Ecological effects on underdominance threshold drives for vector control

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Abstract

Underdominance gene drives are frequency-dependent drives that aim to spread a desired homozygote genotype within a population. When the desired homozygote is released above a threshold frequency, heterozygote fitness disadvantage acts to drive the desired trait to fixation. Underdominance drives have been proposed as a way to control vector-borne disease through population suppression and replacement in a spatially contained and reversible way—benefits that directly address potential safety concerns with gene drives. Here, ecological and epidemiological dynamics are coupled to a model of mosquito genetics to investigate theoretically the impact of different types of underdominance gene drive on disease prevalence. We model systems with two engineered alleles carried either on the same pair of chromosomes at the same locus or homozygously on different pairs at different loci, genetic lethality that affects both sexes or only females, and bi-sex or male-only releases. Further, the different genetic and ecological fitness costs that can arise from genetic modification and artificial rearing are investigated through their effect on the population threshold frequency that is required to trigger the drive mechanism. We show that male-only releases must be significantly larger than bi-sex releases to trigger the underdominance drive. In addition, we find that female-specific lethality averts a higher percentage of disease cases over a control period than does bi-sex lethality. Decreases in the genetic fitness of the engineered homozygotes can increase the underdominance threshold substantially, but we find that the mating success of transgenic mosquitoes with wild-type females (influenced by a lack of competitiveness or the evolution of behavioural resistance in the form of active female mate preference) and the longevity of artificially-reared mosquitoes are vitally important to the success chances of underdominance based gene drive control efforts.

Keywords: gene drive, population genetics, mosquito ecology, epidemiology, mosquito control

1. Introduction

Vector-borne diseases inflict significant levels of human morbidity and mortality, accounting for 17% of the global disease burden. More than half of the world's population are at risk of contracting a vector-borne disease; more than 300M people contract dengue annually [1], and around 200M malaria infections are reported every year, leading to an estimated 429 000 deaths [2]. Genetic vector control technologies may become the first line of defence against diseases for which no vaccine or cure is available, or in

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cases where pathogens have developed resistance to medication. However, there are significant concerns about the safety and viability of these technologies. Thus, the World Health Organisation's Global Vector Response 2017–2030 highlights the need for evidence of the possible impact of new vector interventions [3].

Underdominance refers to traits where heterozygotes are less fit than homozygotes [4]. Consequently, underdominance in a single population is characterised by an unstable equilibrium (the threshold) that acts as a bi-stable switch between the fixation of one allele (in its homozygote genotype) or another. This phenomena may be an economically viable way to ensure an introduced gene reaches fixation if the allele attains a higher frequency than the unstable equilibrium point (through mass-releases of transgenic mosquitoes, for example). This would occur without the need for further investment, through the action of the heterozygote disadvantage. This threshold phenomenon also has two key positive attributes: first, it allows population control efforts to be spatially containable. Migrants from a modified population that enter a wild-type population will be rare (below the threshold) hence the heterozygote disadvantage will drive them to extinction [5–7]. Second, control interventions are naturally reversible: releasing wild-types to a frequency above the threshold will drive them to fixation, thus eradicating the modified population [8]. By contrast, other gene drive-type technologies may be very difficult to contain and are only reversible by releases of further-modified rescue genotypes (although low-fitness varieties of *Medea* and *Wolbachia* should be reversible by large-scale wild-type releases [8]). Given these benefits, amid the potential safety concerns over the use of gene drive systems [9, 10] and the lack of specific and sufficient regulation [11], underdominance drives are a promising candidate as the self-sustaining genetic control technology of choice for insect pests and disease vectors.

Fully understanding both genetic and ecological fitness costs that modified insects may suffer is vital if underdominance is to be utilised successfully for vector population control. Ecological fitness is affected by differences in mating success, larval development and survival between transgenic and wild strains. Genetic fitness is a combination of the cost intended to cause heterozygote disadvantage and the unintended ambient cost due to genetic modification. Implementations of underdominance in the laboratory and field have had mixed results. Insects with underdominant traits have been created in the laboratory using translocations [12], and studies have investigated their use for the control of mosquitoes [13–16] and other insect pests [17–19]. Usually, population replacement rather than suppression is the goal of the control effort, but translocations [20–22] and other chromosomal rearrangements [23] have also been suggested as methods of population suppression. Translocation-bearing insects typically have low fitness compared to wild counterparts [16, 18], and there has been no successful method of linking a payload gene (e.g. a gene causing refractoriness to disease) to a translocation break point [24]—these issues have meant work has moved away from translocations. Underdominant gene drives have more recently been theorised [25] and realised using engineered alleles with versions of a toxin–antidote system (through maternal toxins targeting genes vital for embryonic development [26] and through RNA interference of a haploinsufficient endogenous gene [5]; approaches collectively known as engineered underdominance), which may assuage the high fitness costs that translocation-bearing insects suffer.

Our starting point is the engineered underdominance model developed by Davis *et al.* [25]. Unlike previous extensions of this work [6, 27–29], we avoid simplifying assumptions of hermaphroditic, large (or infinite) populations, where heterozygotes are perfectly non-viable. Instead, we use a continuous-time differential equation ecological model coupled to a population genetics model that governs the offspring proportions. We will account for gender imbalances due to male-only releases and female-specific lethality, imperfect lethality in heterozygotes and genetic fitness costs on the engineered homozygotes. We will also examine the effects of wild-type female mate preference and perturbations to life history parameters of insects carrying engineered alleles. Though the use of underdominance to control disease vector populations has been suggested before, no theoretical study has been undertaken explicitly linking a genetic underdominance model with a mathematical model of a vector-borne disease. We employ an epidemiological model and use it to judge the effects of releasing insects with engineered underdominant alleles.

2. Models

A general engineered underdominance (UD) system consists of two mutually dependent alleles, α and β. Each allele contains a promoter gene that expresses a (partially) lethal toxin, and each also suppresses the toxin expression of the other allele. In this way, heterozygotes of either of the alleles with a wild-type allele suffer a large genetic load, with relative fitness $\phi_{\text{het}} < \phi_{\text{hom}} \leq 1$, where ϕ_{hom} is the fitness of the engineered homozygote (made up of α and β in equal proportions). The wild-type genotype has a relative genetic fitness of unity. The fitness costs of the engineered alleles are assumed to act multiplicatively where they are not suppressed.

We consider two UD systems, as proposed by Davis *et al.* [25]. First, a homologous system, in which α and β are carried on the same pair of chromosomes at the same locus; in this case the wild and engineered homozygote genotypes are AA and $\alpha\beta$, respectively. Second, a non-homologous UD system, in which α and β are carried homozygously on different pairs of chromosomes at different loci; the wild and engineered homozygote genotypes are $AABB$ and $\alpha\alpha\beta\beta$, respectively. The non-homologous system has two subtypes: if a single copy of the α allele sufficiently suppresses the toxin expression of two β alleles (and vice versa), α and β are strongly-suppressed lethal alleles; if two copies of α are required to suppress two copies of β (and vice versa), they are weakly-suppressed lethals. There are six genotypes in the homologous system and nine genotypes in the two non-homologous systems, see table 1 and appendix B **(where the relative fitnesses for all genotypes are given explicitly for both sexes)**. The genetics are linked to a continuous time ecological model of the mosquito lifecycle and a coupled Ross–MacDonald epidemiological model of a vector-borne disease **(see Figure 1 for a schematic overview of this modelling framework)**. We consider the use of underdominance only as a method of vector population suppression, and disregard the possibility of linking payload genes to the engineered underdominance constructs.

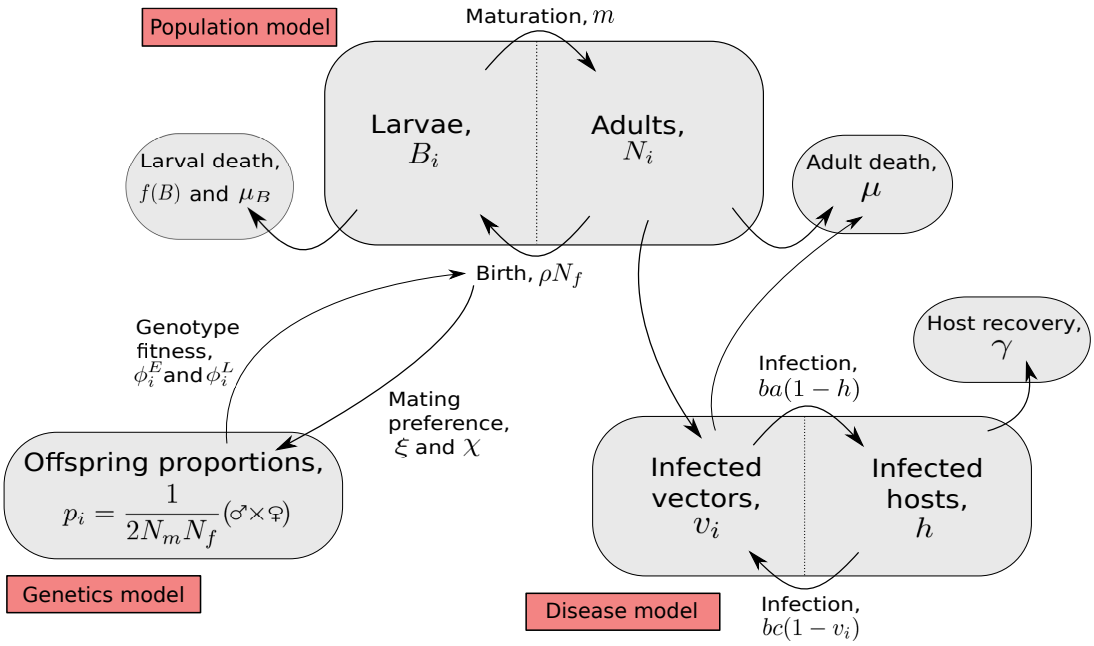


Figure 1: Schematic of the mathematical model defined in (2.1) and (2.9). Arrows depict how variables feed into one another (e.g. larvae feed into adults) or into 'sinks' (e.g. larvae feed into the larval mortality sink). Arrow labels show the parameters that govern the flow between variables. The details of the genetics model, including the implementations of mating preference and genotype fitness, are given in appendix A and appendix B. Parameter and variable definitions are given in table 2 and table 3, respectively.

