Rebuttal

Production team:

When revising your article, we remind you that:
1) Data must be available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad (to pay) or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data;

DONE

2) Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) must be available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused;

DONE

3) Details on experimental procedures must be available to readers in the text or as appendices;

DONE

4) Authors must have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this article declare that they have no financial conflict of interest with the content of this article.";

DONE

5) This disclosure has to be completed by a sentence indicating, if appropriate, that some of the authors are PCI recommenders: “XY is one of the *PCIEvolBiol* recommenders.”.

DONE

by Mathieu Joron, 2020-08-19 14:10
Manuscript: 10.1101/445072 version Version 4
Revision needed

Dear Gabriel Marais
Your revised manuscript was reviewed by three referees, whose comments are pasted below. All referee consider that your manuscript addresses an interesting question and provides a plausible interpretation with solid data and analysis. I agree with them that the manuscript is much improved and that you responded adequately to the earlier comments. Overall I would be glad to recommend your manuscript but two referees and myself still

have a number of suggestions and requests for clarifications which seem important to address before formal acceptance.
1- The analysis of sexual dimorphism as a proxy for sexual antagonism is done adequately. However, the methods are totally absent from the manuscript and the supplementary table S2 is also absent (an excel file apparently). Since the methods are explained in the rebuttal letter, I only became aware of this when reading the actual reviews. Of course table S2 and the corresponding methods section are needed in the revised version.

Table S2 is now available through zenodo. This table is an excel file including the raw data, the methods and analysis and the references.
DOI: 10.5281/zenodo.4003878
https://zenodo.org/record/4003878#.X0esm9NKhgg

2- Both referees 2 and 3 still point to your interpretation (differences in sexually-antagonistic selection explaining differences in recombination suppression) being speculative, even though the ideas are carefully expressed and you acknowledge other processes. In my view, the logic could be more carefully explained. Indeed, looking for differences in sexual selection as a support for a role of SA selection leads the reader to understand that the differences in SA selection could be sufficient to explain the genomic signals, yet this is unclear and merits a careful explanation. SA selection is multifaceted and sexes in mammals differ in a large number of traits, a fraction of which is directly affected by sexual selection (as measured with morphological markers), so clades with little sexual selection might still undergo significant SA selection throughout the development of males vs females. Perhaps the assumptions associated with using sexual selection as a proxy for SA selection should be briefly explained in order to make the underlying logic clearer, and perhaps the conclusions easier to appreciate.

In the ms, we used measures of sexual dimorphism as proxies of SA selection. It is widely recognized that the level of sexual dimorphism should correlate with the intensity of SA selection. Referees 2 and 3 do not seem to question this rationale. They however point to the fact our study is correlational, which we already acknowledge in the ms.
Our measures of sexual dimorphism are assumed to be representative of the level of sexual dimorphism in general in an organism. We have added a sentence to acknowledge this when the analysis on sexual dimorphism is presented.

3- Reviewer 3 has listed a number of additional points, mainly about clarity and logic, and those should be addressed in your revision.

DONE

Reviews
Reviewed by Qi Zhou, 2020-07-22 13:13
I appreciate the authors spent the time to collect sexual dimorphism data and performed the correlation analyses with and without considering the phylogeny. I think their answers addressed my previous questions and the ms should be accepted for publication.

We thank Qi Zhou for his positive feedback.

Reviewed by anonymous reviewer, 2020-07-02 07:10

This manuscript examines sex chromosome evolution in strepsirrhine primates, specifically looking for evidence of changes in the extent of the pseudoautosomal region. They find evidence that this boundary has been very stable over time, suggesting little decrease in the extent of recombination between the X and Y over time, especially compared to other primates. They interpret this finding as being consistent with low levels of sexual antagonism within strepsirrhine primates, and support this interpretation with an analysis showing that the degree of sexual dimorphism is lower in strepsirrhines than in other primates.

