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Source: Journal of Medical Entomology, 49(6):1177-1188. 2012.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/ME11205

FORUM

Mathematical Models as Aids for Design and Development of Experiments: The Case of Transgenic Mosquitoes

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J. Med. Entomol. 49(6): 1177–1188 (2012); DOI: http://dx.doi.org/10.1603/ME11205

ABSTRACT We demonstrate the utility of models as aids in the design and development of experiments aimed at measuring the effects of proposed vector population control strategies. We describe the exploration of a stochastic, age-structured model that simulates field cage experiments that test the ability of a female-killing strain of the mosquito *Aedes aegypti* (L.) to suppress a wild-type population. Model output predicts that choices of release ratio and population size can impact mean extinction time and variability in extinction time among experiments. We find that unless fitness costs are >80% they will not be detectable in experiments with high release ratios. At lower release ratios, the predicted length of the experiment increases significantly for fitness costs >20%. Experiments with small populations may more accurately reflect field conditions, but extinction can occur even in the absence of a functional female-killing construct because of stochastic effects. We illustrate how the model can be used to explore experimental designs that aim to study the impact of density dependence and immigration; predictions indicate that cage population eradication may not always be obtainable in an operationally realistic time frame. We propose a method to predict the extinction time of a cage population based on the rate of population reduction with the goal of shortening the duration of the experiment. We discuss the model as a tool for exploring and assessing the utility of a wider range of scenarios than would be feasible to test experimentally because of financial and temporal restraints.

KEY WORDS *Aedes aegypti*, transgenic female-killing, field cage experiment, experimental design, mathematical model

In entomology, mathematical models have often been used as tools in Integrated Pest Management and Insecticide Resistance Management (Ruesink 1976, Worner 1991, Gould 2010). In this context, they are generally designed for predicting the spatial and temporal population genetics and dynamics of insect pests in the field environment (Stinner et al. 1983, Mayer et al. 1995). Although it is easy to point out the potential for inaccuracies and artifacts in the predictions from these dynamical models, they are generally relied upon when empirical approaches for prediction are too expensive, not operationally feasible, or prohibited for regulatory or ethical reasons. For example, simulation models have been used by the United States Environmental Protection Agency (US EPA) to develop resistance management strategies for transgenic insecticidal crops (US EPA 1998, 2001).

Experimental design is often guided by statistical considerations; for instance, power calculations can guide choice of sample size. Such familiar calculations are based on simple statistical models (e.g., regression or analysis of variance [ANOVA]), but it is less widely appreciated that dynamical models can also be used to assess and improve the design of experiments (Curtis et al. 1976a, b). In this context, models that include the essential parameters in an experiment can be used to tailor the experiment to answer the specific questions of most interest and to predict the limitations of an experiment before resources are invested. Here we demonstrate the application of simulation models for this purpose by describing a model developed to aid field cage testing of a transgenic strain of *Aedes aegypti* (L.), the major mosquito vector of dengue virus (Rosen et al. 1985).

Current strategies for dengue prevention rely on suppression or elimination of local *Ae. aegypti* populations (Gubler 1998, Morrison et al. 2008). There is no licensed, commercially available dengue vaccine, and antiviral drugs are not expected to be used prophylactically (Scott and Morrison 2008, 2010). When implemented properly, mosquito control effectively prevents dengue (Morrison et al. 2008). Unfortunately,

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0022-2585/12/1177–1188$04.00/0

successful dengue vector control programs are the exception and where they do exist they are difficult to sustain. The urgent need to prevent the growing dengue public health problem has led to exploration of novel vector control approaches, including genetic strategies (Gald et al. 2006), among which are methodologies based on the concept of the classical Sterile Insect Release method (Knipling 1955). In this modern genetic approach, mosquito strains are transgenically manipulated so that either all offspring or just the female offspring with a specific transgene will die under field conditions, but can be successfully reared in production facilities where tetracycline is added to the larval diet. Recent publications provide detailed descriptions of this pest control tactic and the molecular genetic methods used for strain development (see Heinrich and Scott 2000; Thomas et al. 2000; Alphey et al. 2008, 2010; Fu et al. 2010).

In our study, we are specifically interested in the utility of female-killing (FK) strains of *Ae. aegypti.* In such strains, female offspring that inherit the FK transgene are unable to develop properly and die before pupation or are effectively removed from the population upon emergence because they cannot find mates or take bloodmeals. One such strain has been developed in which adult female *Ae. aegypti* that inherit the transgene are incapable of flight and therefore incapable of reproducing or obtaining a bloodmeal and transmitting virus (Fu et al. 2010). This effectively lethal flightless condition is intended to affect only females as a result of specific promoter sequences within the transgenic construct. The transgene that incapacitates female flight muscles is only turned on when tetracycline is absent from the diet. Although this success in developing a FK strain is promising, a series of tests must be conducted to ensure safety and effectiveness before genetically modified insects can be released (Benedict et al. 2008).

Results from laboratory cage trials indicate that a wild-type population can be suppressed successfully with regular introduction of FK individuals at a large release ratio (Wise de Valdez et al. 2011). Recently, large field cage trials in Tapachula, Mexico, were conducted to study the ability of these FK individuals to suppress a wild population in a more environmentally natural setting (L. F. et al., unpublished data). The laboratory and field cage trials used a similar experimental design that was aimed at testing whether releases of FK males into a target population could cause extinction under idealized release conditions. To quantitatively assess these experiments and determine what can be learned from their outcomes, we develop a stochastic model that incorporates the biological details of the experiments and enables a quantitative assessment of what can and cannot be learned from the results of these cage trials.

We modify the model and use it to consider a number of other experimental designs and related experiments with the goal of illustrating the model's utility in assisting in the effective design of future cage studies of FK strains in which researchers seek to understand the impacts of mating fitness costs, density-dependent population regulation (Southwood et al. 1972, Legros et al. 2009), and immigration of wild-type individuals into the population being controlled (Dietz 1976, Prout 1978, Barclay 2005). While this model could also be used to conduct a detailed assessment of previous and ongoing experiments as well as to explore alternative experimental designs with rigor, we do not pursue either avenue here.

