Confinement and reversibility of threshold-dependent gene drive systems in spatially-explicit *Aedes aegypti* populations

Héctor M. Sánchez C. [1], Jared B. Bennett [2], Sean L. Wu [1], Gordana Rašić [3], Omar S. Akbari [4], John M. Marshall [1,5,†]

[1] Division of Epidemiology and Biostatistics, School of Public Health, University of California, Berkeley, CA 94720, USA
[2] Biophysics Graduate Group, University of California, Berkeley, CA 94720, USA
[3] Mosquito Control Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia
[4] Cell and Developmental Biology Section, Division of Biological Sciences, University of California, San Diego, CA 92093, USA
[5] Innovative Genomics Institute, Berkeley, CA 94720, USA

[†] To whom correspondence should be addressed:
John M. Marshall
Division of Epidemiology & Biostatistics, School of Public Health
University of California, Berkeley, CA 94720, USA
Email: john.marshall@berkeley.edu

Key words:
Chromosomal translocations, underdominance, metapopulations, population dynamics, population replacement, biosafety, field trials

Abstract:
The discovery of CRISPR-based gene editing and its application to homing-based gene drive systems has been greeted with excitement, for its potential to control mosquito-borne diseases on a wide scale, and concern, for the invasiveness and potential irreversibility of a release. Gene drive systems that display threshold-dependent behavior could potentially be used during the trial phase of this technology, or when localized control is otherwise desired, as simple models predict them to: a) effectively spread into partially isolated populations in a confineable manner, and b) be reversible through releases of wild-type organisms. Here, we model hypothetical releases of two recently-engineered threshold-dependent gene drive systems - reciprocal chromosomal translocations and a form of toxin-antidote-based underdominance known as UD^{MEL} - to explore their ability to be confined and remediated. We simulate releases of *Aedes aegypti*, the mosquito vector of dengue, Zika and other arboviruses, in Yorkeys Knob, a suburb of Cairns, Australia, where previous biological control interventions have been undertaken on this species. We monitor spread to the neighboring suburb of Trinity Park, to assess confinement. Our results suggest that translocations could be introduced on a suburban scale, and remediated through releases of non-disease-transmitting male mosquitoes with release sizes on the scale of what has been previously implemented. UD^{MEL} requires fewer releases to introduce, but more releases to remediate, including of females capable of disease transmission. Both systems are expected to be confineable to the release site; however, spillover of translocations into neighboring populations is less likely.

Introduction:

The discovery of CRISPR and its application as a gene editing tool has enabled gene drive systems to be engineered with much greater ease (1, 2). Recent attention has focused on homing-based drive systems and their potential to control mosquito-borne diseases on a wide scale, either by spreading disease-refractory genes (3) or by spreading genes that confer a fitness load or sex bias and thereby suppress mosquito populations (4, 5). The increased ease of gene editing has also advanced the entire field of gene drive, including systems appropriate during the trial phase of the technology (6). Such systems would ideally be capable of enacting local population control by: a) effectively spreading into populations to achieve the desired epidemiological effect, and b) being recallable from the environment in the event of unwanted consequences, public disfavor, or the end of a trial period.

As gene drive technology has progressed, a number of systems have been proposed with the potential to enact localized population control without spreading on a wide scale (6, 7). Sterile male releases provide one option (8), a recent version of which is based on the same molecular components as CRISPR gene drive systems (8, 9). At the interface between homing-based and localized suppression systems, an autosomal X chromosome-shredding system has been proposed that displays transient drive and suppression before being selected out of the population (10). Population modification drive systems that display transient drive activity before being eliminated by virtue of a fitness cost, could also spread disease-refractory genes into populations in a localized way. Examples of this variety of drive systems include split-gene drive (11), daisy drive (12) and killer-rescue systems (13). Each system has its own strengths and weaknesses, and could be suited to a different situation. In this paper, we theoretically explore the potential for two recently-engineered threshold-dependent gene drive systems to achieve localized and reversible population modification in structured populations - reciprocal chromosomal translocations (14) and a toxin-antidote-based system known as UD^{MEL} (15).

Threshold-dependent gene drive systems must exceed a critical threshold frequency in a population in order to spread. Based on this dynamic, simple population models, in which two randomly mating populations exchange small numbers of migrants with each other, predict that these systems can be released at high frequencies in one population and spread to near-fixation there, but never take off in the neighboring population because they do not exceed the required threshold there (16, 17). These systems can also be eliminated through dilution with wild-type organisms at the release site, making them excellent candidates for the trial phase of a population modification gene drive strategy, or when localized population modification is desired. However, whether these dynamics hold in real ecosystems depends crucially on the dispersal patterns and population structure of the species being considered. First steps towards modeling the spatial dynamics of these systems have been taken by Champer *et al.* (18), who model spatially-structured releases of various threshold-dependent systems without life history, and Huang *et al.* (19), who model engineered underdominance (20) on a grid-based landscape incorporating life history for *Aedes aegypti*, the mosquito vector of dengue, Zika and other arboviruses.

Here, we present a detailed ecological analysis of the expected population dynamics of two recently-engineered threshold-dependent drive systems, translocations and UD^{MEL}, in *Ae. aegypt* in a well-characterized landscape - Yorkeys Knob, a suburb ~17 km northwest of Cairns, Australia (Figure 1C) - where field trials could conceivably be conducted. Yorkeys Knob and the nearby town of Gordonvale were field sites for releases of *Wolbachia*-infected mosquitoes in 2011 (21) and the prevalence of *Wolbachia* infection over time provided information on the number of adult *Ae. aegypti* mosquitoes per household and other mosquito demographic

parameters for that location (22). *Wolbachia* is an intracellular bacterium and biocontrol agent that biases inheritance in its favor if infected females are present, and blocks transmission of dengue and other arboviruses (23). Yorkeys Knob is a partially isolated suburb, separated by a 1-2 km-wide uninhabited, vegetated area from the nearest suburb, Trinity Park, a control site for the *Wolbachia* trial. This allowed us to simulate trials of transgenic mosquitoes in a well-characterized population, while also theoretically exploring their potential of spread to a neighboring community.

A number of other ecological details are relevant to the spread of threshold-dependent gene drive systems that have not been considered in previous modeling studies. Perhaps of greatest importance, the frequency of the introduced transgene is markedly different from the frequency of introduced adults. It is typical to release only male mosquitoes as part of an intervention, as only females are involved in human disease transmission. Life cycle and mating structure therefore become relevant, as immature life stages are not available for mating, and female adults are thought to mostly mate only once soon after emergence (24). This means that many of the released adult males will not find a mating partner, and hence larger releases will be required to exceed threshold frequencies than predicted in simple population frequency models.

The nature of mosquito dispersal behavior is also relevant to the spatial dispersal of transgenes. Our species of interest, *Ae. aegypti*, is understood to display leptokurtic dispersal behavior in a suburban setting, in which mosquitoes tend to remain in the same household for the majority of their lifespan, while a few mosquitoes disperse over larger distances (25). With these landscape, dispersal and life cycle considerations accounted for, we theoretically explore the ability to drive two threshold-dependent systems, translocations and UD^{MEL}, into populations of *Ae. aegypti* in one community, Yorkeys Knob, without them spreading in significant numbers to a neighboring community, Trinity Park, and to be remediated from Yorkeys Knob at the end of the simulated trial period.

