



**Landcare Research**  
**Manaaki Whenua**

# Genetic and reproductive technologies for reintroduction of native species into biodiversity sanctuaries

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**Sustain Genetic Diversity IO – SARB OBI (FRST)**



# Synopsis

- Genetic and reproductive technologies
  - What are they & why valuable?
- Examples of application to native species
  - Assigning gender of frogs
  - Assisted breeding/genetic diversity of skinks
- Relevance to biodiversity sanctuaries
  - Sourcing/selecting animals for reintroduction
  - Monitoring genetic/reproductive “health”



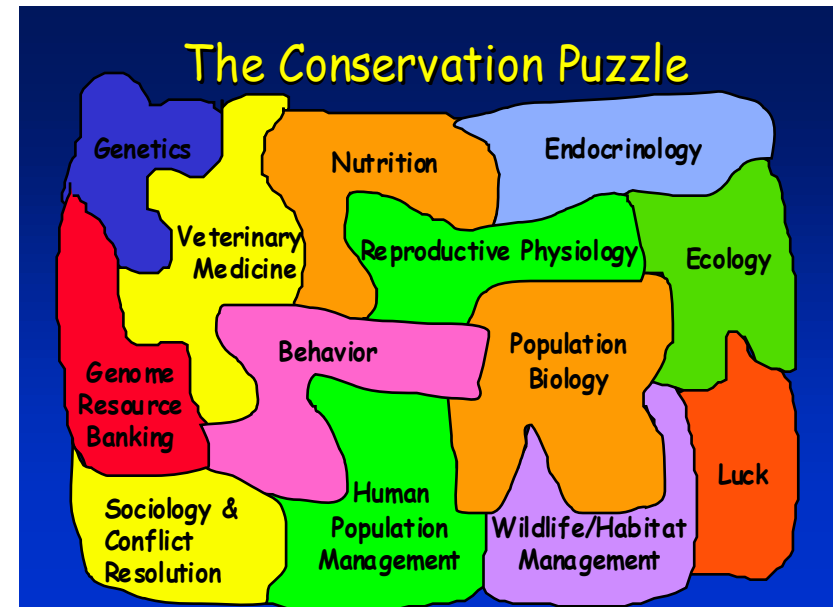
# Technologies – what are they?

- What are they.....**TOOLS!**
  - Assess genetic make-up
    - E.g. microsatellite & mtDNA neutral markers
  - Monitor/control/manipulate reproduction
    - E.g. from behavioural observations to cloning
- Low-tech approaches most useful to date
  - Successful in genetically managing wildlife
    - Non-inv hormone monitoring, AI, sperm GRBs
    - Individuals selected to conserve genetic variation



# Technologies – why valuable?

- Value in understanding:
  - Genetic diversity/distinctiveness of populations
  - Species-specific reproductive mechanisms
- Manage species *ex-situ* & back to nature!
  - Black-footed ferret
  - ‘Conservation-effective’
    - Integrative research
    - Multidisciplinary
    - Partnerships



# Native New Zealand frogs

- All four remaining endemic frogs are declining & nationally threatened
  - Introduced mammalian predators
  - Amphibian chytrid fungus
- Carter Holt Harvey Native Frog Research Centre established 2004 – Auckland Zoo
  - Captive breed Archey's frogs



Photo: Rod Morris

# Breeding Archey's frog

- Achieved over 30 years ago! (Bell 2002)
- Features of reproduction
  - Breed mostly September to November
  - Lay eggs/dorsal brooding
  - Effects of habitat, nutrition, disturbance
  - Key constraint: can't reliably assign gender
    - No sex specific DNA marker available
    - SVL > 31mm are probably females



# Approach 1

- Urinary/faecal hormone analysis
  - T in American toads & E in boreal toads diff between sexes (Szymanski *et al.* 2006)
  - Adapt tech devel in stoats (Molinia *et al.* 2007)
    - Preference to use urine
  - Outputs
    - Non-invasive sex hormone assays
    - Monitor reproduction & stress?

Outcome: much improved breeding success,  
captive management protocols revised

# Approach 2

- DNA testing
  - ID, seq & amplify sex-specific molec markers
    - Establish using tissue from dead frogs first
  - Validate for skin sloughs or buccal swabs (Broquet *et al.* 2007)
  - Outputs
    - Molecular sexing technique
    - Suitable for other genetic techniques (genotyping?)

