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# Greater variability in chimpanzee (*Pan troglodytes*) brain structure among males

Alex R. DeCasien, Chet C. Sherwood, Steven J. Schapiro and James P. Higham

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**Review timeline**
Original submission: 11 December 2019
Revised submission: 18 March 2020
Final acceptance: 24 March 2020

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

# Review History

## RSPB-2019-2858.R0 (Original submission)

## Review form: Reviewer 1

**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?**
Acceptable

**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?**
Yes

**Comments to the Author**
The present manuscript investigates whether male chimpanzees have greater variability in brain structure than females. To do so, they examine the relative and absolute surface areas of several sulci in 91 males and 135 females. Their main findings are 1) males have more variability in brain structures than females; 2) brain structure is heritable and 3) Males exhibit a stronger correlation between sulcal sizes. The MS is overall well written and the analyses seem well performed and adequate to resolve the main question. However, there are some issues that I think should be addressed prior to publication.

My main concern is in the structure of the introduction. Some of the predictions presented in the last paragraph of the introduction are not sufficiently explained beforehand in the introduction. For instance, nothing about heritability is mentioned in the introduction before the presentation of the predictions, although it seems that is one of the predictions the authors want to test.

Also related to the previous point, I find that the 3rd paragraph of the introduction sounds a bit out of place. Initially, I did not understand why devoting an entire paragraph to one of the possible mechanisms (X-linked genes) and even one that was not specific for cognitive traits. However, at the end of the introduction, I understood that this paragraph was related to one of the predictions they wanted to test (#3). Maybe these paragraphs and the 3rd prediction should be closer to each other or try to link them in some way?

In addition, could it be possible to try to link the three predictions? Correct me if I am wrong but after reading the introduction, I get that the main hypothesis to test here is that males should have greater variability in cognitive traits for different reasons and that this has been tested in humans but not in animals, so testing this idea in Chimpanzees is the main novelty here. This refers mainly to prediction 1, but then, do we have different expectations depending on which mechanism is behind the greater variability of males? Do we expect higher heritability if sexual selection is behind the pattern, but not in other mechanisms? Maybe these types of questions could be used to frame predictions 2 and 3 as a complementary analysis to the "main" prediction 1.

Finally, could it be possible to add an initial paragraph or a sentence in the introduction, as well as in the last paragraph of the discussion to try to broaden the scope of the study? In the current version, I feel that the authors go directly to the point of the study -which is not a bad idea- but I think it would be useful for the broader readership of Proceedings B to try to see which are the broader context and implications of this study.

More specific comments:

- Regarding the statistical analysis, I wonder if the authors could specify a threshold to consider that they found a reduced autocorrelation. For instance, 0.1 is often used as such value, following MCMCglmm tutorial guidelines.

- Authors are often referring to P-values as the proportion of simulations that met some criteria (e.g. lower DIC than compared variable). However, the term "P-value" is incorrect here, and it would be more accurate to call it "P" or any other name. It's just to avoid confusing p-value with the real p-value term used in frequentist statistics. That's why the MCMCglmm packages provide pMCMC and not a p-value, where pMCMC is the proportion of samples in the MCMC chain that are above or below zero.

- When authors refer to "motor-control-associated regions" (7th paragraph of the discussion) and to "multiple inhibition-related areas" (8th paragraph in the discussion), it would be useful to mention which areas are they referring to (Maybe in brackets?).

## 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?**
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?**
Yes

**Comments to the Author**
This paper generated new data about the size of cortical sulci in chimpanzees and found that males have greater phenotypic variability than females in this feature. They relate this bimodal sexual selection, singular expression of X chromosome alleles, and extended development in males. In addition, it reports on sulcal size heritability and finds sex differences.
They also found that male sulcal size co-vary more in males than in females.

The main contributions and conclusions of this study are novel and relevant.

General Comments

The Introduction is clear and well organised.

Relevant methodological details are limited to the Supplement. Hardly anything is included about how the new data were generated. Certain details seem crucial and should be included, such as how the anatomical data was labelled.

Further, how was a pedigree determined, and was it available for the entire sample?

The Results section should make it clear that the data acquired were anatomical. In other words, the specific sulci should be listed, but not the presumed functional relevance of those anatomical changes. The associations are not specifically supported by this study, and should be left for the Discussion. Further, there is no analysis referenced to warrant naming the associations in the Results section.

The Discussion section is far too long and references a lot of very high level human neuropsychology and chimpanzee social behaviour which may be a stretch beyond what what actually studied. Further, it is not clear that sulci which do not show greater variability in males lack similar associations.

