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Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex

Valeria Marasco, Winnie Boner, Kate Griffiths, Britt Heidinger and Pat Monaghan

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1st revised submission:	7 August 2019
2nd revised submission:	8 September 2019
Final acceptance:	9 September 2019

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

Review History

RSPB-2019-0922.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?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

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

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
Nice work - very interesting

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?
Excellent

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.
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?
No

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Marasco et al., using captive zebra finches, experimentally investigated the role maternal age and environmental conditions prior to breeding influence offspring telomere length and mass. The prediction was that older females would produce nestlings with shorter telomeres. The implication being that shorter telomeres may indicate shorter lifespans and therefore a potential mechanistic link explaining the "Lansing effect", the phenomenon whereby offspring of older parents tend to have shorter lifespans.
The authors designed an experiment where females bred at 6 months and 3.5 years, but the age of the father was held relatively constant by switching males between breeding events. In addition, females were split into either an experimental group where females experienced unpredictable periods of food rationing prior to breeding to induce stressful conditions or a control group that had ad lib food for the study duration. Indeed, older females produced offspring with shorter telomeres, but environmental effects were sex specific.
The authors suggest that this design rules out paternal age effects on offspring telomere length and that offspring shorter telomere length can only be explained by advanced maternal age. I question whether this is the only explanation. It might be possible that the sudden switch of breeding partner may contribute to offspring telomere length in other unforeseen ways. For instance, the introduction of an unfamiliar male after years paired with another may increase maternal physiological stress and affect parental care. Both can affect offspring telomere length. I would ask the authors to rule out the possible effects of adding a novel male in some form or at the very least clarify and discuss in the discussion. How to do this without running another experiment I am unsure, but the authors may have some data (e.g. female mass or cort measurements before and after introduction) that may indicate other explanations for the result.
The authors note on lines 339-341 that they do not know whether the maternal age effect is linear. If the samples exist, they may not, it could be worth investigating even if reproduction was with the original male. Likewise, if samples exist for birds that aged over the same period but remained with the same male it may shed some light on the problem highlighted above because the effect should still be present?
It would be reassuring to also see that the effect sizes are similar between the two replicates. Recommend that Table S3 be included in the main article because I think its important to view the effect sizes. The reader is currently presented with only p values in the main text.
It is unclear why only maternal age is the focus in the experiment when paternal age has been found to influence offspring telomere length in other species.
On lines 165-166, in addition to the T-test comparing male age to offspring telomere length and the exploration of paternal age in Table S1 (analysis in the period between the maternal breeding events measured) I think checking paternal age has no effect by including in the models presented in Table S3 would be more reassuring. Paternal age ranged between 212-695 days in

the old mother group.
When mentioning environmental stress in the discussion, I think it's worth highlighting that its maternal stress prior to breeding and not a direct effect of environment on offspring.
Line 381-383 I think it would be nice to expand on the trade-off idea here a little.
Line 426 "% telomere reduction".
Line 428 longevity should be replaced with telomere length
Overall, I found the study to be very interesting, well written and highly relevant to advancing our knowledge of both telomere dynamics and the role of parental age on offspring life history. However, I think that the above comments need to be addressed in some form to be considered for publication in Proc. R. Soc. B.

Decision letter (RSPB-2019-0922.R0)

03-Jun-2019

Dear Dr Marasco:

I am writing to inform you that your manuscript RSPB-2019-0922 entitled "Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex" 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.
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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 Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This study presents the results of a simple intergenerational experiment in zebra finches, with intriguing results. The authors measure telomere length in sons and daughters of females produced at 2 different ages (6 months vs 3.5 years) under two treatments (benign vs unpredictalbe food regime), and find strong effects of age, and interactive effects of age x diet treatment on the telomere length of the offspring. The referees both enjoyed the paper, and note that it makes an excellent contribution to the literature. One referee asked (in editorial correspondence) whether there may be "any 'chicken megatelomere' effects described on sex chromosomes in zebra finches that could explain sex effects (i.e., could the different Z and W chromsomes have different number of telomere sequence repeats)?" This is an interesting question, and it would be worth discussion.

