Peer review on manuscript

"Determining causes of genetic differentiation in [●] to identify the need for conservation actions"

by Peer 578

ADDED INFO ABOUT FEATURED PEER REVIEW
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Introduction

This manuscript concerns conservation genetics of [●] in [●]. The authors use population-genetic simulations combined with empirical genetic data to infer past demographic processes (population size, connectivity) of a population that is potentially isolated from the main range by urbanization and agriculture. Using simulations for inference is a powerful approach that is increasingly common in population and theoretical genetics (1,2) but has rarely been employed by applied conservation biologists and conservation geneticists. Therefore, in my opinion, the manuscript contributes substantial insight to this system and the topic of connectivity, and is a valuable framework for objective, quantitative analyses and conclusions in future studies.

Knowledge of population history is important because management interventions and population protection status are most effective when tailored to particular processes, e.g. historic isolation, anthropogenic barriers, etc. (3,4). Simulations can help determine what past processes might have influenced the genetic patterns currently observed in natural populations (1,5). The idea is to produce simulated genetic data under several plausible, competing demographic and genetic scenarios (e.g. fragmentation, bottlenecks, colonization), record statistics summarizing the simulated data under these scenarios, and compare the simulated distribution of summary statistics to statistics calculated on real data (6,7). If the real data "match" the simulated data (i.e. observed genetic signatures are not significantly different from data under a particular simulated scenario), that scenario is a likely candidate for the true population history.

Revision Recommendations

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<th>Question</th>
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After inferring bottlenecks and connectivity reductions, the authors then use additional simulations to forecast future population responses to management intervention (sensu (8,9)), to determine for maintaining genetic diversity.

**Merits**

Conservation and population genetics have traditionally relied on ad hoc interpretations of genetic summary statistics, e.g. inferring a bottleneck if diversity is low relative to another populations/species, often leading to "story-telling," confirmation bias, and overinterpretation of data (2,10,11). As the authors astutely indicate, such subjective conclusions are problematic because low diversity, for example, can stem from numerous processes (12,13).

The simulation approach has facilitated stronger inference over the past ten years in molecular ecology and population genetics. Importantly, I have seen few papers in conservation biology employing simulations for inference; this manuscript is certainly at the forefront of its field.

The introduction provides clear, instructive background on simulations and their utility and connects their study to broader issues in conservation biology (e.g. lines 72-76, 77- 88, 91-92, 132-138, 626-632). The paper is well-structured (logical, step-by-step) and well-written (cohesive paragraphs, articulate statements).

The authors also employ sensitivity analysis (14,15) to explore a range of values for key parameters in the simulation models- migration rates, population sizes and time-scale.

**Critique**

Unfortunately, there are substantial flaws in the simulations and in reporting of simulated data which prevent me from recommending publication, at present.

A crucial flaw is that the authors "hide" the simulation data (on which the major conclusions are based). Appendix-1, comparing simulated and observed data, simply states whether the comparison was "high," "low," or "overlap/non-significant difference". It is necessary for the authors to report (numerically) the simulated data range, to show whether the observed data falls within, and "how close" the real and simulated data are, which is key information for readers. This is often shown graphically (see (16) Fig 3, (17) Fig 3, (18) Fig 6), but the authors could simply replace "high", "low" etc., with the numerical range from the simulations.

Additionally more simulation replicates are vitally needed- only 10 were performed per scenario, while similar investigations typically involve >500-1000 replicates (13,16,19- 24). Fifty is a bare minimum, to obtain 90-95% intervals to compare to the observed data. The authors
(unconventionally) use standard error (line 241) instead of 95% intervals (standard error will likely be much narrower), but a sample of 10 is a tenuous basis for concluding "no significant difference." This is not merely a picky point about "the authors need more data"; unlike in ecology field studies, computer simulations entail no real cost for sufficient sampling (see additional comments for suggestions).

Another principal flaw is absence of discussion of several central caveats/assumptions. The Discussion must admit that the simulations do not employ realistic life history (for example, generational overlap, which affects effective size and genetic drift (25-27)). The authors should also remark that more complex simulations (e.g. Vortex (28,29)) might be a better model for genetic diversity loss and may show different results. Additionally the assumptions of Ne analysis, which are violated (30-32), must be noted (no migration, no generation overlap, no recent demographic change).

Another flaw is the reliance on only diversity-based summary statistics; differentiation-based statistics are not deployed at all, despite "differentiation" appearing in the title and throughout the manuscript. Both diversity- and differentiation-based summary statistics (usually FST) are nearly always used in similar investigation of multiple population simulations. Using STRUCTURE to examine population subdivision is interesting and useful, but not sufficient for comparing simulated and observed data- it is somewhat qualitative, and was used on only three datasets per scenario. The hallmark of this manuscript is the objective, quantitative nature of simulation work, but the interpretation of STRUCTURE results (lines 364-370, 376-378, 389-395, etc.) is entirely subjective (e.g. different cluster membership thresholds per scenario, discussing results in "cherrypicking" fashion, STRUCTURE results not used for scenario 5). Using FST (and/or RST) solves these problems, allowing the same quantitative comparison used on diversity statistics- e.g. does the observed value fall within the range of simulated data or not. Additionally, FIS and M-ratio are valuable summary statistics (33-35) for comparing scenarios, due to the unique information they capture. Testing FST, FIS, and M-ratio (and placing results as columns in Appendix-2) is highly advised.