Specific Comments

p.5 There could be more detailed discussion about the link between brain sulci and cognitive function, within and between species, since this is so essential to the Discussion. Further, the limitations of using sulci as markers for functional areas, particularly across different species, are hardly stated.

p. 10, line 1, which "anatomical regions"?

## Review form: Reviewer 3 (Orlin S. Todorov)

**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.**
Yes

**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**
The paper presents a study exploring the variability of sulcal sizes between sexes in Chimpanzee. It provides a novel and valuable insight into a topic that has attracted a lot of attention – the greater male variability hypothesis – and elucidates a novel neuromophological aspect of this effect, sulcal size variability. The sample size is adequate, and the data is analyzed appropriately with contemporary statistical approaches. Despite the lack of details about the heritability analysis included, and some minor points throughout, I consider that the study is worth publishing after minor revisions. If the alpha level needs to be adjusted due to multiple comparisons, many of the results might not stand, so I think this is an important issue to be addressed too. See my detailed comments:

The manuscript does not include line numbers, which makes reviewing and commenting it not very convenient.

Introduction
Page 1, line 4 "For example, male barn swallow tail length…" – this is a bit odd example, considering that the sentence leading up to it reads "Across mammals…". I'd recommend using an example from mammals.
Page 1, End of the first paragraph - The lek paradox, to my knowledge, is observed in lek mating species, where consistent female preferences are present. I don't think that this is applicable to chimpanzee mating systems, where female preference is not consistent, lekking not present, and mating is mainly promiscuous and opportunistic.
Page 6, Prediction 3 – Is sulcal size related to the X chromosome at all? This prediction needs to be backed up by a statement/study that shows that there is indeed a relationship between any

aspect of sulcal morphology and proteins encoded from X chromosome genes and such relationship is warranted. References 9 and 10 do not give any suggestion as to why sulcal morphology relates to the X chromosome.
Introduction, general – There is no mention of the 'Variability hypothesis' (also know as 'greater male variability hypothesis'). The authors had tried to lay down their own arguments, but this is a well-argued hypothesis with a long history, and it needs to be mentioned as it is.
Heritability is mentioned in passing at the end of the introduction. The authors need to elaborate on the concept and explain what they mean by that and how is it relevant to the question at hand at all.
Materials and methods
Given that age of maturity differs between males and females in chimpanzees it seems reasonable to only include mature individuals. At the same time, senescence has a significant effect on brain morphology, so again, old aged individuals may also be excluded? (I.e. Chen X, Errangi B, Li L, et al. Brain aging in humans, chimpanzees (Pan troglodytes), and rhesus macaques (Macaca mulatta): magnetic resonance imaging studies of macro- and microstructural changes. Neurobiol Aging. 2013;34(10):2248–2260. doi:10.1016/j.neurobiolaging.2013.03.028).
Additionally, average age and SD for both sex groups should be included, along with the already present mean/SD value for the whole sample.
It is interesting to see the boxplots for the outlier analysis, as the 1.5 times beyond the interquartile range rule of thumb is not a golden rule, and if there are specimens very close to that range i.e. (1.6) they might be included too.
When calculating the sex specific variance why wasn't overall brain size/volume/weight or body size/weight corrected for? Sulcal size is a function of overall brain size and approaching the question without controlling for it might just reveal overall greater variance in body/brain size in the larger sulci.
Heritability – this whole section needs more detail and clear explanation as to its necessity in the introduction. There is no explanation as to what heritability analysis is, the formula included is not explained (there are parameters there that need spelling out) and in general the whole section needs to include more detail and clarity as to rationale behind it.
Results
Given the approach of multiple comparisons the alpha level should be adjusted to a more conservative one.
Correlations – This prediction was based on males's homozygosity but still there are 6 sulci that are more correlated in females than in males? Figure S49 does not have a legend explaining the red, green and white spaces.
Discussion
The whole discussion alludes to relationships between brain structure and function, but it does not make clear how variability (high or low) relates to that. For example, it is suggested that behavioral inhibition is an aspect of male's mating and foraging behavior and this somehow should warrant greater fitness benefits from variability. It is interesting to think about how low variability in females is also related to their specific mating and foraging behaviors. The same argument can be applied to other systems the authors are discussing (facial recognition, gaze processing etc).

## Decision letter (RSPB-2019-2858.R0)

18-Feb-2020

Dear Ms DeCasien:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. We all find your manuscript to be of interest and appropriate for Proceedings B. However, the reviewers raise a number of issues, primarily about the structure of the

Introduction and hypotheses, and questions about your statistics that need to be addressed. Their (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. I invite you to revise your manuscript accordingly to address the concerns that they have raised.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please 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 Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the 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.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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
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If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

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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. Please try to submit all supplementary material as a single file.

