

Pest Control by the Release of Insects Carrying a Female-Killing Allele on Multiple Loci

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ABSTRACT With recent advances in genetics, many new strategies for pest control have become feasible. This is the second article in which we model new techniques for pest control based on the mass release of genetically modified insects. In this article we model the release of insects carrying a dominant and redundant female killing or sterilizing (FK) allele on multiple genetic loci. If such insects are released into a target population, the FK allele can become widely spread in the population through the males while reducing the population each generation by killing females. We allow the number of loci used to vary from 1 to 20. We also allow the FK allele to carry a fitness cost in males due to the gene insertions. Using a model, we explore the effectiveness and optimal strategies for such releases. In the most ideal circumstances (no density-dependence and released insects equal in fitness to wild ones), FK releases are several orders of magnitude more effective than equal sized sterile male releases. For example, a single release of 19 FK-bearing males for every two wild males, with the released males carrying the FK allele on 10 loci, reduces the target population to 0.002% of no-release size. An equal sized sterile release reduces the target population to 5% of no-release size. We also show how the effectiveness of the technique decreases as the fitness cost of the FK alleles in males increases. For example, the above mentioned release reduces the target population to 0.7% of no-release size if each FK allele carries a fitness cost in males of 5%. Adding a simple model for density-dependence and assuming that each of the released males carries the FK allele on six loci, we show that the release size necessary to reduce the target population to 1/100 of no-release size in 10 generations of releases varies from 0.44:1 to 4:1 (depending on parameter values). We also calculate the optimal number of loci on which to put the FK allele under various circumstances.

KEY WORDS genetic control, sterile insect technique, multilocus, female-killing, autocidal

NEW INSECT GENETIC engineering techniques (see Atkinson and O'Brochta 1999 for a review) may allow far more powerful genetic control methods than were envisioned by earlier researchers. This is the second article in which we model new approaches to control of insect pests made possible by genetic engineering of insect strains (see Schliekelman and Gould 2000 for further background details). With the flexibility that these new techniques bring, a wide variety of genetic control methods appropriate for various circumstances will be possible. In this article, we model the release of insects carrying alleles that distort gender ratios in favor of males by killing females. Reducing the number of females reduces future population size while the female-killing genes remain in the population through the males. In a recent article, Thomas et al. (2000) described such a genetic system in *Drosophila melanogaster*. They constructed a laboratory strain in which a dominant female-killing trait is expressed in the absence of tetracycline in the diet (Gossen and Bujard 1992). They also showed a simple

model of the release of insects carrying this trait into a target population. Here we show that the true power of this system lies in putting the female-killing trait on multiple loci in a form such that a single allele on any of the loci can cause the trait to be expressed (a fact hinted at in figure 1 of Thomas et al. 2000).

In a previous article (Schliekelman and Gould 2000), we modeled the release of pest insects carrying conditional lethal traits. We showed that a dominant and redundant conditional lethal trait inserted onto multiple genetic loci could be spread widely through a target population in a small number of generations. When the engineered insects are first released, insects at the release site either have no copies of the inserted gene or all of the inserted copies of the gene. As the released genotypes mate with the wild types, linkage disequilibrium decreases and a larger proportion of the population inherits at least a single copy of the gene. Under appropriate circumstances such a release would be orders of magnitude more effective than sterile male releases. Although potentially very effective, this technique has shortcomings and is not ideal for all circumstances. At least four generations from the time of release to the activation of the conditional lethal trait are needed to achieve maximum effectiveness. Because most potential conditional lethal traits are triggered by annual events (e.g., heat shock pro-

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moter or nondiapausing trait), it would be difficult to have a sufficient number of generations with species with fewer than three to four generation per year. Furthermore, the conditional lethal gene is completely removed from the population once it is activated. Thus, a new release must be made to get additional target population reduction.

The advantage of using a female-killing (FK) trait over a conditional lethal trait is that a reduction in pest population can be achieved while the introduced allele remains in the population. Furthermore, the timing of the activation of the trait is not a major issue. Because the allele could remain in the population longer, there may be a greater ultimate reduction in the target population. Because the FK trait can be introduced on multiple loci, we expect that its efficiency in reducing the population should be much higher than a sterile male release. The concept of genetic control using a single insertion of a female killing trait is not new. Foster (1991) showed that a field female-killing system using a Y-linked translocation can achieve higher genetic death rates than the sterile insect technique (SIT) in density-influenced populations. Here we show that using multiple redundant copies of the FK allele is a far more efficient way of driving it into the population than using translocations. This multiple insertion method is made possible (or at least conceivable) by new genetic engineering techniques. Whereas the method of Foster and co-workers uses clever chromosomal rearrangements to drive the FK alleles into the target population, our method simply depends on releasing insects carrying enough copies so that most of their offspring will carry at least one copy.

Questions to be Answered. *How Effective is the Multilocus FK System at Reducing Pest Populations in the Ideal Case?* How do ideal multilocus FK systems compare with ideal sterile insect releases and ideal conditional lethal releases? By an "ideal" release, we mean one in which the released males have fitness equal to the wild males (this does not preclude fitness differences due to the female-killing action of the alleles) and in which various ecological complications (e.g., weather or migration) are not acting. This gives an upper bound on the effectiveness of the method and is useful for comparisons with other pest control methods.

How do Reductions in Fitness Due To Insertion of the FK Alleles Impact the Effectiveness of the Multilocus FK Technique? *How Does This Compare with Conditional Lethal Releases?* It is unlikely that a large number of alleles can be inserted into the genome of an insect without doing some damage to fitness (e.g., insertions within coding regions). Given this, how much genetic load can the released insects sustain and still be useful in spreading the female-killing trait?

What is the Optimal Number of Loci to Use in the Multilocus FK Technique? The probability that the descendants of matings between released- and wild-type insects pass on no copies of the FK allele to their offspring is reduced by each additional locus that the FK allele is inserted on. If the FK allele carries no

fitness cost, then it will be advantageous to use as many loci as possible. However, if each FK allele carries a constitutively expressed fitness cost, then there should be an optimal number of loci that balances the fitness reduction in the released insects with the increase in fraction of offspring carrying the FK allele.

Materials and Methods

We derive a system of deterministic difference equations for the multilocus FK system, assuming infinite population size, nonoverlapping generations, and no population structure.

Model Parameters and Output. The model parameters are as follows: L is the number of loci on which released insects carry the FK allele; s is the per-allele fitness reduction resulting from random damage to the insect genome caused by the insertion of the FK alleles; and I is the release proportion. The release size will be expressed as both *number of released insects: number of wild insects* and release proportion $I = \text{fraction of male population which is released type immediately following the release}$. $I = N / (0.5 + N)$, where N is the number of released insects per wild insect. Both values will usually be given. We model both single and multiple releases. For multiple releases, the release size and proportion usually refer to the size of the release relative to the initial population size (the context should make it clear when it is otherwise).

The primary model output variables are as follows: the frequency of the genotype with no FK alleles and the size of the population relative to that if there had not been a release.

Multilocus Dynamics. We assume that the FK allele is inserted on up to 20 loci on different chromosomes (or functionally unlinked areas of a chromosome). We assume that any fitness reduction associated with the alleles is equal between loci (we will further discuss this assumption later). Since the released population is fixed for the introduced allele on all loci, the allele will start at an equal frequency on all loci. Because selection acts the same and the recombination frequency is equal between all loci ($1/2$), the frequencies of all gamete types with the same number of introduced-type alleles will be equal for all time. Thus, we only have to track frequencies for each of the L gamete "classes" (the set of all gamete types with a given number of introduced-type alleles), instead of the 2^L gamete types (see Schliekelman and Gould 2000 for details).

We assume that the released insects carry an allele (denoted FK) that either kills or sterilizes all females before reproduction. We will first derive the case when the allele kills females, and then show that female killing and female sterilizing are equivalent for our purposes.

