Sex-biased gene expression in an hemimetabolous insect: pattern during development, extent, functions involved, rate of sequence evolution, and comparison with an holometabolous insect

# **Open Access**

**Nadia Aubin-Horth** based on reviews by 2 anonymous reviewers A recommendation of:

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Dynamics of sex-biased gene expression over development in the stick insect Timema californicum

Jelisaveta Djordjevic, Zoé Dumas, Marc Robinson-Rechavi, Tanja Schwander, Darren James Parker (2021), bioRxiv, 2021.01.23.427895, ver. 6 peer-reviewed and recommended by Peer Community in Evolutionary Biology https://doi.org/10.1101/2021.01.23.427895

Submitted: 22 April 2021, Recommended: 25 October 2021

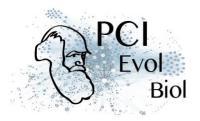
#### Cite this recommendation as:

Nadia Aubin-Horth (2021) Sex-biased gene expression in an hemimetabolous insect: pattern during development, extent, functions involved, rate of sequence evolution, and comparison with an holometabolous insect. *Peer Community in Evolutionary Biology, 100135.* https://doi.org/10.24072/pci.evolbiol.100135

### Recommendation

An individual's sexual phenotype is determined during development. Understanding which pathways are activated or repressed during the developmental stages leading to a sexually mature individual, for example by studying gene expression and how its level is biased between sexes, allows us to understand the functional aspects of dimorphic phenotypes between the sexes.

Several studies have quantified the differences in transcription between the sexes in mature individuals, showing the extent of this sex-bias and which functions are affected. There is, however, less data available on what occurs during the different phases of development leading to this phenotype, especially in species with specific developmental strategies, such as hemimetabolous insects. While many well-studied insects such as the honey bee, drosophila, and butterflies, exhibit an holometabolous development ("holo" meaning "complete" in reference to their drastic metamorphosis from the juvenile to the adult stage), hemimetabolous insects have juvenile stages that look similar to the adult stage (the hemi prefix meaning "half", referring to the more tissue-specific changes during development), as seen in crickets, cockroaches, and stick insects. Learning more about what happens during development in terms of the identity



of genes that are sex-biased (are they the same genes at different developmental stages? What are their function? Do they exhibit specific sequence evolution rates? Is one sex over-represented in the sex-biased genes?) and their quantity over developmental time (gradual or abrupt increase in number, if any?) would allow us to better understand the evolution of sexual dimorphism at the gene expression level and how it relates to dimorphism at the organismic level.

Djordjevic et al (2021) studied the transcriptome during development in an hemimetabolous stick insect, to improve our knowledge of this type of development, where the organismic phenotype is already mostly present in the early life stages. To do this, they quantified whole-genome gene expression levels in whole insects, using RNA-seq at three different developmental stages. One of the interesting results presented by Djordjevic and colleagues is that the increase in the number of genes that were sex-biased in expression is gradual over the three stages of development studied and it is mostly the same genes that stay sex-biased over time, reflecting the gradual change in phenotypes between hatchlings, juveniles and adults. Furthermore, male-biased genes had faster sequence divergence rates than unbiased genes and that female-biased genes.

This new information of sex-bias in gene expression in an hemimetabolous insect allowed the authors to do a comparison of sex-biased genes with what has been found in a well-studied holometabolous insect, Drosophila. The gene expression patterns showed that four times more genes were sex-biased in expression in that species than in stick insects. Furthermore, the increase in the number of sex-biased genes during development was quite abrupt and clearly distinct in the adult stage, a pattern that was not seen in stick insects. As pointed out by the authors, this pattern of a "burst" of sex-biased genes at maturity is more common than the gradual increase seen in stick insects.

With this study, we now know more about the evolution of sex-biased gene expression in an hemimetabolous insect and how it relates to their phenotypic dimorphism. Clearly, the next step will be to sample more hemimetabolous species at different life stages, to see how this pattern is widespread or not in this mode of development in insects.

