Sheltering of deleterious mutations explains the stepwise extension of recombination suppression on sex chromosomes and other supergenes :

Details of the questions addressed, main arguments and breadth of the parameter values explored in simulations in Jay et al (2022)

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Preamble

In the first version of the preprint [1], published on bioRxiv in November 2023, several questions about the methodology that we used in our article [2] were raised. The present document is intended to answer the expressed concerns by explaining in more details why, in our Figure 3C [2], our approach was valid in setting aside :

(1) simulations in which the inversion does not survive the first 20 generations;
(2) simulations corresponding to the case « s=0 » (i.e., all mutations are neutral).

These criticism (1) actually only comes from the criticism (2), as we explain below. The misunderstanding of these aspects of our approach led some of our colleagues to question the generality of our conclusions. Therefore, in the last part of this document, we go through the text of our article to emphasize again which precise question we posed, to show that it is different from the question posed in [1], and to highlight the wide range of parameters under which the sheltering effect has a significant impact in our model.

Several months after the publication of [1] on bioRxiv, most of the content of [1] was published as part of a larger review on recent models of the *sheltering* hypothesis for recombination arrest between sex chromosomes [3]. This review offers a softer and more comprehensive discussion of the assumptions made and conclusions drawn from different models incorporating a sheltering effect. The claim that a comparison with a situation without any deleterious mutations (s=0) is necessary to evaluate the sheltering effect still remains in this new version, and constitutes the main argument for criticizing the two points above. The rationale behind this claim is the following : if fixation probabilities of inversions on the Y chromosome are not higher in the *presence* of deleterious mutations segregating in genomes than in the *absence* of segregating deleterious mutations, we can conclude that the sheltering effect does not provide any *advantage* compared to a simple neutral dynamics and is therefore is unlikely to explain the fixation of inversions on sex chromosomes. At first sight, this claim may seem right and in line with classical approaches in population genetics. However, as we argue below and in Box 3 of our review article [4], we respectfully disagree with the *logic* of this claim, as we detail below: the case s=0 tells you about the fixation probabilities of an inversion in the total absence of deleterious mutations. However, in most species deleterious mutations *do* segregate in genomes. Even if, in a hypothetical similar population free of deleterious mutations, inversion fixation would be higher, it does not imply that deleterious mutations do not contribute to inversion fixations in real natural populations in which deleterious mutations segregate, as we show below. The presence of deleterious mutations indeed changes the whole adaptive lanscape, and their sheltering is not a classic selective advantage as is typically compared to a neutral case in population genetics. **Our question is : *given that* deleterious recessive mutations segregate in genomes, can they explain inversion fixations on sex chromosomes in nature ? The latter challenge is the one we address in our paper.**

Insights on Figure 3C in our article [2]

The rationale for focusing on the simulations in which the inversions survived the first generations.

The criticism on our Figure 3C in [2] was that it was misleading when used to compare the absolute inversion fixation probabilities on Y chromosomes compared to the case s=0. However, this figure was not intended for such a comparison, so that the innuendo of being misleading was unfair. Our Figure 3C was instead intented to illustrate the comparison of inversion fixation probabilities between Y chromosomes and autosomes when the sheltering mechanism acts, i.e. when inversions become frequent (i.e., after 20 generations). In that, the way of plotting the Figure was fully valid and was explained in the legend. Actually, for our comparison between sex chromosomes and autosomes, including the first 20 generations does not change anything to the conclusion, as seen in Figure 2 in [1] when noticing that the different Y scales. The comparison with the case s=0 is irrelevant for the sheltering effect, as we show in the second part below.

