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ORIGINAL ARTICLE OPEN ACCESS

Maximising Eradication Potential of Rat Gene Drives Using a Two-Target Homing Rescue Strategy: Spatial Modelling of Empirical Data

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Received: 21 October 2024 | **Revised:** 24 March 2025 | **Accepted:** 9 April 2025

Handling Editor: Benjamin Sibbett

Funding: This work was supported by Centre for Invasive Species Solutions, New South Wales Government, Government of South Australia.

Keywords: gene drive | invasive species | island conservation | rats | rodents

ABSTRACT

Gene drives are genetic elements with positively biased transmission and may be useful tools to suppress mammalian pests that threaten biodiversity worldwide. While gene drives are progressing in mice, less is known about their potential for invasive rat control. A recent report has provided the first data on germline gene conversion in rats, demonstrating that modest homing rates (up to 67%) can be achieved in females. Here, we apply these empirically derived values to investigate the potential of various gene drive strategies to suppress an island population of 200,000 rats, using our stochastic, spatially explicit, individual-based modelling framework. Standard homing drives embedded in haplosufficient fertility or viability genes failed to eradicate, but achieved permanent population suppression. In contrast, a two-target design with a homing rescue (HR) drive embedded in a haplolethal gene that also targets an independent fertility or viability gene demonstrated considerable suppression potential. Remarkably, an HR drive targeting a haplosufficient female fertility gene showed robust eradication even at the relatively low homing rates previously demonstrated in rats. Interestingly, homing rate had a relatively low influence on eradication probability while cutting efficiency at the haplolethal gene was critical. Further, as long as the latter was similar to the cutting and subsequent knockout of the unlinked female fertility gene, then eradication could be achieved across a range of homing rates. Together, these results suggest that modest homing rates, such as have been demonstrated in rats and other species, can potentially be leveraged for population suppression, offering new opportunities for gene drive development.

1 | Introduction

Invasive alien mammals have extensive impacts on the environment and on human well-being and livelihoods (Bradshaw et al. 2021; Pyšek et al. 2020; Paini et al. 2016). Among invasive alien mammals, rodents are considered to be the main driver of extinctions globally, and continue to threaten hundreds of species, of which ~80% are insular endemics (Doherty et al. 2016; Blackburn et al. 2004). The black rat (*Rattus rattus*) and the brown rat (*Rattus norvegicus*) are significant rodent pests found throughout the world, causing extinctions and ecosystem function collapse through the interruption of pollination, nutrient

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pathways, and seed predation, particularly on islands (Capizzi et al. 2014; Harper and Bunbury 2015; Towns et al. 2006). The *Rattus* sp.'s impact can be particularly severe on species-rich tropical islands, where eradication efforts have been less successful (Russell and Holmes 2015). Native species show a positive response to invasive species eradications by demographic recovery and/or recolonisation of previously extirpated places (Le Corre et al. 2015; Jones et al. 2016).

Rodenticide poisons, particularly anticoagulants, are most commonly used to control rodents worldwide (Capizzi et al. 2014). Anticoagulants are not only disfavoured due to extreme welfare impacts on the target taxa (Mason and Littin 2003), but they have also been detected at trace levels across a wide range of non-target taxa (e.g., Pitt et al. 2015) and at high concentrations in predators of rodents (Lohr 2018); and even in predators with largely non-rodent diets (Pay et al. 2021; Cooke et al. 2023). These results suggest that the impacts of broader, non-rodent routes of transfer of rodenticides in the food chain could be more severe than previously acknowledged. Alternative, non-toxic approaches for invasive rodent suppression are therefore highly desirable.

Gene drives offer the potential for landscape-scale suppression of rodent populations without toxicant-based harms (Prowse et al. 2017; Godwin et al. 2019; Champer, Oakes, et al. 2021; Birand, Cassey, Ross, Russell, et al. 2022; Hay and Guo 2022; Gierus et al. 2022; Bunting et al. 2023). CRISPR 'homing' gene drives are transgenic elements composed of one or more guide RNAs (gRNAs) and a cognate Cas9 endonuclease. Activation of the CRISPR complex in the germline results in cleavage of the homologous chromosome that lacks the transgene. Subsequent repair via homologous recombination enables the drive element to be copied (or 'homed') onto the homologous chromosome, ensuring biased transmission. Alternatively, the cleavage could be repaired by end-joining (non-homologous end-joining [NHEJ] or microhomology-mediated end joining [MMEJ]), which can result in a small deletion or insertion mutation (indel) rendering the target site resistant to further cleavage and inhibiting the drive spread (Champer et al. 2017; Esvelt et al. 2014; Unckless et al. 2017; Prowse et al. 2017). Homing rates > 99% have been reported in mosquitoes (*Anopheles* sp.) (Kyrou et al. 2018); however, gene drive homing in mice has thus far proven to be much less efficient, particularly in males (Weitzel et al. 2021; Pfitzner et al. 2020; Grunwald et al. 2019). Recently, homing rates were quantified in brown rats (*Rattus norvegicus domestica*) for the first time and shown to be considerably higher than in mice Lai et al. (2023). For example, a split drive experiment in which Cas9 was expressed from the endogenous *Ddx4* (*Vasa*) promoter resulted in 66.8% homing of the gRNA expression cassette in *Ddx4*-Cas9 homozygous females. Hemizygous females (possessing a single copy of *Ddx4*-Cas9) exhibited a homing rate of 55.1%. In contrast, male hemizygotes exhibited a homing rate of only 0.9% (homozygous males were infertile).

The homing rate of 66.8% in female rats is the highest demonstrated in a rodent to date. Given the importance of homing efficiency for population suppression, we investigated whether this experimentally derived value was sufficiently high for effective gene drive control of invasive rat populations. We investigated the suppression potential of two gene drive strategies with homing efficiency values in this range and limited to females. First, we explored conventional homing gene drive scenarios, where the drive element is embedded within and disrupts a haplosufficient fertility/viability gene. Second, we investigated a two-target gene drive design with a homing site and distant cutting site, as recently proposed by Faber et al. (2024). The homing site where the drive conversion takes place is essentially a homing rescue (HR) drive (Esvelt et al. 2014; Noble et al. 2017; Kandul et al. 2021; Champer, Yang, et al. 2020) that is embedded in a haplolethal viability gene that generates a loss-of-function mutation and also has an additional 'rescue' component. The distant cutting site is the targeted site for population suppression. With this design, Faber et al. (2024) demonstrated that the main factor determining the suppressive power of the drive was the cutting efficiency at the homing and distant sites rather than the conversion efficiency at the homing site, so this approach could be useful for suppressing species with inefficient drive conversion rates. Our findings indicate that a two-target HR design has much greater potential for population suppression than traditional homing approaches, and that HR drives that target an unlinked female fertility gene can cause eradication at the (modest) homing rates recently demonstrated in rats (Lai et al. 2023).

2 | *In Silico* Modelling

To model the spread and population-level impact of the two gene drive strategies on rats, we used the individual-based and spatially explicit modelling framework presented in Birand, Cassey, Ross, Russell, et al. (2022) and Birand, Cassey, Ross, Thomas, et al. (2022). This is a discrete-time stochastic model with overlapping generations. Individuals are diploid, have genetically controlled autosomal traits and sex chromosomes, and occupy a rectangular array of patches that together form a hypothetical island. Each patch can hold multiple individuals. An individual is not restricted to a single patch, but can utilise multiple patches within a single breeding cycle. Each breeding cycle is considered a model time step, and individuals that survive long enough pass through a number of breeding cycles until they reach a maximum age (age_m).

