SCIENCE OF BIOLOGICAL AND ENVIRONMENTAL RISK MITIGATION APPROACHES

NExTRAC Workshop
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United States Department of Agriculture
Animal and Plant Health Inspection Service
Wildlife Services National Wildlife Research Center

USDA

USDA United States Department of Agriculture

USDA/NWRC Mission

NWRC Mission: is to apply scientific expertise to resolve human-wildlife conflicts while maintaining the quality of the environment shared with wildlife.

- Need tools for detection, monitoring, and control of cryptic and/or elusive species and pathogens
- Apply genetic methods to these issues
- Strong background in non-invasive genetic applications
  - Forensics
  - Mark-recapture
  - eDNA
- Genomics
- Synthetic biology

Safeguarding gene drive experiments in the laboratory

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Potentially stringent confinement strategies for gene drive research

Multiple stringent confinement strategies should be used whenever possible.

TYPE	STRINGENT CONFINEMENT STRATEGY	EXAMPLES
Molecular	Separate components required for genetic drive	sgRNA and Cas9 in separate loci (*8*)
	Target synthetic sequences absent from wild organisms	Drive targets a sequence unique to laboratory organisms (*3*,*4*,*8*)
Ecological	Perform experiments outside the habitable range of the organism	*Anopheles* mosquitoes in Boston
	Perform experiments in areas without potential wild mates	*Anopheles* mosquitoes in Los Angeles
Reproductive	Use a laboratory strain that cannot reproduce with wild organisms	*Drosophila* with compound autosomes*
Barrier	Physical barriers between organisms and the environment	Triply nested containers, >3 doors (*6*)
	•Remove barriers only when organisms are inactive	Anesthetize before opening (*6*)
	•Impose environmental constraints	Low-temperature room, air-blast fans
	•Take precautions to minimize breaches due to human error	Keep careful records of organisms, one investigator performs all experiments (*6*)

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INSIGHTS | PERSPECTIVES

SCIENCE GALLEY

OVERLINE

Core commitments for field trials of gene drive organisms

If followed, they will promote responsible conduct.

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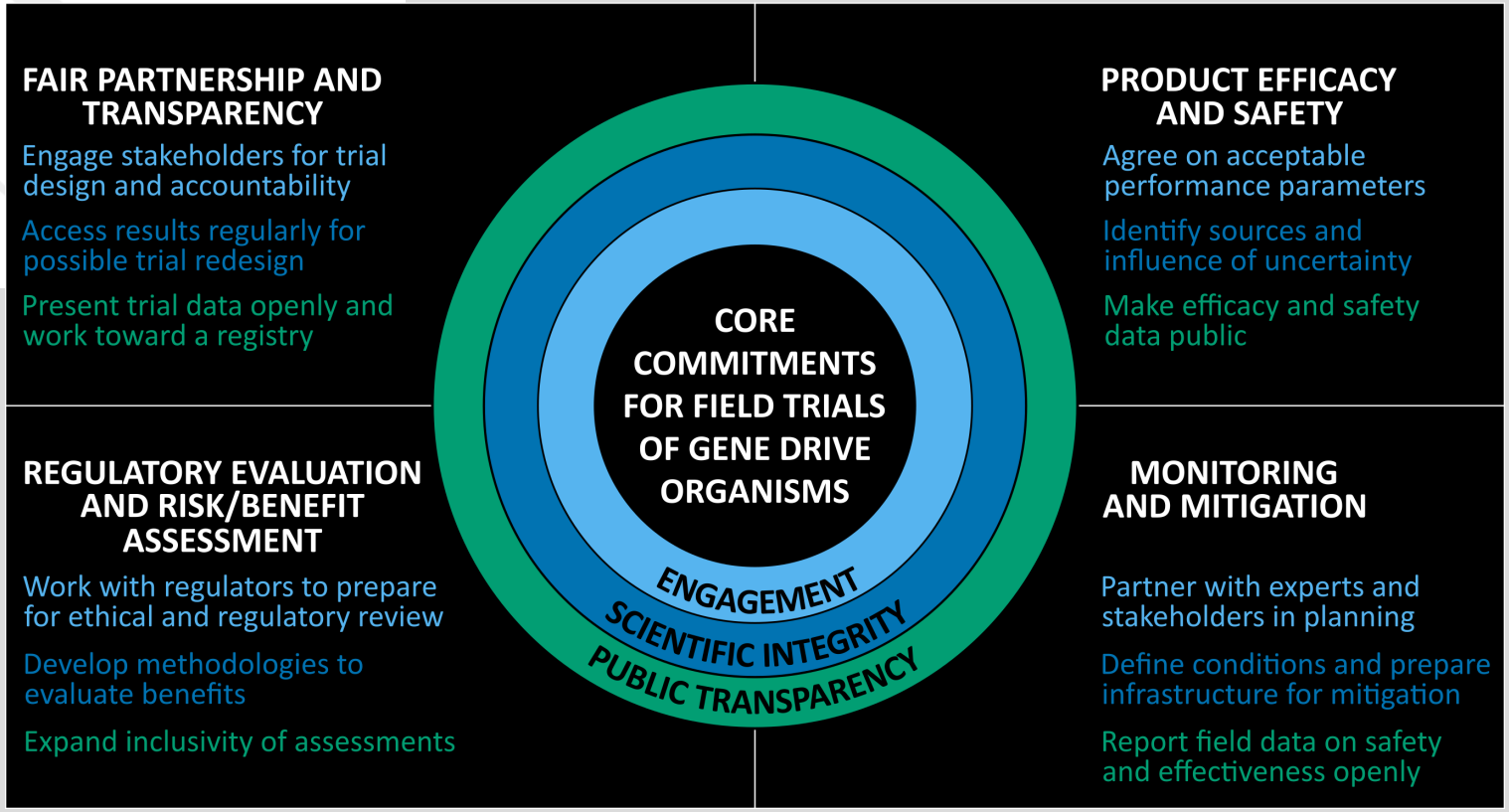
Gene drive organisms (GDOs), whose genomes have been genetically engineered to spread a desired genetic trait through a population, have the potential to transform the way societies address a wide range of daunting public health and environmental challenges. Development, testing, and implementation of GDOs, however, is complex and often controversial. A key challenge is to clarify the appropriate roles of developers and others actively engaged in work with GDOs

