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 and analyses as suggested. We have added an additional collaborating author to expedite the inclusion of a second empirical analysis to the paper. We believe these changes have significantly improved the manuscript. 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.

With kind regards,

Heath Blackmon

**Referee: 1**  
It was not clear HOW this could “test the long-standing hypothesis that SA-fusions are selectively favored for their ability to resolve sexual antagonism”.  The main problem of this approach is to use the formulas in “real life” since SA-fusions are much easier to detect than AA-fusions, therefore, these last ones can be underestimated. Besides, many sex chromosomes are still homomorphic and therefore such a fusion that could look like an AA-fusion could be, in fact, an SA-fusion. Especially among fishes, my main area of expertise, an enormous number of examples can be found of multiple fusions (AA, followed by SA) and sex-turnovers, with translocations turning Autosomes into neo-sex chromosomes among populations of the same species. All the above-mentioned scenarios turn any attempt of modeling these into a complicated and non-accurate process.

We appreciate the hesitancy expressed by the reviewer. In particular we agree that there may be many groups where the application of these approaches would be difficult if not complete folly. However we believe that there are many groups like the two we present in the revised version of the paper where our approach promises a new way forward. We agree that if either comparing two distantly related species simply by chromosome number or if working in a group where homomorphic sex chromosomes are common many events might be missed. However, by applying our test in a group like Drosophila where sex chromosomes are heteromorphic and we can sample densely these concerns should lessen. Our use of probabilistic models of chromosome evolution allow us to infer a rate of fusions that can accommodate many unobserved events and simulation testing has shown that these estimates align well with simulated datasets where the true history of fusions and fissions is known. Our analysis with the Drosophila dataset illustrates this well.

Lines XXX-XXX

Small note: Page 7: First paragraph: Some Mazama species also posses multiple X1X2Y sex chromosomes (Aquino, C. I.; Abril, V. V.; Duarte, J. M B. Meiotic pairing of B chromosomes, multiple sexual systems, and Robertsonian fusion in the red brocket deer Mazama americana (Mammalia, Cervidae). Genetics and Molecular Research, v. 12, n. 3, p. 3566-3574, 2013)

We have added citation to this article

**Referee: 2**  
  
On the one hand, the calculations are exactly what one would do naturally as a side calculation and so do not represent a substantive advance. On the other hand, the result will surprise people: sex-autosome fusions are expected to occur under the null hypothesis at least 25% of the time until the diploid number of chromosomes rises about 16. This deserves highlighting in the abstract.

We have highlighted this in our abstract lines 14-17  
  
My recommendation would be to add more meat to the bones of this paper by analyzing a data set along the lines suggested in Figure 2 (Drosophila?). The authors do reanalyze the case of the jumping spiders, but that’s a simple one given that the autosome counts remain 26 for the transitions examined.  Having two case studies, not just this one, would balance the paper and would be useful to mention in the abstract to motivate the reader. Without this motivation, I don't think the paper is strong enough.

We appreciate this suggestion and have added an analysis of 120 species of drosophila.

abstract lines 17-18

methods lines XXX-XXXX

discussion lines XXXX-XXX

Other major suggestions:  
  
The XY and XO sections are really such straightforward calculations that these two sections should be merged.

We chose to move the entire derivation of the formula to a supplement and simply present the final equations as well as two empirical applications of our work.  
  
The authors note after equation (10) that their calculations apply to multi-XY systems, but that isn’t true.  They only apply to multi-X Y or X multi-Y systems, not to multi-X multi-Y systems.  Although the latter are rare (noted in the discussion), the calculation is not that much more complicated and should be given.

We have modified our equations such that they are now appropriate for XY and ZW systems regardless of the number of Xs or Ys. We also point out that this formulation is appropriate for UV systems given that the number of U and V chromosomes is equal.

Minor comments:  
  
“In an XYY system, X\_S = 2" should read “In an XYY system, X\_S = 1”

This section has been rewritten and this error has been removed.

