

Olfactory receptor repertoire size in dinosaurs

Graham M. Hughes and John A. Finarelli

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Review timeline

Original submission: 15 February 2019

1st revised submission: 17 April 2019

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Final acceptance: 23 May 2019

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

Review History

RSPB-2019-0399.R0 (Original submission)

Review form: Reviewer 1

Recommendation

Major revision is needed (please make suggestions in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

N/A

Is it clear?

N/A

Is it adequate?

N/A

Do you have any ethical concerns with this paper?

No

Comments to the Author

Comments are in the attached file (See Appendix A).

Review form: Reviewer 2

Recommendation

Major revision is needed (please make suggestions in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Acceptable

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

Comments to the Authors

In this manuscript, Hughes and Finarelli compare the olfactory bulb (OB) ratio in extant birds, alligator and dinosaurs to make inferences about the size of the olfactory receptor (OR) repertoire in ancestral nodes and extinct species, and then compare reptilian and avian ORs to make inferences about the diversity of the repertoire of the common ancestor of theropod dinosaurs. The major conclusion is that theropod dinosaurs had relatively large OBs which was probably associated with a sizeable OR repertoire and a keen sense of smell. A novel and interesting result.

The manuscript is divided into two fairly independent portions, the first one is devoted to comparing OB ratios to body size and the number of olfactory receptors in a given genome, and the second one is focused on the diversity of the OR repertoire. The first portion is pretty solid and straightforward: The authors used a comparative approach to make inferences about the olfactory bulb ratio in ancestral nodes and extinct species, and explore how this ratio correlates with body size, the size of the olfactory receptor repertoire in extant species, and diet. The second portion is focused on inferences about the diversity of the OR repertoire is clearly weaker, and the authors are very upfront about the limitations of this section of the analyses. The methods used to reconstruct the diversity of ancestral OR repertoires are a bit crude, the authors include both genes and pseudogenes and do not apply an evolutionary explicit model to pick representative sequences or make inferences about the presence of a given gene in an ancestral node. Further, the authors should probably include sequences from other testudines and crocodylian lineages to this portion of the study. This might be overkill, but it would provide much more solid estimates of the numbers of ORs per family present in the ancestor shown in table 1. The second weakness of this section is that because we have no genomic data for non-avian dinosaurs, their reconstruction can only tell us about the ORs putatively present in the last common ancestor of birds and crocodiles or the last common ancestor of birds, but not the last common ancestor of theropod dinosaurs.

Minor Issues

I think the title is somewhat misleading because the authors can make claims about the inferred size of the olfactory bulb in theropod dinosaurs and from there they can derive expectations about the size of their olfactory receptor repertoire, but their claims about the diversity of this repertoire have limited support.

The authors exclude three bird species in comparisons of the OB ratio because they have unusual

expansions of a particular family of ORs, which seems reasonable to draw inferences about the diversity of ancestral nodes. On the other hand, since they claim that the enlarged bulbs of some members of Tyrannosauroidae are linked to expansions. Are the expansions in chicken, zebra finch and budgerigar associated with increases in the OB ratio?

As a side note, we know that the elephant has the largest number of ORs present in mammals. This finding seems relevant to theirs, and I was wondering whether elephants also have expanded OBs? Or is the large OR repertoire in this group more related to the evolution of their trunk?

The authors should probably define the different taxonomic groups used in the analyses explicitly in the tree, to facilitate comprehension. I would assume most readers will not be intimately familiar with the terms Avialae. In addition, I would use the term sauropsids instead of the term reptiles, since the former is monophyletic and the second one is not.

Supplementary Table S3. The authors provide the inferred size of the OR repertoires for different nodes, but it is not possible to link the results from the table to nodes in the tree in figure 1.

There are 64 references in the text, but only 63 are listed in the literature cited portion of the study.

Decision letter (RSPB-2019-0399.R0)

26-Mar-2019

Dear Dr Hughes:

I am writing to inform you that your manuscript RSPB-2019-0399 entitled "Inferring the Composition of Olfactory Receptor Repertoires in Theropod Dinosaurs" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

- 1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
- 2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.

3) Line numbers in your main document.