Results:

Model framework. We use the Mosquito Gene Drive Explorer (MGDrivE) modeling framework (26) to model the spread of translocations and UD^{MEL} through spatially-structured mosquito populations (Figure 1). This is a genetic and spatial extension of the lumped age-class model of mosquito ecology (27) modified and applied by Deredec *et al.* (28) and Marshall *et al.* (29) to the spread of homing gene drive systems. The framework incorporates the egg, larval, pupal and adult life stages, with egg genotypes being determined by maternal and paternal genotypes and the allelic inheritance pattern of the gene drive system. Spatial dynamics are accommodated through a metapopulation structure in which lumped age-class models run in parallel and migrants are exchanged between metapopulations according to a specified dispersal kernel. Further details of the framework are described in the Materials and Methods section.

Applying the MGDrivE modeling framework to our research questions, we incorporate the inheritance patterns of reciprocal chromosomal translocations and UD^{MEL} into the inheritance module of the model (Figure 1A-B), the life cycle parameters of *Aedes aegypti* (Table S1) into the life history module, and the distribution of households in Yorkeys Knob and Trinity Park along with their expected mosquito population sizes and movement rates between them into the landscape module (Figure 1C). The suburb of Trinity Park served as a control site for field releases of *Wolbachia*-infected mosquitoes, to quantify the extent to which the *Wolbachia* infection could spread from one community to another, and plays a similar role for our simulated releases of threshold-dependent gene drive systems.

The inheritance patterns that result from chromosomal translocations are depicted in Figure 1A. Chromosomal translocations result from the mutual exchange between terminal segments of two nonhomologous chromosomes. When translocation heterozygotes mate, several crosses result in unbalanced genotypes and hence unviable offspring, resulting in a heterozygote reproductive disadvantage. This results in bistable, threshold-dependent population dynamics, confirmed in laboratory drive experiments (14). The inheritance patterns produced by the UD^{MEL} system are depicted in Figure 1B. This system consists of two unlinked constructs, each possessing a maternally-expressed toxin active during oogenesis, and a zygotically-active antidote expressed by the opposite construct. The resulting dynamic, confirmed in laboratory drive experiments (15), is gene drive for allele frequencies greater than ~24%, in the absence of a fitness cost.

Population replacement and remediation for translocations. The use of translocations for transforming pest populations was initially suggested by Serebrovskii (30) and later Curtis (31) for the introduction of disease-refractory genes into mosquito populations. A number of models have been proposed to describe their spread through randomly-mating populations (14, 16, 32, 33); however, with one recent exception addressing spatial structure (18), these have largely ignored insect life history and mating structure. Such models suggest that the translocation need only exceed a population frequency of 50%, in the absence of a fitness cost associated with the translocation, to spread to fixation in a population, which could conceivably be achieved through a single seeding release round. Here, we find that incorporating life history and population structure into mosquito population dynamic models significantly increases release requirements.

In Figures 2-3, based on the precedent set by the World Mosquito Program (https://www.worldmosquitoprogram.org/), we consider weekly releases of 20 adult *Ae. aegypti* males homozygous for the translocation for given durations and coverage levels, where coverage level is the proportion of households that receive the releases. Releases are simulated in the community of Yorkeys Knob, in which prior releases of *Wolbachia*-infected mosquitoes suggested a local population of ~15 adult *Ae. aegypti* per household (22), and for mosquito movement rates inferred from previous studies (25, 34, 35) (Table S1). For a coverage level of 100%, and in the absence of a fitness cost, five weekly releases of 20 *Ae. aegypti* males (~3:1 release to local males) are required for the translocation to spread to fixation in the community (Figure 2), as opposed to the single release expected when ignoring life history and population structure (32). As coverage is reduced to 50%, the required number of releases increases to 11, and for a coverage level of 25%, as seen for the World Mosquito Program in Yorkeys Knob, the required number of releases increases to 20 (Figures 2-3). Although large, these releases are achievable, considering the much larger releases conducted for sterile insect programs (36).

To simulate remediation of a translocation, we consider weekly releases of 20 adult *Ae. aegypti* wild-type males in the community of Yorkeys Knob, whereby the translocation has already reached fixation in that community. In the absence of a fitness cost associated with the translocation, translocations are symmetrical in their threshold dynamics and so, for a coverage level of 100%, five weekly releases are required for the translocation to be completely remediated from the community, and for a coverage of 25%, 20 weekly releases are required for the translocation to be completely remediated (Figures 2-3). Encouraging features of these results are that: i) remediation can be achieved through releases of non-biting, non-disease-transmitting males, ii) release sizes are achievable, and iii) despite the spatial household structure, both replacement and remediation are complete within the community. The time to

replacement is highly dependent on the coverage level and number of releases; but is reasonably quick given sufficient releases. At a coverage of 50%, 20 weekly releases led to the translocation spreading to a frequency >99% within a year of the final release (or within 500 days of the first release). For equivalent wild-type releases, this is the same as the time to >99% elimination.

Population replacement and remediation for UD^{MEL}. UD^{MEL} was the first synthetic gene drive system to be engineered that displays threshold-dependent dynamics (15). The system consists of two unlinked constructs, each possessing a maternally expressed toxin active during oogenesis, and a zygotically active antidote expressed by the opposite construct (Figure 1B). At low population frequencies, the maternal toxin confers a significant fitness cost, leading to elimination, while at high population frequencies, the zygotic antidote confers a selective benefit in the context of a prevalent toxin, leading to fixation. The dynamics of this system in randomly-mating populations have been characterized by Akbari *et al.* (15), suggesting that the system need only exceed a population frequency of ~24%, in the absence of a fitness cost, to spread to fixation, while the wild-type must exceed a population frequency of ~76% to eliminate the construct. Both replacement and remediation should therefore be achievable with 1-2 releases of transgenic and wild-type organisms, respectively; however, as for translocations, we find that incorporating life history and population structure into our models increases release requirements in both cases.

In Figures 2-3, we consider weekly releases of 20 adult *Ae. aegypti* males homozygous at both loci for the UD^{MEL} system in the community of Yorkeys Knob. The lower threshold for UD^{MEL} as compared to translocations means that replacement is much easier to achieve for UD^{MEL}. For a coverage level of 75% or higher, and in the absence of a fitness cost, a single release of 20 *Ae. aegypti* males leads to the UD^{MEL} system spreading to fixation throughout the community (Figure 2). As coverage is reduced to 25%, the required number of releases to achieve fixation increases to three (Figures 2-3). As for translocations, the time to replacement is highly dependent on the coverage level and number of releases. From Figure 2, it is apparent that UD^{MEL} reaches total allele fixation slowly, although the number of individuals having at least one copy of the transgene increases quickly. At a coverage of 50%, 10 weekly releases lead to wild-type individuals falling to a frequency <2% within a year of the final release (or within 500 days of the first release).

Remediation, however, is more difficult to achieve for UD^{MEL} compared to translocations due to the higher threshold that wild-type organisms must exceed to eliminate UD^{MEL}. To simulate remediation, we first consider weekly releases of 20 adult *Ae. aegypti* wild-type males in the community of Yorkeys Knob. This reveals that remediation of UD^{MEL} is not possible with male-only releases (Figure 2), and so we next consider weekly releases of 10 adult *Ae. aegypti* females and 10 adult males. In the absence of a fitness cost associated with the UD^{MEL} construct, and for a coverage level of 75%, 10 weekly releases are required for the UD^{MEL} construct to be completely removed from the community. As coverage is reduced to 50%, the required number of releases increases to 17, and for a coverage level of 25%, remediation is not possible with 20 releases (Figures 2-3). These results make a strong case for translocations as preferred systems to introduce transgenes in a local and reversible way as: i) remediation of UD^{MEL} requires releases of biting, vector-competent females, and ii) release requirements for these biting, vector-competent females are large due to the higher threshold that must be surpassed.

