nature portfolio

Peer Review File

Launching traits and fates into wild populations with Mendelian DNA sequence modifying Allele Sails

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
This paper is a theoretical exposition of a genetic control approach termed 'allele sails'. The technique and its application shares features with some other previously proposed approaches but there are also some novel features. I find the paper to be very well written, with good explanations of scenarios and insightful descriptions of the behaviour of alleles and their frequencies over time. I think this is an important piece of work that will be well cited.
Given that it is just at the concept stage, not all considerations of contingencies can be made at this point (since some outcomes are simply unknown) but I find the discussion of known potential outcomes to be thorough. It will not always be trivial to make LOF mutations or to convert to particular gain of function mutations but that does not weaken the overall concept.
I think some of the discussion around the sex determination systems could be streamlined a little and there are some assumptions on the reader (particularly re: zW systems) that make this section hard to follow in places.

The article is very long and could take some streamlining in places by moving some sections to supplementary info.

Rather than listing all my comments and suggestions I have attached an annotated copy of the manuscript.
I just note here, three other things that need addressing:
1- the 'lower regulatory hurdle' is perhaps overstated, since any release involving an allele sail will still rely on release of transgenic editor alleles (albeit at a lower frequency and with potentially less perdurance)
2- Elements of the system have similarities with Y-linked editors proposed by Austin Burt and colleagues. These similarly produce edits that manifest their effect only in one sex and can have a ratcheting effect in population suppression with repeated releases. This previous work should be cited and discussed.
3- the discussion on potential changes in sex determination systems poses interesting questions from an evolutionary theory point of view but, in terms of the ultimate goal of popn suppression, what changes if transition of SD systems is complete or partial? Obviously there may be regulatory concerns in this scenario. This could be mentioned.

Reviewer #2:
Remarks to the Author:

This paper introduces a potential new approach to manipulate the genetics of free-living animal populations that they call “allele-sail”. They use a stochastic model of a panmictic population to show how the method could be used either to modify populations through introduction of beneficial genes, or to suppress (pest) populations through introducing genes that distort sex-ratio. I am inclined to agree with the authors that the approach may have potential for some applications. However, I did not find it entirely clear how the approach differs from some other “self-limited gene-drive” approaches, and I felt the paper did not do enough to compare approaches and thus highlight the unique strengths, as they see them, of this approach.

I would also have liked a bit more detail in the mechanistic description of the allele-sail approach.

• From the description it was not clear to me how the editor is intended to operate. I’m not a molecular biologist, and would have liked a bit more detail about “DNA sequence modifiers” – how do they work, how readily can they be engineered, etc..
• It would be helpful to give an idea of how much of a technical challenge it would be to build an allele-sail – what is the state of play, what would be the next steps towards developing one? Etc.
• I’m not familiar with the terminology of ‘allele-pump’ – is this the same as a ‘split drive’ (e.g. ())?
• I was hoping the Discussion would include comparison of “allele sails” to other theoretical ideas for self-limiting population manipulation: there are now numerous papers on “Daisy chain”, “Split drives”, “underdominance”, and “Y-linked editors” (and probably others). While a comprehensive review of these ideas is probably beyond the scope of this paper, some discussion of where allele-sails may offer unique advantages and where they may be less useful would strengthen this paper. As it is, I found the focus of the Discussion on potential uses of gene drives - that were largely not specific to allele sails – rather too general.
• A question on Figure 6. Interesting figure but it wasn’t clear to me what factors determined where a simulation ends up on the (Y or Z) “curve”? The legend and text states that “various amounts of Edit” was used – is this the reason the curves span the whole frame? Or is it randomness? Please be more specific on what is meant by “various amounts of Edit”!
• The methods section seems disordered and difficult to read. I suggest starting with describing the fundamental assumptions of the model before giving details of the simulation scenarios. It is not a complicated model so this should be easy to do without too much space. The paragraph on density dependence is unnecessarily confusing – it is odd to write “density dependence” as a solution to an equation (eqn. 1)!? Why not print the Beverton-Holt model equation and then explain how this incorporates DD?
• Very minor comment – noticed a couple of missing parantheses. (Beginning of page 9

end of 1st paragraph; page 29 - “existing sequence polymorphisms)”.