In the following sections of this article, we 1) describe the protocols used in the cage trials, 2) describe the characteristics of the mathematical model built to simulate the population dynamics and population genetics of the cage trials, 3) present results of model simulations, and 4) discuss the specific and broader implications of the model results.

The Experimental Protocol

The FK laboratory and field cage trials use a design in which sets of control and treatment populations are maintained in separate cages throughout the experiment. Control populations consist solely of wild-type mosquitoes, provide baseline information on dynamics of a caged population of *Ae. aegypti*, and are used to calculate input of FK mosquitoes into treatment populations (see equation 1). Treatment populations consist of both wild-type and FK individuals. To reduce the impact of environmental influences on cage dynamics, a treatment population is paired with a control population with similar environmental conditions.

In both cages, wild-type populations are established and allowed to stabilize; that is, reach a stable adult population density. Eggs laid in the cage are collected twice per week, counted, and hatched in a laboratory. At the beginning of each week a specific number, N, of second-instar larvae that hatch from collected eggs is returned to the cage from which they were collected. This is done to maintain a stable adult population size. Larvae are provided with adequate resources to ensure that larval survival and development are not density-dependent. Using this method of population maintenance, a stabilization period of $\approx$13 wk is needed (Wise de Valdez et al. 2011).

In the experiments, once both control and treatment populations are stabilized, the treatment phase of the experiment begins. Each week, in addition to the return of larvae hatched from eggs collected in the cage, rN homozygous FK pupae are introduced into the treatment cage (pupae are introduced rather than larvae for operational reasons). Here, r is the initial release ratio of homozygous FK pupae to wild-type larvae. For example, if a 10:1 initial ratio of homozygous FK pupae to wild-type larvae is desired ($r = 10$), then the number of FK pupae placed into the treatment cage each week is $10N$. (Note that because only male FK mosquito adults are expected to survive, the number of male mosquitoes released each week, assuming a 1:1 sex ratio, is approximately $0.5rN$.) The control cage continues to receive only larvae hatched from eggs collected in the cage.

If FK releases have the desired effect of reducing population size, the number of eggs laid in the treatment cage will decline over time, and so the number of larvae returned to the treatment cage is adjusted to reflect this. This is achieved by setting the input into the treatment cage at the beginning of week w, N_w, equal to the ratio of eggs laid in the treatment and control cages in the previous week (E^{T}_{w-1} and E^{C}_{w-1}, respectively) multiplied by the fixed input into the control cage. That is,

$$N_w = N\frac{E^{\mathrm{T}}_{w-1}}{E^{\mathrm{C}}_{w-1}}. \quad [1]$$

Altering the input into the treatment cage in this manner ensures that the input is directly proportional to the numbers of eggs laid each week, and that population dynamics are not density-dependent.

In the experiments, once mating occurs between FK males and wild-type females, larvae that hatch from the eggs are screened for a physical marker indicating that they bear the FK genetic construct so that the frequency of the FK gene can be monitored. The larvae that are returned to the cage each week are hatched from a random sample of the eggs that are laid in the cage so that the distribution of genotypes of the larvae is expected to reflect that of the eggs laid. This process of input, removal, and screening continues until no eggs are produced in the treatment cage for two consecutive weeks, at which point the population is declared extinct.

Description of the Model

We use a discrete time stochastic model that links population genetics and population dynamics of caged *Ae. aegypti* populations and study expected extinction times and variation of extinction times that result from different experimental designs. For a detailed description of the mathematical details of the model, see Supp. Material 1 (online only). We track integer numbers of the population each day subdivided by age, sex, and genotype. $M_{g,a,d}$ is the number of adult males of genotype g and age a on day d, $F_{a,d}$ is the number of adult females of age a on day d, and $E_{g,d}$ is the number of eggs laid of genotype g on day d. There are three possible genotypes: wild-type ($g = 1$), heterozygous FK ($g = 2$), and homozygous FK ($g = 3$); the only individuals of this latter genotype are the released males. Note that only wild-type females will be viable, so multiple adult female genotypes are not tracked.

Emergence and Survival. Individuals in each larval cohort emerge as adults over several days according to the probability distribution presented in Fig. 1 (L. F., L. V., J. R., and T.W.S., unpublished data). Figure 1 shows the emergence distribution for male larvae. We assume that females emerge 1 d later than males (Christophers 1960, Craig 1967), so female emergence is given by an identical distribution that is shifted by 1 d compared with that of males. Pupae emerge within the first 2 d of being placed into the cage, with a 32% chance of emerging the day after placement, a 65% chance of emerging 2 d after placement, and a 3% chance of dying before emergence (Rueda et al. 1990).

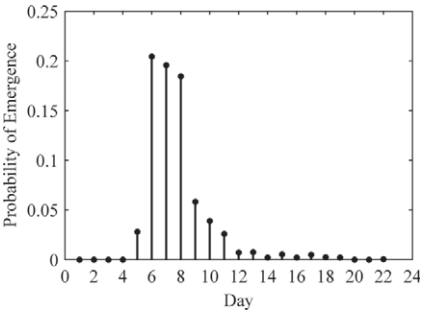

Fig. 1. Probability distribution of adult male emergence times when second-instar larvae are seeded in containers in semifield conditions. The horizontal axis is the number of days after second-instar larvae are placed in the cage on day 0, and the vertical axis is the probability that males will emerge on the day given on the horizontal axis. In this study, female emergence time distribution has the same shape, but is shifted so that female emergence occurs 1 d later than would male emergence. Note that the probability of mortality before emergence is 0.2318 (L. F., L. V., J. R., and T.W.S., unpublished data.).

When mosquitoes are adults, the number that survives from one day to the next is determined by a sex-dependent daily survival probability: s_m for males and s_f for females. In the results presented below, we set $s_m = 0.72$ and $s_f = 0.9$ for all age cohorts (Muir and Kay 2007, Foque et al. 2006). In the Supp. Material 2 (online only), we explore variations in survival parameters (including age-dependent survival patterns), and we show that the effects of age-dependent survival are minimal unless the average lifespan is much longer than assumed here. Knowledge of the lifespan distribution is important in making predictions with this model (see Figs. S1 and S2 in Supp. Material 2 [online only]).

