Answer to editor and reviewers

Editor (Jos Käfer)

Thank you for your submission to PCI Evol Biol. I have received three reviews of your preprint, and all three reviewers agree this is a manuscript of good quality, well-written, and that it is addressing an interesting question. I would thus be delighted to recommend this manuscript on PCI Evol Biol once the comments of the reviewers have been taken into account.

In particular, all three reviewers suggest you include more empirical data on inversions and reversions, and provide some concrete suggestions on how to achieve this.

I also support the suggestion by reviewer 2 to amend the title, as the term "heteromorphic" is ambiguous, and usually applies to differences that can be observed by light microscopy. There indeed appears to be no universal relationship between the size of the non-recombining region and its age (see for example Renner & Müller, Nat. Plants 2021), so old non-recombining regions can be small enough not to be visible under a microscope.

We agree that we are not interested in morphological differences between sex chromosomes, but indeed on the evolution of 'old non-recombining regions'. We followed the useful suggestion to change the title made by reviewer 2.

Reviewer 1 suggests you move figure S1 to the main text. While I understand your choice to focus the main text on the critical evaluation of Jay et al.'s model, I do think that showing what additional processes might lead to long-term maintenance of recombination suppression, such as those modeled by yourself, would help clarifying the debate. To this end, I would suggest transforming lines 284-290 into a subsection of its own.

We followed the suggestion and moved the corresponding section that was given in the supplementary material to the main text, in replacement of the lines 284-290. We moved figure S1 to the main text and also replaced Fig 3 by Fig S3, which is almost identical (just also showing the results of the regulatory model). For simplicity, we also moved Fig S2 to the main text (now Fig 5) so that the appendix now only concerns nested and overlapping inversions.

I furthermore suggest you change title of the paragraph "Modelling sex chromosome evolution" into "Methods" and start the "Results" section with the paragraph that follows ("Very low rates of reversion...").

We followed the suggestion.

I would like you to consider changing "male fitness" into "fitness of the heterogametic sex" in the paragraph starting at line 249 ("The constraint scenario is more likely to lead to..."). There is a large literature on sex-specific fitness mostly related to fertilization success, offspring production etc., where fitness depends on the type of gametes (small: male; large:

female) the individual produces. The fitness effect you're describing occurs in the heterogametic sex, no matter what gametes it produces, and I'm afraid that referring to "male fitness" might be ambiguous for non-specialist readers.

We mentioned at the end of the introduction that we would consider the XX/XY system as an example but that everything would apply to ZZ/ZW species. Indeed, the argument applies to the heterogametic sex. We added a parenthesis to remind the reader about this in this section where, indeed it would be a major misunderstanding if a reader would read literally read 'male' instead of 'the heterogametic sex'.

The sentence at the beginning of this section now reads "When an inversion fixes and starts accumulating deleterious mutations, it depresses male fitness (again, we take the example of XX/XY species, but the argument applies to the heterogametic sex: in ZZ/ZW species, females would show this fitness reduction)."

I am confident that the suggestions made by the reviewers will allow you to improve the manuscript even further, and I'm looking forward to reading a revised version.

Thank you for the feedback and suggestions.

A few typos not mentioned by the reviewers and suggestions on the text:

- line 217 "a first inversions" -> a first inversion

- line 230 "For example, in the example illustrated on Fig 1B...": redundant formulation. Just "For example, in Fig 1B..." would do.

- line 331 "Fig S4 and S5 illustrates" -> illustrate

They were corrected, thank you for pointing them out.

Reviewer 1

A general pattern observed across species with sex chromosomes (XX/XY or ZW/ZZ sex determination) is that the sex-specific chromosome (Y or W) and the paired chromosome (X or Z) evolve to suppress recombination along their length, often via structural rearrangements. This is a key transition in the evolution of sex chromosomes, as this shutdown of recombination allows for (1) divergence between the sex chromosome pair and (2) the eventual accumulation of deleterious mutations and loss of gene content on the sex-specific chromosome ('degradation'). Understanding what causes the initial shutdown of recombination across the sex chromosomes, and what maintains this recombination shutdown, is therefore a key question in the field of sex chromosome evolution.

