Rat eradication comes within a whisker! A case study of a failed project from the South Pacific

W. Amos, H. J. Nichols, T. Churchyard and M. de L. Brooke

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2nd revised submission: 9 March 2016
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Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Note: This manuscript was transferred from another Royal Society journal with peer review.

Review History

RSOS-150556.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

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Have you any concerns about statistical analyses in this paper?
Yes

Recommendation?
Reject

Comments to the Author(s)
When I first read this paper, the authors appeared to make a compelling case for using genetic data analysis to better understand the eradication attempt using genetic data analysis to estimate the relative size of the bottleneck this population endured. However several things troubled me. My first concern was two-fold - why did the authors not use well-established methods to estimate effective population size from two-sample genetic data, specifically the allele frequency variance and linkage disequilibrium estimator methods - both would be appropriate and powerful methods for this question. I also note that allelic diversity (i.e. number of alleles) is the most sensitive indicator of a population bottleneck and could be easily simulated, but this index was not reported. Because the authors did not provide summary diversity statistics for pre- and post-eradication samples (which is an essential yet missing part of the results presentation), I generated them myself from the supplementary data table using the 18 loci retained in the authors analysis.

I was quite surprised to note the following observations from this exercise. Unbiased expected heterozygosity INCREASED by 0.03 following the eradication attempt - as did raw allelic diversity:

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So, I can't see any evidence for a bottleneck signature in this data whatsoever, I'm sure the apparent increases are within the ranges of sampling error, but clearly no evidence for a decline. This suggests to me that the main finding is completely wrong. Even if I were to reverse the pre/post sample labels - there is still no compelling evidence for a reduction in effective population size based on allele frequency or diversity change, noting that the maximum bottleneck of 100 simulated by the authors still falls within the confidence limits of the estimated allele frequency change.

Review form: Reviewer 2 (Andrew Veale)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
The code for the population simulations is currently not included and would be useful in order to replicate the results.
Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
I think the manuscript does add to the literature, and attempts to do a novel task - evaluating the number of survivors after a failed eradication.

I think the simulations are appropriate for the task and the conclusions sound. I think the use of STRUCTURE in this case - while not misleading in terms of survivor/reinvader hypotheses, has some drawbacks, with violations of assumptions, which therefore mean the analysis is less clean. Using population assignment in conjunction with the clustering would probably tidy this up, but I very much doubt any conclusions would be changed. It would just mean there was less noise to explain away. STRUCTURE does the task, showing they are survivors, despite it's inherent problems.

Other than that there are only some small notes I have added. Some of the methods in the software are not clearly stated, and the codes for the simulations are not available. Once these small problems have been addressed I feel the manuscript should be acceptable for publication.

Some small notes:

A sentence in the abstract stating that you also looked at the possibility of toxin resistance would be good.

In the abstract you say 19 microsatellites, when in fact you actually use 18 for the analysis given you removed one due to null alleles.

Line 141 You could make it clearer that there were three multiplex PCRs.

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Line 228. Within these rather than within the.

Line 277. Ne of 50, How would pregnant females affect this Ne?

Line 293. Structure works assuming hardy-weinberg equilibrium in each cluster. If a population has gone through an extreme bottleneck, then the frequency of alleles before and after the bottleneck will likely differ - hence samples combined from before and after will no longer have loci in hardy-weinberg. This may be the cause of your observed multiple groups on henderson. This doesn't change the overall interpretation, just shows that this aspect of the analysis is not really designed for this task. Pairwise assigment using something like geneclass 2 would be a
better way, as previously mentioned by a reviewer. While I do think there are probably better ways to analyse this question, given that STRUCTURE makes assumptions that are likely to be violated in this scenario, I doubt that the results or interpretation would change. The rats are survivors and all analyses show this.

From reading the previous comments, it appears that in a previous iteration of the manuscript you speculate that larger islands may have a similar percentage of rats survive to smaller islands, but since the absolute number of survivors is larger, the chance of some of them finding each other and breeding is larger. (though this section has been deleted). I do think this is a valid speculation to include, as we scale up island eradications this is a point that will need to be addressed.