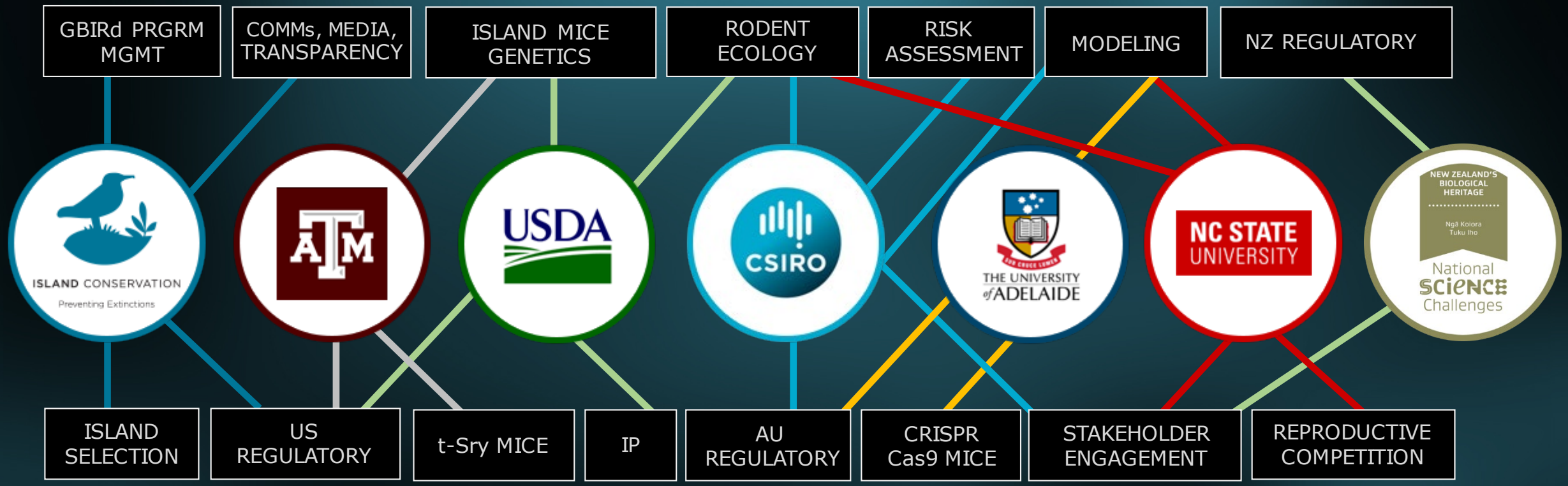
dengue, Chikungunya, and Zika viruses), *Anopheles* spp. (major vectors of malaria parasites), or white-footed mice (carriers of the Lyme disease bacterium). GDOs for suppression of pest populations could also contribute greatly to biodiversity conservation, agricultural productivity, and human and animal well-being.