Outcome: improved breeding success & genetic management *ex-situ*, valuable offspring for release



# Progress

- Key players & collaborators in place
  - Landcare Research, Auckland Zoo, DOC/iwi, Otago & Canterbury University
- Hormone analysis is promising!
  - Assays developed for low T, E and P metabas in urine
  - Sex-based differences in Southern Bell frogs (model)
- Dead specimens have been ID for DNA testing
- DOC permit (iwi consultation-Nov 2007)
- Future partnerships?
  - Researchers, captive breeders, ecosystem restoration
    - USP-Fijian ground frogs, Aus-Corroboree frogs, Karori Wildlife Sanctuary (*L. pakeka* translocation in 2006)

# Native New Zealand lizards

- Skinks & geckos, many with dramatically reduced abundance & distribution
  - Introduced mammalian predators
  - Habitat loss
- Grand & Otago skinks
  - Critically threatened
  - Extinction predicted < 10yrs



Otago skink. Photo: DOC

# McCann's skink

- A suitable 'model' species
  - Taxonomically related (*Oligosoma maccanni*)
  - Still relatively abundant
  - Reproductive cycle in Macraes Flat similar to GAOS skinks (Holmes & Cree 2006)
    - Viviparous & annual breeders
    - Vitellogen/preg length similar
    - Ovulation/parturition earlier



McCann's skink. Photo: Bruce Thomas

# Protocols in development

- Semen collection
  - Develop suitable method
    - Adapt 'massage' technique from Gecko's (Todd)
    - Electroejaculation?
  - Overcome potential contamination
    - Modify collection method
    - Trial different 'washing' strategies

**Output: non-lethal sperm collection protocol**



# Protocols in development

- Semen evaluation
  - Standardise methods
    - Assess sperm volume, concentration, motility & viability
  - Media development
    - Buffers, substrates, cryoprotectants etc
    - Likely different for liquid- & frozen-stored sperm

**Output: functional sperm for AI or GRB**



# Protocols in development

- Artificial insemination
  - Sperm deposition
    - How & where?
    - Adapting approaches used for birds
  - Timing
    - Sperm-storage in females likely occurs for months
    - Test Autumn versus Spring insemination(s)
    - Different for frozen-thawed than fresh sperm?

Output: birth of viable offspring

# Adaptation to threatened lizards

- Refinement of protocols will be required
- Outputs
  - Vastly improved knowledge of reproduction in threatened lizards (e.g. GAOS)
  - Birth of offspring following AI with fresh or frozen-thawed sperm

Outcomes: genetic management *ex-situ*, valuable offspring for release & secure genetic repositories for the future



# Progress

- Key players & collaborators in place
  - Landcare Research, DOC/iwi, Otago University
- Relevant proposals/permits obtained
- Captive colony established in Dunedin
  - 6 males/12 females
    - Semen collection/evaluation & AI this coming summer
- Future partnerships?
  - Researchers, captive breeders, ecosystem restoration
    - Massey University, Mike Kean, COET & Mokomoko Dryland Sanctuary, ONHT & Orokonui Ecosanctuary





