THE ROYAL SOCIETY PUBLISHING

PROCEEDINGS B

Rodent gene drives for conservation: opportunities and data needs

John Godwin, Megan Serr, S. Kathleen Barnhill-Dilling, Dimitri V. Blondel, Peter R. Brown, Karl Campbell, Jason Delborne, Alun L. Lloyd, Kevin P. Oh, Thomas Prowse, Royden Saah and Paul Thomas

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Review timeline

Original submission:	25 July 2019
1st revised submission:	2 October 2019
2nd revised submission:	10 October 2019
Final acceptance:	11 October 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-1606.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
I like this paper. I see benefit that it provides a reader with a succinct summary of the state of rodent control options relating to gene drives primarily within a molecular and modelling context, and with social community considerations.
It may be useful for the authors to supply a summary table of the 'gaps' they see that require to be addressed going forward? Otherwise a reader needs to cherry pick out the gaps across all the text for future use. Just an idea.

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

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Line 55: This review is targeted at a broad and likely unfamiliar audience. However there is no definition of what a gene drive is and why they are theoretically an improvement over existing methods for eradicating rodents from islands. This may be covered in other papers within the proposed edition and thus be unnecessary.

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Line 75: What are the limitations of this approach? With 95% inheritance, why is the t-haplotype not already everywhere? – with corresponding high levels of sterility in wild male mouse populations? If there are (are there?) existing mechanisms to limit the spread of t-haplotype in wild mouse populations, do the authors not believe these to be present in island populations?

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Line 78: There are many types of 'synthetic gene drive' system which do not require HDR to function at all – the authors describe a few of them in the manuscript. Specifically, within the homing drives, a very high level of HDR is only really necessary for drives aimed at population suppression.

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Line 105: Conventional theory on using existing 'natural' gene drives is that if there were no significant impediments to their spread they would already be fixed in populations and conversely, if they aren't fixed then there is probably a reason for this – i.e. there may already be existing resistance mechanisms. This may not be the case in island populations for t-haplotype but the degree to which this is known would constitute a knowledge gap.

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Line 119: As demonstrated in mosquitoes, an X-shredder is only a drive system if it is itself Y-linked. As such, having both of these seems superfluous.

Line 123: Which would be beneficial?

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Line 174-179: Does this suggest that a transgene linked to a novel Y may experience positive selection irrespective of its driving activity? Is it known whether such a system could drive desired phenotypes through an invasive population? Additionally, could this have repercussions for proposed locally-limited drives such as t-sry which are effectively kept from spreading by fitness costs to females?

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Figure 2:

Line 3: There is no prior explanation of what an invasion threshold is.

Decision letter (RSPB-2019-1606.R0)

02-Sep-2019

Dear Dr Godwin

Your manuscript RSPB-2019-1606 entitled "Rodent Gene Drives for Conservation: Opportunities and Data Needs" has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers have indicated that major changes to your manuscript are required, and we would therefore like to invite you to revise your manuscript to address these requirements.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that

the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Professor Loeske Kruuk
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This is an interesting and timely addition to the gene drive literature, with a focus on approaches relevant to invasive rodents on islands, particularly house mice, for which a variety of approaches are being considered. The referees have however several suggestions for improvement.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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Figure 2:

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Author's Response to Decision Letter for (RSPB-2019-1606.R0)

See Appendix A.

Decision letter (RSPB-2019-1606.R1)

03-Oct-2019

Dear Dr Godwin

I am pleased to inform you that your Review manuscript RSPB-2019-1606.R1 entitled "Rodent Gene Drives for Conservation: Opportunities and Data Needs" has been accepted for publication in Proceedings B.

The Associate Editor handling the paper has not recommended any further changes. Therefore, please proof-read your manuscript carefully and accept the tracked-changes, and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

To upload your manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2019-1606.R1 which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor Loeske Kruuk
Editor, Proceedings B
mailto:proceedingsb@royalsociety.org

Decision letter (RSPB-2019-1606.R2)

11-Oct-2019

Dear Dr Godwin

I am pleased to inform you that your manuscript entitled "Rodent Gene Drives for Conservation: Opportunities and Data Needs" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Response to Referees RSPB-2019-1606 (Godwin et al., 2019)

We have responded to referee comments below and uploaded a revised manuscript with changes indicated using MS Word's 'Track Changes' function. The detailed responses to referee comments and questions include both the line number in the original manuscript submission and, in parentheses, the line number(s) in the revised manuscript.