Decision letter (RSOS-150556)

23-Nov-2015

Dear Dr Brooke:

Manuscript ID RSOS-150556 entitled "Rat eradication comes within a whisker! A case study of a failed project from the South Pacific" which you submitted to Royal Society Open Science, has been reviewed. The comments from reviewers are included at the bottom of this letter.

In view of the criticisms of the reviewers, the manuscript has been rejected in its current form. However, a new manuscript may be submitted which takes into consideration these comments.

Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to peer review before a decision is made.

You will be unable to make your revisions on the originally submitted version of your manuscript. Instead, revise your manuscript and upload the files via your author centre.

Once you have revised your manuscript, go to https://mc.manuscriptcentral.com/rsos and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript.

Your resubmitted manuscript should be submitted by 22-May-2016. If you are unable to submit by this date please contact the Editorial Office.

We look forward to receiving your resubmission.

Sincerely,

Matthew Allinson
Royal Society Open Science

on behalf of

Kevin Padian, Royal Society Open Science

openscience@royalsociety.org
Associate Editor Comments to Author (Dr Mike Bruford):

Comments to the Author:

One of the two reviewers of your manuscript thinks they have found a substantial problem with your analysis and have attempted to analyse your data from the supplementary material in a more conventional manner. If their analysis is correct then a clear problem with your interpretation is apparent. I have not attempted to replicate their computation but this warrants further investigation on your part. I have therefore opted for a 'reject may resubmit' decision here to allow you to re-analyse your data as reviewer 1 suggests, revisit their observations and if you remain convinced that your observations are robust, you can resubmit the paper as a new manuscript.

Reviewers' Comments to Author:

Reviewer: 1

Comments to the Author(s)

When I first read this paper, the authors appeared to make a compelling case for using genetic data analysis to better understand the eradication attempt using genetic data analysis to estimate the relative size of the bottleneck this population endured. However several things troubled me. My first concern was two-fold - why did the authors not use well-established methods to estimate effective population size from two-sample genetic data, specifically the allele frequency variance and linkage disequilibrium estimator methods - both would be appropriate and powerful methods for this question. I also note that allelic diversity (i.e. number of alleles) is the most sensitive indicator of a population bottleneck and could be easily simulated, but this index was not reported. Because the authors did not provide summary diversity statistics for pre- and post-eradication samples (which is an essential yet missing part of the results presentation), I generated them myself from the supplementary data table using the 18 loci retained in the authors analysis.

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Reviewer: 2

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Author's Response to Decision Letter for (RSOS-150556)

See Appendix A.

RSOS-160110

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
Yes

Recommendation?
Reject

Comments to the Author(s)
While some of the main findings here are fairly clear (unlikely recolonization from one of the alternate sources sampled, or the evolution or poison resistance) I am unconvinced that the genetic data analysis shows how close to success the treatment was. The data (heterozygosity and more notably number of alleles per locus) do not suggest that the extermination caused an appreciable population bottleneck, and to my thinking, the evidence that the attempt came "within a whisker" is equivocal. I would perhaps be more convinced by the use of well-established two-sample Ne estimators (e.g. the Waples temporal method) as well as more recent bottleneck tests, which are considerably improved. The method developed and applied here has not been validated or independently tested, and I also note that the estimate it produces does not have an upper confidence interval - in fact the observed allele frequency perturbation is within one standard error of the mean (and considerably within a 95% confidence interval) for the weakest bottleneck (100 rats) simulated. I could be more easily swayed if the results were supported by conventional bottleneck or Ne estimation analyses, and there are numerous and improved, accessible and validated ways to do this (e.g. see Do et al. Mol Ecol Res 2014).
Decision letter (RSOS-160110)

04-Mar-2016

Dear Dr Brooke,

The Subject Editor assigned to your paper ("Rat eradication comes within a whisker! A case study of a failed project from the South Pacific") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

Please submit a copy of your revised paper within three weeks (i.e. by the 27-Mar-2016). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. 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 Editors, 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.

