Rebuttal

Our replies are in blue

Editor:
Dear Rylan Shearn and Gabriel Marais
Your manuscript was reviewed by two referees whose comments are attached. Both referees found your study very interesting, remarking that it contributes nicely to sex chromosome research and should be of broad interest to evolutionary biologists. The role of selection in recombination suppression is a timely subject and this study brings new and interesting data using sex chromosome strata formation, and contrasting the known structure of sex chromosomes in haplorrhine primates to their understudied sister group, the Strepsirrhines. However, based on those reviews and my own reading, I think that your manuscript requires a few improvements to lead to a recommendation. Both reviewers find the link between the lack of strata 4 and 5 in Strepsirrhines and the lower level of sexually antagonistic selection acting in this group still tenuous, and would have expected a quantitative evaluation of sexual dimorphism, or a discussion of alternative mechanisms. After all, the two groups may vary in many ways which could affect strata formation. Therefore, it seems essential to improve the argument for or against isolating SA selection as the factor causing differences in chromosome structure. Referee 2 would like more details on the use of male/female SNP density of 0.5 to identify regions with suppressed recombination, and the paper would benefit from a critical discussion of the factors affecting this metric and determining the expected values under recombination suppression. Finally, following Referee 1, providing further details on how the PAB and the segments corresponding to strata 4 and 5 in Humans vary among Strepsirrhines would also improve the manuscript.
I would therefore invite you to submit a revised version taking those suggestions into account, and explaining in details how you have dealt with the points raised by the reviewers. Thank you for sending this interesting work to PCI Evol Biol. I look forward to seeing the revision.
Best regards,
Mathieu Joron

Dear Editor,

First, I would like to apologize for such a long delay in replying to the referees' comments and yours and in submitting a revised version. Several unforeseen events both professional and personal have greatly retarded the work on this manuscript. I have sent news to the PCI chef editors.

Also, while revising the manuscript, we found an error in one of the analysis (the M/F SNP density analysis) and had to re-do it. Since Rylan Shearn is no longer in academia and has very little time for this research, I contacted Alison Wright who is an expert of this kind of analysis and she agreed to do it for us. She duplicated the M/F coverage analysis (and confirmed it) and performed the M/F SNP density one. She is now co-author of this study.

We thank the recommender for the positive feedback and suggestions, and the referees for their comments. We have addressed all their comments. Please find our replies below.

The revised manuscript (both main text and supplementary material) has been deposited on Biorxiv:
MS ID#: BIORXIV/2018/445072

We hope this revised manuscript will be suitable for a PCI Evol Biol recommendation.

Best regards,

Gabriel Marais
On behalf of the co-authors

Referee 1:

Shearn et al conduct a comparative analysis of sex chromosome evolution across two primate groups to test the relationship between sexual dimorphism, a proxy for sexual conflict, and recombination suppression. This is a hot topic at the moment. They find that there is a higher rate of strata formation in haplorrhine primates than strepsirrhines, a direction consistent with the role of sexual conflict in halting recombination. I enjoyed reading the manuscript and think it makes a valuable contribution to sex chromosome research. My comments are below:

Can the authors comment about when sexual dimorphism evolved in haplorrhines? This essential to interpret the results as the argument that sexual conflict promoted the evolution of S4 and S5 only holds if the common ancestor of haplorrhines was sexually dimorphic.

Unfortunately, there is no equivalent of the Petty and Drea study in haplorhines. However, it is well known that sexual dimorphism is very visible in the majority of the haplorhines (Plavcan 2001, 2004, Kappeler & van Schaik 2004). This suggests that pronounced sexual dimorphism and sexually antagonistic selection are probably ancestral in this group. Sexual dimorphism of extent primate species suggests that sexual dimorphism has evolved early in haplorhines, before the split between new world and old world monkeys. Sexual dimorphism became then stronger in old world monkeys and apes (Martin et al. 1994). Such a scenario is supported by the fossil record (Plavcan 2001). Body mass might have increased in both sexes in the early evolution of haplorhines and then sexual dimorphism has evolved consequently (Lindenfors 2002). In haplorhines, male-male competition seems to be the major force underlying the evolution of body mass and canine size dimorphism (Plavcan 2004). There is some evidence that sexual conflicts (and potentially sexually antagonistic selection) are strong in old world monkeys and apes compared to new world monkeys (Zinner et al. 2004). It would be too long to present all the literature about sexual dimorphism in haplorhines, but we added in the main text a sentence saying that current view is consistent with sexual dimorphism being ancestral and even having evolved within haplorhines, although this has not been tested formally. See p 4.

