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Report

# A Synthetic Gene Drive System for Local, Reversible Modification and Suppression of Insect Populations

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## Summary

**Replacement of wild insect populations with genetically modified individuals unable to transmit disease provides a self-perpetuating method of disease prevention but requires a gene drive mechanism to spread these traits to high frequency [1–3]. Drive mechanisms requiring that transgenes exceed a threshold frequency in order to spread are attractive because they bring about local but not global replacement, and transgenes can be eliminated through dilution of the population with wild-type individuals [4–6]. These features are likely to be important in many social and regulatory contexts [7–10]. Here we describe the first creation of a synthetic threshold-dependent gene drive system, designated maternal-effect lethal underdominance ($UD^{MEL}$), in which two maternally expressed toxins, located on separate chromosomes, are each linked with a zygotic antidote able to rescue maternal-effect lethality of the other toxin. We demonstrate threshold-dependent replacement in single- and two-locus configurations in *Drosophila*. Models suggest that transgene spread can often be limited to local environments. They also show that in a population in which single-locus $UD^{MEL}$ has been carried out, repeated release of wild-type males can result in population suppression, a novel method of genetic population manipulation.**

## Results and Discussion

Threshold-dependent, chromosomal gene drive can occur when transgene-bearing chromosomes experience frequency-dependent changes in fitness with respect to non-transgene-bearing homologs such that the former have lower fitness at low frequency and higher fitness at high frequency. These systems behave as a bistable switch, with the transgene-bearing chromosomes spreading to genotype fixation (the transgene is present in all individuals), and in some cases allele fixation (transgenes are present on all versions of the chromosome in the population), when present above a threshold frequency, while being lost from the population if their frequency falls below this threshold [4–6, 11–15]. Most threshold-dependent gene drive mechanisms have not been implemented; for those that have, such as translocations, large fitness costs and the practical difficulty in tightly linking genes of interest to the translocation breakpoint through inclusion within an inversion kept the system from further development [16, 17]. The threshold-dependent gene drive system described here, known as maternal-effect lethal underdominance ($UD^{MEL}$), bears some similarity to an earlier proposed form of purely zygotic engineered underdominance [13]. The $UD^{MEL}$ system utilizes two constructs, each consisting of a maternally expressed toxin and a zygotically expressed antidote. Each antidote is linked with a toxin whose activity it does not rescue (toxin A linked with antidote B and vice versa). As a result, the survival of embryos from mothers carrying one ($UD^{MEL\text{-}1}$) or both ($UD^{MEL\text{-}1}$ and $UD^{MEL\text{-}2}$) kinds of $UD^{MEL}$ chromosomes requires that they inherit the other ($UD^{MEL\text{-}2}$) or both kinds of $UD^{MEL}$ chromosomes, respectively, in order to achieve zygotic rescue (Figures 1A and 1B; see also Figure S1 available online). The likelihood of this happening is frequency dependent and represents a form of underdominant (heterozygous disadvantage) behavior. Finally, because the toxins are only active when present in adult females, $UD^{MEL}$ chromosomes can be passed from males to viable offspring at normal Mendelian ratios. Each construct, consisting of a toxin-antidote pair, can be located at the same position on homologous chromosomes (single-locus $UD^{MEL}$) (Figure 1C). In this configuration, viable transgenics are always transheterozygotes, with half the progeny dying in each generation, as with some naturally occurring balanced lethal systems [18, 19] (Figure S1A). Alternatively, $UD^{MEL}$ constructs can be located on nonhomologous chromosomes (two-locus $UD^{MEL}$) (Figure 1C), in which case many more genotypes are viable (Figure S1B).

We used a simple difference equation framework to model the spread of single- and two-locus $UD^{MEL}$ through a randomly mating population. To investigate the confinement properties of single- and two-locus $UD^{MEL}$, we follow the framework of Marshall and Hay [6], assuming a metapopulation model consisting of two populations, each of which exchanges a fraction of its population, $\mu$, with the other each generation (Figure 2A). This model assumes that population size is constant, being limited by the carrying capacity of the environment, not the number of viable offspring produced per mating pair, as is observed with a majority of agricultural pests and disease vectors [20, 21] (see Supplemental Experimental Procedures for details). Transgenic insects are introduced into population A, whereas population B initially consists of wild-type individuals.

In the absence of other fitness costs, the single-locus system has a release threshold for transheterozygous males equivalent to 78% of the total (transgenic and +/+) population (6.5:1 transgenic males: wild males and females) (Figure 2B). This threshold decreases to a much more reasonable 44% (0.78:1) if releases are spread over two generations (Figure 2C). In contrast, the threshold for a single release of males carrying two copies of an autosomal element and one copy of an X-linked element, a form of two-locus $UD^{MEL}$, is significantly lower, 37% (Figure 2D). A single release of males doubly homozygous for two-locus autosomal $UD^{MEL}$ has an even lower threshold, 24% (Figure 2E). Elements with an additive fitness cost can still drive, though as expected, they have somewhat increased introduction thresholds (illustrated in Figures S2A–S2D for a 10% cost). Interestingly, for all

