

## Note to the editor and reviewers

There are two small errors with this version that I am aware of, but which I can easily fix at a later date. I didn't want to hold up the Special Issue by submitting my article late, and I don't think these errors will interfere with your review, so I hope you will accept my assurance that I will fix these two errors in the next submission.

The errors are:

- I have used the wrong journal's L<sup>A</sup>T<sub>E</sub>Xtemplate. I could not find a template anywhere for *Proceedings B*, but this one is similar, and could be re-formatted if I find the correct one.
- The figures in the supplementary material are not prepared to the same standard as the ones in the main text (e.g. the font is different and the Greek symbols did not render properly when I exported the figures from R). I will fix this later, as it involves a fair bit of manual graphic design work that I am too ill/busy to do this week.

Many thanks in advance for your input, time, and attention.

Luke Holman



Article submitted to journal

**Subject Areas:**

Evolutionary biology, Theoretical modelling, Gene drives

**Keywords:**

Sex chromosomes, Gene drives, Population control, Schistosomiasis, Selfish genes

<sup>1</sup>

**Author for correspondence:**

L. Holman

e-mail: [luke.holman@unimelb.edu.au](mailto:luke.holman@unimelb.edu.au)

## Evolutionary simulations of Z-linked suppression gene drives

Luke Holman<sup>1</sup>

<sup>1</sup>School of BioSciences, The University of Melbourne, Victoria 3010, Australia.

Synthetic gene drives may soon be used to suppress or eliminate populations of disease vectors, pathogens, invasive species, and agricultural pests. Recent proposals have focused on using Z-linked gene drives to control species with ZW sex determination, which include Lepidopteran pests and parasitic trematodes. These proposals include Z-linked ‘W-shredders’, which would suppress populations by cleaving the W chromosome and causing females to produce only sons, as well as Z-linked female-sterilising gene drives. Here I use eco-evolutionary simulations to evaluate the potential of some proposed Z-linked gene drives, and to produce recommendations regarding their design and use. The simulations show that W-shredders are likely to be highly effective at eradicating populations provided that resistance to W-shredding cannot evolve. However, the drive allele is likely to spread rapidly across meta-populations, making it unsuitable for some use cases.

## 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-stranded DNA 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] showed 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 populations can 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 an alternative DNA repair pathway (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](http://www.sculptingevolution.org); at the time of writing, these ideas have not been published elsewhere). 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. Esvelt et al. also proposed that one could suppress populations using  
 55 a  $Z$ -linked locus that caused sterility or lethality in females, either by shredding the  $W$  in somatic  
 56 tissues, or by spreading some other allele that harms females only. If this female-harming allele  
 57 were capable of gene drive in males, or were continually released into the wild, it could perhaps  
 58 reach high enough frequencies to suppress the population. The  $W$ -shredder could be designed  
 59 to also cause gene drive in males. Male gene drive could be accomplished using ‘standard’  
 60 CRISPR-Cas9 gene conversion, whereby the driving  $Z$  allele would convert the wild type locus  
 61 using homing endonuclease activity followed by homology-directed repair, causing heterozygous  
 62 males to produce mostly drive-carrying sperm. Esvelt et al. note that male gene drive might not  
 63 be necessary, since a  $Z$ -linked locus that prevents transmission of the  $W$  may already enjoy a  
 64 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 full description of the simulation is provided as Supplementary Material. In brief, I simulate a  
 82 finite population of dioecious diploids with  $ZW$  sex determination, living in  $j$  discrete habitat  
 83 patches that are arranged linearly in a ring, and examine the demographic and evolutionary  
 84 consequences of releasing  $n_{release}$  homozygote males carrying a  $Z$ -linked gene drive allele,  $Z^*$ .  
 85 The drive allele causes biased inheritance and/or reduced fecundity in females, and optionally  
 86 also causes biased inheritance in heterozygous males (e.g. via gene conversion). Each generation  
 87 proceeds in discrete steps: birth, dispersal between patches, breeding within patches, and death  
 88 of the parental generation. The equilibrium population size was roughly 10,000 in all simulations  
 89 upon release of the gene drive, and the main outcomes of interest are the likelihood and speed of  
 90 extinction. The simulation was written in R 3.4.0 and run on the Spartan cluster at the University  
 91 of Melbourne; Table 1 lists the simulation parameters that were manipulated to study their effects.

92 Each male carries two autosomal loci (termed  $A/a$  and  $B/b$ ) and one  $Z$ -linked locus, while  
 93 females carry both autosomal loci, a single allele at the  $Z$ -linked locus, plus a  $W$  chromosome.  
 94 There are three possible  $Z$ -linked alleles: the drive allele ( $Z^*$ ), a wild-type allele ( $Z^+$ ) that is  
 95 vulnerable to gene drive in  $Z^*Z^+$  males, and a resistant allele ( $Z^r$ ) that is immune to gene  
 96 drive in  $Z^*Z^r$  males. Similarly, there are two types of  $W$  chromosome: a wild-type  $W$  that is  
 97 vulnerable to shredding by the  $Z^*$  allele ( $W^+$ ), and an immune variant ( $W^r$ ). The alleles  $A$  and  
 98  $B$  are dominant ‘trans-acting’ resistance alleles that confer immunity to  $W$ -shredding and gene  
 99 conversion, respectively. The  $Z^*$  allele imposes a cost  $c_f$  on the fecundity of female carriers, and a  
 100 cost  $c_m$  on the mating success of male carriers. The resistance alleles  $W^r$ ,  $Z^r$ ,  $A$  and  $B$  are assumed  
 101 to be cost-free. Setting  $c_f = 1$  allows simulation of a female-sterilising  $Z$ -linked drive (Figure 1D).

102 Females carrying  $Z^*$  (and no  $A$  or  $W^r$  alleles) produce  $\frac{1}{2}(1 + p_{shred})$   $Z$ -bearing gametes and  
 103  $\frac{1}{2}(1 - p_{shred})$   $W$ -bearing gametes, and thus produce mostly sons when  $p_{shred} > 0$ . Secondly,

104  $Z^*Z^+$  males produce  $\frac{1}{2}(1 + p_{conv} - p_{conv}p_{nhej})$  gametes carrying the  $Z^*$  allele,  $\frac{1}{2}(1 - p_{conv})$   
 105 gametes carrying the  $Z^+$  allele, and  $\frac{1}{2}(p_{conv}p_{nhej})$  gametes carrying the  $Z^r$  allele. Thus, gene  
 106 conversion occurs in males if  $p_{conv} > 0$ , meaning that the  $Z^*$  allele is over-represented in the  
 107 gametes of these three male genotypes. The parameter  $p_{nhej}$  represents the creation of resistance  
 108 alleles via non-homologous end joining.

109 Female fecundity depends on the local and/or global density and fitness of other females,  
 110 and the density of males, via functions involving the parameters  $K$ ,  $r$ ,  $\alpha$ ,  $\delta$  and  $\psi$  (Table 1),  
 111 allowing the simulation to capture a variety of different ecologies and life histories. Female and  
 112 male offspring disperse to other patches with probabilities  $x_f$  and  $x_m$  respectively, allowing for  
 113 variable and sex-specific gene flow between patches. Dispersal was either local or global (i.e. to a  
 114 neighbouring patch or a random patch).

### 115 3. Results

#### 116 (a) Three illustrative simulation runs

117 Figure 2 shows three contrasting simulation runs. In Figure 2A, the release of 20  $Z^*Z^*$  males  
 118 at generation 50 resulted in invasion of the  $Z^*$  allele, causing rapid extinction due to a lack of  
 119 females. This simulation run assumed that the  $Z^*$  alleles causes complete  $W$ -shredding, that  $Z^*$   
 120 has minimal fitness costs, and there is no resistance to  $W$ -shredding (Table S3).

121 In Figure 2B,  $Z^*$  invaded but failed to cause extinction, even though it was assumed that  
 122  $p_{shred} = 1$  and  $W$ -shredding was not resistable. However, this simulation did assume that  
 123 individuals carrying at least one  $Z^*$  allele paid heavy fitness costs ( $c_f = 0.5$  and  $c_m = 0.2$ ), and  
 124 that there was no gene drive in males ( $p_{conv} = 0$ ). The assumptions  $p_{shred} = 1$  and  $c_f = 0.5$  could  
 125 imply that the  $W$ -bearing eggs/offspring of  $Z^*W^+$  females are destroyed and not replaced, such  
 126 that  $W$ -shredding increases the proportion but not the absolute number of offspring that inherit  
 127 the  $Z^*$  allele. Essentially  $Z^*$  spreads via ‘spite’ [30], in that it removes  $W$  chromosomes from the  
 128 local population and thereby makes room for more  $Z^*$  alleles, creating indirect fitness benefits.  
 129 However, the net fitness returns of the  $Z^*$  allele’s ‘strategy’ (i.e. sacrificing 20% fitness in males in  
 130 order to remove  $W$  chromosomes in females) decline as females become rarer, halting the spread  
 131 of  $Z^*$ .