Kappeler, P. M., & van Schaik, C. P. (2004). Sexual selection in primates: review and selective preview. Sexual selection in primates: new and comparative perspectives. Cambridge University Press, Cambridge, 3-23.

Lindenfors, P. (2002). Sexually antagonistic selection on primate size. Journal of Evolutionary Biology, 15(4), 595-607.

Martin, R. D., Willner, L. A., & Dettling, A. (1994). The evolution of sexual size dimorphism in primates. The differences between the sexes, 159-200.

Plavcan, J. M. (2001). Sexual dimorphism in primate evolution. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists, 116(S33), 25-53.

Plavcan, J. M. (2004). 13• Sexual selection, measures of sexual selection, and sexual

dimorphism in primates. Sexual selection in primates, 230.

Zinner, D. P., Nunn, C. L., van Schaik, C. P., & Kappeler, P. M. (2004). Sexual selection and exaggerated sexual swellings of female primates. Sexual selection in primates: New and comparative perspectives, 71-89.

The discussion of alternative processes promoting recombination suppression is a bit light. Eg. Úbeda F, Patten MM, Wild G (2015) On the origin of sex chromosomes from meiotic drive. Proc Biol Sci 282:20141932 Matsumoto T, Yoshida K, Kitano J (2017) Contribution of gene flow to the evolution of recombination suppression in sex chromosomes. J Theor Biol 431:25–31

These papers discuss interesting ideas. However, Ubeda et al. proposes a new model based for the origin of sex chromosomes by meiotic drive and is relevant in the context of sex chromosome turn over, not strata formation. We nevertheless found a theoretical paper by Scott and Otto (2017) suggesting meiotic drive could trigger recombination suppression in sex chromosomes. Matsumoto et al. investigates the importance of spatial selection and gene flow in neo-sex chromosome evolution using simulations. Whether gene flow favours not only Y-autosome fusions but also Y inversions remains to be studied.

To explain our results with these models, a systematic difference between haplorhines and strepsirhines in terms of meiotic drivers and/or gene flow should exist. Meiotic drive and population structure should be much stronger in haplorhines than strepsirhines. We could not find any paper on meiotic drive in lemurs. Literature on lemur population genetics indicates that population structure is present and is sometimes strong but the situation can vary greatly from one species to another (see for example Quemere et al. 2010, Perry et al. 2012, Aleixo-Pais et al. 2019). Moreover, to our knowledge there is no available quantification of genetic diversity or population structure being stronger / weaker in strepsirhines than haplorhines now nor during primate evolution (see for example Pecon-Slattery 2014, Lawler 2018, two review papers on primate population genetics and phylogenetics).

We thus felt very difficult to develop on these very speculative ideas and just added a small paragraph saying that other factors than SA differing between strepsirhines and haplorhines could of course explain our results. See page 7.

Aleixo-Pais, I., Salmona, J., Sgarlata, G. M., Rakotonanahary, A., Sousa, A. P., Parreira, B., ... & Minhós, T. (2019). The genetic structure of a mouse lemur living in a fragmented habitat in Northern Madagascar. Conservation Genetics, 20(2), 229-243.
Dec;207(4):1631-1649.

Lawler, R. R. (2018). Emerging and Enduring Issues in Primate Conservation Genetics. Annual Review of Anthropology, 47, 395-415.

Pecon-Slattery, J. (2014). Recent advances in primate phylogenomics. Annu. Rev. Anim. Biosci., 2(1), 41-63.

Perry, G. H., Melsted, P., Marioni, J. C., Wang, Y., Bainer, R., Pickrell, J. K., ... & Pritchard, J. K. (2012). Comparative RNA sequencing reveals substantial genetic variation in endangered primates. Genome research, 22(4), 602-610.

Quemere, E., Crouau-Roy, B., Rabarivola, C., Louis Jr, E. E., & Chikhi, L. (2010). Landscape genetics of an endangered lemur (Propithecus tattersalli) within its entire fragmented range. Molecular Ecology, 19(8), 1606-1621.

