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 substantially 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 and any sections quoted from the paper are italicized.

With kind regards,

Heath Blackmon

**Referee: 1**  
1.1 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. Furthermore, 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 [1]. Our analysis with the Drosophila dataset illustrates the utility of our approach.

Lines 128-168

1.2 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)

This section of the paper no longer exists in the revised version of the manuscript.

**Referee: 2**  
  
2.1 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 23-26.

*We find that over 25% of all chromosomal fusions are expected to join a sex chromosome and an autosome whenever the diploid autosome count is fewer than 16, regardless of sex chromosome system.*

2.2 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.

Lines 26-30, 128-168, 185-200

2.3 The XY and XO sections are really such straightforward calculations that these two sections should be merged.

We agree and have chosen to create a supplement that allows us to illustrate the step by step process of deriving our final equation. In the main manuscript we now just present the final equations with far less exposition. We believe this strikes a good balance between having everything available for someone who might be trying to figure out how to do something similar and making it an enjoyable read for the average person.

Lines 60-112  
  
2.4 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.

Line 104-117

Minor comments:  
  
2.5 “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.

2.6 "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 180-182

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

This section has been rewritten.

2.8 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 an explanation for what would be considered as expected ratios under neutrality.

**Referee: 3**  
3.1 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 helped clarify some of our thoughts on this problem. Although we do draw a distinction that this paper is focused more heavily on trying to determine if fusions with X or Y chromosomes are more common. We mention this in the revised manuscript.

Line 189-191

*Previous work examining SA- fusions in Drosophila have largely focused on the balance between fusions of an autosome with the X versus the Y [2]. Much of this work was done prior to the development of modern comparative approaches today and could not fully incorporate the evolution of chromosome number over the history of Drosophila.*

We do not cite this paper in the introduction. Instead we retain our citation to Charlesworth and Charlesworth 1980. This paper is the true inspiration for developing the approach we have applied. In fact, in this paper the Charlesworth’s verbally state (page 211) the probability of sex chromosome autosome fusions for one specific chromosome number and sex chromosome system.

Unfortunately, in both cases the comparative methods that we employ (stochastic mapping, probabilistic models of chromosome evolution) were unavailable when these papers were written. With the addition of our analysis of drosophila we believe that the revised manuscript is much clearer and the ability to overcome the hurdles that previous researchers faced is well illustrated by our results.

3.2 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 our thinking which briefly is that:

1. If sexual antagonistic variation exists on autosomes it can favour fusions joining autosomes and sex chromosomes
2. Some groups (like *Habronattus*) show what appears to be clear excess of these fusions
3. Evidence suggests that there is sexually antagonistc variation on autosomes in multiple species.
4. Young sex chromosomes show signatures consistent with resolved sexual antagonism
5. People are still debating whether sexual antagonistic variation is a driving force in evolution of genome structure (e.g. fusions, reduction in recombination, etc.)

The text responsive to this is now in lines 36-56 of the manuscript and reads:

*In particular, the fusion of a sex chromosome and an autosome (SA-fusion) has been proposed to resolve sexual antagonism (when an allele is beneficial for one sex and deleterious for the other). Linking sexually antagonistic alleles to sex chromosomes can increase the average fitness of both sexes [3]. Therefore, SA fusions are predicted to be more common than fusions joining two autosomes (AA-fusions) [4]. For example , an apparent surplus in X chromosome autosome fusions in the jumping spider genus, Habronattus, is hypothesized to result from a mechanism of isolating male-beneficial sexually antagonistic alleles on the neo-Y chromosome [5]. However, most evidence for sexually antagonistic variation comes from within species. For instance, empirical studies in fish, human, and flies have found evidence for segregating sexually antagonistic variation (variation with opposite fitness effects in males and females) [6-8]. Furthermore, young sex chromosomes (originating through fusion, translocation, or turnover) exhibit signs consistent with the resolution of sexual antagonism [9-11]. However, there remains significant debate on the ubiquity of sexually antagonistic variation and its potential role in genome evolution [12-14]. A strong measure of the frequency of significant sexually antagonistic variation across genomes would be an excess of SA-fusions relative to AA-fusions across large clades. We derive equations describing the probability of each type of fusion necessary to perform such a test and illustrate two approaches to using these equations with empirical datasets. This approach will provide a method to quantitatively analyze the balance of these two types of fusions in the many groups with a well-documented history of fusion between sex chromosomes and autosomes [15-18].*

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 119-120.

*The intuition that this imbalance is unlikely can be rigorously tested with our null model that the distribution of fusions is determined by chromosome number and sex chromosome system.*

3.3 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 ones from other reviewers we have applied our approach to an analysis of 120 species of *Drosophila*.

Lines 26-30, 128-168, 185-200

3.4 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.

Thank you we have corrected as suggested

Line 179-180

3.5 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 26-30, 128-168, 185-200

We have changed “accounts for” to “takes account of”

line 120

3.6 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. We do plan on delving into the impact of some of these complications in future work. You touch on one of those avenues the result of fusions in species that have sex chromosomes with a PAR. PARs have some of the highest recombination rates ever measured and as such it is possible that fusions with the PAR region may lead to very little if any increase in linkage between sexually antagonistic loci and the sex determining region. However, regardless of these complications it remains that some types of fusion with the sex chromosomes would lead to a fitness benefit due to increased linkage and thus should be favoured. Depending on the strength and ubiquity of sexual antagonism this should lead to an imbalance in the proportion of fusion types. Furthermore, in the revised version of the manuscript we actually discuss the role that achiasmatic meiosis and genome structure may play in driving the unexpected results of our analysis in Drosophila

Lines 191-200

*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 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. Drosophila has relatively few chromosomes such that each chromosome carries many genes. (D. melanogaster 43% of all genes are on autosome 3). 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.*

3.7 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.

