

Engineered reciprocal chromosome translocations drive high threshold, reversible population replacement in *Drosophila*

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4 Engineered reciprocal chromosome translocations drive high threshold,
5 reversible population replacement in *Drosophila*
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22 **Abstract:**

23 Replacement of wild insect populations with transgene-bearing individuals
24 unable to transmit disease or survive under specific environmental conditions
25 using gene drive provides a self-perpetuating method of disease prevention.
26 Mechanisms that require the gene drive element and linked cargo to exceed a
27 high threshold frequency in order for spread to occur are attractive because they
28 offer several points of control: they bring about local, but not global population
29 replacement; and transgenes can be eliminated by reintroducing wildtypes into
30 the population so as to drive the frequency of transgenes below the threshold
31 frequency required for drive. Reciprocal chromosome translocations were
32 proposed as a tool for bringing about high threshold population replacement in
33 1940 and 1968. However, translocations able to achieve this goal have only been
34 reported once, in the spider mite *Tetranychus urticae*, a haplo-diploid species in
35 which there is strong selection in haploid males for fit homozygotes. We report
36 the creation of engineered translocation-bearing strains of *Drosophila*
37 *melanogaster*, generated through targeted chromosomal breakage and
38 homologous recombination. These strains drive high threshold population
39 replacement in laboratory populations. While it remains to be shown that
40 engineered translocations can bring about population replacement in wild
41 populations, these observations suggest that further exploration of engineered
42 translocations as a tool for controlled population replacement is warranted.
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47 **Keywords:**

48 Gene drive, selfish genetic element, vector control, mosquito, malaria, dengue,
49 UDMEL, engineered translocations, self-propagating, unbreakable, public
50 acceptance.
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54 One strategy for disease prevention of insect vector-borne disease, first
55 articulated by Curtis ¹, involves using gene drive to bring about replacement of
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3 wild, disease transmitting insect populations with individuals engineered to be
4 refractory to disease transmission, but still subject to traditional vector control
5 (reviewed in ²⁻⁶). An important appeal of this strategy is that it is species-specific
6 and potentially self-perpetuating. However, gene drive mechanisms must also
7 function within regulatory frameworks ⁷⁻¹⁵. Central to these are issues of
8 confinement and reversibility: can the spread of transgenes to high frequency be
9 limited to locations in which their presence is sought, and can the population be
10 restored to the pre-transgenic state ¹⁵?

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13 High threshold gene drive mechanisms can potentially provide positive answers
14 to these questions. These mechanisms require that transgenes make up a large
15 fraction of the total insect population (important examples range from 15-70%)
16 before they spread to high frequency within a target area in which they are
17 broadly introduced. Below this frequency transgenes are instead actively
18 eliminated from the population. Once replacement has occurred in the primary
19 target area, spread to high frequency in areas connected to this region by low
20 levels of migration is inhibited because the transgene fails to reach the threshold
21 frequency needed for drive. Finally, transgenes can be eliminated from the
22 population if the release of wildtypes throughout the area in which replacement
23 has occurred results in the frequency of transgenics being driven below the
24 threshold required for drive.
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29 A number of gene drive mechanisms that could in principle bring about high
30 threshold population replacement have been proposed. Examples include a
31 number of single locus gene drive mechanisms ¹⁶⁻¹⁸, reciprocal chromosome
32 translocations, inversions and compound chromosomes ¹⁹, and several forms of
33 engineered underdominance ^{18,20-25}. Here we focus on the use of engineered
34 reciprocal chromosome translocations.
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37 A reciprocal chromosome translocation results in the mutual exchange of DNA
38 between two non-homologous chromosomes (reviewed in ²⁶, illustrated in Figure
39 1A). Provided that the translocation breakpoints do not alter the expression
40 and/or function of nearby genes, translocation heterozygotes and homozygotes
41 can in principle be phenotypically normal. For example, phenotypically normal,
42 naturally occurring translocation-bearing individuals are found in populations of
43 many species ²⁷, including humans ^{28,29}. However, translocation heterozygotes
44 are usually semi-sterile, producing a high frequency of inviable offspring. This
45 occurs because meiosis in a translocation heterozygote can generate a variety of
46 different products. Three patterns of segregation are possible: alternate,
47 adjacent-1 and adjacent-2 (Figure 1A). While alternate segregation leads to the
48 production of gametes with a full genome complement, adjacent-1 and adjacent-
49 2 segregation lead to the production of aneuploid gametes, resulting in the death
50 of progeny that inherit an unbalanced chromosome set. In many species
51 alternate and adjacent-1 segregation occur roughly equally, with adjacent-2
52 segregation being rare (reviewed in ^{30,31}). In such species progeny genotypes
53 and survival phenotypes resulting from crosses between translocation-bearing
54 individuals and wildtypes are as illustrated in the Punnett square in Figure 1B.
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3 Progeny with unbalanced genotypes die, while balanced translocation
4 heterozygotes, translocation homozygotes, and homozygous wildtypes survive.
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7 The frequency of a reciprocal translocation lacks a stable internal equilibrium,
8 with either wildtype or translocation-bearing chromosomes spreading to fixation
9 in an isolated population through natural selection. Based on this, Serebrovskii
10 and Curtis proposed that releases of translocation-bearing individuals could be
11 used to alter the chromosomal composition of a population^{1,32}. Curtis went on to
12 note that if a gene beneficial to humans could be linked to the translocation
13 breakpoint, this behavior of translocations could be used to spread the gene to
14 high frequency¹. More recent modeling work has highlighted the potential of
15 translocations for bringing about local, but not global population replacement,
16 and the possibility of reversal to the pre-transgenic state³³. The positive points
17 notwithstanding, it is important to note that wide-scale spread is only expected
18 under a limited set of conditions. Thus, modeling suggests that in spatially
19 distributed populations underdominant alleles must convey a fitness benefit in
20 order to spread from a localized introduction, as traveling waves^{34,35}. These
21 authors also note that the spatial dynamics of bistable systems depend critically -
22 when considering spread from a point source - on factors such as local
23 differences in population density and migration rate. Since it is unlikely that
24 underdominant systems such as reciprocal translocations will confer a fitness
25 benefit to carriers, these observations imply that population replacement
26 strategies involving translocations will need to utilize an alternative approach, in
27 which translocations are distributed more or less uniformly throughout the target
28 area at super threshold levels.
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33 Evolutionary studies show that translocations can become fixed in populations²⁷.
34 However, efforts to directly bring about population replacement using
35 translocations created in the lab have generally not been successful (reviewed in
36 ^{19,30,36,37}). Thus, for example, Robinson and Curtis found that even 9:1
37 introduction ratios of fit-seeming translocation homozygotes into wildtype
38 *Drosophila* populations resulted in elimination of the translocation from the
39 population³⁶. In most other experiments, in a variety of insects, homozygotes
40 were unfit, rare or entirely absent, indicating low fitness^{30,37-39}. This low fitness
41 could sometimes be ameliorated through extensive introgression into wildtype
42 strains⁴⁰, though the introgressed translocations were never tested for ability to
43 bring about population replacement. Field tests of population replacement using
44 *Aedes aegypti* homozygous for a translocation were unsuccessful⁴¹. The CSIRO
45 Entomology group did achieve some success in population reduction with small
46 field trials of translocation-bearing Australian Blow flies, but later larger-scale
47 trials failed and the efforts were ultimately abandoned (reviewed in^{42,43}). Another
48 group, working with the spider mite *Tetranychus urticae*, has shown population
49 replacement with a small fraction of translocations generated. However, this
50 species likely represents something of an exception since its haplodiploid
51 lifecycle (in which males develop from unfertilized eggs) provides a strong
52 selective filter for translocations that are likely to be viable and fit as
53 homozygotes⁴⁴. Several reasons are likely to account for why most
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3 translocations tested fail to drive. First, the translocation-bearing individuals
4 (particularly homozygotes) were generated using X-rays. This can result in a high
5 frequency of background mutations, which can reduce fitness, particularly of
6 homozygotes (reviewed in ^{30,37}). Second, breakpoints may disrupt genes or their
7 regulatory regions. Finally, more recently it has become clear that chromosome
8 positioning and structure in the nucleus can play a role in determining large-scale
9 patterns of gene expression, and that chromosome translocation can result in
10 changes in the patterns of gene expression ^{45,46}. These changes also may result
11 in translocation-bearing individuals experiencing a fitness cost. These latter
12 observations in particular leave it unclear how frequent translocation-bearing
13 individuals of high fitness are. To explore these issues we developed an
14 approach to generate and identify site-specific reciprocal chromosomal
15 translocations. We report the generation of two strains of *Drosophila* carrying
16 engineered chromosome translocations and show they are capable of bringing
17 about threshold-dependent population replacement in competition with a
18 laboratory wildtype strain. Implications of these results and next steps are
19 discussed.
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24 Results

