**SHORT COMMUNICATION**

# Scalability of genetic biocontrols for eradicating invasive alien mammals

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## Abstract

CRISPR-based gene drives offer novel solutions for controlling invasive alien species, which could ultimately extend eradication efforts to continental scales. Gene drives for suppressing invasive alien vertebrates are now under development. Using a landscape-scale individual-based model, we present the first estimates of times to eradication for long-lived alien mammals. We show that demography and life-history traits interact to determine the scalability of gene drives for vertebrate pest eradication. Notably, optimism around eradicating smaller-bodied pests (rodents and rabbits) with gene-drive technologies does not easily translate into eradication of larger-bodied alien species (cats and foxes).

## Keywords

Cat, fox, gene drive, invasive mammals, mice, rabbit, rat, spatial model

## Introduction

Alien vertebrates are some of the costliest invasive alien species worldwide (Diagne et al. 2021), directly causing species extinctions (Bellard et al. 2016) and driving profound environmental change (Pyšek et al. 2020). The risk of new invasive alien species continues to increase (Seebens et al. 2017, 2021), and is intimately linked to growth in globally expanding transportation networks, widespread rapid environmental change, and geopolitical forces - including intercontinental trade agreements. In Australia, mammals are the costliest invasive taxa; with feral cats (*Felis catus*), rodents (house mice *Mus musculus* and rats *Rattus spp.*), pigs (*Sus scrofa*), rabbits (*Oryctolagus cuniculus*), and red foxes (*Vulpes vulpes*) accounting for 95% of the total costs imposed by invasive mammals over the last 50 years (Bradshaw et al. 2021). Despite some notable successes in eradicating vertebrate invasive alien species on islands (Gregory et al. 2014), continental eradications remain elusive, and are greatly hampered by a lack of socio-political resourcing and will (Pluess et al. 2012). New tools are urgently needed.

CRISPR-based gene-drive approaches promise ground-breaking tools for the eradication or suppression of invasive alien species (Esvelt et al. 2014; Webber et al. 2015). By avoiding unwanted consequences to non-target organisms, genetic biocontrols offer many advantages over classical control methods such as poison baiting, trapping or hunting (Howarth 1991). There have been promising developments in laboratories using gene-drive technology in mosquitoes (Gantz et al. 2015; Kyrou et al. 2018), fruit flies (Gantz and Bier 2015; Champer et al. 2020), mice (Grunwald et al. 2019; Weitzel et al. 2021), and proof of principle for CRISPR gene editing has been demonstrated in cats (Brackett et al. 2022). Despite great interest in developing the technology for a range of vertebrate pests (Prowse et al. 2017; Moro et al. 2018; Prowse et al. 2018; Faber et al. 2021), the feasibility of achieving large-scale eradications of these species using gene drives has not been evaluated theoretically.

Here, we investigated how differences in the life-history traits of five invasive mammals (mice, rats, rabbits, feral cats, and red foxes) interact and influence the feasibility of deploying gene-drive technologies for population suppression at large spatial scales. We used an individual-based, spatially explicit, stochastic model that provides realistic estimates of eradication probabilities and expected times to eradication, due to its ability to model large population sizes at a landscape level (see Suppl. material 1: Tables S1, S2; Birand et al. 2022). We explored the effectiveness of a Y-chromosome-linked X-chromosome-shredding drive ("driving-Y"), which targets the X-chromosome for deletion during spermatogenesis with slightly imperfect efficiency (Fig. 1A). Population suppression is achieved by producing disproportionately more male offspring, and thus limiting female numbers. Theoretical models suggest that a driving-Y strategy could be effective for population eradication (Hamilton 1967; Deredec et al. 2008, 2011; Beaghton et al. 2016; Eckhoff et al. 2017; Prowse et al. 2019; Faber et al. 2021; Birand et al. 2022), and proof-of-concept for X chromosome shredding has been demonstrated in mouse zygotes (Zuo et al. 2017). We also modelled a CRISPR homing drive (see Suppl. material 1: Tables S1, S2) targeting female fertility that is predicted to be similarly effective (Prowse et

al. 2017; Birand et al. 2022), but may be challenging to generate (Grunwald et al. 2019; Pfitzner et al. 2020; Weitzel et al. 2021). Our motivation is to explore how species-specific life history and demographic traits influence eradication probabilities and times to eradication, rather than to evaluate the efficiencies of different gene-drive strategies *per se*, which is discussed extensively elsewhere (Champer et al. 2017; Unckless et al. 2017).

Based on density estimates in Australia (*d* in Table 1), we calculated the area (*A*) that each species would occupy, assuming a population size of roughly 200,000 individuals. We then modelled the required area for each species—from 40 km² for mice to 100,000 km² for cats and foxes—as a 64 × 64 grid of patches. We used historical (or experimental) invasion records to estimate the maximum distances ($\Delta_i$) that each species could disperse per breeding cycle. These distance estimates provide reliable representation of the distance each species would cover when the population density is low at the later stages of a successful suppression (Birand et al. 2022). A dispersal function was developed for each species that was both distance and negative density dependent, mimicking the fact that individuals would move long distances to find mates when densities are low (Diffendorfer 1998; Travis and French 2000; Matthysen 2005; Birand et al. 2022).