2.1. Population dynamics

We propose a stage-structured population model for the mosquito, taking into account an aquatic juvenile stage (B) and an adult stage (N). The dynamics are captured by the following set of ordinary differential equations

$$\frac{\mathrm{d}B_i}{\mathrm{d}t} = \rho p_i N_f \phi_i^E - (f_i(B) + m + \mu_B)B_i, \tag{2.1a}$$

$$\frac{\mathrm{d}\hat{B}_i}{\mathrm{d}t} = \rho p_i N_f \hat{\phi}_i^E - (f_i(B) + m + \mu_B)\hat{B}_i, \tag{2.1b}$$

$$\frac{\mathrm{d}N_i}{\mathrm{d}t} = mB_i\phi_i^L - \mu N_i, \tag{2.1c}$$

$$\frac{\mathrm{d}\hat{N}_i}{\mathrm{d}t} = m\hat{B}_i\hat{\phi}_i^L - \mu\hat{N}_i, \tag{2.1d}$$

where ρ is the oviposition rate of the adult females, p_i is the proportion of individuals of each sex that arise from **the i^{th} genetic mating cross** (see appendix A), m is the larval maturation rate and μ and μ_B are the density-independent mortality rates for adults and juveniles, respectively. **The relative fitness of a female (male) of genotype i, ϕ_i ($\hat{\phi}_i$), is constructed multiplicatively from the contributions of each of the alleles that make up the genotype (with wild-type alleles contributing a relative fitness of unity). Thus,**

$$\phi_i = \kappa_{\mathrm{a}}^{m_i}\kappa_\ell^{n_i}, \tag{2.2}$$

where m_i is the total number of trangenes carried by genotype i (imposing an ambient cost c_{a} each) and n_i ($\leq m_i$) is the number of unsuppressed toxin transgenes carried (imposing a partially lethal cost c_ℓ each), with $\kappa_{\mathrm{a}} = 1 - c_{\mathrm{a}}$ and $\kappa_\ell = 1 - c_\ell$. The relative fitnesses ϕ_i^E and ϕ_i^L are mutually exclusive (see below) and account for early- and late-acting fitness costs, respectively. Hats on variables denote male versions that may differ from the female version—for example $\hat{\phi}_i \neq \phi_i$

if the heterozygote lethality is female-specific (e.g. table 1). The function $f_i(B)$ in (2.1a) and (2.1b) accounts for density-dependent mortality at the larval stage, and due to the lack of robust evidence on this ecological process we choose to use the flexible function [30]

$$f(B) = \ln\left[1 + (\nu B)^{\eta}\right], \tag{2.3}$$

where η is the strength of density dependence and ν is the scale parameter of the density-dependent effects. We define ν such that the following relation holds:

$$\nu = \frac{m}{2k^* H\mu}\left(\mathrm{e}^{\{\rho m/(2\mu) - m - \mu_B\}} - 1\right)^{\frac{1}{\eta}}, \tag{2.4}$$

to ensure that the equilibrium wild vector population N_1^* is equivalent to k^*H, the number of wild-type vectors per host at equilibrium (k^*) multiplied by the host population (H, assumed constant). The total population sizes are given by

$$B = \sum_{j=1}^{n_g}(B_j + \hat{B}_j), \qquad N_f = \sum_{j=1}^{n_g} N_j, \qquad N_m = \sum_{j=1}^{n_g} \hat{N}_j, \tag{2.5}$$

where we sum over all genotypes (including both males and females in the larval population B), of which there are n_g. For early-acting lethality, the fitness costs act at reproduction (i.e. in (2.1a) and (2.1b)), thus $\phi_i^E = \phi_i$, $\phi_i^L = 1$, $\hat{\phi}_i^E = \hat{\phi}_i$ and $\hat{\phi}_i^L = 1$; for late-acting lethality, the fitness costs act at maturation to the adult stages (i.e. in (2.1c) and (2.1d)), thus $\phi_i^E = 1$, $\phi_i^L = \phi_i$, $\hat{\phi}_i^E = 1$ and $\hat{\phi}_i^L = \hat{\phi}_i$.

	Genotype							
	Male				Female			
Lethality	AA	$A\alpha$, $A\beta$	$\alpha\beta$	$\alpha\alpha$, $\beta\beta$	AA	$A\alpha$, $A\beta$	$\alpha\beta$	$\alpha\alpha$, $\beta\beta$
BSL	1	$\kappa_\mathrm{a}\kappa_\ell$	κ_a^2	$\kappa_\mathrm{a}^2\kappa_\ell^2$	1	$\kappa_\mathrm{a}\kappa_\ell$	κ_a^2	$\kappa_\mathrm{a}^2\kappa_\ell^2$
FSL	1	κ_a	κ_a^2	κ_a^2				

Table 1: Genotype fitness for the homologous engineered underdominance system for bi-sex lethality (BSL) and female-specific lethality (FSL); $\kappa_\mathrm{a} = 1 - c_\mathrm{a}$ and $\kappa_\ell = 1 - c_\ell$ where c_a is the ambient fitness cost of carrying a single transgene and c_ℓ is the (partially) lethal fitness cost of carrying an unsuppressed toxin gene. Fitness costs are assumed to combine multiplicatively. In particular, the engineered homozygote fitness is $\phi_\mathrm{hom} = (1 - c_\mathrm{a})^2$ and the heterozygote fitness is $\phi_\mathrm{het} = (1 - c_\mathrm{a})(1 - c_\ell)$. Genotypes with the same fitness are grouped. For the non-homologous underdominant systems see tables B.4–B.7 in appendix B.

2.2. Disease dynamics

The basic disease dynamics follow a modified Ross–MacDonald approach [31] such that if the density of infected mosquitoes is

$$\frac{dY}{dt} = bc(N_f - Y)h - \mu Y, \tag{2.6}$$

where each susceptible mosquito (of which there are $N_f - Y$) bites b people per day of which a proportion h are infectious and fraction c of those bites acquires infection then the proportion of infected vectors v is:

$$\frac{\mathrm{d}v}{\mathrm{d}t} = bc(1 - v)h - \left(\mu + \frac{1}{N_f}\frac{\mathrm{d}N_f}{\mathrm{d}t}\right)v. \tag{2.7}$$

The proportion of infected humans is:

$$\frac{\mathrm{d}h}{\mathrm{d}t} = \frac{N}{H} ba(1-h)v - \gamma h, \tag{2.8}$$

where a is the proportion of bites on susceptible humans by infectious mosquitoes that result in infection and γ is the host recovery rate. The dynamics of the expanded vector–disease interaction when GM mosquitoes are released are:

$$\frac{\mathrm{d}h}{\mathrm{d}t} = \frac{1}{H} ba(1-h) \sum_{i=1}^{n_g} N_i v_i - \gamma h, \tag{2.9a}$$

$$\frac{\mathrm{d}v_i}{\mathrm{d}t} = bc(1-v_i)h - \left(\mu + \frac{1}{N_i}\frac{\mathrm{d}N_i}{\mathrm{d}t}\right) v_i, \tag{2.9b}$$

where the adult females in every genotype can be a vector of disease. Parameter definitions and values for the epidemiological model (2.9) and the population model (2.1) are listed in table 2; variable definitions are given in table 3.

Parameter	Description	Default value	Notes
ρ	per adult female oviposition rate	16 per day	[32–34]
m	larval maturation rate	0.1	[35]
μ	adult mosquito death rate	$\ln \frac{10}{9}$ per day	[36–40], chosen to be conservative
μ_B	density-independent larval death rate	0.03 per day	[41]
η	strength of density dependence	0.9	varies from $\eta < 1$ for contest to $\eta > 1$ for scramble
ν	scale of larval density-dependence	eq. (2.4)	ensures $N_1^* = k^* H$
c_{a}	ambient fitness cost of carrying single transgene	variable	fitness cost acts even when the toxin is suppressed
c_ℓ	lethal fitness cost of carrying unsuppressed toxin gene	variable	lethality may not be totally efficient
κ_{a}	relative fitness derived from incurring cost c_{a}	$1 - c_{\mathrm{a}}$	relative to wild type
κ_ℓ	relative fitness derived from incurring cost c_ℓ	$1 - c_\ell$	relative to wild type
$\phi_i / \hat{\phi}_i$	female/male relative fitness of genotype i	$\kappa_{\mathrm{a}}^n \, \kappa_\ell^m$	n, m are genotype-specific numbers of transgenes imposing costs c_{a}, c_ℓ
H	host population	1000	variable
k^*	vectors per host at equilibrium	2	but can be as high as 200 [39, 42–44]
b	mosquito bite rate	2.8 per day	[45]
a	vector to human transmission efficiency	0.084	median value from [36, 37]
c	human to vector transmission efficiency	0.216	median value from [36, 37]
γ	host recovery rate	1/14 per day	variable, assuming a two-week average
ξ	mating preference of wild females for wild males	variable	the proportion of wild females from each encounter with non-wild males that instead choose to mate with wild males
χ	increase in wild-wild matings due to mating preference	$\frac{\xi}{\hat{N}_1} \sum_{i=2}^{n_g} \hat{N}_i$	where $\hat{N}_i$ is the male population of genotype i, $i \in [1, n_g]$ with $i = 1$ being wild-type

Table 2: Parameter definitions and values. *Anopheles gambiae* is used as the model species, with the disease model parameterised using malaria data.

Variable	Description
B_i, $\hat{B}_i$	female and male larvae population of genotype i
N_i, $\hat{N}_i$	female and male adult mosqito population of genotype i
h	porportion of human hosts infected
v_i	proportion of adult female mosquitoes of genotype i infected

Table 3: Definitions of the variables for which we solve as functions of time, t, using the coupled dynamical system defined in (2.1) and (2.9).

3. Results: genetics

In this section we examine different genetic configurations for UD gene drives: bi-sex and male-only releases; variation in fitness costs imposed by the engineered alleles; and female-specific and bi-sex lethality. We use two metrics to aid in the analysis: (i) the threshold frequency that a single initial release must reach in order to trigger the UD drive in favour of the engineered constructs; (ii) the effect on the disease burden of both successful and unsuccessful attempts to trigger the UD drive, quoted as the

percentage of cases averted over a time period compared to a control-free scenario. The number of cases averted is approximated by ([mean no. with no releases – mean no. with releases]/average duration of disease) × no. of days). The percentage of cases averted is then calculated with respect to the control-free scenario.

We define a function, $\Gamma(t)$ as an indicator of whether the UD drive has been successfully triggered after a time t has passed:

$$\Gamma(t) = \frac{N_1(t) - N_{\mathrm{E}}(t)}{\sum_{i=1}^{n_g} N_i(t)}, \tag{3.1}$$

where N_1 is the number of female wild-type homozygotes and N_{E} is the number of female engineered homozygotes. Prior to any releases, $\Gamma = 1$, then, as engineered homozygotes are released, Γ will decrease. Eventually, Γ will stabilise either at $\Gamma = 1$ (transgenic insects die out) if the UD threshold was not reached, or $\Gamma \to -1$ (transgenic insects spread to fixation) if the threshold was successfully reached (the limits may not be reached exactly due to heterozygotes surviving in small numbers). The amount of time that is sufficient to be sure that an attempt to trigger the UD drive has been either a success or a failure is dependent upon how close to the threshold the initial release was: a release very close to the threshold will take a longer time to either fade out or to spread to fixation. Figures 2a–2c show that after 360 days Γ has converged to an approximation of a step function (where the release frequency f has been discretised at the third decimal place), crossing quickly from $\Gamma \approx 1$ to $\Gamma \approx -1$. We choose to use the time horizon of one year ($t_N = 365$ days) in all computations henceforth to ensure that the threshold release frequency has been accurately determined (longer simulations will not provide richer information about the threshold). We use the term release frequency to mean the proportion of the entire population (of the same sex as the released insects) that are engineered homozygotes immediately after an initial release of a given size. The term threshold release frequency means the lowest release frequency that produces a negative Γ value after one year ($\Gamma(t_N) < 0$). The conversion between release ratio (GM mosquitoes per one wild mosquito of the same sex) and release frequency f is shown in fig. 2d: for example, a release frequency of $f = 0.5$ translates to releasing one mosquito for every one wild mosquito of the same sex; $f = 0.9$ translates to releasing nine mosquitoes for every one wild mosquito of the same sex; $f = 0.99$ translates to releasing 99 mosquitoes for every one wild mosquito of the same sex.

