THE ROYAL SOCIETY PUBLISHING

PROCEEDINGS B

Migration behaviour of commercial monarchs reared outdoors and wild-derived monarchs reared indoors

Ayşe Tenger-Trolander and Marcus R. Kronforst

Article citation details
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Review timeline

Original submission:	28 February 2020
1st revised submission:	6 June 2020
2nd revised submission:	13 July 2020
Final acceptance:	14 July 2020

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

Review History

RSPB-2020-0475.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
See attached file

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

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Comments to the Author
Dear Ayse and Marcus,
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2. It seems that you are missing the word 'of' in line 62.
3. There is a typo in the spelling of Asclepias syriaca in line 78.
4. Lastly, you switch back and forth between "wildtype" and "wild-type" throughout the text. Please choose one and make it consistent throughout the entirety of the manuscript.

Abstract: Lines 17-18. This sentence could slightly re-phrased for clarification. I assume you mean that overall/on average/as a whole, the group did not orient south BUT there were a few cases where individuals did do so. Something like "While the majority of commercial monarchs did not fly south, a subset did repeatedly do so, potentially explaining…."

Introduction:
1. Can you articulate why "multiple flight testing" is important in the last paragraph of the introduction? Why is it important that you test the adult monarchs multiple times? Is this because a recent study indicated that monarchs may be able to regain southern orientation over time? If so, you should explain that here. Otherwise, it is unclear to the reader why this matters and why you are doing it. (You state that it can be done and is feasible, but not why it should be done).
2. The results should be saved for the Results section, rather than including them in the Introduction (see lines 66-70).

Methods:
1. The organization of this section should be improved, particularly in 'Animal husbandry.' To paint a clearer picture for the reader, I would start out by explaining each of the four treatment types. For instance, in line 78, you could say: "Once females laid eggs, we washed and transferred the eggs to their respective treatment types..." Then, go on to explain each treatment type and details, and the number of monarchs that eclosed from each type.
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Results: It would be helpful to provide the total number of monarchs (by treatment type) that flew for at least ten minutes in the flight simulator in the text.

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8. A concluding sentence should be added to the second to last paragraph of the Discussion section (following line 255).

Review form: Reviewer 3

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Acceptable

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8) Rather than (or in addition to) citing blog posts on captive rearing in the introduction, the authors could reference official position statements from organizations on monarch captive rearing, including those shared by Journey North

(https://journeynorth.org/tm/monarch/conservation_action_release.pdf), Xerces (https://xerces.org/monarchs/joint-statement-regarding-captive-breeding-and-releasing-monarchs), and NABA (https://www.naba.org/action.html).
9) Line 49 – give reference for previous paper mentioned here.
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12) Methods – line 74 – at what stage were wild monarchs caught, at how many sites, how many individuals were captured?
13) Were all of the monarchs in this study tested for infection by the protozoan OE prior to collecting eggs and running the experiment? This is important, as up to 20% of late summer wild monarchs, and a high fraction of commercial monarchs, can be infected with OE, which can lower monarch survival and body size in captivity, and can also affect flight performance.
14) Line 76 – why order from only a single breeder when there are dozens across the US? And where was the breeder based (what state or region)? How many individuals in total were ordered?
15) Line 77 and elsewhere – pop-up should be hyphenated
16) Line 79 – were the plants greenhouse raised or wild cuttings, potted, etc? Were the plants rinsed or bleached before being fed to monarchs?
17) Why did the outdoor raised monarchs emerge 2 weeks sooner than the indoor reared ones, and what was the temperature experienced outdoors? (could give averages for max-min, and ranges)
18) Line 90- did the authors confirm NPV via microscopy or PCR, is it more suspected? What was the overall % mortality in each treatment group?
19) Did the authors record monarch sex, weight, wing area or wing length, and did this differ among sources and treatment groups?
20) Lines 103-104: Were there attempts to retest ALL individuals, and only some were willing to fly again, and did this account for the 41% of the total were retested? Not clear from how this is worded. Also, what % of original monarchs from each treatment group were tested?
21) Were the flight tests done at the same time each day, and between what hours?
22) Lines 107-110: More information is needed on how flight headings were converted into degree counts and analyzed. Please define 'individual mean vector' and 'vector strength'.
23) Line 111 – this is the first time that data from previous years are mentioned – more information is needed here, and reference a previous paper if these data are published elsewhere.
24) I found the section in lines 110-132 to be difficult to follow. Perhaps this could be better explained using a diagram with figures and side-bar text. I think this section could be both shortened and made more crisp.
25) As suggested earlier, could the authors report on whether other variables such as size differed between the multiple treatment groups?
26) Results – the first paragraph strikes me as belong in the Methods, as it explains what was measured.
27) In the final paragraph of the Results, the first two sentences read more as Methods, and the actual results component of that paragraph would benefit from further development – i.e., what was the % difference observed, and what were the significance values?
28) Table 1 is referenced several times in the Results, but is not provided in the main manuscript file.
29) As noted earlier, the Discussion is highly monarch-centric and would benefit from bringing in perspectives from other species.
30) The authors could cite other work on monarchs showing elements of migratory physiology and orientation (i.e., sun compass work by Taylor et al, and Reppert et al.; work on reproductive diapause and cues that trigger this by Herman, Oberhauser). There is some nice work from other studies that examines the mechanistic basis of migratory behavior in monarchs, but the first paragraph of the Discussion implies that virtually nothing is known.

31) Line 219- avoid starting a paragraph with “However”
32) Line 228 – see point 31. Also, minimum period may be required for what?
33) The paragraph that starts with line 228 is a bit long and could be shortened.
34) A minor point, but there are numerous instances throughout the manuscript where important commas are missing, such as in line 28 (after pitfalls), line 37 (after pressures), line 39 (after Recently), line 42 (after south), line 50 (after sites), line 53 (after wild type, which should be 2 words), line 65 (after feasible), line 67 (after time), and line 68 (after south). *This is not an exhaustive list of places where commas are needed*

Decision letter (RSPB-2020-0475.R0)

@@date to be populated upon sending@@

Dear Ms Tenger-Trolander:

I am writing to inform you that your manuscript RSPB-2020-0475 entitled "Migration behaviour of commercial monarchs reared outdoors and wild derived monarchs reared indoors" 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,
Dr Maurine Neiman
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Thank you for your submission. We received comments from three reviewers. Two of the reviewers have a favorable opinion of the paper overall, one some important critiques. The third reviewer is more critical in terms of the study's importance, clarity, and interpretation.

I am open to a revision that addresses all of the comments in a substantive way. I do expect to send a revised version out for peer review and thus cannot commit that it will ultimately be accepted for publication in this journal. Yet, it does seem that the study could be a valuable one for the general audience that Proceedings of the Royal Society seeks to reach.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
see attached file

Referee: 2

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8. A concluding sentence should be added to the second to last paragraph of the Discussion section (following line 255).

