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# Resistance to genetic insect control: Modelling the effects of space

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1 **Abstract**

2 Genetic insect control, such as self-limiting RIDL<sup>1</sup> (Release of Insects Carrying a Dominant  
3 Lethal) technology, is a development of the sterile insect technique which is proposed to suppress  
4 wild populations of a number of major agricultural and public health insect pests. This is achieved by  
5 mass rearing and releasing male insects that are homozygous for a repressible dominant lethal genetic  
6 construct, which causes death in progeny when inherited. The released genetically engineered ('GE')  
7 insects compete for mates with wild individuals, resulting in population suppression. A previous  
8 study modelled the evolution of a hypothetical resistance to the lethal construct using a frequency-  
9 dependent population genetic and population dynamic approach. This found that proliferation of  
10 resistance is possible but can be diluted by the introgression of susceptible alleles from the released  
11 homozygous-susceptible GE males. We develop this approach within a spatial context by modelling  
12 the spread of a lethal construct and resistance trait, and the effect on population control, in a two  
13 deme metapopulation, with GE release in one deme. Results show that spatial effects can drive an  
14 increased or decreased evolution of resistance in both the target and non-target demes, depending on  
15 the effectiveness and associated costs of the resistant trait, and on the rate of dispersal. A recurrent  
16 theme is the potential for the non-target deme to act as a source of resistant or susceptible alleles  
17 for the target deme through dispersal. This can in turn have a major impact on the effectiveness of  
18 insect population control.

19 **Keywords:** Pest insects, self-limiting RIDL, Sterile insect technique (SIT), Mathematical modelling,  
20 Spatial dynamics

21 <sup>1</sup>RIDL<sup>®</sup> is a registered trademark of Oxitec Limited, UK.

22 **1. Introduction**

23 The control of insect pests is a major area of concern for both public health and agriculture,  
24 as these pests cause widespread harm and economic damage. Diseases such as malaria and dengue  
25 fever, transmitted by *Anopheles* and *Aedes* mosquitoes respectively, are particularly damaging, with  
26 the former being responsible for around 584,000 deaths in 2013 (World Health Organisation, 2014),  
27 whilst insect pests are responsible for a large proportion of the 18% annual crop production losses  
28 attributable to animal pests overall (Oerke, 2006).

29 The sterile insect technique (SIT) is a method that has been used since the 1950s to control  
30 pest populations and involves releasing large numbers of mass reared insects into natural populations  
31 (Knipling, 1955). These insects, having been sterilised by irradiation, compete for mates with  
32 wild insects but do not produce any viable offspring, thus reducing the total number of offspring  
33 contributing to the next generation. If the number of insects released is large enough and released  
34 over a long enough period this can lead to local population suppression or elimination. The technique  
35 has been used to eradicate pests such as the screwworm fly *Cochliomyia hominivorax* in the USA  
36 and Mexico, and the Mediterranean fruit fly ('Medfly') *Ceratitis capitata* from various locations in  
37 the Americas (reviewed by Alphey et al. (2010)).

38 A self-limiting genetic technology, referred to in published literature as RIDL<sup>®</sup> (Release of Insects  
39 carrying a Dominant Lethal), is a development of the sterile insect technique that involves the release  
40 of genetically engineered ('GE') insects homozygous for a dominant lethal genetic construct instead  
41 of being irradiated (Alphey, 2014; Thomas et al., 2000). These insects compete for mates with wild  
42 type insects and pass the construct onto their offspring, causing them to die before they fully mature  
43 (supplementary figure S1). The timing of death during insect development can be engineered in  
44 order to maximise population suppression. For example, early acting lethality is preferable for some  
45 agricultural pests such as Medfly where the larval stages cause the most damage (Schetelig et al.,  
46 2007). Late acting lethality on the other hand is used for insects, such as mosquitoes, that have

47 a density dependent mortality phase during their development; if the lethality acts after this phase,  
48 population suppression is maximised (Atkinson et al., 2007; Phuc et al., 2007).

49 The efficiency of both the traditional sterile insect technique and its genetic variants are greatly  
50 increased if only males are released, as these individuals must then disperse and find wild-type  
51 females to mate with (Rendon et al., 2004). The separation of males and females can be achieved  
52 mechanically on a large enough scale for a limited number of species including screwworm and *Aedes*  
53 *aegypti* mosquitoes, and using classical genetics for Medfly, but is not 100% effective (Alphey et al.,  
54 2010). The need for male only release is particularly pertinent for insects such as mosquitoes where  
55 only females bite and transmit diseases.

56 The evolution of resistance to both chemical insecticides and insecticidal (Bt toxin) crops is al-  
57 ready severely impeding population control efforts in a number of species (Nicholson, 2007; Tabash-  
58 nik et al., 2009) and has the potential to impact severely on the efficacy of the sterile insect  
59 technique. Behavioural resistance to conventional sterile insect technique has already been observed  
60 where selection favours wild-type females that alter their mating preferences to avoid lab reared  
61 males (McInnis et al., 1996). Although none has yet been detected in lab reared or sampled wild in-  
62 sect populations, there exists the distinct possibility that a genetic resistance to the lethal construct  
63 may emerge (Alphey et al., 2011; Gong et al., 2005).

64 The emergence of resistance to an engineered lethal genetic construct could occur through  
65 selection for an existing allele that may be currently undetected due to low frequency, or through  
66 mutation in the genes affecting susceptibility to the construct (Alphey et al., 2011). In either case,  
67 a strong advantage would be conferred once the lethal construct was introduced into a population.  
68 However, unless it conferred some pleiotropic advantage, a resistance trait would not be under any  
69 positive selective pressure in the absence of the lethal construct, and so would likely only be present  
70 at a very low frequency in wild populations. For comparison, naturally occurring insect resistance to  
71 Bt toxins without widespread prior exposure has been found at frequencies of around  $10^{-3}$  (Mahon

72 et al., 2007). As no resistance to a genetic lethal construct has been detected, we must consider its  
73 potential key properties. Hypothetical resistance could provide either complete or partial protection  
74 from the lethal effects. Such a resistance trait could impose costs on the insect, particularly if it  
75 acts by simply increasing metabolism to counter the lethal mechanism.

76 In a previous study (Alphey et al., 2011) we used a combined population genetic and population  
77 dynamic model to investigate potential scenarios for the spread of both the lethal construct and a  
78 pre-existing hypothetical physiological resistance allele. The effect of varying the effectiveness and  
79 cost of resistance, along with the size of the GE release, on allele frequencies and population size  
80 was tested for varying levels of dominance in the resistance gene. We found that the evolution of  
81 the resistant allele frequency was driven by selection pressure from the lethal effects of the construct  
82 as expected. However, the spread of the resistance allele was limited by resistance dilution from the  
83 influx of susceptible alleles from the released modified males, which were homozygous susceptible at  
84 the resistant locus. This dilution effect could prevent the emergence of, or even reverse the spread  
85 of resistance and enable effective population suppression. The model looked at both bisex-lethal GE  
86 strains, where all progeny (of both sexes) are targeted by the construct, and female-lethal strains,  
87 which target females but leave males unaffected (Heinrich and Scott, 2000; Thomas et al., 2000).  
88 These were assumed to be fully dominant and 100% lethal to the target sex, as is usually the case  
89 with lab tested strains that are selected for further development (Ant et al., 2012; Fu et al., 2007;  
90 Gong et al., 2005; Jin et al., 2013; Phuc et al., 2007). The overall effectiveness of GE control was  
91 measured by simulating population size over time, as the number of individuals emerging in each  
92 generation is an effective indicator of economic or public health damage.

93 That model (Alphey et al., 2011) took no account of the spatial distribution of the target and  
94 released populations. We know, however, that spatial structure has the potential to be a major  
95 factor determining the efficacy of GE-SIT and other genetic control methods. Yakob et al. (2008)  
96 used a network approach to model the effect of spatial population clustering on the efficacy of GE-

97 SIT control, finding that more highly clustered insect populations were easier to suppress. This was  
98 because once a local cluster was eliminated it could not easily be repopulated due to its isolation  
99 (Yakob et al., 2008). Another genetic insect control method, using a synthetic homing endonuclease  
100 gene (HEG), was modelled by North et al. (2013) in an individual based simulation, revealing that  
101 control could fail if mosquito resources, and therefore local mosquito populations, were too isolated.  
102 The size of release sites has also been found to impact upon the effectiveness of a genetic or  
103 radiation-based sterile insect technique control. Seirin Lee et al. (2013) modelled a barrier control  
104 method, whereby insects are released in order to halt the invasion of a wild-type wave, finding that  
105 the size of the optimal release region depended on the dispersal rate of the invading population.

106 Here we explore the dynamics of a genetic lethal construct and a corresponding resistance allele  
107 in a spatial model. Specifically, we address the question of how a population targeted with the  
108 application of a treatment (GE release) interacts with a second non-target population, which is  
109 linked to the target population through dispersal. A simple two deme metapopulation model is  
110 sufficient for this investigation of how an asymmetrical interaction between target and non-target  
111 populations affects the evolution of resistance. The opposing forces of selection for, and dilution of,  
112 resistance highlighted in the original non-spatial model could play out in a different manner when  
113 space is taken into account. We hypothesize that gene flow of the GE construct into surrounding  
114 areas, outside the target population, and immigration of individuals from surrounding areas into the  
115 target population, could alter the frequency evolution of the resistance gene and consequently affect  
116 the efficacy of genetic control of the target population. We investigate the influence of resistance  
117 traits and dispersal rates on predicted outcomes for a bisex-lethal genetic system.

## 118 2. Methods

119 The model used in this study is based on that previously published (Alphey et al., 2011). It is  
120 a discrete-generation frequency dependent population genetic and population dynamic model, with  
121 random mating, no mutation, and a 1:1 sex ratio. The original model was of a closed homogeneous

122 population with no immigration or emigration. The underlying mathematical processes described in  
123 2.1 and 2.2 below are essentially the same as those in the original model, while the spatial dynamics  
124 described in 2.3 are new, extending the investigation to explore the effects of spatial heterogeneity.  
125 A summary of the parameters and variables used in this model can be found in table 1.

## 126 2.1. Population genetics

127 Within the insect population there are two genetic loci being modelled, the insertion or absence  
128 of the lethal construct, and the locus of resistance. The specific genotype of insects will affect their  
129 relative fitness and survival.

130 The gene affecting the susceptibility of an individual to the lethal genetic construct is assumed to  
131 have a single autosomal locus. The two alternative alleles for this gene are resistant  $R$  (at frequency  
132  $p$  in the current adult generation) and susceptible  $S$  (at frequency  $q = 1 - p$  in the current adult  
133 generation), meaning that there are three possible genotypes at this locus ( $SS$ ,  $SR$  and  $RR$ ). In all  
134 simulations presented here the initial frequency of the  $R$  allele in the population is  $p_0 = 0.001$ , as  
135 this represents the very low frequency that might be expected of a recent mutation or a pre-existing  
136 allele that is not readily detectable (Alphey et al., 2011).

