Transposable element insertion location bias and the dynamics of gene drive in mosquito populations

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Abstract

Some vector-borne disease control strategies using transgenic mosquitoes require transgene spread to high frequency in populations. Transposable elements (TEs) are DNA sequences that replicate and transpose within the genomes of other organisms and may therefore be represented in the next generation in higher frequencies than predicted by Mendelian segregation. This over-representation has allowed some TEs to spread through natural populations. Transgenes incorporated within a TE sequence are expected to be driven into populations as long as there is a positive balance between fitness costs and over-representation. Models have been used to examine parameters that affect this balance but did not take into account biased insertion of TEs to linked sites in the genome. A simulation model was created to examine the impact of insertion bias on TE spread in mosquito populations. TEs that induce no fitness costs are predicted to increase in frequency over a wide range of parameter values but spread is slower for lower levels of transposition and non-local movement. If TEs are costly, high proportions of local movement can slow or halt spread. To function as a robust transgene drive mechanism a TE should replicate and transpose > 10%/insert/ generation, induce < 1% fitness cost/insert, and move preferentially to unlinked sites in the genome.

Keywords: transposon, gene drive, mathematical model, mosquito, vector-borne disease.

Introduction

Re-emerging vector-borne diseases, such as malaria and dengue, are a significant public health threat. Human malaria, caused by four protozoan parasites in the genus *Plasmodium*, infects up to 500 million people per year and is responsible for almost 3 million deaths annually (Nakajima, 1996). *Plasmodium* parasites responsible for human malaria are obligately dependent on mosquitoes in the genus *Anopheles* for transmission from one human host to the next, with *Anopheles gambiae* being the primary vector (Collins & Paskewitz, 1995). There is no vaccine currently available for malaria treatment and therefore control of the disease is limited to antiparasitic drugs and control of the mosquito vectors (Beaty, 2000). Control of the disease has been hampered by evolution of drug resistance in *Plasmodium* (Talisuna *et al.*, 2004), evolution of insecticide resistance in the mosquito vectors (Hemingway & Ranson, 2000), and lack of basic public health infrastructure to sustain vector control efforts in problem areas (Epstein, 1999). Dengue virus (DV) infections cause more human morbidity and mortality than any other arthropod-borne virus disease (Monath, 1994; Kuno, 1995; Gubler & Kuno, 1997). Currently, DV control is dependent on the reduction or elimination of *Aedes aegypti*. There is no licensed vaccine and no clinical cure currently available. Despite an abundance of studies on *Ae. aegypti* biology, DV vector control programs are often non-existent or ineffective (Reiter & Gubler, 1997).

Due to the failure of traditional control strategies to halt the spread of these diseases, genetic manipulation of mosquitoes is being explored as a potential control method. Most strategies for control of vector-borne diseases by release of transgenic mosquitoes require transgene spread to high frequency (close to or at fixation) in populations (Gould & Schliekelman, 2004). Due to lower expected relative fitness of individuals carrying transgenes, the

transgenes must be actively driven into populations. Several methods are under consideration for driving transgenes into populations including *Wolbachia* symbionts (Rasgon & Scott, 2003; Sinkins, 2004), fitness manipulation (Hahn & Nuzhdin, 2004), multiple independently assorting loci (Schliekelman & Gould, 2000) and transposable elements (TEs) (Boete & Koella, 2002; O'Brochta *et al.*, 2003). TEs are DNA sequences that replicate and transpose within the genomes of other organisms and thus may be represented in the next generation in higher frequencies than predicted by Mendelian segregation. Over-representation can give TEs an advantage in the next generation and allow them to spread through populations in spite of fitness costs (Gould & Schliekelman, 2004). Theory suggests that effector molecules (genes affecting the ability of insects to transmit pathogens) linked to TEs are expected to be driven into populations (Boete & Koella, 2002).