"does not drop below 25% until the diploid autosome count is greater than 16” should read "does not drop below 25% until the diploid chromosome count is greater than 16” or "does not drop below 25% until the diploid autosome count is greater than or equal to 16” [it is 22% with 16 autosomes]

Corrected line 116

The notation in (8) and (11) should be synchronized (SA with or without a subscript).

This section has been rewritten.

Sorry to not be that helpful on this, but this is the type of calculation that could easily be in the early work on chromosome evolution. It is worth double checking White 1977, King 1993 (Species Evolution), and Bull 1983 (Evolution of Sex Determining Mechanisms) to make sure that they didn't cover this calculation.

We worried about this while working on this project but cannot find any similar calculation (we have checked all of your suggested references as well as many others). The closest that we can find is in a 1980 Genetics Research paper by Charlesworth and Charlesworth on page 211 they state “Assuming a chromosome set consisting of four autosomal rods and one rod sex chromosome, purely random fusions should occur in the proportions of four fusions involving the sex chromosomes to six involving only autosomes.”. We also found that the appendix of Charlesworth, Coyne, and Barton 1987 compare some similar ratios but never provide a formulation for what would be considered as expected.

**Referee: 3**  
Comments to the Author(s)

The Introduction fails to cite the first detailed discussion of this question, which also discussed some problems with studying it empirically Charlesworth, B., J. A. Coyne and N. H. Barton, 1987 The relative rates of evolution of sex chromosomes and autosomes. American Naturalist 130: 113-146.

We appreciate you catching this! The appendix of this paper actually motivated some of our initial interest this problem. In Charlesworth et al 1987 the primary issues discussed are 1) the lack of a good phylogeny 2) inability to recognize fusions (other problems discussed focus more on the desire to parse the difference in rates of fusions with Y vs X chromosomes). We have added a citation to this article as well as discussing a bit more about how the approach that we apply overcomes these potential problems.

Lines XXX-XXXX

We have addressed the next four comments in a rewriting of one paragraph of the introduction and so have grouped them below:

-This ms points out, as that paper did, that current information is limited to anecdotal examples of such fusions (the paper just cited includes an appendix that reviewed the data available in 1987). However, the statement in the present ms is not accurate. First, the 2012 paper by Zhou and Bachtrog merely reviews a few cases in Drosophila, but not all the other cases, and this ms does not cite their 2015 paper on the recent fusion in D. busckii, whose admittedly does not suggest that it might be relevant (Ancestral chromatin configuration constrains chromatin evolution on differentiating sex chromosomes in Drosophila. Plos Genetics 11: e1005331). Ideally, if Drosophila is to be discussed in this ms, a better review should be cited, that covers the genus as a whole. Second, the Drosophila examples are certainly not all recent.

-Third, there is no evidence that the autosomes involved in these fusions “are enriched for sexual antagonistic loci” (which should be “sexually antagonistic loci”).

-If this were known, then the question at issue would already have been answered for those cases, at least. The Drosophila americana example was “proported” to involve sexual antagonism (this presumably means proposed, and probably not “reported”, as there is no actual evidence to support this).

-The same is true for the jumping spider analysis, but that study did finally describe a data set with fusions between all chromosome types, and detected an apparent excess of X chromosome autosome fusions, compared with autosomal ones (this ms should make clear what null hypothesis was tested).

All three of these comments are justified and we have rewritten this portion of the introduction to more accurately reflect the fact that empirical examples are consistant with a model of sexual antagonism driving fusion of sex chromosomes and with an alternative model that sexual dimorphism (often assumed to equate to resolved sexual antagonism) evolved after the fusion.

With regard to the null being tested in Habronattus: We have edited our discussion to make it clear what the null being tested was.

line 124.