The number of breeding cycles per year is given by n_c, with the following steps occurring each cycle (for more details, see Birand, Cassey, Ross, Russell, et al. 2022; Birand, Cassey, Ross, Thomas, et al. 2022; Gierus et al. 2022): (1) mate search within a predetermined distance from the individual's central patch; (2) mating, where all fertile females mate if they find a male during mate search with the possibility of polyandrous mating; (3) density-dependent reproduction, where the number of offspring from each mated female is drawn from a Poisson distribution using discrete-time Beverton–Holt model (Kot 2001); (4) distance- and negative-density-dependent natal dispersal; (5) survival of adults; and (6) breeding dispersal of surviving adults as in Step 4.

2.1 | Gene-Drive Strategies

We explored two approaches to investigate the effectiveness of a CRISPR-based drive where homing occurs primarily in

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females (Figure 1). The first approach is a classical homing drive (HD, Figure 1) where we assumed the CRISPR transgene is integrated within a gene required for fertility or embryonic viability. We explored three scenarios where the function of the target gene varies. In the first scenario, the drive construct is positioned within an exon of a haplosufficient female fertility gene (HD-fs), generating a loss-of-function mutation (Burt 2003). The fertility gene is autosomal and is present in both sexes; however, the gene is required only in females. If both copies of the gene are inactivated in somatic cells, the female is considered infertile. In the second scenario, the target gene is a haplosufficient male fertility gene (HD-ms), which is autosomal and is required only in males. Similarly, if both copies of the gene are inactivated, the male is considered infertile. If an infertile male is chosen randomly by a female, the female missed a mating opportunity without producing any offspring in that breeding cycle. If the female is mating polyandrously with two males, and one of which is infertile, then she is assumed to mate with the fertile male with no effect on her litter size. Finally, in the third scenario, the target is a viability gene (HD-v), which is also autosomal, but it is essential in both sexes. If the embryo inherited two nonfunctional copies of the gene, it does not develop, reducing the litter size of the mated female. The litter size is determined a priori based on density-dependent fertility selection (Birand, Cassey, Ross, Russell, et al. 2022). The probability of successful homing of the drive is given by $p_C(1-p_N)$, where p_C is the probability of a successful cut, and p_N is the probability of end-joining (NHEJ or MMEJ; Figure 2). If homing fails due to end-joining, the resulting allele is resistant to further cuts. The resistant allele can be nonfunctional (termed an '*r*2 allele') with probability $p_C p_N p_L$, or retain its function (an '*r*1 allele') with probability $p_C p_N(1-p_L)$, where p_L is the probability of loss of gene function following end-joining. The probability that no DNA cutting occurs (i.e., no gene conversion) is $1-p_C$.

The second approach is an HR drive with a two-target design (denoted as 'HR-KO' drive due to additional knockout at an unlinked target), which was recently proposed by Faber et al. (2024). The CRISPR transgene is positioned in the exon of a haplolethal (or haploinsufficient) viability gene, generating a loss-of-function mutation. The drive contains an additional 'rescue' component, which is a re-coded version of the haploinsufficient viability gene (Figure 1, Noble et al. 2017). The probabilities of successful homing of the drive, and *r*1/*r*2 allele generation are same as the HD above. The drive targets an additional autosomal unlinked gene that is haplosufficient and creates a loss-of-function mutation with probability p_K. We assume that the knockout of the second target gene only generates nonfunctional (r2) alleles, which is easier to achieve at this site than at the homing site with multiplexed gRNAs, since end-joining repair is not of concern at this site (Faber et al. 2024).

We explored three scenarios where the unlinked target genes are responsible for female fertility (HR-KO-fs), male fertility (HR-KO-ms), or viability (HR-KO-v) genes that are required in females, males, or both, respectively. The model is coded using

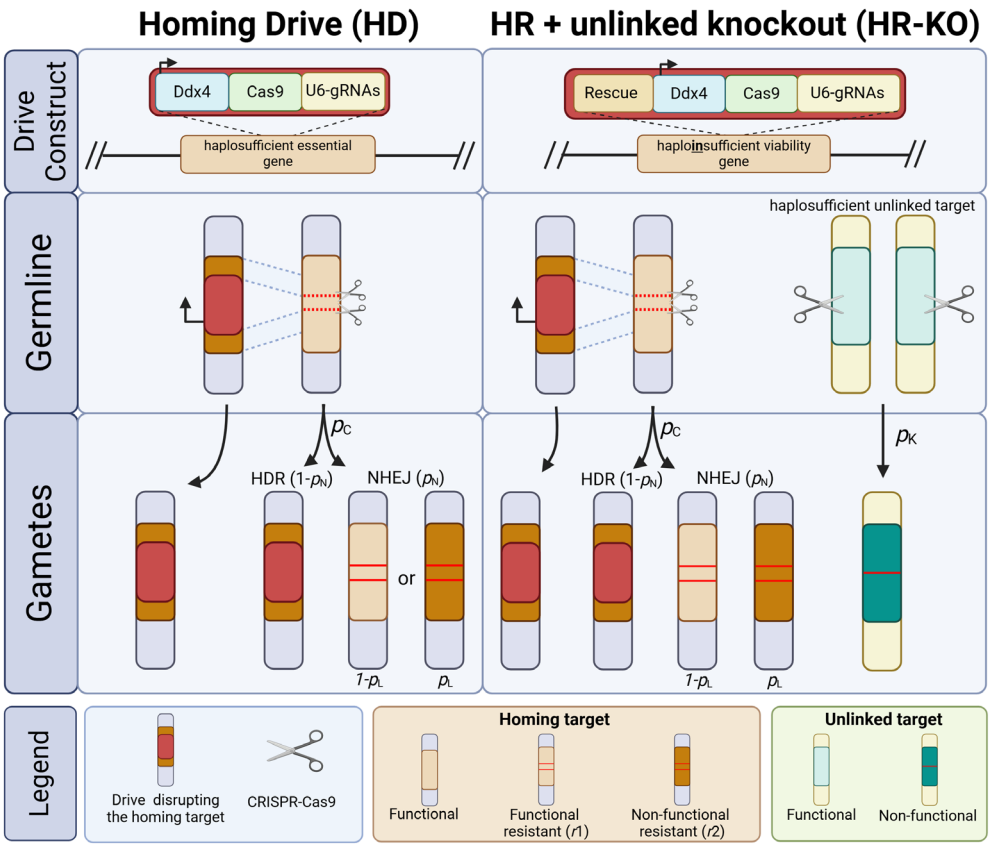

FIGURE 1 | Details of the drive constructs, their activity in the germline and the resulting gametes, where p_C is the probability of a successful cut, p_N is the probability of end-joining, HDR is the homology directed repair $(1-p_N)$, and p_K is the probability of unlinked gene knockout.