The core commitments presented here are intended to address field trials of either non-localized GDOs in ecologically isolated



GBIRd

Genetic Biocontrol of Invasive Rodents



BIOSAFETY/CONTAINMENT/ LOCALIZATION

ETHICS ADVISORY COMMITTEE

SUSTAINABILITY/ FUNDRAISING

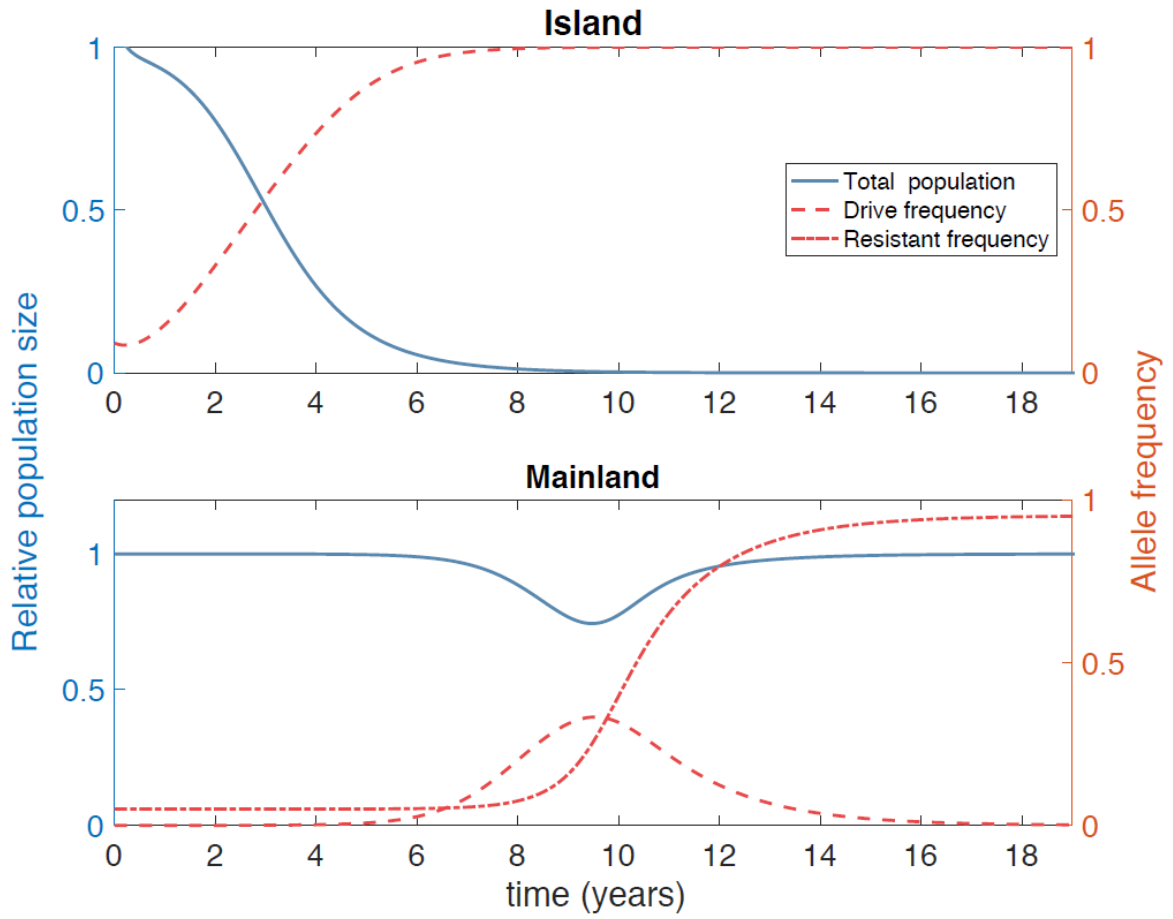
GBIRd: How to limit spread into non-target populations?