25 Engineering Reciprocal Translocations in *Drosophila*

26 Cells or organisms carrying translocations with defined breakpoints have recently
27 been generated using several strategies. One set of approaches begins with two
28 non-homologous chromosomes that each have a different transgene-bearing
29 cassette inserted at a specific position. Recombination between the two
30 chromosomes to generate a translocation is then driven by FLP/FRT
31 recombination ⁴⁷, Cre/loxP recombination ^{48,49}, or homologous recombination
32 following double-stranded break creation within the transgene cassettes using a
33 site-specific nuclease ⁴⁹⁻⁵¹. Translocations have also been generated in
34 completely wildtype backgrounds, following CRISPR/Cas9-mediated cleavage of
35 two otherwise wildtype chromosomes followed by non-homologous end joining
36 ⁵²⁻⁵⁴. In this latter case, PCR-based methods were used to identify pools of cells
37 or individual *C. elegans* carrying translocations.
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41 We sought to create translocations using a variant of the approach described by
42 Egli et al. in which homologous recombination between two chromosomes
43 follows double-stranded break creation using the rare-cutting site-specific
44 nuclease I-SceI ⁴⁹. However, rather than use their approach for identification of
45 potential translocation bearing individuals, which involves scoring for the loss of
46 the marker *y+* in an otherwise a *y-* background, we created a system in which
47 recombination results in the creation of a dominant marker. This approach can be
48 used in otherwise wildtype genetic backgrounds, in diverse species.
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51 Two constructs (A and B) were generated (Figure 2B). Each construct included
52 several components. These were (from left to right) a transformation marker (the
53 *white* gene); a location that could be used as an insertion point of a gene of
54 interest (GOI); a promoter that drives the expression of a dominant fluorescent
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3 marker, either ubiquitously (the Opie2 viral promoter⁵⁵) or in oenocytes⁵⁶; a
4 splice donor site, and two stretches of DNA used as substrates for homologous
5 recombination, annotated as UVW and XYZ, each roughly 670bp in length.
6 These DNA fragments were derived from the mouse IgG locus, and thus lack
7 homology with the *Drosophila* genome. Two target sites for the rare cutting
8 homing endonuclease I-SceI were inserted between UVW and XYZ. To the right
9 of these elements were positioned a splice acceptor, a promoterless reporter
10 gene (GFP or dsRed), and a phiC31 recombination attB site.
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14 These constructs were introduced into flies at three separate attP locations:
15 construct A at 51C on chromosome 2, and construct B at 68E or 70A2 on
16 chromosome 3 (Figure 2A). The attP insertion sites at 51C and 68E lie some
17 distance from annotated genes, while the 70A2 site lies within a cluster of tRNA
18 loci. Both constructs were oriented in the same direction with respect to their
19 centromeres (Figure 2A). The constructs were designed so that flies bearing
20 construct A, located on the second chromosome, would express the svp-driven
21 eGFP marker, while construct B, located on the third chromosome, would
22 express the opie2-driven dsRED marker (Figure 2B). Transgenics for construct B
23 behaved as expected, and were dsRED positive throughout their body. However,
24 transgenics for construct A had no detectable GFP expression. The basis for this
25 is unclear, but could be due to inappropriate splicing of the XYZ-UVW sequence
26 in this construct. Regardless, as illustrated below, one marker is sufficient to
27 identify translocation-bearing individuals.
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31 To generate translocation-bearing individuals we generated stocks doubly
32 homozygous for constructs A and B (51C; 71A2 or 51C; 68E). These were then
33 mated with flies that express I-SceI under the control of the Hsp70 heat shock
34 promoter⁵⁷. Progeny harboring all three transgenes were subjected to multiple
35 rounds of heat shock during larval stages and as adults. Adults were outcrossed
36 to wildtype, and progeny examined under a fluorescent dissecting scope. In a
37 number of individuals strong ubiquitous GFP expression was observed. This is
38 the predicted outcome if I-SceI expression results in cleavage of both transgene-
39 bearing chromosomes (Fig. 2C), followed by homologous recombination between
40 XYZ- and UVW-bearing ends of the two different chromosomes (Fig. 2D,E).
41 Putative translocation heterozygotes ($T_1/+$; $T_2/+$) were individually mated to wild
42 type individuals ($+/+$; $+/+$) to generate males and female translocation
43 heterozygotes (identified as GFP-expressing). These were mated with each other
44 to generate putative translocation homozygotes (T_1/T_1 ; T_2/T_2). PCR and
45 sequencing of products from genomic DNA of these individuals was used to
46 demonstrate that these individuals were homozygous for both translocation
47 products (Methods and Figure 2F).
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52 To explore the genetic behavior of translocation-bearing chromosomes and the
53 fitness of carriers we performed a number of crosses and quantified progeny
54 genotype (Table 1). Stocks consisting of translocation homozygotes appeared
55 generally healthy as adults, and survival from egg to adult was 96% of that
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3 observed for the Canton S (CS) wildtype stock. In contrast, crosses between
4 males or females heterozygous for the translocation and wildtype resulted in
5 semisterility, with only about 50% of progeny surviving to adulthood, and 50% of
6 the survivors being translocation heterozygotes. These are the expected results if
7 alternate and adjacent-1 segregation occur with equal frequency in translocation-
8 bearing individuals during meiosis, resulting in the production of 50% aneuploid
9 gametes (Figure 1B). Finally, for each translocation type we also carried out
10 crosses between male and female translocation heterozygotes. Only 37.5% of
11 progeny are predicted to survive, due to the large fraction of zygotes carrying
12 unbalanced chromosome complements. However, many of the survivors (83%)
13 are predicted to carry one or two copies of the translocation (Figure 1B). The
14 levels of embryo survival and percentage of adults carrying the translocation
15 were in good agreement with these predictions (Table 1). Together, these
16 observations suggest that the translocation-bearing strains are fit
17 (notwithstanding the expected semisterility), at least to a first approximation.
18 These points notwithstanding, fitness measurements such as these are not
19 sufficient to know that frequency-dependent drive will occur. This is well
20 illustrated by the results of Curtis and Robinson, who found that a 2;3
21 translocation strain generated with X-rays, which had homozygous viability and
22 fertility equivalent to wildtype in crosses such as those described above, was
23 unable to drive population replacement, even when introduced at a 9:1
24 translocation:wildtype ratio ³⁶.
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30 For population replacement experiments we first introgressed our translocation-
31 bearing systems, 51C; 70A2 and 51C; 68E flies, with Canton S (CS) for 8
32 generations, so as to minimize background genetic differences between
33 translocation-bearing and wildtype strains. Translocation-bearing individuals
34 were then backcrossed to each other to create homozygous stocks, which were
35 then expanded and maintained for use in population experiments. We initiated
36 population cage experiments by introducing translocation-bearing males and
37 virgin females into cages along with Canton S males and virgin females of similar
38 age. A number of different introduction frequencies were tested, in triplicate.
39 These included frequencies predicted to be super-threshold (80%, 70%, 60%),
40 and sub-threshold (20%, 30%, 40%). Populations were then followed for 14
41 generations, with the frequency of translocation-bearing individuals noted each
42 generation.
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46 Results of these experiments are summarized in Figure 3A,B (solid lines). For
47 both translocation-bearing strains, all nine releases at frequencies lower than
48 50% resulted in elimination of the translocation from the population. Conversely,
49 introductions at frequencies greater than 50% resulted in translocation-bearing
50 genotypes spreading to high frequency. These results are generally consistent
51 with modeling predictions. However, the dynamics of drive are clearly distinct
52 from those predicted for translocations that lack a fitness cost (dotted lines in
53 Figure 3A,B). When translocations were introduced at predicted super-threshold
54 frequencies spread was slower than expected for a translocation with no fitness
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3 cost. Sub-threshold releases also resulted in lower initial translocation
4 frequencies than expected, and this was generally followed in later generations
5 by a modestly decreased time to elimination as compared with a translocation
6 with no fitness cost, except at the 20% introduction frequency.
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9 We explored a number of alternative fitness cost models in order to provide
10 better agreement between the laboratory drive data and theoretical model
11 predictions (Supplementary Text S1). The model that provided the best fit to the
12 observed data was one in which lab-reared individuals homozygous for the
13 translocation and their translocation homozygote offspring had reduced fitness if
14 they were not the result of outbreeding with wild-type individuals (Figure S1).
15 However, significant discrepancies between the observed and predicted
16 dynamics remained, suggesting that further experimental and modeling work will
17 be required to understand these discrepancies and any mechanisms that may be
18 responsible for them.
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21 **Predicted tradeoffs associated with translocation-based gene drive.**

