Can mechanistic constraints on recombination reestablishment explain the long-term maintenance of degenerate sex chromosomes?

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Abstract

Y and W chromosomes often stop recombining and degenerate. Most work on recombination suppression has focused on the mechanisms favoring recombination arrest in the short term. Yet, the long-term maintenance of recombination suppression is critical to evolving degenerate sex chromosomes. This long-term maintenance has been little investigated. In the long term, recombination suppression may be maintained for selective reasons (e.g., involving the emergence of nascent dosage compensation), or due to mechanistic constraints preventing the reestablishment of recombination, for instance when complex chromosomal rearrangements evolve on the Y. In this paper, we investigate these 'constraint' theories. We show that they face a series of theoretical difficulties: they are not robust to extremely low rates of recombination restoration; they would rather cause population extinction than Y degeneration; they are less efficient at producing a non-recombining and degenerate Y than scenarios adding a selective pressure against recombination, whatever the rate of recombination restoration. Finally, whether such very high constraints exist is questionable. Very low rates of recombination reestablishment are sufficient to prevent Y degeneration, given the large fitness advantage to recover a non-degenerate Y or W for the

heterogametic sex. The assumption of a lack of genetic variation to restore recombination seems also implausible given known mechanisms to restore a recombining pair of sex chromosomes.

Introduction

How could the degeneration of whole Y or W chromosomes evolve without being opposed by natural selection? Several theories have been put forward to explain this problem, often separating it into several subproblems: Why does recombination initially stop between sex chromosomes? Why does degeneration occur on a nonrecombining part of a genome? Why is recombination suppression maintained in the long term, once degeneration has already caused substantial degradation of the Y or W? Why isn't recombination eventually reestablished to limit maladaptation? When considering the suppression of recombination, it is useful to distinguish between the initial arrest and its long-term maintenance. We first briefly mention the different ideas that have been proposed for the initial arrest, which has attracted the most attention. We then present the different mechanisms that could lead to the long-term maintenance of recombination suppression, which has been much less investigated. We discuss the XY case throughout the paper, but all arguments apply to ZW systems as well.

The initial recombination arrest on sex chromosomes

Six main ideas have been proposed to explain short-term recombination arrest on sex chromosomes. Even if we focus on the long-term maintenance of recombination suppression, it is useful to list these ideas to clarify the difference between the short and long-term suppression of recombination.

The first proposes that the sex determination (SD) locus happened to arise in a region of the genome where recombination was already suppressed [1,2] or in a non-recombining genome. In achiasmate species, in particular, the heterogametic sex does not recombine (the so-called Haldane-Huxley rule [3,4]), so that, wherever the SD locus is located, the Y or W will not recombine. The direction of causality between the evolution of suppressed recombination on sex chromosomes and the evolution of achiasmy is however difficult to establish [4].

The second idea proposes that recombination is selected against to prevent the production of neuter individuals in species where sex is determined by a combination of a male-sterility and a female-sterility locus [5,6]. This explanation applies to species with such specific combinations of SD loci and is likely to apply to the evolution of separate sexes from dioecy or genetic sex determination from environmental sex determination. However, it cannot account for later events of recombination suppression beyond the region of the genome containing the SD loci.

The third idea is the "sexually antagonistic selection" scenario [6–12], where suppression of recombination is selectively favored due to the occurrence of loci with sexually antagonistic (SA) effects on sex chromosomes. In this case, a non-recombining Y benefits from a selective advantage, by permanently combining male-determining and male-beneficial alleles. A variant of this scenario involves sex differences in the intensity of selection (but not in the direction of selection as considered

in SA theory) against deleterious mutations [13]. There is plenty of evidence for SA variation [14,15], making this hypothesis very plausible. However little evidence for this scenario has been obtained despite intensive research [1,10,16], partly due to the difficulty of definitively establishing the role of SA variation in driving recombination suppression.

The fourth scenario involves ”lucky” inversions on the Y capturing the SD locus [17–19]. Inversions capture at birth a sample of segregating deleterious variation. Some “lucky” inversions can have a selective advantage because they initially capture a portion of the proto-Y that carries fewer or milder deleterious mutations, compared to the population average. Their initial fitness advantage decays through time but can be sufficient to allow them to fix [17,18]. Much of this decay is caused by a deterministic return to the population average load [18,20], but selective interference also contributes. This decay rate is larger for larger inversions [17,18], but inversions with a large initial advantage are likely to be sampled among larger inversions [17]. These opposing effects determine the distribution of fixed inversion sizes, with a mode biased toward smaller sizes compared to their input size distribution [17,18]. Contrary to the fixation of ‘lucky’ inversions on autosomes [20], the fixation of inversions capturing the SD locus on the proto-Y is expected to cause recombination suppression on the inverted segment of the Y [16,21]. This process can involve any Y variant suppressing recombination around the SD locus and closely linked to this region, not only inversions (we use the term inversion only for simplifying the presentation). For instance, it could involve changes in chromatin structure or the loss of recombination hotspots. It solely depends on the presence of deleterious mutations and variation in recombination rates and is thus expected to be widespread. However, due to its recent description, it has not been investigated empirically.

The fifth mechanism involves recombination suppression near the sex-determining locus due to the neutral accumulation of sequence divergence that decreases homology between the X and Y and suppresses recombination as a side effect [22]. When a small non-recombining region is established, then this accumulation continues by slowly moving the boundary of the pseudo-autosomal region. The idea is that strict homology is required for recombination to occur. However, while this effect has been documented in mitotic lineages [23–25, and other references cited in 22], it is not clear whether small amounts of divergence prevent recombination during meiosis. In particular, data from tetraploid rye and from crosses among strains of *Arabidopsis thaliana* with varying levels of divergence suggest that heterozygosity may enhance rather than inhibit crossovers [26–28].

The last mechanism proposes that XY recombination suppression is favored because it increases heterozygosity around the SD locus in the heterogametic sex. Recent versions of this idea proposed that tight linkage between the SD locus and overdominant mutations, or combinations of recessive deleterious mutations (generating pseudo-overdominance) would be favored because these

99 mutations would then be more often present in the heterozygous state. In partially inbred populations,
100 linkage to the SD locus indeed increases heterozygosity, and this can favor recombination suppression
101 in the presence of overdominant mutations [29], while more work is needed for the case of recessive
102 deleterious mutations and random mating [18].

103 The long-term maintenance of recombination arrest on sex chromosomes

104 It has been long established that in the absence of recombination, deleterious mutations will tend to
105 accumulate on the Y due to selective interference [11,12,30–35], which should generate some
106 maladaptation, especially in males. Why then is recombination not reestablished when degeneration
107 becomes too strong? Three main ideas can be distinguished.

108 The first is that SA effects are sufficiently strong to maintain recombination arrest despite the
109 accumulation of deleterious mutations. This has nevertheless been questioned [36–38]. Indeed, the
110 deleterious effect of Y degeneration may eventually offset the selective advantage of linking male-
111 determining and male-beneficial alleles. The restoration of recombination, if it is possible, may then
112 become favorable. However, new SA mutations may continue to appear and accumulate on sex
113 chromosomes, giving time for the population to evolve Y silencing / dosage compensation (DC) limiting
114 maladaptation caused by Y degeneration.

115 The second idea is based on regulatory evolution. Once recombination is suppressed, cis regulators of
116 gene expression may diverge between the X and Y, for genes located in the non-recombining portion
117 of the Y. This regulatory instability may lead to the evolution of early DC (through the joint evolution
118 of cis and trans-acting factors), concomitant with Y early silencing and degeneration. In the model
119 proposed by [17], the emergence of this DC builds up pervasive SA regulatory effects, selectively
120 preventing the long-term reestablishment of recombination. Note that regulatory evolution may lead
121 to the accumulation of deleterious mutations and degeneration even in conditions where selective
122 interference is inoperative [39].