This paper provides a survey of multiple theories that explain the shutdown of recombination, but focuses on determining whether the recently proposed sheltering theory put forward in Jay et al. 2022 is a viable explanation of recombination. The initial step in the Jay et al. 2022 model involves the fixation of a 'less-loaded' or 'lucky' inversion: an inversion

that happens to capture a genetic region with fewer deleterious mutations than the average haplotype. The dynamics of such inversions on autosomes are well-investigated (Nei et al. 1967). If the inverted haplotype is sufficiently more fit than the corresponding haplotypes, it may fix in the population, which restores recombination in the region captured by the inversion (as homozygotes for the inversion may recombine freely). However, if such an inverted haplotype captures a sex-determining locus (e.g. a dominant male-determining factor, such as SRY in mammals), it remains permanently heterozygous even if it reaches fixation because the male-determining factor is never homozygous (i.e., YY individuals are never produced). The fixation of the inverted haplotype therefore induces a shutdown of recombination in inverted region between the X and Y chromosomes.

The idea that lucky inversions can fix on the Y and cause recombination suppression is not specific to Jay et al model. It was previously described in the Lenormand & Roze 2022 paper.

Jay et al. 2022 argue that if the 'less-loaded' inversion haplotype also carries recessive deleterious mutations, its presence in a permanently heterozygote form will be beneficial because these deleterious recessive mutations are sheltered from selection in a heterozygote state. As a result, more and more overlapping inversions of this type will accumulate along the Y chromosome, leading to a chromosome-wide shutdown of recombination.

While we understand that this is indeed what is explained in Jay et al paper, we believe that this description is not entirely accurate (especially the sheltering interpretation). Because it was not directly on the topic of 'constraint theory', we do not develop this point in this paper. Note that the Olito and Charlesworth preprint recently posted also casts doubt on the role of 'sheltering' in the dynamics of Y inversions. This point would be better cover in a separate paper.

However, Jay et al. 2022 assume that the possibility of a reversion of the initial inverted region, which restores recombination between the X and Y, is highly unlikely. This is contrast to a recent model presented in Lenormand and Roze 2022 (the authors of the present paper), which suggested the fixation of such an initial 'lucky' inversion is not sufficient to maintain recombination shutdown between the X and Y, because eventually the non-recombining region on the Y will accumulate enough deleterious mutations that a reversion of the inversion will appear and fix.

The focus of the current paper is to determine the rate at which reversions of the initial inverted haplotype must appear to support the Lenormand & Roze model vs. the Jay et al. model.

Not exactly, as figure S1, S2 and S3 were showing, the evolution of recombination suppression is also much easier in Lenormand & Roze model 2022 with low reversion rates. Low rates of recombination reestablishment are (necessarily) favorable to any theory aiming at explaining Y recombination suppression, not just Jay et al's model. Figure S3 (now Fig 3) for example, is showing the large gap in outcomes between models involving or not regulatory evolution (for the same rate of recombination restoration, whether they are low or not).

In addition, we also show that in the constraint theory, inversions can persist only if reversion rates are extremely low, which, in this model, leads to another problem, namely that the population goes extinct.

Hence, the question is not simply to determine the rate of reversion. We show that irrespectively of this rate, this theory is not sufficient.

In summary, the authors find that this rate of reversion must be very low relative to the rate of inversions in the population for the initial inversion not to be reverted (approximately 4 orders of magnitude lower than the rate of inversions). The authors argue that additional factors (such as the regulatory divergence on the X and Y proposed in their 2022 paper) are therefore required to maintain the presence of an inversion.

This is indeed the overall idea. However, we stress that we consider reversions in a broader sense than Jay et al 2022. Jay et al. specifically and explicitly considered reinversions of inversions. We considered a rate of recombination restoration, independent of the rate of occurrence of events suppressing recombination. In the discussion, we stress that there are other ways to restore recombination than just reinversion of inversion. For instance, moving the sex determining locus to another location (e.g. in the PAR or on an autosome) would completely and immediately restore recombination around the sex-determining locus. Note that this mechanism is still possible even after a series of nested and overlapping inversions, so that it is unlikely that recombination restoration becomes vanishingly small as inversions accumulate on the non-recombining region of the Y (which is a key feature of Jay et al. 2022 model).

Overall, I found this to be a straightforward paper that clarifies why these two recent and superficially similar models reached such different conclusions about the stability of inversions on the sex chromosomes. While the data on the rate of reversions of inversions is quite limited, this paper will presumably direct more attention to empirical work attempting to measure this parameter. I have some suggestions, given below, for how to strengthen the paper.

As just mentioned, the empirical question is not simply about rates of reinversions (even if we agree that better documenting these processes would be useful). Other processes can reestablish recombination, as we mentioned in the discussion. Following the suggestion, we now direct more attention in the discussion to recent empirical work documenting the occurrence of recurrent reinversions (notably Giner-Delgado et al. 2019 and Porubsky et al 2020, now mentioned line 358-359, 419-421).

