nature portfolio

Peer Review Information

Journal: Nature Ecology & Evolution
Manuscript Title: Disease-driven top predator decline affects mesopredator genetic structure
Corresponding author name(s): Andrew Storfer

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:

27th June 2023

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Dr Storfer,

Your manuscript entitled "Disease-driven top predator decline affects mesopredator genetic structure" has now been seen by 3 reviewers, whose comments are attached. The reviewers have raised a number of concerns which will need to be addressed before we can offer publication in Nature Ecology & Evolution. We will therefore need to see your responses to the criticisms raised and to some editorial concerns, along with a revised manuscript, before we can reach a final decision regarding publication.

All three reviewers are broadly positive about your manuscript, and find the results novel, surprising and insightful. One major comment raised by multiple reviewers is the inappropriate use of "trophic cascade" to describe this instance of mesopredator release, and thus we request that this be removed in line with their comments. You will see from the comments that we happened to contact a reviewer (#2) who also reviewed this (positively) at Science. They were unable to spot revisions in line with their previous comments in this version of the manuscript. As part of the revision process for us, we ask that include that original review in your point-by-point response. I have copied the provided version of that review in their comments below.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

* Include a "Response to reviewers" document detailing, point-by-point, how you addressed each reviewer comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the reviewers along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our

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* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

Please use the link below to submit your revised manuscript and related files:

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We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Ecology & Evolution or published elsewhere.

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Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

[REDACTED]

Reviewer expertise:

Reviewer #1: pop ecol, pop gen, spatial ecology

Reviewer #2: landscape genetics, population dynamics

Reviewer #3: ecosystem cascade, biodiversity, mesopredator release

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Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Overview
Beer et al. present landscape analyses based on a reasonably large genomic dataset (345 individuals and 3431 SNPs) of spotted-tailed quolls in Tasmania, Australia. They search for molecular evidence of changes in spotted-tailed quoll populations that correlate with Tasmanian devil density and the number of quoll generations that has passed since the arrival of a devil-affecting disease into the site. The methodology is very original, pairing established landscape genetic approaches with a long-term study of Tasmanian devil populations in order to inform our understanding of competitive effects in Australia's marsupial predators. The study is of high merit and generally well analysed, and contains clear text, graphics, and summary information. I believe that with some minor adjustments to the methodology and interpretation of results, the study will be of high value. Mostly I think the Methods section just requires some clarification of what was done, particularly regarding the GEA analyses, as these are used to support the notion that devil density and time-since-disease-arrival "impose distinct selection pressures", which is, in my view, the result with the strongest and clearest implications.

Broad comments
• The Abstract could be a little bit clearer about what analyses were done and what results were found. Currently for the methods it just states that a "landscape community genomics framework" was involved, which doesn't tell us anything about specific techniques (EEMS, ResistanceGA, pRDA, GDM, RDA, LFMM) used. And I suspect readers prefer to see specific results presented in the Abstract, e.g. "we found xx genes showing signs of selection due to Tasmanian Devils, including xx related to muscle….".
• Data filtering is always a complicated process, but it seems like it was done here with care and due consideration for key biases. Two components that I would like to confirm were included, or hear justification for exclusion, are filters for allele balance ratio and a filter to remove one of any pair of close relatives. In particular, inclusion of close relatives could bias allele frequencies, which could bias the signal of selection. The beta.dosage function of the hierfstat package (Weir & Goudet 2017 / Goudet et al. 2018) is easy to check this.
• Again on data filtering, what is the authors' justification for using a minor allele frequency of 0.03? This cuts out a large proportion of the loci (58,996 to 5,191). My interpretation of that threshold is that it means that about 20 copies of an allele must be present in a dataset of ~345 diploid individuals? I think this could be loosened (e.g. to 15 or maybe even 10 copies), which may substantially increase the number of loci available for testing with the LFMM and RDA methods. See this for consideration (https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13351) – a key point is that "having fewer data points appears to reduce the overall power and effectiveness of GEAs".
• Pertaining to the IBR results, and particularly the Discussion section around Lines 145 to 148: My interpretation of the IBR results is that where devil densities are high, landscape resistance to quolls is reduced, meaning that quolls move more freely in areas of high devil density. I'm taking this from Line 95, where the authors state that quoll resistance is negatively correlated with devil density. Rather than some function of "reduced competition and less dispersal pressure", I would argue that this finding is simply the result of the species being quite similar in their habitat requirements, and so

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quolls disperse less through areas that both species find less preferable (e.g. heavily cleared/modified environments). I.e., where there are lots of devils, the habitat is probably just better quality and so quolls can move more readily through the landscape. As the authors state slightly further down, quolls avoid devils temporally, rather than spatially, so to me this may be a better explanation of the results.

Minor comments/suggestions
Line 20: "top terrestrial predator" probably more accurate.
Line 21: is it multiple cascades, or just a singular cascade? Honestly not sure. Consider that effects that propagate laterally or upward from the main interaction chain should not be thought of as part of the trophic cascade.
Line 44: also state "hereafter, the devil" after the scientific name, as you've done for the spotted-tailed quoll.
Line 57: have you got a citation for "providing sufficient lag time to detect evolutionary effects of trophic cascades."? I agree it's probably correct, but citation also needed.
Line 59: does potential mesopredator release of the eastern quoll factor into this talk of interspecific competition at all? I assume spotted-tailed quolls would probably outcompete eastern quolls, but could be worth including some wording here about other competitors..
Line 67: is it possible to explain a little more clearly what the differences between IBR and IBE are here? I imagine something like IBR being driven by obstacles that hinder movement and gene flow whereas IBE being driven by divergent selection pressures under different environmental conditions.
Line 75: could 'population genomic structure' be used instead of 'genome-wide genetic structure'? It seems like the authors have stuck to using the word 'genetic' throughout the manuscript, whereas 'genomic' or 'population genomic' would probably make interpretation easier in many instances, e.g. 'landscape genomic' instead of 'landscape genetic'.
Line 92: Not sure this is a solid statement, given that there doesn't appear to be a justification for the top ten models being useful for inference. See comment regarding Line 378.
Line 118: Note that authors "should explicitly report the total number of significant associations, the number of unique SNPs that have associations, the total number of associations per SNP, as well as which SNPs have significant associations with which environmental factor" (https://onlinelibrary.wiley.com/doi/full/10.1111/mec.14549). Please ensure all of these are presented (either in this paragraph or in the Extended Data/Supplemental Table).
Line 139: "the top models" should probably be reworded to "some top models".
Line 174: This is a good point. I suggest, to broaden the Discussion beyond Tasmania, adding a sentence that highlights some examples of studies where invasive pests and diseases are known to have direct molecular impacts on native species (e.g.
https://onlinelibrary.wiley.com/doi/10.1111/eva.12226;
https://onlinelibrary.wiley.com/doi/full/10.1111/mec.16680;
https://doi.org/10.1016/j.biocon.2011.04.025).
Line 243: The caption says 'ranked by', does this mean the top model (lowest AICc) is number 1 on the figure? Please be very explicit here. Could even be worth adding the AICc value instead of 1 to 10 underneath panel E.
Line 257: Was the spatial spread of sampling random across the 15-year time period, or were later samples collected in different areas to earlier sampling?
Line 259: "conduct" should be "conducted".
Line 270: A northern quoll reference genome has been available for a while now

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(https://www.dnazoo.org/assemblies/Dasyurus_hallucatus) – would this be more or less appropriate to align spotted-tailed quoll reads to than the Tasmanian devil genome, and why? Presumably something to do with annotation?
Line 268: total number of sequenced reads should be presented (either in Extended Data or here). The percentage of reads successfully assigned to a sample after demultiplexing should also be stated.
Line 290: 50% missing data in one or more individuals is quite high, can the authors remove a small number of individuals with the most missing data to reduce that to say 30%? Or add a histogram of missing data by sample to the extended data section, to show the actual spread of missing data across all samples?
Line 378: The top 10 models are presented in Figure 2, correct? So were these top 10 models those with delta AIC < 2? Please clarify how these 10 were picked. Should be re-stated on Line 91 of the Results section too.
Line 392: Can the authors confirm that these pRDA and GDM analyses do not require no missing data? I'm not very familiar with these analyses.
Line 320: As the authors are discussing two species here, it would be clearer to state that generations diseased is the collection year of a given quoll sample. Readers don't know if devil generations are the same as quoll generations.
Line 325: Following on from the previous comment, I think it would be clearer to change "devil density and generations diseased have independent effects" to "devil density and time since DFTD arrival have independent effects".
Line 457: Can the authors specify the number of latent factors (presumably 3) used here?
Line 459: Wouldn't the authors have used the 'lfmm2.test' function here, which produces p-values? And was there a false discovery rate used for this lfmm? Maybe just need to explain the q values a little more.

Figures and Tables
Line 215: Better to write out the full pRDA title than use the acronym.
Line 217: As above. Also worth explaining what 'generations diseased' means, i.e. time since arrival of disease in Tasmanian devils.
Line 240: better to say "spotted-tailed quoll individuals", as I was initially unsure whether the 189 individuals were quolls or devils.
Line 676: Table caption needs more detail, e.g. which species is being analysed. Also in the table itself, some clarity about thresholds could be improved, e.g. does an individual missingness value of 0.95 mean individuals with less than 95% missing data were removed, and what is "Spatiotemporal SNP missingness"?
Extended Data Figure S3: Can the authors improve resolution and clip the predicted surfaces to the outline of Tasmania? Currently looks like the migration surfaces extends into the ocean.

Data and materials availability:
I recommend that the authors publish all relevant metadata and bioinformatics scripts and R code on Dryad/Zenodo or something similar. Sample locations can be removed or desensitised if necessary. This would be more in line with the journal policy.

Reviewer #2 (Remarks to the Author):

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Dear Authors,
I have been assessing the previous submission, which had been submitted to Science and I am surprised to see only some minor changes to the manuscript when comparing the two different versions. Without any proper response letter to reviewers it is difficult to address which changes have been done and how and if all comments have been addressed sufficiently. I personally feel that is a waste of time and energy of reviewers and should be avoided (obviously the manuscript has been immediately resubmitted given the time line it has been landing on my desk).
I will thus not do another thorough review but like to point out one point I got already confused in the first submission and this is the use of the term "trophic cascade" as introduced by Ripple et al 2016. The paper itself describes cascade as species interactions that originate with predators spreading top down through foodwebs. Authors write in line 34 that changes in top predator densities lead to trophic cascades – but isn´t it a change in the cascade and not a creation of cascade itself? Please correct me if I am wrong here, but I think this needs some clarification at this place and elsewhere as the terminology is not clearly used.
Saying that, I like the landscape community approach and I do think this is a cool and interesting study system and I think the paper is a sound scientific study with interesting results which should be published.

REVIEWER 2 REPORT FROM SCIENCE, PROVIDED BY REVIEWER

Authors here study mesopredator food web dynamics using a landscape community genomic approach in the spotted-tailed quolls and Tasmanian devils. They make use of a long term dataset on the decline oft he Tasmaian devlis following DFTD and its ecological effects on dispersal for the quolls. While landscape genomic studies have somehow become lesss popular in recent years I think this study nicely demonstrate the potential of such an approach, which could also be used by authors. I do think this study thus is worth to be published.
I do have some problems with the use of the term „trophic cascade" by authors. If we use the definition by Ripple 2016 it is described as species interactions that originate with predators spreading top down through foodwebs. Authors describe the effects of on the spotted quoll when tasmanian devil numbers have been reduced shifting the interactions in the foodweb. As currently phrased this means that only now we do have a trophic cascade but not before. My understanding would be that this is rather a change in the current cascade and not creating a novel one?
I am lacking a bit of information why you find three different genetic clusters in the quolls. While the Western one nicely correlates with density of devils I wonder what factors are explaining that? Can you provide more information for the general readership who might be less familiar with the study system? Apparently the north western tip of Tasmania has always been an area with highest density of devils – so I would assume there is also an ecological reason for it.

24: I find that misleading phrased. The disease is resulting in a change of the trophic cascade but not a cascade itself
36: see comment above on the terminology
147 I am not sure I can follow this argument: Are you saying that because there are less devils there is lower pressure to disperse, so more quolls could be in that given area? That would mean that this is not directly a resistance in the landscape ? Do you have data on quoll densities in such areas which

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could support the argument?
190 I like that you are stating this so directly and believe that even such a correlative approch has its merits.
210 isn´t that a too general statement? And how do you see that your results contribute to the conservation of both species in Tasmania?
482 Are Figure panels A and B representing the whole range of the quolls? Looking at figure 1 and Figure 2B gives the impression that the SouthWestern part of Tasmania is not inhabitated largely by both species, right?
589 which are the variables and why did you select those?

Reviewer #3 (Remarks to the Author):

This study links the decline of a dominant scavenger (devils) to genetic changes in a competing mesopredator (quolls). They first describe a variety of known cascading impacts from devil declines and then show new results on how quoll genetic structure changes. To my surprise, I could not find a clear example in the literature of how the loss of a dominant competitor (or predator) altered the genetic structure of a submissive competitor or mesopredator.