1) Careful site selection of islands (e.g., size, isolation, traffic, no human habitation) is critical first step (biocontainment)

2) Targeting spatial limitation of gene drive through exploitation of **locally-fixed alleles**

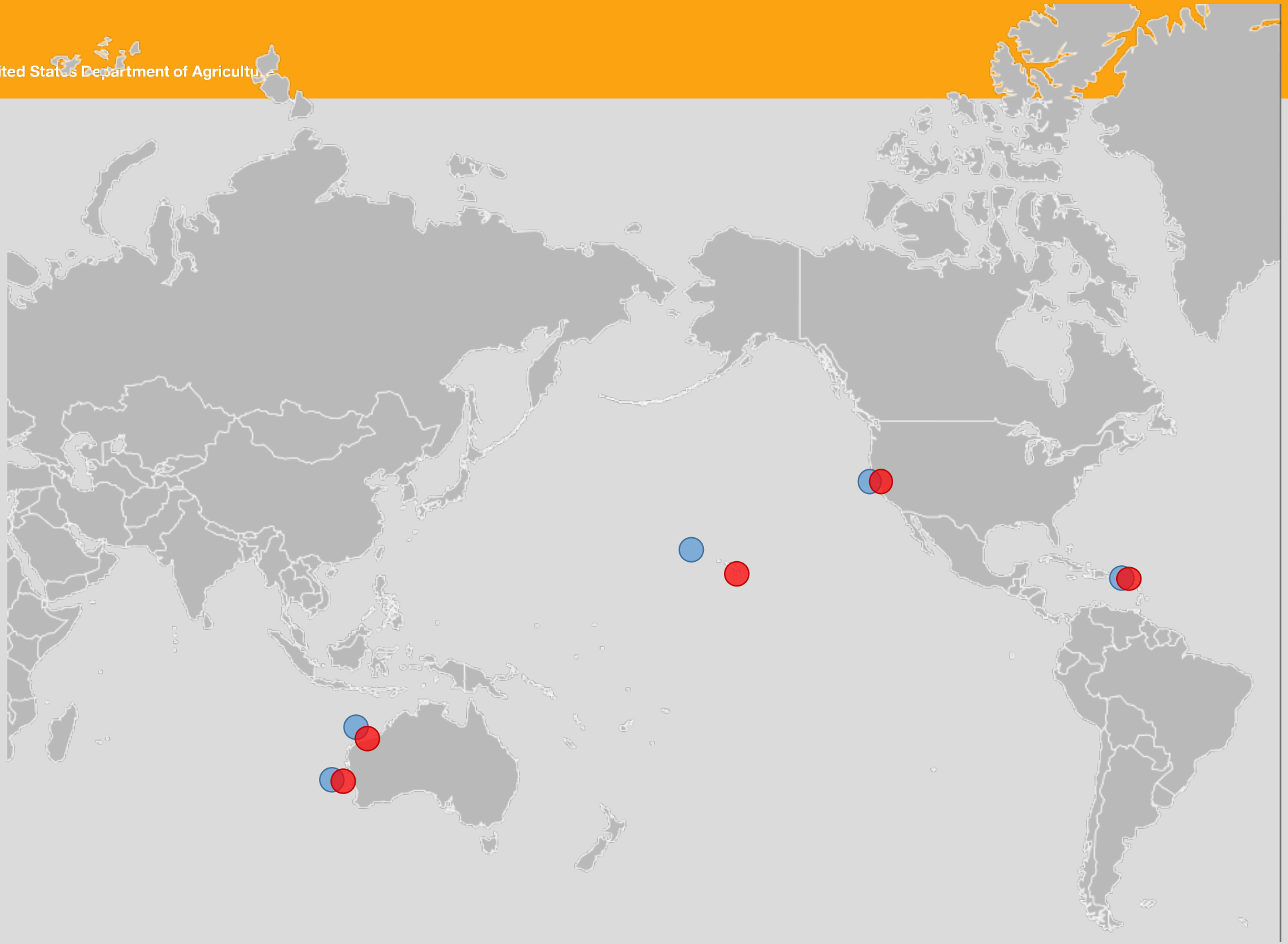
Objective
Population genetic theory would predict that island populations would have lower genetic diversity, higher differentiation (founder effect and genetic drift), and fixation of some alleles –use population genomics to explore **locally-fixed alleles** (frequency, genomic location, off-target activity).

