

Origin of horsetails and the role of whole-genome duplication in plant macroevolution

James W. Clark, Mark N. Puttick and Philip C. J. Donoghue

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Original submission: 15 July 2019
Revised submission: 2 October 2019
Final acceptance: 2 October 2019

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

Review History

RSPB-2019-1662.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?

N/A

Is it clear?

N/A

Is it adequate?

N/A

Do you have any ethical concerns with this paper?

No

Comments to the Author

Review of the manuscript 'Origin of horsetails and the role of whole genome duplication in macroevolution' by James Clark and co-authors submitted for publication in Proceedings of the Royal Society B.

The study reconstructs the occurrence and timing of Whole Genome Duplications (WGDs) in the evolution of Equisetum, and their role in the morphological evolution and diversification of the lineage.

This work addresses an interesting hypothesis, and the combination of transcriptome and fossil data within an explicit phylogenetic framework is highly original. The analyses are well executed and convincingly show the presence of several WDG events in the lineage leading to extant (and extinct) Equisetum species. These events considerably predate the K-Pg mass extinction and they do not account for an increase in morphological variation within horsetails, although they are associated with a movement into a novel area of morphospace.

The paper is clear, convincing and really interesting. I only have a few minor remarks:

L366: 'Calamitaceae achieved greater disparity without WGD'. You cannot be sure that there was no (independent) WDG event in Calamitaceae. The presented analysis convincingly shows that based on extant taxa WDG events can be inferred to have taken place along the branch leading to Equisetaceae. Based on morphological data the extinct Calamitaceae are not part of this clade, but that does not mean that no WDG events occurred there. I would be really careful discussing the absence of WDG events in an extinct group.

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L408: 'species diversity': this was not explicitly tested.

Review form: Reviewer 2 (Kathleen Pryer)

Recommendation

Accept with minor revision (please list in comments)

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Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

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No

Comments to the Author

Please see 2 attached files. (See Appendix A)

Decision letter (RSPB-2019-1662.R0)

24-Sep-2019

Dear Mr Clark

I am pleased to inform you that your Review manuscript RSPB-2019-1662 entitled "Origin of horsetails and the role of whole genome duplication in macroevolution" has been accepted for publication in Proceedings B. Congratulations!

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

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- 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. Please note that PowerPoint files are not accepted.
- 3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g `authorname_procb_ESM_figures.pdf`

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

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

Sincerely,
Professor John Hutchinson, Editor
<mailto:proceedingsb@royalsociety.org>

Associate Editor Board Member: 1

Comments to Author:

Your manuscript has been evaluated by two experts who agree it has the importance and quality required for publication by Proceedings B pending only minor corrections.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

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L408: 'species diversity': this was not explicitly tested.

Referee: 2

Comments to the Author(s)
Please see 2 attached files.

Author's Response to Decision Letter for (RSPB-2019-1662.R0)

See Appendix B.

Decision letter (RSPB-2019-1662.R1)

02-Oct-2019

Dear Mr Clark

I am pleased to inform you that your manuscript entitled "Origin of horsetails and the role of whole genome duplication in plant macroevolution" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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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

This is a great paper. By combining several elegant macroevolutionary and genomic approaches, the authors greatly improve on the estimate of WGD in *Equisetum*, which allows them to better consider the role of WGD in the macroevolution of this important non-angiosperm lineage. It is a cautionary example of “better get the timing right” before you jump to conclusions. I am by no means an expert on the statistical analyses carried out by the authors, but I assume these aspects of the paper will be reviewed by someone who is.

As the authors state in the first paragraph of their introduction, the WGD-K-Pg hypothesis is “dependent on the accuracy and precision of estimates for the timing of WGD events”. In this paper, the authors set out to contest the recent claim by Vanneste et al. (2015) that *Equisetum* underwent WGD that they estimate to have coincided with the K-Pg boundary (age estimate based on K_s between duplicate pairs). WGD is often invoked to explain either species diversification, phenotypic innovation, or extinction resistance. Given that *Equisetum* has only 15 extant species, the link between diversification and WGD is unlikely. Also, it seems unlikely that such a relatively recent WGD had much to do with contributing to the longevity of the lineage, which is about 400 million years old.