123 The third possibility, which we term the “constraint” scenario, involves cases where recombination
124 suppression is maintained in the long term despite the fact that it has become disadvantageous, due
125 to mechanistic constraints preventing the reestablishment of recombination. Most models for the
126 evolution of sex chromosomes ignore the possibility that recombination can be reestablished,
127 implicitly assuming that a constraint maintains recombination arrest in the long term. Few models
128 present a more detailed reasoning about this constraint. For instance, in Jeffries et al.’s simulation
129 model of neutral arrest of recombination [22], crossovers are assumed to be fully suppressed once
130 sequence divergence becomes too high. The constraint emerges from the loss of homology. However,
131 the authors note that, in reality, rare recombination events could occasionally occur at high sequence
132 divergence. While Jeffries et al.'s model does not include deleterious mutations, degeneration would

generate strong selection to restore recombination and these rare events would be highly beneficial. Another idea is that reestablishing recombination might be difficult once complex chromosomal rearrangements have occurred on the Y. For instance, Jay et al.'s model [19] assumes that recombination is suppressed as soon as an inversion occurs and that the occurrence of secondary inversions (overlapping or occurring within a first one) prevents reversion of the first one. Hence, recombination could only be reestablished in a region if all inversions are exactly reinversed, irrespectively of the actual collinearity (or lack thereof) between the X and Y. This can allow lucky inversions to persist in the long term, irrespective of the process of degeneration. Last, an unspecified and unrelated selective advantage could be associated with recombination suppression. This would not be a mechanistic constraint, but we mention it as a possibility. It could for instance be the case for the maintenance of achiasmy in the heterogametic sex, independently of the evolution of sex chromosomes.

In this paper, we revisit this constraint scenario. We focus on the case where the short-term recombination arrest is caused by lucky inversions. The initial arrest is not the factor of interest here, so we use the simplest model (the lucky inversion process only requires the occurrence of deleterious mutations and variation in recombination rates). We contend that explanations based on the mechanistic constraint that recombination cannot be restored on the Y chromosome face several theoretical challenges, rendering them unlikely, in our view, to account for the evolution of sex chromosomes. Furthermore, we argue that mechanistic constraints on recombination restoration may often not be sufficiently strong to lead to stable heteromorphic sex chromosomes.

Methods

We analyze a model of sex chromosome evolution where recombination arrest is caused by lucky inversions and explore the constraint scenario by varying the rate of recombination restoration. Specifically, we use the general model of sex chromosome evolution that we previously introduced to explore the regulatory theory [19,20, which should be consulted for more details], but removing the regulatory effects. This model considers a sex chromosome pair with a large number of genes (here 500) subject to deleterious mutations occurring in their coding sequences. Fitness is determined multiplicatively across loci by the effect of deleterious mutations with a dominance coefficient equal to 0.25, as observed on average for mildly deleterious mutations [40]. For simplicity, the SD locus is located at one extremity of the chromosome. Recombination variation is modeled by introducing mutations suppressing recombination in a region around the SD locus. These mutations can be thought of as being inversions (and we will refer to them as such) although other types of mechanisms are possible, as already mentioned. For instance, the removal of recombination hotspots, or the addition

of a recombination suppressor sequence would work too. Specifically, as described in [17], we assume that inversions occur on the Y at a rate U_{inv} per chromosome per generation (we use $U_{inv} = 10^{-5}$). We only consider inversions that include the SD locus (or extend the non-recombining region of the Y carrying the SD locus). Other inversions are not confined to males and can be fixed in the population, which does not lead to recombination suppression on the Y (homozygous inversions recombine normally). We denote the non-recombining fraction of the Y by *z* (between 0 and 1). This variable is also used to measure the endpoint of each inversion on the chromosome. When $z = 0$, X and Y chromosomes recombine freely, but otherwise X-Y recombination only occurs within the chromosomal segment [*z*, 1] (the SD locus being located at position 0). When $z = 1$, the X and Y do not recombine at all. When a new inversion occurs, its size is drawn as a uniform fraction of the non-recombining part of the Y. Specifically, on a Y where recombination is already stopped between 0 and z_i, after a new inversion *i*+1 the non-recombining region will extend to $z_{i+1} = z_i + (1 - z_i)u$, where *u* is a uniform deviate between 0 and 1. To allow for the possibility that recombination may be reestablished we assume that reversions can also occur (at a rate U_{rev} per chromosome per generation), reverting the last inversion on the non-recombining part of the Y. We investigate the dynamics of Y evolution in this model by supposing that reversion rates are much smaller than inversion rates, with $U_{rev} = 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}$, i.e., from 1 to 4 orders of magnitude lower than the rate of occurrence of inversions ($U_{inv} = 10^{-5}$). At the start of a simulation, each individual carries a pair of fully recombining chromosomes, with the SD locus located at one extremity. Note that we do not perform a full exploration of the parameter space here, but rather use the simulations to illustrate the different points that are developed below concerning the effect of the reversion rate on sex chromosome evolutionary dynamics. Note that parameters scaled by the population size are likely to be the determinants of the evolutionary process so that the scenario can be extended to different population sizes by the appropriate rescaling of mutation rates, selection coefficients, and times. The simulations assume a constant population size (10^4 offspring individuals are drawn each generation, irrespective of the average absolute fitness of male and female individuals in the previous generation). However, the population was considered to be extinct when the average fitness of males became a thousand times lower than the average fitness of females.

Results

Very low rates of reversion can prevent long-term recombination suppression

To evaluate how strong the constraint on reversions should be for recombination suppression to be maintained in the long term, we investigated cases where the rate of reversion was much lower than the rate of inversion. We ran replicated simulations lasting four million generations. With our standard

199 parameters (Table 1), approximately 0.76 inversions are fixed per million generations. Typical
200 outcomes are illustrated on Fig 1 (taken from runs with $U_{rev} = 10^{-8}$). The majority of inversions that
201 reach fixation remain relatively short-lived (Fig 2A) and most of them occur one at a time (i.e., they are
202 reverted before a second one fixes). Before fixation, the marginal fitness of Y inversion decreases
203 through time, as they tend to accumulate deleterious mutations (returning to the equilibrium load and
204 being exposed to selective interference). After they fix, Y inversions continue degenerating because of
205 selective interference, which reduces male fitness and eventually offsets their initial fitness advantage.
206 At this point, they become deleterious, and it is just a matter of time before a reversion occurs that
207 would be selectively favored (Fig 2B shows the marginal fitness at birth and at the time of reversion
208 for fixed inversions, for different reversion rates). This is the example illustrated in Fig 1A. In a few
209 cases, another inversion fixes on top of the first one, before the first has reversed (this corresponds to
210 the example illustrated in Fig 1B). When the rate of reversion is very low, several inversions may stack
211 on the first one. In these cases, the lifespan of the first inversion is prolonged because it can be
212 reversed only after the second one (or third one, etc.) is reversed. This is due to the rather stringent
213 hypothesis of our model that reversions can only occur on the last stratum present on the Y. This
214 assumption protects the first inversion from reversion (until all other strata have reverted), and
215 considerably reduces effective reversion rates (as it becomes zero for all but the last stratum on a Y).
216 Considering reversions that could fully reestablish recombination on the Y at once would greatly
217 reduce this (potentially unrealistic) effect. Whether or not stacking occurs, however, all inversions
218 become reversed at some point (or the population goes extinct as we discuss below). There is no stable
219 long-term maintenance of recombination suppression. Typically, Y chromosomes transiently carry one
220 or two strata (Fig 4) for a relatively short time (Fig 2A). We can therefore conclude that the constraint
221 scenario cannot explain the long-term maintenance of recombination suppression for rates of
222 reversion up to 4 orders of magnitude lower than rates of inversion. This conclusion is very
223 conservative, as our model of reversion does not allow the reversion of a first inversion if a second
224 inversion occurs extending the non-recombining region (the first one can only be reversed after the
225 second one is reversed). Without this constraint, it would be even more difficult to maintain
226 recombination suppression.

