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Altering traits and fates of wild populations with Mendelian DNA sequence modifying Allele Sails

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Population-scale genome modification can alter the composition or fate of wild populations. Synthetic gene drives provide one set of tools, but their use is complicated by scientific, regulatory, and social issues associated with transgene persistence and flow. Here we propose an alternative approach. An Allele Sail consists of a genome editor (the Wind) that introduces DNA sequence edits, and is inherited in a Mendelian fashion. Meanwhile, the edits (the Sail) experience an arithmetic, Super-Mendelian increase in frequency. We model this system and identify contexts in which a single, low frequency release of an editor brings edits to a very high frequency. We also identify conditions in which manipulation of sex determination can bring about population suppression. In regulatory frameworks that distinguish between transgenics (GMO) and their edited non-transgenic progeny (non-GMO) Allele Sails may prove useful since the spread and persistence of the GM component can be limited.

The ability to modify or build resilience into ecosystems is increasingly desirable to confront a range of challenges including vector-borne diseases, agricultural loss, the spread of invasive species, and climate change. One important tool to address these are population-scale genetic alterations. These changes can introduce beneficial traits into a population (population modification) or eliminate a harmful population (population suppression). For example, population modification could promote resilience of an endangered or threatened species by bringing currently beneficial genomic modifications to high frequency. It could also be used to introduce "anticipatory" sequence changes designed to provide a benefit in a likely future environment altered by climate change or the introduction of an invasive disease vector. These possibilities are suggested by the growing number of contexts in which one or a modest number of alleles of large effect can produce significant phenotypic benefits for a population. Examples include a single locus that can confer heat resistance in mussels[1] and cattle[2], fungi resistance in certain plants[3], or varroa mite resistance in bees[4,5]. There are also collections of loci that have been suggested to contribute to coral resistance to heat[6,7], bird resistance to malaria[8], or frog resistance to chytrid fungi[9]. Genetic alterations can also be used to reduce harm, for example by limiting the ability of insects to vector disease[10,11] or reducing the toxicity (poison production) of an invasive species such as cane toads. Finally, genetic alterations can be used to bring about population suppression, either directly[12] or by sensitizing pests to an outside stimulus.

One way to introgress important alleles into a population is via the release of individuals carrying the desired allele. A new allele under positive selection will spread but may take many generations to reach high frequency depending on the size of the benefit and whether it is dominant, additive, or recessive. Alternatively, if the allele is not under positive selection or if the release introduces transgenes meant to suppress the population, large and/or repeated introductions will be needed. These are resource intensive and impractical for many species. Additionally, controlled breeding and the large-scale introduction of these individuals into the target population could increase the frequency of other unintended and possibly harmful alleles. It could also threaten the existing genetic diversity of the target population or subspecies.

These issues can be overcome via the use of synthetic gene-drives (SGD). SGDs are made up of one or more transgenes that bias their own

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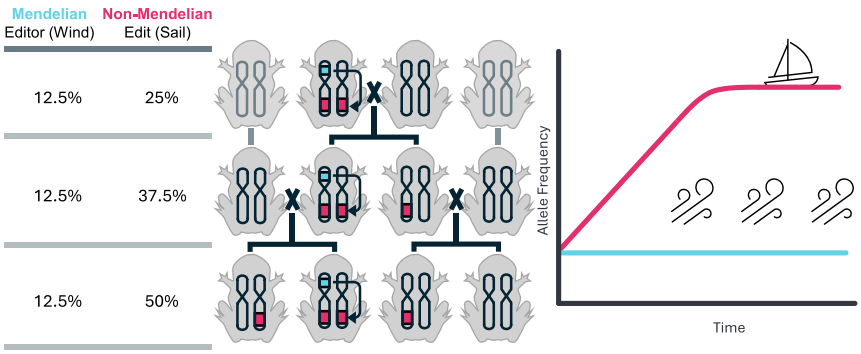

Fig. 1 | Graphical representation of Allele Sail components and behavior. The Wind (an editor, in blue) is inherited in a Mendelian fashion (left panel). When the editor is present in a germline that carries an unedited target locus located elsewhere in the genome, conversion to the edited state occurs (in pink, middle panel). This pushes the Sail (the edited locus, in pink) to higher frequency in the population. The left panel shows the idealized case for 100% editing. The right panel provides a cartoon graphic showing the Wind (blue line), at constant low frequency, pushing the sail (red line) to high frequency.

inheritance by one of several possible mechanisms[13,14], thereby spreading to high frequency. In addition to the genes required for biasing inheritance, SGDs can include one or more genes whose presence is designed to bring about a desired phenotype (cargo). A wide variety of SGDs have been devised (reviewed in refs. 13,14). However, the very features of a gene drive that makes it attractive—that it can rapidly bring to high frequency transgenes that may persist for extended periods in (and in some cases outside) the target region—create regulatory and social hurdles to implementation.

Population-scale genetic alterations with phenotypic consequences can also involve more subtle modifications such as single base changes, or small insertions or deletions. Importantly, in some regulatory environments (Australia provides one example[15]) genome edits present in non-transgenic progeny of a transgenic individual (who express a genome editor such as a Cas9 nuclease) are regulated as non-transgenic. Designation of a population carrying a high frequency of such edits as non-transgenic, provided that some low and perhaps transient level of the editor in the population is acceptable, may facilitate regulatory approval and social acceptance. Here we explore a system that we refer to as an Allele Sail, which can, in the absence of gene drive, bring edits but not the editing transgenes to high frequency for population modification, and cause suppression in certain contexts.

An Allele Sail consists of a chromosomally located genome editor. Some implementations would utilize programmable CRISPR systems such as Cas9 which cause double-strand breaks at locations specified by short guide RNAs. When these breaks are repaired using non-homologous end joining, point mutations, indels or larger deletions can be created. When gRNA multiplexing is used these often create loss-of-function (LOF) alleles of the target gene. Alternatively, nuclease dead or DNA nicking versions of Cas9 or other proteins can recruit base editors to create point mutations. Another option is prime editors which use a linked reverse transcriptase to create a variety of local sequence changes that are templated by an extended gRNA known as a pegRNA (reviewed in ref. 16).

We call this editor the Wind, as it is responsible for pushing edits into the population and is in contrast to an autonomous "drive" which increases its own (transgenic) frequency. The editor is expressed in the germline (though the expression need not be germline specific) and introduces sequence modifications at one or more target sites located anywhere in the genome. The homozygous edited individuals are viable and fertile. While the editor is transmitted in a Mendelian fashion, the edits it creates (we call these edits the Sail) increase in frequency at an arithmetic super-Mendelian rate as the editor encounters new unedited alleles in the germline each generation (Fig. 1).