137 The level of protection that a resistant genotype provides to an individual is governed by the  
138 parameter governing susceptibility to the lethal construct  $\gamma_i$  where  $i$  is the resistant/susceptible  
139 genotype. Susceptible homozygotes have no protection from the effects of the lethal construct  
140 ( $\gamma_{SS} = 1$ ), while homozygote resistant individuals have reduced susceptibility to the construct  
141 ( $\gamma_{RR} < \gamma_{SS}$ ). Resistance can be complete ( $\gamma_{RR} = 0$ ), providing complete protection from the  
142 effects of the lethal construct, or incomplete ( $1 > \gamma_{RR} > 0$ ) with different levels of dominance  
143 affecting the susceptibility of the heterozygote ( $SR$ ) genotype. In this paper a number of different  
144 terms are used to describe the different types of potential resistance studied: dominant complete  
145 ( $\gamma_{SR} = \gamma_{RR} = 0$ ), partially dominant complete ( $1 > \gamma_{SR} > 0$ ,  $\gamma_{RR} = 0$ ), co-dominant complete  
146 ( $\gamma_{SR} = 0.5$ ,  $\gamma_{RR} = 0$ ), and partially-dominant incomplete ( $1 > \gamma_{SR} > \gamma_{RR} > 0$ ) (summarised in

147 table 2).

148 The resistant allele may or may not have associated fitness costs, depending on its mechanism,  
 149 which are represented here by the fitness of genotype  $i$  ( $\psi_i$ ) relative to the fitness of homozygous  
 150 susceptible wild-type insects ( $\psi_{SS} = 1$ ). In this study four different types of resistance were tested:  
 151 no costs ( $\psi_{SR} = \psi_{RR} = 1$ ), 'minor costs' ( $\psi_{SR} = 0.95$ ,  $\psi_{RR} = 0.85$ ), 'fit resistance' ( $\psi_{SR} = 0.9$ ,  
 152  $\psi_{RR} = 0.7$ ), and 'costly resistance' ( $\psi_{SR} = 0.2$ ,  $\psi_{RR} = 0.1$ ) (summarised in table 2). These  
 153 parameter values were chosen to aid comparison with Alphey et al. (2011) where they were found  
 154 to be effective for determining the effect of the magnitude of resistance costs.

155 The lethal genetic construct itself also has a single autosomal locus with the assumption that  
 156 there is no linkage between this and the locus controlling resistance to the construct. The two  
 157 possible alleles at this locus are the dominant  $L$  (at frequency  $l$  in the current adult generation),  
 158 where the construct has been inserted, and the wild-type  $w$  (at frequency  $1 - l$  in the current adult  
 159 generation), where the construct is absent, giving three possible genotypes ( $ww$ ,  $Lw$  and  $LL$ ).  
 160 Where the construct is present it imposes a relative fitness cost  $\epsilon_k$ , where  $k$  indicates either the  
 161 target or non-target sex. In this study a fully bisex-lethal construct is investigated so a maximum  
 162 fitness penalty is always applied to both sexes ( $\epsilon_k = 1$ ). The relative fitness of genotype  $j$  ( $ww$ ,  
 163  $Lw$  or  $LL$ ) is given by:

$$\Omega_j = (1 - \epsilon_k)^{\eta_j} \quad (1)$$

164 where  $\eta_j$  is the number of  $L$  allele copies present ( $0 = ww$ ,  $1 = Lw$ ,  $2 = LL$ ). As the construct is  
 165 bisex-lethal and dominant  $\Omega_{ww} = 1$  and  $\Omega_{Lw} = \Omega_{LL} = 0$ . When susceptibility to the construct ( $\gamma_i$ )  
 166 is included in the equation it acts as a scaling factor on the fitness (survival) penalty  $\epsilon_k$ , while the  
 167 cost of resistance ( $\psi_i$ ) modifies the resulting fitness equation to give:

$$\Omega_{ijk} = \psi_i (1 - \epsilon_k \gamma_i)^{\eta_j} \quad (2)$$

168 The three possible genotypes given by  $i$  ( $SS$ ,  $SR$  and  $RR$ ), the three by  $j$  ( $ww$ ,  $Lw$  and  $LL$ )  
169 and the two sexes means that there are potentially a total of 18 genotypes used in this model. In  
170 practice because only a bisex-lethal construct is modelled, and we assume an equal sex ratio, there  
171 are essentially only 9 genotypes as the male and female genotypes always have equal frequencies.

172 The genetic control being used on the wild insect population is modelled by the addition of adult  
173 males that are homozygous for both the susceptible allele and the lethal construct ( $SSLL$ ) at a  
174 fixed ratio  $d$  to the total number of males in the wild population at that generation. While this  
175 proportional release policy may be difficult to implement in practice, it was included in the original  
176 model as it allowed the change in allele frequencies to be calculated independently of population  
177 size. In a spatial model where dispersal is dependent on population size this rationale is no longer  
178 strictly true, however, the assumption still serves to simplify the calculations involved in the model  
179 and allows direct comparison with previously published results. The addition of GE males occurs as  
180 the current generation in the wild population reaches maturity, prior to mating. Wild and released  
181 insects are assumed to mix homogeneously in the target population and random mating subsequently  
182 occurs between females and all male genotypes.

183 The frequencies of each zygote genotype are calculated post-mating after which Eq. (2) is applied  
184 to calculate their relative fitness and survival. In this way the effects of both the lethal construct  
185 and the costs of resistance act during the larval stage of the insects' life cycle. Those insects that  
186 survive then mature to become the adults of the next generation. The original non-spatial model  
187 used a simulation approach to calculate the changes in allele and genotype frequencies due to the  
188 large number of genotypes which would otherwise result in a complex system of difference equations  
189 that cannot be readily solved analytically. Adding a spatial element further complicates the model,  
190 so a simulation approach is even more essential. Simulations were performed in R (R Core Team,  
191 2016).

## 2.2. Population dynamics

The insect population grows each generation at a rate determined by the basic reproductive rate,  $R_0$ , which is the average number of offspring produced per adult pest insect in its lifetime (a single discrete generation). Where separate female population size is recorded, as it is here, and with an equal sex ratio this can be expressed as the number of female offspring produced per adult female. For the generic agricultural and public health pests (target species suitable for SIT) that are simulated in this model, an  $R_0$  value of 7.5 is used; a plausible  $R_0$  value for *Aedes aegypti* mosquitoes for example would be in the range 3 to 11 (Dye, 1984). In a density-independent population, with the assumption that half of the adults are female, growth is calculated by:

$$N_{t+1} = 2R_0F_t\sigma_t \quad (3)$$

$$F_{t+1} = R_0F_t\sigma_t^{(F)} \quad (4)$$

where  $N_t$  is the adult pest population size at generation  $t$  relative to the initial population size ( $N_0 = 1$ ).  $2R_0$  here is the average number of progeny (male and female) produced per adult female.  $\sigma_t$  and  $\sigma_t^{(F)}$  are the proportion of all offspring and of female offspring respectively, that survive to adulthood (calculated using the fitness values calculated from Eq. (2)). In a bisex-lethal model these values are the same as there are no differential survival rates between the sexes.

All the simulations presented in this study feature density dependent population dynamics in order to represent a realistic field setting. This model adapts an equation from Bellows (1981):

$$\tilde{N}_{t+1} = R_0\tilde{N}_t e^{-\alpha\tilde{N}_t} \quad (5)$$

where  $\tilde{N}$  is the absolute population size,  $\alpha$  is the strength of the density dependence, and  $1/\alpha$  is related to the carrying capacity of the habitat. Because our model uses relative population size rather than the absolute, a substitution of variables is made where population sizes are measured

212 relative to their initial equilibrium value ( $N_t = \tilde{N}_t/\tilde{N}^*$  and  $F_t = \tilde{F}_t/\tilde{F}^*$ ), so that ( $N_0 = N^* = 1$   
 213 and  $F_0 = F^* = 0.5$ ). The non-zero equilibrium for absolute population size from Eq. (5) is:

$$\tilde{N}^* = \frac{\log(R_0)}{\alpha} \quad (6)$$

214 which can be substituted into Eq. (5) giving:

$$\tilde{N}_{t+1} = R_0 \tilde{N}_t R_0^{-N_t} \quad (7)$$

215 As the density dependent term now uses the relative population size  $N_t$  it can now be applied  
 216 to the density-independent Eqs. (3 and 4) to give the population growth used in this model:

$$N_{t+1} = 2R_0 F_t R_0^{-N_t} \sigma_t \quad (8)$$

$$F_{t+1} = R_0 F_t R_0^{-N_t} \sigma_t^{(F)} \quad (9)$$

218 Density dependent mortality is assumed to act before the lethal construct and costs of resistance,  
 219 so the relative survival terms ( $\sigma$ ) are applied to the density dependence formula.

### 220 2.3. Spatial modelling

221 In order to explore spatial dynamics this model uses a two deme metapopulation structure with  
 222 GE release in only one 'target' deme and dispersal between this and the other 'non-target' deme  
 223 (supplementary figure S2). Both demes are of equal size and with an equal and constant rate of  
 224 dispersal  $m$  in each direction (ie.  $m = 0.1$  represents 10% of individuals migrating). The system is  
 225 otherwise closed with no immigration or emigration beyond the two demes.

226 Dispersal occurs after mating but before females have laid their eggs (and therefore before the  
 227 larval phase density dependence and survival calculations). This means that although both sexes  
 228 could disperse in reality, only female dispersal is calculated here as at this point only they can  
 229 contribute to the next generation of insects. Conceptually, mated females may be thought of as

230 containers for fertilised eggs, which either transport those eggs/zygotes to another deme or remain  
 231 in the deme where mating occurred. All 18 zygote genotype frequencies, along with the adult female  
 232 population size, must be adjusted every generation to account for dispersal:

$$233 \quad Z_{t+1}^{(T)} = (1 - m)Z_t^{(T)}F_t^{(T)} + mZ_t^{(NT)}F_t^{(NT)} \quad (10)$$

$$Z_{t+1}^{(NT)} = (1 - m)Z_t^{(NT)}F_t^{(NT)} + mZ_t^{(T)}F_t^{(T)} \quad (11)$$

234 where  $Z$  is a matrix of genotype frequencies,  $(T)$  indicates a variable belonging to the target deme,  
 235 and  $(NT)$  a variable belonging to the non-target deme. The resulting genotype frequencies are then  
 236 rescaled so that  $Z_{t+1}$  sums to 1. The female population size in this previous generation must also  
 237 be adjusted to take into account the migration of adult females:

$$238 \quad \hat{F}_t^{(T)} = (1 - m)F_t^{(T)} + mF_t^{(NT)} \quad (12)$$

$$\hat{F}_t^{(NT)} = (1 - m)F_t^{(NT)} + mF_t^{(T)} \quad (13)$$

239 After dispersal is complete the zygote genotype frequencies in each deme comprise the next  
 240 generation and are adjusted by their individual survival rates. The (adult) population size of that  
 241 next generation is then calculated, with the migration adjusted female population size ( $\hat{F}_t$ ) from the  
 242 previous generation, using Eqs. (8 and 9). In these equations  $N_t = 2\hat{F}_t$  (in the density dependence  
 243 term) as density dependent larval mortality is a function of adult female numbers from the previous  
 244 generation. This process simulates the eggs being laid, the larvae emerging, and their maturation  
 245 to adulthood.