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].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
The reviewers agree, as do I, that this study presents interesting and novel data, and makes a valuable contribution to the literature. There are, however, a number of issues that need to be addressed before the study is ready for publication. In particular, reviewers 1 and 3 raises some valid points about the structure and content of the introduction; addressing these could help to improve the clarity and the flow of the paper. In addition all three reviewers point out a number of areas where further detail on aspects of the methodology and presentation of the results require some clarification. Finally as R3 rightly points out, it is currently not entirely clear how sex differences in the variability of sulcus size relate to the points about brain structure and function in the discussion.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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Referee: 2

Comments to the Author(s)
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They also found that male sulcal size co-vary more in males than in females.

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Further, how was a pedigree determined, and was it available for the entire sample?

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p.5 There could be more detailed discussion about the link between brain sulci and cognitive function, within and between species, since this is so essential to the Discussion. Further, the limitations of using sulci as markers for functional areas, particularly across different species, are hardly stated.

p. 10, line 1, which "anatomical regions"?

Referee: 3

Comments to the Author(s)
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Page 6, Prediction 3 – Is sulcal size related to the X chromosome at all? This prediction needs to be backed up by a statement/study that shows that there is indeed a relationship between any aspect of sulcal morphology and proteins encoded from X chromosome genes and such relationship is warranted. References 9 and 10 do not give any suggestion as to why sulcal morphology relates to the X chromosome.
Introduction, general – There is no mention of the 'Variability hypothesis' (also know as 'greater male variability hypothesis'). The authors had tried to lay down their own arguments, but this is a well-argued hypothesis with a long history, and it needs to be mentioned as it is.
Heritability is mentioned in passing at the end of the introduction. The authors need to elaborate on the concept and explain what they mean by that and how is it relevant to the question at hand at all.

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It is interesting to see the boxplots for the outlier analysis, as the 1.5 times beyond the interquartile range rule of thumb is not a golden rule, and if there are specimens very close to that range i.e. (1.6) they might be included too.
When calculating the sex specific variance why wasn't overall brain size/volume/weight or body size/weight corrected for? Sulcal size is a function of overall brain size and approaching the question without controlling for it might just reveal overall greater variance in body/brain size in the larger sulci.
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Results
Given the approach of multiple comparisons the alpha level should be adjusted to a more conservative one.
Correlations – This prediction was based on males's homozygosity but still there are 6 sulci that are more correlated in females than in males? Figure S49 does not have a legend explaining the red, green and white spaces.

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The whole discussion alludes to relationships between brain structure and function, but it does not make clear how variability (high or low) relates to that. For example, it is suggested that behavioral inhibition is an aspect of male's mating and foraging behavior and this somehow should warrant greater fitness benefits from variability. It is interesting to think about how low variability in females is also related to their specific mating and foraging behaviors. The same argument can be applied to other systems the authors are discussing (facial recognition, gaze processing etc).

# Author's Response to Decision Letter for (RSPB-2019-2858.R0)

See Appendix A.

# Decision letter (RSPB-2019-2858.R1)

24-Mar-2020

Dear Ms DeCasien

I am pleased to inform you that your manuscript entitled "Greater Variability in Chimpanzee (Pan troglodytes) Brain Structure Among Males" 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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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.

Sincerely,
Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

# Appendix A

Manuscript RSPB-2019-2858
Response to Reviewers

**Please find attached a new version of manuscript RSPB-2019-2858, which has been revised in response to reviewer and editor comments at *Proceedings B*. We are very grateful for the constructive feedback, which has significantly improved the manuscript. Below, reviewer and editor comments are presented in italics, while our responses are given in bold font. The line numbers we provide correspond to the revised document (without markup).**

**Associate Editor (Remarks to the Author):**

*The reviewers agree, as do I, that this study presents interesting and novel data, and makes a valuable contribution to the literature.*

**We thank the Editor for this comment and for their constructive feedback.**

*There are, however, a number of issues that need to be addressed before the study is ready for publication. In particular, reviewers 1 and 3 raises some valid points about the structure and content of the introduction; addressing these could help to improve the clarity and the flow of the paper.*

**We have followed feedback from the Editor and Reviewers and have restructured the Introduction.**

**First, we better address the topic of heritability throughout the Introduction. Early in the Introduction, we define heritable traits as "trait[s] for which variation is explained by some genetic, rather than purely environmental, variation" and state that selection can only act on heritable traits (Lines 45-46). We also describe more explicitly why we predict sulcal sizes to be heritable in this case (Lines 122-125).**

