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2019

### A potential new tool for the toolbox: assessing gene drives for eradicating invasive rodent populations

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Campbell, K. J.; Saah, J.R.; Brown, P.R.; Godwin, J.; Howald, G.R.; Piaggio, A.; Thomas, P.; Tompkins, D.M.; Threadgill, D.; Delborne, J.; Kanavy, D.M.; Kuiken, T.; Packard, H.; Serr, M.; and Shiels, A., "A potential new tool for the toolbox: assessing gene drives for eradicating invasive rodent populations" (2019). *USDA National Wildlife Research Center - Staff Publications*. 2235.  
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# A potential new tool for the toolbox: assessing gene drives for eradicating invasive rodent populations

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**Abstract** Invasive rodents have significant negative impacts on island biodiversity. All but the smallest of rodent eradications currently rely on island-wide rodenticide applications. Although significant advances have been made in mitigating unintended impacts, rodent eradication on inhabited islands remains extremely challenging. Current tools restrict eradication efforts to fewer than 15% of islands with critically endangered or endangered species threatened by invasive rodents. The Genetic Biocontrol of Invasive Rodents partnership is an interdisciplinary collaboration to develop and evaluate gene drive technology for eradicating invasive rodent populations on islands. Technological approaches currently being investigated include the production of multiple strains of *Mus musculus* with a modified form of the native t-complex, or a CRISPR gene drive, carrying genes or mechanisms that determine sex. These systems have the potential to skew the sex ratio of offspring to approach 100% single-sex, which could result in population collapse. One goal proposed is to test the ability of constructs to spread and increase in frequency in *M. musculus* populations in biosecure, captive settings and undertake modelling to inform development and potential deployment of these systems. Structured ecologically-based risk assessments are proposed, along with social and cultural engagement to assess the acceptability of releasing a gene drive system. Work will be guided by an external ethics advisory board. Partners are from three countries with significant regulatory capacity (USA, Australia, New Zealand). Thus, we will seek data sharing agreements so that results from experiments may be used within all three countries and treat regulatory requirements as a minimum. Species-specific, scalable, and socially acceptable new eradication tools could produce substantial biodiversity benefits not possible with current technologies. Gene drive innovation may provide such a tool for invasive species management and be potentially transformative and worthy of exploring in an inclusive, responsible, and ethical manner.

**Keywords:** conservation, CRISPR, genetic biocontrol, invasive species, mice, *Mus musculus*, pest management, public engagement, risk assessment, transgenic

## INTRODUCTION

Three *Rattus* species (*R. rattus*, *R. norvegicus*, *R. exulans*) and house mice (*Mus musculus*) are, outside of their native ranges, globally widespread invasive species (Capizzi, et al., 2014). These invasive rodents negatively impact stored foods, crops, and infrastructure and can carry pathogens that impact the health of people and their livestock (Stenseth, et al., 2003; Meerburg, et al., 2009; Banks & Hughes, 2012). Invasive rodents cause population declines and extinctions of island floras and faunas and interrupt ecosystem processes with negative cascading effects (Townes, et al., 2006; Jones, et al., 2008; Kurlle, et al., 2008; Doherty, et al., 2016). To recover endangered populations and restore ecosystem processes, invasive rodents on islands are increasingly targeted for eradication, with at least 650 eradication attempts of introduced *Rattus* spp. populations to-date (Russell & Holmes, 2015). These and other island-based invasive mammal eradications have resulted in positive responses by native species with few exceptions (Jones, et al., 2016).

Anticoagulants are the most common control method for invasive rodents (Capizzi, et al., 2014). Rodent eradication on any island typically >5 ha has relied exclusively on the use of anticoagulant toxicants incorporated into cereal or wax baits (DIISE, 2016). Second generation anticoagulants are most commonly used and have had the highest success

rate (Howald, et al., 2007; Parkes, et al., 2011). However, their broad-spectrum toxicity to vertebrates, duration of persistence, ability to biomagnify, mode of death and negative public perception limit their responsible use (Eason, et al., 2002; Fitzgerald, 2009; Broome, et al., 2015). These features can lead to negative impacts, including for conservation targets (e.g. Rueda, et al., 2016), although significant advances in strategies to mitigate these impacts have been made (e.g. Rueda, et al., 2019). Inhabited islands with children, livestock and pets present significant challenges because eradication is currently limited by a lack of species-specific methods, animal welfare issues, high fixed costs, and socio-political opposition (Campbell, et al., 2015). Hence, even with optimistic assessments for current methods (islands up to 30,000 ha and/or 1,000 people), eradications are possible on fewer than 15% of islands with critically endangered or endangered species threatened by invasive rodents (Campbell, et al., 2015). New species-specific, scalable tools are needed if we are to prevent extinctions.

