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Evolutionary simulations of Z-linked suppression gene drives

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Synthetic gene drives may soon be used to suppress or eliminate populations of disease vectors, pathogens, invasive species, and pests. Recent proposals have suggested that one could use a gene drive carried on the Z chromosome to create male-biased sex ratios in species with ZW sex determination, such as Lepidoptera and the trematodes responsible for schistosomiasis. For example, a Z-linked 'W-shredder' might exhibit gene drive by cleaving the W chromosome and thereby causing carrier females to produce only sons. Here I use eco-evolutionary simulations to investigate W-shredders and other Z-linked gene drives, and thereby identify important design considerations. I conclude that W-shredders are likely to be highly effective at eradicating populations provided that resistance cannot evolve, but it may be hard to confine the drive allele to particular populations or geographic regions.

1. Introduction

Developments in genetic engineering will soon make it feasible to alter or eliminate populations of disease vectors, pathogens, agricultural pests, and invasive species using 'gene drives' [1–6]. Gene drives cause particular alleles (usually transgenes) to propagate through populations via a range of mechanisms, which include gene conversion, poison-antidote systems, segregation distortion, and genetic incompatibility [7–9]. For example, CRISPR-Cas9 gene editing can be used to create a transgenic insertion that is transmitted to almost 100% of the offspring of heterozygous individuals instead of the usual 50%; this type of gene drive functions by inducing a double-strand break at the homologous wild type locus, which is then repaired using the transgene as a template. Gene drives are often categorised into two types: replacement drives, which aim to spread a human-beneficial allele throughout a population (e.g. a mosquito allele that interferes with the transmission of malaria [1,10]), and 'suppression drives', which reduce the size of a population (potentially to extinction). Suppression drives typically work by using non-Mendelian inheritance to spread alleles that cause lethality or sterility [2,5,11], or skew the offspring sex ratio – typically towards males [12–16].

Recent theoretical papers have investigated the feasibility, efficacy, and potential negative consequences of various types of gene drives. For example, Noble et al. [6] used models to show that the basic version of a CRISPR-Cas9 gene drive might be highly invasive and could rapidly spread to fixation across whole species, which is often an undesirable outcome. Conversely, other models have concluded that gene drives are likely to fail if and when populations evolve resistance to their effects [17,18]. The issue of resistance is compounded because the standard implementation of CRISPR-Cas9 gene drive (but perhaps not updated versions; [4,5,18,19]) tends to create its own resistance alleles, e.g. when the double-stranded break induced by Cas9 is repaired using non-homologous end joining (NHEJ) instead of homology-directed repair [1–3,18,20]. Given the potential safety, ethical, and sociopolitical concerns surrounding gene drives, some models have focused on gene drives that would go extinct after a time [15,21,22], would stay confined to particular populations [11,22], and/or could be reversed once they have spread [23].

Here, I focus on the evolutionary dynamics of Z-linked suppression gene drives. The simulation is inspired by proposals for various types of Z-linked gene drives by Kevin Esvelt and colleagues, as well as ongoing efforts to develop these Z drives (see www.sculptingevolution.org; at the time of writing, these ideas have not been published in a journal or preprint). Various Z-linked suppression drives proposed by Esvelt and colleagues are shown schematically in Figure 1. Depending on its design, mode of action and the biology of the target species, Z chromosomes carrying the drive allele (denoted Z^*) might enjoy a transmission advantage in Z^*W females (Figure 1B, and perhaps also 1C), and optionally also in Z^*Z males. Esvelt et al. focus on using Z drives to control the *Schistosoma* trematodes responsible for schistosomiasis, though Z drives could theoretically be used to control any organism with female-heterogametic sex determination, such as Lepidopteran agricultural pests or even invasive populations of birds.

A Z-linked gene drive could suppress populations by biasing gametogenesis in females, for example by inducing double-stranded DNA breaks in the W chromosome in order to inactivate it; such a gene drive would be a 'W-shredder', analogous to the X- and Y-shredders under development for XY species [12,13,15,16,24,25]. Females carrying the gene drive would thus produce relatively few viable W-bearing eggs, and therefore produce mainly drive-carrying sons. Esvelt et al. point out that the evolutionary dynamics of the drive will depend on the fitness of drive carriers relative to wild types, the timing of W-shredding (e.g. in germ cells, ova, or zygotes), and the ecology of the target species. For example, some W-shredder designs might allow drive females to produce roughly the same number of (mostly-male) offspring as a wild-type female provided that the W chromosome is destroyed early enough in oogenesis/development that the lost daughters can be replaced by sons (Figure 1B). Alternatively, drive-carrying females might produce half the number of offspring (or less), e.g. if the drive works by destroying all ova or

53 offspring that carry a W chromosome, and this loss is not compensated by reduced competition
54 on the surviving offspring. As an alternative to W -shredders, Esvelt et al. also proposed that one
55 could suppress populations using a Z -linked locus that caused sterility or lethality in females,
56 either by shredding the W in somatic tissues, or by spreading some other allele that harms
57 females only. If this female-harming allele were capable of gene drive in males (see below), it
58 could perhaps reach high enough frequencies to suppress the population. The W -shredder could
59 be designed to also cause gene drive in males. Male gene drive could be accomplished using
60 'standard' CRISPR-Cas9 gene conversion, whereby the driving Z allele would convert the wild
61 type locus using homing endonuclease activity followed by homology-directed repair, causing
62 heterozygous males to produce mostly drive-carrying sperm. Esvelt et al. note that male gene
63 drive might not be necessary, since a Z -linked locus that prevents transmission of the W may
64 already enjoy a transmission advantage (Figures 1B-1C).

