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RESEARCH ARTICLE

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Developing gene drive technologies to eradicate invasive rodents from islands

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ABSTRACT

Island ecosystems are highly threatened by invasive rats and mice. Currently, the only effective technology for eradicating rodents from islands is toxicants. Though effective, they are expensive and have high failure rates. They are not species-specific and are potentially dangerous to humans. Gene drive technology is one alternative to toxicants for rodent eradication. Gene drive methods of rodent eradication offer an alternative to killing that has the potential to be more species-specific, more humane, and more biologically safe for use around humans. Technologies in development aim to apply either natural meiotic drive or clustered regularly interspersed short palindromic repeats to influence offspring development so that all offspring are phenotypically male, eventually creating a population that is not reproductively viable. Implementing this technology would involve releasing laboratory-developed engineered mice into wild populations. Some areas for further research include assessing the ecological effects of releasing engineered mice, the potential risks for the accidental or deliberate release of genetically modified organisms into mainland mouse populations, and the social, ethical, and regulatory acceptability of the technology.

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1. Introduction

Invasive species are responsible for over 50% of animal extinctions worldwide (Clavero and García-Berthou 2005; Doherty et al. 2016). Island ecosystems are at a greater risk from invasive species than mainland ecosystems (Sax, Gaines, and Brown 2002). While islands compose only 5% of Earth's landmass, they disproportionately contain over 20% of terrestrial animal species (Howald et al. 2007). One of the greatest threats to island plant and animal species today are invasive rats (*Rattus rattus*, *R. norvegicus*, *R. exulans*) and mice (*Mus musculus)* (Campbell et al. 2015). Invasive rodents have been implicated in many island extinctions – they are present on over 80% of islands

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worldwide due to human introduction, and are associated with the extinction of over 60 vertebrate species alone (Towns, Atkinson, and Daugherty 2006; Howald et al. 2007).

The significant biodiversity threat posed by rats and mice makes their removal a critical conservation goal, but both species are difficult to eradicate from many localities using current methods, primarily toxicants (Howald et al. 2007; Campbell et al. 2015). We and other researchers are studying gene drive technologies as another method of controlling or eliminating invasive rodent populations from islands: genetic biocontrol of rodents. Our goals in this paper are to summarize the threats rats and mice pose to biodiversity on islands, to compare toxicants and the genetic biocontrol of rodents for eradicating invasive rodent populations, and to contribute to discussions about ecological risk and social acceptability.

2. Threats posed by invasive rats and mice to biodiversity on islands

Rodents travel commensally with humans and can adapt to a broad range of natural habitats and environments (Singleton and Krebs 2007). Both rats and mice are omnivorous and opportunistic feeders, making them particularly damaging to native island species, especially those that evolved without mammalian predators (Towns, Atkinson, and Daugherty 2006; Howald et al. 2007; Mackay, Russell, and Murphy 2007).

On islands without humans, mouse diets typically range from grains and grasses to invertebrates, depending on availability. On islands, food availability is often seasonal – a study of the feeding habits of invasive mice on a subantarctic island showed their diet consisted of high percentages of invertebrates when grasses and seeds were not available (Le Roux et al. 2002). Population declines and extirpations of both plants and invertebrates increase on islands with invasive mice (Crafford and Scholtz 1987; Angel, Wanless, and Cooper 2009). Mice will also feed on vertebrates, most notably the eggs and nestlings of nesting birds. The starkest example is on Gough Island – lacking sufficient plant and invertebrate food, invasive mice primarily consume bird eggs and chicks (Parkes 2008). Two seabird species, the Tristan albatross and Atlantic petrel, and a native terrestrial bird, the Gough bunting, are listed by the International Union for the Conservation of Nature as endangered or critically endangered, and invasive mice have been implicated as the cause of the declining populations (Parkes 2008).

Invasive mice also induce secondary ecological effects, including decreasing endemic species reproduction. While the adult animals or plants may not be affected by mice, eggs, young, or seeds may be consumed in such abundance that the species is effectively unable to reproduce (Le Roux et al. 2002; Parkes 2008). The presence of mice may also affect the food web through hyperpredation or increasing the threat to native species by attracting non-indigenous predators (Courchamp, Langlais, and Sugihara 2000). For example, on the Farallon Islands (CA, USA), the presence of mice creates an abundant food source for migrating owls and is believed to cause some owls overwinter on the islands instead of continuing migrating as they would do otherwise (South East Farallon Islands EIS 2013). Without sufficient food resources over the winter, the mouse population on the Farallon Islands crashes, and the owls consume ashy storm petrels, a threatened seabird that breeds on the island (South East Farallon Islands EIS 2013).