Considered most broadly, an Allele Sail creates *new sequence changes* at a target locus/loci. The editor can be located on an autosome or on a sex chromosome, and as such editors linked to a Y chromosome could be considered Allele Sails. Thus far Y-linked editors have only been discussed in the context of population suppression, through creation of dominant X- or autosome-linked mutations that reduce the viability/fertility of female but not male offspring, contributing to population suppression[17]. However, they could also be used to introduce beneficial alleles, as discussed herein. In contrast, an Allele Pump uses a site-specific nuclease and homing[18], or Toxin-mediated killing of non-carriers of an Antidote in a Toxin-Antidote system[19,20], to bring about an absolute or relative increase, respectively, in the frequency of *an existing sequence*, usually a transgene located at a separate locus. Though sometimes subtle, these distinctions result in substantially different applications and outcomes for Allele Sails.

We first consider the use of an Allele Sail in population modification, where there are a variety of applications for conservation or infectious disease prevention. We then explore potential uses for population suppression in organisms that have sex determination systems in which the activity of a single gene is required for femaleness[21,22].

Results

Population modification

We consider the dynamics of an editor that introduces edits at one or more sites in the nuclear genome, resulting in progeny that are viable and fertile. The editor is transmitted in a Mendelian manner, while the edits change in frequency as a function of frequency of the editor, frequency of wild-type alleles, fitness costs and editing efficiency. To explore the use of Allele Sails for population modification, we characterize behavior of the components using a discrete-time and generation stochastic model with a panmictic population (see "Methods" for details). This type of model is often used to gain insight into population genetic processes and provides a format that allows comparison of methods for genetically altering populations.

We first consider ideal conditions: the editor alters a target sequence with 100% efficiency, and editing occurs in the male and female germline and in the progeny of a female carrier due to maternal carryover of editing activity, which is the result of maternally deposited editor mRNA and/or protein. Such activity has been demonstrated with Cas9 in both vertebrates[23] and invertebrates[24]. The power of the Allele Sail system can be seen by comparing the frequency of edits over time when introduced in the presence or absence of an editor. These comparisons are shown in Fig. 2A for an editor with no fitness cost introduced at a frequency of 10%, with the edit conferring either no fitness change, or an additive benefit or cost of 5% per allele. Recessive and dominant fitness costs/benefits are considered in Supplementary Fig. 1 and show largely similar but distinct dynamics. In the presence of

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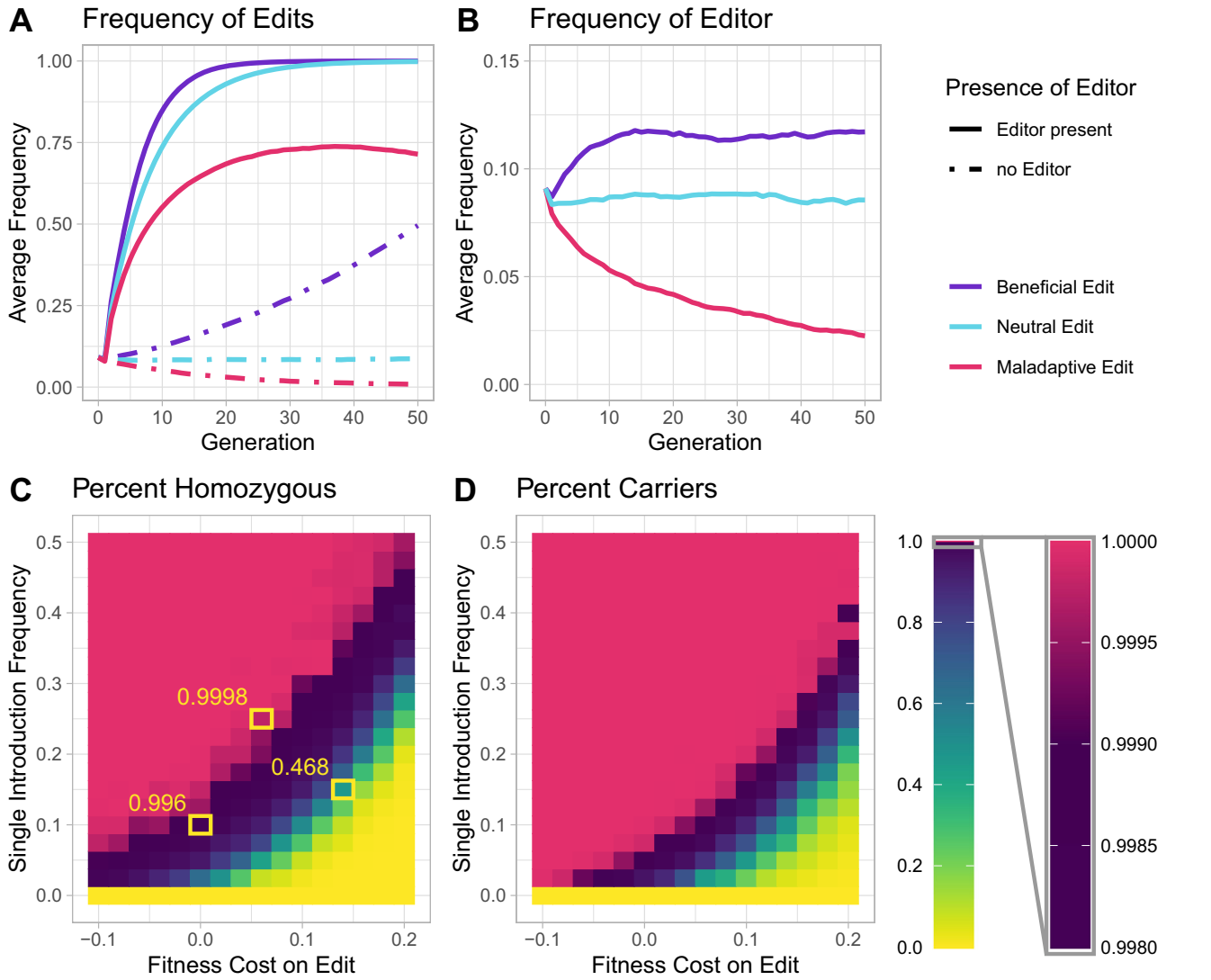

Fig. 2 | Behavior of a neutral editor and non-neutral edit. A The average allele frequency of edits after being introduced at 10% frequency. Introduced individuals are either homozygous for the edit with no editor present, or homozygous for both edit and editor when the editor is present. The fitness benefit (in purple) shown here is an additional 5% chance of survival for each copy of the edit present (additive benefits), where fitness cost (in pink) is a 5% decrease in survival for each copy of the edit present. **B** The average allele frequency of the editor, from the simulations shown in (**A**). For (**C**, **D**), the pink-purple heat map scale bar on the far right is a zoomed-in version of the highest frequency region of purple-yellow heat map scale bar gradient to its left. **C** Average percent of the population that is homozygous for an edit after 50 generations, for various editor introduction frequencies and fitness costs associated with the edit. Pink tiles represent an average allele frequency of more than 99.9% of the population, across 20 simulations. Negative fitness costs represent fitness benefits, and boxed areas highlight specific allele frequencies to provide guidance for interpretation of the heat map colors. **D** Average percent of the population that carries at least one copy of the edit, for various editor introduction frequencies and fitness costs associated with the edit, and for 20 simulations at each point, as in (**C**).

an editor, edits with no cost or a benefit spread rapidly to allele fixation. In contrast, edits conferring a cost rise to high frequency (~75%) and then decline. The peak frequencies of the introduced allele are much higher in all Allele Sail scenarios than those only relying on Mendelian inheritance of the allele.