Genetic biocontrol in the form of gene drives coupled with sex-determining genes to produce single-sex offspring, offers a potentially transformative new tool to add to the rodent eradication toolbox, by offering species-specificity not readily achievable in existing technology (Campbell, et

al., 2015). Gene drives cause a gene to spread throughout a population at a rate higher than would normally occur (Champer, et al., 2016). Gene drives occur naturally and are not recent phenomena (Lindholm, et al., 2016); for example, mice with the native *t*-complex gene drive were first described in 1927 (Schimenti, 2014). Attempts to harness naturally-occurring gene drive systems, primarily for invertebrate pests and disease vectors have had mixed results (Sinkins & Gould, 2006; Champer, et al., 2016). In 2012, the Genetic Biocontrol of Invasive Rodents (GBIRD) partnership was formed between North Carolina State University (NCSU), Island Conservation (IC) and later Texas A&M University (TAMU). GBIRD started exploring opportunities for harnessing the native *t*-complex gene drive in mice to eradicate invasive mouse populations on islands (Kanavy & Serr, 2017; Piaggio, et al., 2017). Other partners were identified through professional networks and during searches for specific skillsets. GBIRD currently includes seven partners in three countries: TAMU, NCSU, University of Adelaide (UA), USA Department of Agriculture's National Wildlife Research Center (NWRC), the Agriculture and Food Business Unit of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Landcare Research (LR), and IC.

Beginning in 2013, a harnessed bacterial immune response system called CRISPR/Cas9 revolutionised the field of genetic engineering. CRISPR/Cas9 can be used to delete, modify or insert new genes more precisely, effectively, time- and cost-efficiently than previous gene editing tools (NASEM, 2016). Multiple genes can also now be edited simultaneously. In 2014, a landmark paper (building upon earlier concepts of Burt, 2003), described how a cassette encoding the CRISPR/Cas9 machinery could be precisely inserted into an organism's DNA, creating a self-replicating gene drive with potential to modify wild populations by design (Esvelt, et al., 2014). Since then, CRISPR/Cas9 gene drives have been developed in yeast *Saccharomyces cerevisiae* (DiCarlo, et al., 2015), fruit fly *Drosophila melanogaster* (Gantz & Bier, 2015) and both *Anopheles stephensi* (Gantz, et al., 2015) and *A. gambiae* (Hammond, et al., 2016) mosquitoes as proof-of-concept demonstrations in biosecure laboratories. This field has become a significant focus of research, and USA and Australian Academies of Science have provided recommendations aimed at guiding its development (NASEM, 2016; AAS, 2017). GBIRD, with its partnership already established, adopted CRISPR as a gene editing and potential gene drive tool.

Gene drives are a technology platform. GBIRD partnership considers *Mus musculus* the logical starting point for developing, exploring, and providing proof-of-concept for a genetics-based invasive vertebrate eradication tool. They are the model vertebrate species for genetics, possess a short generation-time, are small, husbandry is straight-forward, and they are invasive around the world including on many islands (Guénet & Bonhomme, 2003; Phifer-Rixey & Nachman, 2015). Mice are also among the best studied species in terms of mammalian sex determination, reproductive biology, behaviour, genetic manipulation and genetic control of phenotypic traits (Guénet & Bonhomme, 2003; Eggers, et al., 2014; Phifer-Rixey & Nachman, 2015; Singh, et al., 2015). If proof-of-concept, safety, and efficacy are demonstrated in *Mus musculus*, it should be possible to apply this approach to *Rattus* species.

The GBIRD programme (<<http://www.geneticbiocontrol.org/>>) aims to develop multiple gene drive systems in mice for simultaneous evaluation of safety and efficacy, while carefully assessing the social, cultural and policy acceptability of such an approach. Our

staged inclusive approach reflects USA and Australian Academies of Sciences' recommendations (NASEM, 2016; AAS, 2017) that we treat as our minimum standards. The GBIRD partnership aims to provide vital data for conducting risk assessments, determining efficacy, and engaging stakeholders and communities in order to inform and enhance progress, or identify limitations, of future research. A potential longer-term goal is submission of an application to a regulatory agency for release of gene drive constructed mice on a small, biosecure island to test eradication of the wild, invasive mouse population.

This paper provides an overview of the GBIRD programme as it has developed to-date, including the risks and opportunities as they are currently envisioned and understood. These will certainly evolve, and the programme must strategically evolve with them.

## Genetic Biocontrol of Invasive Rodents programme

The programme's guiding principles provide context for decision making:

- Proceed cautiously, with deliberate step-wise methods and measurable outcomes;
- Engage early and often with the research community, regulators, communities and other stakeholders;
- Maintain an uncompromising commitment to biosafety, existing regulations, and protocols as minimum standards (e.g. NASEM, 2016; AAS, 2017);
- Use, and participate in developing best practices;
- Only operate in countries with appropriate regulatory capacity; and
- Be transparent with research, assessments, findings, and conclusions.