Confinement of translocations & UD^{MEL} to release site. Confinement of translocations and UD^{MEL} to partially-isolated populations has previously been modeled by Marshall & Hay (16) and Akbari *et al.* (15). In both cases, two randomly-mating populations were modeled that

exchange migrants at given rates. Population structure was otherwise ignored, as was mosquito life history. Results from these analyses suggest that translocations would spread and remain confined to populations for which the migration rate is less than ~5.8% per mosquito per generation (16), and that UD^{MEL} would remain confined to populations for which the migration rate is less than ~1.6% per mosquito per generation (15). These migration rates are relatively low, however this may be beneficial for the types of landscapes we are considering here, whereby the system may spread between neighboring households, but not from one suburb to another. Recently, Champer *et al.* (18) showed that translocations would remain confined to and persist in a population connected to another by a "migration corridor" under a range of parameter values.

For our landscape of interest - the suburbs of Yorkeys Knob and Trinity Park - it is very unlikely that *Ae. aegypti* mosquitoes will travel from one suburb to another by their own flight. Extrapolating the exponential dispersal kernel used in our simulations, fitted to data from mark-release-recapture experiments collated by Guerra *et al.* (34), suggests these events to be negligible, before accounting for the fact that the intervening vegetated area may serve as a barrier to *Ae. aegypti* flight (37). Furthermore, rare migrant mosquitoes are unlikely to cause the threshold frequency for either drive system to be exceeded, thus making spatial spread due to such movements unlikely. In considering confineability to the release suburb, we therefore model "batch migration," in which several mosquitoes are carried, perhaps by a vehicle, from one community to another at once. Batch migration events could be thought of as several adult mosquitoes being carried at once, or perhaps more likely, as a larval breeding site, such as a tyre, being carried from one household to another, with several adults emerging from the tyre following transport. We model batch migration events as occurring between randomly chosen households, and vary the number of daily migration events and the effective number of adults carried per event. For computational simplicity, we focus on migration events from Yorkeys Knob, in which either system has already reached fixation, to households in Trinity Park, which is initially fixed for wild-type mosquitoes.

In Figure 3E-F, we see that both the number and size of daily batch migration events affect the chance of either system establishing itself in the neighboring suburb, Trinity Park. For translocations, ~20 daily migration events of batches of 5 adults are required for spread in Trinity Park. For batches of 10 adults, ~10 daily migration events are required, and for batches of 20 adults, ~8 daily migration events are required. For UD^{MEL}, ~4 daily migration events of batches of 5 adults are required for spread in Trinity Park, and for batches of 10 adults, ~2 daily migration events are required.

These results continue to make a strong case for translocations as preferred systems to introduce transgenes in a local and reversible way as: i) many more batch migration events are required to lead to spread for translocations as opposed to UD^{MEL}, and ii) the rate of migration events required for translocations to spread is higher than what would be expected between these communities. Specifically, *Wolbachia* releases in Yorkeys Knob in 2011 provide evidence for occasional batch migrations to the nearby suburb of Holloways Beach; however the spatio-temporal pattern of *Wolbachia* spread, as inferred from monitored trap data, suggests only ~1-2 batch migration events over the course of a month, consisting of less than 5 adult females per event (21).

Sensitivity analysis. A theoretical study by Khamis *et al.* (38) on toxin-antidote-based underdominance gene drive systems, similar to UD^{MEL} but for which the toxins are zygotic rather than maternal (20), found that the gene drive threshold frequency is highly sensitive to: i) the

increase in adult mortality rate in organisms having the transgene, ii) the duration of the larval life stage, and iii) parameters determining the character or strength of larval density dependence. In Figure 4 and Figure S2, we explore the sensitivity of our model outcomes of replacement, remediation and confinement for translocations and UD^{MEL} as we vary: i) the duration of the larval life stage, ii) the baseline adult mortality rate, and iii) the fitness cost associated with the gene drive system. For translocations, we model a 10% fitness cost as a 10% reduction in mean adult lifespan for organisms homozygous for the translocation, and a 5% reduction for organisms heterozygous for the translocation. For UD^{MEL}, since its inheritance bias is induced through the action of maternal toxins, we model a 10% fitness cost as a 10% reduction in female fecundity for organisms homozygous for UD^{MEL} at both loci, with 2.5% additive fitness costs contributed by each transgenic allele.

For translocations, the associated fitness cost had the greatest impact on the release scheme required for the system to be fixed or remediated from the population, given the life parameters considered (Figure 4). A 10% fitness cost led to 14 weekly releases at a coverage of 50% being required for the translocation to reach fixation (an increase of 3 releases), while a 20% fitness cost led to 18 weekly releases being required (an increase of 7 releases). Small changes in the duration of the larval life stage and baseline adult mortality had minor impacts on the release requirements, with an increase in larval lifespan of 2 days or a 2% decrease in the adult mortality rate leading to one more weekly release being required for the translocation to reach fixation, and vice versa. Remediation, on the other hand, requires fewer wild-type releases when there is a fitness cost associated with the translocation. A 10% fitness cost led to 8 weekly releases at a coverage of 50% being sufficient to eliminate the translocation (a decrease of 3 releases), and a 20% fitness cost led to 6 weekly releases at a coverage of 50% being sufficient for elimination (a decrease of 5 releases). Small changes in the duration of the larval life stage and baseline adult mortality had minor impacts on the wild-type release requirements for elimination, with an increase in larval lifespan of 2 days or a 2% decrease in the adult mortality rate leading to one fewer release being required.

The sensitivity of our predictions regarding confinement to the release site are of particular interest, as invasion of a neighboring community may be more likely under some parameter values than others. Fortunately, a fitness cost associated with the translocation leads to a higher threshold, and hence more batch migration events required for invasion of a neighboring community. A 10% fitness cost led to ~2-3 additional daily migration events of 10 adults required for spread to Trinity Park, and a 20% fitness cost led to ~6-7 additional daily migration events required (Figure 4). Also noteworthy, a 2% increase in the adult mortality rate led to ~2 fewer daily migration events required for spread to Trinity Park - i.e. ~8 migration events for batches of 10 adults, and ~18 migration events for batches of 5 adults. While still above inferred batch migration rates, this highlights that there could exist parameter sets beyond those explored for which invasion is feasible.

UD^{MEL} displays similar parameter sensitivities regarding fixation, remediation and batch migration outcomes as for translocations, with the exception that these outcomes are less sensitive to fitness costs (Figure S2), likely due to the fact that fitness is accommodated through a reduction in female fecundity rather than an increase in adult mortality. A 20% fitness cost led to ~1 additional weekly release being required for the system to spread to fixation, whether at a coverage of 25% or 50%. Similarly, for an invasion of Trinity Park, a 10% fitness cost required ~1 additional daily migration event of 5 adults, and a 20% fitness cost required ~2 additional daily migration events. Of note, a 2% increase in the adult mortality rate led to ~1 fewer daily

migration event required for spread to Trinity Park, making this now very achievable - i.e. ~3 migration events for batches of 5 adults, and ~2 migration events for batches of 10 adults.

Discussion:

The idea of using threshold-dependent gene drive systems to replace local populations of disease vectors with varieties that are unable to transmit diseases has been discussed for over half a century now, since Curtis (31) famously proposed the use of translocations to control diseases transmitted by mosquitoes. While Curtis had been primarily concerned with introducing and spreading genes into a population, as the technology nears implementation, confining them is becoming of equal concern. As CRISPR-based homing gene drive technology edges closer to field application, concerns are increasingly being raised regarding the invasiveness of these systems (39, 40), and systems such as split drive (11), daisy drive (12) and threshold-dependent underdominant systems (7) are gaining interest, at least during the trial phase of population replacement technology (6). In this paper, we model the introduction of two drive systems, chromosomal translocations and UD^{MEL}, that have been engineered in the laboratory and shown to display threshold-dependent spread (14, 15). While previous papers have described the population dynamics of these two systems in randomly-mating populations ignoring life history (14–16, 31), with one recent paper including spatial structure (18), we present the first analysis of these systems in a spatially-structured population including mosquito life history and reflecting a well-characterized landscape where field trials could conceivably be conducted (21).