22 In real world scenarios other than initial field-testing - in which population
23 isolation will be essential - there will be some level of reciprocal migration
24 between the target area for population replacement and surrounding areas. An
25 important feature of translocations, as distinct from some other proposed forms
26 of underdominance-based gene drive^{16-18,20-22}, is that heterozygotes are viable
27 and fertile, which creates opportunities for the flow of transgenes into neighboring
28 wildtype populations, and wildtype alleles into the replaced population. This
29 behavior has been briefly considered by Marshall and Hay³³. Here we use this
30 framework to consider in more detail the scenario in which the target population
31 for replacement (population 1) and a second, similarly sized population
32 (population 2) are linked by equal levels of reciprocal migration. Previous
33 modeling studies of underdominant systems have noted that the presence of
34 reciprocal migration can result in internal equilibria containing both wildtype and
35 underdominant alleles^{21-23,33,58}. Other studies have explored the fate of
36 underdominant alleles in interacting populations in which alleles are first
37 introduced into a local area and then spread outward^{34,35}. Here we consider the
38 case of reciprocal translocations specifically, in which translocation introductions
39 have initially been carried out throughout population 1.
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44 Figure 3A illustrates a specific scenario, in which translocation homozygotes with
45 no fitness cost are introduced into population 1 at a frequency of 70% for three
46 consecutive generations, and are connected to a similarly sized population 2 by a
47 migration rate of 1%. The translocation increases to high frequency (~99%) in
48 population 1, but not to allele (all are translocation homozygotes) or genotype (all
49 are translocation heterozygotes or homozygotes) fixation, since wildtypes are
50 introduced into population 1 each generation from population 2. Translocation-
51 bearing genotypes are also present at modest levels (<5% (4.99%)) in population
52 2. Figure 3A also illustrates an identical scenario in which the migration rate is
53 now 5%. In this case the translocation equilibrium frequency is <95% (94.67%) in
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3 population 1, <25% (24.49%) in population 2. We ran this model across a range
4 of fitness costs and migration rates to see the general relationship between
5 fitness cost, migration rate and equilibrium frequency in populations 1 and 2
6 (Figures 3B and C, same release conditions as for the single run shown in Figure
7 3A). The highest level of incoming wildtype migration that can be tolerated for a
8 translocation with no fitness cost (~7.0% / generation) results in an equilibrium
9 translocation genotype frequency of ~90% in population 1 and ~25% in
10 population 2. Decreased levels of migration result in correspondingly higher
11 equilibrium frequencies within population 1, which approach fixation as the
12 migration rate falls to zero (Figure 4A), and the converse holds true for
13 population 2. Increased fitness costs result in a minimal decrease in equilibrium
14 translocation frequency for both populations compared to changes in migration
15 rate, as seen by the sharper change in shading along the Y-axis (migration rate)
16 than along the X-axis (fitness cost) (Figure 4B,C).
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21 Population size is also a consideration as an equivalent stochastic model
22 implemented by Marshall and Hay³³ for a translocation with a homozygous
23 fitness cost of 5% and heterozygous fitness cost of 2.5% showed, for two
24 populations of 100 individuals, the system had a ~5% chance of becoming
25 established in both populations for a migration rate of 6.0% per generation;
26 however for two populations of 1,000 individuals, there was only a small chance
27 (~0.1%) that the system became established in both populations for the same
28 migration rate. These observations suggest there is a broad range of conditions
29 under which translocations can spread to a local high frequency, but highlight the
30 tradeoffs associated with increased levels of migration between target and
31 neighboring populations.
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34 Discussion

35 Translocations have previously been generated in animals and plants in several
36 ways using transgenesis, though the fitness of individuals carrying these
37 chromosomes has not been characterized⁴⁷⁻⁵¹. The tools we used to create
38 translocations in *Drosophila* - transgene cassettes located on two different
39 chromosomes, a dominant marker created through the act of translocation, a
40 site-specific nuclease able to bring about breakage within each cassette, and
41 unique sequences that can mediate recombination between the two
42 chromosomes - should be portable to other species (at least ones where
43 sufficient mapping data and genome sequence are available). In particular, future
44 use of the Cas9 system will allow the creation of double-stranded breaks at user-
45 defined sites, which should facilitate the generation of translocations with
46 breakpoints chosen by the developer⁵. The crossing scheme required to
47 generate translocations can also be simplified to a single cross through the use
48 of pairs of chromosomes, one of which carries Cas9, and the other of which
49 carries a gRNA, the combination of which results in site-specific nuclease activity
50 that cleaves a target site present on both transgene-bearing chromosomes (AB,
51 OSA, and BAH, unpublished). These features, coupled with the common genetic
52 behavior of reciprocal translocations in diverse species (semisterility in
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3 heterozygotes), suggest that translocation-based, high threshold and reversible
4 drive may be possible in many species.
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7 Translocations generated in the past, with one exception in a haploid-diploid
8 species ⁴⁴, have not been shown to drive population replacement. This is likely
9 due, at least in part, to the creation of background mutations that compromise
10 fitness in response to the X-irradiation used to create them. Our observations
11 demonstrating population replacement at high but not low introduction
12 frequencies, while limited to two translocations sharing one breakpoint in
13 common, suggest that it may be possible to generate engineered translocations
14 with fitness comparable to wildtype laboratory strains. That said, while the
15 translocations we generated are competitive in a constant laboratory
16 environment, it remains to be shown that these or any other engineered
17 translocations are fit in competition with the diversity of genotypes that would be
18 encountered in complex natural environments.
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22 The population dynamics associated with the spread or loss of our translocations
23 highlight this last point. Both translocations share a common breakpoint and
24 show similar population dynamics. Thus, these dynamics may reflect breakpoint-
25 specific effects on gene expression. Alternatively, and/or in addition, they may
26 reflect the continued segregation of fitness modifiers during drive, since
27 recombination on translocation-bearing chromosomes in *Drosophila* is reduced
28 throughout the involved arms ⁵⁹. Understanding the basis for these dynamics,
29 and whether they are specific to these translocation breakpoints and/or the
30 dominant markers used, will require further study in other genetic backgrounds,
31 and with other engineered translocations, work that is in progress.
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35 Our modeling also illustrates a set of tradeoffs associated with translocation-
36 based gene drive. While an increase in translocation to high frequency can be
37 spatially limited to a single population, this comes with the cost that wildtypes are
38 continuously being introduced into the replaced population, and transgenes are
39 introduced into the neighboring population. This flow keeps the equilibrium
40 frequency of transgene-bearing individuals below 100% in the replaced
41 population and above zero in the neighboring population. These observations
42 suggest that translocation-based gene drive is likely to be most epidemiologically
43 effective, and able to satisfy regulatory requirements relating to the presence and
44 movement of transgene-bearing organisms, in target areas circumscribed by
45 significant barriers to migration.
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49 Related to these points, an important insight gained from other modeling of
50 underdominant systems in spatially distributed populations is that if area-wide
51 population replacement is attempted, attention must be paid to the population
52 dynamics at hybrid zones near borders, as the hybrid front (a traveling wave) can
53 move over time, and depends importantly on the distribution of population
54 densities and migration distances inward and outward ^{34,35}. Thus what constitutes
55 a border may often literally be a moving target. Maintenance of specific borders
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3 will require monitoring and potentially entail additional local releases of the
4 translocation system or wildtype inside or outside the target area, respectively.
5 Modeling of translocation behavior using spatially explicit models based on
6 analysis of real populations in complex environments should provide further
7 insight into the likely behavior of these entities in real populations^{60,61}. Finally,
8 mosquito populations in the wild sometimes consist of multiple chromosomal
9 forms, and may also display some level of reproductive isolation^{62–64}. How
10 engineered translocations will fare in the face of these variants remains to be
11 determined, but can be explored in competition with genetically diverse
12 laboratory strains^{65,66}. While an understanding of the above issues is critical for
13 the success of any population-replacement strategy, the problems may not be
14 intractable, as evidenced by successes in controlling pest populations using non-
15 transgenic⁶⁷ and transgenic inundative population suppression strategies^{68,69}.