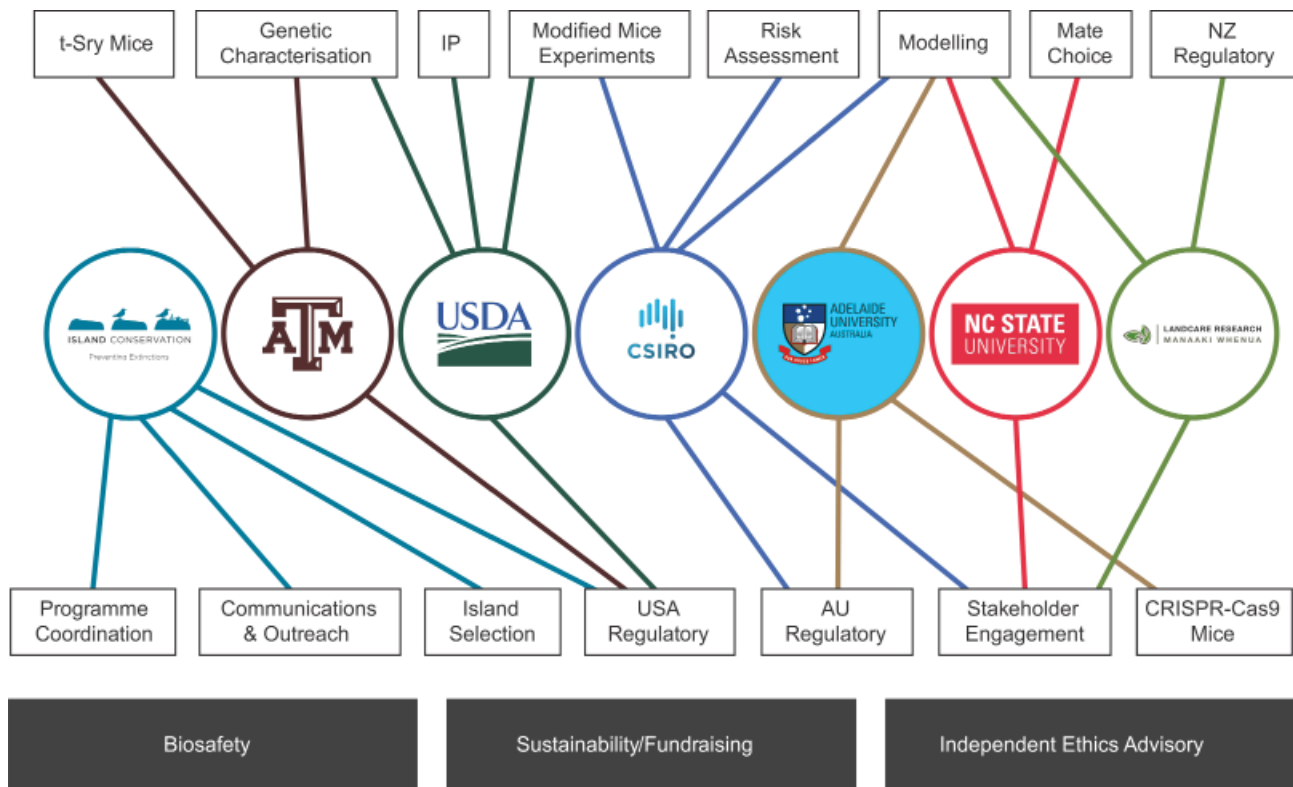
### 1. Governance and Coordination

GBIRD involves seven organisations from Australia, New Zealand and the USA; three universities (NCSU, TAMU, UA), three governmental research (CSIRO, LR, NWRC) and one non-governmental non-profit (IC). Each has specific roles and responsibilities (Fig. 1) as detailed in the memorandum of understanding that formalises the partnership. A steering committee comprised of one or two representatives from each organisation provides direction and decision making, and a programme coordinator facilitates activity. The consortium is inclusive and, indeed, strengthened by a transparent internal dialogue in both the scientific positioning (e.g. Gemmill & Tompkins, 2017) and societal/values realm (e.g. Webber, et al., 2015). GBIRD has 14 component areas and three cross-cutting themes (Fig. 1) being investigated, as follows.

### 2. Gene drives

Three gene drives are currently being investigated; a modified *t*-complex, a CRISPR/Cas9 and a CRISPR/Cpf1 gene drive. The *t*-complex on chromosome 17 in mice is a natural male-transmitted meiotic drive (Lyon, 2003; Schimenti, 2014). The *t*-complex impairs sperm carrying the *t*-complex, leading to an increased frequency of *t*-complex carrying sperm fertilising ova. The frequency of the *t*-complex in natural populations of house mice is typically lower than predicted given the often very strong transmission ratio distortion displayed. This phenomenon is not completely understood (see Lindholm, et al., 2016), but may imply that a sex-biasing system based on the *t*-complex would require ongoing releases to be effective (Backus & Gross, 2016). The *t*-complex haplotype we are using is free of recessive lethals and has a high rate (>95%) of inheritance, also called transmission distortion (Kanavy





**Fig. 1** Programme map, showing 14 component areas being investigated by partners of the Genetic Biocontrol of Invasive Rodents programme. The three components not linked to any organisation are cross-cutting themes.

& Serr, 2017; Piaggio, et al., 2017). The remaining offspring (<5%) would not carry the gene drive or exhibit the phenotypic traits of the genes being driven (Piaggio, et al., 2017).