246 When dispersal is removed from this model ( $m = 0$ ) the target deme functions as a version of  
 247 the original non-spatial model with bisex-lethal GE release, while the non-target deme functions as  
 248 a version with no release of GE insects. In this way the spatial model was validated by confirming  
 249 that results were consistent with results from the underlying non-spatial model. The insect life cycle  
 250 simulated in this model is summarised in supplementary figure S3.

## 251 2.4. Simulations

252 In all simulations the populations were assumed to be naive to the GE construct and did not  
253 contain any  $L$  alleles ( $l_0 = 0$ ). The initially present genotypes are in Hardy-Weinberg equilibrium  
254 ( $SSww = q_0^2$ ,  $SRww = 2p_0q_0$ ,  $RRww = p_0^2$ ) while the other genotypes involving the  $L$  allele only  
255 arise after GE insects have been released and mate with wild females.

256 To illustrate the range of complex dynamics that can arise in a spatially explicit model, a number  
257 of qualitatively and quantitatively different resistance costs and susceptibilities (summarised in 2.1)  
258 were chosen and run in different combinations. Also in order to simplify results a release ratio of  
259  $d = 20$ , a realistic ratio that might be used in a real control programme, was used in most simulations  
260 except for in some early tests (Alphey et al., 2011). All simulations were run for the number of  
261 generations required for equilibrium values to be reached, or if this took a very long period of time,  
262 until the qualitative pattern of results became clear. As the primary purpose of this study was to  
263 investigate spatial dynamics, all simulations were run at three dispersal rates ( $m = 0.01, 0.05, 0.1$ )  
264 with additional rates being tested if this was insufficient to determine the effect of the parameter.  
265 Time series of both the  $L$  and  $R$  allele frequencies along with the relative population size  $N$  at every  
266 generation were recorded for both demes in every simulation run.

267 In the first phase of simulation modelling a non-spatial versus spatial comparison was made using  
268 a strong partially dominant incomplete resistance trait ( $\gamma_{SR} = 0.2$ ,  $\gamma_{RR} = 0.1$ ) with no associated  
269 costs ( $\psi_{SR} = \psi_{RR} = 1$ ). This initial spatial simulation was also run with a low ( $d = 1$ ) and very high  
270 ( $d = 50$ ) release ratio to determine whether this parameter was capable of producing qualitatively  
271 different or unexpected results. Following this, the same simulation (with  $d = 20$ ) was run with each  
272 of the three costs of resistance detailed in 2.1 (minor costs, fit resistance and costly resistance).  
273 The role of various susceptibilities of resistance was then investigated under a variety of these cost  
274 regimes (see table 2 for resistance effects and costs).

275 In all simulations, we consider an equilibrium to have been reached if a value, or the periodic limit

276 of an oscillating value, converges to within 5 decimal places. Furthermore, due to the deterministic  
277 nature of the model, using continuous state variables, the total fixation (or loss) of an allele is  
278 impossible and values instead asymptote at these points (referred to as near fixation).

### 279 3. Results

#### 280 3.1. Spatial vs. non-spatial for a no-cost resistance

281 In the original non-spatial model, a strong, partially dominant, incomplete resistant ( $R$ ) allele  
282 with no fitness costs quickly spreads to reach an equilibrium of  $p \approx 0.515$  in 13 generations (figure  
283 1). This allows the lethal construct ( $L$ ) allele to spread through the population, due to the increased  
284 survival of  $L$ -bearing offspring, and reach near fixation ( $l^* \approx 1.00$ ) in 31 generations. The  $R$  allele  
285 is prevented from reaching fixation due to continuing dilution by the released  $SLL$  males. The  
286 relative population size ( $N$ ) initially falls rapidly from 1.00 to a low of 0.011 in three generations  
287 before recovering, at around the time that  $p$  increases above 0.5 ( $R$  is more common than  $S$ ), to  
288 an equilibrium of  $N^* \approx 0.464$  by generation 25. Therefore population suppression is still achieved,  
289 albeit to a lesser extent. With no resistance the population would be eliminated ( $N^* \approx 0$  to 5 d.p.)  
290 in 10 generations.

291 In the two deme spatial model, the system takes far more generations to reach equilibrium,  
292 (figure 2). For all values of the dispersal rate  $m$  tested (0.01, 0.05 and 0.1) the  $L$  allele does not  
293 reach fixation in either deme, but reaches a much higher equilibrium frequency in the target deme  
294 compared to the non-target deme. In the target deme  $l^*$  is lower with higher values of  $m$ , whereas in  
295 the non-target deme it is higher with higher dispersal; the resultant effect is that the target and non-  
296 target equilibrium frequencies are closer together for higher values of  $m$ . This is due to higher rates  
297 of dispersal shifting more  $L$  alleles from the target to the non-target deme. Instead of increasing to  
298 an equilibrium in a sigmoidal curve, the  $L$  allele frequency with low dispersal ( $m = 0.01$ ) increases  
299 to a local peak before falling gradually to equilibrium.

300 Strikingly, the  $R$  allele reaches a markedly higher equilibrium frequency in the non-target com-  
301 pared to the target deme. This is due to strong positive selection for the no-cost resistance, even  
302 with a low  $L$  allele frequency, combined with only weak dilution from the progeny of released  $SSLL$   
303 males. This resistance dilution in the non-target deme will be even weaker with lower dispersal rates.  
304 As with the  $L$  allele, higher  $m$  values produce closer equilibria between the two demes, with the  
305 effect of increasing  $p$  in the target and decreasing  $p$  in the non-target deme. The rapid spread of  
306 resistance in the non-target deme causes it to act as a source of  $R$  alleles for the target deme; this  
307 effect increases with greater dispersal.

308 Significant population suppression is most clearly observed in the target deme where, as in the  
309 non-spatial model,  $N$  initially drops very rapidly then climbs back to an equilibrium between 0.5  
310 and 0.75. Target population suppression is inferior to that of the non-spatial model (figure 1).  
311 This is partly due to the net migration of insects from the non-target deme, which retains a higher  
312 population size, but it is also due to the slightly higher frequency of resistance in the target deme  
313 (compared to figure 1). In contrast, the non-target deme shows little population suppression due to  
314 a relatively low influx of  $L$  alleles.

315 With higher values of  $m$  the target  $N^*$  is higher (inferior population suppression) but is lower in  
316 the non-target deme. For the target deme this is due to a combination of the lower  $L$  equilibrium,  
317 the higher  $R$  equilibrium, and a greater general loss of insects migrating to the non-target deme.  
318 Again, the two demes' equilibria are closer in value at higher dispersal rates. Simulations conducted  
319 with alternative release ratios ( $d$ ) produced no qualitatively novel results beyond those we have  
320 already described (Alphey et al., 2011) (Supplementary Material section 3.1 and figure S4).

### 321 3.2. Adding costs of resistance

322 Running the same simulations with minor costs of resistance results in very different patterns of  
323 equilibrium frequencies (figure 3) compared to the no-cost model. In contrast to the no-cost model  
324 (compare figures 2 and 3), the  $R$  equilibrium is higher in the target deme than in the non-target,

325 while all  $p^*$  values are lower than in any of the no-cost simulations. The costs of resistance lower  
326 the selective pressure for the  $R$  allele by reducing the number of  $SR$  and  $RR$  individuals surviving to  
327 maturity, particularly in the non-target deme which has lower exposure to the  $L$  allele. Increasing  $m$   
328 still results in deme equilibria being closer in value, due to greater mixing between the populations.  
329 However as the target  $p^*$  is higher than in the non-target deme, increased dispersal now has the  
330 opposite effect on the equilibrium of each individual deme (increasing  $m$  reduces and increases  $p^*$   
331 in the target and non-target demes respectively). The non-target deme now becomes a source of  $S$   
332 alleles for the target deme. The  $L$  allele spreads in the target deme, and to a lower equilibrium with  
333 higher  $m$  as in the no-cost model. However  $L$  barely spreads in the non-target deme with any value  
334 of  $m$  because of the low number of resistant individuals. Due to the lower overall resistance spread,  
335 target population suppression is greater for all values of  $m$  compared to the no-cost model, and is  
336 again superior with a lower  $m$ . Equilibrium frequencies for non-target  $N$  are not markedly different  
337 from those in the no-cost model.

338 Higher resistance costs result in a reduced spread of both the resistant allele and the lethal  
339 construct in both demes (Supplementary Material section 3.2 and figure S5 for intermediate cost  
340 'fit resistance'). In simulations with costly resistance (figure 4) neither the  $R$  nor  $L$  alleles spread  
341 in either deme, while target population suppression is superior to that with any of the other costs  
342 of resistance tested. A new behaviour seen in this set of results, is that of the oscillations in the  
343 non-target  $N$  with  $m = 0.01$ , which are much larger than previously seen and do not converge,  
344 instead reaching a stable period 2 cycle (with limits 0.939 and 1.051 converging to 5 d.p.).

345 We have shown a variety of outcomes that depend on the fitness costs of the resistant allele,  
346 which we have assumed throughout this section to be strong partially dominant and incomplete. If  
347 there are no fitness costs the  $R$  allele spreads through the non-target deme which becomes a source  
348 of  $R$  alleles and negatively affects the suppression of the target population (figure 2). Minor fitness  
349 costs prevent the  $R$  allele from spreading in the non-target deme, which acts as a source of  $S$  alleles

350 and thereby dilutes the resistance in the target deme (figure 3). Resistance is unable to spread in  
351 the target population anyway if its fitness costs are very high (figure 4). To explore the nature of  
352 these shifts in behaviour, we examine plots of equilibria (rather than time dynamics) against the full  
353 spectrum of fitness costs in resistant heterozygotes and in homozygotes.

