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Genetic engineering to eradicate invasive mice on islands: modeling the efficiency and ecological impacts

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Abstract. Invasive rodents are usually eradicated from islands through the application of chemical toxicants that can harm surrounding ecosystems. A recently proposed alternative involves engineering a house mouse (*Mus musculus*) to carry a genetic construct that would cause a majority of its offspring to be male, many of which would be sterile. Releasing these genetically engineered mice to interbreed with an invasive population would reduce the number of fertile female mice until no more remain. We constructed a mathematical model to analyze the population dynamics of eradication with this genetically engineered mouse and determined its eradication efficiency through model analysis and simulations. Because genetically engineered mice would likely have a fitness disadvantage compared to wild mice, we found that they would need to be repeatedly released into the population to ensure complete eradication. However, if genetically engineered mice have a substantial survival advantage, we determined that the genetic construct could theoretically spread and eradicate a population after a single pulsed release onto the target island or after an engineered mouse escapes to a non-target location. Also, while the species specificity of genetic engineering avoids some of the non-target impacts of traditional eradication methods, ecological impacts could manifest indirectly. We compared several metrics to estimate potential transient impacts on the ecosystem and found that there is a trade-off between the speed of an eradication and the intensity of increased disruptive ecological interactions. Together, our results can inform safe and efficient ecological practices for eradication with developing genetic engineering technology.

Key words: eradication; gene drive; genetic engineering; invasive species; mathematical modeling; *Mus;* rodents.

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INTRODUCTION

Island ecosystems often include rare endemic species that are threatened by invasive species (Alcover et al. 1998, Aguirre-Muñoz et al. 2008). Rodents are common among island invasives and contribute to a large number of extinctions (Howald et al. 2007). From a conservation perspective, this creates a strong incentive to eradicate these non-native rodents from islands. Past rodent eradications have mostly been successful at removing the target species from the ecosystem (Howald et al. 2007) and have resulted in subsequent ecological recoveries (Aguirre-Muñoz et al. 2008, Croll et al. 2016, Jones et al. 2016). Unfortunately, eradication is a complex process that comes with a long set of problems and complications, many of which are the result of the most common eradication method that involves exposing islands to chemical toxicants (Campbell et al. 2015). The anticoagulant toxicants that are used can be lethal to non-target organisms (Howald et al. 2007, Campbell et al. 2015) and they kill through slow internal bleeding (Hoare and Hare

2006), which can be a concern for animal welfare and public perception (Fitzgerald 2009, Howald et al. 2010). Also, while rodenticides are effective at eradicating rodents on smaller islands, it can be logistically challenging to scale efforts with increased land area (Howald et al. 2007, Holmes et al. 2015). House mice (*Mus musculus*) are particularly difficult to eradicate with toxicants (Howald et al. 2007), as only 70% of full-island mouse eradications have been completely successful compared to 88% for all other rodent species (DIISE 2015). These numerous drawbacks of rodenticide-driven eradication, especially for mice, motivate the search for alternative eradication techniques (Campbell et al. 2015).

Among the potential new tools for eradication, genetic engineering (GE) provides a promising species-specific, non-lethal alternative. Although GE techniques have been primarily developed to manage insect pest populations (Burt 2003, Deredec et al. 2008, Alphey 2014), they have also recently been considered to manage a wide range of invasive species (Davis et al. 1999, Deredec et al. 2008, Gould 2008, Hodgins et al. 2009, Esvelt et al. 2014, Thresher et al. 2014, Campbell et al. 2015, Johnson et al. 2016, NASEM 2016). Generally, suppressing and eradicating an undesired population with GE technology entails engineering and releasing organisms that would interbreed and skew sex ratios, decrease fertility and fecundity, or impose a fitness cost (Burt 2003, Deredec et al. 2008, Alphey 2014). In theory, these engineered genes can completely eradicate the target population after several generations of release. Populations can be suppressed more efficiently with gene drives, which increase the rate of inheritance above the natural Mendelian rate of 50% (Burt 2003, Esvelt et al. 2014). Increased transmission rates can reduce the number of GE organisms that need to be released and shorten the duration of eradication. Although much of the established theory on GE-assisted population suppression has been developed around the genetic, behavioral, and ecological characteristics of insects, the general theory could also apply to rodent species.

We focus on the eradication of invasive *M. musculus* with a recently proposed engineered genetic construct (Campbell et al. 2015), which we refer to as the *t-Sry* construct. The *t-Sry* mouse is engineered by linking two naturally occurring *M. musculus* genes that are not normally linked in the wild. One of these, the "sex-determining region (of the) Y" (*Sry*) gene, is found on most mammalian Y chromosomes. The gene is an essential component in the development of testes, although it has no known role in spermatogenesis (Goodfellow and Lovell-Badge 1993). Mice with two X chromosomes, which would usually develop as females, can be engineered to develop as males if they carry a copy of the *Sry* gene on an autosome. However, they would also be unable to reproduce lacking the ability to produce sperm (Koopman et al. 1991). The other component of the *t-Sry* construct is the *t*-haplotype (Dobrovolskaia-Zavadskaia and Kobozieff 1927), which distorts transmission in male mice such that fathers with one copy of the *t*-haplotype can pass that copy of the *t*-haplotype to over 90% of their offspring (Schimenti 2000). Linking the *Sry* gene and the *t*-haplotype together would cause most of the offspring of a genetically engineered *t-Sry* father either to carry the *t-Sry* construct or to be sterile (Fig. 1). The *t-Sry* construct should reduce the number of fertile females and fully eradicate the mouse population after several generations of releasing and interbreeding (similar to autosomal X-chromosome shredders in Deredec et al. (2008)).