--- I feel that the paper would be improved by a more detailed discussion of the molecular biology of inversions (and their potential subsequent reversions). Some of this is provided in lines 353-363, but more background on how & why inversions (and reversions) occur would be very helpful. This is particularly the case in terms of the data on reversions from Drosophila, where there is a proposed mechanism in lines 357-358; this mechanism would increase the probability that a reversion occurs with the same (or similar) breakpoints as the initial inversion, which is the point of contention between the two focal models.

We added more explanations in this paragraph and also explicitly mentioned that inversions can repeatedly occur on the same sites (as shown in Giner-Delgado et al. 2019 and Porubsky et al 2020).

--- A key component of the Jay et al. 2022 is the assumption that deleterious mutations are partially recessive, which allows these mutations to be 'sheltered' in their permanently heterozygous form. Here, the dominance of all deleterious mutations is also assumed to be partially recessive (fixed at 0.3). But it would be nice to see a bit more discussion of the implications of this assumption in the paper. For example, if mutations were (on average) even more recessive, would that slow the fitness decline on the inverted region of the Y and allow more time for another inversion to occur (thereby potentially making reversion less likely or slowing the male fitness decline leading to extinction)? Would this also be the case if there was an inverse relationship between h and s as predicted by theories of dominance (e.g. Kacser & Burns 1981, Manna et al. 2011)?

We use h = 0.25, which is the value typically observed for mildly deleterious mutations (as surveyed in Manna et al 2011).

It is true that the fitness decline would be slower for smaller h, but it would not change qualitatively the pattern we describe (ie. will not change orders of magnitude if h is e.g. 0.1). Things could be different with very strong recessivity, but we know that such values are not empirically supported. Note that with very strong recessivity, it would also be much harder to have 'lucky' inversions in the first place (the variance of the marginal fitnesses of newly arising inversions would be much smaller). Note also that Manna et al 2011 do not show that there is an inverse relationship between h and s for mildly deleterious mutation. This trend over all mutations is caused by the dominance of mutations of (very) large effect. In fact, the heterozygous effect of lethal and non-lethal mutations is about the same in both yeast and *Drosophila*, as discussed in Manna et al 2011. This is not a pattern predicted by the metabolic control theory inspired from the work of Kacser and Burns (see also Manna et al 2012 about the yeast deletion data on this question). This empirical fact indicates that the fitness decline (caused by the accumulation of deleterious mutations) would be necessarily similar, if large effect and very recessive mutations were included.

For the effect of basal dominance level on Lenormand and Roze 2022 model, see Fig S6 in the supplementary material of that paper. Recombination suppression and degeneration is faster for higher level of baseline dominance. Indeed, as one would expect, Y silencing would likely play no role if deleterious mutations were already recessive to begin with.

--- Lines 310-313: The authors suggest that the possibility of gene conversions and double-crossovers within the inversions will provide occasional recombination events. Would this then slow the accumulation of deleterious mutations on the Y chromosome? It seems that these occasional recombination events would perhaps stabilize the presence of the inversion on the Y, but simultaneously decrease the rate of degradation of the Y as well (e.g. as in lines 230-240). Further elaboration of the potential impacts of occasional recombination within the inversion on these processes would be helpful.

This is indeed correct: these double crossovers will limit degeneration. Therefore, inversions will tend to stay longer in the population if they were considered. Selection to restore

recombination only occurs when the marginal fitness of the Y inversion falls below the marginal fitness of the corresponding segment on the X. However, the question is to explain both the maintenance of recombination suppression and degeneration. When the marginal fitness of the Y inversion is higher than the marginal fitness of the corresponding segment on the X, there would be, by definition, no degeneration. We added a sentence to clarify the role of double crossovers (l373-374).

---Figure S1 – I would suggest moving this to the main text, if there is space; it summarizes the authors data about reversion rate well.

We followed the suggestion.

--- Lines 360-363: It would be useful to have citations for this point, as the fact recombination may be suppressed outside the inverted region can certainly make the accumulation of secondary inversions more likely and the possibility of reversion therefore more remote, in line with the Jay et al. 2022 model. E.g. from Drosophila melanogaster, this area of reduced recombination outside the inversion can range between 3-12 mb (Koury 2023)

We are repeating here a logical statement that has been mentioned many times in the literature (we added Charlesworth 2017 in addition to Charlesworth 2021 where this point is mentioned). It is relatively easy to accumulate rearrangement in a previously non-recombining region, as these rearrangements do not cause further recombination suppression. Hence, observing a secondary rearrangement cannot be used to infer that recombination suppression was caused by an accumulation of rearrangements. An equally likely possibility (from an empirical standpoint) is that the secondary rearrangement evolved because it was much easier to fix once recombination was suppressed.