# *The grand skink*



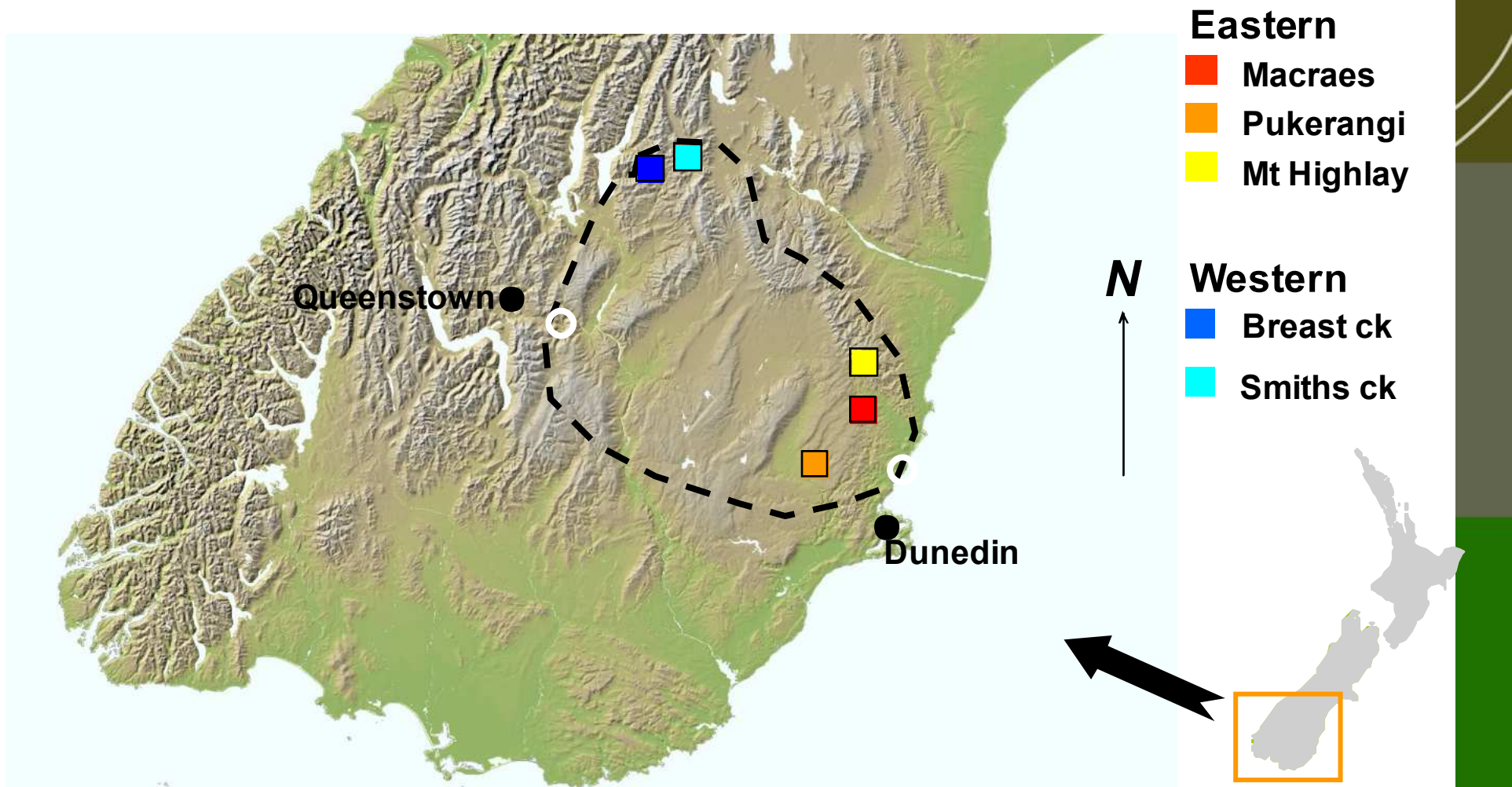
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*Oligosoma grande*