The writing is clear but – for a relatively broad ecology journal like NEE - the main results (including figure 2) need to be more easily interpretable by readers without training in genetics (this would not be required if submitted to Molecular Ecology). There should also be more help interpreting if the effect sizes shown are biologically significant. For example, does the decline of a dominant competitor recreate the magnitude and direction of the genetic impacts induced by human hunting? From habitat loss and fragmentation?

Introduction:

The use of "top predator" for the Tasmanian devil doesn't feel right because they're small (8-10kg) and primarily a scavenger. Devils are not even that much bigger than the quolls there (which often reach 6-8kg). They should more accurately call devils a dominant competitor throughout the manuscript. Referring to cascading impacts from the decline of a dominant competitor is just as novel as how they currently present their results per se. So there's no need to oversell or stretch such terms.
Trophic cascades are a defined term (supposed to be at least) including three trophic levels, with a predator indirectly influencing a species' two-levels down (usually a plant) via their effect on the intermediate species (usually an herbivore). What the authors describe in this paper is the definition of mesopredator release, not a trophic cascade. It is a more general type of ecological cascade but certainly not a trophic cascade. The use of the trophic cascade term should be removed throughout.
Add species diets and weights somewhere to provide biological/ecological rationale for their interactions.

Last sentence is results. This is normally a short paragraph in Nature Comms but this single sentence

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here in NEE doesn't flow.
Results
I really appreciate the framing around specific hypotheses. Well done but would benefit from more laymen's explanation/interpretation of what the genetics changes mean.
Methods: Excellent.
Discussion: bit more help interpreting if the effect sizes shown are biologically significant (see above).
Figures
Overall attractive.
Figure 1 panel C – These smooth PowerPoint curves are more like hypothesized relationships (or idealized trends). Calling them "results" could be misleading.
Figure 2: Help the reader without expertise in genetics understand these.
Figure 2C – need to add that lower AICc is better somewhere. OR maybe put a star over the best model?
Tables: Excellent.

**********END**********

Author Rebuttal to Initial comments

We thank the reviewers for their thorough and thoughtful feedback. Below, please find our point-by-point responses, which also detail where in the manuscript the appropriate changes can be found. Our responses are provided in blue text. Line numbers in our responses refer to the clean copy of our revised manuscript, not the copy that shows tracked changes.

Reviewer #1 (Remarks to the Author):

Overview

Beer et al. present landscape analyses based on a reasonably large genomic dataset (345 individuals and 3431 SNPs) of spotted-tailed quolls in Tasmania, Australia. They search for molecular evidence of changes in spotted-tailed quoll populations that correlate with Tasmanian devil density and the number of quoll generations that has passed since the arrival of a devil-affecting disease into the site. The methodology is very original, pairing established landscape genetic approaches with a long-term study of Tasmanian devil populations in order to inform our understanding of competitive effects in Australia's marsupial predators. The study is of high merit and generally well analysed, and contains clear text, graphics, and summary information. I believe that with some minor adjustments to the methodology

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and interpretation of results, the study will be of high value. Mostly I think the Methods section just requires some clarification of what was done, particularly regarding the GEA analyses, as these are used to support the notion that devil density and time-since-disease-arrival "impose distinct selection pressures", which is, in my view, the result with the strongest and clearest implications.

We thank the reviewer for their time spent providing valuable feedback on our study. We have addressed the reviewer's specific comments below.

Broad comments

• The Abstract could be a little bit clearer about what analyses were done and what results were found. Currently for the methods it just states that a "landscape community genomics framework" was involved, which doesn't tell us anything about specific techniques (EEMS, ResistanceGA, pRDA, GDM, RDA, LFMM) used. And I suspect readers prefer to see specific results presented in the Abstract, e.g. "we found xx genes showing signs of selection due to Tasmanian Devils, including xx related to muscle….".

We agree that simply stating "landscape community genomics framework" in the Abstract is vague, so we have modified the abstract to clarify what this framework entails (i.e., it is a framework to identify environmental drivers of population genomic structure and signatures of selection). This modification (**Lines 22 – 23**) is quoted below (relevant addition in **bold**):

> "… Using a landscape community genomics framework **to identify environmental drivers of population genomic structure and signatures of selection**, we show that..."

We believe this provides sufficient conceptual information regarding the methodology to better inform potential readers about the contents of the manuscript. However, we believe that further allocating substantial text to identifying and describing specific methodology (e.g., pRDA) is not necessary for the Abstract, given that the journal readership is broad.

• Data filtering is always a complicated process, but it seems like it was done here with care and due consideration for key biases. Two components that I would like to confirm were included, or hear justification for exclusion, are filters for allele balance ratio and a filter to remove one of any pair of close relatives. In particular, inclusion of close relatives could bias allele frequencies, which could bias the signal of selection. The beta.dosage function of the hierfstat package (Weir & Goudet 2017 / Goudet et al. 2018) is easy to check this.

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We did not implement an allele balance filter nor a filter for removal of close relatives. Stacks v2 uses the Bayesian genotyping algorithm from Maruki and Lynch (2017), which aggregates evidence for polymorphism across individuals (i.e., it uses population-level sequence read data to inform its Bayesian genotyping algorithm) and also considers read counts of alleles when genotyping individuals (Rochette et al., 2019). Accurate heterozygous genotype calls can occur even when the two alleles have imbalanced representation in an individual's sequence reads because the presence of multiple alleles at that locus is supported by independent sequence data from other individuals. Spurious alleles resulting from sequencing or PCR error are unlikely to be supported by independent sequencing data from multiple individuals, and this will tend to reduce false heterozygote genotype calls. Thus, we did not employ an allele balance filter.

It remains unclear whether removing closely related individuals from population genetic datasets is appropriate. Waples and Anderson (2017) suggest that removal of close relatives can make problems related to family structure in datasets worse; indeed, they show that removing siblings can reduce precision of allele frequency estimates. Peterman et al. (2016) showed that inclusion of close relatives in a dataset has little effect on measures of genetic diversity and genetic distance (as well as patterns characterized on their basis, including isolation-by-distance) in spatial population genetics analyses. Additionally, closely related individuals occur naturally and will be included in a random sample of a population at a rate inversely proportional to the effective population size (Waples and Anderson, 2017); indeed, the presence of closely related individuals is perhaps only a concern when their frequency is higher or otherwise non-representative of the population ("excessive close relatives" as in Wang et al., 2018). We have no reason to believe that we have sampled non-randomly with respect to relatedness. Furthermore, removing closely related individuals decreases sample size and is generally thought to reduce statistical power, given that close relatives often provide only partially redundant information (Waples and Anderson, 2017). Molecular marker-based kinship estimators can also inaccurately characterize relatedness among individuals, such that high kinship coefficients are sometimes estimated for unrelated individuals. Low-density SNP datasets worsen this problem, as does the presence of population genetic structure and admixture (see Goudet et al., 2018). Given these concerns, removing closely related individuals has become less common (e.g., see Gehri et al., 2021; White et al., 2021; Diaz-Suarez, 2022; Forester et al., 2022; Devloo-Delva et al., 2023), and we decided not to remove individuals based on relatedness. We do, however, agree that this decision should be present in the manuscript to improve research transparency; thus, we have added the following statement (**Lines 373 – 375**) in the Materials and Methods:

> "We did not remove individuals on the basis of relatedness, as there is little documented benefit of doing so and the consequences can include reduced statistical precision and power [68,69]."


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References:

Maruki, T., and Lynch, M. 2017. Genotype calling from population genomic sequencing data. G3: Genes, Genomes, Genetics 7: 1393 – 1404.

Rochette, N.C., Rivera-Colón, A.G., and Catchen, J.M. 2019. Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology 28: 4737 – 4754.

Waples, R.S., and Anderson, E.C. 2017. Purging putative siblings from population genetic datasets: a cautionary view. Molecular Ecology 26(5): 1211 – 1224.

Peterman, W., Brocato, E.R., Semlitsch, R.D., and Eggert, L.S. 2016. Reducing bias in population and landscape genetic inferences: the effects of sampling related individuals and multiple life stages. PeerJ 4: e1813.

Wang, J. 2018. Effects of sampling close relatives on some elementary population genetics analyses. Molecular Ecology Resources 18(1): 41 – 54.

Goudet, J., Kay, T., and Weir, B.S. 2018. How to estimate kinship. Molecular Ecology 27(2): 4121 – 4135.

Gehri, R.R., Gruenthal, K., and Larson, W.A. 2021. It's complicated: heterogeneous patterns of genetic structure in five fish species from a fragmented river suggest multiple processes can drive differentiation. Evolutionary Applications 14(8): 2079 – 2097.

White, S.L., Kazyak, D.C., Darden, T.L., et al. 2021. Establishment of a microsatellite genetic baseline for North American Atlantic sturgeon (Acipenser o. oxyrhinchus) and range-wide analysis of population genetics. Conservation Genetics 22: 97 – 992.

Diaz-Suarez, A., Noreikiene, K., Kisand, V., et al. 2022. Temporally stable small-scale genetic structure of Northern pike (Esox Lucius) in the coastal Baltic Sea. Fisheries Research 254: 106402.

Forester, B.R., Murphy, M., Mellison, C., et al. 2022. Genomics-informed delineation of conservation units in a desert amphibian. Molecular Ecology 31(2): 5249 – 5269.

Devloo-Delva, F., Burridge, C.P., Kyne, P.M., et al. 2023. From rivers to ocean basins: the role of ocean barriers and philopatry in the genetic structuring of a cosmopolitan coastal predator. Ecology and Evolution 13(2): e9837.

• Again on data filtering, what is the authors' justification for using a minor allele frequency of 0.03? This cuts out a large proportion of the loci (58,996 to 5,191). My interpretation of that threshold is that it

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means that about 20 copies of an allele must be present in a dataset of ~345 diploid individuals? I think this could be loosened (e.g. to 15 or maybe even 10 copies), which may substantially increase the number of loci available for testing with the LFMM and RDA methods. See this for consideration (https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13351) – a key point is that "having fewer data points appears to reduce the overall power and effectiveness of GEAs".

We selected a minor allele frequency threshold of 0.03 to balance the potential for improved power (i.e., more completely identifying the set of loci experiencing selection) by including SNPs with relatively rare minor alleles with the potential negative impacts of including extremely rare alleles. In Ahrens et al. (2021; the reference linked by the reviewer), the authors investigate how different sampling and filtering parameters influence statistical precision of GEAs (i.e., the number of true positive [TP] detections divided by the total number of SNPs identified as having significant genetic-environment associations). Figure 3 in Ahrens et al. (2021) makes it apparent that a low MAF threshold (retaining SNPs with MAF > 0.01) tends to reduce statistical precision compared to higher MAF thresholds (e.g., retaining SNPs with MAF > 0.05 (see the trends for salmon-colored points/lines compared to comparable datasets using higher MAF filters). Ahrens et al. (2021) note this trend in the Results, where they state on page 1465 "For the Sim calophylla data, a larger MAF generally resulted in a higher proportion of TPs (higher ratio of TP:AA). For the Sim microcarpa data, a MAF of 0.01 generally had the lowest proportion of TPs (lowest ratio of TP:AA), with the highest proportion of TPs varying between MAF 0.05 and 0.1 depending on the program used and amount of missing data." It is inarguable that having more SNPs can improve statistical power (i.e., the fraction of true positives in the entire genome that are detected as having significant GEAs), but including low-MAF SNPs in a dataset appears to increase the relative abundance of false positives among the set of SNPs identified as having significant GEAs. We believe that although we may have filtered out SNPs that truly have GEAs, the set of SNPs we identified as having significant GEAs may be less affected by false positives than if we had used a lower MAF threshold.

Additionally, the math suggesting that the use of a minor allele frequency threshold of > 0.03 requires 21 copies of an allele to be present at a particular SNP in a dataset of 345 individuals is correct for the case when there is no missing data. In our case, the maximum missing data rate observed at any SNP at the end of filtering was 27.25% (see Materials and Methods **Lines 382 – 383**). This adjusts the math such that a minor allele frequency threshold of > 0.03 ensures that ***all*** SNPs have ≥ 16 minor alleles present (345 individuals * 2 alleles/individual * 0.03 * [1 – 0.2725] = 15.05925 = 16 alleles given the **>** 0.03 threshold). Therefore, the SNPs with the lowest minor allele frequency (~ 0.03) have 16 – 21 minor allele copies present in the dataset depending on the missing data rate, which is similar to the reviewer's recommendation of loosening the requirement to 10 – 15 copies. Thus, we do not believe altering the filtering scheme would greatly benefit the results.

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Reference:

Ahrens, C.W., Jordan, R., Brag, J., et al. 2021. Regarding the F-word: the effects of data filtering on inferred genotype-environment associations. Molecular Ecology Resources 21: 1460 – 1474.

