Fitness benefits of dietary restriction

Zahida Sultanova, Edward R. Ivimey-Cook, Tracey Chapman and Alexei A. Maklakov

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Review timeline
Original submission: 10 August 2021
Revised submission: 25 October 2021
Final acceptance: 2 November 2021

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

Review History

RSPB-2021-1787.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?
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?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No
Fitness Benefits of Dietary Restriction

Overall

Sultanova et al have produced a clear and concise manuscript that repudiates the commonly held theory that life extension via dietary restriction (DR) is due to a reallocation of resources from reproduction to somatic maintenance. Instead, the authors suggest that lifespan extension is a result of the reduced direct physiological costs of reproduction and that the impact of DR on lifespan and reproduction can be uncoupled, as suggested through previous works. The authors have designed a straightforward experiment to test whether post-DR, DR worsens reproductive performance comparable to lifetime DR and lifetime fully fed flies. In addition, they tested whether the odour of food, ie neurological signals of “plentiful food” could impact this response. The authors found that, when DR flies were switched to fully fed, reproductive capacity increased to above that of lifetime fully fed flies, resulting in an unchanged reproductive capacity between groups, and this similarly reduced their lifespans back down to that of the fully fed flies. They suggest that this is not due to a “cost” of DR but due directly to the increased cost of mating and reproduction. In addition, they found that DR flies with food odour did have a worse baseline mortality than their DR counterparts.

While I have no major criticisms of this work, I would suggest some changes in the text and figures for clarity. In addition, there appears to be some “lost” supplementary tables and figures that are not mentioned in the text. While the statistics looks sound to me, I am not familiar with all of the algorithms used here so stats consult or review from someone who commonly works with these equations might be helpful.

Finally, I believe there is a need for the addition of diet design and sexual dimorphism in the discussion/introduction. In particular to include some more information on the differences in carbohydrate/protein ratios that have been implicated in both longevity and reproductive success in Drosophila. These ratios also impact the reproductive capacity of flies in a sexually dimorphic manner, in particular while males have improved lifespan and reproduction on high carbohydrate low protein, in females lifespan is improved by high carbohydrate but reproduction by protein. This seems pertinent to their proposed theory that lifespan and reproduction are uncoupled, as this may only be true in females.

Minor Comments

Lines 47-51: This section is well written, but not all of the ideas mentioned apply universally to all organisms. It would be helpful to mention the species the research is referring to for each of the studies referenced.

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Figure 2: For clarity I think this figure would benefit from additional labelling. In particular these figures should be labelled A, B, C, and D and references in the text as such which will make it easier to find what is being referenced. Also headings for “full lifespan” and “post-switch” would be helpful. Otherwise you have to read the figure legend every time you glance at the figure.

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Supplementary Tables: There are lots of supplementary tables not referenced to in the text.

General discussion: It would benefit your discussion and your hypothesis to add some discussion of the potential sexual dimorphism that could be at play here (not explored in your paper but possibly worth repeating in males in the future – maybe a limitation of this study). Jensen et al. found that male and female drosophila have different “optimum” dietary intakes of carbohydrate and protein for reproductive success (and lifespan). This may support your hypothesis that reproduction and lifespan are uncoupled. It is possible that this is only true for females, because in males both lifespan AND reproductive success are optimised by high carbohydrate:low protein but in females while high carbohydrate maximises their lifespan, high protein maximises their reproduction.

This feels similar to some of the results you see here with DR=high carbohydrate and FF=high protein. As you are reducing yeast for your DR, you are mainly constraining protein, which has been shown to be most detrimental to female reproduction but increases lifespan, whereas male reproduction at higher carbohydrate level. When you replace the protein, reproduction increases and lifespan decreases, it would be fascinating to see what happens to males on the same paradigm due to their difference in optima.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4531074/

Review form: Reviewer 2

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Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
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Is the length of the paper justified?
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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 manuscript by Sultanova and coauthors addresses a fundamental question in life history theory: is the extension of lifespan under dietary restriction adaptive? In this study, they provide evidence that females subjected to dietary restriction fully recover their lifetime reproductive success upon re-exposure to food. The experimental design in this study is excellent, and is supported by sophisticated analyses. Further, the discussion was thorough and very interesting. I thoroughly enjoyed this paper, and have only a few minor comments that I hope might help.