Two submission notes: i) It was not clear from the review decision letter whether a 'clean copy' of the revised manuscript should also be uploaded, but we can do this quickly if needed.

ii) Also, the revision upload function did not appear to allow separate uploads of figures 1 and 2 (Figure 1 'disappeared' after uploading Figure 2 – seemingly only one figure file allowed there?). So, these are included together in one pdf file. We can certainly change this if needed, but may need guidance from journal staff on the way to do so.

Referee 1

- This referee was quite positive overall and only suggested it might be useful to include a summary table of knowledge gaps. We appreciate this suggestion, but feel i) it is not possible to accommodate it without having to make significant cuts elsewhere in the manuscript to stay within length limits, and ii) that the benefit realized by inclusion of a table in this case would not be worth these reductions elsewhere.

Referee 2

- This reviewer was also positive about this manuscript, but expressed an interest in seeing it more clearly distinguished from another review by Moro and colleagues published in 2017 that we cite in our manuscript. We feel that our manuscript is different from Moro et al., 2017 in the following respects:
    - Moro et al's focus was broad taxonomically (invasive mammals) and narrow geographically (focused on Australia (an island but clearly continental in scale).
    - Moro et al.'s focus was mostly on ecological factors that could inform ecological risk assessments (*"The application of gene drive technology for invasive species control needs to consider the level of knowledge currently available on population genetics and ecology that can be used as a basis for undertaking the necessary ecological risk assessments and trials."*). By contrast, our manuscript
        - i) briefly reviews understanding of naturally-occurring drives (primarily t-allele), efforts towards synthetic drives including recent and very relevant contributions such as Prowse et al. 2019 and Grunwald et al. 2019. Genetic approaches generally were less advanced in this fast-moving field when the Moro et al. paper was published and these authors had different overall goals in their review. Lastly on this specific topic, we introduce a concept coupling natural gene drive mechanisms such as the t-haplotype with CRISPR-based genome editing effectors that could, in

theory, be extended to naturally-occurring drive mechanisms in other species.
    - ii) discusses the concept of achieving spatial limitation of gene drive function in depth, primarily from the standpoint of assessing the potential utility of locally-fixed alleles in this context,
    - iii) reviews modeling efforts in some detail along with population genetic data and patterns pertinent to modeling efforts,
    - iv) relatedly, it includes consideration of what is known from invasive mouse populations in various parts of the world and ways in which this may be relevant to both modeling and any potential gene drive eradication/control efforts.
  - The Moro et al paper did highlight the importance of understanding what they termed 'translocation' biology and we highlighted this suggestion of theirs (lines 150-154 in original submission). However, these authors did not consider in detail many factors affecting translocation biology as this was generally beyond the scope of the broader scale treatment that was their focus. We delve deeper into what is known about translocation biology for invasive rodents and factors that may affect this in an island gene drive context. We consider this a particularly important topic area when thinking about application of any potential gene drive approach.

- <u>Other detailed comments from Referee #2</u>
- Line 55: This review is targeted at a broad and likely unfamiliar audience. However there is no definition of what a gene drive is and why they are theoretically an improvement over existing methods for eradicating rodents from islands. This may be covered in other papers within the proposed edition and thus be unnecessary.
  - *This will be covered in an introductory chapter for the volume we are assuming.*
- Line 71 (line 71-72 revision): ….crosses <u>between heterozygous males and</u> both laboratory and wild-derived females…
  - *Changed as suggested.*
- Line 75: What are the limitations of this approach? With 95% inheritance, why is the t-haplotype not already everywhere? – with corresponding high levels of sterility in wild male mouse populations? If there are (are there?) existing mechanisms to limit the spread of t-haplotype in wild mouse populations, do the authors not believe these to be present in island populations?
  - *This is an interesting question and has been extensively addressed in previous treatments of the t-haplotype including the first reference cited in this section (37 – Ardlie and Silver, 1998) and further down in references 57-59 (line 113 in original submission, line 128 revision).*
- Line 78 (line 78 revision): insect<u>s</u>
  - *Changed as suggested.*
- Line 78 (line 78 revision): There are many types of 'synthetic gene drive' system which do not require HDR to function at all – the authors describe a few of them in the manuscript. Specifically, within the homing drives, a very high level of HDR is only really necessary for drives aimed at population suppression.
  - *Changed to 'Some synthetic drive systems rely on high rates of homology-directed repair…"*
- Line 79 (line 79 revision): Conventionally it is the parental germline which is converted to a homozygous form, rather than the offspring.
  - *Changed to reflect this appropriate suggestion by the referee.*