#### References

Djordjevic J, Dumas Z, Robinson-Rechavi M, Schwander T, Parker DJ (2021) Dynamics of sex-biased gene expression during development in the stick insect *Timema californicum*. bioRxiv, 2021.01.23.427895, ver. 6 peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/2021.01.23.427895

# **Reviews**

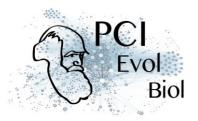
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# Evaluation round #1

29 Jun 2021

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Version of the preprint: 4



## Author's Reply

# Download author's reply Decision by Nadia Aubin-Horth

Dear authors,

Thank you for submitting your preprint "Dynamics of sex-biased gene expression over development in the stick insect *Timema californicum*" to PCI Evol Biol. Your manuscript has been read by two reviewers, whose comments are enclosed. As you will see, the reviews are largely positive, and, based on these reviews as well as my own evaluation, I would recommend your manuscript to be eventually included in PCI Evol Biol. However, before reaching a final decision, I would ask you to revise your manuscript according to the recommendations by the reviewers. Please address the main issues underscored by the reviewers, including the following:

- Addressing the issue of comparing only one species of each developmental type and interpreting it in the context of evolution in a more explicit manner.
- Discussing more explicitly how a whole animal transcriptome approach may affect the data
- Respond to the reviewer's comment about the rationale for not using a fold change cut-off in addition to a statistical significance threshold in the case of studying development while using whole-animal transcriptomes.
- Presenting the statistics supporting some of the claims and modifying some figures to insure that your message is clearly understood by the readers.

I also encourage you to revise your manuscript according to the more minor suggestions from the reviewers, which will certainly improve it.

Best regards,

Nadia Aubin-Horth

## Reviewed by anonymous reviewer, 13 Jun 2021

This manuscript addresses an important knowledge gap in the field of sexual selection, namely how do sex differences in gene regulation manifest through development. Despite the fact that sexually dimorphic phenotypes are likely a product of processes acting through development, gene regulation is typically studied without an ontogenetic perspective. In this regard, this paper is very timely as it focuses on three developmental stages in the stick insect Timema californicum. Furthermore, this insect has hemimetabolous development, where phenotypic sex differences amplify gradually through development, and so provides an important contrast to better studied holometabolous species such as Drosophila. However, there are a number of bold claims made throughout the manuscript that unfortunately I do not think are supported by the results in their current form. I have a number of suggestions, detailed below:

My primary concern focuses on the use of whole body in these analyses. It is likely that tissue composition varies both between males and females, but also through development. This can result in shifts in gene regulation that are then falsely attributed to differential expression in this study (see Montgomery and Mank 2016).



Montgomery SH, Mank JE. Inferring regulatory change from gene expression: the confounding effects of tissue scaling. Mol Ecol. 2016 Oct;25(20):5114-5128

First, this limitation should be fully acknowledged in the discussion. Currently, it is not mentioned at all. Second, Montgomery & Mank recommend using a strict 2x fold change to exclude any genes that might exhibit patterns of regulatory variation arising from allometric shifts. Currently, I believe only a p-value is used to identify sex-biased genes and so many weakly sex-biased genes are included in the analyses that are likely a product of allometric shifts. Although this will obviously reduce the number of sex-biased genes in the study, I would strongly urge the authors to implement this measure to increase confidence in their results. Other studies with this problem have used publicly available data to identify genes post hoc with tissue enriched patterns of expression (Immonen et al 2014). I assume that isn't possible here but may be mistaken.

Immonen, Snook and Ritchie Ecology and Evolution 2014; 4(11): 2186–2201

I also found attempts to link developmental mode to the ontogeny of sex-biased expression weak. Given there is only one hemimetabolous and one holometabolous species, it is impossible to distinguish the effect of developmental mode from species identity. Furthermore, the Drosophila analysis is focused on whole body which is subject to the same allometric problems discussed above. But most importantly, this analysis has already been conducted in Perry et al 2014 arguably to a more robust standard as it studies gene expression at the tissue level. This isn't acknowledged in the paper. I think the authors pose very interesting hypotheses which I understand they want to test. However, I actually think the inclusion of this analysis weakens the manuscript overall. I would limit discussion of the link between expression and developmental mode to the discussion section, where I think the authors can draw conclusions with published data and make some robust hypotheses for future work.