354 The  $R$  allele frequency  $p^*$  in the non-target deme (figure 5) is much higher when there are very  
355 little or no fitness costs (as seen in the rear corner of all figure 5 panels). For most fitness cost  
356 combinations, the resistance goes extinct in the non-target deme ( $p^* = 0$ ), except where there  
357 are low or no fitness costs in  $SR$  heterozygotes (the right edge of all panels in figure 5). In this  
358 relatively small region of parameter space, the non-zero  $R$  allele frequency equilibria are slightly  
359 higher with higher rates of dispersal, while that region of parameter space itself is slightly larger.  
360 Only at, or very close to, zero costs does the  $R$  allele spread significantly in the non-target deme,  
361 with potentially detrimental effects on the pest control programme. Equilibrium plots of  $N^*$  in the  
362 target deme (supplementary figure S6) show worse population suppression in regions where there  
363 are lower fitness costs of resistance, particularly in  $SR$  heterozygotes, with the worst degree of  
364 population control occurring where the  $R$  allele has no fitness costs. The impact of high resistance  
365 frequency in the non-target deme on the effectiveness of target population control is most clearly  
366 seen at higher dispersal rates due to the greater influx of  $R$  alleles from the non-target source.

### 367 3.3. Altering the effectiveness of resistance in a no-cost model

368 A dominant complete resistance with no associated costs (the strongest, most effective resistance  
369 possible) (figure 6) results in the  $L$  allele reaching near fixation in both demes for all values of  $m$ .  
370 The powerful resistance trait at higher frequencies reduces the selection against the  $L$  allele and  
371 allows it to spread to fixation. In the same manner as in the initial no-cost model (figure 2), the  
372  $R$  allele equilibrium is higher in the non-target than in the target deme, and the deme equilibria  
373 are closer in value with a higher  $m$ . The non-target equilibria are also even higher than in figure  
374 2, due to the greater selection for the more effective resistance, while target deme equilibria are

375 very slightly lower. This latter result is most likely due to the reduced difference in population size  
376 between the two demes resulting in the target deme receiving a proportionately lower migratory  
377 influx of  $R$  alleles. The effect of  $m$  on population suppression is similar but the target deme  $N$  is  
378 higher overall due to the greater effectiveness of resistance.

379 Increasing heterozygote resistance susceptibility ( $\gamma_{SR} = 0.4$ ), while keeping homozygote suscep-  
380 tibility at 0, prevents the  $L$  allele reaching fixation in both demes for all  $m$ , although the  $l^*$  values are  
381 still high (figure 7). Heterozygous resistance is no longer effective enough to counter the negative  
382 selection against the lethal construct, the spread of which is therefore limited. In this simulation  
383 it took very much longer to reach equilibria. This is a consequence of the weaker heterozygote  
384 resistance, as previously observed (Alphey et al., 2011).  $R$  allele equilibrium frequencies are higher  
385 in both demes (figure 7a) than they were with dominant complete resistance (figure 6a), with  $R$   
386 nearing fixation in the non-target deme, particularly with a higher  $m$ . It is much more advantageous  
387 for an individual to have homozygous resistance, rather than heterozygosity, and therefore there is  
388 selection pressure for an increase in the  $R$  frequency even at high values of  $p$ . In the dominant  
389 complete model (figure 6) on the other hand, resistance is strong enough to provide good protection  
390 of the population from the genetic control even at lower  $R$  frequencies.

391 With all previously tested parameter combinations, higher dispersal brings the  $L$  allele frequencies  
392 in the two demes closer to each other. Uniquely, in the present case,  $L$  allele equilibria for the two  
393 demes are no longer closer together at higher  $m$  (so there is more going on than simply better mixing  
394 between the two populations), and instead higher dispersal causes lower  $l^*$  in both target and non-  
395 target demes. In this sense the effect of dispersal rate on the non-target deme  $l^*$  has been reversed.  
396 Observing the  $L$  curve for  $m = 0.01$  (figure 7b), we see that in the target deme  $l$  at first appears  
397 to be following earlier patterns (e.g. figure 2b) of declining towards a lower equilibrium point, but  
398 then the frequency suddenly starts increasing again from about the same time when the  $R$  allele in  
399 the non-target deme nears fixation. This suggests that the transient values of  $l$  in the target deme

400 are initially consistent with previous patterns. However, once resistance has spread sufficiently far  
401 in the non-target deme that most individuals are homozygous resistant (and suffer no effects from  
402 the lethal construct), the negative selection against the  $L$  allele in this deme is drastically reduced,  
403 allowing a large increase in its frequency. Due to migration this increase in both  $R$  and  $L$  alleles is  
404 also exported to the target deme. The difference between the  $p^*$  values in the two demes is caused  
405 by a balance between the dispersal rate  $m$  and the relative population sizes of the two demes.

406 Another qualitative difference in this set of simulations is that instead of rising to an equilibrium  
407 from the initial dip as seen previously, the target  $N$  (figure 7c) rises from this dip to a local peak  
408 before falling towards its equilibrium point. This change in the trajectory of  $N$  also appears to  
409 coincide with the rapid increase in  $L$  in this deme. The near fixation of resistance in the non-target  
410 deme drives an increase in the non-target  $l$  which, due to migration, in turn drives an increase in  $L$   
411 alleles in the target deme. Because resistance is not fixed in that deme, this increases suppression  
412 of the target population. In this way the evolution of resistance in the non-target deme indirectly  
413 increases the effectiveness of population control in the target deme. Compared to the dominant  
414 complete model (figure 6),  $N^*$  is lower for all values of  $m$  due to the above process and the overall  
415 lower effectiveness of resistance allowing greater population suppression. Further increasing the  
416 heterozygous susceptibility to achieve a co-dominant complete resistance ( $\gamma_{SR} = 0.5$ ,  $\gamma_{RR} = 0$ )  
417 produces a more extreme pattern of results than seen in figure 7, even further away from those  
418 produced by the dominant complete model (figure 6) due to the lower effectiveness of heterozygous  
419 resistance.

#### 420 3.4. Altering the susceptibility of resistance in a model with resistance costs

421 A dominant complete resistance with minor fitness costs produces a very distinct and unexpected  
422 pattern (figure 8). For  $m < 0.06$ , the  $R$  and  $L$  curves for both demes behave in a qualitatively  
423 similar manner to those in the original minor costs model with strong partially dominant incomplete  
424 resistance (figure 3).  $p^*$  and  $l^*$  in both demes are higher overall due to the more effective resistance

425 and therefore population suppression is inferior. However the curves for  $m \geq 0.06$  behave in a  
426 manner much more reminiscent of those for the no-cost model with dominant complete resistance  
427 (figure 6), with the non-target  $p^*$  increasing above those of the target deme, and  $l^*$  in both demes  
428 reaching fixation. For the  $R$  allele this  $p$  increase in the non-target deme coincides with a smaller  
429 increase in the target deme, while further increasing  $m$  still results in the equilibria in the two demes  
430 being closer in value.

431 This pattern is due to an increase in the dispersal rate increasing the influx of  $L$  alleles to the  
432 non-target deme enough that the (frequency-dependent) benefits of resistance there outweigh the  
433 fitness costs. Passing this threshold results in rapid proliferation of resistance in this deme, for those  
434 values of  $m$ , lowering the average fitness penalty of the  $L$  allele to the point that fixation can occur.  
435 It should be noted that the curves at higher  $m$  values are smooth for the non-target deme but seem  
436 to plateau before increasing again to equilibrium in the target deme. This further implies that it is  
437 the spread of resistance in the non-target deme that is driving these changes, with dispersal causing  
438 a concurrent increase in the target  $p$  and  $l$ , to the point that the latter also reaches fixation. It has  
439 already been established that increasing the costs of resistance lowers the selection for the  $R$  allele  
440 and limits its spread. This increase in resistance costs (compared to figure 6) therefore also limits  
441 the potential for the non-target deme to experience this upwards shifting behaviour in the  $R$  and  $L$   
442 allele frequencies.

443 It is clear that target population suppression with the higher dispersal ( $m$  values) is inferior  
444 (higher  $N^*$ ) compared with the lower  $m$  values, undoubtedly due to the higher levels of resistance  
445 that greater dispersal brings. This coincides with a lower non-target  $N^*$  with the higher  $m$  values  
446 as consequence of net migration from the non-target to the target deme.

447 An overall very similar pattern is seen when the heterozygote susceptibility is slightly increased  
448 to give a partially dominant complete resistance ( $\gamma_{SR} = 0.1$ ,  $\gamma_{RR} = 0$ ) with the same minor fitness  
449 costs (supplementary figure S7). The difference here however is that the upwards shift in the  $R$  and

450  $L$  curves is no longer seen at  $m = 0.06$  but is seen at  $m = 0.11$ . When resistance is less effective  
451 in heterozygotes, a higher dispersal rate is required to cause the critical influx of  $L$  alleles into the  
452 non-target deme required to initiate this upwards shift.

453 Increasing heterozygote susceptibility even further to give a co-dominant complete resistance  
454 ( $\gamma_{SR} = 0.5, \gamma_{RR} = 0$ ) results in resistance and introgression of  $L$  alleles only emerging in the non-  
455 target deme, and only with the lowest dispersal rate  $m = 0.01$  (all other  $R$  and  $L$  curves remain at,  
456 or near, 0 frequency). In this case the dilution of resistance by the susceptible released GE insects  
457 is too strong for resistance to emerge, except in the non-target deme that has the lowest migratory  
458 influx of susceptibles. Large oscillations in the non-target  $N$ , reaching a stable limit cycle, with  
459  $m = 0.01$  are again observed here, as seen in the model with costly resistance (figure 4). This  
460 pattern is almost identically reproduced when the homozygote susceptibility is increased to give a  
461 weak partially dominant incomplete resistance ( $\gamma_{SR} = 0.5, \gamma_{RR} = 0.3$ ), thereby highlighting the  
462 primary importance of heterozygote resistance effectiveness.

#### 463 4. Discussion

464 We have shown by mathematical simulation that incorporating spatial effects into a frequency-  
465 dependent population model of resistance to an engineered dominant lethal genetic construct, in-  
466 troduces a number of interesting and often counter-intuitive dynamic results.

467 We looked for (simulated) evidence of, and conditions for, dilution of resistance to the lethal  
468 construct in a population target for suppression, by susceptible insects from a nearby non-target pop-  
469 ulation. This was in anticipation of parallels with the dilution of resistance to engineered insecticidal  
470 (Bt) crops by susceptible insects from refuges of non-transgenic plants (Alphey et al., 2007; Tabash-  
471 nik et al., 2009). If overall selection for resistance  $\gamma$  is low enough then we did indeed observe that  
472 the non-target population acts as a source of susceptible alleles for the target population, thereby  
473 limiting the spread of resistance in this latter population. This effect arises from a combination of  
474 higher fitness costs and/or a low effectiveness of resistance. The dilution of resistance restricts the

475 adverse effect of such a resistant allele on the degree of control (suppression or local elimination)  
476 of the target population that may be achieved. However, whether this population control is more  
477 or less effective than in a non-spatial model, representing an isolated population, depends on the  
478 magnitude of migration from the relatively unsuppressed non-target population.

