INTRODUCING DESIRABLE TRANSGENES INTO INSECT POPULATIONS USING Y-LINKED MEIOTIC DRIVE—A THEORETICAL ASSESSMENT

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The use of genetic drive mechanisms to replace native mosquito genotypes with individuals bearing antipathogen transgenes is a potential strategy for repressing insect transmission of human diseases such as malaria and dengue. Antipathogen transgenes have been developed and tested, but efficient gene drive mechanisms are lacking. Here we theoretically assess the feasibility of introducing antipathogen genes into wild *Aedes aegypti* populations by using a naturally occurring meiotic drive system. We consider the release of males having both a Y-linked meiotic drive gene and an X-linked drive-insensitive response allele to which an antipathogen gene is linked. We use mathematical models and computer simulations to determine how the post-introduction dynamics of the antipathogen gene are affected by specific genetic characteristics of the system. The results show that when the natural population is uniformly sensitive to the meiotic drive gene, the antipathogen gene may be driven close to fixation if the fitness costs of the drive gene, the insensitive response allele, and the antipathogen gene are low. However, when the natural population has a small proportion of an X-linked insensitive response allele or an autosomal gene that strongly reduces the effect of the drive gene, the antipathogen gene does not spread if it has an associated fitness cost. Our modeling results provide a theoretical foundation for further experimental tests.

KEY WORDS: Autosomal modifier, sex-ratio distortion, transgene, X-chromosome responder, Y-chromosome driver.

Selfish genetic elements spread and persist without adding to an organism's fitness (Burt and Trivers 2006). Since their discovery in the early part of the 20th century (e.g., Wilson 1906; Metz 1926) the evolution and behavior of selfish genetic elements have intrigued biologists. The recent finding that transposons, one class of these selfish elements, comprise approximately 42% of the DNA in the human genome (Deininger and Roy-Engel 2002) makes clear that selfish genetic elements are not rare curiosities. Meiotic drive that results from the action of selfish genetic elements, has long been predicted to alter patterns of genetic diversity in populations and species (Sandler and Novitski 1957). The impacts of meiotic drive and other selfish elements on the evolution of global biodiversity are now receiving increased attention (e.g., Burt and Trivers 2006).

Beyond the importance of selfish genetic elements in affecting the natural processes of evolution, there has been recent

interest in harnessing the evolutionary power of selfish genetic elements to yield benefits to human society. In particular, evolutionary engineering of selfish elements is being considered as a tool in the fight against malaria, dengue, and other insect-vectored diseases (Sinkins and Gould 2006). The general idea is to introduce a pathogen-resistant transgene in the chromosomal region flanking a selfish genetic element (either a natural or a synthesized element) in the disease-vectoring insect. The engineered insects are then released into native populations of the target species, and as the selfish element spreads in the population, the antipathogen transgene also increases in frequency due to tight physical linkage. In specific cases, whether the antipathogen transgene becomes fixed in the population, reaches some intermediate frequency, or is lost depends on the biology of the selfish element and the insect's population genetics and ecology.

In this paper we study a specific Y chromosome meiotic drive mechanism and assess its potential for introducing antipathogen transgenes into natural populations. In most species, individuals of the heterogametic sex produce equal numbers of gametes with chromosomes or loci coding for male and female embryos. In some species, however, a fraction of the individuals produce an unequal sex ratio as a result of a meiotic drive mechanism. In a sex-linked meiotic drive system, the drive gene on a sex chromosome blocks the maturation of gametes carrying a sensitive response allele on the complementary member of the homologous chromosome pair. This causes an increase in the relative frequency of the drive gene in the gametes. Sex-linked meiotic drive has been found in many insect and vertebrate species (James and Jaenike 1990; Jaenike 1999, 2001; Shahjahan et al. 2006).

Male-biased Y chromosome meiotic drive has been reported in *Aedes aegypti*, the primary vector of dengue and yellow fever. Studies of the meiotic drive system in *A. aegypti* have revealed information about factors that can affect the degree of sex distortion (Craig et al. 1960; Hickey and Craig 1966a,b; Sweeny and Barr 1978; Wood 1976; Owusu-Daaku et al. 1997; Suguna et al. 1977). A key factor is the degree of sensitivity of the X-linked response alleles which varies considerably among *A. aegypti* populations. Wood (1976) and Wood and Ouda (1987) studied variation in sex ratios of F_2-generation progeny from the crosses between the Trinidad-derived *A. aegypti* T30 strain and a set of other strains. They suggested that there were at least six different alleles at the response locus with varying degree of sensitivity to the drive gene carried by the T30 male strain. They also found that a modifier gene on chromosome 3 (autosome) of some populations largely counteracted the effect of the drive gene.

Recently Mori et al. (2004) and Cha et al. (2006) have identified a strain T37 with a strong meiotic drive gene at the male-determining locus and an insensitive response allele at the female-determining locus. Their cage trials in which T37 males were released into a population with females from the drive-sensitive

RED strain showed a male-biased sex-ratio among progeny and an increase in frequencies of the drive gene and the insensitive response allele.