Lines 196-206: I found this text a bit hard to follow. I think this is because it compared the DR and FF treatments in addition to the same diet treatments with odour. The primary figures cited in this paragraph (Figures 2 and 3) don’t compare across the treatments with and without odour in this way (this is instead in the supplementary figures). It might make it easier if you discussed the results of figure 2 and 3 (including the diet switching results) first before comparing the no odour/odour treatments from the supp figures.

In the section following Line 241, it would be helpful to remind the reader that this is female mating success. Maybe it’s just me, but I always associate mating success with males.

Is the acronym for lifetime reproductive success (LRS) necessary? The paragraph starting on line 269 already has a lot of acronyms, which I find hard to keep track of.
Dear Dr Maklakov:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (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. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

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:

Research ethics:
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. Please see our Data Sharing Policies (https://royalsociety.org/journals/authors/author-guidelines/#data). 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 http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), 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.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

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,
Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor

Comments to Author:
This manuscript has been assessed by two expert reviewers who are both very positive about the novelty and interest of this manuscript, which uses an experimental approach in outbred Drosophila to test the adaptive nature of dietary restriction effects on lifespan. I agree with the reviewers on their overall appraisal and they both make a number of suggestions that will strengthen this manuscript further. Reviewer 1 provides some excellent suggestions on how to improve the presentation of results both in the main text and supplement; and also recommends that the introduction and/or discussion could include more background on carbohydrate/protein diet ratio studies in Drosophila and sex differences in effects. I appreciate that Proceedings B has strict space constraints but this would be a good contribution - in particular acknowledging that this study has focused on females, and patterns may be different in males. Reviewer 2 makes very minor points which will improve clarity of the manuscript. While also not a fan of unnecessary abbreviations, I appreciate that given the frequency of referring to lifetime reproductive success, in this case it would be acceptable to use the acronym.
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The authors have provided all data used in the analysis, as required by the journal: it would be helpful to also include a README file explaining meta-data (e.g. the different tables and column descriptions). Importantly, following Proceedings B guidelines, the authors should also make the code available at the point of submission: see https://royalsociety.org/journals/authors/author-guidelines/#data Given that the paper describes fairly complex statistics (Bayesian survival analysis), providing the analysis code would be particularly helpful.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

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Overall

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Author’s Response to Decision Letter for (RSPB-2021-1787.R0)

See Appendix A.

Decision letter (RSPB-2021-1787.R1)

02-Nov-2021

Dear Dr Maklakov

I am pleased to inform you that your manuscript entitled "Fitness Benefits of Dietary Restriction" 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.

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

Associate Editor:
Board Member
Comments to Author:
(There are no comments.)
Response to Reviewers:

Comments to Author:
This manuscript has been assessed by two expert reviewers who are both very positive about the novelty and interest of this manuscript, which uses an experimental approach in outbred Drosophila to test the adaptive nature of dietary restriction effects on lifespan. I agree with the reviewers on their overall appraisal and they both make a number of suggestions that will strengthen this manuscript further. Reviewer 1 provides some excellent suggestions on how to improve the presentation of results both in the main text and supplement; and also recommends that the introduction and/or discussion could include more background on carbohydrate/protein diet ratio studies in Drosophila and sex differences in effects. I appreciate that Proceedings B has strict space constraints but this would be a good contribution - in particular acknowledging that this study has focused on females, and patterns may be different in males. Reviewer 2 makes very minor points which will improve clarity of the manuscript. While also not a fan of unnecessary abbreviations, I appreciate that given the frequency of referring to lifetime reproductive success, in this case it would be acceptable to use the acronym.

Thank you very much for this positive and constructive evaluation of our paper. Please find below our point-by-point response to Reviewers’ comments. We now point out in the Discussion that the response can indeed be different in males. We also mention the macronutrient approach but we also mention that males and females can have different dietary preferences and, therefore, different diets (LL 398-404).

On my own reading of the manuscript, I have the following minor suggestions in addition to the reviewer feedback:

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L 67 we suggest, following [17] that detrimental effects of protein on survival could be one specific cost (it has been suggested previously that an excess of protein can be toxic)

L.71 Typo - delete "the" in 'for the developing’

Done.