Our results provide strong support for the use of translocations to implement confineable and reversible population replacement in structured *Ae. aegypti* populations. Regarding reversibility, translocations are preferable to UD^{MEL} as: i) they can be remediated through releases of non-disease-transmitting male *Ae. aegypti*, and ii) required releases sizes are achievable (~10 weekly releases at a coverage level of 50%). UD^{MEL} requires less effort to introduce into a population; but is much more difficult to remove once it has been introduced, requiring a large number of both males and disease-transmitting females to be released. This highlights the benefit of a ~50% threshold for reversible population replacement: the symmetry allows both replacement and remediation to be achieved with similar effort. Extreme underdominance is another example of system with a 50% threshold (20, 41). Regarding confineability, translocations again outperform UD^{MEL} as ~20 daily migration events of batches of 5 *Ae. aegypti* adults are required for translocations to spread to the neighboring suburb of Trinity Park, while UD^{MEL} can spread to Trinity Park given only ~4 daily migration events (or ~3 daily migration events for alternative model parameterizations). The true batch migration rate between suburbs is expected to be smaller than either of these (21), however the rate required for translocations to spread is highly unlikely to be reached, while the rate for UD^{MEL} is conceivable.

As with any modeling study, there are limitations inherent in our analysis. Several of the parameters we assumed to be constant here would indeed be dynamic in a real intervention scenario. At the genetic level, lab experiments suggest non-outbred individuals homozygous for the translocation had a fitness cost that largely disappeared once offspring were produced that were the product of at least one wild-type individual (14). Models fitted to data from UD^{MEL} drive experiments also suggested dynamic fitness costs that depended on the frequency of transgenic organisms in the population (15). At the ecological level, our model of *Ae. aegypti* life history (26), based on the lumped age-class model of Hancock & Godfray (27), assumes a constant population size, and other constant ecological parameters, such as adult death rate and larval development times. These parameters have indeed been shown to vary in space and time,

and in response to local mosquito density (42, 43), which our sensitivity analyses suggest could have significant impacts on release thresholds and gene drive outcomes (38) (Figure 4 & S2). At the landscape level, we have assumed a relatively homogenous distribution of mosquitoes per household, and movement rates between households that depend only on distance and household distribution. Extensive landscape heterogeneities have been shown to slow and alter the spread of *Wolbachia* (44, 45), and would likely impact the spread of translocations and UD^{MEL} as well. Future work that helps to characterize the environmental drivers of mosquito population dynamics will inform iterative model development to address this.

In conclusion, our analysis supports the use of translocations as a threshold-dependent drive system capable of spreading disease-refractory genes into structured *Ae. aegypti* populations in a confineable and reversible manner. If such a system were engineered in *Ae. aegypti*, it would be an excellent candidate for the introduction of disease-refractory genes during the trial phase of population replacement technology, or whenever a localized release were otherwise desired. As the technology nears implementation, further ecological work characterizing the density-dependencies, seasonalities and spatial heterogeneities of *Ae. aegypti* populations will be essential to enhance model predictions in preparation for field trials.

Materials and methods:

To model the expected performance of threshold-dependent gene drive systems - reciprocal chromosomal translocations and UD^{MEL} - at functioning in a confineable and reversible way, we simulated releases of adult *Ae. aegypti* males homozygous for each system in the community of Yorkeys Knob in Queensland, Australia using the MGDrivE simulation framework (26) (https://marshalllab.github.io/MGDrivE/). To simulate remediation, we modeled releases of wild-type adult *Ae. aegypti* into populations in Yorkeys Knob already fixed for the gene drive system. To determine confineability, we simulated batch migration events from Yorkeys Knob (fixed for the gene drive system) to the neighboring community of Trinity Park (initially wild-type). The MGDrivE framework models the egg, larval, pupal and adult (male and female) mosquito life stages implementing a daily time step, overlapping generations and a mating structure in which adult males mate throughout their lifetime, while adult females mate once upon emergence, retaining the genetic material of the adult male with whom they mate for the duration of their adult lifespan. Density-independent mortality rates for the juvenile life stages are assumed to be identical and are chosen for consistency with the population growth rate in the absence of density-dependent mortality. Additional density-dependent mortality occurs at the larval stage, the form of which is taken from Deredec *et al.* (28). Full details of the modeling framework are available in the S1 Text of Sánchez *et al.* (26), and in the software documentation available at https://marshalllab.github.io/MGDrivE/docs/reference/. Parameters describing *Ae. aegypti* life history and the gene drive systems and landscape of interest are listed in Table S1.

The inheritance patterns for reciprocal chromosomal translocations (depicted in Figure 1A) and UD^{MEL} (depicted in Figure S1) are modeled within the inheritance module of the MGDrivE framework (26), along with their impacts on female fecundity and adult lifespan. The distribution of households in Yorkeys Knob and Trinity Park were taken from OpenStreetMap (https://www.openstreetmap.org/) (Figure 1C). We implement the stochastic version of the MGDrivE framework to capture the randomness associated with events that occur in small populations, such as households, which serve as nodes in the landscape modeled here. In the stochastic implementation of the model, the number of eggs produced per day by females follows a Poisson distribution, the number of eggs having each genotype follows a multinomial

distribution, all survival/death events follow a Bernoulli distribution, and female mate choice follows a multinomial distribution with probabilities given by the relative frequency of each adult male genotype in the population.

Acknowledgements:
The authors would like to thank Dr. Gregory Lanzaro, Dr. Yoosook Lee and Ms. Partow Imani for discussions on *Aedes aegypti* life history and dispersal behavior. This work was supported by a DARPA Safe Genes Program Grant (HR0011-17-2-0047), awarded to OSA and JMM, and funds from the Innovative Genomics Institute, awarded to JMM.

Author contributions:
JMM and HMSC conceptualized the study. HMSC, JBB and SLW performed the experiments and visualized the results. GR and OSA contributed to the interpretation of the results. JMM wrote the first draft of the manuscript. All authors contributed to the writing of and approved the final manuscript.

Competing interests:
All authors declare no competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

References:

1. Doudna JA, Charpentier E (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346(6213):1258096.
2. Champer J, Buchman A, Akbari OS (2016) Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nat Rev Genet* 17(3):146–159.
3. Gantz VM, et al. (2015) Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. *Proc Natl Acad Sci U S A* 112(49):E6736–43.
4. Hammond A, et al. (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nat Biotechnol* 34(1):78–83.
5. Kyrou K, et al. (2018) A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nat Biotechnol* 36(11):1062–1066.
6. Marshall JM, Akbari OS (2018) Can CRISPR-based gene drive be confined in the wild? A question for molecular and population biology. *ACS Chemical Biology* 13(2):424–430.
7. Leftwich PT, et al. (2018) Recent advances in threshold-dependent gene drives for mosquitoes. *Biochem Soc Trans* 46(5):1203–1212.
8. Alphey L (2002) Re-engineering the sterile insect technique. *Insect Biochem Mol Biol* 32(10):1243–1247.
9. Kandul NP, et al. (2019) Transforming insect population control with precision guided sterile males with demonstration in flies. *Nat Commun* 10(1):84.
10. Galizi R, et al. (2014) A synthetic sex ratio distortion system for the control of the human malaria mosquito. *Nat Commun* 5:3977.
11. Akbari OS, et al. (2015) Safeguarding gene drive experiments in the laboratory. *Science*