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19 Finally, we address possible sources of failure and ways in which translocation-
20 based drive can potentially overcome them. Pathogens can evolve resistance to
21 the activities conferred by the cargo transgene, and the transgene can mutate to
22 inactivity. These events cannot be prevented, but chromosome-based drive
23 mechanisms such as translocations have the attractive feature that it should be
24 possible to incorporate multiple transgenes near the breakpoints, bringing about
25 redundancy in effector function and thereby increased functional lifetime in the
26 wild. Cycles of population replacement to bring new genes into the population
27 can also be imagined. In one approach, the translocation can first be removed
28 from the population by driving its frequency below the threshold needed for drive
29 throughout the target area, through dilution with wildtypes. This can then be
30 followed by a second release of a new translocation-bearing strain that has the
31 same breakpoints, and a new cargo. Alternatively, if high fitness translocations
32 with distinct breakpoints can be generated routinely, it may be possible to drive a
33 first generation translocation and any remaining wildtypes out of the population in
34 favor of a second, distinct translocation (a point made earlier by³²) in the context
35 of use of translocations for population suppression) carrying a new cargo, as with
36 proposals for cycles of replacement of *Medea*-based gene drive systems^{3,70–72}.
37 The translocation itself is likely to be evolutionarily stable as a drive vehicle since
38 reversion back to the wildtype chromosome configuration is likely to be very rare.
39 However, even if this happened, necessarily in a single rare individual, this
40 chromosome would be eliminated along with other wildtype chromosomes in a
41 population (of this or any other species (see above)) in which the translocation
42 was present at high frequency.
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48 **Materials & Methods:**

49 **Construct Assembly**

50 The Gibson enzymatic assembly (EA) cloning method was used for all cloning⁷³.
51 For both constructs (A and B), translocation allele components were cloned into
52 the multiple cloning site (MCS) of a plasmid⁷⁴ containing the *white* gene as a
53 marker and an attB-docking site. For construct A (Figure 1B), the oenocyte-
54 specific *svp* enhancer⁵⁶ and Hsp70 basal promoter fragments were amplified
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3 from *Drosophila melanogaster* genomic DNA using primers P16 and P17 (*svp*)
4 and P18 and P19 (*Hsp70*). The GFP fragment was amplified from template
5 pAAV-GFP (addgene plasmid #32395) using primers P26 and P27. A Kozak
6 sequence (CAACAAA) directly 5' of the GFP start codon was added with primer
7 P26. The SV40 3'UTR fragment was amplified from template pMos-3xP3-DsRed-
8 attp (addgene plasmid #52904) using primers P28 and P10. The 5' and 3' CTCF
9 insulator fragments⁷⁵ were amplified from *Drosophila melanogaster* genomic
10 DNA using primers P11 and P15 (for the 5' CTCF fragment) and P13 and P14
11 (for the 3' CTCF fragment). The 667 XYZ and 668 UVW homology fragments
12 were amplified as above with primers P22 and P23 (XYZ) and P20 and P21
13 (UVW), from plasmid pFUSE-mlgG1-Fc Invivogen, San Diego). The 5' and 3'
14 splice sites utilized were from a 67bp intron located in the *Drosophila*
15 *melanogaster* Myosin Heavy Chain (MHC) gene ID CG17927. They were added
16 to UVW and XYZ sequences using PCR; the 5' splice site was added to the 5'
17 end of the UVW fragment via PCR with primer P24, and the 3' splice site was
18 added to the 3' end of fragment XYZ via PCR with primer P25. Two I-SceI
19 recognition sequences Two 18bp I-SceI recognition sequences
20 (ATTACCCTGTTATCCCTA-CTAG-TAGGGATAACAGGGTAAT) were added to
21 the 3' end of the UVW fragment with primer P21 and the 5' end of the XYZ
22 fragment with primer P22. The construct was assembled in two steps, as above,
23 with the first (5') CTCF, the *svp* and *hsp70* fragments, the UVW fragment, and
24 the XYZ fragment cloned in via a first EA cloning step, and the GFP fragment,
25 the SV40 3'UTR fragment, and the second (3') CTCF cloned in via a second EA
26 cloning step. For construct B (Figure 1B), the *opie2* promoter fragment was
27 amplified from plasmid pIZ/V5-His/CAT (Invitrogen) using primers P1 and P2.
28 The XYZ and UVW homology fragments were amplified from plasmid pFUSEss-
29 CHlg-mG1 using primers P3 and P4 (XYZ) and P5 and P6 (UVW). Two 18bp I-
30 Scel recognition sequences (ATTACCCTGTTATCCCTA-CTAG-
31 TAGGGATAACAGGGTAAT) were added to the 3' end of the XYZ fragment and
32 the 5' end of the UVW fragment in inverse orientation to each other separated by
33 a 4bp linker sequence (CTAG) using primers P4 (for XYZ) and P5 (for UVW).
34 The 5' and 3' splice sites utilized were from a 67bp intron located in the
35 *Drosophila melanogaster* Myosin Heavy Chain (Mhc) gene ID CG17927; the 5'
36 splice site was added to the 5' end of the XYZ fragment via PCR with primer P7,
37 and the 3' splice site was added to the 3' end of fragment UVW via PCR with
38 primer P8. The dsRed fragment, together with the SV40 3'UTR, were amplified
39 from template pMos-3xP3-DsRed-attp (addgene plasmid #52904) using primers
40 P9 and P10, with a Kozak sequence (CAACAAA) directly 5' of the DsRed start
41 codon added with primer P9. The 5' and 3' CTCF insulator fragments⁷⁵ were
42 amplified from *Drosophila melanogaster* genomic DNA using primers P11 and
43 P12 (for the 5' CTCF fragment) and P13 and P14 (for the 3' CTCF fragment).
44 The construct was assembled in two steps. First, the *Drosophila melanogaster*
45 attB stock plasmid⁷⁴ was digested with *Ascl* and *XbaI*, and the first (5') CTCF,
46 the *opie-2* promoter, the XYZ fragment, and the UVW fragments were cloned via
47 EA cloning. Then, the resulting plasmid was digested with *XhoI*, and the dsRed-
48 SV40 3'UTR fragment and the second (3') CTCF were cloned in via EA cloning.
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All sequences were analyzed with NNSPLICE 0.9 (available at http://www.fruitfly.org/seq_tools/splice.html) to confirm strength of splice signals and to check for cryptic splice sites. A list of primer sequences used in the above construct assembly can be found in Supplementary Table 1.

