**Response letter to reviewer comments**

Dear Dr. Chris Foote, Editor in Chief, *Ecology and Evolution*,

Dear Associate Editor and Reviewers,

Thank you for the time and effort spent on reviewing our manuscript titled "Skull shape of a widely-distributed, endangered marsupial reveals little evidence of local adaptation between fragmented populations"; especially for the rapid response given despite the pandemic difficult times we are all living. Thank you also for giving us the opportunity to submit a revised draft at this stage. We appreciate the valuable feedback and are grateful to the reviewers for their insightful comments. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. The changes are highlighted within the manuscript and commented one by one below.

Here is our response on a point-by-point basis to the associate editor’s and reviewers’ comments and concerns. To facilitate readership, their comments are italicized, and our response is below each comment in blue.

**Comments from Associate Editor**

* **Comment 1:** *Two expert reviewers assessed your manuscript and provided detailed comments aimed to strengthen the manuscript and increase the clarity. Both reviewers found the manuscript interesting, but diverged in their view of completeness of the analyses and aspects of interpretation. Specifically, reviewer 2 has requested new or expanded analyses; I think they make a strong argument that the high amount of unexplained variation may be due to limitations of explanatory variables. Both reviewers analysis suggestions merit consideration.*
* **Comment 2:** *Both reviewers and I thought the paper was well written. However, the reviews highlight a few places where additional clarity would help readers. For example, one reviewer missed the map in Figure 2; another asked for more detail on previous genetic studies as part of broader framing.*
* **Comment 3:** *Minor. Title for Supplementary Figure 4- remove “Supplementary Figure 5”; adjust other legends and within text citations as needed.*

Done.

**Comments from Reviewer 1**

* **Comment 1:** *I enjoyed reading “Skull shape of a widely-distributed, endangered marsupial reveals little evidence of local adaptation between fragmented populations”. This is a nice manuscript that investigated morphological variation among populations/localities of a marsupial species. Authors have found that most of the shape variation remained unexplained, and among the explained portion, allometry contributed more to explain shape than differences among sexes or populations. The article is well written and easy to understand. I found the methods sound and I agree with the results and its interpretation.*

Thanks. We appreciate the encouraging comments.

* **Comment 2:** *My main suggestions are for the inclusion of two aspects ignored in this manuscript: size variation and morphological disparity.*

Please refer to responses of comments 3 and 4.

* **Comment 3:** *Size was used as a predictor for shape, but it could as well be the response variable. I think it would be interesting to explore if there are any differences in size among populations and sexes. In fact, some papers have found that, even when shape differences among populations were small, size variation was higher than shape variation, and some of the size variation was attributable to environmental differences even while shape was not (https://doi.org/10.1111/jbi.12815; https://doi.org/10.1111/j.1466-8238.2008.00432.x). Moreover, the Island Rule predicts that overall size should change when island colonization occurs, even in short periods of time, which would be interesting to know for this marsupial species that occurs in islands and continental regions.*

We completely agree with this statement. We included results of linear models of variables on size variation on supplementary figure 4 and supplementary table 2. We found that females were smaller than males, island specimens were smaller than continental specimens and a size variation among populations depicted in Supp. Fig. 4. However, we did not include them in the manuscript (OR DID WE?)………….

Ok, so size might be independent and vary more or less than shape. We did find some correlation between shape and size, but the non-correlated part may well behave differently. Can we also maybe use predict size with the environmental variables with the variation partitioning model?

Rainfall can be used as a proxy for habitat productivity, which

inﬂuences body mass via food availability (Chapman &

Chapman, 1990; Dunbar, 1990; Barrett & Henzi, 1997;

Chapman & Balcomb, 1998).

Rainfall can be used as a proxy for habitat productivity, which

inﬂuences body mass via food availability (Chapman &

Chapman, 1990; Dunbar, 1990; Barrett & Henzi, 1997;

Chapman & Balcomb, 1998).