These experimental results suggest a few possible strategies for driving desirable transgenes into insect populations using the natural meiotic drive system. One strategy that can be envisioned is the release of males with a Y-linked meiotic drive gene and an X-linked insensitive response allele to which an antipathogen transgene is linked. If the natural population is uniformly sensitive to the drive gene and there is no significant fitness detriment to the drive gene or to the antipathogen gene, their frequencies are expected to increase in the natural population (Mori et al. 2004; Cha et al. 2006).

However, transgenes often bear a substantial fitness cost and natural populations are not always uniformly sensitive to the effects of the drive gene. There may be X-linked and autosomal genes in natural populations, which diminish the effect of the drive gene. The complexity in the combined effects of these factors makes it hard to give a straightforward prediction of the dynamics of the antipathogen transgenes. In this paper, we will use mathematical models and computer simulations to study the population genetics of this specific engineered system and examine whether and when the antipathogen transgene can go to fixation.

The Models

We consider the release of males carrying a Y-linked meiotic drive gene and an X-linked construct created by coupling an antipathogen transgene with a drive-insensitive response allele. Certain species like *A. aegypti* have no clearly differentiated sex chromosomes. In this case we denote the chromosomes with the male- and female-determining alleles as Y and X, respectively, for convenience. We distinguish a few specific scenarios to build population genetic models that track the post-introduction dynamics of the desirable transgene. In all models, we assume that the insect population is infinitely large and consists of nonoverlapping generations. The mating among individuals is assumed to be random.

Scenario I. We consider the release of drive males into a uniformly sensitive natural population (with a single response allele that is sensitive to the effect of the drive gene). In this case, there are two types of Y chromosome, Y^D and Y^d, which represent, respectively, the chromosomes with a drive gene (D) and the normal chromosomes. There are also two types of X chromosomes X^{it} and X^{sn} with it as an abbreviation for the insensitive allele and transgene construct and sn as an abbreviation for the sensitive natural response allele. The genotype of the released males is then $X^{it}Y^D$. The genotype of the wild males and females are, respectively, $X^{sn}Y^d$ and $X^{sn}X^{sn}$. After the F_2 generation there will be four male genotypes: $X^{it}Y^D$, $X^{it}Y^d$, $X^{sn}Y^D$, and $X^{sn}Y^d$, and three female genotypes: $X^{it}X^{it}$, $X^{sn}X^{it}$, and $X^{sn}X^{sn}$. We assume that an

$X^{sn}Y^D$ male produces Y^D-bearing sperm with probability $(1/2 + d_{sn})$ (with $0 < d_{sn} \leq 0.5$) and $X^{sn} -$ bearing sperm with probability $(1/2 - d_{sn})$. The other types of males and all females show normal segregation.

We further assume that Y^D incurs a fitness cost C_{dr} (with $0 \leq C_{dr} \leq 1$). Individuals carrying the X^{it} are also assumed to suffer a fitness cost; individuals carrying a pair of X^{it} have a relative fitness $1 - C_{it}$ (with $0 \leq C_{it} \leq 1$), whereas individuals carrying only one copy of X^{it} have a relative fitness $1 - hC_{it}$ (where $0 < h \leq 1$ is the degree of dominance). The fitnesses of the four male genotypes $X^{it}Y^D$, $X^{it}Y^d$, $X^{sn}Y^D$, and $X^{sn}Y^d$ are v_{11}, v_{12}, v_{21}, and v_{22}, respectively, which are defined as follows:

$$v_{11} = (1 - hC_{it})(1 - C_{dr}), \quad v_{12} = 1 - hC_{it}$$
$$v_{21} = 1 - C_{dr}, \quad v_{22} = 1$$

whereas the fitnesses of the three female genotypes $X^{it}X^{it}$, $X^{sn}X^{it}$, and $X^{sn}X^{sn}$ are u_{11}, u_{12} (or u_{21}), and u_{22}, respectively, which are defined as

$$u_{11} = 1 - C_{it}, \quad u_{12} = u_{21} = 1 - hC_{it}, \quad \text{and} \quad u_{22} = 1.$$

Let the frequencies of gametes X^{it} and X^{sn} be x_{f1} and x_{f2} in eggs and be x_{m1} and x_{m2} in sperm ($x_{f2} = 1 - x_{f1}$, $x_{m2} = 1 - x_{m1}$). Let the frequencies of gametes Y^D, Y^d be y_1 and y_2 in sperm ($y_2 = 1 - y_1$). The notations are listed in Table 1. Table 2 shows the expected gametic frequencies from specific male and female genotypes. Based on this table we have the following recursion equations which will be referred to as model 1:

$$x'_{f1} = \frac{u_{11}x_{f1}x_{m1} + (1/2)[u_{12}x_{f1}x_{m2} + u_{21}x_{f2}x_{m1}]}{u_{11}x_{f1}x_{m1} + u_{12}x_{f1}x_{m2} + u_{21}x_{f2}x_{m1} + u_{22}x_{f2}x_{m2}} \quad (1)$$

$$x'_{m1} = \frac{v_{11}x_{f1}y_1 + v_{12}x_{f1}y_2}{v_{11}x_{f1}y_1 + v_{12}x_{f1}y_2 + (1 - 2d_{sn})v_{21}x_{f2}y_1 + v_{22}x_{f2}y_2} \quad (2)$$

Table 1. Parameters and their meanings.