In your revision process, please take a second look at how open your science is; our policy is that all data involved with the study should be made openly accessible-- see: <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/>
Insufficient sharing of data can delay or even cause rejection of a paper.

To upload a resubmitted manuscript, log into <http://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor John R. Hutchinson, Editor
Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:

Two expert reviewers have now seen your manuscript, and as you will see, both are positive towards the findings you have presented and both feel that they represent a significant, interesting and useful contribution. However both have a number of reservations about your manuscript that need to be addressed prior to publication, particularly regarding the inferences that can be drawn about the diversity of the OR repertoire in extinct species. This and several other issues raised by the two reviewers require a through reconsideration prior to publication.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Comments are in the attached file.

Referee: 2

Comments to the Author(s)

In this manuscript, Hughes and Finarelli compare the olfactory bulb (OB) ratio in extant birds, alligator and dinosaurs to make inferences about the size of the olfactory receptor (OR) repertoire in ancestral nodes and extinct species, and then compare reptilian and avian ORs to make inferences about the diversity of the repertoire of the common ancestor of theropod dinosaurs. The major conclusion is that theropod dinosaurs had relatively large OBs which was probably associated with a sizeable OR repertoire and a keen sense of smell. A novel and interesting result.

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with body size, the size of the olfactory receptor repertoire in extant species, and diet. The second portion is focused on inferences about the diversity of the OR repertoire is clearly weaker, and the authors are very upfront about the limitations of this section of the analyses. The methods used to reconstruct the diversity of ancestral OR repertoires are a bit crude, the authors include both genes and pseudogenes and do not apply an evolutionary explicit model to pick representative sequences or make inferences about the presence of a given gene in an ancestral node. Further, the authors should probably include sequences from other testudines and crocodylian lineages to this portion of the study. This might be overkill, but it would provide much more solid estimates of the numbers of ORs per family present in the ancestor shown in table 1. The second weakness of this section is that because we have no genomic data for non-avian dinosaurs, their reconstruction can only tell us about the ORs putatively present in the last common ancestor of birds and crocodiles or the last common ancestor of birds, but not the last common ancestor of theropod dinosaurs.

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As a side note, we know that the elephant has the largest number of ORs present in mammals. This finding seems relevant to theirs, and I was wondering whether elephants also have expanded OBs? Or is the large OR repertoire in this group more related to the evolution of their trumpet?

The authors should probably define the different taxonomic groups used in the analyses explicitly in the tree, to facilitate comprehension. I would assume most readers will not be intimately familiar with the terms Avialae. In addition, I would use the term sauropsids instead of the term reptiles, since the former is monophyletic and the second one is not.

Supplementary Table S3. The authors provide the inferred size of the OR repertoires for different nodes, but it is not possible to link the results from the table to nodes in the tree in figure 1.

There are 64 references in the text, but only 63 are listed in the literature cited portion of the study.

Author's Response to Decision Letter for (RSPB-2019-0399.R0)

See Appendix B.

RSPB-2019-0909.R0

Review form: Reviewer 1

Recommendation

Accept as is

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

Good

General interest: Is the paper of sufficient general interest?

Excellent

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

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

After reviewing the revised article and response to criticisms, I have concluded my criticisms were adequately addressed which improved the quality of the manuscript. I have no further comments and recommend this article for publication.

Review form: Reviewer 2

Recommendation

Accept with minor revision (please list in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

This is a revised version of RSPB-2019-0399, and the authors have done a good job addressing most of the issues I listed in the previous version.

Here are some remaining issues.

First, the authors provide the wrong citation for the olfactory repertoire of the African elephant, *Loxodonta africana*. The actual paper is the following:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4158756/>, by Niimura, Matsui and Touhara.

Secondly, when the authors discuss that a semi-aquatic lifestyle is associated with smaller OR repertoire, they should probably cite the work done on mammals on the subject, of which Ema Teeling is a corresponding author.

Finally, in their response letter, the authors indicate that 'the correlation between OB size and OR gene repertoire size has been noted in birds. There is a paucity of literature on whether such a relationship is observed in the crocodylian/testudine lineages, so were hesitant to include these taxa.' This statement would require more discussion, as dinosaurs are outside birds, and might exhibit relationships more typical of testudines or crocodylians. The central assumption for their inferences of olfactory receptor repertoire size is based on the assumptions that the relationship between OB ratio and OR repertoire size are fairly constant, and their statement seems to contradict their assumption to a certain extent. At the very least, this needs to be acknowledged.