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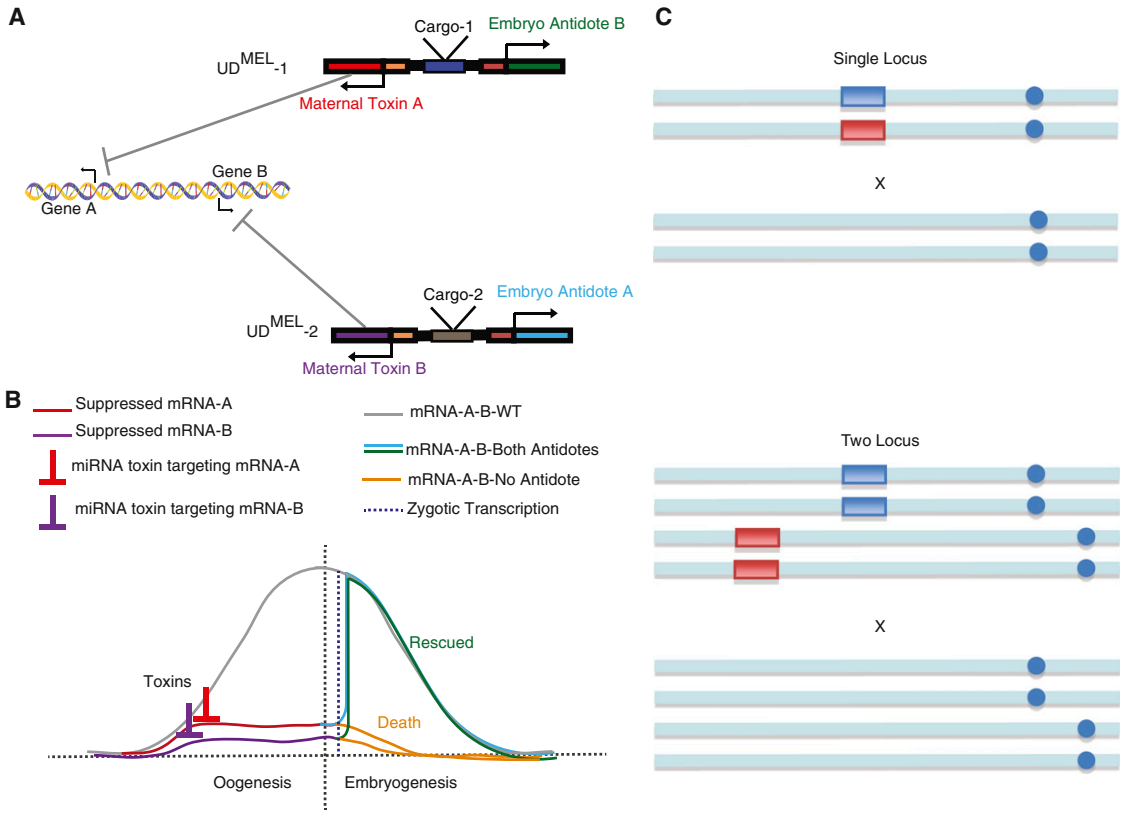



Figure 1. Schematic of the UD$^{MEL}$ Drive System and Illustration of How UD$^{MEL}$ Rewires Developmental Gene Expression

(A) The UD$^{MEL}$ system is composed of two constructs. UD$^{MEL-1}$ consists of maternal toxin A (red) and embryo antidote B (green), and UD$^{MEL-2}$ consists of maternal toxin B (purple) and embryo antidote A (light blue).