**Second, we provide more background about the relevant evolutionary and developmental mechanisms early in the Introduction, so that sex chromosome effects are not discussed disproportionately (selection: Lines 43-49; developmental timing: Lines 50-57; sex chromosomes: Lines 58-44). We also now explicitly mention that the X chromosome is likely to play a role in the development of sulcal morphology (Lines 118-120). Finally, we better link our prediction regarding higher inter-regional correlations in males (Prediction 4) back to the described sex chromosome effects (Lines 133-137).**

**Third, we more clearly link our different predictions to each other and to the evolutionary and developmental mechanisms discussed. Specifically, we: 1) frame our Prediction 1 (i.e., greater male variability in sulcal size) as the "main" prediction; 2) note that Predictions 2-4 are complementary analyses that are relevant to teasing apart possible underlying mechanisms and link each prediction back to these mechanisms; and 3) add an analysis of sex differences in heritability to better address one of the mechanisms discussed (i.e., whether longer developmental periods in males make them more susceptible to environmental effects) (Lines 101-137).**

*In addition all three reviewers point out a number of areas where further detail on aspects of the methodology and presentation of the results require some clarification.*

**We have followed feedback from the Editors and Reviewers and have clarified our methods and results. Specifically, we have: 1) included details regarding the 0.1 cutoff for autocorrelation (Lines 192-194); 2) provided more details regarding the calculation of heritability and defined all variables (Lines 206-213); and 3) present two-sided (versus one-sided) permutation tests as our primary analysis (Lines 198-203); and 4) also presented results corrected for multiple comparisons using the Benjamini-Hochberg procedure (Lines 203-204).**

*Finally as R3 rightly points out, it is currently not entirely clear how sex differences in the variability of sulcus size relate to the points about brain structure and function in the discussion.*

**We have followed Reviewer 3's feedback and have more explicitly linked sex differences in the variability of sulcus size to brain function.**

**Specifically, we state that:**

**"If sulcal surface areas represent investment in the size of related cortical areas and, therefore, certain cognitive abilities, disruptive/balancing selection on male behavior may lead males to vary more than females in the size of related sulci." (Lines 308-311).**

**"While males using different mating strategies are likely to differ in their socio-cognitive and combat abilities, females may not be expected to show as much variation. Accordingly, greater male variability at the superior temporal, fronto-orbital, occipital lateral and superior parietal sulci may reflect disruptive selection on male social and visual information processing skills, which may be under more stabilizing selection among females." (Lines 323-328)**

**"Similarly, different mating strategies may place varying demands on male motor and inhibitory control, since successful male-male combat necessitates movement regulation and subordinate males often need to prevent themselves from feeding and mating in the presence of dominant individuals [81]. By contrast, we may not expect these skills to vary as much among females… Accordingly, greater male variability at the fronto-orbital and inferior frontal sulci may reflect disruptive selection on male motor control… [and] greater male variability at the inferior and middle frontal sulci may reflect disruptive selection on male inhibitory control." (Lines 329-339).**

**<u>Reviewer #1 (Remarks to the Author):</u>**

*The present manuscript investigates whether male chimpanzees have greater variability in brain structure than females. To do so, they examine the relative and absolute surface areas of several sulci in 91 males and 135 females. Their main findings are 1) males have more variability in brain structures than females; 2) brain structure is heritable and 3) Males exhibit a stronger correlation between sulcal sizes. The MS is overall well written and the analyses seem well performed and adequate to resolve the main question.*

**We thank the reviewer for this comment and for their constructive feedback.**

*However, there are some issues that I think should be addressed prior to publication. My main concern is in the structure of the introduction. Some of the predictions presented in the last paragraph of the introduction are not*

*sufficiently explained beforehand in the introduction. For instance, nothing about heritability is mentioned in the introduction before the presentation of the predictions, although it seems that is one of the predictions the authors want to test.*

*Also related to the previous point, I find that the 3rd paragraph of the introduction sounds a bit out of place. Initially, I did not understand why devoting an entire paragraph to one of the possible mechanisms (X-linked genes) and even one that was not specific for cognitive traits. However, at the end of the introduction, I understood that this paragraph was related to one of the predictions they wanted to test (#3). Maybe these paragraphs and the 3rd prediction should be closer to each other or try to link them in some way?*

*In addition, could it be possible to try to link the three predictions? Correct me if I am wrong but after reading the introduction, I get that the main hypothesis to test here is that males should have greater variability in cognitive traits for different reasons and that this has been tested in humans but not in animals, so testing this idea in Chimpanzees is the main novelty here. This refers mainly to prediction 1, but then, do we have different expectations depending on which mechanism is behind the greater variability of males? Do we expect higher heritability if sexual selection is behind the pattern, but not in other mechanisms? Maybe these types of questions could be used to frame predictions 2 and 3 as a complementary analysis to the "main" prediction 1.*