We explore several aspects of *t-Sry* mouse population dynamics to address some new concerns about the emerging use of GE for eradicating invasive rodents. First, we investigate how differences in engineered mouse survival rates can change the success and long-term dynamics of GE-driven eradication. In most situations, GE organisms would be expected to have a lower survival rate than their wild counterparts. This is because engineered genes tend to impose a fitness cost on individuals that carry them (Catteruccia et al. 2003, Marrelli et al. 2006) and because the *t-Sry* construct would be engineered in laboratory mice that would not be adapted to surviving in island ecosystems (Miller et al. 2000). Some of these costs could be reduced by backcrossing laboratory-derived *t-Sry* mice with wild-derived varieties. However, *t-Sry* mice would likely lack some of the learned social and ecological behaviors that would be necessary to survive in a new environment. Thus, the *t-Sry* construct would most likely be lost from a population over time if it is not sustained by repeated releases of *t-Sry* mice, a quality referred to as self-limitation (Gould et al. 2008, Alphey 2014).

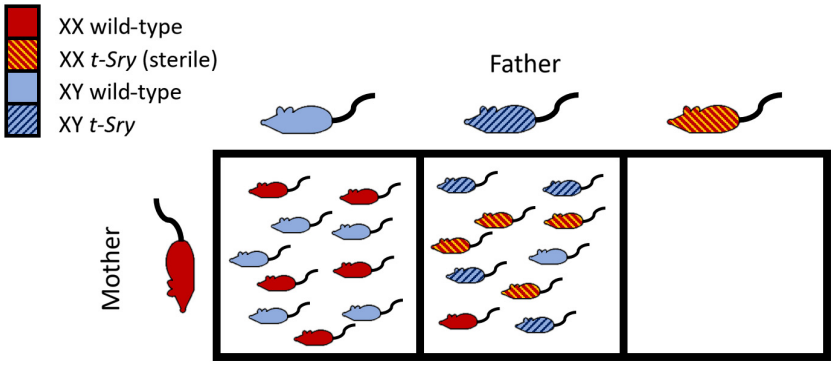

Fig. 1. Diagram showing how the *t-Sry* construct spreads to offspring. All newborn mice must have a wild-type mother. When the father is wild-type, all offspring would be wild-type. Half of these would be male (XY) and the other half would be female (XX). When the father is a fertile *t-Sry* mouse (XY), more than half of the offspring ($\tau = 0.9$ in this example) would also carry the *t-Sry* construct. As before, half of all of these offspring would be XY and the other half would be XX. XX mice that carry the *t-Sry* construct would be phenotypically male, but would be sterile.

Alternatively, we also consider the theoretical possibility that the *t-Sry* construct could be self-sustaining, in the sense that the GE construct would remain in the population without additional releases. There are a wide variety of wild *M. musculus* populations throughout the world, each with unique genetic traits (Miller et al. 2000, Chalfin et al. 2014), including differences in lifespan, maturation rates (Miller et al. 2002), and competitive ability (Cunningham et al. 2013). Additionally, some *M. musculus* populations could be theoretically less susceptible to climate stress, predators, or pathogens. A *t-Sry* mouse that is backcrossed to carry these beneficial traits could theoretically be more fit than other wild *M. musculus* populations. In such a situation, it might be possible to eradicate a population with only a single pulsed release of *t-Sry* mice. An unintentional escape of a mouse carrying a self-sustaining suppressive gene drive construct into a non-target population could cause widespread suppression and extinction, even to the ancestral *M. musculus* population (Esvelt et al. 2014). This emphasizes the need to understand how the fitness of a GE mouse can affect the persistence of an engineered gene in wild populations.

While direct population suppression from a GE construct would be species-specific (if no closely related rodent species are present), releasing *t-Sry* mice could disrupt the island ecosystem in other ways. *Mus musculus* have an adaptive diet that has allowed them to exploit island ecosystems by preying on endemic plants, invertebrates, reptiles, and even seabird chicks (Angel et al. 2009). Invasive species can also attract and support populations of non-native predators that would otherwise prey on rarer endemic species (Roemer et al. 2002, Howald et al. 2007). Eradication with the *t-Sry* construct would require adding more house mice into the ecosystem where wild house mice are already invasive, further intensifying these antagonistic interactions. Moreover, the number of GE mice that would need to be released to suppress a population could be considerable. The magnitude of these transient impacts would likely vary depending on the survival of *t-Sry* mice and their rate of release into the population.

Even though the increase in mouse population density would only be temporary during the beginning of the eradication process, its resulting effects on the ecosystem could be permanent (David et al. 2013, Esvelt et al. 2014), especially if the temporary increase in mouse density results in the extinction of a rare species. Namely, short-term impacts can have long-term ramifications. Despite this, while some have explored the ecological impacts of similar GE-driven population suppression techniques (Scott et al. 2002, Gould 2008, Bonsall et al. 2010, Esvelt et al. 2014), few have considered the transient ecological impacts of forcing a population above natural levels

(David et al. 2013). This is possibly because previous work has primarily focused on mosquitoes, which are usually thought to have a reasonably small role in their ecosystems (Godfray 2013). Comparatively, invasive rodents can be very disruptive to non-native ecosystems (Howald et al. 2007), bringing into question what damage they could cause at greater than usual densities. Therefore, understanding how the relative survival and release rate of GE mice affect the population dynamics of a GE-assisted mouse eradication could help to limit temporary impacts on the ecosystem.

In this article, we address these issues using a mathematical model to explore the dynamics and ecological impacts of eradicating a population of invasive mice with genetically engineered *t-Sry* mice. First, we identify the conditions under which this technique can successfully eradicate a population. In doing so, we consider the effects of survival on the long-term dynamics of *t-Sry* mice. This helps to determine the situations in which the construct is self-limiting or self-sustaining. Also, we create and analyze several metrics to quantify the temporary negative ecological impacts that would ensue from the release of *t-Sry* mice. We compare these metrics over a number of different release strategies, varying the mortality and release rates of the *t-Sry* construct.