Mating and Reproduction. We assume that adult males begin mating 2 d after emergence and that females mate 1 d after emergence with only one mate and do not mate again (Christophers 1960), although there is evidence that polyandrous mating occurs at a low rate (Williams and Berger 1980, Young and Downe 1982). A mating pair distribution (i.e., the distribution of mate genotypes) is determined for each female cohort on the day that it mates; this distribution determines the genotypes of the offspring of that cohort. In the Supp. Material 2 (online only), we further explore the effects that mating age and polyandrous mating might have on cage experiments.

We assume that FK males can incur a fitness disadvantage in the form of reduced mating competitiveness. While fitness disadvantages could also manifest themselves through a reduced number of offspring or survival disadvantages, we feel that studying fitness disadvantages only through reduced mating competitiveness captures the most likely effects of fitness disadvantages for this system. We assume that the mating fitness cost, if incurred by FK males, is additive, and denote this fitness cost by c. We define

the mating fitness of each genotype as $\Phi_1 = 1$, $\Phi_2 = 1 - \frac{c}{2}$, and $\Phi_3 = 1 - c$. Hence on day d a female chooses a male of genotype k with probability $p_{k,d}$, where

$$p_{k,d} = \frac{\Sigma_a \Phi_k M_{a,k,d}}{\Sigma_a \Sigma_{k'} \Phi_{k'} M_{a,k',d}}. \quad [2]$$

We assume that females begin laying eggs 5 d after they mate and continue to lay eggs until their death. We assume that the daily number of eggs laid by a female mosquito is independent of age. (Age-dependent fecundity, which reflects changes in daily fecundity related to gonotrophic cycles, is explored in the Supp. Material 2 [online only]). We assume the number of eggs laid by a cohort of age a each day follows a Poisson distribution with mean $\lambda \cdot F_{a,d}$ where λ is the mean number of eggs that an individual female lays each day. Throughout this analysis, we set $\lambda = 10$ (Harrington et al. 2001, Styer et al. 2007). The genotypes of the offspring in eggs laid by each cohort on a given day are determined by the distribution of genotypes of the mating pairs formed on the day the cohort mated and the Mendelian probability, $P_{g,k}$, that an offspring of genotype g results from the mating of a wild-type female and male of genotype k. That is, the probability that an offspring produced by a female who mated on day d is of genotype g is $v_{g,d} = \Sigma_k p_{k,d} P_{g,k}$.

The distribution of genotypes among eggs laid is then used to determine the distribution of the genotypes of larvae input on a weekly basis as described in the experimental protocol. In the model, we select the number of larvae of each genotype by sampling from a multinomial distribution.

Density Dependence. Density regulation of *Ae. aegypti* populations is a complicated process that is not yet well understood. Changes in population density can affect larval development time, larval survival, and adult size, and the impacts on these aspects of growth and development can depend upon factors such as container size, food availability, and temperature (Barbosa et al. 1972, Gilpin and McClelland 1979, Agnew et al. 2002, Braks et al. 2004, Padmanabha et al. 2011). While the impacts of all of these aspects of density dependence on cage experiments are important to understand, the study of most of them would require drastic alterations of the current experimental protocol. Here, we focus on investigating the influence of density dependence on larval survival because such a study can be done using a simple variant of the current experimental protocol.

In this analysis, all aspects of the current design are maintained, with two exceptions. We consider the return of pupae rather than larvae to consider density-dependent regulation that occurs during the larval stages, and we put pupae into the cages according to the following function, adapted from Bellows (1981):

$$N_w(E_{w-1}) = \gamma E_{w-1} e^{-\alpha E_{w-1}^{\beta}}. \quad [3]$$

$N_w(E_{w\text{-}1})$ is the number of input pupae returned to the cage each week, determined as a function of the number of eggs, $E_{w\text{-}1}$, collected during the previous week. Parameters α, β, and γ determine the functional description of density-dependent larval survival. Although the parameter space available for exploration is vast, we focus on three parameter sets to provide a brief illustration of one way in which density dependence can be studied in the cage experiments. We choose these parameter sets because they give rise to three different descriptions of density dependence (see Fig. 2; values of the parameters α, β, and γ are given in the figure caption). In the absence of FK individuals, all three input curves give rise to an equilibrium at approximately $E_w^* = 4025$, which corresponds to the average weekly egg production in density-independent cage populations where the weekly pupal input is $N = 200$. The three forms differ primarily by location of the maximum relative to this equilibrium. Form 1 has a maximum near the equilibrium, so the number of pupae returned does not differ much when the number of eggs collected is near the equilibrium value. The maximum input for Form 2 results from a number of eggs being laid that is well beyond what is expected to be observed in cages. Pupal input for populations regulated by this form of density dependence can increase when the number of eggs collected surpasses the equilibrium value. The maximum value of Form 3 occurs below the equilibrium, so the number of pupae returned generally decreases when the number of eggs collected increases; however, as the number of eggs collected decreases from the equilibrium, the number of pupae returned first increases, but then decreases again as the number of eggs collected gets closer to zero. We assume that during a field release, the homozygous FK individuals produced for release will be provided with adequate re-

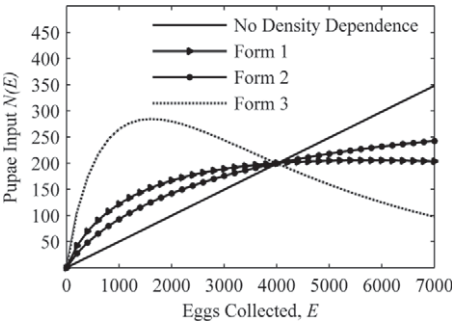

Fig. 2. Functional forms for density-dependent survival. These forms determine the number of pupae placed into the cage each week in terms of the number of eggs collected in the previous week, mimicking density-dependent larval survival. For the function $N_w(E_{w-1}) = \gamma E_{w-1} e^{-\alpha E_{w-1}^{\beta}}$, Form 1 (triangles): $\alpha = 0.0522$, $\beta = 0.4370$, and $\gamma = 0.3540$; Form 2 (circles): $\alpha = 0.0516$, $\beta = 0.4021$, and $\gamma = 0.2125$; and Form 3 (dotted): $\alpha = 0.0089$, $\beta = 0.6896$, and $\gamma = 0.7516$. The solid line represents input in the absence of density-dependent survival. Because a population resulting from regular input of 200 larvae will produce, on average, 4,025 eggs in a week, populations resulting from each of the four input types have an equilibrium point at $E = 4025$, $N(E) = 200$.

sources, so their survival is not regulated by density dependence in the model.