• Pertaining to the IBR results, and particularly the Discussion section around Lines 145 to 148: My interpretation of the IBR results is that where devil densities are high, landscape resistance to quolls is reduced, meaning that quolls move more freely in areas of high devil density. I'm taking this from Line 95, where the authors state that quoll resistance is negatively correlated with devil density. Rather than some function of "reduced competition and less dispersal pressure", I would argue that this finding is simply the result of the species being quite similar in their habitat requirements, and so quolls disperse less through areas that both species find less preferable (e.g. heavily cleared/modified environments). I.e., where there are lots of devils, the habitat is probably just better quality and so quolls can move more readily through the landscape. As the authors state slightly further down, quolls avoid devils temporally, rather than spatially, so to me this may be a better explanation of the results.

We thank the reviewer for their insightful comment. We agree that the negative association between quoll landscape resistance and devil density may partly be explained by shared habitat preferences. However, habitat preferences of quolls and devils are divergent with respect to some qualities. E.g., quolls are considerably more arboreal than devils and prefer habitat that is forested or otherwise lower-visibility than habitat preferred by devils (see Jones and Barmuta, 2000). This preference is also borne out in diet data, which shows quolls consume a greater proportion of arboreal species than devils (Jones and Barmuta, 1998; Jones and Barumuta, 2000). Although the spatial overlap between quolls and devils is considerable (88.38% overlap) in terms of broad-scale home ranges (95% utilization distribution derived from movement-based kernel density estimation [MKDE]), they differ more substantially (40.45% overlap) in terms of the core geographic areas they use within home ranges (50% utilization distribution derived from MKDE; Andersen et al., 2020). The decreased spatial overlap between devils

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and quolls in the core home range relative to the broad home range is consistent with broad geographic overlap but incompletely shared habitat preferences or resource use. The possibility of shared habitat preferences explaining the IBR results and the nuance pertaining to that possibility (as described above) were not originally conveyed in our manuscript, so we have modified the text (**Lines 176 - 182**) as follows (relevant changes in **bold**):

> "… Thus, dispersing quolls that encounter areas of low devil density and therefore low interspecific competition may not continue dispersing elsewhere, leading to low devil densities impeding gene flow and thereby increasing landscape resistance. **Alternatively, telemetry data show that quoll and devil home ranges overlap at broad scales (88.4% overlap)[20], although core home ranges with higher usage intensity overlap less extensively (40.5% overlap)[20] likely because quolls prefer forested or otherwise lower-visibility habitat than devils[19]. High devil densities likely occur where habitat is highly suitable for devils, and this habit is likely highly suitable for quolls as well given broad overlap in the two species' space use. Highly suitable habitat may be relatively permissive to quoll dispersal and consequent gene flow, which may partly explain the negative correlation between quoll landscape resistance and devil density.** Quolls may also avoid areas with high densities of feral cats, which increase in abundance concomitantly with declines of devils,[16] although we have little evidence for quoll—cat interactions."

The references we used our response to this comment (in order of appearance; excluding citations in pasted/quoted text from the manuscript) include:

Jones, M.E., and Barmuta, L.A. 2000. Niche differentiation among sympatric Australian dasyurid carnivores. Journal of Mammalogy 81(2): 434 – 447.

Jones, M.E., and Barmuta, L.A. 1998. Diet overlap and abundance of sympatric dasyurid carnivores: a hypothesis of competition? The Journal of Animal Ecology 67: 410 – 421.

Andersen, G.E., Johnson, C.N., and Jones, M.E. 2020. Space use and temporal partitioning of sympatric Tasmanian devils and spotted-tailed quolls. Austral Ecology 45: 355 – 365.

Minor comments/suggestions

Line 20: "top terrestrial predator" probably more accurate.

We thank the reviewer for their suggestion and have made the recommended change (**Line 20**).

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Line 21: is it multiple cascades, or just a singular cascade? Honestly not sure. Consider that effects that propagate laterally or upward from the main interaction chain should not be thought of as part of the trophic cascade.

Although we removed this sentence describing the Tasmanian trophic cascade from the Abstract, we have revised the manuscript elsewhere (**Line 52**) to refer to the trophic cascade involving the Tasmanian ecological community as a singular cascade, which is consistent with recent work characterizing the community ecological effects of Tasmanian devil declines cited throughout our study (e.g., Cunningham et al., 2021).

Line 44: also state "hereafter, the devil" after the scientific name, as you've done for the spotted-tailed quoll.

We agree that explicitly clarifying our use of the word "devil" would be beneficial, and we have made the recommended change (**Lines 47 – 48**).

Line 57: have you got a citation for "providing sufficient lag time to detect evolutionary effects of trophic cascades."? I agree it's probably correct, but citation also needed.

We agree that this claim should have had a reference in the original version of the manuscript. We have added the reference Landguth et al. 2010 (Quantifying the lag time to detect barriers in landscape genetics; Molecular Ecology 19:19, 4179 – 4191), which uses simulation to show that genetic data can reflect changes to landscape resistance in relatively few generations (~15 generations). We also recognize that this does not *guarantee* that the lag time between the onset of devil declines and our sampling of quolls is sufficient for detecting any population genetic consequence for quolls (e.g., due to idiosyncrasies in the biology of any particular study system). Accordingly, we have also attenuated the sentence to reflect this uncertainty and avoid implying that we have existing in-system data to support this claim. The sentence (**Lines 61 – 63**) now reads as follows:

> "Devil populations typically remain at low densities following DFTD-mediated declines [11], such that the quoll has experienced altered resource availability and behavior for up to 25 generations, **likely** providing sufficient lag time to detect evolutionary effects of mesopredator release**.** [19]"

Reference:

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Landguth, E.L, Cushman, S.A., Schwartz, M.K., McKelvey, K.S., Murphy, M., and Luikart, G. 2010. Quantifying the lag time to detect barriers in landscape genetics. Molecular Ecology 19:19, 4179 – 4191.

Line 59: does potential mesopredator release of the eastern quoll factor into this talk of interspecific competition at all? I assume spotted-tailed quolls would probably outcompete eastern quolls, but could be worth including some wording here about other competitors.

We appreciate the thoughtful comment from the reviewer regarding the ecology of the Tasmanian mammalian community. Although the eastern quoll partially overlaps in diet with the spotted-tailed quoll, they show greater habitat separation than the devil and spotted-tailed quoll (Jones and Barmuta, 2000), so the intensity of competition may be reduced between eastern and spotted-tailed quolls compared to spotted-tailed quolls and devils. Furthermore, the eastern quoll has become relatively rare (indeed, the eastern quoll is classified as endangered by the IUCN) likely due to climatic events several decades ago (Fancourt et al., 2015), and there is no evidence of behavioral or demographic release of the eastern quoll in association with devil declines. In contrast to the observed association between behavioral variation and diet in spotted-tailed quolls and devil densities (Cunningham et al., 2018, 2019), there is no data concerning behavioral variation of spotted-tailed quolls in association with variation in eastern quoll densities. Indeed, comparisons of ecological characteristics of the spotted-tailed quoll and Tasmanian devil (as well as ecological changes in association with devil declines) have been studied more thoroughly than the eastern quoll (e.g., recent studies compare diet, space use, and temporal niche of the spotted-tailed quoll and devil but omit the eastern quoll, perhaps due to the rarity of the eastern quoll; Andersen et al., 2017; Andersen et al., 2020; Cunningham et al., 2018; Cunningham et al., 2019). Thus, we have little *a priori* information concerning whether and how eastern and spotted-tailed quolls compete currently, and we have accordingly omitted details regarding the eastern quoll.

References (in order of appearance) used in our response to this comment:

Jones, M.E., and Barmuta, L.A. 2000. Niche differentiation among sympatric Australian dasyurid carnivores. Journal of Mammalogy 81(2): 434 – 447.

Fancourt, B.A, Hawkins, C.E., Cameron, E.Z., Jones, M.E., and Nicol, S.C. 2015. Devil declines and catastrophic cascades: is mesopredator release of feral cats inhibiting recovery of the eastern quoll? PLoS ONE 10(3): e0119303.

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Cunningham, C.X, Johnson, C.N., Barmuta, L.A., Hollings, T., Woehler, E.J., and Jones, M.E. 2018. Top carnivore decline has cascading effects on scavengers and carrion persistence. Proceedings of the Royal Society B 285: 20181582.

Cunningham, C.X., Scoleri, V., Johnson, C.N., Barmuta, L.A., and Jones, M.E. 2019. Temporal partitioning of activity: rising and falling top-predator abundance triggers community-wide shifts in diel activity. Ecography 42: 2157 – 2168.

Andersen, G.E., Johnson, C.N., Barmuta, L.A., and Jones, M.E. 2017. Dietary partitioning of Australia's two marsupial hypercarnivores, the Tasmanian devil and the spotted-tailed quoll, across their shared distributional range. PLoS ONE 12(11): e0188529.

Andersen, G.E., Johnson, C.N., and Jones, M.E. 2020. Space use and temporal partitioning of sympatric Tasmanian devils and spotted-tailed quolls. Austral Ecology 45: 355 – 365.

Line 67: is it possible to explain a little more clearly what the differences between IBR and IBE are here? I imagine something like IBR being driven by obstacles that hinder movement and gene flow whereas IBE being driven by divergent selection pressures under different environmental conditions.

We thank the reviewer for their suggestion and agree that it can be challenging to clearly distinguish IBR and IBE. We have updated the text (**Lines 74 – 79**) to more clearly differentiate the genetic patterns the two methodological frameworks aim to characterize:

> "We used three general approaches to address these goals: 1) tests for a population genomic pattern of isolation-by-resistance (IBR) [20], 2) tests for a population genomic pattern of isolation-by-environment (IBE) [21], and 3) landscape genomic tests for selection at individual SNPs based on genetic-environment associations (GEAs) [22]. **Tests for IBR characterize how environmental conditions across the landscape between locations affect population genomic structure by impeding or facilitating gene flow (e.g., certain environmental conditions may act as obstacles to dispersal between locations),[20] whereas tests for IBE characterize how at-location environmental conditions influence population genomic structure as a consequence of selection against migrants from locations with different environmental conditions or as a result of environmentally biased dispersal.[21]**"

Line 75: could 'population genomic structure' be used instead of 'genome-wide genetic structure'? It seems like the authors have stuck to using the word 'genetic' throughout the manuscript, whereas

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‘genomic’ or ‘population genomic’ would probably make interpretation easier in many instances, e.g. ‘landscape genomic’ instead of ‘landscape genetic’.

We agree that “population genomic structure” is a suitable alternative to “genome-wide genetic structure,” and we agree that unifying the terminology throughout the manuscript would aid interpretation. Thus, we now use “population genomic structure” throughout the manuscript to replace “genetic structure,” “genome-wide genetic structure,” and “genome-wide genetic differentiation.”

However, the terms “landscape genomic” and “landscape genetic” are used variably throughout our manuscript because they reflect methodology unique to different sub-fields of population genomics; i.e., landscape genomics focuses on the characterization of loci related to environmental adaptation, whereas landscape genetics focuses on understanding the effects of the environment on gene flow (as inferred using estimates of population genomic differentiation), respectively. We agree that the similarity in the terms “landscape genomic” and “landscape genetic” is unfortunate, but we believe they improve clarity of the text for experts in the two sub-fields with relatively little propensity for causing confusion to non-experts. Thus, we have elected to retain variable use of the terms “landscape genetic” and “landscape genomic” throughout the manuscript.

Line 92: Not sure this is a solid statement, given that there doesn’t appear to be a justification for the top ten models being useful for inference. See comment regarding Line 378.

We thank the reviewer for pointing out a lack of clarity regarding our ResistanceGA analyses, and we agree additional detail was necessary in this paragraph. Specifically, we now indicate that the model containing landcover classes and temperature diurnal range has the best (lowest) AICc of all models, and that none of the other models have $\Delta AICc < 2$ relative to this model.

The visualization of performance statistics for only a fraction of the models in Figure 2 was a matter of practicality, as plotting all 100 models results in a very complex figure (although note that we did plot performance statistics for all 100 models in Extended Data Figure S4); we believe that this is still justified, as the vast majority of the 100 models perform poorly while a subset with low AICc values demonstrate the variability in marginal R-squared metrics among models, which may be of interest to some readers. We agree that referring to the ten plotted models as the “top 10 models” could misleadingly imply that these models perform similarly in terms of AICc, so we remove the use of the term “top model(s)” in this paragraph in favor of more explicitly describing performance statistics. The legend for Figure 2 now notes that the ResistanceGA models are a subset of the models, in addition to a sentence we had in the original manuscript indicating that a plot for all 100 models can be found in Extended Data Figure S4.

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We note here that ResistanceGA also outputs the fraction of bootstrap replicates in which a given model has the lowest AICc, which may be a useful statistic for understanding the contribution of different environmental factors to IBR (see Winiarski et al., 2020). We did not previously report this statistic in favor of reporting only *mean* AICc and marginal R-squared values across bootstraps. As noted in Winiarski et al. (2020), the best practices for model selection in landscape genetics are still under development, so we believe this statistic is also worth reporting in the results. Accordingly, the Results paragraph concerning testing for isolation by resistance using ResistanceGA (**Lines 106 – 112**) now reads as follows (additions **in bold** and removed text crossed out):

> "… Resulting models indicated the greatest support (i.e., lowest mean AICc across 10,000 bootstraps with 70/30% training/test data split) for a model containing landcover classes and temperature diurnal range~~, and eight of the top 10 models included at least one of the four temporally lagged devil density variables~~ (Fig. 2C-E**); this model had the best (i.e., lowest) AICc in 90.93% of the bootstrap replicates and explained substantial variance in genetic distances among quolls (i.e., marginal R-squared = 64.36%). All other models had a mean ΔAICc > 2 relative to this model. A model containing temperature diurnal range and devil density lagged by 15 generations had worse mean AICc support but had the best AICc in 6.34% of bootstrap replicates; this model explained similar variance in genetic distances among quolls (marginal R-squared = 66.61%) compared to the model with the lowest mean AICc**."