TEs can transpose to sites on the chromosome closely linked to the original insertion (local hopping) or to unlinked sites on the same chromosome or to sites on other chromosomes (non-local hopping). There is experimental evidence to suggest that local hopping is a common phenomenon for many transposable elements (Tower *et al.*, 1993; Zhang & Spradling, 1993; Golic, 1994; Machida *et al.*, 1997; Newfeld & Takaesu, 1999; Ladeveze *et al.*, 2001; Semiarti *et al.*, 2001; Guimond *et al.*, 2003). Local hopping does not give the TE a significant replication advantage in the next generation because tightly linked inserts will rarely segregate during meiosis and will therefore be inherited similarly to a single insert (Gould & Schliekelman, 2004). Furthermore, each insertion into a new chromosomal location may interfere with the normal function of one or more of the host organism's genes and thereby cause a decrease in fitness (Mackay, 1989). It is possible that a high degree of local hopping may prevent the spread of TEs into vector populations.

Specific applied models have been developed to assess the spread of 'loaded' TEs (TEs with linked effector molecules) into vector populations for disease control (Ribeiro & Kidwell, 1994; Kiszewski & Spielman, 1998; Boete & Koella, 2002). These models have been used to investigate the conditions under which TE-based gene drive strategies are likely to be successful. While these modelling efforts have proven useful for investigating the general dynamics of TE spread in mosquitoes, for simplicity they did not take into account critical features of TE postintegration behaviour such as local/non-local transposition bias. More general models of TE population genetics have also been developed (Charlesworth & Charlesworth, 1983; Langley *et al.*, 1983; Ohta, 1984, 1985; Kaplan *et al.*, 1985; Brookfield, 1986; Charlesworth & Langley, 1989; Brookfield & Badge, 1997). Many of these models examine TE equilibrium copy number distribution and assume that insertion sites are unlinked (Charlesworth & Langley, 1989) or, if assuming some linkage and recombination along chromosomes, that TEs insert randomly throughout the genome (Brookfield, 1982; Charlesworth & Charlesworth, 1983; Ohta, 1984, 1985; Quesneville & Anxolabehere, 1998).

We created a stochastic simulation model to examine the impact of TE insertion location bias on element spread in mosquito populations (Fig. 1). Although our model has similar structural details to several previously developed models (Brookfield, 1982; Charlesworth & Charlesworth, 1983; Quesneville & Anxolabehere, 1998), it differs from previous efforts in that we explicitly examine the effect of local/non-local transposition bias on the dynamics of TE invasion. Parameters that can be varied include: i, probability of replicative transposition/insert/generation; s, relative fitness of an individual carrying a single insert; b, rate of local transposition and r, recombination rate between adjacent loci on the same chromosome (Table 1, Fig. 1). In our simulations, we did not vary the recombination rate between adjacent loci on the same linkage group, but rather kept r constant at 0.05 to allow for some degree of insert assortment by recombination. Released transgenic mosquitoes were homozygous at a single TE locus on chromosome one (total: 2 inserts per genome) and were released at a rate of 10%. We assumed: (1) all TE movement is replicative (no cut-and-paste); (2) complete linkage between the TE and effector molecule; (3) no excision of TEs once inserted; (4) no truncation of TEs that alter their transposition activity; (5) no TE or effector molecule silencing; (6) no population structure and (7) random mating. Our results can be generalized to other transgenic insect systems of public health or agricultural importance with the caveat that the underlying genetic structure of the model may have to be altered.