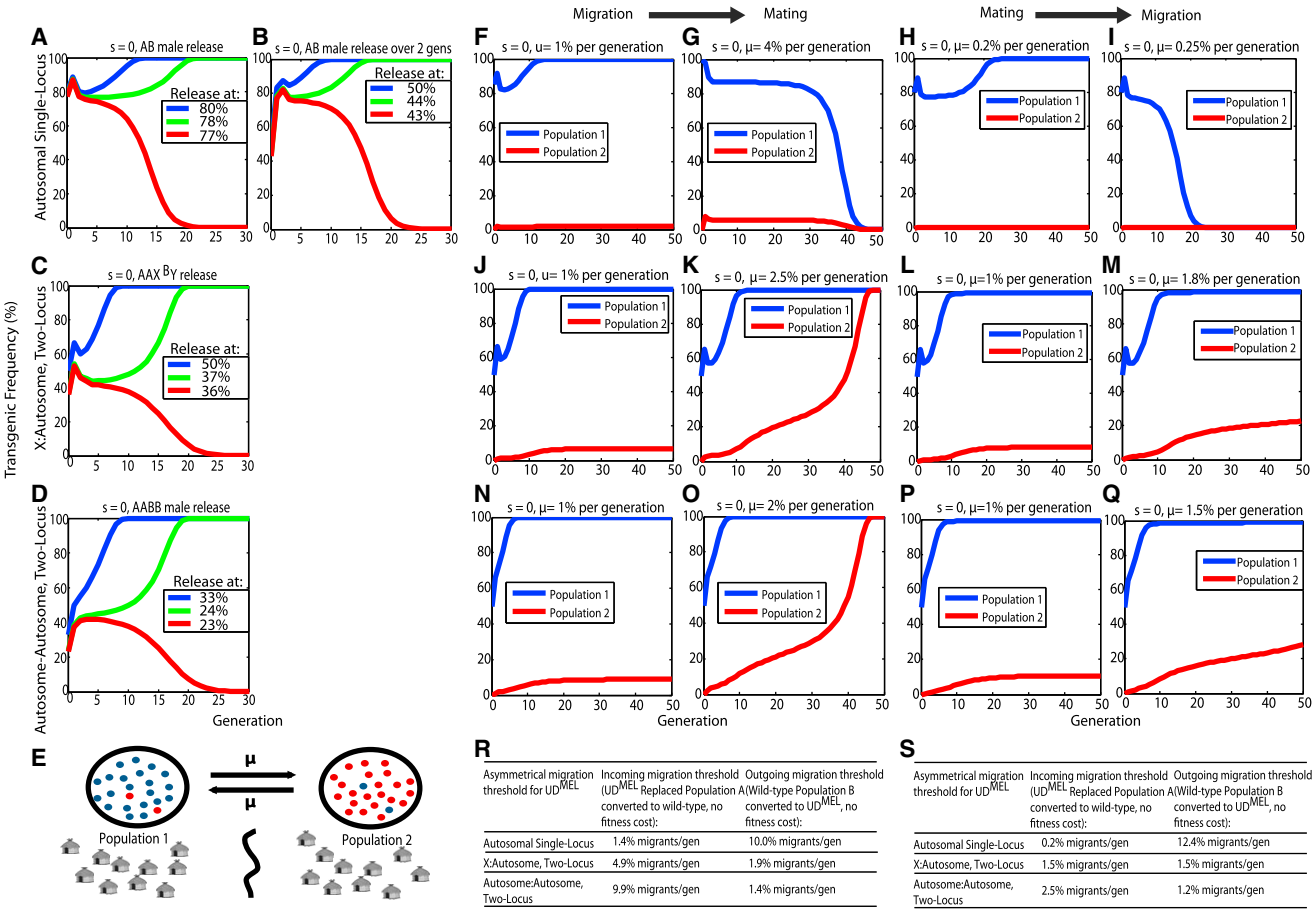
(B) In wild-type mothers, maternal transcripts from gene A and gene B (gray line) are required for normal embryonic development. The toxins, multimers of miRNAs, degrade one or both of these mRNAs (red line for UD$^{MEL-1}$ toxin A targeting gene A; purple line for UD$^{MEL-2}$ toxin B targeting gene B), to which they are complementary. Embryos lacking one or both mRNAs and/or their products (orange lines), depending on whether the mother is heterozygous or transheterozygous, respectively, for the miRNA multimers, die. Progeny inheriting the other construct, or both constructs, survive because they express miRNA-resistant versions of the mRNAs (blue and green) in the early zygote at levels sufficient to rescue embryonic development. The dotted dark blue line corresponds to the initiation of zygotic transcription.

(C) Single- and two-locus UD$^{MEL}$ configurations are illustrated, with the two different constructs being indicated by the blue and red boxes, and homologous and nonhomologous chromosomes being indicated by the positions of their centromeres (blue circles). Wild-type chromosomes lack boxes.

reasonable fitness costs (up to 50% in the two-locus systems; data not shown), when spread occurs, it results in fixation of the transgenic allele (illustrated in Figures S3A–S3D for a 10% fitness cost).

In a system such as UD$^{MEL}$, in which progeny survival depends on parental and progeny genotypes, it matters whether mating occurs before (ma-mi) or after (mi-ma) migration, because the genotype distributions within the two populations will often be quite different. Here we consider the two extreme cases, in which all mating occurs before or after migration, to illustrate the range of possible outcomes. In single-locus UD$^{MEL}$, when migration precedes mating and occurs at a rate of 1% per generation (the migration rate of *Anopheles gambiae* between neighboring villages in Mali [22]), transgenes spread to near fixation at the release site (the failure to fix reflecting back migration of wild-types from population 2), and transgenics reach a frequency of 2.0% in neighboring populations (Figure 2F). Higher migration rates, up to 3.9% per generation, also fail to show drive in neighboring populations, with the frequency of transgenics peaking at 7.1% for a migration rate of 3.5% per generation. When the migration rate is 4% or higher, transgenics are eliminated from both populations (Figure 2G) because migration of wild-type individuals into the release population results in the frequency of transgenics falling below the threshold required for spread. When mating precedes migration, the dynamics are qualitatively similar (Figures 2H and 2I), but the threshold for loss from both populations decreases dramatically, to 0.25% (Figure 2I), with transgenics reaching a maximum frequency of 0.1% in population 2. In consequence, the single-locus system is highly confineable and is only able to show drive in isolated populations.