65 Here, I present an evolutionary simulation that can accommodate all of these hypothetical Z -
66 linked drives. I aimed to test which properties of the gene drive and the ecology of the target
67 species are critical to determining the likelihood and speed of extinction. For example, the gene
68 drive will presumably spread faster if it can bias transmission in both sexes, but perhaps a
69 simpler female-only drive would be perfectly adequate. Also, since the population will become
70 more male-biased as the gene drive invades, there will be eco-evo feedback that might affect the
71 evolutionary outcome in non-intuitive ways. For example, the altered sex ratio might intensify
72 the fitness advantage accruing to any resistant W chromosomes or autosomal modifiers that
73 prevent W -shredding (due to Fisherian selection for an even sex ratio; [26]), relative to that
74 observed in earlier models focusing on gene drives carried on autosomes [17,18]. Moreover, the
75 change in sex ratio could affect the ecology and evolution of the population, particularly if males
76 and females contribute differentially to density-dependent population growth [27,28], or have
77 different dispersal rates [29]. The model incorporates the possibility that Z -linked resistant-to-
78 drive alleles are sometimes created by NHEJ in heterozygote males, to test whether resistance is
79 just as problematic as for autosomal drives [1-3,18,20].

80 2. Methods

81 (a) Overview

82 I model a finite population of dioecious diploids with ZW sex determination, living in j discrete
83 habitat patches that are arranged linearly in a ring. The model considers the demography and
84 evolution of a population into which $n_{release}$ males carrying a Z -linked gene drive allele (Z^*) are
85 released. The drive allele causes either W -shredding or sterility in females, and optionally also
86 causes gene drive in heterozygous males (e.g. via gene conversion). Each generation proceeds
87 as follows: birth, dispersal between patches, breeding within patches, and death of the parental
88 generation. The species has 3 loci with 2-3 alleles each, some of which potentially show non-
89 Mendelian inheritance. The equilibrium population size was roughly 10,000 in all simulations
90 upon release of the gene drive, and the main outcomes of interest are the likelihood and speed of
91 extinction. The model is a stochastic individual-based simulation written in R 3.4.0; it was run on
92 the Spartan cluster at the University of Melbourne.

93 (b) Loci and alleles

94 Each male in the simulation carries one Z -linked locus and two autosomal loci, each with two
95 alleles. Each female carries a single allele at the Z -linked locus plus a W chromosome, as well as
96 two alleles at both of the autosomal loci.

97 There are three possible Z -linked alleles: a gene drive allele (Z^*), a wild-type allele (Z^+) that
98 is vulnerable to gene drive in Z^*Z^+ males, and a resistant allele (Z^r) that is immune to gene
99 drive in Z^*Z^r males. Similarly, there are two possible types of W chromosomes: a wild-type W

100 chromosome (W^+) that is vulnerable to gene drive by the Z^* allele, and a resistant W chromosome
 101 (W^r) that is immune to gene drive.

102 The two autosomal loci, denoted A/a and B/b , control immunity to W -shredding and gene
 103 conversion respectively. A/a and B/b are ‘trans-acting’ resistance loci, since they are at a different
 104 locus (indeed, a different chromosome) to the gene drive allele, in contrast to the ‘cis-acting’
 105 resistance conferred by the Z^r and W^r alleles. The A allele is dominant to a and confers immunity
 106 to Z -linked gene drive (e.g. W -shredding) in females. The B allele is dominant to b and confers
 107 immunity to Z -linked gene drive (e.g. gene conversion) in males.

108 (c) Calculating fitness

109 Wild-type individuals (i.e. those lacking drive or resistance alleles) have fitness $w = 1$, while other
 110 genotypes have $0 \leq w \leq 1$. The fecundity of females carrying Z^* is reduced by a factor $1 - c_f$.
 111 Small c_f implies minimal costs (e.g. because mothers replace lost gametes/offspring and/or sib-
 112 sib competition is intense), $c_f = 0.5$ could represent the case where all daughters die and are
 113 not replaced, and $c_f = 1$ means that females carrying Z^* are completely sterile. Setting $c_f = 1$
 114 allows us to model a female-sterilising Z -linked drive (not necessarily a W -shredder, though W -
 115 shredding in somatic tissues is one way to produce female-specific lethality/sterility). Similarly,
 116 the fitness of males carrying the gene drive is reduced by a factor $1 - c_m$; male fitness determines
 117 mating success (see below). For simplicity, I assume that the resistance alleles W^r , Z^r , A and B
 118 are cost-free. Also, the costs of Z^* to males were assumed to be dominant, such that Z^*Z^+ males
 119 and Z^*Z^* males had equal fitness.

120 (d) Gamete production and gene drive

121 I assume that the A/a and B/b loci segregate independently during meiosis and display standard
 122 Mendelian inheritance. Inheritance of the sex chromosomes is also Mendelian, except for certain
 123 genotypes carrying one Z^* allele.

124 Firstly, $Z^*W+aaBB$, $Z^*W+aaBb$, and $Z^*W+aabb$ females produce a fraction $\frac{1}{2}(1 + p_{shred})$ of Z -
 125 bearing gametes and $\frac{1}{2}(1 - p_{shred})$ W -bearing gametes. Therefore, these three female genotypes
 126 produce >50% sons when $p_{shred} > 0$, due to the shortage of W chromosomes in their gametes.
 127 The gamete frequencies of Z^*W^r females, or of females carrying at least one A allele, conform to
 128 the standard Mendelian expectations due to resistance.

129 Secondly, Z^*Z^+AAbb , Z^*Z^+Aabb , and Z^*Z^+aabb males produce a fraction $\frac{1}{2}(1 + p_{conv} -$
 130 $p_{conv}p_{nhej})$ of gametes carrying the Z^* allele, $\frac{1}{2}(1 - p_{conv})$ gametes carrying the Z^+ allele, and
 131 $\frac{1}{2}(p_{conv}p_{nhej})$ gametes carrying the Z^r allele. Thus, gene conversion occurs in males if $p_{conv} > 0$,
 132 meaning that the Z^* allele is over-represented in the gametes of these three male genotypes. The
 133 parameter p_{nhej} represents the creation of resistance alleles via non-homologous end joining, in
 134 which the gene drive fails to copy itself to the homologous chromosome, and instead induces an
 135 indel mutation that creates a resistant allele. The gamete frequencies of Z^*Z^r males, or of males
 136 carrying at least one B allele, conform to the standard Mendelian expectations due to resistance.

