We thank the reviewers for providing thoughtful comments that have helped us to improve this manuscript. We have incorporated changes that address the reviewer’s concerns, and significantly improved the clarity of the manuscript. We hope that you find the revised manuscript suitable for publication. Below we have included each of the reviewer's comments and our responses in red.

**Reviewer 1**

A main issue is that the authors praise their current study to go deeper than a former study that looked at the impact of holocentricity at the level of orders (line 75) and indeed the authors use several genera per order. However, the results amalgamate the inferences again to an order level (same in the abstract - line 26). It would be important to assess and discuss the variation within each order also because such variation may provide hints about the underlying processes. This has been shown for Lepidoptera in De Vos et al. (2020 Philosophical Transactions of the Royal Society B) where the authors compared rates of speciation in relation to chromosomal variation.

XXX

Another potential issue I see is that chromosome numbers per genus can be very variable. The authors tried to get around with this by sampling from the distribution (lines 238/239), but how robust is this and how does such variation relate to the variation in R?

XXX

Line 27: Here and throughout the manuscript it would be important to state in which orders polyploidy is common. It is for example highly debated if polyploidisation occurred in Lepidoptera – the authors only highlight that there were ancient whole genome duplications (line 186-188) but even that has been debated (ref 41 in the manuscript). See also Lukhtanov et al (2015, Proceedings of the Royal Society B).

XXX

Line 48 onwards: It is important to note that these processes described here, e.g. Robertsonian translocations, are based on monocentric chromosomes. I would suggest to expand this section to also indicate processes involved for holocentric chromosomes (reviewed in ref 21 in the manuscript).

XXX

Line 63: However, many species are often not variable at all in terms of chromosome numbers, such as Lepidoptera where most known species show a karyotype close to the putative ancestral state (ref 36 in the manuscript and De Vos et al. 2020).

XXX

Line 70 onwards: This argument makes only sense if holocentricity would result in fission events, yet fusion is also possible.

XXX

Line 101: Perhaps remind the reader what rate you are looking at.

XXX

Line 106: Clarify that you refer to the tree of Misof et al.

XXX

**Reviewer 2**

First, the title conclusion is too broad given the scope of the paper. The title should be changed to:  
  
Chromosome number evolves at equal rates in holocentric and monocentric insects.

We agree that the title was a bit broad originally. We have changed it to “Chromosome number evolves at equal rates in holocentric and monocentric clades”. We do not believe that it is necessary to specify insects in the title. The information that has informed our understanding of the differences in monocentric and holocentric chromosomes comes from both plants, insects, and non insect animals and the segregation behavior in all of these groups is largely similar.

Second, the machinery for rate estimation is too opaque. In particular, the authors should provide a simplified version of the core likelihood equation that is being used.

XXX

Some clarification should be provided on how the polyploidization rate is estimated. In particular, would a polyploidization even be inferred when there is a doubling of chromosome number inferred in an ancestor? Presumably there is no other karyotypic signal identified. What signal in the phylogeny provides the support for these estimates? Some brief discussion would be helpful.

XXX

Finally, since the machinery for estimating fusion, fission and polyploidization rates is novel, it would be worth running some simulations on the tree that is being used, with fixed rates on subclades, followed by rate estimation on the simulated tip values for chromosome number. The degree to which the machinery used is good at estimating rates from simulated data is important to know.

XXX

**Reviewer 3**

My main concern about the paper is that not enough attention is devoted to the underlying  
HMM model, and to the differences in results between models with and without polyploidy.  
Although the model is explained in an earlier publication (Blackmon et al. 2019), the current  
manuscript would perhaps be easier to understand for the reader if at least a minimal  
conceptual schematic was provided, showing how the eight parameters of the full model are  
related to each other.

XXX

In the order-level analysis, the exclusion of the polyploidy parameter  
leads to an opposite conclusion. The basic reason for it seems obvious and there is some  
discussion in lines 185-193. However, what is the relation between size of a clade, rate of  
polyploidy and number of chromosomes?

XXX

Lines 188: “Even a small number of polyploidy events  
[…] could lead to much higher variance […]” – how small and how much higher?  
Furthermore, as multiple alternative explanations are provided in the Discussion, how difficult  
would it be to incorporate them into the current model? Since the authors offer “a way  
forward” (line 223 how close are we to evaluating other factors discussed? Are the data there?

XXX

4,393 are many species, but not that many among insects. How well are different clades  
represented? Is the distribution of sampled species relatively even across the  
phylogeny? Just something to clarify.

XXX

line 101: what is the extent of difference between the two phylogenies? “Some clades”  
is vague.

XXX

Line 132-144: when discussing “intermdiate”, “lower” and so on rates, why not report  
the means and/or confidence intervals of those?

XXX

Line 133: why is 20 the cutoff?

XXX

line 143 takeN

XXX

line 236: a 100 trees is not many for a posterior. How much variability Is there among  
them?

XXX

Lines 260-265: if high rates are not “biologically realistic” (BTW – is there a reference for  
this assumption?), why not limit the uniform prior to low rate values?

XXX

Line 291: perhaps should be: “…statistic, where …”

XXX

Line 293 – monocentric clades evolve slower?

XXX

The authors should be commended for reproducibility of the analysis. However, everything  
hinges on the R package chromePlus. Yet its repository  
([https://urldefense.com/v3/\_\_https://github.com/coleoguy/chromePlus\_\_;!!KwNVnqRv!SJMwQ5KcSvFhwbhRc3AQzSw3KnfRZl7MAxncX6NYuNJuNVYfl3Gq6g1fb38SNsp-$](https://urldefense.com/v3/__https:/github.com/coleoguy/chromePlus__;!!KwNVnqRv!SJMwQ5KcSvFhwbhRc3AQzSw3KnfRZl7MAxncX6NYuNJuNVYfl3Gq6g1fb38SNsp-$) ) states “This package is in the early stages of  
development and should not be used for any analysis at this point.” Not encouraging!

XXX

100 trees is not much for posterior: how much variability was there in those anyway?

XXX

In terms of methods and data, the paper relies heavily on previous work by the same authors  
(references 5, 6). This is fine, but especially when it comes to the Markov model, a little more  
information on the guiding principles behind the model would be helpful to the reader.  
Incidentally, the website for the R package containing the statistical model states “This  
package is in the early stages of development and should not be used for any analysis  
at this point.”, which is unhelpful for anyone interested in further developments suggested at  
the end of the Discussion.

XXX