Rats' effects on islands are similar, if not greater, compared to mice (Harper and Bunbury 2015). The damages rats can cause are well documented in New Zealand,

where they have been present for hundreds of years. On a single island in New Zealand, Little Barrier Island, rats were documented to threaten a number of native plants, seabirds, invertebrates, and reptiles before being eradicated (Towns, Atkinson, and Daugherty 2006). On the Lord Howe Island Group, off the coast of Sydney, Australia, rats have brought on the extinction of many endemic species, including 5 species of birds, 2 species of plants, and 13 species of invertebrates (Wilkinson and Priddel 2011). Some species of invertebrates and birds now only exist in small populations of islands where rats are not present, and the population of many species of plants are in decline where rats are present (Wilkinson and Priddel 2011). Due to their size, rats are more threatening than mice to vertebrates and able to prey on adult birds, eggs, and chicks, as well as on other mammals (Towns, Atkinson, and Daugherty 2006; Angel, Wanless, and Cooper 2009; Harper and Bunbury 2015).

While the focus of this assessment is on the conservation threats posed by invasive rodents, it should be noted that rodents also represent threats to health and livelihoods on islands with human populations. Though these threats are not conservation-oriented, it is worth mentioning the potential future benefits of improved rodent pest management for humans living in areas impacted by rodents, as pressing human health and agriculture concerns could help drive the development of the technology. Rodents can consume vast quantities of grain and rice crops, causing loss of both a food source and a source of income. Rice farmers in Asia experience a 20–30% loss of crops yearly on average, with some farmers losing 50–100% of their crops to rodent pests (Singleton 2003). The potential damage from rodents also prevents many farmers from planting more rice crops per year. Taken together, these losses amount to enough rice to have fed 180 million people for a year (Singleton 2003). Similar effects are seen in Australia. One study found that damage from mice to cereal, rice, soybean, and maize crops in one month caused losses ranging from 14% to 66% (Brown and Singleton 2000). Additionally, mouse irruptions lead to damage to electrical equipment, animal housing, and human dwellings and food supplies (Brown and Singleton 2000). Rodent pests in agricultural areas also increase the potential for disease – over 60 zoonotic diseases are carried by rodents, some of which can cause death (Singleton 2003). Considering the threats to human health and agriculture, the potential benefits of improved rodent pest management to humans is significant.

3. Methods of rodent eradication

Currently, toxicants are the only effective technology for eradicating rodents from islands greater than 5 ha (Campbell et al. 2015). Second-generation anticoagulants, also known as 'superwarfarins,' are the toxicant of choice (Ishizuka et al. 2008; Campbell et al. 2015). Developed after rodents displayed resistance to the similar but less potent toxicant warfarin, these anticoagulants cause death through internal hemorrhaging (Ishizuka et al. 2008). Brodifacoum, the most widely used superwarfarin for rodent eradications, is palatable to rodents and is easily dispensed through bait stations or aerial broadcasting (Howald et al. 2007). It is lethal with the consumption of only a few grams and has a delayed effect, discouraging invasive rodents from associating the toxicant bait with illness and death (Howald et al. 2007).

Genetic pest management offers a potentially attractive alternative to using toxicants for rodent eradication, especially because the methods being proposed are non-lethal. The most promising of these new technologies are gene drive systems. Gene drives are selfish genetic elements that can replicate their genetic sequence and insert it into the genetic sequence of the gametes of a sexually reproducing species. By manipulating a gene drive and adding in a desired trait, humans could theoretically spread the desired trait and, in the case of invasive rodents, suppress wild populations. Most genes have normal Mendelian inheritance, or a 50% chance for each of two alleles for a gene being passed on to the next generation. Gene drive systems have super-Mendelian inheritance, which is broadly defined as an allele having greater than the 50% inheritance pattern expected. Accordingly, they have the potential to spread quickly through a population (Lyttle 1991).

Current research on genetic biocontrol of rodents is confined to mice due to the relative ease in manipulating the mouse genome in comparison to rats. Mice have a naturally occurring gene drive on Chromosome 17 called the t-haplotype (Willison and Lyon 2000), which is being developed for the genetic biocontrol of mice. Male mice heterozygous for the t-haplotype pass it on to greater than half of their offspring, with some variants of the t-haplotype having transmission rates of over 90% (Bauer et al. 2005). Sperm containing the t-haplotype are more successful than sperm without the t-haplotype, leading to an increased proportion of eggs being fertilized by sperm with the t-haplotype (Bauer et al. 2005). Mice homozygous for the t-haplotype usually die before birth from accumulated mutations in the t-haplotype. The t^{w2} variant of the t-haplotype is being used in developing genetic biocontrol for mice since males homozygous for the t-haplotype are sterile instead of dying before birth. Female mice with the t^{w2} variant have normal fertility (Lyon 2003).