- Line 83 (line 83 revision): I would recommend replacing NHEJ here and elsewhere with just end-joining or perhaps error-prone end joining as it is increasingly demonstrated that other repair mechanisms are involved and NHEJ may actually be a minority contributor.
    - *We adopted this suggestion by the referee and have changed NHEJ to just 'end joining' here and below in the manuscript.*
- Line 87: One way to potentially distinguish this review would be to expand this molecular section to include literature that is known or unknown about how relevant homology-based DNA repair pathways function in mammals/rodents and, concurrently, a discussion of why timing Cas9 expression to particular times in the cell-cycle or germ cell stage would be desirable and how this might be achieved.
    - *We appreciate this suggestion by the reviewer, but space constraints precluding this sort of more in-depth treatment while also addressing the other key points we wish to cover in the manuscript. This subject matter is also not as directly related to the primary focus of our manuscript as other material that is included.*

[Please note that MS Word 'decided' to jump here from line 85 to 100 in only 8 lines of text for some reason and I cannot seem to fix this. No text is missing, but it is potentially confusing. Just noting it here to hopefully avoid that confusion.]

- Line 95 (lines 99-104 now): It would also be critical to evaluate the fitness of various end joining mutants at the target site. Also, cite Burt 2003 for first proposed use of multiple gRNAs.
    - *We have now changed this paragraph to reflect both suggestions from the referee here.*

- Line 98: A knowledge gap that would be beneficial to explore is the degree to which different CRISPR enzymes function at different temperatures. Some may be more useful within a mammalian context depending on their activity within typical rodent body temperatures.
    - *While we agree with the reviewer this is an interesting question, this general area of function in mammals is also currently the subject of an extraordinary amount of research because of the interest in employing CRISPR-based approaches to gene therapy in humans. Given that, we do not feel it would be a good use of the limited space available to address the issue in this manuscript.*
- Line 101 (~line 110 revision): Has this not largely been discussed earlier with linking t-haplotype to SRY? The authors suggest that this should be achievable so what advantage do these other non-homing systems provide? Are there other proposed 'cargo' genes for such non-driving systems? I.e. that modify a seasonal behaviour? Or is this also a knowledge gap?
    - *There could be a great many potential cargos for such a non-homing type system. We mention this in the context of the t-allele as it has not previously been described to our knowledge. This being particularly true for the idea of the t-allele (or potentially some other selfish element) providing the 'drive' with the endonuclease and gRNAs being inserted solely for the purpose of editing (rather than providing gene drive). We also feel this suggestion is also different and useful here because it would introduce the possibility of achieving spatial limitation through targeting of locally fixed alleles (by appropriately designed gRNAs) while using this naturally-occurring drive mechanism (not likely to be possible with introducing Sry or some other direct effector gene).*
- Line 105 (line 112 revision): Conventional theory on using existing 'natural' gene drives is that if there were no significant impediments to their spread they would already be fixed in populations and conversely, if they aren't fixed then there is probably a reason for this – i.e. there may already be existing resistance mechanisms. This may not be the case in island populations for t-haplotype but the degree to which this is known would constitute a knowledge gap.
    - *As noted above, this has been addressed in some depth by other authors who are cited here and it is our understanding that this will also be an important point of emphasis in other papers in this issue (as the referee is definitely correct that this is an important and interesting issue).*
- Line 116 (line 131 revision): and<u> are</u> widespread
    - *Changed as suggested.*