During an actual release of transgenic mosquitoes, it is critical that the ability of the TE to spread be assessed within the first few years postrelease even if the trait has not reached fixation by this time. Loaded TEs must reach high

Parameter	Definition	Value for simulations
s	Relative fitness of individual carrying a single insert	1.0, 0.99, 0.95
i	Tranposition rate/insert/generation	0.03, 0.1, 0.2
b	Rate of intra-chromosomal (local) transposition	0.05, 0.95
r	Recombination rate between adjacent loci on the same linkage group	0.05
n	Total number of possible insertion sites per individual	30
x	Initial release frequency of transgenic mosquitoes	0.1

Table 1. Model variables, definitions and values used in simulations
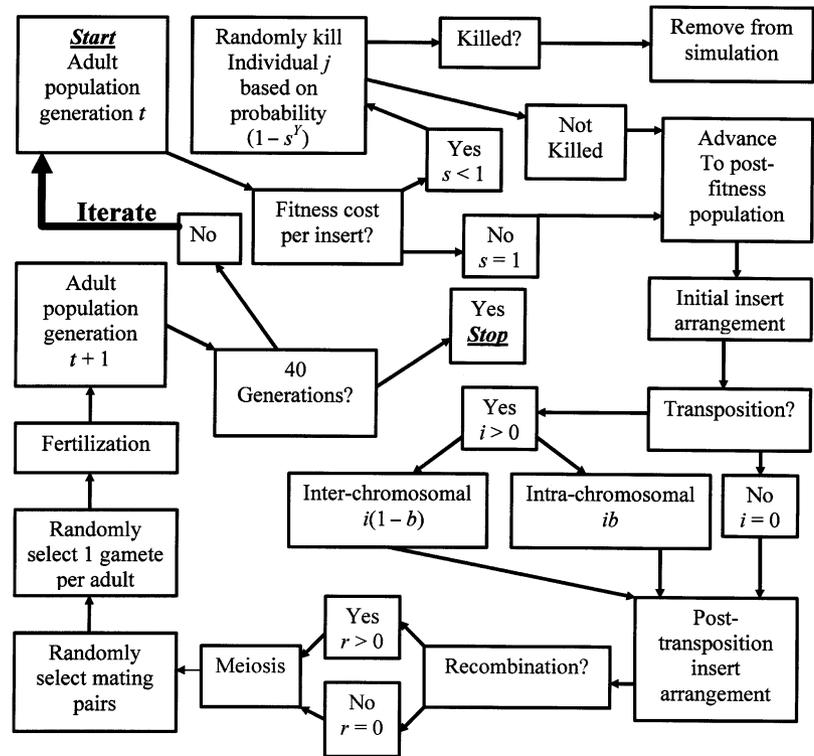
Figure 1. Model flowchart.

frequency rapidly to demonstrate the disease-reducing efficacy of the transgenic intervention and to minimize the probability that the parasite or vector will evolve to negate the effectiveness of the antiparasite molecule. For the purpose of our analyses, we define a successful introduction as an increase in the frequency of individuals carrying at least one TE copy from an initial level of 10% to ≥ 80% by the end of the simulation (40 generations, or approximately 5 years). TEs that do not reach this frequency within this time frame are considered inadequate for use as transgene drivers, although they may eventually reach high frequency in a longer time frame. We do not address the critical frequency that the TE must reach to halt pathogen transmission, as this value is likely to vary substantially temporally and geographically within and between disease systems and is beyond the scope of this analysis.

Results

Validation of model genetic structure

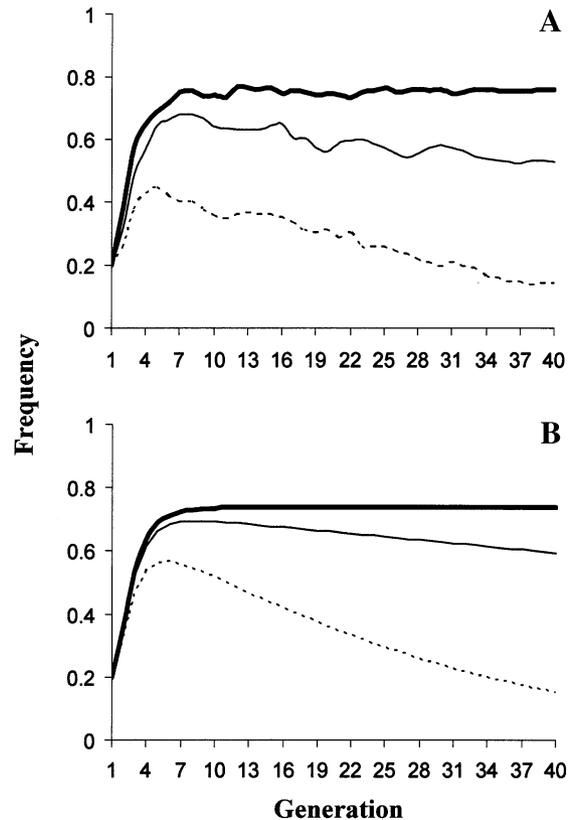We validated the underlying genetic structure of the model by comparing our model output to that of the General