The dynamics of edit frequency can be understood by considering the fate of the editor (Fig. 2B). When the presence of edits has no effect on fitness the editor remains at its introduction frequency, continually generating new edits until fixation is reached. When the presence of the edit confers a benefit the editor increases in frequency. This occurs because the editor spends more time in the presence of the higher fitness edited genotype (which it creates) than does its counterpart wild-type allele. The frequency of the editor plateaus when the edits are ubiquitous (allele fixation) because at this point all individuals have equal fitness. Conversely, when the edit results in a cost to carriers the frequency of the editor declines continuously, since it now spends increased time in lower fitness edited individuals (so long as these never reach fixation) than does its wild-type allele counterpart, leading to its loss through natural selection.

The general relationship between introduction frequency and edit fitness costs/benefits on the frequency of edits is shown in heat maps which plot the frequency of edit homozygotes (Fig. 2C) and carriers (homozygotes and heterozygotes) (Fig. 2D) at the 50 generation time point. There is a large region of parameter space in which edits are pushed to very high frequency, and increasing the introduction frequency has the general effect of increasing the rate of spread, as well as the time spent at high frequency for those edits that do not reach allele fixation. There is also a sharp transition where relatively small changes in fitness result in substantially different outcomes. Considering that the precise fitness impacts of actual Allele Sail components are unlikely to be well characterized in advance, and variables such as migration and population density are often important, repeated releases may be

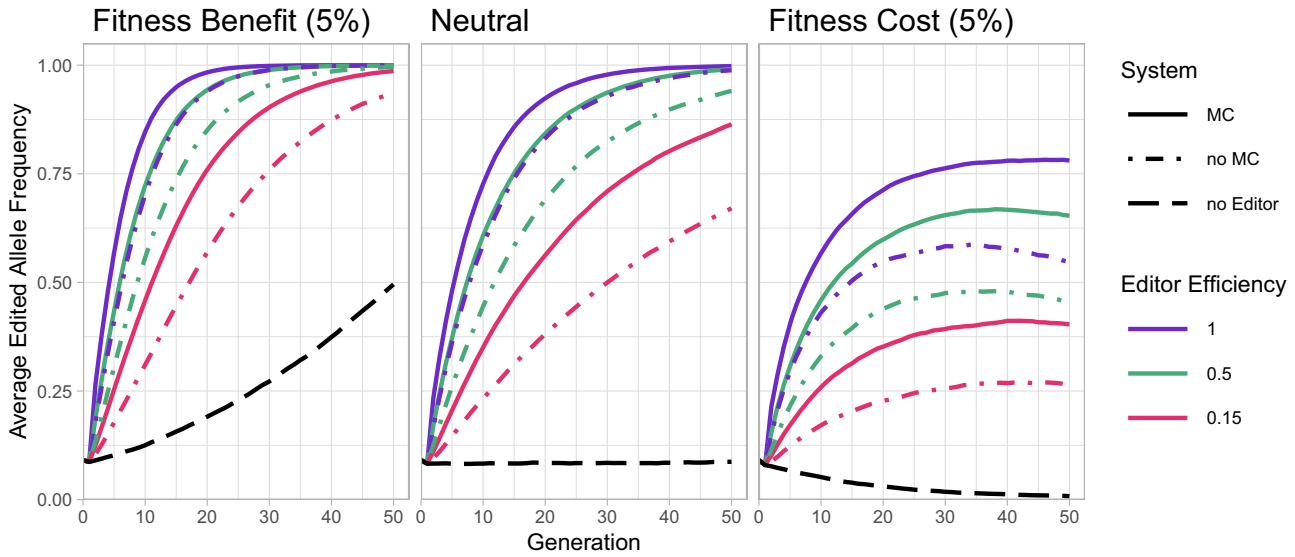

Fig. 3 | Consequences of reducing editing efficiency for population modification. Comparison of edit frequencies over time when using editors with different efficiencies (15% in pink, 50% in green, 100% in purple, and no editor in black), for various cost/benefits and presence or absence of maternal carryover (MC). Each line represents the average of 20 simulations. Fitness benefits and costs are additive and reflect a 5% increase or decrease in the chance of survival for an individual, respectively. Costs are associated with the edit, and the editor has neutral fitness.

necessary to efficiently alter the target population. Finally, we note that the dynamics of edit-bearing genotypes in the population (the frequencies of heterozygotes and homozygotes) depends to some extent on whether editing occurs in the germline with maternal carryover (illustrated above), in the germline only, or in the germline and somatic cells, as well as whether the edit results in a dominant, additive or recessive fitness effects (illustrated for a fitness cost in Supplementary Fig. 2).

The presence of the editor may result in some fitness cost to carriers[25–27]. As such, we explored the context in which the editor but not the edits result in fitness costs. This scenario is illustrated in Supplementary Fig. 3, which shows edit frequency at generation 50 as a function of introduction frequency and additive editor fitness costs, up to 10% per allele. Costs on the editor cause its eventual loss from the population and thus reduce the parameter space in which a single introduction can push edits to high frequency, though increased introduction frequencies and/or multiple releases can compensate. In some cases a guarantee of eventual loss may be desirable, such as when regulatory approval requires that transgenes do not persist in the population. In other contexts, in which the spread of edits to high frequency at minimal cost is the dominant consideration, it may be possible to take advantage of next generation editors that have increased specificity and reduced toxicity[27–30] to reduce any editor-associated fitness costs.

Consequences of altering editing efficiency

We have thus-far assumed the editor is100% efficient. CRISPR nucleases such as Cas9 can cleave and create loss of function (LOF) alleles (especially when using multiple gRNAs) in the *Drosophila* germline or the plant *Arabidopsis thaliana* at frequencies near 100%[19,31–38]. While base and prime editors also have significant levels of editing activity in *Drosophila*, they are not 100%; instead closer to 36% for a prime editor[39], and >90% for a base editor[27]. These encouraging rates notwithstanding, it is important to note that the editing enzymes used—nuclease, reverse transcriptase, deaminase, and uracil-DNA glycosylase—are often derived from organisms that live in temperature ranges very different from those in which their use is intended, which may result in significantly reduced activity in the target species[27,40,41]. To explore these less-than-ideal scenarios, we model several representative examples in which the editor is introduced at a frequency of 10% into the wild-type population and has no associated fitness costs, for various editor efficiencies (between 15–100%), both with and without maternal carryover. These results are compared with a scenario in which edits are introduced directly into the population in the absence of the editor.