CRISPR/Cas9 gene drives are capable of >94% inheritance (Gantz, et al., 2015; Hammond, et al., 2016). Once inserted within one individual's genome, a gene drive can work in one of two ways. A zygotic gene drive works when that individual's ova or sperm are fertilised. If the gene drive cassette is activated in the fertilised egg (zygote), the guide RNA (gRNA) directs Cas9 to produce a double-stranded break in the DNA at the target site in the chromosome lacking the gene drive. This triggers the cell's repair mechanism to repair the break using the gene drive-containing chromosome as a template resulting in self-replication of the gene drive. Alternatively, in a germline gene drive, germ cells can be targeted as the stage for self-replication of the gene drive.

### 3. Targeted genes

Genes can be targeted for deletion, modification or insertion of new genes in conjunction with a gene drive to increase inheritance of specific traits. Investigations currently focus on the appropriateness of two target genes (*Sry*, *Sox9*) to be inserted and one chromosome to be deleted (Y-'shredder'), each in coordination with a gene drive. The *Sry* gene is found on the Y chromosome and is considered the master sex-determining gene in most mammals (Kashimada & Koopman, 2010; Eggers, et al., 2014). Another key component of the testis pathway is the autosomal gene *Sox9*, which acts immediately downstream of *Sry* (Eggers, et al., 2014). Both genes drive the development of male testes in mammals and sex reversal has been demonstrated in transgenic female (XX) mice (Koopman, et al., 1991; Vidal, et al., 2001; Eggers, et al., 2014). A Y-shredder (Adikusuma, et al., 2017)

promotes solely offspring with one (XO) or two X (XX) chromosomes, i.e. females. Initial developments focus on *t*-complex with *Sry* inserted (*t-Sry*), and CRISPR/Cas9 and CRISPR/Cpf1 gene drives with *Sox9* and Y-shredder.

As of June 2018, partners attempting to incorporate *Sry* into a *t*-complex drive have been challenged by the large construct size of *Sry*. If that technological hurdle can be overcome, these mice are expected to produce >95% phenotypically male offspring (Kanavy & Serr, 2017; Piaggio, et al., 2017). The mice currently under development in Australia are expected to test the functionality of a split CRISPR/Cas9 gene drive that uses phenotypic coat markers as genetic 'cargo'. A 'split gene drive system' has the gene drive in two separate 'cassettes' (DiCarlo, et al., 2015). This design is a safety feature for laboratory testing where the separation of the cassettes results in drive components being inherited separately even if a drive carrier were to escape, thus preventing drive function (since both are necessary for function). Development of CRISPR/Cpf1 gene drives and incorporating *Sox9* and the Y-shredder are underway.

### 4. Spatial control of gene drive

Spatially or temporally limiting drive function is one of the major research challenges for CRISPR gene drives, e.g. restricting a gene drive to affect only a single island's rodent population. Our programme is investigating genome-level targeting of population-specific locally-fixed alleles as a potential spatial control mechanism. It is likely that through the process of invasion, founder effects and population bottlenecks, certain alleles across the genome have become fixed in any island population (Britton-Davidian, et al., 2000; Hartl & Clark, 2006). This pattern of fixation is likely a unique genomic signature in every genetically isolated island population. Similar to the molecular confinement strategy being implemented in the laboratory

(see *Biosafety*), population-specific locally-fixed alleles (and their sequence) could act as unique gRNA targets for a CRISPR gene drive that will not function outside the island population. Others are investigating alternative approaches to temporally and/or spatially contain gene drives and their relative effectiveness (e.g. Dhole, et al., 2018).

### 5. Biosafety

Multiple biocontainment strategies accompany all laboratory work and are part of our staged testing pathway (following the recommended approach by NASEM, 2016). Recommended containment standards for gene drives include at least two stringent confinement strategies wherever possible, in addition to containment (Akbari, et al., 2015; NASEM, 2016), and our programme exceeds these standards. For example, the CRISPR gene drive studies are using physical containment at the currently required level (PC2) (AAS, 2017) and three containment/confinement methods; a ‘split gene drive system’ as explained above (DiCarlo, et al., 2015); coat colour (white or black) to identify the zygotic homing in offspring – white mice (Cas9-positive) are less likely to survive in the wild (Vignieri, et al., 2010); and gRNA exclusively targeting a synthetic sequence not present in wild mice, providing molecular confinement to transgenic laboratory mouse populations. For scaled laboratory trials, CSIRO and NWRC state-of-the-art facilities provide the opportunity to safely conduct trials with colonies of mice that could originate from islands.

### 6. Safety and efficacy experiments

Experiments demonstrating that constructs work effectively and efficiently, are species-specific and safe to the environment are needed. Data needs for risk assessments and field trial applications have yet to be determined in conjunction with regulatory agencies, and this will dictate minimum requirements for experiments. Experiments will inform risk assessments to reduce uncertainty surrounding outcomes and probabilities. Phased testing and experiments are viewed as part of the development process, and occur at each tier (i.e. molecular level, individual mice, mouse population, ecological community). This phased development process incorporates feedback loops to developers, and evaluates efficiency, stability, specificity and safety to determine whether a specific construct proceeds to the next stage (e.g. molecular to insertion in a mouse or going from individual mice to a colony). Constructs that pass will go on to more rigorous testing, and those that don’t will either be dropped or modified and then re-evaluated. No functional CRISPR drives have yet been reported for vertebrates. Attempting development of multiple combinations of gene drives and gene targets within our programme increases the likelihood of success, and, if successful, would provide opportunities for comparative analyses and risk assessments. High-quality data for modelling and risk analyses will be necessary.