137 (e) Calculating female fecundity

138 In the breeding phase of the lifecycle, the simulation first determines the number of offspring
 139 produced by each female. The expected fecundity of female i (F_i) is affected by three factors:
 140 the female’s genotype, the density of males and females in the local patch and/or in the full
 141 population, and some global parameters in the model, as follows:

$$F_i = (1 + w_i r(1 - (D_i/K)^\alpha)) \quad (1)$$

142 where D_i is the ‘density’ experienced by female i , w_i is her fitness, K is the carrying capacity,
 143 and r and α are constants that control the maximum possible fecundity and the shape of density-
 144 dependence respectively (function from [30]).

145 To ensure that the simulation captures various possible types of life history and ecology, I
 146 calculated density D_i in various ways in different simulation runs. First, I define the 'global
 147 density' d_g , which acts equally on every female in every patch, as

$$d_g = \sum_{i=1}^{N_f} w_i + \delta N_m \quad (2)$$

148 where N_f and N_m are the numbers of females and males across all patches, the first term
 149 is the summed fitnesses of all these females, and δ is a constant (range: $0 - \infty$) that scales the
 150 effect of each male on d_g relative to a female with fitness $w_i = 1$. This formulation means that
 151 females with high relative fitness (i.e. fecundity) have a stronger effect on the global density than
 152 do low-fitness females. I also assume that each male contributes a fixed amount to the global
 153 density, irrespective of his genotype/fitness (since I assume that male fitness only affects male
 154 mating success; see below). The parameter δ represents sex differences in ecological niche use
 155 and behaviour. For example, we might expect $\delta < 1$ in species where males and females utilise
 156 very different environmental niches, or $\delta > 1$ in species where males are harmful to females.

157 Second, I define the 'local density' d_j , which is experienced by every female in patch j , as

$$d_j = \sum_{i=1}^{n_{f,j}} w_i + \delta n_{m,j} \quad (3)$$

158 where $n_{f,j}$ and $n_{m,j}$ are the numbers of females and males in patch j . As before, this
 159 formulation means that d_j depends on the fitnesses of the females in the patch, as well as the
 160 number of males (scaled by the constant δ).

161 Finally, the overall density experienced by female i in patch j (D_i) is a weighted sum of
 162 the global and local densities given by $D_i = \psi d_g + (1 - \psi) d_j$, where the parameter ψ weights
 163 the importance of global and local density to female fecundity. When $\psi = 0$, only local density
 164 matters and selection on females is entirely 'soft', while when $\psi = 1$ only global density matters
 165 and selection on females is completely 'hard' (as in [31]). Intermediate values of ψ produce a
 166 mixture of hard and soft selection on females.

167 After calculating the expected fecundity of each female (F_i), we generate the realised fecundity
 168 of the female by randomly sampling from a Poisson distribution with $\lambda = F_i$ (allowing for
 169 stochastic variation in fecundity between females with equal F_i). If the resulting number of
 170 offspring exceeded the global carrying capacity K , the model randomly selects K surviving
 171 offspring.

172 (f) Competition between males

173 After determining how many offspring each female produces, we determine the fathers of each
 174 of these offspring. We assume that all breeding occurs within patches, such that males only
 175 compete for matings/fertilisations with males in the same patch. If the patch contains k different
 176 male genotypes and there are n_1, n_2, \dots, n_k males of each genotype, the probability that a male of
 177 genotype k is the father of any given offspring is

$$p_j = \frac{n_k w_k}{\sum_{i=1}^k n_i w_i} \quad (4)$$

178 such that relatively common and/or high-fitness male genotypes are more likely to sire
 179 offspring. This formulation means that both sexes potentially reproduce with multiple partners.

180 (g) Reproduction, mutation and dispersal

181 After picking the parents, the model randomly generates each offspring's genotype according to
 182 its parents' expected gamete (and thus zygote) frequencies. Offspring are born in the same patch
 183 as their parents, and the parental generation is replaced by the offspring generation.

When an offspring is created, each Z^+ allele it carries has a chance μ_Z to mutate to a Z^r allele, and *vice versa* (i.e. mutation in both directions is equally probable). Similarly, each W^+ allele has a chance μ_W to mutate to a W^r allele, and *vice versa*.

Female and male offspring disperse to another patch with probabilities x_f and x_m respectively. We model two types of dispersal, in separate simulations: local dispersal, in which offspring move to one of the two neighbouring patches with equal probability (recalling that the patches are arranged in a ring), or global dispersal, in which dispersing offspring can land in any of the other patches.

(h) One compete run of the simulation

The model first initialises a population of 10,000 individuals (the carrying capacity, K) with low or zero frequencies of Z^r , W^r , A and B alleles, higher frequencies of the wild type Z^+ , W^+ , a , and b alleles, and zero Z^* gene drive alleles. It then runs 50 generations of burn-in to allow the population to reach demographic and genotypic equilibrium. Next, $n_{release}$ males with the genotype Z^*Z^*aabb are added to the population just before fathers are selected, representing the release into the wild of a laboratory-reared strain homozygous for the driving Z . In some simulations, all the Z^*Z^*aabb males were released in a single patch, while in others the $n_{release}$ males were randomly and evenly divided across all k patches. The model continued until either A) the driving Z^* allele went extinct, B) the population went extinct, C) the W^r chromosome went to fixation (making population suppression impossible), D) the Z^* allele fixed, but failed to cause population extinction, or E) 900 generations had elapsed. The model recorded which of these five outcomes occurred, as well as the allele frequencies, population size, and sex ratio at each generation.

(i) Investigating the parameter space

For each of the parameters in Table 1, I selected two or more possible parameter values (e.g. high versus low rates of W -shredding p_{shred} ; many versus few patches k). I then ran the model once for all possible combinations of these parameter values ($n = 6,000,000$ model runs). The aim was to measure the effect of each parameter across a background of assumptions for the other parameters, as well as to investigate all 2-way interactions between the parameters.

3. Results

(a) Three illustrative simulation runs

Figure 2 shows three contrasting simulation runs. In Figure 2A, the release of 20 Z^*Z^* males at generation 50 was followed by the rapid invasion of the Z^* allele, causing rapid extinction due to a lack of females. This simulation run assumed that the Z^* alleles causes perfect W -shredding ($p_{shred} = 1$), that Z^* has minimal fitness costs, and there is no resistance to W -shredding (Table S3).