Immigration. We explore another variant of the current experimental protocol that could be used to study the potential effect of immigration of wild-type individuals into a population that is being controlled by releases of FK individuals. To study introduction of larvae that could occur as a result of movement of containers, we introduce additional wild-type larvae into both cages each week. These introductions occur for the duration of the experiment, including the stabilization period. In the Supp. Material 2 (online only), we consider the immigration of newly emerged, unmated adults and 3-d-old, mated adult females and mating males.

Simulations. We simulate a number of different experimental designs by varying the baseline wild-type input, N, and release ratio, r. We study the impacts that these experimental designs have on the wild-type population under various scenarios (e.g., considering fitness costs, immigration, and density dependence) by observing population decline and extinction. We focus primarily on mean time to extinction and variation in extinction times, which allows us to predict the range of total experiment times (i.e., stabilization period + time to extinction) that could result from different experimental designs. Throughout the results and discussion, we present the extinction time as the number of days until the treatment population reaches extinction following the initial FK release. Under some scenarios (e.g., with density dependence and immigration), population extinction does not always occur. In those cases, we observe reduction in population density as measured by the percentage of wild-type adult females remaining a given number of weeks after the initial FK release relative to the number of wild-type adult females present the day before releases begin. For illustrative purposes, we choose to measure the reduction 14 wk after the initial release (14 wk postrelease).

Note that variability in our results is due only to the components outlined in the model description. There are a number of other potential sources of variability that we do not consider, such as that caused by environmental factors or individual-based variability in survival, mating, or reproduction. Unless otherwise stated, for each parameter set, we run 1,000 simulations for a maximum of 1,000 d each.

Results

Treatment cage dynamics from one simulation are shown in Fig. 3. The population in each cage was stabilized before releases were started, and population numbers oscillated with a period of 7 d, which reflects the weekly release schedule. Females reached higher pre-FK release densities because they had a higher probability of survival than males. About 3 wk after the first introduction of FK individuals, adult males heterozygous for FK began to appear, which indicated successful matings between homozygous FK males and wild-type females. At the same time, the population of wild-type adults in the treatment cage began to decline, which followed the decline in the number of wild-type eggs. Comparison of the model output to data from Wise de Valdez et al. (2011) shows that the model captured the general dynamics observed in experiments (Fig. S1 [online only]); see the Supp. Material 2 (online only) for further discussion.

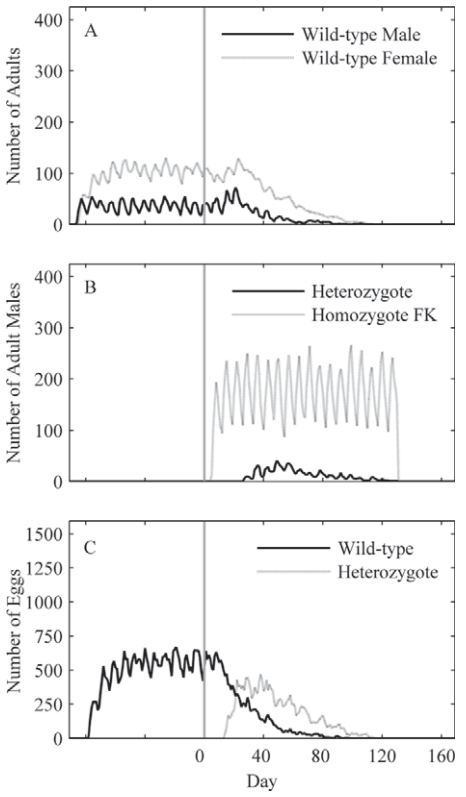

Fig. 3. Treatment cage dynamics of the stabilization and postrelease period obtained from a single simulation. (A) The number of wild type adults, (B) the numbers of heterozygous and homozygous FK males, and (C) the number of eggs laid. Here, $N = 200$, $r = 5$, $\lambda = 10$, $c = 0$, $s_m = 0.72$, $s_f = 0.9$. The releases of FK individuals begin at the time marked by the vertical gray line.

While assessing the results presented here, it is important to remember two components of the experimental design that influence the extinction time in all of the results. First, heterozygote adults typically began appearing 3 wk after the initial input of FK males (see Fig. 3). This indicates that the presence of FK males began impacting the adult population about 3 wk after the initial release. Second, extinction time is defined as in the experiments by Wise de Valdez et al. (2011): A population is considered to be extinct when no eggs have been laid for two consecutive weeks. With the influence of these two components, the very minimum extinction time will be >35 d postrelease.

Baseline Wild-Type Input. We varied the baseline wild-type input, N, over a wide range of values. We found that as N increased, the mean time to extinction

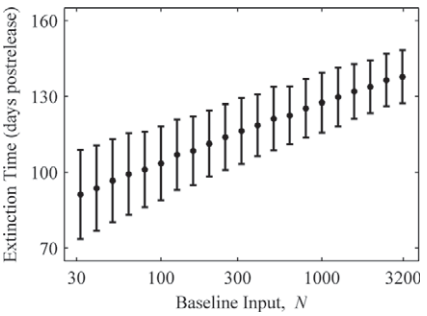

Fig. 4. Extinction time (postrelease) for different values of baseline input, *N*. Circles represent mean time to extinction and error bars represent mean ± SD. Here, $r = 10$, $\lambda = 10$, $c = 0$, $s_m = 0.72$, $s_f = 0.9$. Note the horizontal axis is on a log scale.

increased gradually, and the variance generally decreased (Fig. 4). Even though mean extinction time did increase with larger values of *N*, the difference from $N = 100$ to $N = 1{,}000$ was only ≈24 d on average with a 3 d difference in standard deviation. An increase in *N* by 100 individuals did not cause more than a few days change in average extinction time when $N \geq 400$, but decreasing *N* from high values to low values (e.g., from 1,500 to 50) did lead to a reduction of a few weeks in extinction time. We note, however, that small baseline input may lead to populations being incapable of persisting in the absence of FK introductions. We return to this point in a subsequent subsection of the results.

