A CRISPR endonuclease gene drive reveals distinct mechanisms of inheritance bias

Sebald A. N. Verkuijl1,2,5, Estela Gonzalez2,4,5, Ming Li3, Joshua X. D. Ang2,4, Nikolay P. Kandul3, Michelle A. E. Anderson2,4, Omar S. Akbari3, Michael B. Bonsall1 & Luke Alphey2,4

CRISPR/Cas gene drives can bias transgene inheritance through different mechanisms. Homing drives are designed to replace a wild-type allele with a copy of a drive element on the homologous chromosome. In Aedes aegypti, the sex-determining locus is closely linked to the white gene, which was previously used as a target for a homing drive element (wGDe). Here, through an analysis using this linkage we show that in males inheritance bias of wGDe did not occur by homing, rather through increased propagation of the donor drive element. We test the same wGDe drive element with transgenes expressing Cas9 with germline regulatory elements sds3, bgcn, and nup50. We only find inheritance bias through homing, even with the identical nup50-Cas9 transgene. We propose that DNA repair outcomes may be more context dependent than anticipated and that other previously reported homing drives may, in fact, bias their inheritance through other mechanisms.

Received: 16 December 2021
Accepted: 4 November 2022
Published online: 21 November 2022

Check for updates

Genetic modification of wild populations through gene drive may be a means of addressing some of the most pressing public health challenges in the world. Gene drive is the ability of a genetic element to bias its own inheritance, allowing it to spread a genetic change throughout a population1. There are many examples of natural gene drives that act through different inheritance biasing mechanisms2. Some types of gene drive function through the action of enzymes that create sequence-specific DNA breaks (DNA endonucleases), and various context-dependent cellular repair mechanisms exist to resolve DNA breaks3. Correspondingly, nuclease-based gene drives can function through different mechanisms including inheritance bias through a copying mechanism (homing drives) and drives that cause the loss of non-drive bearing gametes or offspring (here referred to as meiotic drive).

Generally, in diploid organisms, each parent contributes one chromosome of each homologous pair and each allele has a 50% chance of being passed on to a given progeny (Mendelian inheritance). Synthetic homing and meiotic endonuclease gene drives both rely on selectively creating double-strand DNA breaks on the non-drive-bearing homologue. Through different mechanisms, this results in an inheritance bias of an allele or genomic region and, for meiotic drive, potentially the entire chromosome. Meiotic endonuclease drives lower the inheritance of the competing chromosome within a pair by damaging it, such that gametes carrying the non-drive chromosome are eliminated during gametogenesis or, in some cases, produce non-viable offspring. This includes the disruption of specific essential genes in toxin-antidote meiotic drives4–6, or through more structural damage, such as chromosome ‘shredder’ meiotic drives7,8. Natural sex-linked meiotic drive systems have been reported in Aedes and Culex mosquitoes9,10. Synthetic shredder endonuclease meiotic drives have generally sought to exploit large-scale, potentially repeating sequence differences between chromosome pairs to increase the damage done to the chromosome that does not carry the drive7,8.

For most reports of synthetic homing drives, the method of quantifying inheritance bias (phenotypic scoring of progeny carrying a marker gene in the drive allele) cannot differentiate between the underlying inheritance bias mechanism. However, a small subset of reports of homing drives have had marked chromosomes11–16, especially pre-CRISPR17–20, which may allow homing and meiotic
inheritance bias to be differentiated. Through the use of a coincidental chromosomal marker, we observed evidence for meiotic drive in male A. aegypti with a homing CRISPR gene drive design reported by Li et al.12.

Li et al. tested the inheritance biasing ability of a set of homing split drive systems comprising a guide RNA (gRNA) expressing element inserted into the white gene (w<sup>GDe</sup>) and one of five secondary site transgene insertions expressing Cas9 under the control of various promoters from genes expressed in the mosquito germline. The white gene is tightly linked to the sex-determining region of A. aegypti which allows the sex of the progeny to function as a chromosomal marker (donor/recipient) in the progeny of male drive carriers. While three of the Cas9 regulatory regions resulted in drive activity in females, only nup50 expressing Cas9 resulted in a statistically significant increased inheritance of the drive from male drive parents15. We re-analysed the results of Li et al. for nup50 males taking into account the sex linkage and found that the observed inheritance bias in males seemingly proceeded exclusively through meiotic drive.

We set out to test the hypothesis that the meiotic drive observed with the nup50 expression pattern is a more general phenomenon and also occurs with other A. aegypti gene drives that show activity in males. We repeated the w<sup>GDe</sup> and nup50-Cas9 crosses with lines provided by the original authors, and performed crosses with Cas9 expression under the control of putative transcription regulatory regions of two additional A. aegypti germline genes. The first, suppressor of defective silencing 3 (sds3) has been shown, by dsRNA-induced knockdown in Anopheles gambiae, to be necessary for normal development of the ovarian follicles and testes, without other obvious defects16. The second, benign gonial cell neoplasm protein (bgcn) is involved in the regulation and promotion of gametogenesis in both sexes17 and has been described in the context of gene drive in Drosophila melanogaster with the Fscel nuclease18.

For each line that expresses Cas9, we report the degree of inheritance bias of the w<sup>GDe</sup> element for both sexes and, in males, the mechanism of inheritance bias. For sds3, bgcn, and nup50-Cas9, we find an increase in recombination events indicative of homing. Furthermore, by scoring somatic eye phenotypes, we also find strong evidence of zygotic/somatic expression, maternal deposition and an effect of the Cas9 carrying grandparent’s sex on w<sup>GDe</sup> inheriting grand-offspring phenotypes.

**Results**

**Inheritance of w<sup>GDe</sup> is biased by bgcn, sds3 and nup50-Cas9**

To assess the degree and, in males, the mechanism of inheritance bias, we bred transgenic A. aegypti mosquitoes to create and analyse a split drive arrangement. In this split drive, the w<sup>GDe</sup> allele expresses a gRNA targeting the wildtype white gene (w<sup>+</sup>) at the site corresponding to where the drive element has been inserted and disrupts its protein coding sequence (Fig. 1a and Supplementary Fig S1). The white gene is located on chromosome one, near the dominant acting male determining allele M such that males are M/m and females m/m.