Unless we misunderstand the comment, the empirical observation made by Koury (and others) is not directly relevant to our simulations : it is the size of non-recombining fragments that is directly drawn in our simulations. It is possible to think about these sizes as being the size of the inversion plus the size of the neighboring outside region that shows recombination suppression.

Thank you for the useful comments and suggestions.

Reviewer 2

I greatly enjoy reading this simulation study, aiming to address whether mechanistic constraints alone on recombination restoration can maintain long-term sex chromosome recombination suppression. Overall, I found the simulation results convincing within the reasonable rate of inversion and reversion they use in the study. There are a few points I wish the authors to consider for further clarification, perhaps a bit better integrate with current empirical evidence too.

1. Title requires slight modification. ‘Heteromorphic sex chromosomes’ *per se* do not necessarily imply long-term recombination suppression and degeneration of sex chromosomes, as the authors intend to show in this study. Because heteromorphic sex

chromosomes can be due to fusion of instant homomorphic sex chromosome and autosome, or expanded W or Y chromosomes, which are largely due to TE accumulation and remain rather early stage of sex chromosome evolution. I'd suggest avoiding this confusion, perhaps something like 'Can mechanistic constraint on recombination reestablishment explain the long-term maintenance and degeneration of sex chromosomes?', or something along this line.

Indeed, we were not thinking about this possible confusion and we entirely agree that changing the title along the line suggested would address this issue. Thank you very much for the suggestion.

2. I wonder whether it is a good idea to explicitly separate the theories in relation to drive initial recombination suppression between sex chromosomes and the further stepwise degeneration. Sex chromosome differentiation and degeneration is a continuous progress, the separation seems to suggest the two are distinct processes and require possible various selections or mechanisms to act upon.

a. I can see pros and cons in either argument. I remain open for either way, yet, the current theories listing in this study needs further clarification and integration for certain theories.

There are indeed pro and cons. As all the literature usually ignore the issue of long-term maintenance of recombination suppression, we believe, overall, that taking this angle is more stimulating and thought provoking for the reader.

b. Clarification: The theory #2 to explain initial recombination suppression suggests male-sterility and female-sterility locus combination acts as a selection force. I am not sure this is so different from all other sex determining locus system. The first step is to require a SD locus via mutation, but this alone does not suggest recombination suppression. Maybe I miss something here, please clarify.

We are not sure to see the issue mentioned here. We say that "recombination is selected against to prevent the production of neuter individuals", not that male-sterility and female-sterility locus combination acts as a selection force. The difference from other sex determining system is the fact that this case involves two loci. If recombination occurs between these two loci, neuter/intersex individuals can be produced which is unfavorable. In a system with just one sex-determining locus, this problem is necessarily absent.

c. Clarification: The theory #5 to explain initial recombination suppression suggests recombination suppression near the PAR region boundary due to neutral accumulation of sequence divergence between X and Y and suppression as a side effect. This is unclear. This assumes it has already evolved somewhat evolutionary strata and PAR region supposedly. Also, the example from *Arabidopsis thaliana* plant does not even has sex chromosomes is very confusing.

Indeed, this theory relies initially on the accumulation of sequence divergence near the sex-determining locus. When a small non recombining region is established, then this accumulation continues by slowly moving the PAR boundary. We changed the sentence to avoid a possible ambiguity. The example from A thaliana is not on sex chromosome, it is a

paper indicating that meiotic recombination is not inhibited by sequence divergence (contrary to the assumption made in this theory), as the sentence is reporting.

d. Better integration certain theories on each process (initial recombination suppression, and long-term heteromorphic sex chromosome degeneration). 1) Jeffries *et al*'s neutral arrest of recombination model was only mentioned during the maintenance of recombination suppression, but this model also explains the initial recombination suppression of sex chromosomes.

We did mention Jeffries et al neutral arrest for the initial recombination suppression (case #5).

2) The pre-existing non-recombination (theory #1) was only mentioned for initial sex chromosome recombination suppression, not discussed in the later phase. Also, this theory could largely extend to various exaggerated heterochiasmy systems which are more widespread, not only for the achiasmy systems. 3) Both SA selection and Jay *et al*'s sheltering of deleterious mutations explain both initial recombination suppression and further stepwise degeneration.