In the first sentence of the last paragraph, the authors probably mean complement rather than compliment.

Decision letter (RSPB-2019-0909.R0)

13-May-2019

Dear Dr Hughes

I am pleased to inform you that your manuscript RSPB-2019-0909 entitled "Olfactory Receptor Repertoire Size in Dinosaurs" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation

It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:

- DNA sequences: Genbank accessions F234391-F234402
- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

If you wish to submit your data to Dryad (<http://datadryad.org/>) and have not already done so you can submit your data via this link

[http://datadryad.org/submit?journalID=RSPB&manu=\(Document not available\)](http://datadryad.org/submit?journalID=RSPB&manu=(Document%20not%20available)) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/> for more details.

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

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

Sincerely,

Professor John R. Hutchinson, Editor

Proceedings B

mailto:proceedingsb@royalsociety.org

Associate Editor

Board Member

Comments to Author:

Both expert reviewers have now read your revised manuscript, and have considered your responses to their comments and criticisms, and I am pleased to inform you that both are very satisfied with your revision. Only Reviewer 2 has a few further minor suggestions for improvement and these should be addressed prior to publication (which should not be a particularly onerous task).

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

After reviewing the revised article and response to criticisms, I have concluded my criticisms were adequately addressed which improved the quality of the manuscript. I have no further comments and recommend this article for publication.

Referee: 2

Comments to the Author(s).

This is a revised version of RSPB-2019-0399, and the authors have done a good job addressing most of the issues I listed in the previous version.

Here are some remaining issues.

First, the authors provide the wrong citation for the olfactory repertoire of the African elephant, *Loxodonta africana*. The actual paper is the following:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4158756/>, by Niimura, Matsui and Touhara.

Secondly, when the authors discuss that a semi-aquatic lifestyle is associated with smaller OR repertoire, they should probably cite the work done on mammals on the subject, of which Ema Teeling is a corresponding author.

Finally, in their response letter, the authors indicate that 'the correlation between OB size and OR gene repertoire size has been noted in birds. There is a paucity of literature on whether such a relationship is observed in the crocodylian/testudine lineages, so were hesitant to include these taxa.' This statement would require more discussion, as dinosaurs are outside birds, and might exhibit relationships more typical of testudines or crocodylians. The central assumption for their inferences of olfactory receptor repertoire size is based on the assumptions that the relationship between OB ratio and OR repertoire size are fairly constant, and their statement seems to contradict their assumption to a certain extent. At the very least, this needs to be acknowledged.

In the first sentence of the last paragraph, the authors probably mean complement rather than compliment.

Author's Response to Decision Letter for (RSPB-2019-0909.R0)

See Appendix C.

Decision letter (RSPB-2019-0909.R1)

23-May-2019

Dear Dr Hughes

I am pleased to inform you that your manuscript entitled "Olfactory Receptor Repertoire Size in Dinosaurs" has been accepted for publication in Proceedings B.

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

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

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

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Electronic supplementary material:

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

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

Congratulations!!

Sincerely,

Professor John R. Hutchinson, Editor

Proceedings B

<mailto:proceedingsb@royalsociety.org>

Appendix A

Inferring the composition of olfactory receptor repertoires in theropod dinosaurs

Overall

These analyses attempted to reconstruct and estimate the olfactory receptor repertoire of extinct theropod dinosaurs. They used OR counts from extant archosaurs and olfactory bulb size ratios to model and predict OR sizes. I think the methods were appropriate and the conclusions were supported by the analyses. I found this work particularly interesting and relevant to the scientific community. However, there are some issues in the current draft that need to be addressed prior to publication. I look forward to seeing a revised and improved version of this report.

Major comments

1. Please make a whole separate methods section about the construction of the dinosaur tree. It was a little tricky finding the description of the tree in the olfactory bulb ratios section, I think it would be better as a separate section with a little more detail.

