Why and how do Y chromosome stop recombining?

INVITED COMMENTARY

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Sex chromosomes either carry a factor like the male-determining factors of mammals (Berta et al., 1990) or the plant *Silene latifolia* (Westergaard, 1958), or function in balanced sex-determining systems, as in Drosophila (Bridges, 1925) and *Caenorhabditis elegans* (Meyer, 2022). The sex chromosome pairs of these and several other species include physically extensive fully Y-linked, or male-specific, regions ('MSY') that have undergone 'genetic degeneration' and lost most of the genes that are carried on the X (X-linked genes are then hemizygous in males). Similar losses of recombination, and similar extensive degeneration, have occurred in the evolution of sex chromosomes in species with female heterogamety, including birds and Lepidoptera, respectively, reviewed by Wang et al. (2014) and Fraisse et al. (2017). The lack of recombination is probably not a direct consequence of carrying the sex-determining genes, or of having an essential role in sex determination, since several unrelated fish have physically small sex-determining regions located within recombining regions (Koyama et al., 2019; Kuhl et al., 2021; Nacif et al., 2022; Reichwald et al., 2015), and the completely sex-linked regions of some plants are smaller than 1 Mb (Akagi et al., 2014; Harkess et al., 2020). These situations can be explained in several ways, for example they could reflect recent evolution of separate sexes, or recent appearance of a sex-determining factor in a genome region in a so-called turnover event reviewed by (Vicoso, 2019).

Species whose chromosome pair has undergone changes in which formerly recombining regions stopped recombining with a previously established completely sex-linked region are therefore important, because they tell us that recombination became suppressed after their sex-determining locus evolved. The first case to be discovered was in Eutherian mammals. The earliest enlargement of the MSY involved fusion of the Y with a chromosome, that is an autosome in Marsupials (Waters et al., 2001). This occurred around 115 million years ago (Cortez et al., 2014). Further enlargements caused by subsequent recombination suppression events produced 'evolutionary strata' with wide differences in sequence divergence between the Y-X pairs in different regions of the chromosome (Figure 1). Based on the genetic map estimated in females (which probably represents the ancestral order of genes on the added chromosome), divergence stopped most recently near the region that still recombines, the pseudo-autosomal region, or 'PAR' (Lahn & Page, 1999; Skaletsky et al., 2003). In the two most recently evolved strata in humans, the median synonymous site divergence values are 14% and 26%, almost as high as for the rest of the added region (Sayres & Makova, 2013). Very varied Y-X divergence, perhaps reflecting strata, is also seen in the plant *Silene latifolia*, in the carnation family (Bergero et al., 2007; Papadopulos et al., 2015). In the threespine stickleback, whose sex chromosomes evolved without fusions with any autosomes, Y-X divergence shows clear strata like the two in Figure 1b (Peichel et al., 2020), and strata have also been inferred in the ZW pair in Neognathous birds (Wang et al., 2014).

A lack of recombination between sex chromosomes may not always reflect such suppressed recombination (Charlesworth, 2019). However, cases when recombination suppression has evolved (shrinking the PAR and changing its boundary with the completely sex-linked region) demand explanation. Several hypotheses have been proposed. One idea is that sexually antagonistic polymorphisms can become established in the PAR (e.g. a male-benefit allele that would reduce fitness in females is more likely to spread if closely linked to the MSY than one with the same fitness effects that is autosomal). Should such a mutation establish a polymorphism, this generates selection for closer linkage with the MSY (Rice, 1987). This idea is difficult to test because, if a non-recombining stratum has evolved, the mutation will be present only in the fully Y-linked region, making genetic analysis impossible. Although such male-benefit mutations might be detectable from expression differences

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between the sexes at the genes involved (perhaps higher in males), genetic degeneration is expected eventually to reduce expression of Y-linked genes in old evolutionary strata. Another approach is to test for population genomic evidence of balanced polymorphisms that show associations between the alleles and the sex-determining alleles (Dagilis et al., 2022; Qiu et al., 2013). However, if such a signal is found in partially sex-linked region, it still does not tell us whether selection will in the future lead to the evolution of suppressed recombination. Another approach is to search for alternatives that could also explain the evolution of suppressed recombination and ask if they can be ruled out. As described below, some new ideas have recently been proposed. Testing the predictions of these new hypotheses (Figure 2 below) requires studies of newly evolving strata of completely sex-linked genes, and of the PAR boundary regions of genomes, and such studies are becoming possible, using a combination of genome sequencing and genetic mapping.