Fly Culture and Strains

Fly husbandry and crosses were performed under standard conditions at 25°C. Rainbow Transgenics (Camarillo, CA) carried out all of the fly injections. Bloomington Stock Center (BSC) fly strains utilized to generate translocations were attP lines 68E (BSC #24485: y^1 M{vas-int.Dm}ZH-2A w^{*}; M{3xP3-RFP.attP}ZH-68E), 51C (BSC #24482; $y[1]$ M{vas-int.Dm}ZH-2A w^{*}; M{3xP3-RFP.attP}ZH-51C), and 70A2 (BSC #9741: $y[1]$ w[1118]; PBac{y[+]-attP-9A}VK00023). Fly Stock BSC#6935 ($y[1]$ w^{*}; P{ry[+t7.2]=70FLP}23 P{y[+t1.8]=70I-Scel}4A/TM) was used as the source of heat shock induced I-Scel. For balancing chromosomes, fly stocks BSC#39631 (w^{*}; wg[Sp-1]/CyO; P{ry[+t7.2]=neoFRT}82B Isn[SS6]/TM6C, Sb[1]) BSC#2555 (CyO/sna[Sco]) were used. For introgression into a wild type background we used the Canton-S stock BSC#1. Translocation construct A was inserted at site 51C, and construct B was inserted at 68E and 70A2 using phiC31 mediated attP/attB integration. These site combinations allowed for the generation of two distinct translocation types, 51C;68E and 51C;70A2. Stocks homozygous for both constructs were then mated with flies that express I-Scel under the control of the Hsp70 heat shock promoter⁵⁷. Progeny carrying all three transgenes were subjected to 5 rounds of heat shock during larval stages and as adults. Heat shocks were conducted by submerging fly vials in a water bath set to 38°C for one hour. Adults were outcrossed to w⁻, and progeny examined under a fluorescent dissecting scope for ubiquitous GFP expression, indicative of translocation generation.

Homozygous translocation-bearing stocks were generated for both 51C;68E and 51C;70A2 site combinations by crossing translocation heterozygotes and identifying homozygous progeny by eye color (light orange eyes for homozygotes versus yellow for heterozygotes for the 51C;68E site combination; light red eyes for homozygotes versus orange for heterozygotes for the 51C;70A2 site combination). After confirming homozygous viability, translocations were introgressed into a Canton-S genetic background. First, CS females were crossed to translocation-bearing males so as to bring the CS mitochondrial genotype into the translocation background. Subsequently, translocation heterozygote females were outcrossed to CS males for 8 generations. Heterozygous translocation-bearing males and virgin females were then crossed to each other to generate homozygous stocks in the CS background for each site combination. Homozygosity was confirmed by outcrossing. Drive experiments for these stocks were set up against CS as the wildtype stock.

Embryo and Adult viability determination

For embryo viability counts (Table 1), 2-4 day old adult virgin females were mated with males of the relevant genotypes for 2-3 days in egg collection

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3 chambers, supplemented with yeast paste. On the following day, a 3hr egg
4 collection was carried out, after first having cleared old eggs from the females
5 through a pre-collection period on a separate plate for 3hrs. Embryos were
6 isolated into groups and kept on an agar surface at 25°C for 48-72 hrs. The %
7 survival was then determined by counting the number of unhatched embryos.
8 One group of 100-300 embryos per cross was scored in each experiment, and
9 each experiment was carried out in biological triplicate (total number of offspring
10 scored is presented in Table 1). The results presented are averages from these
11 three experiments. Embryo survival was normalized with respect to the %
12 survival observed in parallel experiments carried out with the Canton-S wild-type
13 strain, which was 93.00% + 1.82%. For adult fly counts (Table 1), individual flies
14 for each genotype cross were singly mated. For each genotype cross, we set up
15 10-15 individual fly crosses, and the results presented are averages from all
16 these experiments (total number of offspring scored is presented in Table 1). χ^2
17 statistical analyses were carried out for both embryo and adult fly counts to
18 compare expected and observed values, and no statistically significant
19 differences were observed (p values shown).
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24 **Population cage experiments**

25 All population cage experiments were carried out at 25°C, 12 hour-12 hour day
26 night cycle, with ambient humidity in 250 ml bottles containing Lewis
27 medium supplemented with live, dry yeast. Starting populations for drive
28 experiments included equal numbers of virgins and males of similar ages, for
29 each genotype. Translocation-bearing homozygotes were introduced at
30 population frequencies of 60%, 70%, and 80% (T_1/T_1 ; T_2/T_2) for above threshold
31 drive experiments, and 20%, 30%, and 40% (T_1/T_1 ; T_2/T_2) for below threshold
32 drive experiments. CS virgin females and males (+/+; +/+) of similar age as the
33 translocation-bearing individuals made up the remainder of the population. The
34 total number of flies for each starting population was 100. All experiments were
35 conducted in triplicate. After being placed together, adult flies were removed after
36 seven days. After another seven days, progeny (typically 200-250, depending on
37 the replicate) were collected and divided arbitrarily into two equally sized groups.
38 For one group the fraction of translocation-bearing individuals (T_1/T_1 ; T_2/T_2 or
39 $T_1/+$; $T_2/+$) was determined, while the other group was placed into a new bottle to
40 initiate the next generation. No significant evidence of crowding in the 250 ml
41 bottles was observed.
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46 **Theoretical Framework**

47 We apply the model of Curtis and Robinson ⁷⁶ to describe the spread of
48 reciprocal translocations through a population. This is a discrete-generation,
49 deterministic population frequency model assuming random mating and an
50 infinite population size. We denote the first chromosome with a translocated
51 segment by " T " and the wild-type version of this chromosome by " t ." Similarly, we
52 denote the second chromosome with a translocated segment by " R " and the wild-
53 type version of this chromosome by " r ." As a two-locus system, there are nine
54 possible genotypes; however, only individuals carrying the full chromosome
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complement are viable, which corresponds to the genotypes $TTRR$, $TtRr$ and $ttrr$, the proportion of the k th generation of which are denoted by p_k^{TTRR} , p_k^{TtRr} and p_k^{ttrr} . The four haplotypes that determine the genotype frequencies in the next generation – TR , tR , Tr and tr – are described by the following frequencies:

$$\begin{aligned} f_k^{TR} &= p_k^{TTRR}(1-s) + 0.25p_k^{TtRr}(1-hs) \\ f_k^{tR} &= f_k^{Tr} = 0.25p_k^{TtRr}(1-hs) \\ f_k^{tr} &= p_k^{ttrr} + 0.25p_k^{TtRr}(1-hs) \end{aligned}$$

Here, s denotes the reduced fecundity of $TTRR$ individuals and hs denotes the reduced fecundity of $TtRr$ individuals relative to wild-type individuals, where $h \in [0,1]$. By considering all possible mating pairs, the genotype frequencies in the next generation are:

$$\begin{aligned} p_{k+1}^{TTRR} &= (f_k^{TR})^2 / \sigma_k \\ p_{k+1}^{TtRr} &= 2(f_k^{TR} f_k^{tr} + f_k^{tR} f_k^{Tr}) / \sigma_k \\ p_{k+1}^{ttrr} &= (f_k^{tr})^2 / \sigma_k \end{aligned}$$

where σ_k is a normalizing term given by,

$$\sigma_k = (f_k^{TR})^2 + 2(f_k^{TR} f_k^{tr} + f_k^{tR} f_k^{Tr}) + (f_k^{tr})^2$$

We evaluated a number of fitness cost models in terms of their ability to replicate the dynamics observed in the laboratory drive experiments. These included: a) constant fitness costs, b) fitness costs that varied with translocation population frequency, c) fitness costs that decreased with time (either linearly, exponentially or sigmoidally), and d) an introgression model, in which lab-reared individuals homozygous for the translocation and their translocation homozygote offspring had reduced fitness if they were not the result of outbreeding with wild-type individuals. These models are described in Supplementary Text S1.

For our three-population models, there are three sets of the above equations to represent each population. We let m represent the migration rate per generation. After genotype frequencies for all three populations are calculated for a given generation, a proportion m is removed from each genotype from populations 1 and 3 and added to population 2, and a proportion $2m$ is removed from each genotype from population 2, half of which is added to population 1 and the other half of which is added to population 3.