We also note here that we added three new panels to Extended Data Figure S4 (panels D – F) with IBR models ordered by decreasing marginal R-squared to facilitate understanding of this model statistic.

References:

Winiarski, K.J., Peterman, W.E., and McGarigal, K. 2020. Evaluation of the R package 'ResistanceGA': A promising approach towards the accurate optimization of landscape resistance surfaces. Molecular Ecology Resources 20: 1583 – 1596.

Line 118: Note that authors "should explicitly report the total number of significant associations, the number of unique SNPs that have associations, the total number of associations per SNP, as well as which SNPs have significant associations with which environmental factor" (https://onlinelibrary.wiley.com/doi/full/10.1111/mec.14549). Please ensure all of these are presented (either in this paragraph or in the Extended Data/Supplemental Table).

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We thank the reviewer for their comment. In the main text, we reported the number of unique SNPs that have significant genetic-environment associations (GEAs) in **Line 136**. Information regarding the number of SNPs detected in relation to each environmental factor is provided in Extended Data Table S3. Information regarding the total number of associations per SNP as well as the corresponding environmental factors are included in Supplementary Table 1.

Line 139: "the top models" should probably be reworded to "some top models".

We agree and have changed the text as recommended (**Line 159**).

Line 174: This is a good point. I suggest, to broaden the Discussion beyond Tasmania, adding a sentence that highlights some examples of studies where invasive pests and diseases are known to have direct molecular impacts on native species (e.g. https://onlinelibrary.wiley.com/doi/10.1111/eva.12226; https://onlinelibrary.wiley.com/doi/full/10.1111/mec.16680; https://doi.org/10.1016/j.biocon.2011.04.025).

We thank the reviewer for their suggestion, and we agree that broadening the Discussion will help situate our work in the context of the wider literature concerning evolutionary impacts of biotic factors. We have adjusted the Discussion paragraph containing Line 174 to include the following text (**Lines 223 – 230**; additions in **bold**):

> "Notably, our results represent some of the first evidence for selection driven by indirect ecological interaction between a pathogen and a non-host taxon, a phenomenon that is expected to be widespread but difficult to study [6]. **In addition to increasing evidence for selection resulting from direct interactions between species (e.g., pathogen-imposed selection on hosts[46]; selection on predators associated with lethally toxic, novel pests [47]), we suggest that indirect ecological interactions may also broadly shape adaptive genomic variation in many species**."

This paragraph now uses one of the references provided by the reviewer (Von Takach et al., 2022; full citation below). Given that the paragraph in question focuses on signatures of selection we detected in our analyses, we believe this publication (which provides evidence for signatures of selection in the northern quoll in association with the spread of the invasive, lethally toxic cane toad) is most suitable, although the other suggested publications are interesting in their own right.

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Relatedly, Von Takach et al. (2022) is another example of a landscape community genomics study, which we missed when we originally wrote our manuscript. We now refer to this publication in our Discussion section concerning landscape community genomics, which reads as follows (**Lines 250 – 253**):

> "Notable insights from such an approach that would otherwise go undescribed include, for example, that spatiotemporal variation in the biotic factor of DFTD is more important than abiotic factors in shaping patterns of adaptive genomic variation in devils ~~themselves~~[46] **and signatures of selection in northern quolls associated with lethally toxic cane toads spreading across the Australian mainland**.[47] …"

References:

Von Takach, B., Ranjard, L., Burridge, C.P., et al. 2022. Population genomics of a predatory mammal reveals patterns of decline and impacts of exposure to toxic toads. Molecular Ecology 31: 5468 – 5486.

Line 243: The caption says 'ranked by', does this mean the top model (lowest AICc) is number 1 on the figure? Please be very explicit here. Could even be worth adding the AICc value instead of 1 to 10 underneath panel E.

We thank the reviewer for their helpful feedback. The X-axis is shared across panels C-E in Figure 2, such that Model 1 corresponds to the leftmost AICc value in panel C, the leftmost Marginal R-squared value in panel D, and the leftmost column in panel E. Thus, the relative AICc values of Models 1-10 were shown in the original version of this figure. To improve clarity of interpretation, we have modified the legend for Figure 2 to read as follows (**Lines 301 – 312**; with relevant changes in **bold**):

> "Figure 2. Population genomic structure of the spotted-tail quoll across Tasmania... C-E) **A subset of** linear mixed effects models with maximum likelihood population effects evaluating the contribution of abiotic and biotic variables to isolation by resistance, a spatial genomic pattern generated by environmental conditions between locations contributing to spatially variable resistance to gene flow across the landscape. **Panels C-E share the X-axis specified under panel E, such that Model 1, which includes geographic distance, landcover, and TDR, is characterized by the leftmost AICc value and marginal R-squared value in panels C and D. Models are ordered left-to-right by increasing (worsening) average AICc based on 10,000 bootstrap replicates; i.e., Model 1 has the lowest AICc and is thus the best-performing model** C) **Mean AICc of the ten models with the lowest AICc** along with the null model of geographic distance. D) Marginal R-squared of the top-ranking models…"

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Line 257: Was the spatial spread of sampling random across the 15-year time period, or were later samples collected in different areas to earlier sampling?

There was little geographic bias in the timing of sampling. Neither latitude nor longitude explain substantial variation in collection years in a linear model (Adjusted R-squared = 0.021; F = 4.697, p < 0.01), indicating that locations across Tasmania were often sampled repeatedly across the 15-year time period. This is now indicated in the text in **Lines 326 – 330**:

> "Samples span 15 generations (2004–2019; quolls have a generation time of approximately one year) and are geographically widespread**; broad geographic areas were generally repeatedly sampled over time, resulting in little geographic bias in the timing of sampling (linear model of collection year regressed against latitude and longitude; adjusted R-squared = 0.021, F = 0.4697, p < 0.01).**"

Line 259: "conduct" should be "conducted".

We thank the reviewer for identifying the grammatical mistake, and we have corrected the issue (**Line 333**).

Line 270: A northern quoll reference genome has been available for a while now (https://www.dnazoo.org/assemblies/Dasyurus_hallucatus) – would this be more or less appropriate to align spotted-tailed quoll reads to than the Tasmanian devil genome, and why? Presumably something to do with annotation?

The Tasmanian devil and spotted-tailed quoll belong to sister genera within the family Dasyuridae (noted on **Line 348** in our manuscript; Westerman et al., 2016), suggesting that aligning sequence reads to the devil genome is unlikely to be problematic. To thoroughly address this comment, we aligned the sequence reads of a random subset of 50 individual spotted-tailed quolls to the northern quoll genome for comparison with the same individuals' data when aligned to the devil genome. Per-sample, the number of mapped reads with MAPQ > 10 increased only marginally when using the northern quoll genome compared to the devil genome (mean change = +0.60%; standard deviation = 0.78%). Additionally, one of our motivations for using the current devil genome (MSarHar1.11; Stammnitz et al., 2023) is that it has substantially higher contiguity (devil genome contig N50: 63.34 Mb; quoll genome contig N50: 91kb). Indeed, the current devil genome is composed of 445 contigs and 7 scaffolds corresponding to chromosomes; in contrast, the northern quoll genome is composed of 479,471 contigs

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in 418,623 scaffolds. Thus, the devil genome likely gives us higher-quality positional information for our SNPs and nearby genomic features such as genes.

This information is now found in **Lines 348 – 356,** which read as follows (changes in **bold**):

> "The Tasmanian devil and quoll belong to sister genera within the family Dasyuridae, suggesting that directly aligning sequence reads to the devil reference genome is a viable strategy for inferring RAD loci [66]. Supporting this expectation, processed sequence reads for each sample mapped robustly to the devil genome (mean proportion of reads aligning with MAPQ10 = 89.3%; SD=2.5%); **using the northern quoll (*Dasyurus hallucatus*) genome only marginally increased the per-sample number of reads with MAPQ10 (mean change + 0.60%; SD=0.78%). Furthermore, the devil reference genome has substantially higher contiguity (contig N50 = 63.34Mb; 445 contigs; 7 scaffolds) than the northern quoll genome (contig N50 = 91kb; 479,471 contigs; 418,623 scaffolds), and thus the devil genome likely provides higher quality positional information.**"

Reference:

Westerman, M., Krajewski, C., Kear, B.P., et al. 2016. Phylogenetic relationships of dasyuromorphian marsupials revisited. Zool. J. Linn. Soc. 176: 686–701.

Stammnitz, M.R., Gori, K., and Kwon, Y.M., et al. 2023. The evolution of two transmissible cancers in Tasmanian devils. Science 380 (6642): 283-293.

Line 268: total number of sequenced reads should be presented (either in Extended Data or here). The percentage of reads successfully assigned to a sample after demultiplexing should also be stated.

In the Materials and Methods, we have added the total number of raw sequence reads generated, as well as the number (and percentage) that were retained following demultiplexing. This information is now found on **Lines 340 – 344**, which read as follows:

> "… **Sequencing generated a total of 6,122,805,452 raw reads.** We trimmed the first two bp from raw forward and reverse sequence reads using Cutadapt [55]. Next, we demultiplexed and cleaned the sequence data using the *process_radtags* module of Stacks v2.52 [56], with the r, c, q, and bestrad options specified. **5,046,173,116 reads (82.42% of the total raw reads) were retained following demultiplexing and cleaning**…"

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Line 290: 50% missing data in one or more individuals is quite high, can the authors remove a small number of individuals with the most missing data to reduce that to say 30%? Or add a histogram of missing data by sample to the extended data section, to show the actual spread of missing data across all samples?

Retaining individuals with ≤ 50% missing genotypes is common among recent population genomics studies of wildlife (e.g., Kozakiewicz et al., 2019; LaCava et al., 2021; Beer et al., 2022), so we do not believe that further filtering and subsequent re-analysis is necessary. To further support this position, we review the missing data statistics presented in the Materials and Methods below.

In **Lines 380 – 381** we note that individual missing data ranged from 2.36 – 49.99%, meaning all individuals were at a minimum genotyped at ~1,715 SNPs. Several of our analyses relying on pairwise individual genetic distances did not require imputation, including our test for isolation-by-resistance using ResistanceGA, as well as our implementation of generalized dissimilarity modeling to test for isolation-by-environment. Thus, those analyses used genetic distances based only on the non-missing SNPs shared between the two individuals in a pair; in response to this comment, we calculated the minimum number of SNPs any individual pairwise genetic distance was calculated from and found that value to be 926 SNPs. Thus, we expect genetic distances estimated between individuals with even the highest missing genotype rates to be high quality.

The previous paragraph illustrated the basis for estimating genetic distances in the worst case. The *average* individual missing genotype rate was 13.24% (standard deviation 13.29%; noted in **Line 380**), and only 52 individuals out of 345 (~15%) had missing genotype rates that exceeded 30%. Thus, typical per-individual missing data is relatively low, and individuals with relatively high missing genotype rates are relatively uncommon in our dataset. Although we agree that understanding the distribution of missing data may be useful for readers, we believe that reallocating a figure (we have already reached the figure limit for both the main text and extended data) to visualize the distribution of missing data is not necessary. However, we acknowledge that the range, mean, and standard deviation of individual and SNP missing data rates may be insufficient for understanding their distributions. Accordingly, we have now added the 50th and 90th percentile of individual and SNP missing data rates to the Materials and Methods in **Lines 380 – 384**, which read as follows:

> "Individual-level missing data was generally low (mean = 13.24%; SD = 13.29%; range = 2.36 – 49.99%) **and positively skewed such that relatively few individuals had missing data rates near 50% (50th percentile = 6.56%; 90th percentile = 35.95%).** SNP-level missing data were also

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generally low (mean = 13.24%; SD = 4.92%; range = 1.45- 27.25%) **but more symmetrically distributed around the mean (50th percentile = 13.04%; 90th percentile = 20.00%)**."

Additionally, in **Lines 382 - 383** we note that per-SNP missing data did not exceed 27.25%. Together, the SNP- and individual-level missing data rates combine such that 13.24% of the genotype matrix (dimensions = number of individuals * number of SNPs) contained missing data. Thus, the majority of the genotype matrix was not imputed for analyses requiring no missing data, and we believe the overall level of missing data is unlikely to have greatly influenced our results. The total percentage of missing data in the genotype matrix is now reported in **Lines 384 – 385**, which reads as follows:

"**In terms of the genotype matrix (with dimensions equaling 345 individuals by 3,431 SNPs), 13.24% of genotypes were missing."**

References used for our response to this comment:

Kozakiewicz, C.P. Burridge, C.P., Funk, W.C, et al. 2019. Urbanization reduces genetic connectivity in bobcats (Lynx rufus) at both intra- and interpopulation spatial scales. Molecular Ecology 28(23): 5068 – 5085.