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

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

143 Figure 3 shows the effects of each parameter for simulations of a  $W$ -shredder that potentially  
 144 also benefits from gene drive in  $Z^*Z$  males. Figure 4 shows the importance of each main effect  
 145 and two-way interaction term to the extinction probability, while Figure S1 shows the effect of  
 146 each parameter on the number of generations until extinction. Under favourable assumptions,  
 147 extinction occurred around 20 generations after releasing  $Z^*$ , though it often took longer (Figure  
 148 S1). Tables S1-S2 give the relative frequencies of the various possible outcomes (e.g. extinction of  
 149 the population, or loss of  $Z^*$ ).

150 In Figure 3, the parameters are arranged in order of their importance to extinction probability  
151 (see also Figure 4). By far the most important predictors of extinction were the efficiency of *W*-  
152 shredding in females ( $p_{shred}$ ) and the existence of resistance against *W*-shredding: extinction  
153 never occurred unless  $p_{shred}$  was high and autosomal alleles conferring resistance to *W*-  
154 shredding (allele *A* in the model) were absent. This makes sense because a *W*-shredder cannot  
155 cause extinction unless  $Z^*$ -carrying females produce a strongly male-biased sex ratio and  
156 resistance to *W*-shredding cannot readily evolve. Extinction also occurred more quickly when  
157  $p_{shred}$  was 1 rather than 0.95 (Figure S1), further highlighting efficient *W*-shredding as an  
158 important design consideration.

159 The strength of gene drive in  $Z^*Z$  males ( $p_{conv}$ ; colours in Figure 3) also predicted extinction  
160 probability. However,  $p_{conv}$  was less important than  $p_{shred}$ , and the *W*-shredder frequently  
161 caused extinction even when it showed normal Mendelian inheritance in males, or if resistance  
162 to male gene drive was common. The effect of male gene drive on extinction depended on other  
163 factors in the model (Figures 3, 4 and S2); for example, male gene drive was at its most beneficial  
164 when resistance to it could not evolve (either through pre-existing genetic variation, or the  
165 creation of resistant  $Z^r$  alleles through NHEJ). Although its effects on extinction probability were  
166 somewhat small, male gene drive did hasten extinction considerably (Figure S1). For example,  
167 assuming perfect *W*-shredding, adding male gene drive with  $p_{conv} = 0.95$  reduced the expected  
168 time to extinction from around 75 to 22 generations.

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

180 Some of the ecological variables also affected extinction probability. Chief among these was the  
181 shape parameter of the density-dependence function,  $\alpha$ . Setting  $\alpha < 1$  causes female fecundity to  
182 decline at a decelerating rate with increasing population density, such that per-female fecundity  
183 only approaches its maximum value when the population is heavily depleted, making extinction  
184 more likely. Conversely for  $\alpha > 1$ , fecundity declines at an accelerating rate with increasing  
185 density, making extinction less likely due to the immediate increases in female fecundity that  
186 manifest once the population begins to shrink. Unsurprisingly, populations in which females  
187 have a higher maximum possible fecundity ( $r$ ) were less likely to go extinct. Also, extinction  
188 was slightly more probable when female fecundity was determined by local density more than  
189 global density ( $\psi$ ). This is because local density can remain high (and thus, per-female fecundity  
190 can remain low) even in meta-populations that are declining due to the spread of the  $Z^*$  allele in  
191 some of their sub-populations.

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

200 Populations that are split into many semi-isolated patches were more difficult to drive extinct  
201 than those comparatively free of spatial structure, though the effect on extinction rate was small.

202 The likely reason is that a highly-structured population creates refuges from the gene drive allele.  
203 The frequency and sex bias in dispersal was relatively unimportant to extinction probability,  
204 though there was a slight tendency for higher dispersal rates to stave off extinction, presumably  
205 because dispersal allows recolonisation of patches emptied by the gene drive. Similarly, it did  
206 not matter whether dispersal carried individuals to any patch, or only to neighbouring patches.  
207 Finally, there was no effect of the release strategy, suggesting that it may be unnecessary to  
208 release a W-shredding gene drive across the species' entire range provided that there is gene  
209 flow between patches. An additional implication of this result is that we cannot expect Z-linked  
210 gene drives to remain confined to their release sites, as previously found for autosomal drives [6].

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

212 I also used the model to examine the evolution of a Z-linked allele that causes gene drive in males  
213 and also causes total sterility in females ( $c_f = 1$ ; Figures S3-S6). This alternative type of gene drive  
214 was also effective at causing extinction, but only under the assumption that the population has  
215 little or no resistance to gene drive in males. For example, extinction never occurred if even 1% of  
216 the progeny of  $Z^*Z$  males inherited a resistant  $Z^r$  allele created by non-homologous end joining  
217 [c.f. 18]. Extinction also required that gene drive in males was strong (high  $p_{conv}$ ), and that there  
218 were no autosomal resistance alleles to male gene drive. The effects of the other parameters in the  
219 model were similar as for a W-shredder, and extinction (when it occurred) took a fairly similar  
220 number of generations (around 25-30).

### 221 (d) Interactions between model parameters

222 Many of the model parameters interacted in their effects on extinction probability (Figures  
223 4 and S2). For W-shredders, increasing  $p_{shred}$  only increased extinction probability provided  
224 that resistance to W-shredding was absent from the population, reaffirming the importance of  
225 resistance. Male gene drive was most beneficial when  $Z^*W$  females had half the fecundity of wild  
226 types (i.e.  $c_f = 0.5$ ) and when  $p_{shred}$  was high, but male gene drive made little difference when  
227  $c_f \leq 0.1$  or  $p_{shred}$  was low. The demographic parameters  $\alpha$  and  $r$  were important to extinction  
228 rate only when  $p_{shred} \leq 1$ ; for  $p_{shred} = 1$ , the W-shredder was likely to cause extinction regardless  
229 of the ecological assumptions. For female-sterilising Z drives, the most important interaction  
230 terms underscored the importance of efficient and unresistable male gene drive (Figures S5-S6).

## 231 4. Discussion

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

236 One design consideration is whether to engineer W-shredders that are also capable of gene  
237 drive in males, e.g. by including guide RNAs that target the Z as well as the W chromosome. In  
238 the model, W-shredders very often caused extinction even without male gene drive (i.e. when  
239  $p_{conv} = 0$ ), provided that females carrying the W-shredder had comparable fecundity to wild  
240 type females, and that carrier females produce very few daughters (as in Figure 1B). Conversely  
241 if W-shredder females had low fecundity (around half that of a wild type, or below; Figure  
242 1C) or produced some daughters, male gene drive was often essential for the W-shredder to  
243 cause extinction, or at least for extinction to occur rapidly enough to be useful. Although male  
244 gene drive was not always essential to extinction, it did reduce the number of generations until  
245 extinction occurred, sometimes substantially. Therefore, I conclude that it would almost certainly  
246 be worth the effort to incorporate a male-acting gene drive if developing a W-shredder for species  
247 with long generation times, such as invasive birds. Conversely, the rate of population decline  
248 may be adequate even without male gene drive for species that have multiple generations per

249 year, such as Lepidoptera and *Schistosoma* parasites. Foregoing male drive could simplify the  
250 design of W-shredders since they would only need to target the W chromosome (and not also the  
251 Z), particularly because male-acting gene conversion drives seem more challenging to develop  
252 than female-acting ones in some taxa (due to sex differences in DNA repair; [31]). Conversely,  
253 strong male gene drive was always essential to extinction for female-sterilising suppression drives  
254 (Figure 1D). Z-linked alleles that drive in males and cause sterility in females were effective at  
255 causing extinction, but were very vulnerable to the evolution of resistance to male gene drive  
256 (e.g. via drive-resistant alleles created by NHEJ; [18]).