Referee: 3

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11) Line 66 – what is meant by "strength" here – I don't think you tested how strong the monarchs were in terms of pulling or speed, so this needs clarification. Does it mean consistent orientation? It would help to more clearly define the orientation variables quantified in this study in the Methods.
12) Methods – line 74 – at what stage were wild monarchs caught, at how many sites, how many individuals were captured?
13) Were all of the monarchs in this study tested for infection by the protozoan OE prior to collecting eggs and running the experiment? This is important, as up to 20% of late summer wild monarchs, and a high fraction of commercial monarchs, can be infected with OE, which can lower monarch survival and body size in captivity, and can also affect flight performance.
14) Line 76 – why order from only a single breeder when there are dozens across the US? And where was the breeder based (what state or region)? How many individuals in total were ordered?
15) Line 77 and elsewhere – pop-up should be hyphenated
16) Line 79 – were the plants greenhouse raised or wild cuttings, potted, etc? Were the plants rinsed or bleached before being fed to monarchs?
17) Why did the outdoor raised monarchs emerge 2 weeks sooner than the indoor reared ones, and what was the temperature experienced outdoors? (could give averages for max-min, and ranges)
18) Line 90- did the authors confirm NPV via microscopy or PCR, is it more suspected? What was the overall % mortality in each treatment group?

19) Did the authors record monarch sex, weight, wing area or wing length, and did this differ among sources and treatment groups?
20) Lines 103-104: Were there attempts to retest ALL individuals, and only some were willing to fly again, and did this account for the 41% of the total were retested? Not clear from how this is worded. Also, what % of original monarchs from each treatment group were tested?
21) Were the flight tests done at the same time each day, and between what hours?
22) Lines 107-110: More information is needed on how flight headings were converted into degree counts and analyzed. Please define 'individual mean vector' and 'vector strength'.
23) Line 111 – this is the first time that data from previous years are mentioned – more information is needed here, and reference a previous paper if these data are published elsewhere.
24) I found the section in lines 110-132 to be difficult to follow. Perhaps this could be better explained using a diagram with figures and side-bar text. I think this section could be both shortened and made more crisp.
25) As suggested earlier, could the authors report on whether other variables such as size differed between the multiple treatment groups?
26) Results – the first paragraph strikes me as belong in the Methods, as it explains what was measured.
27) In the final paragraph of the Results, the first two sentences read more as Methods, and the actual results component of that paragraph would benefit from further development – i.e., what was the % difference observed, and what were the significance values?
28) Table 1 is referenced several times in the Results, but is not provided in the main manuscript file.
29) As noted earlier, the Discussion is highly monarch-centric and would benefit from bringing in perspectives from other species.
30) The authors could cite other work on monarchs showing elements of migratory physiology and orientation (i.e., sun compass work by Taylor et al, and Reppert et al.; work on reproductive diapause and cues that trigger this by Herman, Oberhauser). There is some nice work from other studies that examines the mechanistic basis of migratory behavior in monarchs, but the first paragraph of the Discussion implies that virtually nothing is known.
31) Line 219- avoid starting a paragraph with "However"
32) Line 228 – see point 31. Also, minimum period may be required for what?
33) The paragraph that starts with line 228 is a bit long and could be shortened.
34) A minor point, but there are numerous instances throughout the manuscript where important commas are missing, such as in line 28 (after pitfalls), line 37 (after pressures), line 39 (after Recently), line 42 (after south), line 50 (after sites), line 53 (after wild type, which should be 2 words), line 65 (after feasible), line 67 (after time), and line 68 (after south). *This is not an exhaustive list of places where commas are needed*

Author's Response to Decision Letter for (RSPB-2020-0475.R0)

See Appendix A.

RSPB-2020-1326.R0

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?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

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

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

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Comments on revised Proceedings B paper
Andy Davis, University of Georgia

I have read through the latest version of Tenger-Trolanger and Kronforst's study on navigation of captive-reared monarchs, and I am pleased with the latest improvements. I was generally in favor of the prior version and had only comments for improvement. I have a few remaining comments that I hope there would be time and energy to address.

- The new supplemental videos are awesome to visually show the difference between a southward-orienting monarch and one that has no directionality. But I don't see these two videos referenced in the text, nor are they labelled in a meaningful way. I assume the "spinning" monarch is one that was reared indoors and the southward one was a wild monarch? If so, then make this clear, both in the title of the videos, and somewhere in the text. These videos will likely be widely shared. I might also recommend you add a big label directly on the video above the monarch, if you can.

- A couple of places use the words "captive-bred", instead of "captive-reared". For some reason, these terms are getting confused by people. Stick to "captive-reared".

- I'm still not satisfied with the wishy-washiness of the statements at the end about captive-rearing (line 339 onward). From all of the research thus far, it seems that captive-rearing and releasing is really causing damage to the population, by weakening migration ability. Why not say this, exactly as I just stated? Or recommend that people severely limit their rearing, or ONLY rear outside (as opposed to what you stated, "rear outdoors when possible"). I know that you want to soften the blow here, but you are also undermining the take-home message of this paper by encouraging people to continue indoor-rearing. Also, nowhere in this paper was there any discussion of the main argument I had made in the Davis et al 2020 paper – that captive-rearing bypasses the typical natural selection that would occur in the wild, so that weaker individuals enter the population that would never have made it on their own. So again, I recommend you conclude with a firm statement pointing out the risks of this practice. And, I would also suggest you include a similar ending statement in the abstract, which is likely to be read by most people

Well done!

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

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

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

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

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

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

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

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Dear authors,

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L263-264: Is it correct to say that you found that commercial monarchs are a mix of migratory and non-migratory individuals? We do not have evidence that southern directional orientation in a flight simulator predicts migratory behavior, correct? I think the only way we can know this for sure is with tagging data. It would be really interesting to test this in the future! We know that some commercial monarchs have indeed made it to Mexico, but we do not know that monarchs in this study would have if provided the opportunity. Thus, I would change the wording from migratory individuals to southern-orienting individuals (or something to that effect).

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L293-296: Split this sentence into two for clarity. Also, I would change the term 'directional migratory adult' for reasons stated in my comments to lines 263-264 above. Again, just because an individual orients southward on average in the simulator, doesn't mean it would migrate successfully.

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L300-310. Great job summarizing the study's findings here! In line 307, you may want to clarify which direction when you say, "once directionally oriented." (I know you mean oriented southward, so I would say that to be entirely clear.)

Decision letter (RSPB-2020-1326.R0)

07-Jul-2020

Dear Ms Tenger-Trolander

I am pleased to inform you that your manuscript RSPB-2020-1326 entitled "Migration behaviour of commercial monarchs reared outdoors and wild-derived monarchs reared indoors" has been accepted for publication in Proceedings B.

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

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

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

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

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

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

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

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

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

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

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

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

Sincerely,
Dr Maurine Neiman
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
Please see positive reviews from two reviewers with some suggestions for improvement. I'll be able to judge if you (the authors) have adequately addressed these suggestions in a revised version of the manuscript, i.e., do not need to send out again for review.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
Comments on revised Proceedings B paper
Andy Davis, University of Georgia

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Well done!

Referee: 2

Comments to the Author(s).
Dear authors,

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Author's Response to Decision Letter for (RSPB-2020-1326.R0)

See Appendix B.

Decision letter (RSPB-2020-1326.R1)

14-Jul-2020

Dear Ms Tenger-Trolander

I am pleased to inform you that your manuscript entitled "Migration behaviour of commercial monarchs reared outdoors and wild-derived monarchs reared indoors" 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

Your article has been estimated as being 9 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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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.