In Figure 2B, Z^* invaded but failed to cause extinction, even though it was assumed that $p_{shred} = 1$ and W -shredding was not resistable. However, this simulation run did assume that individuals carrying at least one Z^* allele paid heavy fitness costs ($c_f = 0.5$ and $c_m = 0.2$), and that there was no gene drive in males ($p_{conv} = 0$). The assumptions $p_{shred} = 1$ and $c_f = 0.5$ could imply that the W -bearing eggs/offspring of Z^*W^+ females are destroyed but not replaced, such that W -shredding increases the proportion but not the absolute number of offspring that inherit the Z^* allele. Essentially Z^* spreads via ‘spite’ [32], in that it removes W chromosomes from the local population and thereby makes room for more Z^* alleles, creating indirect fitness benefits. However, the net fitness returns of the Z^* allele’s ‘strategy’ (i.e. sacrificing 20% fitness in males in order to remove W chromosomes in females) decline as females become rarer, allowing the fitness costs of Z^* to halt its spread.

Lastly, Figure 2C shows a case where the invasion of Z^* was reversed by the evolution of autosomal and Z-linked resistance alleles. Following the introduction of the Z^* allele, resistant Z^r mutants were created via non-homologous end joining, and then Z^r spread to fixation due to its immunity to gene conversion in males. The autosomal resistance allele A also spread; A confers resistance to W-shredding and was initially present in the population at 5% frequency. The spread of A caused the sex ratio to revert to normal, preventing extinction, and Z^* went extinct due to its direct fitness costs no longer being outweighed by the benefits of W-shredding and gene conversion. Incidentally, the resistant allele A was favoured over a because the male-biased population sex ratio created by Z^* favours the production of daughters, and AA and Aa females produce more daughters than aa females in populations where Z^* is present.

(b) Effects of each parameter on the evolution of a W-shredder

Figure 3 shows the effects of each model parameter, for models of a Z-linked W-shredder that potentially also benefits from gene drive in Z^*Z males. Figure S1 is similar but instead shows the number of generations until extinction on the y-axis. Under favourable assumptions, extinction occurred around 20 generations after releasing Z^* , though it often took longer (Figure S1). Tables S1-S2 give the relative frequencies of the various possible outcomes.

In Figure 3, the parameters are arranged in approximate order of their importance to extinction probability. By far the most important predictors of extinction were the efficiency of W-shredding in females (p_{shred}) and the existence of resistance against W-shredding: extinction never occurred unless p_{shred} was high and autosomal alleles conferring resistance to W-shredding (allele A in the model) were absent. This makes sense because a W-shredder cannot cause extinction unless Z^* -carrying females produce a strongly male-biased sex ratio and resistance to W-shredding cannot readily evolve. Extinction also occurred more quickly when p_{shred} was 1 rather than 0.95 (Figure S1).

The strength of gene drive in Z^*Z males (p_{conv} ; colours in Figure 3) also predicted extinction probability. However, p_{conv} was less important than p_{shred} , and the W-shredder frequently caused extinction even if it did not drive in males, or if resistance to male gene drive was common. The effect of male gene drive on extinction depended on other factors in the model (Figures 3, 4 and SXX); for example, male gene drive was at its most beneficial when resistance to it could not evolve (either through pre-existing genetic variation, or the creation of resistant Z^r alleles through NHEJ). Although its effects on extinction probability were somewhat small, male gene drive did hasten extinction considerably (Figure S1). For example, assuming perfect W-shredding, adding male gene drive with $p_{conv} = 0.95$ reduced the expected time to extinction from around 75 to 22 generations.

The cost of the Z^* allele to female fitness also affected extinction probability, and its effect interacted with the strength of gene drive in Z^*Z males. Specifically, assuming that the Z^* allele halves female fitness ($c_f = 0.5$) negates the fitness benefits of segregation distortion for the Z^* allele, and so extinction could only occur when $c_f = 0.5$ if there was gene drive in males. Reassuringly, increasing c_f from 0.01 or 0.1 had almost no effect on the likelihood of extinction, meaning that W shredders might be an effective means of population control even if females carrying the gene drive suffer a 10% fitness cost. Similarly, assuming that Z^* was costly to male carriers had little effect on extinction probability: extinction occurred almost as frequently when the reduction in male mating success was 20% rather than 1%. Both c_f and c_m were positively correlated with the time to extinction, particularly when there was no gene drive in males (Figure S1).

Several of the ecological variables also affected the extinction probability. Chief among these was the shape parameter of the density-dependence function, α . Setting $\alpha < 1$ causes female fecundity to decline at a decelerating rate with increasing population density, such that per-female fecundity only approaches its maximum value when the population is heavily depleted, making extinction more likely. Conversely for $\alpha > 1$, fecundity declines at an accelerating rate with increasing density, making extinction less likely due to the immediate increases in female

fecundity that manifest once the population begins to shrink. Unsurprisingly, I also found that populations in which females have a higher maximum possible fecundity (r) are more difficult to extirpate. Also, extinction was slightly more probable when female fecundity was determined by local density more than global density (ψ). This is because local density can remain high (and thus, per-female fecundity can remain low) even in meta-populations that are declining due to the spread of the Z^* allele in some of their sub-populations.

Extinction probability also increased with δ , the parameter that determines how male density affects female fecundity. When δ is high, female fecundity is constrained from increasing as the drive allele spreads by the ever-increasing proportion of males, contributing to extinction. Conversely, lower values of δ mean that male numbers are relatively unimportant in determining female fecundity, making extinction less likely because the shortage of females created by the gene drive alleviates competition on the remaining females. This result highlights that it is worth considering the ecology and population dynamics of target species when designing suppression drives that eliminate one sex.