### 7. Mate choice

Behavioural barriers to mating success and resulting gene flow must be considered, as to how (or if) a gene drive will successfully spread through a population, and if understood and used correctly may provide significant advantage. Key characteristics influencing male reproductive success in mice include aggressive dominance for securing territories, and a preference among females for unfamiliar males (Gray & Hurst, 1998; Cunningham, et al., 2013). Promiscuity of male mice and their ability to inseminate many females provides males the potential to disproportionately influence the genetic makeup of future

generations. Experiments in the 1980s introducing Isle of Eday mice to the Isle of May (57 ha) demonstrate the power of selecting appropriate stock for facilitating introduced individuals ‘invading’ another population (Berry, et al., 1991; Jones, et al., 1995). A Y-chromosome (i.e. male) linked marker spread across the Isle of May site within six months and in 18 months only hybrids could be detected (Berry, et al., 1991; Jones, et al., 1995). The 42 Isle of Eday males introduced were estimated at <5% of May’s resident mouse population, demonstrating differential success of introduced versus resident males (Berry, et al., 1991; Jones, et al., 1995). We aim to rank the ‘invasibility’ of males from laboratory strains, selected islands and mainlands so that appropriate stock may be selected for backcrossing in gene drives and their cargo. Initial trials involve *t*-complex carrying laboratory mice (C57BL/6/129 strain), Southeast Farallon Island, and F1 hybrid Farallon-laboratory mice in small cages with single males and females, to determine if mating would occur (Serr & Godwin, 2019). (Note: Southeast Farallon Island is not considered a potential site for field trials at this time). Larger arenas were used to determine mate choice and male competition where males from different populations would have to compete for females and resources (Serr & Godwin, 2019).

Behavioural experiments to-date indicate that *t*-complex carrying lab mice can successfully mate with island mice in captivity (Serr & Godwin, 2019). Other mate competition results indicate that male F1 hybrid Farallon-laboratory mice may be able to outcompete male Farallon island mice.

### 8. Island selection

As part of our staged, stepwise approach, if biosecure laboratory studies support safety and efficacy in biasing sex ratios and suppressing test populations, the next stage will involve studies in natural settings under conditions where dispersal or persistence of the organisms outside the evaluation area is restricted (NASEM, 2016). We have identified a suite of ecological criteria for initial selection of potentially appropriate islands for trials, including 1. the island is biosecure (i.e. closed to public or infrequent/controlled visitation; and remote enough (>1 km from other land masses) to avoid unassisted immigration or emigration), 2. no significant challenges exist to treatment using traditional toxicant-based methods to eradicate mice (e.g. no major non-target species, regulatory environment allows the use of brodifacoum bait products, single land manager), 3. *M. musculus* are the only rodent present or could be introduced, and 4. the island is reasonably economical and feasible to visit year-round (see Harvey-Samuel et al., 2019 for a more detailed account and rationale). By selecting islands where the use of traditional eradication methods could readily be used to eradicate all rodents (Howald, et al., 2007) a contingency (i.e. exit strategy) explicitly exists. However, these ecological criteria are just a first filter and additional steps would be required prior to any field trial, including engagement with stakeholders (e.g. land managers, local communities) and regulators to determine final approval (Harvey-Samuel et al., 2019).

### 9. Population genetic characterisation

Genetic characterisation of mouse populations from islands selected for potential trials will occur using next-generation sequencing technologies (e.g. Illumina MiSeq). Analyses of these data will inform the feasibility of using population-specific fixed allele sequences as gRNA targets to provide spatial control of any gene drive trialled. They will also provide baseline assessments of genetic characteristics of target island populations, and potentially inform future strategies.

## 10. Modelling

Modelling can be used to inform broad strategies, such as male or female biasing gene drives and, within those strategies, to identify heritable traits or environmental conditions that provide disproportionate advantages (Bax & Thresher, 2009; Backus & Gross, 2016). Modelling is contemplated at each development stage (i.e. molecular, individual mouse, mouse population, ecological community), incorporating data from experiments and trials, and providing feedback to developers and trial designs. It aims to predict outcomes, reduce the number of animals required in experiments and trials and provide insight on strategies. At the molecular level, for example, the efficiency and stability of homing and non-homologous end joining for Cas9 and Cpf1 zygotic and germline homing approaches can be modelled based on data from experiments informing on likelihood of failure (Prowse, et al., 2017). Models also consider individual mouse characteristics and the effects these may have at the population level. A population model would estimate the number of gene drive mice with certain characteristics required for release to a specific island, the optimal frequency, timing and location of releases, and time until eradication. The impacts of changes to specific mouse characteristics (or other variables) can then be estimated. As data sets accumulate, the accuracy and sophistication of models will increase. The opportunity exists to leverage a 30+ year dataset and existing mouse population models, which will facilitate sophisticated analyses and allow the development of advanced deployment strategies that optimise seasonal and climatic variation (Singleton, et al., 2005; CSIRO, unpub. data). The use of these and other models will be critical in the development of robust ecologically-based risk assessments.