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10 **Conflicts of Interest**

11 A.B, O.S.A and B.A.H have a patent pending related to population control using
12 engineered translocations.
13

14 **Author Contribution(s)**

15 A.B., O.S.A, B.A.H conceived and designed experiments. T.I, J.M.M, A.B., O.S.A
16 performed all mathematical, molecular and genetic experiments. All authors
17 analyzed the data, contributed to the writing, and approved final manuscript.
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20 **Supporting Information**

21 Includes Supplementary Methods describing the general theoretical framework of
22 the modelling used and the specifics and comparison of various investigated
23 fitness cost models, as well as Supplementary Figure 1 and Supplementary
24 Table 1.
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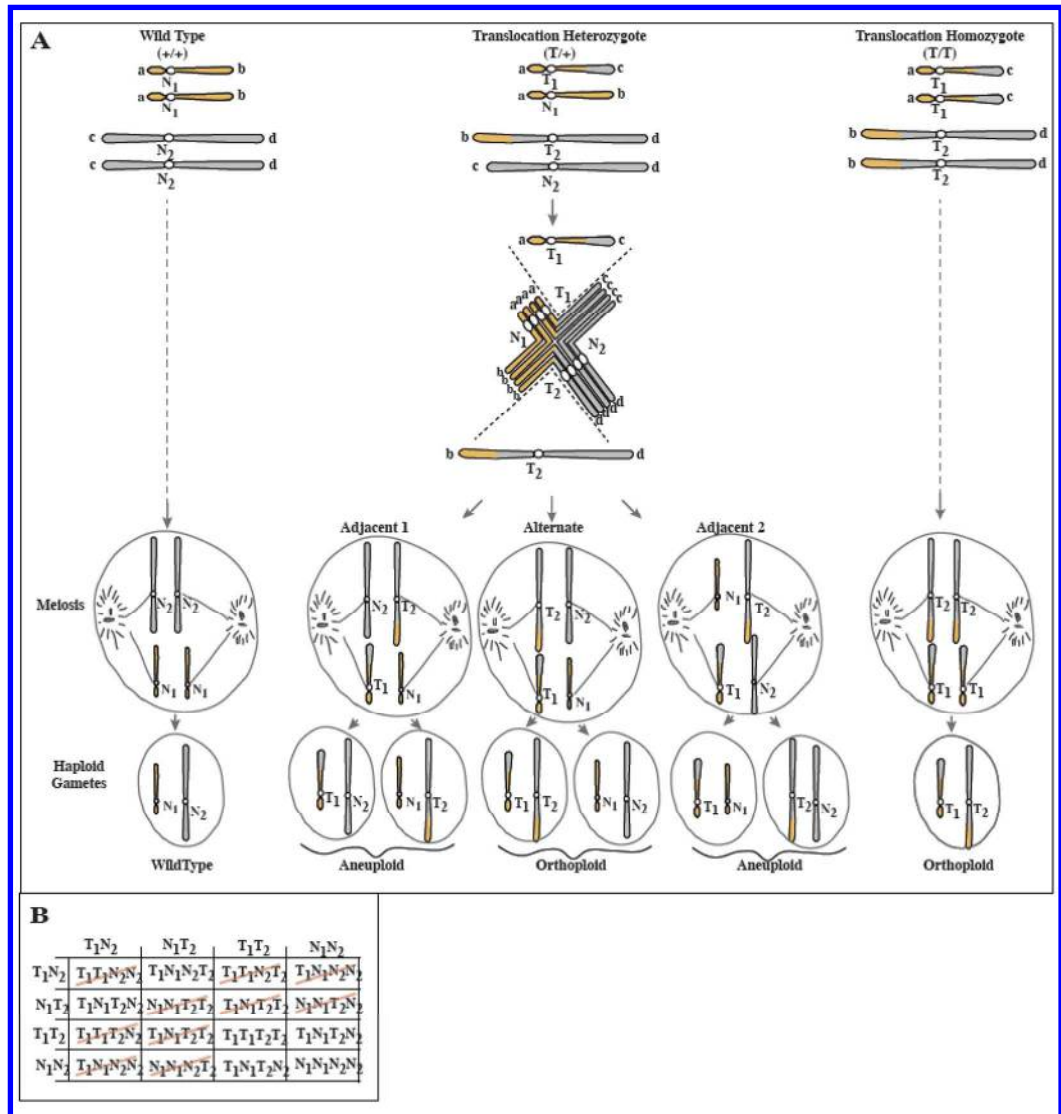


Figure 1. Gamete and zygote genotypes associated with the presence of a reciprocal translocation. Wildtype chromosomes N_1 and N_2 , and translocation chromosomes T_1 and T_2 , are indicated. (A) One chromosome type (a) is indicated in yellow. A second chromosome type (b) is in gray. Gamete types generated by wildtype (+/+), translocation heterozygotes (T/+), and translocation homozygotes (T/T) are indicated. (B) Gamete and zygote genotypes possible in crosses involving a translocation are indicated. Inviabile genotypes are indicated by a red line.

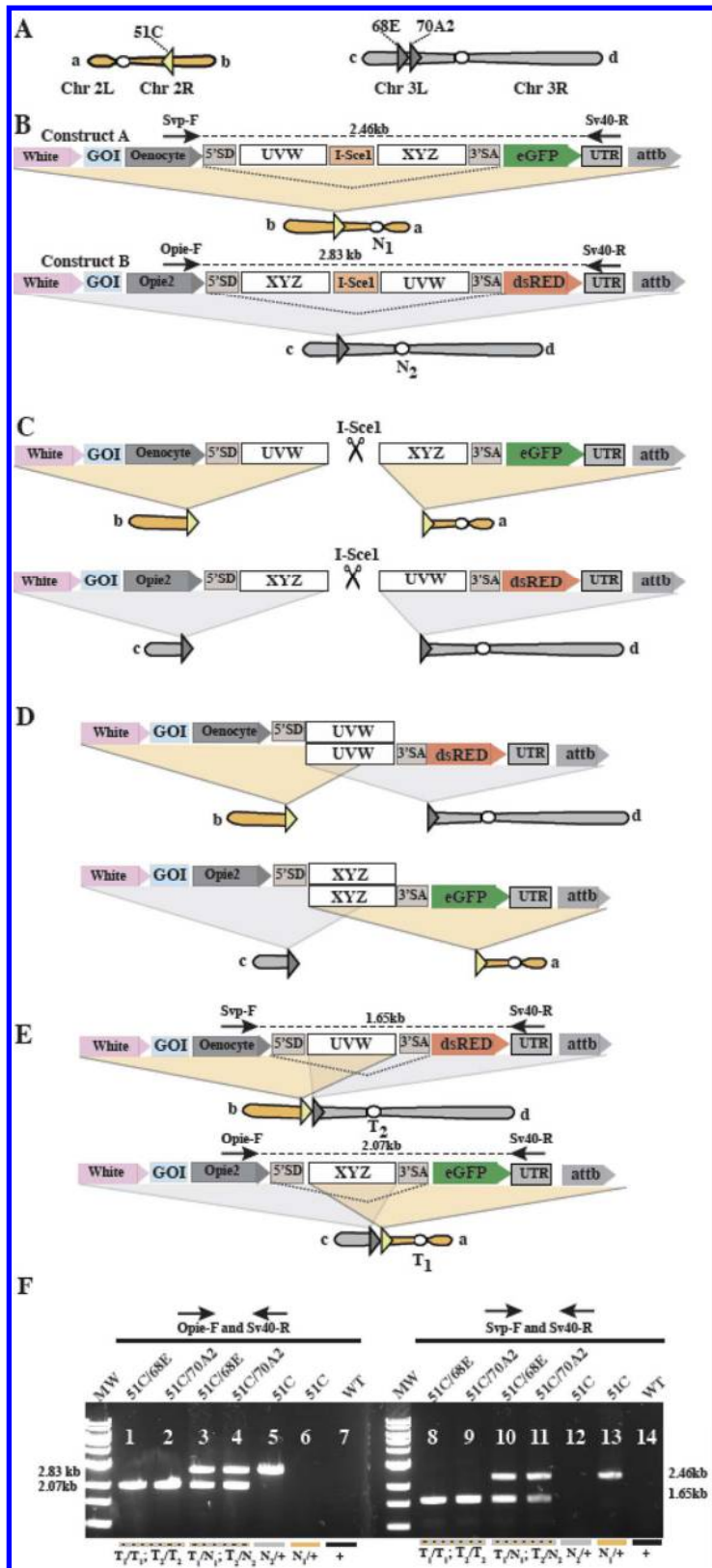


Figure 2. Generation of reciprocal translocations in *Drosophila*. (A) Approximate location of the attP sites used for transgene insertion; orientation with respect to