This ms re-visits this case, and supports that previous conclusion, which is a small, but worthwhile, contribution that will show others how this should be done. Zhou, Q., and D. Bachtrog, 2015 Ancestral chromatin configuration constrains chromatin evolution on differentiating sex chromosomes in Drosophila. Plos Genetics 11: e1005331. It is rather misleading to cite evidence that sexually antagonistic selection has been inferred to be common throughout the genome (as at the bottom of p. 2), because only in situations where such selection leads to the maintenance of sexually antagonistic polymorphism does the selection create selection for closer linkage of the locus with the sex-determining locus. It is important to differentiate such situations from other sexually antagonistic selection, because polymorphism is generated in only a very limited set of situations.  The text uses the phrase “debate on the ubiquity of sexually antagonistic variation”, but it might be better to be more explicit, and simply say “sexually antagonistic polymorphism”.

We appreciate the critiques of our discussion of and coverage of the previous work in Drosophila. In response to this comment and one from other reviewers we have applied our approach to an analysis of 120 species of Dropsophila.

Lines XXXXX

The text in question is poorly written and needs the word “that” — at present it reads “In figure 1, we show when the autosome number is small ….” And can be shortened, as just written.

Corrected

Line XXX

This could be related to the lack of a good study using Drosophila, as obviously one reason for this lack may simply be that people chose not to attempt a test, because of the small chromosome numbers in these species. In Drosophila, fusions involving autosomes can be detected cytogically, and have been described (unlike many other taxa) so the genus might seem to be good for asking the question posed here, but the analysis in this ms is discouraging. It might be good to say explicitly in the introduction section that testing whether the proportion of fusions involving sex chromosomes is unexpectedly high, which might suggest selection favoring such fusions, requires a quantitative null model that takes account of the chromosome number and sex chromosome system (NOT accounts for, which means “explains).

We have expanded the study to include an analysis of 120 species of drosophila.

Lines XXXXX

Changed accounts for to takes account of line 120

However, the exercise is a purely probabilistic calculation, and seems to ignore biological context. One place in the ms where this may cause misunderstanding is in discussing “XXY systems (see below). Another instance is that the ms does not mention differences such as whether crossovers occur only in one sex, or both. This has very important consequences for whether any sexually antagonistic polymorphism on the autosome involved becomes completely associated with one sex. In species where males don’t have crossovers, both X-A and Y-A fusions lead to the former autosome becoming co-inherited along with the Y, which means that it will not recombine, allowing complete association with maleness. In species where males recombine, such an autosomal polymorphism might become Y-linked, but this seems most likely to happen if it is close to the fusion point, and the fusion is with the Y, not the X (because the rearrangement could inhibit chromosome pairing in this region.

We agree that our model does ignore some biological reality (type of meiosis for instance). We have added a short discussion of the impact of meiosis type and also the potential importance of the precise type of fusion (with regard to the PAR region in chiasmatic species). More broadly we discuss the possible impact of meiosis type in the discussion when interpreting our results from the empirical analysis of Drosophila.  
  
Overall, it would be better to use the text to describe biological results, and put the derivations in an Appendix (in as short and clear forms as can be achieved). At the very least, the “XXY” one should be removed from the main text.

We have moved the vast majority of the derivation to a supplement and now focus much more on the empirical applications.

Lines XXXX

The authors assume that every chromosome is equally likely to be involved in a fusion event. The text after equation (1) can be shortened by mentioning that, for the probability of an A-A fusion, one autosome is chosen at random, and another non-homologous one is chosen without replacement, to exclude fusions between homologous chromosomes. This includes fusions between an X and Y chromosome, so in the first section about the model (the XY case) it is unclear what is meant by the case when the two chromosomes are both sex chromosomes (SS-fusions). It would be clearer (and could shorten the text) if the paragraph introducing the models explained that fusions between homologous chromosomes are excluded in the first models discussed, but will be used later, when examining the case of multi-XY systems.

We have rewritten this section and have altered the text as suggested. The majority of the derivation is now in the supplement.  
  