Figure 3 shows that decreasing the rate of editing from 100% to 50% or 15% still results in the rapid spread of a beneficial or neutral edit to high frequency by generation 50. A deleterious allele also undergoes a significant increase in frequency, though there is ultimately a decline (which also occurs when edits are created 100% of the time) when the frequency of the editor is so low it no longer generates edits faster than they are lost through natural selection. Even so, the peak frequency and time to decline can always be improved by increasing the editor introduction frequency (Supplementary Fig. 4).

Consequences of genetic linkage between editor and edit

Thus far we have considered scenarios in which the editor and edit site are unlinked. In some cases, particularly if multiple changes are desired, some degree of linkage between the editor and one of the edits may be present. To explore the consequences of linkage we consider the extreme scenarios in which the edit and editor are either tightly linked (are always co-inherited) or unlinked (have equal chance of being co-inherited or not), the editor has a 50% probability of germline editing, and there is no maternal carryover. We utilize a lower rate of cleavage because when editing rates are 100% there is no difference between the linked and unlinked scenarios; all progeny inherit an edit from the carrier parent regardless of linkage. As shown in the heat maps in Fig. 4, for a neutral editor and deleterious edit an absence of linkage is beneficial for spread of the edit (Fig. 4). This is because an unlinked editor encounters more non-edited alleles than a linked editor. When fitness costs are assigned to the editor transgene, the unlinked version still performs slightly better than the linked (Supplementary Fig. 5). However, the differences are much smaller and more difficult to see, and beneficial edits spread rapidly regardless of linkage status. In summary, linkage can decrease rates of editing for neutral and deleterious alleles, but the effects are only significant when linkage is tight and editing rates are substantially below 100%.

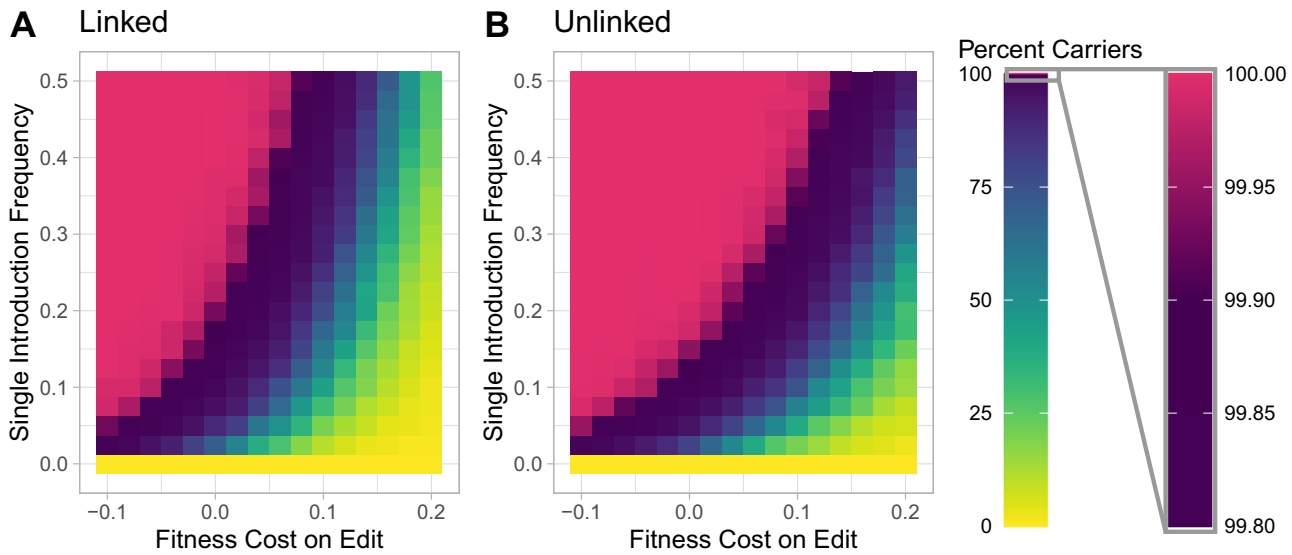

Fig. 4 | Effects of linkage between editor and edit on population modification. The frequency of editing is set to 50%, fitness costs are associated only with the edit, and there is no maternal carryover of editing. The pink-purple legend on the right is a zoomed-in version of the highest percentages of the purple-yellow bar. **A**, **B** The percentage of the population that has at least one edited allele at generation 50 averaged over 20 simulations. For higher fitness costs, tight linkage between editor & edit (**A**) results in reduced ability to push edits to high frequency as compared with the case in which editor & edit are unlinked (**B**).

Population suppression

Transgene-based population suppression strategies take several forms. In one, a self-sustaining gene drive utilizes high frequency homing to spread into (thereby inactivating) a haplosufficient gene required in somatic cells for female sexual identity, fertility, or viability. This drives the population toward a homozygous genotype that is fit in males but unfit in females, leading to a population crash[12,42,43]. There are also suppression strategies that do not rely on drive; Non-drive transgene-based approaches utilize periodic inundation of males to, by one mechanism or another, reduce the frequency of female progeny, of progeny generally, or of fertile females[17,44–48].

Here we explore how an Allele Sail could be used for population suppression by causing sex ratio distortion. Our focus is on species in which expression of a single gene is needed for femaleness, and whose loss results in conversion of these individuals into fertile males. The Transformer gene in medfly *Ceratitis capitata* provides one example (reviewed in ref. 49). Aromatase, encoded by the cyp19a1a gene, plays a similar role in a number of vertebrates. Aromatase converts androgens to estrogens and its loss through chemical inhibition or mutation converts genetic females to fertile males[22,50,51]. This, combined with the fact that estrogen agonists can promote femaleness in genetic males[51] argues that aromatase activity is necessary for femaleness in these species and knocking it out results in males.

Here we model the composition and fate of a population in which an editor such as Cas9 is introduced, creating LOF alleles (the edit) in the aromatase gene. Because single locus sex specification occurs in species with diverse chromosomal systems, we model suppression in species with XX female/XY male and ZW female/ZZ male sex chromosome systems. We also remove maternal carryover from these simulations, which allows the editor to persist in the female line for longer. While maternal carryover of Cas9 is observed with many germline promoters in insects, engineering of regulatory elements can reduce these effects (reviewed in refs. 13,14). Other strategies for limiting carryover, such as attaching a degron to the editor or expression of an inhibitor of editing activity, are under active investigation. We first consider the consequences of single releases and then multiple releases.