To generate individuals in which drive can occur, the w<sup>GDe</sup> element is combined with the other component of the split drive, a separate transgene that expresses Cas9 under the control of regulatory sequences from an endogenous germline-specific gene, either nup50, bgcn, or sds3. Individuals carrying a single copy of both the w<sup>GDe</sup> and Cas9 transgenes (double heterozygotes) were generated in two ways: by crossing parental F<sub>0</sub> female white w<sup>GDe</sup> homozygotes to male Cas9 individuals (Fig. 1b Top) or with the reciprocal cross (Fig. 1b Bottom). The double heterozygous offspring (F<sub>1</sub>) were in turn crossed to the Liver-pool wild type strain, and their progeny (F<sub>2</sub>) were collected and their fluoresence and phenotype scored (Supplementary Tables S1–S7).

For each condition, Fisher’s Exact tests were performed comparing the w<sup>GDe</sup> inheritance rates to those in the absence of any Cas9 element for male (52%, 620/1203) or female (51%, 308/605) parents (Supplementary Table S8). All Cas9 expressing lines were able to bias the inheritance of the w<sup>GDe</sup> element in at least one cross (Supplementary Table S9 and Fig. 1c).

For sds3, F<sub>1</sub> drive females with maternal Cas9 propagated the w<sup>GDe</sup> element to 67% (118/176, p value: 0.050) of their progeny (Supplementary Table S1) and for bgcn, F<sub>1</sub> drive males with maternal Cas9 the propagation rate was 66% (257/389, p value: 0.010) (Supplementary Table S2). For nup50 (Supplementary Table S3), all four crosses had significantly increased inheritance rates, and to a similar degree as reported to the identical crosses in ref. 12. The nup50 double heterozygous males passed along the w<sup>GDe</sup> element to 64% (1159/1819, p value: 0.001***) of their progeny with paternal Cas9 and to 63% (1852/2926, p value: <0.001***) of their progeny with maternal Cas9. For nup50 drive females the propagation rate was 69% (932/1377, p value: <0.001***) for paternal Cas9 and 70% (1055/1501, p value: <0.001***) for maternal Cas9.

For nup50-Cas9, the progeny were collected individually from F<sub>1</sub> parents (Supplementary Table S4–S7). There was considerable variation between the inheritance rate from different parents carrying the same drive (Fig. 1c), a notable feature that has been reported in other articles on homing drives12,20–22. Due to this overdispersion, we cannot reliably determine if there is a statistical difference in the inheritance rate between the different Cas9 regulatory elements.

However, because this overdispersion is expected only to occur if the drive is functional, our method for determining a difference from the control remains valid, albeit with a potentially inflated false negative rate.

**Eye phenotype reveals the source of nuclease activity**

All progeny were evaluated for eye pigment defects that may result from embryonic or later somatic biallelic disruption of the white gene by the w<sup>GDe</sup> element and NHEJ mutations. Since the double heterozygote drive-carrying parents were crossed to wildtype individuals, each progeny inherited at least one dominant functional white allele from the non-drive parent, and, if the w<sup>GDe</sup> element is not inherited, potentially an additional one from the drive parent. The biallelic loss of function of the white gene must therefore occur through deposition into, or somatic expression by, F<sub>2</sub> individuals. Consistent with this, the progeny of the–Cas9 control crosses did not present with a white phenotype (Supplementary Table S8).

For male double heterozygote sds3-Cas9 crosses, of the F<sub>2</sub> progeny (♂ and ♀ pooled) that inherited both the w<sup>GDe</sup> and the Cas9 element, 86% (111/129) presented with a mutant somatic phenotype if the Cas9 carrying F<sub>0</sub> grandparent was male, or 98% (61/62) if the Cas9 carrying F<sub>0</sub> grandparent was female (F<sub>2</sub>♂, −Cas9 in Fig. 1d and Supplementary Table S10). For bgcn-Cas9 this was 7% (14/196) or 17% (22/129), and for nup50-Cas9 this was 95% (586/615) or 98% (924/946). However, if only the w<sup>GDe</sup> element was inherited, no cross had more than 1% of the pooled ♂ and ♀ progeny present with a somatic phenotype, presumably resulting from the lack of paternal Cas9 deposition into the sperm (F<sub>2</sub>♂, −Cas9 in Fig. 1d and Supplementary Table S10). For each cross, this was a significant difference (Supplementary Table S10) indicating somatic expression, without substantial paternal deposition of Cas9/Cas9:gRNA*. In the case of the <0.1% rate observed in the progeny of F<sub>1</sub> drive males, in the contrasts to female double heterozygotes where only the w<sup>GDe</sup> element was inherited, 40% (39/98) of progeny presented with a somatic phenotype if the Cas9 carrying F<sub>0</sub> was male, while 95% (124/131) if the Cas9 carrying F<sub>0</sub> grandparent was female. An astounding 99% (75/76) or 100% (61/61) of the sds3 and 100% (462/462) or 99% (528/533) of the nup50 progeny presented with somatic phenotypes (F<sub>2</sub>♂, −Cas9 in Fig. 1d and Supplementary Table S11). This indicates strong maternal deposition of Cas9/Cas9:gRNA*. For each cross, this was a significant difference (Supplementary Table S11).

Maternal Cas9 deposition without substantial paternal deposition has been reported for many other drive systems12,13,21–24.
Grandparent enhanced somatic phenotype

Surprisingly, in the $w^{GDe}$ inheriting progeny, we observed a trend where a higher fraction of the progeny exhibited a somatic phenotype when the Cas9-carrying grandparent was female as opposed to male ($F_0^w$ vs $F_0^m$ in Fig. 1d). Contrasting each male $F_0$ Cas9 carrying grandparent cross with the equivalent cross with a female $F_0$ Cas9 (each row in Fig. 1d) showed, for female $F_0$ Cas9, an average 5.2% (sd:14.4%) percentage point increase in white/mosaic eyed phenotype among +/− $w^{GDe}$ $F_2$ progeny. While maternal deposition from a Cas9-carrying grandparent may increase the number of $w^{GDe}$ and NHEJ mutated alleles passed along by the $F_1$ parental generation to $F_2$ progeny, this should not, in contrast to what we observe (Fig. 1d), influence the phenotype of the progeny that inherit the $w^{GDe}$ element. If the $w^{GDe}$ element is inherited there is no opportunity to inherit a germline NHEJ mutation that was created due to deposition from the grandparent into the parent. We created a generalised linear model that included Cas9 promoter, $F_2$ Cas9 status, $F_1$ drive gene, $F_2$ progeny, initially of the $GDe$ genotype, that display a mosaic or total loss of eye pigment phenotype due to disruption of their white gene which is tightly linked to the sex-determining region ($M$ or $m$). A sex-specific model was used to fit to the data, with the $GDe$ status of the $F_1$ drive parent and the sex of the $F_2$ progeny as factors. Sex of the $F_2$ progeny reveals the mechanism of inheritance bias in A. aegypti, the white gene is tightly linked to the sex-determining locus. This locus comprises two forms, a dominant male determining allele $M$ and a corresponding $m$ allele, such that males are $M/m$ and females $m/m$. While the molecular basis of sex determination in this mosquito is not fully understood, $M$ is associated with Nix, a gene shown to be involved in sex determination. Analogous to an XY chromosome system, male offspring of an $M/m$ male always carry the paternal $M$ allele and female offspring the parental $m$, with no such distinction between the two allelic states of the mother. For the male