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit https://royalsociety.org/journals/ethics-policies/media-embargo for more information.

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

Dear Editor,

We thank you and the reviewers very much for your valuable feedback on our manuscript. In general, we very much agreed with comments and suggestions. We believe our new manuscript reflects those important changes. We are re-submitting our revised manuscript for your review. Please see below for a detailed response to comments. Our responses are in italics.

Thank you,

Ayse Tenger-Trolander and Marcus R. Kronforst

Referee: 1

Review of RSPB-2020-0475 March 12, 2020 Andy Davis, University of Georgia General This paper describes a study conducted to determine if monarch butterflies reared inside are as capable of orienting properly as wild monarchs during the fall migration. It is a follow up to an earlier project spearheaded by the same authors. I enjoyed reading this paper, and learning of these new data. The authors should be commended for conducting this work, which was largely born out of the tremendous amount of discussion that came from the prior, very, splashy paper. And in doing so, it seems that this new paper quite effectively dispels much of the earlier critiques. While I do have a number of comments and suggestions for improvement, the scope of these only reflects my keen interest in this subject, and not that the paper was lacking.

Specific points - Introduction section

o There is a recurring issue that comes up in the introduction as well as throughout the paper, such as in the headings of the methods. There are many references to "migration behavior" and "flight tests" throughout. Using these terms, it sounds like the tests were on the ability to fly, or flying speed, or something akin to a flight mill that tests actual flying ability or endurance. What you really tested was the orientation ability, or orientation behavior. Where this issue first comes up is in the introduction, and here you should at least briefly describe the flight tests, for those who have not read the prior paper. And in the rest of the paper, use more specific language to indicate your flight tests were looking at flight direction, or navigation.

We have changed references to 'flight tests' to 'directional orientation tests' or 'orientation tests'. We also changed references to migratory behavior to directional orientation.

o The rationale for the paper could be strengthened a bit. The introduction describes how this study came about because some reared monarchs do in fact make it to Mexico, and you wanted to test this. But really, you knew of this fact during the prior study as well, so this seems a little vague. From my reading of this and the prior study (and the hoopla it created), it sounds like this new project was undertaken specifically to address the criticisms of the last one, with one of

the most often-cited being that the "indoor" reared monarchs were reared in an incubator, and not really "indoors". There was a very prominent rebuttal paper that Karen Oberhauser wrote in Nature, that raised this and other issues. I wonder if it would be pertinent to include some discussion of that paper, plus some of the hoopla, in the introduction here. I see you did cite that paper, but it is rather tangential. And you did point out that the prior study did not raise the monarchs indoors, but also in passing. By bringing the critiques up specifically, it would basically make the argument for you, i.e. that this paper was intended to address those critiques.

We have completely re-written the introduction, lines 26-67, to better motivate the rationale behind our work.

While we recognize that the publication of our first paper on directional orientation in monarchs created a stir in the broader monarch community, our motivation for this work did not stem from the controversy. In late 2018, when we realized rearing monarchs in an environmental chamber wouldn't reproduce directional orientation, we started planning additional experiments. This work was the next step in the science but was not motivated by these conversations directly.

- Methods section

o In the bit about the flight simulator, it does not say whether the testing was done blind, so that the observer did not know the origin of the monarchs. Given that the system is set up to record the data autonomously, this may not matter. But if that's the case, just state that instead.

We did not conduct testing blind since the observer only starts the automatic recording. We have included this information in the methods at lines 130-131.

o Line 103 - Why were there additional flight tests on some butterflies, and why were the number of additional test variable? If it states this later, ok, but this statement here seems out of place, or not fully explained.

We focused testing on the outdoor reared individuals since we wanted to assess the overall direction an individual chose. The variation in testing comes from restrictions imposed by time of day, weather, emergence date of the individual, willingness to fly in the simulator, etc. We have re-organized the methods section, and detailed information on orientation testing is now included under 'Flight Simulator and Testing', lines 109 -118. We have also included a new supplemental table (Table S1) which gives the ID of each monarch tested, how many times it was tested, and how many successful tests it had (including those that never flew in the simulator).

o Line 105 – if these flights were recorded on video, it would be really nice to include an example video in the online supplemental files. Or even better, perhaps one video of a monarch that did not fly south, and one that does. One of the things that was questioned a lot of the prior study was this orientation device. Perhaps if people could see it in action, it would be more informative.

We have included two supplemental videos of monarchs flying in the simulator. Spinning_monarch.mp4 is a 1-minute example of a monarch switching directions and South_orienting_monarch.mp4 a 1-minute example of a monarch flying consistently south.

o Line 111 – wait, there is data in here from the prior study? While this seems ok to do (especially given the sample size issue), the statement here also comes out of nowhere. This is something that should have been mentioned earlier if it is an important part of the study, like in the introduction and maybe even the abstract.

We only use the previous data to create a migratory flight group from which we can randomly sample. We have moved this section to the methods section and described the data from the previous work and where it can be found, lines 154-164.

o The sample size issue is one limitation of this study, but the authors did a commendable job to combine the data here with other data, and moreover, the results seems pretty concrete even with this issue.

We have re-written the section of the methods on sample size to improve clarity, lines 119-131.

o Line 112 to 116 – I have read and re-read this sentence and am still having trouble following the procedure here. Maybe clarify and expand on this sentence.

We have significantly modified the 'Random Re-sampling' section to improve clarity, please see lines 154-180.

o The end of the methods section left me hanging. I was reading how the migratory monarch flights were handled, and then it stopped. What about the commercial monarchs? What was it that you were actually comparing here between the groups? Vectors? How were the comparisons made, i.e. statistics? Some of this can be pieced together from reading the results, but this is a glaring hole. The end of any methods section should sum up the key tests and leave the reader wanting to get to the results. –

As suggested, we have re-written and re-organized the methods section significantly.

Results section

o The results section could be enhanced by breaking it up into sections, with subheadings. And, at the end of each section, add a last statement that summarizes the paragraph or section

We have re-organized the results into two different sections with subheadings, starting on lines 193 and 234.

o Line 142 – again, use of the terms flight behavior is not accurate. Flight direction or orientation behavior is more appropriate

We have changed all flight behavior to directional orientation behavior, in this particular instance, this sentence has been removed completely.

o Line 171-174 – the reference to "a number of monarchs did not fly south…" could use an actual number and perhaps a percentage, since this part seems like a key takeaway of the paper.

We have added the counts and percentages to the results, lines 204-209 (commercial) & lines 242-244 (indoor).

- Discussion section

o Line 179 – "…whether monarchs fly south." should be something like "whether monarchs orient south on a flight mill". The prior sounds like you tested actual flying and/or tag recoveries.

We have changed the sentence as suggested to "Our earlier work suggested changes in long-term selection pressures and short-term developmental conditions can affect whether monarchs orient south in a flight simulator[8]." Now on lines 258-260.

o Paragraph beginning line 192. I generally agree with the thoughts raised in this section here, and would add one of my own. The idea that monarchs with poor migration ability would simply die anyway (and therefore not be a problem) may not be entirely true, at least anymore. While there is little data on this yet, it is entirely possible that these poor-migrators don't die, but rather they fail to keep up with the main cohort and then become stragglers that wind up on Gulf coast or south Atlantic coast regions, where they eke out the winter and avoid freezes. These stragglers are becoming more abundant each year, and with warmer climates, these failed migrants could actually end up reproducing, especially if there is tropical milkweed nearby, which there usually is these days.

