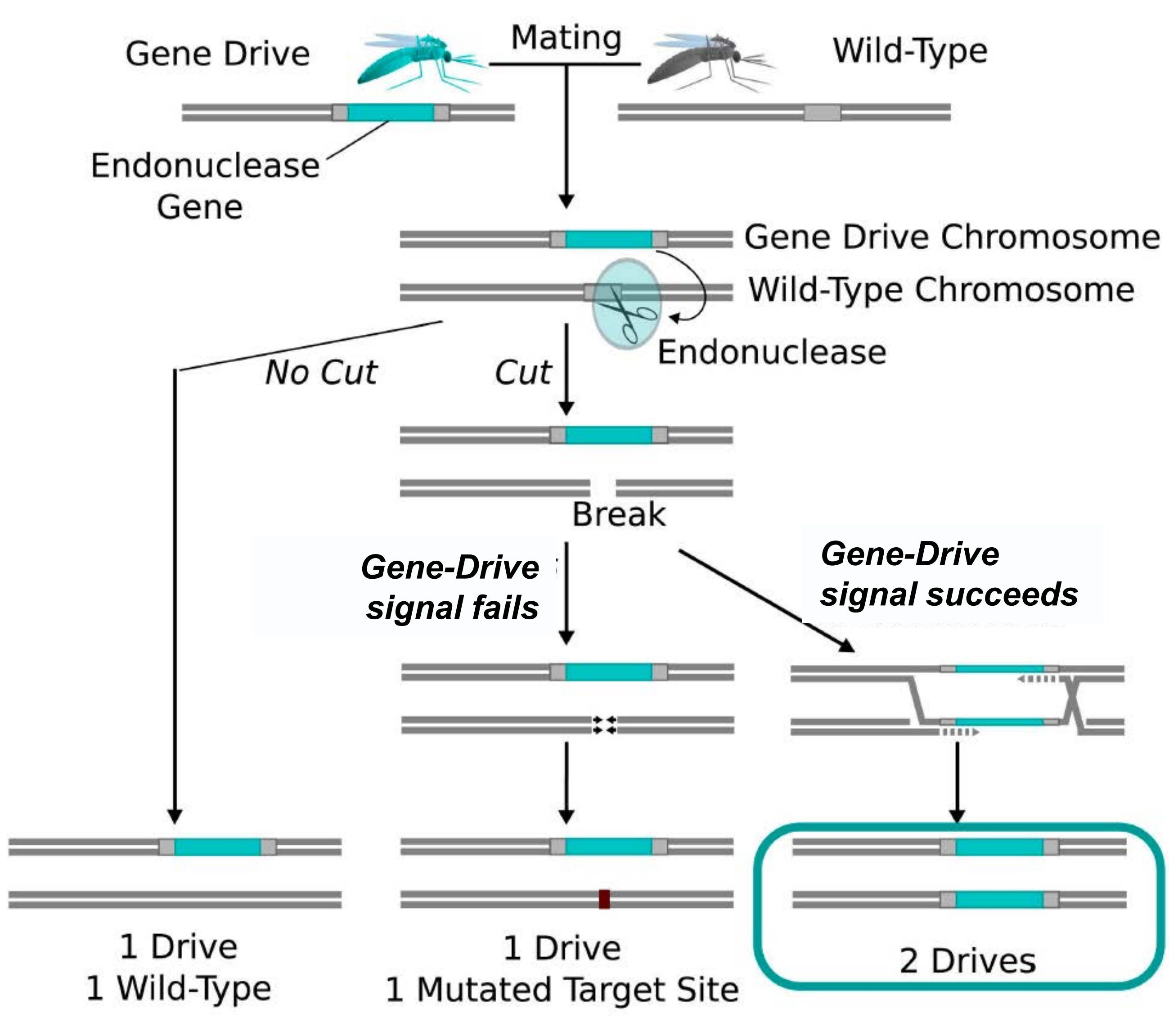
Multiple single guide sgRNAs
- Maintain drive activity
- Target host gene function