![Diagram](https://example.com/diagram1.png)

**Fig. 1** Gene drive element ($w^{GDe}$) inheritance and somatic eye phenotype in the progeny of double heterozygote split drive carriers. a An illustration of gRNA:Cas9 split drive system. The gene drive element $w^{GDe}$ is inserted into, and disrupts, the white gene which is tightly linked to the sex-determining region ($M$ or $m$). b Breeding schemes for the four crosses per Cas9 expression variant. The solid boxes indicate the $F_1$ genotypes that may bias the inheritance of $w^{GDe}$ in their germline. The upper family tree shows the double heterozygous $F_1$ with paternally contributed Cas9 and maternally contributed $w^{GDe}$, $m$-linked in both $F_1$ males and females. The bottom family tree shows the double heterozygous $F_1$ with paternally contributed $w^{GDe}$, $M$-linked in male and $m$-linked in female $F_2$s, and maternally contributed Cas9. c $F_2$ drives parent germline inheritance bias of $w^{GDe}$ when combined with a $sds3$, $bgcn$ or $nup50$ Cas9 expressing element. The horizontal dotted line indicates the expected Mendelian 50% inheritance. For $nup50$, individual crosses were performed, and each circle represents the percentage of $w^{GDe}$ positive progeny from an individual parent. Data are presented as mean values with the Wilson confidence intervals for the binomial proportion calculated for the pooled progeny count, which does not take into account the potential lack of independence due to parent-by-parent batch effects. Stars indicate the p value thresholds from two-sided Fisher’s exact tests to the matched drive sex $F_1$–Cas9 condition. The $p$ values and number of progeny scored are presented in Supplementary Table S9. Source data are provided as a Source Data file. d The percentage of $w^{GDe}$ inheriting $F_2$ progeny, initially of the $w^{GDe}$ genotype, that display a mosaic or total loss of eye pigment phenotype due to disruption of their $w$ allele. The circle size indicates the number of progeny, and circle colour indicates if the Cas9 carrying $F_0$ grandparent was male (Blue) or female (Orange). Progeny from $F_1$ drive females is indicated with ‘Maternal Deposition’. Progeny that inherited both a $w^{GDe}$ allele and Cas9 element are indicated with ‘Somatic Expression’. White phenotype rates for the $F_2$ progeny that did not inherit $w^{GDe}$ are shown in Supplementary Fig S3.
parent, if the initial linkage of \(w^{GDe}\) to \(m\) or \(M\) is known (determined by the sex of the \(w^{GDe}\)-carrying grandparent), the sex of the progeny can be used as an indication of whether an observed inheritance bias is due to new recombination events (homing), or increased inheritance of the original drive carrying chromosome (meiotic drive) (Fig. 2a). To this end, we stratified the \(w^{GDe}\) inheritance by the sex of the \(F_2\) progeny for each of the double heterozygous male parents (Fig. 2b).

The background recombination rate of \(w^{GDe}\) and sex in the absence of any Cas9 element was 1.08% (13/1203) (Supplementary Table S8) and was compared by Fisher’s Exact tests to the recombination rate from \(w^{GDe}\) Cas9 male double heterozygotes (Supplementary Table S13). As reported above, only one cross each of the \(sds3\) and \(bgcn\) double heterozygotes showed a significant increase in overall \(w^{GDe}\) inheritance. However, quantifying conversion with marked
In line with Li et al., we expression and, unexpectedly, an effect of the Cas9 carrying chromosome inheritance, was detected (Supplementary Table S15). For 48% of progeny (216/450) inherited the recipient chromosome as <0.001*** of progeny were female, >99% of which were maternally contributed Cas9 67% (1371/2059 progeny were consistent with a meiotic drive mechanism where some of the non-

Another significant increase in inheritance of the w<sup>Dde</sup> element, there was no evidence of an increased recombination rate: 0% (3/690 p-value: <0.001**) for paternal Cas9 and 0% (3/688 p-value: <0.001**) respectively. This large difference in the rate of homing between crosses with maternal vs. paternal F<sub>0</sub> Cas9 suggests that for bgcn maternally deposited Cas9 may contribute more to homing than autonomously expressed Cas9. Low expression with high maternal deposition by bgcn-Cas9 is also consistent with the observed phenotype rates of white (Fig. 1d).

For nups0 double heterozygote males with paternal Cas9 contribution, 24% (20/869 p-value: <0.001**) of the recipient chromosomes were converted by homing. For maternally contributed Cas9 this was 23% (315/1387 p-value: <0.001*) of recipient chromosomes. We also performed this analysis on the nups0 crosses reported by Li et al. (Supplementary Table S14). Despite a significant increase in inheritance of the w<sup>Dde</sup> element, there was no evidence of an increased recombination rate: 0% (3/690 p-value: 1.0**) for paternal Cas9 and 0% (3/688 p-value: 1.0**) for maternal Cas9 contribution. Instead, there was a significant bias in favour of the w<sup>Dde</sup> linked sex corresponding to the donor chromosome. For maternally contributed Cas9, 65% (1306/1996 p-value: <0.001**) of progeny were female, >99% of which were w<sup>Dde</sup> positive. For maternally contributed Cas9 67% (1371/2059 p-value: <0.001**) of progeny were male, >99% of which were w<sup>Dde</sup> positive (Supplementary Table S15). This sex bias should not occur through homing, instead, this is consistent with a meiotic drive mechanism where some of the non-w<sup>Dde</sup> chromosomes are lost, or conversion of a very large region encompassing both w<sup>Dde</sup> and the sex-determining region (Fig. 2a). For the crosses performed for this study, including the nups0 line, no significant difference in sex, and by extension recipient vs donor chromosome inheritance, was detected (Supplementary Table S15). For bgcn with maternal F<sub>0</sub> Cas9, 59% of all F<sub>2</sub> inherited the donor chromosome (male), but this did not reach our significance threshold due to the relatively low number of progeny scored for this cross.