Very true, we have added this as another potential problem on lines 277-278. We have also expanded the section on resident populations and disease, lines 283-290.

o Line 205 – probably not correct to say that indoor monarchs did not orient south. You found that most do not. There is a real difference, and the people who rear monarchs are always pointing to how one or more of their reared monarchs was found in Mexico.

We have changed the sentence to "Wild-type monarchs reared indoors with full exposure to natural autumn sun did not consistently orient south, though their genetic background is identical to the wild types reared outdoors." Now on lines 291-293.

o Two paragraphs in the discussion begin with the word However, which is poor grammar.

We have replaced both of these sentences. Those two paragraphs now begin on line 311 and line 327 respectively.

o The paragraphs describing the Wilcox et al study were well-placed here, since that study did appear, at least at first glance, to throw a wrench into these results here. But, as you pointed out, that study suffered greatly from a lack of control (wild) monarchs. In fact, from my interpretation

of that paper, it looks like the reared monarchs they tracked using the backpacks were actually flying in the wrong direction – their mean flight headings were southeast, which would put them heading toward New Jersey. You could point this out here if need be, to further underscore how that paper was flawed. To me, the lack of controls was egregious. Also, I like how you rebutted their case by looking at outdoor exposure time vs flight direction (Table S1), but I also wonder if you could bring up something from your last experiment. In that study, you had examined monarchs at different ages, and in some cases they were several weeks old, I recall (I looked at the supplemental data). It seems to me that those data also refute this idea that the monarchs simply need more time to acclimate to outdoors. This was something that I don't think anyone realized from the last paper, or maybe you didn't play it up enough.

You are correct. In our previous experiments on indoor-reared monarchs, we did follow the same procedure (i.e. bringing all tethered butterflies outdoors whenever testing). Some of them would have had prolonged outdoor exposure at the time of their test. Unfortunately, I only recorded the ID and time for individuals tested on a given day (not all that experienced the outdoors). If a monarch flew continuously for ten minutes, I would freeze the individual – leaving us with data from only 17 orientation tests (no re-testing in 2018), and the minimum time spent outdoors could be a vast underestimate (I could have brought an individual outdoors several times without ever testing it). We think that including the data here would only serve to confuse readers.

o There is something in the discussion that seems missing here, which needs to be brought up somewhere. The Oberhauser rebuttal was very critical of the earlier study because your incubator did not vary the daylength to truly mimic fall environmental conditions, and she then suggested that rearing next to a window would do that. A lot of enthusiasts then used her argument to disregard the entire PNAS paper. But here, you effectively quashed that argument – rearing next to a window, and with declining daylength, still did not result in effective migrators. Perhaps you could add some text or a paragraph here, where you are also discussing other work.

The final paragraph now begins with "While many people hope that captive rearing is helping a declining population, the cumulative data available suggest that captive breeding of monarchs has negative consequences for migration behaviour, and that monarchs reared indoors are not as well equipped to survive migration as those left in the wild[8,13,38]." Now on lines 339-342.

o Line 254 – I didn't know this – very cool.

The release and re-captures are reported in the paper, but the distances flown were not compared. We had to use release and recovery sites reported to determine the minimum distance flown by each individual. We also now have additional data from the author that increases the sample sizes to 10 indoor reared and 6 wild, lines 333-338.

o Paragraph beginning line 256 – while I know that you may want to soften the blow here by stating this (not dissuade people from rearing), I think you, and others who suggest the same should take a firmer stance here. There is a lot of evidence now that the biggest threat to

monarchs in the east is a failure to migrate, so we as a society should not be doing anything to make it more difficult for the migrators. Rearing appears to do just that – make it more difficult. I would encourage you to strengthen this argument, while still being polite. But at the very least, I think you should offer a suggestion for how many monarchs people could safely rear without harming the population. That has been sorely lacking in all of the discussion over rearing. I would argue that 10 monarchs per year is a safe number. This allows people the opportunity to experience the process, and even contribute to some citizen science, but then it may not hurt. My fear is that if this paper comes out and it indicates that rearing monarchs is ok because at least some reared monarchs are successful (which is the current thinking), then it will galvanize people to simply rear more. They'll just do the mental math – if 5% of reared monarchs orient properly, then I'll just rear 500, so that 25 reach Mexico.

We agree and certainly do not want people to think that they should rear even more monarchs to make up for the fraction that do not migrate. We have revised this paragraph to include more specific advice, but have stayed away from citing a specific number of monarchs to rear. The new conclusion paragraph begins on line 339. Specific advice starts on line 344.

- Figures

o I know that the orientation device was described in the prior study, but for anyone reading this who had not read that, I wonder if Figure 1 could have a separate panel showing the device schematic.

The flight simulator schematic is now included in Figure 1. We have separated Figure 1 into two figures. The indoor rearing orientation data are now in Figure 4.

o Figure 3 would benefit from adding labels of each monarch treatment/rearing type, directly above the circles

We have added genetic background to the plots. Since all of the individuals in Figure 3 were reared outdoors, this is now written in the first sentence of the figure text rather than on each plot.

Referee 2:

Dear Ayse and Marcus,
It is great that you have explored this topic further, building on your last publication. I like that you've added the natural light treatment and that you are trying to understand the role timing plays in southern orientation. My main suggestions for the manuscript include improving clarity, consistency, providing missing key information, and further discussing some of the limitations of the study. See my comments regarding each specific section outlined below. (I have also attached a PDF of the comments in case the formatting does not work out here.)

General comments: There are a few places throughout the manuscript in which the wording needs to be cleaned up.
1. The word 'multiply' should be removed in line 52.

We have removed this and re-worded the sentence to reflect that we tested individual monarchs multiple times. Please see the new introduction lines 42 – 48.

2. It seems that you are missing the word 'of' in line 62.

We have changed the introduction such that this sentence no longer appears.

3. There is a typo in the spelling of Asclepias syriaca in line 78.

We have corrected the error in spelling.

4. Lastly, you switch back and forth between "wildtype" and "wild-type" throughout the text. Please choose one and make it consistent throughout the entirety of the manuscript.

We used 'wildtype' as a noun and 'wild-type' as an adjective through-out the text. We did mistakenly use 'wildtype' as the noun in our first submission; all references to 'wildtype' are now 'wild type.' Any use of 'wild-type' as an adjective remains hyphenated.

Abstract: Lines 17-18. This sentence could slightly re-phrased for clarification. I assume you mean that overall/on average/as a whole, the group did not orient south BUT there were a few cases where individuals did do so. Something like "While the majority of commercial monarchs did not fly south, a subset did repeatedly do so, potentially explaining…."

As suggested, we have changed this in text to "While as a group the commercial monarchs did not fly south on average, a subset of individuals did orient south over multiple tests, potentially explaining the discordance between flight simulator assays and the recovery of tagged commercial monarchs at overwintering locations." See line 17. We have also added the phrase "as a group" to lines 39 and 41 of the introduction.