To revise your manuscript, log into http://mc.manuscriptcentral.com/rsos 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. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to each of the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections before the reference list:

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.
If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link: http://datadryad.org/submit?journalID=RSOS&manu=RSOS-160110

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Yours sincerely,
Andrew Dunn
Senior Publishing Editor, Royal Society Open Science
openscience@royalsociety.org

Comments to Author:

Associate Editor's comments (Dr Mike Bruford):
Associate Editor
Comments to the Author:
Your revision has been seen by the original reviewer who, while acknowledging that the heterozygosity issue has been dealt with, remains sceptical about the strength of the results you have obtained. They have asked for conventional bottleneck tests to be applied and if you are able to do this, I think this would strengthen your conclusions considerably.

Reviewers' Comments to Author:
Reviewer: 1

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Author's Response to Decision Letter for (RSOS-160110)
See Appendix B.

RSOS-160110.R1 (Revision)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No
Recommendation?
Accept as is

Comments to the Author(s)
I think the authors missed my point about the upper confidence interval (on the bottleneck size, not on the allele frequency change), yes I see that standard errors (and not CIs) are reported around the estimate of the allele frequency change, however the point estimate for the allele frequency change of the weakest bottleneck still falls within an SE of the point estimate. Therefore the method as implemented does not provide an upper confidence interval for the size of the bottleneck.

Decision letter (RSOS-160110.R1)

16-Mar-2016

Dear Dr Brooke,

I am pleased to inform you that your manuscript entitled "Rat eradication comes within a whisker! A case study of a failed project from the South Pacific" is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article within approximately 10 working days. Please contact the production office (openscience_proofs@royalsociety.org) to let us know if you are likely to be away from e-mail contact during that period. Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

Royal Society Open Science operates under a continuous publication model (http://bit.ly/cpFAQ). Your article will be published straight into the next open issue and this will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

In order to raise the profile of your paper once it is published, we can send through a PDF of your paper to selected colleagues. If you wish to take advantage of this, please reply to this email with the name and email addresses of up to 10 people who you feel would wish to read your article.

On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Best wishes,
Dr Matthew Allinson
matthew.allinson@royalsociety.org
http://rsos.royalsocietypublishing.org/

Associate Editor Comments to Author:
Associate Editor: 1
Comments to the Author: The final referee has recommended publication of your manuscript and I am happy to accept that recommendation. You may, however, wish to clarify the CI point in your final version.
Reviewer(s)' Comments to Author:
Reviewer: 1

Comments to the Author(s)
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Reviewers' Comments to Author:
Reviewer: 1

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As explained in the cover letter, our earlier estimates of the heterozygosity change were based on a silly error. We are eternally grateful to the referee for spotting this. However, as explained in an expanded section of the MS (lines 160-190), there are good reasons for being cautious about the heterozygosity approach, especially when it suggests no population change in total contradiction to all the field evidence pointing to a reduction in rat numbers of at least 98 percent. Accordingly, we now just present data from the change in allele frequency.

Reviewer: 2

Comments to the Author(s)
I think the manuscript does add to the literature, and attempts to do a novel task - evaluating the number of survivors after a failed eradication.

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No action taken

Other than that there are only some small notes I have added. Some of the methods in the software are not clearly stated, and the codes for the simulations are not available.

Code now available as Supplementary material

Once these small problems have been addressed I feel the manuscript should be acceptable for publication.

Some small notes:

A sentence in the abstract stating that you also looked at the possibility of toxin resistance would be good.

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In the abstract you say 19 microsatellites, when in fact you actually use 18 for the analysis given you removed one due to null alleles.

Corrected

Line 141 You could make it clearer that there were three multiplex PCRs.
Clarification added (l. 142-143)

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Line 228. Within these rather than within the.

Within the seems correct. Retained

Line 277. Ne of 50, How would pregnant females affect this Ne?

This is a complexity that is seldom dealt with! If females are pregnant at the time of sampling this would be equivalent to sampling the population slightly later in time (if one counts the offspring) but should not impact the estimated size of the bottleneck.