**We have followed Reviewer 1's feedback and have restructured the Introduction as outlined above in our response to the Editor. Specifically we better address the topics of heritability, sex chromosome effects, and worked to improve our prediction structure. We outlined these changes in detail in response to the Editor, above, but paste that text again here for ease of reference:**

**First, we better address the topic of heritability throughout the Introduction. Early in the Introduction, we define heritable traits as "trait[s] for which variation is explained by some genetic, rather than purely environmental, variation" and state that selection can only act on heritable traits (Lines 45-46). We also describe more explicitly why we predict sulcal sizes to be heritable in this case (Lines 122-125).**

**Second, we provide more background about the relevant evolutionary and developmental mechanisms early in the Introduction, so that sex chromosome effects are not discussed disproportionately (selection: Lines 43-49; developmental timing: Lines 50-57; sex chromosomes: Lines 58-44). We also now explicitly mention that the X chromosome is likely to play a role in the development of sulcal morphology (Lines 118-120). Finally, we better link our prediction regarding higher inter-regional correlations in males (Prediction 4) back to the described sex chromosome effects (Lines 133-137).**

**Third, we more clearly link our different predictions to each other and to the evolutionary and developmental mechanisms discussed. Specifically, we: 1) frame our Prediction 1 (i.e., greater male variability in sulcal size) as the "main" prediction; 2) note that Predictions 2-4 are complementary analyses that are relevant to teasing apart possible underlying mechanisms and link each prediction back to these mechanisms; and 3) add an analysis of sex differences in heritability to better address one of the mechanisms discussed (i.e., whether longer developmental periods in males make them more susceptible to environmental effects) (Lines 101-137).**

*Finally, could it be possible to add an initial paragraph or a sentence in the introduction, as well as in the last paragraph of the discussion to try to broaden the scope of the study? In the current version, I feel that the authors*

*go directly to the point of the study -which is not a bad idea- but I think it would be useful for the broader readership of Proceedings B to try to see which are the broader context and implications of this study.*

**We have followed Reviewer 1's feedback and now mention the broader implications of this study in the Introduction and Discussion. In the Introduction, we state that "Questions surrounding the mechanisms by which trait variation is produced and maintained over time are at the heart of evolutionary biology" (Lines 34-35). In the Discussion, we state that "Altogether, this line of inquiry will contribute to our understanding of intraspecific variation in neuroanatomy and behavior" (Lines 375-4376).**

*More specific comments:*
*- Regarding the statistical analysis, I wonder if the authors could specify a threshold to consider that they found a reduced autocorrelation. For instance, 0.1 is often used as such value, following MCMCglmm tutorial guidelines.*

**We thank Reviewer 1 for pointing this out. We did use the 0.1 threshold to detect low autocorrelation, and this is now described in the manuscript (Lines 192-194).**

*- Authors are often referring to P-values as the proportion of simulations that met some criteria (e.g. lower DIC than compared variable). However, the term "P-value" is incorrect here, and it would be more accurate to call it "P" or any other name. It's just to avoid confusing p-value with the real p-value term used in frequentist statistics. That's why the MCMCglmm packages provide pMCMC and not a p-value, where pMCMC is the proportion of samples in the MCMC chain that are above or below zero.*

**We have followed Reviewer 1's feedback and have replaced "p-value" with "pPERM" or "pSIM" throughout the manuscript to reflect that: 1) for variance ratios – pPERM represents the proportion of permuted test statistics (absolute value) greater than the observed ratio (absolute value); 2) for heritability estimates – pSIM represents proportion of simulations with lower dDIC values than the observed dDIC value. This is now described in the manuscript (Lines 202-203, 222-223).**

*- When authors refer to "motor-control-associated regions" (7th paragraph of the discussion) and to "multiple inhibition-related areas" (8th paragraph in the discussion), it would be useful to mention which areas are they referring to (Maybe in brackets?).*

**We have followed Reviewer 1's feedback and now discuss the specific brain areas in close proximity to their associated cognitive abilities throughout the Discussion (Lines 317-339). Please see our response to the Editor comments above for more details on this.**

**<u>Reviewer #2 (Remarks to the Author):</u>**

*This paper generated new data about the size of cortical sulci in chimpanzees and found that males have greater phenotypic variability than females in this feature. They relate this bimodal sexual selection, singular expression of X chromosome alleles, and extended development in males. In addition, it reports on sulcal size heritability and finds sex differences. They also found that male sulcal size co-vary more in males than in females.*