Methods

Model

Our model occurs over continuous time and continuous state space with overlapping generations. It is also density-dependent with polygamous random mating, no migration, and no mutation. This model consists of a mouse population separated into four distinct groups based on whether or not individuals carry a Y chromosome and whether or not they carry the *t-Sry* construct. The state variables are the population densities of XX wild-type mice, $W_X(t)$; XY wild-type mice, $W_Y(t)$; XX *t-Sry* mice, $G_X(t)$; and XY *t-Sry* mice, $G_Y(t)$. The sum of all of these groups is the total population density, defined as $N(t) = W_X(t) + W_Y(t) + G_X(t) + G_Y(t)$. Because the *t-Sry* construct contains an *Sry* gene, which is partially responsible for male development in mice, the terms "male" and "female" could be somewhat ambiguous. Throughout the rest of this study, we use "male" to refer to mice that carry at least one functional copy of the *Sry* construct in some form (W_Y, G_X, and G_Y) and "female" to refer to mice that contain no copies of the *Sry* gene (W_X).

Without any genetically engineered mice, the population dynamics of this model should reflect a simplified natural mouse population. Therefore, this model is a standard logistic growth model (Verhulst 1838) when it contains only wild-type mice. Several studies demonstrate that mouse birth rates decrease with greater population densities (Vandenbergh 1987, Nathan et al. 2015). Specifically, when a large number of female mice are in close proximity, they will go into estrus less often (Vandenbergh 1987). Thus, $a_1 > 0$ is the baseline per capita birth rate of females (or males) and $a_2 > 0$ is the rate at which this per capita birth rate declines with increasing female density. Additionally, the per capita death rate should increase as the total population density increases because of overcrowding and resource limitation. Then, $b_1 > 0$ is the baseline per capita death rate and $b_2 > 0$ is the rate at which the per capita death rate increases with increasing density. At an equal sex ratio, the equivalent logistic growth parameters are the growth rate $r = a_1 + b_1$ and the carrying capacity $K = 2\big((a_1 - b_1)/(a_2 + 2b_2)\big)$ (Appendix S1).

Adding *t-Sry* mice into the model requires further manipulations from basic two-sex logistic growth (Fig. 1).

1. The genotype of any newborn mouse depends on the parental genotypes. All mice are born from a wild-type mother. However, both wild-type and *t-Sry* XY mice could be potential fathers. To focus on other dynamics, we do not consider mating preference in this model. With this simplification, the frequency of newborn mice with wild-type fathers is equal to the proportion of fertile male mice that are wild-type, $W_Y/(W_Y + G_Y)$. Similarly, the frequency of *t-Sry* fathers is $G_Y/(W_Y + G_Y)$. Hereafter, the frequency of *t-Sry* mice in the reproductive male population is defined as $\phi(t) = G_Y(t)/(W_Y(t) + G_Y(t))$.
2. The XX *t-Sry* mouse is sterile and does not directly contribute to future births.
3. When the father carries the *t-Sry* construct, a biased proportion $0.5 \leq \tau \leq 1$ of offspring

also inherit the construct. The other $1 - \tau$ inherit the wild-type counterpart allele.

4. The fitness of *t-Sry* mice is likely to be different from the wild-type mice of the island. This fitness difference could manifest in many ways (such as modifying the birth rate or chance of mating), but, for simplicity, we only alter the death rate of GE mice. Additionally, resource competition between wild and *t*-Sry mice could be modeled more explicitly (Russell et al. 2014), but we simplify these dynamics by assuming that each genotype's competitive ability is incorporated in its relative death rate. The change in the death rate of *t-Sry* mice is represented as c. Most likely, this would occur as an increase in the death rate ($c > 0$). If *t-Sry* mice could be backcrossed with a highly competitive wild mouse, we are also interested in exploring the possibility of *t-Sry* mice with neutral ($c = 0$) or increased survival ($c < 0$).
5. New *t-Sry* XY mice are continuously added into the population at a rate of $\mu \geq 0$ per month.

If there is initially an equal sex ratio of wild-type mice, the model can be simplified into three equations. With some algebra (Appendix S1), the final model is

$$\frac{dN}{dt} = 2\left(\left(a_1 - a_2 W_X\right) W_X - \left(b_1 + b_2 N\right)\left(W_X + (c+1)\left(\frac{N}{2} - W_X\right)\right)\right) + \mu$$

$$\frac{dW_X}{dt} = \left((a_1 - a_2 W_X)(1 - \tau\phi) - (b_1 + b_2 N)\right) W_X$$

$$\frac{d\phi}{dt} = -\left((1-\tau)(a_1 - a_2 W_X) + c(b_1 + b_2 N)\right) \times (1-\phi)\phi + \mu \frac{(1-\phi)^2}{W_X}$$

Analysis

Using this model, we determine both the long-run dynamics and transient impacts of releasing *t-Sry* mice into wild population. All analyses and simulations are conducted in Maple 18 (Maplesoft 2014) and MATLAB 2015b (Mathworks 2015), with a simple theoretical sample island beginning with a stable population at carrying capacity. Natural demographic parameters (Table 1) for wild-type mice (a_1, a_2, b_1, and b_2) are loosely derived from Nathan et al. (2015) where they were estimated for an experimental house mouse invasion, although we adjusted the density-dependent terms so the island would have a carrying capacity of $K = 1000$. The transmission distortion from the *t*-haplotype could have a wide range of values. However, we did not focus on this parameter here because it does not have a large effect on the qualitative behavior of the model. This analysis uses a constant higher transmission distortion $\tau = 0.95$, roughly based on current laboratory studies (D. Threadgill and D. Kanavy, *personal communication*; Table 1). Instead, model analysis focuses on manipulating the mortality and release rate of *t-Sry* mice. These parts of the model represent aspects that can be more easily influenced by human control. Additionally, both mortality and release rate have a large effect on the qualitative behavior. Therefore, the model is analyzed over a wide range of *t-Sry* mouse death rates (c) and release rates (μ; Table 1).

