

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.
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.
  - 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.
  - 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.
  - 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. 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.
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.

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?
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.