More precisely, our model proposes two phases, as explained in more details in [4]. In the first phase, an inversion less-loaded than the population average, occurring on a single genome at first, manages to increase in frequency because it has fewer deleterious mutations than average in the population. Of course, when fluctuations due to genetic drift are taken into account, few of such inversions will succeed in this (short) phase, as in the classical Wright-Fisher with directional selection framework. **The second phase is when the sheltering effect starts acting:** when an inversion is successful, *i.e.*, it has managed to rise in frequency (which we translated into « has managed to survive 20 generations », passing from one copy to whichever strictly positive number of copies it has in generation 20), it now faces the hurdle that its full mutational load will be expressed if it appears in a homozygous state. **Figure 3C is meant to illustrate the property that the inversions having reached the second phase now have a higher probability of becoming fixed in the population when they are linked to a permanently heterozygous allele (like the Y allele) compared to the case when they are *not* linked to a permanently heterozygous allele (like autosomal alleles). This is the sheltering effect.** The first phase of our model was illustrated and analysed in other figures in [2].

By contrast, the computations carried out in [1] mostly concern the first phase, during which the contradictory effects of the « lower-load » positive directional selection and of the stochastic fluctuations due to genetic drift oppose each other and lead either to a very quick extinction of the inversion, or to its increase in frequency. The calculations performed in [1] make use of the classical weak selection regime where the selection coefficient is inversely proportional to population size N, and therefore lead to probabilities of « fixation » (that is, of an initial increase in frequency during our first phase, when drift has the strongest effect) of order $1/N$ in both the autosomal and Y chromosome scenarios, which is also of comparable order with the « neutral » case. This is akin to standard population genetics results on the effect of weak selection. In Figure 3 (A,B,C) in [2], our point is that, even though the probabilities of increasing in frequency during this first phase are of similar magnitude in the sex-chromosome and the autosome cases, the sheltering effect then starts acting and prevents nearly all the initially successful inversions from fixing when they are on autosomes, while it protects a substantial fraction of the Y-linked inversions surviving the first phase from expressing their full mutation load during the second phase, leading to their eventual fixation in the subpopulation of Y chromosomes (note that Figures 3A and 3B do not have restrictions on the minimal number of generations that the inversions should survive). In a forthcoming paper, we show that considering all inversions (including those going extinct in the first generations) does not change our conclusions : for a large majority of parameter sets, fixation probabilities are larger on Y chromosomes than on autosomes because of the sheltering effect. One should be able to read this result from Figure 2 in [1] (by comparing the pairs of curves with identical colors in the top and bottom rows), but the authors

used a scale ten times larger to represent fixation probabilities on Y chromosomes (bottom row) than the one used for fixation probabilities on autosomes (top row), and this renders the reading of actual fixation probabilities on Y-chromosomes very difficult.

The reason why comparing our results to the « s=0 » scenario brings nothing to the understanding of the efficiency of the sheltering effect.

As a reminder, the question we address is the following : « Given that deleterious recessive mutations segregate in genomes (a property which is widely accepted), can the sheltering of these mutations due to their association with a permanently heterozygous allele contribute to explain the observation that non-recombining regions can extend on sex chromosomes ? »

The sheltering effect is not a classical directional selective force giving an advantage to some allele (s>0) compared to the situation where all alleles would not have this advantage (s=0). It is a protection against the expression of the full mutational load of a set of recessive mutations when they are linked to a permanently heterozygous allele. To answer the question whether, given the presence of segregating deleterious mutations in genomes, being linked to a permanently heterozygous allele has a positive effect on the fixation of an inversion requires to compare what happens when the inversion *is* linked to a permanently heterozygous alleles to what happens when it *is not* linked to such an allele. This is why we compared autosomes to a Y-like chromosome. Removing the deleterious character of the mutations deeply alters the whole fitness distribution (and thus the « genomic environment ») in which these inversions evolve and answers another, distinct question : does being linked to a permanently heterozygous allele have positive effect on the fixation of an inversion when there are deleterious mutations in genomes compared to a situation where there are none. Again, the sheltering effect only *offsets* the disadvantage of deleterious mutations when they become frequent, so comparing to a case without any deleterious mutations cannot inform on its contribution to inversion fixation on Y chromosomes in natural populations. We show in [2] that the presence of deleterious mutations in genomes does impair inversion fixation on autosomes, but that the sheltering effect protects inversions on Y-like chromosomes from this disadvantage, thereby effectively contributing to inversion fixation on Y-like chromosomes. Removing deleterious mutations removes the sheltering effect, but also the disadvantage it protects from; therefore, it is not a valid comparison to analyse whether the sheltering effect contributes to explaining the evolution of sex chromosomes in natural populations in which deleterious mutations segregate. **The question is not, as stated in [1] and [3], whether deleterious mutations provide an advantage to inversions compared to a case where there are no deleterious mutations. Instead, our question is whether, given that deleterious mutations segregate and incur a disadvantage to inversions rising in frequency, the sheltering effect can promote their fixation.** Similarly, the lower load advantage arises from the fitness landscape changed by the presence of deleterious mutations. And here too, the question is not whether deleterious mutations provide an advantage to inversions compared to a case where there are no deleterious mutations. The question is instead whether, given the fitness landscape with deleterious mutations, which provides a lower disadvantage to the less loaded inversions compared to inversions with a higher load, this lower load can contribute to the fixation of inversions in natural populations.