In two-locus X-autosome UD$^{MEL}$, when migration precedes mating and occurs at a rate of 1% per generation, transgenes spread to near fixation at the release site, and transgenics reach a frequency of 8% in neighboring populations (Figure 2J; also ~8% when ma-mi, Figure 2L). However, in contrast to the case of single-locus UD$^{MEL}$, two-locus X-autosome UD$^{MEL}$ shows (in the absence of fitness costs) a clear migration threshold, above which the system becomes established in both populations (2% when mi-ma, Figure 2K; 1.7% when ma-mi, Figure 2M). Two-locus autosome-autosome UD$^{MEL}$ shows characteristics similar to those of two-locus X-autosome UD$^{MEL}$, though higher frequencies of transgenics are




R

| Asymmetrical migration threshold for UD$^{MEL}$ | Incoming migration threshold (UD$^{MEL}$ Replaced Population A converted to wild-type, no fitness cost): | Outgoing migration threshold (Wild-type Population B converted to UD$^{MEL}$, no fitness cost): |
|---|---|---|
| Autosomal Single-Locus | 1.4% migrants/gen | 10.0% migrants/gen |
| X:Autosome, Two-Locus | 4.9% migrants/gen | 1.9% migrants/gen |
| Autosome:Autosome, Two-Locus | 9.9% migrants/gen | 1.4% migrants/gen |

S

| Asymmetrical migration threshold for UD$^{MEL}$ | Incoming migration threshold (UD$^{MEL}$ Replaced Population A converted to wild-type, no fitness cost): | Outgoing migration threshold (Wild-type Population B converted to UD$^{MEL}$, no fitness cost): |
|---|---|---|
| Autosomal Single-Locus | 0.2% migrants/gen | 12.4% migrants/gen |
| X:Autosome, Two-Locus | 1.5% migrants/gen | 1.5% migrants/gen |
| Autosome:Autosome, Two-Locus | 2.5% migrants/gen | 1.2% migrants/gen |

Figure 2. UD$^{MEL}$ Single- and Two-Locus Systems Are Predicted to Show Threshold-Dependent Gene Drive and Bring about Local Population Replacement

(A and B) The threshold frequency above which a UD$^{MEL}$ drive system spreads into a population and below which it is eliminated from the population was calculated using a deterministic model and graphed. Release thresholds are calculated for two single-locus scenarios: a single, all-male release of transheterozygotes (A) and two all-male releases of transheterozygotes in the first and second generation (gens) (B), for elements with zero fitness cost (s).

(C and D) For X-autosome two-locus UD$^{MEL}$ (C) and autosome-autosome two-locus UD$^{MEL}$ (D), single releases of doubly homozygous males are illustrated. Transgenic frequency represents the fraction of individuals in the total population carrying at least one UD$^{MEL}$ construct.

(E) Two-way migration occurs between population 1, illustrated as a group village of houses, which has undergone population replacement, and population 2, which is separated from population 1 by a barrier (vertical line) and is initially all wild-type.

(F–Q) Plots depict the dynamics of single- and two-locus UD$^{MEL}$ under two-population models in which migrants are exchanged between population 1 (blue line), which has been seeded with transgenics, and population 2 (red line), which initially consists only of wild-types. Migration occurs either prior to mating (F, G, J, K, N, and O) or after mating (H, I, L, M, P, and Q).

(F and G) For single-locus UD$^{MEL}$, when migration occurs before mating and the migration rate is 1%, transgenics spread to high levels in population 1 and reach a frequency of 2.0% in population 2 (F); when the migration rate is 4%, transgenes are ultimately eliminated from both populations (G).

(H and I) When mating occurs before migration and the migration rate is 0.2%, transgenics spread to high frequency in population 1 and reach a frequency of ~1% in population 2 (H); a migration rate of 0.25% or higher results in loss of transgenes from both populations (I).

(J and K) For X-autosome two-locus UD$^{MEL}$, when migration occurs before mating and the migration rate is 1%, transgenics spread to high frequency in population 1 and reach a frequency of 8% in population 2 (J); a migration rate of 1.8% or higher (2.5% is illustrated) results in spread to fixation in both populations (K).

(L and M) When mating occurs before migration and the migration rate is 1%, transgenics spread to high frequency in population 1 and reach a frequency of 8% in population 2 (L); a migration rate of 1.7% or higher (1.8% is illustrated) results in spread to fixation in both populations (M).

(N and O) For autosome-autosome, two-locus UD$^{MEL}$, when migration occurs before mating and the migration rate is 1%, transgenics spread to high frequency in population 1 and reach a frequency of 8.8% in population 2 (N); a migration rate of 1.65% or higher (2% is illustrated) results in spread to fixation in both populations (O).

(P and Q) When mating occurs before migration and the migration rate is 1%, transgenics spread to high frequency in population 1 and reach a frequency of 11% in population 2 (P); a migration rate of 1.4% or higher (1.5% is illustrated) results in spread to fixation in both populations (Q).

(R) Asymmetrical migration thresholds required to eliminate UD$^{MEL}$ from a replaced population or to drive population replacement in a neighboring wild-type population are indicated for single-locus UD$^{MEL}$, X-autosome two-locus UD$^{MEL}$, and autosome-autosome two-locus UD$^{MEL}$. Migration occurs before mating.