Reproducing females carry no copies of the introduced-type allele. Thus, the genotype of females with fitness greater than zero is always known, and we only have to explicitly track male genotypes. Furthermore, we only have to consider the paternal gamete in zy-

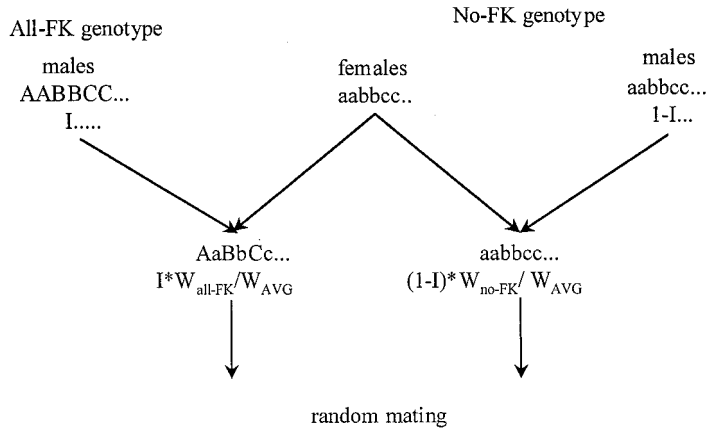


Fig. 1. Matings in the release generation. I is the fraction of the total male population that are released males. W_{all-FK} is the fitness of the genotype with FK alleles in homozygous form at all loci, W_{no-FK} is the fitness of the genotype with no FK alleles, and W_{avg} is the average fitness of the population. Uppercase letters represent the FK alleles (A is locus 1, B is locus 2, and so on) and lower case letters represent the absence of the FK allele.

gote formation, since the maternal gamete is always known. At each locus, a male will either have no copies of the introduced-type allele or he will be hemizygous for it. This is represented in the example below, where 1 indicates an FK allele and 0 indicates the absence of such an allele. (The term hemizygous is used in place of heterozygous to indicate that there is no alternate allele to the FK allele.)

male zygote	gamete
$\begin{pmatrix} 1 & 0 \\ 0 & 0 \\ 0 & 0 \\ 1 & 0 \\ 1 & 0 \end{pmatrix}$	$\Rightarrow \begin{pmatrix} 1 \text{ or } 0 \\ 0 \\ 0 \\ 1 \text{ or } 0 \\ 1 \text{ or } 0 \end{pmatrix}$

The left column of the zygote represents paternally derived alleles and the right column represents maternally derived alleles. There is a 1/2 chance of an FK allele being segregated at the first, fourth, and fifth loci (since each locus is on a different chromosome). Thus, the number of FK alleles in the gamete is distributed binomially. The probability $P(X \rightarrow Z)$ that a male with X FK alleles produces a gamete with Z FK alleles is then as follows:

$$P(X \rightarrow Z) = \binom{X}{Z} \left(\frac{1}{2}\right)^X \quad [1]$$

where $\binom{X}{Z}$ is known as a binomial coefficient. This is the number of ways that Z unordered objects can be chosen from X objects without replacement ($Z \leq X$). In addition, because all FK alleles are inherited from the father, the frequency of zygotes with Z FK alleles equals the frequency of male gametes with Z FK alleles:

$$F_t(Z) = G_t(Z), \quad [2]$$

where $G_t(Z)$ is the frequency of male gametes with Z FK alleles uniting with female gametes to form a

zygote in generation t and $F_t(Z)$ is the frequency of zygotes with Z FK alleles in generation t .

The Release. We assume that the released population consists entirely of males carrying the introduced allele in homozygous form on L loci. Thus, the matings in the release generation will be as shown in Fig. 1.

Selection. We explore the effect of selection against the introduced-type alleles due to damage to the insect genome resulting from the gene insertions. Mackay et al. (1992) studied the effects of P-element insertions on viability of *Drosophila meonogaster*. They found that each insertion decreased viability of insects by an average of 5.5% for heterozygotes and 12.2% for homozygotes. Measurements of decrease in viability may underestimate the decrease in fitness. However, Mackay et al. studied random insertion events; these insertions can be screened to find the ones causing the least fitness reduction. Regression of viability on number of insertions yielded an expression with significant linear and quadratic terms. The resulting quadratic expression is reasonably approximated by a multiplicative expression of the form

$$W(X) = (1 - s)^X \quad [3]$$

where $W(X)$ is the fitness of the male genotype with X introduced-type alleles. Note that this fitness function applies only to the selection within the male population due to insertion-caused fitness reduction. It is in addition to the selection that results from the female-killing action of the FK alleles.

We will make comparisons between FK and sterile male releases. To make a fair comparison, we must include the fitness reductions caused by the process of sterilization. Laboratory studies have shown that sterilized males have fitnesses of 20–50% of the fitness of unsterilized males (e.g., Holbrook and Fujimoto 1970, Hooper and Katiyar 1971, Ohinata et al. 1971). Fitness here means the ability to achieve matings, and obviously does not include the sterility. We assume 50%

fitness for sterile males. Thus, the results will be conservative with respect to the advantage of the FK technique over sterile male releases.

Iteration Equations. Because males are the only source of FK alleles in zygote formation, all offspring of no-FK genotype males will have the no-FK genotype. Furthermore, all offspring of a male with no FK allele at a particular locus will have no FK allele at that locus. The probability that a male with one FK allele will segregate a gamete with no FK alleles is one-half. The probability for a male with two FK alleles is one-fourth, and so on. Therefore the frequency of males with no FK alleles in generation $t + 1$ is given by

$$F_{t+1}(0) = F_t(0) + \frac{1}{2} F_t(1) + \frac{1}{4} F_t(2) + \dots + \frac{1}{2^L} F_t(L), \quad [4]$$

where $F_t(Z)$ is the frequency of males with Z FK alleles. Modifying this equation for selection against the FK alleles in males, we get

$$\bar{w}_t F_{t+1}(0) = F_t(0) + \frac{f}{2} F_t(1) + \frac{f^2}{4} F_t(2) + \dots + \frac{f^L}{2^L} F_t(L), \quad [5]$$

where \bar{w}_t is the average fitness of males in generation t and $f = 1 - s$ is the fitness reduction due to one FK allele (see equation 3 above).

Using equation 1, we see that the iteration equation for general $F_t(Z)$ is given by

$$\bar{w}_t F_{t+1}(Z) = \binom{0}{Z} F_t(0) + \binom{1}{Z} \left(\frac{f}{2}\right) F_t(1) + \binom{2}{Z} \left(\frac{f}{2}\right)^2 F_t(2) + \dots + \binom{L}{Z} \left(\frac{f}{2}\right)^L F_t(L) \quad [6]$$

These equations are solved in *Appendix 1*. The solutions take the form

$$\prod_{i=0}^t \bar{w}_i F_i(0) = 1 - I + If^{2L}(-1)^L \left(\frac{f}{2-f}\right)^L \cdot \left(1 - \sum_{x=1}^L (-1)^{x-1} \binom{L}{x} \left(\frac{f^x}{2^x}\right)^t\right) \quad [7]$$

$$\prod_{i=0}^t \bar{w}_i F_i(Z > 0) = If^{2L}(-1)^{L-1} \left(\frac{f}{2-f}\right)^{L-Z} \binom{L}{Z} \cdot \sum_{x=Z}^L (-1)^{x-1} \binom{L-Z}{x-Z} \left(\frac{f^x}{2^x}\right)^t \quad [8]$$

We can get genotype frequencies by taking ratios of these equations (and thus canceling out the factors of

$$\prod_{i=0}^t \bar{w}_i).$$

Population Size. Only male gametes with no FK alleles form viable female offspring. Therefore, the reproducing female population is a fraction $F_t(0)$ of the female population that is born. If mortality is density independent, the number of reproducing females is reduced by this fraction each generation. Therefore, the population size in generation t is given by

$$N_{t+1} = R_t F_t(0) N_t$$

where R_t is what the per-capita growth rate would be in generation t for an all wild-type population. Then

$$N_t = F_t(0) R_t F_{t-1}(0) R_{t-1} F_{t-2}(0) R_{t-2} \dots F_0(0) R_0 = R_t R_{t-1} R_{t-2} \dots F_t(0) F_{t-1}(0) F_{t-2}(0) \dots N_0$$

With no release, $F_t(0) = 1$ for all t . The population size relative to no release is then

$$\text{relative size} = \frac{R_t R_{t-1} R_{t-2} \dots F_t(0) F_{t-1}(0) F_{t-2}(0) \dots N_0}{R_t R_{t-1} R_{t-2} \dots N_0} = F_t(0) F_{t-1}(0) F_{t-2}(0) \dots$$

Or,

$$\text{relative population size} = \prod_{s=0}^{t-1} G_s(0). \quad [9]$$

This gives the population size relative to what it would be if there had been no release.