It could be argued that a neutral scenario (i.e. without any deleterious mutations) is a good control for the effect of drift. However, it is not in this particular case, as it also removed the disadvantage against which the sheltering effect protects, as explained above. Nevertheless, it is indeed relevant to test whether drift alone could explain the effects we analyse, but we need the right control for this. Genetic

drift accounts for the fluctuations due to the stochasticity in reproduction, and its magnitude is commensurate with (effective) population size. Therefore, an appropriate control for the effect of drift in the Y chromosome population is to compare with the fate of an inversion in an autosomal population with the same effective population size as the Y chromosome population, so that the fluctuations in allele frequencies in the Y-linked and in the autosomal cases have the same variance. In a forthcoming paper, we show that for a large set of parameter values in our model, drift alone cannot explain the higher fixation probabilities of non-recombining regions on the Y chromosome compared fixation probabilities on autosomes, highlighting the impact of the sheltering effect.

Note that, as advertised in [1], in the course of the project we had run simulations for the case « s=0 », before realising that they were not the good control for the sheltering effect. These simulations were included in the supplementary data associated with our article simply because we forgot to remove them. Because they were useless for our analysis, we have not discussed them in the text, and neither have we discussed what would happen in another scenario where all mutations would be beneficial. These were not the questions we were addressing. Now, following the principles of reproducible research, any reader interested in the fate of inversions in a purely neutral scenario can access these simulations and analyse them as they want.

The question is whether the lower load advantage and sheltering effect contribute to the fixation of a few lucky inversions on Y-like chromosomes

In [1] and [3], there were additional misunderstandings on the questions we addressed in [2]. Indeed, our question in [2] was whether the evolutionary strata observed in nature on sex chromosomes can be explained by a combination of lower load and sheltering effect. Evolutionary strata, resulting from successive steps of recombination suppression, are rare in natural populations. For instance, only five of them appeared on the mammalian Y chromosome across 180 millions years. Explaining this pattern only requires a few lucky inversions fixing on a Y chromosome, and the question is whether the lower load and sheltering effect do contribute to the fixation of these few lucky inversions (not if such inversions can frequently fix, or whether they would fix more or less often without any deleterious mutations segregating). Yet, [1] and [3] reasoned in terms of average genomic backgrounds and parameter ranges. Even if we deemed valid the comparison with the s=0 case (although we insist that it is *not* valid), the conclusions in [1] and [3] would still be incorrect. There are indeed combinations of parameter values for which the simulations with deleterious mutations lead to higher frequencies of inversion fixation than under s=0, and this is sufficient to explain a few lucky inversions across millions of years that would carry mutations with these combinations of parameter values. Note also that the analysis carried out in [1] and [3] used values for the dominance parameter which are the less conducive for our theory.

Details of the questions addressed, main arguments and breadth of the parameter values explored in simulations in Jay et al (2022)

In this part, we go through the text of the article [2] to emphasize again the main elements of our reasoning and methodology, as well as to emphasize the large range of parameter values explored in our analysis.