227 Very low rates of reversion can prevent degeneration

228 Many old Y chromosomes are largely non-recombining and degenerate, mutations having
229 accumulated up to the point where genes have become nonfunctional or have been lost. In our model,
230 degeneration corresponds to the situation where a gene has accumulated deleterious mutations up to
231 a maximum fitness effect of s_{max} (corresponding to the fitness drop caused by the loss of function of a
232 gene, here set to 0.3). When reversion rates are low, some inversions can fix and persist in the

population for some time, especially when secondary inversions also occur prolonging the lifespan of the first one. Usually, this does not correspond to a large fraction of the Y, but on rare occasions, this can be significant (in particular, when several fixed inversions are stacked, which reduces the rate of reversion of all but the last one of them, as explained above). For example, in Fig 1B, about a third of the Y stopped recombining for nearly a million generations. Even in these extreme cases, however, degeneration remains moderate: even with extremely low rates of reversion (10^{-9}), almost no gene accumulates deleterious mutations up to s_{max}. Yet, we use a relatively high rate of deleterious mutation per gene ($U_g = 2 \times 10^{-4}$), a distribution of fitness effects of mutations with a relatively high mean ($s_{mean} = 0.05$), and a large proportion of small effect mutations (the distribution of effects is exponential). In the vast majority of cases, almost no loss of function is detectable (Fig 5A). The reason for this lack of loss of function is that many weakly deleterious mutations accumulate in all genes present on a fixed inversion. Collectively, their impact on the marginal fitness of the inversion starts to be strong long before any gene in particular becomes fully nonfunctional. Hence, inversions become selectively disfavored (and therefore selectively eliminated as soon as a reversion arises), long before they exhibit any gene loss. We can therefore conclude that the constraint scenario cannot explain strong degeneration for rates of reversion up to 4 orders of magnitude lower than rates of inversion. Degeneration may occur under very low rates of reversion if carrying nonfunctional genes on the Y would only cause a very small fitness cost for males (a situation that would be represented by setting s_{max} to a small value in our model). This situation seems unlikely in the absence of a mechanism silencing impaired genes on the Y, however, while letting gene expression evolve would lead to the regulatory scenario, under which recombination arrest can be maintained even in the absence of any constraint on recombination restoration [17].

The constraint scenario is more likely to lead to extinction than Y degeneration

It may be argued that reversion rates are even smaller than the ones we considered, making the constraint scenario a possibility, at least theoretically. There is a strong argument against this possibility. 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). Initially, a lucky inversion is selectively favored because it captures a fraction of the Y carrying fewer deleterious mutations (or deleterious mutations with smaller effects) compared to the average Y population. Deleterious mutations start accumulating within the inversion because the inversion tends to return towards the average mutation load [18], and to selective interference. As explained above, a first threshold is reached when the accumulation of deleterious mutations depresses the marginal fitness of this portion of the Y below the average marginal fitness of the homologous portion of the X in the population.

267 Reversions then become selectively favored, and it is just a matter of time before one occurs and
268 eliminates the inversion. If the reversion rate is extremely low, this can indeed take a long time.
269 However, a second threshold will be reached relatively quickly, corresponding to the non-viability or
270 sterility of males, and hence to the extinction of the population/species. This cannot happen in our
271 model as we assume a constant population size, i.e., the absolute number of individuals in the
272 population does not depend on the fitness of individuals (soft selection), but the simulations show a
273 crash in male fitness relative to female fitness when inversions are maintained for a sufficiently long
274 time (Fig 5B). It is not easy to determine the male fitness threshold that would lead to population
275 extinction in nature. We used a relatively conservative threshold equal to 10^{-3} (meaning that the fitness
276 of males is three orders of magnitude lower than the fitness of females). Such a distortion of male vs.
277 female fitness would be particularly conspicuous in natural conditions. In the few cases where some
278 degeneration occurs (e.g., when a large inversion unfortunately fixes), the population reaches this limit
279 quickly and becomes extinct. Reversions can rescue the population and prevent extinction, but if they
280 are too rare, they do not occur quickly enough to prevent it. For instance, with very low rates of
281 reversions ($U_{rev} = 10^{-9}$), this threshold was often reached in our simulations (extinction occurred in 70%
282 of cases, 14 replicates out of 20 within the first 4 million generations of evolution). This estimate is
283 conservative, since considering that the number of males in the population may be much lower than
284 assumed under our soft selection regime would lead to an even faster accumulation of deleterious
285 mutations on the Y (due to stronger drift). Note that our model does not include back mutations, which
286 would eventually stop the decline in fitness caused by deleterious mutation accumulation. However,
287 previous work has shown that in the absence of recombination, mean fitness reaches very low values
288 even when back mutations do occur, unless the mean fitness effect of deleterious mutations is
289 extremely weak [41,42]. Hence, a theory based on constraints alone cannot explain both degeneration
290 and the persistence of populations/species. For degeneration and persistence to occur, Y silencing and
291 DC must also evolve, which can be a powerful selective mechanism that stabilizes recombination
292 arrest.

293 Comparing the constraint scenario to a scenario including a selective pressure against 294 recombination

295 It is not because reversion rates are low (representing strong constraints on recombination
296 restoration) that explanations of long-term recombination suppression solely based on constraints are
297 likely to hold. Quantitatively, the question is rather, for given reversion rates, to determine the most
298 likely scenario for observing Y chromosomes with non-recombining and degenerate strata within a
299 realistic timeframe. To illustrate this point, we simulated the evolution of Y chromosomes under the
300 same low reversion rates used above, but allowing regulators to evolve using the model described in

301 [17], which should be consulted for more details. We use the same simulations as above, but we
302 consider that the expression of each gene is controlled by a cis-regulator and two trans-regulators (one
303 only expressed in males and the other only expressed in females). These regulators determine
304 quantitative traits that control the total and allele-specific level of expression of each gene. Total gene
305 expression is supposed to be under stabilizing selection for all genes. Fitness is determined
306 multiplicatively across loci by the effect of deleterious mutations (whose dominance depends on the
307 relative strength of cis-regulators in heterozygotes, with a baseline dominance in the absence of cis-
308 regulatory variation equal to 0.25) and by the departure from optimal expression at each locus. Cis and
309 trans regulators mutate at a fixed rate per generation (with Gaussian variation in trait values). Table 1
310 indicates the additional parameters and their value for the simulations with the evolution of
311 regulators.