The XY case can be explained in a much shorter manner, which would also be clearer. Similarly, for the X0 system, though it seems odd not to mention that in this case we can have X-A, but not Y-A fusions, as explained in Charlesworth and 1980 Charlesworth, which also mentions some other caveats about attempting tests in Drosophila (also, the phrase “assume that males and females make equal contributions to possible fusions” is rather unclear — does it mean that the fusion could occur in either sex?). The end of the latter section (“Hence, this result is accurate for both XO and XY sex chromosome systems” simply means “Hence, this result applies to both XO and XY systems”.  
  
We have rewritten this section and have altered the text as suggested. The majority of the derivation is now in the supplement.

The term “XXY system” is odd, and presumably means one where a fusion has already become established. Such cases are usually described as X1X2Y systems, where X1 is an ancestral X, and X2 is a former autosome that is now a neo-X because its homolog fused to the former Y.

We have clarified the description of these multi sex chromosome systems. In many groups this description (XXY or X1X2Y) is used to describe a system where an autosome has fused with the Y chromosome and there are now two unfused X chromosomes that segregate opposite a single fused Y chromosome CITATIONS. In cases where the a single X and single Y segregate but there is evidence of a fusion such that the X and Y now include a fused autosome we see the term NeoXY as being the most common CITATIONS. This is the terminology that we use for instance in the analysis of Drosophila.  
  
The ms also seems not to be familiar with the development of ideas in the area. Bachtrog et al. 2014 did not discover the sex chromosome systems of haploid plants, but simply proposed calling what had previously been called the Y (male-determining) chromosome V and the female-determining one U (it was previously called an X chromosome). An earlier paper should be cited, e.g.  
Allen, C. E., 1935 The Genetics of Bryophytes. Botanical Review 1: 269-291.  
If Bachtrog et al. is to be cited, please indicate clearly that this is a review article. In addition, the ms appears to say nothing about UV systems, other than that the equations derived do not apply in this case. I believe that sex chromosome-autosome fusions are not uncommon among bryophytes. So why not work out the chances for this case also? I think this is simpler than the ones that are included.

We didn’t intend to imply that the UV system was discovered or even proposed to be called a UV system by the Bachtrog paper. We have retained our citation to the review and synthesis paper as we feel that it offers a good introduction for readers to the possible systems documented but we now make it clear this is simply a review and we have added a citation to the paper first describing the UV system.

Lines XXX-XXX  
  
The phrase “across the entire clade” is confusing when it refers to a hypothetical case.

We have clarified that we are referring the the jumping spider clade, we have clarified this fact line 133.

The last part of the text describes how the equations might be applied when data exist for a group of organisms includes not just XY or X0 systems, but different systems in different lineages. This is worth mentioning, but could be shortened, given that there are no data sets currently, and the approach has already been developed for other characters. In my opinion, this short paper will be much more likely to be read, and lead to advances in understanding, if it is shortened and made more readable — at present, parts are long-winded and make heavy weather of rather simple stuff.

We believe the edits that we have made in response to other comments have largely dealt with these issues in particular we have greatly shortened the derivations and added additional empirical analyses.

Some comment about ZW and Z0 systems should be added to the text. In my opinion, it is not sufficient merely to say that these are in the supplement. Presumably, the results are similar to those for XY and X0 systems, and, if so, why not say that. If not, a brief mention of why a difference arises would be helpful.

We have corrected this issue and made it clear that the results are identical for XY and ZW systems assuming that all else is equal.

Lines XXX-XXX  
  
Referee: 4  
  
Comments to the Author(s)  
Anderson & Blackmon present a model to test the probability of sex chromosome-autosome fusion given their importance in sexual antagonism. Understanding the role of chromosome fusions in chromosomal evolution is in fact one of the most intriguing questions in biology. Several hypotheses have been proposed, from the hybrid dysfunction model (White 1969; King 1993) to the suppressed recombination models of speciation (Navaro and Barton 2003; Faria and Navarro 2010; Brown and O’Neill 2010; Farre et al. 2013; Faria et al 2019). Therefore, the area of research where the paper is focused on is of interest.  
  