479 Conversely, a key finding from our model is the potential for a high selective pressure for resistance  
480 to drive a very high equilibrium frequency of resistance in the non-target population, where resistance  
481 dilution from the homozygous susceptible males is limited. A critical strength of net positive selection  
482 can be achieved through a combination of low costs and a high effectiveness of resistance. In these  
483 cases the non-target population becomes a source of resistant alleles and increases the frequency  
484 of resistance in the target population also, particularly with higher dispersal rates between demes.  
485 This in turn reduces the effectiveness of population control.

486 It is not simply the case that the resistant allele's fitness properties assign the non-target deme  
487 irrevocably and unequivocally to be either a source of  $R$  alleles or a source of  $S$  alleles and thus seal  
488 the fate of a planned programme of genetic population control. Interesting dynamic behaviours can  
489 emerge from the interplay of genetic traits and spatial dynamics, with dispersal both into and out of  
490 the target population playing a role over time. For example, where (partially dominant) resistance  
491 is complete in homozygotes, but incomplete in heterozygotes, the dispersal of the genetic construct  
492 away from the release site, and low fitness costs of resistance, can allow a very high equilibrium  
493 frequency of resistance to emerge in the non-target population. As resistance reaches near fixation,  
494 a critical population protection threshold is crossed which drives a rapid increase in the frequency  
495 of the lethal construct in this deme. Migration then drives a rapid increase in the frequency of  
496 the lethal construct in the target population, which ultimately results in stronger suppression of the  
497 target population, a favourable outcome from a programme manager's perspective. This process  
498 implies that spatial effects could make an insect population with a partially dominant and complete  
499 resistance relatively amenable to genetic control (as long as the non-target population is not also of

500 economic or public health importance).

501 The dynamics observed in this spatial model could have different implications for the use of  
502 genetic control depending on the type of control being implemented. If the release strategy is static,  
503 meaning that it is only ever applied in a single target population, then many of the processes described  
504 in these results could come into play. For example, if a very effective resistance with low costs  
505 emerges, then this resistance could proliferate in the (non-target) populations in the surrounding area,  
506 and then indirectly decrease the efficacy of genetic control in the target population through migration.  
507 This process may not be detectable without detailed population genetic monitoring of resistance  
508 frequencies in both the target and the non-target populations. A rolling control programme, on  
509 the other hand, where modified insect releases are first applied to a target population before being  
510 extended to surrounding populations, raises additional questions. For instance, if a high frequency of  
511 resistance evolves in a non-target population, could this evolution be slowed or reversed by resistance  
512 dilution once genetic control is extended and applied directly to this population? Resistance in any  
513 of these scenarios could also be combated by switching to alternative engineered strains which are  
514 not affected by the field-evolved resistance (i.e. no cross-resistance).

515 In a related concurrent study, Thompson (2015) used a constant-number release policy and ab-  
516 solute population sizes in a non-spatial model, finding qualitatively similar results to the proportional  
517 release model (Alphey et al., 2011). This constant release assumption, arguably closer to practical  
518 reality, merits further investigation in a spatial model. However with the insights provided by our  
519 model, the qualitative explanations for our findings are still expected to hold true (albeit at different  
520 values and thresholds). For example, no-cost, modestly effective resistance has a major advantage  
521 in the non-target deme against the lethal construct, which leaks in from the target deme, and has  
522 no selective disadvantage. Resistance therefore spreads extensively in the non-target deme, which  
523 then acts as a source of  $R$  alleles to the target deme. Very high-cost resistance on the other hand  
524 will go extinct in both demes. It is reasonable to anticipate that both of these insights would remain

525 true in a constant-release model, although how 'modestly effective', and how 'high' the costs of,  
526 resistance must be to fall within that region of outcome, would most likely be different under the  
527 two assumptions.

528 We do not simulate dynamics after cessation of releases, where the only benefit of resistance  
529 would be against legacy  $L$  alleles. Unless the  $R$  allele is fixed (unlikely in the target deme, which was  
530 flooded with released susceptible insects), resistance with associated fitness costs should fade away,  
531 and at a faster rate with greater costs. The  $L$  allele should also die out, unless there is complete  
532 resistance remaining in the population. Experiments with the olive fruit fly *Bactrocera oleae* and  
533 diamondback moth *Plutella xylostella*, bearing female-lethal transgenic constructs, showed that the  
534 construct frequency fell by approximately 50% each generation (Harvey-Samuel et al., 2014). This  
535 is as expected due to the construct killing a half of its carriers, with female offspring dying but males  
536 surviving. The decrease in frequency could also potentially be faster if the construct had significant  
537 fitness costs in males. Transgenes at an initial frequency of 0.25 in populations of 200 insects went  
538 extinct in 11 generations or fewer, however the presence of a resistant allele would be expected to  
539 slow this decay. Given the sometimes non-intuitive nature of the spatial effects in our model, it is  
540 difficult to make predictions about the relative rates of allele extinction in the two demes.

541 When judging the relative effectiveness of genetic control in these results it is important to  
542 consider what level of population suppression is actually required to meet the goals of the programme.  
543 In all our simulations, the target equilibrium population size is not as low as that which would be  
544 achieved if no resistance were present. However, population suppression might still be sufficient to  
545 limit damage from an agricultural crop pest below the threshold for economic harm. Similarly, for  
546 pests that act as vectors for disease, the population could potentially still be suppressed below the  
547 entomological threshold to sustain disease transmission. While the scenarios presented here that  
548 result in superior population suppression are more likely to meet these criteria, additional studies, that  
549 include the use of economic and epidemiological models, would be required for formal predictions

550 to be made.

551 Stable oscillations in the population size of the non-target deme, with a low dispersal rate,  
552 were observed in some simulations (costly partially dominant incomplete resistance, figure 4; or  
553 co-dominant complete resistance). This phenomenon is a consequence of the formula for density  
554 dependence used in this model creating a bifurcation surface for population size under certain condi-  
555 tions. Bifurcations of this kind are typically produced by changing the effective rate of reproductive  
556 increase and/or the strength of the density dependent feedback (Hassell et al., 1976). The popu-  
557 lation growth parameter ( $R_0$ ) is kept constant in this model, but the effective population growth  
558 rate is also influenced by the effective ratio of fertile to 'genetically sterile' males, which reduces  
559 reproduction by reducing the fraction of matings that are successful. That fraction is modified by the  
560 extent to which resistance protects individuals from inherited lethal genes. The survival advantage  
561 and the associated fitness penalty (particularly if very costly) of a resistant allele, thus have the  
562 potential to alter the strength of the density dependent feedback, especially in the non-target deme  
563 which has a higher population size. Dispersal also has the potential to produce complex dynamics,  
564 although such effects depend on the model construct (Dey et al., 2014; Sinha and Parthasarathy,  
565 1994); the Bellows model has been shown to exhibit periodic dynamics under emigration (Agarwal  
566 and Sinha, 2005). Additional study of the specific mathematical processes involved (i.e. stability  
567 analysis) would be needed to shed further light on this topic and whether it is of mathematical  
568 and/or ecological significance.

569 We have not explicitly presented simulations for a few alternative combinations of homozygous  
570 and heterozygous resistance effectiveness (e.g. recessive complete resistance,  $\gamma_{SR} = 1$ ,  $\gamma_{RR} = 0$ ).  
571 However, it is clear that the parameter combinations used in this study are sufficient for determining  
572 the general impact of the effectiveness of resistance and the relative importance of homozygous vs.  
573 heterozygous effectiveness. The effectiveness of heterozygous resistance is the major driver behind  
574 the observed dynamics, in accordance with our previous non-spatial findings (Alphey et al., 2011).

575 In some circumstances the homozygous effectiveness has been shown also to play an important role  
576 (figure 7).

577 The type of genetic construct investigated here (bisex, late acting lethality) is applicable to GE  
578 strains such as OX513A *Aedes aegypti*, which targets the main mosquito vector of dengue fever  
579 (Phuc et al., 2007), but could be altered in further studies to tailor results for alternative strains  
580 and target species. Our non-spatial model (Alphey et al., 2011) found qualitatively similar results  
581 from simulations with bisex and female-specific lethality, although this might not necessarily be the  
582 case in a spatial model.

583 The point in the insect life cycle at which dispersal predominantly occurs could differ in some  
584 species. If dispersal were modelled to occur before mating instead, there could be a large influx of  
585 released GE males into the non-target population also. However the assumption in our model, of  
586 limited dispersal occurring after mating, is applicable to a number of key target insect species. For  
587 example, diamondback moths mate at dusk on the day they emerge (Talekar and Shelton, 1993).  
588 The vast majority of males and females only make short trivial flights, remaining on their natal crops,  
589 although a small proportion ( $< 1\%$ ) travel further and long-distance seasonal migrations have been  
590 observed (Furlong et al., 2013). For *Aedes aegypti* mosquitoes, closely adjoining neighbourhoods  
591 would be part of a single population (requiring an area-wide release programme across that whole  
592 district), but male mosquitoes do not disperse across open spaces, or travel far at all, unless necessary  
593 to find resources such as food and mates (Harrington et al., 2005; Hemme et al., 2010; Trpis et al.,  
594 1995). In defining the landscape scale, and planning the releases, we may therefore reasonably  
595 assume that two distinct populations are connected by only a small fraction of adults at the high  
596 end of the dispersal scale distribution.

597 The use of continuous state variables in our deterministic model, means that complete fixation or  
598 loss of an allele cannot occur mathematically. Extending this model to include stochastic dynamics  
599 would therefore be a useful development and might reveal any important differences. For example, if

600 the initial suppression of a population is good enough, potentially the population could be eliminated  
601 though stochastic effects before resistance emerges and limits the effectiveness of control. Similarly,  
602 at very low frequencies the resistant ( $R$ ) or susceptible ( $S$ ) alleles, or the wild type ( $w$ ) absence  
603 of transgene at the locus of the lethal construct, could be lost from the population, while the  
604 relevant corresponding allele would reach fixation. In a spatial model, an allele would have to be  
605 lost, or a population would have to be eradicated, from both demes in order to disappear entirely,  
606 as migration will quickly reintroduce alleles and individuals. Stochasticity could also have a major  
607 impact on dynamics where critical thresholds exist that can drive alternative outcomes.