<u>Abstract</u>

(1) *We show here, by mathematical modeling and stochastic simulation, that recombination suppression on sex chromosomes and around supergenes can expand under a wide range of parameter values simply because it shelters recessive deleterious mutations, which are ubiquitous in genomes*

⇨ As stated in this sentence, we find that the fixation probability of an inversion is strictly positive under a wide range of parameter values. We do not claim that it expands more easily than when s=0. What we say is that, in the *presence* of deleterious recessive mutations (a common feature of genomes), the sheltering hypothesis works for a wide set of parameter values for the selection strength, dominance coefficient, mutation rate and inversion sizes. In biological populations, large inversions are much more often loaded with deleterious recessive mutations than neutral, which is why we focus on the scenario where deleterious mutations are present (see also excerpt (6) below). Maybe the case s=0 leads to similar fixation rates for some parameter sets in our model, but it does not mean that the sheltering effect does not play a role in the fixation of inversions on Y chromosomes in nature.

Introduction

(2) *We use mathematical modeling and stochastic simulations to test the hypothesis that permanently heterozygous alleles, such as male-determining alleles in XY systems, protect linked chromosomal inversions against the expression of their recessive mutation load, potentially leading to an accumulation of inversions around permanently heterozygous alleles, generating evolutionary strata.*

⇨ Same here, nothing is compared to the case s=0. What we claim is that, under the assumption that deleterious recessive mutations are present in genomes, being linked to a permanently heterozygous gene can facilitate the fixation of an inversion compared to *not* being linked to a permanently homozygous gene.

(3) *Inversions capturing fewer deleterious variants than the population average for the region concerned have a fitness advantage and should, therefore, increase in frequency. Such inversions are advantageous due to associative overdominance, i.e., the inversion itself is neutral but it captures a combination of alleles that is advantageous when heterozygous [28,29]. However, as the frequency of an inversion increases, homozygotes for this inversion become more common. Homozygotes are at a strong disadvantage due to the recessive deleterious variants carried by these inversions, and selection against homozygotes would therefore be expected to prevent such inversions from reaching high frequencies (Fig 1B). Now, consider an inversion that, by chance, captures a permanently heterozygous allele, such as the male-determining allele in an XY system. If this Y-linked inversion captures fewer deleterious variants than the population average, it should increase in frequency without ever suffering the deleterious consequences of having its load expressed. The recessive deleterious mutations captured by the sex-linked inversion are, indeed, fully associated with the permanently heterozygous, male-determining allele, and will, therefore, never occur as homozygotes. Unlike autosomal inversions, Y-linked inversions retain their fitness advantage with increasing frequency (Fig 1C). Hence, Y-linked inversions with a lower load than average would be expected to spread, becoming fixed in the population of Y chromosomes, resulting in a suppression of recombination between the X and Y chromosomes in the region covered by the inversion.*

⇨ The « Phase 1 » and « Phase 2 » described in the introduction of this document are not explicitly mentioned in the main text of the article, as we came up with this terminology later, but the two phases are distinctly explained in this paragraph early in the introduction and in Figure 1. Fig 1A depicts Phase 1 (by chance the inversion carries less mutations than average on this portion of genome, and therefore has a chance to increase in frequency) and Fig 1B/C depict Phase 2 and the difference between autosomes and sex chromosomes during this phase.

(4) *The accumulation of deleterious mutations following recombination suppression has been extensively studied [30–33], but we investigate here its converse: that deleterious mutations could be a cause, and not only a consequence, of recombination suppression.*

⇨ Here again, we insist on the idea that the presence of deleterious mutations segregating in genomes, through the heterogeneous fitness distribution they generate, could explain why non-recombination regions extend by strata.

Results

(5) *Each of these recombination modifiers, which was assumed to be neutral in itself, appeared in a single haplotype, and was, thus, in linkage disequilibrium with a specific set of mutations, such that its fitness was exclusively dependent on the number of deleterious alleles within the segment captured. We first compared the dynamics of inversion-mimicking mutations in an autosome with those capturing a male-determining allele in an XY system (males are XY and females are XX, the male-determining allele being permanently heterozygous), and we then considered other types of recombination modifiers and heterozygosity rules.*

⇨ In line with what we had announced in the introduction, we compare the fate of a recombination modifier (=an inversion) in linkage disequilibrium with (=containing) a random number of deleterious recessive mutations when it appears on an autosome to its fate when it appears tightly linked to (=also capturing) a permanently heterozygous allele such as the male-determining Y allele.