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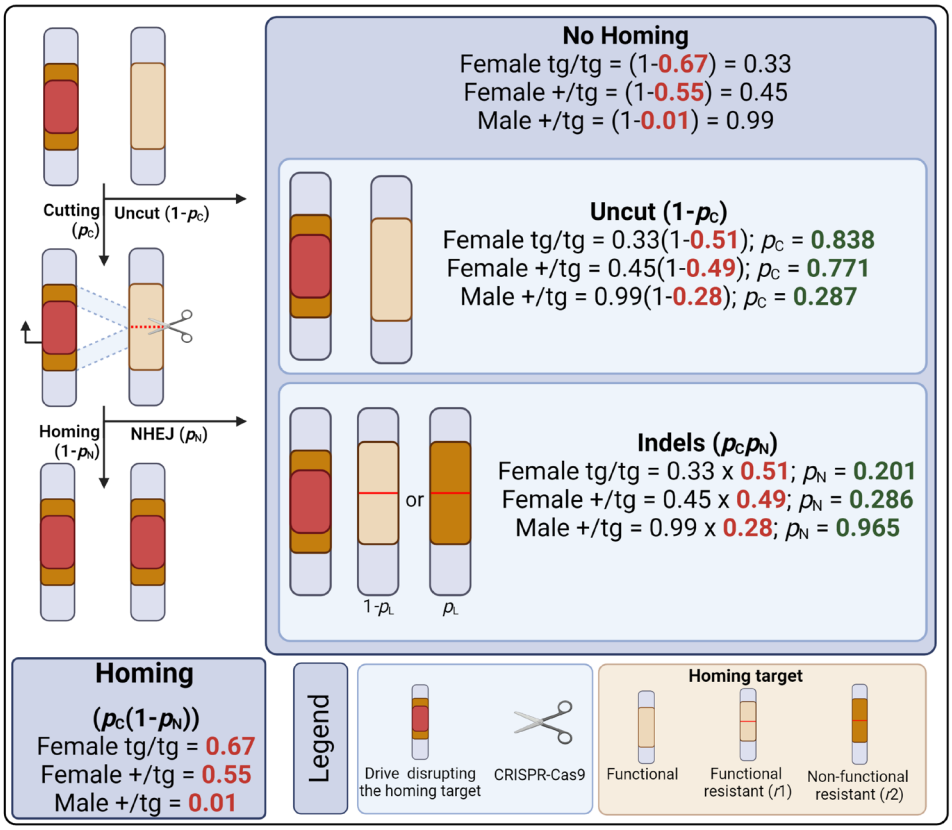
a)

Values from Lai *et al.* (2023)			
Sex	**Ddx4-Cas9**	**homing rate**	**p. indels**
Female	Homozygous (tg/tg) Heterozygous (+/tg)	0.67 0.55	0.51 0.49
Males	Homozygous (tg/tg) Heterozygous (+/tg)	NA 0.01	NA 0.28

b)



c)

Values used in model			
Sex	**Ddx4-Cas9**	**Cutting (p_C)**	**NHEJ (p_N)**
Female	Homozygous (tg/tg) Heterozygous (+/tg)	0.838 0.771	0.201 0.286
Males	Homozygous (tg/tg) Heterozygous (+/tg)	NA 0.287	NA 0.965

FIGURE 2 | (a) Empirical values for the homing rate and the proportion of indels in female and male rats reported by Lai et al. (2023), (b) corresponding gene drive parameters in the model, and (c) their values.

the C programming language and is available at the Github platform (see Data Availability Statement).

2.2 | Genetic and Demographic Parameters

Parameters related to gene drive efficiency p_C and p_N in each sex are based on empirical results obtained by Lai et al. (2023). Note that Lai et al. (2023) reported homing rates in female and male rats (given by $p_C\left(1-p_N\right)$) and the proportion of individuals with indels among all individuals where homing failed (Figure 2). The proportion of individuals with indels where homing fails is $p_C p_N$, and proportion of individuals with no indels where homing fails is $\left(1-p_C\right)$. The corresponding values for the gene drive parameters used in the model are given in Figure 2c. The cutting and subsequent knockout of the unlinked gene p_{K-f} with the HR-KO approach was based on Lai et al.'s (2023) estimate for the cutting of the homing target (p_{C-f}); however, we also explored higher values for p_{K-f}. Finally, we assumed that there were no fitness costs associated with the drive, even though the homozygous *Ddx4*-Cas9 configuration used by Lai et al. (2023) was associated with reduced female fertility and male sterility.

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Parameters related to life history traits and inoculation scenarios (Table 1) are based on empirical data where available (Birand, Cassey, Ross, Thomas, et al. 2022). We assumed that the average number of offspring (b) produced by a female rat in the absence of density-dependent regulation in each breeding cycle is 4 (Moro et al. 2018). The survival probability per breeding cycle is ($\omega = 0.62$, Harper and Bunbury 2015), and the maximum age (age_m) that an individual rat could reach is assumed to be 2 years (Murphy and Nathan 2021). Based on the observed intervals between litters, we assumed that the number of breeding cycles in a year (n_c) is 6 (Murphy and Nathan 2021; Moro et al. 2018). The actual number of offspring a female produces during her lifetime depends on the number of breeding cycles she survives, and the random offspring number drawn from the Poisson distribution with density dependence each breeding cycle (Birand, Cassey, Ross, Russell, et al. 2022 and Birand, Cassey, Ross, Thomas, et al. 2022). When the random number drawn is zero, which is often the case near carrying capacity, the female skips a breeding cycle without producing offspring. If there are multiple males in a female's breeding patch, females can mate with up to two males ($n_\text{m} = 2$) in a breeding cycle with probability $p_\text{m} = 0.68$ (King et al. 2014). Offspring produced are assumed to be adults in the next breeding cycle (see Table S1 for the population growth parameter estimates in a sample simulation initiated with a few individuals).

2.3 | Simulated Landscape and Initial Conditions

Our hypothetical island is 200 km^2 with ~200,000 rats (based on density estimate: ~1000 per km^2, Murphy and Nathan 2021).

TABLE 1 | Parameters related to the life history of rats, gene drive efficiency, and inoculation scenarios.

Parameter	Base value	SA
Life history		
Average number of offspring (b)	4	4
Maximum age (age_m)	2	2
Number of breeding cycles in a year (n_c)	6	6
Dispersal coefficient (a)	1	1
Density coefficient (c)	1	1
Dispersal and mate-search distance (D)	8	8
Drive fitness (ω_d)	1	1
Probability of survival (ω)	0.62	0.62
Carrying capacity per patch (K)	12	1
Probability of multiple mating (p_m)	0.68	0.68
Maximum number of males mated per breeding cycle (n_m)	2	2
Gene drive parameters		
Females		
Probability of successful cut ($p_{\text{C}-\text{f}}$)	0.77–0.83*	$U(0.0, 1.0)$
Probability of end-joining ($p_{\text{N}-\text{f}}$)	0.2–0.29*	$U(0.0, 1.0)$
Probability of unlinked gene knockout ($p_{\text{K}-\text{f}}$)	0.77–1.0	$U(0.0, 1.0)$
Probability of loss of function after end-joining ($p_{\text{L}-\text{f}}$)	1.0	$U(0.99, 1.0)$
Males		
Probability of successful cut ($p_{\text{C}-\text{m}}$)	0.29*	$U(0.0, 1.0)$
Probability of end-joining ($p_{\text{N}-\text{m}}$)	0.97*	$U(0.0, 1.0)$
Probability of unlinked gene knockout ($p_{\text{K}-\text{m}}$)	0.29	$U(0.0, 1.0)$
Probability of loss of function after end-joining ($p_{\text{L}-\text{m}}$)	1.0	$U(0.99, 1.0)$
Inoculation		
Number of inoculation sites	256	256
Number of gene-drive carrying individuals released (N_i)	1	1
Number of releases over time (n_t)	1	1

Note: Empirical values (*) reported by Lai et al. (2023) are used as base values for the gene drive parameters in most of the simulations. For sensitivity analyses (SA), parameter combinations are drawn from a uniform distribution (U) using Latin hypercube sampling. For detailed sensitivity analysis of life history parameters, see Birand, Cassey, Ross, Russell, et al. (2022) and Birand, Cassey, Ross, Thomas, et al. (2022).