349(6251):927–929.
12. Noble C, et al. (2019) Daisy-chain gene drives for the alteration of local populations. *Proc Natl Acad Sci U S A*. doi:10.1073/pnas.1716358116.
13. Gould F, Huang Y, Legros M, Lloyd AL (2008) A killer-rescue system for self-limiting gene drive of anti-pathogen constructs. *Proc Biol Sci* 275(1653):2823–2829.
14. Buchman AB, Ivy T, Marshall JM, Akbari OS, Hay BA (2018) Engineered reciprocal chromosome translocations drive high threshold, reversible population replacement in *Drosophila*. *ACS Synth Biol* 7(5):1359–1370.
15. Akbari OS, et al. (2013) A synthetic gene drive system for local, reversible modification and suppression of insect populations. *Curr Biol* 23(8):671–677.
16. Marshall JM, Hay BA (2012) Confinement of gene drive systems to local populations: A comparative analysis. *Journal of Theoretical Biology* 294:153–171.
17. Altrock PM, Traulsen A, Reeves RG, Reed FA (2010) Using underdominance to bi-stably transform local populations. *J Theor Biol* 267(1):62–75.
18. Champer J, Zhao J, Champer S, Liu J, Messer PW Population dynamics of underdominance gene drive systems in continuous space. doi:10.1101/449355.
19. Huang Y, Lloyd AL, Legros M, Gould F (2011) Gene-drive into insect populations with age and spatial structure: a theoretical assessment. *Evol Appl* 4(3):415–428.
20. Davis S, Bax N, Grewe P (2001) Engineered underdominance allows efficient and economical introgression of traits into pest populations. *J Theor Biol* 212(1):83–98.
21. Hoffmann AA, et al. (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476(7361):454–457.
22. Ritchie SA, Montgomery BL, Hoffmann AA (2013) Novel estimates of *Aedes aegypti* (Diptera: Culicidae) population size and adult survival based on *Wolbachia* releases. *J Med Entomol* 50(3):624–631.
23. Walker T, et al. (2011) The *wMel Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476(7361):450–453.
24. Hartberg WK (1971) Observations on the mating behaviour of *Aedes aegypti* in nature. *Bull World Health Organ* 45(6):847–850.
25. Schmidt TL, et al. (2017) Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti*. *PLoS Biol* 15(5):e2001894.
26. Sánchez C. HM, Wu SL, Bennett JB, Marshall JM MGDrivE: A modular simulation framework for the spread of gene drives through spatially-explicit mosquito populations. doi:10.1101/350488.
27. Hancock PA, Godfray HCJ (2007) Application of the lumped age-class technique to studying the dynamics of malaria-mosquito-human interactions. *Malar J* 6:98.
28. Deredec A, Godfray HCJ, Burt A (2011) Requirements for effective malaria control with homing endonuclease genes. *Proc Natl Acad Sci U S A* 108(43):E874–80.
29. Marshall JM, Buchman A, Sánchez C HM, Akbari OS (2017) Overcoming evolved resistance to population-suppressing homing-based gene drives. *Sci Rep* 7(1):3776.
30. Serebrovskii AS (1940) On the possibility of a new method for the control of insect pests. *Zool Zhurnal* 19:618–630.
31. Curtis CF (1968) Possible use of translocations to fix desirable genes in insect pest populations. *Nature* 218(5139):368–369.

32. Curtis CF, Robinson AS (1971) Computer simulation of the use of double translocations for pest control. *Genetics* 69(1):97–113.
33. Wright S (1941) On the probability of fixation of reciprocal translocations. *The American Naturalist* 75(761):513–522.
34. Guerra CA, et al. (2014) A global assembly of adult female mosquito mark-release-recapture data to inform the control of mosquito-borne pathogens. *Parasit Vectors* 7:276.
35. Harrington LC, et al. (2005) Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *The American Journal of Tropical Medicine and Hygiene* 72(2):209–220.
36. Carvalho DO, et al. (2015) Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. *PLoS Negl Trop Dis* 9(7):e0003864.
37. Schmidt TL, Filipović I, Hoffmann AA, Rašić G (2018) Fine-scale landscape genomics helps explain the slow spatial spread of *Wolbachia* through the *Aedes aegypti* population in Cairns, Australia. *Heredity* 120(5):386–395.
38. Khamis D, El Mouden C, Kura K, Bonsall MB (2018) Ecological effects on underdominance threshold drives for vector control. *Journal of Theoretical Biology* 456:1–15.
39. Marshall JM (2009) The effect of gene drive on containment of transgenic mosquitoes. *J Theor Biol* 258(2):250–265.
40. Noble C, Adlam B, Church GM, Esvelt KM, Nowak MA (2018) Current CRISPR gene drive systems are likely to be highly invasive in wild populations. *Elife* 7. doi:10.7554/eLife.33423.
41. Maselko M, Heinsch SC, Chacón JM, Harcombe WR, Smanski MJ (2017) Engineering species-like barriers to sexual reproduction. *Nat Commun* 8(1):883.
42. Hancock PA, et al. (2016) Density-dependent population dynamics in *Aedes aegypti* slow the spread of *w Mel Wolbachia*. *Journal of Applied Ecology* 53(3):785–793.
43. Ross PA, Endersby NM, Yeap HL, Hoffmann AA (2014) Larval competition extends developmental time and decreases adult size of *wMelPop Wolbachia*-infected *Aedes aegypti*. *Am J Trop Med Hyg* 91(1):198–205.
44. Hancock PA, Godfray HCJ (2012) Modelling the spread of *Wolbachia* in spatially heterogeneous environments. *J R Soc Interface* 9(76):3045–3054.
45. Hancock PA, et al. Predicting the spatial dynamics of *Wolbachia* infections in *Aedes aegypti* arbovirus vector populations in heterogeneous landscapes. doi:10.1101/458794.