Figure 2. Model validation. (A) Results from stochastic model simulating 20% introduction of individuals homozygous for three stably inserted TEs on separate chromosomes (six inserts/genome). Solid thick line: $s = 1.0$; solid thin line: $s = 0.99$, dotted thin line; $s = 0.95$. Each line is the mean of 20 model runs. (B): Results from general three-locus selection model simulating 20% introduction of individuals homozygous for a stably inserted TE at three loci (six inserts/genome). Solid thick line, $s = 1.0$; solid thin line, $s = 0.99$; dotted thin line, $s = 0.95$.

Frequency   **Mean Copy Number**
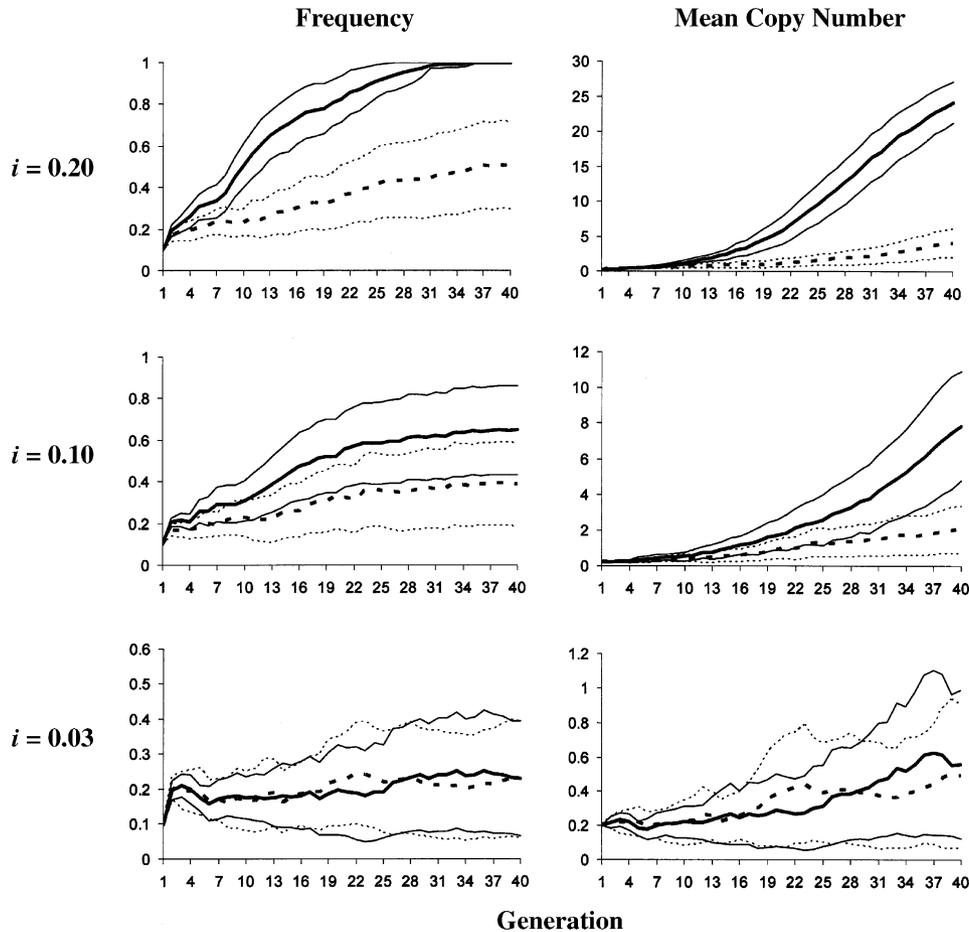
Figure 3. Changes in frequency of individuals with ≥ 1 copies of the TE and changes in the population mean copy number as a function of *i* and *b*, where there are no fitness costs associated with TE inserts (*s* = 1.0). Solid lines, *b* = 0.05; dotted lines, *b* = 0.95. Thick lines represent the mean of 20 model runs. Thin lines represent 95% confidence intervals. Note differences in *y*-axis range.