Being said that, the authors base their hypothesis on an assumption that is simply not correct. They assume that all chromosomes have an equal probability of being involved in fusions. But extensive literature in cytology, cell biology and genomics fields have demonstrated that chromosomes do not distribute randomly inside the nucleus. In fact, a layer of complexity is provided by the compartmentalization of the nucleus. The genome is organized into discrete, three-dimensional chromosomal territories or domains. It is known that this organization is non-random; gene-rich chromosomes and active euchromatin tend to reside in the inner portion of nuclei, while gene-poor regions and genetically inert heterochromatin are located at the nuclear periphery. And this distribution is strongly species-dependent based on the cell type, the number and morphology of chromosomes among other factors. From the Rabl (or Rabl-like) distribution in plants and yeast to centromere clustering in A. thaliana. So, chromosomes do not have equal probability of being involved in rearrangements. And this is especially true for sexual chromosomes, whose nuclear occupancy tends to be more towards the periphery in same species (at least in mammals). There is also evidence that certain properties of local DNA sequences together with the epigenetic state of the chromatin could promote the change of chromatin to an open configuration and this can contribute the origin of chromosomal reorganizations. Therefore, authors need to reformulate their model based on experimental evidence.

We agree that the assumption of all chromosome having equal probability is a strong assumption but we feel that this is a necessity to develop a null model of this sort. Existing methods could and have been used to test whether this assumption is violated for instance a Monte Carlo simulation approach where we ask whether the number of times that a specific chromosome is involved in a sex chromosome autosome fusion is beyond a null expectation CITATION.

Moreover, I would also suggest to the author to provide a more thoughtful view on the role of chromosome reorganization in evolution in the introduction section. Not only fusions and fissions are strong driving forces, but also inversions.

We have added a bit about inversions but since our model and the focus of this paper is fusions we have kept this to a minimum.

Lines XXXX-XXXX  
  
It is also not clear which taxonomic group are they referring to (i.e., mammals, insects, all taxa?).

We are not sure what this is in reference to. However, we have hopefully fixed any lack of clarity in our edits to the revised manuscript.  
  
It will be helpful also to provide a framework for the importance of autosome-sex chromosome fusions. Different sexual chromosome systems (which are indeed diverse and complex) need to be properly introduced in early stage of the paper. Some of them are named in the discussion but the audience might not be familiar with them. Are ZW systems also considered?

We have improved our introduction to possible sex chromosome systems in the introduction.

Referee 1 questions whether the model is useful in terms of application across diverse taxa, as well as for testing the hypothesis of SA-fusions and sexual antagonism. Referee 2 suggests emphasizing the expectations of SA fusions based on the number of chromosomes. I agree that the abstract needs to be carefully rewritten in a manner that identifies the novelty of this null model. I also agree with this referee’s recommendation to analyze an additional set of data. Referee 3 provides detailed recommendations that I feel will improve the manuscript, and I suggest that the authors carefully address these comments. Referee 4 also discusses the need for validation of the model. This referee challenges the assumption that all chromosomes have an equal probability of fusion. I understand this argument, but it seems to me that a null model would use such an assumption. Perhaps the authors can provide a better argument for this assumption. All referees highlight various areas where the paper can be improved, and revision should start with the abstract and introduction. The challenge presented to the authors relates to the overall novelty of this approach and how it will contribute to ideas about chromosomal antagonism. As written, the paper falls short of highlighting the overall significance of this model.

We appreciate you taking the time to summarize your evaluation of the received reviews this was very helpful in formulating our responses.  
  
Here are a few extra recommendations. It would be nice to define sexual antagonism in the introduction. References should be numerical designations and listed in order of citing rather than alphabetically by author. The following paper seems germane: Matsumoto and Kitano. 2016. The intricate relationship between sexually antagonistic selection and the evolution of sex chromosome fusions. Journal of Theoretical Biology 404:97-108.

We have edited the introduction to clearly define sexual antagonism line 27-29 and have also included a citation to the suggested article.

Line XXX-XXX