Inversions less loaded than average are frequent in genomes

(6) *Under realistic parameter values, the vast majority of large chromosomal regions therefore carry several deleterious mutations. For example, considering s = 0.001, h = 0.1, μ = 10−9, and n = 2 Mb, more than 99.999% of chromosomal fragments carry at least 1 mutation, the mean number of mutations being nq = 20.*

⇨ We argue here about the reason why we place ourselves in the scenario where deleterious mutations segregate in the population within the genomic region of interest.

(7) *Therefore, a substantial fraction of inversions occurring in genomes are beneficial when they form (i.e., when rare enough not to occur as homozygotes). For example, with h values ranging from 0 to 0.5 and s values ranging from 0.001 to 0.25, between 36% and 98% (mean = 70%) of the 2-Mb inversions occurring in the genome are beneficial, carrying fewer recessive deleterious mutations than average (Fig 2B). Simulations in finite populations of different sizes confirmed that most inversions (mean = 66% in the range of parameter values studied) had a fitness advantage upon formation. The simulations also showed that inversions could be favored if they captured mutations that were rarer than average (S2 and S3 Figs).*

⇨ The values of h that we consider in Fig 2B (infinite populations) range from 0 to 0.25 and the values of s range from 0+ to 0.1 (here, 0+ means that we consider arbitrarily small but strictly positive values for s; the case s=0 is excluded as our calculations with selection are not appropriate in this case). In Figures S2 and S3 (finite populations), we consider all inversions (not restricted to those surviving at least 20 generations) and we consider values of h ranging from 0.001 to 0.1, and values of s ranging from 0.01 to 0.1. Figure S2 does show that some inversions survive longer than 1000 generations when they appear on autosomes (we do not speak about fixation here), but long-lasting inversions are less numerous on autosomes than on sex chromosomes (Fig S3, with the same range of parameter values). The difference between Y chromosomes and autosomes is all the more visible as s decreases and as h decreases.

Less-loaded inversions are much more likely to fix when they capture a Y-like male determining locus

(8) *Based on this model, and initially assuming that inverted and noninverted segments no longer accumulate deleterious mutations after their formation, i.e., WII, WNI, and WNN are fixed parameters (this strong assumption is relaxed latter), we simulated the evolutionary trajectory of inversions on autosomes and of inversions capturing the male-determining allele on the Y chromosome under a wide range of parameter values. We found that the frequency of less-loaded inversions tended to remain low in autosomes, whereas these inversions became fixed in the population of Y chromosomes (Fig 2C), as expected according to our hypothesis.*

⇨ The expressions that we compute in this section (in infinite populations) are valid for all possible positive values for h and s. In the corresponding (first) part of the Methods section, we insist on the fact that we do not work with the classical approximation(s) for the probability of a given site to be mutated, but instead we use the full expression for this probability in order to cover all cases as rigorously as possible. What we obtain is that, in this model for infinite populations, inversions can fix on autosomes only if they carry less than a certain threshold of deleterious mutations, and the value of this threshold is computed. It is lower than the threshold for such an inversion to fix on Y chromosomes, which is also computed :

(9) *Thus, contrary to the argument proposed in a previous study that only mutation-free inversions can become fixed [35], we found that inversions can carry deleterious mutations and nevertheless become fixed in the population, provided that they carry fewer than qhn/(1−h) mutations if they are located on autosomes and fewer than nq mutations if they capture a permanently heterozygous allele (on the Y chromosome, for example). […] With a realistic range of parameters, inversions are much more likely to become fixed if they capture the male-determining allele on the Y chromosome than if they are unlinked to this allele (e.g., on an autosome; Figs 2C and 2D and S4 to S9). For example, with μ = 10−9, h = 0.1, s = 0.001, and n = 2 Mb, 47% of inversions occurring on the Y chromosome would be expected to become fixed, versus only 0.000045% of inversions on autosomes.*

⇨ The selection coefficient in these simulations ranges from 0+ to 0.5 and the dominance coefficient ranges from 0 to 0.5, for two mutation rates (mu=10^{-8} and 10^{-9}).