Selection Model (Mettler *et al.*, 1988) with three unlinked loci and two alleles per locus (insert presence/absence). For the stochastic model, we assumed that TEs were stably inserted (*i* = 0) and inherited as di-allelic loci. We placed one locus on each linkage group. We simulated releases of mosquitoes homozygous at all three loci (6 inserts/ genome) at a 20% introduction frequency where inserts were neutral (*s* = 1.0), or caused a 1% (*s* = 0.99) or 5% fitness (*s* = 0.95) cost/insert. Each simulation was run 20 times. There was close agreement between the results of the General Selection Model and our stochastic model regarding the frequency of individuals with at least one copy of the insertion (Fig. 2).

No fitness cost due to TE

In the absence of fitness costs, simulations indicate that TEs can spread under a wide range of parameter values for transposition rate (*i*) and local vs. non-local movement (*b*). High transposition rates (*i* = 0.2) coupled with non-local bias in movement (*b* = 0.05) can result in rapid element invasion

(reaching fixation in less than 40 generations). TEs that transpose at rates below 0.2, or frequently move to linked sites can still spread, but require longer than 40 generations to reach high frequency (≥ 0.8) and thus may not be adequate for transgenic mosquito release strategies (Fig. 3).

Small fitness costs (1%/insert)

Simulations indicate that small fitness effects (1%/insert) will slow TE invasion into the population. Invasion can still occur fairly rapidly (reaching a frequency of at least 0.8) with high transposition rates (*i* = 0.2) coupled with biased transposition to unlinked sites (*b* = 0.05). Lower rates of transposition or local insertion bias can make TE invasion unacceptably slow or can halt spread (Fig. 4).

Large fitness costs (5%/insert)