Introduction:
1. Can you articulate why "multiple flight testing" is important in the last paragraph of the introduction? Why is it important that you test the adult monarchs multiple times? Is this because a recent study indicated that monarchs may be able to regain southern orientation over time? If so, you should explain that here. Otherwise, it is unclear to the reader why this matters and why you are doing it. (You state that it can be done and is feasible, but not why it should be done).

This is an excellent point. We have modified the introduction and now explain why testing an individual monarch multiple times is important, lines 40 – 50.

2. The results should be saved for the Results section, rather than including them in the Introduction (see lines 66-70).

We have removed this section from the introduction.

Methods:
1. The organization of this section should be improved, particularly in 'Animal husbandry.' To paint a clearer picture for the reader, I would start out by explaining each of the four treatment types. For instance, in line 78, you could say: "Once females laid eggs, we washed and transferred the eggs to their respective treatment types..." Then, go on to explain each treatment type and details, and the number of monarchs that eclosed from each type.

As suggested, we have re-organized the method section. Animal husbandry has been separated it into two new sections: 'Animal Husbandry' and 'Treatments'. See lines 71-84 for 'Animal Husbandry' and lines 86 – 101 for 'Treatments.'

2. Lines 78-79: Did you rear the eggs individually in the small outdoor mesh cages (1 per cage) or together? Can you please clarify that in the manuscript?

Caterpillars were reared in groups; this is now explicitly stated on line 79 of the 'Animal Husbandry' section.

3. Lines 78-79: Was the milkweed (Asclepias Syriacs) wild collected at the time of the study or grown in a greenhouse? You should note this in the methods as well.

We collected milkweed from the wild. We have added this information at line 80-81.

4. What was the timing of eggs laid by adults? Why did commercial monarchs eclose so much later than the wild-type monarchs? You should explain this.

The commercial and wild-type outdoor monarchs emerged in overlapping windows; however, the wild-type monarchs reared indoors emerged later. We have included a more detailed description of our suspected NPV outbreak that led to the later ecolsion of the indoor group, lines 97 – 101 of the 'Treatments' section.

Though the indoor group eclosed late (Sept 24th – Oct 28th), outdoor-reared monarchs in 2016 that eclosed between Oct. 7-20th and monarchs in 2018 that eclosed between Sept 7th-18th, both flew south – these data are published in Tenger-Trolander et al. 2019.

5. Lines 99-101: Did you also provide nectar in these outdoor cages?

Yes, please see line 83 in 'Animal Husbandry.'

6. You indicate that you included flight data from 2016 and 2018 for wild-type outdoor-reared monarchs. You should provide further detail on those monarchs in the methods section as well – were they reared in the same conditions that you used in 2019? What was the timing of collection and emergence for those adults?

The 2018 and 2016 outdoor-reared, wild-type monarch data (ecolsion date and directional orientation) are published in Tenger-Trolander et al. 2019 in the supplemental data file 'Dataset_S01.xlsx' which we have now indicated in the methods. They were reared in the same conditions which is also now explicit in the methods. We have added the emergence dates for those two years as well. Please see lines 157-163 under 'Random Re-sampling of Migratory Flight Data'.

Results: It would be helpful to provide the total number of monarchs (by treatment type) that flew for at least ten minutes in the flight simulator in the text.

We realize that our descriptions of the sample sizes were quite confusing. We have included a new description at lines 119 – 130 of the 'Flight Simulator and Testing' section that explicitly states the number of fliers by treatment and population. We have also included a new supplemental table (Table S1) which includes the ID of each monarch tested, how many times it was tested, and how many successful tests it completed (i.e. flew continuously for 10 minutes).

Discussion:
1. Lines 182-184. I think the language could be softened here to something like "migration loss MAY BE a result of a combination of….." This study did not necessarily test each of those factors and therefore, does not know that it is for sure a combination of all of those things. (For instance, how do you know that small breeding size affects migration loss?)

We have completely removed this from the manuscript.

2. Lines 201-203: I think you could expand on this statement. What is the significance of

year-round breeding increasing in this scenario? It is likely that year-round breeding would really only occur in the presence of Asclepias curassavica (tropical milkweed), which is indeed present in the southern US. Thus, these two issues are sort of coupled, along with the transmission of Ophryocystis elektroscirrha (Oe). If year-round breeding increases because of non-migratory alleles and the presence of tropical milkweed, so may the transmission of Oe.

As suggested, we have expanded upon the significance of year-round breeding on increased disease transmission, see lines 282 – 289 of the discussion.

3. Lines 216-217: Please distinguish between wild-caught and wild-type monarchs in this sentence. I assume you are comparing the flight of the wild-type reared monarchs to those collected as adults from the wild (wild-caught). Please indicate if this is correct and clarify it in the paper.

Yes, that is correct. We have clarified the text, please see lines 304-306.

4. Line 218: Please expand on what exactly you mean by "until they experience a bout of cold and re-orient north." A few more details here would be helpful.

We have slightly modified this sentence to indicate that northern orientation is part of the spring re-migration, lines 306-309.

5. The topics presented in lines 228-244 should have been introduced in the methods and results sections -- the discussion should not be the first time you mention that analysis. In lines 234-236, you say that increased time spent outdoors does not correlate with southern orientation. That test should be presented in Methods and the statistics of the test should be reported in the Results section.

We have moved the discussion of time spent outdoors to the 'Results' section, lines 247-251. We have added a sub-section, 'Outdoor Exposure and Southern Orientation,' at line 181 detailing the method and statistical tests used.

6. I think there are a few other topics regarding the study design that warrant discussion in the manuscript. First, the monarch conservation community wants to know if rearing monarchs in their homes will cause monarchs to lose orientation/migration ability. I think it is worth noting that your study does not exactly or fully answer this question. The greenhouse treatment most closely mimics home conditions in the fall, which are likely warmer during the day and cooler at night. You found that neither greenhouse-reared nor

lab-reared monarchs orient south, but only 12 monarchs were reared in the greenhouse. Based on Figure 1, it looks like a total of 12 indoor-reared monarchs were tested, and this appears to be greenhouse-reared + lab-reared monarchs. Thus, I am guessing the number of greenhouse-reared monarchs flight tested was even smaller than 12. Therefore, I would like to see this limitation discussed in the paper.

We recognize that the way in which we reported sample size was quite confusing; we re-organized this substantially in the manuscript. There were in fact 18 greenhouse-reared individuals tethered and tested of which 15 flew (at least once) and 4 lab-reared of which 3 flew at least once. These changes can be found at lines 119–130 of 'Methods' section and lines 196-199 and lines 238-239 of the 'Results' section.

We have discussed the limitations of the testing of indoor-reared individuals on lines 292-295 of the 'Discussion' section.

7. It seems a bit concerning that many of the monarchs were tested so late (early November). Many monarchs have already reached the overwintering grounds by early November. The sun angle and temperature would have been different than testing in September, when monarchs typically begin their migration from the Midwest. The outdoor conditions that you monarchs experience (written about in lines 230-236), would have varied based on the monarch tested (because the dates varied so much). This should be discussed in the Discussion section.

While we understand the concern, we have tested outdoor reared wild types even later in the autumn (between the dates of Oct 31st – Nov. 9th) and found that as a group they flew directionally south. The results of these orientation tests are reported in Tenger-Trolander et al. 2019. Interestingly, once a monarch becomes directionally oriented south, it does not alter that behavior (Perez et al. 2004) until it experiences a sufficient period of relative cold as documented in Guerra et al. 2013.