Payload gene construct with biological impact e.g.
- Sex bias
- Susceptibility

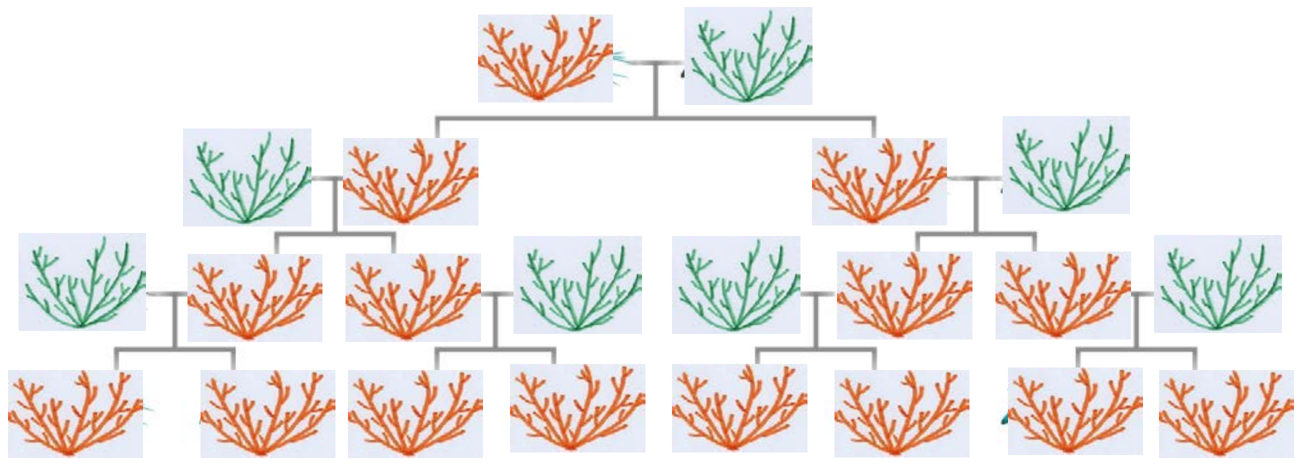
Promoter for Cas9
- Active post fertilization
- in germ line
- inducible



Gene drive mode of action – so far only tested in mosquitos



Cas9-based gene drives – hypothetical weed scenario



- Assumes gene drive (red) efficiency of 2 = 100% success – (mosquito data suggests real efficiency 1.2 - 1.9)
- Potential for gene-drive to spread into all members of the population – even with small fitness loss

Gene drive requirements/constraints

- Identified target genes – need gene annotation
- Sexual reproduction – and ideally fast reproducing
- Short live cycle
- High construct heritability efficiency
- Few individual fitness costs

Gene drive target mechanism?????

- Daughterless gene-drive for pest animals?
- Remove self compatibility in weeds ?
- Induce ♀ sterility and ♂ fertility?
- Increase susceptibility to a benign chemical
  = new specific but ecoharmless pesticide?
- Inducible disease susceptibility gene
  = new biopesticide?

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 are inherited (6)

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Outline

1. **Mediterranean snails (collaboratior: l'Université Cadi Ayyad, Maroc)**
2. Dung beetles
3. Sowthistle

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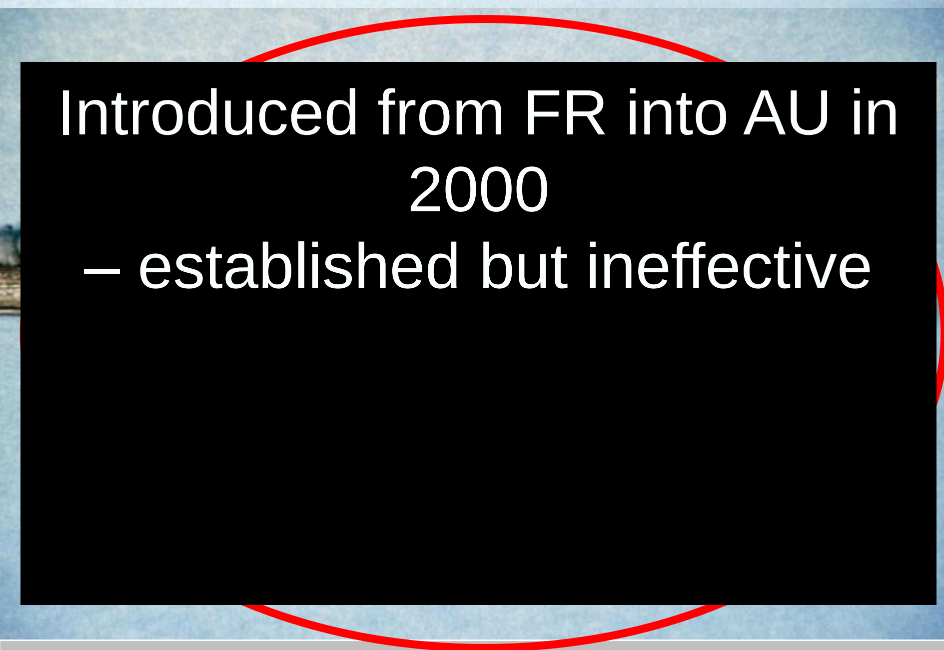
White & Conical Snails