Gene editing tools are being used at Texas A&M University to create 'daughterless' mice – in other words, mice that are unable to bear female offspring – by inserting the *Sry* gene sequence into the t-haplotype. The *Sry* gene is normally located on the Y-chromosome and controls the development of male characteristics. Because the t-haplotype has meiotic drive and is located on an autosome, in order to spread the *Sry* gene to both XX and XY offspring, the *Sry* gene has to be copied and inserted into the t-haplotype. This ensures that all offspring receiving the t-allele with the *Sry* gene, regardless of chromosomal sex, would be phenotypically male (Figure 1; Piaggio et al. 2017). Targeting a construct containing *Sry* to the t-haplotype should result in nearly all offspring inheriting the *Sry* gene. Releasing male genetically engineered (GE) mice with the *Sry*/t-haplotype into a population of wild mice, where they could breed with wild females, could be effective in spreading the *Sry*/t-haplotype through a population and increasing the relative proportion of male mice in a population.

Currently, the best gene drive alternative to the *Sry*/t-haplotype construct would be using a clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 gene drive system. Using CRISPR as a gene drive mechanism could enable the genetic pest management strategy to be applied to more species, as it does not rely on a native gene drive mechanism. The *Sry*/t-haplotype system is currently being explored because both components naturally exist in mice and there is a better understanding and ability to manipulate the mouse genome.

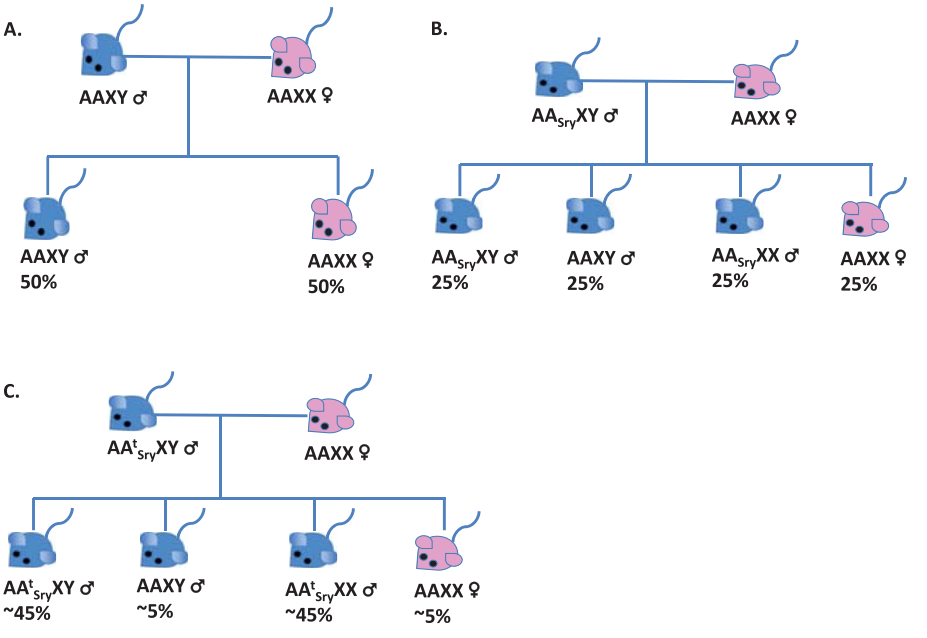

Figure 1. *Sry* gene drive: to skew sex ratios in naturally breeding populations, the male determining gene (*Sry*), normally found on chromosome (Chr) Y, can be inserted into a naturally occurring gene drive element on Chr 17 called the t-complex. The t-complex is passed down to greater than 90% of the offspring through the paternal side. XX and XY indicate the sex chromosomes and A indicates any of the 22 autosomes. A_{Sry} is the *Sry* gene inserted into an autosome and A^{t}_{Sry} is *Sry* inserted into the t complex. (**A**) In normal breeding scenarios, the *Sry* gene is only located on Chr Y and therefore only mice inheriting Chr Y are male, resulting in approximately 50% of the offspring are XY (male) and 50% are XX (female). (**B**) In a breeding scenario where the *Sry* gene has been added to any autosome, approximately 75% of the offspring will be male and 25% will be female. (**C**) In breeding scenarios where the male carries the *Sry* gene within the t-complex, over 90% of offspring will inherit the t-complex containing autosome. It is predicted that fewer than 10% of the offspring will be XX (female), with the remaining being phenotypically male, including either XY (male) or XX (sterile male).