Perry JC, Harrison PW, Mank JE (2014) The ontogeny and evolution of sex-biased gene expression in Drosophila melanogaster. Molecular Biology & Evolution 31: 1206-1219

dn/ds is calculated using a pairwise comparison between T. californicum and T. poppensis. This means that it is impossible to attribute rates of change to either lineage and therefore weakens power to test for the relationship between rates of sequence evolution and sex-bias in T. californicum. Ideally, an outgroup should be added so that dn/ds can be calculated falong the branch leading to T. californicum after the split with T. poppensis. This approach obviously relies on having a reference genome for another closely related species.

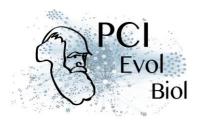
The manuscript tests if genes that are more sex-biased have higher rates of coding sequence evolution. There are a number of factors that influence the rate of coding sequence evolution and need to be accounted for. This includes expression level, which the authors already control for, but also tissue-specificity and GC bias which should be included in the analysis.

Fidel Botero-Castro, Emeric Figuet, Marie-Ka Tilak, Benoit Nabholz, Nicolas Galtier, Avian Genomes Revisited: Hidden Genes Uncovered and the Rates versus Traits Paradox in Birds, Molecular Biology and Evolution, Volume 34, Issue 12, December 2017, Pages 3123–3131

Richard P. Meisel, Towards a More Nuanced Understanding of the Relationship between Sex-Biased Gene Expression and Rates of Protein-Coding Sequence Evolution, Molecular Biology and Evolution, Volume 28, Issue 6, June 2011, Pages 1893–1900

L36 I don't think this statement is supported by the results. It is impossible to distinguish species differences from differences in development when only two species are compared.

L45 Seems inappropriate to cite Chauhan et al here in isolation. I suggest only citing the review paper (Mank 2017) or a more exhaustive list of empirical papers.



L49 Seems odd to single out these two papers when the majority of transcriptional studies focus on adults. I would simply cite Mank 2017 here.

#### L51 Missing citations:

Mank JE, Nam K, Brunström B, Ellegren H. Ontogenetic complexity of sexual dimorphism and sex-specific selection. Mol Biol Evol. 2010 20142440.

Perry JC, Harrison PW, Mank JE. The ontogeny and evolution of sex-biased gene expression in Drosophila melanogaster. Mol Biol Evol. 2014;31(5):1206-1219.

Hale MC, Xu P, Scardina J, Wheeler PA, Thorgaard GH, Nichols KM. Differential gene expression in male and female rainbow trout embryos prior to the onset of gross morphological differentiation of the gonads. BMC Genomics. 2011;12:404.

Zhao M, Zha X-F, Liu J, Zhang W-J, He N-J, Cheng D-J, Dai Y, Xiang Z-H, Xia Q-Y. Global expression profile of silkworm genes from larval to pupal stages: toward a comprehensive understanding of sexual differences. Insect Sci. 2011;18:607–618.

L65 Cite Mank 2017

L75 Cite Zhao M, Zha X-F, Liu J, Zhang W-J, He N-J, Cheng D-J, Dai Y, Xiang Z-H, Xia Q-Y. Global expression profile of silkworm genes from larval to pupal stages: toward a comprehensive understanding of sexual differences. Insect Sci. 2011;18:607–618.

L372 Similar to points raised above, I do not think there is sufficient evidence to claim this.

L485 Specify the model used in PAML. I assume codeml?

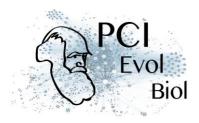
L508 Remove 'the'

## Reviewed by anonymous reviewer, 21 Jun 2021

In their pre-print "Dynamics of sex-biased gene expression over development in the stick insect Timema californicum", Djordjevic and colleagues used RNA-sequencing in three developmental stages of the hemimetabolous stick insect, T. californicum, as well as previously published RNA-seq data from D. melanogaster, which is holometabolous, in order to examine how sex-biased gene expression varies over developmental time and to compare how the dynamics of sex-biased gene expression vary between hemimetabolous and holometabolous insects. The authors found that in T. californicum the proportion of sex-biased genes gradually increased over developmental time, with the direction of sex bias generally remaining consistent during developmental progression, but that T. californicum had less sex bias than in D. melanogaster, where sex-biased gene expression abruptly increased for the adult stage. In general, the preprint is well-written and well-organized, with the objectives of the study well-outlined and the study itself nicely presented within the context of previous research in the field. However, there are a few issues that I think should be addressed (outlined in the major and minor comments below).