Approach
Sampled both invasive mouse island populations and potential "source" mainland populations. Pooled whole-genome resequencing ('pool-seq') to identify population-specific locally fixed alleles with CRISPR/Cas9 binding sites and conduct population genetic analyses.

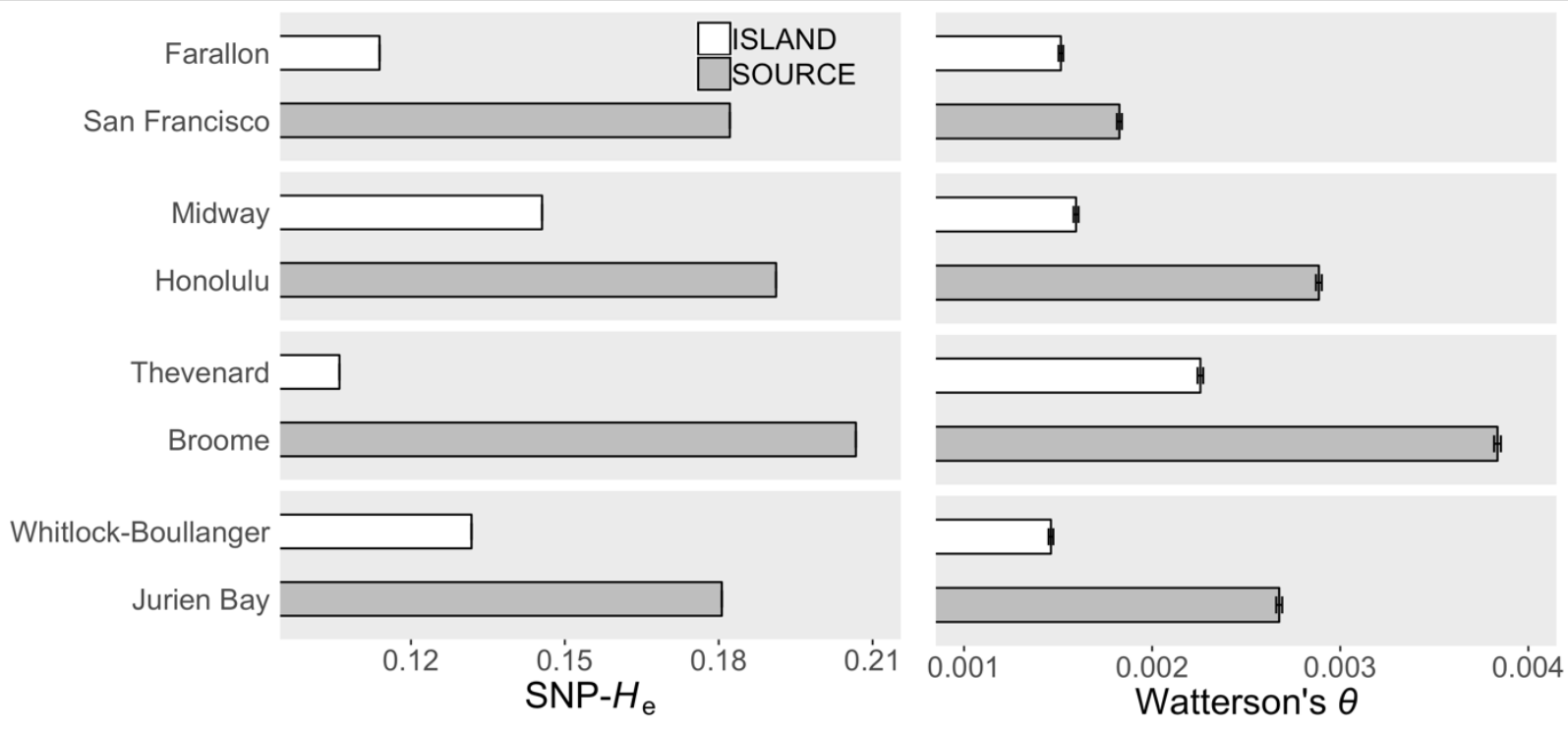


Locally Fixed Alleles: A method to localize gene drive to island populations

Jaye Sudweeks [a], Brandon Hollingsworth [b], Dimitri V. Blondel [c], Karl J. Campbell [d], Sumit Dhole [e], John D. Eisemann [f], Owain Edwards [g], John Godwin [c,h], Gregg R. Howald [d], Kevin Oh [f,i], Antoinette J. Piaggio [f], Thomas A. A. Prowse [j], Joshua V. Ross [j], J. Royden Saah [d,h], Aaron B. Shiels [f], Paul Thomas [k], David W. Threadgill [l], Michael R. Vella [b], Fred Gould [e,h] and Alun L. Lloyd [a,b,*]