**Discussion**

In this study, we report the efficiency and mechanisms of three CRISPR-Cas9 nuclease gene drives targeting the white gene, expanding the set of tools to develop genetic control strategies for the public-health-relevant A. aegypti mosquito. In our study, sds3, bgcn and nups0 expressed Cas9 each resulted in increased inheritance of the w<sup>Dde</sup> drive element, with the primary mechanism being homing. Additionally, for each promoter, we find evidence of maternal deposition and somatic expression and, unexpectedly, an effect of the Cas9 carrying grandparent’s sex on the grand-offspring phenotypes that we termed GESP. In line with Li et al., we find the white locus to be a good drive target, allowing for efficient transmission bias and convenient readout of an easily-scored visible recessive phenotype<sup>1</sup>. In addition, the inversion site allows for effective transgene expression from a sex-linked locus, which may be of particular use for future drives and other genetic control approaches. For the bgcn drive in males, the recipient chromosome conversion rate was much higher with maternally contributed Cas9 (19%) compared to paternally contributed Cas9 (5%). These results suggest that, in at least males, the bgcn drive may substantially function through maternally contributed Cas9. Homing through Cas9 deposition in the absence of expressed Cas9 (Shadow drive) has been reported for other drives<sup>11,13,34</sup>, but to our knowledge, not as the primary means of inheritance bias for a drive. We find nups0 and sds3-Cas9 capable of directing transmission bias in females and males, and we did not find that maternal deposition from the Cas9-carrying grandmother negatively influenced the homing rate observed in males. It is important to note that in our crosses only Cas9 could be maternally deposited into the F<sub>1</sub> double heterozygotes, maternal deposition of Cas9 protein and the gRNA simultaneously may be much less conducive to shadow drive<sup>4</sup>.

For all drives, the almost complete absence of any somatic phenotype in individuals that did not inherit the w<sup>Dde</sup> element (Supplementary Fig S3) could indicate that, while maternal deposition of the Cas9 occurs, the gRNA<sup>+</sup> or gRNA−:Cas9 complex are either not deposited or are rapidly degraded. However, progeny that did not inherit the w<sup>Dde</sup> element instead inherited the (initially) w<sup>-</sup> allele from the double heterozygous parent. For white eye phenotypes to occur in these individuals, up to two functional w<sup>-</sup> alleles may need to be disrupted by deposition instead of one; direct comparison of the rates of somatic mutation between offspring that do and do not inherit the gRNA<sup>+</sup> transgene are therefore potentially misleading. Furthermore, some non-w<sup>Dde</sup> progeny may have inherited a white allele that contained a functional, but cut resistant, NHEJ mutation (type-I resistant mutation) which would make biallelic disruption impossible.

For the w<sup>Dde</sup> F<sub>2</sub> progeny, maternal deposition from the F<sub>0</sub> grandparent could increase their probability of inheriting a mutated w<sup>-</sup> allele. As such, GESP does not apply and only refers to + w<sup>Dde</sup> F<sub>2</sub> progeny where the sex of the w<sup>Dde</sup> or Cas9-carrying grandparent see-mingly influences their propensity to present with a somatic phenotype. Although deposition from an F<sub>0</sub> grandparent may explain a change in the quantity of w<sup>Dde</sup> alleles passed along by the F<sub>1</sub> drive parent, it does not appear to explain a change in the phenotype of those F<sub>2</sub> progeny that inherited a drive element. One possible explanation for GESP may be an increased maternal deposition rate of Cas9:gRNA complexes from increased gRNA expression in w<sup>Dde</sup> homozygous germ line cells compared to w<sup>Dde</sup> heterozygous germ line cells. Consistent with this, for bgcn-Cas9 the w<sup>Dde</sup> homing rate was higher when the Cas9-carrying F<sub>0</sub> grandparent was female. A similar analysis of a single drive element (containing both Cas9 and a gRNA) found that maternal deposition rates were lower when drive conversion in the maternal germline was less<sup>27</sup>. However, in our split drive system, only the gRNA-expressing element is biased, the Cas9-expressing element remains heterozygous regardless if homing has occurred or not. It may be that different mechanisms, such as genomic imprinting or transgenerational persistence of deposited Cas9 mRNA/protein, contribute to GESP.
For nup50, the overall inheritance biasing rate and somatic/embryonic drive activity closely match those reported by Li et al.3 and underscore its potential utility for systems such as precision-guided STI.1 However, an important finding of our work is the propensity of this drive to function through two different mechanisms. The selective inheritance or elimination of a chromosome is generally achieved by creating multiple DNA breaks on the target chromosome5–10 (e.g., X-shredder) or by disrupting an essential gene11. Meiotic drive through a single cut in a non-essential gene as found by Li et al. and reported here is noteworthy. An explanation could be the chromosomal location of the induced double-stranded break. A single cut has been shown to be sufficient for inheritance bias through the loss of a chromosome in yeast when it is targeted to a centromere, while nearby sites were not sufficient.12 Chromosome loss has also been found to be a frequent outcome of allele-specific editing of a pericentromeric site in human embryos.13 The white gene is located relatively near the centromere. However, a centromere effect does not explain the difference in results from this study and that of Li et al., which instead suggests subtle differences in the rearing conditions or background genetics of the mosquito strains may have a significant influence on the underlying mechanisms. Gene drive assessment performed in D. melanogaster with different genetic background has revealed differences in drive activity but changes in the underlying mechanism were not investigated.10 The nup50-Cas9 and wDx transgenic lines used in this study are derived from those described in Li et al., but the crosses to assess homozygotes were made to Liverpool (LVP) strains maintained for a long period of time in different insectaries. Mosquito colonies maintained in laboratories can suffer from founder and drift effects, affecting their genetic background and reducing their heterozygosity.14 Moreover, genetic variability in A. aegypti colonies of the same strain but reared in different laboratories has been documented.14 There may also be methodological factors that could allow the same biological processes to manifest differently (e.g., different screening timings with genotype-dependent mortality rates).