REVIEWER COMMENTS

We thank the reviewers for their insightful comments and have made numerous changes to the manuscript to address their concerns. See below for a point by point response.

Reviewer #1 (Remarks to the Author):

This paper is a theoretical exposition of a genetic control approach termed 'allele sails'. The technique and its application shares features with some other previously proposed approaches but there are also some novel features. I find the paper to be very well written, with good explanations of scenarios and insightful descriptions of the behaviour of alleles and their frequencies over time. I think this is an important piece of work that will be well cited.

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I think some of the discussion around the sex determination systems could be streamlined a little and there are some assumptions on the reader (particularly re: zW systems) that make this section hard to follow in places.

Response: We changed how sex determination systems are first introduced into the manuscript so that it is clear which sex typically corresponds to which chromosomes. We also added a small plot to figure 6 to further provide clarity on XY vs ZW systems.

The article is very long and could take some streamlining in places by moving some sections to supplementary info.

Response: We have dramatically shortened the discussion. Originally this walked the reader through several possible use cases initially noted in the introduction. This has all been eliminated as they are mentioned in the introduction.

Rather than listing all my comments and suggestions I have attached an annotated copy of the manuscript.

- Page 1, commented on "a multi-gene cassette" : "Not necessarily, and not always, multi-gene"

Response: We have changed this to transgene cassette

- Page 6, caption of Figure 1, commented on "conversion to the edited state occurs at *some frequency*": "The example frequency is 100% conversion - it would be prudent and potentially less misleading to state as much. This is the best case scenario"

Response: We have now noted in the figure legend that the numbers shown to the left are for the idealized case of 100% modification. We also note that the right panel is a cartoon graphic, not the results of modeling.

- Page 8, commented on "There is a *large region* of parameter space": "this is fair but it is also true, is it not, that there is quite a sharp and large 'cliff' where, at a given introduction frequency, with small changes in fitness cost the desired edit can reach very high final frequency or really very low final frequency? I take this to mean that in some paramerer spaces there could be considerable uncertainty linked to estimations of actual fitness cost. I think this is worthy of some discussion"

Response: This is a very good point. With many gene drives there are regions in which one is near a threshold. Simple models give a somewhat artificial view of the level of confidence

one should have about outcomes near these thresholds. The same points are true for an allele sail.

We have now added to this section the following statement:

"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 necessary to efficiently alter the target population."

- Page 10, Figure 2, commented on color legend: "it took me a while to realise that these two are the same scale, with the right being just a magnification of the top end. Maybe just state as much in the legend? "

Response: This is a great point; we have now added text to this effect, and edited the figure.

- Page 11, Figure 2's figure caption, commented on "boxed areas highlight specific allele frequencies to provide guidance " : "a very helpful touch"

Response: Thank you!

- Page 12, Figure 3, reviewer suggests adding "(5%)" behind the fitness benefit and cost labels

Response: We have now added this to the title of the relevant figure panels.

- Page 13, reviewer highlighted but did not comment on "When fitness costs are associated with the editor "

Response: We are not quite sure what was intended here, but have changed the phrase to "when fitness costs are assigned…" which may be less ambiguous.

- Page 16, commented on "We also *remove maternal carryover* from these simulations," : "some discussion of the feasibility of having full maternal carryover versus zero maternal carryover, is warranted. Were it so easy to modulate..."

Response: We appreciate the reviewers comment that "dialing in" the presence or absence of maternal carryover is an ongoing research area, particularly with respect to insects. We now include a statement to the effect that maternal carryover is common (at least in insects using the current germline promoters), but that it can be modulated, and remains an ongoing topic of investigation.