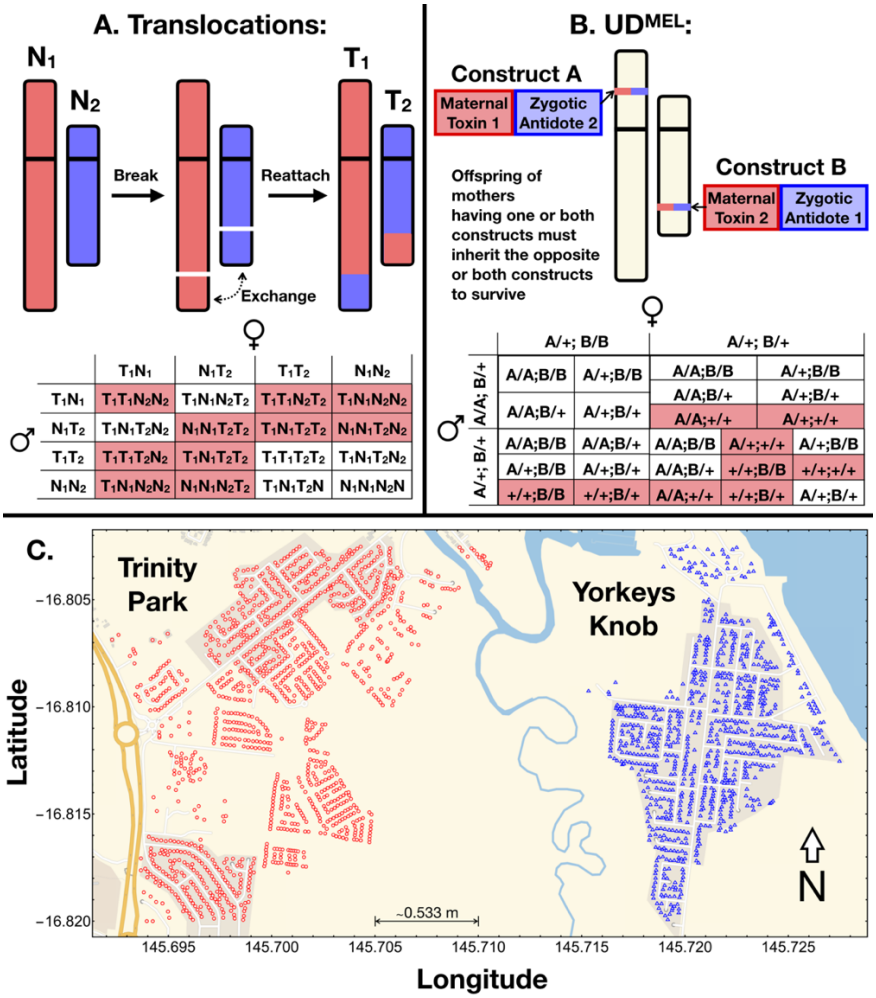

Figure 1. Inheritance and landscape features of the modeling framework. *(A)* Reciprocal translocations (T1 and T2) result from the mutual exchange between terminal segments of two non-homologous chromosomes (N1 and N2). The cross here depicts possible parental gametes, with respect to the translocation, and offspring that result from matings between them. Matings between wild-type organisms or translocation homozygotes result in viable offspring; but translocation heterozygotes produce unbalanced gametes, and many of the resulting offspring are unviable (shaded). This results in a heterozygote disadvantage and threshold-dependent population dynamics. *(B)* UD^{MEL} is composed of two unlinked constructs (here referred to as A and B), each consisting of a maternally-expressed toxin and a zygotically-expressed antidote for the toxin on the opposite construct. The cross here represents matings between two of the nine possible parental genotypes ("+" represents the wild-type allele, and "A" and "B" represent alleles corresponding to the two UD^{MEL} constructs). The complete inheritance pattern is depicted in Figure S1. Offspring lacking the antidotes to the maternal toxins produced by their mother are unviable (shaded). At high population frequencies, the selective advantage on the constructs, by virtue of the antidotes, outweighs the fitness load due to the toxins, and hence results in frequency-dependent spread. *(C)* Distribution of households in Yorkeys Knob (blue) and Trinity Park (red) in Cairns, Queensland, Australia. Households serve as *Aedes aegypti* metapopulation nodes in our simulations, with movement of adult *Ae. aegypti* between them. Yorkeys Knob serves as a simulated release site, and Trinity Park as a simulated control site.

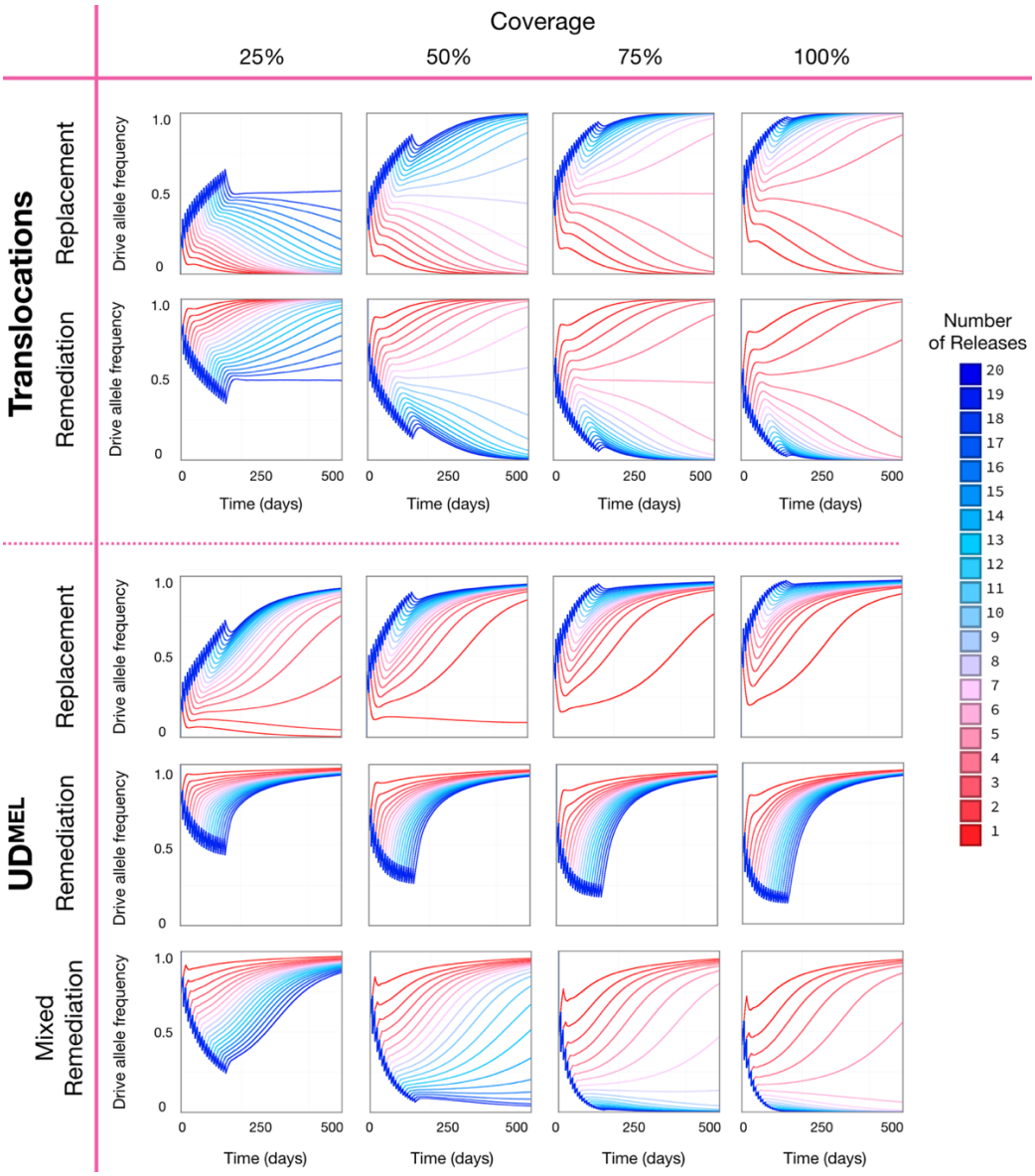

Figure 2. Replacement and remediation results for translocations and UD^{MEL}. Time-series results are shown for a given number of weekly releases of 20 adult *Ae. aegypti* per household with the intent of population replacement or remediation in the community of Yorkeys Knob (Figure 1C), and at given coverage levels, where coverage is the proportion of households that receive the releases. For population replacement, releases are of males homozygous for the translocation or UD^{MEL} into a wild-type population. For remediation, releases are of wild-type males into a population homozygous for the translocation or UD^{MEL}. *(Top)* Replacement and remediation are symmetric for translocations. At a coverage of 50%, 11 or more releases result in the translocation being driven to fixation or remediated from the population. *(Bottom)* For UD^{MEL}, remediation is not possible through releases of males only, and so "mixed remediation" is considered, in which releases consist of 10 females and 10 males. Release requirements for UD^{MEL} are smaller for population replacement, but larger for mixed remediation. At a coverage of 50%, two or more releases result in UD^{MEL} being driven to fixation; however, 17 releases of both females and males are required to remediate UD^{MEL} from the population.