Introduced from FR into AU in 2000
– established but ineffective

Snail Host (*C. acuta*) Phylogenetic Tree CO1 Mit.-Gene

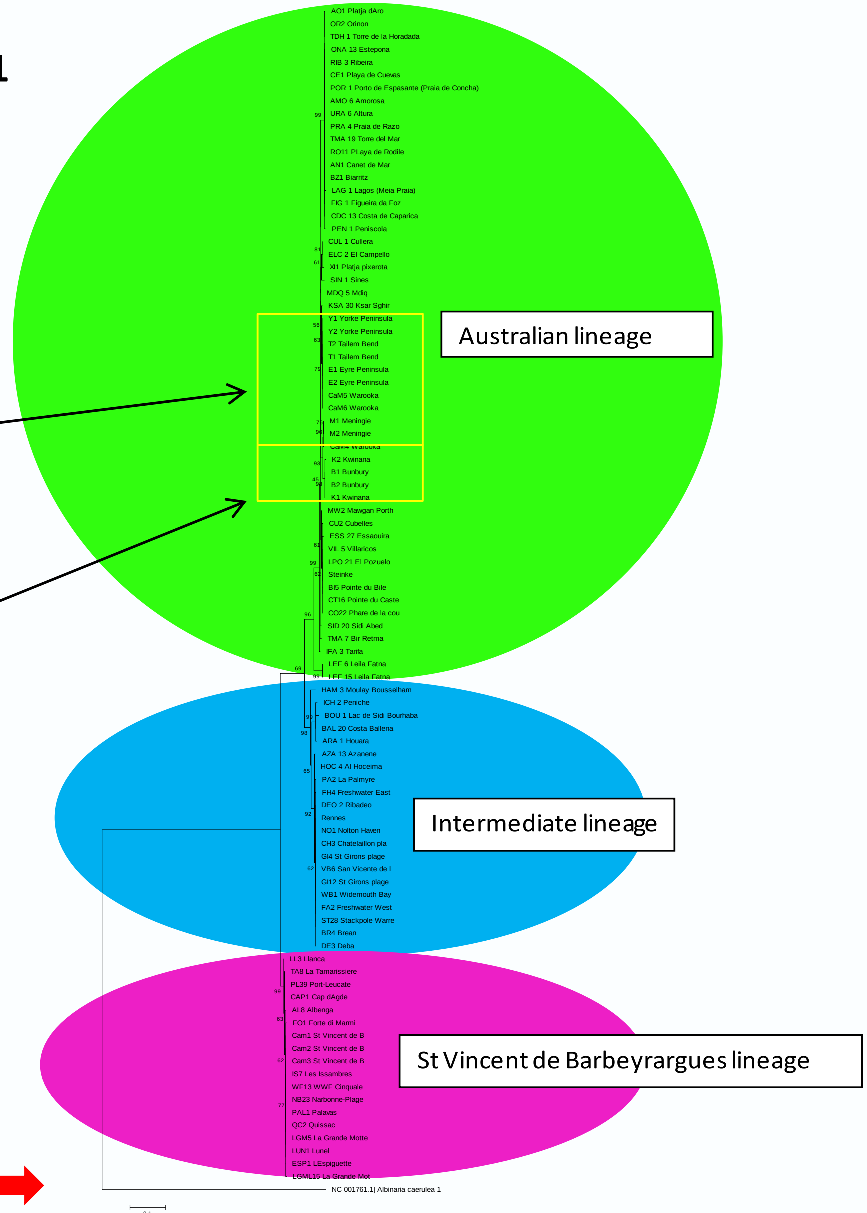
(similar tree for 16S)