Drift and mutation accumulation do not prevent Y-linked inversion fixation

(10) *In finite populations, low-frequency or permanently heterozygous inversions tend to evolve under a Muller's ratchet-like dynamic, with the mean fitness of inversions decreasing in a stepwise manner due to the sequential loss, by drift, of the inverted haplotypes with the lowest mutational load. Following their formation, autosomal and Y-linked inversions tend to accumulate more mutations than the population average, contrasting with predictions for infinite populations (S13 Fig). Only inverted segments reaching relatively high frequencies in autosomes eventually recombine when homozygous, and their dynamics of mutation*

accumulation therefore involve a mixture of a Muller's ratchet-like regime (when rare, at the start of their spread) and a mutation-selection-drift regime with recombination (when they reach intermediate frequencies). Little is known about the transition between these regimes [40,41]. We therefore used individual-based simulations to study the fate of inversions accumulating deleterious mutations.

⇨ Indeed, we could have explained more clearly that Figure 3C was only illustrating the impact of mutation accumulation in the second regime (or phase) described in this paragraph. Note however that Figures 3A and B do not have the restriction of inversions having to survive at least 20 generations (as not surviving 20 generations means that the inversion frequency quickly falls to 0). These panels clearly show that, for the parameter set used there (N=1000, mu=10^{-8}, h=0.1 and s=0.01), over 10,000 independent simulations, no inversions fixed on autosomes (they all go to extinction in less than 2.000 generations without reaching high frequency), and 49 of the 10,000 inversions fixed on Y chromosomes.

(11)Over most of the parameter space, inversions are much more likely to spread if they capture the sex-determining allele on the Y chromosome than if they are located on autosomes (Figs 3 and S10 to S16).

⇨ Figures 3A and B represent the full fate of 10,000 inversions for a set of parameters (N=1000, mu=10^{-8}, h=0.1 and s=0.01), and they are complemented by Figures S10-S14, exploring different combinations of parameters, with h being equal to 0.001, 0.01 and 0.1, and s being equal to 0.001, 0.01 and 0.1 (N=1000 as in Figure 3). In Figure S10, we see that, when s=0.001 (very weak selection), some inversions *fix* on Y chromosomes (that, is, the inversion reaches frequency 0.25 in the whole population of males and females) and some inversions can *remain at nonzero frequencies* without fixing on autosomes (fixation happening when the inversion frequency reaches 1) or on X chromosomes (fixation happening when the inversion frequency reaches 0.75 in the whole population). Fixation of an inversion happens on autosomes *when h=0.01 and s=0.1* (and for only 2 out of 10.000 realisations, compared to 123 out of 10,000 realisations in which the inversion fixes on the Y chromosome), and *when h=0.1 and s=0.1* (the less favourable scenario for our theory) 4 out of 10,000 inversions fix on autosomes, and 7 out of 10,000 inversions fix on Y chromosomes. For all other sets of parameter values, some inversions fix on Y chromosomes and no inversions fix on autosomes *(out of 10,000 independent simulations)***.** Other variations of parameter values are explored in subsequent supplementary figures (N=10,000, in particular), in which inversions are shown to be helped by being linked to the Y allele in all cases (even when they eventually go extinct in all simulations, they can reach higher frequencies before going extinct).

(12) Many autosomal inversions carrying a mutation load segregated for hundreds of generations. For example, with N = 1,000, s = 0.01, h = 0.1, and μ = 10^{-8}, 73 of 10,000 inversions of 2 Mb in length continued to segregate after 500 generations. However, all these autosomal inversions were lost at the end of the simulations (i.e., after 10,000 generations, Fig 3A).