A limitation of our study is that we cannot rule out that the sex bias we report for Li et al. nup50-Cas9 is due to copying of the estimated 45Mbp14–16 region comprising both the wDx and the sex-determining region (Fig. 2a). However, the large distance between the wDx drive and the M/m locus leads us to believe that this is unlikely, as co-inversion in similar contexts is generally reported to be on the scale of 100s of base pairs17,24–26. Furthermore, a substantial fraction of conversion tracts have been reported to be unidirectional in A. aegypti.17–20 This suggests that even if large-scale co-inversion was favoured, some repair events should still have caused recombination between wDx and the sex-determining locus if co-inversion occurred primarily in the other direction relative to the sex-determination locus. Finally, several studies have reported partial homing events12,27,30–32. These partial homing events are seemingly due to sequences in the drive element (such as the gRNA gene) having undesired homology to the recipient chromosome (shown for wDx in Supplementary Fig S1) and result in only part of the drive element being copied over. These reports of partial homing are inconsistent with a single DNA break inducing large-scale homing beyond the (immediately) adjacent regions of homology.

There are additional phenomena that can lead to biased inheritance with a sex-linked transgene. In particular, alleles with sex-specific lethal effects may be clustered within the neighbourhood of the sex-determining region in A. aegypti and can become linked to a transgene.2 However, the meiotic drive we report shows a reciprocal sex bias depending on the linkage of the wDx element with the M or m locus and the use of a split drive system demonstrates that the effects depend on Cas9 activity and are not simply due to the wDx insertion or a linked allele. A more comprehensive analysis of (even more distal) sequence differences between donor and recipient chromosomes after DNA repair may further inform the exact mechanism of inheritance bias. However interpretation of such data must be done with caution, donor chromosome sequences (including the drive element) may incorrectly appear homozygous when NHEJ mutations cause the binding sites of a PCR primer to be blocked on the recipient chromosome. This issue has been raised in several analyses12,15–16 and highlights potential pitfalls for identifying homing events with these types of molecular assays. We highlight these cases specifically because we believe such genetic assays are worth pursuing, but should be informed by this prior work to reduce the chance of misinterpretation.

To our knowledge, for gene drives designed to function through homing, recipient/donor chromosome markers have been used with non-CRISPR nucleases in D. melanogaster17,19 and A. gambiae20 and with CRISPR-Cas9 in D. melanogaster12, A. aegypti11 and M. musculus.13–14 There may be additional cases in which a split drive element can coincidentally act as a chromosome marker. In D. melanogaster, some studies have noted a reduced inheritance of the recipient chromosome, however, these may be attributable to genotype-specific fitness effects instead of DNA damage-induced loss of the recipient chromosome.11,14 Xu et al have performed the most extensive investigation of homing drives with marked chromosomes and found a mix of homing and bias through chromosome damage20.

In light of our results, re-evaluation of the A. gambiae I-sel gene drive reported by Windbichler et al. may suggest that a meiotic drive effect in homing drive designs is more widespread in mosquitoes20. Their drive-carrying line had a small marker (NotI restriction site) located ∼0.7 kilobases from the I-sel cut-site on the recipient chromosome, but not on the donor drive chromosome. They reported 86% inheritance of the drive element from heterozygote males. However, drive alleles that included the NotI site only accounted for around half the increased drive allele inheritance. The authors attributed this discrepancy to co-conversion, where homing of the drive element also replaced the nearby NotI marker. A combined meiotic drive and homing effect would seem to provide an alternative explanation. In the M. musculus drive reported by Grunwald et al. the recipient chromosome had a linked coat colour marker that allowed the homing events to be precisely tracked.15 In females, vas-Cre induced CAG-Cas9 expression resulted in homing rates of 42% (36/86) and 11% (5/47) depending on the Cas9 insertion site. In males, no homing was observed with any drive. However, for the iast drives, males passed along the donor drive chromosome to 63% (45/71) and 54% (49/91) of offspring. It should be noted that detecting meiotic drive using this method is less sensitive than detecting homing, and more progeny would need to be scored to have confidence in this trend. Together, these results suggest that a meiotic mechanism in drives intended to function through homing may be more common than currently realised. Distinguishing these mechanisms requires linked markers; for some organisms, this type of in-depth investigation may best be reserved for drives that after initial tests warrant further development.

Our work further expands the Cas9 expression patterns that have been tested in the context of mosquito gene drives. It is notable that the drives with a homing design reported in Anopheles mosquitoes A. gambiae13–15,18 and A. stephensi16, almost invariably have a dramatically higher conversion rate than those found in A. aegypti.17–20 It is not clear what underlies this difference. However, the fact that the modest conversion rate for nup50-Cas9 males remains stable despite a change in the mechanism may limit possible explanations. This stability suggests that the factors that negatively affect the conversion rate in A. aegypti are not specific to either homing or meiotic drive. Moreover, it also indicates that the difference in conversion rate observed between mosquito species is probably not due to the species favouring one mechanism over the other. Yet, the difference in mechanism between homing and meiotic drive through gamete destruction has important practical implications: first, the loss of gametes through a meiotic-drive
mechanism may negatively affect mating competitiveness by lowering the number of viable gametes, though in some cases gametes may be produced in sufficient excess for this not to be significant. The homing mechanism functions through conversion and should not affect gamete numbers. For the $\text{nupsO}$ meiotic drive reported by Li et al., male $\text{nupsO-Cas9}$ fecundity was tested and found to not differ from wildtype.\textsuperscript{62} Second, on a ‘per cut’ basis, meiotic drive is moderately less efficient than homing. When meiotic drive removes a non-drive gamete/embryo, it thereby benefits the remaining gametes/embryos. These may, in addition, to drive carrying gametes, include other wildtype and cut-resistant allele-carrying gametes that were not destroyed. In contrast, homing converts a non-drive gamete to a drive gamete, which does not benefit any of the leftover non-drive gametes making homing more efficient. Third, the linkage between different drive components may vary significantly depending on the mechanisms: for instance, if in a split drive system the Cas9 is located near the gRNA element homing would still only increase the number of gRNA alleles, but not the Cas9 alleles. However, meiotic drive would increase the inheritance of both the gRNA and Cas9 elements. This could theoretically cause a split drive or aaisy-chain drive\textsuperscript{48} to spread more than anticipated. Locating each element on separate chromosomes would prevent this, and our data suggest that this may be a wise precaution to increase the predictability of their invasiveness. Although, if anticipated or identified in early-stage field trials, a meiotic drive-induced linkage between elements could also be leveraged, lowering the required release frequencies\textsuperscript{48}. Nonetheless, in regards to risk-assessment of rare recombination events, the genomic distance at which two split drive elements become strongly linked is presumably still much more permissive for a meiotic drive mechanism as opposed to a homing mechanism. Last, in the case of Li et al.’s white targeting $A.\ aegypti$ drive, its linkage to the sex-determining locus caused an otherwise neutral replacement drive to act, in males, like a sex-biasing suppression drive. This might be desirable for some applications, but surely detrimental if the intended application were different. Most of these concerns apply even if the actual mechanism is co-conversion/copy-grafting of a large chromosome segment as opposed to meiotic drive.