If density dependence is important, then there will be compensation in the growth rate for any population reduction due to a release. In such cases, the effect of a single release will diminish with time. To explore the effects of density dependence, we use a simple threshold model to simulate pesticide spraying triggered by insect density. Each time the threshold is exceeded, the population is subjected to one-time pesticide mortality. This model is fairly reasonable for a pest population subject to spraying.

Female Sterilizing. If the number of available male matings equals or exceeds the number of available female matings, then an allele that sterilizes females has the same effect as an allele which kills females before mating if there is no density dependence. In this case, all available matings with fertile females occur. As long as mating is random, the proportion of fertile female matings to males with and without female sterilizing alleles will not be altered by the presence of females that are genetically sterile. Therefore, in the absence of density dependence, the impact of a female killing and a female sterilizing allele should be equal. If the number of available female matings is greater than the number of available male matings, then some available matings with fertile females will not occur. In this case, the sterile females act as a trap for wild-type males and female killing and sterilization are not equivalent. However, there are few insect populations in which the number of available female matings exceeds the number of available male matings. Furthermore, an FK release distorts sex ratios in favor of males.

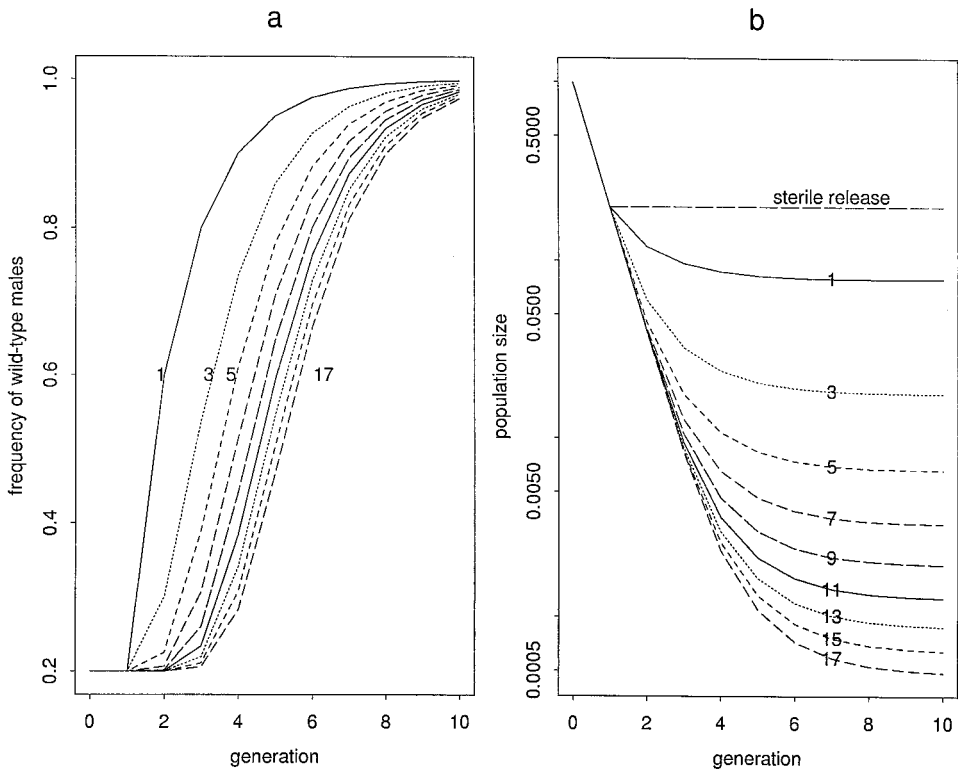


Fig. 2. Simulation output for a single release of size 2:1 ($I = 0.8$) with no fitness cost to the FK alleles in males. Fig. 2a shows the frequency of males with the genotype having no FK alleles. Fig. 2b shows the population size with a 2:1 release relative to the population size when there is no release (assuming no density-dependence). Numbers on the curves indicate the number of loci in the released insects that carry the FK allele. Note that the relative population sizes are on a log scale.

In a density dependent setting, the presence of the sterile females can affect density response of the population. Thus, female killing and sterilizing are not equivalent. We will limit density-dependent cases to female killing alleles only.

Results

No Selection in Males. It is clear from equation 4 that the FK allele is rapidly removed from the population after the release of the FK-bearing males. Because each FK allele has a $\frac{1}{2}$ chance of being lost during the segregation of a male gamete (see the derivation of equation 4), the FK alleles disappear quickly. Fig. 2a shows the frequency of the no-FK genotype plotted against generations for a 2:1 release for a range of L values. The no-FK genotype frequency starts from 0.2 and steadily increases. Although the no-FK genotype approaches fixation by the 10th generation, the FK allele does stay in the population long enough to cause a major population reduction. Examining equation 7 with $f = 1$ and $\bar{w}_t = 1$, we see that all terms inside the summation except the first two will quickly become negligible. Thus, after the first few generations we have.

$$F_t(0) \approx 1 - \frac{L}{2^t}.$$

Increasing L then causes an approximately linear decrease in the no-FK frequency in a given generation. Recalling that the frequency of no-FK males in one generation is the frequency of reproducing females in the following generation (see equation 9), we see that this linear reduction will be multiplied in a geometric product over generations when determining population size. Thus, increasing L causes a large reduction in the eventual population size. See Fig. 2b. In 10 generations the population is reduced to under 0.7% of the no-release size for $L = 5$, to under 0.2% for $L = 9$, and to under 0.05% for $L = 17$. Compare this with a population reduction to 20% of no-release size for an ideal (no fitness reduction in sterile males) sterile male release of the same size (shown in Fig. 2).

Effect of Size of Release. The release proportion, I (fraction of males in the population that are of the released type immediately after release), appears linearly in equation 7. Thus, increasing the size of the release causes a linear decrease in the frequency of no-FK males in a given generation and in the reproducing females in the following generation. Again, this linear decrease will be multiplied in a geometric product over generations to determine population size. Fig. 3 shows the no-FK frequency and population size plotted against generations for a 19:2 ($I = 0.95$) size release and a 1:2 ($I = 0.5$) size release. By the 10th

