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Dear Associate Editor Fay-Wei Li,

Thank you very much for your e-mail (January, 16th, 2019) about the revision of the manuscript number MPE\_2018\_645 formerly entitled **“Inferring hypothesis-based transitions in clade-specific models of chromosome number evolution along phylogenies”**.

We have reviewed the manuscript as suggested, considering all comments by the reviewers. Revisions and suggestions were indeed helpful and have contributed to improve the quality of the manuscript. Please find below the responses (red font) to the main points made by both reviewers. Also, find highlighted (track changes option enabled) some modifications in the manuscript based on the reviewers’ comments.

We hope you will consider this new and improved version of the manuscript acceptable for publication in Molecular Phylogenetics and Evolution.

Thank you very much in advance

Sincerely yours,

José Ignacio Márquez-Corro, Santiago Martín-Bravo, Daniel Spalink,Modesto LuceñoandMarcial Escudero

-**Reviewer 1**

Comments:

You've pulled down a lot of sequence data to infer the phylogeny for Cyperaceae, but you spend very little time talking about this in the Results and the Discussion. Did you recover the relationships that are consistent with the current taxonomy? What did bootstrap support look like for the major groups? How much missing data did you have?

Phylogenetic relationships are consistent with previous phylogenetic studies and mostly congruent with current taxonomy. The cited congruence between our phylogeny and previously published phylogenies suggest that missing data does not interfere with the macroevolutionary scope of the study. The bootstrap values range from moderate to high (ca. 40% of nodes with more than 70% boostrap support).

In Table 1, it would help to have an extra column with a brief explanation of the interpretation for each model. This is what was done in O'Meara et al. (2006) for their comparison of models. I think this will be a nice summary of the models compared and what it means in the context of chromosome evolution in the Cyperaceae.

We thank the reviewer for this suggestion since we agree it will allow an easier interpretation of the data included in table 1. Done.

The first Highlight says that complex models of chromosome evolution fit better in

megaphylogenies. The work that you've done in this paper does not really address this issue, so I think that this claim cannot be made. The title has a similar issue in that you are only testing models in Cyperaceae, so you cannot generalize their success without doing simulations or a comparison across more groups. The title could be something like: "Inferring hypothesis-based transitions in clade-specific models of chromosome number evolution in the Cyperaceae."

Done. We have modified highlights and title accordingly.

The way that you've worded the Abstract makes it sound as if polyploidy is a form of chromosomal rearrangement, which I wouldn't consider to be true. In the Introduction you include more detail that I think sets up the distinction correctly: chromosomal evolution and rearrangement after polyploidy is very common. However, whole genome duplication itself is not a rearrangement. Having the first sentence be something like: "Large-scale changes in chromosome number have been associated with diversification shifts in many lineages of plants."

Done.

Along these same lines, have people shown that polyploidy, per se, promoted angiosperm diversification. It is likely associated with it, but I don't know that we can definitively say that polyploidy caused the diversification of angiosperms. If there are citations that can clarify this a bit more then please include them.

This sentence has been briefly expanded, and is further explained in the second paragraph of the Introduction.

It's really great that you've included your code on GitHub! I did just want to say that I think it would help more to have the individual source code and data files, rather than zipped files, in the repository.

Done. We will publish our GitHub repository with a citable doi using zenodo (https://zenodo.org/record/2553838).

The legend for Table 2 could use more description. I'm not entirely sure what it is showing.

Done. We have added an additional explanatory line in the description.

-**Reviewer 2**  
  
General feedback:

1) To me the current title suggested a new method to test the locations of transitions in the mode of chromosome evolution along phylogenies, yet the paper is really focused on a single specific empirical case study. It would be exciting to see the exploration of a probabilistic model that allows for detecting different "regimes" of chromosome evolution along different branches of the same phylogeny, but that is not what is done in this paper. Instead the paper applies different models to different predetermined subclades of Cyperaceae and discusses the differences detected. I suggest adding "in Cyperaceae" to the title to make it clear that this paper is empirically focused.