L.85 Would mention in methods section too that flies are outbred (as seems important to mention in the Abstract, Introduction but then non-Drosophila person may wonder what this means in terms of laboratory stock)

Added to the first sentence (L88).

---

Appendix A

Response to Reviewers:

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l.111 'Excess yeast particles' to return the DR to FF diet seems somewhat vague for study replication: are these measured out to be the same across treatments? (Or is this a standard approach to generating such diets?)

Added: “We added eight granules of yeast particles per vial, which is more than flies could consume until they were transferred to a new vial.” (LL 118-119)

Fig 4 - Use same colour scheme for experimental treatments, and it might be helpful to have vertical line for the point of diet switch on the right-hand graphs.

Done.

The authors have provided all data used in the analysis, as required by the journal: it would be helpful to also include a README file explaining meta-data (e.g. the different tables and column descriptions). Importantly, following Proceedings B guidelines, the authors should also make the code available at the point of submission: see https://royalsociety.org/journals/authors/author-guidelines/#data Given that the paper describes fairly complex statistics (Bayesian survival analysis), providing the analysis code would be particularly helpful.

Done.

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Referee: 1

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Thank you, we believe Reviewer referred to the trace plots for Basta analyses. We now mention them in the text (e.g. L 175).

Finally, I believe there is a need for the addition of diet design and sexual dimorphism in the discussion/introduction. In particular to include some more information on the differences in carbohydrate/protein ratios that have been implicated in both longevity and reproductive success in Drosophila. These ratios also impact the reproductive capacity of flies in a sexually dimorphic manner, in particular while males have improved lifespan and reproduction on high carbohydrate low protein, in females lifespan is improved by high carbohydrate but reproduction by protein. This seems pertinent to their proposed theory that lifespan and reproduction are uncoupled, as this may only be true in females.
We now refer to this in the Discussion. Indeed, the situation could be different for males and because the two sexes can have very different optimal diets in carbohydrate:protein space, this would have to be taken into account. However, we note that because sexes can have different dietary preferences, they may have very different diets in nature. Studies suggest that even if dietary choice is partly constrained by intra-locus conflict (which is debated), there is still sexual dimorphism in diet choice (Maklakov et al 2008, Rapkin et al 2017).

Minor Comments
Lines 47-51: This section is well written, but not all of the ideas mentioned apply universally to all organisms. It would be helpful to mention the species the research is referring to for each of the studies referenced.

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Line 226: There’s no FF-DR in Figure S3 but this is referenced in the text.

Done.

Line 338: “Despite using a different DR regime to the previous work, we also found that food odour increased the mortality of DR flies” – true but I think figures S1 and S2 could do something to make this clearer, maybe overlaying DR and DRod. This is a super interesting finding and it would be great if it was clearer in the figure.

We found that DR(od) has higher baseline mortality rate (b0) but lower Gompertz’s rate parameter (b1) (See Figure S2) than DR. (LL 410-411)

Figure 2: For clarity I think this figure would benefit from additional labelling. In particular these figures should be labelled A,B,C and D and references in the text as such which will make it easier to find what is being referenced. Also headings for “full lifespan” and “post-switch” would be helpful. Otherwise you have to read the figure legend every time you glance at the figure.

Done

Figure 3: As with figure 2 I think some additional labelling would and make this clearer and easier to refer to in the manuscript.

Done
Figure S1: It would be helpful to have FF and DR-FF and DR and FF-DR (or vice versa) on the same graphs. Please also add the x-axis labels of days to the top bar of graphs - it’s a little difficult to compare them.

We added supplementary figures: Figure S8A compares DR-to-FF and FF; Figure S8B compares D Rod-to-FF vs. FF; Figure S9A compares FF-to-DR and FF, Figure S9B compares FF-to-DRod and FF.

The x axis label is added in the new SM. See Figure S1.

Figure S2. The difference between b0 and b1 not explained in figure legend and it also would be nice if they had a non-mathematical label. It would also be easier to reference in the MS if these graphs were labelled A and B.

Done.