8. A concluding sentence should be added to the second to last paragraph of the Discussion section (following line 255).

We have added a concluding sentence to this paragraph, see lines 335-337.

Referee: 3

Comments to the Author(s)

General strengths:
This study examines that migratory orientation behavior of captive-reared monarchs, and contrasts both monarch source (wild or commercial) and rearing conditions (indoors or outdoors). The authors test the prediction that commercial monarchs do not show consistent directional orientation (even when reared outdoors). The authors also test the idea that wild-type monarchs reared indoors with ambient light show less directional orientation than outdoor reared wild monarchs. Importantly, the authors examine the repeatability of individual monarch orientation behavior across treatments. Findings here show that commercially sourced monarchs and wild monarchs reared indoors do not show consistent southward orientation behavior, in contrast to wild sourced outdoor reared monarchs.

Directional orientation is a key element of the migratory syndrome of monarchs (and many other migratory species). Testing how captive rearing affects monarch flight orientation is timely and of conservation relevance. Given that monarchs are heavily used in classrooms, outreach activities and citizen science programs, captive rearing has scientific and educational value. Because thousands of people also rear large numbers of monarchs with the goal of protecting them from sources of natural mortality, this goal of boosting monarch population size through mass rearing can have multiple downsides – including those shown here – that reared monarchs might not sufficiently orient during the fall migration. Understanding the mechanistic basis for these differences is helpful to inform future monarch rearing and conservation activities, and is also important for understanding the evolution and persistence of migratory behavior.

General concerns:
My concerns about the paper, outlined below, can be addressed by (1) better contextualizing the motivation and findings of this study beyond monarchs, in the Introduction and Discussion, (2) more clearly outlining the methods and description of analyses, and (3) including additional variables beyond orientation angle (such as adult weight, wing area, and wing color) in the analyses. Revising the paper to account for these concerns would improve the general interest, quality and importance of the study.

These are excellent points, and we have worked hard to address them. Responses to specific comments are below.

In terms of general interest, the authors could do a better job broadening the contextual importance of this work beyond monarchs as a single species. Acclimation and genetic adaptation to captive conditions is a concern for many wild species of conservation concern

– for example, see Frankham, R., 2008. Genetic adaptation to captivity in species conservation programs. Molecular ecology, 17(1), pp.325-333. The authors could note whether captive rearing is known to affect migratory behavior or orientation in other species, especially for hatchery-reared salmonids, where there should be some literature to draw upon. What other behavioral components crucial for survival in the wild are impacted by captive rearing? At present, the introduction, discussion and literature cited focus almost exclusively on monarchs, which is a limitation of the current manuscript.

We have re-written the introduction, lines 27 -67, to broaden general interest and place our results within the context of captive breeding as a solution to population decline more generally. In terms of the discussion, we have chosen to keep the focus on monarchs to adequately address the literature and controversy surrounding directional orientation in commercial and captive-reared individuals.

A methodological concern in the study is the small sample size and high mortality during rearing, discussed below. Beyond this, the Methods section would benefit from additional details of experimental protocols and methods for data analysis. The second on circular statistics is overly brief, and the section on random re-sampling is hard to follow. Similarly, the Results section could be better organized, and the authors could more consistently report on significance and % differences among the treatments.

We have re-written the section on sample sizes, lines 119 – 128. We have added detail and explanation to the 'Data Analysis and Circular Statistics' section of the methods as well, lines 134 – 151. We have also re-written the 'Random Sampling' section to improve clarity of the analysis. For the results section, we have broken up the results into two subsections and improved consistency of reporting on differences between the treatments/populations.

To strengthen the findings reported here, it would help for the authors to examine 2-3 additional variables important for migration that differ among sources and rearing treatments. In particular, body size, thorax size, weight, wing area, and wing color can differ between migratory and non-migratory monarch populations and have also been shown to be important for flight in other butterfly species. It would be interesting to know whether the groups examined here showed similar differences. Adding this to the manuscript would not require much additional length, and could even be done in a supplement.

While we agree that the addition of any number of the traits mentioned above would be interesting, measuring directional orientation is destructive to the specimens, especially when testing repeatedly (as we did in this case).

Over repeated testing and time spent in the outdoor cages, the monarchs lose scales and eventually chunks of wing. In our previous publication (Tenger-Trolander et al 2019), we were able to examine wing shape and size because we only tested each individual once and then immediately froze the specimen. In that case, the wings were in decent condition. We found that the commercial monarch's wings were significantly different in shape, but not size. The wings of the specimens from this work are very tattered. Unfortunately, we cannot assess body mass either due to the tethering method. Additionally, the specimens are not age matched at time of freezing/death.

In addition to these general concerns, other comments are provided below:

1) In the Abstract, line 9 add "of" between group and commercially-derived

Missing 'of' has been added, now on line 10.

2) Lines 15-17 – does this refer to variation in strength and direction among individuals, or within individuals?

We have removed this part of the sentence in the abstract, see line 15 - 16. Instead, on lines 214-218 of the results, we describe that the difference is within individuals – so variance in the vector magnitudes from the individual's multiple tests- compared between the two populations.

3) Lines 18-19 – Reword the first part of this sentence to state that the commercially-sourced monarchs as a group did not orient in a southward direction on average… .

We have changed the text (now on line 17) to reflect that the group did not fly south on average.

4) Line 20 – add the word "when" between even and raised

We have added the missing 'when' on line 20.

5) As a key word, add 'directional orientation'

We have added the new keyword, line 25.

6) Introduction – as noted earlier, broaden this opening paragraph to consider captive rearing in conservation or management more broadly, and whether insights from other species can inform the way that acclimation and adaptation to captive conditions influence behaviors critical for survival in the wild.

We have re-written the introduction with this suggestion in mind. Please see the new introduction, lines 27-67.

7) In considering motivation for captive rearing of monarchs, it's important to note that several citizen science programs have data recorded from captive raised monarchs (Monarch Watch, Monarch Health, MLMP-tachinid project). Also, a key motivation for captive rearing is to protect monarch eggs from predators and parasitoids, as past work showed that >90% of eggs do not reach the adult stage in the wild.

While we do not directly address citizen science in the introduction, we now write about citizen science projects in our discussion, see lines 346-349.

8) Rather than (or in addition to) citing blog posts on captive rearing in the introduction, the authors could reference official position statements from organizations on monarch captive rearing, including those shared by Journey North (https://journeynorth.org/tm/monarch/conservation_action_release.pdf), Xerces (https://xerces.org/monarchs/joint-statement-regarding-captive-breeding-and-releasing-monarchs), and NABA (https://www.naba.org/action.html).

In re-writing the introduction, we have completely removed this section.

9) Line 49 – give reference for previous paper mentioned here.

This sentence is no longer part of the manuscript; however, any mention of our prior work is now cited.

10) Line 65 –reword to state that "we find that repeated testing of flight orientation in individual monarchs is feasible…"

We have removed this sentence and now discuss the importance of multiple testing on lines 40-50.

11) Line 66 – what is meant by "strength" here – I don't think you tested how strong the

monarchs were in terms of pulling or speed, so this needs clarification. Does it mean consistent orientation? It would help to more clearly define the orientation variables quantified in this study in the Methods.