Methods

DNA constructs

The sequence and insertion site of the 3xP3-\textit{tdTomato} carrying gRNA element (Supplementary Fig S1) and $\text{nupsO}$ lines are described in ref.\textsuperscript{12} and the $\text{bgcn-Cas9}$\textsuperscript{8} and $\text{sds3-Cas9}$ constructs were produced by making several alterations to those original plasmids, provided by Omar Akbari.\textsuperscript{41} These plasmids contain, within \textit{piggyBac} terminal sequences, Cas9 expressed by $\text{nupsO}$ followed by a T2A self-cleaving peptide and EGFP and an OpIE2-DSRed cassette. To improve the visibility of the fluorescent marker, this was replaced with PUB-mCherry-SV40 for $\text{bgcn}$ and $\text{sds3}$. To reproduce the germline-specific expression patterns predicted for these genes, the Cas9:EGFP coding sequence is preceded and followed by the non-coding sequences flanking the endogenous $\text{bgcn}$ or $\text{sds3}$ gene’s open reading frame, followed by an additional P10 3′UTR. The $\text{bgcn}$ and $\text{sds3}$ constructs use a Cas9 that is insect codon optimised\textsuperscript{12}. The $\text{nupsO}$ line makes use of a human codon optimised Cas9$^\text{4}$.

Mosquito lines

No ethical approval was required for working with the insect lines used in this study. $A.\ aegypti$ Liverpool strain (WT) was a gift from Jarek Krzywinski. The $\text{nupsO-Cas9}$ and white gRNA expressing element $\text{w}^{\text{bgcn}}$ ($\text{w}^{\text{bgcn-Cas9}}$) lines were provided by Omar Akbari.\textsuperscript{41} The $\text{sds3-Cas9}$ line was generated by standard embryo microinjection with a hyper-active \textit{piggyBac} transposase helper\textsuperscript{11}. At Pirbright, the $\text{nupsO-Cas9}$ line was maintained as a mix of homozygotes and heterozygotes with periodic selective elimination of wildtypes; the $\text{w}^{\text{bgcn}}$ element line was provided as homozygous and maintained in our facilities by screening for the white eye phenotype (homozygous knockout of \textit{white}) and the fluorescent marker. Cas9 expressing lines generated at the Pirbright facilities were maintained as heterozygotes, usually by crossing transgenic males to WT females and selecting for the fluorescent marker.

All mosquito lines were reared in an insectary facility under constant conditions of 28°C, 65–75% relative humidity and 12:12 light/dark cycle (1h dawn/1h dusk). Larvae were fed ground TetraMin flake fish food (TetraMin) while adults were provided with 10% sucrose solution ad libitum. Defibrinated horse blood (HB034, TCS Bioscience) was provided using a Hemotek membrane feeding system (6W1 system, Hemotek Ltd) covered with Parafilm (HS234526A, Bemis).

Crosses for homing assessment

Male and female adults, homozygous for $\text{w}^{\text{bgcn}}$ were crossed with mosquitoes of the Cas9 lines. Their progeny were screened as late larvae under fluorescence using a Leica MZ165FC microscope. The eye phenotype was also evaluated. Double heterozygous mosquitoes carrying both transgenes were then crossed to WT mosquitoes. Inheritance of the transgenes as well as eye phenotype, was again assessed under a fluorescence microscope. For $\text{nupsO-Cas9}$, double heterozygotes were individually crossed. For $\text{bgcn-Cas9}$ and $\text{sds3-Cas9}$ multiple double heterozygotes were crossed simultaneously to WT of the opposite sex. The exact number and phenotype of the progeny of each cross are shown in Supplementary Tables S2–S3. The individual cross data for $\text{nupsO-Cas9}$ are shown in Supplementary Table S4–S7. In some cases, F1 double heterozygotes produced from the same cross presented with a different fluorescent marker or eye pigment phenotypes. In each case, these were noted in the cross tables, and examples of the phenotypes are shown in Supplementary Fig S2.

Statistical analysis of $\text{w}^{\text{bgcn}}$ inheritance bias

For each F1 sex, the $\text{w}^{\text{bgcn}}$ inheritance rate in the absence of a Cas9 expressing element (Supplementary Table S8) was used as the baseline inheritance. This was 52% (620/1203) for males and 51% (308/605) for females. These rates were used as the expected outcome in a two-sided Fisher’s exact test with the $\text{w}^{\text{bgcn}}$ inheritance from F1 parents that carried the $\text{w}^{\text{bgcn}}$ and one of the Cas9 expressing elements. A significant difference in $\text{w}^{\text{bgcn}}$ inheritance is taken as evidence for drive activity. See Supplementary Table S9.

Statistical analysis of somatic expression and parental deposition

For each Cas9 line, the fraction of mosaic-eyed (ME) or white-eyed (WE) progeny among the F2 offspring inheriting $\text{w}^{\text{bgcn}}$ but not the Cas9 (+/−) from F1 drive males served as a control for the frequency of such phenotypes in the absence of somatic expression or maternal deposition. For somatic expression, the ME/WE fraction of the F2 progeny harbouring both the Cas9 and $\text{w}^{\text{bgcn}}$ elements from F1 drive males was compared to the control cross using a two-sided Fisher’s exact test (Supplementary Table S10). For maternal deposition, the F2 progeny harbouring only the $\text{w}^{\text{bgcn}}$ element from F1 drive females as compared to the control (Supplementary Table S11).