3.1. Male-only releases

Mosquito vector control interventions generally release males to disrupt a wild population. Previous theoretical studies of underdominance as a population control technique have generally not considered the problem of sex, considering instead genderless genetic models or releases composed of both sexes. This neglects the stumbling block of releasing biting insects and potential vectors of disease.

We find that male-only releases require a higher threshold to be reached than bi-sex releases, for both the homologous and non-homologous UD systems (compare figs. 3a and 3b). This is due to the requirement for male releases to establish a self-sustaining population: that heterozygotes survive to breed in order to produce female engineered homozygotes. The strongly-suppressed non-homologous UD

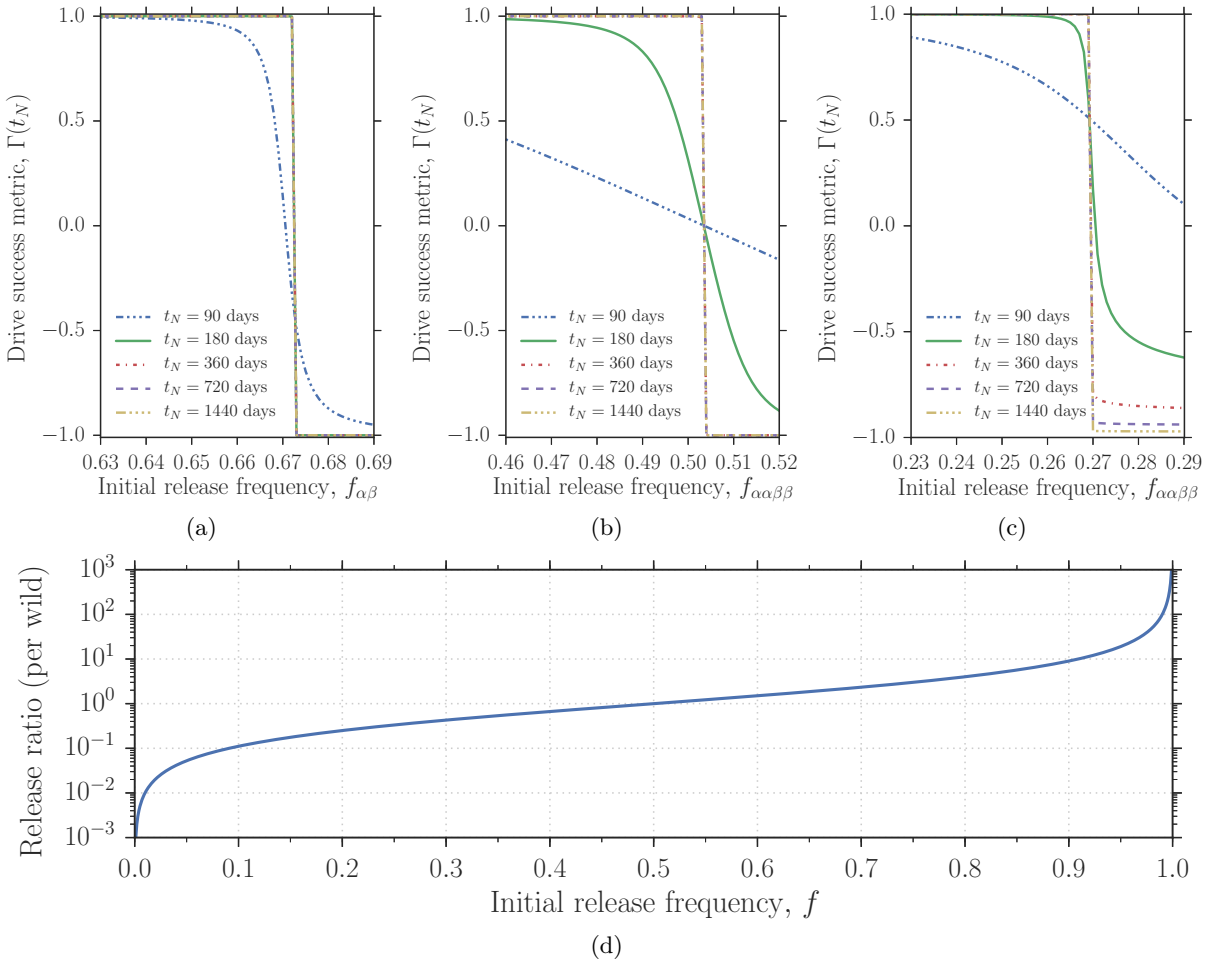
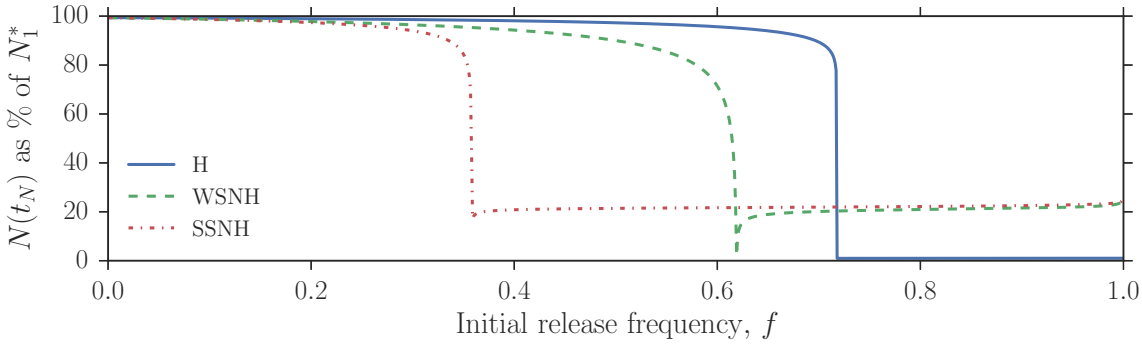
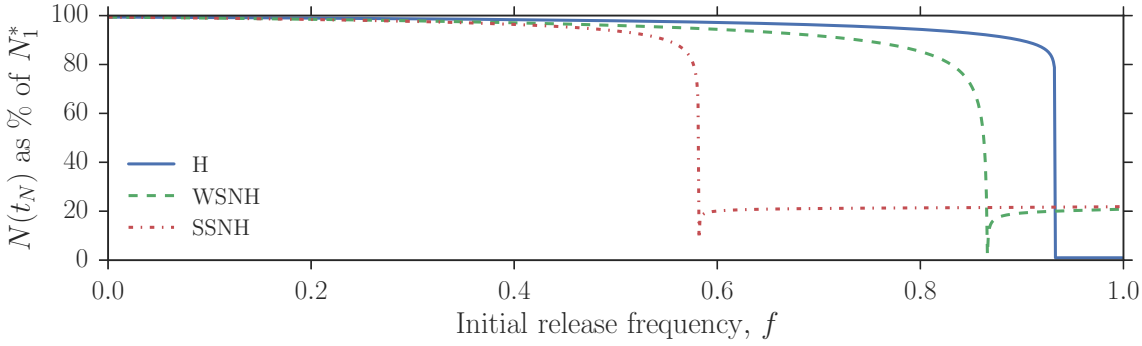


Figure 2: The threshold frequency is accurately resolved by running simulations for a time horizon of $t_N \geq 360$ days, as $\Gamma(t_N)$ tends towards a step function changing between $\Gamma(t_N) \approx 1$ (wild-type dominant) and $\Gamma(t_N) \approx -1$ (engineered genotypes dominant), centred on the threshold release frequency. Plots show (a) homologous, (b) weakly-suppressed non-homologous and (c) strongly-suppressed non-homologous underdominance, for a bi-sex release with early-acting bi-sex lethality. (d) The conversion between initial release frequency and number of transgenic mosquitoes released for every one wild mosquito of the same sex, calculated using $n_{pw} = f/(1-f)$, where n_{pw} is the number released per wild-type of the same sex. Parameters as in table 2 with fitness costs $c_a = 0$ and $cc_\ell = 0.99$.



(a) Bi-sex release



(b) Male-only release

Figure 3: Using male-only rather than bi-sex releases increases the required release threshold for a successful underdominance drive, but only marginally alters the outcome of a successful drive. Plots show the total vector population $N(t_N)$ at the end of one year ($t_N = 365$ days) as a percentage of the wild-type female equilibrium population, N_1^*, as the initial release frequency is varied, for the homologous (H), weakly-suppressed non-homologous (WSNH), and strongly-suppressed non-homologous (SSNH) underdominance systems. Top plot (a) shows a bi-sex release; bottom plot (b) shows a male-only release. Genetic fitness costs are $c_a = 0.05$, $c_\ell = 0.9$. Other parameters as in table 2, for an initial release with early-acting bi-sex lethality.

system has the lowest threshold frequency; the homologous system has the highest threshold release ratio.

The population suppression caused by the non-homologous UD systems peaks (strongly) when releasing exactly at the threshold frequency (fig. 3). The release of 'extra' mosquitoes partly counteracts the population suppression that occurs in the transition period between wild-type fixation and transgenic fixation.

The homologous UD system generally leads to greater population suppression than the two non-homologous UD systems (due to having fewer viable genotypes), almost eradicating the active vector population (fig. 3); this system acts more in the spirit of 'removal' than replacement, leaving the 'replaced' population greatly diminished. Conversely, the non-homologous systems may be useful for population replacement (though they also enact substantial suppression when $c_a > 0$, e.g. fig. 3b, and the level of suppression is strongly dependent on c_a).

3.2. Effects of genetic fitness costs

An underdominance system has two forms of genetic fitness cost: (i) the desirable toxic fitness cost responsible for lethality in heterozygotes and (ii) the ambient cost on individuals carrying a transgene that could arise, for example, through incomplete suppression of toxin expression due to imperfect binding of the suppressor protein to the promoter [25] ('leaky' toxicity), or through other unforeseen genetic complications.

It is possible to achieve an underdominance drive with a relatively small toxic transgene fitness cost (c_ℓ) and changes in the heterozygote fitness affect the UD threshold only slightly (fig. 4a). However, a high heterozygote relative fitness (i.e. low c_ℓ) adversely affects the resulting disease suppression of a successfully triggered UD drive (fig. 4c). Perfect or near-perfect lethality is detrimental to the UD drive process for a male-only release, as in order to establish a population of female transgenic homozygotes ($\alpha\beta$ or $\alpha\alpha\beta\beta$ for homologous or non-homologous UD, respectively), the heterozygote offspring of engineered males and wild-type females must survive and mate. It is worth noting that the perfectly lethal underdominant system initially proposed by Davis *et al.* [25] would be impossible to engineer: in breeding the transgenic line, the first transgenic insect would necessarily be heterozygote and hence would be nonviable.

Fitness costs on the engineered homozygotes (through 'leaky' toxicity causing $c_a > 0$, for example) can increase the drive threshold significantly (fig. 4b). The non-homologous UD systems are particularly affected, with the threshold frequency overtaking that of the homologous system at $c_a \approx 0.1$ for the weakly-suppressed system and $c_a \approx 0.24$ for the strongly-suppressed system (fig. 4b). Interestingly, even high ($c_a < 0.26$) unintended fitness costs associated with genetic modification are associated with a higher percentage of cases averted (fig. 4d) when a UD drive is successfully triggered at its threshold (above $c_a \approx 0.26$ the threshold frequencies exceed $f = 0.999$, a release ratio of around $1000:1$, which we define here as 'out of bounds' due to being practically unattainable). Increasing c_a has only a marginal effect on the percentage of cases averted by the homologous system (fig. 4d).