LaCava, M.E.F, Gagne, R.B., Gustafson, K.D., et al. 2021. Functional connectivity in a continuously distributed, migratory species as revealed by landscape genomics. Ecography 44(7) 987 – 999.

Beer, M.A., Kane, R.A., Micheletti, S.J., Kozakiewicz, C.P., and Storfer, A. 2022. Landscape genomics of the streamside salamander: Implications for species management in the face of environmental change. Evolutionary Applications 15(2): 220 – 236.

Line 378: The top 10 models are presented in Figure 2, correct? So were these top 10 models those with delta AIC < 2? Please clarify how these 10 were picked. Should be re-stated on Line 91 of the Results section too.

We thank the reviewer for identifying the lack of clarity concerning model selection pertaining to isolation-by-resistance. As noted in Winiarski et al. (2020), the best practices for model selection in landscape genetics are still evolving, so we consider multiple types of evidence for different models. As noted earlier, in addition to evaluating the mean AICc of a model across bootstraps, ResistanceGA also outputs the fraction of bootstrap replicates in which a given model has the lowest AICc. Winiarksi et al. (2020) suggest that this may be a useful statistic (in addition to mean AICc) for understanding the contribution of different environmental factors to IBR; this statistic has been used in some recent

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landscape genetics literature (e.g., Kozakiewicz et al. 2020). We have updated the Materials and Methods (**Lines 472 – 476**) to read as follows (additions **in bold**):

> "… We used 10,000 bootstrap iterations, retaining 70% of the data for training in each iteration. The model with the lowest average AICc across bootstraps was taken as the top model; **any models within ΔAICc < 2 of the top model were considered as having similar statistical performance. We also used the percentage of bootstrap replicates for which a model had the lowest AICc to consider evidence for alternative models that do not necessarily have the lowest mean AICc [42].**"

As we noted in our response to a previous comment, the visualization of performance statistics for only a fraction of the models in Figure 2 in the Results section was a matter of practicality, as plotting all 100 models results in a very complex figure (although note that we did plot performance statistics for all 100 models in Extended Data Figure S4). Readers may also vary in their preference for model selection statistic (e.g., AICc vs R-squared), so we believe it is useful to display multiple models with varying marginal R-squared even though one model has the lowest mean AICc. To improve clarity of the results, we now more thoroughly explain the relative support of different models of isolation-by-resistance in **Lines 103 – 115**, which read as follows (additions in **bold**):

> "Resulting models indicated the greatest support (i.e., lowest mean AICc across 10,000 bootstraps with 70/30% training/test data split) for a model containing landcover classes and temperature diurnal range (**statistical performance of a subset of 10 models with the lowest mean AICc are plotted in Fig. 2C-E; ; the total set of 100 models is plotted in Extended Data Fig. S4); this model had the best (i.e., lowest) AICc in 90.93% of the bootstrap replicates and explained substantial variance in genetic distances among quolls (i.e., marginal R-squared = 64.36%). All other models had a mean ΔAICc > 2 relative to this model. A model containing temperature diurnal range and devil density lagged by 15 generations had worse mean AICc support but had the best AICc in 6.34% of bootstrap replicates; this model explained similar variance in genetic distances among quolls (marginal R-squared = 66.61%) compared to the model with the lowest mean AICc.** Notably, a model including devil density lagged by 10 quoll generations and mean annual temperature had the highest mean marginal R-squared (67.7%), although it did not have the lowest mean AICc. In models containing devil density, devil density was negatively correlated with landscape resistance for the quoll (Extended Data Fig. S4)."

References:

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Winiarski, K.J., Peterman, W.E., and McGarigal, K. 2020. Evaluation of the R package ResistanceGA: A promising approach towards the accurate optimization of landscape resistance surfaces. Molecular Ecology Resources 20(6): 1583 – 1596.

Kozakiewicz, C.P., Lauren, R., Patton, A.H., et al. 2020. Comparative landscape genetics reveals differential effects of environment on host and pathogen genetic structure in Tasmanian devils. Molecular Ecology 29(17): 3217 – 3233.

Line 392: Can the authors confirm that these pRDA and GDM analyses do not require no missing data? I'm not very familiar with these analyses.

We thank the reviewer for identifying the lack of clarity regarding data imputation in the Materials and Methods sections concerning isolation-by-resistance and isolation-by-environment. Throughout these sections, we now note explicitly whether an analysis used imputed data, including the following locations (changes in **bold**):

> Lines 449 – 452: "… To characterize pairwise individual genetic distances, we used the *propShared* function in adegenet to calculate the proportion of shared alleles between individuals and subsequently calculated the pairwise individual genetic distance D_{PS}.[73] **D_{PS} was calculated without imputing missing data**."
>
> Lines 493 – 498: "… Prior to carrying out pRDA, we carried out spatial PCA (sPCA) using the *spca* function in the R package adegenet [64]. We used a distanced-based connection network with the maximum distance specified as 50% of the maximum observed distance between samples to account for the expectation that quolls are unlikely to disperse across the entire study area while still ensuring robust connectivity among nodes in the network. **Missing data were imputed by the *spca* function using mean-value imputation**…"
>
> Lines 515 – 518: "… GDM fits regression models to test for associations between pairwise genetic distances (here, D_{PS}) and environmental distances, while accommodating nonlinear relationships using spline functions. [30] **As described previously, we calculated D_{PS} without imputing missing data.**"

Line 320: As the authors are discussing two species here, it would be clearer to state that generations diseased is the collection year of a given quoll sample. Readers don't know if devil generations are the same as quoll generations.

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We thank the reviewer for pointing out the ambiguity in this paragraph regarding whether "generations diseased" refers to quoll or devil generations. Accordingly, we have altered the methods to read "quoll generations" when this variable is introduced. These changes are found in **Lines 410 – 411**, which now read as follows (changes in **bold**)"

> "We additionally collected data for two biotic variables: devil density and the number of **quoll** generations DFTD has existed at a given location, a variable we refer to as "generations diseased."

We also updated the Introduction section to more explicitly define the generations diseased variable in the main text, as not all readers will pursue the technical details of our study in the Materials and Methods. We do so in **Lines 63 – 65**, which read as follows:

> "We hypothesize that declines in devil density and **the duration of DFTD presence (in quoll generations; hereafter simply "generations diseased")** will alter quoll population genomic structure…"

Note that generations diseased is not the collection year of a given quoll sample. Rather, generations diseased is calculated as the collection year of the quoll sample minus the year of DFTD arrival, with negative values converted to zeroes. This procedure was described in the original manuscript and is documented in **Lines 413 – 415**.

Line 325: Following on from the previous comment, I think it would be clearer to change "devil density and generations diseased have independent effects" to "devil density and time since DFTD arrival have independent effects".

We agree with the reviewer and have made the following change, which parallels the text we added to the Introduction in our response to the previous comment (**Lines 419 – 421**):

> "Indeed, devil density and **duration of DFTD presence (in the present study, "generations diseased")** have independent significant effects on the Tasmanian mammalian community."

Line 457: Can the authors specify the number of latent factors (presumably 3) used here?

We indeed used K = 3 latent factors in LFMM. We have updated the text (**Lines 559 – 560)** to read as follows:

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> "LFMM uses several latent factors (equal to the K genetic clusters represented in the data**; herein, K = 3 latent factors**)…"

Line 459: Wouldn't the authors have used the 'lfmm2.test' function here, which produces p-values? And was there a false discovery rate used for this lfmm? Maybe just need to explain the q values a little more.

We agree that greater clarity regarding the genetic-environment association tests would improve the manuscript. We used lfmm_test() to produce a p-value for each SNP. Then, we used the function qvalue in the R package qvalue to estimate q-values from the p-values. Then, SNPs with q-values < 0.01 were considered as showing significant GEAs. We added the above details to the manuscript **in Lines 559 – 564**, which now read as follows:

> "We implemented LFMM 2 using the R package lfmm. LFMM uses several latent factors (equal to the K genetic clusters represented in the data; herein, K = 3 latent factors) to account for population genomic structure when testing for associations between alleles and environmental factors. **We used the function *lfmm_test* to output a p-value for each SNP; we used the function *qvalue* in the R package qvalue to estimate q-values based on p-values. SNPs with q-values < 0.01 were identified as being significantly associated with environmental variation."**

Figures and Tables

Line 215: Better to write out the full pRDA title than use the acronym.

We have revised the titles of both Table 1 and Table 2 to include the full names of the associated analyses. The text has changed as follows:

> Line 272: "Table 1. **Partial redundancy analysis** (pRDA) variable importance for population genomic isolation by environment."

We also note here that in relation to our analysis of isolation-by-environment (IBE) using partial redundancy analysis, in the Methods section, we mistakenly reported the maximum variance inflation factor (VIF) of any retained variable as 9.82. The corrected value is that all retained variables had a VIF < 10 except for longitude (VIF = 11.46). We opted to retain longitude because it represents geography, which serves as a null model for identifying IBE. Longitude was included in the conditioning matrix of our pRDA model, and thus the effects of longitude were removed prior to estimating environmental effects. We thus expect that our estimates of environmental effects are conservative relative to estimates we

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would have obtained had longitude been removed on the basis of its VIF. This correction is present in **Lines 505 – 510**, which read as follows (additions in **bold**):

> "We defined an initial model using all environmental factors and calculated variance inflation factors (VIFs) to evaluate multicollinearity; specifically, we calculated VIFs, removed the factor with the maximum VIF, and recalculated VIFs until all retained variables had VIF < 10. Ultimately, all retained variables had a VIF < 10 **except for longitude (VIF = 11.46), and we opted to retain this variable because it represents geography and has its effects removed prior to estimating environmental effects.**"

Line 217: As above. Also worth explaining what 'generations diseased' means, i.e. time since arrival of disease in Tasmanian devils.

We have modified the title of Table 2 to include the full name of the associated analysis. **Line 277**: "Table 2. **Generalized dissimilarity modeling** (GDM) variable importance for population genomic isolation by environment."

We have also added a table legend to Table 1 (**Line 274**) that explains the "generations diseased" variable, which reads **"† Generations diseased refers to the number of quoll generations that DFTD has affected devils at a given time point and location."**

Line 240: better to say "spotted-tailed quoll individuals", as I was initially unsure whether the 189 individuals were quolls or devils.

We agree that exceptional clarity is required in a study involving multiple species. We have updated the figure legend in question (**Lines 301 – 304**) to read as follows:

> "Figure 2. Population genetic structure of the spotted-tail quoll across Tasmania. A) Map of FastStructure ancestry proportions for all **345 individual quolls** based on inference of three genetic clusters (i.e., K = 3). B) Individual FastStructure ancestry proportions for the **189 individual quolls** used in ResistanceGA plotted over devil density lagged by 10 generations."

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Line 676: Table caption needs more detail, e.g. which species is being analysed. Also in the table itself, some clarity about thresholds could be improved, e.g. does an individual missingness value of 0.95 mean individuals with less than 95% missing data were removed, and what is "Spatiotemporal SNP missingness"?

We have updated the title (**Line 802**) of Extended Data Table S1 to state the species being analyzed, as follows:

> "Extended Data Table S1. Iterative filtering of **spotted-tailed quoll** genomic data." We have also updated the header of the fourth column to reiterate this information, as follows: "Individual **quolls** remaining."

We have also added a table legend beneath the filtering procedure table to clarify what "individual missingness" and "spatiotemporal SNP missingness" mean. The table legend reads as follows:

> **"† Individual missingness refers to the removal of individuals that have a missing genotype rate higher than the specified value.**
>
> **‡ Spatiotemporal SNP missingness refers to the removal of a SNP when its missing data rate exceeds the specified value in at least one of nine spatiotemporal groups of samples (see Materials and Methods)."**

We also note that "spatiotemporal SNP missingness" was not explained in detail in the Materials and Methods of our original manuscript. Accordingly, we have more thoroughly explained the procedure in **Lines 362 – 373**, which read as follows:

> "We note that although studies often remove SNPs that have high missing data in the global dataset, we sought to prevent spatiotemporal bias in missing data. Accordingly, we removed SNPs that had high missing data in at least one of nine spatiotemporal groups of samples. **These nine spatiotemporal groups of samples were determined by first distributing samples into three spatial groups based on Universal Transverse Mercator Zone (UTM) Zone 55S Eastings; samples were assigned to spatial groups by dividing the range of observed Eastings into three bins of equal width (i.e., samples between 299,982m and 403,135m, samples between 403,135m and 506,288m, samples between 506,288m and 609,441m). Next, samples within each of these three spatial groups were distributed into three temporal groups (leading to a total of nine spatiotemporal groups) by dividing the range of observed collection years into three bins of equal width (i.e., samples from 2004 – 2009, 2009 – 2014, and 2014 - 2019).** Samples lacking GPS coordinates were removed prior to iterative filtering."

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Extended Data Figure S3: Can the authors improve resolution and clip the predicted surfaces to the outline of Tasmania? Currently looks like the migration surfaces extends into the ocean.