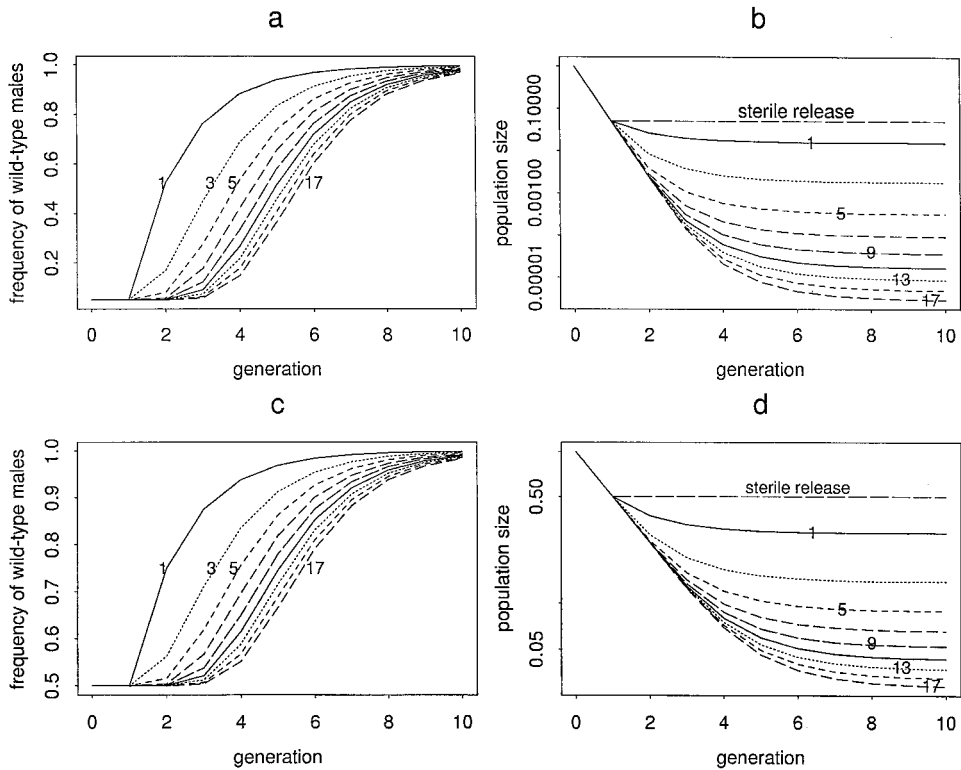


Fig. 3. Effect of release size. (a and b) Simulation results for a single release of size 19:2 ($I = 0.95$). (c and d) A release of size 1:2 ($I = 0.5$). Left-hand panels show the frequency in males of the genotype with no FK alleles. Right-hand panels show the population size relative to that with no release. Note that these graphs are on a log scale. Impact of sterile releases of the same release size are also shown.

generation for the 19:2 release, the population is reduced to 0.04% of no-release size for $L = 5$, 0.004% for $L = 9$, and 0.0003% for $L = 17$. An ideal 19:2 sterile male release reduces the population to 5% of its original size. For the 1:2 ($I = 0.5$) FK release, the population is reduced to 9, 4, and 3%, respectively, for $L = 5, 9$, and 17. The 1:2 sterile male release reduces the population to 50% of no-release size.

Effect of Selection Against FK Alleles in Males. The released insects carry $2L$ copies of the FK allele and their F_1 descendants carry L copies. Thus, their fitness is very low if sL is high. This is reflected in Fig. 4, which shows releases with s values of 0.05 (4 a and b) and 0.025 (4 c and d). The frequency of no-FK males mating in the release generation ranges up to nearly 0.6 (for $L = 16$) when $s = 0.05$, compared with 0.2 when $s = 0$. For high L , most FK alleles are carried in individuals with many other FK alleles. If there is a per-allele fitness cost, the FK alleles will “drag each other down,” and they are removed very quickly from the population. Thus, unlike the case with no fitness cost to the FK alleles, the no-FK frequency increases more quickly for higher L than for lower L . We see from Fig. 4b that a release with $L = 4$ achieves the greatest population reduction. The population is reduced to 5% of no-release size in this release. Thus, the genetic load caused by the gene insertion process

greatly reduces the effectiveness of the release. For $s = 0.025$, the greatest population reduction is to 2%, for $L = 7$. By comparison, the “realistic” sterile male release (with 50% fitness of sterilized males) achieves a reduction to 33% of no-release population size.

Interaction of Effects of Release Proportion and s . Fig. 5 shows contour plots of the optimal value of L (5a) and the population size at that L in the F_{10} generation (5b) plotted against I and s . The optimal L is the value that gives the lowest population size. For example, with $s = 0.04$ and $I = 0.6$ (point A in Fig. 5) the optimal is between 2 and 4, but if I is increased to 0.9 (point B) the optimal L is between 4 and 6. We see that the optimal value of L decreases with increasing s —increasing s raises the costs of the FK alleles. Optimal L also increases with I (except for unrealistically small I). Increasing the release proportion decreases the amount of favorable genetic variation and thus decreases the effectiveness of selection against the FK allele. There is a very steep gradient (infinite, in fact) in optimal L with respect to s as s goes to zero, and with respect to I as I goes to 1. At these limits, selection against the FK alleles in males goes to zero and the optimal L goes to infinity. However, optimal L decreases to 1 when selection is at its maximum for high s and small to intermediate release proportions.

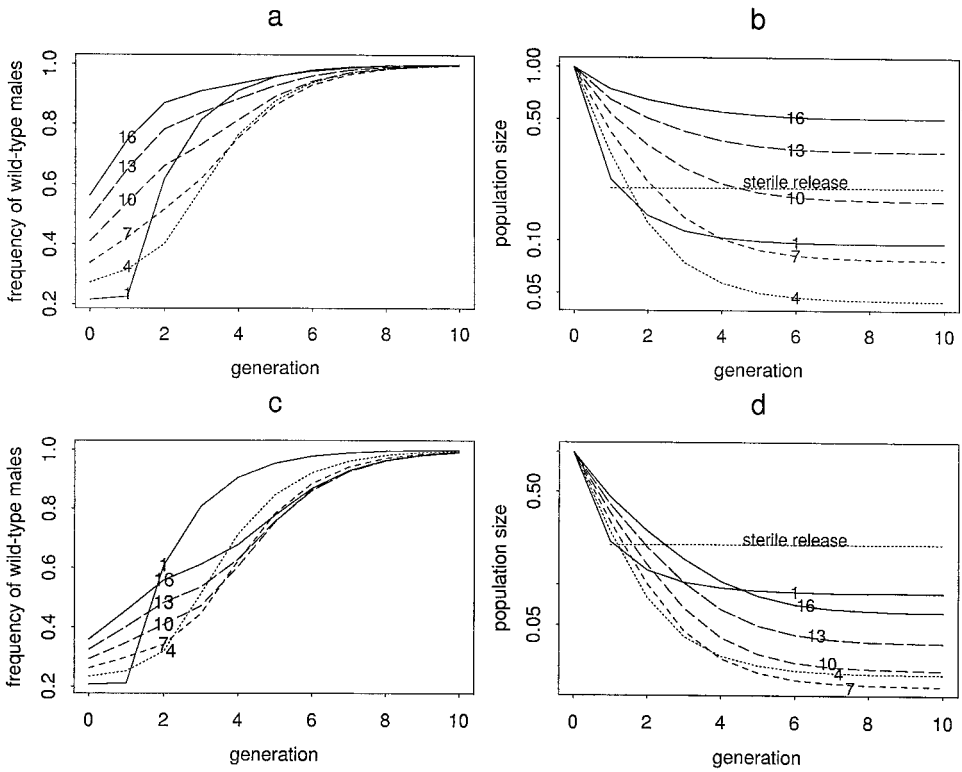


Fig. 4. Effect of a fitness cost of the FK allele in males. (a and b) Simulation results for a single 2:1 release with each FK allele carrying a 5% fitness cost. (c and d) A 2:1 release with a FK alleles carrying a 2.5% fitness cost. Numbers on the curves indicate the number of loci in the released insects that carry the FK allele. Note that the population size graphs are on a log scale.

As expected, the surviving population (Fig. 5b) increases as s increases and I decreases. The gradient in s is steepest for intermediate release proportion, when selection is strongest. There is also a steep gradient with respect to I as I goes to 1. This is because the ratio of released to wild insects (which equals $I/2/[1-I]$) changes very rapidly as I approaches one. Thus, it is an artifact of the way in which the results are presented and is not a property of the dynamics of the system.