## Results and discussions

We initially simulated various spatial gene-drive release strategies and compared the simulated times to eradication for mice (Fig. 1B, C) to find an optimal release strategy that is fast, spatially expansive, but also conservative in terms of the laboratory effort required to produce gene-drive carrying individuals for release into the wild. The number of individuals released influenced the simulated time to eradication more than the spatial release strategy used. For example, releasing 4 gene-drive carrying individuals to 16 evenly-spaced patches had the same effect as releasing 1 individual to 64 evenly-spaced patches, except when the total number of individuals released was very low (less than 16, Fig. 1B), or when the dispersal distances were small (Fig. 1C). For the remainder of our study, we assumed an achievable release size of 256 individuals released into 256 evenly-spaced patches (i.e., one individual released per patch).

In order to capture the uncertainty in some of the demographic and dispersal parameters in our simulations, we generated uniform distributions based on the parameter ranges of the probabilities of survival ($\omega$) and polyandry ($p_m$), and for dispersal distances (*D*) (Table 1, also see Suppl. material 1: Tables S1, S2). We used these distributions to generate 1,000 unique parameter combinations for each species using Latin hypercube sampling (randomLHS, R package *lhs*, Carnell 2020). We ran one simulation for each parameter combination for 500 breeding cycles (Prowse et al. 2016), and calculated the times to eradication in years based on the estimates of number of breeding cycles ($n_c$) in a year for each species.

The probability of eradication for small-bodied species (0.97, 1.0, 1.0, respectively for mice, rats, and rabbits) was higher than for large-bodied species (0.50, and 0.89 respectively for cats and foxes, Fig. 1D). The probabilities were lower with the homing
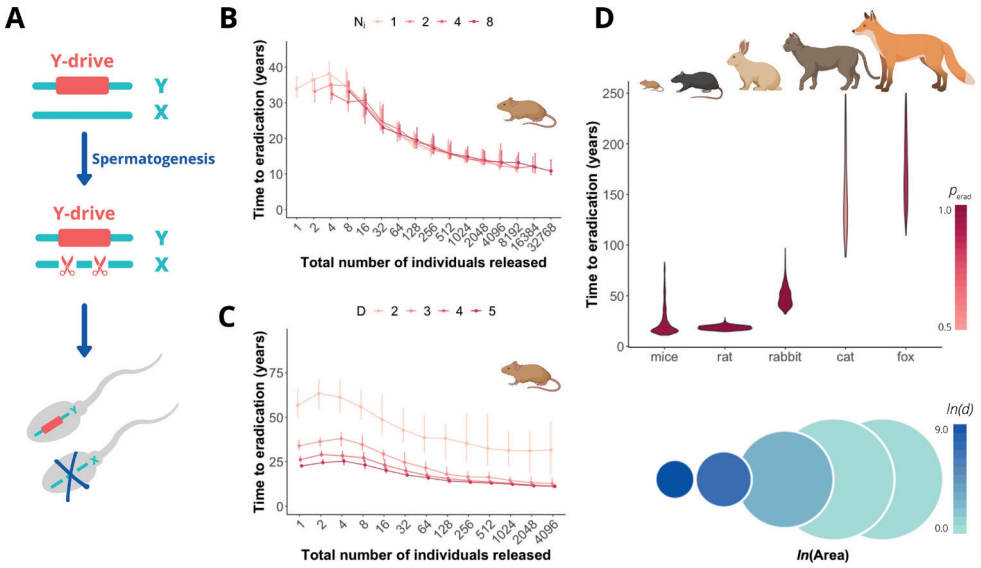
**Figure 1.** Times to eradication with various release strategies in mice and other invasive mammals using Y-drive. **A** the X-chromosome shredding Y-drive is located on the Y chromosome, and cuts the X chromosome at multiple locations during spermatogenesis (with probability $p_x = 0.96$). The X-bearing sperm are destroyed and eggs are predominantly fertilized by Y-bearing sperm, causing disproportionately more male offspring **B** interquartile ranges for the time to eradication of mice with various spatial release strategies when the number of individuals released per patch, $N_i$, is varied and the maximum dispersal distance $D = 3$ patches, and **C** when $N_i = 1$ and $D$ is varied (100 simulations for each combination) **D** violin plots showing the distributions of simulated times to eradication (1000 simulations for each species) and circles representing areas that each species with roughly 200,000 individuals would occupy. The colors of violin plots and circles represent probabilities of eradication and density estimates, respectively.