C_{dr}	Fitness cost of the drive gene (Y^D)
C_{it}	Fitness cost of the insensitive gene and transgene (X^{it})
C_{in}	Fitness cost of the insensitive allele in the natural population (X^{in}) (for model 2)
C_{sn}	Fitness cost of the sensitive allele in the natural population (X^{sn}) (for model 2)
h	Dominance parameter
d_{in}	Sex-ratio distortion for Y^DX^{in} (for model 2)
d_{sn}	Sex-ratio distortion for Y^DX^{sn}
d_{mn}	Sex-ratio distortion for $Y^DX^{sn}A^mA^m$ (for model 3)

Table 2. A list of genotypes and their frequencies, relative fitnesses, and the probabilities of gametes they produce. The fitnesses v_{ij} and u_{ij} are defined in the text.

Genotype	Frequency	Fitness	X^{it}	X^{sn}	Y^D	Y^d
$X^{it}Y^D$	$x_{f1} y_1$	v_{11}	$1/2$		$1/2$	
$X^{it}Y^d$	$x_{f1} y_2$	v_{12}	$1/2$			$1/2$
$X^{sn}Y^D$	$x_{f2} y_1$	v_{21}		$1/2 - d_{sn}$	$1/2 + d_{sn}$	
$X^{sn}Y^d$	$x_{f2} y_2$	v_{22}		$1/2$		$1/2$
$X^{it}X^{it}$	$x_{f1} x_{m1}$	u_{11}	1			
$X^{it}X^{sn}$	$x_{f1} x_{m2}$	u_{12}	$1/2$	$1/2$		
$X^{sn}X^{it}$	$x_{f2} x_{m1}$	u_{21}	$1/2$	$1/2$		
$X^{sn}X^{sn}$	$x_{f2} x_{m2}$	u_{22}		1		

$$y'_1 = \frac{v_{11}x_{f1}y_1 + (1 + 2d_{sn})v_{21}x_{f2}y_1}{v_{11}x_{f1}y_1 + v_{12}x_{f1}y_2 + (1 + 2d_{sn})v_{21}x_{f2}y_1 + v_{22}x_{f2}y_2} \quad (3)$$

The primes indicate the frequencies of gametes in the next generation. The model is similar to that of Hall (2004) in which an X-chromosome drive system was considered and different fitness assumptions were made.

Scenario II. We consider the release of drive males ($X^{it}Y^D$) into a population in which there are two natural X-linked response alleles, one is less sensitive or insensitive to the drive gene (X^{in}) and the other is more sensitive to the drive gene (X^{sn}). X^{in} is initially rare. The corresponding degrees of sex distortion for these two X-linked response alleles are, respectively, d_{in} and d_{sn} (see Table 1). We assume that the X-linked response allele linked to the transgene is always insensitive. The corresponding model describing the recursion of gametic frequencies in this specific scenario is given in Appendix A. The model will be referred to as model 2.

Scenario III. We consider the release of drive males ($X^{it}Y^D$) into a natural population in which a fraction of individuals carry an autosomal modifier gene that diminishes response of the X-linked response allele to the drive gene. The autosomal modifier allele and its alternative, inactive form are denoted as A^m and A^o, respectively. We assume that there is only one natural X-linked sensitive response allele (X^{sn}). The male genotype $X^{sn}Y^D$ is assumed to produce a fraction $0.5 - d_{sn}$ of sperm bearing X^{sn} if the autosomal modifier is absent and a fraction $0.5 - d_{mn}$ of sperm bearing X^{sn} if the autosomal modifier is present. We assume that the autosomal modifier diminishes the sensitivity of the X-linked response allele to the drive gene (and thus reduces the degree of sex distortion), so $0 \leq d_{mn} < d_{sn} \leq 0.5$. The detailed description is given in Appendix B. The model will be referred to as model 3.

Results

THE DYNAMICS OF THE TRANSGENE IN SCENARIO I

To find out how the post-introduction dynamics of the transgene are affected by the degree of sex distortion (d_{sn}) and the fitness costs of the drive gene and the transgenic construct (i.e., C_{dr} and C_{it}) in this specific scenario, we analyze the equilibrium stability and bifurcations in model 1. The model has four corner equilibria $(x_{f1}, x_{m1}, y_1) = (0, 0, 0)$, $(0, 0, 1)$, $(1, 1, 0)$, $(1, 1, 1)$, one internal equilibrium in the boundary plane $y_1 = 1$, and one true internal equilibrium. The stability and bifurcations of these equilibria are summarized in Figure 1 in which C_{dr} and d_{sn} are the control parameters. The (C_{dr}, d_{sn}) parameter space consists of five different regions in general (see Figure 1b).

- In region 1 where $d_{sn} < C_{dr}/(2(1 - C_{dr}))$, the equilibrium $(x_{f1}, x_{m1}, y_1) = (0, 0, 0)$ is stable, meaning that both the drive gene (i.e., Y^D) and the transgene (i.e., X^{it}) go extinct. Note that

$$d_{sn} = \frac{C_{dr}}{2(1 - C_{dr})} \qquad (4)$$

  is the critical condition at which the selection for the drive gene is neutral. When this critical condition is met, $(0, 0, y_1)$ is an equilibrium for any y_1.