3.1. Drawbacks of eradication technologies

There are downsides to both types of technologies being examined for rodent eradications (Table 1). Many of the downsides are related to the removal of rodents from an ecosystem, regardless of the technology used to do so. Biomathematical modeling has been helpful in addressing these types of ecological questions. Whether using current methods or gene drive, the ecological 'hole' that is created when an entire species is removed has been addressed by modeling for invasive rodents (Zavaleta, Hobbs, and Mooney 2001). The presence of an invasive species, especially species that are food sources for predators, can attract other species in search of food, as seen on the Channel Islands and the Farallon Islands (Collins, Latta, and Roemer 2009; South East Farallon Islands EIS 2013). If the invasive food source is removed too quickly, the predator may turn to consuming endemic species rather than leave the island (Courchamp, Woodroffe, and Roemer 2003; Collins, Latta, and Roemer 2009). In the case of invasive rodents, if both mice and rats are present, they can have additional interactions that make removal difficult. When trying to suppress one or both populations, the overall reduction in individuals can cause a

Table 1. Comparing toxicants and gene drives.

Method	Function	Effectiveness	Cost (US dollars)	Pros	Cons	Containment and reversal
Toxicants	Primarily Brodifacoum. Ingested toxicant causes internal hemorrhaging[h]	5–10% failure rates for rats, up to 60% failure rate for mice[a,g]	Millions of dollars per island[f]	– Established protocol[g] – High success rate for rats[g]	– Inhumane[c] – Secondary effects for endemic species[g] – Not suitable for use around humans[c] – Not as effective for mice[a] – High fixed costs[f]	– Geographical containment – Toxicant degrades over time[h] – Only reversal is to re-introduce invasive rodents to the system
Gene Drives	Using either the t-haplotype (mice) or CRISPR (mice and rats) to ensure all offspring are phenotypically male (Figure 1)	Unknown – modeling predicts high success potential if minimum release rate is surpassed[b]	Millions of dollars to develop, likely to decrease over time[d]	– Species-specific[e] biologically safe to use around humans – Non-lethal method[c]	– Social acceptability unknown – Questions of containment, especially for CRISPR[e,k]	– Geographic containment – Threshold dependent[b] – Target the engineered sequence and/or release resistant rodents back into population[i,j]

Note: Though much of what is known regarding the implementation of gene drives in an invasive rodent context is hypothetical, comparisons with toxicants are possible on many key points. This table summarizes the primary points for how each technology works, its cost, how effective it is, its advantages and disadvantages, and whether or not the technology can be contained or reversed.

[a]Angel, Wanless, and Cooper (2009).
[b]Backus and Gross (2016).
[c]Campbell et al. (2015).
[d]Backus et al. (2016).
[e]Esvelt et al. (2014).
[f]Holmes et al. (2015).
[g]Howald et al. (2007).
[h]Ishizuka et al. (2008).
[i]Manser, König, and Lindholm (2015).
[j]Min, Smidler, and Esvelt (Forthcoming).
[k]NASEM (2016).

rapid population growth of one or both species (Caut et al. 2007). Fortunately, the use of modeling can help in planning species removal timing in order to more closely restore the food web to pre-invasive interactions (Courchamp, Woodroffe, and Roemer 2003; Caut et al. 2007; Caut, Angulo, and Courchamp 2009; Collins, Latta, and Roemer 2009).

There are also downsides specific to the technology being used. Brodifacoum is a biologically efficacious method for eradicating invasive rodents, but eradication campaigns using toxicants can still fail. Though reported instances of toxicant resistance are low, failed eradication campaigns increase the risk for mice and rats to develop resistance to currently used anticoagulants (Ishizuka et al. 2008). Mutations in the Vkorc1 protein can lead to resistance to anticoagulants and many eradications using toxicants now test for mutations in Vkorc1 before proceeding (Didion, Threadgill, and Pardo-Manuel de Villena 2012). Hybridization with native, closely related species resistant to Brodifacoum has also been observed in mice (Song et al. 2011), suggesting that there are multiple avenues for rodents to develop resistance to Brodifacoum. Resistance to Brodifacoum and other toxicants can make future eradication campaigns more difficult and can also cause problems in controlling rodents in urban areas if the resistant rodents spread. Although rat eradication campaigns using Brodifacoum and similar toxicants have been highly successful and have only a 5–10% failure rate, the same methods have a failure rate close to 40% when applied in the context of mouse eradications (Howald et al. 2007; Angel, Wanless, and Cooper 2009). Brodifacoum was developed after rodent resistance to Warfarin was widespread (Ishizuka et al. 2008), and if resistance to Brodifacoum reaches similar levels, a new toxicant would also have to be developed.

Explaining these higher failure rates for mice is difficult, but mice have smaller home ranges and different foraging behavior than rats, which may decrease mouse encounters with toxicants (Howald et al. 2007). Rodent eradications using toxicants typically involve a single release of toxicants across an island, which is then monitored in the following years for the presence of rodents (Howald et al. 2007). Failure of an eradication is often not detected until multiple years have passed. The toxicants do not persist long in the environment before degrading, and by the time failure is detected, a new campaign has to be developed (Howald et al. 2007).