For the long-term maintenance of recombination suppression, we do mention the accumulation of SA loci (case #1), and the constraint on reversion (case #3). We are therefore unsure what the reviewer is referring to. We added in the constraint scenario that recombination suppression in one sex may be maintained for another reason to cover the possibility mentioned by achiasmy and strong heterochiasmy (line 140-144).

3. The main concern for this simulation model is how widespread and what rate the restoration of inversion on sex chromosomes is from empirical data. Indeed, the authors acknowledge that this empirical data is rare, and has used quite conservative values in the simulation. I would suggest incorporating more empirical data on inversion restoration from sex chromosome turnover studies, which occurs in many fish, reptile and amphibian lineages, as well as sex reversal restoring recombination between sex chromosomes in amphibians, fish etc. such as Nicolas Perrin's Fountain of Youth theory and empirical studies he demonstrated. These more wide-spread events might better support the restoration of sex chromosome recombination leads to young/non-degenerated sex chromosomes in these lineages. In mammals and most birds, no such turnover occurs to restore recombination between sex chromosomes, they indeed progressive degenerate and evolve DC mechanism. I think the authors mention this here and there, perhaps can rephrase and integrate these in a better way.

This is indeed what we say in the paragraph in the discussion about change of location of SD loci (now l400-413). We already cited models of turnover made by Perrin et al (former refs 37, 38), studies mentioning change of location of the SD locus (former refs 65-67), a paper about recombination restoration by sex reversal (former ref 36), and a review about turnover (former ref 71).
To follow the suggestion, we added three references to Perrin's lab : (1) the Rodrigues et al 2018 paper about empirical sex reversal and recombination restoration. (2) The original

Perrin 2009 paper about the fountain of youth. (3) The Beukeboom and Perrin book surveying cases of turnovers.

4. One another thing I have a bit concern is the evolution of DC as a sole selection pressure to maintain and drive long-term sex chromosome degermation. If this is specifically listed, so far, as the only to-be-tested workable theory to explain the long-term sex chromosome degeneration, (because the standard DC evolve to counteract the gene loss and balance the copy number variation between sexe) do we explicitly should predict all degenerated sex chromosome would evolve DC mechanisms (local or global)? How does this look like for UV system, mate-type chromosomes?

Indeed, a prediction of this theory is that long-term sex chromosome degeneration is associated to global or local DC. We emphasized better this point as suggested (line 465-467).
Regarding UV or mating-type chromosomes, our preliminary results indicate that very similar conclusions should hold, but as we mention in the perspective, a specific study should be done to cover this case in details.

5. The term 'knock-out' is a bit misleading in this context, better switch to gene loss for a widely accepted term in this field.

Done.

Thank you for these helpful comments and suggestions.

Reviewer 3

Summary The authors present a review of models relating to the long term maintenance of recombination suppression on sex chromosomes, and complement this with simulations exploring the 'constraint hypothesis' whereby recombination remains restricted due to a low rate of reversion. This paper is very clearly written, and provides a compelling argument for the limitations of the constraint scenario alone to drive long-term stability of recombination suppression on sex chromosomes. They also nicely summarise recent competing models by clearly delineating the possible short term causes of initial recombination arrest, and three main proposed mechanisms for the long-term maintenance of that initial arrest. Simulation results show that even rare reversions can be sufficient to remove non-recombining sex chromosomes from the population, given strong selection against Y chromosomes that accumulate a greater deleterious load. While the argument is in general clearly laid out and compelling, we have minor comments the authors should consider addressing. General comments.

1) A key goal of this paper is to evaluate what factors can drive the long-term maintenance of recombination suppression on the sex chromosomes despite Y degeneration. Furthermore, a major result of the simulations is that the constraint scenario is unlikely to be the explanation for the long-term suppression of recombination. However, it would be worth clarifying the timescales required to be considered 'long term'. Clearly mammalian sex chromosomes are ancient, but there is growing evidence that this may be the exception. Given that the simulation results show inversions that can persist for a million generations

(and this clearly depends on the exact parameters that haven't been fully explored) the constraint scenario actually presents a quite interesting possible scenario that might well be consistent with what is observed in many taxa. In particular, many sex-linked regions may have relatively young origins, with heterogeneity across the chromosome in the timing of recombination suppression that might reflect variation in the accumulation of genetic load/the distribution of fitness effects, differences in rates and/or the precision of reversion, and other factors. In other words, a flip side to the authors' results is that this transient recombination suppression they show in their simulations might be a very important source for the recurrent turnover of sex linkage and degeneration seen in a growing number of taxa. This point would be useful to spell out more clearly- i.e. the mechanisms described in Jay et al could drive recurrent gains/losses of sex-linked regions over evolutionary time, a phenomenon that may in fact be quite common.