## 11. Risk assessment

There is the possibility that releases of gene drive-modified organisms will lead to unpredicted and undesirable side effects. Ecologically-based risk assessments (EBRA) aim to reduce some types of uncertainty surrounding outcomes and probabilities (NASEM, 2016; AAS, 2017). They are used to estimate the probability of immediate and long-term environmental and public health harms. EBRA allow alternative strategies to be compared (e.g. traditional use of toxicants), incorporate the concerns of relevant publics, and can be used to identify sources of uncertainty, making them well-suited to inform research directions and support public policy decisions about emerging gene drive technologies. EBRA provide the ability to trace cause-and-effect pathways and the ability to quantify the probability of specific outcomes. We regularly consult with risk assessment experts leading other gene drive EBRA and plan to apply specific tools to identify where, within our development process, additional studies are required to reduce uncertainties, complementing regulatory requirements. The large existing body of work on rodent eradications, including the potential ecological impacts from toxicant use (Broome, et al., 2015) and probability of success of traditional methods (DIISE, 2016), along with meta-data analyses on the ecological impacts of removing invasive rodents (Jones, et al., 2016) will facilitate rigorous EBRA. Our staged experimental approach prior to any potential release would culminate in trials within biosecure simulated natural environments with colonies of mice imported from the target island(s) with the most efficacious gene drive mice. This allows simulations of various ecological scenarios and increases the power of predictive analyses, resulting in increased levels of certainty around potential outcomes and ecological impacts.

## 12. Social engagement

The emergence of gene drives and other genetic technologies will force not only technologists, but conservationists, other environmentalists and the public to “negotiate with unfamiliar interest groups and perhaps compromise on deeply held positions if they are going to succeed in a complex world of contradictory perspectives” (McShane, et al., 2011, p. 969). We hope to develop guiding principles to establish dialogue between these disparate groups to identify and eventually negotiate trade-offs, things that should not be traded off, and also to “render explicit the relevant justice dimensions and principles at play in particular contexts” (Martin, et al., 2015, p. 176). The programme aims to establish a transparent process that both encourages public participation and offers a trustworthy and responsible decision pathway for making decisions about releases of gene drive organisms.

Specifically, members of our team have developed a three-part plan for social engagement. First, we will conduct a stakeholder landscape analysis to understand the mix of interests, priorities, concerns, and hopes of diverse stakeholders that surround the programme. Second, we will convene a stakeholder workshop to create a forum for discussion, provide feedback to the technical project team, and strategise the design of community engagements. Third, we propose to organise community focus groups near potential island release sites to engage relevant publics sufficiently early to influence technological innovation and field trial research (see Chapter 7, NASEM, 2016). Importantly, the international nature of our partnership will foster the sharing of best practices – and challenges – of social engagement across different cultural contexts.

To-date, engagements have occurred with publics, scientists, conservationists, indigenous groups and other stakeholders (including those opposing gene drive research, Borel, 2017; Reese, 2017), but more work is required.

## 13. Communications and outreach

The investigation requires clear, concise, and transparent communications to ensure public perceptions by target audiences are based on facts, and not unduly influenced by scientifically-unsubstantiated fears and hyperbole. Communicating to stakeholders, researchers, communities, and decision-makers interested in this evaluation is the foundation of the programmatic principle of transparency. Coordinated external communications by the partnership’s representatives through media, in peer-reviewed publications, presentations, and one-on-one outreach have and will continue to be core to our mission. Informing stakeholders and decision-makers in fora such as the IUCN’s World Conservation Congress and the United Nations’ Convention on Biological Diversity encourages public discourse about this innovation, engages thought leaders in making our investigations more robust, ensures that fact-based concerns can be addressed while unsubstantiated fears can be allayed, and helps guide decision-makers in developing policies and guidelines complementary to the precautionary, stepwise research guiding principle, even as the technology is being developed.

## 14. Ethics

There are considerable potential benefits of this technology and we are committed to exploring it in a responsible and inclusive manner. But the question remains, if the technology works, should it be used? This key ethical question is best answered once robust EBRA have been completed and in the context of rigorous social and regulatory engagement. The USA and Australian



Academies of Science recommend that research continue and decisions to release gene drives continue to be made on a case-by-case basis following a comprehensive environmental risk assessment that includes ecological and evolutionary modelling (NASEM, 2016; AAS, 2017). We have volunteered our programme as a case study for discussion at various fora, including ethical deliberations amongst ethicists and peers (e.g. NCSU Genetic Engineering and Society Center, 2016; Leitschuh, et al., 2018), on national radio (Barclay, 2017) and for the USA National Academies of Sciences Engineering, and Medicine's report on gene drives (case study 4, NASEM, 2016). Emulating the Target Malaria partnership (<<http://targetmalaria.org/>>), an independent ethics advisory board has been established to provide advice on ethical matters and identify issues for the partnership's consideration.