Rainfall can be used as a proxy for habitat productivity, which

inﬂuences body mass via food availability (Chapman &

Chapman, 1990; Dunbar, 1990; Barrett & Henzi, 1997;

Chapman & Balcomb, 1998

* **Comment 4:** *Morphological variability/disparity measured within each population could reveal which population harbors the higher phenotypic variability. This is a different test than what was done; and despite the differences in shape being low among populations, some of them could have more morphological variability than others, which could have implications for conservation. This would be closely aligned with conservation genetics studies (mentioned in the Introduction), which usually explore genetic variability within localities.*

Add morphological disparity between populations. Add island v mainland disparity in methods.

* **Comment 5:** *It would be nice to see a geographic map with the specimens/populations’ localities.*

Figure 2… make a different Figure.

* **Comment 6:**

*Specifics:  
Lines 212-217: How many PCNMs entered in the variation partitioning analysis? How was the PCNM selection made?*

Help from Gabriele

*Lines 218-219: Why these two environmental variables and not others?*

Other variables might have autocorrelation problems (e.g. Aridity index and precipitation). What other available variables could I use? Added land cover/vegetation type, distance to coast, primary productivity

PRIMARY PRODUCTIVITY

Cardini 2007 cited by reviewer 1 shows similar amounts of unexplained variation (20-40%). Martinez y cola, 2011 explains 20%.

Martinez y cola: From these 21 variables, before running the models, we selected only 10 environmental variables in order to not over-parameterize our niche models with redundant climatic information (Table 2).

Bergmann’s rule?

Rainfall can be used as a proxy for habitat productivity, which inﬂuences body mass via food availability (Chapman &Chapman, 1990; Dunbar, 1990; Barrett & Henzi, 1997;Chapman & Balcomb, 1998)

*Lines 221-223: I did not understand this. Variation partitioning is based on RDA, so why was this last test conducted?*

Is this a wording issue? Gabriele.

*Table 1. Formulas could appear in regular notation instead of in R language.*

Is this really a problem? Look for papers… only found one from Ariel… or just change the table…

**Comments from Reviewer 2**

* **Comment 1:** *The manuscript explores the skull shape of Northern quolls and relates it to few putative drivers of shape variation across populations. Despite previous ecological and genetic studies on this species, the morphological variation seems to be neglected so far (according to the authors), which could provide important information about adaption to distinct environments. Although this is a valuable argument to address skull variation, there are many critical aspects in the text that need to be carefully revised. In particular, the data have been over-interpreted and many parts of the discussion are not supported by the data presented and in some cases are even contradictory.*

PCA in main text to show that it is a gunshot and that we did not come up with our interpretation…

This isn’t just about p-values but about the relative contribution of variables…

There are differences between populations but these are very subtle, the interlandmark distances between population mean shapes was very low. The contribution of population factor to shape was very low despite its significant p-value. Problem here is most significances with shape have similar F and Z values.

There may be a practical difference but this one seems to be very low…

* **Comment 2:** *The discussion is filled with assumptions on a lack of morphological adaptation, genetic factors, and developmental constraints (lines 350-359). In some cases show even contrasting conclusions. For example, at the beginning of the discussion, you said that differences in skull shape do not reflect local adaptation (lines 349-351) or that quoll populations have not evolved discrete morphotypes (329-331). Then in the next paragraphs say the variation mirrors adaptations in mastication to particular diets (lines 370-372) and that animals in drier areas have distinct skull shapes that might be related to dietary differences (lines 373-376). Later on, the authors discussed that differences can be related to male "mating bite" (lines 381-398). However, all the analyses were carried out combining males and females, so how can one know that those cranial differences are only associated with male individuals.*

This last sentence is true. But for the first part of the paragraph, we are just setting the ground with all sorts of hypothesis. We are worried about sample size but this is the results… semantic issue, phrase that this happens in animals and can also happen in quolls…