(S) Asymmetrical migration thresholds as in (R), but for the case in which mating occurs before migration.

found in neighboring populations (8.8% when mi-ma, Figure 2N; 11% when ma-mi, Figure 2P) and the migration threshold is lower (~1.65% when mi-ma, Figure 2O; 1.4% when ma-mi, Figure 2Q). Thus, although versions of two-locus UD$^{MEL}$ can be confined to isolated populations with low migration rates, the thresholds for spread are relatively low, and

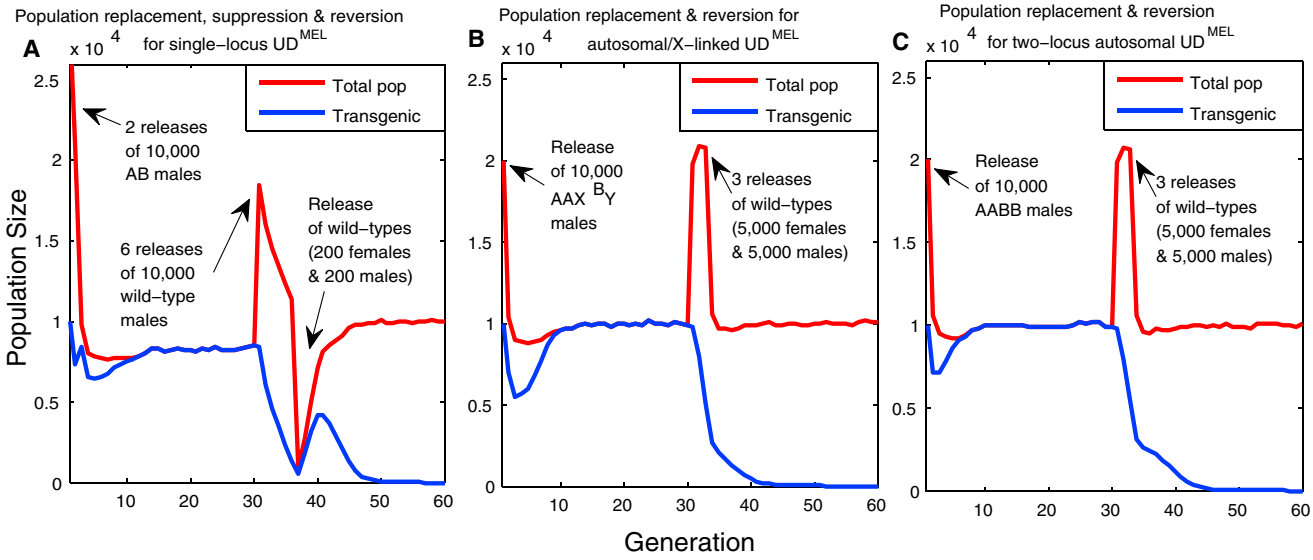



Figure 3. Introduction of Wild-Type Individuals into UD$^{MEL}$-Replaced Populations Can Result in Population Suppression and/or Loss of Transgenes from the Population

(A) Two releases of 10,000 males transheterozygous for single-locus UD$^{MEL}$ constructs with no fitness cost into a wild-type population (pop) of 10,000 result in UD$^{MEL}$ transgene fixation at approximately generation 12 (blue line). Six releases of 10,000 wild-type males into this population during generations 31–36 result in a population crash. Subsequent release of 200 wild-type males and females into the wild at generation 37 results in recovery of the total population to its wild-type, pretransgenic numbers within 12 generations (red line). This is associated with loss of the UD$^{MEL}$ chromosomes, which have fallen below their threshold frequency for spread.

(B and C) One release of 10,000 AAX$^{B}$ Y males (B) or AABB males (C) into a wild-type population of 10,000 results in population replacement. Release of 5,000 males and 5,000 females into the replaced population during generations 31 and 33 results in loss of transgenes from the population but does not lead to population suppression.

a more detailed demographic analysis will be necessary to predict actual thresholds for spread in specific ecological settings.

Because migration may often be asymmetrical, either into or out of a replaced population, we also examined two limit cases: the incoming wild-type migration rate needed to convert a replaced population back to wild-type, and the outgoing transgenic migration rate needed to bring about replacement of a neighboring wild-type population (mi-ma, Figure 2R; ma-mi, Figure 2S). Replacement with single-locus UD$^{MEL}$ is disrupted by low rates of wild-type migration into a replaced population, whereas high levels of migration from a single-locus UD$^{MEL}$-replaced population are required for successful invasion of a neighboring wild-type population. In contrast, both versions of two-locus UD$^{MEL}$ show the opposite behavior, with higher levels of wild-type migration being required to disrupt a UD$^{MEL}$-replaced population and lower levels of UD$^{MEL}$ migration being sufficient to bring about replacement of a neighboring population.