312 In this case, we observe much faster Y recombination suppression and degeneration than in the
313 absence of regulatory evolution, for all reversion rates investigated. This is probably also true for
314 scenarios involving SA loci, which generate a selective pressure against recombination accelerating the
315 process. Low reversion rates are favorable to any theory on the maintenance of recombination arrest,
316 not only those solely based on constraints. Indeed, low reversion rates give more time for other SA loci
317 to accumulate, or nascent DC to emerge, and more time for degeneration to happen. In our model of
318 regulatory evolution, and with very low reversion rates, almost any fixed inversion has time to develop
319 nascent DC, generating sexually antagonistic regulatory effects that effectively disfavor recombination.
320 Fig 4 shows that many more strata accumulate on the Y in this case than in the absence of regulatory
321 evolution, while Fig 5C shows that most of these strata are long-lived and fully degenerated (while
322 almost none is degenerated for the same parameter values in the absence of regulatory evolution, Fig
323 5A). Fig 5D shows that this degeneration is not associated with a large drop in male-to-female fitness
324 ratio, while this drop is considerable in the absence of regulatory evolution. When regulators evolve,
325 reversions occur but are not selectively favored. Indeed, the reestablishment of X-Y recombination on
326 a given stratum causes X cis-regulators to move to the Y, creating recombinant (low fitness) Y that
327 cause a departure from optimal gene expression in males. This is a case of an evolved selective
328 constraint. Strata are selectively stabilized because DC emergence creates sex antagonistic regulatory
329 effects on expression levels, as well as silencing of the deleterious mutations accumulating on the Y.
330 Hence, strata become permanently stabilized and can persist indefinitely, in contrast to the constraint
331 theory where strata are never stable. The ultimate cause of long-term recombination suppression is
332 not the absence of genetic variation for reestablishing recombination once an inversion has fixed
333 (mechanistic constraint), but that it is selectively unfavorable to reestablish it. Overall, for the
334 parameter values considered here, we see that after 2 million generations a large fraction of the Y has

335 stopped recombining and degenerated in the presence of regulatory evolution with the lowest
336 reversion rates (for high reversion rates the process is still ongoing with only a few small stabilized and
337 degenerated strata, Fig 3). Nothing nearly comparable occurs without regulatory evolution, where at
338 most, in a few cases with extremely low rates of reversion, some recombination suppression evolves
339 and drives the population to extinction without leading to significant Y degeneration (Fig 3). We
340 conclude that even if reversion rates were extremely low so that a scenario solely based on constraints
341 could produce partial and transient degeneration and recombination suppression, a scenario involving
342 a selective pressure maintaining recombination arrest is orders of magnitude more likely to produce
343 complete and permanent recombination suppression and degeneration, without a major drop in male
344 fitness.

345 Discussion

346 The arguments and results presented in this article imply that, in the absence of regulatory evolution,
347 the decreased fitness of the heterogametic sex due to mutation accumulation on the Y should lead to
348 two possible outcomes: (i) the restoration of recombination if reversions can occur, even at very low
349 rates, or (ii) the extinction of populations if constraints on reversions are sufficiently strong. In the
350 following, we discuss the constraint theory in the light of those results and possible mechanisms of
351 recombination reestablishment, before indicating future avenues for theoretical and empirical
352 research concerning the initial steps of recombination suppression, the mechanisms responsible for
353 the long-term maintenance of recombination suppression, and the extension of these theories.

354 What is the degree of constraint on recombination reestablishment?

355 The possibility of recombination restoration should depend on the mechanism of recombination
356 arrest. Recent theoretical work has emphasized the possible role of inversions in suppressing
357 recombination on sex chromosomes [17–19,43], although these models also apply to other
358 mechanisms of stepwise recombination arrest. Inversions do indeed occur frequently within
359 populations and may be caused by ectopic recombination between repeated sequences [44–47]. For
360 this reason, they often tend to occur on the same sites and sometimes repeatedly [48–50]. They are
361 often observed on sex chromosomes [10,51–53], although some of these inversions may have
362 occurred after recombination arrest [10]. Inversions are well known to reduce recombination rates in
363 heterokaryotes. This reduction is not necessarily because inversions inhibit homologous pairing. If
364 inversions are sufficiently large, pairing can occur, and inversions form loops allowing for a local
365 alignment of the two homologous chromosomes. These loops can directly inhibit chiasma formation,
366 especially near the breakpoints of the inversion [54,55, but see 56]. However, the suppression of
367 recombination is also strongly mediated by the fact that an odd number of crossovers within the

368 inversion loop leads to the production of unbalanced chromosomes. Such unbalanced chromosomes
369 usually cause a fitness reduction and are thus eliminated (however, as explained in the appendix, this
370 may be less true when the chromosome is degenerated). Finally, it is important to note that
371 recombination may still occur when the number of crossovers falling within the inversion is even
372 (especially in the case of relatively large inversions where crossover interference is weaker [57–60]),
373 while gene conversion events may also allow for genetic exchanges between inverted and non-
374 inverted segments [54,60]. Such exchanges would limit degeneration and thus allow for longer
375 persistence time of inversions compared to the situation modeled in our simulations.

376 In a previous model of inversion dynamics on sex chromosomes, Jay et al. [19] consider that
377 recombination restoration is possible only when an inversion with exactly the same breakpoints
378 ("reinversion") restores the exact original gene order before another inversion overlaps or occurs
379 within the first one. If a second nested or overlapping inversion occurs, it can also be reversed, but
380 only before a third nested/overlapping inversion occurs, and so on. In this model, the chance of
381 reinversion becomes vanishing low as the number of breakpoints increases. Indeed, with N
382 breakpoints on the chromosome and with a first inversion spanning k breakpoints, the chance of
383 reinversion is $\sim 1/N^2$, while the chance of a second overlapping / nested inversion is $\sim k/N$. In the
384 results we presented, this level of constraint is achieved for the very low rates of reversions. For
385 instance, with N = 100 breakpoints and a first inversion spanning k = 10 breakpoints, reinversions are
386 3 orders of magnitude less likely than inversions. An interesting feature of this model is that it
387 mechanistically represents inversions and reinversions. It also captures the idea that reestablishing the
388 exact gene order with random inversions becomes increasingly difficult as they accumulate. This
389 phenomenon may indeed occur and constrain the reestablishment of recombination.

390 However, several processes could largely limit the constraint imposed by the accumulation of
391 overlapping and nested inversions. First, recombination may occasionally occur even if gene
392 collinearity is not exact, as shown with ectopic recombination [61–63], especially after a second
393 inversion restoring the original direction on a portion of the chromosome. Fig S1 and S2 in the appendix
394 illustrate such possibilities for nested or overlapping inversions. With imperfect collinearity,
395 recombined chromosomes have low fitness in general, but here, with a partially degenerated Y, the
396 question is more subtle, as the loss of some (already) degenerated genes may be compensated by the
397 acquisition of non-degenerated portion of the X. What matters is the relative fitness of recombined Y
398 compared to the current (partially degenerated) low fitness Y. Hence, many favorable cases of
399 imperfect recombination could occur and be favored, which could largely increase rates of
400 recombination reestablishment.

401 Another possibility is that recombining sex chromosomes may be reestablished by moving the SD locus
402 out of the non-recombining region. This mechanism can always occur, even when complex
403 rearrangements have taken place on the Y. This may occur for instance by recruiting a new master
404 switch gene for sex-determination or following a duplication of the existing SD locus into a new
405 location. Gene duplications are frequent events in eukaryotic genomes [64]. Rates in the range 10^{-5} –
406 10^{-7} per gene per generation have been reported in animals [65–67], i.e. a much higher rate than the
407 rate of reversions that we investigated. If the SD locus moves to another (recombining) location on the
408 sex chromosome, a new Y recently re-derived from the X (and fully recombining) can evolve easily.
409 Examples of such events are often reported [68–70], indicating that they may be common. The SD
410 locus may also move to another autosomal pair, leading to the evolution of a new pair of fully
411 recombining sex chromosomes. Such turnovers of sex chromosomes have also been reported in
412 several species [71,72] and are predicted to be favored when deleterious mutations have accumulated
413 due to the lack of recombination [37,38]. In some species, another possibility for restoring
414 recombination on the Y involves environmental sex reversal when recombination is sex-dependent
415 [36,73,74].