- Line 119 (line 134 revision): As demonstrated in mosquitoes, an X-shredder is only a drive system if it is itself Y-linked. As such, having both of these seems superfluous.
    - *We were not clear exactly as to the point the referee is making here. We discuss a Y-shredder for mice as this has been demonstrated both in vitro and in vivo and modeled (as noted and referenced), but a X shredder mechanism thus far has not. For the same reason, it could be useful in mosquitoes (male-biasing and decreased reproduction overall), we briefly raise this possibility for mice.*
- Line 123 (lines 138-9 revision): Which would be beneficial?
    - *The referee appears to be asking what the benefit would be here, so we added the following to the end of this paragraph " and therefore be potentially more effective for reducing invasive mouse populations."*
- Line 134 (~line 150 revision): To what extent is the LFA concept scalable? Is this a realistic design if drives require re-specification on each new island they are used on?
    - *This could be discussed potentially at considerable length and from a variety of perspectives (genetic, 'business models' for eradications, etc) and would be a particularly salient point with some previous genome editing approaches where 're-targeting' represented a major undertaking (ZFNs, TALENs, etc). However, the relative ease of programming editing with CRISPR-based methods should make the necessary 'tailoring' to LFA feasible.*
- Line 155 (line 172 revision): <u>Translocation</u>? Instead of translation?
    - *The referee is correct and this has been changed.*
- Line 158-162 (lines 173-7 revision): To what extent is this resistance to introgression a consequence of behavioural differences between established rats and incomers, or due to these small islands being at carrying capacity already? If the latter is contributing then reducing densities with conventional tools prior to deployment may aid gene drive spread.
    - *We completely agree with the referee here that a combination of conventional and genetic tools could aid spread (or, for example, introducing drive carriers during low points for highly cyclical invasive rodent populations). While interesting and important to explore in the future, both space constraints and the scope of this manuscript prevent an exploration of these possibilities here.*
- Line 170 (line 186 revision): As stowaways on Viking vessels, presumably?
    - *This is suggested in the papers referenced here by Britton-Davidian et al (2007) and Forster et al (2009).*
- Line 174-179: Does this suggest that a transgene linked to a novel Y may experience positive selection irrespective of its driving activity? Is it known whether such a system could drive desired phenotypes through an invasive population? Additionally, could this have repercussions for proposed locally-limited drives such as t-sry which are effectively kept from spreading by fitness costs to females?
    - *This is a very interesting suggestion, but the case of a Y-linked transgene not been modeled (at least for mammals) to our knowledge. Our knowledge of the phylogeographic patterns described is primarily descriptive at this point (other than the Isle of May example where an experimental introduction was conducted) and limited to the presence and frequency of the Y chromosome sequences in these populations. It is not known, to our knowledge at least, whether the apparently greater introgression success also leads to (as the referee suggests) driving other phenotypes through these populations. As we note in the next paragraph (original line 183, line 199 revision), this general area of inquiry is one where research is needed before any gene drive mechanism could be deployed.*
- Line 180 (line 196 revision): could be an important <u>consideration</u> ?
    - *This did need 'consideration' added and we have done so, as suggested by the referee.*
- Line 190 (lines 208-212 now): A very specific form of resistance (evolution of non-targetable alleles at the cutting site) was mentioned in the previous section. However, there are many other conceivable ways in which resistance could evolve to a gene drive system for example, inbreeding, behavioural avoidance of gene drive carriers, selection for higher levels of polygyny (in Y-shredder designs) to name a few. These all constitute novel knowledge gaps for deploying these technologies in these more behaviourally complex mammalian species and could be expanded upon.

- *This point is well taken and we have added the following to the end of this paragraph: " We described the impressive degree of behavioural adaptation rodents show in island ecosystems at the beginning of this section. Such behavioral adaptations could represent mechanisms of resistance to gene drive spread as well in the form of inbreeding, mate choice, and patterns of multiple mating (see ref 36 for polyandry and Manser et al., in press for polygyny)."*