257 Another aim when designing W-shredders should be to ensure that female carriers produce  
258 as few daughters as possible (ideally none), while producing a large number of drive-carrying  
259 sons (ideally as many as the total offspring produced by non-carriers). This implies that one  
260 should ideally design a construct that cleaves the W chromosome early in gametogenesis or  
261 development, to increase the chance that the number of surviving progeny produced by each  
262 female is unaffected. Cleavage of the W should also be restricted to the female germ line, to  
263 minimise fitness losses due to the loss of the W in somatic cells. For some species, this may  
264 be as simple as placing the W-shredder under the control of a promoter such as *nanos* [32,33],  
265 assuming that females are able to replace lost W-bearing oocytes before they are provisioned with  
266 limiting resources. Even if the lost daughters are not replaced with sons, the  $Z^*$  allele might still  
267 exhibit drive because the surviving  $Z^*$  sons will experience reduced competition (somewhat like  
268 *Medea* [34]). In Lepidoptera, juvenile density is often strongly negatively correlated with survival,  
269 and there are various maternally-transmitted endosymbionts that drive through populations  
270 by killing males to lessen competition on their infected sisters (e.g. [35,36]); these observations  
271 suggest that W-shredder alleles might invade Lepidopteran populations even if  $Z^*W$  females  
272 produced half as many viable eggs, though male gene drive would certainly help the invasion.

273 The W-shredding mechanism should also be designed in a way that makes it difficult for W-  
274 linked or *trans*-acting resistance to shredding to evolve. One way to do this would be to use a  
275 single guide RNA that targets high copy number W-specific sequences, or to use multiple guide  
276 RNAs that target multiple W-linked sequences [32,37]. This way, multiple changes to the reference  
277 sequence would be required for a W chromosome to acquire resistance to cleavage by the W-  
278 shredder. To ensure that the targets of cleavage do not become resistant as a result of indels  
279 induced by NHEJ, one can ensure that the guide RNA's target lies within an essential gene where  
280 an indel would be selectively disadvantageous, preventing resistant alleles from accumulating in  
281 the population [32,37]. This may not be necessary if the W-shredder targets many W-linked loci,  
282 but it is an important design consideration for the male component of the gene drive, because  
283 the evolution of Z-linked resistance completely nullified the usefulness of male gene drive in the  
284 simulation (echoing [18]). Recent work demonstrated the feasibility of arrays containing many  
285 guide RNAs separated by spacers [38], suggesting it may soon be easier to create gene drives  
286 with multiple guide RNAs.

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

298 The model further showed that W-shredders can fail to cause extinction if carrier individuals  
299 have low fitness, although extinction was frequently observed even if these fitness costs were  
300 substantial. Populations in which females can become highly fecund as the population shrinks

(i.e. low  $\alpha$  and high  $r$ ) were also less likely to go extinct, though extinction tended to occur anyway provided  $p_{shred} = 1$ . The model also highlighted that W-shredders, and indeed any gene drive that creates a male-biased sex ratio, are most effective in suppressing species in which the density of males is an important determinant of population growth, e.g. because males use resources that females need [28]. By contrast, if male density is not very important to population growth (e.g. because females are limited by a resource that is not consumed by males), female fecundity increases as females become rarer, slowing the decline in population size caused by the W-shredder and potentially staving off extinction. Interestingly, the sexes are very different in the *Schistosoma* trematodes responsible for schistosomiasis, which have been proposed as candidates for control using a W-shredder by Kevin Esvelt and colleagues. Female *Schistosoma* live inside the body of the much larger male, who feeds on the host's blood and passes some of it to the female. Presumably, this means that the number of males (not females) is the primary determinant of whether a host or habitat is saturated, making *Schistosoma* a good candidate for control with W-shredders. In Lepidoptera and birds – two other ZW taxa that could potentially be controlled with W-shredders – males and females generally have similar ecological niches, such that W-shredders should be effective. Other ecological parameters like the patchiness of the population ( $k$ ), the frequency and sex bias of dispersal ( $x_f$  and  $x_m$ ), and the scale of competition ( $\psi$ ) had relatively little effect on the probability of extinction.

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

335 Data Accessibility. A website presenting all R scripts used to run the simulation and analyse the data  
336 can be found at [https://lukeholman.github.io/W\\_shredder/](https://lukeholman.github.io/W_shredder/).

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

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

339 Funding. This project was stimulated by an ESEB *Progress Meetings in Evolutionary Biology* meeting, funded  
340 by grants from ESEB (European Society for Evolutionary Biology) and from the Swiss National Science  
341 Foundation.

342 Acknowledgements. I thank the organisers (Anna Lindholm and Tom Price), funding bodies (European  
343 Society for Evolutionary Biology; Swiss National Science Foundation), and attendees of the 2018 ESEB *Progress*  
344 *Meetings in Evolutionary Biology*, which provided the impetus for this paper. I also thank Kevin Esvelt and  
345 colleagues for describing their ongoing research on a personal webpage; their ideas were instrumental to this  
346 paper.

## 347 References

- 348 1. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. 2015  
349 Highly efficient Cas9-mediated gene drive for population modification of the malaria vector  
350 mosquito *Anopheles stephensi*. *PNAS* **112**, E6736–E6743.
- 351 2. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker  
352 D, Marois E, Russell S et al.. 2016 A CRISPR-Cas9 gene drive system targeting female  
353 reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology* **34**, 78.
- 354 3. Wang Z, Pan Q, Gendron P, Zhu W, Guo F, Cen S, Wainberg MA, Liang C. 2016 CRISPR/Cas9-  
355 derived mutations both inhibit HIV-1 replication and accelerate viral escape. *Cell Reports* **15**,  
356 481–489.
- 357 4. Prowse TA, Cassey P, Ross JV, Pfitzner C, Wittmann TA, Thomas P. 2017 Dodging silver  
358 bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates. *Proc. Roy.  
359 Soc. B* **284**, 20170799.
- 360 5. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, Nolan T, Crisanti A. 2018  
361 A CRISPR-Cas9 gene drive targeting *doublesex* causes complete population suppression in  
362 caged *Anopheles gambiae* mosquitoes. *Nature Biotechnology*.
- 363 6. Noble C, Adlam B, Church GM, Esvelt KM, Nowak MA. 2018 Current CRISPR gene drive  
364 systems are likely to be highly invasive in wild populations. *eLife* **7**, e33423.
- 365 7. Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson  
366 H, Knief U, Kokko H, Larracuente AM, Manser A, Montchamp-Moreau C, Petrosyan VG,  
367 Pomiąkowski A, Presgraves DC, Safranova LD, Sutter A, Unckless RL, Price TAR. 2016 The  
368 ecology and evolutionary dynamics of meiotic drive. *Trends in Ecology & Evolution* **31**, 315–326.
- 369 8. Champer J, Buchman A, Akbari OS. 2016 Cheating evolution: engineering gene drives to  
370 manipulate the fate of wild populations. *Nature Reviews Genetics* **17**, 146.
- 371 9. Oberhofer G, Ivy T, Hay BA. 2019 Cleave and Rescue, a novel selfish genetic element and  
372 general strategy for gene drive. *PNAS* **116**, 6250–6259.
- 373 10. Marshall JM, Akbari OS. 2015 Gene drive strategies for population replacement. In *Genetic  
374 Control of Malaria and Dengue*, pp. 169–200.
- 375 11. Maselko M, Heinsch SC, Das S, Smanski MJ. 2018 Genetic incompatibility combined with  
376 female-lethality is effective and robust in simulations of *Aedes aegypti* population control.  
377 *bioRxiv* p. 316406.
- 378 12. Windbichler N, Papathanos PA, Crisanti A. 2008 Targeting the X chromosome during  
379 spermatogenesis induces Y chromosome transmission ratio distortion and early dominant  
380 embryo lethality in *Anopheles gambiae*. *PLOS Genetics* **4**, e1000291.
- 381 13. Galizi R, Doyle LA, Menichelli M, Bernardini F, Deredec A, Burt A, Stoddard BL, Windbichler  
382 N, Crisanti A. 2014 A synthetic sex ratio distortion system for the control of the human malaria  
383 mosquito. *Nature Communications* **5**, 3977.