Discussion

The authors use simulation methods to infer processes influencing genetic data, which represents a paradigm shift in conservation genetics methodology. However, the authors do not use sufficient simulation replicates to establish convincing conclusions, do not report summaries of simulation data, and do not calculate sufficient summary statistics. It is therefore not possible to judge, as yet, the reliability of the conclusions. Fortunately, these deficiencies are easily rectified.
References


47. Beerli P. Migrate Documentation. 2010;

Additional comments for authors

First some additional key comments (important!), and then line-by-line edits (less important)…

Regarding the additional replications needed for each scenario. I recognize that hundreds of simulations are computationally demanding, but they are within the capabilities of even small computer clusters or even on individual computers (over the time of days to weeks). If the authors
do not have access to their own or a government/university cluster, there are numerous institutions in Canada, the USA, and Europe which offer use of their computer cluster at small or no cost (http://rna.urmc.rochester.edu/bioinfo.html, http://cse.ucsd.edu/node/251, http://www.biotech.uconn.edu/bf/, http://www.bioinformatics.ku.edu/resources/biocluster, http://cagt.bu.edu/page/Bcluster, note: I am not advertising my own institution in this list in order to maintain neutrality). The time spent on additional simulations will be very much worth the small additional effort.

The authors should note in discussion that Approximate Bayesian Computation ((6,34,36,37)) is a more formal and advisable method for comparing simulated and observed data than the "grid" method used (which however was sufficient for the task).

I think the Methods is unbalanced regarding the main focus of the paper- the authors spend more than three pages describing the bottleneck and effective population size methods, which are a minor component of the study, and much of which could be placed in the appendix. More space should be devoted in the main manuscript to the simulations (parameter space, assumptions, sensitivity analysis), which only receive one paragraph currently. I understand that simulations are explained in appropriate detail in the Appendix 1, but unfortunately many readers do not utilize appendices and online material, so please attempt to place as much of this information as possible within the main manuscript Methods- several paragraphs at least.

You monitor heterozygosity as the response over the next hundreds of years with □□, but why not monitor number of alleles or inbreeding coefficient (or level of relatedness), which might be more sensitive to low population size? These might also show "thresholds" or "vortexes" in which loss accelerates more than linearly, perhaps.

Again, I highly emphasize that using only within-population diversity measures for comparing simulated and observed data is unconvincing, and would set a poor precedent if the authors with this paper to be a model for future work (line 636)… As per this, note that other options for among-population diversity measures (other than FST/RST) are number of shared alleles, Goldstein's delta mu squared statistic, and Paetkau's mean assignment likelihood (38-41).

I think the Introduction is a bit lengthy, and is a bit heavy with citations- could be reduced some; same for the Discussion. Don't remove any points, just try to make the sentences tighter, use less repetition, etc..

A series of papers (24,42-44) have looked at the power and error rates of the M-ratio and heterozygote excess test to detect real bottlenecks, and some of their findings should be mentioned when interpreting the bottleneck results, in terms of possibility of false positives and also "missed" bottleneck signals.
It seems that (1) should be cited, as it is probably the most comprehensive recent review of the use of simulations in ecology and evolution and the most thorough resource of existing simulation software (see also (45)).

There is also insufficient/unclear explanation of how this paper and the data therein. There is also insufficient/unclear explanation of how this paper and the data therein overlaps with other recent papers on these populations… Some information on this can be garnered throughout the manuscript, but it is scattered. Please include several sentences that clearly state, in one paragraph, what data previous studies collected and what methods they used (did any use Structure or simulations?), and state clearly what the new study does provide.

If the authors continue to use STRUCTURE results, they should set some quantitative means to compare observed and simulated data, such as mean q values- the discussion of STRUCTURE results does not seem very quantitative. Q values are mentioned but it seems a different "threshold" was used each scenario rather than determining if the observed value fell within the standard deviation of the simulated. Of course, FST and the other statistics will help here. Importantly, the lengthy, somewhat qualitative presentation of STRUCTURE results for each simulated scenario is a substantial "bump" in the otherwise nice flow of the paper.

In some PVA studies of large mammals, it has been suggested that [●], as the authors allude to in the abstract. The authors only tested [●], and I think it is worth testing if [●]. Line 338-339 seems to imply that the authors did test [●] but no results are reported regarding this (not included in Fig 4.3)… In any case please clarify.

Following are some small in-line changes

Line 68 change "and" to "or"

Line 86- suggest to use another term than "non-natural"… a very vague term… what is "natural?"
Same in line 456

Line 188 "small" is relative… in many parts of the [●], this size population would not be considered "small", so I suggest changing to "relatively small"

Line 227- should be "in order to"?

Line 296- "we can note that for microsatellite data, the SMM or TPM with pg= 90% are usually more appropriate"… I think you meant 10%?
Line 492- what do you mean by "performing better"?

Line 605- you mean "phenotypic polymorphism" or "genetic"? Regarding source sink discussion on page 23, you could confirm this with the software MIGRATE (46,47) which should reveal asymmetries in migration rates.

Figure 4.1- please zoom in the figure 4, as the sampling zone only takes up maybe 20% of this figure, the rest is basically empty so the part we want to look at is tiny. It is hard to see the sampling symbols (and I have a young person's eyes). Also, the WMU boundaries (wildlife management units??) make it cluttered and hard to see the symbols. Maybe remove them? Lastly, numbers might be easier to read than the small triangles etc. is hard to find, the symbols for and etc.

Figure 4.2- a bit confusing especially regarding the bottleneck- it doesn't look like a population reduction, it looks like a wavy line.