3 Clades

CO1

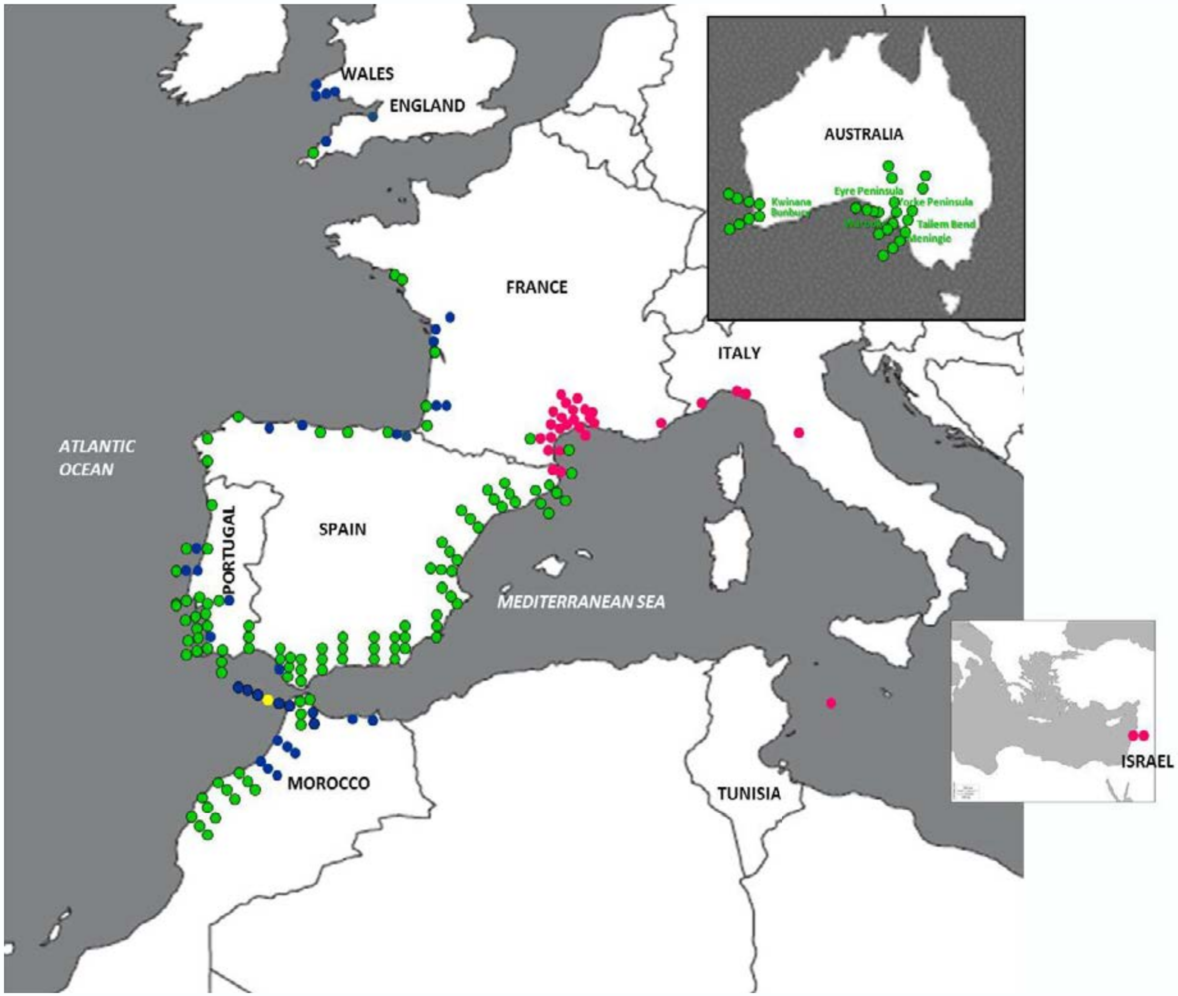
S. Aust.

W. Aust.



Outgroups Map Here
(only 1 species shown)

<u>Host</u> *C. acuta* (CO1 & 16S) geographic distribution of 3 clades .