Populations that are split into many semi-isolated patches were more difficult to drive extinct than those comparatively free of spatial structure, though the effect on extinction rate was small. The likely reason is that a highly-structured population creates refuges from the gene drive allele. The frequency and sex bias in dispersal was relatively unimportant to extinction probability, though there was a slight tendency for higher dispersal rates to stave off extinction, presumably because dispersal allows recolonisation of patches emptied by the gene drive. Similarly, it did not matter whether dispersal carried individuals to any patch, or only to neighbouring patches. Finally, there was no effect of the release strategy, suggesting that it may be unnecessary to release a W -shredding gene drive across the species' entire range provided that there is gene flow between patches. An additional implication of this result is that we cannot expect Z -linked gene drives to remain confined to their release sites, as previously found for autosomal drives [6].

306 (c) Effects of each parameter on a female-sterilising Z drive

I also used the model to examine the evolution of a Z -linked allele that causes gene drive in males and also causes total sterility in females ($c_f = 1$; Figure S2). This alternative type of gene drive was also effective at causing extinction, but only under the assumption that the population has little or no resistance to gene drive in males. For example, extinction never occurred if even 1% of the progeny of Z^*Z males inherited a resistant Z^r allele created by non-homologous end joining [c.f. 18]. Extinction also required that gene drive in males was strong (high p_{conv}), and that there were no autosomal resistance alleles to male gene drive. The effects of the other parameters in the model were similar as for a W -shredder (Figure S2), and extinction (when it occurred) took a fairly similar number of generations (around 25–30; Figure S3). As before, the Z^* allele sometimes went extinct, typically because the strength of gene drive in males was not sufficiently strong to overcome the fitness costs to drive carriers (Table S2).

318 (d) Interactions between model parameters

Many of the model parameters interacted in their effects on extinction probability (Figures 4 and SXX). For example, increasing p_{shred} only increased extinction probability provided that resistance to W -shredding was absent from the population, reaffirming the importance of resistance. Male gene drive was most beneficial when Z^*W females had half the fecundity of wild types and when p_{shred} was high, but male gene drive made little difference when $c_f \leq 0.1$ or p_{shred} was low. The demographic parameters α and r were important to extinction rate only when $p_{shred} \leq 1$; for $p_{shred} = 1$, the W -shredder was likely to cause extinction regardless of the ecological assumptions.

4. Discussion

The model shows that *W*-shredders are, in principle, very effective at eliminating populations, especially if Z^*W females produce no daughters ($p_{shred} = 1$) and resistance to *W*-shredding cannot evolve. The results have implications for the design of *Z*-linked *W*-shredders and female-sterilising suppression drives.

One design consideration is whether to engineer *W*-shredders that are also capable of gene drive in males, e.g. by including guide RNAs in the gene drive cassette that target the *Z* as well as the *W* chromosome. In the model, *W*-shredders very often caused extinction even without male gene drive (i.e. when $p_{conv} = 0$), provided that females carrying the *W*-shredder had comparable fecundity to wild type females, and that carrier females produce very few daughters (as in Figure 1B). Conversely if *W*-shredder females had low fecundity (around half that of a wild type, or below; Figure 1C) or produced some daughters, male gene drive was often essential for the *W*-shredder to cause extinction, or at least for extinction to occur rapidly enough to be useful. Although male gene drive was not always essential to extinction, it did reduce the number of generations until extinction occurred, sometimes substantially. Therefore, I conclude that it would almost certainly be worth the effort to incorporate a male-acting gene drive if developing a *W*-shredder for species with long generation times, such as invasive birds. Conversely, the rate of population decline may be adequate even without male gene drive for species that have multiple generations per year, such as Lepidoptera and *Schistosoma* parasites. Foregoing male drive could simplify the design of *W*-shredders since they would only need to target the *W* chromosome (and not also the *Z*), particularly because male-acting gene conversion drives have proved more challenging to develop in some taxa (due to sex differences in the use of homology-directed repair versus NHEJ; [33]). Conversely, strong male gene drive was always essential to extinction for female-sterilising suppression drives (Figure 1D). *Z*-linked alleles that drive in males and cause sterility in females were effective at causing extinction, but were very vulnerable to the evolution of resistance to male gene drive (e.g. via drive-resistant alleles created by NHEJ; [18]).

Another aim when designing *W*-shredders should be to ensure that female carriers produce as few daughters as possible (ideally none), while producing a large number of drive-carrying sons (ideally as many as the total offspring produced by non-carriers). This implies that one should ideally design a construct that cleaves the *W* chromosome early in gametogenesis or development, to increase the chance that the number of surviving progeny produced by each female is unaffected. Cleavage of the *W* should also be restricted to the female germ line, to minimise fitness losses due to the loss of the *W* in somatic cells. For some species, this may be as simple as placing the *W*-shredder under the control of a promoter such as *nanos* [34,35], assuming that females are able to replace lost *W*-bearing oocytes before they are provisioned with limiting resources. Even if the lost daughters are not replaced with sons, the Z^* allele might still exhibit drive because the surviving Z^* sons will experience reduced competition [somewhat like *Medea*; 36]. In Lepidoptera, juvenile density is often strongly negatively correlated with survival, and there are various maternally-transmitted endosymbionts that drive through populations by killing males to lessen competition on their infected sisters [e.g. 37,38]; these observations suggest that *W*-shredder alleles might invade Lepidopteran populations even if Z^*W females produced half as many viable eggs, though male gene drive would certainly help the invasion.

The *W*-shredding mechanism should also be designed in a way that makes it difficult for *W*-linked or *trans*-acting resistance to shredding to evolve. One way to do this would be to use a single guide RNA that targets high copy number *W*-specific sequences, or to use multiple guide RNAs that target multiple *W*-linked sequences [34]. This way, multiple changes to the reference sequence would be required for a *W* chromosome to acquire resistance to cleavage by the *W*-shredder. To ensure that the targets of cleavage do not become resistant as a result of indels induced by NHEJ, one can ensure that the guide RNA's target lies within an essential gene where an indel would be selectively disadvantageous, preventing resistant alleles from accumulating in the population. This may not be necessary if the *W*-shredder targets many *W*-linked loci, but it is an important design consideration for the male component of the gene drive, because the

379 evolution of Z-linked resistance completely nullified the usefulness of male gene drive in the
380 simulation (echoing [18]). Recent work demonstrated the feasibility of arrays containing many
381 guide RNAs separated by spacers [39], suggesting it may soon be easier to create gene drives
382 with multiple guide RNAs.