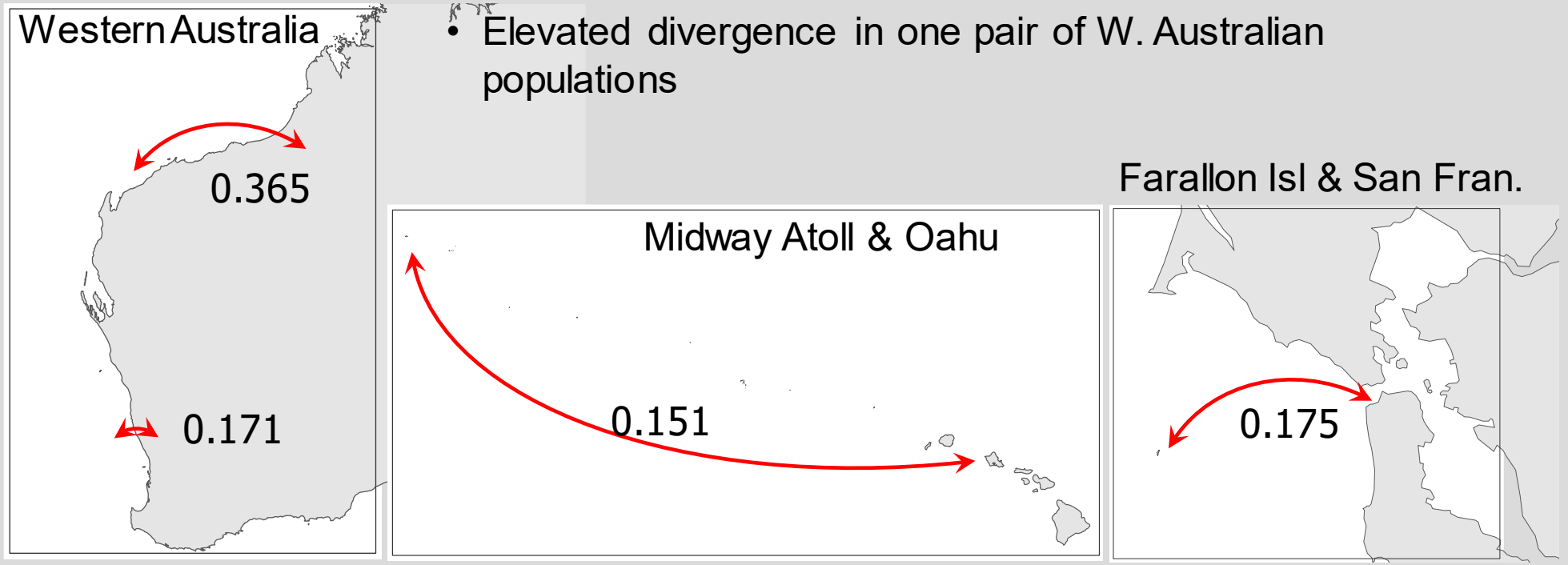
Reduced allelic diversity in remote isolated islands vs. ‘source’ populations



Based on ~38 million autosomal single-nucleotide polymorphisms (SNPs)