We also examined the effect of early vs late-acting lethality (i.e. whether the fitness costs are incurred in the egg or pupal stage) on the efficacy of the control. We found that the UD threshold is insensitive to the timing of the heterozygote lethality, but late-acting lethality has a marginally greater impact on the resulting disease burden.

3.3. Female-specific lethality

Many genetic constructs in development have sex-specific action, such as X-shredders [46, 47] (which act to distort the offspring sex ratio), female killing or sterilising alleles [48–50] and female-specific flightless phenotype transgenes [51, 52] (which cause death indirectly via flightlessness). Female-specific lethal (FSL) systems are in principle more efficient than standard SIT [48], and may have benefits related to resistance management when used in an integrated vector management programme [53, 54].

Using a construct that kills only females has interesting effects in an underdominance system. First, the UD drive threshold is increased when compared to a bi-sex lethal (BSL) gene (fig. 5a), in particular for the homologous UD system for which the threshold release ratio is more than doubled from $33:2$ for BSL to $89:2$ for FSL when $c_a = 0.1$. Second, the disease suppression that is achieved by an FSL release is generally greater than for a BSL release of the same initial release frequency (fig. 5b), except in narrow regions where the BSL threshold has been met, triggering a successful UD drive, but the release frequency is still below the FSL threshold. Importantly, at release frequencies below the threshold for both FSL and BSL systems, FSL releases avert a higher percentage of disease cases over the year than do BSL releases (fig. 5b).

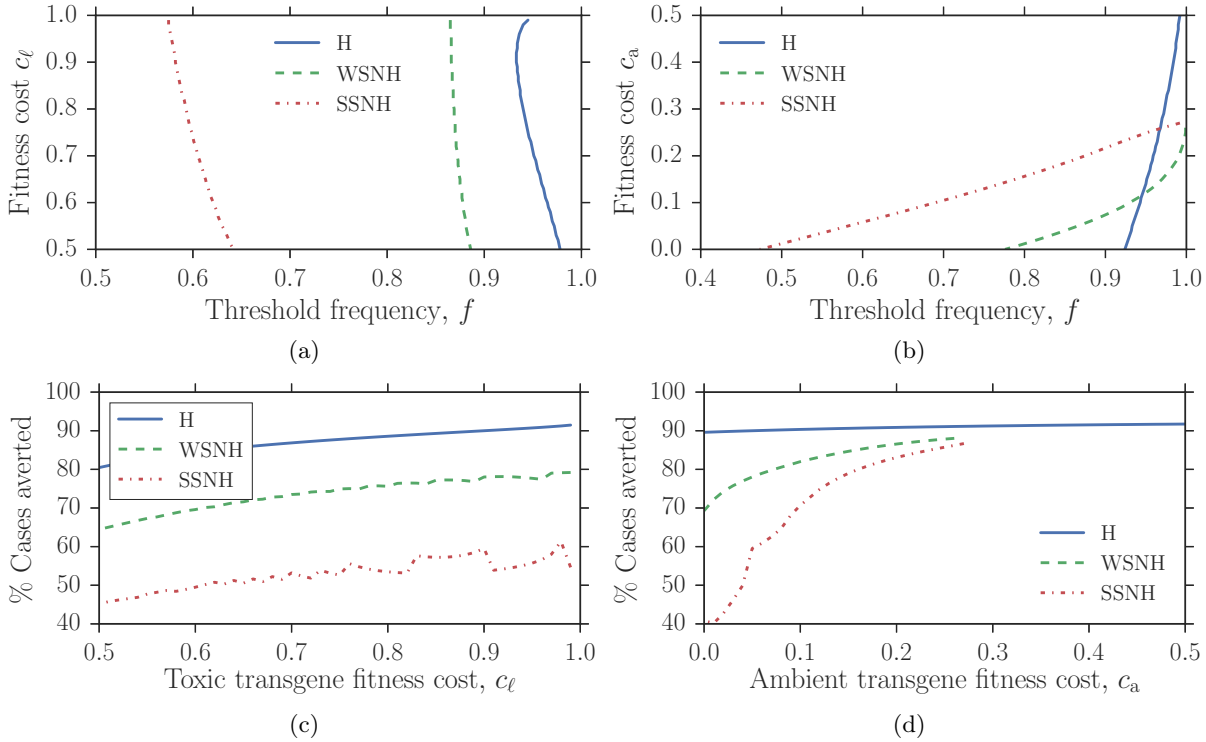


Figure 4: The performance of a control effort using non-homologous engineered underdominance is influenced greatly by the ambient transgene fitness cost, c_a, and to a lesser extent by the toxic transgene fitness cost, c_ℓ. The homologous underdominance system is less sensitive to changes in the transgene fitness costs. Top row shows the threshold release frequency when changing the (a) toxic and (b) ambient transgene fitness costs for the homologous (H), weakly-suppressed non-homologous (WSNH), and strongly-suppressed non-homologous (SSNH) underdominance systems. Bottom row shows the percentage of cases averted over one year for a given (c) toxic and (d) ambient transgene fitness cost when a single release is made at the threshold frequency. In (c) $c_a = 0.05$; in (d) $c_\ell = 0.9$. Other parameters as in table 2, for initial releases with early-acting bi-sex lethality.

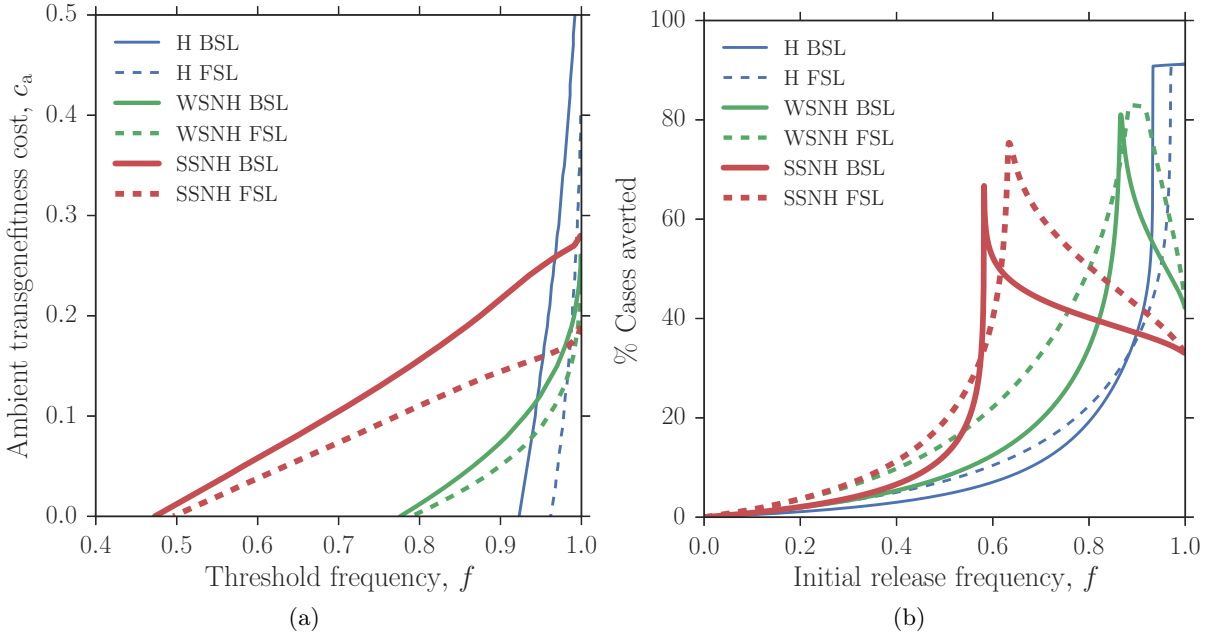


Figure 5: Female-specific lethality increases the underdominance threshold over that of bi-sex lethality, but has a greater impact on the disease burden for both successful and unsuccessful attempts to trigger the underdominance drive. (a) The underdominance threshold frequency as c_a is varied with $c_\ell = 0.9$ held constant, for homologous (H) and weakly-suppressed non-homologous (WSNH) and strongly-suppressed non-homologous (SSNH) underdominance systems that employ female-specific lethality (FSL), or bi-sex lethality (BSL). (b) Percentage of cases averted during the year compared to the control-free scenario, as initial release frequency f is varied, with $c_a = 0.05$ and $c_\ell = 0.9$. Parameters as in table 2, for a male-only initial release with late-acting lethality.

4. Results: ecology

4.1. Mating preference

We model the mating success of transgenic mosquitoes as the level of phenotypic preference of wild females to mate proportionally more with wild males than with any other genotype (see appendix A.2). Figure 6a shows that modest female mating preference can have a large effect on the UD threshold: if one in five wild females chooses to mate with a wild male rather than any other heterozygote or homozygote genotype (an 80% mating success rate for the transgenic genotypes), the threshold release frequency for the homologous system increases to $f_{\alpha\beta} = 0.996$, corresponding to the requirement of releasing almost 250 males for every one wild male—a seventeen-fold increase over the random mating case. The weakly-suppressed non-homologous system suffers a two-fold increase in threshold release ratio when mating success is reduced from 100% to 80%. The strongly-suppressed non-homologous system is most able to tolerate lower mating success (having the most viable genotypes of the systems tested), with the threshold release ratio increasing to only $3:1$ at 80% mating success. Stronger mating preference on the part of the wild females for wild males (translating to a smaller percentage mating success for the transgenic males) has a negative impact on the percentage of disease cases averted throughout the year for a single initial release that successfully triggers an underdominance gene drive at its threshold frequency (fig. 6b).

4.2. Ecological fitness costs

We wish to distinguish between the genetic fitness cost imposed by the toxins of the α and β alleles and changes in life history parameters that could be caused by lab rearing, loss of 'wild' phenotypic behaviours and any manifestation of genetic load caused by the UD constructs that are not directly associated with heterozygote lethality or ambient genetic transgene fitness cost [55–57]. To this end, we consider the effects of an increased scale of larval competition (ν), slower maturation (m), a higher rate of adult (μ) and larval (μ_B) density-independent mortality and a greater strength of density dependence (η). These costs are applied to all non-wild genotypes. Importantly, changing ecological parameters for all genotypes equally does not affect the UD threshold, which relies on the relative, rather than absolute, strengths of each genotype (see appendix C for an analytical investigation). (The implications for disease and population suppression do depend on the absolute values of the life history parameters.)

Increases in the adult mortality rate have the greatest effect on the UD drive threshold (see fig. 7, which shows results for the strongly-suppressed non-homologous system only; the other systems show qualitatively similar behaviour), with a 20% increase in μ above the wild-type value being sufficient to quadruple the required release ratio to over $109:2$ for the homologous system. The threshold release ratio for the weakly-suppressed non-homologous system more than triples to 20:1; for the strongly-suppressed system the threshold more than doubles to 7:2. The maturation rate and strength of the larval density dependence have a moderate effect on the thresholds. Increasing the density-independent mortality or the scale of the density-dependent mortality of the larvae has only a minor effect on the threshold frequency (fig. 7).