Dynamics of alleles and chromosomes following a single release

Results of a single release of an editor active only in the germline at a frequency of 10% of carrying capacity are shown in Fig. 5A. We compare these to results of a single release of males carrying transgenes that implement female specific Repressible Inducible Dominant Lethal (fsRIDL), a system in which an autosomal transgene, transmitted in a Mendelian manner, causes death or sterility of female progeny carriers[52]. The suppression effects of fsRIDL are immediate, but the total population also increases back to carrying capacity very rapidly. In contrast, release of an Allele Sail editor results in a relatively prolonged reduction in population size, which is particularly prominent in the ZW system (Fig. 5A). To understand why a single release of an Allele Sail with no associated fitness costs only modestly reduced population size rather than collapsing it entirely, we first examined the XY system and the frequency of the editor, the edit, and the Y chromosome over time. The initial release is of XX males homozygous for the editor and edit. When XX males mate with XX females, only female progeny are produced, since the offspring will have inherited one functional copy of the target gene, and the editor is germline specific, which allows for functional aromatase expression in the typical pattern which is primarily from somatic ovarian granulosa and luteal cells[53]. This increase in females leads to a transient spike in population numbers, as their progeny include both males and females. Interestingly, the editor undergoes a rapid decrease in frequency, even as the frequency of the edit increases dramatically and the population slowly returns to its carrying capacity (Fig. 5B–F).

These dynamics (also observed when XY males are released; Fig. 5D) can be explained by considering how the frequency of males in the population changes over time. As edits accumulate, males (many now XX) constitute an increasing fraction of the population (Fig. 5A, dashed lines) and the editor is also disproportionately present in males (Supplementary Fig. 6). Since each female only mates once, males are in excess and the probability that an editor-bearing male will participate in reproduction is reduced. In consequence, the frequency of the editor declines while the edit stabilizes at an intermediate frequency, thereby preventing further population suppression. The Y chromosome (Fig. 5B) is lost from the population for similar reasons. As edits accumulate, XX males make up a larger fraction of the (increased) male

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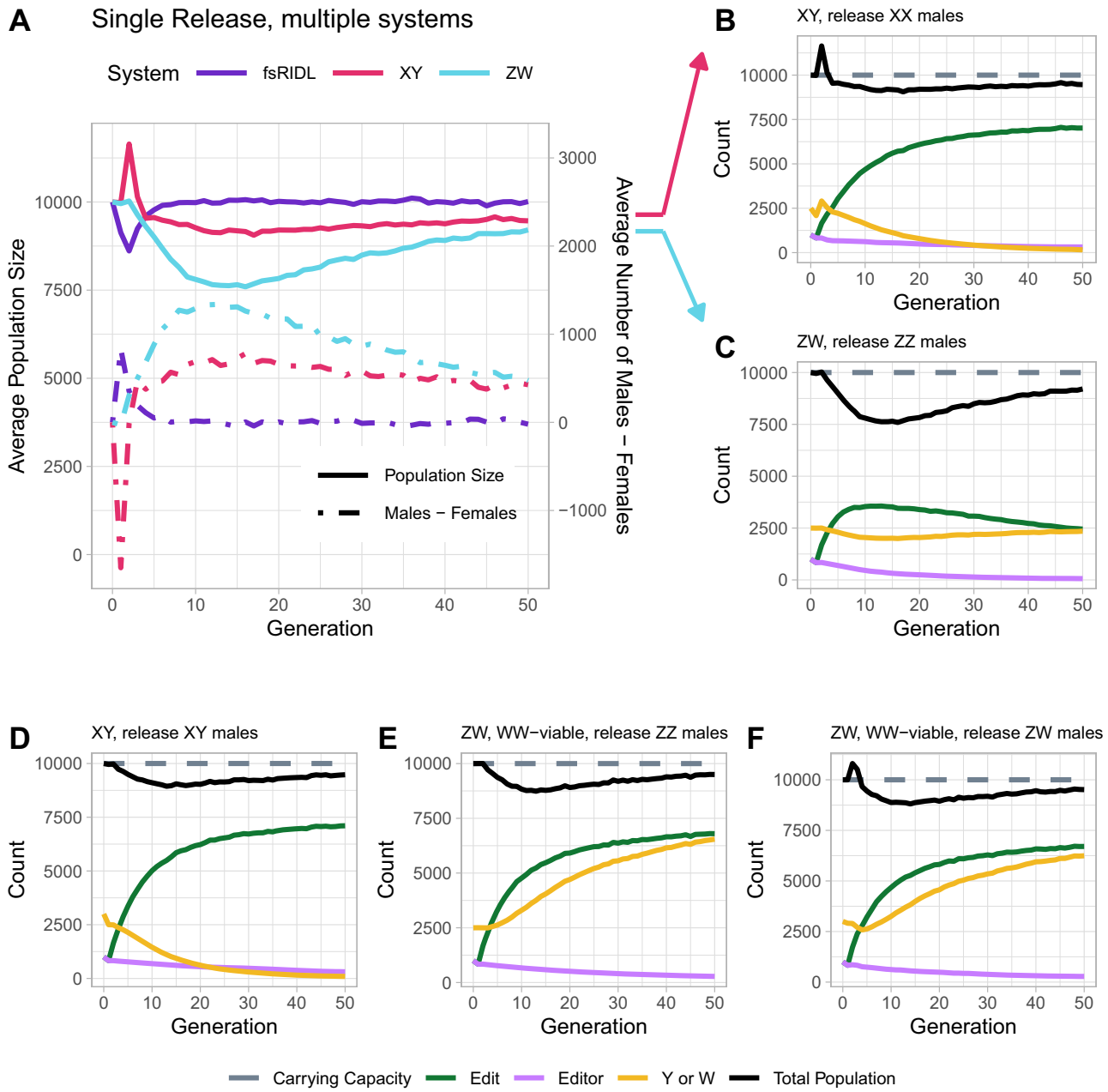

Fig. 5 | Single release of Allele Sail for population suppression. Values are averaged over 20 stochastic simulations, after a single release of transgenic individuals, with introductions being 10% of the carrying capacity. The counting of population size occurs before this additional release, so those individuals are not counted here. Additionally, there is no maternal carryover occurring in these simulations. **A** An overview of the total population over time, for XY (pink) and ZW (blue) systems using Allele Sail for suppression, compared to fsRIDL (purple). **B**–**F** A breakdown of allele frequency over time, graphed as a proportion of the total population and compared to the carrying capacity. The edit is in green, the editor in purple, the sex chromosome (either Y or W) is in yellow. Total population is shown in black, and the carrying capacity is in gray. **B** For an XY system, releasing editor+/editor+, edit+/edit+, XX males. The introduction of additional X chromosomes leads to an initial spike in population, further explored in Supplementary Fig. 8. **C** For a ZW system, releasing editor+/editor+, edit+/edit+, ZZ males. The increase in Z alleles only increases the effects of our male-skewing editor. WW individuals are considered non-viable, and die off. **D** Same as (**B**), but releasing XY males, which does not result in a population spike. **E** The same as (**C**), but WW individuals are viable, leading to an overall increase in W alleles in the population. **F** Same as (**E**) but releasing ZW males instead of ZZ, causing a population spike.

population, resulting in a corresponding decrease in the probability that Y-bearing males participate in reproduction.