I was not one of the original reviewers of this manuscript, but after reading their comments and the authors' response to them, it seems like the revised version has successfully addressed the criticisms that were raised in the first round of review. I did feel that the last sentence of the abstract is a bit strongly worded ("Our work supports the view that sexually antagonistic mutations have influenced the evolution of sex chromosomes in primates"), but the main text provides a more nuanced view and acknowledges that other explanations are possible.

Unfortunately I was not able to find any information about the analysis of sexual dimorphism and testes size anywhere other than the response to reviewers. Table S2 with the results of the analysis was missing from the supplementary information files, and there was no detailed description of how the analysis was carried out. This is presumably an oversight on the part of the authors, but it's a shame since this seems like it was a nice addition to the manuscript, and I currently can't evaluate the robustness of these results.

Uploading table S2 in Biorxiv has somewhat failed. We apologize for this problem. Table S2 is now available in zenodo:
DOI: 10.5281/zenodo.4003878
https://zenodo.org/record/4003878#.X0esm9NKhgg

Reviewed by anonymous reviewer, 2020-07-02 08:07

This is an interesting analysis of the evolution of the pseudo-autosomal region in primates, which demonstrates that the suppression of recombination between the X and the Y chromosomes has proceeded along different trajectories in the two main lineages of primates. In haplorrhines, there has been further suppression of recombination leading to the formation of two additional strata, while in strepsirrhines, there has been no further suppression of recombination since these lineages diverged. The patterns of recombination suppression are consistent with the presence of high levels of sexual dimorphism in haplorrhines and low levels of sexual dimorphism in strepsirrhines. Using levels of sexual dimorphism as a proxy for the intensity of sexually antagonistic selection, the authors conclude that selection for linkage between the sex-determination locus and alleles with sexually antagonistic effects might have driven suppression of recombination and the formation of the two additional strata in haplorrhines. I find this an intriguing hypothesis that lays the groundwork for additional studies. Although these conclusions remain speculative, the authors are careful in their conclusions and clear about the limitations. They have responded well to the previous reviews.

Nonetheless, the manuscript could be more clearly written in places, and so I provide some suggestions here for revisions to improve the clarity of the presentation.

1 P4, L25 - P5, L1: change “a ratio of 0.5 the X-specific region” to “a ratio of 0.5 should

indicate the X-specific region"

DONE

2 P5, L15 and P6, L2: perhaps change "human strata 4, 5 and PAR1" to "PAR1 and human strata 4 and 5"

DONE

3 P5, L17: change "and agreeing with" to "in agreement with"

DONE

4 P5, L23 - P6, L1: the sentences here seem out of order. Perhaps start by saying why the fragmented assembly cannot identify the size of the PAR or autosomal to PAR translocations, and then state that an improved, de novo assembly based on long-read sequencing would allow you to confirm one of these alternatives.

DONE

5 P6, L3: "the location of the PAB is conserved" – conserved to what? The PAB is different in grey mouse lemur and humans. Either delete this phrase or be more specific.

DONE

6 P6, L5: change "only available one" to "only one available"

DONE

7 P6, L12: change "PAR1 and strata 4 and 5 in humans"

DONE

8 P6, L13: Interestingly, in Figure S2, the location of the PAB seems to differ between lorises and lemurs, but I assume this is simply due to differences in the X assembly?

Correct. Figure 1A (lemurs) and Figure S1 (lorises) clearly show that the PAB in lorises and lemurs is the same. It corresponds to the boundary between stratum 4 and 3 of humans. This is now explained in Figure S2 legend. Main text was also slightly changed.

9 P6, L14: change "no recombination suppressing event" to "no suppression of recombination between the X and the Y"

DONE

10 P6, L16 - P7, L15: here, I suggest changing the order of presentation; currently it is very confused. First, I would present the density of SNPs to show that you did not

miss any strata. Simply move this paragraph up. Then, I would have a separate paragraph describing the rates of strata formation, with the conclusion that the rate is higher in haplorrhines. Finally, you can end with a paragraph describing the analyses of sexual dimorphism and say that the higher levels of sexual dimorphism in haplorrhines is consistent with sexual antagonism driving higher rates of strata formation in this group. For me, this is a nice logical presentation that builds the results to a clear statement of your major conclusions at the end.