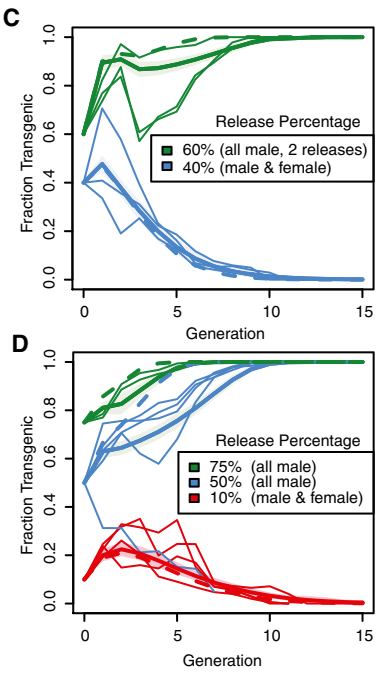
Finally, we note that single-locus and two-locus UD$^{MEL}$ provide realistic opportunities for transgene removal from the population (transgene recall) and/or population suppression. Thus, single-locus UD$^{MEL}$, as well as several versions of single-locus zygotic underdominance [4, 5, 13] (data not shown), has the property that when replacement has gone to allele fixation, repeated introductions of wild-type males can bring about suppression of the replaced population. This occurs because matings between UD$^{MEL}$ females and wild-type males produce only progeny with the wrong antidote, which are therefore inviable. This results in a population suppression effect similar to that seen in sterile male release programs [20]. If suppression is then followed by reintroduction of wild-type males and females, the population recovers, but the transgenes are lost because their frequency is now below that required for drive (Figure 3A). Population suppression cannot be carried out through a similar mechanism for X-autosome and autosome-autosome versions of two-locus UD$^{MEL}$. However, three consecutive releases of wild-type males and females, totaling ~3× the wild population, are sufficient to drive the frequency of the UD$^{MEL}$ chromosomes below the threshold for spread, resulting in transgene elimination and reversion to a wild-type population (Figures 3B and 3C).

Each toxin in the UD$^{MEL}$ system consists of a maternal germline-specific promoter driving the expression of a multimer of synthetic microRNAs (miRNAs) designed to silence expression of one of three different genes: *discontinuous actin hexagons* (*dah*, CG6157), required for cellular blastoderm formation [23]; *O-fucosyltransferase 1* (*o-fut1*, also known as *neurotic*, CG12366), required for Notch signaling [24, 25]; or *myd88* (CG2078), required for Toll-dependent embryonic dorsoventral pattern formation [26, 27]. Each of these genes is expressed maternally, with the product being essential for normal early embryonic development but not oogenesis. Zygotic rescue is achieved using versions of these transcripts, recoded so as to be invisible to the synthetic maternally expressed miRNAs and expressed under the control of a transient, early-zygote-specific promoter.

One version of UD$^{MEL}$ utilizes silencing and rescue of *dah* and *myd88* (chromosomes bearing constructs UD$^{MEL}$-*dah*$^{T}$-*myd88*$^{A}$ or UD$^{MEL}$-*myd88*$^{T}$-*dah*$^{A}$) and is implemented in a single-locus format on chromosome three. The second utilizes silencing and rescue of *dah* and *o-fut1* (chromosomes bearing constructs UD$^{MEL}$-*dah*$^{T}$-*o-fut1*$^{A}$ or UD$^{MEL}$-*o-fut1*$^{T}$-*dah*$^{A}$) and is

**A**

| | Embryo | | | Adult |
|---|---|---|---|---|
| Female | $UD^{MEL}/+$ | $UD^{MEL}/+$ | +/+ | +/+ |
| Male | $UD^{MEL}/+$ | +/+ | $UD^{MEL}/+$ | $UD^{MEL}/+$ |
| Maternal Toxin (#) | 1 | 1 | 0 | 0 |
| Expected Survival (%) | 0 | 0 | 100 | - |
| Expected Fraction Transgenic (%) | - | - | - | 50 |
| $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$ (%) | 0 | 0 | 94.8 ± 3.21 | 47.0 ± 4.92 |
| $UD^{MEL}$-myd88$^{T}$-dah$^{A}$ (%) | 0 | 0 | 98.1 ± 2.51 | 44.9 ± 3.85 |
| $UD^{MEL}$-dah$^{T}$-myd88$^{A}$ (%) | 0 | 0 | 95.2 ± 1.53 | 47.85 ± 3.35 |
| $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$ (%) | 0 | 0 | 99.2 ± 1.52 | 50.0 ± 2.40 |
| $UD^{MEL}$-myd88$^{T}$-dah$^{A}$ (%) | 0 | 0 | 97.0 ± 3.78 | 55.0 ± 4.08 |
| $UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$ (%) | 0 | 0 | 95.6 ± 5.0 | 52.8 ± 1.89 |