Large fitness effects (5%/insert) will hamper the effectiveness of TE drive into mosquito populations. Even with high rates of non-local transposition (*i* = 0.2, *b* = 0.05) elements will not reach high frequency within the selected time
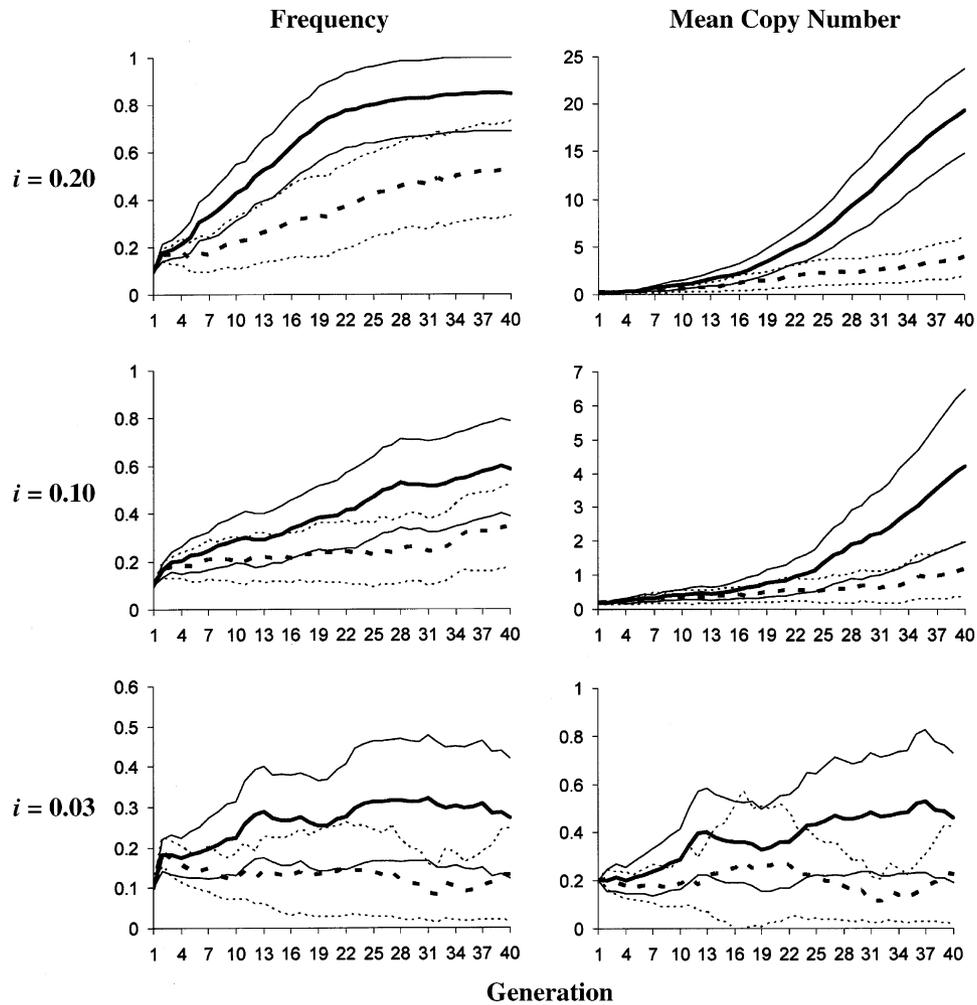
Figure 4. Changes in frequency of individuals with ≥ 1 copies of the TE and changes in the population mean copy number as a function of *i* and *b*, where there are small (1%/insert, *s* = 0.99) fitness costs associated with TE inserts. Solid lines, *b* = 0.05; dotted lines, *b* = 0.95. Thick lines represent the mean of 20 model runs. Thin lines represent 95% confidence intervals. Note differences in *y*-axis range.

frame. Biased transposition to linked sites can seriously slow or completely eliminate element spread even with moderately high transposition rates ($i = 0.1$) because the transmission advantage conferred by infrequent recombination events is not enough to counteract the cumulative negative fitness costs of multiple inserts (Fig. 5).

Discussion

Our analyses show that TE postintegration behaviour can seriously affect efficacy of TE drive. TEs that transpose preferentially to linked sites in the genome do not accumulate insert copies rapidly, and only gain an inheritance advantage from infrequent recombination events between closely linked inserts, in comparison to TEs that transpose to unlinked sites. Thus, under many circumstances, locally biased transposition can halt the spread of TEs or make them take an unacceptably long time to spread. This effect

becomes more pronounced when there are fitness costs associated with TEs or effector molecules. Costly TEs that move to closely linked sites express cumulative fitness costs but do not gain a great advantage from transposition.