An important consequence of a single modest release of an editor in an XY system is that the Y is completely lost from the population even as the ratio of males to females approaches 1:1 (Fig. 5B). This result implies that sex is now determined through a different mechanism. The aromatase loss-of-function edit plateaus at a frequency of 75%, indicating that the aromatase locus is now the primary sex determining locus, with males being edit+/edit+ and females edit+/edit−. This behavior is predicted by earlier modeling of switching of the heterogametic sex through intermediate states in which multiple sex-determination systems are active[54,55].

This impact on sex-determination can be further understood by considering the plots in Fig. 6, which show outcomes when populations are seeded with various frequencies of XX or WW aromatase knockout individuals and then followed for 500 generations. Figure 6A

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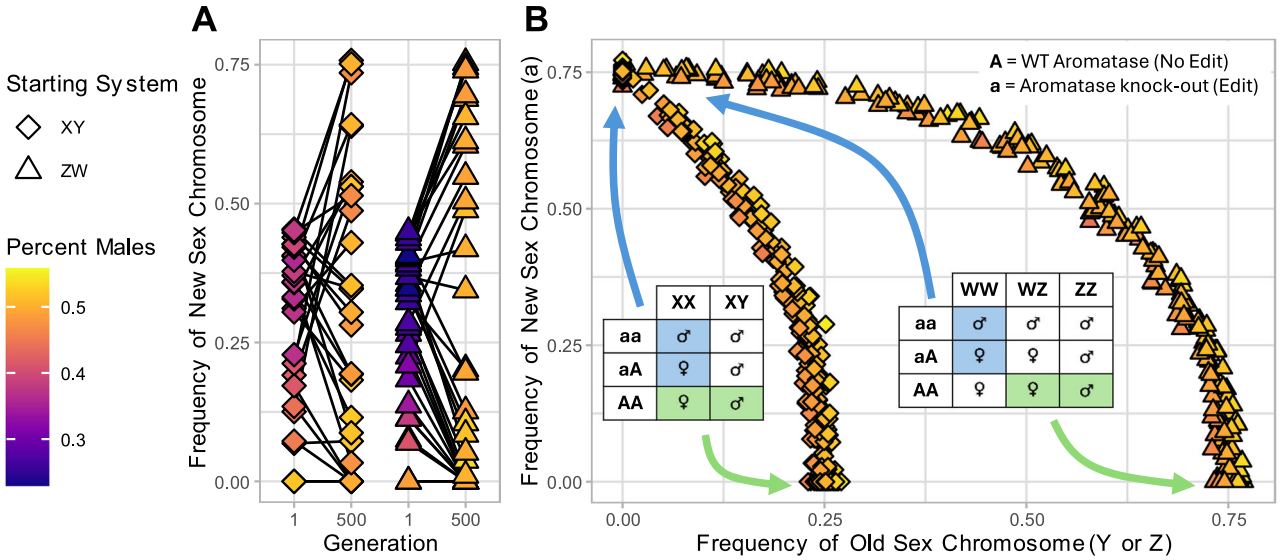

Fig. 6 | Paths to sex chromosome turnover. A The frequency of the new sex chromosome, an Aromatase knock-out, is shown at generations 1 and 500 for 120 simulations. Frequency is plotted for generations 1 and 500, with lines connecting individual start and end frequencies. Simulations started with a wild-type population of 2000 individuals, to which aromatase knockout individuals (both male and female) were added at the indicated frequency (between 0 and 75%; the y-value at generation 1 is the introduction frequency / 1 + the introduction frequency). **B** The frequency of the new sex-determining chromosome (aromatase knockout) plotted against the frequency of the old sex-determining chromosome (either Y or Z) at generation 500, for >500 simulations. A population of the given system, either XY (diamonds) or ZW (triangles), was seeded with individuals carrying the new sex determination system, varying from 0 to 75%, with step size 0.5%. The old sex-determining chromosome is either Y or Z depending on the system. The tables show all possible genotypes within each sex determination system, and their sex. For example, an aaWW individual will be male. Each point shows the allele frequencies and percent males of each simulation after 500 generations. The frequency of the old sex chromosome (either Y for XY, or Z for ZW) is plotted as the X-coordinate, and the frequency of the new sex chromosome, (knocked out aromatase) is plotted as the Y-coordinate. WW individuals are viable, allowing for complete turnover from a ZW system in which edits are absent, to a WW system in which the presence or absence of the edit determines sex. The genotypes of these endpoints are shown in the green and blue boxes. The curves approximated by all points represent continuous paths of equilibria connecting two different sex determining systems in which sex ratios remain near 1:1 males:females. Genotypes not highlighted by colored boxes only occur during transitions from one sex determination system to the other (points on the arcs other than the endpoints).

shows that the frequency of the aromatase knockout in generation 500 (present anywhere from 0 to 75% frequency) is only weakly related to the introduction frequency, which ranges from 0 to 75%. Regardless of the final frequency of the aromatase knockout, all simulations end with a sex ratio that approaches the Fisherian 1:1[56] (Fig. 6A).

The basis for this behavior can be understood from the results shown in Fig. 6B. Each point represents a simulation endpoint (beginning from diverse introduction frequencies) after 500 generations. The frequency of the original sex chromosome (either Y for XY, or Z for ZW) is plotted as the X-coordinate, and the frequency of the new sex chromosome, (knocked out aromatase) is plotted as the Y-coordinate. These points form a curved line, which represents a path of equilibria by which sex can transition from male heterogametic to female heterogametic without a change in sex ratio[54,55].

In the case of the XY to aromatase knockout turnover, this path is relatively short. In such a system, to complete a sex determination system turnover the Y allele need only drop from 25% frequency to 0%, while the aromatase knockout must reach 75%. With sufficient editor this turnover is easily achieved and sex determination becomes solely dependent on the status of the aromatase locus. The consequences of changes to a sex determination system are unknown and likely highly species specific as the genetics of sex determination along with the divergence between sex chromosomes are highly variable in fish[57] and amphibians[58]. The loss of a sex chromosome in some may significantly impact male phenotypes, while other species without sex-specific genes and recombination between sex-chromosomes are less likely to be impacted[59,60]. How such a transition may affect a particular pest species impacts warrants further study.

We now consider a ZW system in which the W chromosome is required but not sufficient for femaleness/female viability (e.g., certain birds[61], crustaceans[62], and amphibians[63,64]) (Fig. 5A, C). Release of ZZ males brings about a gradual but transient decrease in population size (Fig. 5A, C), coupled with a transient increase in the male:female ratio (Fig. 5A). These effects are due to a rise and subsequent fall in the frequency of edits (Fig. 5C). The editor is ultimately lost for the same reasons as in the XY system: it is present more often in males than females (Supplementary Fig. 6), reducing its likelihood of participating in mating. When the WW genotype is inviable, the edits also ultimately decrease in frequency because they find themselves in inviable WW progeny from ZW female and ZW male crosses more often than do the non-edited alleles. Placing the editor on the Z-chromosome can mitigate this effect, leading to increased suppression (Supplementary Fig. 7). In the XY system there is no equivalent lethal genotype since the creation of XX males drives the population toward an all XX genotype in which sex is now determined by the presence or absence of a functional aromatase allele. Also unlike the XY system, the W sex chromosome (in contrast to the Y) is not lost from the population because it is required (but not sufficient) for femaleness. Because of this, and because the Z is required for viability, neither can be lost and heterogametic sex chromosome flipping cannot occur. In such a system the editor is quickly lost, edits decrease over time, and the W allele returns to a frequency of 25% (Fig. 5C).