Rodents can also be resistant to gene drives, both behaviorally and because the individuals carrying the gene drives often have lower fitness than the non-drive carriers. The t-haplotype is naturally occurring in *Mus musculus*, but only at low rates – only 6–25% of the wild mice have the t-haplotype (Carroll et al. 2004). Most strains have a recessive lethal allele associated with being homozygous for the t-haplotype. Females who carry the t-haplotype also tend to avoid males who are also heterozygous for the t-haplotype (Lindholm et al. 2013). It is not possible to contain the t-haplotype itself. However, it is likely that over time the autosomal *Sry* gene would be naturally selected against and only the natural t-haplotype without the *Sry* gene would remain in wild populations. Selection also naturally occurs against the t-haplotype, which should limit its drive (Carroll et al. 2004). Additionally, using an autosomal *Sry* involves inserting a large piece of DNA, which could decrease the fitness of the mouse, potentially inhibiting its ability to mate and thereby diminishing the effectiveness of the eradication campaign. It is possible that the translocated *Sry* gene will impair an important function of a gene or cause the mouse to not be able to compete as effectively as the native mice on the island. For these

reasons, for the t-haplotype to be used as an effective drive it would likely need multiple releases (Backus and Gross 2016).

There is also a concern for secondary effects of these technologies. Second-generation anticoagulants have a greater impact on non-target species than warfarin, either through direct consumption or through consumption of toxicant-laced rodents or rodent bodies. While the biggest non-target effects are on birds, other native vertebrates are also impacted (Ishizuka et al. 2008). On islands with humans, pets, and livestock, toxicants pose an additional threat (Campbell et al. 2015). Not only do eradications on islands with humans require additional planning, adding to the lengthy years-long planning process, the financial cost also increases when incorporating protections for human populations (Campbell et al. 2015; Holmes et al. 2015). Eradication campaigns must also be timed to the cyclical patterns of rodent populations, particularly for islands in higher latitudes where food and water availability fluctuate (Mackay, Russell, and Murphy 2007). Eradication campaigns cost millions of dollars, and require a large number of people to implement them successfully (Howald et al. 2007; Holmes et al. 2015). If a campaign fails, financial and ecological costs are accrued again.

Gene drive technologies also have secondary effects, though many of them are still hypothetical while the technology is being developed in the lab. The primary secondary effect being examined is the temporary rodent population increase that happens when releasing the GE rodents. The use of a gene drive mechanism to eradicate rodent populations would initially involve increasing the overall population with the introduction of GE rodents. During this transitory period, ecological interactions can be intensified, potentially leading to permanent ecological consequences, such as further impacts to the ecosystem, potentially including some of the endemic species that are being protected (David et al. 2013; Esvelt et al. 2014; Backus and Gross 2016). These damages could potentially increase the overall cost of the eradication as well.

While neither technology being examined here is free from biological concerns, there are also social issues to consider. Eradicating invasive species can be a socially contentious issue that leads to a lack of support from local communities and may contribute to the failure of eradication campaigns (Howald et al. 2007; Campbell et al. 2015). Rather than causing a quick and painless death, anticoagulants like Brodifacoum kill over a period of days, and related animal welfare concerns can lead to public resistance (Howald et al. 2007; Campbell et al. 2015). The lack of social support, the risks toxicants pose to non-target species including humans and livestock, and the high financial and time costs provides strong impetus for consideration of newer, safer, and more humane technologies for removing invasive rodents from islands. The development of these new technologies also calls for an examination and integration of social opinion, which we discuss later on.

3.2. Containment and reversal of gene drive systems

Because gene drive systems, unlike toxicants, can spread on their own, biosecurity is a major concern (Esvelt et al. 2014). Containment and biosecurity must be addressed at various levels, from laboratory to wild settings. Many safe laboratory practices, such as secondary containment and security protocols, already exist for rodents, as mice and rats are frequently used for biomedical research. The need for proper containment of gene drive systems and proposed methods has also been addressed in laboratory settings by

Akbari et al. (2015) and Min, Smidler, and Esvelt (Forthcoming). Nevertheless, the deliberate release of gene drive biotechnologies subverts the very the concept of 'containment,' given that the technology is designed to spread (Min, Smidler, and Esvelt, Forthcoming). While the waters surrounding island ecosystems offer a geographic hurdle, rodents have a long history of uninvited travel on human sea vessels, and rats, in particular, are capable of swimming up to 1 km and for 3 days straight (Russell, Towns, and Clout 2008; Harper and Bunbury 2015). Additionally, both mice and rats are difficult to catch and can fit through holes smaller than 2.5 cm in diameter, which makes preventing escape (or reinvasion) difficult (Centers for Disease Control and Prevention 2016).