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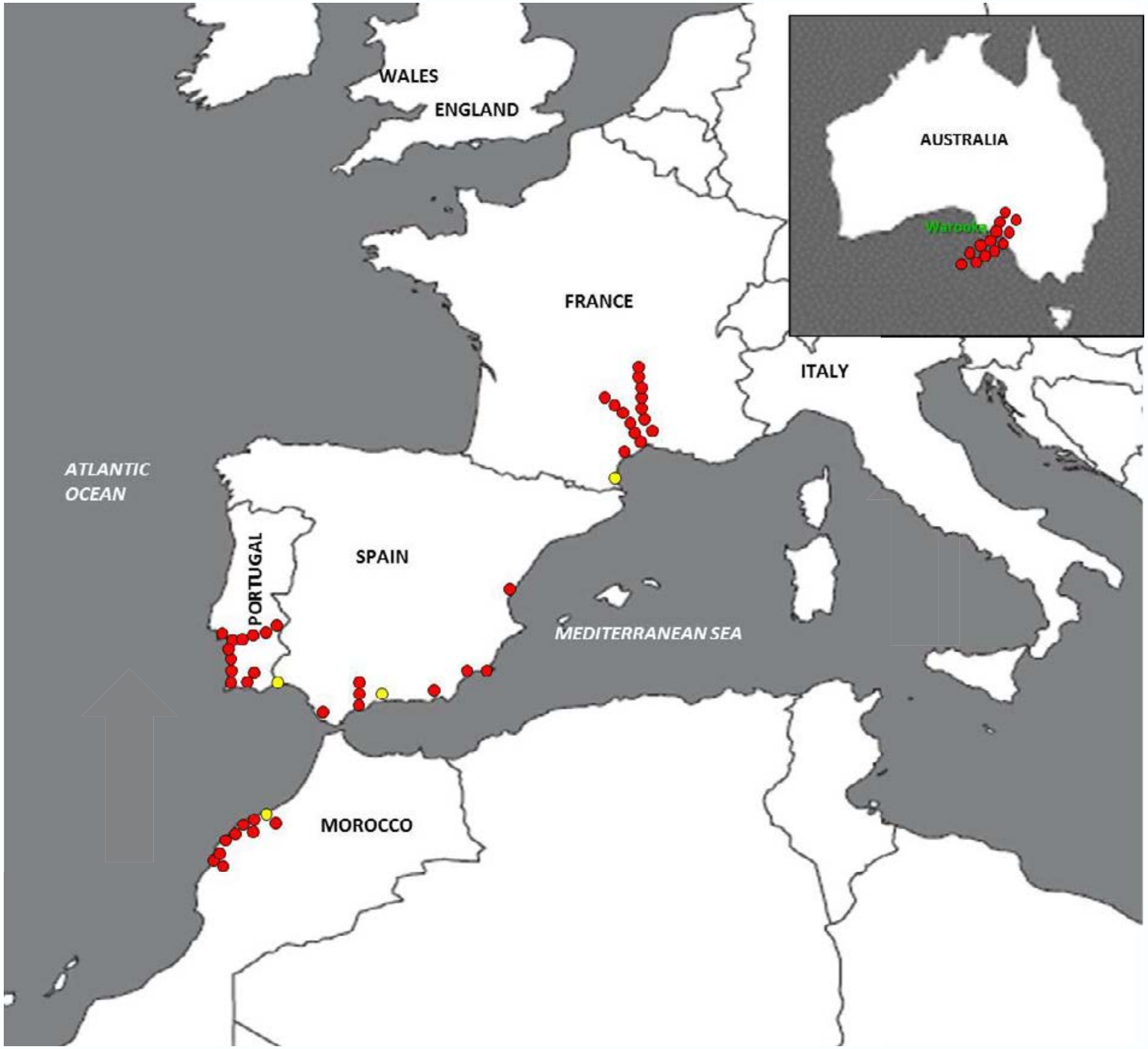
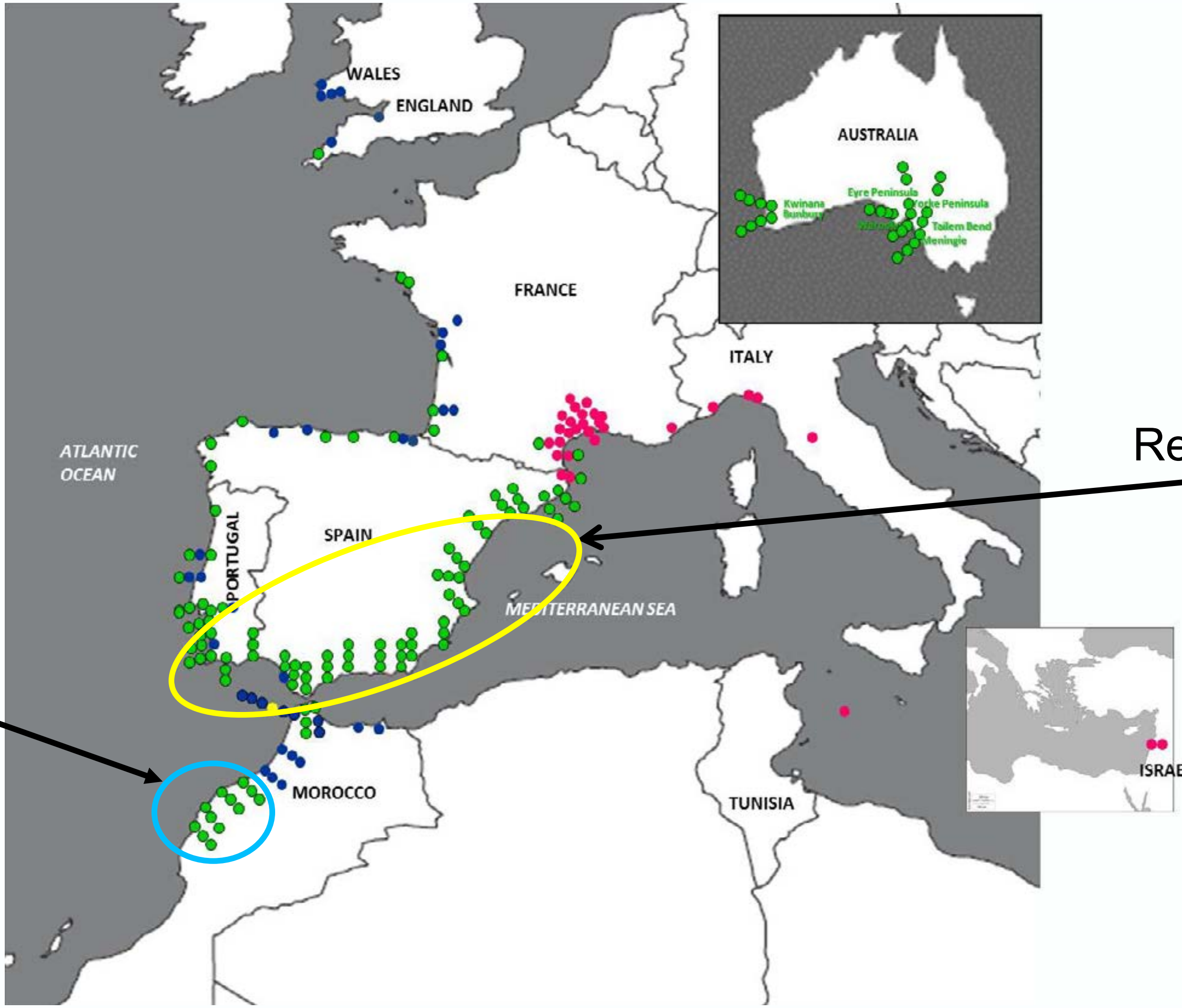
<u>Parasite</u> *S. villeneuveana* (CO1) geographic distribution of 2 clades (n=69).