* **Comment 3:** *Furthermore, there is a large variation unexplained that can be related to a set of factors not tested, such as biomes, food preference, genetic divergence, other than bio1 and bio12 climate factors. The data presented is limited to conclude or rule out adaptability. For example, a more generalist skull shape (one-to-many mapping) might be advantageous under distinct climate conditions.*

Biome (check worldclim and my spreadhsheet), food preference (this is quite hard..., they are opportunistic...) , genetic divergence (this might be a good idea, but how to get this info and how do I integrate it?… amalgamate genetic distances with the NJ tree?? Quolls in different years might eat different things…

* **Comment 4:** *It is mentioned in the introduction that genetic differentiation between populations is well-understood, but in any part of the discussion, the authors try to relate their findings to the genetic evidence available. Therefore, several questions remain open. Do the morphological patterns parallel the genetic evidence? What is the source mainland population of the island individuals? Do the island individuals are more morphological similar to their mainland source population than to other populations?*

Do we integrate genetic evidence? Build tree and parallel it with genetic literature

Mainland source of island populations? In the literature and doable but limited sample size of islands is a problem here.

* **Comment 5:** *The population data are presented quite succinctly, without giving the reader any clue about the environment/biome or ecological traits (e.g., dietary preference) experienced for each population. Additional map and tables (showing sample size) should complement the presentation of the sampling and provide more information that justifies the expectation of the authors about possible differences in the skull among populations.*

Opportunistic forager behaviours (no real dietary preference. I can, however highlight some environment differences they may encounter. But not sure if I can do this very specifically. Additional map with sample sizes…

* **Comment 6:** *In Lines 111-114. Your expectation that variation will increase with geographic distance cannot be related to adaptation to the local environment, but rather to genetic drift. Second, by using only two metrics (annual temperature and precipitation) as proxies of environmental differences, you are likely overlooking all possible differences related to vegetation, seasonality, and other climate effects.*

ok, I will look into other bioclimatic variables and integrate them in the model, I actually just added these two to make it simpler and because I hypothesized those were the ones mostly influencing shape

* **Comment 7:** *Lines 116-117. If local adaptation is prevented by a constraint due to the quoll’s early birth, most shape variation is expected along a size gradient or should be unexplained. Many other mechanisms could be related to a lack of difference between populations. For example, the skull shape of quolls may represent a generalist form able to exploit diverse resources, in this case, one shape may be advantageous under distinct habitats. By pinpointing the early birth as the only possible evolutionary process that leads to a lack of variation surely overlooks other mechanisms.*

(did not understand well this comment, but I get that other variables might be overlooked) stabilizing selection could be another explanation…

* **Comment 8:** *Lines 118-120 Lastly, it is also possible that most variation relates to the biomechanical use of the cranium in feeding and – particularly in males – biting. This would result in a mostly uniform distribution of shape variation across geographic range of northern quolls. I do not follow the rationale here. The authors mentioned earlier that distinct populations of quolls have different dietary preferences (also showed by Dunlop et al 2017). Thus, if skull shape relates to biomechanical feeding than one would expect distinct populations to show uneven skull shapes reflecting their dietary preference.*

Fix text, wording issue

* **Comment 9:** *Lines 179-182. We did, however, test whether there were differences in shape variation between island and mainland populations, which would occur if divergent selection on the different islands shaped each population differently. Are those islands close? How can you be sure that each island is under the same selective pressure? Did you compare the skulls among islands?*

Sample size of islands is a problem here.