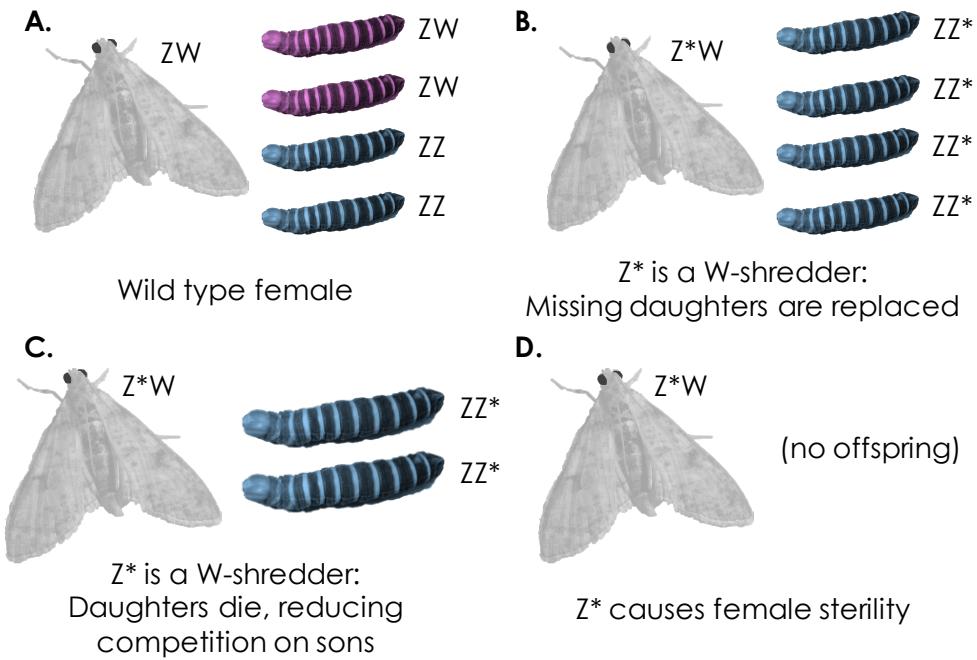
- 384 14. Beaghton A, Beaghton PJ, Burt A. 2017 Vector control with driving Y chromosomes: modelling  
385 the evolution of resistance. *Malaria Journal* **16**, 286.
- 386 15. Burt A, Derec A. 2018 Self-limiting population genetic control with sex-linked genome  
387 editors. *Proc. Roy. Soc. B* **285**, 20180776.
- 388 16. Papathanos PA, Windbichler N. 2018 Redkmer: An assembly-free pipeline for the  
389 identification of abundant and specific X-chromosome target sequences for X-shredding by  
390 CRISPR endonucleases. *The CRISPR Journal* **1**, 88–98.
- 391 17. Drury DW, Dapper AL, Siniard DJ, Zentner GE, Wade MJ. 2017 CRISPR/Cas9 gene drives in  
392 genetically variable and nonrandomly mating wild populations. *Science Advances* **3**, e1601910.
- 393 18. Unckless RL, Clark AG, Messer PW. 2017 Evolution of resistance against CRISPR/Cas9 gene  
394 drive. *Genetics* **205**, 827–841.
- 395 19. Esveld KM, Smidler AL, Catteruccia F, Church GM. 2014 Emerging technology: concerning  
396 RNA-guided gene drives for the alteration of wild populations. *eLife* **3**, e03401.
- 397 20. Gantz VM, Bier E. 2015 The mutagenic chain reaction: a method for converting heterozygous  
398 to homozygous mutations. *Science* p. aaa5945.
- 399 21. Min J, Noble C, Najjar D, Esveld KM. 2017 Daisyfield gene drive systems harness repeated  
400 genomic elements as a generational clock to limit spread. *BioRxiv* p. 104877.
- 401 22. Noble C, Min J, Olejarz J, Buchthal J, Chavez A, Smidler AL, DeBenedictis EA, Church GM,  
402 Nowak MA, Esveld KM. 2019 Daisy-chain gene drives for the alteration of local populations.  
403 *PNAS* p. 201716358.
- 404 23. Vella MR, Gunning CE, Lloyd AL, Gould F. 2017 Evaluating strategies for reversing CRISPR-  
405 Cas9 gene drives. *Scientific Reports* **7**, 11038.
- 406 24. North A, Burt A, Godfray HCJ. 2013 Modelling the spatial spread of a homing endonuclease  
407 gene in a mosquito population. *Journal of Applied Ecology* **50**, 1216–1225.
- 408 25. Prowse TA, Adikusuma F, Cassey P, Thomas P, Ross JV. 2019 A Y-chromosome shredding gene  
409 drive for controlling pest vertebrate populations. *eLife* **8**, e41873.
- 410 26. Holman L, Price TA, Wedell N, Kokko H. 2015 Coevolutionary dynamics of polyandry and  
411 sex-linked meiotic drive. *Evolution* **69**, 709–720.
- 412 27. Rankin DJ, Kokko H. 2007 Do males matter? The role of males in population dynamics. *Oikos*  
413 **116**, 335–348.
- 414 28. Li XY, Kokko H. 2019a Intersexual resource competition and the evolution of sex-biased  
415 dispersal. *Frontiers in Ecology and Evolution* **7**, 111.
- 416 29. Li XY, Kokko H. 2019b Sex-biased dispersal: a review of the theory. *Biological Reviews* **in press**.
- 417 30. Gardner A, West SA. 2006 Spite. *Current Biology* **16**, R662–R664.
- 418 31. Grunwald HA, Gantz VM, Poplawski G, Xu XrS, Bier E, Cooper KL. 2019 Super-Mendelian  
419 inheritance mediated by CRISPR-Cas9 in the female mouse germline. *Nature* **566**, 105.
- 420 32. Champer J, Liu J, Oh SY, Reeves R, Luthra A, Oakes N, Clark AG, Messer PW. 2018 Reducing  
421 resistance allele formation in CRISPR gene drive. *PNAS* **115**, 5522–5527.
- 422 33. Zhang Z, Niu B, Ji D, Li M, Li K, James AA, Tan A, Huang Y. 2018 Silkworm genetic sexing  
423 through W chromosome-linked, targeted gene integration. *Proceedings of the National Academy  
424 of Sciences* **115**, 8752–8756.
- 425 34. Hay BA, Chen CH, Ward CM, Huang H, Su JT, Guo M. 2010 Engineering the genomes of wild  
426 insect populations: challenges, and opportunities provided by synthetic Medea selfish genetic  
427 elements. *Journal of Insect Physiology* **56**, 1402–1413.
- 428 35. Jiggins F, Hurst G, Jiggins C, vd Schulerburg J, Majerus M. 2000 The butterfly *Danaus*  
429 *chrysippus* is infected by a male-killing *Spiroplasma* bacterium. *Parasitology* **120**, 439–446.
- 430 36. Jiggins FM. 2003 Male-killing *Wolbachia* and mitochondrial DNA: selective sweeps, hybrid  
431 introgression and parasite population dynamics. *Genetics* **164**, 5–12.
- 432 37. Oberhofer G, Ivy T, Hay BA. 2018 Behavior of homing endonuclease gene drives targeting  
433 genes required for viability or female fertility with multiplexed guide RNAs. *PNAS* **115**,  
434 E9343–E9352.
- 435 38. Kurata M, Wolf NK, Lahr WS, Weg MT, Kluesner MG, Lee S, Hui K, Shiraiwa M, Webber  
436 BR, Moriarity BS. 2018 Highly multiplexed genome engineering using CRISPR/Cas9 gRNA  
437 arrays. *PLOS ONE* **13**, e0198714.

## 331 5. Tables

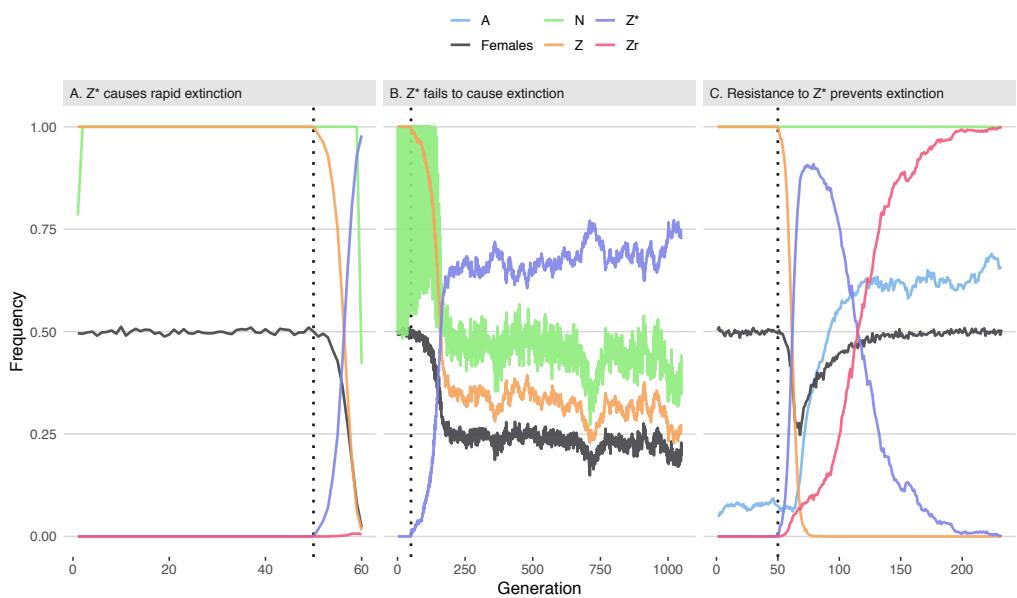
332 **Table 1:** List of variables, and their corresponding parameter(s) in the model, which were varied  
 333 in order to study their effects on extinction.