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We assumed that the island is comprised of 64×64 = 4096 patches, each of which roughly corresponds to a 220×220 m space and adjusted dispersal distance D accordingly (Russell et al. 2005; Harper and Bunbury 2015; Birand, Cassey, Ross, Thomas, et al. 2022). We initiated each patch with 20 males and 20 females, and allowed the population to reach ~ 200,000 individuals during a burn-in period of 2 years before introducing gene-drive carrying individuals. In order to keep the overall population size on the island stable under different demographic parameterisations before inoculation, we adjusted the per-patch carrying capacity in the discrete-time Beverton–Holt model (Kot 2001), which only affects population size through reproduction. We introduced one drive carrying female ($N_i = 1$) to each of 256 patches evenly distributed in the landscape. Since all females that have males present within their mating ranges are assumed to mate in the model and that females choose among all available males, which implies that some males do not mate, we solely modelled the release of drive-carrying females. We ran simulations for a maximum of 500 breeding cycles and compared the efficacy of the gene drive approaches mentioned above.

2.4 | Scenario Modelling and Sensitivity Analysis

Lai et al. (2023) reported that the heterozygous *Ddx4*-Cas9 configuration was slightly less efficient than the homozygous configuration, which we treated as the lower and the upper bounds for the value estimates, respectively. We generated multiple parameter combinations within these ranges and ran 20–60 simulations for each parameter combination. For the drive strategies that resulted in eradication, we extended that range of values reported by Lai et al. (2023) and ran additional simulations to carry out a global sensitivity analysis. We investigated the relative influence of parameters on the probability of successful eradication and the time to eradication (as outlined by Birand, Cassey, Ross, Russell, et al. 2022). For each successful strategy, we created 3000 unique parameter combinations from parameter ranges given in Table 1 using Latin hypercube sampling (randomLHS, R package *lhs*, Carnell 2020) and carried out a single simulation for each parameter combination (Prowse et al. 2016). Finally, we examined the influence of parameter inputs using Boosted Regression Tree (BRT) models (R package *dismo*, Hijmans et al. 2011) that we fitted to the simulation outputs using the function gbm.step from the R package *dismo* (learning rate: 0.01; bag fraction: 0.75; tree complexity: 3; and fivefold cross-validation Elith et al. 2008). We used a binomial error distribution after scoring whether simulated eradication attempts were successful or unsuccessful (Elith et al. 2008).

3 | Results

3.1 | HD Can Suppress but Not Eradicate

With the parameter ranges reported by Lai et al. (2023), where homing is inefficient (0.55 − 0.67) and is limited to females, the HD was incapable of eradicating the simulated rat population regardless of the target gene function (eradication failed in all simulations ($p_{erad} = 0$)). However, up to 75% permanent suppression was possible in ~20 years if the target gene was a female fertility gene (Figure 3). Suppression was less effective if the target gene was a male fertility gene, or a viability gene. If the loss of function of the target gene after end-joining could not be guaranteed ($p_{L-f} = 0.999$), suppression failed for all approaches due to evolution of functional resistant $r1$ alleles, and population sizes in all simulations bounced back to initial population sizes with all individuals carrying $r1$ alleles. Eradication was only possible when we explored homing rates higher than reported by Lai et al. (2023) (when $p_{N-f} < 0.1$ and $p_{C-f} = 1$; Figure S1).

3.2 | Eradication Is Possible With the HR-KO-fs

In contrast to the classical HD, with relatively inefficient drive conversion rates limited to females (as reported by Lai et al. (2023)), the HR drive that targets an additional unlinked essential gene (HR-KO) could still suppress, and even eradicate, rat populations. The function of the unlinked target gene strongly influenced the outcome: complete eradication was achieved when the unlinked target gene was a female fertility gene (HR-KO-fs, Figures 4 and 5), and significant permanent suppression was achieved when the unlinked target genes were male fertility (~62%) and viability genes (~69%, Figure S2).

3.2.1 | Generation of $r1$ Alleles and the Role of Fertile Females With HR-KO-fs

Given that the release of rats carrying the HR-KO-fs drive could eradicate the simulated rat population with the parameter ranges reported by Lai et al. (2023), we provide a more detailed analysis of this strategy. The probability of eradication was very high ($p_{erad} \sim 1$, Figure 5) for the drive conversion rates ($p_{C-f} = [0.77,\ 0.83]$) reported by Lai et al. (2023). Increasing the cutting efficiency reduced the expected time to eradication from > 30 years to ~20 years. Surprisingly, the eradication probabilities were largely independent of the probabilities of end-joining in females (p_{N-f}; Figure 5) when the cutting and subsequent knockout of the unlinked gene p_{K-f} was the same as the cutting of the homing target (p_{C-f}). However, when the cutting and subsequent knockout of the unlinked target gene was more efficient than the cutting of the homing target (see when $p_{K-f} = 1$ in Figure 5; $p_{K-f} = [0.85, 1]$ in Figure S3), p_{N-f} (and the subsequent loss of function after end-joining, ($p_{L-f,m}$) in either sex) became critical and hindered the eradication probabilities with the evolution of resistant ($r1$) alleles. When the knockout of the distant female fertility gene was more efficient than the cutting of the homing target ($p_{K-f} > p_{C-f}$), fewer fertile females carried the drive, which slowed the spread of the drive and, hence, the population suppression. Higher population sizes created more opportunities for functional resistant ($r1$) alleles to emerge. Evolution of $r1$ alleles was not an issue when p_{K-f} increased in tandem with p_{C-f} (Figures 5 and S5). Under the best-case scenario when both $p_{C-f} = 1$ and $p_{K-f} = 1$, the probabilities of eradication were very high, and the expected time to eradication was reduced considerably (Figure 5).

3.3 | Sensitivity Analysis for the HR-KO

The results of the sensitivity analysis for the HR-KO-fs and HR-KO-ms drives showed that homing rates $(1 - p_{N-f,m})$ generally had a low influence on the probability of eradication (Figure 6,

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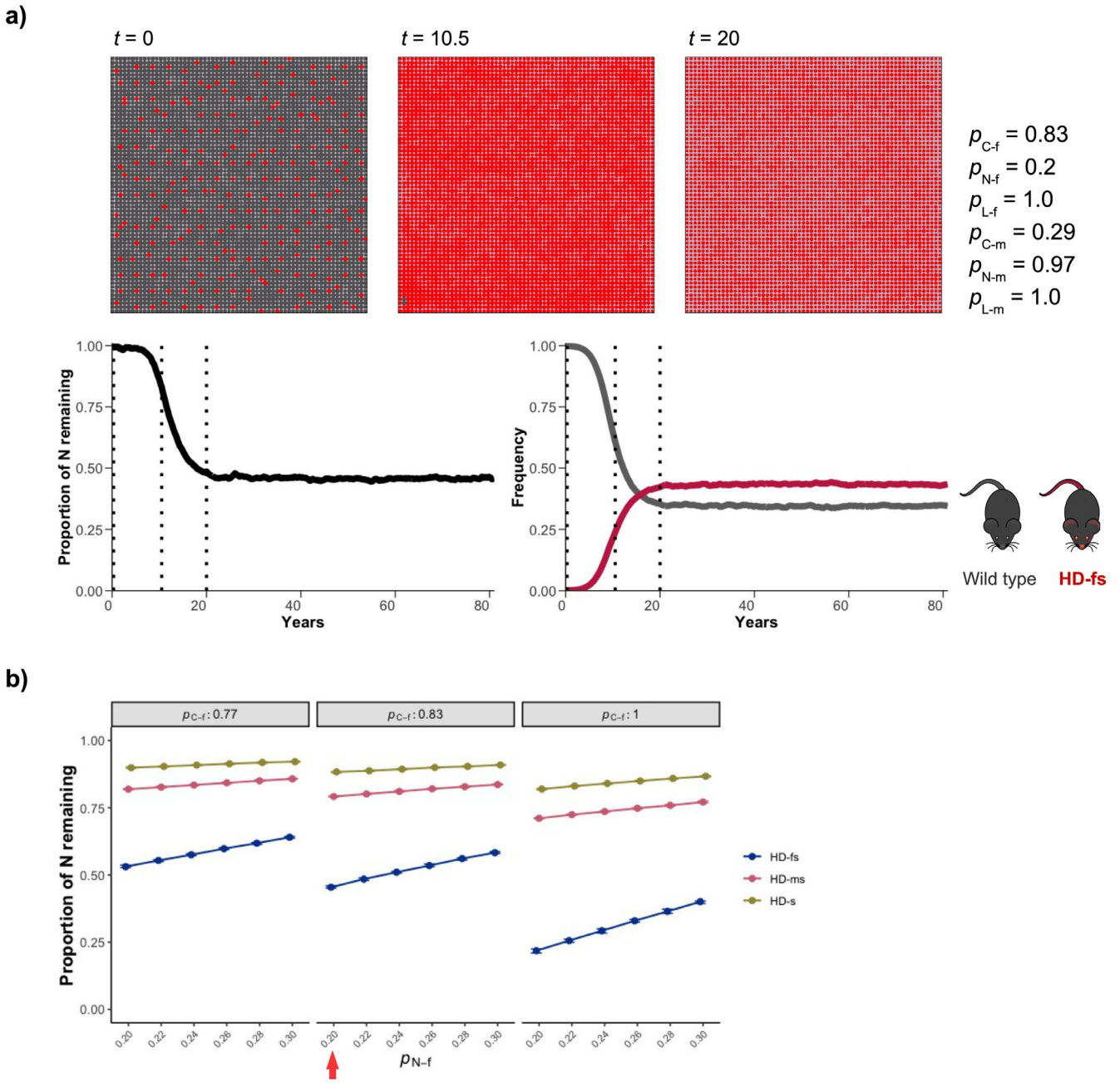