In the transient analysis, the minimum release rate needed for eradication, μ^*, is numerically solved for each set of parameter values. Eradication is then simulated with applicable release rates of *t-Sry* mice into a wild-type population at carrying capacity. These simulations continue until females are considered eradicated. Because the wild-type mice can only approach complete eradication but not actually reach it with this model, we consider wild-type mice to be eradicated when their density is below a small threshold ($W_X < 0.05$).

For each simulation, a variety of metrics are calculated (Fig. 2a). The first metric, t_{erad}, is the time from the beginning of *t-Sry* release to the time that females are considered eradicated. Second, the total density of *t-Sry* mice that need to be released to complete eradication is calculated as μt_{erad}. Third, the maximum density of the population throughout a successful eradication is determined. Last, we create another metric, referred to as population excess, that combines both the time and magnitude of increasing the population density above its carrying capacity into a single quantity. For this, t_0 is the time that the first *t-Sry* mice are released and t_K is last time that the population is above carrying

Table 1. Model parameters and default values or range of values.

Parameter	Description	Values
a_1	Baseline per capita birth rate	0.7 per month
a_2	Strength of density dependence on birth rate	9×10^{-4} (mice/unit area)$^{-1} \times$ month^{-1}
b_1	Baseline per capita death rate	0.2 per month
b_2	Strength of density dependence on death rate	5×10^{-5} (mice/unit area)$^{-1} \times$ month^{-1}
c	Change in death rate of *t-Sry* mice	−0.2 to 0.2
τ	Transmission distortion of the *t*-haplotype	0.95
μ	Release rate of fertile *t-Sry* mice	0 to 200 (mice/unit area) $\times$ month^{-1}

capacity. The population excess is then defined as $\int_{t_0}^{t_K}(N(t)-K)dt$ and is calculated with the trapezoidal method function in MATLAB (trapz).

Results

Long-term dynamics

The long-run outcomes (stable equilibria) of the model demonstrate the conditions in which releasing *t-Sry* mouse can eradicate a mouse population. The number of long-run outcomes depends on the change in death rate, c, and the release rate, μ, of *t-Sry* mice. While all of these equilibria can be solved analytically, the formulae for most are complicated and do not provide much insight. Therefore, we present written and graphical descriptions.

When *t-Sry* mice are present, there can be either one or two potential long-run outcomes. If there are two stable equilibria, the model is bistable and the long-run outcome depends on initial conditions. One outcome, eradication, occurs when there are no female mice in the population and all males are *t-Sry* mice. Some mice would remain in the population, but because there are no females, the population would only be sustained from the continuous release rate of *t-Sry* mice, $\mu > 0$. Upon reaching this point, a population manager would stop releasing mice and the population would be completely eradicated after the remaining mice naturally die. This is a potential outcome for all parameter values. Another outcome consists of a stable resident population of *t-Sry* mice coexisting with the wild-type population.

Overall, eradication is always successful when *t-Sry* are released above a critical release rate μ^*, and eradication fails (approaching the coexistence outcome) when the release rate is below μ^*. In general, the value of μ^* is greater when *t-Sry* mice have a greater death rate. We describe three distinct types of long-run behavior in order of increasing values of c.

First, if *t-Sry* mice have a much lower death rate than wild-type mice, any release of *t-Sry* mice would lead to eradication (Fig. 3a). Specifically, there is a critical value for the change in mortality $c_1^* < 0$ below which the *t-Sry* construct is self-sustaining and it approaches fixation over time. Thus, if $c < c_1^*$, then $\mu^* = 0$ and any positive release rate results in eradication. With a single small pulsed release of *t-Sry* mice, the construct would spread through the population, leading to complete eradication even if no more *t-Sry* are released after the initial release pulse (Fig. 4a, b).

Next, there is another critical value for the change in mortality c_2^* (where $c_1^* \le c_2^* \le 0$). When c is between c_1^* and c_2^*, a low release rate of *t-Sry* mice cannot eradicate a wild-type population that is initially at carrying capacity. In this case, $\mu^* > 0$, and when the release rate is below μ^*, the model is bistable. Under these conditions, an eradication attempt would result in a stable coexistence of both wild-type and *t-Sry* mice. With a larger release rate $\mu > \mu^*$, the wild-type population would be eradicated (Fig. 3b). However, as long as the change in death rate is between c_1^* and c_2^*, the *t-Sry* construct is still self-sustaining. Thus, if a population manager were to stop releasing mice after a small initial release, the *t-Sry* mice would remain in the population indefinitely, although the GE construct would not eradicate the wild-type population.

When the change in *t-Sry* mouse death rate is increased above c_2^*, the *t-Sry* construct is self-limiting. This contrasts with the previous case because the gene construct would eventually be lost from the population if it is not sustained by