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	$\mu_W$
Frequency of Z-linked resistance mutations and NHEJ	$\mu_Z$ and $p_{nhej}$
Frequency of autosomal resistance alleles	$\mu_A$ and $\mu_B$
Patchiness of the population	$k$
Dispersal rate of males and females	$x_m$ and $x_f$
Global versus local density-dependence of female fecundity	$\psi$
Contribution of males relative to females in density-dependence	$\delta$
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	$\alpha$

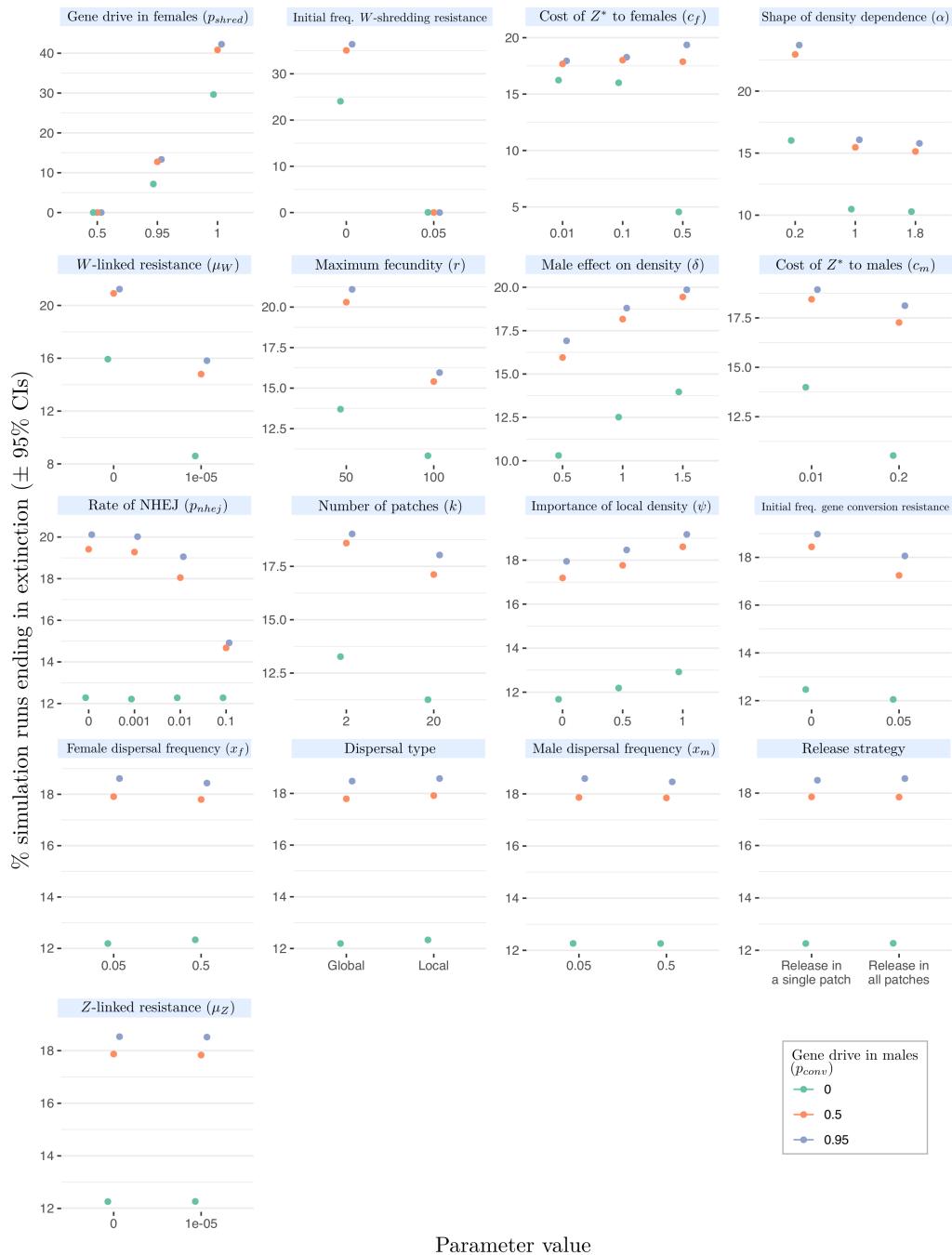
## 334 6. Figures



**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 (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<sup>r</sup>* 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.



**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 I assumed  $p_{shred} = 0.5$ , 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 S3 gives a similar plot for simulations of a female-sterilising  $Z^*$  allele.



**Figure 4.** Relative parameter importance in the simulations of  $W$ -shredders, for the top 25 most important main effects or two-way interactions (from a binomial GLM that included all the main effects and all their two-way interactions). Each predictor variable was scaled before running the model, meaning that the absolute effect size indicates how important each parameter is to the extinction probability, given the range of values plotted in Figure 3. Figure S5 gives a similar plot for simulations of a female-sterilising  $Z^*$  allele.

# Supplementary Material

From: Holman 2019, *Evolutionary simulations of Z-linked suppression gene drives*

The R scripts used to run the model and generate these figures and tables can be viewed at [https://lukeholman.github.io/W\\_shredder/](https://lukeholman.github.io/W_shredder/), along with annotations explaining the code.

## Supplementary Methods

### Calculating fitness

Individuals with no  $Z^*$  alleles have an intrinsic fitness of  $w = 1$ , while other genotypes have  $0 \leq w \leq 1$ . The fecundity of females carrying  $Z^*$  is reduced by a factor  $1 - c_f$ . Small  $c_f$  implies minimal costs (e.g. because mothers replace lost gametes/offspring and/or sib-sib competition is intense),  $c_f = 0.5$  could represent the case where all daughters die and are not replaced, and  $c_f = 1$  means that females carrying  $Z^*$  are completely sterile. Setting  $c_f = 1$  allows simulation of a female-sterilising Z-linked drive. Similarly, the fitness of males carrying  $Z^*$  is reduced by a factor  $1 - c_m$ ; male fitness determines mating success (see below). For simplicity, I assume that the resistance alleles  $W^r$ ,  $Z^r$ ,  $A$  and  $B$  are cost-free. Also, the costs of  $Z^*$  to males were assumed to be dominant, such that  $Z^*Z^+$  males and  $Z^*Z^*$  males had equal fitness.

### Gamete production and gene drive

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

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

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

### Calculating female fecundity

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

$$F_i = (1 + w_{ir}(1 - (D_i/K)^\alpha)) \quad (1)$$

where  $D_i$  is the ‘density’ experienced by female  $i$ ,  $w_i$  is her fitness,  $K$  is the carrying capacity, and  $r$  and  $\alpha$  are constants that control the maximum possible fecundity and the shape of density-dependence respectively (function from [@fowler1981de]).

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

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

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

Second, I define the local density  $d_j$  experienced by every female in patch  $j$ , as

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

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

Finally, the overall density experienced by female  $i$  in patch  $j$  ( $D_i$ ) is a weighted sum of the global and local densities given by  $D_i = \psi d_g + (1 - \psi)d_j$ , where the parameter  $\psi$  weights the importance of global and local density to female fecundity. When  $\psi = 0$ , only local density matters and selection on females is entirely ‘soft’, while when  $\psi = 1$  only global density matters and selection on females is completely ‘hard’ (as in [@li2018ev]). Intermediate values of  $\psi$  produce a mixture of hard and soft selection on females.

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

### Competition between males

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

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

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

## Reproduction, mutation and dispersal

After picking the parents, the model randomly generates each offspring's genotype according to its parents' expected gamete (and thus zygote) frequencies. Offspring are born in the same patch 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  $\mu_Z$  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  $\mu_W$  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.

## 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 without causing extinction, or E) 1000 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.

## 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 ( $n = 6,287,654$  model runs). The aim was to measure the effect of each parameter across various assumptions for the other parameters, as well as to investigate all 2-way interactions between the parameters. To gauge the relative importance of the various features of the  $Z^*$  allele and the species' ecology to the extinction probability, I fit a binomial generalised linear model (GLM) with extinction as the dependent variable, and all the model parameters and their 2-way interactions as predictors. The predictors were scaled and centred before running the GLM, allowing for a meaningful ranking of the predictors by their absolute effects on extinction.