This sentence has been removed completely. We now use the phrase 'vector magnitude' in place of strength which was confusing. A more detailed discussion of the orientation variables is now available in the 'Data Analysis and Circular Statistics' section, lines 134-151.

12) Methods – line 74 – at what stage were wild monarchs caught, at how many sites, how many individuals were captured?

We caught approximately 20 monarchs at a single site as adults in July 2019. We have added this information in the 'Animal Husbandry' section of the methods, lines 72-74.

13) Were all of the monarchs in this study tested for infection by the protozoan OE prior to collecting eggs and running the experiment? This is important, as up to 20% of late summer wild monarchs, and a high fraction of commercial monarchs, can be infected with OE, which can lower monarch survival and body size in captivity, and can also affect flight performance.

While OE does affect flight performance, it does not affect the directional orientation of monarchs. Only monarchs not infected with OE were kept and bred, please see lines 74-75.

14) Line 76 – why order from only a single breeder when there are dozens across the US? And where was the breeder based (what state or region)? How many individuals in total were ordered?

We ordered from the same breeder we had previously identified as having a non-directional population. Since we already knew that this specific population exhibited unusual behaviour which we wanted to further investigate, we chose to use the same population. We have added that we ordered 20 individuals from the breeder on line 73 of the methods.

15) Line 77 and elsewhere – pop-up should be hyphenated

We have corrected the mistake here and throughout the paper.

16) Line 79 – were the plants greenhouse raised or wild cuttings, potted, etc? Were the plants rinsed or bleached before being fed to monarchs?

We fed caterpillars a diet of wild-collected Asclepias syriaca that we bleached, washed, and replenished daily. This is now stated on lines 80-81 of 'Animal Husbandry'.

17) Why did the outdoor raised monarchs emerge 2 weeks sooner than the indoor reared ones, and what was the temperature experienced outdoors? (could give averages for max-min, and ranges)

The wild-type monarchs reared indoors emerged later due to a suspected NPV outbreak. We have included a more detailed description of our suspected NPV outbreak that led to the later ecolsion of the indoor group, lines 97 – 101 of 'Treatments.'

18) Line 90- did the authors confirm NPV via microscopy or PCR, is it more suspected? What was the overall % mortality in each treatment group?

We did not confirm with PCR or microscopy. The symptoms were consistent with NPV infection. We do not know the % mortality in each treatment group as monarch were reared in groups, now stated on line 79.

19) Did the authors record monarch sex, weight, wing area or wing length, and did this differ among sources and treatment groups?

We recorded sex which does not vary between treatment groups. Unfortunately, repeated testing monarchs for directional orientation is quite destructive. As we mention above, over the course of testing, the monarchs lost wing scales and chunks of their wings making it impossible to accurately assess wing length, area, or color for these individuals retroactively.

20) Lines 103-104: Were there attempts to retest ALL individuals, and only some were willing to fly again, and did this account for the 41% of the total were retested? Not clear from how this is worded. Also, what % of original monarchs from each treatment group were tested?

We have now included detailed information on the counts and percents of monarchs tested in the 'Flight Simulator and Testing' section, lines 119-131, as well as a new supplemental table (S1) that lists the total number of times each monarch was tested and how many of those tests were successful (i.e. flew continuously for 10 minutes) by population and treatment.

21) Were the flight tests done at the same time each day, and between what hours?

Yes, flight testing was performed in the same window of time, between 10am-2:30pm. We have added this information at line 111-112.

22) Lines 107-110: More information is needed on how flight headings were converted into degree counts and analyzed. Please define 'individual mean vector' and 'vector strength'.

We have substantially revised the 'Data Analysis and Circular Statistics' section, lines 134-151.

23) Line 111 – this is the first time that data from previous years are mentioned – more information is needed here, and reference a previous paper if these data are published elsewhere.

We use the previous data to create a pool of known migrator tests from which we can randomly sample. We have added this as well as where the data can be found under 'Random Re-sampling of Migratory Flight Data,' lines 154-164.

24) I found the section in lines 110-132 to be difficult to follow. Perhaps this could be better explained using a diagram with figures and side-bar text. I think this section could be both shortened and made more crisp.

We have substantially revised this section to improve clarity, please see lines 154-180.

25) As suggested earlier, could the authors report on whether other variables such as size differed between the multiple treatment groups?

Please see response to comment 19.

26) Results – the first paragraph strikes me as belong in the Methods, as it explains what was measured.

We have removed this section completely from the results. Relevant information is now included in the 'Methods' section, lines 143-148.

27) In the final paragraph of the Results, the first two sentences read more as Methods, and the actual results component of that paragraph would benefit from further development – i.e., what was the % difference observed, and what were the significance values?

We have removed the first sentence and added that information to the 'Methods' section under 'Data analysis and circular statistics.' We have also revised the 'Results' section. Lines

200-218 report on the differences between commercial and wild outdoor reared. Lines 240-252 report on the differences between wild outdoor and indoor reared groups. Both include new statistics.

28) Table 1 is referenced several times in the Results, but is not provided in the main manuscript file.

Table 1 was part of the draft proof we received from Proceedings B; however, we noticed it is on the final page with the page number listed as 14 of 13. While other reviewers did not note the absence, we wonder if perhaps the table was not available in reviewer's version? We apologize for this and will make sure the draft includes all tables and figures.

29) As noted earlier, the Discussion is highly monarch-centric and would benefit from bringing in perspectives from other species.

Please see the new introduction, lines 27-67.

30) The authors could cite other work on monarchs showing elements of migratory physiology and orientation (i.e., sun compass work by Taylor et al, and Reppert et al.; work on reproductive diapause and cues that trigger this by Herman, Oberhauser). There is some nice work from other studies that examines the mechanistic basis of migratory behavior in monarchs, but the first paragraph of the Discussion implies that virtually nothing is known.

We have changed this sentence to reference work on diapause and navigation in migratory monarchs, lines 255-258. We have also switched the word migration to directional orientation which is a more accurate description of the migratory phenotype to which we are referring. Very little is understood about developing and maintaining southern/directional orientation.

31) Line 219- avoid starting a paragraph with "However"

We have removed the 'howevers.'

32) Line 228 – see point 31. Also, minimum period may be required for what?

We have completely removed this line.

33) The paragraph that starts with line 228 is a bit long and could be shortened.

We have shortened this text and separated the paragraph into two, now starting at line 311.

34) A minor point, but there are numerous instances throughout the manuscript where important commas are missing, such as in line 28 (after pitfalls), line 37 (after pressures), line 39 (after Recently), line 42 (after south), line 50 (after sites), line 53 (after wild type, which should be 2 words), line 65 (after feasible), line 67 (after time), and line 68 (after south). *This is not an exhaustive list of places where commas are needed*

We have addressed this by adding all missing commas in the manuscript and correcting the misspelling of wildtypes to wild types.

Appendix B

Dear Editor,

We are submitting our revision (RSPB-2020-1326.R1) for your review. Please see below for a detailed response to reviewer comments. Our responses are in blue italics. We thank you and the reviewers again for valuable feedback on our manuscript.