**B**

| | Inherited by the Oocyte: Maternal Toxin (toxin,#) | Inherited by the Embryo: Zygotic Antidotes (antidote,n,%) | Expected Embryo Survival % | Embryo Survival % |
|---|---|---|---|---|
| 2/3 Autosomal:Two-locus | | | | |
| yw; $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$/+; +/+ X yw; +/+; $UD^{MEL}$-dah$^{T}$o-fut1$^{A}$/+ | o-fut1 (1) | o-fut1 (1) and dah (1),25<br>o-fut1 (1), 25<br>dah (1), 25<br>0,25 | 50 | 48.32 ± 3.78 |
| yw; $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$/+; $UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$/+ X yw; $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$/+; $UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$/+ | o-fut1 (1)<br>dah (1) | o-fut1 (2) and dah (2),6.25<br>o-fut1 (2) and dah (1), 12.5<br>o-fut1(1) and dah(2),12.5<br>o-fut1(1) and dah(1), 25<br>o-fut1(1), 12.5<br>o-fut1(2), 6.25<br>dah(1), 12.5<br>dah(2), 6.25<br>0, 6.25 | 56.25 | 49.39 ± 1.52 |
| yw; $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$/$UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$; $UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$/$UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$ X yw; +/+; +/+ | o-fut1 (2)<br>dah (2) | o-fut1 (1) and dah (1),100 | 100 | 98.01 ± 1.73 |
| yw; $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$/$UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$; $UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$/$UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$ X yw; $UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$/$UD^{MEL}$-o-fut1$^{T}$-dah$^{A}$; $UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$/$UD^{MEL}$-dah$^{T}$-o-fut1$^{A}$ | o-fut1 (2)<br>dah (2) | o-fut1 (2) and dah (2),100 | 100 | 98.08 ± 3.0 |
| 3/3 Autosomal: Single-locus | | | | |
| yw; $UD^{MEL}$-myd88$^{T}$-dah$^{A}$/+ X yw; $UD^{MEL}$-dah$^{A}$-myd88$^{T}$/+ | myd88 (1) | myd88 (1) and dah (1), 25<br>myd88(1), 25<br>dah(1), 25<br>0, 25 | 50 | 45.13 ± 5.68 |
| yw; $UD^{MEL}$-myd88$^{T}$-dah$^{A}$/$UD^{MEL}$-dah$^{T}$-myd88$^{A}$ X yw; $UD^{MEL}$-myd88$^{T}$-dah$^{A}$/$UD^{MEL}$-dah$^{T}$-myd88$^{A}$ | myd88 (1)<br>dah (1) | myd88 (1) and dah (1), 50<br>myd88(2), 25<br>dah(2), 25 | 50 | 48.6 ± 2.51 |
| yw; $UD^{MEL}$-myd88$^{T}$-dah$^{A}$/$UD^{MEL}$-dah$^{T}$-myd88$^{A}$ X yw; +/+; +/+ | myd88 (1)<br>dah (1) | myd88 (1), 50<br>dah (1), 50 | 0 | 0 |

* For each cross the female genotype is on the top and male genotype is on the bottom.

**Figure 4. Synthetic $UD^{MEL}$ Chromosomes Show Maternal-Effect Lethal and Zygotic Rescue, Underdominant Behavior, and Drive Population Replacement**

(A) Crosses between parents of specific genotypes, either wild-type or heterozygotes for the same $UD^{MEL}$ construct (indicated in the two leftmost columns), were carried out, and progeny survival to crawling first-instar larvae was quantified (rightmost six columns). + indicates wild-type. The chromosome each $UD^{MEL}$ construct was inserted on is indicated by color of the horizontal line (second chromosome, green; third chromosome, blue).

*(legend continued on next page)*

implemented in a two-locus format on chromosomes two and three (see Supplemental Experimental Procedures for details).

Matings between males heterozygous for each of the four $UD^{MEL}$ constructs ($UD^{MEL}$/+) and homozygous +/+ females resulted in high levels of embryo viability, and ~50% of the adult progeny carried the $UD^{MEL}$ construct, as expected for an element with Mendelian segregation and high fitness throughout the fly life cycle (Figure 4A). In contrast, when $UD^{MEL}$/+ females were mated with +/+ males, or with $UD^{MEL}$/+ males heterozygous for the same construct, all progeny died as unhatched embryos or (very rarely) as early first-instar larvae (Figure 4A). This indicates that a single maternal copy of each $UD^{MEL}$ construct is sufficient to bring about complete maternal-effect killing of progeny in the absence of the appropriate antidote. Transheterozygotes for the single- and two-locus $UD^{MEL}$ systems were generated through crosses between $UD^{MEL}$/+ individuals heterozygous for the two different constructs. Approximately 50% of all embryos from these crosses hatched, and all adult progeny were transgenic, again indicating that one copy of the toxin is sufficient to kill and that one copy of the appropriate antidote is sufficient for rescue (Figure 4B).

For single-locus $UD^{MEL}$, surviving adults from a cross between $UD^{MEL}$/+ individuals carrying different $UD^{MEL}$ chromosomes should be transheterozygous for the two $UD^{MEL}$ constructs. Consistent with this, crosses between transgenic female progeny of such a cross and +/+ resulted in no viable progeny. Importantly, however, crosses between putative transheterozygotes (in which females express two distinct toxins) resulted in ~50% progeny survival.

For two-locus $UD^{MEL}$, crosses between transheterozygotes for both constructs resulted in ~50% embryo survival, close to the expected frequency, indicating that both antidotes are sufficient to rescue lethality due to expression of both toxins in mothers. This point is reinforced by the observation that in a cross between females doubly homozygous for both $UD^{MEL}$ constructs and +/+ males, in which mothers express two copies of both toxins and all progeny inherit one copy of each antidote, embryo survival is close to 100%. Finally, crosses between doubly homozygous $UD^{MEL}$ males and females also resulted in high levels of embryo viability, indicating that inheritance of two copies of each antidote does not compromise embryo survival (Figure 4B).