A new study of *Silene latifolia* and *S. dioica* (closely related species that hybridize in nature and have homologous XY sex chromosomes), recently published in Journal of Evolutionary Biology (Filatov, 2022), examines such a newly evolved stratum. Y- and X-linked alleles can nevertheless be ascertained by identifying male-specific variants in gene sequences, using samples of males and females from natural populations. This approach allowed the Y-X sequence divergence analyses mentioned above that showed that recombination in parts of the *S. latifolia* Y chromosome pair became suppressed after the establishment of its male-determining factor. Clear step changes like those in Figure 1 are not seen, and the numbers of genes in the PAR and the MSY strata are unknown, since these species' genomes are

as large as the human genome and have not yet been assembled. However, genetic mapping does not suggest an extremely high recombination rate in the *S. latifolia* PAR, which may thus differ from mammalian PARs, with crossover events concentrated in physically very small regions (Rouyer et al., 1986). Some genes have male-specific variants in *S. latifolia*, but not in *S. dioica*, suggesting that *S. latifolia* has evolved a new stratum of completely sex-linked genes (and a smaller PAR than *S. dioica*; Campos et al., 2016). The new study ascertained more genes from the recombining end of the *S. latifolia* X chromosome genetic map and studied them in detail. 22 were fully sex-linked in both *Silene* species studied (they had male-specific variants in samples from natural populations), and 20 were classified as PAR genes in both species, but 15 PAR genes in *S. dioica* appear to be fully sex-linked in *S. latifolia*, forming a young stratum (much younger than the youngest ones in humans described above) suitable for testing ideas about recombination suppression.

One recent idea is that a completely sex-linked region (MSY) might gradually expand as sequence divergence near a sex-determining locus or PAR boundary with the MSY hinders pairing of very closely linked sequences in meiosis (Jeffries et al., 2021). MSY-X sequence divergence should then increase with distance from the PAR boundary into the MSY region (Figure 2). A slight trend is seen for Y-X synonymous site divergence in the new *S. latifolia* stratum, but divergence from the outgroup species *S. vulgaris* also increases (Filatov, 2022), so mutation rate differences may be responsible (HKA tests, which correct for this effect, detected elevated diversity in PAR genes suggests that the present PAR might have a higher mutation rate than the completely sex-linked regions). Interestingly, Y-X divergence increases
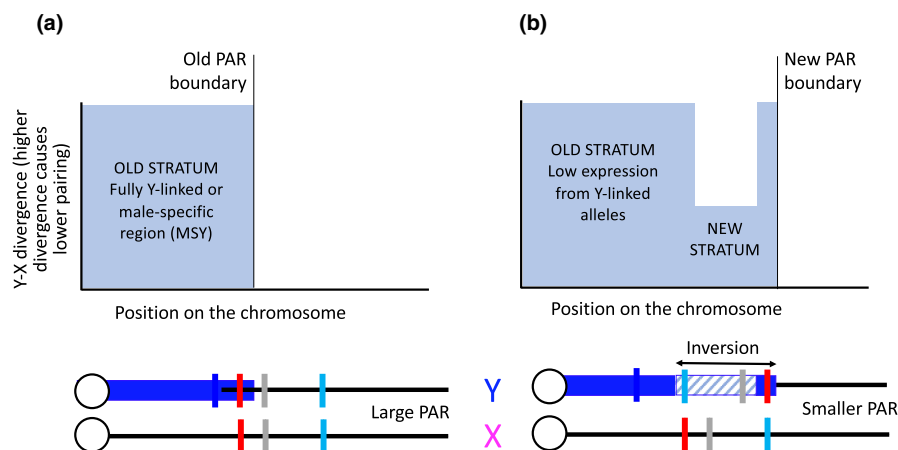