- In region 2 where $d_{sn} > C_{dr}/(2(1 - C_{dr}))$ and

$$d_{sn} < \frac{hC_{it}(3 - hC_{it})}{2(1 + hC_{it})} \qquad (5)$$

  the equilibrium $(x_{f1}, x_{m1}, y_1) = (0, 0, 1)$ is stable. The drive gene thus goes to fixation, but the transgene goes extinct.

- In region 3, an internal equilibrium in the boundary plane $y_1 = 1$ is stable. The Y-linked drive gene thus goes to fixation, whereas the X-linked genes (X^{it} and X^{sn}) go neither to fixation nor extinct.

- In region 4, an internal equilibrium is stable. Both the X-linked genes (X^{it} and X^{sn}) and the Y-linked genes (Y^D and Y^d) will go neither to fixation nor extinct (see an example time series in Fig. 2f).

- In region 5, the frequency of the drive gene and the frequency of the transgene exhibit stable periodic or quasi-periodic oscillations (see Figs. 2b and d for the example time series of the stable periodic oscillations). When parameters cross the boundary between regions 4 and 5, the internal equilibrium in region 4 bifurcates, giving rise to a stable periodic cycle in region 5. The boundary curve is derived by the numerical continuation softwarev CONTENT.

(a). C_{it}=0.05

(b). C_{it}=0.2
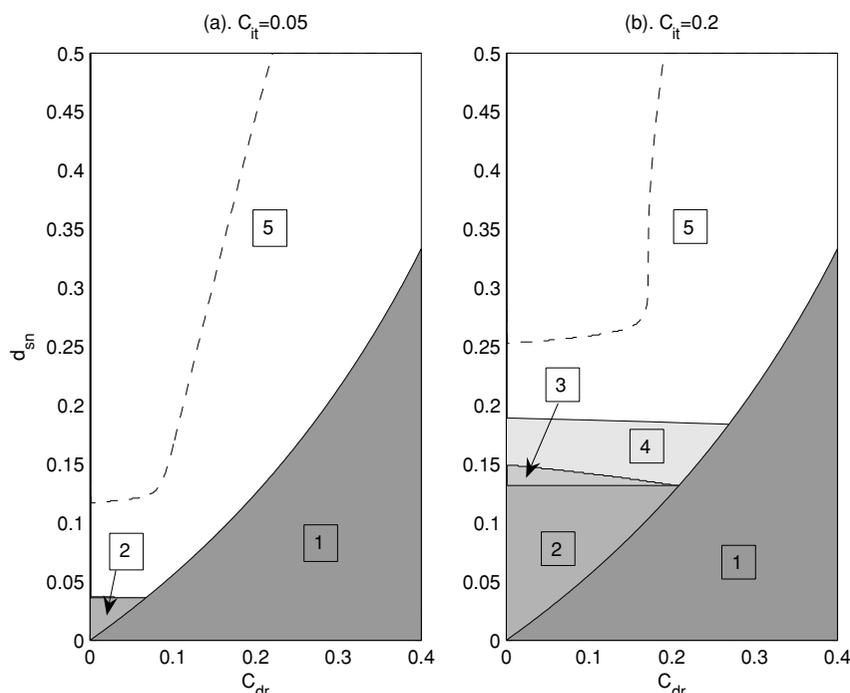
Figure 1. Two-parameter bifurcation diagrams in scenario I describing the dynamics of the transgene as functions of the parameters C_{dr} and d_{sn}. For each diagram, the equilibrium $(x_{f1}, x_{m1}, y_1) = (0, 0, 0)$ is stable in region 1. In region 2 the equilibrium $(0, 0, 1)$ is stable. In region 3 an internal equilibrium in the boundary plane $(y = 1)$ is stable. In region 4 an internal equilibrium is stable. In region 5 an internal periodic or quasi-periodic cycle is stable. In the upper left part of region 5, the frequency of X^{it} reaches nearly 100%. The detailed biological interpretations are given in the text. The degree of dominance for the fitness costs associated with the X-linked genes is $h = 0.5$.
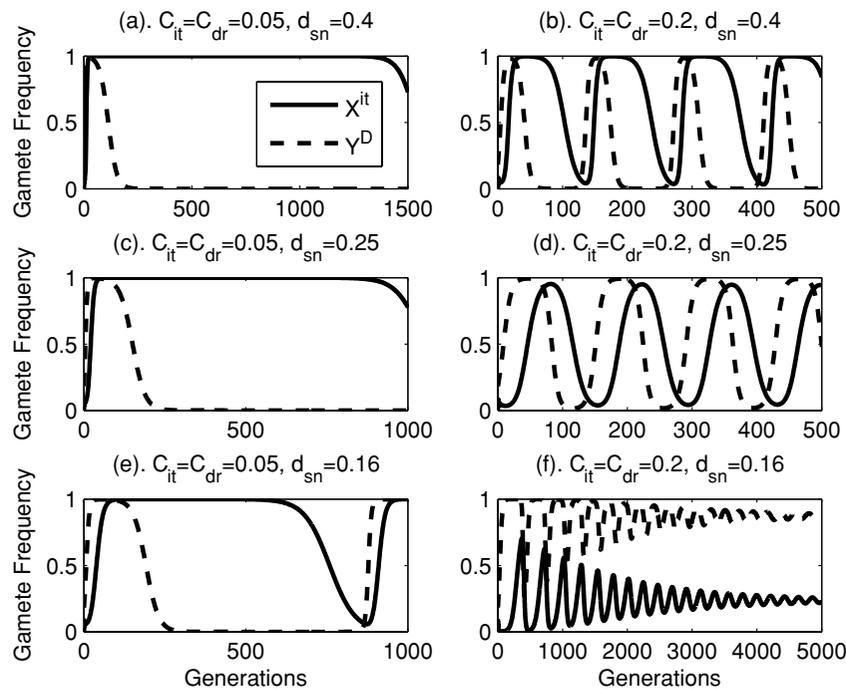
Figure 2. Short-term time series of the frequency of the gametes X^{it} and Y^D in scenario I. This figure is short time segment of Figure 2. The released drive males are initially 10% of the total population. $h = 0.5$.