Effect of Population Dynamics. If the population is density regulated, then the population growth rate will increase to compensate for the population reduction due to a release and the population will eventually return to a "no-release" state. However, most mass release strategies aim to eradicate target populations. Multiple releases are usually necessary to achieve this goal. For our discussions of the effect of density regulation, we use the threshold model for density dependence discussed in the methods section.

With our model for density dependence, we can easily calculate the release proportion necessary to achieve eradication with a sterile male release: If I is the release proportion (fraction of males which are sterile immediately after the release), R is the per-capita growth rate, and W_s is the mating fitness of the sterile males, then the condition to achieve a decrease in population size with one release is

$$(1 - I)/(IW_s + (1 - I)) < \frac{1}{R}.$$

Since the population growth rate is constant except when the spraying threshold is exceeded, then releases once per generation of this size will continually decrease the population and eventually eradicate it. For $W_s = 1$, this relationship reduces to $I > 1 - (1/R)$. If the release proportion does not meet this criterion in the first generation, then it never will (assuming that $R > 1$).

The situation for the FK release is more complicated. Because each FK release is equivalent to multiple sterile releases, it is possible for the frequency of FK alleles to build over several generations to a level sufficient to cause the population to decrease in size. Fig. 6 demonstrates such a situation. In this figure an FK release of constant size is repeated every generation. The release size is such that the initial release is of size 1:2 ($I = 0.5$). The population grows through the F_2 generation for all values of L . We see that the frequency of the no-FK genotype (the only reproducing genotype) is greater than $1/R$ ($=1/6 = 0.166667$) through this generation for all values of L . However, the no-FK frequency dips below $1/R$ in the F_3 generation for the larger values of L . Once this occurs, the population size begins to decrease. For L

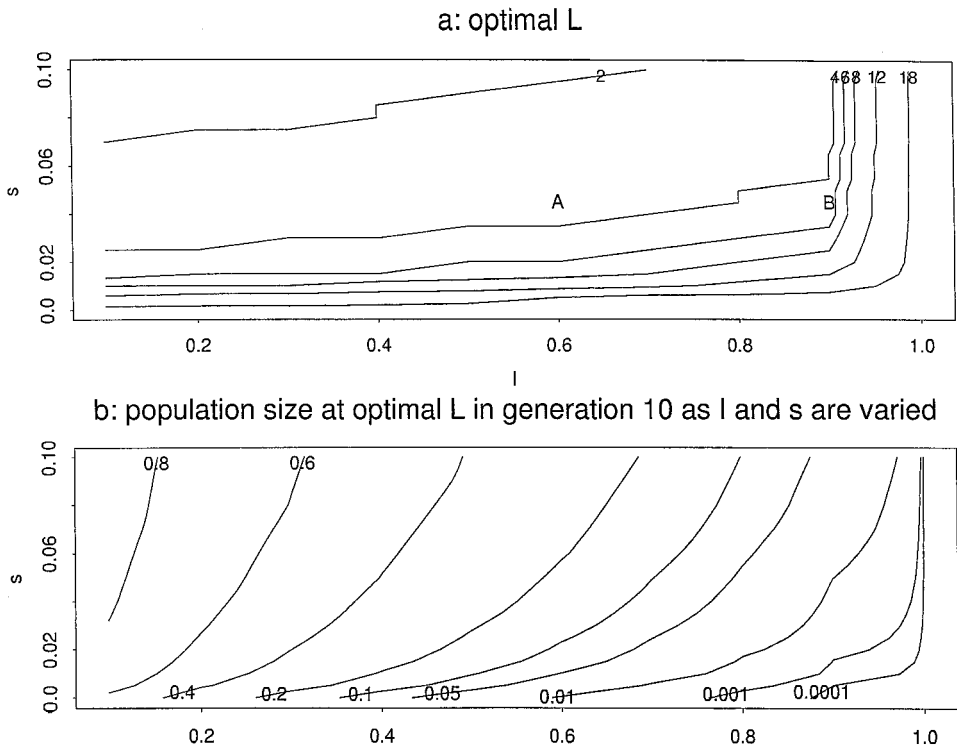


Fig. 5. Contour plots of the optimal L and the generation 10 population size at that L as functions of release proportion I and cost s of the FK alleles in males. I is the fraction of males that are released-type immediately after the single release. This is related to the ratio of released to wild insects by $N = I/[2(1 - I)]$, where N is the number of released insects to each wild insect.

of 14 and 17, the population is reduced to eradication levels within about eight generations. Fig. 7 shows a release with the same parameters except that the release population is of size such that the initial release is 2:1 ($I = 0.8$). For higher L , the multiple releases drive the population to eradication levels within five generations of releases.

The effect of $s > 0$ (Fig. 8) is predictable. We see once again that selection against the FK alleles overpowers the benefit of increasing L as L becomes large. However, we see that the optimal value of L is much larger. Recall that the optimal value of L increased with release size for a single release (see Fig. 5). The same effect is acting here. Because the release is of the same size each generation, it becomes very large relative to the target population as the target population is reduced. Selection is inefficient in later generations due to the low amount of genetic variation. Thus, it becomes better to use high values of L . The optimal value of L changes drastically depending on which generation we examine—8 in generation 6, 10 in generation 8, and 16 in generation 10 for this example. This reflects the rapidly decreasing size of the target population.

Table 1 is an attempt to summarize the effects of per capita growth rate R and selection against the FK allele. It shows the approximate minimum release size necessary to reduce the population to 1/100th of no-

release size for $L = 6$ by generation 10 for various values of s and R . Table 1 also shows the same information for SIT releases with sterile male fitness values of 1 and 0.5. Comparing the “ideal” FK release to the “ideal” SIT release (both with released insects with no fitness reduction), we see that the SIT release size necessary for reduction to 1/100th is in the range 4–7 times that for FK. FK is more favored for lower population growth rates. The number of insects required to achieve this reduction with a “realistic” (reduced fitness) sterile release is in the range of 4–9 times that necessary for a “realistic” ($s = 0.025$ – 0.05) FK release. Recall that a fitness of sterile males of 0.5 is the upper end of the range for the observed fitness values of sterilized males. Thus, this gives a conservative estimate of the relative effectiveness of FK releases. Note also that the FK release would be more favored for higher values of L .

Effect of Assumption of Equal Fitness Contribution Across Loci. Because the selection against the FK allele in males is due to random damage to the genome caused by the insertion of the FK alleles, the assumption of equal fitness contribution across loci that underlies the model is unrealistic. To explore that impact of this assumption, we have developed a model that allows two types of FK loci. We compare two releases: (1) a release with L_1 loci of fitness cost $s_1 = 0$ per allele and L_2 loci of cost $s_2 = 0.05$ per allele,