“A composite phylogeny of all 76 species was generated for these phylogenetic comparative analyses (Abelisauridae [44], Allosauroidea [45], Ankylosauridae [46], Aves [47], Dromeosauridae [48], Ornithomimosauria [49], Therizinosauria [50], Tyrannosauroidea [51]), with lengths inferred using the Grafen method [52]”

This wasn't a very informative description to me.

2. It's not until the results before you discuss the different OR subfamilies. I think a description of the subfamilies and Class I/Class II discussion should be moved from the results section to the introduction. Further, I think you need to explicitly state how you assign ORs to different subfamilies in the methods.

3. There were a lot of issues in the supplemental materials, particularly the supplemental figures.

a) Two figures, supplementary Figure S3 and S5 could not be displayed. I don't know if that's a journal error or an author error, but I couldn't view either of those two figures.

b) Sup Fig 4 looks like a copy of Sup Fig 1 instead of an original figure.

c) What metric was used for body mass in Sup Figs S1 and S2? I'm assuming grams, but that's not presented in the figure, and I'm pretty sure a T-rex doesn't weigh 10 kg... Please clarify.

d) I don't like seeing fasta formatted sequences in a pdf table. It's kind of a pain for others to gather sequences from a pdf. I'd recommend making a separate fasta file with OR sequences.

4. Is this statement on line 181 “showed that piscivory was significantly associated with larger OB ratios ($p = 0.004$)” missing a test statistic, i.e. r ? How was that test made, I couldn't find it in the methods.

More importantly, this is kind of an interesting result, and I didn't see much discussion about it. May be worthwhile to elaborate.

Minor comments

Minor typos throughout need to be fixed.

Khan et al. 2015 and Zelenitsky et al 2009 should probably be cited on line 82 because they also correlated OR gene number to olfactory bulb size.

This is really minor, but the stats reporting varies and it's a little distracting. L 176 has the r 1st and p value 2nd, whereas in line 182, the p value is reported first. Make sure all of that is consistent.

I don't think it would hurt to mention that an estimated 497 ORs in dinosaurs isn't an absurd estimate given that most terrestrial vertebrates hover around the 400-600 OR range including snakes, crocodylians and some birds.

Also in regards to the discussion about herbivores not having smaller OR repertoires, I think it would be worth mentioning the elephant has the largest OR repertoire discovered up to this point.

The statement "We further explored OR gene families with known de-orphaned odorant ligands in human and mouse genes to identify the potential odorant-space [56-58] that was perceivable by extinct dinosaur taxa." isn't very clear to me, I may not be understanding what de-orphaned means.

Citation 64 doesn't exist in the bibliography, there are only 63 articles in the bibliography. Citation 63 in text doesn't sync to citation 63 in the bibliography. Citation 62 doesn't exist in text. Make sure your citations are proper.

Figure 1: I'm a little confused. The alligator and other crocodylians have ~500 intact OR genes, so where does the 1077 come from? I'm assuming that number includes pseudogenes, but you were ignoring pseudogenes right? Were analyses conducted with the 1077 or the more appropriate ~500? Please check that.

Appendix B

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

Comments are in the attached file.

Overall

These analyses attempted to reconstruct and estimate the olfactory receptor repertoire of extinct theropod dinosaurs. They used OR counts from extant archosaurs and olfactory bulb size ratios to model and predict OR sizes. I think the methods were appropriate and the conclusions were supported by the analyses. I found this work particularly interesting and relevant to the scientific community. However, there are some issues in the current draft that need to be addressed prior to publication. I look forward to seeing a revised and improved version of this report.

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This wasn't a very informative description to me.

>>We have now added a separate section: ‘Phylogenetic tree topology’, where we detail how we constructed the tree topology.

2. It's not until the results before you discuss the different OR subfamilies. I think a description of the subfamilies and Class I/Class II discussion should be moved from the results section to the introduction. Further, I think you need to explicitly state how you assign ORs to different subfamilies in the methods.

>>We have added more text about the OR gene classes and subfamilies to the introduction. We give information on their function and detail that they are distinct monophyletic gene families. We also emphasise the usage of Hidden Markov Models to assign OR genes to these specific subfamilies in the materials and methods.

3. There were a lot of issues in the supplemental materials, particularly the supplemental figures.

a) Two figures, supplementary Figure S3 and S5 could not be displayed. I don't know if that's a journal error or an author error, but I couldn't view either of those two figures.