Done. See also reviewer #1's comment about the title above.

2) The authors test two hypotheses: (i) that changes in the mode of chromosome evolution accompany shifts in diversification rates, and (ii) that chromosome changes are constant across the entire tree. Another highly likely hypothesis seems to have been mostly overlooked and untested by the authors: that some changes in the mode of chromosome evolution might occur without a corresponding change in diversification rates. Perhaps these shifts in the mode of chromosome evolution are linked to phenotypic changes, changes in habitat, etc. The discussion mentions briefly that the development of C4 photosynthesis might be linked to a chage in chromosome evolution (something the BiChrom model would test directly, see comments below), but many other factors could be possible. More discussion up front in the introduction about these possibilities seems warranted.

We have tested alternative hypotheses of shifts in models of chromosome number evolution against the null hypothesis of constant or single model of chromosome evolution in the whole phylogeny. We specifically tested possible transitions of models of chromosome evolution in nodes where shifts in diversification rates were previously detected in previous studies. While we can test alternative hypotheses of transitions of chromosome number evolution against the null hypothesis and conclude that there are three shifts in models of chromosome evolution, we cannot demonstrate a causal relationship between the shift in chromosome evolution and the diversification rates. Our concern from the beginning was exactly that. Therefore we have only suggested a possible relationship between chromosome number model transition and diversification rates shifts and we have added alternative explanatory possibilities (i.e. the C4 photosynthetic pathway) when possible. We agree that the influence of many other factors in chromosome number evolution could be possible and, although this was already stated in the previous version of the manuscript, we have added more discussion up front in the introduction about these possibilities in the current version. As far as we are concerned BiChrom model would test whether or not there is a relationship between two trait states (i.e. C3 and C4 or two different diversification rates) and two models of chromosome evolution but again causal relationship would be difficult to demonstrate. The two main differences between our analytical approach and BiChrom are that (i) our approach tests transitions in chromosome models of evolution only in specific nodes of the phylogeny whereas BiChrom tests whether or not there are two models of chromosome evolution associated to trait states with independence of the distribution of the two trait states across the phylogeny and (ii) whereas BiChrom only deals with two trait states (although three or more trait states –or even hidden states- could be used but not empirically tested yet), our approach may deal with several trait states (in our case up to five different models associated to five different subtrees). We have added some lines at the end of the introduction regarding BiChrom and ChromoSSE and their model alternatives. Also, see the response to the next point.

3) The section in the introduction regarding existing chromosome evolution models is a bit inaccurate -- it is not true that noone has considered more than one model throughout the phylogeny. "Freyman and Hohna (2018) expanded ChromEvol functions with the ChromoSSE package in revBayes (Höhna et al., 2014). This software allows detecting shifts in the mode of chromosome evolution during cladogenesis associated with diversification rate shifts or binary phenotypic character evolution (BiChroM; Zenil-Ferguson et al. 2017). However, none of these new approaches considers the possibility of more than one model of chromosome evolution throughout the phylogeny." Sorry for being picky about this (I wrote ChromoSSE) but ChromoSSE is a reimplementation of the ChromEvol models that allow for a greatly expanded range of chromosome evolution models to be tested. Most significantly it allows for (i) both cladogenetic and anagenetic changes in chromosome number (original ChromEvol only allowed for anagenetic), (ii) for difersification rate shifts to be associated with chromosome changes, and (iii) for BiChrom type models to be integrated with all these other models.

We thank reviewer #2 for its thorough explanation, and we apologize for our misunderstanding. We have improved the explanation of ChromoSSE and other chromosome evolution models in the introduction. We were not fully aware of the versatility of ChromoSSE (although this is not empirically tested yet. See more details below).