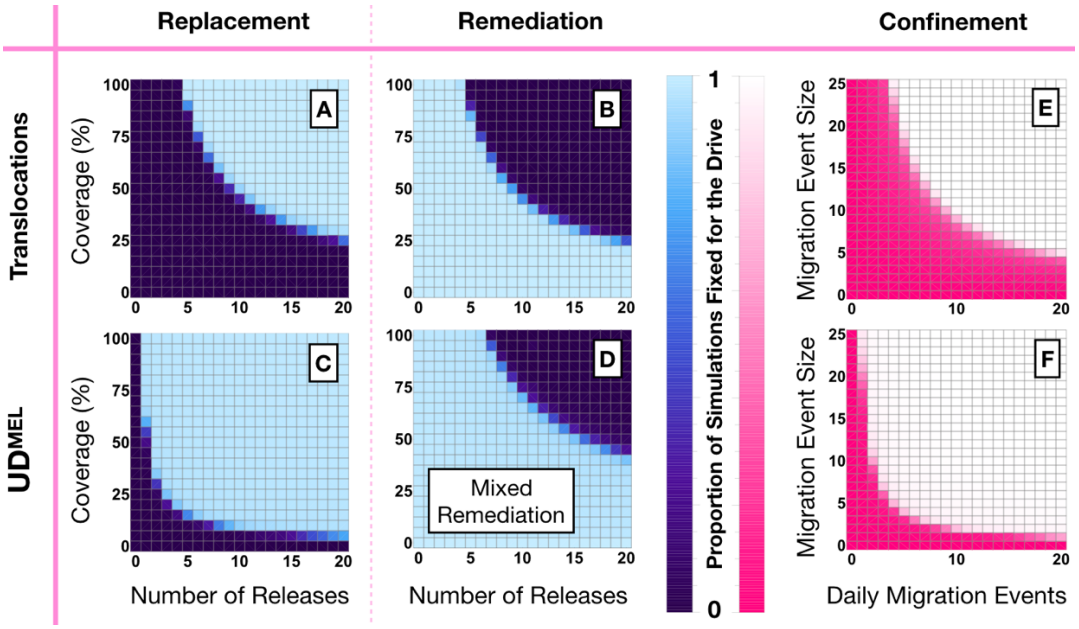

Figure 3. Replacement, remediation and confinement outcomes for translocations and UD^{MEL}. Outcomes are depicted for the proportion of 50 stochastic simulations of population replacement, remediation and confinement of translocations and UD^{MEL} that result in fixation of each system. *(A-D)* For replacement and remediation, each cell corresponds to a given number of releases (horizontal axis) and coverage level (vertical axis), given 20 adult *Ae. aegypti* per household per release. For replacement, releases are of males homozygous for the system into a wild-type population. For remediation of translocations, releases are of wild-type males into a population homozygous for the translocation, and for mixed remediation of UD^{MEL}, releases are of wild-type females and males into a population homozygous for UD^{MEL}. Light blue cells represent cases where all simulations result in fixation of the system, and dark blue cells represent cases where the wild-type is fixed in all simulations. *(E-F)* For confinement, each cell corresponds to a daily number of batch migration events (horizontal axis) of a given size (vertical axis) from Yorkeys Knob, where the system is fixed, to Trinity Park, where the system is initially absent. White cells represent cases where all simulations result in fixation of the system in Trinity Park, and dark pink cells represent cases where the wild-type is fixed in all simulations. These results are encouraging for translocations as systems for introducing transgenes in a local and reversible way as: i) they can be remediated through an achievable number of male-only releases, and ii) they require more batch migration events to spread to neighboring communities.

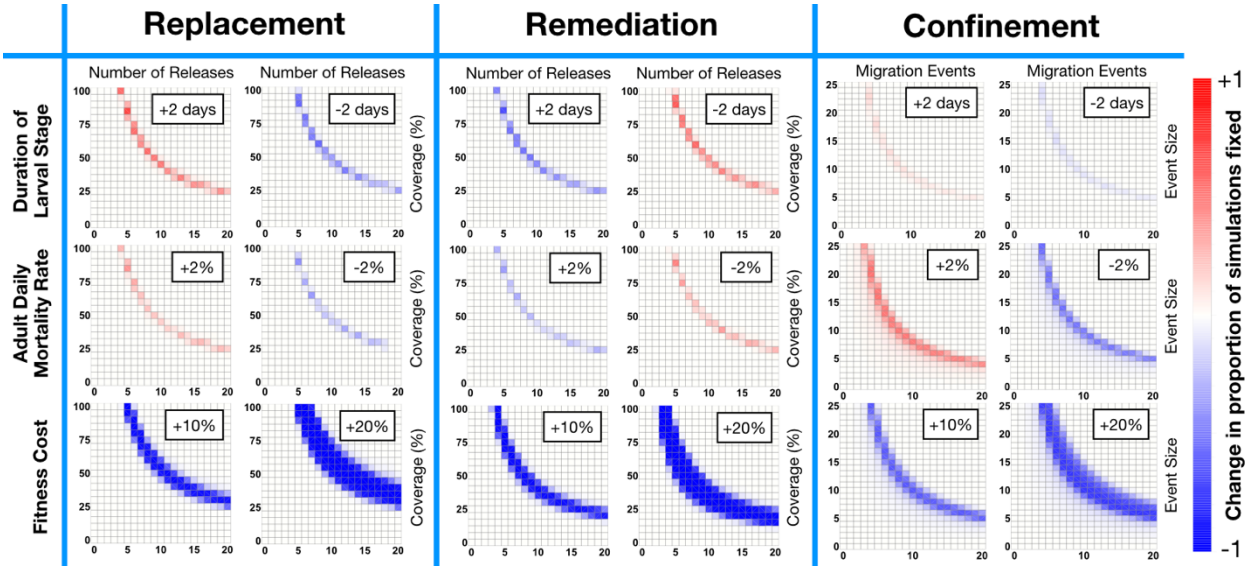

Figure 4. Sensitivity of model outcomes for replacement, remediation and confinement of translocations. Changes are depicted in the proportion of 50 stochastic simulations that result in fixation for replacement, remediation and confinement of translocations. Proportions are compared to those in the first row of Figure 3 as we vary: i) the duration of the larval life stage (+/- 2 days), ii) the adult daily mortality rate (+/- 2%), and iii) the fitness cost associated with being homozygous for the translocation (+10% or +20%). Fitness costs have the greatest impact on the release scheme required for the system to be fixed or remediated from the population, given the life parameters considered. Fitness costs also lead to more batch migration events being required for invasion of Trinity Park. A small increase in the baseline adult mortality rate leads to slightly fewer batch migration events being required for invasion of Trinity Park; however, comparison to migration rates inferred from field data suggests that confinement is still expected.