The 2016 National Academies of Sciences, Engineering, and Medicine (NASEM) report on gene drives suggests a detailed phased-testing pathway from conception of an idea to application in the field and post-application monitoring that incorporates both containment strategies as well as feedback from scientists, stakeholders, community members, and the general public (NASEM 2016). Modeled on the phased approach that the World Health Organization proposed for testing genetically modified mosquitoes (WHO 2014), this pathway would involve thoughtful evaluation at each step of the process, including checkpoints that determine whether research should move to the next phase. We recommend adopting this testing pathway (or something similar) for further development of a gene drive mouse because the proposed pathway is thorough in addressing containment issues and biological risk, transparent to the public and welcoming to their input (an issue that we discuss in more detail in Section 5), and goal-oriented, with a clear end-point.

Many questions of biological containment are already being considered in the development of a gene drive mouse. An ideal gene drive system would be able to spread through and eradicate a target island population but not spread through any mainland populations where they might unintentionally escape. One way to achieve this is to specifically design a threshold system where the gene of interest would only spread through population when the gene is above some sufficiently high threshold (Curtis 1968; Davis, Bax, and Grewe 2001; Akbari et al. 2013). Alternatively, a self-limiting gene drive like the *Sry*/t-haplotype mouse could behave like a threshold system if gene drive organisms are repeatedly released into the population above some minimum release rate (see Sections 3.1 and 4). In this situation, the *Sry*/t-haplotype construct would be forced to spread and eradicate the wild population even though the gene construct would be expected to be lost from the population when the release rate is not maintained (Backus and Gross 2016). If any mice carrying this *Sry*/t-haplotype construct were to escape the island, they would not be maintained at a high enough frequency to eradicate the mainland population (Backus and Gross 2016).

If CRISPR is used as an artificial gene drive, there are a number of ways to ensure that it would not spread to non-target species or to mainland rodent populations. It could be targeted to a very species-specific locus in the genome to ensure that it would not easily move laterally between species. Synthetic site targeting, or first inserting a non-coding sequence into a wild, invasive population and then targeting that sequence for *Sry* insertion would be possible using CRISPR, though not using the t-haplotype (Min, Smidler, and Esvelt, Forthcoming). This would help ensure that only the targeted population containing the specific non-coding sequence could receive the gene drive. A split drive system could also be used, where the construct of interest is broken up into multiple parts and inserted separately, which allows for monitoring at every stage and lessens the chance of accidental escape (Min, Smidler, and Esvelt, Forthcoming).

Regardless of the type of gene drive mechanism, an inducible system could be used to turn *Sry* function on or off, such as with the *tet* regulatory system controlled by doxycycline. The system can be designed such that when the mice are provided doxycycline in their water, the desired gene is turned either on or off through transcription regulation. This could be built into the *Sry* construct where the presence of doxycycline would turn on the *Sry* gene and lead to male development. If a mouse were to escape the island, then the *Sry* gene would be inactive. If that mouse were to reproduce, it would spread the inactive gene, and all of that mouse's offspring would develop according to their inherited sex chromosomes. The challenge would be to have doxycycline available to the mice on the island of interest, as eradication could prove unsuccessful if not all mice consume it.

A way of reversing the production of male-only offspring if either the t-haplotype or CRISPR were used would be to introduce wild type males and females resistant to the drive system back into the population. Because success of the t-haplotype system is dependent on being above a certain frequency in the population, releasing wild type mice back into the system and not releasing any more GE mice should be enough to re-establish an invasive mouse colony. Though this has not been tested directly, there is ample evidence that female mice prefer mice without the t-haplotype (Carroll et al. 2004; Lindholm et al. 2013; Manser, König, and Lindholm 2015), and if there are enough male mice without the t-haplotype reintroduced, the wild females are likely to preferentially mate with those males. If using CRISPR as a gene drive system, the most effective way to reverse the system would be targeting an innocuous sequence found only in the engineered mice to override the current system, instead of targeting the *Sry* gene directly. Targeting the *Sry* gene would target both the autosomal *Sry* as well the *Sry* gene present on the Y-chromosome, which would also disrupt normal male-female ratios. While the resulting mice would still have autosomal *Sry* genes, their function would be disrupted and they would have normal male to female ratios in their offspring.

Regardless of the method used, proper monitoring and enforcing physical containment can help mitigate the risk of accidental escape and spread to the mainland population. Questions of monitoring and containment are addressed using the NASEM (2016) phased-testing pathway. Included in the phased-testing pathway would be a field trial using just the gene drive mechanism with no linked gene. A trial of this nature is important to ascertain the effectiveness of the gene drive method, as well as to test out components of biosecurity and how quickly a gene would spread. We address additional research methods in the following section to predict how different drive systems will work and the risks they may pose to non-target populations.