Release Ratio. Figure 5 shows the effects of release ratio of FK to wild-type individuals on extinction time. We varied the release ratio, *r*, from 0.10 to 400, and we found that the mean and variance of time to extinction decreased as the release ratio increased. These decreases were most rapid for low release ratios and became more gradual with higher release ratios. In fact, in our simulations, the mean extinction time differed by ≈209 d between $r = 0.1$ and $r = 1$ with a 51 d difference in standard deviation, whereas there was only a difference of 8 d between average extinction times for $r = 25$ and $r = 400$ with a 2 d difference between the standard deviations.

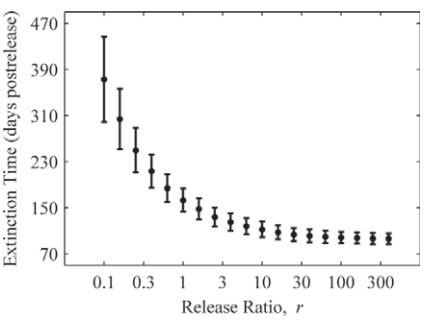

Fig. 5. Extinction time (postrelease) for different release ratios, *r*. Circles represent mean time to extinction and error bars represent mean ± SD. Here, $N = 200$, $\lambda = 10$, $c = 0$, $s_m = 0.72$, $s_f = 0.9$. Note the horizontal axis is on a log scale.

Fitness Cost. We used the model to predict the effects that mating fitness cost, taken to vary from 0 to 0.9, could have on extinction time under four different release ratios ($r = 0.1$, 1, 10, and 100). With all four release ratios, there were increases in the mean and variance of extinction times as the fitness cost was increased (Fig. 6). The differences in mean extinction time caused by increasing fitness costs were much greater with lower release ratios. As an example, under a high release ratio of $r = 100$, average extinction time differed by ≈13 d between $c = 0$ and $c = 0.9$ (Fig. 6d), while for the low release ratio of $r = 1$ the average extinction time increased by 236 d between these two fitness costs (Fig. 6b). The standard deviations of extinction times exhibited similar patterns, increasing by just 3 d in the $r = 100$ case, but quadrupling in the $r = 1$ case. When $r = 10$, as in the laboratory and field cage experiments previously conducted (Wise de Valdez et al. 2011; L. F. et al., unpublished data), average extinction times differed by ≈25 d between $c = 0$ and $c = 0.8$, with the average extinction time for $c = 0$ falling within the range of extinction times predicted by the model when $c = 0.8$ (98–199 d postrelease).

When fitness costs are high and release ratios are low (e.g., Fig. 6a; when $c = 0.8$ or 0.9 and $r = 0.1$), extinction in the treatment cage did not always occur within the 1,000 d period allotted for the experiment. For example, when $r = 0.1$ and $c = 0.8$ or 0.9, extinction occurred in fewer than 32% of the simulations, and wild-type population persistence was observed in a handful of simulations for fitness costs as low as $c = 0.4$. These simulations indicate that extinction of populations in cages in which FK individuals are released at low ratios may take several years if released mosquitoes have a high reduction in fitness because of the presence of the FK gene.

Population Size and Natural Extinction. To date, cage experiments have used high population densities resulting from high baseline input values. Population densities of adult *Ae. aegypti* in many dengue-endemic areas can be rather low (Morrison et al. 2004, Koenraadt et al. 2008, Jeffery et al. 2009), so it would be informative to know how lower density populations react to introductions of FK individuals. Because the small cage populations needed to mimic low density natural populations are at greater risk than larger populations of going extinct even without the introduction of an FK population, it is important to know how small a population can be maintained in the cage experiment without it being likely to go extinct because of environmental or demographic causes. We examined the capacity for small cage populations to persist in the absence of FK introductions by simulating experiments with baseline wild-type input numbers as low as $N = 25$. We simulated a wild-type cage population for 1 yr after a 13-wk stabilization period and calculated the proportion of 1,000 simulations that went extinct before the end of that year (Fig. 7). The proportion decreased with increased larval input, with 42% of the simulated cages going extinct when $N = 25$, and <1% going extinct when $N = 100$.