The stable periodic cycles in region 5 may have very large amplitudes in which the frequency of the transgene (X^{it}) reaches nearly 100% (see Figs. 2a, c, and e for the example time series). In the upper left area of region 5 bounded by the dashed line, for instance, the maximum frequencies of the transgene are larger than 99.9%. In a finite population, the transgene with such a "very" high frequency might go to fixation due to stochastic effects. However, the fixation in this case is unstable because both equilibria $(1, 1, 0)$ and $(1, 1, 1)$, in which the transgene is fixed, are unstable (unless $C_{dr} = C_{it} = 0$). The frequency of the transgene is thus likely to decline if gene mutation or immigration occurs over time.

When the fitness cost of the transgene is small (Fig. 1a) the parameter regions 3 and 4 are too small to be seen. Compared to the diagram for $C_{it} = 0.2$ (Fig. 1b), region 2 is smaller, but region 5 is larger.

Figure 3 presents a short time segment of Figure 2 in which we can examine the initial dynamics of the drive gene and the transgene in detail and understand how the transgene can reach a sufficiently high level in wild populations for a period of time that is relevant to disease control.

When the drive males $X^{it}Y^D$ are released into a sensitive population in which the frequency of X^{sn} is high, the frequency of Y^D increases if the selection for the drive gene is positive (i.e., when $d_{sn} > C_{dr}/(2(1 - C_{dr}))$). As the drive gene Y^D increases in frequency, the advantage for X^{it} becomes greater and its frequency therefore increases. When the fitness costs of X^{it} and Y^D are small (Figs. 3a, c, and e), X^{it} can be brought close to fixation

by a strong drive. As X^{it} approaches fixation, X^{sn} becomes rare and the frequency of Y^D declines due to its fitness disadvantages over the nondrive gene Y^d. When the fitness costs of Y^D and X^{it} are relatively large (Figs. 3b, d, and f), the frequency of Y^D may quickly decline to almost zero after reaching its maximum, and so may the frequency of X^{it}.

THE DYNAMICS OF THE TRANSGENE IN SCENARIO II
In this subsection we examine the effects of polymorphism at the X-linked response locus in the natural population on the dynamics of the transgene. For this purpose we examine the dynamics of the transgene under specific parameter conditions. There are three qualitatively different outcomes for the frequency of the transgene

1. When X^{it} and X^{in} are both completely insensitive to the drive gene and the fitness cost of X^{it} is larger than the fitness cost of X^{in}, the frequency of the transgene increases initially, but then declines (Fig. 4a). The introduction of Y^D leads to an initial increase of both X^{it} and X^{in} and a reduction of the initially common X^{sn}. As the frequencies of X^{it} and X^{in} increase, the viability advantage of the natural allele (X^{in}) over the introduced allele (X^{it}) causes the loss of the introduced (transgene carrying) one.

2. When X^{it} and X^{in} are both insensitive to the drive gene and have the same fitness costs, their frequencies increase initially and then stay constant for a long time before declining (Fig. 4b). The introduction of Y^D leads to an initial increase of both X^{it} and X^{in} and a reduction of the initially common
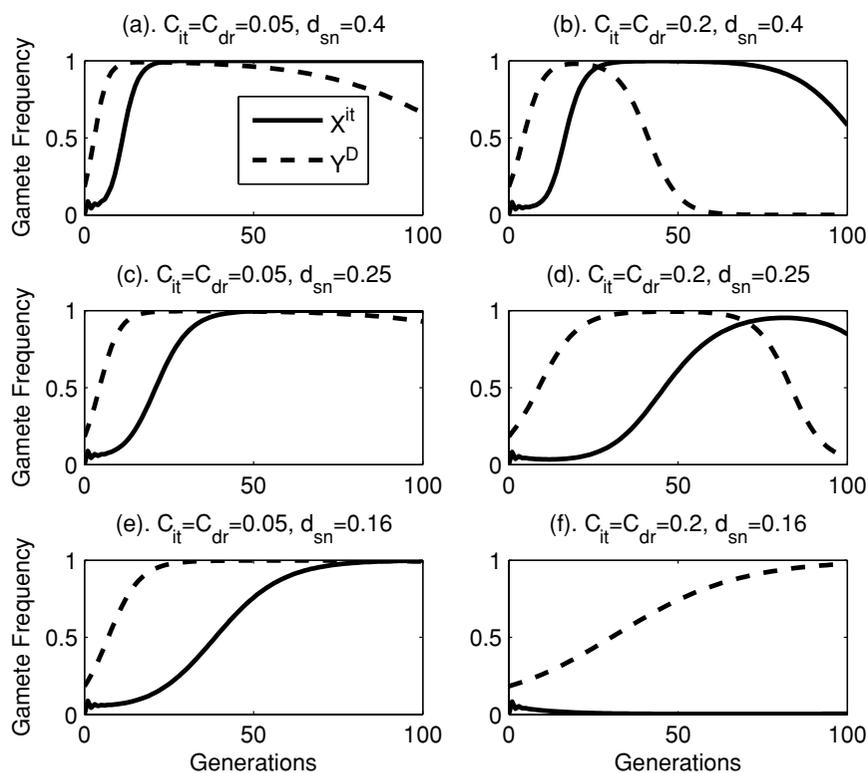
Figure 3. Time series for the frequencies of gametes X^{it} and Y^D in scenario I. Note that in this figure, X^{it} represents the frequency of the transgene in females (i.e., x_{f1} in model 1) and Y^D is the frequency of the drive gene (i.e., y_1 in model 1). The released drive males are initially 10% of the total population. $h = 0.5$.