Referee 2 proposed an intriguing alternative explanation for the results. I do not expect you conduct further experiments to disentangle their alternative from your interpretation -- but if the data is already available to do some form of analysis to disentangle these processes, please do so. Otherwise, please include this alternative in your discussion in the manuscript. This referee also noted that there was substantial variation in male age, and that this factor could be included in the main models in Table 3, to rule out any effect. This referee made a series of other valuable comments, each of which require careful attention.

Please respond to all of the Referees comments within a revised manuscript, and cover letter.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Nice work - very interesting

Referee: 2

Comments to the Author(s)
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The authors suggest that this design rules out paternal age effects on offspring telomere length and that offspring shorter telomere length can only be explained by advanced maternal age. I question whether this is the only explanation. It might be possible that the sudden switch of

breeding partner may contribute to offspring telomere length in other unforeseen ways. For instance, the introduction of an unfamiliar male after years paired with another may increase maternal physiological stress and affect parental care. Both can affect offspring telomere length. I would ask the authors to rule out the possible effects of adding a novel male in some form or at the very least clarify and discuss in the discussion. How to do this without running another experiment I am unsure, but the authors may have some data (e.g. female mass or cort measurements before and after introduction) that may indicate other explanations for the result.
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Author's Response to Decision Letter for (RSPB-2019-0922.R0)

See Appendix A.

RSPB-2019-1845.R0

Review form: Reviewer 1

Recommendation
Accept as is

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

General interest: Is the paper of sufficient general interest?
Excellent

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

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
NA

Decision letter (RSPB-2019-1845.R0)

03-Sep-2019

Dear Dr Marasco

I am pleased to inform you that your Review manuscript RSPB-2019-1845 entitled "Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex" has been accepted for publication in Proceedings B.

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. 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 me know immediately.

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3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
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 see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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

Sincerely,
Professor Gary Carvalho
mailto:proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
Many thanks for providing a careful revision that addressed each of the referees' comments. Referee 2 has now reviewed your revision, as have I. The revision is suitable for publication in its current form, and will make an excellent addition to the literature. Well done.

Decision letter (RSPB-2019-1845.R1)

09-Sep-2019

Dear Dr Marasco

I am pleased to inform you that your manuscript entitled "Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex" 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.

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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.

Sincerely,

Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

POINT-BY-POINT RESPONSE TO REVIEWERS' COMMENTS

Title: Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex

>> We would like to thank the Editor and the Reviewers for their constructive comments. We have revised the manuscript accordingly. We believe that the revision has substantially improved the manuscript. Below, our point-by-point responses in italics.

03-Jun-2019

Dear Dr Marasco:

I am writing to inform you that your manuscript RSPB-2019-0922 entitled "Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex" 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.

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 Gary Carvalho

mailto: proceedingsb@royalsociety.org

Associate Editor

Board Member: 1

Comments to Author:

This study presents the results of a simple intergenerational experiment in zebra finches, with intriguing results. The authors measure telomere length in sons and daughters of females produced at 2 different ages (6 months vs 3.5 years) under two treatments (benign vs unpredictalbe food regime), and find strong effects of age, and interactive effects of age x diet treatment on the telomere length of the offspring. The referees both enjoyed the paper, and note that it makes an excellent contribution to the literature. One referee asked (in editorial correspondence) whether there may be "any 'chicken megatelomere' effects described on sex chromosomes in zebra finches that could explain sex effects (i.e., could the different Z and W chromsomes have different number of telomere sequence repeats)?" This is an interesting question, and it would be worth discussion.

>> We thank the Reviewer and the Editor for their positive comments and for suggesting the possibility of differences in telomeres on the sex chromosome. Chromosome specific telomere lengths have not been studied in birds. However, the ultra-long (Class III) telomeres have been found in some birds, including the chicken, Japanese quail and great tit (Meyne et al 1990; Nanda et al 2002; McPherson et al 2014; Atema et al 2019), but are absent, or only minimally present in many others such as in the zebra finch (Delany et al 2000; Haussmann et al 2003). While an interesting idea, this is not applicable for this species. We have added the following to the Discussion (lines 382-383):

"It is also possible that the sex-specific effect could relate to differences in telomere dynamics in the sex chromosomes, but nothing is known about this in birds."

