Dear reviewers

We would like to thank the handling editor and the reviewers for the feedback and the useful comments on our manuscript "*The Probability of Fusions Joining Sex Chromosomes and Autosomes*". We have taken the advice of reviewers to revise the manuscript as suggested with the exception of the additional empirical analysis (explained below). We appreciate the careful and detailed comments that we have received and have responded to each comment. Our replies are given in red following each comment and any sections quoted from the paper are italicized. Line numbers refer to the new version of the manuscript with tracked changes.

With kind regards,

Heath Blackmon

Handling Editor / Board Member Comments to Author:

1.1 Both the Board Member and I agree with referee 4's request for testing this hypothesis with vertebrates. This would make their model useful for examining a broader group of taxa. It would take little effort to consider and would make the paper far more complete and, in the long term, far more influential.

We agree that an analysis of all amniote vertebrates would provide significant insight into the probability of sexual antagonistic variation across the tree of life. However, this is well beyond the scope of the current project and article format. Even if we were to limit ourselves to mammals (a small fraction of amniote vertebrates), a much more nuanced analysis and discussion would be necessary than is possible in the short form of Biology Letters. For instance, within mammals, the marsupials have achiasmatic sex chromosomes that may impact the fate of fusions as we mention in the current manuscript. Even in eutherians, achiasmatic meiosis has evolved on several occasions. An analysis of mammals describing the methods used to deal with this variation and interpreting the results of several different forms of the model applied in multiple clades will require far more space than we have in this journal.

Regarding the effort involved we would also call attention to the fact that for each clade on which this approach is to be used a number of steps are necessary before analysis. The most time consuming of which is collection of sex chromosome and chromosome number data from the literature. I have personally been responsible for this type of data collection in several projects [1–5]. For each clade it is necessary to first perform extensive literature searches (often

including waiting weeks or months for difficult to access dissertations or monographs) followed by a slow process of name resolution matching cytogenetic records that go back to the early 1900s with currently accepted names that can be matched to phylogenies.

We are not sure how to interpret the statement that this would “make our model useful for examining a broader group of taxa”. The model as presented can be applied across all taxa with virtually any known sex chromosome systems.

Our manuscript illustrates how a long standing hypothesis put forth by the Charlesworths 40 years ago and cited more than 200 times can finally be quantitatively tested across large clades [6]. We have illustrated this approach in two clades. Our lab and others can and will use this approach to investigate evidence for natural selection in favor of sex chromosome autosome fusions across the tree of life in the coming months and years.

Referee: 3

3.1 The initial analysis is generalised to include the biologically plausible case when the sexes differ in the probability that fusions will be transmitted to the progeny. This is not explained, and I think it should be explained briefly why the sexes might differ — is it the “mutation” rate at which chromosomes fuse, or the chance that a fusion product is transmitted through meiosis, or something else?

We have edited the text to make it clear that it is exactly these possibilities that led us to include this parameter in the general form of our equation. We have retained the citation to Pennell 2015 since it includes a theoretical analysis in the supplement that shows how imbalances in these probabilities can lead to different expectations with regard to the frequency with which different forms of multiple sex chromosome systems are observed.

Lines 78-80

It is quite possible that the sexes may make unequal contributions to the fusions entering a species. These imbalances could stem from common processes such as meiotic drive or mutation rate differences [7].

3.2 The analysis of Habronattus spiders suggests that they display excess of SA-fusions, and a more sophisticated analysis of Drosophila (which is clearly explained) suggested that there is no excess, and the ms proposes that this is because the fusion genotypes suffer from some disadvantage. The disadvantage proposed, that the cessation of recombination due to male

achiasmate meiosis will lead to rapid genetic degeneration, with loss of lineages with fusions, is rather speculative, and I would have liked to see evidence that degeneration can be fast enough to prevent the fixation of the new karyotype.

We are currently developing population genetic models to test this hypothesis. To our knowledge this has never been modeled. We have moderated our discussion of this hypothesis to make it clear that this is something that demands further exploration.

Lines 200-211

The scarcity of SA-fusions that we document suggests that in Drosophila SA-fusions are more likely to have deleterious effects than fusions that join two autosomes. One possible explanation for apparent selection against SA-fusions in Drosophila may lie in the joint action of genome structure and lack of recombination in males (achiasmatic meiosis). In species with achiasmatic meiosis, when an SA-fusion occurs, the entire Y chromosome is immediately subject to population genetic forces (e.g., Muller's ratchet) that lead to the loss of functional genes [8]. Drosophila has relatively few chromosomes such that each chromosome carries many genes. (D. melanogaster 43% of all genes are on autosome 3) [9]. Therefore, while an SA-fusion may initially provide a fitness benefit, the fitness benefit may quickly decay due to the "target size" for deleterious mutations on the Y chromosome precluding the fusion's fixation. Testing this hypothesis across multiple clades with variation meiotic mechanisms should reveal whether this is a general pattern.