The key point is whether degenerate strata are kept for long. It is relatively easy to maintain fixed inversion in the population, provided their marginal fitness is not substantially reduced compared to the equivalent fragment on the X chromosomes. Take for instance the example shown on Fig 1A. Indeed, the inversion remains in the population for quite some time (more than half a million generations), but it does not accumulate a strong load (see the orange curve for sY in the panel 'degeneration'; note that this variable reaches 0.3 when genes are degenerate on the stratum).

From a theoretical stand point, what we mean by long term is a stable situation, not a situation that is maintained only because the suitable mutation has not yet occurred. In the simulation with regulators, recombination suppression is maintained indefinitely. In the 'constraint' simulation, in contrast, it is always a question of waiting time (see Fig 2A). We changed the text to highlight this (l218, 327-330)

We agree however, that empirically speaking, it is very difficult to distinguish the two situations. At least as a 'thought experiment', the key difference is that strata have a higher or lower marginal fitness compared to the equivalent segment on the X in the two theories. It may become feasible to experimentally switch these portions of chromosome and actually investigate the fitness effect of this switch in males and females. Our prediction is that switching would reduce male fitness (whether it also reduce female fitness is less critical to prevent recombination restoration). If, on the contrary the experiment reveals that male fitness is improved by switching, then one could conclude that it is maintained by a form of constraint. In principle, these are clear cut predictions that can falsify each of these theories, provided some degeneration has taken place (i.e. the switch does change fitness). This is the relevant test. We added a mention of this idea line l433-440.

Otherwise, the description made in this comment is already what we describe starting line 380. "[In the absence of regulatory evolution], the accumulation of deleterious alleles caused by recombination arrest should eventually lead to population extinction or to the re-establishment of recombination (via reinversion(s) or sex chromosome turnover), rather than the long-term maintenance of degenerate Y or W chromosomes." We added the words in square brackets for clarity in the revision.

We propose in this paper that the key factor explaining whether a species is in a regime of turnover or of stable degenerate sex chromosome is whether regulatory evolution / DC can easily evolve, not whether recombination restoration is constrained. This is our main conclusion (last sentence of the paper). This is very different from what Jay et al say (they suggest that constraints on reinversions can explain the evolution of stable and degenerate sex chromosomes).

2) Further empirical support for the authors' arguments about the plausibility of reversion of inversions can be found in the recent literature. For example, Giner-Delgado et al. 2019 (doi: 10.1038/s41467-019-12173-x.) and Prubsky et al 2020 (https://doi.org/10.1038/s41588-020-0646-x) provide evidence for inversion hotspots whereby the same inversion polymorphisms have arisen multiple times independently, suggesting the rate of back-mutation may be higher than often assumed. This is worth adding in the discussion (around line 353).

Thanks a lot for pointing out these references. We (incidentally) have been in contact with authors of the Giner-Delgado et al 2019 paper, since we submitted this paper, and we were planning to add a mention of these results (especially those shown on their sup fig 3), which are indeed highly relevant (added line 358-359, 419-421). We also added a mention to the Porubsky et al 2020 paper, as suggested. We already cited Porubsky et al 2022 (a related paper).

3) A recent preprint from Olito and Charlesworth (https://doi.org/10.1101/2023.11.27.568803) suggests that the conclusion from Jay et al that Y-linked inversions have a fixation advantage needs to be reconsidered, given the approach used to estimating fixation probabilities. Although the focus here is on the long-term maintenance of recombination suppression, this issue is probably worth mentioning in a revised version.

We read this preprint with interest. We agree that several results in the Jay et al paper need to be reconsidered. Regarding our main point, this is not critical, we believe, as we are confident that Y lucky inversions can indeed fix, as we already showed before Jay et al, in our 2022 paper (see appendix 1 in that paper). However, we agree that the detailed interpretation, the quantitative result and the interpretation probably requires a careful re-evaluation. This is not the topic of this paper, focused on the constraint hypothesis, and we believe these questions would be better addressed in separate papers.

Minor comments 44: Typo. "shot" -> short

Corrected.

Thank you for these helpful comments.