**Table 1.** Parameters that are related to the demography and life-history traits for each species, along with the areas that the species are assumed to occupy with roughly 200,000 individuals, based on density estimates obtained from literature. We note that these density estimates are used for area (A) calculation only, and due to the stochastic nature of the simulations, densities change through time and also across simulations.

| Species | $b$ | $n_c$ | $age_m$ | $\omega$ | $p_m$ | $d$ | $A$ | $\Delta_i$ | $D$ |
|---|---|---|---|---|---|---|---|---|---|
| Mouse | 6 | 6 | 2 | [0.48, 0.58] | [0.41, 0.51] | 5000 | 40 | 0.4 | [2, 4] |
| Black rat | 4 | 6 | 2 | [0.62, 0.67] | [0.63, 0.73] | 1000 | 200 | 2 | [7, 9] |
| Rabbit | 4 | 4 | 3 | [0.82, 0.87] | [0.15, 0.25] | 25 | 8000 | 12.5 | [7, 9] |
| Cat | 4 | 2 | 5 | [0.85, 0.90] | [0.20, 0.30] | 2 | 100000 | 25 | [3, 5] |
| Fox | 4 | 2 | 5 | [0.88, 0.93] | [0.71, 0.81] | 2 | 100000 | 45 | [7, 9] |

$b$: average number of offspring per breeding cycle; $n_c$: number of breeding cycles in a year; $age_m$: maximum age (years); $\omega$: probability of survival to the next breeding cycle; $p_m$: probability of multiple mating; $d$: density (km$^{-2}$); $A$: area (km$^2$); $\Delta_i$: invasion distance per breeding cycle (km); $D$: corresponding distances (number of patches) in the model.
References: Brothers et al. (1985); Williams (1996); Bowen and Read (1999); Say et al. (1999); Read and Bowen (2001); Abbott (2002); Devillard et al. (2003); Baker et al. (2004); Mutze (2009); Russell (2012); Cox et al. (2013); King et al. (2014); Shiels et al. (2014); Elliott et al. (2015); Harper and Bunbury (2015); Legge et al. (2017); Barnett et al. (2018); Moro et al. (2018); Fairfax (2019); Porteus et al. (2019); Murphy and Nathan (2021).

drive than with the Y-drive (0.88, 0.90,0.91, 0.57, and 0.87; for mice, rat, rabbit, cat, and fox, respectively) due to efficient DNA repair mechanisms resulting in the evolution of functionally resistant alleles. Based on sensitivity analysis results across all species, survival ($\omega$) had the highest influence (50.45%) on the simulated eradication probabilities with the Y-drive, followed by dispersal (46.71%) and probability of polyandry (2.83%) (Suppl. material 1: Table S1). Cats had the lowest eradication probability, and also had low dispersal ($D$) in the model (the relative influence of dispersal in probability of eradication in cats increased to 73.10%, Suppl. material 1: Table S1). Polyandry had higher influence in simulated times to eradication (28.95%), in which the relative influence of dispersal was reduced to 26.69%, and survival remained at similar levels (44.35%). We expect that polyandry might have a higher impact on eradication probabilities if the competitive ability of sperm from gene-drive carriers is reduced further than has been assumed here (Manser et al. 2020; Birand et al. 2022).

Median eradication times of roughly 200,000 individuals with the X-chromosome shredding drive were 17.7 years for mice, 18.5 years for rats, 48.0 years for rabbits, 142.3 years for cats, and 169.0 years for foxes, with nearly 90% population suppression achieved at half that time (Suppl. material 1: Table S2) (median times to eradication with the homing drive: 13.2, 14.3, 40.8, 121.5, and 110.5 years in the same species order). Simulated eradication times were much longer and also more uncertain in cats and foxes; in comparison to the shorter-lived species tested (Fig. 1D). Shorter dispersal distances resulted in longer times to eradication (e.g. Fig. 1C) mostly due to the emergence of "chase dynamics" where successive waves of local extinction and re-colonization by wild types prolonged the eradication attempt (Champer et al. 2021; Birand et al. 2022). In fact, the effect of dispersal in simulated times to eradication can override the effect of survival, and its relative influence in the time to eradication increase in species with low dispersal (Suppl. material 1: Table S1). For example, estimated eradication times for mice were more uncertain than those for rats (Fig. 1D). This is because, although mice have faster life histories than rats, they also have lower dispersal abilities so chase dynamics are more likely to arise. Similarly, lower dispersal in cats resulted in higher uncertainty in estimated eradication times compared to foxes. The relative influence of polyandry on simulated times to eradication also increased in species with high polyandry rates (Suppl. material 1: Table S1). It is certain that reliable estimates of the time to eradication will rely on a thorough understanding of movement and mating behaviors, particularly at low densities when gene drives begin to take effect.

## Conclusion

Our results are in agreement with theoretical models suggesting that gene drives could eradicate large populations of short-lived mammals successfully, within reasonable time periods, and could be an attractive alternative to current lethal control methods (Prowse et al. 2017, 2018; Prowse et al. 2019; Champer et al. 2021; Birand et al. 2022). In contrast, delayed eradication times for large and long-lived species could

render gene drives less attractive as control tools for such species. However, due to their lower densities, even if time to eradication using gene drives is high, control of long-lived species is potentially possible over very large areas.

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## Supplementary material 1

**Tables S1, S2**
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Data type: Pdf file
Explanation note: Methods.

Link: https://doi.org/10.3897/neobiota.74.82394.suppl1