>>We believe that this was an issue with the original document. We created a single PDF containing all of the supplemental figures, and have also made these available via Figshare (<https://figshare.com/s/41d98e65fb5899c261da>). This ensures that all of our data is easily and openly accessible.

b) Sup Fig 4 looks like a copy of Sup Fig 1 instead of an original figure.

>>Again, we believe that this was related to the display issues in the original document that we mentioned above. Supplemental Figure 1 is a plot of body mass vs OB ratio while the (original) Supplemental Figure 4 was a phylogeny showing ancestral node estimates for OB ratios using PICs. We hope that the single PDF, and making these available via Figshare solves this issue.

c) What metric was used for body mass in Sup Figs S1 and S2? I'm assuming grams, but that's not presented in the figure, and I'm pretty sure a T-rex doesn't weigh 10 kg... Please clarify.

>>Both Supplemental Figures S1&S2 measure body mass in Kg. We have made this explicit in both figure captions.

d) I don't like seeing fasta formatted sequences in a pdf table. It's kind of a pain for others to gather sequences from a pdf. I'd recommend making a separate fasta file with OR sequences.

>>We have removed this table and the sequence data have been added as a separate ASCII text file, per reviewer's suggestions. It is available on Figshare at: <https://figshare.com/s/41d98e65fb5899c261da>

4. Is this statement on line 181 "showed that piscivory was significantly associated with larger OB ratios ($p = 0.004$)" missing a test statistic, i.e. r ? How was that test made, I couldn't find it in the methods. More importantly, this is kind of an interesting result, and I didn't see much discussion about it. May be worthwhile to elaborate.

>> This comparison across diets was carried out using generalized least squares, correcting for phylogeny. This is detailed in the materials and methods. However, these data are categorical, so there is no correlation coefficient. We have expanded our text in the results and discussion of this section to be clearer about the results.

Minor comments

Minor typos throughout need to be fix.

>>We have addressed this throughout

1. Khan et al. 2015 and Zelenitsky et al 2009 should probably be cited on line 82 because they also correlated OR gene number to olfactory bulb size.

>>This was an oversight on our part. These papers are now cited.

2. This is really minor, but the stats reporting varies and it's a little distracting. L 176 has the r 1st and p value 2nd, where as in line 182, the p value is reported first. Make sure all of that is consistent.

>>The reporting of our statistics has been standardized throughout.

3. I don't think it would hurt to mention that an estimated 497 ORs in dinosaurs isn't an absurd estimate given that most terrestrial vertebrates hover around the 400-600 OR range including snakes, crocodilians and some birds.

>>This is a good point, and we have included this in our discussion.

4. Also in regards to the discussion about herbivores not having smaller OR repertoires, I think it would be worth mentioning the elephant has the largest OR repertoire discovered up to this point.

>>We agree, and have now added a comment on the elephant OR repertoire in our discussion.

5. The statement “We further explored OR gene families with known de-orphaned odorant ligands in human and mouse genes to identify the potential odorant-space [56-58] that was perceivable by extinct dinosaur taxa.” isn’t very clear to me, I may not be understanding what de-orphaned means.

>>We have now changed the wording of this statement, defining the term ‘de-orphaned’.

6. Citation 64 doesn’t exist in the bibliography, there are only 63 articles in the bibliography. Citation 63 in text doesn’t sync to citation 63 in the bibliography. Citation 62 doesn’t exist in text. Make sure your citations are proper.

>>We have corrected this

7. Figure 1: I’m a little confused. The alligator and other crocodylians have ~500 intact OR genes, so where does the 1077 come from? I’m assuming that number includes pseudogenes, but you were ignoring pseudogenes right? Were analyses conducted with the 1077 or the more appropriate ~500? Please check that.

>>The reviewer is correct, the number “1077” is the full OR gene repertoire, including intact OR genes and OR pseudogenes, based on Green *et al.*, 2014 (supplemental material). We expect the olfactory bulb size to be a function of absolute OR gene repertoire size (both intact and non-functional ORs Steiger *et al.*, 2008 . Khan *et al.* 2015 and Zelenitsky *et al.* 2009). As such, we used the absolute repertoire size (1077, not ~550) for modeling.