Scott MF, Otto SP. Haploid Selection Favors Suppressed Recombination Between Sex Chromosomes Despite Causing Biased Sex Ratios. Genetics. 2017

I don't quite follow the use of SNP density to identify recent strata. Why is the sum of SNPs divided by four? Do you really expect 0.5 for the old strata? I am bit perplexed by the results in the SI. I agree that in regions where the Y has largely degenerated, we might expect SNP density to be lower in males as the X is effectively hemizygous in males. But this will depend on the level of standing genetic variation in the population, and whether females are homozygous for the X. I don't understand how all of the X has a SNP density ratio of 0.5? I would expect the ratio to be much closer to 1.

After double-checking the M/F SNP density analysis, we found an error in the script that we wrote for this analysis. We contacted Alison Wright, an expert of this kind of analysis and she agreed to do the analysis for us. Consequently, Alison is now co-author of this work. The methodology that she followed is presented in the Method section (p 11). The new figure (Figure S3) is presented in the Supplementary material. Importantly, the conclusions remain unchanged. Alison also added some references, updated some parts of the text citing new research that was published since the submission of our manuscript and double-checked the English of the manuscript.

For the methods further detail is necessary on:
a. Whether any trimming steps on the reads was taken
b. What thresholds were imposed on SNP calling ie minor allele frequency

See reply to previous comment.

The colour scheme of Fig 1 need to be improved for clarity.
a. Firstly, mPAR needs to be defined in the figure legend, I assume mPAR refers to the ancestral PAR (what does the m mean)? At the moment it is red, which is the same colour as s5. Therefore, it looks like s5 is at the beginning of the X for the 7 species studied when I believe the authors are trying to show this is ancestral PAR.
b. Similarly, misA needs to be defined, I assume it means misassembled autosomal scaffolds? But currently it is grey which makes these regions look like PAR.

We have changed figures 1 and S1 and legends accordingly.

Abstract L13 'Moved' seems a bit unspecific in this sentence, I would replace with extended PAR.

We changed this sentence in the abstract.

P3 L12 I'm not aware of any direct evidence that recombination between the primate X and Y stopped instantaneously as 'at once' suggests. I would remove this.

Done.

P3 L14 I am not sure what process is referred to in this sentence.

An inversion on the Y and not on the X will be always heterozygous in males. Such situation is well known to suppress recombination, and inversions are considered important recombination modifiers (suppressors in fact). There is evidence that such inversions occurred on the human sex chromosomes in the literature that is cited (see for example

Lemaitre et al. 2009 for strata 4 and 5).
Once recombination is suppressed. The X and the corresponding inverted-Y regions evolve independently and diverge. Different inversions will create different regions with different level of X-Y divergence, reflecting the time when recombination stopped. This in humans is very clear (the term “evolutionary strata” was even proposed in a human paper, see Lahn & Page 1999).
We added the definition of evolutionary strata in the text to be clearer. See page 3.

P7 L12 I believe Wright et al showed extension of recombination suppression not additional strata

Our sentence was imprecise. What we meant is the strata are indeed larger in some populations. This was changed.

P7 L17 What does ‘process of erosion’ refer to?

This term used by Van Laere et al. is attrition erasure. We changed the sentence.

P8 L24 Typo – should be in not on

Done.

P9 L6 Typo – should be cell

Done.

P9 L10 Typo – should be samples

Done.

P9 L14 Typo – should be on not onto

Done.

P10 L4 Should be gap after bracket

Done.

P10 L6 Remove ;

Done.

P10 L15 Remove)

Done.

Fig 1 Legend Missing of after Identification

It is strange. We see the full Figure legend in the pdf available in Biorxiv.

Fig 1 Legend What does the sentence starting ‘Strata in humans using definition of strata shown …’ mean? Why does coverage ratio approach 1.5 in some species?

Strata number and boundaries are from the Page lab. Some authors think the old strata 1, 2 and 3 according to Page are composed of more strata (see Pandey et al. 2013). We changed this to be clearer in Figure 1, S1 and S2.
As always when sequencing genomes especially in re-seq analysis, coverage varies along the chromosomes and also between individuals. Our normalisation procedure (using coverage in one autosome, see Methods) improves things but does not solve completely the problem. In some cases, the observed ratios are a bit higher or a bit lower than the expected values. This has also been observed in other papers that have used this approach.