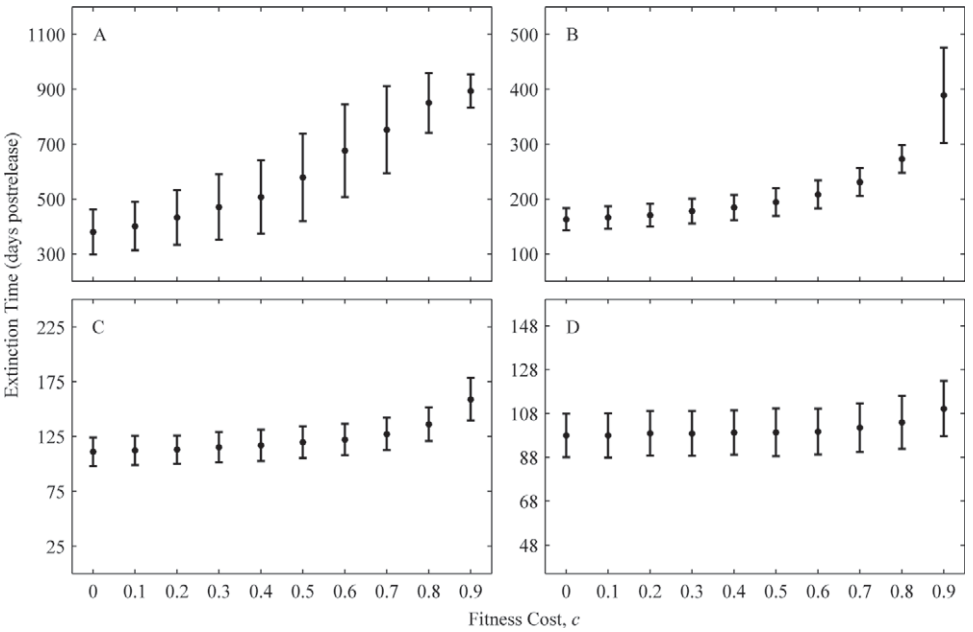

Fig. 6. Extinction times (postrelease) for different fitness costs combined with different release ratios. Here, $N = 200$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$. Release ratios are $r = 0.1$ (A), $r = 1$ (B), $r = 10$ (C), and $r = 100$ (D). Solid circles indicate mean of 1,000 simulations and error bars represent mean ± SD. Note each panel has a different scale on the vertical axis, with panel (A) having the largest range and each subsequent panel having half the range of the previous one. Recall that we restrict the duration of each simulation to 1,000 d after a release; this leads to the reduced variation in extinction time for high values of c that is observed in panel (A).

Density Dependence. We studied the effects of density-dependent survival, using the three descriptions of density dependence given above, under four different release ratios, $r = 0.1$, $r = 1$, $r = 10$, and $r = 100$. Density dependence had a marked effect on extinction times for the lower release ratios. For $r = 0.1$, there was no extinction within the 1,000 d experimental time frame in any of the 1,000 simulations for populations subject to any of the three forms of density dependence considered here. There was also no extinction for populations subject to Form 3 density dependence when $r = 1$. For all other combinations of release ratios and forms of density dependence, extinction was the typical outcome.

Because not all populations subject to density dependence went extinct in our simulated experiments, we compared the effects of density dependence on cage experiments by observing population reduction (see Fig. 8). We studied the percentage of the wild-type adult female population remaining 14 wk postrelease, as previously defined. For populations subject to any of the three forms of density dependence con-

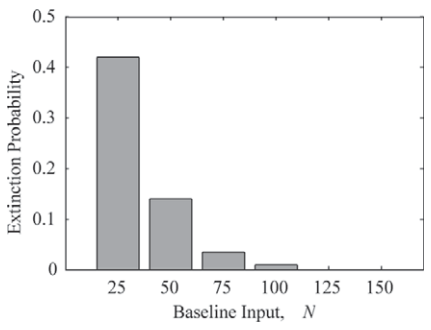

Fig. 7. Extinction probability of a wild type population in the absence of FK introduction. Bars represent the proportion of 1,000 simulations in which the population went extinct within 1 yr when low-density populations were simulated. Here, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$.

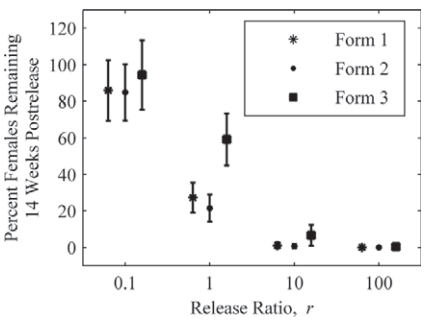

Fig. 8. Effects of density-dependent input for different release ratios. Vertical axis is the percentage of the wild-type adult female population remaining 14 wk postrelease. Here, $c = 0$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$. Form 1, Form 2, and Form 3 density dependence from Fig. 2 are represented by asterisks, circles, and squares, respectively. Solid asterisks, circles, and squares indicate mean of 1,000 simulations and error bars represent mean ± SD.

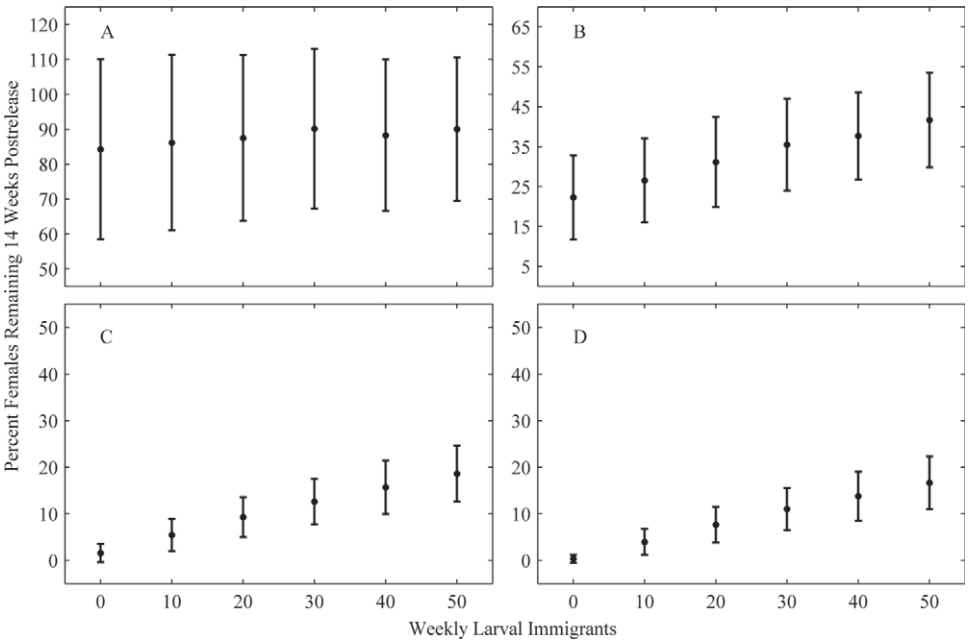

Fig. 9. The effect of weekly larval immigration on wild-type adult female population reduction. The horizontal axis is the number of immigrants per week, and the vertical axis is the percentage of the wild-type adult female population remaining 14 wk postrelease. Here, $N = 200$, $c = 0$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$. (A) $r = 0.1$, (B) $r = 1$, (C) $r = 10$, (D) $r = 100$. The solid circles indicate the mean of 1,000 simulations and error bars represent mean ± SD.

sidered here there was little population reduction when the release ratio was $r = 0.10$. When we considered larger release ratios, however, the populations were generally reduced. When $r = 1$, the mean percentage of females remaining 14 wk postrelease ranged from 21.6% for density dependence described by Form 2 to 59.1% for that under Form 3. For $r = 10$, populations subject to density dependence of Forms 1 and 2 were at or near extinction after 14 wk, whereas a mean of 6.7% of the female population remained when density dependence was described by Form 3. For releases with $r = 100$, all populations were reduced to, or very near to, extinction 14 wk postrelease.