X^{sn}. With no fitness differences, both the natural and the introduced insensitive response alleles increase in frequency until the sensitive response allele (X^{sn}) disappears.

3. when X^{it} is completely insensitive to the drive gene whereas X^{in} is slightly sensitive to the drive gene, the frequency of X^{it} exhibits stable periodic oscillations (Figs. 4c and d). Note that in Figure 4d, the amplitudes of the oscillations of the transgene are very large. If the fitness cost of X^{it} is larger than the fitness cost of X^{in}, the maximum of X^{it} is generally smaller than that of X^{in}.

Essentially we found that a small fraction of the low-cost natural insensitive allele in a wild population could compete with the introduced insensitive allele and block the spread of the transgene in the wild population.

THE DYNAMICS OF THE TRANSGENE IN SCENARIO III
In this section we examine the impact of an initially rare autosomal modifier gene in a wild population on the dynamics of the transgene. To this end, we plot a set of time series of the gametic frequencies for some specific parameter values (Fig. 5) and compare them to model 1.

The dynamic consequences of the autosomal modifier gene can be examined by comparing Figure 5 to Figure 2. When C_{it}

and C_{dr} are small (Figs. 5a, c, and e) the dynamics of the drive gene and transgene do not qualitatively differ from those without the modifier (Fig. 2a), unless d_{mn} is very small. When C_{it} and C_{dr} are large (Figs. 5b, d, and f) the modifier gene has a significant effect on the dynamics of the drive gene and the transgene (Fig. 2b); the transgene either goes extinct after a transient increase in frequency, or simply does not spread.