Referee 2 (Qi Zhou):
Sex chromosomes show a dynamic composition even between related species regarding their regions with or without recombination. The sex-linked regions with recombination is called pseudoautosomal regions (PAR), and this work by Rylan et al. found that strepsirrhines (lemurs and lorises) share a longer PAR than other primates (apes and monkeys), and they explained this pattern by suggesting a lower level of sexual antagonistic selection in the former than the latter. It is a great effort to characterize the PAR of all seven species, and the topic should be of broad interest to evolutionary biologists. However, I hope the authors provide more quantitative measurements of the level of sexual antagonistic (SA) selection ongoing in the strepsirrhines. Because after all, the experimental evidence for SA selection causing the recombination loss is quite rare. Guppy is an excellent example because the previous comparison by Alison et al was performed between two closely related populations with a very clear indication of sexual selection (the color), thus the association between the expanded recombination suppression region vs. different color (different degree of SA selection) can be interpreted as the suggestive evidence. The author talked briefly in the introduction about sexual size dimorphism, and how exactly was the degree of strepsirrhines compared to that of haplorrhines? How about the mating-system in these two groups of haplorrhines? I only know the mating system is associated with the relative testis size of primates, which is indicative of the strength of sexual selection.

There is currently no global proxy encompassing all aspects of sexual dimorphism across primates (e.g. males and females can differ in body size, morphological traits such as teeth size, color and physiology, see Plavcan (2004) for a full discussion). Thanks to a colleague in Ecology Jean-François Lemaître, who is now co-author of this manuscript, we collected relevant data as sex-differences in canine height, testes mass, sex specific body mass and social system for all the species in our dataset (see new supplementary table S2).
All statistical analyses were conducted with the R statistical software (R Core Team, 2019). Sexual dimorphism based on body mass (SSD, size-based sexual dimorphism) or on canine length (CSD, canine height based sexual dimorphism) was quantified as the logarithm of the ratio of the male to the female values (for instance, SSD = ln(male body mass/female body mass), Plavcan, 2004). The relative testes mass (RTM) was computed as the residual of the linear regression ln(*combinedtestesmass*)~ln(*malebodymass*).

In a first approach, the phylogenetic architecture underlying the data was ignored and we simply compared the average dimorphism value between the two groups (haplorhines vs strepsirhines). In a second stage, we accounted for the underlying phylogenetic architecture using phylogenetic contrasts in a classical phylogenetic generalized least square analysis (see Symonds and Blomberg, 2014). Two evolutionary models were investigated: a simple Brownian motion (BM) and the Ornstein-Uhlenbeck model (OU) that includes stabilizing selection. The results based on the latter (OU) model should however be considered cautiously as this analysis is certainly over-parameterized considering the very small sample size (between n=11 and n=13 species). Analyses accounting for phylogenetic architecture in

the data used the following specialized R packages:adephylo (Jombart and Dray, 2010), ape (Paradis and Schliep, 2018), geiger (Harmon et al., 2008) and phytools (Revell, 2012). Sexual dimorphism based on body mass (SSD, mean ± standard error) was 0.378±0.097 in haplorhines and 0.062±0.017 in strepsirhines. This difference based on n=13 observations was statistically significant only when ignoring phylogenetic inertia (p=0.043) but no longer significant when considering phylogenetic inertia with a Brownian motion model (p=0.66). Analysis involving an OU model would lead to a significant difference between the two groups (p=0.043) but this analysis may either be over-parameterized or suffer from the lack of phylogenetic signal in our data as revealed by the low Pagel's $\lambda<0.001$ (not significantly different from 0) estimated in the Brownian motion model. In such a case, non phylogenetically-corrected analyses should be reported (Freckleton, 2009).

Sexual dimorphism based on canine height (CSD) showed the same kind of pattern: the mean is 0.385±0.076 in haplorhines and 0.045±0.016 in strepsirhines. This difference based on n=12 observations is only significant when ignoring the underlying phylogeny (p=0.013) but no longer significant (p=0.39) when phylogeny is accounted for with a Brownian motion model (leading to a non different from 0 estimate of Pagel's λ). The OU model leads to a significant difference between groups (p=0.013).

Based on our n=11 observations, the average relative testes mass did not significantly differ between haplorhines (0.18±0.24) and strepsirhines (–0.21±0.32). In order to avoid using residuals of a generalized least square model, we also compared testes mass in an analysis of covariance model (see Lemaître et al., 2009, for an example) including the male body mass as a covariate using the following statistical model in R: ln(combinedtestesmass)~ln(malebodymass)+group. The results were however qualitatively unchanged (the p-value associated with the "group" factor was p=0.4).