383 The model also indicated that extinction does not require the release of large numbers of
384 individuals: releasing just 20 Z^* males was often enough to eliminate a spatially-structured
385 metapopulation of 10,000 individuals in a few generations. On the one hand, this is advantageous
386 because W-shredders would be cheap and easy to deploy once they are developed, and they
387 are likely to extirpate whole metapopulations even if gene flow is weak. However, such high
388 invasiveness is not always desirable, because it makes the gene drive more difficult to restrict
389 to a particular area, country, or population. This could limit the usefulness of W-shredders to
390 control species like Lepidoptera and birds, where one may wish to eradicate only invasive or
391 agriculturally damaging populations, while leaving other populations untouched. Modifications
392 to gene drive design – such as the self-limiting ‘daisy drive’ system – are being developed to
393 address this important concern [21,22].

394 The model further showed that W-shredders can fail to cause extinction if carrier individuals
395 have low fitness, although extinction was frequently observed even if these fitness costs were
396 substantial. Populations in which females can become highly fecund as the population shrinks
397 (i.e. low α and high r) were also less likely to go extinct, though extinction tended to occur
398 anyway provided $p_{shred} = 1$. The model also highlighted that W-shredders, and indeed any gene
399 drive that creates a male-biased sex ratio, are most effective in suppressing species in which
400 the density of males is an important determinant of population growth, e.g. because males use
401 resources that females need [28]. By contrast if male density is not very important to population
402 growth (e.g. because females are limited by a resource that is not consumed by males), female
403 fecundity increases as females become rarer, slowing the decline in population size caused by the
404 W-shredder and potentially staving off extinction. Interestingly, the sexes are very different in the
405 *Schistosoma* trematodes responsible for schistosomiasis, which have been proposed as candidates
406 for control using a W-shredder by Kevin Esvelt and colleagues. Female *Schistosoma* live inside the
407 body of the much larger male, who feeds on the host’s blood and passes some of it to the female.
408 Presumably, this means that the number of males (not females) is the primary determinant of
409 whether a host/population is saturated, making *Schistosoma* a good candidate for control with
410 W-shredders. In Lepidoptera and birds – two other ZW taxa that could potentially be controlled
411 with W-shredders – males and females generally have very similar ecological niches, such that W-
412 shredders should be effective. Other ecological parameters like the patchiness of the population
413 (k), the frequency and sex bias of dispersal (x_f and x_m), and the scale of competition (ψ) had
414 relatively little effect on the probability of extinction.

415 Finally, I note that W-shredders might in general be easier to develop than X-shredders. Efforts
416 to develop an X-shredder in *Anopheles* mosquitos were initially hindered because the I-PpoI
417 protein that cleaves the X was paternally transmitted to the embryo inside sperm, causing all
418 embryos to die (not just daughters) due to cleavage of the maternally-inherited X. Although
419 this technical issue was later mitigated [13], such intergenerational effects would not trouble
420 a W-shredder since the W chromosome is unique to females (provided that the W-shredding
421 protein was not expressed in males and/or was not transmitted in their sperm). Additionally,
422 W-shredders might sometimes be easier to develop than gene drives that work by deleting genes
423 that are essential to female (but not male) fitness [e.g. 15]. This is because one could design a
424 prototype W-shredder based only on sequence data from the sex chromosomes, while identifying
425 genes with female-specific fitness effects requires more detailed data (e.g. expression profiling or
426 knockout studies) that are often unavailable.

427 5. Tables

428 **Table 1:** List of variables, and their corresponding parameter(s) in the model, which were varied
 429 in order to study their effects on the likelihood of population extinction.

Variable	Parameter(s)
Strength of gene drive in females (e.g. W-shredding)	p_{shred}
Strength of gene drive in males (e.g. gene conversion)	p_{conv}
Cost of gene drive allele to female fecundity	c_f
Cost of gene drive allele to male mating success	c_m
Frequency of W-linked resistance mutations	μ_W
Frequency of Z-linked resistance mutations and NHEJ	μ_Z and p_{nhej}
Frequency of autosomal resistance alleles	μ_A and μ_B
Patchiness of the population	k
Dispersal rate of males and females	x_m and x_f
Global versus local density-dependence of female fecundity	ψ
Contribution of males relative to females in density-dependence	δ
Number of gene drive carrier males released	$n_{release}$
Release strategy: all in one patch, or global	-
Fecundity of females at low population densities	r
Shape of density dependence	α