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3 the centromere are indicated by triangles. (B) Components of each starting
4 transgene cassette. Construct A is inserted on the second chromosome and
5 construct B on the third chromosome. Components are as indicated in the text.
6 (C) I-Sce-dependent cleavage results in a double-stranded break in each
7 transgene-bearing chromosome. (D) Alignment of broken chromosome ends
8 occurs using homologous sequences UVW and XYZ. (E) Recombinant
9 chromosomes are generated by homologous recombination using sequences
10 UVW and XYZ. (F) Agarose gel image is shown of PCR amplification products
11 generated from different genotypes: translocation homozygotes (T1/T1; T2/T2);
12 translocation heterozygotes (T1/N1; T2/N); individuals carrying only the 51C
13 starting chromosome insertion (N1/+); or the 68E and 70A2 starting chromosome
14 insertion (N2/+). Primers used, and expected amplification product sizes, are
15 indicated in B and E.
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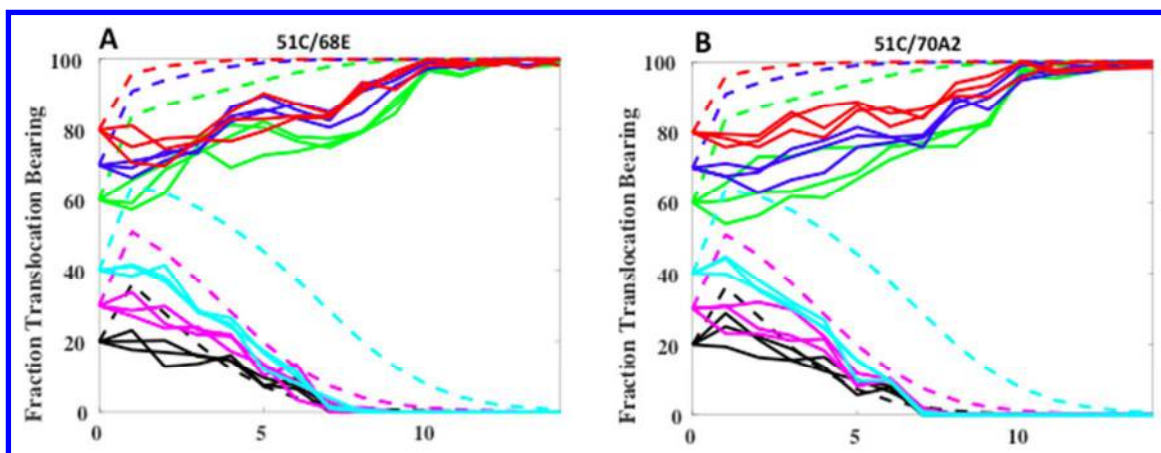


Figure 3. Dynamics of translocation-based population replacement, and model predictions in the absence of fitness costs. Population frequency of the adult population having the indicated translocation is plotted versus generation number for a number of homozygous translocation release ratios: 80%, 70%, 60%, 40%, 30% and 20%. Solid lines indicate observed population frequencies, and dashed lines indicate predicted translocation-bearing genotype frequencies for an element with no fitness cost.

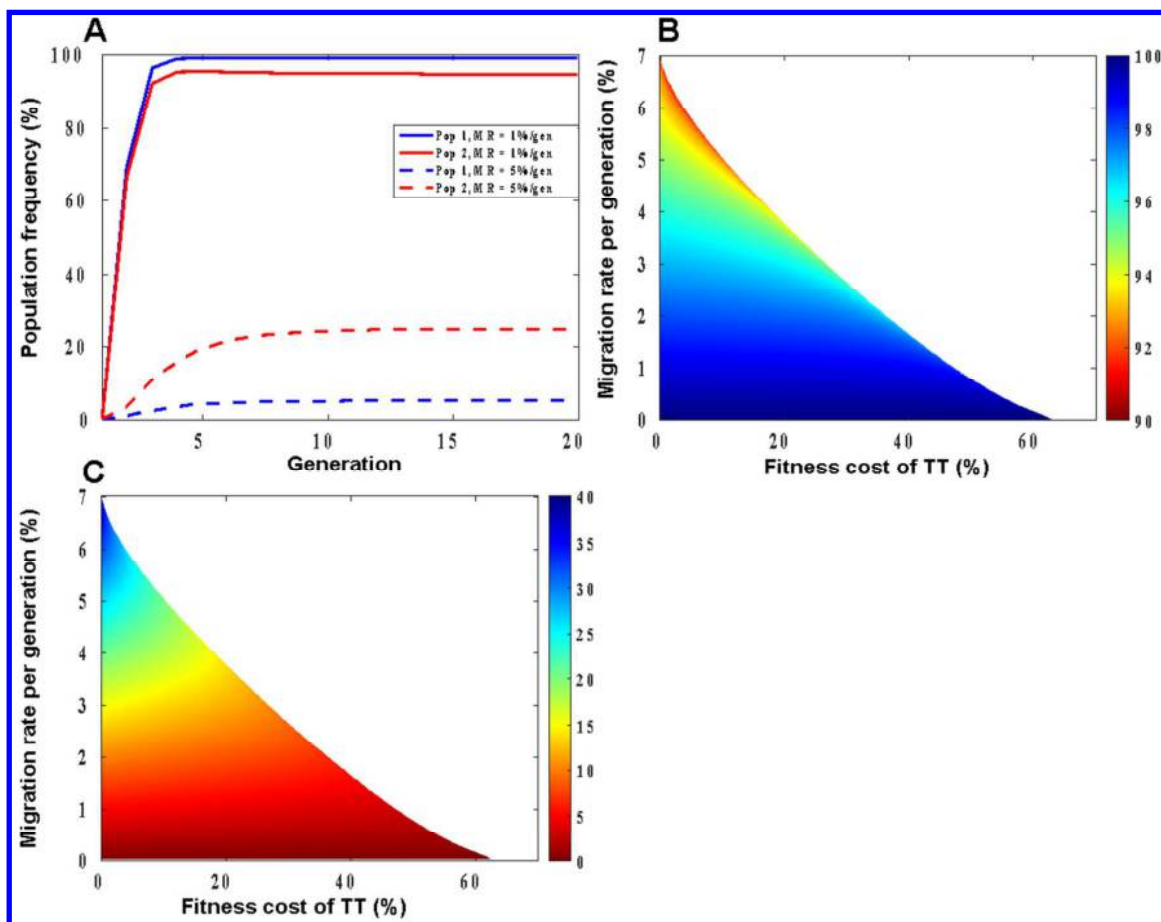


Figure 4. Translocation dynamics in a two population migration model. (A) Population frequency of a translocation with no fitness cost, introduced into population 1 using three consecutive releases of translocation-bearing homozygotes. Populations 1 and 2 are linked by a migration rate of 1% (solid lines) or 5% (dashed lines). (B,C) Equilibrium frequency of translocation bearing individuals over a range of fitness costs and migration rates for population 1 (B) or population 2 (C). For both populations increasing fitness cost has little effect on the equilibrium frequency at low migration rate and increased effects at higher migration rates. In contrast, migration rate has a much stronger effect on equilibrium frequency independent of fitness cost as seen by the color gradient shifts. Note that the equilibrium frequency varies between 90-100% and 0-25% in the target population (population 1) and population 2, respectively.