## Supplementary tables

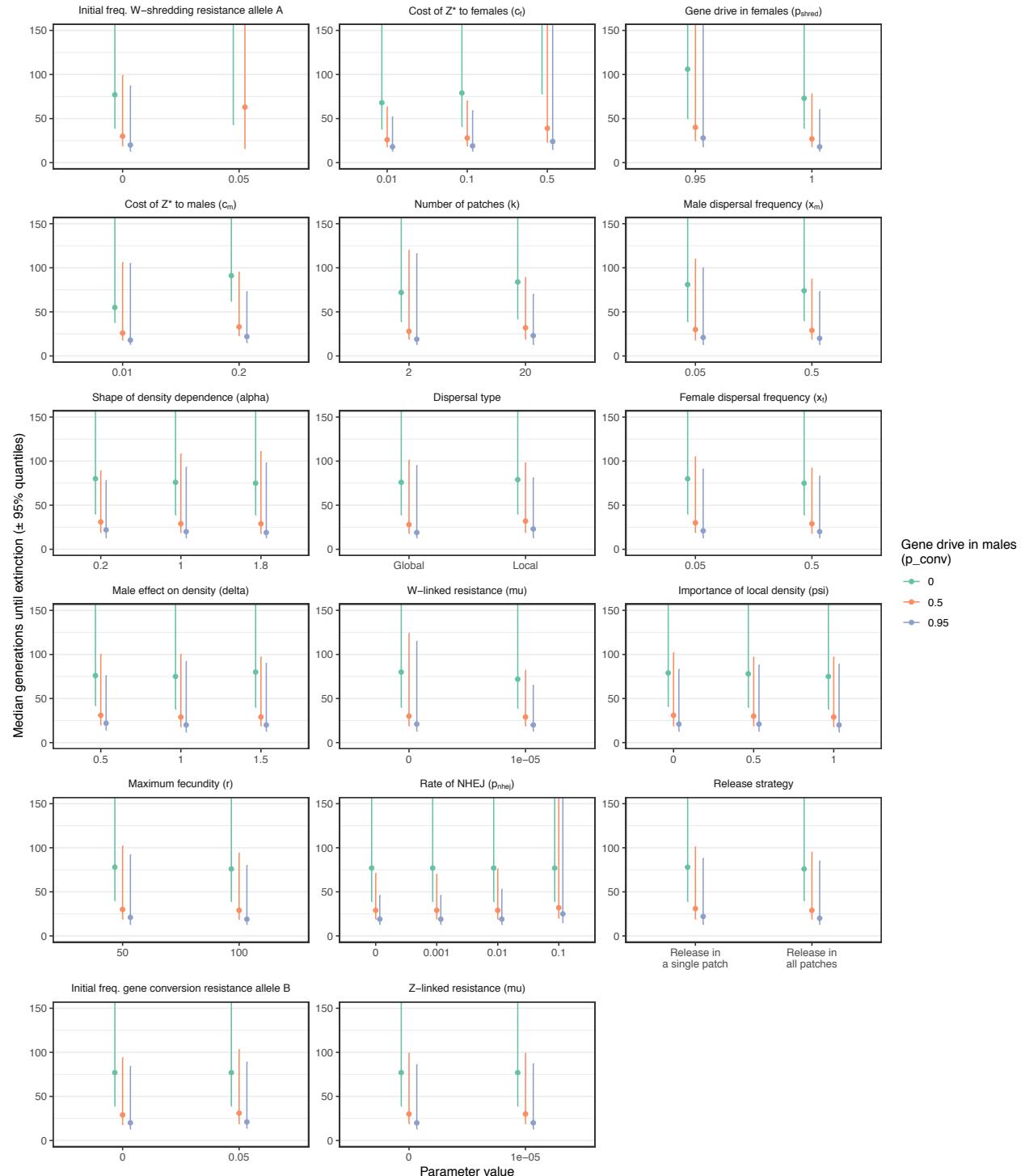
**Table S1:** The number and percentage of simulation runs (out of 5,657,700 total) that ended with the five possible outcomes, for the subset of simulation runs focusing on a *W*-shredder gene drive.

Outcome	Number of simulations	%
Z* fixed without causing extinction	2,330,324	41.2
Z* went extinct	1,584,409	28.0
Population went extinct	917,328	16.2
Wr fixed	689,487	12.2
Timer expired	136,152	2.4

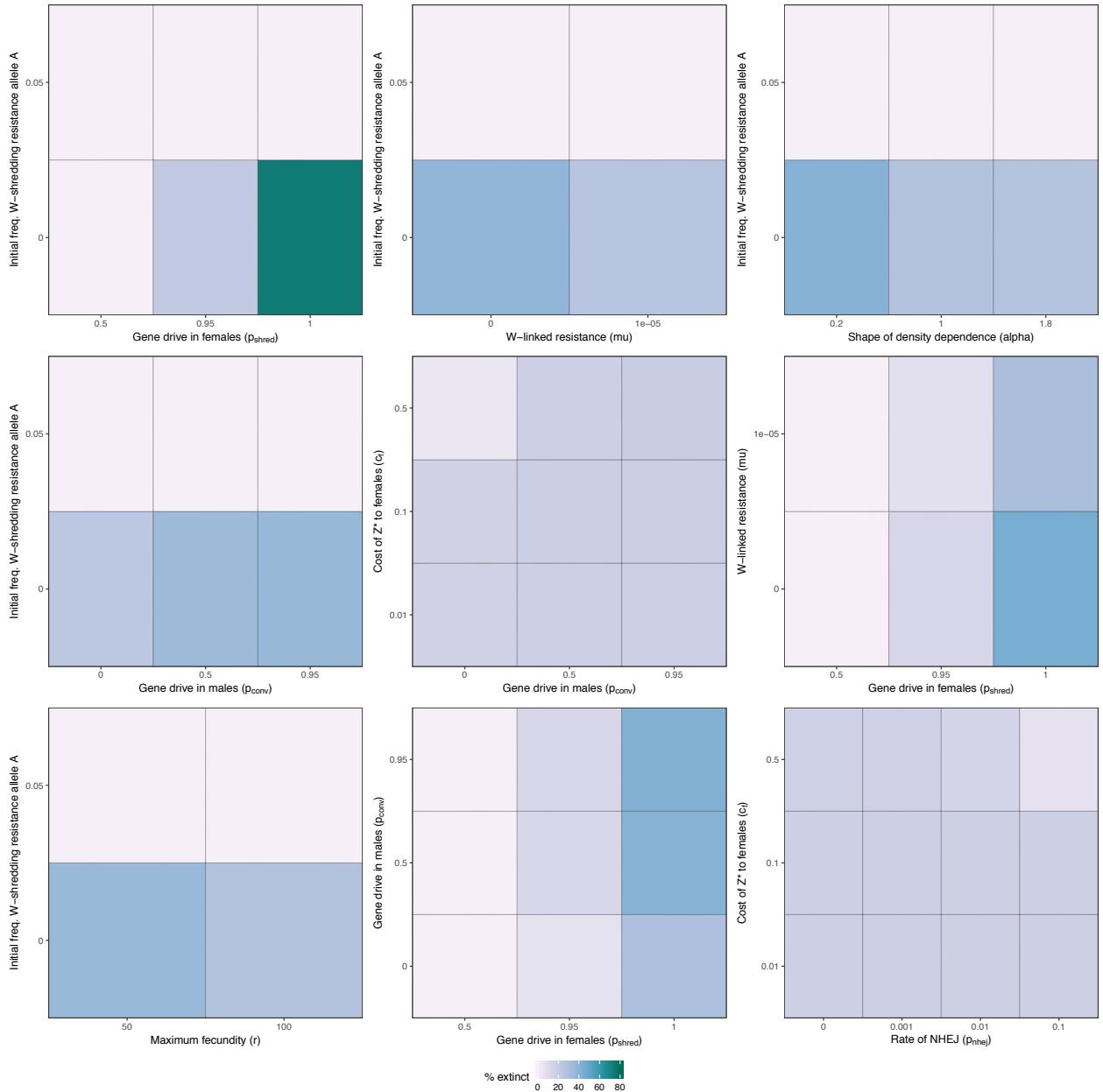
**Table S2:** The number and percentage of simulation runs (out of 629,954 total) that ended with the five possible outcomes, for the subset of simulation runs focusing on a female-sterilising *Z*-linked gene drive.