Island x 'source' population genetic differentiation

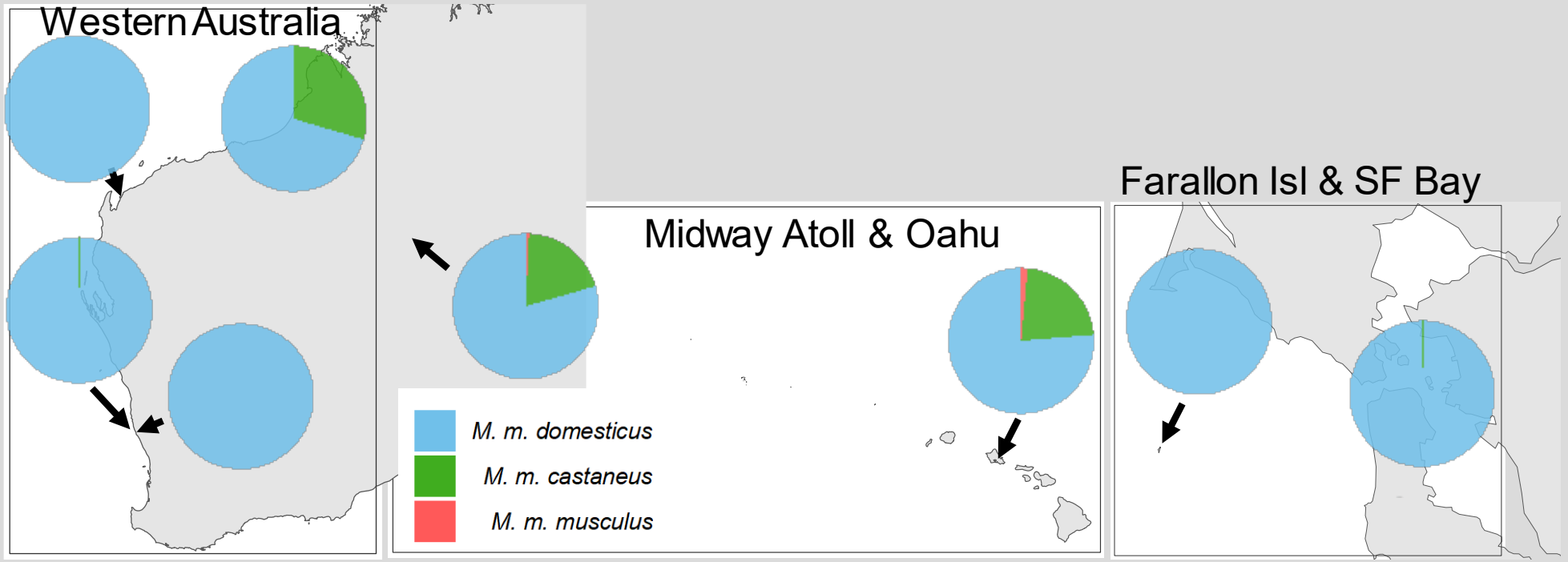
- Measured using fixation index (F_{ST})
- All island populations showed low to moderate genetic differentiation from putative source populations
- Elevated divergence in one pair of W. Australian populations



Based on ~38 million autosomal single-nucleotide polymorphisms (SNPs)

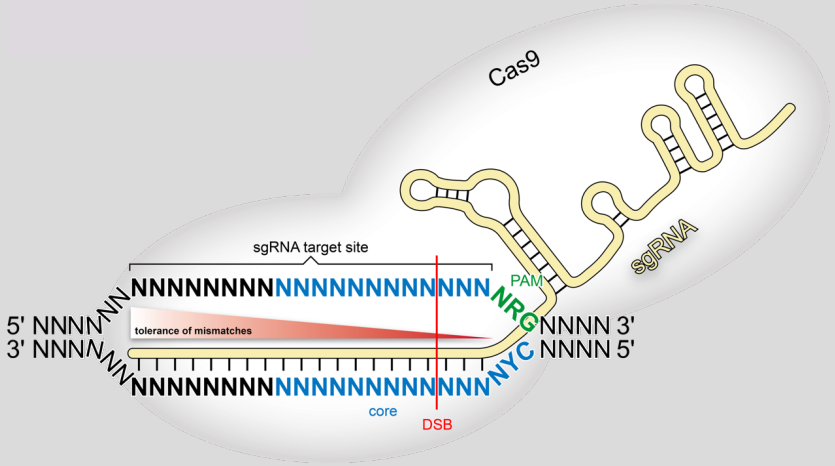
Admixture analysis revealed presence of multiple subspecies