The basic BiChrom model is basically two different models of chromosome evolution nested in one larger model which allows different rates of chromosome evolution in different clades of the tree. In RevBayes the basic BiChrom model can be expanded to more than a binary shift -- you could apply as many different regimes of chromosome evolution as are computationally possible all on the same tree. The models have been implemented and described but not yet explored empircally. Using such models may allow one to detect the location of shifts in the mode (submodel) of chromosome evolution instead of a priori picking the locations as the authors did for the sedges.

We were only aware of the basic BiChrom model: basically two different models of chromosome evolution nested in one larger model which allows different rates of chromosome evolution in different clades of the tree. We did not know that the basic BiChrom model can be expanded in RevBayes to more than a binary shift. Nevertheless, although implemented and described, these models have not been yet explored empirically. We are then reticent to use them right now (although see below).

Reviewer #2 also stated that "Using such models may allow one to detect the location of shifts in the mode (submodel) of chromosome evolution instead of a priori picking the locations as the authors did for the sedges". If we have a phenotypic trait with two or several states in the phylogeny, we could code the trait states of the species in the phylogeny and check whether or not there are two or more chromosome evolution models in the phylogeny. In this study we are not testing whether or not there are two or more models of chromosome evolution associated to two or more trait states across the phylogeny. We are mapping chromosome number model transitions in nodes of the phylogeny. If we run BiChrom coding the species in the phylogeny in concordance with the nodes in which the species are nested, we would be also "a priori picking the locations". The only possibility could be that we use the hidden states option mentioned in the manual of BiChrom. But this does not seem to be empirically tested yet.

After reading reviewer's comments (thank you very much! They have been very helpful) we have realized that the combined used of BiChrom and ChromoSSE is more versatile than we previously thought and could have been optimal to search for different models of chromosome evolution across the phylogeny. We assume that it will be very tricky to make a search of multiple chromosome number models of evolution associated to multiple hidden states across of all nodes and tips of the phylogeny. In addition, these complex models (multistate phenotypes with hidden states) have not been empirically tested yet (not even separately). We would like to explore them in depth in the near future and hopefully in collaboration with William A. Freyman and Sebastian Höhna (authors of ChromoSSE).

Minor comments:

Line 66: change "implemented on ChromEvol 2.0 software" to "implemented in the ChromEvol 2.0 software"

Done.

Line 89: Roalson 2008 cited 3 times

Corrected.

Line 154: I have found that using the most frequent count per species rather than using the full range of counts dramatically decreases uncertainty in inferences. Of course this is true for any type of phylogenetic model when one incorporates intraspecific variability. Perhaps it is outside the scope of this manuscript to rerun the analyses completely, but do you have any idea how well your results hold up when incorporating intraspecific variability in chromosome count?

Based on our large previous experience with ChromEvol (Escudero et al. 2014, PLoS One; Escudero et al. 2018, Plant Biology…), including the intraspecific variation usually affect largely to the ancestral state chromosome number reconstruction but the inferred model changes little if any. We have also found that using the most frequent count per species rather than using the full range of counts dramatically decreases uncertainty in inferences.

-**Reviewer 3**  
  
What this paper needs most—what would make it more citable, more broadly interesting—as a revision for clarity of writing. The story does not come out as it should. This is a fascinating story: the transition from chromosome stasis to polyploidy to agmatoploidy, and the potential effects of each on diversification rates. The authors will find this paper more readable and probably more impactful if they tell the story aloud, as though they were telling a packed room full of naturalists. Tell your grandmother this story, and let her imagine she is walking down the branches of the tree of life, seeing chromosomal innovations arise and fall. The reader should feel as though her or she were tunneling down the millions of years of evolutionary history that make sedges who they are. This is a *great* story: as written, it falls short of its narrative potential, and consequently it not as likely to grab even the scientific reader as strongly as it should. Well cited papers are almost always well written, and they are often quite narrative.