*The main contributions and conclusions of this study are novel and relevant.*

*General Comments*
*The Introduction is clear and well organised.*

**We thank the reviewer for this comment and for their constructive feedback.**

*Relevant methodological details are limited to the Supplement. Hardly anything is included about how the new data were generated. Certain details seem crucial and should be included, such as how the anatomical data was labelled.*

**We have followed Reviewer 2's suggestion and have moved some of the details regarding sulci labelling in from the Supplementary Material to the Methods section in the main text (Lines 152-160).**

*Further, how was a pedigree determined, and was it available for the entire sample?*

**Within each colony, pedigrees were well documented from the founder animals (for which parentage data is not available) to the current populations. For all offspring, the mothers were known, and paternity tests confirmed the fathers of most animals in this study. This is now described in the manuscript (Lines 186-187).**

*The Results section should make it clear that the data acquired were anatomical. In other words, the specific sulci should be listed, but not the presumed functional relevance of those anatomical changes. The associations are not specifically supported by this study, and should be left for the Discussion. Further, there is no analysis referenced to warrant naming the associations in the Results section.*

**We have followed Reviewer 2's feedback and have removed functional descriptions from the Results section (Lines 247-265).**

*The Discussion section is far too long and references a lot of very high level human neuropsychology and chimpanzee social behaviour which may be a stretch beyond what actually studied. Further, it is not clear that sulci which do not show greater variability in males lack similar associations.*

**We have followed Reviewer 2's feedback and have shortened the Discussion by limiting our discussion of the cognitive functions that may be associated with the various cortical sulci. In addition, we now introduce possible functional implications as interesting points to consider rather than necessarily characterize it as explicit evidence of sexual selection. Specifically, we state that: "…we find that males exclusively exhibit more variable sulcal surface areas than females... Interestingly, the specific regions that exhibit more variability in males may be associated with cognitive abilities that are likely to facilitate inter- and intrasexual selection." (Lines 292-300).**

*Specific Comments*
*p.5 There could be more detailed discussion about the link between brain sulci and cognitive function, within and between species, since this is so essential to the Discussion. Further, the limitations of using sulci as markers for functional areas, particularly across different species, are hardly stated.*

**We now acknowledge the limitations of using sulci as markers for functional areas. Specifically, we state that: "Sulcal organization may reflect some aspects of cortical cytoarchitectonic organization [31], since certain functional areas can be consistently located using specific sulci, both across (e.g. [32]) and within species [33-35]; however, this relationship is likely to be limited to certain areas [33-36]." (Lines 86-89).**

**Also, we now provide a more detailed discussion of the possible links between sulci and cognitive function. Specifically, we state that: "Given that the surface areas of specific cortical areas are positively correlated with certain abilities (e.g. planum temporale and**

**music skill: [37]), larger sulcal surface areas may, in some cases, provide additional surface area for related cortical areas and, therefore, the elaboration of certain cognitive abilities. We should note that cortical volume is the product of thickness and surface area, and it is unclear how much each component contributes to cortical volume variation [38]. Nevertheless, sulcal variation has been linked to inter-individual differences in behavior (e.g. handedness: [39-40]) in primates, and even correlates with the severity of some neurological disorders in humans (e.g. [41-42]). Additionally, motor and cognitive outcomes in humans are positively correlated with the surface areas of specific sulci early in development [43].” (Lines 89-97).**

*p. 10, line 1, which "anatomical regions"?*

**These words have been removed from the manuscript. The sentence now reads: “Correlation analyses were used to assess whether pairs of sulci were correlated, and if there was a difference in the strength of these correlations between males and females.” (Lines 232-233).**

**Reviewer #3 (Remarks to the Author):**

*The paper presents a study exploring the variability of sulcal sizes between sexes in Chimpanzee. It provides a novel and valuable insight into a topic that has attracted a lot of attention – the greater male variability hypothesis – and elucidates a novel neuromophological aspect of this effect, sulcal size variability. The sample size is adequate, and the data is analyzed appropriately with contemporary statistical approaches. Despite the lack of details about the heritability analysis included, and some minor points throughout, I consider that the study is worth publishing after minor revisions. If the alpha level needs to be adjusted due to multiple comparisons, many of the results might not stand, so I think this is an important issue to be addressed too. See my detailed comments:*

*The manuscript does not include line numbers, which makes reviewing and commenting it not very convenient.*

**We have reformatted the manuscript to include line numbers. We apologize for the inconvenience on the prior version.**

*Introduction*
*Page 1, line 4 “For example, male barn swallow tail length…” – this is a bit odd example, considering that the sentence leading up to it reads “Across mammals…”. I’d recommend using an example from mammals.*