We have improved the resolution of all figures in our submission. EEMS generates a triangular lattice on which individuals/demes are placed, and the exact configuration of this lattice depends on the boundaries of the geographic area input to EEMS. Buffering the outline of Tasmania was necessary to ensure that the lattice did not miss large areas of land area (e.g., if we had used the exact outline of Tasmania as the boundaries of the geographic area input to EEMS, coastal land may not have been represented by vertices in the lattice). The publication associated with EEMS (Petkova et al., 2016) similarly buffered geographic regions of interest in their case study. Nevertheless, we created a new boundary that more closely tracks the coast of Tasmania while still providing a slight buffer. This principally affected peninsular areas, but inference of effective migration patterns remains largely unchanged (indicating that the migration patterns are largely insensitive to the boundary used to generate the lattice). In addition, we subsequently clipped the migration surfaces to the outline of Tasmania, as requested by the reviewer. Effective migration surfaces for 250-, 500-, and 1000-deme lattices for our re-run of EEMS are presented in Extended Data Figure S3, although we now use a 2x2 panel figure rather than the original 3x1 to make the panels easier for readers to see.

Reference:

Petkova, D., Novembre, J., and Stephens, M. 2016. Visualizing spatial population structure with estimated effective migration surfaces. Nature Genetics 48(1): 94 – 100.

Data and materials availability: I recommend that the authors publish all relevant metadata and bioinformatics scripts and R code on Dryad/Zenodo or something similar. Sample locations can be removed or desensitised if necessary. This would be more in line with the journal policy.

We have published our metadata and analysis scripts in a GitHub repository (https://github.com/marcabeer/stquoll_landscape_genomics).

This is now noted in the Code Availability statement (**Lines 788 - 790**).

Reviewer #2 (Remarks to the Author):

Dear Authors,

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I have been assessing the previous submission, which had been submitted to Science and I am surprised to see only some minor changes to the manuscript when comparing the two different versions. Without any proper response letter to reviewers it is difficult to address which changes have been done and how and if all comments have been addressed sufficiently. I personally feel that is a waste of time and energy of reviewers and should be avoided (obviously the manuscript has been immediately resubmitted given the time line it has been landing on my desk).

I will thus not do another thorough review but like to point out one point I got already confused in the first submission and this is the use of the term “trophic cascade” as introduced by Ripple et al 2016. The paper itself describes cascade as species interactions that originate with predators spreading top down through foodwebs. Authors write in line 34 that changes in top predator densities lead to trophic cascades – but isn´t it a change in the cascade and not a creation of cascade itself? Please correct me if I am wrong here, but I think this needs some clarification at this place and elsewhere as the terminology is not clearly used.

Saying that, I like the landscape community approach and I do think this is a cool and interesting study system and I think the paper is a sound scientific study with interesting results which should be published.

We thank the reviewer for their time spent reviewing our manuscript and providing valuable feedback. We appreciate the positive comment regarding the novelty of the study. The reviewer’s feedback concerning the use of the term “trophic cascade” is addressed below, as are their other comments.

REVIEWER 2 REPORT FROM SCIENCE, PROVIDED BY REVIEWER

Authors here study mesopredator food web dynamics using a landscape community genomic approach in the spotted-tailed quolls and Tasmanian devils. They make use of a long term dataset on the decline oft he Tasmaian devlis following DFTD and its ecological effects on dispersal for the quolls. While landscape genomic studies have somehow become lesss popular in recent years I think this study nicely demonstrate the potential of such an approach, which could also be used by authors. I do think this study thus is worth to be published.

We thank the reviewer for their kind comment.

I do have some problems with the use of the term „trophic cascade“ by authors. If we use the definition by Ripple 2016 it is described as species interactions that originate with predators spreading top down

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through foodwebs. Authors describe the effects of on the spotted quoll when tasmanian devil numbers have been reduced shifting the interactions in the foodweb. As currently phrased this means that only now we do have a trophic cascade but not before. My understanding would be that this is rather a change in the current cascade and not creating a novel one?

We thank the reviewer for their feedback regarding our use of the term "trophic cascade," which was not originally consistent with the definition provided by Ripple et al. (2016). Upon revisiting Ripple et al. (2016), and particularly the section concerning the detection of trophic cascades, we agree that presenting devil declines as **causing** a trophic cascade is erroneous. Rather, devil declines have **revealed or allowed the detection** of a trophic cascade within the Tasmanian mammalian community. We have updated the text to reflect this change in understanding. Specifically, we have updated the following lines:

> Lines 36 – 37: "Changes in top predator densities often lead to changes in densities and/or behaviors of species at lower trophic levels, **thus revealing the occurrence of trophic cascades**."
>
> Lines 52 – 53: "Devil declines have ~~**caused**~~ **revealed** a trophic cascade involving density- and trait-mediated effects on several species, but the evolutionary effects remain unknown."

I am lacking a bit of information why you find three different genetic clusters in the quolls. While the Western one nicely correlates with density of devils I wonder what factors are explaining that? Can you provide more information for the general readership who might be less familiar with the study system? Apparently the north western tip of Tasmania has always been an area with highest density of devils – so I would assume there is also an ecological reason for it.

We thank the reviewer for their feedback regarding interpretation of the inference of three genetic clusters and the lack of clarity regarding the connection between the genetic clustering analysis and other results in the manuscript. Most species will exhibit > 1 genetic cluster for a variety of reasons, including dispersal limitation. That is, individuals typically do not disperse across the entirety of their geographic range (e.g., due to geographic distances between locations simply exceeding dispersal capacity or environmental conditions limiting dispersal); this results in groups of individuals occupying different parts of the range being partially genetically isolated and thus accumulating genetic differences from one another (which will lead to inference of multiple genetic clusters).

Our isolation-by-resistance (IBR) and isolation-by-environment (IBE) analyses suggest that both geographic distance and environmental factors contribute to population genomic structure among quolls across Tasmania, and these factors thus likely contribute to genetic clustering results as well. We

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appreciate that this connection between the genetic clustering results, estimated effective migration surfaces, and our evaluation of how specific environmental factors contribute to IBR was not very clear in the original manuscript. We have revised the first section of the Results to improve clarity of the connections between these analyses. This section (**Lines 86 – 99**) now reads as follows (relevant changes in bold):

> "To understand the influence of the environment on quoll population genomic structure, it is first necessary to identify the number of genetic clusters represented in the dataset. FastStructure [23] and DAPC [24] showed strong agreement for K = 3 genetic clusters among our samples across Tasmania (Extended Data Fig. S1), with 98% of individuals sharing the same cluster of majority assignment in both methods (Fig. 2A; Extended Data Fig. S2A, B). **The three genetic clusters were broadly oriented from east – west (Fig. 2A).** Consistent with the broad results of the genetic clustering methods, an analysis of Estimated Effective Migration Surfaces (EEMS) [25] identified a **geographic region characterized by** low effective migration **(i.e., a statistic reflecting both local effective population size as well as gene flow)** separating the eastern and western halves of Tasmania (Extended Data Fig. S3). **Spatial variation in effective migration was also evident throughout Tasmania (Extended Data Fig. S3), which evidences a genetic pattern of isolation-by-resistance (IBR). IBR occurs when environmental conditions on the landscape between locations impede or facilitate dispersal and consequent gene flow.[20]**
>
> Next, we used ResistanceGA[26] to **further evaluate evidence for IBR** and quantify the extent to which specific environmental factors contribute to IBR…"

24: I find that misleading phrased. The disease is resulting in a change of the trophic cascade but not a cascade itself

We have updated the abstract to keep the language in line with the changes we made in response to the first comment by this reviewer. The phrase in question has been removed to make room for a conceptual description of methodology associated with the "landscape community genomics" framework.

This change is apparent in **Lines 19 – 20** (removed portion crossed out):

> "Tasmania's top terrestrial predator, the Tasmanian devil, is declining due to a lethal transmissible cancer~~, resulting in trophic cascades~~."

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36: see comment above on the terminology

We have altered this line (now **Lines 36 – 37**) to be consistent with updated language we detailed earlier in our response to reviewers. The sentence now reads as follows:

> "Changes in top predator densities often lead to changes in densities and/or behaviors of species at lower trophic levels, **thus revealing the occurrence of trophic cascades**."

147 I am not sure I can follow this argument: Are you saying that because there are less devils there is lower pressure to disperse, so more quolls could be in that given area? That would mean that this is not directly a resistance in the landscape ? Do you have data on quoll densities in such areas which could support the argument?

A genetic pattern of isolation by resistance (IBR) results from environmental conditions across the landscape facilitating or impeding gene flow between locations. Gene flow (and effective migration estimated by EEMS in our study) reflects the composite of local effective population sizes/densities and migration rates (i.e., the effective number of migrants, Ne*m in population genetic theory), so our results cannot evaluate the extent to which Ne and/or m are individually impacted by environmental conditions. The mechanism(s) by which environmental conditions generate landscape resistance (e.g., do they influence Ne and/or m, and how?) that we consider in the Discussion are simply hypotheses. The hypothesis in Line 147 (now **Line 169**) is that dispersing quolls that encounter areas of low devil density and therefore reduced competition may favor those areas over continuing to disperse elsewhere. This would be borne out analytically by two locations separated by a low devil density region showing higher genomic differentiation (owing to less dispersal and consequent gene flow between the locations) than locations separated by higher devil densities; this is the pattern we identified in our ResistanceGA analyses of IBR. Unfortunately, we lack data concerning spotted-tailed quoll densities across Tasmania, so we are unable to further evaluate support for this hypothesis. We have adjusted the relevant text to improve the clarity regarding this hypothesis. The changes are found in **Lines 168 – 176** and read as follows:

> "The negative relationship between devil density and landscape resistance in IBR models indicates that quolls disperse less through areas of low devil density, possibly because such areas offer reduced competition and less dispersal pressure. That is, devil declines likely increase habitat quality for quolls because both species are nocturnal predators and scavengers with relatively high dietary overlap [17,40]. Low devil density increases the amount of carrion available to quolls [14] and simultaneously may reduce the chance of aggressive encounters between quolls and devils. **Thus, dispersing quolls that encounter areas of low devil density**

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> **and therefore low interspecific competition may not continue dispersing elsewhere, leading to low devil densities impeding gene flow and thereby increasing landscape resistance…**"

Note also that we have included additional alternative hypotheses in this section, including another interpretation of the results offered by Reviewer #1. The aforementioned paragraph now continues on as follows (new additions in **bold**):

> "… **Alternatively, telemetry data show that quoll and devil home ranges overlap at broad scales (88.4% overlap)[20], although core home ranges with higher usage intensity overlap less extensively (40.5% overlap)[20] likely because quolls prefer forested or otherwise lower-visibility habitat than devils[19]. High devil densities likely occur where habitat is highly suitable for devils, and this habit is likely highly suitable for quolls as well given broad overlap in the two species' space use. Highly suitable habitat may be relatively permissive to quoll dispersal and consequent gene flow, which may partly explain the negative correlation between quoll landscape resistance and devil density.** Quolls may also avoid areas with high densities of feral cats, which increase in abundance concomitantly with declines of devils,[16] although we have little evidence for quoll—cat interactions…"

190 I like that you are stating this so directly and believe that even such a correlative approch has its merits.

We appreciate the kind comment and positive feedback.

210 isn´t that a too general statement? And how do you see that your results contribute to the conservation of both species in Tasmania?

In our original Nature Ecology and Evolution submission, Line 210 corresponds to Line 205 (now **Line 262** following revisions). This line previously read "Genetic studies of the evolutionary impacts of the changing abiotic environment, including climate and land use change, have informed conservation efforts." We agree that this sentence could be made more specific. This sentence now reads

> "Genetic studies of the evolutionary impacts of the changing abiotic environment, including climate and land use change, have informed conservation efforts **aimed at maintaining genetic diversity and gene flow across the landscape.**[8]"

Thus, the sentence now provides specific aims of conservation efforts that are informed by landscape genetics studies that have largely focused on the abiotic environment. The remainder of the paragraph

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(unchanged from the original Nature Ecology and Evolution submission) explains why the inclusion of the biotic factors in future landscape genetics and landscape genomics studies (as we have done in our present study) would be beneficial.

We do not argue that our results concerning the population genetics of quolls will contribute to the conservation of the Tasmanian devil; the conservation relevance of our results to the spotted-tailed quoll is described in **Lines 231 – 238** of our revised manuscript. We suggest that the association between the biotic factors included in our study (devil densities and duration of presence of DFTD) and evolutionary forces in quolls may lead to repeated perturbation of quoll genetic structure in the future because devils and DFTD may experience population cycles. This provides an aim for future monitoring efforts. In **Lines 219 – 224** we state that a fertility-related gene showing a signature of selection in the quoll may suggest currently undocumented changes to quoll population densities, providing another aim for future monitoring efforts. Thus, we believe our original version of this manuscript satisfactorily explains the potential conservation importance of our research study and the landscape community genomics framework in general.

482 Are Figure panels A and B representing the whole range of the quolls? Looking at figure 1 and Figure 2B gives the impression that the SouthWestern part of Tasmania is not inhabitated largely by both species, right?