The authors implement sophisticated molecular clock methods to investigate the timing of the WGD. Given the rich fossil history for *Equisetum*, the authors also use likelihood model-based methods to reanalyze a recently published matrix of 43 equisetalean species (15 extant, 28 extinct) with an extensive combination of morphological and molecular characters (Elgorriaga et al. 2018) that had previously been analyzed using only parsimony approaches. They go even further and perform a morphological clock analysis using only the morphological data set to examine rates of phenotypic evolution, and the extent of phenotypic disparity. Finally, a genome size analysis was implemented (which even estimated 1C-values for fossil taxa!).

Together, these very thorough methods result in revealing that not just one, but two, WGD events occurred well-before the K-Pg—one in the Carboniferous, and the other in the Triassic. Their Bayesian analysis of the combined molecular and morphological dataset are mostly congruent with, but also much improve upon, results from the parsimony methods used by Elgorriaga et al. 2018. The authors go beyond expectation in this paper, using quite novel approaches to place the WGD within the context of the gross historical diversity of the lineage. This study sets the bar high for other studies that should more thoroughly test existing hypotheses that propose a link between WGD and the K-Pg mass extinction event.

Minor quibbles, in addition to minor edits made on the manuscript (see attached):

- I would prefer a more explicit manuscript title that reveals that the WGD observed in *Equisetum* does not appear to be linked with any apparent macroevolutionary consequences. The current title initially led me to think that WGD did have a macroevolutionary role in *Equisetum* (perpetuating the idea that WGD events are agents of macroevolutionary change). With a more explicit title, it is more likely to be cited in future to caution that WGD events do not necessarily precipitate changes on a macroevolutionary scale.

- The authors could refer to the figures more often throughout the text to help guide the reader, and they could use more informative labelling on many of the figures (e.g., Fig. 4, label the 2 extant subgenera, and adding dates would be helpful to the x-axis. Note in this figure that the “l” has dropped out of *E. fluviatoides* and *E. fluviatile*, for some reason; see also Fig. 6). Also, more details in the figure legends would be useful in many cases, but perhaps this is a constraint by the journal?
- That the more recent shift toward larger genomes (Fig. 6) does not appear to be associated with a WGD event is interesting and the discrepancy in genome sizes between the 2 extant subg. of *Equisetum* is notable. It would be interesting to know how the authors might explain for the subg. *Equisetum* having apparently jettisoned ca. half its genome in the Paleogene/Neogene? (Fig. 6).

Appendix B

Responses

Reviewer 1

L366: 'Calamitaceae achieved greater disparity without WGD'. You cannot be sure that there was no (independent) WDG event in Calamitaceae. The presented analysis convincingly shows that based on extant taxa WDG events can be inferred to have taken place along the branch leading to Equisetaceae. Based on morphological data the extinct Calamitaceae are not part of this clade, but that does not mean that no WDG events occurred there. I would be really careful discussing the absence of WDG events in an extinct group.

I have changed the text.

L407: I am not sure what you mean with 'coincide with the origin of new taxonomic lineages'. I assume that the WDG events mark clades that coincide with taxonomic groups? How relevant is that?

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I have changed the text

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I have changed the text

Reviewer 2

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We feel that the original title provided a more broad appeal. While this paper does focus on the role of WGD in horsetails, we believe that the methods and approaches within will be of interest to people working in wider fields, which is reflected in our choice of title.

The authors could refer to the figures more often throughout the text to help guide the reader, and they could use more informative labelling on many of the figures (e.g., Fig. 4, label the 2 extant subgenera, and adding dates would be helpful to the x-axis. Note in this figure that the "l" has dropped out of *E. fluviatoides* and *E. fluviatile*, for some reason; see also Fig. 6). Also, more details in the figure legends would be useful in many cases, but perhaps this is a constraint by the journal?

I have changed the figure and have added more references to figures throughout the text

That the more recent shift toward larger genomes (Fig. 6) does not appear to be associated with a WGD event is interesting and the discrepancy in genome sizes between the 2 extant subg. of Equisetum is notable. It would be interesting to know how the authors might explain for the subg. Equisetum having apparently jettisoned ca. half its genome in the Paleogene/Neogene? (Fig. 6).

This is interesting, though I think beyond the scope of the present study and present page limits!

The differences in genome size between the two subgenera could be interpreted either as a dramatic loss in subg. Equisetum or a gain of genomic material in subg. Hippochaete. The mechanisms and reasoning behind this remain to be explored, though it is likely due to the gain/jettisoning of repeats and 'junk' DNA. The two subgenera occupy different ranges, so it is possible that the difference has some ecological explanation too.