**We now begin the sentence with “across the animal kingdom” (Line 35) since the pattern of greater male variability has been observed in many non-mammalian species, including insects and birds (Pomiankowski & Moller 1995).**

*Page 1, End of the first paragraph - The lek paradox, to my knowledge, is observed in lek mating species, where consistent female preferences are present. I don’t think that this is applicable to chimpanzee mating systems, where female preference is not consistent, lekking not present, and mating is mainly promiscuous and opportunistic.*

**We have followed Reviewer 3’s suggestion and have removed references to the lek paradox in this paragraph.**

*Page 6, Prediction 3 – Is sulcal size related to the X chromosome at all? This prediction needs to be backed up by a statement/study that shows that there is indeed a relationship between any aspect of sulcal morphology and proteins encoded from X chromosome genes and such relationship is warranted. References 9 and 10 do not give any suggestion as to why sulcal morphology relates to the X chromosome.*

**We have followed Reviewer 3's suggestion and included a reference that suggests that X chromosome genes are likely to affect sulcal morphology (Lines 118-120).**

*Introduction, general – There is no mention of the 'Variability hypothesis' (also know as 'greater male variability hypothesis'). The authors had tried to lay down their own arguments, but this is a well-argued hypothesis with a long history, and it needs to be mentioned as it is.*

**We have followed Reviewer 3's feedback and now explain the greater male variability hypothesis in more detail. Specifically, we state that "Across the animal kingdom, males tend to exhibit greater behavioral and morphological variability than females, known as the greater male variability hypothesis [1-2]… Throughout the human literature, the "greater male variability hypothesis" [2] has sparked debate regarding its validity and potential causes [11]. For example, while some question the existence of this pattern (e.g. [22]), others have proposed that these differences reflect extended male development [25], sex chromosome effects [10,24], or sexual selection processes [9]; however, mechanisms of sexual selection are hard to measure in modern humans." (Lines 35-37, 68-73).**

*Heritability is mentioned in passing at the end of the introduction. The authors need to elaborate on the concept and explain what they mean by that and how is it relevant to the question at hand at all.*

**We have followed Reviewer 3's feedback and now better address the topic of heritability throughout the Introduction. Early in the Introduction, we define heritable traits as "trait[s] for which variation is explained by some genetic, rather than purely environmental, variation" and state that selection can only act on heritable traits (Lines 45-46). We also describe more explicitly why we predict sulcal sizes to be heritable in this case (Lines 122-125).**

*Materials and methods*
*Given that age of maturity differs between males and females in chimpanzees it seems reasonable to only include mature individuals. At the same time, senescence has a significant effect on brain morphology, so again, old aged individuals may also be excluded? (I.e. Chen X, Errangi B, Li L, et al. Brain aging in humans, chimpanzees (Pan troglodytes), and rhesus macaques (Macaca mulatta): magnetic resonance imaging studies of macro- and microstructural changes. Neurobiol Aging. 2013;34(10):2248–2260. doi:10.1016/j.neurobiolaging.2013.03.028).*

**Previous work in humans suggests sex differences in neuroanatomical variability are present across the lifespan (Wierenga et al. 2020). Additionally, the degree of cortical folding reaches adult levels by early childhood and does not decrease with aging (Zilles et al. 1997), and relative sulcal surface areas do not appear to change substantially with age (Glasel et al. 2011). Accordingly, we included individuals of all ages in our study to obtain the largest possible sample size. This is now described in the manuscript (Lines 146-150).**

*Additionally, average age and SD for both sex groups should be included, along with the already present mean/SD value for the whole sample.*

**We have followed Reviewer 3's feedback and have included the mean and standard deviation for sex-specific ages (Line 145).**

*It is interesting to see the boxplots for the outlier analysis, as the 1.5 times beyond the interquartile range rule of thumb is not a golden rule, and if there are specimens very close to that range i.e. (1.6) they might be included too.*

**We have included sex-specific adjusted boxplots here as an attachment. It appears that most of the outliers exist quite far beyond 1.5 times the IQR. We initially implemented the 1.5 times rule in order to be consistent across all sulci and prevent cherry picking.**

*When calculating the sex specific variance why wasn't overall brain size/volume/weight or body size/weight corrected for? Sulcal size is a function of overall brain size and approaching the question without controlling for it might just reveal overall greater variance in body/brain size in the larger sulci.*

**We did control for overall cortical folding area to explicitly test for differences in relative surface area. In addition, total sulcal area scales nearly isometrically with total brain volume (Fish et al. 2017), so overall folding area is likely to be capturing the same variation and overall brain size. This is now mentioned in the manuscript (Lines 182-184).**