However, we realize that this confusion was likely caused by our not stating this clearly enough. We have modified our text in the introduction and materials and methods sections. We have also added this to the caption for Figure 1.

Referee: 2

Comments to the Author(s)

In this manuscript, Hughes and Finarelli compare the olfactory bulb (OB) ratio in extant birds, alligator and dinosaurs to make inferences about the size of the olfactory receptor (OR) repertoire in ancestral nodes and extinct species, and then compare reptilian and avian ORs to make inferences about the diversity of the repertoire of the common ancestor of theropod dinosaurs. The major conclusion is that theropod dinosaurs had relatively large OBs which was probably associated with a sizeable OR repertoire and a keen sense of smell. A novel and interesting result.

The manuscript is divided into two fairly independent portions, the first one is devoted to comparing OB ratios to body size and the number of olfactory receptors in a given genome, and the second one is focused on the diversity of the OR repertoire. The first portion is pretty solid and straightforward: The authors used a comparative approach to make inferences about the olfactory bulb ratio in ancestral nodes and extinct species, and explore how this ratio correlates with body size, the size of the olfactory receptor repertoire in extant species, and diet.

The second portion is focused on inferences about the diversity of the OR repertoire is clearly weaker, and the authors are very upfront about the limitations of this section of the analyses.

1. The methods used to reconstruct the diversity of ancestral OR repertoires are a bit crude, the authors include both genes and pseudogenes and do not apply an evolutionary explicit model to pick representative sequences or make inferences about the presence of a given gene in an ancestral node.

>>Our decision to include both functional and non-functional OR genes when estimating ancestral OR repertoires is based on previous studies (Please see the final response to Reviewer 1's comments). In addition, Hughes et al. (2018, *MBE*: citation [10] in main text) found that the absolute number of OR genes (functional and non-functional) is a good indicator of expansion/contraction of the OR repertoire in an evolutionary lineage. Our method of ancestral state reconstruction is a phylogenetically-explicit model across the topology

As such, reconstruction of an ancestral gene sequence is not the aim of our study. Rather the character of interest that we are tracking along the phylogeny is the number of OR gene copies along each lineage, both in the aggregate and within specific gene families, documenting expansion and contraction across time and phylogeny. We have applied ancestral state reconstruction methods based on numeric (counts) data with our phylogeny. To complement this, we have also used comparative genomics methods to identify bona-fide orthologs shared across the genomes of both Aves and non-avian sauropsids.

When mapping orthologs from "reptile" species (*Alligator sinensis*, *Alligator mississippiensis*, *Chrysemys picta*, *Anolis carolinensis*, *Python molurus*) to avian data, within-sauropsid orthologs were clustered to avoid double-mapping the same OR. Within clusters of 80% shared amino acid identity, the longest sequence was chosen as representative for each cluster. An evolutionary model

was deemed unnecessary at this point as each sequence shared at least 80% identity, so sequence length, representing maximum protein information, was used to choose a representative mapping protein. Because the goal was to quantify a minimum size of the ancestral OR repertoire, not the nucleotide or amino acid sequence composition, we believe this method is appropriate.

A gene that is determined to be present in both one extant avian species and one non-avian sauropsid, using our 65% shared identity threshold, would suggest that this gene, or some paralog of it, was present at the node defining the most recent common ancestor of all Sauropsida. We therefore use this as a means of determining a minimum OR repertoire size in the Sauropsida MRCA.

2. Further, the authors should probably include sequences from other and crocodylian lineages to this portion of the study. This might be overkill, but it would provide much more solid estimates of the numbers of ORs per family present in the ancestor shown in table 1.

>>In estimating our model ($\text{repertoire_size} = 5.41 \cdot \text{OB_ratio} + 260.77$) we only included extant birds, because the correlation between OB size and OR gene repertoire size has been noted in birds. There is a paucity of literature on whether such a relationship is observed in the crocodylian/testudine lineages, so we were hesitant to include these taxa.

There is also a lack of OB ratio data specific to these taxa. We did find OB ratio for *A. mississippiensis*, and we did model the OB-ratio/OR repertoire with the Alligator, with predicted repertoire results in supplemental table 5. We have made this more explicit in our materials and methods.