It is difficult to differentiate between the pairwise comparisons and diet switches as they are represented by – and –> could you find a clearer way to represent this.

Done.

Figure S3: Is there an explanation for the large drop in survival for DR(od) to FF and particularly FF(od)? It seems quite strange.

We acknowledge that there was a large drop in survival on these days. The exact reason is not clear.

I think it would improve clarity if you match colours/line types between Figure S1 and Figure S3. It makes comparing them a little confusing.

Done.

Could you explain if the P-value on panel B is the log rank test for trend between all three groups? Or just between FF(od) and DR(od)-FF.

It was comparison of all three groups. If there is no overall difference between the three, there is no need to do individual comparisons.

Figure S4: Just wanted to say I like this a lot - especially the unpaired differences - it illustrates your point well.

Thank you!

Figure S6-S8: You have a figure S6-S8 but no reference to it in the text. Additionally, S6 needs a label for the key. Also I am a little confused how Age (Weeks) and Lifespan (Weeks) are different, could you explain this in the legend.
We removed S6 but left S7 and S8, which are now S6 and S7, as they provide some additional information regarding BASTA analyses.

Supplementary Tables: There are lots of supplementary tables not referenced to in the text.

Done and checked throughout.

General discussion: It would benefit your discussion and your hypothesis to add some discussion of the potential sexual dimorphism that could be at play here (not explored in your paper but possibly worth repeating in males in the future – maybe a limitation of this study). Jensen et al. found that male and female drosophila have different “optimum” dietary intakes of carbohydrate and protein for reproductive success (and lifespan). This may support your hypothesis that reproduction and lifespan are uncoupled. It is possible that this is only true for females, because in males both lifespan AND reproductive success are optimised by high carbohydrate:low protein but in females while high carbohydrate maximises their lifespan, high protein maximises their reproduction.

Thank you for this suggestion, indeed males and females can maximise fitness in different C:P ratios as was shown by Maklakov et al 2008 and later by Jensen et al. 2015 in Drosophila. Jensen et al. did not find a sex difference in intake ratio that maximises lifespan but did find a difference in reproduction. We now mention the C:P ratio in the discussion but we also mention that because males and females likely have different dietary preference and different diets, it is not directly relevant to our main conclusion. (LL 396-402)

This feels similar to some of the results you see here with DR=high carbohydrate and FF=high protein. As you are reducing yeast for your DR, you are mainly constraining protein, which has been shown to be most detrimental to female reproduction but increases lifespan, whereas male reproduction at higher carbohydrate level. When you replace the protein, reproduction increases and lifespan decreases, it would be fascinating to see what happens to males on the same paradigm due to their difference in optima. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4531074/

Yeast has slightly more protein (45%) than carbs (40%) but we cannot say that we mainly constrain protein when we don’t add yeast.

Referee: 2

Comments to the Author(s)
This manuscript by Sultanova and coauthors addresses a fundamental question in life history theory: is the extension of lifespan under dietary restriction adaptive? In this study, they provide evidence that females subjected to dietary restriction fully recover their lifetime reproductive success upon re-exposure to food. The experimental design in this study is excellent, and is supported by sophisticated analyses. Further, the discussion was thorough and very interesting. I thoroughly enjoyed this paper, and have only a few minor comments that I hope might help.
Thank you!

Lines 196-206: I found this text a bit hard to follow. I think this is because it compared the DR and FF treatments in addition to the same diet treatments with odour. The primary figures cited in this paragraph (Figures 2 and 3) don’t compare across the treatments with and without odour in this way (this is instead in the supplementary figures). It might make it easier if you discussed the results of figure 2 and 3 (including the diet switching results) first before comparing the no odour/odour treatments from the supp figures.

We switched the second and the first paragraphs of the Results.

In the section following Line 241, it would be helpful to remind the reader that this is female mating success. Maybe it’s just me, but I always associate mating success with males.

Thank you, we added “female” in the subtitle.

Is the acronym for lifetime reproductive success (LRS) necessary? The paragraph starting on line 269 already has a lot of acronyms, which I find hard to keep track of.

We use LRS several times throughout the text, so it reduces word count, also LRS is an established term in evolutionary biology/ecology so we have retained it to streamline the text. We hope this is OK.