New sampling regions based on native clade of host that is found in Australia



Renewed Effort

Moroccan PhD student

CSIRO

Field % Parasitism of *C. acuta* by *Sarcophaga* flies

Native Range Surveys

- **% parasitism Spain -**11% (May), 15% (July) & 25% (Sept) - Max 83%
- **% parasitism Maroc** – 20% (May), 19% (July) & 15% (Sept) – Max 41%
- - Most flies *Sarcophaga villeneuveana* > 300 confirmed morphologically by R. Richet.
- 31% hyper-parasitism (Spain)
- 67% died of unknown causes

Australian - Yorke Peninsula Collections

2015 6,200 snails sent to Montpellier, 122 French *Sarcophaga* sp. emerged (2.0%)
2016 3,800 snails sent to Montpellier 80 French *Sarcophaga* sp. emerged (2.0%)

French v Spanish Fly performance on AU/F/E snails?

Lab experiments significant results

French flies

Host Snails (*C. acuta*)	France	Australia
% adult fly emergence	30%	6.5%
% parasitism (n=3200)	48%	30%

Spanish flies

Host Snails (*C. acuta*)	Spain	Australia
% adult emergence	6.5%	28%
% parasitism (n= 1080)	28%	36%

French vs Spanish flies - on Australian snails ?