We compared the extinction time of cages subject to density-dependent input to those of experiments run with density-independent input. Here, we considered only $r = 10$ and $r = 100$. Extinction times when $r = 10$ were not much different when survival was density-dependent with the exception of populations subject to density dependence described by Form 3, where the average extinction time was >40 d greater. (Extinction times ranged between 80–167 d with no density dependence and between 81–393 d when density dependence was described by Form 3.) Mean extinction times in our simulations for populations subject to Forms 1 and 2 density dependence were ≈13 d greater and 2 d less, respectively, than when the population was not subject to density dependence. (Extinction times ranged between 82–193 d for Form 1 and 76–172 d for Form 2.) When $r = 100$, the difference between density-dependent and density-independent cases was not as great; the mean extinction time was about the same for populations regulated by Form 3 density dependence, while populations subjected to Forms 1 and 2 density dependence were actually extinct a few days earlier, on average, than density-independent populations. (Extinction times ranged between 77–143 d when there was no density dependence, 73–153 d when density dependence was described by Form 1, 71–152 d for Form 2, and 69–163 d for Form 3.)

Wild-Type Immigration. For this analysis we assumed that larval input was not subject to density dependence. We considered the flow of 10, 20, 30, 40, or 50 larval immigrants per week under four release ratios ($r = 0.1, 1, 10$, and 100). We found that the mean percentage of the wild-type adult female population remaining after 14 wk increased approximately linearly with the weekly immigration rate (see Fig. 9). The variance decreased for the cases where $r = 0.1$ as the number of immigrants increased, but for all other release ratios, the variance increased with the number of immigrants. For higher release ratios ($r = 10$, $r = 100$) where the population, in the absence of immigration, was often extinct or nearly extinct 14 wk postrelease, the percentage remaining corresponded roughly to the percentage of immigrants to the wild-type baseline (e.g., for the case of 10 immigrants per week, when $N = 200$ and $r = 100$, the mean percentage remaining was 7.64%, slightly larger than 10/200 = 0.05, or 5%). This indicates that the population was being maintained primarily by immigrants.

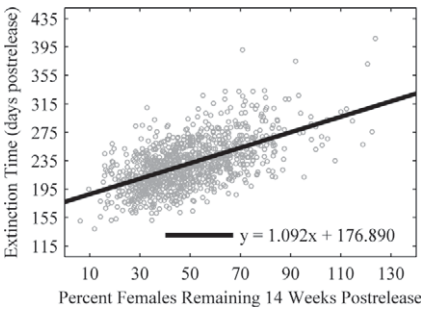

Fig. 10. Scatter plot of days of treatment cage extinction time (postrelease) versus population reduction as measured by the percentage of wild-type adult females remaining 14 wk postrelease. The equation for the line shown here is obtained from a linear regression model (coefficient of determination $R^2 = 0.339$). For these simulations, $N = 200$, $r = 1$, $\lambda = 10$, $c = 0.7$, $s_m = 0.72$, $s_f = 0.9$.

An Alternative Measure for Assessment of Population Reduction. In the previous two sections we studied population reduction that occurred in the first 14 wk postrelease. Although extinction time is an important measure of the overall efficacy of a release strategy, extinction could take months or even years in some cases. By using other measures of efficacy and defining a different endpoint of the experiment, overall experiment time can be shortened, and more experiments can be conducted.

As an illustration, we analyzed the model output for the scenario in which $c = 0.7$ and $r = 1$ (as presented in Fig. 6b) to assess the relationship between mean extinction time and wild-type adult female population reductions after 14 wk. We obtained the correlation coefficient between time to extinction and percentage of the wild-type adult female population remaining 14 wk postrelease (here, $n = 1{,}000$ simulations was the total sample size). Figure 10 shows a scatter plot of extinction time versus population reduction along with the line of best fit obtained via simple linear regression. The correlation between the percentage of the wild-type adult female population remaining after 14 wk and extinction time was 0.582 ($R^2 = 0.339$; $P < 0.0001$).

In this case, the population reduction 14 wk postrelease as defined here could be a good indication of cage extinction time. The time at which population reduction is measured (for the purposes of predicting extinction time) will depend upon the experimental setup chosen. For instance, if a larger release ratio is chosen, one may wish to observe the population reduction only a few weeks after the initial FK release rather than waiting 14 wk because many of the cages will have reached extinction within 14 wk postrelease (as in Fig. 6d). In general, one must be sure that the time chosen to observe reduction is neither before reductions in population size have begun nor after extinctions have begun. We further discuss how correlation depends on the time chosen to observe reduction in the Supp. Material 2 (online only).

Discussion

This study raises and addresses a series of important questions regarding the information that can (and cannot) be obtained about the qualities of FK transgenic mosquitoes from specific field cage experiments. Under which experimental designs can fitness costs be detected? Can populations of both low and high densities be studied in cages? Can information regarding the efficacy of FK introductions be obtained without waiting for extinction? As we noted, the experimental design that has been used so far leaves unanswered many important questions that will be need to be addressed before open field releases occur. How will immigration impact the population suppression ability of FK strains? Will density-dependent effects hinder population suppression, and how can such effects be overcome? What influence can cage experiments have on open field releases? Our study proposed and simulated variants of the current experimental design that could at least partially address these questions, although a more radical change to the experimental setup, and hence to the model, would be required to fully explore some of these questions, such as the complexities of density dependence. Such questions could easily be overlooked when temporal, financial, and personnel restraints limit the number and variety of experiments that are conducted, but models can suggest which issues will be important to address in field cage experiments before open field releases are conducted. Models can provide guidance that will make the process of designing and implementing further experiments more directed and efficient.