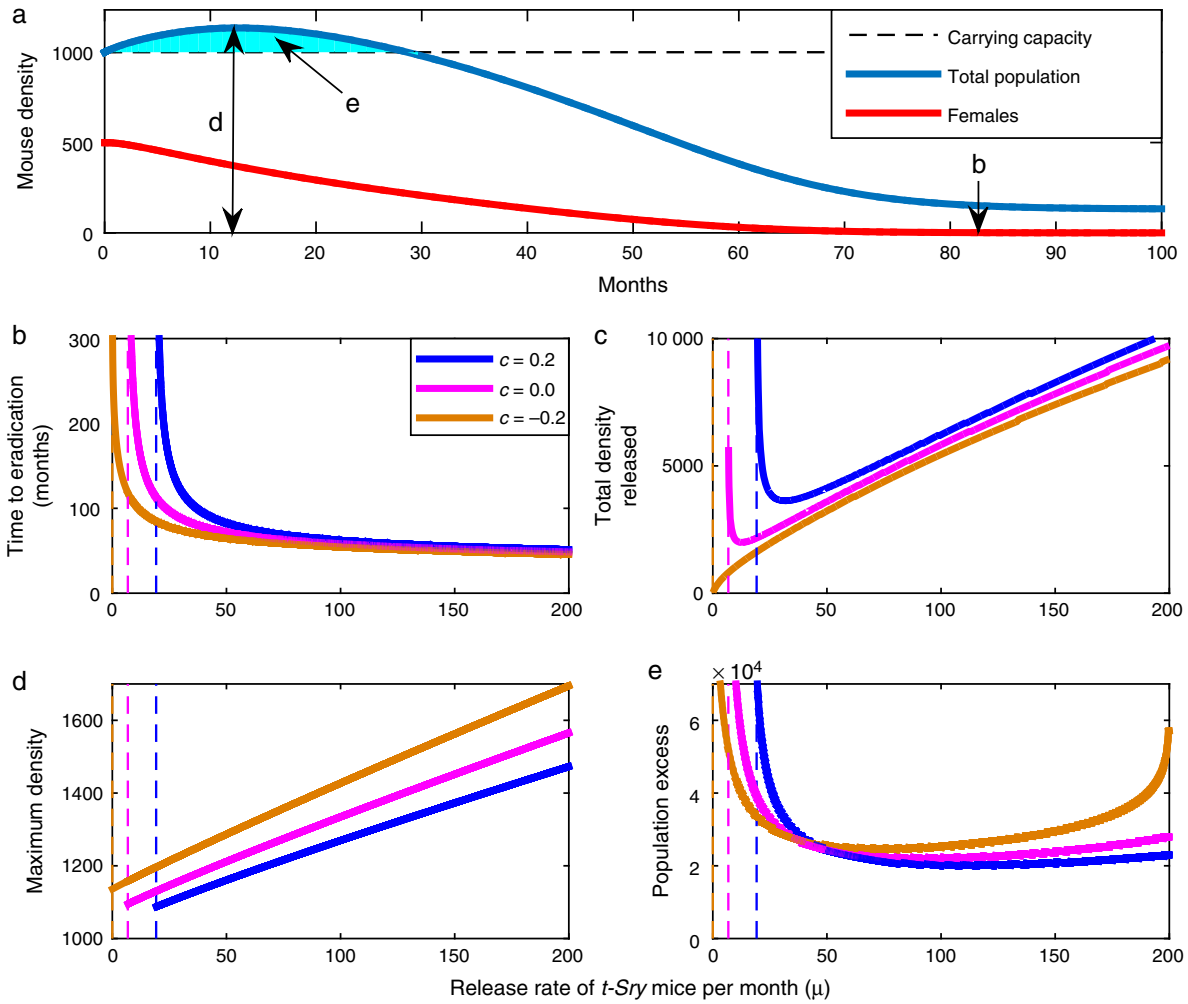

Fig. 2. The efficiency and ecological impact of an eradication depend on both the change in death rate, c, and the release rate, μ, of *t-Sry* mice. (a) An example eradication via *t-Sry* mouse release illustrates the time to eradication, the maximum density, and the population excess. (b) The time to eradication decreases when the release rate increases and when *t-Sry* mice have greater mortality. (c) The total number of mice necessary to release before eradication is minimized for intermediate release rates. As *t-Sry* mouse mortality increases, so does the number of mice that need to be released. (d) The maximum density of mice increases as the release rate of *t-Sry* mice increases and decreases when *t-Sry* mortality increases. (e) Population excess is minimized for intermediate release rates. The dashed lines in (b–e) represent the critical release rate μ^*, below which eradication is impossible, and are colored to match their respective values of c.

repeated releases. The self-limiting case is most likely to be realized as it occurs when *t-Sry* mice have a greater death rate or even a slightly smaller death rate than wild mice. Similar to the previous case, a smaller release $\mu < \mu^*$ would not result in eradication, and it could even lead to an increase in the total population density as long as release continues (Fig. 4c, d). To reach eradication, one would need to release *t-Sry* mice at a high rate of $\mu > \mu^*$ (Fig. 4e, f). The value of μ^* increases as the death rate of *t-Sry* mice increases (Fig. 3c–e).

Transient analysis

The amount of time needed for a successful eradication decreases with greater release rates

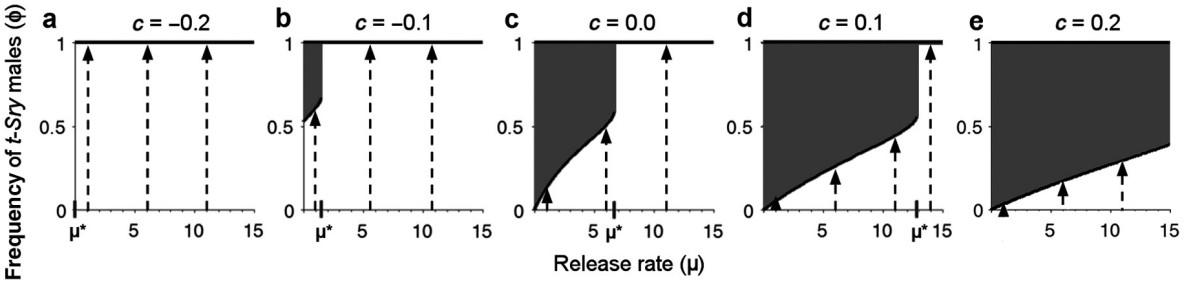

Fig. 3. Simplified equilibrium diagrams in ϕ (frequency of *t-Sry* males) over μ (release rate of *t-Sry* mice) with different values of c (survival cost to *t-Sry* mice). Thick solid lines represent stable, attracting equilibria. In this example, $c_1^* = -0.18$ and $c_2^* = -0.06$. An eradication attempt with a constant release rate μ would begin at the bottom of these diagrams (where $\phi = 0$). The population would change over time, following the dashed lines, by moving upward on this diagram until it reaches the first solid line stable equilibrium. It would remain there unless the release rate is changed. In (a), $c < c_1^*$ and the population would be eradicated in the long run for any release rate. In (b), $c_1^* < c < c_2^*$, so the population would approach the coexistence state when $\mu < \mu^*$. As the *t-Sry* construct is self-sustaining in this case, stopping release ($\mu = 0$) after some genetic engineering mice are in the population would result in the *t-Sry* mice remaining in the population indefinitely. The population would be eradicated if $\mu > \mu^*$. Each of (c–e) represents cases where $c > c_2^*$. The population would approach coexistence state when $\mu < \mu^*$. The *t-Sry* construct would be lost from the population if *t-Sry* release was later stopped. When $\mu > \mu^*$, the population would be eradicated. Because $\mu^* = 19.2$ when $c = 0.2$, it is not shown in (e).