3.3 The fairly recent paper by Dupim et al. should probably be cited.
Dupim, E., G. Goldstein, T. Vanderlinde, S. Vaz, F. Krsticevic et al., 2018 An investigation of Y chromosome incorporations in 400 species of Drosophila and related genera. PLoS Genetics 14: e1007770. doi: 10.1371/journal.pgen.1007770

This is a fascinating paper, but we have very little room in the main manuscript to discuss it. In response to this and a comment by another reviewer (4.3) we have included a new section of the supplement that discusses the role of a variety of mechanisms in restructuring the genomes.

Supplement lines 11-36

While we have focused this work on fusions, we recognize that many types of chromosomal rearrangements, such as inversions, fusions, fissions, and translocations, can all dramatically reshape the genomic landscape of species, their sexes, and populations. In fact, in a study by

Bush in 1977 it was found that speciation was strongly positively correlated with the rate of chromosomal evolution in mammals [10]. This correlation was further supported in a recent study of chromosomal fusions and fissions in Lepidoptera [11]. Since early studies in Drosophila by Sturtevant, inversions have been shown to segregate among populations and provide evidence of population structure. While some of these inversions are neutral and do not provide a known benefit, other inversions link alleles beneficial to survival due to the biotic and abiotic environment a population experiences, resulting in local adaptation [12–15] and eventual speciation, as seen across introgression studies in Drosophila and mosquitoes [16–19]. Outside of their role in replenishing the pseudoautosomal region of sex chromosomes and formation of neo-sex chromosomes [20–22], fusions, like inversions, reduce recombination between loci, by bringing genes which were previously on separate chromosomes together, providing a similar role in local adaptation as described above [14,23,24]. In fact, the models developed by Guerroro and Kirkpatrick for the role of fusions in local adaptation also apply to more specific types of fusions (end-to-end, tandem, fusions of holocentric chromosomes) as well as to other types of chromosomal rearrangements, such as Robertsonian translocations and reciprocal translocations [23]. Translocations involving sex chromosomes, however, can dramatically reshape sex linkage more than inversions as seen in frog, Rana temporaria [25]. Just as the structure of chromosomes is not static the position of individual genes is also labile. In particular there is abundant evidence that gene duplication can produce both tandem copies closely linked to the original gene copy as well as duplicates on entirely different chromosomes. These distant duplicates often show a pattern that suggests that movement onto or off of sex chromosomes is beneficial [26–29]

MINOR COMMENTS

Line 73 onwards can be simplified, as using one minus the quantity of interest is such a common ‘trick’ that it can be described briefly, perhaps as “Given that a fusion has occurred, we are interested in the probability that it is an SA-fusion, which can be found by calculating the expected proportion of all fusions that do not involve a sex chromosome:

Lines 72-75 changed as suggested

We denote our three possibilities as events , , and , respectively. Given that a fusion has occurred, we are interested in the probability that it is an SA-fusion, which can be found by calculating the expected proportion of all fusions that do not involve a sex chromosome:

Referee: 4

4.1 But I still feel the authors can provide a better argument for (i) the assumption that chromosomes have an equal probability of fusion and (ii) for the overall significance of this model.

We have made minor edits to the text in several places and included a new sentence (pasted below) addressing both points 1 and 2. More broadly we have tried to clarify that we are not proposing that all fusions are equally likely but that this is a standard approach for the construction of a null model used to detect deviations from an equal expectation (e.g., T-test assumes equality of means for two groups)

Lines 117-119
The model that we have developed will allow for the identification of clades that exhibit significant deviations from a neutral expectation that all fusions are equally likely.

4.2 It is a nice improvement that authors now provide two empirical applications of their approach: Habronattus and Drosophila. But this might not represent the variety of chromosomal systems described in nature. Besides, these two genera are characterized by either no recombination in sex chromosomes (Drosophila) or distal/terminal chiasmata (Habronattus). The idiosyncrasy of recombination in these species can also influence the occurrence (and the final fate) of chromosomal rearrangements among populations.