608 We assume a single resistant gene in the population, with constant trait values for the effec-  
609 tiveness of resistance and its fitness costs. A further interesting study could start with a high  
610 cost and relatively ineffective resistance, and explore the potential evolution of fitness modifiers by  
611 modelling a number of resistant alleles with different values, and allowing mutation between types.  
612 This could model the time dynamics of evolutionary change in the resistant allele simultaneously  
613 with the population dynamics of the genetic control method. This might be done, for example, by  
614 adapting evolutionary ecology methods that apply Price's equation to model evolutionary change in  
615 a pathogen population coupled with epidemiological dynamics (Day and Gandon, 2006).

616 Further development of this model could focus on varying the spatial population structure and  
617 patterns of dispersal. In reality a target population may be surrounded by a larger non-target  
618 population or group of populations, a scenario that could be simulated by simply increasing the  $N_0$   
619 of the non-target deme (or by expanding to a network model). The study of source/sink population  
620 dynamics, or a directional bias in migration, between the two demes could also prove fruitful and  
621 would increase the relevance of this model for a wider variety of ecological settings. Furthermore,  
622 the flight potential of wild type and transgenic insects can differ (Bargielowski et al., 2012), a  
623 scenario that could be modelled by using different dispersal rates for these two groups of genotypes  
624 when calculating migration between the demes. Rather than a constant proportion of a population

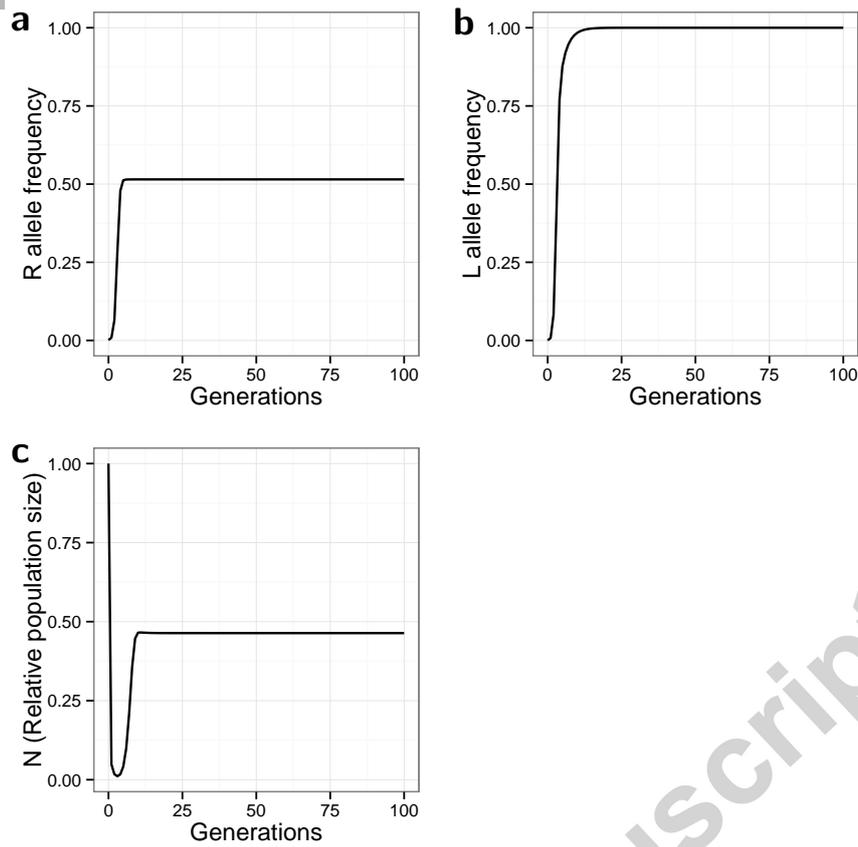
625 dispersing to another deme, the departure of insects could be density dependent. This is a promising  
626 area for further study, as there are a large number of additional factors that could have a significant  
627 effect on the spatial evolution of resistance.

628 Pest insects are notorious for developing resistance, physiological or behavioural, to diverse  
629 forms of pest management. Genetics-based methods, like any other effective control, impose strong  
630 selection pressure in favour of any allele that confers resistance to the control mechanism. The  
631 built-in resistance dilution (through releasing susceptible males) may lessen the risk somewhat, by  
632 presenting an evolutionary barrier that weak unfit resistant alleles are unable to surpass. However, it  
633 is still likely that the self-limiting genetic strategy modelled here would be implemented as part of an  
634 integrated pest management or integrated vector control programme. Deployment of multiple pest  
635 management tools with independent modes of action, for example engineered male releases with  
636 biopesticides such as Bti, serves to slow the evolution of resistance to any one of the components.

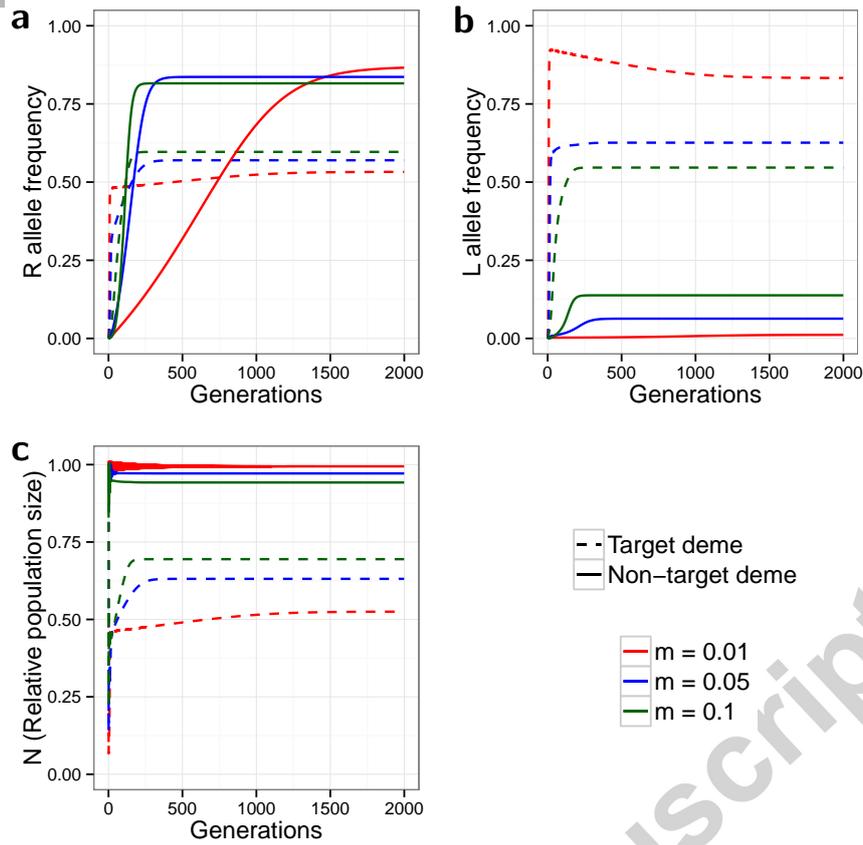
637 In conclusion, we have highlighted the importance of spatial effects in the evolution of resistance  
638 to a self-limiting genetic insect control method based on the sterile insect technique. Depending  
639 on the nature of the resistance and the rate of dispersal, spatial dynamics can drive an increased or  
640 decreased evolution of resistance in both the target and non-target population, compared with that  
641 predicted for otherwise identical resistance in an isolated target pest population. This evolution could  
642 have a significant impact, positive or negative, on the effectiveness of genetic population suppres-  
643 sion, through a variety of interacting population genetic and population dynamic processes. These  
644 considerations could influence priorities for research and development, for example, which species to  
645 prioritise for product development, and how much effort to devote to measuring dispersal behaviour  
646 in field settings. Our findings also have practical implications for field trials and implementation  
647 programmes. For example, the number and placement of traps in and outside of the focal area for  
648 entomological surveillance, and knowledge of how changes in nearby populations (e.g. the stability  
649 of population dynamics in the field, or performance of wild-caught insects in laboratory tests for

650 resistance) might presage evolutionary effects in the population that has been targeted for control.  
651 Spatial interactions between a target population and insects in a non-target area can have a range  
652 of possible outcomes, from providing extra dilution to further slow or even prevent the spread of  
653 resistance in the target population, thus improving the efficacy of the control programme, to at the  
654 other extreme exacerbating the evolution of resistance.