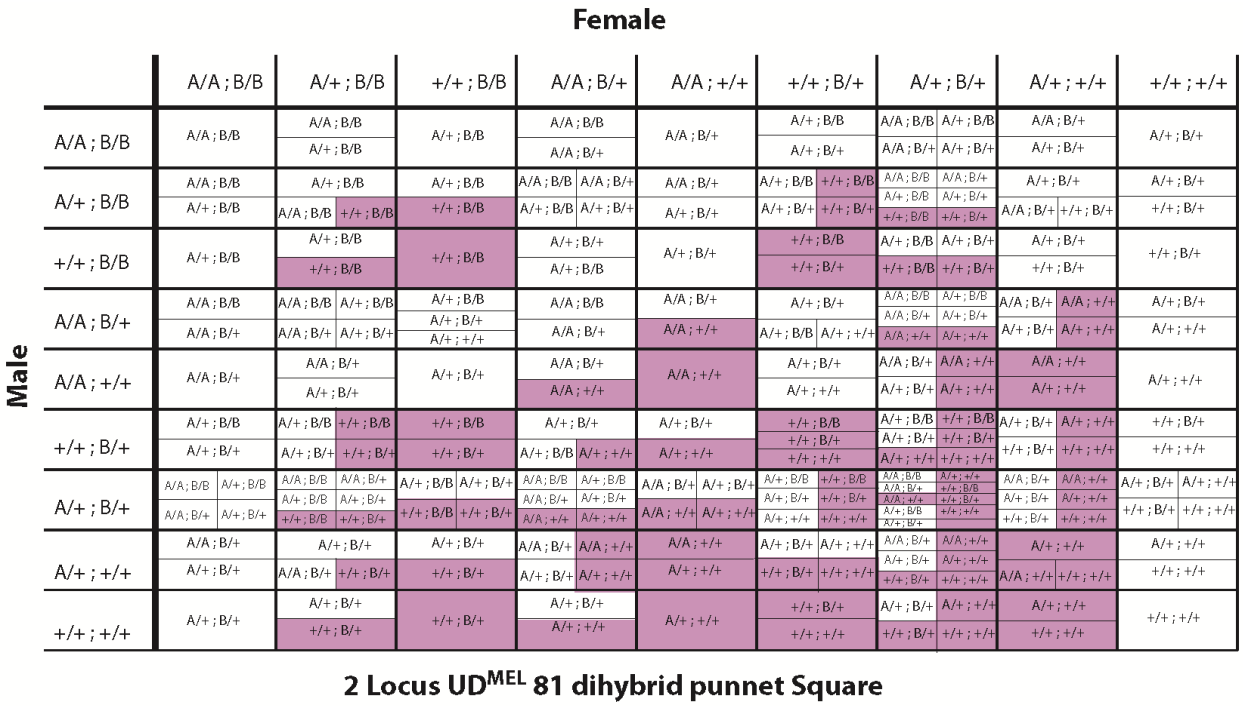

Figure S1. Complete inheritance pattern of UD^{MEL}. UD^{MEL} is composed of two unlinked constructs (here referred to as A and B), each consisting of a maternally-expressed toxin and a zygotically-expressed antidote for the toxin on the opposite construct (see Figure 1B). The cross here represents matings between all nine possible parental genotypes ("+" represents the wild-type allele, and "A" and "B" represent alleles corresponding to the two UD^{MEL} constructs). Offspring lacking the antidotes to the maternal toxins produced by their mother are unviable (shaded). At high population frequencies, the selective advantage on the constructs, by virtue of the antidotes, outweighs the fitness load due to the toxins, and hence results in frequency-dependent spread.

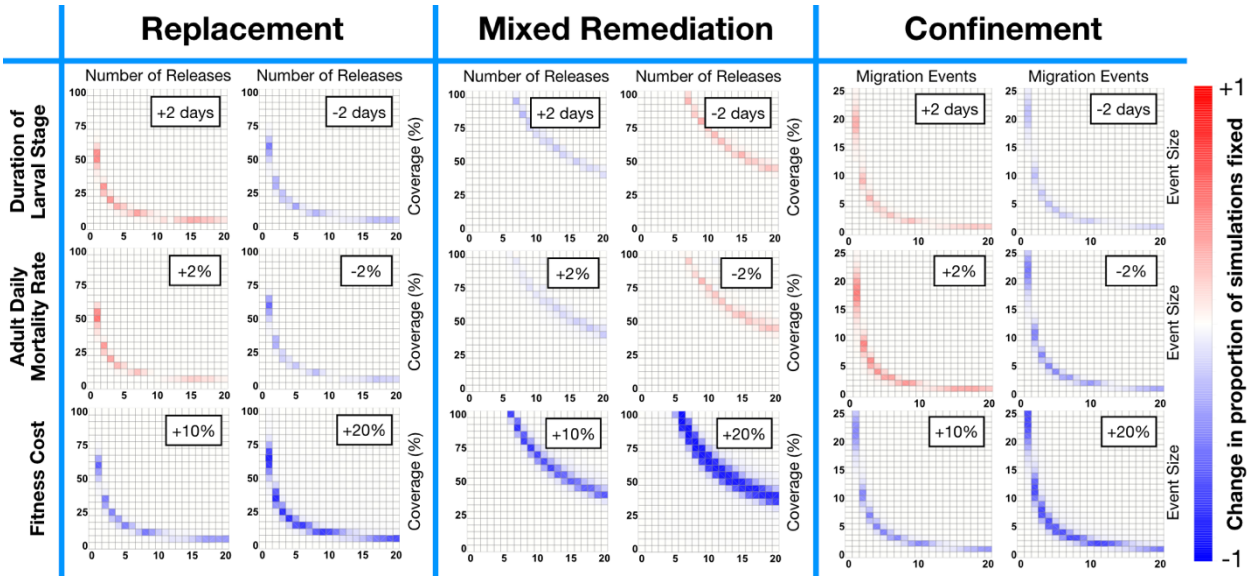

Figure S2. Sensitivity of model outcomes for replacement, remediation and confinement of UD^{MEL}. Changes are depicted in the proportion of 50 stochastic simulations that result in fixation for replacement, remediation and confinement of UD^{MEL}. Proportions are compared to those in the second row of Figure 3 as we vary: i) the duration of the larval life stage (+/- 2 days), ii) the adult daily mortality rate (+/- 2%), and iii) the fitness cost associated with being homozygous for the UD^{MEL} (+10% or +20%). UD^{MEL} displays similar parameter sensitivities regarding fixation, remediation and batch migration outcomes as for translocations (Figure 4), with the exception that these outcomes are less sensitive to fitness costs, likely due to the fact that fitness is accommodated through a reduction in female fecundity rather than an increase in adult mortality.

Table S1. Life history, population size and movement parameters for *Aedes aegypti* in Cairns, Australia.

Parameter	Symbol	Value	Reference
Egg production per female (day^{-1})	β	20	(1)
Duration of egg stage (days)	T_E	5	(2)
Duration of larval stage (days)	T_L	6	(2)
Duration of pupa stage (days)	T_P	4	(2)
Daily population growth rate (day^{-1})	r	1.175	(3)
Daily mortality rate of adult stage (day^{-1})	μ_M	0.090	(4–6)
Adult population size per household	N_H	15	(7)
Daily probability adult leaves household	p	0.28	(8, 9)
Mean dispersal distance of adult	d	54.1	(10)

1. Otero M, Solari HG, Schweigmann N (2006) A stochastic population dynamics model for *Aedes aegypti*. *Bull Math Biol* 68(8):1945–1974.
2. Christophers SR (1960) *Aëdes Aegypti (L.), the Yellow Fever Mosquito: Its Life History, Bionomics, and Structure* (Cambridge University Press).
3. Simoy MI, Simoy MV, Canziani GA (2015) The effect of temperature on the population dynamics of *Aedes aegypti*. *Ecological Modelling* 314:100–110.
4. Focks DA, Haile DG, Daniels E, Mount GA (1993) Dynamic life table model for *Aedes aegypti*: analysis of the literature and model development. *J Med Entomol* 30(6):1003–1017.
5. Horsfall WR (1955) *Mosquitoes: Their Bionomics and Relation to Disease*.
6. Fay RW (1964) The biology and bionomics of *Aedes aegypti* in the laboratory. *Mosq News* 24:300–308.
7. Ritchie SA, Montgomery BL, Hoffmann AA (2013) Novel estimates of *Aedes aegypti* (Diptera: Culicidae) population size and adult survival based on *Wolbachia* releases. *J Med Entomol* 50(3):624–631.
8. Schmidt TL, Filipović I, Hoffmann AA, Rašić G (2018) Fine-scale landscape genomics helps explain the slow spatial spread of *Wolbachia* through the *Aedes aegypti* population in Cairns, Australia. *Heredity* 120(5):386–395.
9. Harrington LC, et al. (2005) Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *Am J Trop Med Hyg* 72(2):209–220.
10. Guerra CA, et al. (2014) A global assembly of adult female mosquito mark-release-recapture data to inform the control of mosquito-borne pathogens. *Parasites & Vectors* 7(1):276.