DONE

11 Discussion, P7, L21: delete “and this is validated by a statistical test”. Also, in this paragraph, the biggest caveat of all these analyses is that it is a comparative study, and correlation does not equal causation! Even if you had a large phylogenetic study, this remains a caveat.

DONE

12 P8, L6-9: the evidence for expansion of loss of recombination on the guppy Y chromosome in high predation populations is not supported by the work of the Charlesworth group, and I think this needs to be acknowledged here (e.g. Bergero et al. 2019 PNAS).

We do cite the major findings of Bergero (2019 paper) but are hesitant to go into further details about the discordance between their results and Wright et al. 2017. First, Wright et al 2019 showed that Bergero et al's failure to replicate the loss of recombination expansion was in fact due to lack of statistical power. Please see Wright et al. (2019 PNAS) for specifics. Second, since this paper was submitted to PCI a new study has replicated the results of Wright et al. 2017 using linked-read data and correcting for a large inversion in the reference genome (Almedia et al 2020 bioRxiv). The pattern of suppressed Y recombination in the high predation populations now appears to be more pronounced than previously thought. We have updated the sentence to reflect this.

In guppies, while the Y chromosome exhibits low levels of divergence from the X (Wright et al. 2017, Bergero et al. 2019, Darolti et al. 2019), populations exhibiting stronger sexual dimorphism seem to have a larger non-recombining region (Wright et al. 2017, Wright et al. 2019, Almedia et al 2020).

Divergence and Remarkable Diversity of the Y Chromosome in Guppies
Pedro Almeida, Benjamin A. Sandkam, Jake Morris, Iulia Darolti, Felix Breden, Judith E. Mank
bioRxiv 2020.07.13.200196; doi: https://doi.org/10.1101/2020.07.13.200196

Wright AE, Darolti I, Bloch NI, Oostra V, Sandkam B, Buechel SD, Kolm N, Breden F, Vicoso B and Mank JE (2019) On the power to detect rare recombination events. Proceedings of the National Academy of Sciences, USA. 116:12607-12608.

13 P8, L13: please define “a process of attrition” and change “DNA repeats

accumulation" to "accumulation of DNA repeats".

DONE (attrition means reduction in size).

14 P8, L14-15: this is a challenging sentence to read. Perhaps change to "In Microbotryum violaceum, strata are found on the mating type chromosomes despite the fact that this species only has mating types and not sexes, such that sexual antagonism is absent".

DONE

15 P8, L22-23: What is the evidence that there is more suppression of recombination in red-bellied lemur than in the other species? I do not see this in Figure 1 or the supplementary figures?

The sentence is "The red-bellied lemur showed no more evidence for recombination suppression than the other species studied here." It thus says the contrary to what the referee has read. The sentence was slightly changed to make it clearer.

16 P9, L24-25: Perhaps cite a nice recent paper in Science that identifies the genetic basis of sexual dichromatism in canaries (Gazda et al Science 12 June 2020)

DONE

17 In Figure 1 (as well as supplementary figures), can you add the common names of the species (used in the manuscript) to the scientific names (used in the figures) so that the reader can easily find the appropriate panel without needing to look at Table S1.

The scientific names are already present in the main text (Material and Methods). We added them also in the Introduction.

18 P12, L5-6: How can a major allele frequency be 0.3x the site coverage? For example, if the site coverage is 10, then the major allele frequency would be 3. This does not make sense. Also, these analyses do not seem very robust, as the older strata are also not identified these analyses. Most of the SNP densities across the X chromosome seem to fall within the autosomal range?

Thanks for spotting this typo. We changed major by minor.