**Critically threatened**

*“...the species now occurs in only 8% of its former range...”*

Whitaker & Loh 1995





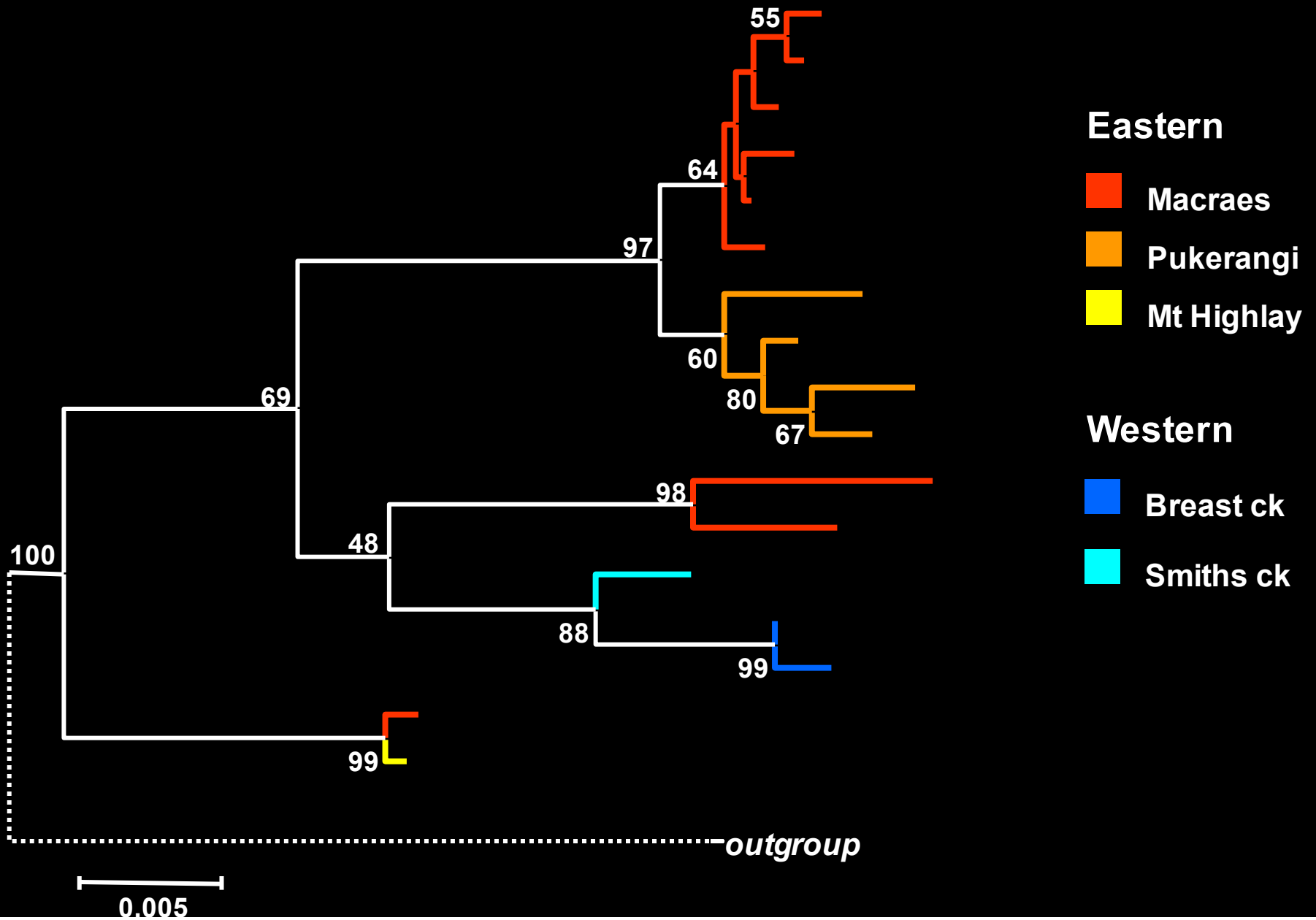
*western*



*eastern*



# Control Region mtDNA phylogeny



# Sourcing & selecting animals

- Genetic considerations BEFORE reintroduction
  - What & Where to release
    - Dictated by size/location of sanctuary
  - How many populations to establish
    - >2 demographically & environmentally independent
  - How many individuals to release
    - Depends on breeding system/pop growth rate (~30-50)
  - Age & sex of individuals
    - More females than males - max pop growth & limit genetic drift
  - Which & how many source populations to use
    - High genetic diversity
    - Multiple sources perhaps if species recently fragmented



# Sourcing & selecting animals

- Role of reproductive technologies
  - Sourcing the numbers
    - Finite numbers in the wild can be translocated
    - Greater demand likely from captive breeding stocks
    - Techniques can improve breeding success
  - Selecting the individuals
    - Animals bred to maximise genetic variability of offspring produced for reintroduction
    - Ensure they are reproductively functional
    - Assisted breeding when animals don't/can't mate



# Monitoring

- Crucial for success but seldom done!
- Genetic considerations AFTER reintroduction
  - Determine reproductive contribution of individuals
  - Is genetic diversity being maintained?
  - Guide decisions about adding/moving animals within or between sanctuaries
- Role of reproductive technologies
  - Reproductively functional or stressed?
  - Sourcing/selecting new “stocks”
    - E.g. move gametes (sperm) not animals?



# Do you want this?

- Genetic/Repro technologies are important for ensuring successful reintroduction and survival
  - Sparse on the website
- Would this information be useful to YOU?
  - Translocation/monitoring sections of website
  - Flow diagram approach
    - Ins & outs of sourcing/selecting/monitoring animals
    - Key contacts (DOC, Recovery/Captive Management Groups, Breeders, Researchers etc)
- Funding?
  - TFBIS, Biodiversity Advice Fund, Others?





# Take home messages

- Genetic/Repro technologies .....**TOOLS!**
  - Genetic diversity/distinctiveness of populations
  - Species-specific reproductive mechanisms
  - Improved genetic management of wildlife for reintroduction
- Relevance to biodiversity sanctuaries
  - Sourcing/selecting animals for reintroduction
  - Monitoring genetic/reproductive “health”
  - **Your support to get this information out there!**