⇨ Based on this paragraph, we agree that the reader may wonder whether the number of inversions surviving the first 20 generations may be much higher on autosomes than on Y chromosomes, as we do not discuss the number of inversions surviving at least 20 generations on Y chromosomes. This information is nevertheless available in Figure S10 for a relatively large range of parameter values. Additionally, observe first that in the main text, we do not discuss proportions or probabilities of inversions going to fixation, we *compare total counts* of inversions going to fixation or not (as in panels A and B of Figure 3). Therefore, even if the quick reader could have misunderstood Figure 3C as representing absolute fixation probabilities starting from 1 copy of the inversion, the paragraph explaining these results does not infer the advantage of being linked to the Y allele in terms of fixation probabilities based on Figure 3C :

(13)By contrast, substantial fractions of less-loaded inversions capturing the permanently heterozygous sex-determining allele on the Y chromosome spread until they became fixed in

the Y chromosome population; this was the case even for inversions that were not mutation-free (Figs 3 and S10 to S16). For example, for s = 0.01, h = 0.1, μ = 10^{-8}, and N = 1,000, ***49 of the 10,000 Y linked inversions*** *(2 Mb) became fixed in the Y chromosome population, whereas* ***all inversions on the autosome were lost****. New mutations occurred on Y-linked inversions, but they did not accumulate rapidly enough to prevent these 49 inversions from spreading and reaching fixation (Figs 3A and 3B and S13).*

⇨ Observe that if the number of inversions surviving 20 generations was really higher on autosomes than on Y chromosomes, the global result of Figure 3 (A,B,C) would be even more striking as none of these numerous autosomal inversions would survive in the long term, whereas a certain number of the « few » inversions surviving 20 generations on a Y chromosome would eventually fix, demonstrating a large advantage of Y-linked inversions over autosomal inversions. Conversely, Fig 3A shows that all fixation events on Y chromosomes take much longer than 20 generations to occur, and are therefore not due to a strong effect of drift allowing inversions to fix on Y chromosomes more easily than on autosomes during the very first generations.

(14)As shown above, we found that neutral recombination modifiers spread in a very large range of conditions if they captured permanently heterozygous alleles (Figs 3C, S15 and S16). As expected, inversions were more easily fixed on the Y chromosome when the deleterious mutations segregating in the genome were more recessive (Figs 3C, S15 and S16). […] The probability of Y-linked inversion fixation thus increases with increasing inversion size, mutation recessiveness, and mutation rate (Figs 3C, S15, S16 and S19).

⇨ The combination of trajectories of inversion frequencies (Figs. 3A and S10-14) and of conditional fixation probabilities (Figs. 3C, S15-S16) leads to the conclusion we claim : inversions loaded with deleterious mutations can fix more easily when they occur on Y chromosomes than on autosomes. However, it would have been better to also cite Fig 3 as a whole as well as the supplementary figures showing trajectories of inversion frequencies (Figs. S10-14) in this paragraph when discussing the result that inversions were more easily fixed on Y-chromosomes. This conclusion of the higher rate of fixation of inversion due to the sheltering effect is robust when exploring « other systems with permanently heterozygous alleles, other recombination modifiers, and sex chromosome-autosome fusion », hence the claim that this result is very general. This assertion about the generality of our results is supported by the wide range of parameter values and variations of the model considered in the supplementary figures depicting stochastic (finite population) simulations that we recall here :