We found one past example in which deterministic simulation models were used to assess experiments and examine how different release schedules within cages would affect experimental outcomes (Curtis et al. 1976a, b). In contrast, our stochastic model explored a range of experimental parameters that transcends that which may be financially and temporally feasible to explore in field cage experiments, and considered at least some of the sources of variability that such experiments would experience.

For example, our model predicted that the size of the target population being studied in cages might not have a great effect on overall experiment time. The small differences that we found in extinction time because of population size can be beneficial from two perspectives. If one needs to reduce the effort of rearing large numbers of mosquitoes for an experiment, then it is useful to know that using a smaller population of mosquitoes will provide a similar result, in terms of overall experiment time, as using a larger population. In contrast, if one is interested in testing high-density populations, a population that is increased by 10-fold in size by changing N from 100 to 1,000 can be driven to extinction without a substantial increase in time. Although experiment times may not differ much when large and small population densities are studied, one must be cautious when studying small population densities, because our model indicated that interpreting results of such experiments could be

complicated by extinction that is due solely to demographic stochasticity. In cases where studying small populations is desired, such as when using cage studies to assess the ability of the released strain to find mates in the wild, short-term experiments that are not affected by population fluctuations could be more appropriate.

For a genetic control strategy to be successful, genetically modified mosquitoes must be able to compete with wild-type individuals for mates. The importance of mating competitiveness of males has been studied extensively in mathematical models for the sterile insect technique (see Barclay 2005, Ito and Yamamura 2005). Mating competitiveness similarly is expected to influence strategies involving a FK mechanism (Schliekelman and Gould 2000). Results from our simulations of the impact of fitness costs predicted that a large range of costs will not be detected in field cage experiments that use high release ratios. This study revealed that when experiments are conducted with release ratios similar to those of laboratory and field cage experiments that have been conducted to date, fitness costs may not be detectable unless they are >0.80. If an important goal of a field cage experiment is to assess fitness costs then low release ratios should be used.

The potential impact of density dependence on genetic control strategies has long been realized (Prout 1978, Foster et al. 1988, Schliekelman and Gould 2000). Within the constraints of the existing experimental design, we proposed a way to explore one facet of density dependence, namely larval survival. We found that when populations were subject to density dependence and FK releases occurred at low release ratios, eradication of the cage populations did not always occur within the 1,000 d allotted for the modeled experiments. High release ratios could overcome the density-dependent regulation of the population and ultimately drive the wild-type population to extinction, but the time to extinction will be greater than when the population is not regulated by density dependence. Because density dependence in *Ae. aegypti* populations can be difficult to accurately quantify (Legros et al. 2009), we explored different forms of density-dependent survival in the model and found that the extent to which density dependence interferes with population extinction in the simulations depends on the particular form of density dependence used. Our results showed that the model can be used to determine if, for a given population, more accurate assessment of density dependence would be desirable. As mentioned previously, larval survival represents just one component of density-dependent population regulation. A more complete exploration of the impact of density dependence would likely entail radical changes to the experimental design and model, but would provide invaluable information to inform field releases.

Immigration of juveniles into a population subject to control by FK releases provided another scenario in which wild-type populations did not go extinct in our model, even in the presence of forceful control measures. Our simulations indicated that immigration of larvae could result in the wild-type population being maintained, regardless of how many FK individuals were being introduced (see Prout 1978).

Once cage experiments have established that introduction of a specific FK strain can drive a population to extinction under ideal conditions, further experiments could be carried out to investigate the relationship between more realistic ecological factors and FK releases. Our model predicted that some of these more realistic experiments could take months or even years if extinction time is taken as the endpoint of the experiment. We found that as an alternative to the extinction endpoint, the efficacy of FK releases in cages could be assessed by observing the population reduction after a given time frame has passed. In our simulations, the proportion of the wild-type adult female population remaining 14 wk postrelease was a good predictor in some instances of time to extinction and could lead to shorter and more manageable experiments. In other cases, population reduction after 14 wk might not be a good predictor of extinction time, but as we show in the Supp. Material 2 (online only), our model can be used to determine beforehand the scenarios in which population reduction could provide information on extinction time.

Literature on previous field cage experiments indicates that population densities and release ratios used were often determined based on an intuitive feel for what would be logistically reasonable. In our exploration of the cage model, we illustrated why it is critical to design field cage experiments differently depending on goals for specific experiments. Clearly, an experiment aimed at assessing the impact of fitness costs should not use the same release ratio as might be considered optimal in the field because low or mid-range fitness costs may not be detectable when the wild-type population is inundated with FK males. Similarly, long-term field cage experiments may be inappropriate for assessing impacts of releases into low-density populations because of the effects of demographic stochasticity.

Our study of the impact that average lifespan may have on cage extinction time as well as our efforts to compare model output to data collected from laboratory experiments (see Supp. Material 2 [online only]) highlight the need to collect data on survival, fecundity, and emergence times before using this model to predict or assess experimental results. A detailed study of environment-specific values for these types of demographic parameters should be carried out before an attempt is made to use this type of model to make predictions.

The modeling exercise presented here was focused on assessment of FK release strategies. The broader message from this work is that for long-term experiments aimed at evaluating population dynamics, simulation models can provide useful insights that lead to substantial resource savings by fine tuning experimental designs to most effectively and efficiently address specific questions.

Acknowledgments

This work is funded by National Institutes of Health (NIH) grant R01AI091980-01A1, a grant to the Regents of the University of California from the Foundation for the NIH through the Grand Challenges in Global Health initiative. Our analysis was improved by discussions fostered by the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science and Technology Directory, Department of Homeland Security, and Fogarty International Center, NIH. M. A. Robert was supported in part by the North Carolina State University Mathematics Research Experience for Graduate Students (NSF grant DMS-EMSW21-MCTP-0943855). L.V. was supported by a Pasteur Institute—Cenci Bolognetti Foundation grant. We extend our gratitude to two anonymous reviewers and Steven Juliano for comments that improved the content of this article.

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Received 19 September 2011; accepted 5 September 2012.