If authors want their paper to be influential it might be helpful to test their hypothesis also in Amniote vertebrates (represented by mammals and sauropsids i.e. birds/reptiles), which are characterized by both distal and interstitial chiasmata. In fact, there is a well-documented significant variation in chromosome number and morphology among marsupials and eutherian mammals, with diploid numbers ranging from 2n=6 to 2n=102. And recombination is mainly interstitial (with few exceptions). Within mammals rodents also show atypical sex chromosome systems, such as XX/ X*X/ X*Y females and XY males in African pygmy mice and lemmings or XY males and XO females in creeping voles. This is also the case of autosome/sex chromosomal translocations in some primate species.

Please see response 1.1 above

Specific comments:
4.3 Introduction: I still suggest to the authors to provide a more thoughtful view on the role of chromosome reorganization in evolution.

We lack space in the manuscript to include a discussion of additional mechanisms of chromosome reorganization; however, we included a new section in the supplement discussing the role of a variety of mechanisms in genome reorganization.

Supplement lines 11-36

Quoted text is available above in response to comment 3.3

4.4 Empirical applications: It will be helpful to introduce an initial paragraph that justifies the use of these two systems and no others. Background on jumping spiders is rather short.

We have made it clear in a short introduction to the empirical section why these clades were chosen. Including additional background material on Habronattus would be difficult as we are already at the maximum word count. In lieu of this we have included an additional citation that discusses this group.

Lines 123-126

To demonstrate the utility of our approach we apply our equations in two empirical systems. The first is the jumping spiders genus Habronattus that has been suggested to show a large excess of SA-fusions [30,31], and the second is Drosophila which has served as a model system for much of our understanding of sex chromosomes [6,32,33].

Lines 128-141: This paragraph would fit better in the Habronattus section.

We have moved this section as suggested.

Lines 191-200: This paragraph needs references.

We have added citations in the section of this paragraph below.

Line 203-207

In species with achiasmatic meiosis, when an SA-fusion occurs, the entire Y chromosome is immediately subject to population genetic forces (e.g., Muller's ratchet) that lead to the loss of functional genes [8]. Drosophila has relatively few chromosomes such that each chromosome carries many genes. (D. melanogaster 43% of all genes are on autosome 3) [9].

Bibliography

1. Blackmon H, Ross L, Bachtrog D. Sex determination, sex chromosomes, and karyotype evolution in insects. J Hered. 2017;108: 78–93. doi:10.1093/jhered/esw047
2. Blackmon H, Demuth JP. Coleoptera Karyotype Database. The Coleopterists Bulletin. 2015;69: 174–175.
3. Blackmon H, Hardy NB, Ross L. The evolutionary dynamics of haplodiploidy: Genome architecture and haploid viability. Evolution. 2015;69: 2971–2978. doi:10.1111/evo.12792
4. Ross L, Blackmon H, Lorite P, Gokhman VE, Hardy NB. Recombination, chromosome number and eusociality in the Hymenoptera. J Evol Biol. 2015;28: 105–116. doi:10.1111/jeb.12543
5. Perkins RD, Gamboa JR, Jonika MM, Lo J, Shum A, Adams RH, et al. A database of amphibian karyotypes. Chromosome Res. 2019;27: 313–319. doi:10.1007/s10577-019-09613-1
6. Charlesworth D, Charlesworth B. Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes. Genet Res. 1980;35: 205–214.
7. Pennell MW, Kirkpatrick M, Otto SP, Vamosi JC, Peichel CL, Valenzuela N, et al. Y fuse? Sex chromosome fusions in fishes and reptiles. PLoS Genet. 2015;11: e1005237. doi:10.1371/journal.pgen.1005237
8. Bachtrog D, Charlesworth B. Reduced adaptation of a non-recombining neo-Y chromosome. Nature. 2002;416: 323–326. doi:10.1038/416323a
9. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, et al. The genome sequence of Drosophila melanogaster. Science. 2000;287: 2185–2195. doi:10.1126/science.287.5461.2185
10. Bush GL, Case SM, Wilson AC, Patton JL. Rapid speciation and chromosomal evolution in mammals. Proc Natl Acad Sci USA. 1977;74: 3942–3946. doi:10.1073/pnas.74.9.3942
11. de Vos JM, Augustijnen H, Bätscher L, Lucek K. Speciation through chromosomal fusion and fission in Lepidoptera. Philos Trans R Soc Lond B Biol Sci. 2020;375: 20190539. doi:10.1098/rstb.2019.0539

12. Kirkpatrick M, Barton N. Chromosome inversions, local adaptation and speciation. Genetics. 2006;173: 419–434. doi:10.1534/genetics.105.047985