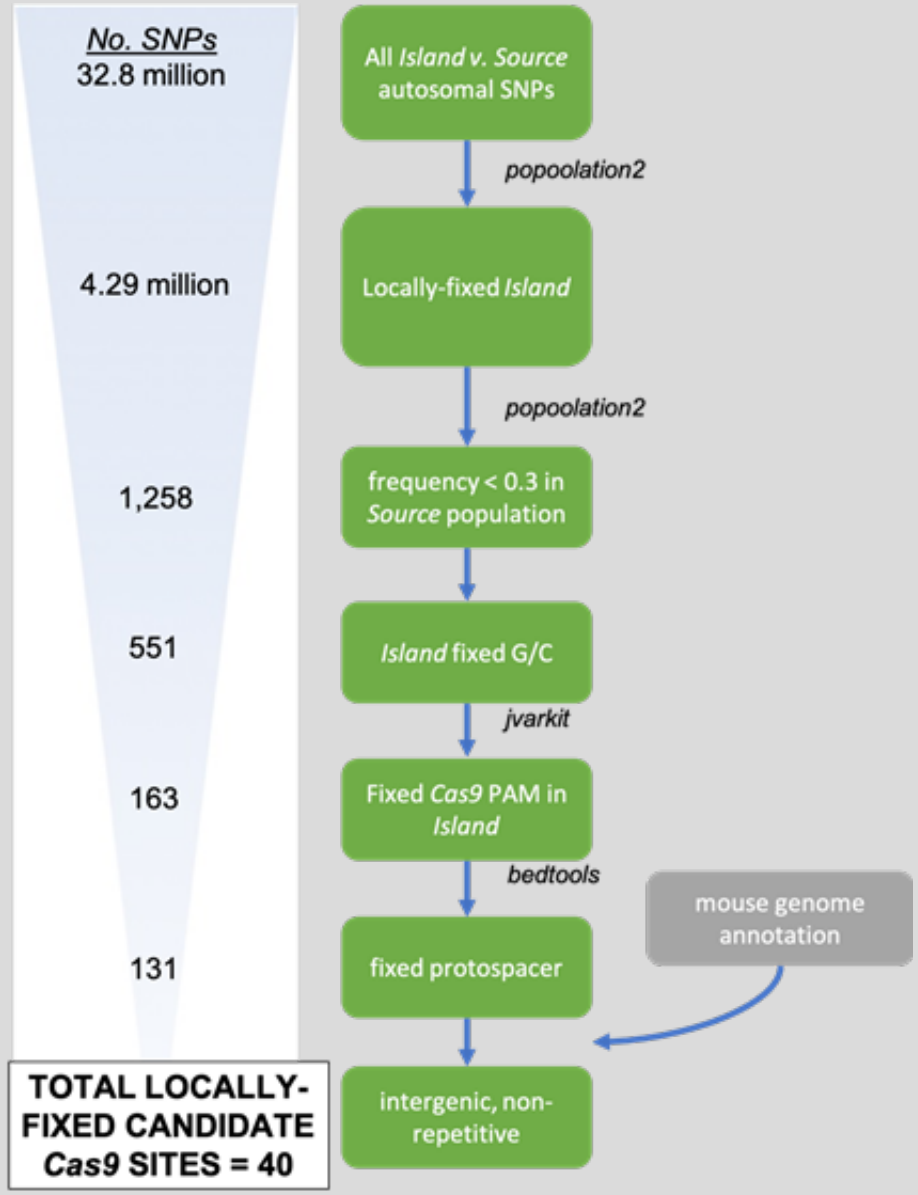
- Estimated admixture coefficients for 3 primary *M. musculus* subspecies
- Elevated divergence in NW Australia due to presence of *M. m. castaneus*



Scans for Locally-Fixed Alleles (*Midway v. Oahu*)

- Putative Cas9 PAM binding sites (NGG)
- 19.6% had additional SNPs in protospacer
- 69.5% overlapped with genes





Summary

- The characterization of population genetics of a target population is critical and knowledge gap that takes years to fill and is key for minimizing risk of spread (e.g., subspecies, levels of genetic diversity, genetic connectivity).
- Further the lack of basic biology for our target systems, such as mouse breeding ecology, which can vary per ecosystems a critical knowledge gap that also takes years to fill
- Synthetic gene drives hold potential as a future alternative method for population suppression of invasive rodents that can be targeted, humane, and low environmental burden.

What steps have been taken to help ensure containment of future GE trials at NWRC?

- Ship embyos and not live gene drive mice

A multi-tiered physical containment plan (Barrier): 1º (Arena), 2º (traps), 3º (room), 4º (bldg.), 5º (bait stations) with staff training, protocols, and campus biosecurity plan

- Series of trials and mouse observations to demonstrate physical containment & biosecurity
- Environmental Assessment (EA) solicit public comment for 60 days on GE trials, risk, and our planned confinement strategies

Molecular containment via:

- Private alleles (Ft Collins *Mus musculus* will not be used)
- Additional molecular stops (target synthetic sequences, split drives, etc.)

Questions

Camazotz