Thank you,

Ayşe Tenger-Trolander and Marcus R. Kronforst

Associate Editor
Board Member
Comments to Author:
Please see positive reviews from two reviewers with some suggestions for improvement. I'll be able to judge if you (the authors) have adequately addressed these suggestions in a revised version of the manuscript, i.e., do not need to send out again for review.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
Comments on revised Proceedings B paper
Andy Davis, University of Georgia

I have read through the latest version of Tenger-Trolanger and Kronforst's study on navigation of captive-reared monarchs, and I am pleased with the latest improvements. I was generally in favor of the prior version and had only comments for improvement. I have a few remaining comments that I hope there would be time and energy to address.

- The new supplemental videos are awesome to visually show the difference between a southward-orienting monarch and one that has no directionality. But I don't see these two videos referenced in the text, nor are they labelled in a meaningful way. I assume the "spinning" monarch is one that was reared indoors and the southward one was a wild monarch? If so, then make this clear, both in the title of the videos, and somewhere in the text. These videos will likely be widely shared. I might also recommend you add a big label directly on the video above the monarch, if you can.

We have provided more details on the individuals flying in both videos in the description of both videos in the 'Electronic Supplementary Document'. We have also added descriptions in text in the videos as suggested. We have added a reference to both videos in text at lines 114-

115 of the 'Flight Simulator and Testing' section of the methods.

- A couple of places use the words "captive-bred", instead of "captive-reared". For some reason, these terms are getting confused by people. Stick to "captive-reared".

We have changed both occurrences of captive-bred to captive-reared for clarity as suggested.

- I'm still not satisfied with the wishy-washiness of the statements at the end about captive-rearing (line 339 onward). From all of the research thus far, it seems that captive-rearing and releasing is really causing damage to the population, by weakening migration ability. Why not say this, exactly as I just stated? Or recommend that people severely limit their rearing, or ONLY rear outside (as opposed to what you stated, "rear outdoors when possible"). I know that you want to soften the blow here, but you are also undermining the take-home message of this paper by encouraging people to continue indoor-rearing. Also, nowhere in this paper was there any discussion of the main argument I had made in the Davis et al 2020 paper – that captive-rearing bypasses the typical natural selection that would occur in the wild, so that weaker individuals enter the population that would never have made it on their own. So again, I recommend you conclude with a firm statement pointing out the risks of this practice. And, I would also suggest you include a similar ending statement in the abstract, which is likely to be read by most people

While we agree that captive rearing of monarchs is more than likely negatively affecting migration in the North American population, we also understand there is not complete consensus on the issue. For instance, no one has found direct evidence that commercial monarch genetics are present in wild populations. The evidence we do have suggests that captive-reared monarchs will survive at seriously reduced rates given their non-adaptive phenotypes for migration, but it is not entirely clear what that does to the wild population. While we also suspect releases of captive-reared monarchs affect wild populations, we just do not know whether that is the case. We also have no information on enthusiast rearing, how many individuals are taken from the wild every year? How many are released? How many are bought and released? Without estimates, we find it difficult to argue that people should only rear a specific number.

We have changed the sentence on lines 346-348 to "For those who love rearing monarchs, we advise the following: rear caterpillars individually in clean enclosures, rear outdoors when possible (especially in late summer and autumn), limit the total number reared, avoid purchasing, and participate in citizen science projects."

Well done!

Thank you!

Referee: 2

Comments to the Author(s).
Dear authors,

Thank you for your substantial edits to the manuscript. You have greatly improved the organization and clarified your intent, methods, and results. The subsections have added much clarity to the manuscript. I am pleased to see the addition of Table S1 and the simulator videos. Please see my comments below regarding some minor edits to clarify sections of the Discussion.

L263-264: Is it correct to say that you found that commercial monarchs are a mix of migratory and non-migratory individuals? We do not have evidence that southern directional orientation in a flight simulator predicts migratory behavior, correct? I think the only way we can know this for sure is with tagging data. It would be really interesting to test this in the future! We know that some commercial monarchs have indeed made it to Mexico, but we do not know that monarchs in this study would have if provided the opportunity. Thus, I would change the wording from migratory individuals to southern-orienting individuals (or something to that effect).

As suggested, on line 265, we have changed the wording here to, "We found that the commercial monarchs are a mix of southern-orienting and non-southern orienting individuals."

L266-270. "Without annual selection against weak migrators plus small population sizes, long-term captive rearing could lead to stochastic increase in the frequency of non-migratory alleles which do not respond to the correct environmental cues or perhaps alter the reaction norm of the population making responses to the environment more variable. Additionally, migration may even be actively selected against in captivity." The first sentence here is lengthy and unclear. What is removing the annual selection against weak migrators in this case (commercial breeding?) and by what mechanism? You should clarify those things here. Furthermore, how would selection be actively selected against in captivity? Can you explain that further?

We have re-written this section on lines 268-273 of the current manuscript as follows:

"In commercial facilities, the difficulties of flying thousands of kilometers, finding the overwintering ground, and surviving till spring are no longer barriers to successful breeding. Add to that small population sizes, inherent to commercial breeding, and long-term captivity could lead to stochastic increase in the frequency of non-migratory alleles that do not respond to the correct environmental cues or alter the reaction norm of the population making responses to the environment more variable.'

We removed the sentence about active selection against migration in captivity as we feel a full discussion would require several additional sentences and is not critical to the discussion.

L264-266. The wording of this sentence could be slightly improved to something like: "While the effect of commercial releases on the North American monarch population is currently unknown, it may be ultimately inconsequential if natural selection purges the wild population of non-migratory individuals."

We have taken the advice of the reviewer and changed the sentence to the above, now starting on line 276. Thank you for the suggestion.

L279. Do you have any references to support this sentence (regarding the recessive and polygenic nature of migration genetics)? That would make this stronger.

The migration syndrome is composed of morphological, physiological, and behavioral phenotypes. Monarchs alter their metabolism, body fat content, directional orientation, reproductive output, etc. These many different traits make it difficult to presume a single gene could be responsible for migration. As for the recessive nature, we do have a reference which we have now included (line 281). Crosses between wild North American and commercial monarchs resulted in directionally southern orienting adult hybrids.

L286: I would use the word "rates" instead of "numbers" when referring to Oe prevalence in resident populations.

We have switched the word numbers to rates (line 289).

L293-296: Split this sentence into two for clarity. Also, I would change the term 'directional migratory adult' for reasons stated in my comments to lines 263-264 above. Again, just because an individual orients southward on average in the simulator, doesn't

mean it would migrate successfully.

We have changed 'migratory' to directional, and split the sentence as suggested. The sentence starting on line 295 now reads,

"That being said, our results do not fully answer the question of what degree of "naturalness" is required to rear a directional adult. As we have only 5 indoor-reared individuals with multiple tests, we do not know if some proportion of the indoor-reared individuals are directional."

L297: Again, the term "directionally oriented migrators" is maybe not appropriate. See comments above. I'd recommend replacing with a term like southern-oriented individuals if that matches your intent.

We have changed the word 'migrators' to 'monarchs' in this sentence (line 299).

L300-310. Great job summarizing the study's findings here! In line 307, you may want to clarify which direction when you say, "once directionally oriented." (I know you mean oriented southward, so I would say that to be entirely clear.)

Thank you, we have clarified by changing 'directionally oriented' to 'oriented south' (line 309).