Results indicate that TEs with transposition rates less than 0.2/insert/generation are unlikely to invade the population effectively in the case of large fitness costs and/or local transposition bias. Research should be focused on identifying or engineering TEs with ideal characteristics for gene drive. Such a TE should transpose frequently ($i > 0.1$), cause negligible fitness costs ($s > 0.99$) and move preferentially to unlinked sites in genome. A TE with these characteristics can potentially reach high frequency in the population in an epidemiologically relevant time period (≤ 40 generations) from relatively low introduction levels (~10%). Current candidate TEs in mosquitoes do not meet these requirements (O'Brochta *et al.*, 2003). Performing laboratory experiments in parallel with theoretical, ecological
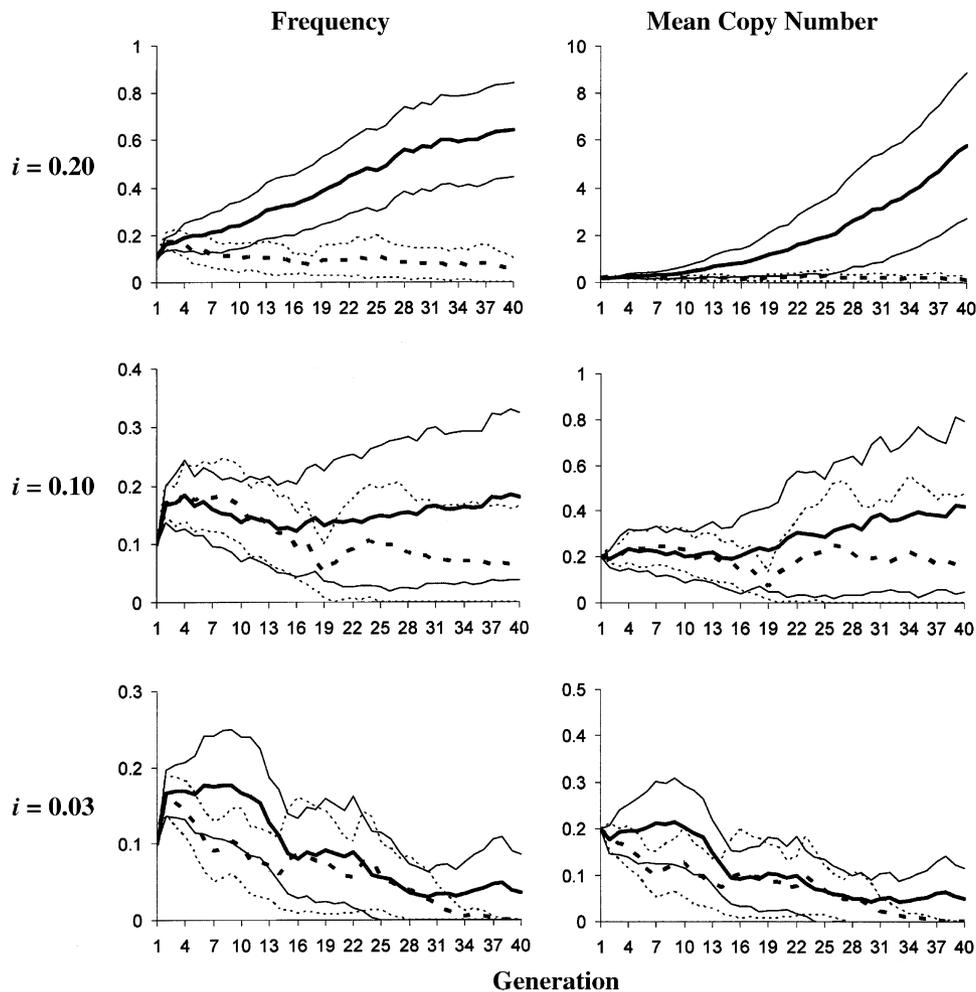
Figure 5. Changes in frequency of individuals with ≥ 1 copies of the TE and changes in the population mean copy number as a function of *i* and *b*, where there are large (5%/insert, *s* = 0.95) fitness costs associated with TE inserts. Solid lines, *b* = 0.05; dotted lines, *b* = 0.95. Thick lines represent the mean of 20 model runs. Thin lines represent 95% confidence intervals. Note differences in *y*-axis range.

and population-level studies will increase the likelihood of a successful release of genetically modified mosquitoes for disease control.