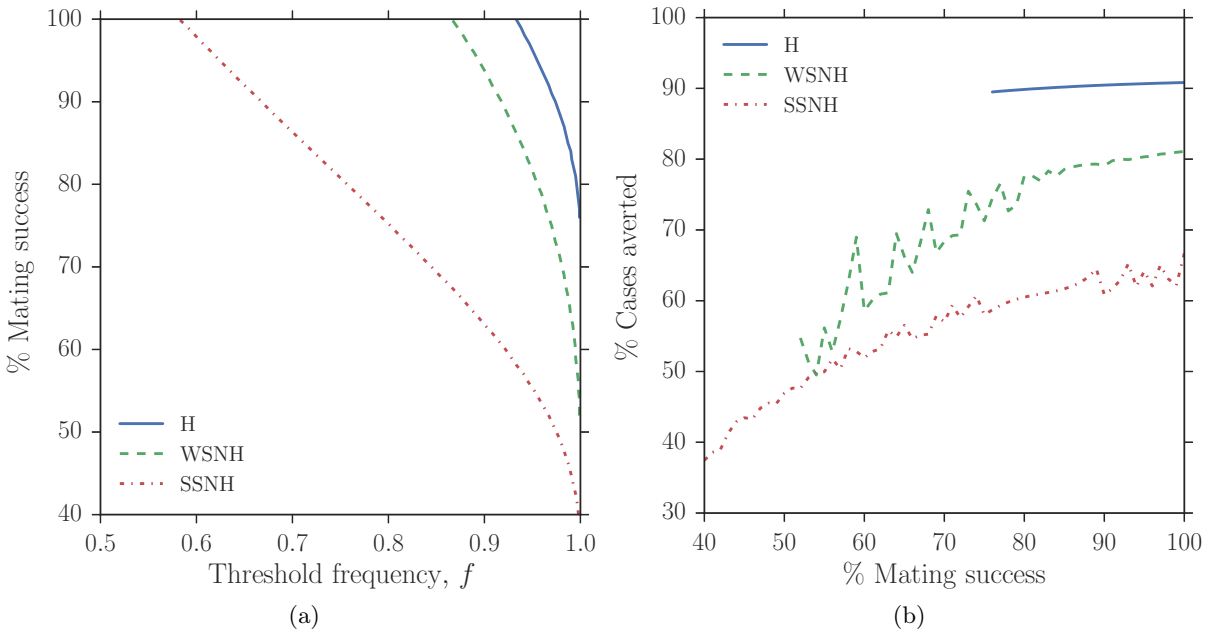


Figure 6: Reducing the mating success of engineered male mosquitoes with wild females, who proportionally prefer wild males, greatly affects the threshold required to trigger a successful underdominance gene drive. (a) The change in threshold frequency for homologous (H), weakly-suppressed non-homologous (WSNH) and strongly-suppressed non-homologous (SSNH) underdominance systems as the mating success of engineered males with wild females is varied. (b) The effect of mating success on the percentage of cases averted over a year compared with a control-free scenario, when an underdominance drive is successfully triggered by a single release at its particular threshold frequency. Parameters as in table 2 with $c_a = 0.05$ and $c_\ell = 0.9$, for a male-only initial release with late-acting bi-sex lethality. Note, jaggedness in the WSNH and SSNH lines in (b) is due to the sensitivity of these releases to the accuracy with which the threshold frequency is met—the 'narrow region' of efficacy discussed in section 3.1 (see, e.g., sharp troughs in fig. 3). Threshold frequencies are found correct to the third decimal place.

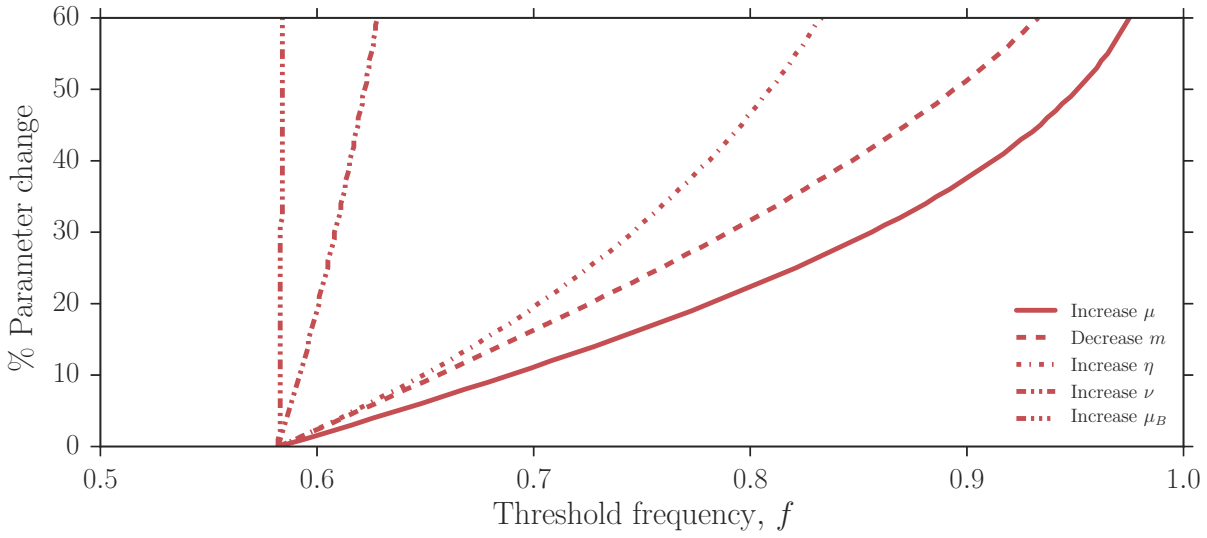


Figure 7: Engineered mosquitoes that have a greater adult mortality rate (μ) than wild-types face a substantially increased threshold frequency. The larval maturation rate (m) and character (or strength) of the larval density dependence (η) also play an important role in determining the threshold frequency, while the density-independent larval mortality rate (μ_B) and scale of density dependence (ν) play a negligible role. The threshold frequency is plotted as life history parameters of transgene-carrying insects are perturbed, for the strongly-suppressed non-homologous underdominance system. All mosquitoes that contain one or more of the engineered alleles α or β are taken to be equally affected by the parameter changes (which change relative to the wild-type value). Unchanged parameters as in table 2 with $c_a = 0.05$ and $c_\ell = 0.9$, for a male-only initial release with late-acting bi-sex lethality.

5. Discussion

We have theoretically investigated the use of underdominance as a method of vector population and disease suppression, using coupled models of mosquito ecology, genetics and epidemiology. We show that ecological barriers, that may manifest as decreased mating competitiveness or an increased rate of adult mortality, are the most important factors in determining the success chances of a control attempt (figs. 6a and 7). Low fitness of the engineered homozygotes (through 'leaky' toxicity from underdominant alleles, for example) can lead to high underdominance thresholds (fig. 4b) and hence could be economically costly (although it should be possible to engineer underdominant transgenes with a low ambient fitness cost). The efficiency of heterozygote lethality via toxin expression is less important (fig. 4a).

Previous investigations of underdominance drives as vector control only considered bi-sex releases. However, it may be hard to justify the release of biting insects and potential disease vectors. Our results suggest that population suppression can be achieved by releasing males carrying two homologous engineered underdominance constructs (though with a substantial increase in the release frequency threshold required to trigger the underdominance drive). Another partial solution to the problems associated with bi-sex releases (and one that we do not investigate here) is the proposal to link disease refractory genes to the engineered underdominance constructs, thus reducing the vectorial capacity of GM females [8, 58]. For mosquito control, male-only releases are more plausible from a regulatory standpoint, so their increased threshold requirements (fig. 3) need to be accounted for.

Once an underdominant construct is released, population suppression occurs during the transition towards the new stable state. If this transitional period is in fact the gradual elimination of the engineered construct (i.e. the initial allele frequency was below that required to trigger the underdominant drive in the favour of the engineered construct), the temporary population suppression may be large and have tangible socioeconomic benefits (as noted by Serebrovsky [20], who theorised that pest populations controlled with temporary translocation strains would be "dealt a severe blow"). The population suppression resulting from a successful underdominance drive using the homologous system is so great that it may push the vector population below the entomological threshold necessary to transmit disease. For example, fig. 3b shows a situation where the homologous system control effort reduces the total vector population to less than 1% of the wild-type equilibrium, corresponding to $k = 0.02$ vectors per host—lower than the $k = 0.05$ boundary that a simple R_0 analysis of the disease model (2.9) gives as the minimum number of vectors per host required for the disease to spread. Similarly large population suppression was observed by Vanderplank [59, 60] in field trials of tsetse fly control by underdominant-type methods (utilising the discovery that cross-breeds of two tsetse species exhibited reduced fecundity; the field trial results are published in [61]). Thus it should be possible to use certain underdominance systems as disease suppression technologies without the need to link anti-pathogen effector genes to the underdominant constructs.

Population eradication may not be the most beneficial result of a control programme due to the

possibility of secondary vectors invading the abandoned niche [62]. This is certainly a possibility in some regions of the Americas where *Ae. aegypti* and *Ae. albopictus* live in close proximity and actively compete [63, 64]. Approximating the minimum size of a primary vector population such that it could successfully occupy an ecological niche at the expense of a secondary vector would be an interesting problem for those aiming to implement genetic vector control technologies. Population replacement, rather than population suppression, may be the answer to such concerns (where the new population is genetically engineered to have a lower vectorial capacity), but is not without its own risks (as discussed above). The two non-homologous underdominance systems tested here show potential as agents of population replacement, with the amount of population suppression resulting from a successful underdominance gene drive depending on the value of the ambient transgene fitness cost, which governs the relative fitness of the engineered homozygotes (fig. 3).

Female-specific lethality acts to push underdominance thresholds higher (fig. 5a), suggesting that a greater initial investment is required. However, release attempts that fail to trigger the underdominance drive perform better (i.e. avert more infections) with female-specific, rather than bi-sex, lethality (fig. 5b). Due to the high threshold frequencies of male-only releases (for the homologous system in particular) and the possible difficulties in encouraging released mosquitoes to disseminate from the release site [65, 66] and assimilate into the wild breeding population [67, 68], it may be a common occurrence during a control programme for a drive threshold not to be reached with a single bulk release. In this case, the economic and social benefit of averting a higher percentage of infections may outweigh the extra expense, in the form of extra releases, required to subsequently push a female-specific lethal release over the drive threshold compared with an bi-sex lethal release.

The mating success of the engineered homozygotes and heterozygotes with wild-type females is vitally important. Even a modest degree of sexual selection could generate strong behavioural resistance to the gene drive. This could take the form of mate choice by wild females or mating competition on the part of the wild males and could render large, expensive releases useless by increasing the underdominance threshold higher than had been accounted for (fig. 6a). In the control of species other than mosquitoes, behavioural resistance has been observed within a few generations [69, 70] and has resulted in control programmes being abandoned [71]. There are many reports of mosquito control field trials producing poor results due to reduced mating competitiveness of transgenic mosquitoes [13, 68], and in most cases it is apparent that the ability to compete successfully for mates in laboratory conditions does not transfer perfectly to the field [56, 57, 67, 72, 73]. However, recent trials of self-limiting male-releases have shown more promise [74, 75]. To determine how problematic behavioural resistance could be for the successful establishment and persistence of underdominance drives, and gene drives more generally, an important parameter to understand is the degree to which male mating success is determined by female choice. Variance in male quality has been the focus of mating behaviour research, whereas the role of female choice has largely been ignored. There is a need, then, for behavioural experiments aimed at providing insights into the importance of female mating preference and the speed with which preference can develop

or change under strong selection pressure.