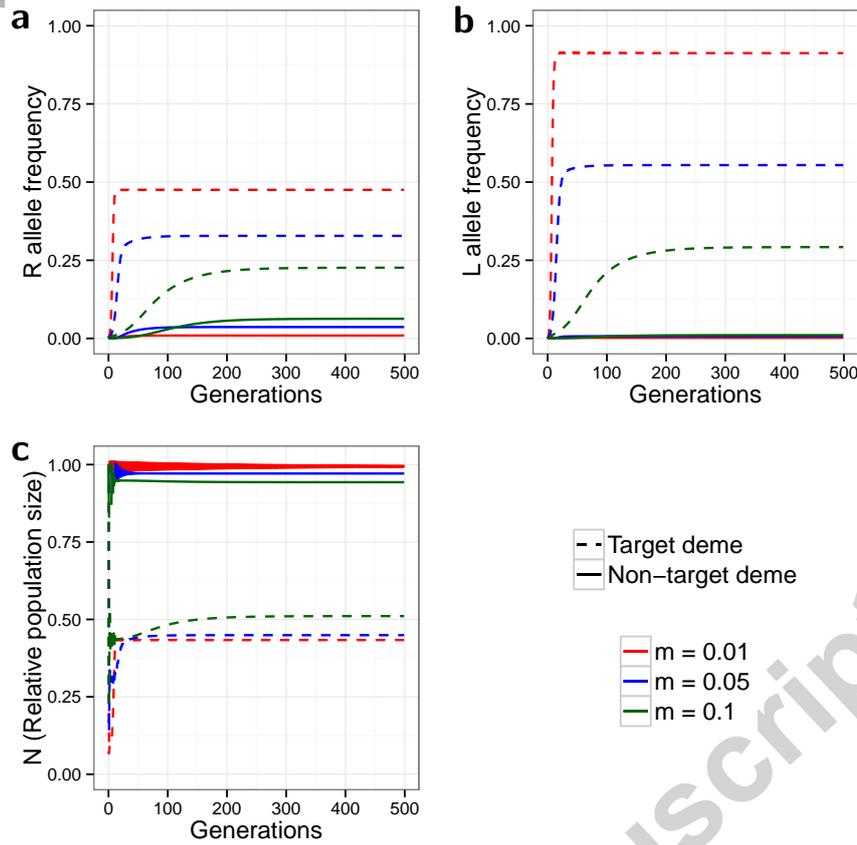
Accepted manuscript



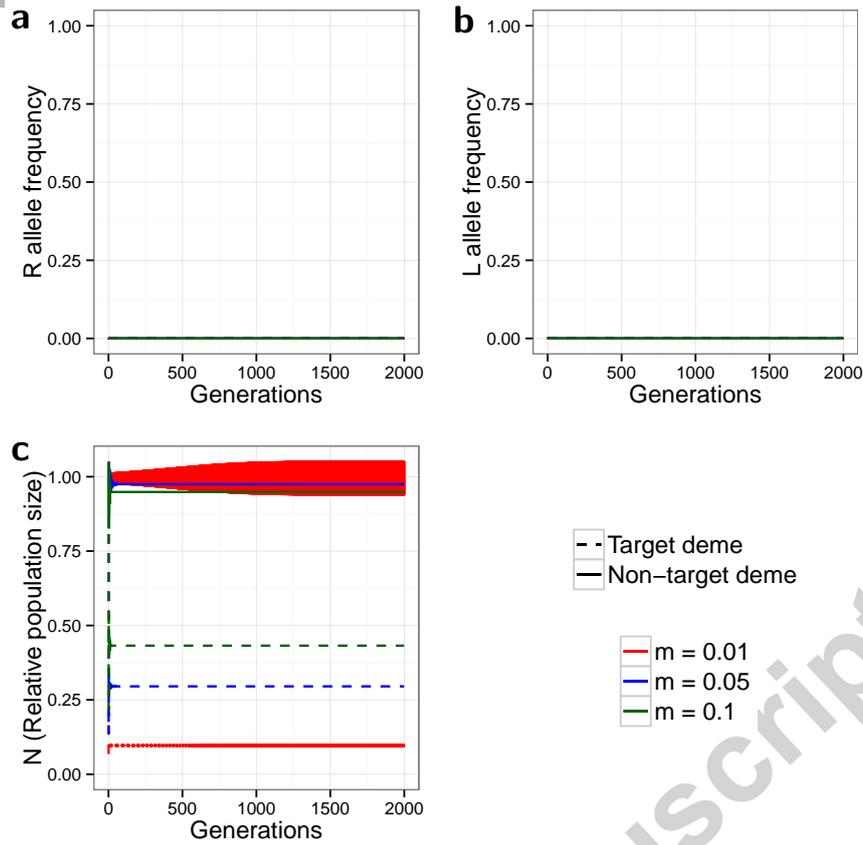
**Figure 1: Non-spatial model** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model has a release ratio of  $d = 20$ , and a strong partially dominant incomplete resistance ( $\gamma_{SR} = 0.2$ ,  $\gamma_{RR} = 0.1$ ) with no associated costs ( $\psi_{SR} = \psi_{RR} = 1$ ). The spread of resistance leads to fixation of the lethal construct, and reduces the effectiveness of population control.



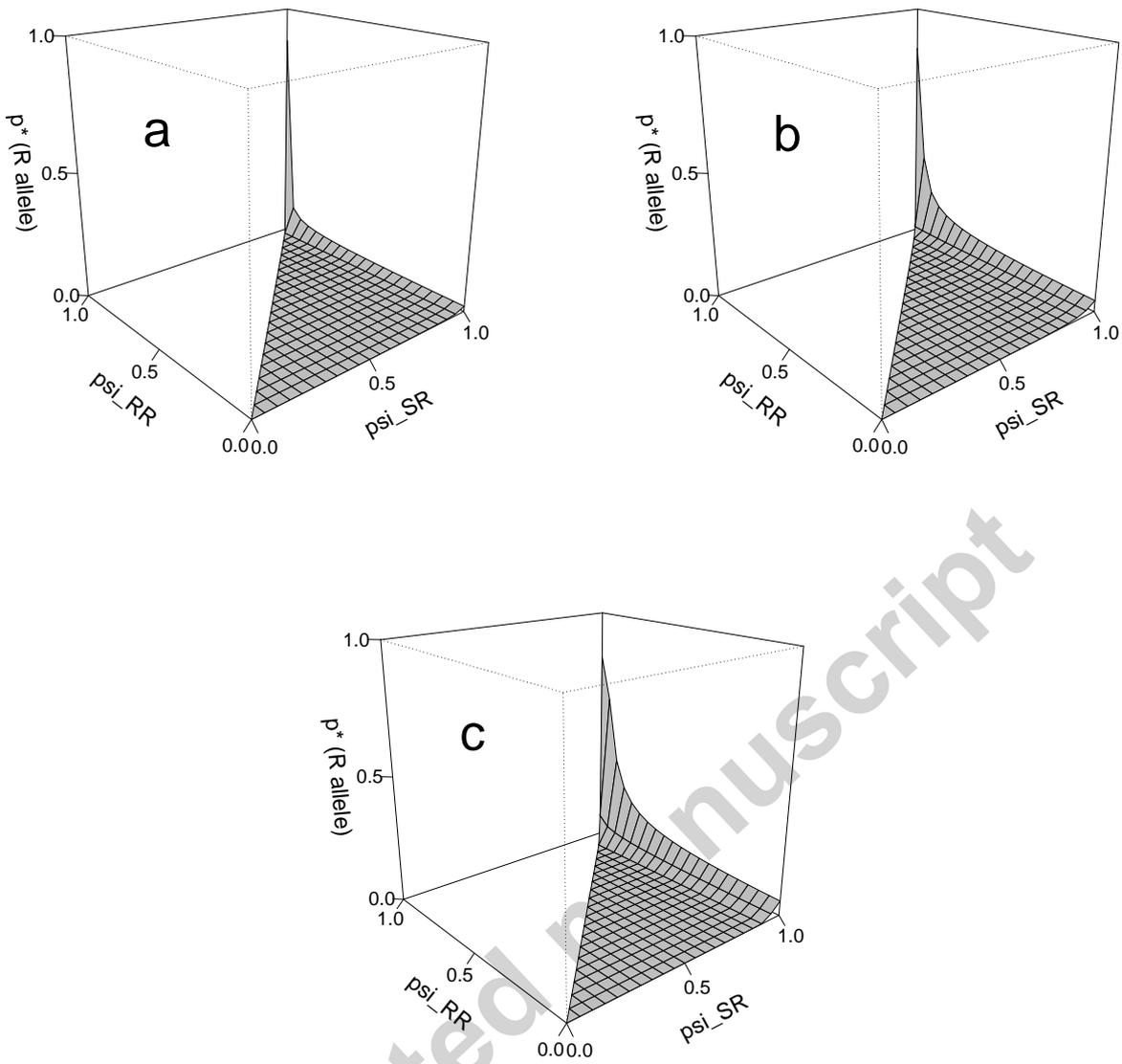
**Figure 2: Spatial model, no-cost resistance** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model is spatial, with release ratio  $d = 20$ , and a strong partially dominant incomplete resistance ( $\gamma_{SR} = 0.2$ ,  $\gamma_{RR} = 0.1$ ) with no associated costs ( $\psi_{SR} = \psi_{RR} = 1$ ). Dashed lines indicate the target deme, solid lines indicate the non-target deme, and the line colours indicate the simulated dispersal rate (see legend). 2000 of the 3000 simulated generations are shown. The non-target deme acts as a source of  $R$  alleles, which in turn reduces the effectiveness of control in the target deme. Higher dispersal increases the magnitude of this impact.



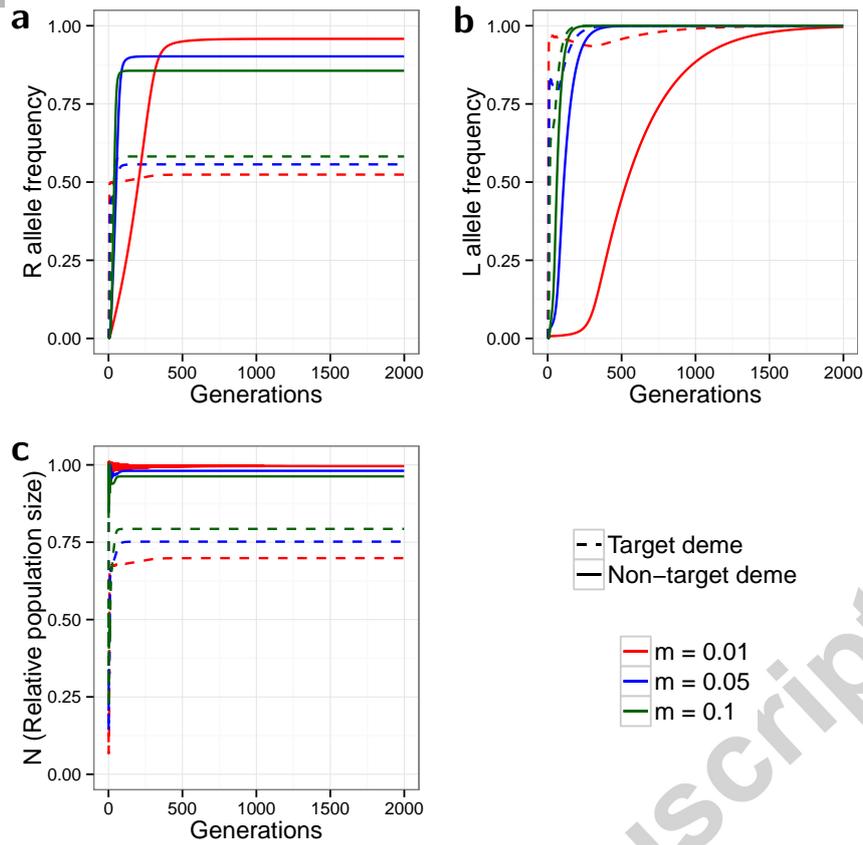
**Figure 3: Minor costs of resistance** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model is spatial, with release ratio  $d = 20$ , and a strong partially dominant incomplete resistance ( $\gamma_{SR} = 0.2$ ,  $\gamma_{RR} = 0.1$ ) with minor costs ( $\psi_{SR} = 0.95$ ,  $\psi_{RR} = 0.85$ ). Dashed lines indicate the target deme, solid lines indicate the non-target deme, and the line colours indicate the simulated dispersal rate (see legend). The non-target deme acts as a source of  $S$  alleles, thereby enhancing the effectiveness of target population control.