Outcome	Number of simulations	%
Z* went extinct	539,978	85.7
Timer expired	69,942	11.1
Population went extinct	12,992	2.1
Wr fixed	7,042	1.1

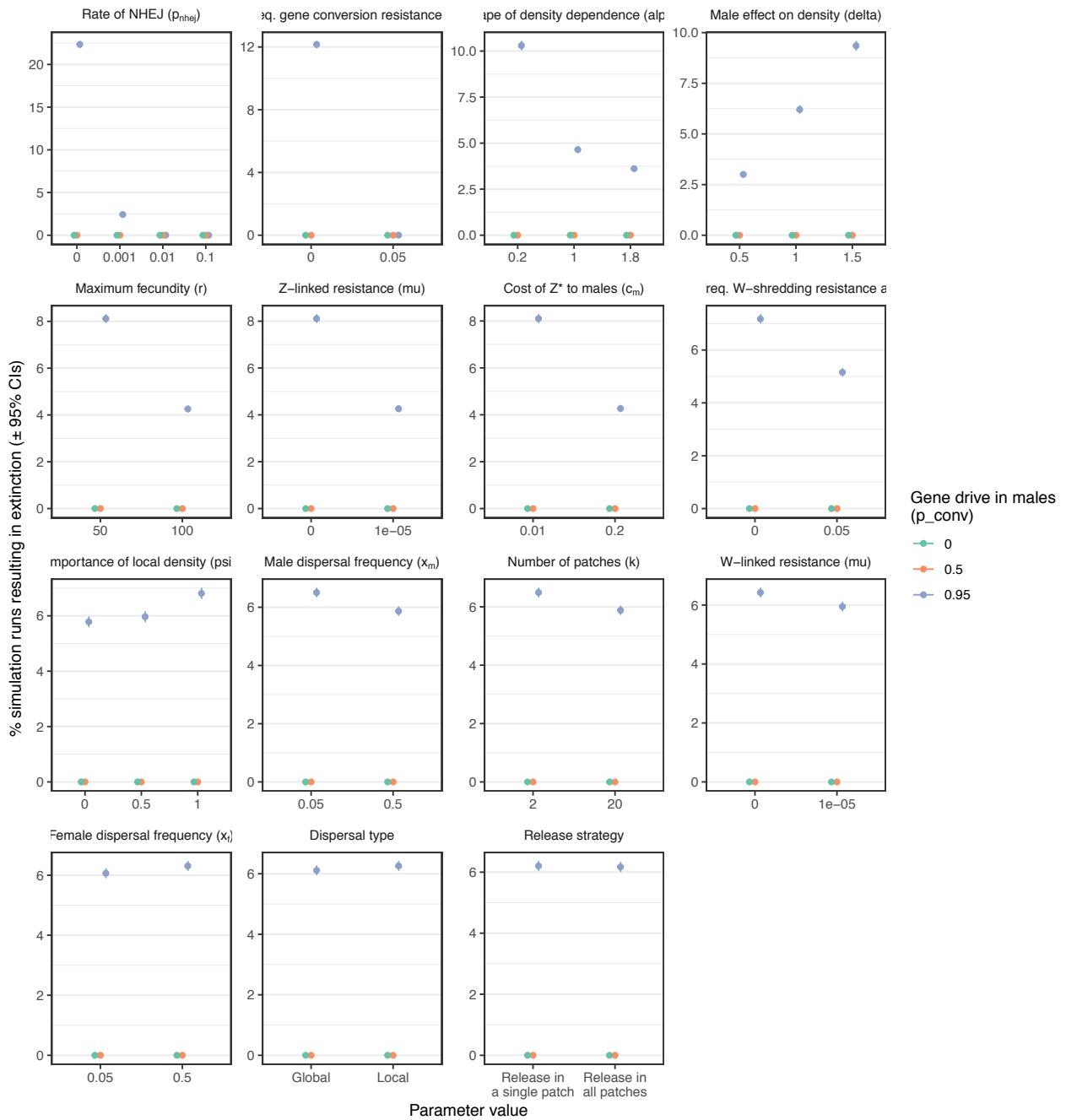
## Supplementary figures



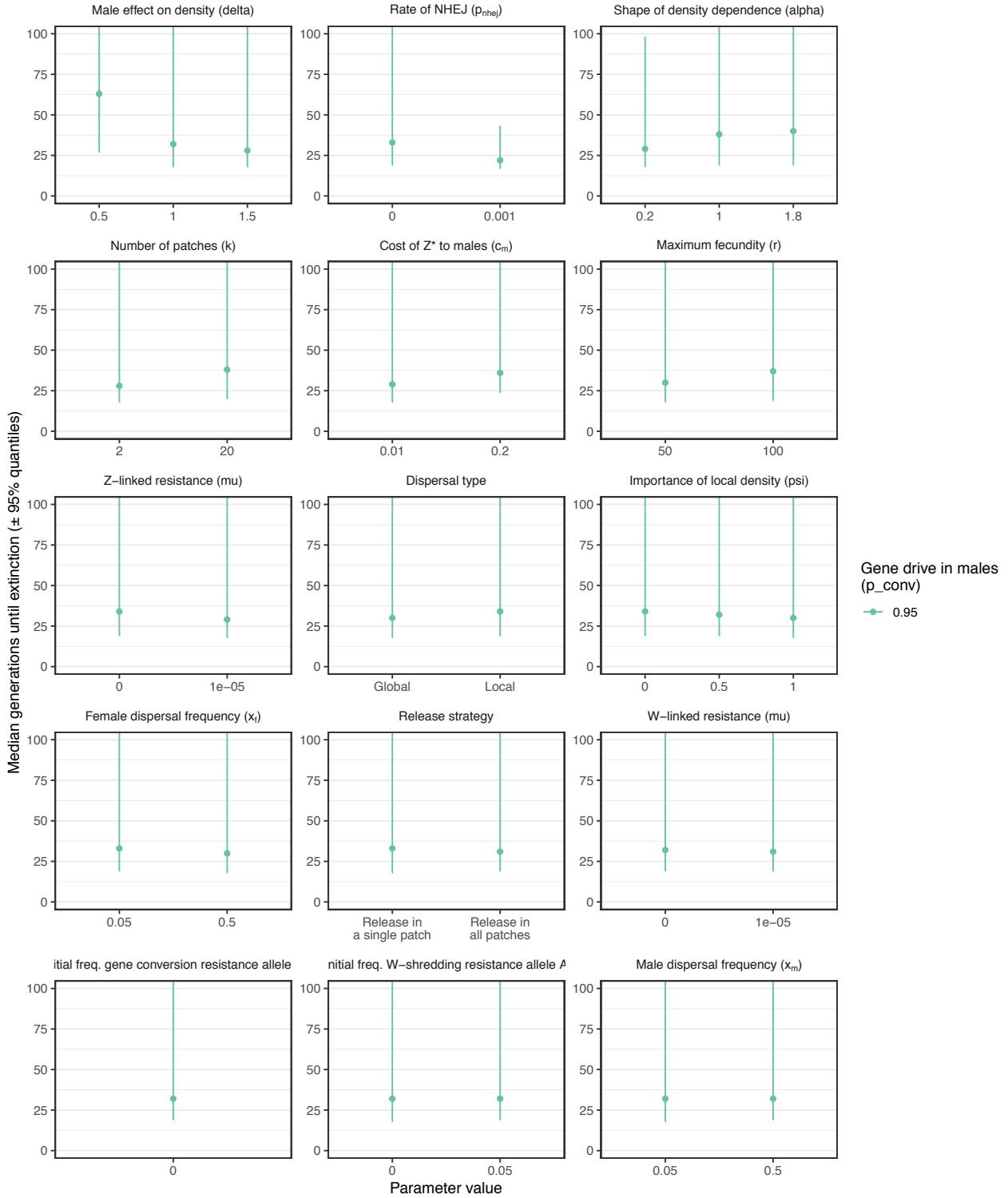
**Figure S1:** Similar plot to Figure 3, except that the y-axis shows the median number of generations until the *W*-shredder caused extinction, among just the subset of simulations in which extinction actually occurred ( $n = 917,328$  simulation runs). The median was only calculated if at least 40 simulation runs reached extinction, and the y-axis is truncated at 150 generations.