FIGURE 3 | (a) Progression of a simulation with the homing drive (HD) with female fertility gene as the target gene. The upper panel shows the distribution of the rat populations across the island through time, starting with the inoculation of gene-drive carrying individuals. Populations in patches are represented as circles, the size of which is proportional to the population size in that patch. The grey circles represent patches that contain wild-type individuals only. The colour changes to red as soon as the population has at least one individual with gene drive. In the lower panel, the plot on the left shows the population size relative to the initial population size on the island through time, and the plot on the right shows the frequencies of wild-type and gene-drive carrying individuals. In both plots, the dashed lines correspond to the times of simulation snapshots above. At the time of inoculation ($t = 0$), gene-drive carrying individuals dispersed from their inoculation patches before breeding. Within a decade, the gene drive had spread throughout the entire island, and suppressed the population to $< 50\%$ of its initial size in 20 years. (Parameter values are given on the right; also see the Supporting Information Online for a video of the simulation). (b) Proportion of population size remaining at the end of simulations when eradication attempt failed with the HD, where the target gene was a female fertility gene (blue), a male fertility gene (pink), or a viability gene (yellow). Up to 75% suppression was possible with HD-fs when $p_{N-f} = 0.2$, and $p_{C-f} = 1$. Red arrow points at the parameter combination for simulation presented in (a). (Slight offset in the *x*-values for this and the following plots are for display purposes; based on 60 simulations for each parameter combination).

also see Figure S6 for an additional set of simulations assuming that $p_{L-f,m} = 1$, where HR-KO-v was also successful in eradication). The cutting efficiency of the homing target (p_C) had a higher relative influence, since it is required for homing to occur. Moreover, higher levels of p_C contributed to creating non-functional resistant alleles (*r*2) that are not viable when homing

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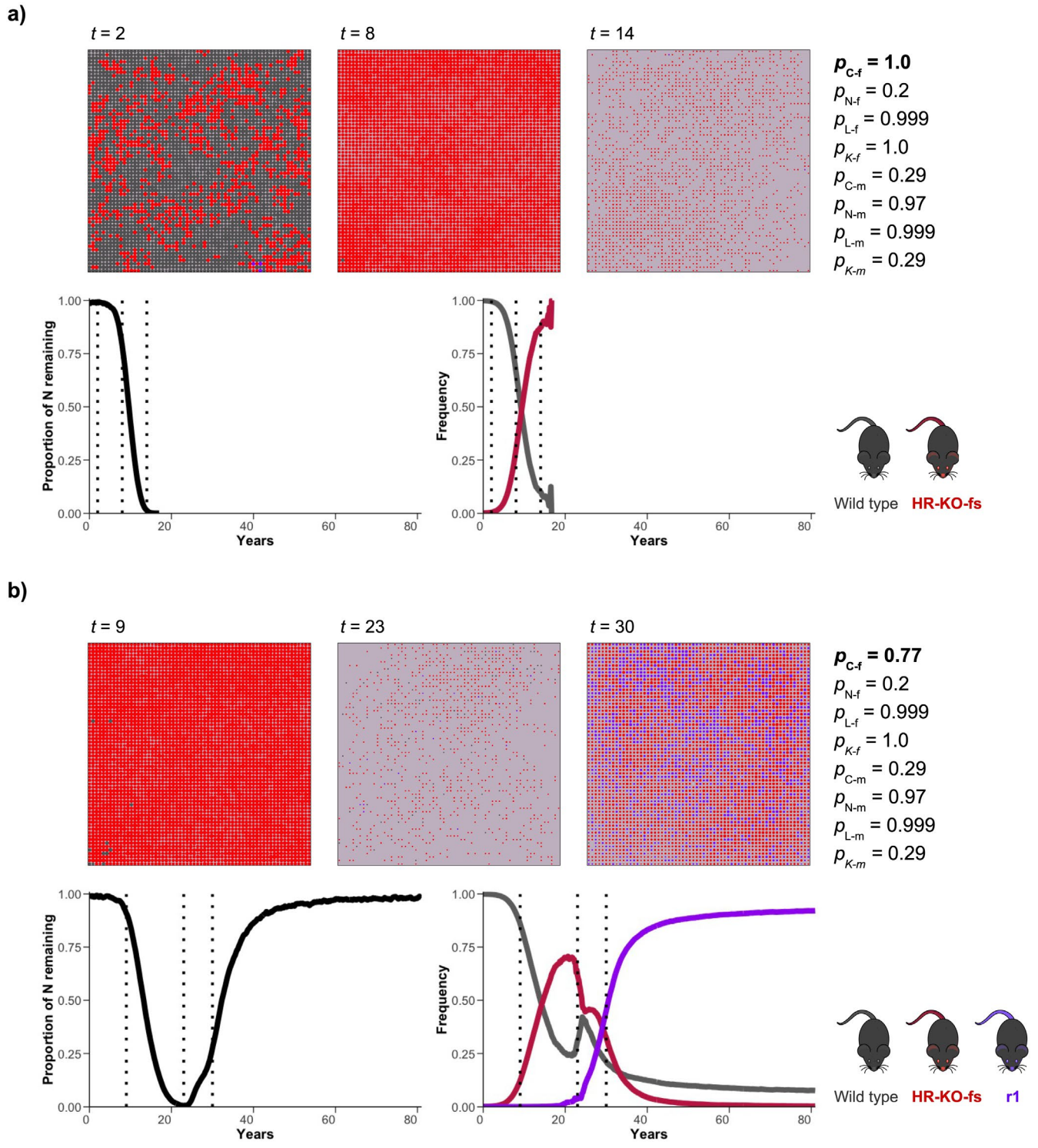

FIGURE 4 | Progression of two simulations with the HR-KO drive where the unlinked target gene was the female fertility gene (refer to the caption of Figure 3 for the description of the plots). (a) Population is eradicated in 17 years after inoculation with drive-carrying individuals (with $p_{C-f} = 1.0$). (b) Population is nearly eradicated ($N_{min} = 1236$) within two decades after inoculation with drive-carrying individuals (with $p_{C-f} = 0.77$), which is also when the functional resistant ($r1$) alleles emerge, and population bounces back to pre-inoculation levels with resistant alleles. Note that only the value for p_{C-f} differed between the two simulations in (a) and (b) (see the Supporting Information Online for the videos of the simulations.)

failed, which also aided in generating bias towards higher transmission of the drive. Since suppression was achieved through targeting fertility genes, unlinked gene knockout probabilities p_{K-f} and p_{K-m} had the highest influence in eradication probabilities with HR-KO-fs and HR-KO-ms, respectively. Their relative influences on the expected times to eradication were even higher than their influence on the probability of eradication (Figure S7).