We thank the reviewer for pointing out a lack of information regarding the spotted-tailed quoll's distribution across Tasmania. The distributions of both the spotted-tailed quoll and Tasmanian devil include southwestern Tasmania (i.e., both species are found island-wide). However, the terrain in southwestern is rugged and largely inaccessible for fieldwork to collect samples; thus, southwest Tasmania is not represented by samples in our dataset. We have now added this information to the Materials and Methods in **Lines 330 – 332**, pasted below:

> **"Both quolls and devils are present in southwestern Tasmania, but rugged terrain makes the area largely inaccessible for fieldwork to collect samples; thus, southwestern Tasmania is not represented by samples in our dataset."**

Relatedly, we reworded **Lines 87 – 88** to explicitly state that there is evidence for three genetic clusters *represented by our samples*, as unsampled quolls in southwestern Tasmania may represent another genetic cluster (changes in **bold**):

> "FastStructure [23] and DAPC [24] showed strong agreement for K = 3 genetic clusters **among our samples** across Tasmania…"

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589 which are the variables and why did you select those?

We thank the reviewer for their comment. Line 589 of the version of our manuscript we previously submitted to Science corresponds to Line 337 (now **Line 431** following revisions) of the present submission to Nature Ecology and Evolution; this line is within the Materials and Methods section concerning isolation by resistance (section titled "Isolation by resistance: effects of environmental variation between sites"). We note that we described the environmental variables we tested and explained the rationale for selecting them in the preceding section titled "Environmental data processing" in **Lines 396 - 422**. A summary of these environmental factors and which analyses they are used in is also provided in Extended Data Table S2 (noted on **Line 422**). Therefore, we do not believe it is necessary to reiterate this information in the section concerning isolation by resistance. Instead, we have modified **Lines 431 - 432** to refer readers to the section "Environmental data processing." The updated sentence reads as follows (changes in **bold**):

> "We evaluated the contributions of specific environmental variables **(described above and in Extended Data Table S2)** to population genomic structure using a circuit-theory based approach implemented in the R package ResistanceGA.[28]"

Reviewer #3 (Remarks to the Author):

This study links the decline of a dominant scavenger (devils) to genetic changes in a competing mesopredator (quolls). They first describe a variety of known cascading impacts from devil declines and then show new results on how quoll genetic structure changes. To my surprise, I could not find a clear example in the literature of how the loss of a dominant competitor (or predator) altered the genetic structure of a submissive competitor or mesopredator.

The writing is clear but – for a relatively broad ecology journal like NEE - the main results (including figure 2) need to be more easily interpretable by readers without training in genetics (this would not be required if submitted to Molecular Ecology). There should also be more help interpreting if the effect sizes shown are biologically significant. For example, does the decline of a dominant competitor recreate the magnitude and direction of the genetic impacts induced by human hunting? From habitat loss and fragmentation?

We thank the reviewer for the time and effort they spent providing feedback on our study. We broadly agree that allocating more text to improving the interpretability of the results and situating our results

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in the broader literature (e.g., by comparing effect sizes to those found in similar studies) would be beneficial; this feedback is addressed in our responses to specific comments below.

Introduction:

The use of "top predator" for the Tasmanian devil doesn't feel right because they're small (8-10kg) and primarily a scavenger. Devils are not even that much bigger than the quolls there (which often reach 6-8kg). They should more accurately call devils a dominant competitor throughout the manuscript. Referring to cascading impacts from the decline of a dominant competitor is just as novel as how they currently present their results per se. So there's no need to oversell or stretch such terms.

We thank the reviewer for their feedback regarding the ecology of devils and quolls.

Although the Tasmanian devil is a relatively small carnivore compared to other examples globally (e.g., big cats), the devil is the apex predator of Tasmania (the now-extinct Thylacine was formerly the apex predator of Tasmania). A substantial body of research has shown that devils play an important role in structuring the Tasmanian mammal community, such that their decline has revealed a trophic cascade (Hollings et al., 2014; Hollings et al., 2016; Cunningham et al. 2020) through predation and by driving a "landscape of fear" (e.g., by inducing vigilance behavior in the quoll [Andersen et al., 2016] and the brushtail possum [Hollings et al., 2015; Cunningham et al., 2019]). Accordingly, the body of peer-reviewed ecological research concerning the Tasmanian devil has consistently referred to the devil as the top predator of Tasmania's ecological community (Hollings et al., 2014; Hollings et al., 2016; Cunningham et al., 2019; Cunningham et al. 2020). Our text remains consistent with this body of research, and thus we respectfully disagree with the reviewer's recommendation to alter our use of the term "top predator" when describing the Tasmanian devil. The aforementioned references are cited throughout our manuscript.

With respect to body size *ranges* (based on both males and females), the spotted-tailed quoll and Tasmanian devil may overlap somewhat (quolls: 1.5 – 5kg; devils 5 – 10+ kg; Jones & Barmuta 1998), but the *typical* body sizes of the devil and quoll are quite different. The quoll's average adult body size is substantially smaller than the devil's average adult size (quolls: mean 1.7kg females and 3.2kg males; devils: mean 6.4kg females and 8.4kg males) (Jones & Barmuta, 1998; Jones & Barmuta, 2000; Belcher, 2003; Belkhir et al., 2022; Hamilton, unpublished).

The interaction between the devil and the quoll is indeed characterized largely by competition (e.g., partial dietary overlap, competition for daily temporal niche). Our original submission acknowledged competitive interactions between the two species throughout the manuscript, but we have revised the

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Abstract, Introduction, and Discussion to more clearly state this information. Alterations to the manuscript are listed below, with changes in **bold**:

> Lines 41 – 44: "… The large home ranges and dispersal capabilities of top predators mean that "eco-evolutionary trophic cascades [7]" **and evolutionary feedbacks associated with other species interactions (e.g., competition)** should be studied at the landscape scale, but most studies at this scale focus on abiotic factors that impact gene flow and local adaptation [8,9]."
>
> Lines 28 – 30: "Our results provide some of the first evidence of the evolutionary impacts of **competition between a top predator and a mesopredator species in the context of a** trophic cascade."
>
> Lines 54 – 60: "We focus on the spotted-tailed quoll (*Dasyurus maculatus*; hereafter, the quoll), **), a smaller (quolls: mean 1.7kg females and 3.2kg males[17,18]; devils: mean 6.4kg females and 8.4kg males[17,19]), competitively subordinate mesopredator that experiences mesopredator release characterized by** shifts in ecological resource use and activity timing in association with devil declines (Fig. 1B) [14,18]. At pre-disease devil densities, devils and quolls exhibit **partial** dietary [14,18,19] and **substantial** temporal [15,20] niche partitioning **indicative of interspecific competition**, both of which become weaker following devil declines."
>
> Lines 154 – 156: "Using multiple complementary approaches, we show that declines in devil densities resulting from the spread of a lethal transmissible cancer have significant effects on evolutionary processes in the spotted-tailed quoll, a mesopredator **and subordinate competitor**."
>
> Lines 156 – 157: "Prior work has shown that quolls benefit from mesopredator release via ~~**devil decline-induced trophic cascades**~~ **the decline of the competitively dominant devil."**
>
> Lines 162 – 164: "Thus, disease-induced declines of Tasmania's top predator contribute to patterns of gene flow and selection in the quoll, thereby providing novel evidence of the evolutionary impacts of ~~**a trophic cascade**~~ **altered competitive interactions occurring within the broader context of a trophic cascade."**

References used in our response to this comment (in order of appearance):

Hollings, T., Jones, M., Mooney, N. and Mccallum, H. Trophic Cascades Following the Disease-Induced Decline of an Apex Predator, the Tasmanian Devil. *Conserv. Biol.* **28**, 63–75 (2014).

Hollings, T., Jones, M., Mooney, N. and McCallum, H. Disease-induced decline of an apex predator drives invasive dominated states and threatens biodiversity. *Ecology* **97**, 394–405 (2016).

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Cunningham, C.X., Johnson, C.N., and Jones, M.E. 2020. A native apex predator limits an invasive mesopredator and protects native prey: Tasmanian devils protecting bandicoots from cats. Ecology Letters 23(4): 711-721.

Andersen, G.E., Johnson, C.N., and Jones, M.E. 2016. Sympatric predator odour reveals a competitive relationship in size-structured mammalian carnivores. Behavioral Ecology and Sociobiology 70(11): 1831 – 1841. Proceedings of the Royal Society B 282: 20150124.

Hollings, T.,. McCallum, H., Kreger, K., Mooney, N., and Jones, M.E. 2016. Relaxation of risk-sensitive behaviour of prey following disease-induced decline of an apex predator, the Tasmanian devil.

Cunningham, C.X., Johnson, C.N., Hollings, T., Kreger, K., and Jones, M.E. 2019. Trophic rewilding establishes a landscape of fear: Tasmanian devil introduction increases risk-sensitive foraging in a key prey species. Ecography 42(12): 2053 – 2059.

Jones, M.E., and Barmuta, L.A. 1998. Diet overlap and relative abundance of sympatric dasyurid carnivores: a hypothesis of competition. Journal of Animal Ecology 67(3): 410 – 421.

Jones, M.E., and Barmuta, L.A. 2000. Niche differentiation among sympatric Australian Dasyurid carnivores. Journal of Mammalogy 81(2): 434 - 447.

Belcher, C.A. 2003. Demographics of the tiger quoll (Dasyurus maculatus maculatus) populations in south-eastern Australia. Australian Journal of Zoology 51: 611 – 626.

Belkhir, S., Hamede, R., Thomas, F., Ujvari, B., and Dujon, A.M. 2022. Season, weight, and age, but not transmissible cancer, affect tick loads in the endangered Tasmanian devil. Infection, Genetics, and Evolution 98: 105221.

Trophic cascades are a defined term (supposed to be at least) including three trophic levels, with a predator indirectly influencing a species' two-levels down (usually a plant) via their effect on the intermediate species (usually an herbivore). What the authors describe in this paper is the definition of mesopredator release, not a trophic cascade. It is a more general type of ecological cascade but certainly not a trophic cascade. The use of the trophic cascade term should be removed throughout.

We thank the reviewer for their helpful feedback. We agree that our study focuses on the competitive interactions between devils and quolls (and mesopredator release given the decline of the devil). We have made several revisions to our manuscript to more clearly describe the interaction between devils and quolls as competition and not a trophic cascade. However, the devil – quoll interaction and ecological changes to quolls associated with devil declines are accurately described in our study as


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occurring *in the broader context of a trophic cascade* involving numerous species across three trophic levels in the Tasmanian mammal community (see Cunningham et al., 2020). We believe that describing this broader ecological context of the study system is relevant background for our manuscript. Therefore, we have maintained the use of the term trophic cascade at several places in the manuscript but have accordingly changed text that originally implied that the devil – quoll interaction itself was a trophic cascade.

Specifically, we note the following locations where the former version of our manuscript erroneously described (or implied) that the devil – quoll interaction itself is a trophic cascade (changes in **bold**, with former text crossed out):

> Lines 28 – 30: "Our results provide some of the first evidence of the evolutionary impacts **of ~~a trophic cascade~~ competition between a top predator and a mesopredator species in the context of a trophic cascade."**
>
> Lines 61 – 63: "Devil populations typically remain at low densities following DFTD-mediated declines [11], such that the quoll has experienced altered resource availability and behavior for up to 25 generations, likely providing sufficient lag time to detect evolutionary effects of **mesopredator release~~trophic cascades~~** [21]."
>
> Lines 156 – 157: "Prior work has shown that quolls benefit from mesopredator release via **~~devil decline-induced trophic cascades~~ the decline of the competitively dominant devil."**
>
> Lines 162 – 164: "Thus, disease-induced declines of Tasmania's top predator contribute to patterns of gene flow and selection in the quoll, thereby providing novel evidence of the evolutionary impacts of **~~a trophic cascade~~ altered competitive interactions occurring within the broader context of a trophic cascade."**

Greater clarity and focus on the competitive interactions between quolls and devils has been introduced into the manuscript in our response to the previous comment. The term trophic cascade now appears in our manuscript only where it describes the broader ecological context of the devil – quoll interaction.

Reference:

Cunningham, C.X., Johnson, C.N., and Jones, M.E. 2020. A native apex predator limits an invasive mesopredator and protects native prey: Tasmanian devils protecting bandicoots from cats. Ecology Letters 23: 711 – 721.

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Add species diets and weights somewhere to provide biological/ecological rationale for their interactions.

We thank the reviewer for their suggestion. We have added species' masses to the Introduction in **Lines 54 – 57**, which reads as follows:

> "We focus on the spotted-tailed quoll (*Dasyurus maculatus*; hereafter, the quoll), a smaller (quolls: mean 1.7kg females and 3.2kg males[17,18]; devils: mean 6.4kg females and 8.4kg males[17,19]), competitively subordinate mesopredator that experiences mesopredator release characterized by shifts in ecological resource use and activity timing in association with devil declines (Fig. 1B) [14,18]."