In conclusion, this analysis supports the idea that strepsirrhines and haplorrhines differ in sexual dimorphism, and validate the comparison of strata formation vs. sexual dimorphism between two groups shown in Text S2. This is presented p 6. However, with n = 13, it is not feasible to perform an analysis of the co-variation of sexual dimorphism variables with number of strata and controlling for phylogeny. This is discussed p 6.

Freckleton, R. P. 2009, The seven deadly sins of comparative analysis. Journal of evolutionary biology **22**:1367–1375.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008, Geiger: investigating evolutionary radiations. Bioinformatics **24**:129–131.

Jombart, T. and S. Dray. 2010, adephylo: exploratory analyses for the phylogenetic comparative method. Bioinformatics **26**:1907–1909.

Lemaître, J.-F., S. A. Ramm, R. A. Barton, and P. Stockley. 2009, Sperm competition and brain size evolution in mammals. Journal of evolutionary biology **22**:2215–2221.

Paradis, E. and K. Schliep. 2018, ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics **35**:526–528.

Plavcan, J. M. 2004, 13• sexual selection, measures of sexual selection, and sexual dimorphism in primates. Sexual selection in primates 230.

R Core Team. 2019, R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Revell, L. J. 2012, phytools: an r package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution **3**:217–223.

Symonds, M. R. and S. P. Blomberg. 2014, A primer on phylogenetic generalised least squares. *In* Modern phylogenetic comparative methods and their application in evolutionary biology, 105–130, Springer.

Second, I am not sure if the author has identified a scaffold that shows a switch between the M:F ratio of 1 vs. 0.5. The authors mentioned scaffolds that show a M:F ratio of 1 correspond to strata 4 & 5 in human, this only indicate there were no recombination loss after strata 3 formed in strepsirrhines. A process called attrition, which as mentioned by the authors is the gradual erosion of PAR by repetitive elements has been reported in cow, and in fact, many mammalian PABs are characterized by insertion of TEs (reviewed by Terje et al. 2015). Has the author found a similar pattern? Or are the PABs of the seven species actually aligned to each other? Finally, just curious, has the author managed to assemble any Y-linked genes, and are they any different from those that have been assembled for other mammals?

We changed erosion by attrition and now cite the review paper by Terje Raudsepp et al. We prepared a new figure zooming on the PABs of the different lemurs (new Figure S2). It seems that the PAB is quite conserved, at least between otolemur, nycticebus and galago, and between microcebus, daubentonia, Prolemur and Eulemur. We do not see clear evidence for attrition in those species.

But only looking at the sequences could really tell and we agree that it would be nice to study more precisely the PABs in lemurs. However, the Y reads from our re-seq data (PE 150 bp) are impossible to assemble. To do what the referee is suggesting, we would need to have a reference sequence for both the X and the Y chromosomes. For all the species, we have studied, we only have a X chromosome (derived from a female reference genome).

Raudsepp T, Chowdhary BP. The Eutherian Pseudoautosomal Region. Cytogenet Genome Res. 2015;147(2-3):81-94.

Some other minor comments:
introduction: line 1, 'strongly' maybe changed to 'very';

Done.

line 9, the master male-determining gene in Eutherian mammals, as platypus does not share the same sex chromosome and master sex determining gene;

We changed mammals to therian mammals (placentals + marsupials).

line 15, I suggest to emphasize here that all eutherian mammals share strata 1-3;

Done.

page 4, line 21, the same copy number of autosomes;

Done.

page 5, line 16, are there any read pairs that span the X-specific region and the autosome like region? If so, there should help to clarify if there are potential recent fusion or just

assembly errors;

Unfortunately, the boundaries between the X-specific regions and the autosome-like regions are made of repeats, not genes. It is thus easy to find read pairs that span them; we usually find several such pairs, which makes it difficult to conclude.

page 6, I am not sure about the N50 of gray mouse lemur, but there is also a blue-eyed black lemur genome published with about 400kb N50 size, would it be better to use that as reference?

The gray mouse lemur genome that we used has a N50 of 3.7 Mb for scaffolds (and N50 of 182 Kb for contigs), and thus probably of similar quality as that of the blue-eyed black lemur. Information about genome versions, which was missing is now included p 10.