- **S10 :** Described in (11) above (3 orders of magnitude for h and s, N=1000, mu=10^{-8})
- **S11 :** Similar to Fig 3A and 3B but with *N* = 10,000, s=0.01, h=0.1, $\mu = 10^{-8}$
- **S12 :** Similar to S11 Fig but with *s* = 0.001
- **S14 :** Similar to Fig 3A but with a locus with 2 permanently heterozygous alleles (instead of one in the case of the XY system) and with other recombination suppressors than chromosomal inversions. N=1000, h=0.1, 0.01, 0.001, s=0.01, 0.05, 0.1
- **S15 :** Similar to Fig 3 but considering different sizes of inversions. N=1000, s=0.001, 0.01, 0.1, 0.25, 0.5, inversion size= 0.5Mb, 1Mb, 2Mb, 5Mb. Note that « similar to Fig 3 » indicates that the same procedure as for Fig 3C, including the 20 generations rule, applies here.
- **S16 :** Similar to Figs 3 and S15 but with *N* = 10,000 and for different sizes of inversions. Same range of inversion sizes and selection coefficients as in S15, and same comment about the repetition of the same procedure.
- **S17 :** Similar to Figs 3 and S15 but considering that mutations segregating in the genome have their fitness effects drawn from a gamma distribution with a shape of 0.2, and their dominance coefficient *h* randomly sampled among 0, 0.001, 0.01, 0.1, 0.25, 0.5 with uniform probabilities. Simulations were run considering that the mean

of the selection coefficient values (mean of the gamma distribution) was either 0.001, 0.01, 0.1, or 0.5. Mutation rates mu= 10^{-9}, 10^{-8}.

- **S18 :** Similar to Fig 3A but considering the fusion-mimicking mutations instead of inversions. Simulations were performed with N = 1,000, $\mu = 5\times10^{-8}$, and $s = -0.01$, h=0.01, 0.1, 0.25. […] Only fusions not lost after 20 generations are displayed. (This point was recalled here because the procedure in the case of chromosome fusion had to be adapted from the procedure used to derive Fig 3, and so we gave the full details of this adaptation in the caption.)
- **S19** : Fraction of Y-linked inversions spreading or fixed after 10,000 generations depending on the mean number of mutations segregating in the region where they appear. N=1000, h=0.001, 0.01, 0.1, 0.5, s=0.001, 0.01, 0.1, inversion size=0.5Mb, 1Mb, 2Mb, 5Mb.

<u>Evolution of nonrecombining sex chromosomes with evolutionary strata despite possible reversions</u>

(15)We studied the formation of such strata by the sequential occurrence of multiple inversions by simulating the evolution of large chromosomes experiencing the occurrence of multiple chromosomal inversions that can overlap with each other, under parameter values typical of those observed in mammals (Figs 4, 5 and S24). […] The dominance coefficient of each mutation was chosen uniformly at random from a wide set of values (see Methods for details).

*(16)*In Methods : *We simulated randomly mating populations of N = 1,000 and N = 10,000 individuals. Point mutations appeared at a rate of $\mu = 10^{-9}$ per bp, and their individual selection coefficients were determined by sampling a gamma distribution with a mean of −0.03 and with a shape of 0.2; these parameter values were set according to observations in humans [22,81]. For each new mutation, a dominance coefficient was chosen from the following values, considered to have uniform probabilities: 0, 0.001, 0.01, 0.1, 0.25, 0.5.*

⇨ As can be read in these paragraphs, we also allowed parameter values to stochastically vary in the course of each simulation, showing that our conclusions are robust to variations in the selective and dominance coefficient of the mutations occuring in genomes.

<u>Discussion</u>

(17)Our results show that recombination suppression on sex chromosomes and other supergenes can evolve simply because genomes harbor many partially recessive, deleterious variants. Our model for the evolution of sex chromosomes, and supergenes in general, is based on simple and widespread phenomena.

⇨ This first sentence exactly matches the content of excerpt (1) and (2) above, taken from the abstract and introduction of the paper.

(18)On autosomes, inversions maintained at low frequencies because of their homozygote disadvantage tend to be lost rapidly because they accumulate further deleterious mutations. On the Y chromosome, contrary to previous suggestions [28], we show that the accumulation of further deleterious mutations following the formation of an inversion is generally too slow to prevent the fixation of less-loaded inversions.

⇨ This assertion is proved by all figures showing trajectories of inversion frequencies (like Fig. 3A), even when we put aside the (coherent) results shown by the figures displaying the fractions of inversions surviving 20 generations that manage to reach fixation (like Fig. 3C).

The rest of the discussion gives suggestions to test the predictions of our theory in real populations, which have been largely extended in our review paper [4].

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