When using shared sequence homology to estimate OR numbers, we include sequences for several non-avian taxa (*Alligator sinensis*, *A. mississippiensis*, *Chrysemys picta*, *Anolis carolinensis*, *Python molurus*), as this analysis does not rely on OB ratios. We have made this point clearer in the text.

3. The second weakness of this section is that because we have no genomic data for non-avian dinosaurs, their reconstruction can only tell us about the ORs putatively present in the last common ancestor of birds and crocodiles or the last common ancestor of birds, but not the last common ancestor of theropod dinosaurs.

>>This is the unfortunate reality of inferences of genome composition in non-avian dinosaurs (or almost all extinct taxa). Given the diversity of OR genes among extant taxa, and the fact that OR gene subfamilies contain a multitude of paralogs, it is not possible to use ancestral sequence reconstruction to reconstruct a meaningful gene sequence, as might be possible for a more highly-conserved, single-copy gene with little-to-no serial duplication. Therefore we can only report an inference of the quantity of OR genes in the ancestral repertoire, not the sequence composition.

Minor Issues

1. I think the title is somewhat misleading because the authors can make claims about the inferred size of the olfactory bulb in theropod dinosaurs and from there they can derive expectations about the size of their olfactory receptor repertoire, but their claims about the diversity of this repertoire have limited support.

>>**We have taken this suggestion on board, and have modified our title to “Olfactory Receptor Repertoire Size in Dinosaurs” to better represent the study.**

2. The authors exclude three bird species in comparisons of the OB ratio because they have unusual expansions of a particular family of ORs, which seems reasonable to draw inferences about the diversity of ancestral nodes. On the other hand, since they claim that the enlarged bulbs of some members of Tyrannosauoidea are linked to expansions. Are the expansions in chicken, zebra finch and budgerigar associate with increases in the OB ratio?

>>**We include below the OB ratios and OR gene counts for each of the three excluded species in comparison to their sister taxa in our phylogeny and also other species with similar OB ratios (* indicates excluded species, + indicates closest sister taxon):**

<i>*Taeniopygia guttata</i>	OB: 9.7, ORs: 688
+ <i>Geospiza fortis</i>	OB: 9.7 ORs: 182
<i>Manacus vitellinus</i>	OB: 9.7 ORs: 227
<i>Acanthisitta chloris</i>	OB: 9.7 ORs: 222
<i>Colius striatus</i>	OB: 9.7 ORs: 292
<i>Pelecanus crispus</i>	OB: 9.7 ORs: 330

<i>*Melopsittacus undulatus</i>	OB: 6.2, ORs: 484
+ <i>Nestor notabilis</i>	OB: 8 ORs: 239
<i>Corvus brachyrhynchos</i>	OB: 5 ORs: 229

<i>*Gallus gallus</i>	OB: 15.4 ORs: 674
+ <i>Meleagris gallopavo</i>	OB: 13.5 ORs: 313
<i>Charadrius vociferous</i>	OB: 16.1 ORs: 393
<i>Merops nubicus</i>	OB: 14.5 ORs: 252

We find that the OR repertoire size in the sister taxon to each anomalous species is more similar to other taxa of similar OB size. Moreover, it has been noted that these taxa have experienced their expansion principally in OR gene family 14. This would imply that the expansion observed in family 14 in these species occurred independently after their divergence from their sister taxa in this phylogeny, and appear to be independent of OB ratio. As such, we excluded these three taxa (see also: Khan *et al* (2015; reference 19 in main text).

The cause of such dramatic expansions in this particular gene family, which appears to be convergently achieved in at least three lineages should be investigated. But it is unlikely that this corresponds to the more general rule of OB expansion/contraction observed across all of Aves.

However, the reviewer makes a good point that this can appear as cherry-picking of our data. We have carried out all analyses of OB-ratio/OR repertoire size with and without these three outlier taxa. We show that their inclusion in the analyses does not have a significant on the results.

3. As a side note, we know that the elephant has the largest number of ORs present in mammals. This finding seems relevant to theirs, and I was wondering whether elephants also have expanded OBs? Or is the large OR repertoire in this group more related to the evolution of their trunk?