In the case where WW homozygotes are viable[62,64,65] (as might be the case with a newly derived sex chromosome) heterogametic sex flipping can occur[55], but is harder to achieve than in the XY case. The dynamics that support this conclusion are shown in Figs. 5E, F and 6, focusing on the behavior of the W chromosome. Figure 5E, F shows the consequences of a single release of ZZ or ZW individuals, respectively. In both cases the transient drop in population size is coupled with a substantial and persistent rise in the frequency of the edits and the W chromosome. Both plateau as the editor is lost from the population through the mechanisms discussed above.

The reason the population returns to its carrying capacity even while edits remain at high frequency is due to the fact that WW individuals are viable and can be either male or female depending on the status of the aromatase gene. This point is illustrated in Fig. 6A, B, which shows that there is a large region of parameter space in which ZZ males and ZW females can coexist at a near 1:1 sex ratio with ZW males and WW females. The presence of editors and edits drives the population toward a WW state (with aromatase status again determining sex), but a much higher frequency of edits is required than with the XY system, since the Z allele must decrease in frequency from 75% to 0% (as opposed to a drop of the Y from 25% to 0%) for the transition to be complete. Thus, a modest single release is insufficient and leaves the population in a state with mixed sex determination systems. These results correspond to previous findings that modeled a scenario termed mildly male-determining XY to ZW turnover[54,66].

Dynamics of alleles and chromosomes following multiple releases

Here we model the effects of repeated releases—once every generation. As illustrated in Fig. 7, repeated releases of an aromatase editor at a frequency of 10% eventually causes population collapse (Fig. 7A). This contrasts with repeated releases of fsRIDL, which at the same introduction frequencies only decrease the total population size (Fig. 7A). To explore multiple release scenarios in more depth we investigated the time to collapse. Plots of the average generation to collapse versus introduction frequency are shown in Fig. 7B. An editor is much more effective than fsRIDL for low-frequency releases, causing collapse for releases between 10% and 20%, while fsRIDL only results in collapse when the release frequency is 25%, and even then, only after roughly an additional 10 generations. For higher introduction frequencies, the time to collapse is comparable (Fig. 7B). Reduced editing efficiencies can still lead to population collapse under some scenarios. For example, an XY system can still be collapsed faster than fsRIDL (assuming 100% female killing with fsRIDL) when the editing efficiency is 90%, but not when the editing efficiency is 80%. Meanwhile, in a ZW system in which WW individuals are non-viable, an editing efficiency of 80% still promotes population collapse faster than with fsRIDL (Supplementary Fig. 9).

Finally, we asked what the effects on population suppression are if the editor, in addition to knocking out a gene required for femaleness, also creates LOF alleles in a haplosufficient gene required in somatic cells for female viability or fertility—a strategy sometimes referred to as

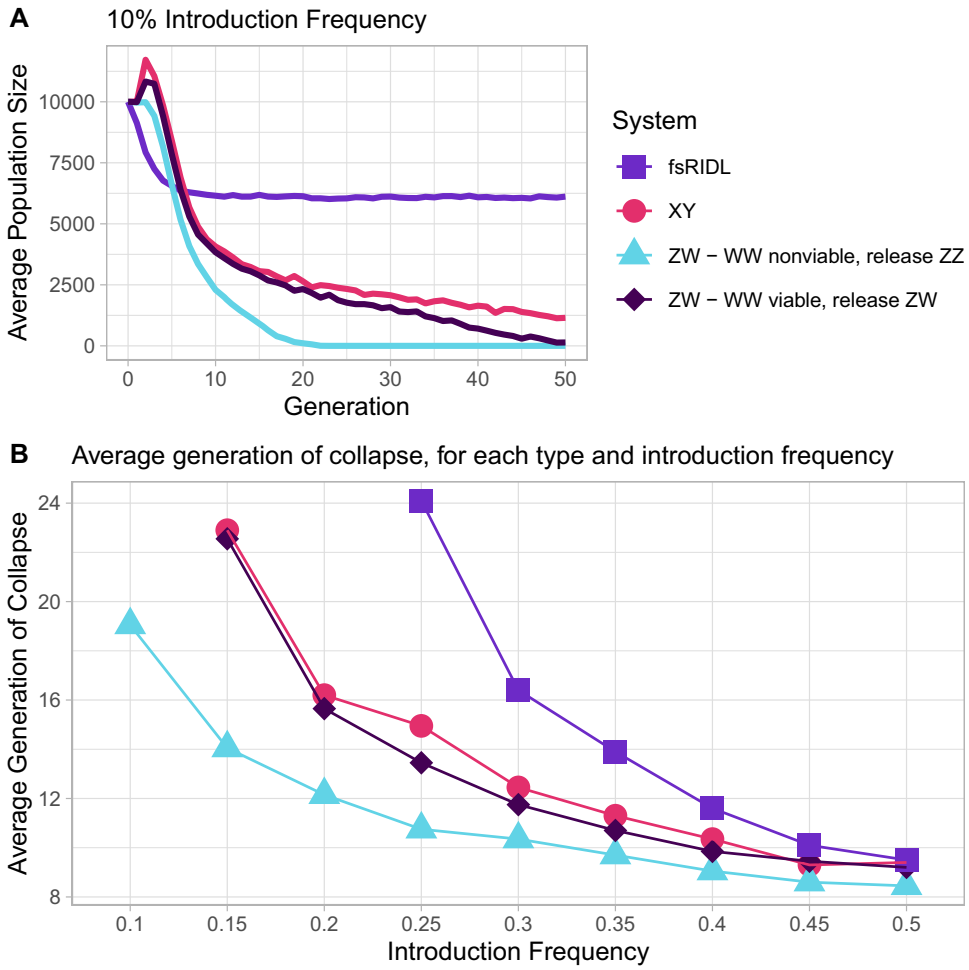

Fig. 7 | Comparison of population suppression using multiple releases of an Allele Sail or fsRIDL. A Total population over time, averaged over 20 simulations. Transgenic individuals are introduced at 10% of carrying capacity, at the start of every generation. The total population count shown here does not include this additional population, only counting the number of surviving offspring. The XY system (pink) shown here introduces XX males, and the ZW introduces ZZ, with WW offspring being either non-viable (blue) or viable (dark purple). **B** The average generation of collapse for various introduction frequencies. Points plotted here are the average of 20 simulations, where all 20 simulations went to collapse within 50 generations. If all 20 simulations did not collapse within 50 generations, the corresponding point is not plotted. Transgenic individuals were introduced at the indicated introduction frequency, once every generation, until collapse. For introduction frequencies below 25%, an editor can collapse a population where fsRIDL (light purple) cannot. At higher frequencies, the ability to collapse a population and time to collapse are comparable for strategies using fsRIDL or an editor.

sterilizing sex conversion[67]. As illustrated in Supplementary Fig. 10, sterilizing sex conversion leads to faster collapse, bringing about elimination of a population using repeated releases of only 5% of the starting population (Supplementary Fig. 10).