Artificial rearing and genetic engineering can lead to other barriers to success for an underdominance control programme by affecting the life history parameters of the transgenic insects. Field trials of transgenic mosquitoes have shown evidence of increased mortality in adults and larvae, longer development times to reach adulthood, lower fertility and a reduced range of dispersal [55–57, 65, 66]. We identify adult longevity as an important attribute for allowing a threshold drive to succeed (fig. 7) as a longer reproductive window gives the greatest chance that desirable genes will spread. Larval maturation rate and the strength and scale of density-dependent competition pressure are shown to have a lesser effect, independently, on the underdominance threshold. However, a lesser ability to compete for resources at the larval stage may lead to a lower maturation rate, and smaller, less developed larvae may emerge from pupation as stunted adults doomed to a shortened life (and with a lesser ability to compete for mates). Thus, the problems of ecological fitness costs manifested as altered life history parameters are likely to be compounded and attempts to establish an engineered population capable of triggering the underdominance threshold drive may be compromised. Indeed, field trials of translocation-bearing insects have reported many disadvantages compared with wild types [73]. Engineered underdominance systems may impose a lesser genetic load on individuals than translocations, but this type of gene drive system has thus far only been engineered in *Drosophila* [5, 26], and no underdominance system has yet been engineered that is capable of carrying a payload gene in an organism of medical or agricultural importance [8].

Future modelling work is required to investigate more fully the ramifications of our findings. For instance, capturing the evolution of mate choice as a dynamic process inside an ecological model would help determine whether behavioural resistance would develop quickly enough to prevent significant population suppression. Also, spatially explicit dynamics are vital for predicting the spread or containment of a control effort—understanding how threshold drives perform when movement between subpopulations occurs is necessary in judging their safety and viability. While previous studies have shown that small amounts of migration between a target and non-target population does not endanger the non-target population when an underdominance drive is used (in contrast to a homing gene drive), these models used pure population genetics approaches [6, 28]. Ecological or environmental stochasticity might plausibly lead an underdominance threshold to be triggered by migration into a non-target population. It is clear, though, that understanding and accounting for the ecological and evolutionary effects – be they decreased adult longevity or the development of behavioural resistance via sexual selection – of any gene drive technology is vital if they are to be used successfully for mosquito vector control.

Competing interests

We have no competing interests.

Authors' contributions

DK, CEM, KK and MBB conceived the study. DK performed the mathematical analysis and drafted the manuscript. CEM, KK and MBB helped draft the manuscript. All authors gave final approval for

publication.

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Appendix

A. Genotypic offspring proportions

A.1. Random mating

Working through the population genetic matings for the homologous underdominance system leads to the following proportions of each sex in each genotype at any given time:

$$p_1 = \frac{1}{2N_mN_f}\left(N_1\hat{N}_1 + \frac{1}{2}(N_1\hat{N}_2 + \hat{N}_1N_2) + \frac{1}{2}(N_1\hat{N}_3 + \hat{N}_1N_3) + \frac{1}{4}N_2\hat{N}_2 + \frac{1}{4}N_3\hat{N}_3 + \frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3)\right), \tag{A.1a}$$

$$p_2 = \frac{1}{2N_mN_f}\left(\frac{1}{2}(N_1\hat{N}_2 + \hat{N}_1N_2) + \frac{1}{2}(\hat{N}_1N_4 + N_1\hat{N}_4) + (\hat{N}_1N_5 + N_1\hat{N}_5) + \frac{1}{2}N_2\hat{N}_2 + \frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{4}(\hat{N}_2N_4 + N_2\hat{N}_4) + \frac{1}{2}(\hat{N}_2N_5 + N_2\hat{N}_5) + \frac{1}{2}(N_3\hat{N}_5 + \hat{N}_3N_5) + \frac{1}{4}(\hat{N}_3N_4 + N_3\hat{N}_4)\right), \tag{A.1b}$$

$$p_3 = \frac{1}{2N_mN_f}\left(\frac{1}{2}(N_1\hat{N}_3 + \hat{N}_1N_3) + \frac{1}{2}(\hat{N}_1N_4 + N_1\hat{N}_4) + (N_1\hat{N}_6 + \hat{N}_1N_6) + \frac{1}{2}(N_2\hat{N}_6 + \hat{N}_2N_6) + \frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{4}(\hat{N}_2N_4 + N_2\hat{N}_4) + \frac{1}{2}N_3\hat{N}_3 + \frac{1}{2}(N_3\hat{N}_6 + \hat{N}_3N_6) + \frac{1}{4}(\hat{N}_3N_4 + N_3\hat{N}_4)\right), \tag{A.1c}$$

$$p_4 = \frac{1}{2N_mN_f}\left(\frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{4}(\hat{N}_2N_4 + N_2\hat{N}_4) + \frac{1}{2}(N_2\hat{N}_6 + \hat{N}_2N_6) + \frac{1}{4}(\hat{N}_3N_4 + N_3\hat{N}_4) + \frac{1}{2}(N_3\hat{N}_5 + \hat{N}_3N_5) + (N_5\hat{N}_6 + \hat{N}_5N_6) + \frac{1}{2}(\hat{N}_5N_4 + N_5\hat{N}_4) + \frac{1}{2}(\hat{N}_6N_4 + N_6\hat{N}_4) + \frac{1}{2}N_4\hat{N}_4\right), \tag{A.1d}$$

$$p_5 = \frac{1}{2N_mN_f}\left(\frac{1}{4}N_2\hat{N}_2 + \frac{1}{4}(\hat{N}_2N_4 + N_2\hat{N}_4) + \frac{1}{2}(N_2\hat{N}_5 + \hat{N}_2N_5) + \frac{1}{4}N_4\hat{N}_4 + \frac{1}{2}(\hat{N}_5N_4 + N_5\hat{N}_4) + N_5\hat{N}_5\right), \tag{A.1e}$$

$$p_6 = \frac{1}{2N_mN_f}\left(\frac{1}{4}N_3\hat{N}_3 + \frac{1}{4}(\hat{N}_3N_4 + N_3\hat{N}_4) + \frac{1}{2}(N_3\hat{N}_6 + \hat{N}_3N_6) + \frac{1}{4}N_4\hat{N}_4 + \frac{1}{2}(\hat{N}_6N_4 + N_6\hat{N}_4) + N_6\hat{N}_6\right), \tag{A.1f}$$

where p_1, p_2, p_3, p_4, p_5 and p_6 relate to genotypes AA, $A\alpha$, $A\beta$, $\alpha\beta$, $\alpha\alpha$ and $\beta\beta$ respectively. Each genotype is split explicitly into males ($\hat{N}_i$) and females (N_i), though gender imbalance may only occur through female-specific lethality or in the $\alpha\beta$ genotype through releases of male mosquitoes (i.e. offspring are generated with a $1:1$ sex ratio, giving the factor of $1/2$ outside the brackets in each p_i definition).

For the non-homologous underdominance system, the offspring proportions of each sex in each genotype are given by