To understand the dynamics of the transgene described above we analyze the interactions among the Y-linked drive gene, the X-linked insensitive-allele/transgene, and the autosomal modifier gene. There is intense selection for these alleles when the driver-caused sex distortion is strong and the X-linked sensitive response allele is abundant. The frequencies of these three genes thus increase initially. When the frequency of the insensitive allele is greater than that of the sensitive allele, the selection on the modifier becomes weak. The frequency of the autosomal modifier then increases slowly or stays constant. When both the frequency of the drive gene and the frequency of the insensitive allele increase again due to the oscillatory mechanism, the frequency of the autosomal modifier gene increases again, too. The frequency of the autosomal modifier therefore never decreases during the entire time course. As the frequency of the autosomal modifier becomes higher, the effect of the drive gene becomes weaker. This may
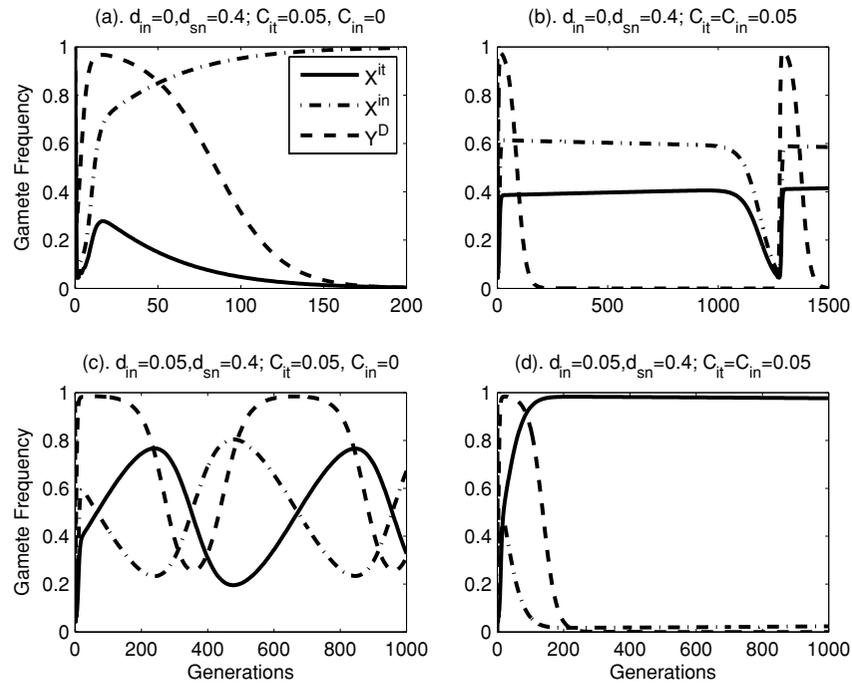
Figure 4. Time series for the frequencies of the gametes X^{it}, X^{in}, and Y^D in scenario II. In each panel, X^{it} and Y^D have the same meaning as before, whereas X^{in} represents the frequency of the natural insensitive response allele in females. The sensitive response allele has no fitness cost. Initially, X^{in} is 10% of the natural population. The released drive males are 10% of the total population. Fixed parameters are $C_{dr} = 0.05$, $h = 0.5$.
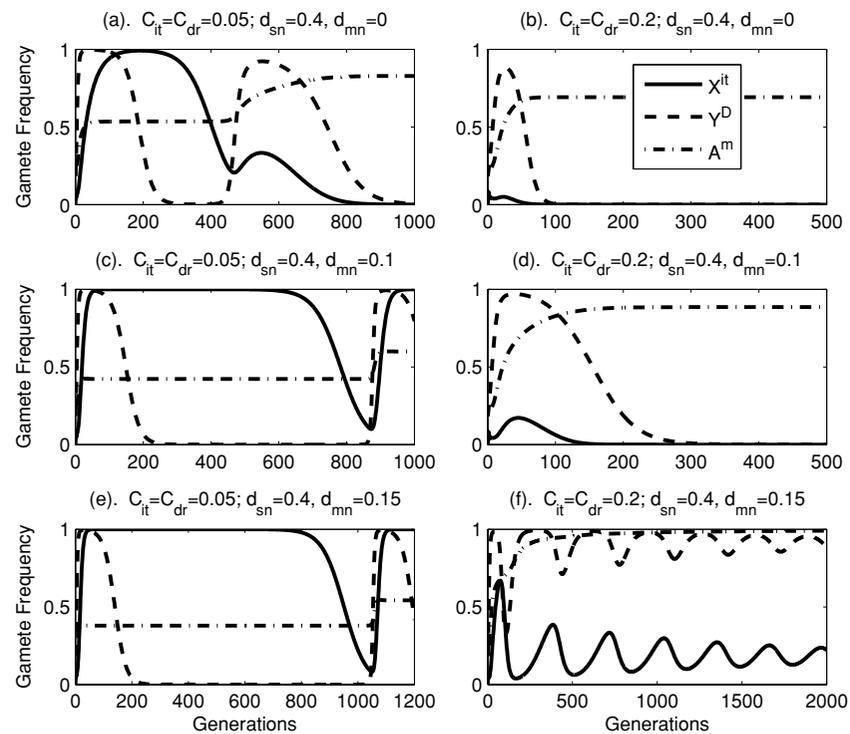
Figure 5. Time series for the frequencies of the gametes X^{it}, Y^D, and A^m in scenario III. In each panel, X^{it} and Y^D have the same meaning as before. A^m represents the frequency of the autosomal modifiers in females. There is no fitness cost associated with either the X-linked sensitive response allele or the autosomal modifier gene. Initially, the autosomal modifier is 20% of the natural population. The released drive males are 10% of the total population. $h = 0.5$.

eventually cause the frequency of the transgene to approach an equilibrium (Figure 5f).

Discussion

In this article, we evaluated the feasibility of using Y-linked meiotic drive as a means of introducing an antipathogen transgene into a natural Aedes aegypti population. As we were concerned about both the short-term suppression and long-term elimination of pathogens vectored by this mosquito species, we have tracked and analyzed both the short-term and long-term dynamics of the antipathogen gene after an initial introduction. We found that there are three possible outcomes for the transgene: short-term fixation (with very high allelic frequency), oscillations, or extinction.

As long as the natural population is uniformly sensitive to the drive gene and the fitness costs of the associated genes are low, we always expect the first outcome. The short-term fixation can meet the goal of suppressing outbreaks of dengue and yellow fever. However, it is important to be aware that even if the transgene becomes fixed due to stochastic effects, stability in this specific meiotic drive system is not assured; when gene mutation or immigration introduces even a small fraction of an X-linked sensitive allele, the frequency of the transgenic construct is expected to decline.

The instability of transgene fixation is largely due to the assumption that the transgene would impose a persistent fitness cost to the transgenic strain. If a transgenic strain can be engineered without introducing a fitness cost or if it can evolve to become more fit, the fixation of the transgene will be stable and could achieve the long-term goal of disease elimination.

Our model has assumed a strong linkage between the X-linked insensitive response allele and the antipathogen transgene so that recombination between these two genes was ignored. This assumption would be reasonable if the transgene could be stably inserted directly flanking the X-linked response allele. This is far from assured with the current transgenic techniques. Therefore, recombination between the two genes may occur. If the recombination rate is very small, short-term disease suppression is still feasible, but even a small recombination rate could reduce the effect of this strategy for disease control. The general dynamics of the transgene due to recombination would resemble the dynamics in scenario II in which a small fraction of natural insensitive response allele was present in the wild population. The exact dynamics of the transgene would depend on the relative fitness costs separately associated with the insensitive response allele and the antipathogen gene and, in particular, on the extent of recombination. Thus, any strain developed for this purpose would need to be rigorously examined to determine the recombination rate between the response allele and the antipathogen gene.