We completely agree with the reviewer #3. We have always tried to make papers as easier to read as possible. Nevertheless, sometimes this is quite difficult, especially in studies like this with many methodological, statistical and technical aspects. We have made the maximal effort to take this recommendation into account, especially in the Discussion. For example, we have changed the subtree nomenclature to clearly identify the clade or lineage that we were describing. We believe that this change and others included in the present revision will make the manuscript more readable. Thanks!

Going along with this, I encourage the authors to revisit figure 2. What in the Sam Hill is Linear\_Rate\_Demi\_Est to the uninformed reader? I work on this stuff, and I had to look it up! Avoid jargon when you don’t need to use jargon. And all those statistics on the right side? Those belong in a caption or a table. You have SUCH A GREAT STORY! Give the reader a figure to salivate over!!

Thank you very much for this encouraging comment! Since the parameters results to which the reviewer refers (previously shown on the right part of figure 2) are also present in the Appendix E, we opted for dropping them off the figure, as suggested by the reviewer. We have added a brief explanation instead about what those values mean, in order to make the figure more understandable for the broad audience. We believe that the new, revised version of the figure will fulfil the reviewer#3´s view.

Overall, very nice work. I look forward to seeing this in press. A few specific comments follow:

**ABSTRACT**

It’s not clear what models you are pitting against each other, and consequently what alternative hypotheses you are testing. In a sentence or two, give the reader information about what hypotheses and / or models you are evaluating. “Alternative hypotheses of chromosome evolution are significantly supported against the null hypothesis of a single model” is too vague… and not even strictly correct. A heterogeneous model, one where each of many clades has a complex model that differs from the others, is itself a single model, just a more complicated one.

Done.

Lines 25-26: “inferred for the complete phylogeny” -- I would change this to “inferred to be homogeneous in rate and process across the complete phylogeny”

Done.

**INTRODUCTION**

line 41: “are related to” s/b “are in many cases correlated with”

Done.

line 45: “linkage disequilibrium” s/b “linkage”

Done.

line 46: “amount of gene content” s/b “gene content”

Done.

line 66: “on” s/b “in the” … though I would not turn to software so quickly here. Present the methods and models, not the implementations. Imagine a paper saying in paragraph 3, “Rapid statistical phylogenetic inference has recently been implemented in RaxML.” In your case, wouldn’t it be more informative to say, “Statistical methods of inferring the history of lineage diversification and trait evolution have been only recently adapted to chromosome data,” or something of the sort? *Then* you can introduce implementations later on.

Done.

Lines 75-75: Again, I wouldn’t characterize this as multiple models: you just introducing model heterogeneity, where parameters drop in and out on different branches of the phylogeny.

Done.

Line 84: Delete “Remarkably”

Done.

Line 89: I think the citation after the chromosome number range s/b Hipp et al. 2009, not Hipp 2007. Also, check your Roalson 2008, which is printed thrice.

Done.

Line 97: “named” s/b “termed”

Done.

Lines 121-122: Not clear what you mean here by “different mechanisms of adaptation.” Be clearer about mechanisms you have in mind, if you can be.

Done.

**MATERIALS AND METHODS**

Lines 160-161: do you just mean the likelihood of the model? The wording “determines the probability… along the phylogeny” is odd. I would say the approach uses maximum likelihood to estimate parameters [described in lines 162 ff.].

Done.

Line 180: “asumptions” is missing an “s”

Done.

**RESULTS**

Lines 222, 225, 231: “relationship” in all three of these lines should be “rate”, I believe.

Done.

**DISCUSSION**

Line 242: “than previous” s/b “as previous”

Done.

Line 244: New methodology? I don’t think anything new is being done methodologically… this is good old-fashioned model testing. The new application was in O’Meara et al. 2006, who formalized the censored test, but that was 12 years ago.

Done.

Line 248: “on the sedges’ family” s/b “on the sedge phylogeny”

Done.

Lines 249–251: This sentence (“Thus, our approach… as well”) does not follow from the previous. Nothing about this work demonstrates that it is appropriate for finer evolutionary levels. Most certainly it is! But you present it as though the conclusion followe

Done.