*Heritability – this whole section needs more detail and clear explanation as to its necessity in the introduction. There is no explanation as to what heritability analysis is, the formula included is not explained (there are parameters there that need spelling out) and in general the whole section needs to include more detail and clarity as to rationale behind it.*

**More details regarding heritability have been included, both in the Introduction and the Methods.**

**In the Introduction, we define heritable traits as "trait[s] for which variation is explained by some genetic, rather than purely environmental, variation" and state that selection can only act on heritable traits (Lines 45-46). We also describe more explicitly why we predict sulcal sizes to be heritable in this case (Lines 122-125).**

**In the Methods, we state that "…we estimated the ratio of additive genetic to phenotypic variance for each sample of the posterior distribution ($h^2 = V_A/V_P = V_A/(V_A+V_R)$; $h^2$ = heritability; $V_A$ = additive genetic variance; $V_P$ = phenotypic variance; $V_R$ = residual (i.e., non-additive genetic) variation). This was done using the output from MCMCglmm, which provides the additive genetic variance as the posterior distribution of variance for the pedigree random effect (i.e., the 'animal' term) in addition to the residual variance (i.e., 'unit' term). We extracted mean estimates and 95% highest posterior density intervals from these distributions." (Lines 206-213).**

*Results*
*Given the approach of multiple comparisons the alpha level should be adjusted to a more conservative one.*

**We now present both unadjusted and adjusted multiple correction values are shown in Tables 1 and S1. We used the Benjamini-Hochberg procedure to control the false discovery rate (i.e., correct for multiple comparisons). We also updated our permutation tests to represent two-sided (versus one-sided) tests of sex differences in variability as our primary analysis. This is now described in the manuscript (Lines 202-204, 225-226).**

*Correlations – This prediction was based on males's homozygosity but still there are 6 sulci that are more correlated in females than in males? Figure S49 does not have a legend explaining the red, green and white spaces.*

**Reviewer 3 is correct that some pairs of regions show higher correlations in some regions among females; however, we only predicted that more pairs of regions would show significantly stronger correlations in males (Lines 136-137), in line with findings in humans (Wierenga et al. 2018, 2020).**

**Supplementary Figure notes are available in the Supplementary Material. For both Figures 2 and S49, we previously noted that "Green colors indicate stronger correlations in males, while purple indicates stronger correlations in females. Darker colors indicate larger sex differences in correlation values… [and] pairs of sulci [that] show significant sex differences… are represented by filled boxes below the diagonal". To better clarify the meaning of the color scheme used, we added "(i.e., unfilled, white boxes represent pairs of sulci that exhibit non-significant sex differences)" to our notes for both Figures 2 and S49.**

*Discussion*
*The whole discussion alludes to relationships between brain structure and function, but it does not make clear how variability (high or low) relates to that. For example, it is suggested that behavioral inhibition is an aspect of male's mating and foraging behavior and this somehow should warrant greater fitness benefits from variability. It is interesting to think about how low variability in females is also related to their specific mating and foraging behaviors. The same argument can be applied to other systems the authors are discussing (facial recognition, gaze processing etc).*

**We have attempted to address Reviewer 3's concerns by more explicitly stating how sulcal variability may relate to behavioral/cognitive variability.**

**We have given this text in our response to the Editor and to Reviewer 1, but for ease of reference, we have added the following text:**

**"If sulcal surface areas represent investment in the size of related cortical areas and, therefore, certain cognitive abilities, disruptive/balancing selection on male behavior may lead males to vary more than females in the size of related sulci." (Lines 308-311).**

**"While males using different mating strategies are likely to differ in their socio-cognitive and combat abilities, females may not be expected to show as much variation. Accordingly, greater male variability at the superior temporal, fronto-orbital, occipital lateral and superior parietal sulci may reflect disruptive selection on male social and visual information processing skills, which may be under more stabilizing selection among females." (Lines 323-328)**

**"Similarly, different mating strategies may place varying demands on male motor and inhibitory control, since successful male-male combat necessitates movement regulation and subordinate males often need to prevent themselves from feeding and mating in the presence of dominant individuals [81]. By contrast, we may not expect these skills to vary as much among females… Accordingly, greater male variability at the fronto-orbital and inferior frontal sulci may reflect disruptive selection on male motor control… [and] greater male variability at the inferior and middle frontal sulci may reflect disruptive selection on male inhibitory control." (Lines 329-339).**

We hope that with the above changes you find our manuscript suitable for publication, and look forward to hearing from you.

Sincerely,

Alex DeCasien, Chet Sherwood, Steve Schapiro, & James Higham