Like all models, our model makes simplifying assumptions that may affect the interpretation of the results. In reality, all of these simplifying assumptions may not hold true. Experimental evidence indicates that cut-and-paste transposition is frequently observed, excision events are common, linkage between the inserted gene and the TE may not be maintained, TEs may be truncated or otherwise modified during the invasion process, gene silencing may occur, mating may not be random, or the population may exhibit spatial structure, age structure, and/or overlapping generations (Reisen *et al.*, 1985; Gorrochotegui-Escalante *et al.*, 2000, 2002; Okanda *et al.*, 2002; O'Brochta *et al.*, 2003; Rasgon & Scott, 2004). Modifications in these assumptions will lower the likelihood of TE invasion, so our predictions of TE invasion and disease control success are optimistic.

Experimental procedures

The model is constructed as an Excel spreadsheet using the Poptools plug-in (http://www.cse.csiro.au/poptools), which implements stochastic processes using the Mersenne Twister algorithm (Matsumoto & Nishimure, 1998) for generation of pseudo-random numbers. The model is based on mosquito genetics and assumes that individuals are diploid with three pairs of chromosomes (Clements, 1992). The population size is set at 100 individuals at the beginning of each run, which is within the estimated range for effective population size in *Ae. aegypti* (Gorrochotegui-Escalante *et al.*, 2000). Each simulation was replicated 20 times. The model structure is based on empirical data for *P* element (Carareto *et al.*, 1997). The maximum number of transgene inserts that an individual chromosome can carry is set at five, for a total of 30 maximum inserts across the genome. Each chromosome is represented as a vector of 1s and 0s, where '1' indicates presence of a transgene insert and '0' indicates absence of the insert at that locus. Locus position is numbered from top to bottom. For example,

$$\begin{pmatrix}1\\0\\0\\0\\0\end{pmatrix}\begin{pmatrix}1\\0\\0\\0\\0\end{pmatrix}\begin{pmatrix}0\\0\\0\\0\\0\end{pmatrix}\begin{pmatrix}0\\0\\0\\0\\0\end{pmatrix}\begin{pmatrix}0\\0\\0\\0\\0\end{pmatrix}\begin{pmatrix}0\\0\\0\\0\\0\end{pmatrix}$$

represents an individual homozygous for a single insert at locus position 1 on chromosome pair 1. The inheritance of chromosomes is Mendelian. For inserts on the same chromosome, the recombination rate between any two adjacent loci can vary between 0 (complete linkage) to 0.5 (free recombination). The variables in the model are listed in Table 1.

The model assumes that fitness costs induced by transgenes are multiplicative. Each individual has a certain number of inserts Y and is randomly selected to live based on the probability s^Y. For example, if the relative fitness of an individual with a single insertion is 0.95, then an individual with three copies of the TE would have a relative fitness of $0.95^3 = 0.857$ and will die with probability $1 - s^Y = 0.143$. Individuals that survive are advanced to the next model step (Fig. 1).

Each individual that survives possesses a particular number and arrangement of elements inserted throughout its genome. Each element randomly transposes with probability i. The proportion of transposition events to a location on the same chromosome is denoted by b. Thus, a copy of an element can move randomly to an unoccupied site on the same chromosome (local, or intrachromosomal movement) with probability ib, and to a randomly selected, unoccupied site on one of the other five chromosomes (non-local, or interchromosomal transposition) with probability $i(1 - b)$. Recombination occurs between sister chromosomes, with recombination events between adjacent loci randomly occurring with probability r. Gamete formation then takes place. Mating pairs are randomly selected (with replacement) and one gamete is randomly selected from each parent. Fertilization occurs, and the population is advanced to generation $t + 1$. This procedure is completed iteratively for 40 generations to assess the ability of TEs to increase in frequency in a rapid, epidemiologically relevant time period. We define successful TE invasion as a frequency of individuals carrying ≥ 1 TE copy of at least 80% by the end of the simulation (40 generations, or approximately 5 years).

Acknowledgements

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