Statistical analysis of the influence of factors on the fraction of mosaic and white-eyed progeny

A generalised linear model with binomial errors was created that included Cas9 promoter ($\text{sds3}$, $\text{bgcn}$, $\text{nupsO}$), F2 Cas9 status (+/−), F2 sex (♂/♀), F1 drive parent sex (♂/♀), and F0 Cas9 carrying grandparent sex (♂/♀). The response variable was the proportion of ME and WE progeny among all the F2 progeny from that cross and F2 sex (48 conditions). The analysis was performed in R version 4.0.2 using the glm function. See Supplementary Table S12.
Statistical analysis of homing and meiotic drive

For homing, the background recombination rate (calculated from the $F_1 + w^{Cas}$—Cas9 male cross Supplementary Table S8) is used as the expected outcome in a two-sided Fisher’s exact test. For the control cross (in the absence of possible Cas9-mediated inheritance bias) the $w^{Cas}$ allele was provided by the male F0 grandparent and therefore M-linked to the F1 males. In the absence of recombination, all F2 males should be $w^{Cas}$ positive, and all F2 females should be $w^{Cas}$ negative. Out of the I203 progeny scored, we saw 13 (1.08%) recombination events. 2 out of 609 $F_2$ males were $w^{Cas}$ positive, and 11 out of 581 $F_2$ females were $w^{Cas}$ positive. For the crosses including a Cas9 element, a statistically significant increase in recombination rate between the recipient/donor chromosome marker and the drive element was taken as evidence of homing (Supplementary Table S13). For meiotic drive, a statistically significant difference in the inheritance of either the recipient or donor chromosome (i.e., $F_2$ sex) is taken as evidence for meiotic drive (Supplementary Table S15). The progeny sex ratio is compared to the sex ratio in the absence of a Cas9-expressing element (Supplementary Table S8).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All the datasets generated during the current study are included in the supplementary information/source data file. The Li et al. data used in this study are available in the supplemental files of the original article https://doi.org/10.7554/E1Life.31701.

Code availability

R version 4.0.2 was used for data analysis. The script for analysis and additional files are available at: https://osf.io/q4e3j/.