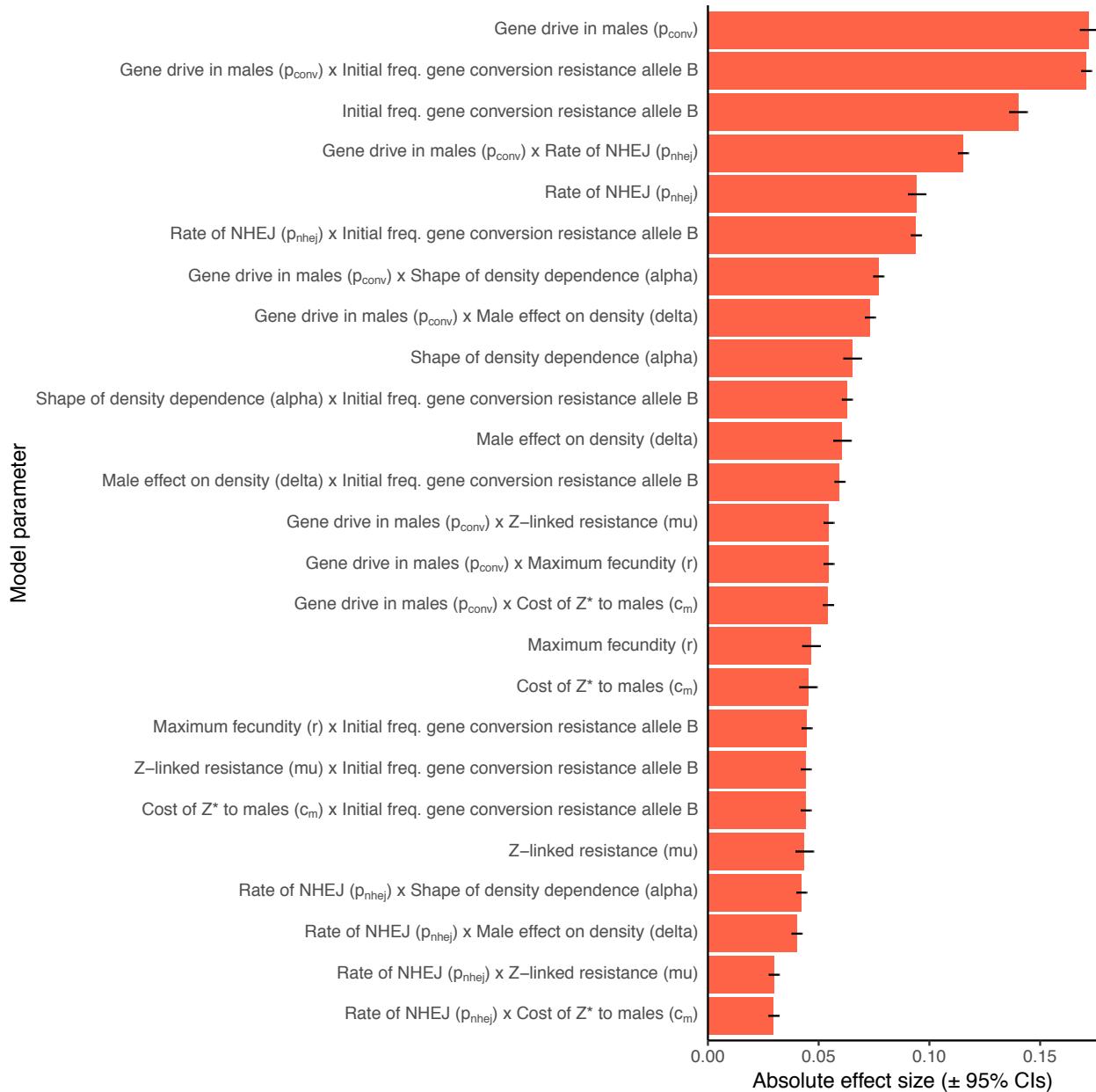
**Figure S2:** Heatmap illustrating the twelve strongest two-way interactions for simulations of a *W*-shredder, as determined by the effect sizes from the GLM plotted in Figure 4 ( $n = 5,657,700$  simulation runs).



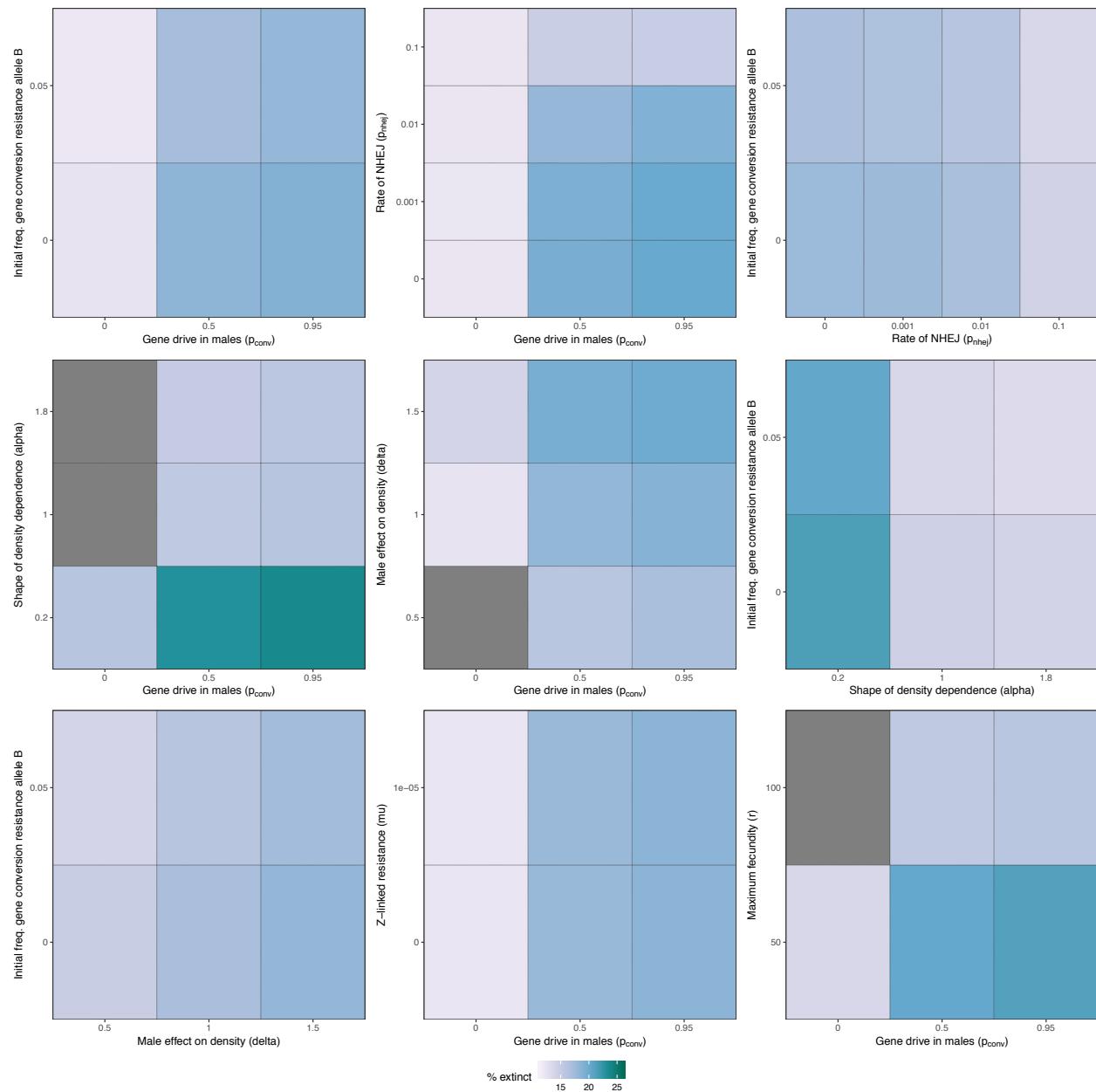
**Figure S3:** Analogous information to Figure 3, but showing the results for a female-sterilising  $Z^*$  allele instead of a  $W$ -shredder ( $n = 629,954$  simulation runs).



**Figure S4:** Analogous information to Figure S1, but showing the time to extinction for a female-sterilising  $Z^*$  allele instead of a  $W$ -shredder. Note that a median was only calculated if at least 40 simulation runs reached extinction ( $n = 12,992$  simulation runs), and extinction only occurred when gene drive in males was strong ( $p_{conv} = 0.95$ ).



**Figure S5:** Relative parameter importance in the simulations of *Z*-linked female-sterilising gene drives, for the top 25 most important main effects or two-way interactions (from a binomial GLM that included all the main effects and all their two-way interactions;  $n = 629,954$  simulation runs). Each predictor variable was scaled before running the model, meaning that the absolute effect size indicates how important each parameter is to the extinction probability, given the range of values plotted in Figure S3.



**Figure S6:** Heatmap illustrating the twelve strongest two-way interactions for simulations of a female-sterilising gene drive, as determined by the effect sizes from the GLM plotted in Figure S5 ( $n = 629,954$  simulation runs).