#### Major comments:

1. Lines 113–115, page 6: Did the authors perform any statistical test to support this statement that "sex-biased gene expression gradually increased over the three developmental stages"?



- 2. Supp Fig 2 is confusing. In lines 127–128, pages 6–7, the authors claim this figure show "genes sex-biased at earlier stages generally and remaining sex-biased in the same direction at later stages", but this is not evident from this figure as in several places on the heat map there are genes that clearly switch from red to blue or vice versa, especially between the juvenile and adult stages. I assume these genes were not significant?
- 3. Lines 130–133, page 6 and page 14 lines 245–249: While it is nice to see that there is some overlap in sex-biased genes among stages, I think a more informative way to characterize similarities in sex bias across developmental stages would be to calculate the correlation of sex bias between stages and that the authors should consider adding this as well. This would also allow the author to more directly and thoroughly compare sex-biased gene expression dynamics between the two species.
- 4. Related to point 3 above. In the Fig 3 legend, the authors state "The number of genes shared between all three stages was greater than expected by chance". Did the authors make this comparison for any of the individual stage comparisons?
- 5. Fig 4: I think that it is misleading to present and test for significant differences in tau for each developmental stage. Here, tau is a measure of the stage specificity of gene expression and is based on the gene expression in all stages (see comment 6 below). Thus, there is only one tau value calculated for each gene and the tau values for each stage are largely overlapping. I think it would be more appropriate for the authors to simply present and test for significant differences in tau for males and females for each gene category rather than also include the developmental stage.
- 6. Related to point 5 above. Lines 474–480, page 24: I think that it would be nice for the reader if the authors included how tau is calculated in this section. I admit that this is a little pedantic, but tau is actually originally a measure of tissue-specificity for gene expression, which is how it is proposed and used in Yanai et al, which the authors cite. In recent years it has also successfully been applied as a measure of stage specificity.
- 7. Lines 238–241, page 14 and Fig 8: It would be nice if the authors tested for significant differences in the proportion of sex-biased genes in T. californicum versus D. melanogaster here to provide statistical support for their claims that T. californicum shows lower levels of sex bias. It would also be nice if they tested for significant differences in the proportion of sex biased genes between stages within each species to better support their observations of how sex-biased gene expression changed differently over time between the two species.
- 8. Page 19, lines 360–364: While it is nice that the authors acknowledge that gonad size may play a role, sex bias varies depending upon tissue and it would be nice if they expanded a bit more about how using whole bodies versus individual tissues may affect their findings.

Minor comments:



- 1. Lines 46–49, page 3: I agree with the authors and there are a lot of studies focusing on this topic in adult stages in various species. Therefore, it would be nice if the authors provided a few additional examples here.
- 2. Line 60, page 3: I think that the "?" at the end of this line should be a ".".
- 3. Line 80, page 4: I think that "in hemimetabolous insects" should be "in a hemimetabolous insect".
- 4. Lines 92–101, page 5: This is a purely stylistic suggestion: I think that it helps with the flow and reader understanding when the final paragraph of the introduction ends with a brief summary of the major results and their take-home message and the authors could consider doing this.
- 5. Line 112, page 6: I think that "over development" should either be "during development" or "over developmental time".
- 6. Figs 2, 6, and 7a: Perhaps it is my computer, but the colors for the different categories within each sex are a bit difficult to distinguish.
- 7. Lines 119, 121, 122 (page 6), 243, and 245 (page 14): I find the inclusion of P-values here confusing. In their methods the authors already state the p-value threshold that they used to assess significance. When I initially saw them here, I thought that they were referring to a statistical test.
- 8. Fig 5: Why are the M-biased genes NA for the hatchling stage? Could you please explain this in the figure legend?
- 9. Fig 6: I think it would be nice to label each box with the sex in addition to the stage.
- 10. Page 21, lines 399–401: Did the authors do any kind of quality control to ensure that the inclusion of DNA in their RNA samples during library prep did not affect their results?