"While maternal carryover of Cas9 is observed with many germline promoters in insects, engineering of regulatory elements can reduce these effects (reviewed in ([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"

- Page 16, commented on "since the editor is germline specific." : "i think this could be phrased to make it more clear to reader - that all XX genotypes will presnt as female beacuse even if they receive the edit will still have a functional allele on the other chromosome"

Response: This is a good point. We have now reworded this section of the paragraph as follows:

"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, the editor is germline specific, and aromatase is primarily expressed in somatic ovarian granulosa cells. This increase in females leads to an transient spike in population numbers, as their progeny include both males and females. "

- Page 18, highlighted but did not comment on "The consequences of such transitions during attempts (particularly failed attempts) to suppress a population warrant further study. "

Response: We have altered this passage to now focus more on highlighting that the outcomes will be species specific to some extent:

"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[53] and amphibians[54]. 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[55,56]. How such a transition may affect a particular pest species impacts warrants further study."

- Page 22, commented on "release is insufficient and leaves the population in a state with mixed sex determination systems." : "while these are interestijng questions from

an evolutionary theory point of view , in terms of the ultimate goal of popn suppression, what changes if transition of SD systems is complete or partial?"

Response: There is minimal effect if the transition is partial or complete. If the transition is partial, then the population now has mixed sex determination. This corresponds to points on the curves in Figure 6A and 6B in which the sex ratio settles close to 1:1. In this situation–which corresponds also to the first few generations of a continuous release strategy–release of males that edit and inactivate a gene required for femaleness still drives population suppression by pushing away from the 1:1 ratio, and removing females. A complete transition should also have negligible effects - instead of knocking out a novel sex-determining locus, we would instead be knocking out the standing sex-determining locus,. For suppression all that matters is the frequency of the gene required for femaleness, regardless of the sex chromosome system.

- Page 29, commented on "seeding an increased susceptibility to certain toxicants before introducing said toxicants into a population,": "potential for confusion in reader between toad-produced toxciants and, in a separate use case, more genral toxciants for removal of a particular pest"

Response: We have now removed this part of the discussion

- Page 31, commented on "is nearly eliminated by generation 50. Introduction of the same frequency of editors into a ZW system did not yield a switch, but could (when WW is viable) do so under multiple release scenarios in which population elimination failed. The ZW to edit+/edit- switch is a much slower process,

highlighted by the neutral equilibrium path lengths of these two systems. ” : “see above comment re: consequences for ultimate goal - suppression”

Response. We have deleted this part of the discussion. But, to the reviewers point, the above text relates to changes in sex chromosomes, but these changes do not have any consequences for population suppression, as noted above.

- Page 32, commented on “me other editor should never significantly increase in the population. In countries like Australia, offspring that only inherit an edit, but not an editor transgene, could be considered non-GMO, reducing regulatory hurdles” : “but any releaae is still a ‘transgenic release’ so it is still not clear, nor does it follow, that regulatory hurdles would be lower”

Response.: See below

I just note here, three other things that need addressing:

1- the 'lower regulatory hurdle' is perhaps overstated, since any release involving an allele sail will still rely on release of transgenic editor alleles (albeit at a lower frequency and with potentially less perdurance)

Response: We agree with the reviewer that this point was a bit overstated. The revised manuscript now discusses how the transient nature of the editor plus the ‘non-GM’ status of the remaining edits may result in reduced regulatory hurdles. These points are made at multiple points in the manuscript.. In several this is presented in the context of alternatives (i.e., gene-drives). We believe this is likely to be the case as non-persisting GM systems (e.g. RIDL) have been permitted. We also believe it is worth pointing out in the manuscript as the Allele Aail idea

provides a context in which regulators and others might reasonably consider if a modest level of transgenes–in the service of a high frequency of a non-transgenic edit–merits any special consideration.

2- **Elements of the system have similarities with Y-linked editors proposed by Austin Burt and colleagues. These similarly produce edits that manifest their effect only in one sex and can have a ratcheting effect in population suppression with repeated releases. This previous work should be cited and discussed.**

Response: We have updated the introduction to more clearly distinguish Y-linked editors from Allele Sails and added the relevant citations.

3- **the discussion on potential changes in sex determination systems poses interesting questions from an evolutionary theory point of view but, in terms of the ultimate goal of popn suppression, what changes if transition of SD systems is complete or partial? Obviously there may be regulatory concerns in this scenario. This could be mentioned.**

Response: Please see our responses to your comment about pg 18 of our manuscript above which address this issue.