parental genotypes			embryo survival %		transgene bearing adults %	
male	female	progeny genotype (%)	predicted	observed*	predicted	observed*
$T_1/T_1; T_2/T_2$	$T_1/T_1; T_2/T_2$	$T_1/T_1; T_2/T_2$ (100%)	100	96.9 ± 1.8 (n = 588, p = 0.80) 96.9 ± 0.3 (n = 579, p = 0.87)	100	100 ± 0.0 (n=507, p=1) 100 ± 0.0 (n=592, p=1)
$T_1/T_1; T_2/T_2$	+/+; +/+	$T_1/+; T_2/+$ (100%)	100	94.6 ± 2.2 (n = 569, p=0.62) 98.2 ± 2.6 (n=536, p=0.88)	100	100 ± 0.0 (n=516, p=1) 100 ± 0.0 (n=469, p=1)
+/+	$T_1/T_1; T_2/T_2$	$T_1/+; T_2/+$ (100%)	100	90.1 ± 1.6 (n=759, p=0.22) 92.5 ± 4.8 (n=410, p=0.33)	100	100 ± 0.0 (n=507, p=1) 100 ± 0.0 (n=504, p=1)
$T_1/+; T_2/+$	+/+; +/+	$T_1/+; T_2/+$ (25%) $T_1/+; +/+$ (25%)** $+/+; T_2/+$ (25%)** $+/+; +/+$ (25%)	50	51.2 ± 1.6 (n=820, p=0.90) 50.4 ± 1.3 (n=576, p=0.95)	50	49.3 ± 3.4 (n=825, p=0.99) 49.5 ± 2.4 (n=938, p=0.99)
+/+; +/+	$T_1/+; T_2/+$	$T_1/+; T_2/+$ (25%) $T_1/+; +/+$ (25%)** $+/+; T_2/+$ (25%)** $+/+; +/+$ (25%)	50	48.3 ± 2.8 (n=441, p=0.77) 48.3 ± 3.9 (n=805, p=0.65)	50	49.4 ± 2.2 (n=877, p=0.99) 48.5 ± 3.4 (n=941, p=0.99)
$T_1/+; T_2/+$	$T_1/+; T_2/+$	$T_1/T_1; T_2/T_2$ (6.25%) $T_1/T_1; T_2/+$ (12.5%)** $T_1/T_1; +/+$ (6.25%)** $T_1/+; T_2/T_2$ (12.5%)** $T_1/+; T_2/+$ (25%) $T_1/+; +/+$ (12.5%)** $+/+; T_2/T_2$ (6.25%)** $+/+; T_2/+$ (12.5%)** $+/+; +/+$ (6.25%)	37.5	36.2 ± 1.8 (n=568, p=0.84) 32.4 ± 4.0 (n=503, p=0.22)	~83%	80.4 ± 6.5 (n=519, p=0.99) 80.8 ± 5.8 (n=463, p=0.99)

* Translocation 51C/68E (top) and 51C/9741 (bottom)
** These genotypes are not viable.

Table 1. Behavior of translocations in crosses to various genotypes. Crosses between parents of specific genotypes - wild-type (+/+; +/+), translocation heterozygotes ($T_1/+; T_2/+$), and translocation homozygotes ($T_1/T_1; T_2/T_2$), were carried out. Embryo survival (fifth column from right) and percentage of translocation-bearing adults (rightmost column) were independently quantified; χ^2 statistical analysis (p-values from which are shown) was carried out to determine if differences between expected and observed values were significant. The top number in each column shows results for the 51C/68E translocation; the bottom number shows the results for the 51C/70A2 translocation. ** Indicates unviable genotypes. Embryo survival was normalized with respect to percent survival (\pm SD) observed in the w^{1118} stock used for transgenesis (methods).

Supplementary Table 1. List of primer sequences used in this study.

Primer name	Primer sequence, 5' to 3'	Source
P1	CCTAACAACTCACACCTTGCAGCGCCACCTG GCCCTAGAGATCCACCAACTTTTTGCACTG C	pIZ/V5- His/CAT (Invitrogen)

P2	ATTCCTAAGCATCAGTGGTTGAACCTACCTTG TTGGCGTGACCAGAGACAGGTTGCGGCG	
P3	AGGTTCAACCACTGATGCTTAGGAATAGGCC ATGTGAAGCTGAAGGAATC	pFUSEss- CHlg-mG1 (Invivogen)
P4	TATTACCCTGTTATCCCTACTAGTAGGGATAA CAGGGTAATACTAGAATCCCTGGGCACAATT T	
P5	CTAGTATTACCCTGTTATCCCTACTAGTAGGG ATAACAGGGTAATAGTGGTTGTAAGCCTTGC A	pFUSEss- CHlg-mG1 (Invivogen)
P6	AAAGGATAAGAATTAGGGTTAGTCGTTTCGG TGTGCCTAGTTTACCAGGAGAGTGGGAGA	
P7	CGCCCACGCCATCCAACCGCCGCCGCAACC TGTCTCTGGTCACGCCAACAAAGGTAGGTTC	P3/P4 XYZ PCR
P8	ATGACGTTCTTGGAGGAGCGCACCATTTTGT TGCTAAAGGAAAGGATAAGAATTAGGGTT	P5/P6 UVW PCR
P9	AAACGACTAACCCCTAATTCTTATCCTTTCCTTT AGCAACAAAATGGTGCGCTCCTCCAAG	pMos-3xP3- DsRed-attP (addgene plasmid #52904)
P10	AATGGAACCTCTTCGCGGCCAGGTGGCGCTG CAAGGCTCGAGGGTCTGACTGATCATAATCA	
P11	GGATCCGGGAATTGGGAATTGGGCAATATTT AAATGGCGGCCTTGCAGCGCCACCTGGCC	Drosophila genomic DNA
P12	AGCGTGTTTTTTTTGCAGTGCAAAAAGTTGGT GGATCTCTAGGGCCAGGTGGCGCTGCAA	
P15	CCAACGCATTTTCCAAGCTTGTTTAAACGTGG ATCTCTAGGGCCAGGTGGCGCTGCAAGG	
P13	TACAAATGTGGTATGGCTGATTATGATCAGTC GACCCTCGAGCCTTGCAGCGCCACCTGG	Drosophila genomic DNA
P14	GAGACCGTGACCTACATCGTCGACACTAGTG GATCTCTAGGGCCAGGTGGCGCTGCAAGG	
P16	CCTTGCAGCGCCACCTGGCCCTAGAGATCCA CGTTTAAACAAGCTTGAAAATGCGTTGG	Drosophila genomic DNA
P17	CGAAGCGCCTCTATTTATACTCCGGCGCTCG TTTAAACAAAGTGGCAGGGCCCATGTGTT	
P18	GAGTGGAGCACAAACACATGGGCCCTGCCA CTTTGTTTAAACGAGCGCCGGAGTATAAAT	Drosophila genomic DNA

P19	AAGCATCAGTGGTTGAACCTACCTTGTTGGC GTGTCTGATGCAGATTGTTTAGCTTGTTG	
P20	GCCAACAAGGTAGGTTCAACCACTGATGCTT AGGAATAGGCGTGGTTGTAAGCCTTGCAT	pFUSEss- CHlg-mG1 (Invivogen)
P21	CCCTGTTATCCCTACTAGTAGGGATAACAGG GTAATACTAGTTTACCAGGAGAGTGGGAG	
P22	TATTACCCTGTTATCCCTACTAGTAGGGATAA CAGGGTAATACATGTGAAGCTGAAGGAA	pFUSEss- CHlg-mG1 (Invivogen)
P23	AAAGGATAAGAATTAGGGTTAGTCGTTTTCGG TGTGCCTAGAATCCCTGGGCACAATTTTC	
P24	CAAGCGCAGCTGAACAAGCTAAACAATCTGC ATCAGACACGCCAACAAGGTAGGTTCAAC	P20/P21 UVW PCR
P25	ACCTACATCGTCGACACTAGTGGATCTCTAG CTCGAGCTAAAGGAAAGGATAAGAATTAGGG	P22/P23 XYZ PCR
P26	CCCTAATTCTTATCCTTTCCTTTAGGAATTCC AACAAAATGGTGAGCAAGGGCGAGGAGC	pAAV-GFP (addgene plasmid #32395)
P27	TTCACTGCATTCTAGTTGTGGTTTGTCCAAAC TCATCAATGTTTACTTGTACAGCTCGTC	
P28	GCCGCCGGGATCACTCTCGGCATGGACGAG CTGTACAAGTAAACATTGATGAGTTTGGAC	pMos-3xP3- DsRed-attp (addgene plasmid #52904)

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5 Engineered reciprocal chromosome translocations drive high threshold, reversible
6 population replacement in *Drosophila*
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9 Anna B. Buchman^{*#}, Tobin Ivy^{*}, John M. Marshall^{\$}, Omar S. Akbari^{*#&}, and Bruce A.
10 Hay^{*&}
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