Line 293. Structure works assuming hardy-weinberg equilibrium in each cluster. If a population has gone through an extreme bottleneck, then the frequency of alleles before and after the bottleneck will likely differ - hence samples combined from before and after will no longer have loci in hardy-weinberg. This may be the cause of your observed multiple groups on henderson. This doesn't change the overall interpretation, just shows that this aspect of the analysis is not really designed for this task. Pairwise assignment using something like geneclass 2 would be a better way, as previously mentioned by a reviewer. While I do think there are probably better ways to analyse this question, given that STRUCTURE makes assumptions that are likely to be violated in this scenario, I doubt that the results or interpretation would change. The rats are survivors and all analyses show this.

We tend to agree with the reviewer that STRUCTURE performs adequately. The key point is that the putative three clusters found on Henderson are extremely tenuous, with weak and variable assignment of each rat to each cluster. The differences in allele frequency before and after are slight (though significant). We feel that the STRUCTURE analysis is certainly good enough to show what is needed and benefits from being accessible to a wide audience. We would of course add the geneclass 2 analysis if the Editor feels this is necessary, but in our opinion it is not.

From reading the previous comments, it appears that in a previous iteration of the manuscript you speculate that larger islands may have a similar percentage of rats survive to smaller islands, but since the absolute number of survivors
is larger, the chance of some of them finding each other and breeding is larger. (though this section has been deleted). I do think this is a valid speculation to include, as we scale up island eradications this is a point that will need to be addressed.

After earlier referee prompting, we thought it best to delete the speculation about whether there is any threshold density below which the survivors are likely to fail to meet each other, reproduce, and restore the population. Although this is an intriguing line of thought, it depends critically on whether the survivors are spatially clustered or uniformly distributed across an island.
Associate Editor’s comments (Dr Mike Bruford):

Your revision has been seen by the original reviewer who, while acknowledging that the heterozygosity issue has been dealt with, remains sceptical about the strength of the results you have obtained. They have asked for conventional bottleneck tests to be applied and if you are able to do this, I think this would strengthen your conclusions considerably.

Do et al. Mol Ecol Res 2014 tests applied as requested. See further comments below

Reviewers’ Comments to Author:
Reviewer: 1

Comments to the Author(s)
While some of the main findings here are fairly clear (unlikely recolonization from one of the alternate sources sampled, or the evolution or poison resistance) I am unconvinced that the genetic data analysis shows how close to success the treatment was. The data (heterozygosity and more notably number of alleles per locus) do not suggest that the extermination caused an appreciable population bottleneck, and to my thinking, the evidence that the attempt came "within a whisker" is equivocal.

We apply the Ne estimator suggested. See new Table 2 in revised MS. It and the simulation method we develop converge reassuringly on an Ne value around 50 survivors, representing very roughly 0.1 percent of the starting population. It doesn’t seem outrageous to describe the project as within a whisker of success.

I would perhaps be more convinced by the use of well-established two-sample Ne estimators (e.g. the Waples temporal method) as well as more recent bottleneck tests, which are considerably improved. The method developed and applied here has not been validated or independently tested, and I also note that the estimate it produces does not have an upper confidence interval –

The estimated average change in individual microsatellite allele frequency between rats sampled before and after the eradication program, shown in Fig 3, DOES have standard errors shown above and below the estimate.

in fact the observed allele frequency perturbation is within one standard error of the mean (and considerably within a 95% confidence interval) for the weakest bottleneck (100 rats) simulated.

The parametric 95% CI for the NeEstimator estimates are reasonably tight (new Table 2) while both our simulated CIs and those of the jackknife are somewhat broader. Despite this variation between methods in the size of the confidence intervals, the strong agreement between methods in the point estimate leads us to believe that the true bottleneck was of the order of Ne=50.

I could be more
easily swayed if the results were supported by conventional bottleneck or Ne
estimation analyses, and there are numerous and improved, accessible and
validated ways to do this (e.g. see Do et al. Mol Ecol Res 2014).

Do et al. Mol Ecol Res 2014 tests applied as requested (new Table 2), with results
reassuringly similar to those generated by simulation (Fig 3).