Reviewer #2 (Remarks to the Author):

This paper introduces a potential new approach to manipulate the genetics of free-living animal populations that they call “allele-sail”. They use a stochastic model of a panmictic population to show how the method could be used either to modify populations through introduction of beneficial genes, or to suppress (pest) populations through introducing genes that distort sex-ratio. I am inclined to agree with the authors that the approach may have potential for some applications. **However, I did not find it entirely clear how the approach differs from some other “self-limited gene-drive” approaches, and I felt the paper did not do enough to compare approaches and thus highlight the unique strengths, as they see them, of this approach.**

Response: We have now expanded the introduction to more clearly distinguish Allele Sails from other self-limited approaches like Y-linked editors and allele pumps. To summarize, the key features of an allele sail is that it involves the spread of edits, not transgenes, and that those edits end up in individuals who are viable and fertile. Y-linked editors behave similarly, but the focus in the literature has been on their use to create dominant mutations that make genetic females unfit. In contrast, a allele pump, of whatever variety, is designed to drive the spread of an existing transgene cassette to high frequency. So, the key distinctions are between edits and transgenes, and the intent of the edits.

I would also have liked a bit more detail in the mechanistic description of the allele-sail approach.

Response: We updated the introduction to provide more details overall and included more information on genome editors.

• From the description it was not clear to me how the editor is intended to operate. I'm not a molecular biologist, and would have liked a bit more detail about "DNA sequence modifiers" – how do they work, how readily can they be engineered, etc..

Response: We added to the introduction, as commented above.

• It would be helpful to give an idea of how much of a technical challenge it would be to build an allele-sail – what is the state of play, what would be the next steps towards developing one? Etc.

Response: We have altered our introduction and discussion to provide more information about the current state of the art while also mentioning that a limitation for implementing Allele Sails is that transgenesis methods have not been developed for many threatened species which could benefit from the technology.

• I'm not familiar with the terminology of 'allele-pump' – is this the same as a 'split drive' (e.g. ())?

Response: The introduction has been updated to clarify what an allele pump is. A split drive is a version of an allele pump, and often this is the context referred to in the literature.

• I was hoping the Discussion would include comparison of "allele sails" to other theoretical ideas for self-limiting population manipulation: there are now numerous papers on "Daisy chain", "Split drives", "underdominance", and "Y-linked editors" (and probably others).

While a comprehensive review of these ideas is probably beyond the scope of this paper, some discussion of where allele-sails may offer unique advantages and where they may be less useful would strengthen this paper. As it is, I found the focus of the Discussion on potential uses of gene drives - that were largely not specific to allele sails – rather too general.

Response: We have re-written the discussion in total, including a lot of text that is eliminated. Here and earlier we also point out the appeal of an allele sail, which has to do with the fact that edits are being spread into the population, not transgenes. There are features of the dynamics of allele sails versus the many other systems the reviewer notes. That said, we would prefer not to get into the quantitative details of these dynamics here, instead keeping the focus on the qualitative differences.

"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 and persists indefinitely (such as daisy chain, split drives, and underdominance). Regulatory approvals have been made for release of transgenic mosquitoes carrying a transgene that brings about a fitness cost to 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[71]."

• A question on Figure 6. Interesting figure but it wasn't clear to me what factors determined where a simulation ends up on the (Y or Z) "curve"? The legend and text states that "various amounts of Edit" was used – is this the reason the curves span the whole frame? Or is it randomness? Please be more specific on what is meant by "various amounts of Edit"!

Response: We added a bit more to the text, the figure, and the figure caption to try and better explain. While the "amount of edit" (the percent of new-sex-determination-individuals we added to a standing wildtype population) varies from 0% to 75%, it does not have a large impact on where on the curve a simulation might end. The curve denotes an area in which a population will have a 1:1 sex ratio. As such, a population that exists anywhere on that line will be relatively happy, and our simulations saw many populations drifting up and down that line over time. As you suggested, the curves span the whole frame due to randomness! As a part of our response we have added a new set of panels to show how little relationship the introduction frequency has for the final frequency, though all end up at a 1:1 sex ratio.