Biological control projects

1. Mediterranean snails
2. **Dung beetles (collaborator University Montpellier III)**
3. Sowthistle

Two species introduced from France/Spain to Australia in 2015

Bubas bubalus

Onthophagus vacca

History

- 80M tonnes of dung produced each year by Australian livestock is valued at A$13B.
- CSIRO's major importation program from 1960s and 1990s established 23 from 45 imported species
- Gaps still remain – need to finish the job

Benefits - unquantified

1. Improved soils - nutrients, mixing aeration, carbon & water storage
2. Pasture health meat productivity
3. Reduced dung GHG emissions & nutrient pollution run-off into waterways
4. Livestock gut parasite worm infection cycle disruption - reducing veterinary chemical drenching
5. Reduced Australian bushfly breeding in dung - improved animal health, rural lifestyles, and tourism potential
6. Reduced buffalo flies livestock disease vectors in dung

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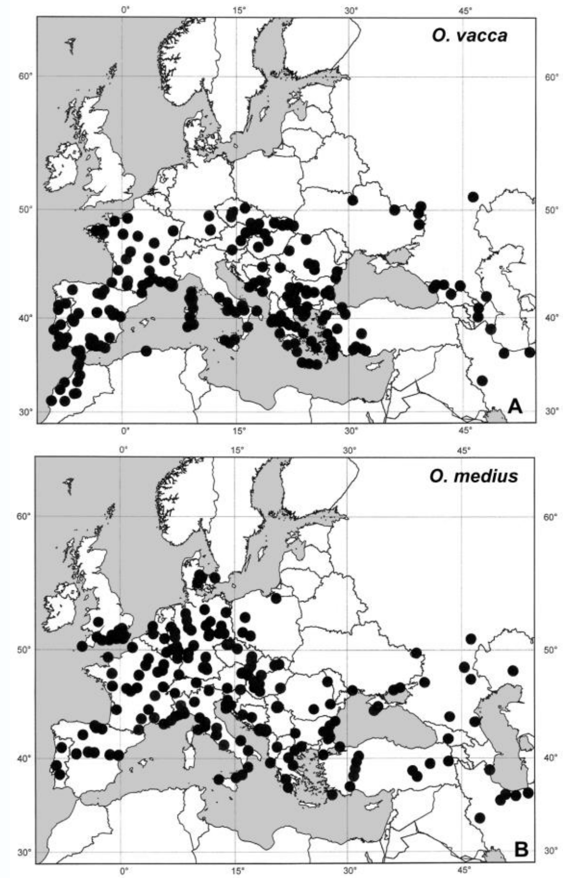
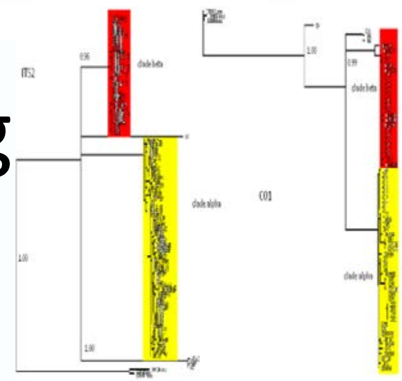
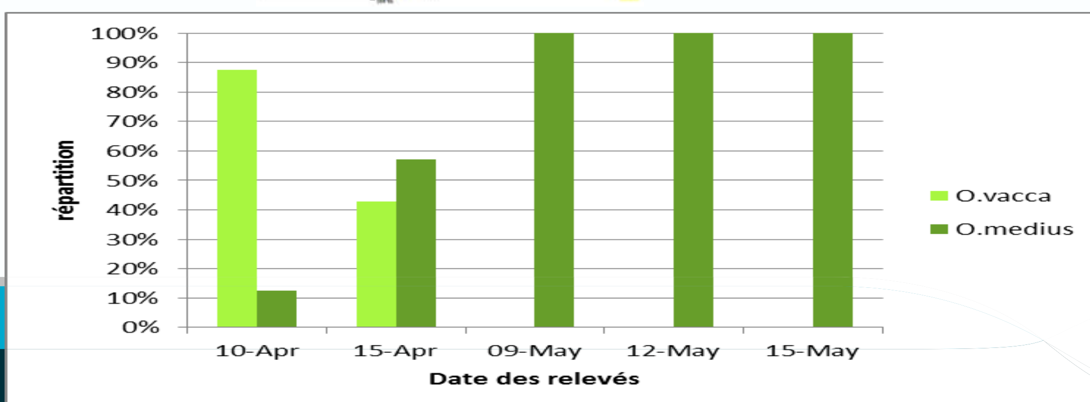
Montpellier activities