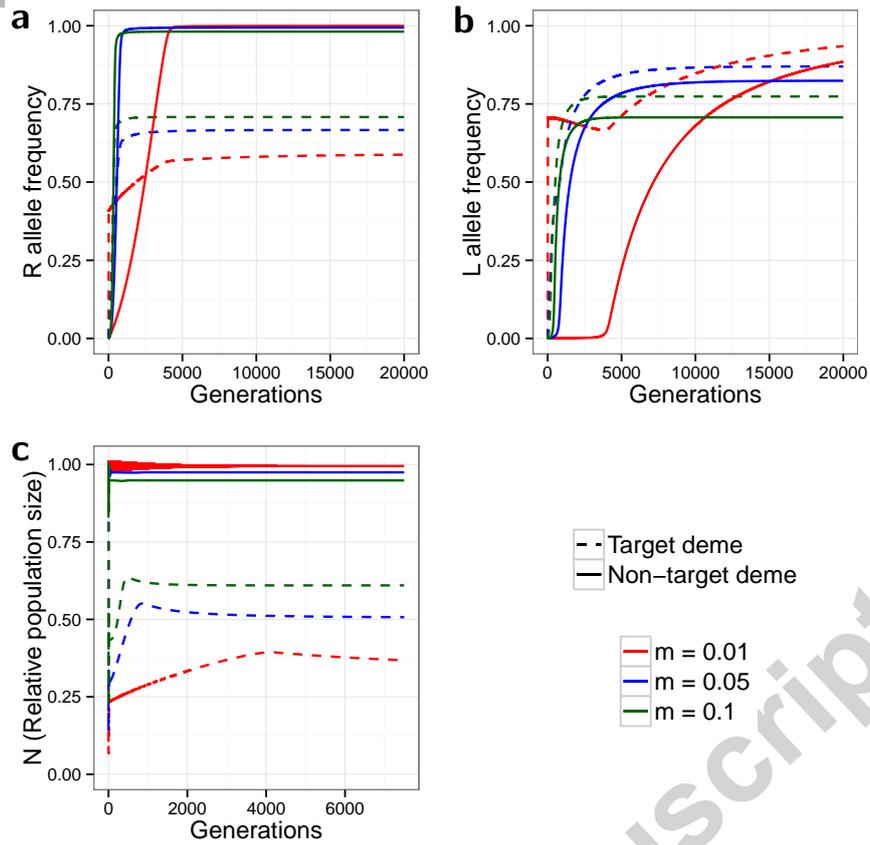
**Figure 4: Greater costs of resistance** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model is spatial, with release ratio  $d = 20$ , a strong partially dominant incomplete resistance ( $\gamma_{SR} = 0.2$ ,  $\gamma_{RR} = 0.1$ ), and costly resistance ( $\psi_{SR} = 0.2$ ,  $\psi_{RR} = 0.1$ ). Dashed lines indicate the target deme, solid lines indicate the non-target deme, and the line colours indicate the simulated dispersal rate (see legend). Note the longer time scale (2000 generations) than shown in earlier figures. The high-cost resistance goes extinct in both demes; control of the target population is only tempered by immigration from the non-target deme.



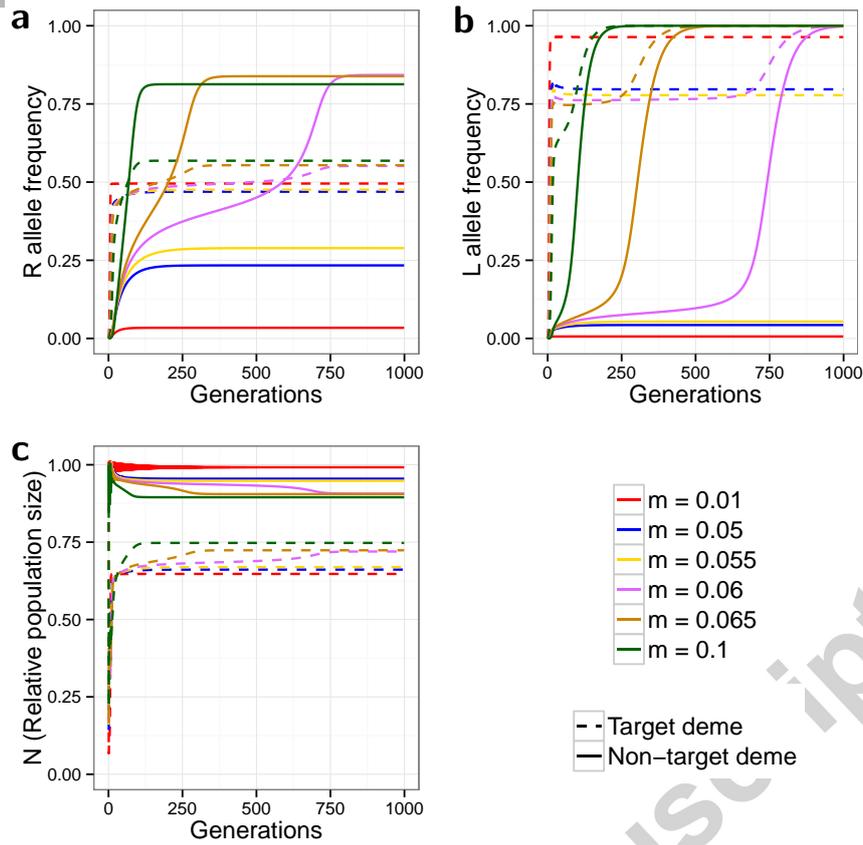
**Figure 5:** The effect of  $\psi_{SR}$  and  $\psi_{RR}$  on  $p^*$  (the equilibrium  $R$  allele frequency) in the non-target deme. The model is spatial, with release ratio  $d = 20$ , and a strong partially dominant incomplete resistance ( $\gamma_{SR} = 0.2$ ,  $\gamma_{RR} = 0.1$ ). Dispersal rates  $m = 0.01$  (a),  $m = 0.05$  (b) and  $m = 0.1$  (c) are used. Only points where  $\psi_{SR} \geq \psi_{RR}$  (so that heterozygote resistance is always less costly than homozygote resistance) are shown. The threshold increase in  $p^*$  can clearly be seen at high  $\psi_{SR}$  and  $\psi_{RR}$  values (low costs of resistance).



**Figure 6: Dominant, complete, no-cost resistance** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model is spatial, with release ratio  $d = 20$ , and a dominant complete resistance ( $\gamma_{SR} = \gamma_{RR} = 0$ ) with no associated costs ( $\psi_{SR} = \psi_{RR} = 1$ ). Dashed lines indicate the target deme, solid lines indicate the non-target deme, and the line colours indicate the simulated dispersal rate (see legend). 2000 of the 3000 simulated generations are shown. The highly effective resistance spreads and allows the lethal construct to reach fixation in both demes. Even in this worst-case scenario, some suppression of the target population is observed.



**Figure 7: Less dominant, complete, no-cost resistance** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model is spatial, with release ratio  $d = 20$ , and a partially dominant complete resistance ( $\gamma_{SR} = 0.4$ ,  $\gamma_{RR} = 0$ ) with no associated costs ( $\psi_{SR} = \psi_{RR} = 1$ ). Dashed lines indicate the target deme, solid lines indicate the non-target deme, and the line colours indicate the simulated dispersal rate (see legend). Note the longer time scale (20000 generations) than that shown in earlier figures. Only 7500 generations are shown for c to highlight the local peak in the target population size. The near fixation of resistance in the non-target deme drives an increase in the frequency of the lethal construct in that deme. Through migration, this increases the  $L$  allele frequency in the target deme also, which ultimately increases the effectiveness of target population control.



**Figure 8: Introducing minor costs of dominant, complete resistance** - Evolution of the  $R$  allele frequency (a), the  $L$  allele frequency (b), and the change in the relative population size over time (c). The model is spatial, with release ratio  $d = 20$ , and a dominant complete resistance ( $\gamma_{SR} = \gamma_{RR} = 0$ ) with minor costs ( $\psi_{SR} = 0.95$ ,  $\psi_{RR} = 0.85$ ). Dashed lines indicate the target deme, solid lines indicate the non-target deme, and the line colours indicate the simulated dispersal rate (see legend). Greater influx of  $L$  alleles through dispersal into the non-target deme selects for a large increase in the frequency of resistance. This causes the non-target deme to become a source of  $R$  alleles for the target deme, which decreases the effectiveness of target population suppression.

**Table 1:** Parameters and variables used in the mathematical model

Symbol	Parameter/Variable Description	Constraints/Values
$p$	Frequency of resistant $R$ allele in current adult generation	$0 \leq p \leq 1$
$q$	Frequency of susceptible $S$ allele	$0 \leq q \leq 1, p + q = 1$
$p_0$	Initial $R$ allele frequency	0.001
$p^*$	Equilibrium $R$ allele frequency	
$l$	Frequency of the (GE) lethal genetic construct $L$	$0 \leq l \leq 1$
$i$	Genotype at $S/R$ locus	$SS, SR$ or $RR$
$j$	Genotype at $L/w$ locus ( $w$ is the wild-type absence of the lethal construct)	$LL, Lw$ or $ww$
$\psi_i$	Relative fitness of larvae of genotype $i$ (costs of resistance)	$0 \leq \psi_{RR} \leq \psi_{SR} \leq \psi_{SS} = 1$
$\epsilon_k$	Fitness penalty of lethal construct (to both sexes)	$\epsilon_k = 1$
$\gamma_i$	Susceptibility to the lethal construct (scaling factor applied to fitness penalty $\epsilon$ )	$0 \leq \gamma_{RR} \leq \gamma_{SR} \leq \gamma_{SS} = 1,$ $\gamma_{RR} \neq \gamma_{SS}$
$\eta_j$	Number of copies of the lethal construct	0 for $ww$ , 1 for $Lw$ , or 2 for $LL$
$\Omega_{ijk}$	Relative fitness of larvae of genotype $i, j, k$	$0 \leq \Omega_{ijk} \leq 1$
$d$	Release ratio of GE males to the total number of males in the wild population at that generation	1, 20, or 50
$R_0$	Average number of female progeny produced per adult female in its lifetime (a single generation)	7.5
$N_t$	Population size of mature adults at generation $t$ relative to the initial population size	$N_0 = 1$
$F_t$	Relative population size of mature females at generation $t$	$F_0 = 0.5$
$\hat{F}_t$	Migration adjusted female population size	
$\sigma_t$	Simulated proportion of all offspring that survive to maturity	
$m$	Dispersal rate between demes (the proportion of the resident population emigrating)	$0 \leq m \leq 1$
$Z_t$	Matrix of zygote genotype frequencies in generation $t$	

**Table 2:** Designated parameter values for the resistance trait.  $\gamma_{SS} = \psi_{SS} = 1$  in all simulations. Relative fitnesses for each combination of parameter values by genotype are detailed in supplementary table S1.

Parameter designation	Constraints/Values
dominant complete	$\gamma_{SR} = \gamma_{RR} = 0$
partially dominant complete	$1 > \gamma_{SR} > 0, \gamma_{RR} = 0$
co-dominant complete	$\gamma_{SR} = 0.5, \gamma_{RR} = 0$
partially dominant incomplete	$1 > \gamma_{SR} > \gamma_{RR} > 0$
no costs	$\psi_{SR} = \psi_{RR} = 1$
minor costs	$\psi_{SR} = 0.95, \psi_{RR} = 0.85$
fit resistance	$\psi_{SR} = 0.9, \psi_{RR} = 0.7$
costly resistance	$\psi_{SR} = 0.2, \psi_{RR} = 0.1$

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