3.8 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.  
  
3.9 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.

3.10 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 changed the wording from XXY to X1X2Y. We also provide a reference to the book *The Evolution of Sex Determination* by Beukeboom and Perrin in this section as it does an excellent job of laying out some of the variety observed. In cases where there is a single X and single Y segregating but there is evidence of a fusion we use the term NeoXY. This is the terminology that we use for instance in the analysis of Drosophila.  
  
3.11 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.

In reworking on manuscript to reduce the derivation section and include another empirical analysis the referenced part of the manuscript is no longer included. As mentioned above we do provide a citation to a book that does a nice job of presenting some of the diversity of sex chromosomes systems.

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

We have clarified that we are referring to the jumping spider clade

Line 131.

*However, during the evolution of the Habronattus clade, a variety of karyotypes must have existed.*

3.13 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 an additional empirical analysis.

3.14 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 105-107

*This formulation can be converted for use in ZW sex chromosome systems by exchanging and , replacing with , replacing with , and replacing with .*

**Referee: 4**  
4.1 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. We believe the benefits of applying a simplified null model like this is illustrated in our analysis of Drosophila. Our recognition that Drosophila violate our expectation gives us the opportunity to think deeply about what violations might produce the pattern that we do observe. In this case we discuss this a bit towards the end of the paper.

Lines 192-201

*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 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. Drosophila has relatively few chromosomes such that each chromosome carries many genes. (D. melanogaster 43% of all genes are on autosome 3). 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.*

4.2 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 agree that inversions are a key process involved in genome evolution but the method that we are presenting is focused specifically on assessing fusions. With the space requirements of the article we do believe that we have space to discuss the role of fusions in genome evolution.

4.3 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.  
  
4.4 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.

Editors Comments

5.1 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.  
  
5.2 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 37-38 and have also included a citation to the suggested article line 38.

[1] Blackmon, H., Justison, J., Mayrose, I. & Goldberg, E.E. 2019 Meiotic drive shapes rates of karyotype evolution in mammals. *Evolution* **73**, 511-523.

[2] Charlesworth, B., Coyne, J. & Barton, N. 1987 The relative rates of evolution of sex chromosomes and autosomes. *Am Nat* **130**, 113-146.

[3] Matsumoto, T. & Kitano, J. 2016 The intricate relationship between sexually antagonistic selection and the evolution of sex chromosome fusions. *Journal of theoretical biology* **404**, 97-108.

[4] Charlesworth, D. & Charlesworth, B. 1980 Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes. *Genet Res* **35**, 205-214.

[5] Maddison, W.P. & Leduc-Robert, G. 2013 Multiple origins of sex chromosome fusions correlated with chiasma localization in Habronattus jumping spiders (Araneae: Salticidae). *Evolution* **67**, 2258-2272. (doi:10.1111/evo.12109).

[6] Cheng, C. & Kirkpatrick, M. 2016 Sex-specific selection and sex-biased gene expression in humans and flies. *PLoS genetics* **12**, e1006170.

[7] Innocenti, P. & Morrow, E.H. 2010 The sexually antagonistic genes of Drosophila melanogaster. *PLoS biology* **8**, e1000335.

[8] Roberts, R.B., Ser, J.R. & Kocher, T.D. 2009 Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. *Science* **326**, 998-1001.

[9] Kitano, J., Ross, J.A., Mori, S., Kume, M., Jones, F.C., Chan, Y.F., Absher, D.M., Grimwood, J., Schmutz, J. & Myers, R.M. 2009 A role for a neo-sex chromosome in stickleback speciation. *Nature* **461**, 1079-1083.

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[11] Zhou, Q. & Bachtrog, D. 2012 Sex-specific adaptation drives early sex chromosome evolution in Drosophila. *Science* **337**, 341-345.

[12] Ironside, J.E. 2010 No amicable divorce? Challenging the notion that sexual antagonism drives sex chromosome evolution. *BioEssays* **32**, 718-726.

[13] Kasimatis, K.R., Ralph, P.L. & Phillips, P.C. 2019 Limits to genomic divergence under sexually antagonistic selection. *G3: Genes, Genomes, Genetics* **9**, 3813-3824.

[14] Ponnikas, S., Sigeman, H., Abbott, J.K. & Hansson, B. 2018 Why do sex chromosomes stop recombining? *Trends in Genetics* **34**, 492-503.

[15] Blackmon, H., Ross, L. & Bachtrog, D. 2017 Sex Determination, Sex Chromosomes, and Karyotype Evolution in Insects. *J Hered* **108**, 78-93. (doi:10.1093/jhered/esw047).

[16] Traut, W., Sahara, K. & Marec, F. 2008 Sex chromosomes and sex determination in Lepidoptera. *Sexual Development* **1**, 332-346.

[17] White, M.J.D. 1977 *Animal cytology & evolution*. 3rd ed. Cambridge, University Press.

[18] Sylvester, T., Hjelmen, C.E., Hanrahan, S., Lenhart, P., Johnston, J.S. & Blackmon, H. 2020 Lineage-specific patterns of chromosome evolution are the rule not the exception in Polyneoptera insects. *Proceedings of the Royal Society B: Biological Sciences* **in press**.