* **Comment 10:** *Lines 191-193. How many permutations?*

999

* **Comment 11:** *Lines 193 / 250-251. then performed permutation-based pairwise comparisons between the shape and centroid size least squares means of each population. I strongly advise you to assess pairwise comparisons using the shape variance in addition to the shape mean. This additional metric provides important insight into within-group variation and might shed light on some ecological drivers. For example, populations with a larger shape variance might suggest relaxed selection, source population, many-to-one mapping mechanism; whereas, populations with a limited shape variance may be under stronger environmental constraints, which exclude skull forms with lower fitness, represent only a subset of the potential morphospace. In addition, the results of disparity analysis were only briefly reported between island x mainland individuals.*

Disparity differences integrated

* **Comment 12:** *Line 194. I found hard to get a complete picture of shape changes between populations through the heatmaps presented. Mainly because there are only pair-pair comparisons, lacking a general view between changes across all populations. An alternative and more informative approach is to build a Neighbor-joining tree of skull shape per population (see Maestri et al. 2016. J. Biogeography. 43(6)10.1111/jbi.12718; Feijó et al. 2020. Biological Invasions. 22. 10.1007/s10530-019-02085-8). Through the NJ tree, the authors can assess which populations have more similar skulls. It can also help visualize whether island populations are closer to the founder populations (inferred from the genetic evidence) and whether island animals are closer to each other and clustered apart from the mainland. In this case, we could infer that they are under similar environmental pressure.*

build a tree? We can do this, though I am particularly worried about its reliability) (we stand with heatplots because it gives the biological story that a tree doesn’t contain) we had considered using a tree, show the tree… do UPGMA, upgma works on averages so this solves difficulties of dimensionality

UPGMA, ask Gabriele why

We don't do Neighbour joining for Gabriele's reasons

Clustering by population is possible but there is so much more variation in the sample that population differences only make a very small part of hte overall variation, and so taht clustering is unreliable (perhaps demonstrate in the supplementaries that the branch lengths are very small)

Clustering specimens makes more sense but probably reveals a mess

Use coloration

* **Comment 13:** *Line 212. Instead of a partition analysis through the varpart function that requires transforming the variables in distance matrix, why not just simply perform a Procrustes ANOVA with size, sex, spatial and climate together? In addition, because of the nature of the geometric morphometric data that has a high dimension, statistical significance is better assessed using permutation.*

First part of the question, ask Gabriele.

Second part, permutations, yes.

* **Comment 14:** *Lines 218-219. Why limit the climatic variables to only bio1 and bio12?*

*Because of what I hypothesized, but can add other variables, no worries. We just need to take care of autocorrelatedness.*

* **Comment 15:** *Figure 1. Why the different colors and sizes of arrows? The blue biggest arrow seems to point to a less variable area.*
* **Comment 16:***Discussion. Lines 331-334. Surprisingly, however, we found little structure in northern quoll shape variation (~76% of shape variation remains unexplained) and no strong evidence that any of the populations have evolved into discrete, possibly locally adapted, morphotypes. The fact you found a large portion of unexplained variation doesn't mean that there is no structure among populations, it just indicates your models do not include the main drivers. In addition, the Proc. Anova significantly differentiated all populations of quolls. So it seems contrary your conclusion that there is no discrete morphotypes. Now, whether the morphotypes are adaptive is another more complex issue to be proven that will need more evidence, for example, from genetic data. What genetic evidence says about population relationships?*
* **Comment 17:** *Lines 336-339 In particular, populations and sex differences have low effect sizes. What does it mean?*

Check this out. But in general, that the populations and sex differences contribute little to shape differences.

* **Comment 18:** *Lines 336-339 And explain less variation than size, meaning that similarly-sized individuals from opposite ends of the biogeographic distribution are likely to be similar in shape, even if they are of different sex. I don't follow this interpretation. The analyses are poorly presented to support this sentence. Where are the full results from the proc Anova including the effect of each factor instead of just show the interaction results as in Table 1. In addition, Figure 2 clearly shows statistic differences between populations when controlled for allometry, even between nearby populations.*

This is true. We chose to interpret those p-values stricter… P-values are not the only parameter to look at to arrive to a conclusion with the data. The impact of the actual shape differences is quite low, we checked it visually . Figure 2 shows the slight differences that these populations have but they are very low, as we can see from the PCA.

* **Comment 19:** *Lines 362-366 These lines should be allocated in the results section.*

ok