416 Often, the idea that recombination restoration is strongly constrained stems from the idea that
417 'reinversions' are not observed. While the rate of reinversion is probably low, this view is not entirely
418 accurate. Reinversions have been occasionally shown to occur in laboratory populations of Drosophila
419 [75] at rates 10^{-3} – 10^{-4}: while most of them were caused by X-ray irradiation [76,77], one occurred
420 spontaneously in a stock population [78,79] and it was proposed that reinversion may be favored by
421 the physical proximity of the breakpoints during loop formation [77]. Recent comparative genomics
422 has also highlighted that inversions and reinversions occur frequently and repeatedly at particular
423 breakpoints, although estimating the corresponding rates has not been done [49,50]**.** More empirical
424 work on this issue is needed to assess to what extent such a process may occur (and at which rate)
425 despite the repression of crossing-over in the vicinity of inversion breakpoints. However, the
426 observation of an accumulation of nested and overlapping inversions alone is not an indication that
427 the absence of recombination restoration was due to a constraint or to a selection pressure against
428 recombination. Chromosomal rearrangements can secondarily accumulate in a non-recombining
429 region [1,10].

430 Hence, while the accumulation of complex rearrangements is certainly a way to suppress
431 recombination, the maintenance of recombination arrest on the Y by a constraint alone requires a very
432 high level of constraint, given the maladaptation caused by mutation accumulation on the Y. This level
433 of constraint is, in fact, a rather strong assumption. It requires that reinversions are very rare, that rare
434 recombination events involving imperfectly colinear chromosomes do not occur, and that the SD locus

435 cannot move outside the non-recombination region. In any case, determining whether recombination
436 suppression is maintained selectively or by a mechanistic constraint is likely to be empirically difficult.
437 A key difference between these two cases is that Y strata have a higher or lower marginal fitness
438 compared to the equivalent segment on the X. If it was feasible to experimentally switch these portions
439 of chromosome and investigate the fitness effect of this switch, the two cases would lead to opposite
440 predictions. In the constraint theory, the switch should increase male fitness, while the opposite is
441 expected if recombination suppression is selectively maintained, provided some degeneration has
442 occurred.

443 Conclusion and perspectives

444 After recombination suppression, the accumulation of deleterious mutations on the Y generates a
445 selective pressure to restore recombination and purge those alleles. This selective pressure becomes
446 stronger as male fitness declines, soon making recombination restoration events highly favorable. This
447 explains that even with extremely low rates of recombination reestablishment, recombination
448 suppression cannot persist in the long term when only deleterious mutations are considered. In this
449 case, recombination restoration events are rescuing the population from extinction, and even if they
450 are rare, play a disproportionate role in the outcome. This issue is even more acute in models where
451 recombination arrest is caused by neutral divergence [22] as recombination suppression occurs
452 gradually, rather than quickly as in the lucky inversion scenario. This is the central theoretical argument
453 against theories of sex chromosome evolution solely based on mechanistic constraints [19,22]: In the
454 absence of regulatory evolution, the accumulation of deleterious alleles caused by recombination
455 arrest should eventually lead to population extinction or the reestablishment of recombination (via
456 reinversion(s), or via a change of location of the sex-determining locus, either to the PAR or to an
457 autosome as with a sex chromosome turnover), rather than the long-term maintenance of degenerate
458 Y or W chromosomes.

459 Several issues remain to be investigated in more detail. The different processes possibly involved in
460 the evolution of Y chromosomes need to be better integrated. In particular, the conditions allowing
461 the long-term maintenance of recombination suppression under the SA theory should be investigated
462 further [following 37,38] and combined with models of regulatory evolution. Better integration of the
463 different mechanisms of regulatory variation may also be useful, such as mechanisms based on
464 imprinting [80] or based on the reallocation of transcription factors to X-linked genes [11]. While the
465 widespread occurrence of DC [80,81] indicates that it is needed at least for some genes, it would be
466 interesting to introduce heterogeneity among genes in selection on dosage, and varying the genetic
467 architecture of DC (local vs global). Investigating whether DC always evolves, at least for dosage-
468 sensitive genes, for old degenerate sex chromosomes would also be interesting, to confirm the key

role of DC in their long-term stability. Several cases of interest should be investigated further, notably cases involving non-random mating [18,29,82] or UV and mating-type chromosomes. Analytical models are needed to generalize the recent findings that have mostly been explored by simulation. Finally, from an empirical perspective, more data are needed on patterns of recombination suppression, degeneration, and mechanisms of early DC evolution in young sex chromosome systems. Overall, the level of constraint on recombination restoration may not be the key parameter to understand why heteromorphic or homomorphic sex chromosomes occur in a given species. The ease of evolving dosage compensation is likely to be the main driver: if regulatory evolution is difficult, sex chromosomes will remain homomorphic (recombination will be restored in some way or the species will go extinct). If regulatory evolution and DC can evolve relatively easily, stable heteromorphic sex chromosomes may persist in the long term [17].

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Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article. TL and DR are recommenders for PCI Evol. Biol.

Code availability

The simulation code is available at:

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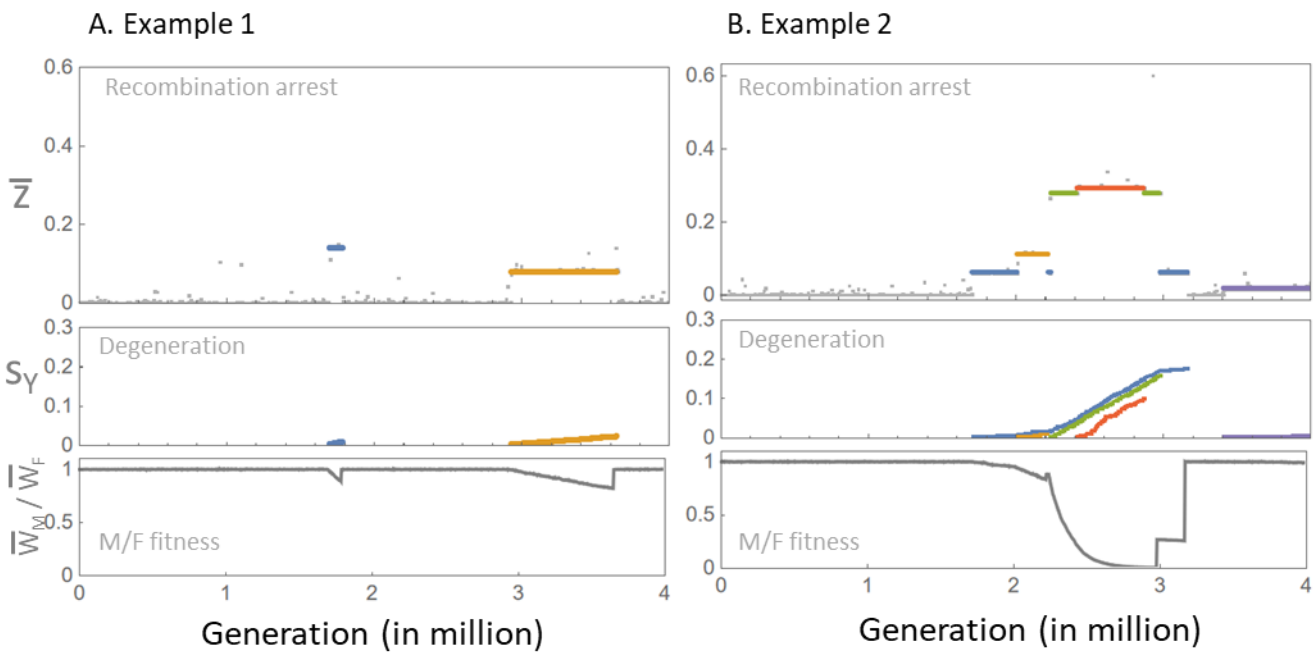


Fig 1. Examples of inversion-reversion dynamics. Each example is illustrated with three panels. The top panel shows the average nonrecombining fraction of the Y in the population ($\overline{z}$) through time (x-axis in million generations). Colored lines correspond to fixed inversions (i.e., all inversions reaching a frequency of 1 during the simulation); different fixed inversions have different colors. The colored line extends between the time of occurrence of the inversion and the time when it becomes extinct. The middle panel shows the (per gene) average cumulative fitness effect of deleterious mutations on these inversions through time (same color code as in the top panel). The bottom panel shows the ratio of the average fitness of males/females in the population through time.