Dietary data are comparatively more complex, and we believe that specific dietary details are not necessary to include in our manuscript to understand its contents. Interested readers may pursue the peer-reviewed research articles regarding dietary comparisons of quolls and devils that we have cited throughout the manuscript, including the following references:

Andersen, G. E., Johnson, C. N., Barmuta, L. A. & Jones, M. E. 2017. Dietary partitioning of Australia's two marsupial hypercarnivores, the Tasmanian devil and the spotted-tailed quoll, across their shared distributional range. *PLOS ONE* **12**, e0188529.

Cunningham, C. X. *et al.* 2018. Top carnivore decline has cascading effects on scavengers and carrion persistence. *Proc. R. Soc. B Biol. Sci.* **285**, 20181582.

Jones, M. E. & Barmuta, L. A. 1998. Diet overlap and relative abundance of sympatric dasyurid carnivores: a hypothesis of competition. *J. Anim. Ecol.* **67**, 410–421.

Jones, M. E. & Barmuta, L. A. 2000. Niche differentiation among sympatric australian dasyurid carnivores. *J. Mammal.* **81**, 14.

Last sentence is results. This is normally a short paragraph in Nature Comms but this single sentence here in NEE doesn't flow.

We agree that the final sentence in the Introduction described results, and we have removed it so that our manuscript is more in-line with recently published articles in Nature Ecology and Evolution.

Results


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I really appreciate the framing around specific hypotheses. Well done but would benefit from more laymen's explanation/interpretation of what the genetics changes mean.

Thank you. A similar comment was made by Reviewer 1 and Reviewer 2, and we believe our responses to those comments address this comment as well. Below, we document changes we made to the manuscript to improve clarity and facilitate interpretation by a broader audience:

1. We have added detail to the introduction to more clearly explain what isolation-by-resistance and isolation-by-environment are, as well as what processes can generate these patterns (**Lines 71 – 79**, added text in **bold**):
    a. "We used three general approaches to address these goals: 1) tests for a population genomic pattern of isolation-by-resistance (IBR) [20], 2) tests for a population genomic pattern of isolation-by-environment (IBE) [21], and 3) landscape genomic tests for selection at individual SNPs based on genetic-environment associations (GEAs) [22]. **Tests for IBR characterize how environmental conditions across the landscape between locations affect population genomic structure by impeding or facilitating gene flow (e.g., certain environmental conditions may act as obstacles to dispersal between locations),[20] whereas tests for IBE characterize how at-location environmental conditions influence population genomic structure as a consequence of selection against migrants from locations with different environmental conditions or as a result of environmentally biased dispersal.[21]**"
2. We have improved the text of the Results section by more explicitly stating the connections between different analyses, which we believe will aid interpretation by a broader audience. In the section pertaining to isolation-by-resistance (IBR), we have modified **Lines 86 – 99** to read as follows (additions in bold):
    a. "To understand the influence of the environment on quoll population genomic structure, it is first necessary to identify the number of genetic clusters represented in the dataset. FastStructure [23] and DAPC [24] showed strong agreement for K = 3 genetic clusters among our samples across Tasmania (Extended Data Fig. S1), with 98% of individuals sharing the same cluster of majority assignment in both methods (Fig. 2A; Extended Data Fig. S2A, B). **The three genetic clusters were broadly oriented from east – west (Fig. 2A).** Consistent with the broad results of the genetic clustering methods, an analysis of Estimated Effective Migration Surfaces (EEMS) [25] identified a **geographic region characterized by** low effective migration **(i.e., a statistic reflecting both local effective population size as well as gene flow)** separating the eastern and western halves of Tasmania (Extended Data Fig. S3). **Spatial variation in effective migration was also evident throughout Tasmania (Extended Data Fig. S3), which evidences a genetic**

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pattern of isolation-by-resistance (IBR). IBR occurs when environmental conditions on the landscape between locations impede or facilitate dispersal and consequent gene flow.[20] Next, we used ResistanceGA[26] to **further evaluate evidence for IBR** and quantify the extent to which specific environmental factors contribute to IBR…"

3. We have improved the clarity of the legend for Figure 2, described in our response to another comment below.

However, we note here that our results are largely composed of descriptions of spatial genetic *patterns* that we identified, and the interpretation of these patterns are hypotheses of the *processes* that generated the observed patterns. We have therefore been careful to restrict explanations of processes that may have generated these genetic patterns to the Discussion section to avoid conflating interpretation of the results with the results themselves.

Methods: Excellent.

We thank the reviewer for their kind comment.

Discussion: bit more help interpreting if the effect sizes shown are biologically significant (see above).

The reviewer's earlier comment to compare effect sizes found in other studies (which is our interpretation of their comment "For example, does the decline of a dominant competitor recreate the magnitude and direction of the genetic impacts induced by human hunting? From habitat loss and fragmentation?") is an excellent suggestion that we agree would assist interpretation and tie in the broader landscape genetics literature. However, we note that rigorous comparisons across species and studies are difficult to achieve because idiosyncrasies of a species' biology can greatly impact the existence of phenomena such as isolation by resistance (IBR) and isolation by environment (IBE); furthermore, methodological decisions such as spatiotemporal sampling design can greatly impact the statistical detection of these genetic patterns.

In the Discussion section, we have added several sentences comparing the genetic variance explained by our IBR and IBE analyses to similar studies of other relatively vagile, mammalian carnivores. In general, the estimates we found in relation to quolls are of similar magnitude to the results of other studies that

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report analogous statistical metrics derived from similar methodology. These comparisons are found near the relevant Discussion paragraph for each class of analysis (i.e., IBR and IBE).

In relation to IBR, we have added the following comparison (**Lines 184 – 190**; relevant additions in **bold**):

> "… **. Interestingly, the marginal R-squared achieved by the best-performing ResistanceGA model of IBR for quolls in our study (64.36%) is substantially higher than the marginal R-squared estimated in a study concerning IBR in Tasmanian devils (37.9%; driven largely by roads)[45]; this suggests that landscape resistance imposed by the environment may influence quoll gene flow more strongly than in its dominant competitor, although we note that the considerably greater spatial extent of our study may facilitate statistical detection of environmental drivers of genomic variation."**

In relation to IBE, we have added the following comparisons/context (**Lines 200 - 299**; relevant additions in **bold**):

> "… **Although the statistically significant effect estimated for generations diseased (which uniquely explains 1.44% of deviance in our final GDM model) is small, it is comparable to the variance explained by individual abiotic environmental factors in other vagile, mammalian carnivores (e.g., statistically significant abiotic environmental drivers of IBE in the American badger each uniquely explain < 1% of genetic variance) [44]. Indeed, our final models for pRDA and GDM returned estimates for genetic variance explained collectively by environmental factors (pRDA: adjusted R-squared = 13.7%; GDM: deviance explained 14.5%) that are similar to other mesopredator species including bobcats (23.6% of genetic variance explained by variation in landcover)[45] and the American badger (8.15% of genetic variance explained collectively by topography and several abiotic environmental factors)[44]."**

Full references used in the added text:

Kozakiewicz. 2020. Comparative landscape genetics reveals differential effects of environment on host and pathogen genetic structure in Tasmanian devils (*Sarcophilus harrisii*) and their transmissible tumor. Molecular Ecology 29(17): 3217 – 3233.

Cancellare, I.A., Kierepka, E.M., Janecka, J., Weckworth, B., Kazmaier, R.T., Ward, R. 2021. Multiscale patterns of isolation by ecology and fine-scale population structure in Texas bobcats. PeerJ 9: e11498.

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Kierepka, E.M., and Latch, E.K. 2016. High gene flow in the American badger overrides habitat preferences and limits broadscale genetic structure. Molecular Ecology 25: 6055 – 6076.

Figures

Overall attractive.

Thank you.

Figure 1 panel C – These smooth PowerPoint curves are more like hypothesized relationships (or idealized trends). Calling them "results" could be misleading.

We agree that the relationships between variables depicted in Figure 1 Panel C are better characterized as trends rather than results strictly speaking, as real quantitative data are not graphed. We note here that the general relationships depicted on the graphs are accurate (e.g., the IBE asymptotic curve depicted in Fig.1C is based on the real asymptotic curve identified by GDM and plotted in Extended Data Figure S6). We have altered Figure 1 to read "Present study" next to Panel C (formerly labelled "present results"), and we have altered the figure legend (**Line 288**) to characterize Panel C as depicting trends as follows:

> "… **C) Trends identified in the present study** based on genomic data include identification of significant contributions of devil density to… "

Figure 2: Help the reader without expertise in genetics understand these.

We thank the reviewer for their helpful feedback and agree that greater explanation of the figure contents in the figure legend would benefit a broader readership. We have adjusted the figure legend in the following ways in reference to this specific comment:

1. Brief indication that K = 3 refers to inference of three genetic clusters.
2. Brief explanation that isolation by resistance (IBR) is a genetic pattern generated by environmental conditions between locations contributing to spatially variable resistance to gene flow across the landscape.

Figure 2's legend now reads as follows in **Lines 301 – 307** (note that this figure legend was also adjusted in response to a previous reviewer's comment, and relevant changes from the original manuscript are **bolded**):

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> "Figure 2. Population genetic structure of the spotted-tail quoll across Tasmania. A) Map of FastStructure ancestry proportions for all 345 individual quolls **based on inference of three genetic clusters (i.e., K = 3)**. B) Individual FastStructure ancestry proportions for the 189 individual quolls used in ResistanceGA plotted over devil density lagged by 10 generations. C-E) A subset of linear mixed effects models with maximum likelihood population effects evaluating the contribution of abiotic and biotic variables **to isolation by resistance, a spatial genomic pattern generated by environmental conditions between locations contributing to spatially variable resistance to gene flow across the landscape…**"

Figure 2C – need to add that lower AICc is better somewhere. OR maybe put a star over the best model?

We agree that indicating that lower AICc is better would make Figure 2 clearer, and we have made this change in the figure legend (**Lines 301 – 311**), pasted below (relevant changes in **bold**).

> "Figure 2. Population genetic structure of the spotted-tail quoll across Tasmania… C-E) A subset of linear mixed effects models with maximum likelihood population effects evaluating the contribution of abiotic and biotic variables to isolation by resistance, a spatial genomic pattern generated by environmental conditions between locations contributing to spatially variable resistance to gene flow across the landscape. Panels C-E share the X-axis specified under panel E, such that Model 1, which includes geographic distance, landcover, and TDR, is characterized by the leftmost AICc value and marginal R-squared value in panels C and D. **Models are ordered left-to-right by increasing (worsening) average AICc based on 10,000 bootstrap replicates; i.e., Model 1 has the lowest AICc and is thus the best-performing model**…"

Tables: Excellent.

We thank the reviewer for their feedback.

Decision Letter, first revision:

9th October 2023

Dear Dr. Storfer,

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nature portfolio

Thank you for submitting your revised manuscript "Disease-driven top predator decline affects mesopredator population genomic structure" (NATECOLEVOL-23051112A). It has now been seen again by the original reviewers and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Ecology & Evolution, pending minor revisions to satisfy the reviewers' final requests and to comply with our editorial and formatting guidelines.

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We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Ecology & Evolution. Please do not hesitate to contact me if you have any questions.

[REDACTED]

Reviewer #1 (Remarks to the Author):

My thanks to the authors for their detailed responses to my concerns and comments. They appear to have adequately and thoroughly edited the manuscript and I am happy for the manuscript to progress for publication.

Reviewer #2 (Remarks to the Author):

Dear Authors,
this version has now been significantly improved and suggestions from all three reviewers addressed.

Our ref: NATECOLEVOL-23051112A

27th October 2023

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Dear Dr. Storfer,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Ecology & Evolution manuscript, "Disease-driven top predator decline affects mesopredator population genomic structure" (NATECOLEVOL-23051112A). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us immediately if you anticipate it taking more than two weeks to submit these revised files.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Ecology & Evolution's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Disease-driven top predator decline affects mesopredator population genomic structure". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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Reviewer #1:
Remarks to the Author:
My thanks to the authors for their detailed responses to my concerns and comments. They appear to

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have adequately and thoroughly edited the manuscript and I am happy for the manuscript to progress for publication.

Reviewer #2:
Remarks to the Author:
Dear Authors,
this version has now been significantly improved and suggestions from all three reviewers addressed.

Author Rebuttal, first revision:

Reviewer #1:
Remarks to the Author:
My thanks to the authors for their detailed responses to my concerns and comments. They appear to have adequately and thoroughly edited the manuscript and I am happy for the manuscript to progress for publication.

We thank the reviewer for their feedback, and we are pleased to hear that we have satisfactorily addressed their comments and concerns.

Reviewer #2:
Remarks to the Author:
Dear Authors,
this version has now been significantly improved and suggestions from all three reviewers addressed.

We thank the reviewer for their feedback, and we appreciate the time they spent reviewing the manuscript.

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nature portfolio

Final Decision Letter:

2nd November 2023

Dear Dr Storfer,

We are pleased to inform you that your Article entitled "Disease-driven top predator decline affects mesopredator population genomic structure", has now been accepted for publication in Nature Ecology & Evolution.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Ecology and Evolution style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required

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Yours sincerely,

Patrick Goymer, DPhil
Chief Editor
Nature Ecology & Evolution

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