Discussion

We have introduced Allele Sails as a tool for population scale genome modification, and used agent-based modeling to explore their performance. Allele Sails consists of a genome editor transgene, transmitted via standard Mendelian inheritance. It introduces changes to a target locus which are both homozygous viable and fertile. Our modeling shows that a single release of Allele Sail carriers can push an allele to very high frequency across a wide range of fitness costs/benefits and editor efficiencies.

The Allele Sail is a relatively simple system that utilizes genome editing tools that are already functional in many species. Importantly its mechanism of action is not dependent on homology directed repair, as with homing-based gene drive. Although the editor transgene could theoretically persist indefinitely under idealized conditions in which there are no fitness costs associated with its presence, and recent work shows that Cas9 expression can incur minimal direct fitness costs[25], its ultimate removal could be ensured via strategies that include integration into a haplosufficient recessive lethal locus or inclusion of a co-expressed transgene that imposes a fitness cost.

A possible concern about the utility of Allele Sails may be that modern genome editors can only make minimal changes to the genome. However, even point mutations or small indels at one or a modest number of loci can have a large effect. As examples, point mutations have been found that contribute to plant disease resistance[3], animal heat tolerance[1,2], and honeybee sensitivity to Varroa mites[4,5]. Also, as of 2019, half of the pathogenic variants in the human ClinVar database were point-mutations, and almost 90% of clinically relevant insertions and deletions were less than 30 bp[68]. Finally, recent work shows that larger fragments of DNA (which would albeit be considered transgenes) can be copy-pasted from one location to another using engineered retrotransposons[69,70]. These observations suggest it may soon be possible to push larger fragments of DNA into a population in a self-limiting manner, without the need for homing, using an editing locus transmitted in a Mendelian manner.

The deployment of Allele Sails may be facilitated by emerging regulatory environments which increasingly view base-edits and indels as indistinguishable from non-GM. An Allele Sail that introduces changes to a population using a low-frequency transgene which does not indefinitely persist may therefore be easier to deploy than other self-limiting approaches where the transgene spreads to high-frequencies. These include 2-component homing-based split drives[18,71–73], many component homing-based split drives (daisy drives[74]), and split drives that utilize a toxin-antidote mechanism of action such as killer-rescue[20,75] and split ClvR[19]). Conditional release permits have been granted for release of transgenic mosquitoes[76–78] and diamondback moths[79,80] carrying a transgene that brings about a fitness cost in female but not male carriers, thereby promoting population suppression (along with the ultimate loss of the transgene). These successes suggest that scenarios involving low frequency releases of self-limiting transgenes are plausible.

The advent of programmable CRISPR based molecular tools enable targeted genetic and transcriptomic manipulation with unprecedented ease[81]. They have also resulted in the development of an array of tools for modifying wild populations. Most have focused on how to eliminate pests[13], while transgenesis methods for many threatened and invasive species are still needed. As our capabilities for genetic manipulation continue to expand, we believe that Allele Sails will prove to be particularly useful options for genetic rescue and mitigating the harm of pests while providing a powerful option for population suppression in species with ZW sex-determination.

Methods

Fundamental model assumptions

All modeling was done using a python program taking in command line arguments. The simulation within is a stochastic, discrete-generation, agent-based simulation. It assumes that the population is panmictic, that all females mate once, and that females are monogamous when they mate. Population growth is assumed to be logistic, and dependent on the adult population density—specifically, the adult population at the *start* of the generation, before additional individuals are released. We choose this implementation because the competition between individuals as they grow drains resources, and the additional individuals can be released immediately prior to mating, which does not put a strain on resources and therefore does not affect density-dependent growth. The expected population size is modeled using the Beverton–Holt model, to produce logistic growth.

Simulation function

For each simulation, we begin with a completely wild-type population of size K, the carrying capacity. Transgenic individuals are added in addition, as a percentage of the carrying capacity. For example, a 10% release of transgenic males when $K = 10{,}000$ would start with a population of 5000 wt females, 5000 wt males, and 1000 transgenic males. Each female in this starting population mates with a randomly chosen male, generating a list of all possible offspring and their probability of being produced. The number of offspring to produced is pulled from a Poisson distribution, with an expected value $\lambda = o * \omega_m * \omega_f$, where o is a pre-set average number of offspring, and ω_m and ω_f are the fertilities of the parents, which range from 0 (infertile) to 1 (completely fertile). Once offspring have been chosen at random and assigned a sex (as defined by their genotype), each offspring i has a chance of surviving $s_i = [2/o] * f_i$, where f_i is the fitness of the individual. An individual with a "fitness cost" of 5% has f_i of 0.95, where an individual with "fitness benefit" of 5% has f_i of 1.05. To incorporate density-dependence, we calculate the expected number of individuals in the next generation using the Beverton–Holt model, $P_{t+1} = g * P_t / [1 + (g-1) * (P_t/K)]$, where g is the low-density growth rate of the population and P_t is the size of the adult population at generation t[82,83]. For our model P_t does not include released individuals. The chance that an offspring i survives, s_i, is multiplied by P_{t+1}/P_t to get the relative growth or decline of the population, and therefore whether more or less offspring than expected should survive. All surviving offspring become the adults of the next generation, and the cycle repeats.

Scenario details

For this paper, models were run using $K = 10{,}000$ and $o = 100$ unless otherwise noted.

Some simulations included maternal carryover modification and some did not—generally, all modification simulations used maternal carryover of the editor except where noted otherwise, while suppression simulations did not. These differences are noted in the text and figure legends. Maternal carryover, when present, was assumed to be 100%, meaning that if the mother carried an editor, both target alleles in the offspring would be edited regardless of whether the father carried an editor.

The simulation and more information about the code and how to use it can be found at https://github.com/HayLab/AlleleSail.

Reporting summary

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

Data availability

All simulation data have been deposited in our github repository: https://github.com/HayLab/AlleleSail. This has also been archived on Zenodo, at https://doi.org/10.5281/zenodo.11205748[84].

Code availability

All code used in this manuscript has been deposited in our github repository: https://github.com/HayLab/AlleleSail and archived on Zenodo at https://zenodo.org/records/11205748[84].

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Author contributions

Conceptualization: M.L.J., B.A.H. and M.M. Experimental design: M.L.J., B.A.H. and M.M. Coding, modeling, and figure creation: M.L.J. Writing: M.L.J., B.A.H. and M.M.

Competing interests

The authors declare no competing interests.

Additional information

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