$$\begin{aligned}
p_1 = \frac{1}{2N_mN_f}\Bigg(&N_1\hat{N}_1 + \frac{1}{2}(N_1\hat{N}_2 + \hat{N}_1N_2) + \frac{1}{2}(N_1\hat{N}_3 + \hat{N}_1N_3) + \frac{1}{4}(N_1\hat{N}_4 + \hat{N}_1N_4) + \frac{1}{4}N_2\hat{N}_2 \\
&+\frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{8}(N_2\hat{N}_4 + \hat{N}_2N_4) + \frac{1}{4}N_3\hat{N}_3 + \frac{1}{8}(N_3\hat{N}_4 + \hat{N}_3N_4) + \frac{1}{16}N_4\hat{N}_4\Bigg), \qquad \text{(A.2a)} \\
p_2 = \frac{1}{2N_mN_f}\Bigg(&\frac{1}{2}(N_1\hat{N}_2 + \hat{N}_1N_2) + \frac{1}{4}(N_1\hat{N}_4 + \hat{N}_1N_4) + (N_1\hat{N}_5 + \hat{N}_1N_5) + \frac{1}{2}(N_1\hat{N}_7 + \hat{N}_1N_7) \\
&+\frac{1}{2}N_2\hat{N}_2 + \frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{4}(N_2\hat{N}_4 + \hat{N}_2N_4) + \frac{1}{2}(N_2\hat{N}_5 + \hat{N}_2N_5) + \frac{1}{4}(N_2\hat{N}_7 + \hat{N}_2N_7) \\
&+\frac{1}{8}(N_3\hat{N}_4 + \hat{N}_3N_4) + \frac{1}{2}(N_3\hat{N}_5 + \hat{N}_3N_5) + \frac{1}{4}(N_3\hat{N}_7 + \hat{N}_3N_7) + \frac{1}{8}N_4\hat{N}_4 + \frac{1}{4}(N_4\hat{N}_5 + \hat{N}_4N_5) \\
&+\frac{1}{8}(N_4\hat{N}_7 + \hat{N}_4N_7)\Bigg), \qquad \text{(A.2b)} \\
p_3 = \frac{1}{2N_mN_f}\Bigg(&\frac{1}{2}(N_1\hat{N}_3 + \hat{N}_1N_3) + \frac{1}{4}(N_1\hat{N}_4 + \hat{N}_1N_4) + (N_1\hat{N}_6 + \hat{N}_1N_6) + \frac{1}{2}(N_1\hat{N}_8 + \hat{N}_1N_8) \\
&+\frac{1}{2}N_3\hat{N}_3 + \frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{8}(N_2\hat{N}_4 + \hat{N}_2N_4) + \frac{1}{2}(N_2\hat{N}_6 + \hat{N}_2N_6) + \frac{1}{4}(N_2\hat{N}_8 + \hat{N}_2N_8) \\
&+\frac{1}{4}(N_3\hat{N}_4 + \hat{N}_3N_4) + \frac{1}{2}(N_3\hat{N}_6 + \hat{N}_3N_6) + \frac{1}{4}(N_3\hat{N}_8 + \hat{N}_3N_8) + \frac{1}{8}N_4\hat{N}_4 + \frac{1}{4}(N_4\hat{N}_6 + \hat{N}_4N_6) \\
&+\frac{1}{8}(N_4\hat{N}_8 + \hat{N}_4N_8)\Bigg), \qquad \text{(A.2c)} \\
p_4 = \frac{1}{2N_mN_f}\Bigg(&\frac{1}{4}(N_1\hat{N}_4 + \hat{N}_1N_4) + \frac{1}{2}(N_1\hat{N}_7 + \hat{N}_1N_7) + \frac{1}{2}(N_1\hat{N}_8 + \hat{N}_1N_8) + (N_1\hat{N}_9 + \hat{N}_1N_9) \\
&+\frac{1}{4}(N_2\hat{N}_3 + \hat{N}_2N_3) + \frac{1}{4}(N_2\hat{N}_4 + \hat{N}_2N_4) + \frac{1}{2}(N_2\hat{N}_6 + \hat{N}_2N_6) + \frac{1}{4}(N_2\hat{N}_7 + \hat{N}_2N_7) \\
&+\frac{1}{2}(N_2\hat{N}_8 + \hat{N}_2N_8) + \frac{1}{2}(N_2\hat{N}_9 + \hat{N}_2N_9) + \frac{1}{4}(N_3\hat{N}_4 + \hat{N}_3N_4) + \frac{1}{2}(N_3\hat{N}_5 + \hat{N}_3N_5) \\
&+\frac{1}{2}(N_3\hat{N}_7 + \hat{N}_3N_7) + \frac{1}{4}(N_3\hat{N}_8 + \hat{N}_3N_8) + \frac{1}{2}(N_3\hat{N}_9 + \hat{N}_3N_9) + \frac{1}{4}N_4\hat{N}_4 + \frac{1}{4}(N_4\hat{N}_5 + \hat{N}_4N_5) \\
&+\frac{1}{4}(N_4\hat{N}_6 + \hat{N}_4N_6) + \frac{1}{4}(N_4\hat{N}_7 + \hat{N}_4N_7) + \frac{1}{4}(N_4\hat{N}_8 + \hat{N}_4N_8) + \frac{1}{4}(N_4\hat{N}_9 + \hat{N}_4N_9) \\
&+(N_5\hat{N}_6 + \hat{N}_5N_6) + \frac{1}{2}(N_5\hat{N}_8 + \hat{N}_5N_8) + \frac{1}{2}(N_6\hat{N}_7 + \hat{N}_6N_7) + \frac{1}{4}(N_7\hat{N}_8 + \hat{N}_7N_8)\Bigg), \qquad \text{(A.2d)} \\
p_5 = \frac{1}{2N_mN_f}\Bigg(&\frac{1}{4}N_2\hat{N}_2 + \frac{1}{8}(N_2\hat{N}_4 + \hat{N}_2N_4) + \frac{1}{2}(N_2\hat{N}_5 + \hat{N}_2N_5) + \frac{1}{4}(N_2\hat{N}_7 + \hat{N}_2N_7) + \frac{1}{16}N_4\hat{N}_4 \\
&+\frac{1}{4}(N_4\hat{N}_5 + \hat{N}_4N_5) + \frac{1}{8}(N_4\hat{N}_7 + \hat{N}_4N_7) + N_5\hat{N}_5 + \frac{1}{2}(N_5\hat{N}_7 + \hat{N}_5N_7) + \frac{1}{4}N_7\hat{N}_7\Bigg), \qquad \text{(A.2e)} \\
p_6 = \frac{1}{2N_mN_f}\Bigg(&\frac{1}{4}N_3\hat{N}_3 + \frac{1}{8}(N_3\hat{N}_4 + \hat{N}_3N_4) + \frac{1}{2}(N_3\hat{N}_6 + \hat{N}_3N_6) + \frac{1}{4}(N_3\hat{N}_8 + \hat{N}_3N_8) + \frac{1}{16}N_4\hat{N}_4 \\
&+\frac{1}{4}(N_4\hat{N}_6 + \hat{N}_4N_6) + \frac{1}{8}(N_4\hat{N}_8 + \hat{N}_4N_8) + N_6\hat{N}_6 + \frac{1}{2}(N_6\hat{N}_8 + \hat{N}_6N_8) + \frac{1}{4}N_8\hat{N}_8\Bigg), \qquad \text{(A.2f)} \\
p_7 = \frac{1}{2N_mN_f}\Bigg(&\frac{1}{8}(N_2\hat{N}_4 + \hat{N}_2N_4) + \frac{1}{4}(N_2\hat{N}_7 + \hat{N}_2N_7) + \frac{1}{4}(N_2\hat{N}_8 + \hat{N}_2N_8) + \frac{1}{2}(N_2\hat{N}_9 + \hat{N}_2N_9) \\
&+\frac{1}{8}N_4\hat{N}_4 + \frac{1}{4}(N_4\hat{N}_5 + \hat{N}_4N_5) + \frac{1}{4}(N_4\hat{N}_7 + \hat{N}_4N_7) + \frac{1}{8}(N_4\hat{N}_8 + \hat{N}_4N_8) + \frac{1}{4}(N_4\hat{N}_9 + \hat{N}_4N_9) \\
&+\frac{1}{2}(N_5\hat{N}_7 + \hat{N}_5N_7) + \frac{1}{2}(N_5\hat{N}_8 + \hat{N}_5N_8) + (N_5\hat{N}_9 + \hat{N}_5N_9) + \frac{1}{2}N_7\hat{N}_7 + \frac{1}{4}(N_7\hat{N}_8 + \hat{N}_7N_8)
\end{aligned}$$

$$+\frac{1}{2}(N_7\hat{N}_9+\hat{N}_7N_9)\Big), \tag{A.2g}$$

$$p_8=\frac{1}{2N_mN_f}\Big(\frac{1}{8}(N_3\hat{N}_4+\hat{N}_3N_4)+\frac{1}{4}(N_3\hat{N}_7+\hat{N}_3N_7)+\frac{1}{4}(N_3\hat{N}_8+\hat{N}_3N_8)+\frac{1}{2}(N_3\hat{N}_9+\hat{N}_3N_9)$$
$$+\frac{1}{8}N_4\hat{N}_4+\frac{1}{4}(N_4\hat{N}_6+\hat{N}_4N_6)+\frac{1}{8}(N_4\hat{N}_7+\hat{N}_4N_7)+\frac{1}{4}(N_4\hat{N}_8+\hat{N}_4N_8)+\frac{1}{4}(N_4\hat{N}_9+\hat{N}_4N_9)$$
$$+\frac{1}{2}(N_6\hat{N}_7+\hat{N}_6N_7)+\frac{1}{2}(N_6\hat{N}_8+\hat{N}_6N_8)+(N_6\hat{N}_9+\hat{N}_6N_9)+\frac{1}{2}N_8\hat{N}_8+\frac{1}{4}(N_7\hat{N}_8+\hat{N}_7N_8)$$
$$+\frac{1}{2}(N_8\hat{N}_9+\hat{N}_8N_9)\Big), \tag{A.2h}$$

$$p_9=\frac{1}{2N_mN_f}\Big(\frac{1}{16}N_4\hat{N}_4+\frac{1}{8}(N_4\hat{N}_7+\hat{N}_4N_7)+\frac{1}{8}(N_4\hat{N}_8+\hat{N}_4N_8)+\frac{1}{4}(N_4\hat{N}_9+\hat{N}_4N_9)+\frac{1}{4}N_7\hat{N}_7$$
$$+\frac{1}{4}(N_7\hat{N}_8+\hat{N}_7N_8)+\frac{1}{2}(N_7\hat{N}_9+\hat{N}_7N_9)+\frac{1}{4}N_8\hat{N}_8+\frac{1}{2}(N_8\hat{N}_9+\hat{N}_8N_9)+N_9\hat{N}_9\Big), \tag{A.2i}$$

where p_1, p_2, p_3, p_4, p_5, p_6, p_7, p_8 and p_9 relate to genotypes $AABB$, $A\alpha BB$, $AAB\beta$, $A\alpha B\beta$, $\alpha\alpha BB$, $AA\beta\beta$, $\alpha\alpha B\beta$, $A\alpha\beta\beta$ and $\alpha\alpha\beta\beta$, respectively.

A.2. Wild-type mating preference

Consider the simple case of extreme underdominance, where two alleles A and a form three genotypes AA, Aa and aa, and take AA to be the wild type. Without mating preference the proportions p_i of the next generation that belong to each genotype will be

$$p_{AA}=\frac{1}{2N_mN_f}\left(N_{AA}\hat{N}_{AA}+\frac{1}{2}\left(N_{AA}\hat{N}_{Aa}+N_{Aa}\hat{N}_{AA}\right)+\frac{1}{4}N_{Aa}\hat{N}_{Aa}\right), \tag{A.3a}$$

$$p_{Aa}=\frac{1}{2N_mN_f}\left(\frac{1}{2}\left(N_{AA}\hat{N}_{Aa}+N_{Aa}\hat{N}_{AA}\right)+N_{AA}\hat{N}_{aa}+N_{aa}\hat{N}_{AA}+\frac{1}{2}N_{Aa}\hat{N}_{Aa}\right. \tag{A.3b}$$
$$\left.+\frac{1}{2}\left(N_{Aa}\hat{N}_{aa}+N_{aa}\hat{N}_{Aa}\right)\right),$$

$$p_{aa}=\frac{1}{2N_mN_f}\left(\frac{1}{4}N_{Aa}\hat{N}_{Aa}+\frac{1}{2}\left(N_{Aa}\hat{N}_{aa}+N_{aa}\hat{N}_{Aa}\right)+N_{aa}\hat{N}_{aa}\right). \tag{A.3c}$$

We model mating preference in the wild-type female population by defining the parameters ξ and χ. A proportion ξ of the wild-type females from each encounter with males who are not wild type will choose instead to mate with wild-type males. The number of resulting matings between wild-type males and females increases by a related proportion χ. With mating preference we find

$$\bar{p}_{AA}=\frac{1}{N_mN_f}\left((1+\chi)N_{AA}\hat{N}_{AA}+\frac{1}{2}\left((1-\xi)N_{AA}\hat{N}_{Aa}+N_{Aa}\hat{N}_{AA}\right)+\frac{1}{4}N_{Aa}\hat{N}_{Aa}\right), \tag{A.4a}$$

$$\bar{p}_{Aa}=\frac{1}{N_mN_f}\left(\frac{1}{2}\left((1-\xi)N_{AA}\hat{N}_{Aa}+N_{Aa}\hat{N}_{AA}\right)+(1-\xi)N_{AA}\hat{N}_{aa}+N_{aa}\hat{N}_{AA}+\frac{1}{2}N_{Aa}\hat{N}_{Aa}\right. \tag{A.4b}$$
$$\left.+\frac{1}{2}\left(N_{Aa}\hat{N}_{aa}+N_{aa}\hat{N}_{Aa}\right)\right),$$

$$\bar{p}_{aa}=\frac{1}{N_mN_f}\left(\frac{1}{4}N_{Aa}\hat{N}_{Aa}+\frac{1}{2}\left(N_{Aa}\hat{N}_{aa}+N_{aa}\hat{N}_{Aa}\right)+N_{aa}\hat{N}_{aa}\right). \tag{A.4c}$$

With wild-type mating preference, we expect $\bar{p}_{AA}>p_{AA}$, $\bar{p}_{Aa}<p_{Aa}$ and $\bar{p}_{aa}=p_{aa}$. The constraint $\sum_i p_i=1=\sum_i \bar{p}_i$ must also still hold. Taking these sums over the proportions and then subtracting one

from the other we may find an expression for χ in terms of ξ:

$$\chi = \frac{\frac{1}{2}\hat{N}_{Aa} + \frac{1}{2}\hat{N}_{Aa} + \hat{N}_{aa}}{\hat{N}_{AA}}\,\xi, \tag{A.5}$$

where the fraction is simply the sum of males in non-preferred matings over the number of males in the preferred mating. Thus we may use (A.5) to define the mating success parameter χ, for a given ξ, in our engineered underdominance models with six and nine genotypes,

$$\chi = \frac{\sum_{i=2}^{n_g} \hat{N}_i}{\hat{N}_1}\,\xi, \tag{A.6}$$

where n_g takes the value six and nine, respectively.

B. Genotype fitness from transgene fitness costs

Here we tabulate how the relative fitness of each genotype is constructed multiplicatively from the costs imposed by the constituent alleles of the genotype (with wild-type alleles imposing no fitness cost). The non-homologous underdominance system is treated in tables B.4–B.7 . The fitness table for the homologous underdominance system is repeated here for completeness, table B.8.