430 6. Figures

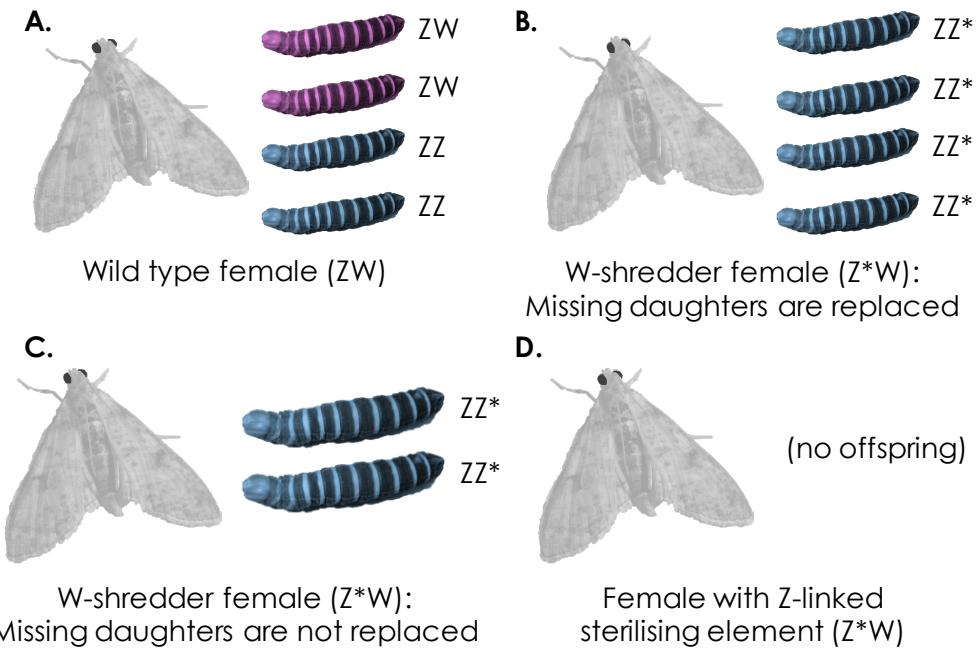


Figure 1. Some hypothetical Z-linked suppression drives considered in this study. Panel A illustrates normal inheritance of sex chromosomes in a wild type ZW female (assumed to be mated to a wild type ZZ male; not shown): the offspring sex ratio is even. In panel B, the female carries a *W*-shredder allele (Z^*) that kills gametes or offspring early enough that missing daughters are replaced with more Z^* -bearing sons. In panel C, the lost daughters are not replaced, though their absence increases the survival probability of the sons somewhat (shown by their larger size), causing super-Mendelian inheritance of the Z^* allele. Lastly, panel D shows a Z-linked female-sterilising allele (e.g. an allele that cleaves the *W* chromosome or a female-essential gene in somatic cells); since it is strongly disadvantageous in females, such an allele would go extinct unless it benefits from gene drive in heterozygous males.

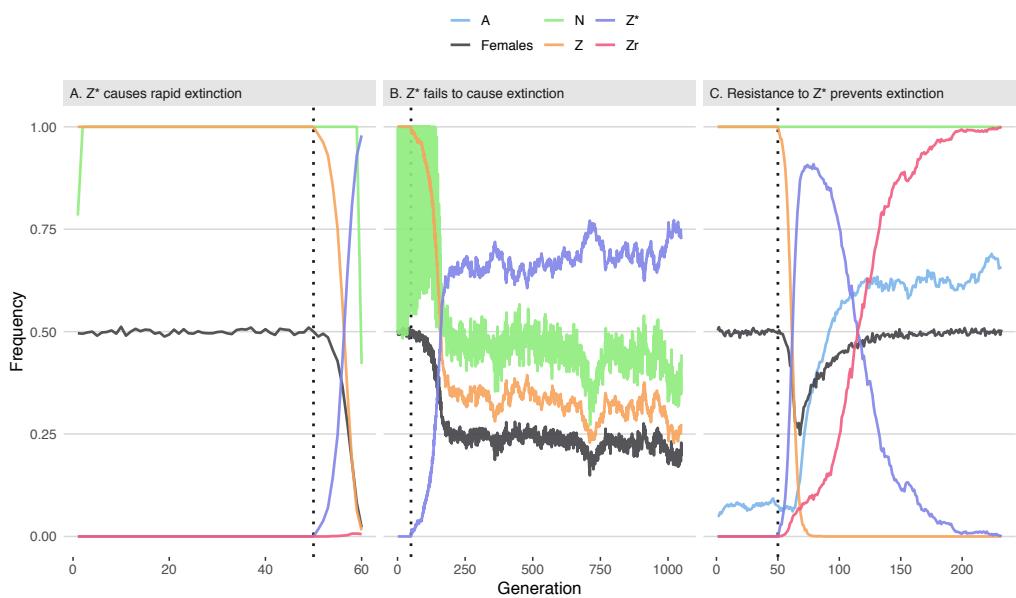


Figure 2. Three illustrative runs of the simulation, showing evolution in response to the introduction of 20 males carrying a W -shredder at Generation 50 (marked by dotted line). In panel A, the driving Z^* allele fixed very quickly, causing population extinction through a shortage of females. In panel B, the Z^* allele spread until its fitness costs began to negate its transmission advantage, causing the population to persist at a reduced size. In panel C, the Z^* allele invaded, which selected for the resistance alleles A and Z^r and caused Z^* to go extinct. The population size N is shown as a fraction of its maximum value of 10,000. Table S3 gives the parameter spaces used for these three runs.

431 Data Accessibility. A website presenting and describing all scripts used to run the data can be found at
432 XXX. Data underlying all figures can be downloaded from XXX.