(Fig. 2b). Eradication time is most sensitive to changes in the release rate when the release rate is only slightly above the critical value μ^*. For larger release rates, increasing the release rate further still decreases the time to eradication, but with diminishing effect. These trends hold regardless of the death rate of the *t-Sry* mouse. However, releasing *t-Sry* mice with reduced death rates should eradicate a population more quickly than releasing *t-Sry* mice with an increased death rate. Thus, the quickest eradication would occur with highly competitive GE mice released at a high rate.

Changing the release rate can have varying effects on the total number of *t-Sry* mice that are necessary to release (Fig. 2c). Overall, an intermediate release rate above μ^* would minimize the number of mice that need to be released (except when $c < c_1^*$, where a single *t-Sry* mouse could theoretically lead to eradication). Additionally, the number of *t-Sry* mice needed for eradication is greater when *t-Sry* mice have increased mortality and less when they have decreased mortality.

The maximum population density increases almost linearly as the release rate increases (Fig. 2d). This near-linear relationship occurs regardless of the death rate of *t-Sry* mice. However, the maximum population density is greater when engineered mice have a lower mortality and less when they higher mortality. Therefore, the maximum density is minimized when *t-Sry* mice have increased death rates and are released into a population slowly.

Changing the release rate also has varying effects on the population excess. Overall, intermediate values for the release rate minimize the population excess of *t-Sry* mice (Fig. 2e). The change in mortality has a different effect depending on the release rate of *t-Sry* mice. In particular, when *t-Sry* mice have a greater death rate, lower release rates will have high population excess. Because of the *t-Sry* mice's reduced survival, the population must be forced above the carrying capacity (via continuous release of *t-Sry* mice) for a longer amount of time before the population density begins to decrease. However, this lower survival also decreases the population excess at higher release rates. As the population reaches a sufficient number of *t-Sry* mice more rapidly, the excess mice will die off below the carrying capacity quickly.

Discussion

The use of gene drives for population suppression is not new (Burt 2003, Deredec et al. 2008,