13. Kirkpatrick M. How and why chromosome inversions evolve. PLoS Biol. 2010;8. doi:10.1371/journal.pbio.1000501

14. Tigano A, Friesen VL. Genomics of local adaptation with gene flow. Mol Ecol. 2016;25: 2144–2164. doi:10.1111/mec.13606

15. Christmas MJ, Wallberg A, Bunikis I, Olsson A, Wallerman O, Webster MT. Chromosomal inversions associated with environmental adaptation in honeybees. Mol Ecol. 2019;28: 1358–1374. doi:10.1111/mec.14944

16. Lohse K, Clarke M, Ritchie MG, Etges WJ. Genome-wide tests for introgression between cactophilic Drosophila implicate a role of inversions during speciation. Evolution. 2015;69: 1178–1190. doi:10.1111/evo.12650

17. Powell JR, Petrarca V, della Torre A, Caccone A, Coluzzi M. Population structure, speciation, and introgression in the Anopheles gambiae complex. Parassitologia. 1999;41: 101–113.

18. della Torre A, Merzagora L, Powell JR, Coluzzi M. Selective introgression of paracentric inversions between two sibling species of the Anopheles gambiae complex. Genetics. 1997;146: 239–244.

19. Kulathinal RJ, Stevison LS, Noor MAF. The genomics of speciation in Drosophila: diversity, divergence, and introgression estimated using low-coverage genome sequencing. PLoS Genet. 2009;5: e1000550. doi:10.1371/journal.pgen.1000550

20. Blackmon H, Demuth JP. The fragile Y hypothesis: Y chromosome aneuploidy as a selective pressure in sex chromosome and meiotic mechanism evolution. Bioessays. 2015;37: 942–950. doi:10.1002/bies.201500040

21. Bachtrog D. The temporal dynamics of processes underlying Y chromosome degeneration. Genetics. 2008;179: 1513–1525.

22. Satomura K, Tamura K. Ancient Male Recombination Shaped Genetic Diversity of Neo-Y Chromosome in Drosophila albomicans. Mol Biol Evol. 2016;33: 367–374. doi:10.1093/molbev/msv221

23. Guerrero RF, Kirkpatrick M. Local adaptation and the evolution of chromosome fusions. Evolution. 2014;68: 2747–2756. doi:10.1111/evo.12481

24. Wellband K, Mérot C, Linnansaari T, Elliott JAK, Curry RA, Bernatchez L. Chromosomal fusion and life history-associated genomic variation contribute to within-river local adaptation of Atlantic salmon. Mol Ecol. 2019;28: 1439–1459. doi:10.1111/mec.14965

25. Toups MA, Rodrigues N, Perrin N, Kirkpatrick M. A reciprocal translocation radically reshapes sex-linked inheritance in the common frog. Mol Ecol. 2019;28: 1877–1889. doi:10.1111/mec.14990

26. Dupim EG, Goldstein G, Vanderlinde T, Vaz SC, Krsticevic F, Bastos A, et al. An investigation of Y chromosome incorporations in 400 species of Drosophila and related genera. PLoS Genet. 2018;14: e1007770. doi:10.1371/journal.pgen.1007770

27. Betrán E, Thornton K, Long M. Retroposed new genes out of the X in Drosophila. Genome Res. 2002;12: 1854–1859. doi:10.1101/gr.6049

28. Emerson JJ, Kaessmann H, Betrán E, Long M. Extensive gene traffic on the mammalian X chromosome. Science. 2004;303: 537–540. doi:10.1126/science.1090042

29. Zhang YE, Vibranovski MD, Landback P, Marais GAB, Long M. Chromosomal redistribution of male-biased genes in mammalian evolution with two bursts of gene gain on the X chromosome. PLoS Biol. 2010;8. doi:10.1371/journal.pbio.1000494

30. Leduc-Robert G, Maddison WP. Phylogeny with introgression in Habronattus jumping spiders (Araneae: Salticidae). BMC Evol Biol. 2018;18: 24. doi:10.1186/s12862-018-1137-x

31. Maddison WP, Leduc-Robert G. Multiple origins of sex chromosome fusions correlated with chiasma localization in Habronattus jumping spiders (Araneae: Salticidae). Evolution. 2013;67: 2258–2272. doi:10.1111/evo.12109

32. Zhou Q, Bachtrog D. Sex-specific adaptation drives early sex chromosome evolution in Drosophila. Science. 2012;337: 341–345. doi:10.1126/science.1225385

33. Ironside JE. No amicable divorce? Challenging the notion that sexual antagonism drives sex chromosome evolution. Bioessays. 2010;32: 718–726. doi:10.1002/bies.200900124