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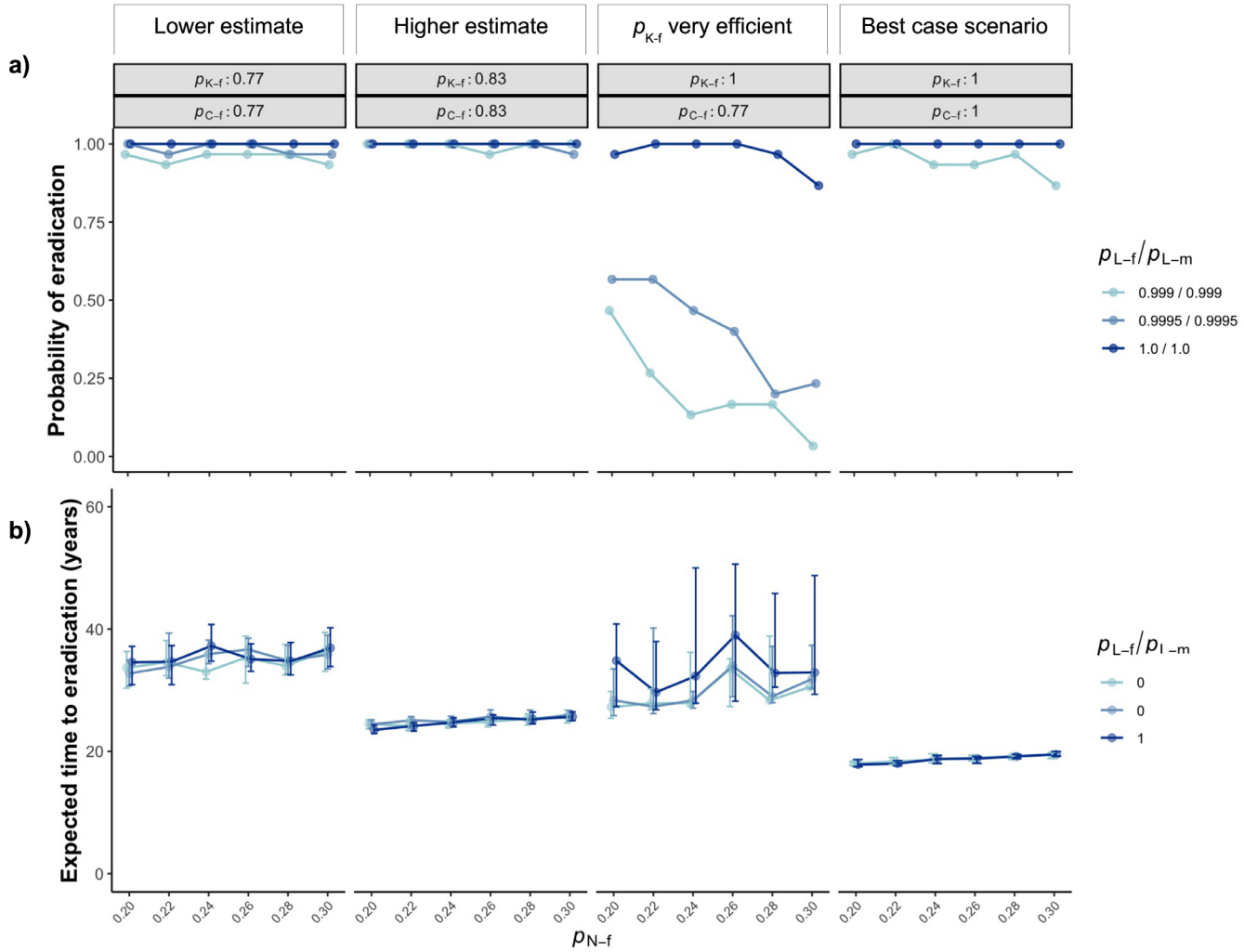

FIGURE 5 | (a) The probability of eradication with HR-KO-fs for the parameter ranges observed by Lai et al. (2023) ('lower' and 'higher') with two other scenarios where: Only the cutting and subsequent knockout of the unlinked female fertility gene is very efficient ('p_{K-f} very efficient', and the 'best-case scenario' where both cutting of the homing target and knockout of the female fertility gene are efficient. The probability of loss of function after end-joining (p_L) in either sex affected the eradication probabilities only when the knockout of female fertility gene was very efficient. (b) The expected time to eradication with interquartile ranges using HR-KO-fs drive when the eradication attempts were successful in simulations presented in (a). Expected time to eradication was shortest under the best-case scenario when $p_{C-f} = p_{K-f} = 1$ (based on 30 simulations for each parameter combination).

The predictions using the best number of trees derived from the BRT models fitted to the sensitivity analysis (Figure 7) supported the results obtained by brute-force simulations that eradication is possible with the HR-KO-fs drive for the gene conversion parameter estimates obtained by Lai et al. (2023). Increasing the cutting efficiency in both sexes $p_{C-f,m}$ increased eradication probabilities (Figure 7a,b). If the cutting in females is not very efficient ($p_{C-f} < 1$), the unlinked gene knockout probabilities should not be too high (Figure 7b); otherwise, there are fewer fertile females carrying the drive. For the same reason, unlinked gene knockout probabilities in both sexes should not be too high simultaneously (Figure 7c). If the loss of function after end-joining cannot be guaranteed ($p_{L-f,m} < 1$), a narrower range of gene drive conversion values produced eradication (cf. Figures 7 and S8). With HR-KO-ms, eradication could potentially be achieved if the unlinked knockout was greatly improved $p_{K-m} > 0.8$ (Figure S9).

4 | Discussion

Despite the relatively modest gene drive conversion in female rats obtained by Lai et al. (2023), our results show that these rates can potentially be leveraged to suppress a large population of invasive rats on islands using a number of different gene drive configurations. Permanent suppression could be achieved with conventional HD approaches; however, the evolution of functional resistant alleles prevented suppression when loss of function after end-joining was not guaranteed (i.e., $p_L < 1$).