	Genotype					
Lethality	$AABB$	$A\alpha BB$ $AAB\beta$	$A\alpha B\beta$	$\alpha\alpha BB$ $AA\beta\beta$	$\alpha\alpha B\beta$ $A\alpha\beta\beta$	$\alpha\alpha\beta\beta$
BSL	1	$\kappa_{\mathrm{a}}\kappa_\ell$	κ_{a}^2	$\kappa_{\mathrm{a}}^2\kappa_\ell^2$	$\kappa_{\mathrm{a}}^3\kappa_\ell$	κ_{a}^4
FSL	1	κ_{a}	κ_{a}^2	κ_{a}^2	κ_{a}^3	κ_{a}^4

Table B.4: Male genotype fitness for the weakly-suppressed non-homologous underdominance system for bi-sex lethality (BSL) and female-specific lethality (FSL); $\kappa_{\mathrm{a}} = 1 - c_{\mathrm{a}}$ and $\kappa_\ell = 1 - c_\ell$ where c_{a} is the ambient fitness cost of carrying a single transgene and c_ℓ is the (partially) lethal fitness cost of carrying an unsuppressed toxin gene. Fitness costs are assumed to combine multiplicatively.

	Genotype					
Lethality	$AABB$	$A\alpha BB$ $AAB\beta$	$A\alpha B\beta$	$\alpha\alpha BB$ $AA\beta\beta$	$\alpha\alpha B\beta$ $A\alpha\beta\beta$	$\alpha\alpha\beta\beta$
BSL/FSL	1	$\kappa_{\mathrm{a}}\kappa_\ell$	κ_{a}^2	$\kappa_{\mathrm{a}}^2\kappa_\ell^2$	$\kappa_{\mathrm{a}}^3\kappa_\ell$	κ_{a}^4

Table B.5: As in table B.4: female genotype fitness for weakly-suppressed non-homologous underdominance system.

	Genotype					
Lethality	$AABB$	$A\alpha BB$ $AAB\beta$	$A\alpha B\beta$	$\alpha\alpha BB$ $AA\beta\beta$	$\alpha\alpha B\beta$ $A\alpha\beta\beta$	$\alpha\alpha\beta\beta$
BSL	1	$\kappa_{\mathrm{a}}\kappa_\ell$	κ_{a}^2	$\kappa_{\mathrm{a}}^2\kappa_\ell^2$	κ_{a}^3	κ_{a}^4
FSL	1	κ_{a}	κ_{a}^2	κ_{a}^2	κ_{a}^3	κ_{a}^4

Table B.6: As in table B.4: male genotype fitness for strongly-suppressed non-homologous underdominance system.

	Genotype					
Lethality	$AABB$	$A\alpha BB$ $AAB\beta$	$A\alpha B\beta$	$\alpha\alpha BB$ $AA\beta\beta$	$\alpha\alpha B\beta$ $A\alpha\beta\beta$	$\alpha\alpha\beta\beta$
BSL/FSL	1	$\kappa_{\mathrm{a}}\kappa_\ell$	κ_{a}^2	$\kappa_{\mathrm{a}}^2\kappa_\ell^2$	κ_{a}^3	κ_{a}^4

Table B.7: As in table B.4: female genotype fitness for strongly-suppressed non-homologous underdominance system.

	Genotype							
	Male				Female			
Lethality	AA	$A\alpha$, $A\beta$	$\alpha\beta$	$\alpha\alpha$, $\beta\beta$	AA	$A\alpha$, $A\beta$	$\alpha\beta$	$\alpha\alpha$, $\beta\beta$
BSL	1	$\kappa_{\mathrm{a}}\kappa_\ell$	κ_{a}^2	$\kappa_{\mathrm{a}}^2\kappa_\ell^2$	1	$\kappa_{\mathrm{a}}\kappa_\ell$	κ_{a}^2	$\kappa_{\mathrm{a}}^2\kappa_\ell^2$
FSL	1	κ_{a}	κ_{a}^2	κ_{a}^2				

Table B.8: Genotype fitness for the homologous engineered underdominance system for bi-sex lethality (BSL) and female-specific lethality (FSL). Genotypes with the same fitness are grouped.

C. Analytical investigation of underdominance thresholds

We may recast the population model in terms of proportions: if $n_i = N_i/N$ where $N = \sum_j^{n_g} N_j$ where n_g is the number of genotypes, then

$$\frac{\mathrm{d}n_i}{\mathrm{d}t} = \frac{1}{N}\frac{\mathrm{d}N_i}{\mathrm{d}t} - n_i\frac{1}{N}\frac{\mathrm{d}N}{\mathrm{d}t}. \tag{C.1}$$

Using (C.1), our governing equations for the proportions of adults (n_i) and larvae (b_i) in each genotype are

$$\frac{\mathrm{d}b_i}{\mathrm{d}t} = \frac{\rho N_f}{B}\tilde{p}_i\phi_i^E - F_i(B)b_i - b_i\left[\frac{\rho N_f}{B}\sum_j^{n_g}\tilde{p}_j\phi_j^E - \sum_j^{n_g}F_j(B)b_j\right], \tag{C.2a}$$

$$\frac{\mathrm{d}n_i}{\mathrm{d}t} = \frac{mB}{2N_f}b_i\phi_i^L - \mu n_i - n_i\left[\frac{mB}{2N_f}\sum_j^{n_g}b_j\phi_j^L - \mu\right], \tag{C.2b}$$

where we have used the fact that $\sum_i n_i = 1$, and we have defined $F_i(B) = \log\left[1 + (\nu_i B)^\eta\right] + m + \mu_B$. We implicitly assume that all genotypes share the same ecological parameters except for the possibility of competing unequally for resources at the larval stage. The $\tilde{p}$ are the proportion of offspring falling into each genotype, and N_f is the sum of all adult females. We are interested in thresholds of the system (C.2). These are internal system states where all viable genotypes k have $n_k \neq 0$ and $b_k \neq 0$. A small perturbation around the state will trigger a drive due to underdominance in favour of the positively perturbed homozygote (to the detriment of the negatively perturbed homozygote). We define

$$\Phi^L = \sum_j^{n_g} b_j\phi_j^L, \qquad \Phi^E = \sum_j^{n_g}\tilde{p}_j\phi_j^E, \qquad \Theta = \sum_j^{n_g}F_j(B)b_j, \tag{C.3}$$

and investigate the non-trivial equilibria of (C.2), which satisfy

$$0 = \frac{\rho N_f}{B}\left(\tilde{p}_i\phi_i^E - b_i\Phi^E\right) + b_i\left(\Theta - F_i(B)\right), \tag{C.4a}$$

$$0 = b_i\phi_i^L - \Phi^L n_i. \tag{C.4b}$$

C.1. Equal larval competition

We first investigate the simple case in which all genotypes compete equally at the larval stage, $\nu_i \equiv \nu$ $\forall i$, hence $F_i(B) \equiv F(B)$ and

$$\Theta = F(B) \sum_i^{n_g} b_i = F(B) \tag{C.5}$$

since $\sum_i^{n_g} b_i = 1$. Under this assumption, (C.4) collapse into one equation for the adult genotype frequencies,

$$\tilde{p}_i \phi_i^E \phi_i^L - \Phi^E \Phi^L n_i = 0, \tag{C.6}$$

which displays symmetry between early-acting and late-acting fitness costs. Summing (C.6) over i in either the early- or late-acting regime produces $\Phi = \sum_i^{n_g} \tilde{p}_i \phi_i$ where the relation holds if the costs ϕ act either before hatching or during pupation. Thus we find the dispersion relation

$$n_i - \frac{\tilde{p}\phi_i}{\sum_j^{n_g} \tilde{p}_j \phi_j} = 0, \tag{C.7}$$

from which we immediately see that the equilibrium genotype frequencies under the assumption of equal larval competition decouple from the effects of density-dependent mortality. Further, if all genotypes share the same ecological parameters (maturation rate, mortality rate etc.) then these parameters drop out of the calculation for the underdominance threshold.

If we simplify further by considering the six-genotype homologous underdominance system and assuming, as Davis *et al.* [25] did, that only the wild type AA and the engineered construct $\alpha\beta$ are viable, then we can deduce from (C.7) that

$$n_1 = \frac{1}{2} n_2 \phi_2, \tag{C.8}$$

(where we have re-numbered such that $i = 1$ is wild type and $i = 2$ is the construct, and non-viable genotypes have been discarded) and using the normalisation condition $n_1 + n_2 = 1$ we find that for $\phi_2 = 1$, $n_1 = 1/3$ and $n_2 = 2/3$. These are genotypic proportions rather than allelic proportions [as in, e.g., 76], but we may compare these results to those found by Davis *et al.* [25] (wherein purely genetic crosses in non-overlapping generations were considered) by converting their results: the threshold lies at the point where the alleles A, α and β are equally common; each α (and similarly β) allele is converted into one $\alpha\beta$ gene, whereas two A alleles are required to make a single AA gene, thereby making the genotypic proportions 2/3 for $\alpha\beta$ and 1/3 for AA, matching our results.

C.2. The effect of density dependence

Now we relax the assumption that all genotypes compete equally during the larval stage. First, consider the case where the genetic constructs impart an early-acting fitness cost, such that $\phi_i^L = 1$ $\forall i$ and $\Phi^L = 1$. From (C.4b) we find $b_i = n_i$; substituting for b_i in (C.4a) leads to a new dispersion relation for the genotypic proportions,

$$n_i - \frac{\frac{\rho N_f}{B} \tilde{p} \phi_i^E}{\frac{\rho N_f}{B} \sum_j^{n_g} \tilde{p}_j \phi_j^E - \left(\sum_j^{n_g} F_j(B) n_j - F_i(B) \right)} = 0. \tag{C.9}$$

If we again simplify down to two viable genotypes, AA and $\alpha\beta$, we can find closed-form expressions for the threshold values. Using $\tilde{p}_1 \to n_1^2$ and $\tilde{p}_2 \to \frac{1}{2}n_2^2$ in the two-genotype limit (from (A.1a) and (A.1d)), the dispersion relation (C.9) reduces to the linear relation

$$n_1 = \frac{1}{2}n_2\phi_2^E + \frac{B}{\rho N_f}\left(F_1(B) - F_2(B)\right), \tag{C.10}$$

which, together with the normalisation condition $n_1 + n_2 = 1$ is enough to fully determine the threshold genotypic proportions as

$$n_1 = \frac{1}{1+\frac{1}{2}\phi_2^E}\left(\frac{1}{2}\phi_2^E + \frac{B}{\rho N_f}(F_1(B) - F_2(B))\right), \tag{C.11a}$$

$$n_2 = \frac{1}{1+\frac{1}{2}\phi_2^E}\left(1 - \frac{B}{\rho N_f}(F_1(B) - F_2(B))\right). \tag{C.11b}$$

(C.11c)

Increasing ν_2 relative to ν_1 or decreasing ϕ_2^E below unity decreases the n_1 threshold value below the $1/3$ which a purely genetic analysis predicts [25], making a population replacement in favour of n_2 (the engineered homozygote $\alpha\beta$) more costly. Thus, where ecological parameters differ between genotypes the underdominance threshold shifts; ecological effects that affect every genotype equally do not play a role in determining the underdominance threshold.