Referee 2 proposed an intriguing alternative explanation for the results. I do not expect you conduct further experiments to disentangle their alternative from your interpretation -- but if the data is already available to do some form of analysis to disentangle these processes, please do so. Otherwise, please include this alternative in your discussion in the manuscript. This referee also noted that there was substantial variation in male age, and that this factor could be included in the main models in Table 3, to rule out any effect. This referee made a series of other valuable comments, each of which require careful attention. Please respond to all of the Referees comments within a revised manuscript, and cover letter.

>> Please see below our detailed answers to the Referee 2 regarding these aspects.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

Nice work - very interesting

Referee: 2

Comments to the Author(s)

Marasco et al., using captive zebra finches, experimentally investigated the role maternal age and environmental conditions prior to breeding influence offspring telomere length and mass. The prediction was that older females would produce nestlings with shorter telomeres. The implication being that shorter telomeres may indicate shorter lifespans and therefore a potential mechanistic link explaining the "Lansing effect", the phenomenon whereby offspring of older parents tend to have shorter lifespans.

The authors designed an experiment where females bred at 6 months and 3.5 years, but the age of the father was held relatively constant by switching males between breeding events. In addition, females were split into either an experimental group where females experienced unpredictable periods of food rationing prior to breeding to induce stressful conditions or a control group that had ad lib food for the study duration. Indeed, older females produced offspring with shorter telomeres, but environmental effects were sex specific.

The authors suggest that this design rules out paternal age effects on offspring telomere length and that offspring shorter telomere length can only be explained by advanced maternal age. I question whether this is the only explanation. It might be possible that the sudden switch of breeding partner may contribute to offspring telomere length in other unforeseen ways. For instance, the introduction of an unfamiliar male after years paired with another may increase maternal physiological stress and affect parental care. Both can affect offspring telomere length. I would ask the authors to rule out the possible effects of adding a novel male in some form or at the very least clarify and discuss in the discussion.

>> We agree with the Reviewer that the sudden introduction of an unfamiliar male after years paired with the same male could induce maternal stress. However, as described in the Methods, our experimental females were housed in single-sex groups for most of their lives and were paired with a young-adult male only during the age-specific breeding events, which lasted approximately 2 months - we have now stressed this at several points in the Methods (lines 127-131 and lines 156-157). Our experimental design aimed at minimising any potential mate familiarity and none of our females experienced a sudden change of partner. We also point out that all the females had not been housed and bred with any male for a relatively long time before the old-mother breeding event (ca. 1.7 years). Thus in our experiment the potential effect of a new partner on maternal stress levels and/or maternal care is very likely to be negligible. We mentioned this now in the Discussion as suggested (lines 365-371):

"All our experimental females were housed in single-sex groups and were paired with a young-adult male only during the age-specific breeding events to minimise mate familiarity. We can thus exclude the possibility that the reduction in offspring telomere length during the old-mother breeding event could be attributable to increased maternal stress due to the sudden introduction of an unfamiliar male after years being paired with the same male, thereby having formed a long-term pair bond which is broken."

How to do this without running another experiment I am unsure, but the authors may have some data (e.g. female mass or cort measurements before and after introduction) that may indicate other explanations for the result. The authors note on lines 339-341 that they do not know whether the maternal age effect is linear. If the samples exist, they may not, it could be worth investigating even if reproduction was with the original male. Likewise, if samples exist for birds that aged over the same period but remained with the same male it may shed some light on the problem highlighted above because the effect should still be present?

>> As the Reviewer acknowledged we would need to run a different experiment to test such possibility. We cannot do the comparison that the Reviewer suggests - i.e keeping females with

the same male through the experiment, since the male would also grow old. What could be done is to change the partner of females in relatively quick succession without the females getting substantially older - however, they would inevitably have got a bit older. As mentioned in our answer earlier we have now acknowledged the possibility of mate change effects in the Discussion.

It would be reassuring to also see that the effect sizes are similar between the two replicates. Recommend that Table S3 be included in the main article because I think its important to view the effect sizes. The reader is currently presented with only p values in the main text.