433 Authors' Contributions. LH performed the analyses and wrote the manuscript.

434 Competing Interests. The author declares no conflict of interest.

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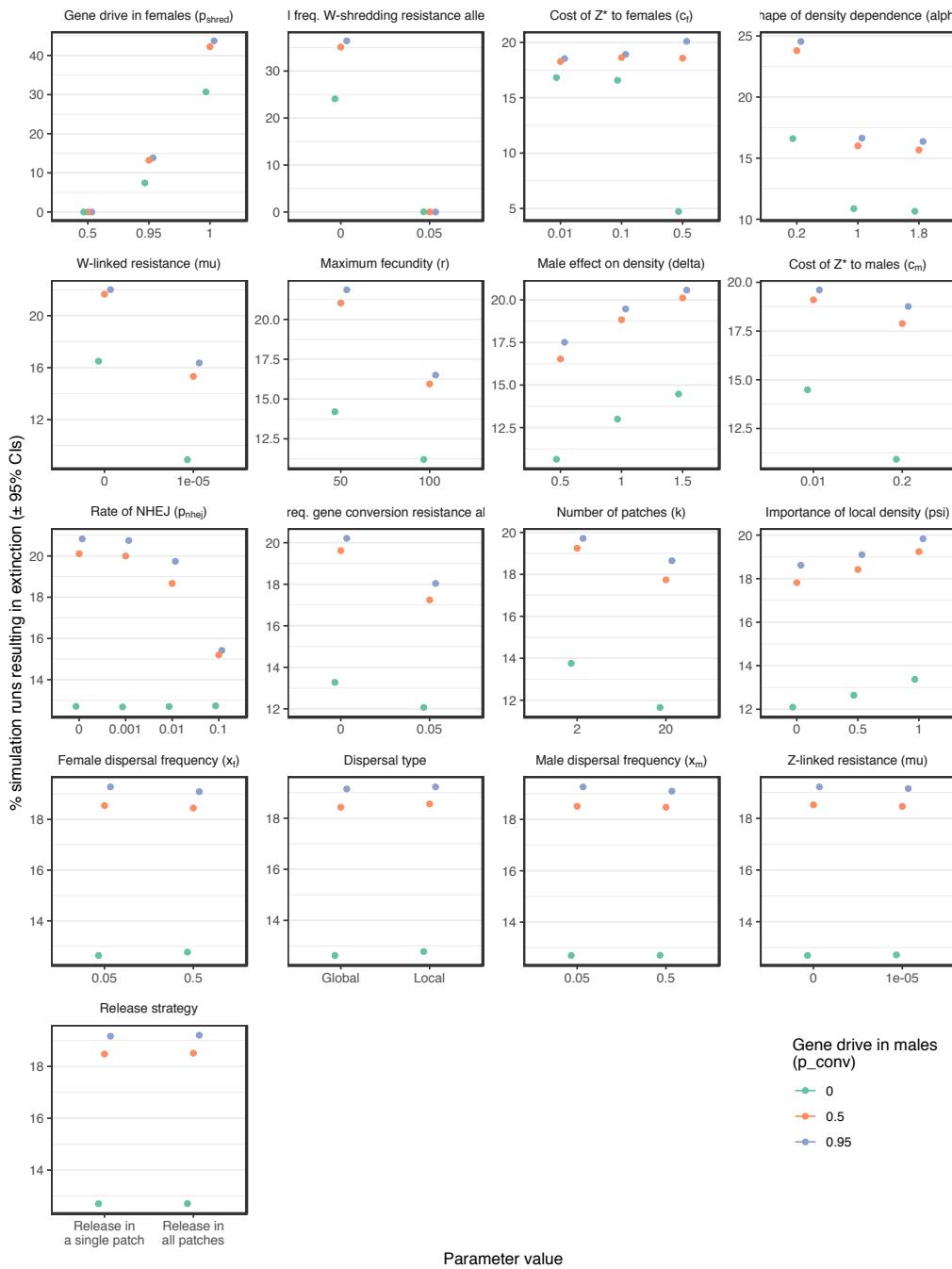


Figure 3. The percentage of simulations of a *W*-shredder that ended in extinction, for all runs with a particular value (shown on the *x*-axis) for a given parameter (shown in the panels). For example, there were no extinctions in any of the thousands of runs for which $p_{shred} = 0.5$, while 60% of runs where $p_{shred} = 1$ resulted in extinction. The panels are ordered by the range of the *x*-axis, which indicates the relative importance of each variable to extinction probability. Figure SXXX gives a similar plot for simulations of a female-sterilising Z^* allele.

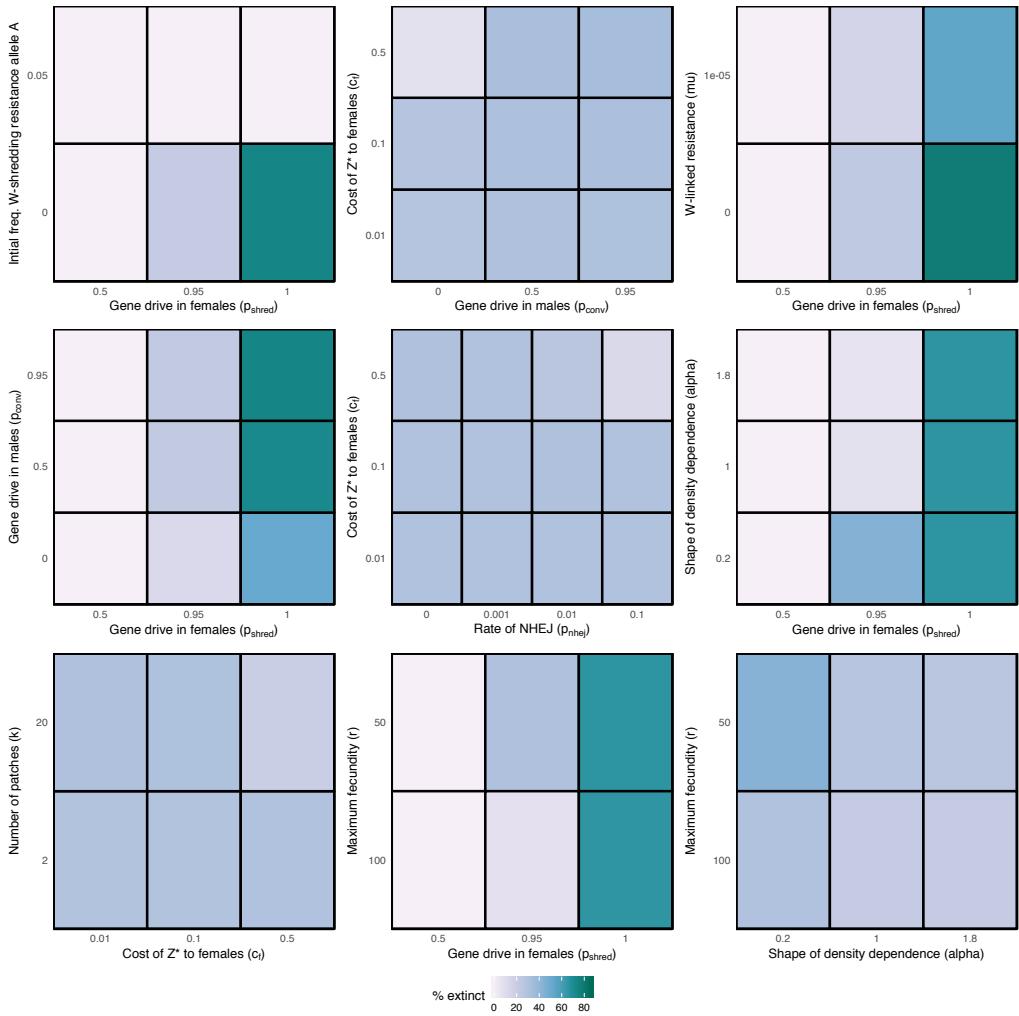


Figure 4. Heatmap showing the nine strongest interactions between pairs of parameters in the model, as determined by the GLM plotted in Figure SXXX.