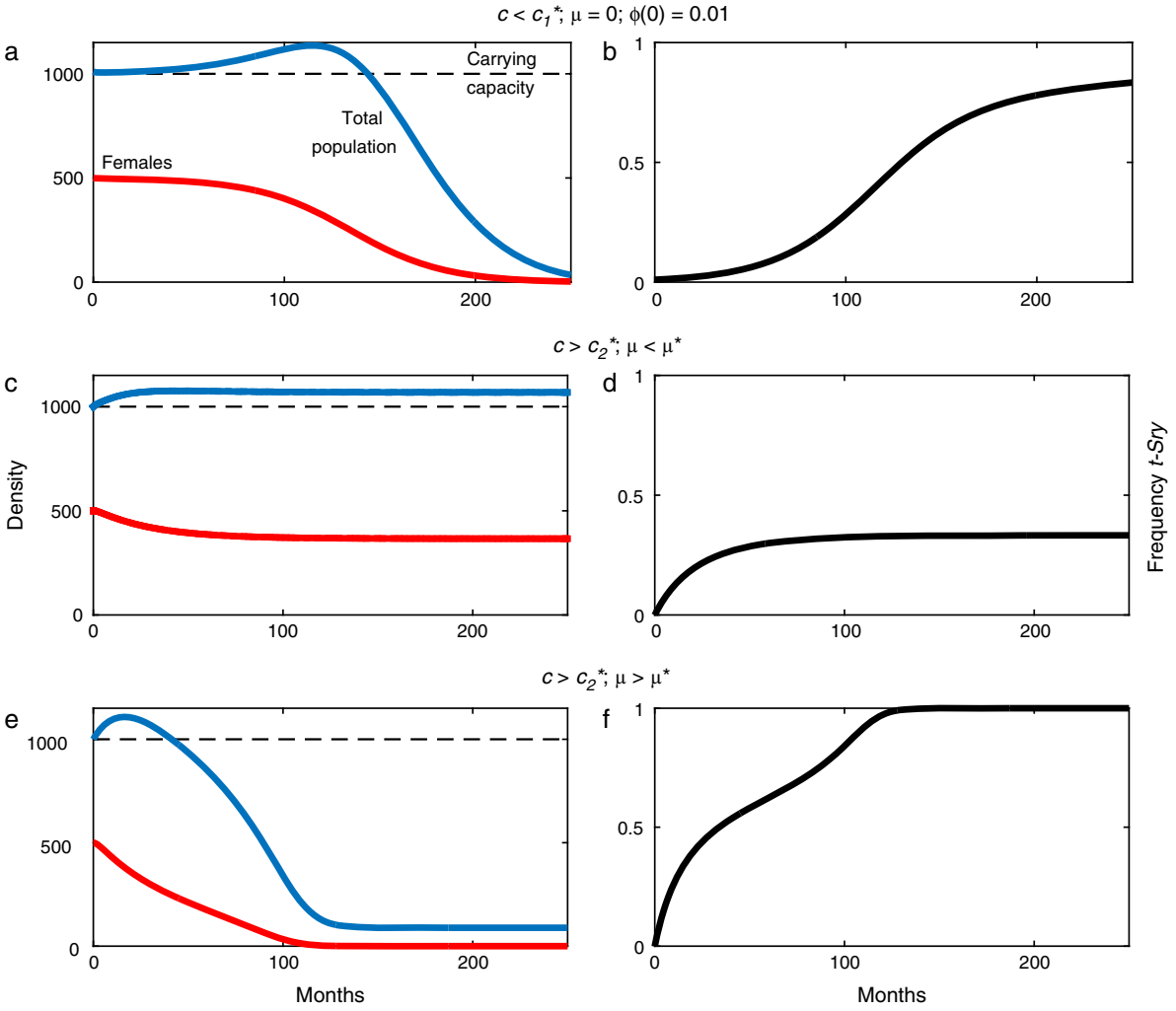

Fig. 4. The success of three potential eradication attempts depends on the change in death rate, c, and the release rate, μ, of *t-Sry* mice. (a, b) A *t-Sry* mouse with a largely reduced death rate, $c = -0.2 < c_1^*$, can cause the population to be eventually eradicated with a single pulsed release. (c, d) With increased mortality $c = 0.1 > c_2^*$ and a low release rate of μ = 8 mice per month, the population maintains a small stable frequency of *t-Sry* mice and lowers the number of female mice, but does not eradicate the population. (e, f) An increased death rate $c = 0.1 > c_2^*$ and a high release rate of μ = 20 > μ* mice per month increase the frequency of *t-Sry* males until ϕ = 1 and decrease the number of wild-type females until they are no longer present ($W_X \approx 0$). This leads to eventual eradication.

Alphey 2014), but we have presented and analyzed one of the first models that considers this technology specifically for a mammalian species. We have shown in theory that genetically engineered *t-Sry* mice can eradicate entire invasive populations of *M. musculus*. In this model, when *t-Sry* mice are released into a population, they interbreed with wild island mice and produce mostly *t-Sry* offspring. Initially, the population density increases as *t-Sry* are added. Over enough time, there would theoretically be no females left in the population because very few fertile female offspring are sired from increasingly common *t-Sry* fathers. Because the *t-Sry* construct would likely impose a survival cost and because transmission distortion will not be 100%, the gene construct would have a selective disadvantage and probably be lost from the

population if *t-Sry* mice are not repeatedly released. In these situations, the *t-Sry* construct is self-limiting and *t-Sry* mice can only eradicate a population if they are released above a critical release rate. In some situations, this critical release rate might be considerable, but it can be reduced by increasing the survival of *t-Sry* mice or by increasing the transmission distortion of the *t*-haplotype. If the survival of *t-Sry* mice could be greatly improved by backcrossing them with highly competitive wild strains, however, the *t-Sry* construct could theoretically be self-sustaining. In these cases, the *t-Sry* construct could spread on its own and eradicate the population without sustained releases.

While these general qualitative trends are similar to previous models of insect gene drives, invasive rodents present a new and important set of ecological characteristics that must be considered as this technology develops. Because mice disrupt the ecosystems where they invade (Howald et al. 2007, Angel et al. 2009), it is worthwhile to determine release strategies that could reduce the additional ecological stress that comes from adding more GE mice to an island. We found that there is no optimal strategy for releasing *t-Sry* mice that would minimize all potential ecological impacts. High release rates of *t-Sry* mice with greater survival can eradicate a population of invasive mice relatively quickly, but also result in a large increase in the density of an ecologically disruptive population. A lower release rate and higher mortality of *t-Sry* mice, on the other hand, can reduce the maximum population density of mice throughout the eradication, although eradication might then take several additional years to complete. Thus, there is a trade-off between the duration and intensity of the transient ecological impacts of GE-assisted mouse eradication. Consequently, there is no "one size fits all" release strategy that applies to every island. Whether GE-assisted eradication should be faster or less disruptive would depend on the ecological sensitivities of threatened species in an island's ecological community.

We demonstrate how differences in the fitness of the GE mice, through modifying their death rate, could theoretically lead to striking differences in the ability of the *t-Sry* construct to spread and to be controlled. The dynamical consequences of *t-Sry* mice with substantially increased survival are particularly notable. Although this case is less likely to be realized outside of theory (at least initially), the potential for increased survival through backcrossing makes this a worthwhile consideration. With enough of a survival advantage, we have shown that the *t-Sry* construct could theoretically be self-sustaining. Without the need for repeated releases, a self-sustaining *t-Sry* could eradicate a population more cheaply and quickly. Although this increased efficiency might seem appealing, this increased persistence would make eradication much more difficult to control. Worryingly, if a highly fit *t-Sry* mouse escapes from the target island, or even a breeding facility, the escaped GE mouse might be able to cause widespread suppression and extinction of non-target *M. musculus* populations. This presents a significant ecological, regulatory, and social risk to non-target areas (Esvelt et al. 2014). Thus, it would be necessary to contemplate measures to make a self-sustaining *t-Sry* construct more controllable. For example, carefully designed physical, molecular, and ecological barriers could limit spread of a gene construct to avoid accidental releases during development and production (Esvelt et al. 2014, Akbari et al. 2015). Also, currently established biosecurity measures to prevent rodent reinvasions after eradication (Russell et al. 2008, Harris et al. 2012) could be adapted to prevent *t-Sry* escape during an eradication. Additionally, a separate "reversal" gene drive could be engineered alongside the *t-Sry* construct to be released in case of an unintentional spreading event (Esvelt et al. 2014). Mice carrying this reversal drive would need to breed into the population to overwrite and nullify the suppressive effects of the *t-Sry* construct before eradication (Esvelt et al. 2014). In the more likely case where *t-Sry* mice have increased mortality, the *t-Sry* construct would be self-limiting. Without the ability to spread indefinitely on its own, a self-limiting construct would present far fewer risks. In short, a self-limiting construct is inherently reversible and therefore more easily controlled.