FIGURE 1 Diagram illustrating how a gradual expansion of the MSY might be caused by an inversion of part of the PAR. In part a, the lower part diagrams the X and Y chromosomes or chromosome arms. The X is shown as a black horizontal line, with a centromere at one end. Three vertical lines in three colours symbolize three genes or genetic markers. The Y differs from the X by having a male-determining factor (the blue vertical line) within a non-recombining 'evolutionary stratum' (the thick blue region). In the old stratum, the Y sequences are diverged from the X-linked ones, as shown in the upper part, and Y-linked alleles may become degenerated or lost. The rest of the arm is a large region similar to the X, the recombining 'pseudo-autosomal region' or PAR. Part b shows the effect of an inversion that includes the old PAR boundary. If the inversion prevents crossing over, part of the PAR will become a new fully Y-linked stratum, symbolized by the hatched blue bar in the chromosome diagram below, with a part of the old stratum at the new PAR boundary (this would not be present in the inversion were wholly within the PAO). The upper diagram shows the situation after enough time for sequence differences to have accumulated on the Y, making the new stratum detectable. Expression from Y-linked alleles may also have declined, creating female-biased expression until dosage compensation evolved.
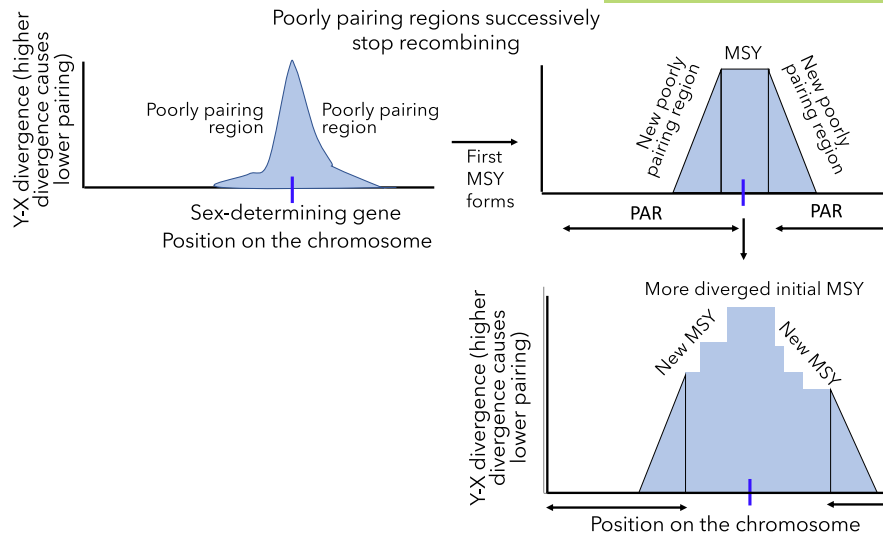
FIGURE 2 Diagram illustrating the ideas underlying the new models for expansion of fully Y-linked regions. An MSY might evolve on an initially recombining chromosome because of mismatches due to Y-X differences near the male-determining gene (indicated by a blue vertical line on the x axis). Mismatches and/or poor pairing might hinder recombination, and a region of low recombination might expand gradually near a boundary with the remaining PARs (Jeffries et al., 2021). This model predicts that Y-X sequence divergence in the new MSYs should increase with sequences' distance from the PAR boundary, either gradually (in the model of Jeffries et al., 2021), or in small steps associated with inversions (as illustrated), which would be predicted by the two other new models for recombination suppression outlined in the text.

sharply between the *S. latifolia*-specific MSY region and that shared with *S. dioica*, without any such change in divergence from the outgroup species, suggesting a possibly discontinuous change between strata, as in Figure 1b. The small divergence time between *S. latifolia* and *S. dioica* also makes it seem unlikely that loss of pairing could have produced an expansion across a region that includes at least 15 genes (probably at least 500 kb, as the gene density on the *S. latifolia* X is estimated to be 34 genes/Mb). The threespine stickleback XY pair includes a stratum with slightly higher synonymous site divergence (Ks), but still only 3% or 4%, and more than 500 X-linked genes (Peichel et al., 2020). Again Ks does not increase near the boundary with the older stratum, as predicted. The same is true in the plant papaya, in which divergence is high near the PAR boundary (Wang et al., 2012), consistent with a chromosomal inversion (Figure 1).