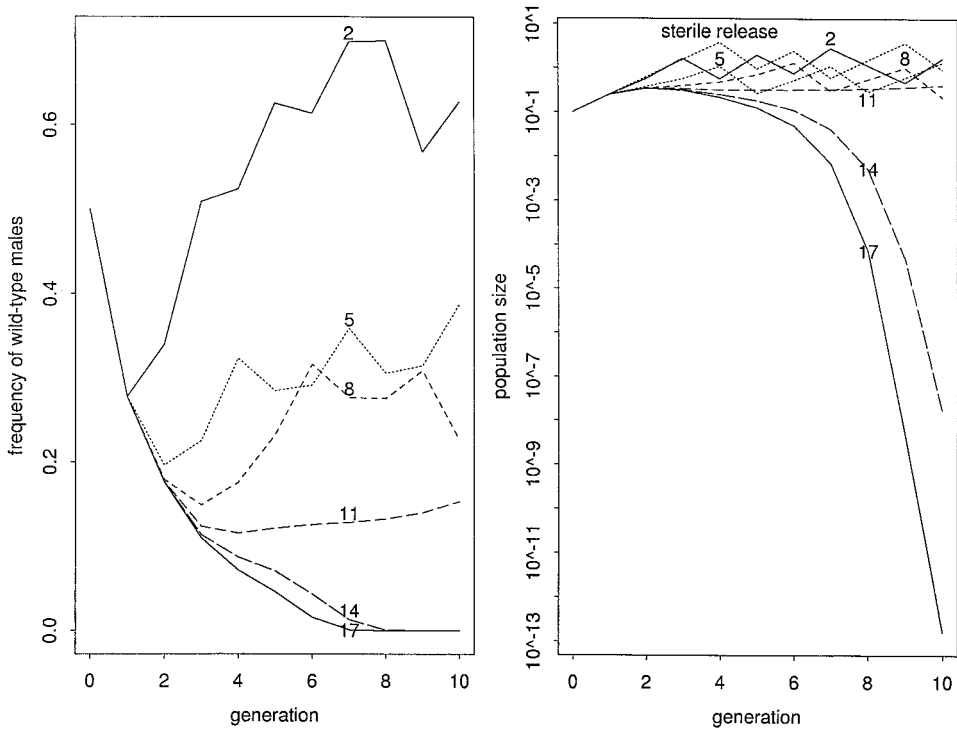


Fig. 6. Multiple releases with an initial release of size 1:2. A new release is made each generation. Releases are of constant absolute size. Because the size of the target population is changing, subsequent releases will be of different relative size. Population size graph is on a log scale. There is no fitness reduction in the released insects (FK-bearing or sterile).

and (2) a release with $L_1 + L_2$ loci with fitness cost $1 - \sqrt{(1 - s_1)(1 - s_2)}$. The cost function in (2) is midway between s_1 and s_2 and makes the all-FK genotype have the same fitness in both cases (see Schliekelman and Gould 2000). Thus, we test how well a release with two allele types is approximated by a release with one allele type of the average effect. Fig. 9 shows three such model runs with $L_1 = L_2 = 1$, $L_1 = L_2 = 3$, and $L_1 = L_2 = 6$. In each case, the two curves are almost indistinguishable. This indicates that the exact distribution of the fitness components among loci does not impact the results significantly, and thus that these results are not sensitive to the assumption of equal fitness contribution.

Discussion

How Effective is the Multilocus FK System at Reducing Pest Populations in the Ideal Case? Mass release strategies suffer from many complications, including migration of insects, poor timing of release, weather conditions, and a multitude of other factors. It is not possible to include all of these factors in modeling new genetic control strategies. We do, however, have decades of data on sterile male releases in a variety of field conditions. We can make comparisons between models of idealized sterile male releases and other genetic control strategies to get a sense of how these strategies will perform in the field.

Figures 2 and 3 show that if released insects are equal in competitiveness to wild ones (a considerable idealization) then multilocus FK releases are orders of magnitude more effective than sterile male releases. The population size in each generation is reduced by the fraction that the FK genotype males make up of the whole male population. In a SIT release, the population is reduced by the fraction that the sterile males make of the full male population in the generation of release. The sterile males are gone after the release generation and have no more effect. In an FK release, FK-bearing males remain for multiple generations. Thus, one multilocus FK release has the same effect as doing a sterile release each generation for 5–10 generations (albeit a smaller release each generation). In insects where a high L is possible (i.e., insects with many chromosomes or high recombination rates), we can conceive of reducing a target population to near eradication levels with a single release.

Populations that are strongly density-dependent and are capable of high growth rates can recover quickly from a single release. The population numbers stay low as long as the frequency of the FK allele is high, but can increase rapidly once the FK allele is gone. Either a large initial release or multiple smaller releases can be used to keep the FK allele frequency high long enough to eradicate the target population. We have shown here that SIT requires 4–7 times more insects than a 6 locus FK release in a multiple release

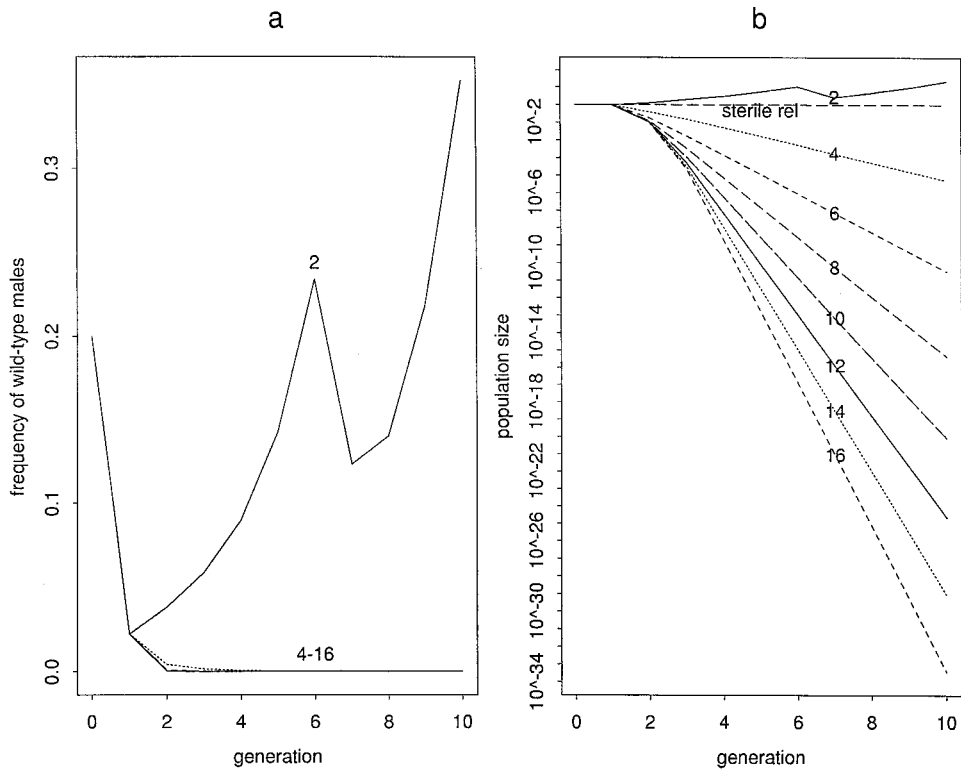


Fig. 7. Multiple releases with an initial release of size 2:1. A new release is made each generation. The releases are of constant absolute size. Because the size of the target population is changing, subsequent releases will be of different relative size. The population size graph is on a log scale. The sterile release population size is constant because the population growth is sufficient to exceed the spraying threshold each generation. Thus, the population is reduced to the same level at the time of the population census each generation.

scheme. The differential would be even greater if geneticists are eventually able to produce insects with less than the 2.5–5% per FK allele genetic load that we assume here.

One important issue that we have ignored is that of gene silencing. Gene silencing occurs when individuals carrying multiple copies of a gene do not express the trait because of interference in the transcriptional or posttranscriptional process. We assume that this does not occur. See Schliekelman and Gould (2000) for a discussion of this issue.

How Do Reductions in Fitness Due To Insertion of the FK Alleles Impact the Effectiveness of These Techniques? In comparing SIT to control strategies using genetically engineered insects, it is useful to separate fitness reductions due to genetic manipulation from fitness reductions due to laboratory-rearing. Undesired selection by laboratory conditions is likely to be an inescapable feature of mass-release strategies. However, damage to the insect’s genome caused by the insertion of new alleles is, at least in principle, under the control of the geneticist. It may be possible to minimize such damage by improving techniques or by screening many insertion events for those with the least fitness cost.

The fitness reduction in SIT caused by irradiation (the “genetic manipulation” component) alone is on the order of 50–80% (see references in the introduction). This percentage translates directly to the reduction in effectiveness of SIT. The picture for other genetic control techniques is more complicated, because the fitness reduction depends on the number of insertions made into the insect’s genome and because the selection occurs over multiple generations instead of just one.

Figure 5 shows that the surviving population increases rapidly as the cost s of the FK alleles increases. Increasing s from 0 to 0.01 makes an order of magnitude difference in the size of the surviving population. The steep gradient in s near $s = 0$ in Fig. 5b indicates that it will be necessary to get s very low (under 0.01) to approach the “ideal” release in efficiency. This sensitivity decreases as s increases.