- Line 194: A discussion of spatial factors e.g. dispersal rates/patterns and movement in complex habitats and especially as densities and sex ratios alter would be useful in this behavioural and ecological section as they can make or break drive success in the real world. First steps have been taken at modelling these complex systems in mosquitoes (Eckhoff et al., 2017 PNAS).
    - *This is a very good point, but we do note that " The knowledge gaps for gene drive modeling in rodents remain numerous and only major ones will be addressed below" (original lines 217-218; lines 240-241 revision), that ' incorporating the ecological and social system variation known from invasive mouse populations will be useful and these data will likely be important to obtain for a given target population before any field trial" (original lines 220-222; lines 244-245 revision) as well as noting further below that territoriality and the social structuring into reproductive demes could be particularly important for understanding and predicting gene drive function (lines 224-226; lines 247-249 revision). Clearly there is a lot that could be discussed here, but we strongly prefer to highlight those points we have made thus far, particularly given the relevant space constraints.*
- Line 204 (line 225 revision): Incorporating multiple gRNAs has been shown to work theoretically but a major knowledge gap in the gene drive field as a whole is whether such a system would work in vivo the way we assume it might. This has not yet been shown conclusively in any system.
    - *As this section is describing the results of the modeling efforts by Prowse et al., 2017, we added 'in these simulations' to the end of this sentence to make it clearer that this is only an in silico result at this point.*
- Line 209 (line 230 revision): Is it known what the level of polygyny is in the field?
    - *There is unfortunately very little information on this that we are aware of. Polyandry is easier to approach since embyros can be genotyped, but even for polyandry there is little information available from field populations. An interesting feature as well that is beyond the scope of this manuscript that any available data would be for populations exhibiting the usual ~1:1 sex ratio presumably and things could clearly change with a strong male- or female-bias in the sex ratio.*
- Line 212 (line 233 revision): Does this not imply that LFA designs will be extremely susceptible to reverse migration (i.e. from mainland to the targeted island) as an escaped drive allele will effectively be selecting for massive resistance in the mainland population? Are levels of gene flow between mainland and island populations always one-way? or equal? This would be a necessary piece of knowledge for an LFA design to be successfully deployed? On a similar note, what frequencies of pre-existing non-targetable alleles within the island population are permitted for a LFA drive system to still succeed? And if this is very low, are there existing technologies which would allow researchers to accurately assess whether these alleles existed in a mouse population prior to release?
    - *These are all interesting questions and addressed to some extent in the Sudweeks et al. paper that is cited in this section (ref 67). In short, a LFA design would likely be extremely susceptible to 'reverse migration', but the same is true with likely any eradication approach and certainly is for rodenticide-based approaches. The models indicate that a locally-fixed allele would indeed need to be locally fixed. That brings up the important question the referee raises of precisely how one would go about rigorously assessing that the target was indeed fixed in the population of interest, but we feel this is beyond the scope of this manuscript.*
- Line 253 (lines 277-280 revision): One area I am not aware of having been explored in a rodent context is the degree to which conventional tools e.g. baiting etc. could be combined with various gene drive designs. In vertebrates this has been most widely looked at for fish and can dramatically improve the feasibility of genetic control in this group. A discussion of existing models describing rodent control on islands (which I am assuming are well developed) and what it would take to integrate novel tools such as gene drives would be a novel inclusion.
    - *This is an interesting point and one that has been the basis of some discussion, but we are aware of no efforts yet to combine modeling of conventional and genetic approaches and a meaningful*

treatment of how to do so is beyond the scope of this manuscript in our opinion. We did add a short paragraph to the end of the modeling section addressing the reviewer's suggestion in a limited way that could either remain or be deleted based on the editor's input (either option would be acceptable to us).

- Line 294 (line 326 revision): Another highly developed and successful organisation working in this field is Target Malaria. Perhaps a knowledge gap would be assessing how this organisation has gone about various aspects of engagement etc., translating this to a mouse/rat context and what can be learnt or avoided from these lessons.
    - *This is a useful general suggestion, but is also the general subject of a much more in-depth treatment in this same issue by George et al. and is cited here for that reason (together with the other references cited that bear strongly on these general questions). For the briefer treatment in this section, we elected to highlight examples directly relevant to rodents and still feel this is preferable in this context.*

- Figure1: I think this legend needs substantially more explanation. For example it may be unclear to the reader why editing t such that it includes a synthetic copy of SRY is an example of a 'natural drive mechanism' but a CRISPR element linked to the same t is now a 'Hybrid system'. Also, I am not sure that readers unfamiliar with these systems would be clear on how t-SRY and t-CRISPR would function as gene drives from looking at 1B and 1C (indeed the same applies for the homing drive depicted in 1A). Perhaps a simple graphic showing how non-t sperm are excluded from the mating pool and how this biases the next generation for SRY or CRISPR inheritance would be more informative?
    - *In order to address the referee's concerns, we have modified both the labeling of this figure and the figure legend.*
- Figure 2: Line 3: There is no prior explanation of what an invasion threshold is.
    - *Based on the referee's suggestion, we have changed this line to read "This scenario models a drive with no invasion threshold, meaning there is no minimum frequency that a drive must reach in order to spread."*