>> Rizvanovic et al. (2012;<https://doi.org/10.1093/chemse/bjs097>) describe the absolute size of the African elephant olfactory bulb is given as 59.9-62.4cm³. This is described as being far greater than a non-proboscidian mammal. However this paper uses volumes of the olfactory bulb, not the OB ratio, which is a ratio of chords across the forebrain. There appears to be a difference in convention for describing relative olfactory bulb size between mammal (volume) and “reptile”/bird researchers (OB ratios). Additionally, they do not investigate OR gene repertoire size in this study. Therefore we have decided to avoid including their metrics directly, as combining linear and volumetric ratios might be misleading. We do now refer to this study and the large size of the elephant olfactory bulb in our discussion.

4. The authors should probably define the different taxonomic groups used in the analyses explicitly in the tree, to facilitate comprehension. I would assume most readers will not be intimately familiar with the terms Avialae. In addition, I would use the term sauropsids instead of the term reptiles, since the former is monophyletic and the second one is not.

>>We now have highlighted Avialae on the tree in Figure 1, and explicitly define it at its first mention in the main text. We have also changed the term ‘reptile’ to sauropsid and we specify “*non-avian sauropsids, referred to from here simply as ‘sauropsids’*”.

5. Supplementary Table S3. The authors provide the inferred size of the OR repertoires for different nodes, but it is not possible to link the results from the table to nodes in the tree in figure 1.

>>We have now added an additional column to this table detailing the node that it represents facilitating these results being linked to Figure 1.

6. There are 64 references in the text, but only 63 are listed in the literature cited portion of the study.

>>See the same comment by reviewer 1 above. This has been corrected.

Appendix C

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

After reviewing the revised article and response to criticisms, I have concluded my criticisms were adequately addressed which improved the quality of the manuscript. I have no further comments and recommend this article for publication.

Referee: 2

Comments to the Author(s).

This is a revised version of RSPB-2019-0399, and the authors have done a good job addressing most of the issues I listed in the previous version.

Here are some remaining issues.

1. First, the authors provide the wrong citation for the olfactory repertoire of the African elephant, *Loxodonta africana*. The actual paper is the following: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4158756/>, by Niimura, Matsui and Touhara.

>>We now provide the appropriate citation for this statement (Niimura *et al.* 2014).

2. Secondly, when the authors discuss that a semi-aquatic lifestyle is associated with smaller OR repertoire, they should probably cite the work done on mammals on the subject, of which Ema Teeling is a corresponding author.

>>We now include a citation for Hayden *et al.* 2010 when discussing the aquatic lifestyles, as suggested.

3. Finally, in their response letter, the authors indicate that ‘the correlation between OB size and OR gene repertoire size has been noted in birds. There is a paucity of literature on whether such a relationship is observed in the crocodylian/testudine lineages, so were hesitant to include these taxa.’ This statement would require more discussion, as dinosaurs are outside birds, and might exhibit relationships more typical of testudines or crocodylians. The central assumption for their inferences of olfactory receptor repertoire size is based on the assumptions that the relationship between OB ratio and OR repertoire size are fairly constant, and their statement seems to contradict their assumption to a certain extent. At the very least, this needs to be acknowledged.

>>We now acknowledge that we make the assumption that avian lineages, rather than crocodylian/testudine lineages, reflect that of theropod lineages. We now highlight this both in our Materials and Methods and Discussion:

Supplementary Materials and Methods

“Given the paucity of literature on whether or not an OB-ratio/OR repertoire size correlation exists in the crocodylian or testudine lineages, and the general lack of OB ratios for taxa for which genome sequences are available, we only included the American alligator as an outgroup. For the PGLS modeling, we make the assumption that theropod dinosaurs reflect scaling patterns more similar to the avian lineage rather than crocodylian/testudine lineages.”

Discussion

“The PGLS models assume that dinosaurs reflect avian OB-ratio/OR repertoire scaling, rather than that of the crocodylian or testudine lineages, and therefore may not completely reflect OR repertoire size among theropods. However, the other estimates do not rely on this assumption about OB-ratio/OR repertoire correlations, and the minimum estimates we reconstruct across methods are consistent with the 400-600 OR gene range found in many extant vertebrates”

4. In the first sentence of the last paragraph, the authors probably mean complement rather than compliment.

>>The reviewer is correct and we have now replaced compliment with complement.