We view our study in this paper as a case study that demonstrates that the population genetic analyses are likely to be essential in the future as the tools of genetic engineering become more sophisticated and more generally applicable to nonmodel organisms. Until recently, the targets of genetic engineering were specific strains of bacteria and crop plants that could not perpetuate themselves in nature. In these cases population genetic analyses were restricted to predicting the evolutionary response of native species to the engineered organisms (e.g., Gould 1991; Bates et al. 2005). Now that the goals of genetic engineering include the release of organisms intended to proliferate in the environment, there are new needs for population genetic analyses including predictions of the feasibility of the direct goals of the genetic engineers, and assessment of unintended effects such as cross-species gene flow.

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Appendix A
THE MODEL FOR SCENARIO II

Let x_{fi} and $x_{mi}(i = 1, \ldots, n)$ be the relative frequencies of X-linked response allele i in eggs and sperm, respectively, $y_j(j = 1, 2)$ be the relative frequencies of the Y-linked meiotic drive allele ($j = 1$) and the nondrive allele ($j = 2$) in sperm. Let u_{ij} be the fitnesses of females carrying X-linked response alleles i and j in their homologous sex chromosomes, v_{ij} be the fitnesses of males carrying X-linked response allele i and Y-linked drive or nondrive allele j. A male individual carrying a Y-linked allele j and an X-linked response allele i is assumed to produce X chromosomes with a probability $(1 - 2d_{ij})/2$ and Y chromosomes with a probability $(1 + 2d_{ij})/2$ (with $0 \leq d_{ij} \leq 0.5$. So d_{ij} measures the combined effect of the drive allele j and the response allele i on the segregation of X and Y chromosomes in males. Incorporating the above assumptions and assuming that mating is random, the recursive equations for the gametic frequencies have the following form:

$$\overline{W}_{xf} x'_{fi} = (1/2) \sum_{j=1}^{n} (u_{ij} x_{fi} x_{mj} + u_{ji} x_{fj} x_{mi}), \quad i = 1, \ldots, n \quad (A1)$$

$$\overline{W}_{xm} x'_{mi} = (1/2) \sum_{j=1}^{2} (1 - 2d_{ij}) v_{ij} x_{fi} y_j, \quad i = 1, \ldots, n \quad (A2)$$

$$\overline{W}_y y'_j = (1/2) \sum_{i=1}^{n} (1 + 2d_{ij}) v_{ij} x_{fi} y_j, \quad j = 1, 2, \quad (A3)$$

where

$$\overline{W}_{xf} = \sum_{i,j} u_{ij} x_{fi} x_{mj} \quad (A4)$$

$$\overline{W}_{xm} = (1/2) \sum_{i,j} (1 - 2d_{ij}) v_{ij} x_{fi} y_j \quad (A5)$$

$$\overline{W}_y = (1/2) \sum_{i,j} (1 + 2d_{ij}) v_{ij} x_{fi} y_j. \quad (A6)$$

Note that

$$\sum_{i=1}^{n} x_{fi} = \sum_{i=1}^{n} x_{mi} = \sum_{j=1}^{2} y_j = 1 \quad (A7)$$

Appendix B
THE MODEL FOR SCENARIO III

For scenario III described in the main text, there will be 12 different male genotypes and nine female genotypes after the F_2

generation. Each genotype consists of a pair of sex-determining chromosomes and a pair of autosomes. For instance, the male genotype $X^{it}Y^DA^mA^o$ consists of a pair of sex chromosomes $X^{it}Y^D$ and a pair of autosomes A^mA^o where m and o indicate the presence and absence of a modifier, respectively. We assume that there are no fitness costs associated with the autosomal modifiers. So the fitness of genotype $X^{it}Y^DA^oA^m$, for instance, is just v_{11} which has been defined in scenario I.

The 12 male genotypes produce eight different types of male gametes: $X^{it}A^o$, $X^{it}A^m$, $X^{sn}A^o$, $X^{sn}A^m$, and Y^DA^o, Y^DA^m, Y^dA^o, Y^dA^m. The nine female genotypes produce four different types of female gametes: $X^{it}A^o$, $X^{it}A^m$, $X^{sn}A^o$, $X^{sn}A^m$. Sex-ratio distortion occurs if the drive gene is present and the response allele is sensitive. The degree of sex-ratio distortion is assumed to decrease if the modifier is present. More precisely, we assume

- $X^{sn}Y^DA^oA^o$ produces a fraction $0.5 - d_{sn}$ of gametes $X^{sn}A^o$ and a fraction $0.5 + d_{sn}$ of gametes Y^DA^o.
- $X^{sn}Y^DA^mA^m$ produces a fraction $0.5 - d_{mn}$ of gametes $X^{sn}A^m$ and a fraction $0.5 + d_{mn}$ of gametes Y^DA^m. Here $0 \leq d_{mn} < d_{sn} \leq 0.5$.
- $X^{sn}Y^DA^oA^m$ produces a fraction $(0.5 - d_{mn})/2$ of gametes $X^{sn}A^o$ and $X^{sn}A^m$, respectively, and a fraction $(0.5 + d_{mn})/2$ of gametes Y^DA^o and Y^DA^m, respectively.

Other genotypes are assumed to show normal segregation. Based on these assumptions, a system of nine recursive equations for the gametic frequencies can be developed in a similar way to model 1. We omit the details here.