>> We found no effect of replicate either on offspring body mass (line 283) or offspring telomere length (line 314) as indicated by the negligible model estimates (Table S3). We had to present full statistics in Table format and we needed to include Table S3 in the Supplementary Material to conform with the Journal length restrictions.

It is unclear why only maternal age is the focus in the experiment when paternal age has been found to influence offspring telomere length in other species.

>> In the Introduction we mentioned that while the importance of paternal age in influencing offspring telomere length has been established across different species (lines 94-98), the contribution of maternal age in influencing offspring telomere length remains unclear as majority of the studies to date are correlative and could not control for a series of relevant factors, including differential survival of females and assortative mating (lines 98-106). We stressed this now in the Introduction to clarify our aims (lines 106-107).

On lines 165-166, in addition to the T-test comparing male age to offspring telomere length and the exploration of paternal age in Table S1 (analysis in the period between the maternal breeding events measured) I think checking paternal age has no effect by including in the models presented in Table S3 would be more reassuring. Paternal age ranged between 212-695 days in the old mother group.

>> We are unable to add in the same main model both maternal age and paternal age as these two are correlated between the two breeding events. This is because in the old-mother breeding event the experimental females were paired with fathers slightly older (but still young males) than during the young-mother breeding event as mentioned in the manuscript (lines 163-169). As the Reviewer pointed out, however, paternal age was relatively variable only within the old-mother breeding event and we found no effect of paternal age on offspring telomere length. This was assessed in a separate General Linear Mixed Model taking into account all the main factors involved in our experimental design (lines 169-171 and Table S1 in the Supplementary). We edited the main text to clarify this (Methods: lines 171; Discussion: lines 354-357).

We note that paternal age ranged between 212-699 days in the old-mother breeding event (mean 464 days), we have now corrected the typo in the main text (lines 167-168).

When mentioning environmental stress in the discussion, I think it's worth highlighting that its maternal stress prior to breeding and not a direct effect of environment on offspring.

>> Good point. Done (line 324 and lines 329-330).

Line 381-383 I think it would be nice to expand on the trade-off idea here a little.

>> Done (lines 388-391).

Line 426 “% telomere reduction”.

>> Corrected.

Line 428 longevity should be replaced with telomere length

>> We did not alter this sentence as the study cited here (Noguera et al 2018) did report a strong effect of parental age on offspring longevity (Figure 1), which is relevant to the interpretation of our results.

Overall, I found the study to be very interesting, well written and highly relevant to advancing our knowledge of both telomere dynamics and the role of parental age on offspring life history. However, I think that the above comments need to be addressed in some form to be considered for publication in Proc. R. Soc. B.

>> Once again we thank the Reviewer for the comments that helped us to improve our manuscript substantially.

Cited literature:

Atema E, Mulder EGA, van Noordwijk A, Verhulst S (2019) Ultra-long telomeres shorten with age in nestling great tits but are static in adults and mask attrition of short telomeres. *Molecular Ecology Resources* 19(3):648-658; doi: 10.1111/1755-0998.12996.

Delany ME, Krupkin AB, Miller MM (2000) Organization of telomere sequences in birds: evidence for arrays of extreme length and for in vivo shortening. *Cytogenetics and Cell Genetics* 90(1-2):139-45; doi: 10.1159/000015649.

Haussmann MF, Winkler DW, O'Reilly KM, Huntington CE, Nisbet ICT, Vleck CM (2003) Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proc Biol Sci.* 270(1522): 1387-1392. doi: 10.1098/rspb.2003.2385.

McPherson MC, Robinson CM, Gehlen LP, Delany ME (2014) Comparative cytogenomics of poultry: mapping of single gene and repeat loci in the Japanese quail (*Coturnix japonica*). *Chromosome Research* 22: 71-83; doi: 10.1007/s10577-014-9411-2.

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wurster-Hill DH, Yates TL, Moyzis RK (1990) Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequence in vertebrate chromosomes. *Chromosoma* 99 (1): 3-10.

Nanda I, Schrama D, Feichtinger W, Haaf T, Schartl M, Schmid M (2002) Distribution of telomeric (TTAGGG)(n) sequences in avian chromosomes. *Chromosoma* 111: 215-227; doi: 10.1007/s00412-002-0206-4.