4. Addressing ecological influence and risk

Addressing ecological risks of using genetic biocontrol of rodents is difficult, as the technology is still in development and not easily studied. However, the authors of this study believe that performing an ecological risk assessment is imperative to safe implementation in the field, a view that is in line with the recent recommendations of the NASEM (2016) gene drive report. The NASEM report recommends both that risk assessments be performed prior to the implementation of gene drive technologies, and that field trials be conducted for ecological applications to ensure that there is enough information for a risk assessment. Given that there has not yet been an ecological risk assessment performed

for a field trial of gene drive technologies, it is especially important that one be performed before (and after if necessary) a field trial of genetic biocontrol of rodents.

An important component of defining risk is acknowledging uncertainty. The NASEM (2016) report on gene drives defines two types of uncertainty: linguistic, or normative, uncertainty, and epistemic, or fact-based, uncertainty. There are high levels of both types of uncertainty when examining the potential use of genetic biocontrol of rodents. Linguistic uncertainty relates to more general normative understandings of new genetic technologies and difficult-to-agree-upon terminology that exists in ecology as a discipline, while epistemic uncertainty stems from the technology still being in development (NASEM 2016). In seeking to restore ecosystems, a persistent normative question relates to how success should be evaluated. For example, there is room for disagreement in determining what would make a field trial sufficiently 'adequate,' depending on whether the trial's goal is to test the ability of genetic biocontrol strategies to curb one versus many successive generations of rodents. It can also be difficult for field trials to speak to how successive releases of GE could affect other areas of a complex ecosystem over various lengths of time. More broadly, when eradication campaigns are applied, should success be determined when invasive species are eliminated, or merely curtailed, and if the latter, what would count as curtailed, and for how long would the curtailment need to last? Similarly, is the goal to restore the ecosystem to the state it was 5 years ago, 10 years ago, or at some other point in time? And again, for how long would the ecosystem need to stay that way?

Regarding epistemic uncertainty, much of the biological risk has to do with containment. There is a possibility, however slight, that an engineered rodent could mate with a closely related, non-invasive species and spread the gene drive system (Esvelt et al. 2014). Quantifying this possibility is difficult – even with present-day advanced genetic analysis tools, when and why species hybridize and the ability to hybridize is still not well understood (Harrison and Larson 2014). Engineered rodents on a contained island could also escape to mainland populations and potentially affect more than the intended target, a risk noted by Esvelt et al. (2014), who also propose first using a CRISPR gene drive mechanism to introduce an 'innocuous' sequence to help mitigate the risk of unintended spread. This would be a genetically inert sequence that is not found in other organisms, which could be used as a target sequence for inserting the functional gene drive system (Esvelt et al. 2014). Targeting a specifically designed sequence could reduce the biological risk of the gene drive mechanism spreading to other populations of mice or related species of rodents. Especially in cases when an engineered gene is not self-limiting, the potential for global population suppression or extinction could become an issue of both conservation and international concern. To understand how a gene drive would behave in the field and develop proper containment measures, we, along with other researchers, are looking at gene drive systems that have already been implemented in insects, performing behavioral and genetic experiments on rodents in the lab, and using ecological modeling. However, it is impossible to eliminate epistemic uncertainty regarding how the technique would work in the field without field trials in a specific environment.

There is also epistemic uncertainty regarding how the fitness of the engineered rodents would compare to that of wild, invasive rodents. There are numerous genetic (individual and population level), neurological, and behavioral differences between laboratory and wild rodents, with laboratory rodents generally showing a narrower range of phenotypes than their wild counterparts (Koolhaas et al. 2010; Fonio, Benjamini, and Golani 2012;

Chalfin et al. 2014). Invasive rodents can also undergo morphological changes while establishing a population on an island (Parkes 2008; Pergams et al. 2015), as they need to adapt to a variety of habitats quickly in order to survive (Berry 1996; Harper et al. 2015). Wild rodents also exhibit mating strategies such as polyandry and assortative mating, (Oakeshott 1974; Dean, Ardlie, and Nachman 2006; Manser, König, and Lindholm 2015) and have seasonal population fluctuations (Singleton et al. 2001), while laboratory rodents have very controlled reproductive environments. Thus, as rodent gene drive systems are being developed in laboratory strains, a primary research focus is whether laboratory rodents can survive in wild environments and compete reproductively with uniquely adapted wild rodents (also see Backus et al. 2016). Examining adaptations to different environments raises the question of how unique to the specific environment and context the engineered rodents would need to be to integrate into the invasive wild population. Would each island require backcrossing with unique wild stock rodents, or could a more general wild stock be backcrossed with engineered rodents to be used across islands? It is important to answer this question to understand the potential impact engineered rodents will have on the environments into which they are released.