• The methods section seems disordered and difficult to read. I suggest starting with describing the fundamental assumptions of the model before giving details of the simulation scenarios. It is not a complicated model so this should be easy to do without too much space. The paragraph on density dependence is unnecessarily confusing – it is odd to write "density dependence" as a solution to an equation (eqn. 1)!? Why not print the Beverton-Holt model equation and then explain how this incorporates DD?

Response: We appreciate the suggestions, and found them quite helpful. We re-wrote the methods section based on the suggestion, starting with fundamental assumptions, moving into

some of the math behind density dependence, following the suggestion of printing the Beverton-Holt model first. Finally, the section ends with the details of the simulation scenarios.

• Very minor comment – noticed a couple of missing parantheses. (Beginning of page 9 end of 1st paragraph; page 29 - "existing sequence polymorphisms)".

Response: We check for and added missing parentheses

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
yes I am happy with the revisions made. It was already a very good and useful article and I believe it will be more penetrable to a wider audience, with the revisions now made. I appreciated the inclusion of a manuscript copy with tracked changes.

Reviewer #2:
Remarks to the Author:
I am reviewer 2 from the previous submission. I find the manuscript is much improved (particularly, the Methods are clearer, and the Discussion is improved) and I only have a few relatively minor comments that I think still need addressing.

• From my understanding and from reading your revised Introduction, I don't quite agree that Y-linked editors are entirely distinct from allele sails, but rather that Y-linked editors are a specific kind of allele sail. Your description of allele sails seems general enough to include them? (The edits may be deleterious or not, they may be intended for suppression or not – what is the specific feature of a YLE that is not covered by your description of an AS?). If you consider there to be a distinction, you should be clearer in the Introduction as to what sort of edits are included in the category of Allele Sail edits – or else define a YLEs as a sub-category.
• A minor point but I found the wording of this sentence a bit odd, suggest re-wording : "SGDs are selfish genetic elements that spread to high frequency by biasing their own inheritance; they can include 'cargo' in addition to transgenes essential for the SGD's function.".
◦ Perhaps this is a matter of taste/semantics, but I would consider the "cargo" to be a part of the transgene in a classic homing based gene drive construct (if this is what you are referring to?). Also, why "transgenes" in the plural?
• I am happy with the revised Discussion, except that you should give citations for the three self-limiting systems that are mentioned (daisy chain, split drives, and underdominance) AND, importantly, a citation for the statement: "Regulatory approvals have been made for release of transgenic mosquitoes carrying a transgene that brings about a fitness cost in female but not male carriers".

REVIEWER COMMENTS

We thank the reviewers for their insightful comments and have made numerous changes to the manuscript to address their concerns. See below for a point by point responses in blue.

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yes I am happy with the revisions made. It was already a very good and useful article and I believe it will be more penetrable to a wider audience, with the revisions now made. I appreciated the inclusion of a manuscript copy with tracked changes.

- Thank you. The reviewer comments were extremely helpful to make it a manuscript that should be more broadly understandable.

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-The challenge with addressing this point has been that despite having a very broad name. Y-linked editors, have been defined in the literature as introducing dominant mutations that either sterilize females or make them non-viable, and Allele Sails rely on mutations that are both homozygous viable and fertile. We have addressed directly addressed this in the introduction.

• A minor point but I found the wording of this sentence a bit odd, suggest re-wording : "SGDs are selfish genetic elements that spread to high frequency by biasing their own inheritance; they can include 'cargo' in addition to transgenes essential for the SGD's function.".

◦ Perhaps this is a matter of taste/semantics, but I would consider the "cargo" to be a part of the transgene in a classic homing based gene drive construct (if this is what you are referring to?). Also, why "transgenes" in the plural?

- We have reworded this to: *SGDs are made up of one or more transgenes that bias their own 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).

• I am happy with the revised Discussion, except that you should give citations for the three self-limiting systems that are mentioned (daisy chain, split drives, and underdominance) AND, importantly, a citation for the statement: "Regulatory approvals have been made for release of transgenic mosquitoes carrying a transgene that brings about a fitness cost in female but not male carriers".

-References have been added for these.