In this analysis, we focused on the dynamics that ensue when GE mice are released at a steady continuous rate into a population of entirely wild-type mice. Under most scenarios (specifically, when $c > c_1^*$, or when GE mice do not live substantially longer than wild-type mice), both

eradication and coexistence of GE and wild-type mice are possible, although the eventual outcome will depend on the initial conditions. This bistability suggests the following possibility. Any successful eradication effort must begin with a release rate that exceeds the critical release rate, μ^*. However, as the proportion of fertile GE males grows, the release rate of GE mice can eventually be reduced without jeopardizing the success of the eradication, as long as the dynamics stay within the basin of attraction of the eradication equilibrium. In other words, it is not absolutely necessary to maintain the initial high release rate throughout the eradication effort for the eradication to succeed. In practical terms, however, reducing the release rate without jeopardizing the eradication requires a confident knowledge of the exact location of the boundary that separates the basin of attraction for the eradication equilibrium from the basin of attraction for the coexistence equilibrium. We have not mapped that boundary here because it will depend sensitively on biological details that are specific to a particular eradication scenario. However, our simplified model at least suggests that a more flexible release schedule is possible. The flexibility to modify the release rate as the eradication unfolds contrasts with rodenticide-driven eradications, which tend to have rigid deployment schedules (Howald et al. 2007, 2010).

This model is based on the assumption that an undisturbed invasive mouse population would remain constant over time. However, many mouse population densities often increase throughout a breeding season, followed by declines once breeding subsides (Ferreira et al. 2006). Rodenticide-driven eradications can capitalize on these population cycles (Howald et al. 2007, 2010, Russell et al. 2011), as fewer individuals need to be eradicated when rodents are naturally less abundant. Sterility-based biological control methods in other rodents have also been shown to benefit from optimal timing throughout natural population cycles (Shi et al. 2002). Population cycles will likely influence the optimal timing of GE-assisted eradications as well. For example, releasing *t-Sry* into a population during the lowest point of an annual cycle would likely result in a greater proportion of *t-Sry* mice than if they were released at other times. Additionally, these GE male mice would be exposed to less competitive interference during lower points, making them less likely to die before mating. Optimal timing of GE mouse release could reduce the total eradication time while also decreasing the impact on the rest of ecosystem.

The release of GE mice could also be combined with other control methods in an integrated eradication strategy. There is precedent to this, as disease-driven biological control has successfully been combined with toxicants to eradicate other vertebrate species from islands (Parkes et al. 2014, Springer 2016). An integrated approach with GE might begin by spreading rodenticide bait onto an island to reduce the population density without the intention of full eradication. Usually, rodenticide needs to be applied heavily over a wide area to ensure that bait is available to the full distribution rodents for enough time (Pott et al. 2015). However, because rodenticide would not need to guarantee 100% efficacy, fewer mice and non-target organisms would need to be killed than in a pure rodenticide eradication. Following rodenticide, *t-Sry* mice could be released onto the island in areas where the population is still extant to gradually eradicate the remaining population. Starting the release of *t-Sry* mice at a lower population density, the eradication would have a shorter duration and lower levels of population excess than a pure GE approach. Overall, both rodenticide and GE mice could reduce the negative impacts of the other. Despite the advantages of an integrated approach, its main barriers are likely to be regulatory. Rodenticide eradications are subject to strict regulation (Eason et al. 2010, Campbell et al. 2015), often requiring several years of planning to navigate (Howald et al. 2010). Bringing the release of GE animals into this framework would introduce additional complex regulatory standards into the process (Campbell et al. 2015). Also, this integrated approach would require some poisoning and killing of mice, partially negating the major animal welfare motivations behind developing *t-Sry* in the first place. A more detailed analysis into this integrated approach could provide more insight into whether this would be economically and ecologically viable.

We focused on the *t-Sry* mouse in this article because it is currently in development. However, our model can also inform rodent eradications

with other gene drive systems. Among the alternative gene drives, CRISPR/Cas9 is one of the most promising (Jinek et al. 2012, Esvelt et al. 2014, Gantz and Bier 2015). Unlike the *t*-haplotype, CRISPR would be applicable to more invasive rodents than just *M. musculus* (Esvelt et al. 2014, Campbell et al. 2015), likely including the rat species *Rattus rattus*, *R. norvegicus*, and *R. exulans*. CRISPR and other engineered gene drives can also have greater transmission rates, increasing their ability to spread. Applying this to our model, we would expect these gene drives to reduce both the critical release rate and potential temporary ecosystem impacts. Additionally, compared to other gene drives, CRISPR can be fairly precise and manipulable despite being a relatively small gene construct (Jinek et al. 2012). This specificity and smaller size can decrease the survival cost that would normally be imposed on GE organisms, again increasing the ability of a construct to spread into a population. Both increased transmission distortion and lower fitness costs would even make it easier for these engineered gene drives to be self-sustaining.

Overall, gene drive-assisted rodent eradication methods provide a targeted, non-lethal alternative to toxicants. With our model, we have addressed some basic ecological questions concerning the ability of GE construct to spread, while also demonstrating a trade-off in the potential impacts on the ecosystem. Before this technology could be considered a viable alternative to rodenticides, future ecological studies will need to further explore the seasonality of mouse population dynamics, the spatial dynamics of release and the subsequent spread of gene constructs, and the trophic community dynamics on each particular targeted island. Additionally, while the spread of a GE construct to other rodent species is unlikely in the timeframe of eradication (through hybridization or horizontal gene transfer (Snow et al. 2005)), the consequences of such an event could be severe enough to lead to the extinction of a non-target species, warranting further research and investigation. Moreover, pathogens are more likely than genes to cross species boundaries. Because introducing laboratory-bred rodents to an island could unintentionally spread new pathogens to naïve endemic species, future research in this area would be critical.

These ecological questions exist alongside a set of new and complex genetic, evolutionary, behavioral, social, and regulatory ideas that come from engineering and releasing GE rodents for eradication. Even if complications or setbacks in any of these research areas could limit the future of the *t-Sry* mouse or any other suppression gene drive rodent, the intersection of synthetic biology and conservation is only in its infancy and could have countless applications moving forward (Redford et al. 2013, Esvelt et al. 2014, Johnson et al. 2016, NASEM 2016). Therefore, our analysis of the temporary and long-term ecological impacts of *t-Sry*-driven mouse eradication should help contribute and inform the ongoing evaluation of these issues.

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