Interestingly, an HR drive that targets an additional unlinked female fertility gene (HR-KO-fs) works as an efficient eradication drive even when the gene drive conversion is modest in female rats and is almost zero in male rats (Lai et al. 2023). Eradication failed if the unlinked target genes were male fertility or viability genes. With HR-KO-fs, eradication was possible

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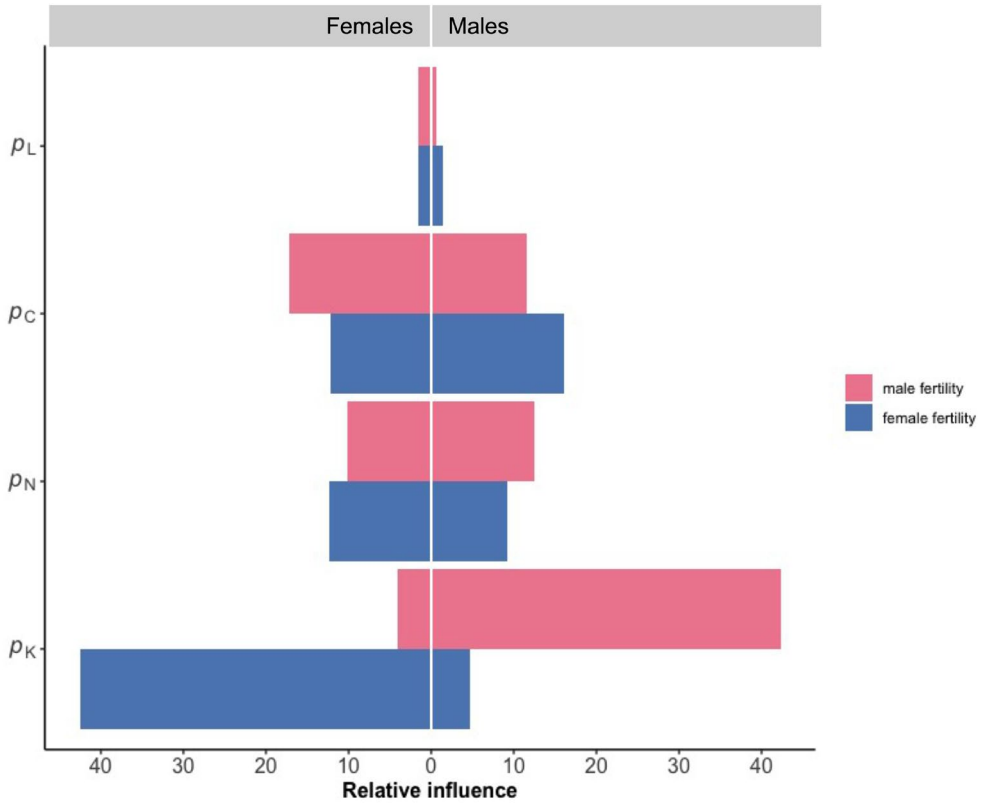

FIGURE 6 | The relative influence of gene drive parameters in females (left) and males (right) on the probability of eradication using HR-KO drive targeting unlinked female fertility (blue) or male fertility (pink) from Boosted Regression Tree models fit to the sensitivity-analysis output. Results are based on 3000 simulations for each approach (9000 simulations in total); eradication was successful in 1 out 3000 simulations when the unlinked target gene was a viability gene; therefore, it was not included in the analysis.

in approximately 20 years, and was largely independent of the probabilities of end-joining in females. The probability of a successful cut at the homing target (p_{C-f}) in females had a higher influence since (p_{C-f}) cutting is required for drive conversion. However, p_C also aided in drive's success by generating nonviable embryos when ($p_L < 1$). Nonfunctional resistant alleles ($r2$) at the homing target can be enhanced using a multi-gRNA approach or targeting critical amino acids (Prowse et al. 2017; Champer, Oh, et al. 2020). The formation of large quantities of $r2$ alleles, however, can slow the rate of spread of the drive (Metzloff et al. 2022; Yang et al. 2022).

Faber et al. (2024) recently proposed this two-target HR-KO drive and showed that with guaranteed loss of function after end-joining ($p_L = 1$) and efficient cutting ($p_C \geq 0.9$), eradication was possible (figure 5A in Faber et al. 2024). The evolution of functional resistant alleles at the target locus reduced eradication probabilities when $p_L < 0.99$ (with $p_C = 1.0$ and $p_N < 0.1$, figure 7 in Faber et al. 2024). Here, we expand on those results by investigating the efficiency of cutting at the homing target (p_C) and knockout of the distant gene (p_K) separately, which provided counter-intuitive but useful new insights into the evolution of functional resistant ($r1$) alleles at the homing target when $p_C < p_K$ (Faber et al. 2024, assumed $p_C = p_K$). Surprisingly, the probability of end-joining (p_{N-f}) at the homing target became critical when the cutting and subsequent knockout of the unlinked target (p_{K-f}) was more efficient than cutting at the homing target in females (p_{C-f}). Efficient unlinked gene knockout created a dearth of fertile females carrying the drive, slowing population suppression and increasing the opportunity for the evolution of resistant ($r1$) alleles when the loss of function after end-joining was not guaranteed (i.e., $p_L < 1$). However, it is reasonable to expect that cutting and unlinked target gene knockouts rate will be similar (i.e., $p_C \sim p_K$). If necessary, these could be empirically optimised using different gRNA sequences.

Improving unlinked gene knockout rates in males (p_{K-m}) when knockout rates were already efficient in females ($p_{K-f} \sim 1$) reduced eradication probabilities for the same reason. Otherwise, HR-KO-fs' eradication potential is largely independent of p_{K-m} (or vice versa when the target is male fertility; Figure S9). This asymmetry arises since the only way that an offspring could inherit a nonfunctional allele for the female fertility gene from its mother is if the mother has a single copy of the nonfunctional allele (heterozygous), or the allele has become nonfunctional in the germline due to the HR-KO drive (with probability p_{K-f}). However, the offspring could inherit a nonfunctional allele for the female fertility gene from a father who could be homozygous for the nonfunctional alleles; hence, the value of p_{K-m} is not as critical (unless knockouts in both sexes are very efficient, see below). Others have also observed that the target gene needs to affect the fertility of the sex in which gene drive conversion occurs (Deredec et al. 2008, 2011; Champer, Kim, et al. 2020; Liu and Champer 2022).

Our extrapolation of the cutting and homing values reported by Lai et al. (2023) to model the HD and HR-KO drive strategies in rats has important caveats. Lai et al. (2023) used the *endogenous*