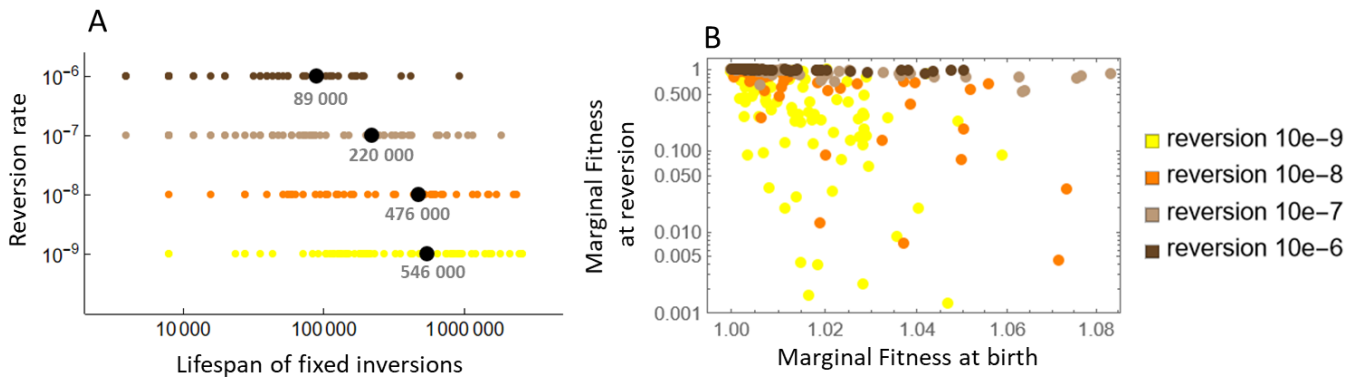


Fig 2. Characteristics of inversions under the constraint scenario. Panel A indicates the lifespan of fixed inversions (taken on 20 replicates) for different reversion rates (on the y-axis). Time was cut off at the end of the simulation (after 4 million generations) or if the population became extinct (by reaching a male/female fitness ratio < 0.001). Panel B shows the marginal fitness at birth of inversions (x-axis) versus marginal fitness at the last recorded time (y-axis, log scale). The latter most often corresponds to a reversion, but in a few cases, it corresponds to the end of the simulation or population extinction. Color codes correspond to different reversion rates as indicated in the legend.

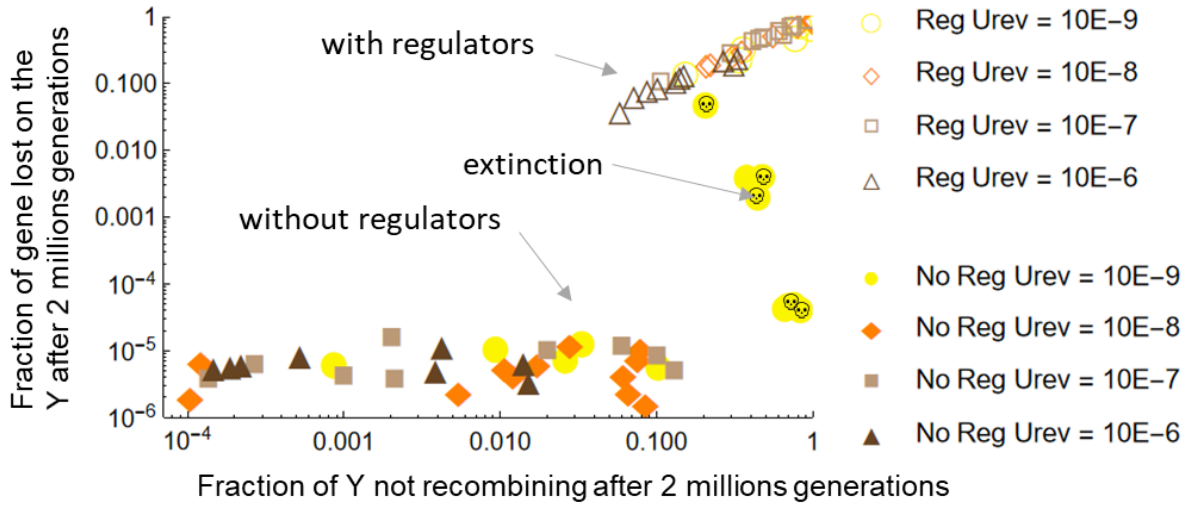
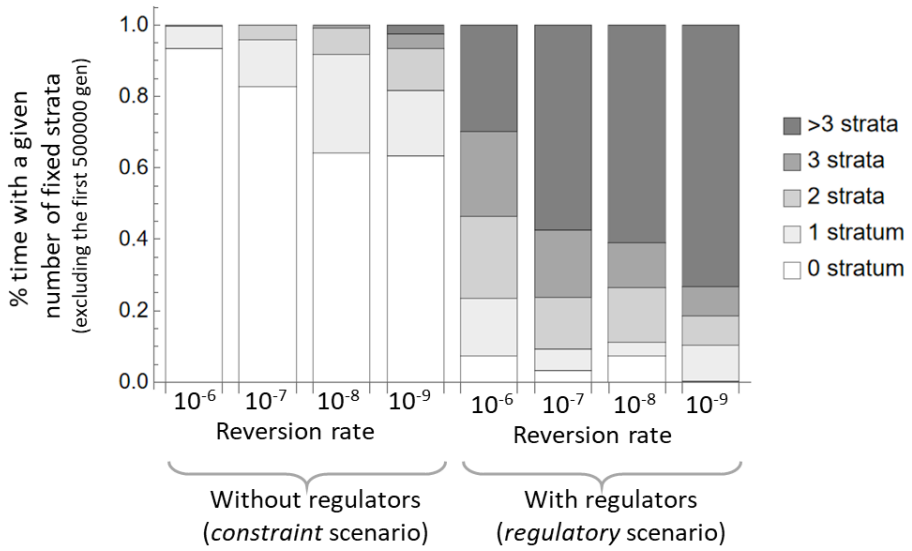


Fig 3. Overall evolution of the Y chromosome under the constraint scenario. The x-axis (in log scale) gives the fraction of the Y that is nonrecombining (averaged over all males in the population) after two million generations. The y-axis (in log scale) gives the fraction of genes lost on the Y after two million generations (averaged over all males in the population). A loss is defined as a gene having accumulated deleterious mutations up to s_{max} = 0.3. Each dot represents a replicated population. Open symbols: regulators evolve (regulatory scenario); filled symbol: regulators do not evolve (constraint scenario). Color codes indicate different rates of reversion (U_{rev}). The rate of inversion is 10^{-5} in all cases. In a few cases (with U_{rev} = 10^{-9} in the constraint scenario, filled yellow disks) the population became extinct before 2 million generations. In these cases (marked with a skull), the x and y axes values are taken at the time of extinction.

723



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725

726 **Fig 4. Fraction of the time during which the Y carries a given number of fixed strata.** During a replicate
727 simulation, there are times during which an inversion is fixed, perhaps including several strata, and
728 times during which no fixed inversion is present (see examples in Fig 1). The bar chart gives this %time,
729 across all replicates when a given number of strata are fixed in the population, for different reversion
730 rates (without regulator evolution as in the constraint scenario, four bars on the left, or with regulator
731 evolution as in the regulatory scenario, four bars on the right). This % is computed excluding the first
732 500 000 generation (to cut the initial phase influenced by the initial condition where the chromosome
733 starts fully recombining and without any fixed inversion). The gray level corresponds to the number of
734 fixed strata present as indicated in the legend on the right. For instance, in the constraint scenario with
735 a reversion rate equal to 10^{-6}, there is no fixed inversion 93.5% of the time, and one fixed inversion
736 6.3% of the time. In contrast, in the regulatory scenario with the same reversion rate, there is no fixed
737 inversion 7.3% of the time, one fixed inversion 16% of the time, 2 fixed inversions 23% of the time, 3
738 fixed inversions 23.7% of the time and more than 3 fixed inversions 30% of the time (over the 3.5
739 million last generations of a simulation).