The results of Mackay et al. (1992) quoted in the methods section indicate that a value of 0.05 for s should be attainable. A single 2:1 ($I = 0.8$) release with $s = 0.05$ reduces the target population to 5% of the no-release size, compared with 33% for a “realistic” sterile male release with 50% fitness of sterile males. Even if geneticists are never able to do better than this,

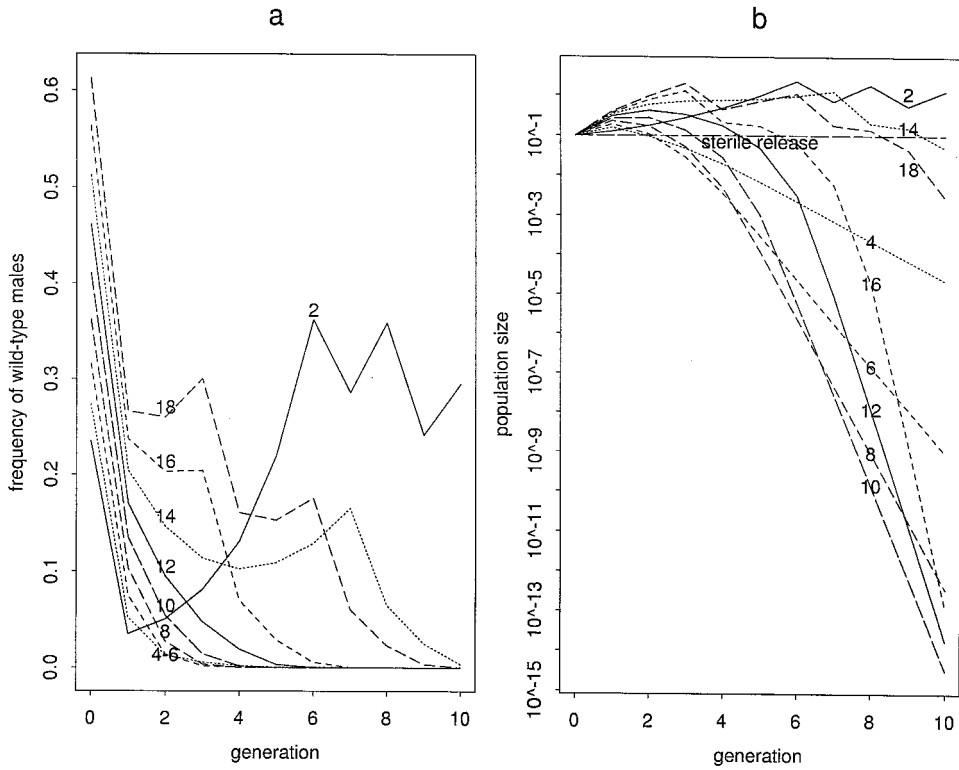


Fig. 8. Multiple releases with fitness cost to the FK alleles. Releases of equal absolute size are made each generation such that the initial release is size 2:1. FK allele carries a 5% fitness cost in males.

the multilocus FK technique is much more efficient than SIT.

The impact of FK allele fitness reductions is reduced as the release size increases. If FK releases are done on the same scale as SIT releases (which are often of sizes 100+ :1), the genetic load associated with the FK alleles would not be important. This is evident in the results for multiple releases shown here. Assuming that the FK allele frequency reaches the "eradication threshold," releases with larger values of *L* are always superior in the long run. As the release size increases, the relative advantage of FK releases over SIT releases increases.

Table 1. Size of release needed to reduce the population to 1/100th of no-release size if a release is made once per generation

	FK release with <i>L</i> = 6			Sterile release	
	<i>s</i> = 0	<i>s</i> = 0.025	<i>s</i> = 0.05	st. fitness = 1	st. fitness = 0.5
<i>R</i> = 4	0.44:1	0.57:1	0.76:1	2.7:1	5.4:1
<i>R</i> = 6	0.93:1	1.2:1	1.6:1	4.3:1	8.6:1
<i>R</i> = 8	1.42:1	1.9:1	2.6:1	6.0:1	11:1
<i>R</i> = 10	2.0:1	2.7:1	3.7:1	7.4:1	15:1

The left-hand column shows the per-capita growth rate *R* of the population. Columns correspond to the fitness cost *s* to the FK allele or the fitness of the sterilized insects. Ratio given is the ratio released: wild insects needed just after the release to reduce the population to 1/100th of the no-release size by the *F*₁₀ generation.

Because all insects of the *F*₁ generation and beyond have a wild type mother, any deleterious recessive traits that have become widespread in the released population due to lab-rearing conditions will not be expressed. Thus, the impact of such traits will be identical between SIT and multilocus FK. The apparent prevalence of such traits in lab-reared populations makes this a crucial advantage of FK releases over conditional lethal (CL) releases and other genetic control strategies for which it is possible for both parents to be descendants of released insects.

The processes for producing insects for SIT releases have generally favored quantity over quality. Thus, SIT releases have depended on "overflooding" the target population with masses of insects. Because FK releases would require far fewer insects, it might be beneficial to develop production systems that emphasize the quality of the insects.

What is the Optimal Number of Loci to Use in the FK Methods? The optimal value of *L* is highly variable, particularly for low *s* and high *I*. It is in the range 3-7 for intermediate values of *s* (i.e., 0.02-0.05) and intermediate release size. It increases quickly for *s* < 0.02, becoming infinite as *s* goes to zero. Optimal *L* also is high (>15) for very large releases (i.e., on the scale that SIT releases are often done). For multiple releases of equal size, the optimal *L* will increase as the target population is reduced in size by successive re-

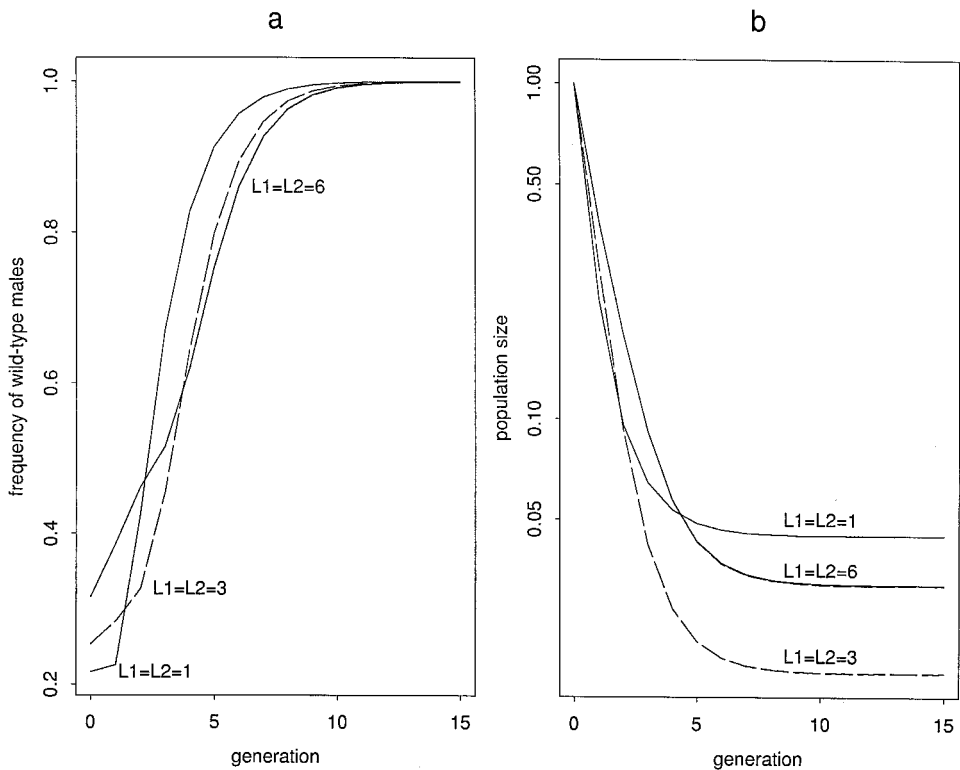


Fig. 9. Effect of assumption of equal fitness cost across loci. Each curve in the figure is actually two overlapping curves: one showing a simulation run for which the L_1+L_2 loci all have $s = 1 - \sqrt{0.95} = 0.0253$, and one for which L_1 loci have $s = 0$ and L_2 loci have $s = 0.05$ (see text for explanation). The overlapping curves are indistinguishable, but not identical.

leases. It would be optimal to release insects with more copies of the FK allele as time goes on. The first release (when the population had the maximum variation and selection is most efficient) would use insects with a few insertions that have little effect on fitness, whereas later releases (when variation is reduced and selection is less efficient) could have increased numbers of FK alleles. If a single L is to be chosen, then it is best to go with higher values.