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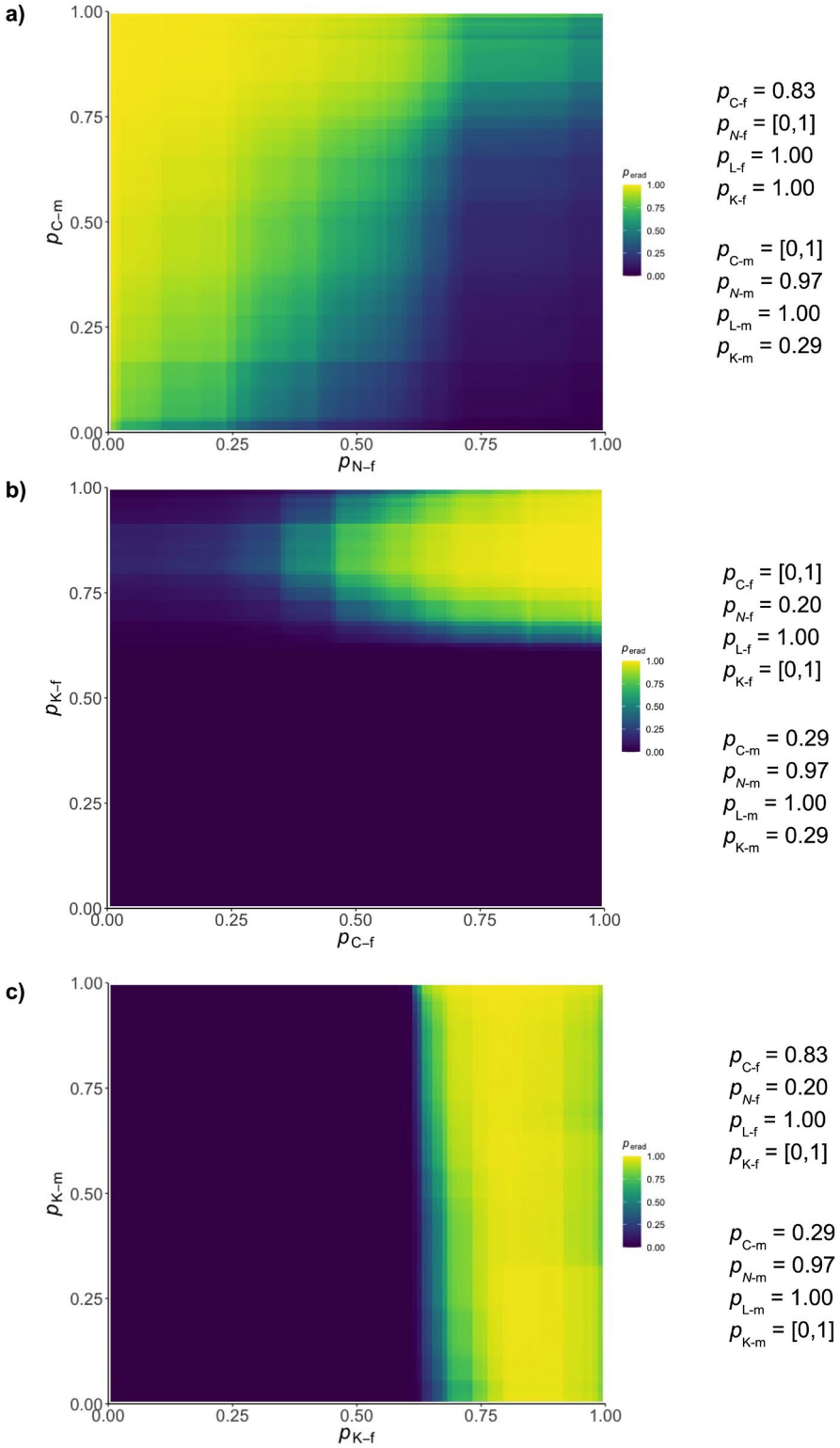

FIGURE 7 | The expected probability of successful eradication (p_{erad}) using HR-KO-fs drive targeting an unlinked female fertility gene estimated from the Boosted Regression Tree model used for sensitivity analysis varying (a) p_{N-f} and p_{C-m}, (b) p_{C-f} and p_{K-f}, and (c) p_{K-f} and p_{K-m} when the loss of function after end-joining could be guaranteed $p_{L-f,m=1.0}$ (for $p_{L-f,m=0.999}$, see Figure S8). Other parameters are given in tables next to the plots (based on 3000 simulations).

(*Ddx4*) promoter to drive Cas9. In contrast, we propose constructs that use a *Ddx4* promoter fragment to drive Cas9. The expression of Cas9 from the endogenous locus may differ from that of an exogenous transgene in terms of level and spatio-temporal regulation, factors that are known to impact homing efficiency (Weitzel et al. 2021). We also note that the highest homing efficiency in Lai et al. (2023) (67%) was generated using a single gRNA that likely cut at two distinct sites at the target locus, which may have increased the homing efficiency at this locus. If required, generating multiple cuts at the target locus

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could easily be accommodated into our designs by multiplexing gRNAs. More generally, it is important to note that while there are many factors that can influence homing efficiency, it appears that some species have an inherently higher propensity for homing than others. For example, homing in *Anopheles* mosquitoes can exceed 99% (Gantz et al. 2015; Hammond et al. 2016; Kyrou et al. 2018) while homing in *Aedes* mosquitoes is typically 70%–80% (Li et al. 2020; Anderson et al. 2023). Thus, we view the 67% homing reported by Lai et al. (2023) as an indication of what is possible in rats—indeed, it is possible that higher rates can be achieved given that only a handful of homing experiments have been performed. Cutting and homing efficiency could potentially be improved through the use of different promoters to control Cas9 timing and expression. Two different male and female germline-specific promoters could also be used to modulate and further control Cas9 timing and expression. An interesting feature of mammalian reproductive biology might also be exploited to enhance drive performative. A previous study in mice has shown that litter size can be partially rescued if lethality occurs prior to implantation (Douglas et al. 2021). Thus, integrating the HR construct within a haploinsufficient viability gene required for preimplantation development may allow a greater proportion of offspring harbouring homing alleles to be born, enhancing the spread of the drive construct.

The efficiency of the HR-KO drive in a spatially explicit model is reassuring (cf. Faber et al. 2024). The importance of including spatial dynamics when evaluating the eradication potential of gene drives has been underlined in previous studies (Champer, Kim, et al. 2021; Birand, Cassey, Ross, Russell, et al. 2022). Drive constructs that are predicted to have high eradication probabilities could fail in spatially explicit populations or get caught in endless cycles of local extinction and re-invasion by wild types, also known as 'chase-dynamics', due to low dispersal abilities of the organism studied. The effect of life history parameters, such as survival rates, polyandry rates, and dispersal abilities, on the efficiency of gene drives are now well known and have been investigated extensively (e.g., Birand, Cassey, Ross, Russell, et al. 2022; Birand, Cassey, Ross, Thomas, et al.2022; Champer, Kim, et al. 2021). Higher survival rates tend to extend the expected time to eradication and hence affect the probabilities of eradication within a certain time frame (Birand, Cassey, Ross, Thomas, et al. 2022; Moro et al. 2018). Higher polyandry rates could further reduce the drive efficiency if the drive carrying sperm has some competitive disadvantage (Manser et al. 2020; Birand, Cassey, Ross, Russell, et al. 2022; Gierus et al. 2022). Reliable data on life history, demography, and movement behaviour, specifically from potential target sites, are essential to enhance our understanding of the eradication potential of the gene drive strategies proposed. Even though additional factors (e.g., fitness cost, polyandry and sperm competition, embryonic activity of the maternally deposited Cas9) could impede the success of the proposed drive, the preliminary drive conversion rates reported by Lai et al. (2023) are still promising for developing gene drives in rats that could modify, suppress, or even eradicate large populations in reasonable time frames comparable to mice (Gierus et al. 2022). Several female fertility genes have already been identified in mice as potential targets for population suppression (Clark et al. 2024), some of which have sequence conservation that could allow them to be targeted in rats as well. Using genetic biocontrol might be particularly critical for eradication efforts on large islands since increasing island area was strongly associated with rat eradication failures (Holmes et al. 2015).

In summary, our data suggest that the modest drive conversion rates observed in vertebrates (and some non-vertebrates) may not be a barrier to the development of effective genetic biocontrol suppression systems. We also show that careful optimisation of gRNA cleavage rates, as well as maximising loss-of-function mutations locus through multiplexing, is critical for generating drive systems with maximal suppression potential.

Author Contributions

All authors designed the research. A.B. developed the computer code and produced the results. A.B. and T.A.A.P. carried out the analyses of data. A.B. and L.G. wrote the initial draft of the manuscript, with additional revisions by all authors. All authors provided comments and gave final approval for publication.

Acknowledgements

The authors would like to acknowledge the Traditional Owners of the land where we live and work, and we respect and value their past, present, and ongoing connection to the land and cultural beliefs. This research was supported by the Government of New South Wales, the Centre for Invasive Species Solutions, and the Government of South Australia. This work was also supported with supercomputing resources provided by the Phoenix High Performance Computing (HPC) service at the University of Adelaide. The authors would also like to thank Jackson Champer and two anonymous reviewers for their valuable comments that significantly improved our manuscript. Open access publishing facilitated by The University of Adelaide, as part of the Wiley - The University of Adelaide agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

C code is available from the Github platform https://github.com/abirand/HomingRescueInRats.

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Supporting Information

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