The other models propose MSY expansions involving chance spread of inversions that prevent recombination between a set of PAR genes and the MSY. This requires an advantage that can outweigh any fertility disadvantages in inversion heterozygotes, including production of gametes with duplications or deficiencies when crossovers occur in paired regions of large inversions. One version (Jay et al., 2022; Olito et al., 2022) proposes that linkage to the male-determining factor prevents new deleterious mutations becoming homozygous in males. This effect is strongest for mutations with large and highly recessive deleterious effects. Accumulation of mutations after the inversion arises can prevent the inversion from fixing in this population, so it must rapidly replace the ancestral Y chromosome population (initially with the X arrangement). The process therefore works best in small populations, and it is not clear whether it can account for observations on the time-course of recombination suppression and evolutionary strata formation. As small inversions are most

likely to be involved, such data may not distinguish this process from the previous one—both predict that Y-X divergence within the MSY will increase gradually with distance from the current PAR boundary.

Only a few examples of inversions coinciding with evolutionary stratum boundaries have so far been discovered. The two recent strata in humans probably involved inversions, and the others may have done (Lemaitre et al., 2009). In both cases, only a few genes became fully sex-linked (10 genes in stratum IV and seven in the younger stratum V), suggesting that new stratum formation does not require large numbers of genes. Another case (involving 16 genes) was detected in papaya (Wang et al., 2012). Clearly, more detailed studies of such regions will be helpful. However, rearrangements, including inversions, can accumulate after recombination stops in a genome region (Charlesworth et al., 1994), and were detected within human strata (Lemaitre et al., 2009).

The third model proposes that alleles that become Y-linked rapidly decrease in expression, in a dosage compensation process that simultaneously increases their X-linked alleles' expression, even before major deterioration of the coding regions of Y-linked genes. This can prevent inversions reverting to the standard arrangement (Lenormand & Roze, 2022). Female-biased expression was indeed observed in the new *S. latifolia* stratum (Filatov, 2022), perhaps reflecting the assumed decreased expression of Y-linked alleles, while expression of X-linked ones has not yet increased enough to restore expression in males. This model predicts that female expression bias should quickly decrease after sex-linkage evolves. Because dosage compensation evolves as part of the process, strongly female-biased expression should be seen near the boundary of the new stratum with the PAR, but expression of many genes in new (and older) strata should be similar in both sexes. However, the bias in *S. latifolia* appears

to change in the opposite way in the new stratum, increasing to reach the same level as that of genes that are fully sex-linked in both species (in which females have almost double the level in males, a significant difference from PAR genes, which, as expected, show little bias). The bias in the older stratum is consistent with genetic degeneration after recombination had stopped (reviewed in Bachtrog, 2008). Genes with changes that have not yet been fully compensated by evolving higher expression of the X-linked alleles in males will show female-biased expression, as observed in *S. latifolia*, in which dosage compensation seems to be incomplete (e.g. Papadopulos et al., 2015). It is interesting that degeneration may already have begun in the new stratum genes studied, which have Y-linked alleles as well as X-linked ones, and Y-X sequence divergence of only a few percent.

Under the sexually antagonistic polymorphism hypothesis outlined above, one might expect expression differences between the sexes at partially sex-linked gene(s) involved (perhaps often higher in males, the opposite of the pattern predicted by the model just described), while genetic degeneration should reduce expression of most Y-linked genes. Overall, *S. latifolia* sex chromosome evolution appears to be most likely to be explained by this model, since the other models do not fit the observations. Filatov's new and larger data set also supports previous studies suggesting elevated diversity of PAR genes (using HKA tests, which correct for mutation rate differences), so the present PAR may include sexually antagonistic variants. Whether or not these conclusions will be confirmed once a genome assembly becomes available, this study is valuable for suggesting how an integrated set of increasingly accessible kinds of data may allow empirical tests of the different models in this, and potentially other species with young strata. For instance, in the threespine stickleback, gene expression has been compared between the sexes in somatic tissues, and sex-linked genes were over-represented among biased genes. 155 genes were in the older XY stratum and 47 in the younger one, but the study did not test for a relationship with the genes' positions in the younger stratum (Kitano et al., 2020).

PEER REVIEW

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DATA AVAILABILITY STATEMENT

This is a commentary and there are no data.

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