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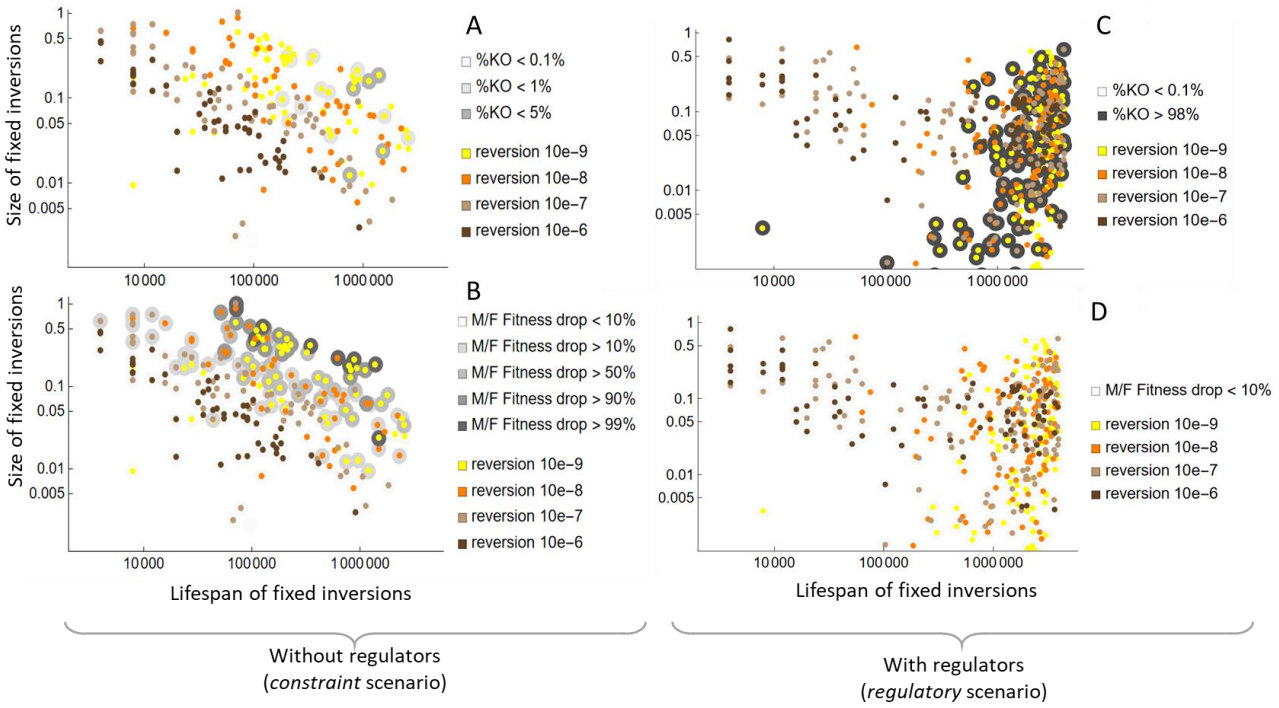

Fig 5. Detailed characteristics of fixed inversions in the constraint (panels A, B) and regulatory (panels C, D) scenarios. In all panels, the x-axis gives the lifespan of inversions (as defined in Fig 2A) and the y-axis the size of the inversion (both in log scale). Each dot represents a different fixed inversion that occurred across all replicates. The color of the dot indicates the reversion rate of the simulation during which the inversion was observed (as given in the legend). On panels A and C, a gray disk is added around each inversion. The gray level indicates the % of gene lost (%KO) on that inversion at the last time when the inversion is observed (i.e., just before it is reversed, at the end of the simulation at 4 million generations, or at population extinction). On panel A (constraint scenario), the gray level is light as this %KO never exceeds 5%. On panel C (regulatory scenario), this gray level is darker as this %KO reaches very high values (being either close to zero or above 98%). On panels B and D, a gray disk is also added around each inversion, this time representing the drop in the male/female fitness ratio caused by this inversion between the first and last time it is observed. Noting $r(t)$ this male / female ratio at time t, this drop is computed as $r(t_1)/r(t_2)$ between times t_1 and t_2. When several inversions are simultaneously present in a given time interval, the log(drop) is portioned proportionally to the relative size of each inversion $s_1/(s_1+s_2)$, i.e., with two inversions of size s_1 and s_2, the drop accrued to the first inversion is Exp($s_1/(s_1+s_2)$ Log $r(t_1)/r(t_2)$). For instance, with two inversions of equal size, each is assigned the square root of the fitness drop on the interval (so that the product of the fitness drop of each inversion gives the overall fitness drop). With this correction, the fitness drop associated with an inversion is more representative of what is happening on this inversion (rather than being caused by the presence of another inversion). On panel B (constraint scenario), these fitness drops can reach large values (more than a 99% reduction in the male/female fitness ratio), but they remain very low in the regulatory scenario (never exceeding 10% on panel D).

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Parameters	Values
Population size	10000
Number of genes	500
Average effect of deleterious mutations (the distribution is exponential)	0.05
Dominance coefficient of deleterious mutations	0.25
Maximum fitness drop caused by mutations in a gene, corresponding to a gene loss (s_{max})	0.3
Mutation rate of genes	0.0002
Recombination rate between consecutive genes (R_g)	0.0005
Rate of inversion mutations	0.00001
Rate of reversion mutations (10 to 10^4 lower than the rate of inversions)	variable
Standard deviation of mutational effects on cis and trans-regulatory traits	0.2
Number of gene, cis-regulator, male-limited, and female-limited trans-regulators	500
Intensity of stabilizing selection on expression levels	0.1
Mutation rates on cis-regulators	0.0002
Mutation rates on trans-regulators	0.0001
Recombination rate between cis-regulator and its gene (R_c)	0.00005

766 **Table 1. Parameter values used in simulations.** In grey, parameters are only used when regulators can
767 evolve (in the constraint theory mutation rates on regulators are all set to zero).

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Appendix

Recombination reestablishment after secondary nested or overlapping inversions

A difficulty to model recombination evolution by inversions and reinversions is that it is difficult to model the fact that the recombination process is not 'perfect' in the sense that it can occur between regions that are only locally homologous, as in the case of ectopic recombination. In the case of Y evolution, recombination may occasionally occur even in the absence of exact collinearity between the X and Y. This is illustrated in Fig S1 for an overlapping inversion that includes the SD locus, and in Fig S2 for nested inversions. In both cases, the secondary inversion has a homologous region on the X and can pair at meiosis, allowing recombination to occur around the SD locus. If a crossover occurs within this region, a new Y can be produced which may not carry the exact complement compared to the original chromosome (with either deleted or duplicated positions). In each case, such a crossover will eliminate parts of the former Y on the first stratum, i.e., regions that may have already accumulated a load of deleterious mutations. This recombined Y could be particularly favorable (compared to the degenerated Y), even if some sequences are duplicated or missing compared to the X (see appendix and Figs S1, S2). Indeed, whether the recombined Y can invade depends on its marginal fitness relative to the marginal fitness of the potentially highly degenerated Y chromosomes present in the population (and not to the marginal fitness of a hypothetical mutation-free Y chromosome with full gene content). Furthermore, the recombined Y may be "improved" in further steps since it can now recombine more easily with the X around the SD locus after this first recombination event. In particular, a second crossover near the SD locus can further improve collinearity with the X and eliminate further degenerated parts of the Y from the first stratum that are still present (see Figs S1, S2). Alternatively, recombination may also be restored if an inversion arises on the X, facing the inversion on the Y [17]. Again, more empirical work is needed to assess whether recombination may indeed occur in such scenarios despite non-perfect collinearity. However, the occurrence of ectopic recombination between repeated sequences indicates that it is possible in principle [45,62], while the results of the present article show that even very low rates would be sufficient to maintain recombination in the long term.