We initiated population replacement (drive-in) and transgene recall (drive-out) experiments by combining single-locus transheterozygotes and two-locus double homozygotes with wild-type (+/+) individuals in several different ways (Figures 4C and 4D). We released only transgene-bearing males for superthreshold drive-in experiments because for many insect vectors, it is only females that bite and transmit disease. For drive-out experiments, we utilized populations consisting of transgene-bearing males and females because an established transgenic population in the field will consist of both sexes. For single-locus $UD^{MEL}$, we released males twice, during the first two generations, each time at a frequency of 60% with respect to the total postrelease population (1.5:1 transheterozygous males: +/+ males and females; predicted release threshold of 44%). Population replacement occurred in each of three experiments such that within 11 generations, all individuals in the population were transgenic (Figure 4C). For the drive-out experiments, transheterozygous males and females were combined with +/+ males and females in a ratio of 2:3, equivalent to releasing 60% +/+ into the replaced transgenic population. Both males and females were released because wild-type females are needed in order for the population to produce non-transgenic progeny. In each of three replicates, the transgenes were completely lost from the population by generation 11 (Figure 4C).

For two-locus $UD^{MEL}$, we initiated two superthreshold release experiments, with transgenic males provided at a frequency of 75% (a 3:1 ratio of doubly homozygous $UD^{MEL}$ males: +/+ males and females, two replicates) or 50% (a 1:1 ratio, five replicates). Population replacement occurred in all cages but one, in which the frequency of transgenics decreased rapidly in the second generation, perhaps due to poor mating efficiency with +/+ females. For the four replicate drive-out experiments, double-homozygous males and females were combined with +/+ at a ratio of 1:9 (a double-homozygote release ratio of 10%). In each case, the population was rendered transgene-free within 15 generations (Figure 4D).

## Conclusions

Here we describe the first fully synthetic, threshold-dependent gene drive mechanism, $UD^{MEL}$, able to bring about local and reversible population replacement. Modeling predicts that both single-locus and two-locus $UD^{MEL}$ drive systems should spread to transgene fixation and be confineable to local populations when migration rates are low. Our gene drive experiments support these predictions and demonstrate clear threshold dependence, implying that population replacement with $UD^{MEL}$ is reversible. Transgene removal can be achieved by dilution with wild-type males and females, as illustrated by the experiments described in Figure 4. Modeling suggests that it can also be accomplished in single-locus $UD^{MEL}$ through a combined strategy in which wild males are released first, to drive down population numbers through killing of heterozygotes, followed by the release of small numbers of wild-type males and females to restore the wild population, if desired (Figure 3).

The components needed to generate the $UD^{MEL}$ system—maternally expressed genes whose products are required for embryogenesis but not oogenesis, maternal- and early-zygote-specific promoters, and miRNAs—are also needed to build synthetic *Medea* selfish genetic elements, which have been shown to drive population replacement in *Drosophila* [28, 29] and are predicted to function as an invasive gene drive mechanism [30]. Genes and promoters with the desired

(B) Crosses between parents of different genotypes (indicated to the left) were carried out. The maternal copy number of Bicaudal C (BicC)-driven miRNAs targeting *myd88*, *dah*, or *o-fut1* (toxin, n) and the zygote copy number of the *bnk*-driven, miRNA-resistant versions of *myd88*, *dah*, or *o-fut1* (antidote, n) are indicated, as are the predicted and observed rates of embryo survival. The genotypes of embryos expected to survive are indicated in black, and those of embryos expected to die in red. Embryo Survival % data were normalized to that of wild-type ($w^{1118}$), which was 93.80% ± 3.19%.
(C and D) Plots depict frequency of transgenics over generations in populations of +/+ (wild-types) into which single-locus $UD^{MEL}$ transheterozygous (C) or two-locus $UD^{MEL}$ double homozygotes (D) were introduced. Release percentage indicates the fraction of the total population, postrelease, consisting of $UD^{MEL}$ individuals. All populations were followed for 15 generations, or until transgenic individuals were lost from the population. Thin lines represent experimental data. The thick line and surrounding shading represent a best-fit analysis of the data. The dashed line represents the predicted behavior of elements carrying no fitness cost.

characteristics are likely to exist in all insects but largely remain to be identified in pest species of interest. Our work suggests that, once in hand, these components could be used to create a range of gene drive systems that are more or less confinable and reversible through dilution with wild-types, with single-locus $UD^{MEL}$ > X-autosome two-locus $UD^{MEL}$ > autosome-autosome two-locus $UD^{MEL}$ > *Medea*. This diversity may prove useful in allowing, to some extent, drive characteristics to be tailored to specific social, regulatory, and physical environments in which population replacement is being considered.

Finally, as the intrinsic introduction thresholds for the above-mentioned drive mechanisms increase, so does the selection for mutations that silence toxin expression and/or activity, which would allow the reappearance of wild-types. An important challenge is to identify molecular strategies that can best forestall the eventual breakdown of these elements and allow for cycles of replacement when failure occurs. Multimerization of toxin-encoding genes, and the toxin-encoding miRNAs expressed by each gene, will limit the possibility that inactivation of individual miRNA units, or polymorphisms at their target sites, results in a loss of killing, providing one strategy. In addition, because second-generation $UD^{MEL}$ elements can in principal be generated that utilize toxin-antidote combinations distinct from those of first-generation elements, it may be possible to carry out multiple cycles of population suppression if first-generation elements fail. Nonetheless, even with these and other strategies in place, it is possible that the use of very high-threshold drive mechanisms such as single-locus $UD^{MEL}$ will be limited to populations that are relatively small, and/or for which replacement or suppression is only needed temporarily.

#### Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.02.059.

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