References

1. Alphey, L. S., Crisanti, A., Randazzo, F. F. & Akbari, O. S. Opinion: standardizing the definition of gene drive. Proc. Natl Acad. Sci. 117, 30864–30867 (2020).
2. Burt, A. & Trivers, R. Genes in Conflict: The Biology of Selfish Genetic Elements, vol. 1 (Harvard University Press, 2006).
3. Nambiar, T. S., Baudrier, L., Billon, P. & Ciccia, A. CRISPR-based genome editing through the lens of DNA repair. Mol. Cell 82, 348–388 (2022).
4. Oberhofer, G., Ivy, T. & Hay, B. A. Gene drive and resilience through renewal with next generation Cleave and Rescue selfish genetic elements. Proc. Natl Acad. Sci. 117, 9013–9021 (2020).
5. Oberhofer, G., Ivy, T. & Hay, B. A. Cleave and Rescue, a novel selfish genetic element and general strategy for gene drive. Proc. Natl Acad. Sci. 116, 6250–6259 (2019).
6. Champer, J. et al. A toxin-antidote CRISPR gene drive system for regional population modification. Nat. Commun. 11, 1082 (2020).
7. Windbichler, N., Papathanos, P. A. & Crisanti, A. Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryonic lethality in anopheles gambiae. PLoS Genet. 4, e1000291 (2008).
8. Galizi, R. et al. A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nat. Commun. 5, 3977 (2014).
9. Hickey, W. A. & Craig, G. B. J. Genetic distortion of sex ratio in a mosquito, Aedes aegypti. Genetics 53, 1177–1196 (1966).
10. Sweeny, T. L. & Barr, A. R. Sex ratio distortion caused by meiotic drive in a mosquito, Culex pipiens L. Genetics 88, 427–446 (1978).
11. Guichard, A. et al. Efficient allelic-drive in Drosophila. Nat. Commun. 10, 1640 (2019).
12. Ly, M. et al. Development of a confinable gene drive system in the human disease vector Aedes aegypti. eLife 9, 1–40 (2020).
13. Grunwald, H. A. et al. Super-Mendelian inheritance mediated by CRISPR-Cas9 in the female mouse germline. Nature 566, 105–109 (2019).
14. Terradas, G. et al. Inherently confinable split-drive systems in Drosophila. Nat. Commun. 12, 1480 (2021).
15. Xu, X.-R. et al. Active genetic neutralizing elements for halting or deleting gene drives. Mol. Cell 80, 246–262 (2020).
16. Weitzel, A. J. et al. Meiotic Cas9 expression mediates gene conversion in the male and female mouse germline. PLOS Biol. 19, e3001478 (2021).
17. Chan, Y.-S., Huen, D. S., Glauert, R., Whiteway, E. & Russell, S. Optimising homing endonuclease gene drive performance in a semi-refractory species: the Drosophila melanogaster experience. PLoS One 8, e54130 (2013).
18. Simoni, A. et al. Development of synthetic selfish elements based on modular nucleases in Drosophila melanogaster. Nucleic Acids Res. 42, 7461–7472 (2014).
19. Chan, Y.-S. et al. The design and in vivo evaluation of engineered l-Onul-based enzymes for HeG gene drive. PLoS One 8, e74254 (2013).
20. Windbichler, N. et al. A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. Nature 473, 212–215 (2011).
21. Magnusson, K. et al. Transcription regulation of sex-biased genes during ontogeny in the malaria vector anopheles gambiae. PLoS One 6, e21572 (2011).
22. Bauer DuMont, V. L., Flores, H. A., Wright, M. H. & Aquadro, C. F. Recurrent positive selection at Bgcn, a key determinant of germ line differentiation, does not appear to be driven by simple coevolution with its partner protein Bam. Mol. Biol. Evol. 24, 182–191 (2007).
23. Hammond, A. M. et al. The creation and selection of mutations resistant to a gene drive over multiple generations in the malaria mosquito. PLOS Genet. 13, e1007039 (2017).
24. Oberhofer, G., Ivy, T. & Hay, B. A. Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs. Proc. Natl Acad. Sci. 115, E9343–E9352 (2018).
25. López Del Amo, V. et al. A transcomplementing gene drive provides a flexible platform for laboratory investigation and potential field deployment. Nat. Commun. 11, 352 (2020).
26. Gantz, V. M. et al. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc. Natl Acad. Sci. 112, E6736–E6743 (2015).
27. Champer, J. et al. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLOS Genet. 13, e1006796 (2017).
28. Pfitzner, C. et al. Progress toward zygotic and germline gene drives in mice. CRISPR J. 3, 388–397 (2020).
29. Kandul, N. P., Liu, J., Bennett, J. B., Marshall, J. M. & Akbari, O. S. A confinable home-and-rescue gene drive for population modification. eLife 10, 24377–24383 (2021).
30. Adolfi, A. et al. Efficient population modification gene-drive rescue system in the malaria mosquito Anopheles stephensi. Nat. Commun. 11, 5553 (2020).
31. Pham, T. B. et al. Experimental population modification of the malaria vector mosquito, Anopheles stephensi. PLOS Genet. 15, e1008440 (2019).
32. Hammond, A. et al. Regulating the expression of gene drives is key to increasing their invasive potential and the mitigation of resistance. PLOS Genet. 17, e1009321 (2021).
33. Champer, J. et al. Molecular safeguarding of CRISPR gene drive experiments. eLife 8, 1–10 (2019).
34. Kandul, N. P. et al. Assessment of a split homing-based gene drive for efficient knockout of multiple genes. G3 Genes|Genomes|Genet. 10, 827–837 (2020).
35. Aryan, A. et al. Nix alone is sufficient to convert female Aedes aegypti into fertile males and myo-sex is needed for male flight. Proc. Natl Acad. Sci. 117, 17702–17709 (2020).
36. Xu, H. et al. Chromosome drives via CRISPR-Cas9 in yeast. Proc. Natl Acad. Sci. 115, 5522–5527 (2018).
37. Zuo, E. et al. CRISPR/Cas9-mediated targeted chromosome elimination. Genome Biol. 18, 224 (2017).
38. Adikusuma, F., Williams, N., Gruzné, F., Hughes, J. & Thomas, P. Targeted deletion of an entire chromosome using CRISPR/Cas9. Mol. Ther. 25, 1736–1738 (2017).
39. Ross, P. A., Endersby Harshman, N. M. & Hoffmann, A. A. A comprehensive assessment of inbreeding and laboratory adaptation in Aedes aegypti mosquitoes. Evol. Appl. 12, 572–586 (2019).
40. Zuccaro, M. V. et al. Allele-specific chromosome removal after Cas9 cleavage in human embryos. Cell 183, 1650–1664 (2020).
41. Ang, M. A. E. et al. A CRISPR-Cas9 gene drive targeting doublesex gene drive variants in the mosquito Aedes aegypti via single generation crosses and modeling. G3 Genes|Genomes|Genetics https://doi.org/10.1093/g3journal/jka280 (2022).
42. Zuccaro, M. V. et al. Allele-specific chromosome removal after Cas9 cleavage in human embryos. Cell 183, 1650–1664 (2020).
43. Verkuijl, S. A. N., Ang, J. X. D., Alphey, L., Bonsall, M. B. & Anderson, M. A. E. The challenges in developing efficient and robust synthetic homing endonuclease gene drives. Front. Bioeng. Biotechnol. 10, 856981 (2022).
44. Kandul, N. P. et al. Transforming insect population control with precision guided sterile males with demonstration in flies. Nat. Commun. 10, 84 (2019).
45. Matthews, B. J. et al. Improved reference genome of Aedes aegypti. Genet. Mol. Biol. 40, 225–232 (2017).
46. Giraldo-Calderón, G. I. et al. VectorBase: an updated bioinformatics resource for invertebrate vectors and other organisms related with human diseases. Nucleic Acids Res. 43, D707–D713 (2015).
47. Hammond, A. M. The Development of Gene Drives for Genetic Control of the Malaria Mosquito. Ph.D. thesis, Imperial College London (2016). https://doi.org/10.25560/53131.
48. Ang, J. X. D. et al. Considerations for homology-based DNA repair in mosquitoes: Impact of sequence heterology and donor template diversity of laboratory strains and implications for research: the case of Aedes aegypti. PLOS Negl. Trop. Dis. 13, e0007930 (2019).
49. Ang, M. A. E. et al. A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nat. Biotechnol. 36, 1062–1066 (2018).
50. Hammond, A. M. The Development of Gene Drives for Genetic Control of Malaria. Ph.D. thesis, Imperial College London (2016). https://doi.org/10.25560/53131.
51. Ang, M. A. E. et al. A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nat. Biotechnol. 36, 1062–1066 (2018).
52. Reitano, S. A. N., Ang, J. X. D., Alphey, L. & Anderson, M. A. E. The challenges in developing efficient and robust synthetic homing endonuclease gene drives. Front. Bioeng. Biotechnol. 10, 856981 (2022).
53. Kandul, N. P. et al. Transforming insect population control with precision guided sterile males with demonstration in flies. Nat. Commun. 10, 84 (2019).
54. Adikusuma, F., Williams, N., Gruzné, F., Hughes, J. & Thomas, P. Targeted deletion of an entire chromosome using CRISPR/Cas9. Mol. Ther. 25, 1736–1738 (2017).
55. Zuo, E. et al. CRISPR/Cas9-mediated targeted chromosome elimination. Genome Biol. 18, 224 (2017).
56. Ang, J. X. D. et al. Considerations for homology-based DNA repair in mosquitoes: Impact of sequence heterology and donor template diversity of laboratory strains and implications for research: the case of Aedes aegypti. PLOS Negl. Trop. Dis. 13, e0007930 (2019).
57. Matthews, B. J. et al. Improved reference genome of Aedes aegypti informs arbovirus vector control. Nature 563, 501–507 (2018).
58. Giraldo-Calderón, G. I. et al. VectorBase: an updated bioinformatics resource for invertebrate vectors and other organisms related with human diseases. Nucleic Acids Res. 43, D707–D713 (2015).
59. Hammond, A. M. The Development of Gene Drives for Genetic Control of the Malaria Mosquito. Ph.D. thesis, Imperial College London (2016). https://doi.org/10.25560/53131.
60. Zuo, E. et al. CopyCatches are versatile active genetic elements that detect and quantify inter-homolog somatic gene conversion. Nat. Commun. 12, 2625 (2021).
61. Li, M. et al. Germline Cas9 expression yields highly efficient genome engineering in a major worldwide disease vector, Aedes aegypti. Proc. Natl Acad. Sci. 114, E10540–E10549 (2017).