How do Multilocus FK Releases Compare in Effectiveness to Multilocus Conditional Lethal Releases? In a previous article (Schliekelman and Gould 2000), we modeled the release of insects carrying a conditional lethal trait on multiple loci. Comparisons with that work show that conditional lethal releases are an order of magnitude more effective in the ideal case and moderately more effective with $s > 0$, if there are sufficient generations for the conditional lethal allele to spread before it is activated. For example, a 2:1 ($I = 0.8$) conditional lethal release with $s = 0.0$ and the allele activated in the F_4 generation reduces the target population to 0.002% of no-release size. The same release with $s = 0.05$ reduces the population to 1% of no-release size (Schliekelman and Gould 2000). However, the effectiveness of conditional lethal releases is sensitive to the number of generations available before the conditional lethal allele is activated. FK re-

leases have no such constraints and are more robust. However, FK releases are more sensitive to population dynamics. Until the conditional lethal allele activates, CL-carrying insects undergo the same population dynamics as the wild-type insects. Within-season population dynamics thus have little impact on the spread of the conditional lethal allele. The population dynamics only play a role after the conditional lethal allele has become lethal.

In conclusion, the work of Thomas et al. (2000) and others (e.g., Handler et al. 1998) shows that more effective autocidal control strategies are on the horizon. The key uncertainty is the degree of success that molecular geneticists will have at inserting the desired alleles into insect genomes. We have shown in this and the previous paper (Schliekelman and Gould 2000) that the effectiveness of these control strategies increases dramatically as the number of loci is increased, but can decrease dramatically as the fitness cost associated with the loci increases. It may well turn out that the difficulties of inserting alleles on multiple loci while maintaining high fitness are too great, and the potential of these control strategies is not realized. However, if molecular geneticists are able to develop techniques to produce strains with multiple insertions of the desired alleles at low fitness cost, then autocidal control strategies may be very powerful. Results of this

theoretical analysis suggest that confined greenhouse experiments must be performed on each single insertion line to estimate impacts of each insertion on male fitness. Realistic environmental conditions must be used in such experiments. Once a tentative set of FK insertion lines is selected, pedigree crosses can be used to produce lines with multiple insertions. These multiple insertion lines will also need to be tested to make sure that there are no epistatic interactions causing excess decrease in male fitness. Although fitness testing will require a significant investment of time and money, it could have a major impact on the efficacy of this autocidal control technique.

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Appendix 1. Solution of Iteration Equations

Applying equation 6 gives the system

$$\begin{pmatrix} F_{t+1}(0) \\ F_{t+1}(1) \\ F_{t+1}(2) \\ \vdots \\ F_{t+1}(L-1) \\ F_{t+1}(L) \end{pmatrix} = \begin{bmatrix} 1 & \frac{f}{2\bar{w}_t} & \frac{f^2}{4\bar{w}_t} & \frac{f^3}{8\bar{w}_t} & \bullet & \frac{\binom{n}{0} f^n}{2^n \bar{w}_t} & \bullet & \frac{1f^L}{2^L \bar{w}_t} \\ 0 & \frac{f}{2\bar{w}_t} & \frac{f^2}{2\bar{w}_t} & \frac{3f^3}{8\bar{w}_t} & \bullet & \frac{\binom{n}{1} f^n}{2^n \bar{w}_t} & \bullet & \frac{\binom{L}{1} f^L}{2^L \bar{w}_t} \\ 0 & 0 & \frac{f^2}{4\bar{w}_t} & \frac{3f^3}{8\bar{w}_t} & \bullet & \bullet & \bullet & \frac{\binom{L}{2} f^L}{2^L \bar{w}_t} \\ 0 & 0 & 0 & \frac{f^3}{8\bar{w}_t} & \bullet & \bullet & \bullet & \bullet \\ 0 & 0 & 0 & 0 & \bullet & \bullet & \bullet & \bullet \\ 0 & 0 & 0 & 0 & 0 & \frac{\binom{n}{n} f^n}{2^n \bar{w}_t} & \bullet & \bullet \\ \bullet & \bullet & \bullet & \bullet & \bullet & \bullet & \bullet & \bullet \\ 0 & 0 & 0 & 0 & 0 & 0 & \bullet & \frac{f^L}{2^L \bar{w}_t} \end{bmatrix} \begin{pmatrix} F_t(0) \\ F_t(1) \\ F_t(2) \\ \vdots \\ F_t(L-1) \\ F_t(L) \end{pmatrix} \tag{A.10}$$

This system is linear if there is no selection in males (then $f = 1$ and $\bar{w}_t = 1$). With selection, we have

$$\vec{F}_{t+1} = \frac{A}{\bar{w}_t} \vec{F}_t = \frac{A^2}{\bar{w}_t \bar{w}_{t-1}} \vec{F}_{t-1} = \dots = \frac{A^t}{\prod_{i=0}^{t-1} \bar{w}_i} \vec{F}_0 \quad [\text{A.11}]$$

$$\left(\prod_{i=0}^t \bar{w}_i \right) \vec{F}_{t+1} = A^t \vec{F}_0 \quad [\text{A.12}]$$

where A is the matrix in equation A.10. The right hand side of this equation is linear. Thus, we can easily calculate the quantity $(\prod_{i=0}^t \bar{w}_i) \vec{F}_{t+1}$. Because the term $\prod_{i=0}^t \bar{w}_i$ multiplies each component, the ratios of components the left hand side of are the same as the ratios of the components of \vec{F}_t equation A.12. Thus, in particular,

$$F_i(Z) = \frac{\left(\prod_{i=0}^{t-1} \bar{w}_i \right) F_t(Z)}{\sum_{X=0}^L \left(\left(\prod_{i=0}^{t-1} \bar{w}_i \right) F_t(X) \right)} \quad [\text{A.13}]$$

We can solve equation A.12 using standard techniques for linear systems. Applying the initial condition

$$\begin{pmatrix} 1 - I \\ 0 \\ \bullet \\ \bullet \\ 0 \\ I f^{2L} \end{pmatrix} \quad [\text{A.14}]$$

in the F_1 generation (these equations are not valid in the release generation because all individuals are not offspring of no-FK mothers) and proceeding in the standard way, we get solutions of the form

$$\prod_{i=0}^t \bar{w}_i F_t(0) = 1 - I + I f^{2L} (-1)^L \left(\frac{f}{2-f} \right)^L \cdot \left(1 - \sum_{X=1}^L (-1)^{X-1} \binom{L}{X} \left(\frac{f^X}{2^X} \right)^t \right) \quad [\text{A.15}]$$

$$\prod_{i=0}^t \bar{w}_i F_t(Z > 0) = I f^{2L} (-1)^{L-1} \left(\frac{f}{2-f} \right)^{L-Z} \binom{L}{Z} \cdot \sum_{X=Z}^L (-1)^{X-1} \binom{L-Z}{X-Z} \left(\frac{f^X}{2^X} \right)^t \quad [\text{A.16}]$$