### 15. Regulatory

Our regulatory engagement strategy is to ensure transparent and early engagement with the regulatory agencies responsible for the oversight and review of the program. Varying regulatory maturity exists around the world, with Australia and New Zealand having possibly the most developed and mature biotechnology regulatory review processes. The USA is revising regulatory guidelines through the Coordinated Framework for the Regulation of Biotechnology (Barbero, et al., 2017). Currently, in the USA it is likely the Food and Drug Administration will lead regulatory review of GBIRd.

Regulatory data-sharing agreements for registration of pesticides exist between Australia, New Zealand, and USA, and we anticipate that this will carry over to review of biotechnology. The design, execution, and data collection will be compliant with all three countries' regulatory agency requirements or under data sharing agreements.

The regulatory oversight and testing is intended to demonstrate efficacy and safety of the construct, i.e. does it work and what are the ecological consequences. Managing risks associated with its potential release, including capacity to "shut off" *in vivo* in case of unanticipated consequences is one hallmark of our programme. Testing will take place in a step-wise manner, laboratory development and characterisation, laboratory testing, pen trials and field trials. With the lack of clarity of regulatory pathways at this time, we are engaging regulators early, and have done so in Australia, New Zealand and USA to inform and ideally strengthen regulatory standards, while ensuring open dialogue and regulatory awareness of GBIRd exists.

### 16. Intellectual property

A patent for RNA-guided gene drives was filed in 2014 and two competing patents exist over CRISPR gene editing technology (Egelie, et al., 2016; AAS, 2017). However, there may be little scope for commercialisation for CRISPR/Cas9 gene drives for conservation and public health purposes (AAS, 2017). The intent of our partnership is to safely and effectively develop and assess this technology in a socially responsible manner that democratises the science involved with the innovation. Our partnership is composed of organisations that are dedicated to the public good potential of this technology. We intend for intellectual property to be secured in a manner that prevents unintended use but allows maximum benefit for communities and environments in need. The mechanisms with which to do this have not yet been identified.

### 17. Financial

Budget estimates until completion of experimental biocontained trials are uncertain until refinement of

constructs to ensure appropriate characteristics is clear. Technical issues may arise, and data needs for risk assessments and field trial applications have yet to be determined in conjunction with regulatory agencies. The timeline for completion of experimental biocontained trials is also uncertain as not all funding has been secured, processes are of uncertain duration in some cases and requirements for experiments have not yet been determined in conjunction with regulators. Considering these caveats, we estimate US\$16–22M will be needed over the next 4–5 years to complete experimental biocontained trials.

All programme areas are unfunded or partially funded at this time. We are actively pursuing opportunities for complementary funding.

## DISCUSSION

Unlike incremental advancements in current technology or tools, the development of transformative applications cannot be undertaken within existing rodent eradication projects on islands or as part of rodent control on mainlands. Transformative innovations require deliberate intent and focussed programmes. GBIRd includes interdisciplinary scientists, varied experience, backgrounds and viewpoints. An analysis of the hazards associated with a hypothetical split gene drive is underway. If proof of concept of the gene drive can be established in laboratory populations, and suitable target populations can be identified, funding will be sought to perform a risk assessment building on the results of the hazard analysis. GBIRd is also engaging with independent external ethicists to develop best practice ethical conduct for gene drives. Indeed, as a programme we have attempted to maintain a balanced approach and wish to inform future decisions with the best science at that time. This does not preclude pursuing a pathway to broader deployment of this type of technology if, indeed, it proves to be safe, efficacious, and socially accepted.

In addition to impacting biodiversity on islands, invasive rodents also negatively impact the health of people and their livestock, and greatly reduce agricultural productivity, stored food stocks and damage infrastructure. In the future, these problems may also benefit from the application of gene drive systems in invasive rodents. However, the GBIRd programme is currently focussed on the development and evaluation of gene drives in invasive rodents on islands to prevent biodiversity loss. We are committed to a deliberate and step-wise approach following National Academies' recommendations (NASEM, 2016; AAS, 2017).

Eradication is a biological extreme involving all individuals in a population (Parkes & Panetta, 2009). Populations hold a diversity of genes that provide plasticity in behaviours and susceptibilities (e.g. Buckle & Prescott, 2012; Cunningham, et al., 2013). Eradication of a population requires that eradication method(s) overcome this variability (Parkes & Panetta, 2009). That we are looking to develop an eradication (i.e. complete and permanent removal of a population), and not a control (i.e. frequent removal of a portion of a population for perpetuity) tool, is intentional and strategic. Eradication provides permanent solutions and for invasive species is nearly always desirable when it can be achieved (Parkes & Panetta, 2009). Eradication methods may be used for control, but not necessarily vice-versa. Our methods must be robust enough to eradicate populations independent of their variability but specific enough, or controlled in some way, that the global population (especially native populations) are not at risk. The concept of eradication units is a useful way to think of this (Robertson & Gemmill,



2004). Are there alleles shared by all individuals (i.e. fixed) within invasive populations that are not found in the native population, or only a subset of individuals have? Gene drive could be contained under either of these scenarios. GBIRD is attempting to identify island-specific locally-fixed alleles that would provide molecular confinement of the gene drive to the target island population. If this is possible, potential exists for the approach to be scaled (e.g. where locally-fixed alleles can be identified for archipelagos, or for invasive but not native populations). Further, our programme is also researching differential mating success of males between populations to be able to select the most effective stock for transmitting a gene drive and associated genes to a target population.