1. Collecting & shipping beetles to AU
2. Basic biology & taxonomy
3. Developing rearing methods for resynchronisation







New Dung beetle program proposal ($22.7M) due to start in Dec 2017

1. **Selection, importation, release and distribution - import 4 new dung beetle species by 2020**
2. Quantification of the complex, multiple dung beetle benefits – to justify practice change on-farm
3. Dynamic National Dung Beetle Distribution and Impact database and management information through a Smartphone App. to assist on-farm decisions – augmented by citizen science and validated by monitoring
4. National and regionally-specific dung beetle services program to over a network to >1000 producers

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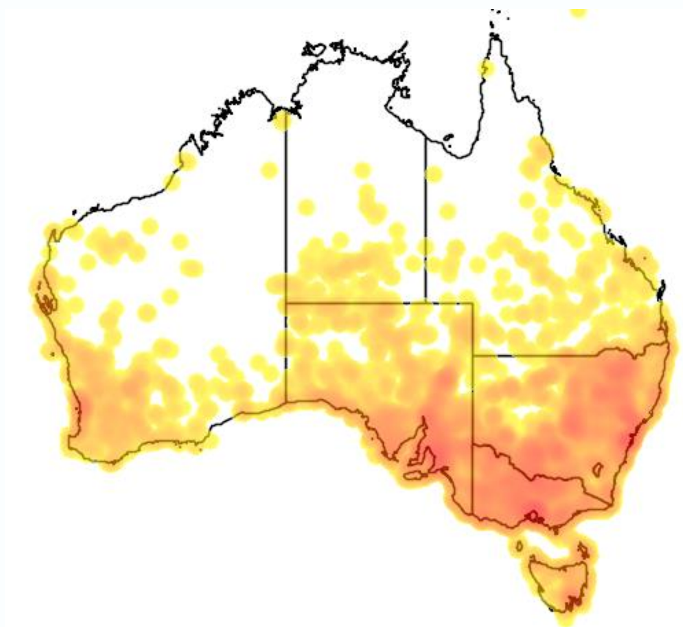
Biological control projects

1. Conical snails
2. Dung beetles
3. **Sowthistle (collaborators SupAgro & CBGP)**



New AU weed biocontrol program ($16M)

10 new targets:

Cropping – , ***Sonchus* spp. ,** *Conyza* spp. Solanum Sliverleaf Nightshade

Grazing – African boxthorn, Mother-of-Millions, Ox-eye daisy, Giant rat's tail grass, Prickly acacia

Aquatic – Cabomba, Sagittaria

Sowthistle history :

- Canada surveys of northern Europe (Peschken 1982-1984)



1. *Cystiphora sonchi* - cecidomyid
2. *Tephritis dilacerata* – tephritid
3. *Liriomyza sonchi* - agromyzid

- CSIRO surveys in Southern France, 2004 - Jourdan & Scott unpublished

  - *Aceria* sp.
  - *Miyagia pseudosphaeria*


Current status

Literature review and historic surveys:

- 59 ± ? Pathogens found on *Sonchus* spp. - *Bremia lactucae, B. sonchi, Cercospora sonchifolia* & *Entyloma bullulum*.
- 83 arthropods (49 generalists + 34 specific to tribe or Sonchus) – *Tephritis dilacerate, Contarina sonchi, Botanophila sonchi* & *Aceria sonchi*

Activities planned 2017-2020 :

- Genetic diversity of Sonchus oleraceus & S asper at 26 sites in both EU and AU based on CLIMEX and likely origins in AU
- Natural enemy surveys and risk assessment on appropriate genotypes
- **New research scientist 4 yrs on project employed by SupAgro (Mar 2017)**

Take home messages

- 110 years Classical Biological Control experience allows Australians to analyse and understand risks of exotic organism releases and still reap the control benefits
- Gene technologies are now available that could provide next-generation biological control solutions - more reliable & effective
- RNA interference – a non-GM option, but still hard to deliver & may have limited commercial application for pest management
- Gene-drive technologies now available to drive deleterious genes into pest populations – but GM
- Can we build on our experience with classical biological control to safely exploit these opportunities?

CSIRO

Thank you

Andy Sheppard

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