Even once these questions are answered and an engineered rodent is shown to be able to survive and reproduce on an island, epistemic uncertainty would remain around how the spread of the gene drive construct itself would interact with a variety of genetic and ecological factors. Theoretical models suggest that the proposed *Sry*/t-haplotype system for mice would be unlikely to drive itself into a population with a single release (Backus and Gross 2016; or see the similar technique of autosomal X-chromosome shredders: Deredec, Burt, and Godfray 2008). As long as the genetic construct functions as expected and ecological conditions are not entirely unfavorable, the *Sry*/t-haplotype construct should be able to effectively suppress a population through multiple releases if the GE mice are released at a high enough rate relative to the wild population (Deredec, Burt, and Godfray 2008; Alphey 2014; Esvelt et al. 2014; Backus and Gross 2016). As long as the releases continue, the population should be suppressed unless resistance to the gene drive evolves. This resistance could take the form of mating behaviors that allow wild females to avoid producing offspring with gene drive males or mutations that disrupt the function of either the autosomal *Sry* or the t-haplotype (Burt 2003; Esvelt et al. 2014; Bull 2015; Lindholm et al. 2016). Other factors will come into play when using a system such as CRISPR/Cas9, which would likely result in a lower reduction in fitness (Esvelt et al. 2014; Min, Smidler, and Esvelt, Forthcoming). Testing these systems and rodents in the laboratory and the field is contingent on the development of the technology.

5. Social acceptability

As outlined above, genetic biocontrol of rodents would need to be sufficiently effective, minimize non-target impacts, eliminate or reduce animal welfare concerns, be affordable, and employ additional measures to maintain biosecurity before being considered a viable option. However, the potential application of genetic biocontrol of rodents will depend not only on technical assessment, but on the technology's acceptability in specific socio-cultural contexts. Historically, the decision to apply a new technology has often relied on technical assessments that quantify and compare various options (e.g. cost–benefit analysis, traditional risk assessment) (Hill and Sendashonga 2003). More recent scholarship has

emphasized the importance of more participatory methods that engage a wide variety of stakeholders (groups of people with direct professional or personal interest) and communities (e.g. groups of people who live on or near the island) in decision-making (NASEM 2016). In addition to advancing procedural justice (Besley and McComas 2005), deliberative engagement can allow researchers and developers to gain insights that make research – and the technologies it yields – more effective, producing knowledge that would not otherwise be gained (Bates et al. 2005; Sharpe 2014). When communication and public engagement are conducted in a flexible way that adapts to site- and audience-specific priorities, it is possible to uncover risks that would not be addressed by quantified technical assessments (Lavery et al. 2010), as well as potential areas for new research and development.

Acceptability regarding the application of a rodent gene drive may differ from previous biotechnology applications (Levidow, Carr, and Wield 2005). For example, because genetic biocontrol may be at odds with conventional moral norms that direct how humans should interact with the natural world, systems for controlling invasive rodent populations may be especially prone to criticism as proposed deployment sites are typically construed as wild places (NASEM 2016). The use of GE organisms may be viewed as unsuitable or incompatible with places viewed as wilderness and untouched by humans. In addition, some research suggests that cisgenic transformations (i.e. transformations using genetic material from the same rodent species) may be preferable to conventional transgenic transformations (i.e. inserting genes from a species of an unrelated taxa) among potential European consumers of agricultural genetically modified organisms (Delwaide et al. 2015). However, further investigation is warranted before assuming that cisgenic rodents would be favorably received.

More broadly, even if inclusive deliberations are undertaken to consider the potential application of these technologies, the scope of the questions considered will affect the outcome of any decision. For instance, the appeal of framing genetic biocontrol as an application specific to islands may stem in part from a desire for island applications to serve as proof of concept for genetic interventions in other locales, including mainland habitats. Accordingly, it would be valuable for further research to broaden the scope of this framing to consider issues such as containment, reversal, and ecological implications in non-island ecosystems, thereby offering points of comparison for the consideration of both scientific and regulatory audiences as well as stakeholders and communities. Given the uncertainties surrounding genetic biocontrol as well as the controversies that have been associated with past eradication campaigns, it may be equally valuable to expand the scope of public deliberations to examine what types of technology applications would be unacceptable, as well as acceptable, rather than focusing discussions on the narrower question of whether to pursue genetic biocontrol strategies. As gene drives and other emerging biotechnologies increasingly stretch the limits of current regulatory systems, opening up the scope of deliberation to include broad considerations, including questions about ethics and the human–nature relationship, could potentially help to establish a more adequate system of governance (Stirling 2008; Kuzma 2016).

6. Conclusions

Using gene drive technology to control or eradicate invasive rodent populations on islands holds promise. However, both the design of the engineered mouse and the research needed to assure its efficacy are only in the earliest stages. The limitations of the currently used

toxicant approaches make development of gene drive technologies a potential alternative for conservation. Some of the advantages of using genetic biocontrol methods include that they may be considered more humane than toxicants, as the methods being proposed are non-lethal methods, and may require less time to implement and maintain than toxicants. At the same time, a number of areas of potential concern warrant consideration, including ecological risk, social acceptance, regulatory scrutiny, and ethical questions.

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