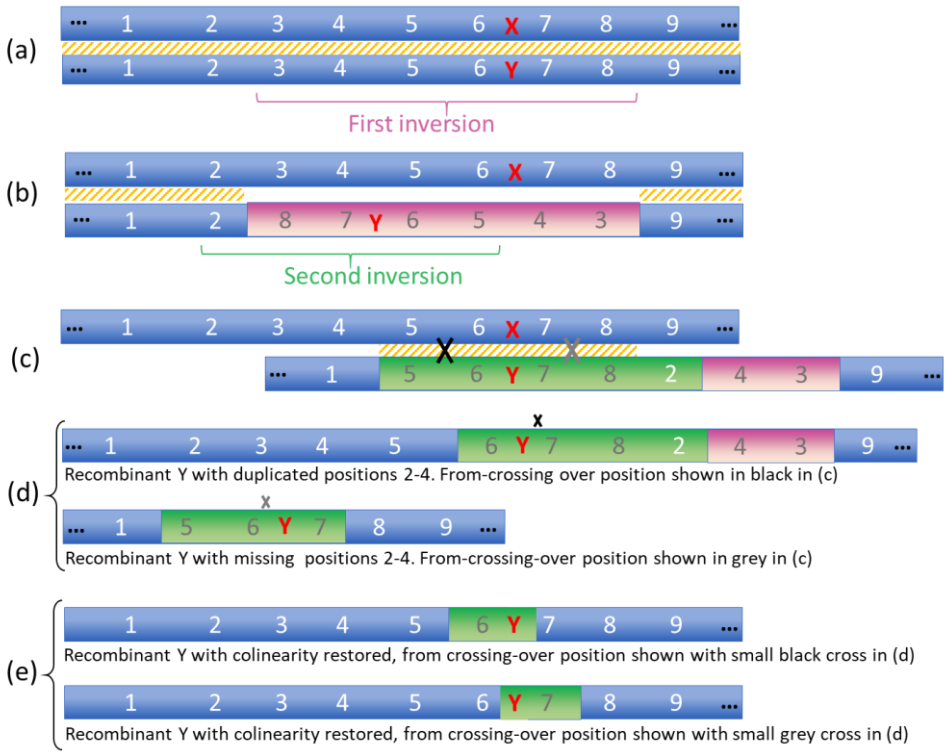

Fig. S1. Restoration of recombination on the Y with overlapping inversions. (a) depicts the fully recombining XY pair with the SD locus in red. The orange dashing indicates where homologous pairing is possible. A first inversion occurs (purple) on the Y between positions 3 and 8 leading to the situation in (b). Pairing and recombination do not occur around the SD locus. This first stratum on the Y chromosome can start degenerating in the absence of recombination. This is shown by the brown color of the position numbers. A second overlapping inversion occurs (green) on the Y between positions 2 and 5. The resulting Y in (c) can now pair with the X between positions 5 and 8. Crossing-over on the left of the SD locus (black cross) can generate recombinant Y with duplicated positions 2-4 (note that this Y recovers functional copies in positions 3-5, and has two functional copies in position 2). Crossing-over on the right of the SD locus (gray cross) can generate recombinant Y with missing positions 2-4 (note that the lack of these positions may not reduce fitness a lot if they were degenerated). (e) In both cases, a second crossover with the X occurring near the SD locus (shown with a small cross) can restore full collinearity with the X and get rid of other degenerated copies (on the right and left position of the Y, respectively).

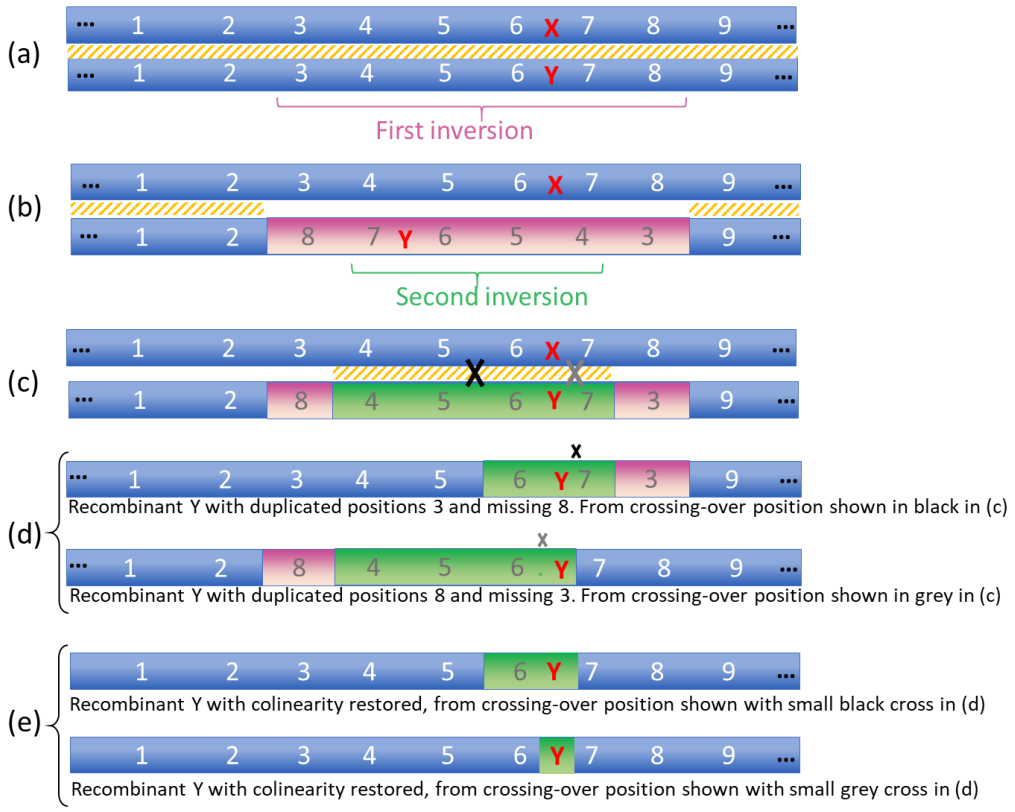

Fig. S2. Restoration of recombination on the Y with nested inversions. (a) depicts the fully recombining XY pair with the SD locus in red. The orange dashing indicates where homologous pairing is possible. A first inversion occurs (purple) on the Y between positions 3 and 8 leading to the situation in (b). Pairing and recombination do not occur around the SD locus. This first stratum on the Y chromosome can start degenerating in the absence of recombination. This is shown by the brown color of the position numbers. A second nested inversion occurs (green) on the Y between positions 4 and 7. The resulting Y in (c) can now pair with the X between positions 4 and 7. Crossing-overs on the left of the SD locus (black cross) can generate a recombinant Y with duplicated positions 3 and missing 8. Note that this Y can have a higher fitness by recovering functional copies at positions 3-5. Position 8 is missing, but this may not be consequential since it was a degenerated position. Crossing-over on the right of the SD locus (gray cross) can generate a recombinant Y with duplicated position 8 and missing 3. Again, the fitness of this Y may increase, since it recovers functional copies at positions 7-8 while losing a degenerated copy at position 3. (e) In both cases, a second crossover with the X occurring near the sex-determining locus (shown with a small cross) can reconstitute a Y chromosome fully colinear with the X.