CRISPR has transformed gene editing and CRISPR gene drives are providing similar transformational opportunities for genetic pest management (Webber, et al., 2015; Harvey-Samuel, et al., 2017). Our partnership was formed prior to these revolutionary tools, providing a ready foundation upon which we expanded our partnership and incorporated these tools, increasing the number of technical approaches and likelihood of success. CRISPR, as an editing tool, has also increased the efficacy of inserting large genetic sequences (e.g. 10kb *Sry*) and due to its precision, efficacy and high success rate has often reduced the number of animals required compared to previous approaches. We anticipate there will be other opportunities, technological or otherwise, that emerge throughout the life of our programme.

CRISPR has been shown to be able to edit DNA in a range of taxa (NASEM, 2016; AAS, 2017) and a CRISPR gene drive has advantages when developing a technology platform, when compared to the *t*-complex drive which may not be effective in species other than mice. However, the *t*-complex provides options and, being naturally occurring in mice, may increase social acceptability, or be technically more appropriate for certain situations. Having multiple gene drives and target genes or mechanisms allows for many potential combinations and simultaneous comparisons in efficacy, safety and acceptability. We are currently investigating various combinations of gene drive mechanisms (i.e. *t*-complex, CRISPR/Cas9, CRISPR/Cpf1) and target genes or deletion mechanisms (i.e. *Sry*, *Sox9*, Y-shredder), providing multiple potential combinations.

Spatial control and remediation of CRISPR/Cas9 gene editing and gene drives has been a major concern and is the focus of significant research. We are keeping abreast of advances in this field and will look to incorporate mechanisms developed where appropriate. Recent research identified CRISPR/Cas9 inhibitors that can block genome editing, providing a means to spatially, temporally, and conditionally control Cas9 activity (Pawluk, et al., 2016; Rauch, et al., 2017). As a nascent field, it is understandable that not all technological concerns have yet been addressed (NASEM, 2016; AAS, 2017), but a significant amount of research is underway to do so.

Few, if any, people are opposed to preventing extinctions but there is mixed opinion about the methods by which this is done. Rodent eradication on islands of any significant size can currently only be implemented with toxicants, the least publicly accepted of all control methods (Fitzgerald, 2009). Gene drives hold promise as putting an additional tool in the practitioner's toolbox that could increase the feasibility and scale of conservation efforts. In contrast to toxicant-based invasive rodent eradication campaigns characterised by a short duration of implementation and high fixed costs (Howald, et al., 2007; Holmes, et al., 2015), gene drive approaches could provide

an alternative and flexible financial model. Alternative financial mechanisms such as endowments covering annual costs instead of single campaigns costing tens of millions of dollars may be feasible. If the anticipated species specificity holds true, risks from methods to non-target species (e.g. raptors, Rueda, et al., 2016) would be eliminated and the ability for non-specialists to implement projects would increase. Animal welfare concerns over the mode of death of rodents and non-target species from toxicants could be alleviated by gene drives that bias the sex of invasive populations as no animals would be killed (Dubois, et al., 2017). This approach could also facilitate potential future developments with other invasive mammals beyond rodents, including foxes (*Vulpes vulpes*) and rabbits (*Oryctolagus cuniculus*) in Australia (Kinnear, et al., 2016; AAS, 2017), brushtail possums (*Trichosurus vulpecula*), and stoats (*Mustela erminea*; Owens, 2017) in New Zealand. New Zealand has set a goal of eradicating invasive mammal predators from their country ('Predator Free New Zealand 2050' – New Zealand, 2016). One interim 2025 goal in this strategy is to develop a scientific breakthrough capable of removing at least one small mammalian predator from New Zealand entirely (New Zealand, 2016), and gene drive is one of a suite of potential innovations currently being considered. Globally, invasive rodents are linked to 30% of all extinctions (Doherty, et al., 2016), and currently threaten 88% of all insular critically endangered or endangered terrestrial vertebrates (TIB Partners, 2014). New, scalable, species-specific tools are needed to prevent further extinctions. The opportunity that gene drives as a transformative technology may bring to invasive species management is significant and worthy of exploring in a responsible and inclusive manner.

## ACKNOWLEDGEMENTS

This programme's financial support has come from CSIRO, IC, LR, NCSU, NWRC, TAMU, UA, US National Science Foundation (to NCSU; NSF IGERT grant # 000166685), and The Seaver Institute (to IC). Thanks to N. Holmes, G. Baxter and two anonymous peer reviewers for suggestions that improved the manuscript.

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