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Opinion

Why Do Sex Chromosomes Stop Recombining?

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It is commonly assumed that sex chromosomes evolve recombination suppression because selection favours linkage between sex-determining and sexually antagonistic genes. However, although the role of sexual antagonism during sex chromosome evolution has attained strong support from theory, experimental and observational evidence is rare or equivocal. Here, we highlight alternative, often neglected, hypotheses for recombination suppression on sex chromosomes, which invoke meiotic drive, heterozygote advantage, and genetic drift, respectively. We contrast the hypotheses, the situations when they are likely to be of importance, and outline why it is surprisingly difficult to test them. Lastly, we discuss future research directions (including modelling, population genomics, comparative approaches, and experiments) to disentangle the different hypotheses of sex chromosome evolution.

Highlights

Why sex chromosomes evolve suppressed recombination remains elusive.

Sexual antagonism is the prevailing hypothesis for the evolution of recombination suppression, but meiotic drive, heterozygote advantage, and genetic drift are often-neglected counterhypotheses.

Despite a long history of research on sex chromosomes, direct tests of any of these hypotheses are surprisingly rare and the evidence often indirect.

Sex chromosome systems are now being characterised at an increasing rate. This work uncovers interesting heterogeneities between lineages and enables comparative analyses to understand sex chromosome evolution.

Recombination Suppression: A Characteristic Feature of Sex Chromosomes

Sex chromosomes (see Glossary) have evolved from **autosomes** many times throughout the history of life [1–4]. Ever since their discovery, these specialised chromosomes have fascinated researchers because of their obvious involvement in fundamental aspects of life, such as **sex determination** and sexual reproduction. Despite this long-lasting fascination, and steady theoretical and empirical progress, important aspects of the biology of sex chromosomes remain unclear, in particular concerning their seemingly ubiquitous evolution of **recombination suppression**, which is typically followed by degeneration and gene loss of the nonrecombining part of the sex-limited chromosome (Y or W) [5–8]. This process often progresses until recombination is absent over a large part of the sex chromosomes, with the exception of the **pseudoautosomal region** (PAR), which is needed for proper segregation, and where recombination between the gametologs still occurs in the heterogametic sex (XY males or ZW females). Recombination suppression can be advantageous, for instance, if it brings together beneficially interacting genes, but reduced recombination also carries costs: linkage constrains the accumulation of beneficial mutations and the efficiency by which mildly deleterious mutations are being removed [1–4]. There are other costs and benefits as well. For example, when recombination suppression evolves together with the evolution of dioecy in hermaphrodites, the establishing sex-determining mutation may pay a cost caused by skewing the sex ratio, becoming associated with the most common sex and experiencing lower relative fitness [2–4].

It is widely assumed that repressed recombination between the sex chromosomes is driven by **sexual antagonism** or more specifically by selection favouring linkage between the sex-determining gene(s) and nearby sexually antagonistic loci. That this has become the prevailing view is not surprising given that the role of sexual antagonism has strong support from a large body of theory [9–13], going back to Fisher's early work in 1931 [14]. Moreover, influential experiments constraining evolution to a single sex, and population genomic analyses of sex chromosomes, have shown that sexually antagonistic mutations accumulate fairly readily on

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sex chromosomes [15–20]. However, these studies do not provide conclusive support for the sexual antagonism hypothesis, as one cannot exclude that the antagonism accumulated after recombination ceased [6,10,21]. Likewise, obvious signs of sexual antagonism are lacking in some sex chromosome systems [22,23]. Moreover, several alternative hypotheses for why sex chromosomes stop recombining have been proposed, which are based on **meiotic drive** [24–26], **heterozygote advantage** [27,28], and **genetic drift** [29–31], respectively. Consequently, one should be cautious with routinely assuming that the sexual antagonism hypothesis applies to every sex chromosome system.

Here, we contrast the sexual antagonism hypothesis with the alternative hypotheses, outline why the support for any of them is surprisingly scarce, and, lastly, discuss future research directions to help disentangle how sex chromosomes evolve.

Hypotheses for the Evolution of Recombination Suppression

Sexual Antagonism

Sexual antagonism is hypothesised to favour the evolution of recombination suppression on sex chromosomes, because linkage between sexually antagonistic and sex-determining genes can resolve sexual conflicts by making it possible for the sexes to evolve towards their respective fitness optima [9,11–13,32].

Charlesworth and Charlesworth [9] concluded via modelling that sex chromosomes can evolve decreased recombination during the evolution of **dioecy** from **hermaphroditism** or **monoecy**, through a two-step mutation process where each mutation causes sterility in one sex (Figure 1A). The first mutation is likely to create females through a male-sterility mutation and thus to create **gynodioecy** (rather than to create males through a female-sterility mutation; **androdioecy**). In particular, females can be favoured to avoid inbreeding depression in partially selfing populations, which sometimes occurs in hermaphrodites [9]. Sexual antagonism is central to the second step of this model as it constitutes the selective pressure to convert hermaphrodites into males (i.e., investment in improved male function by avoiding an expensive female function). A sexually antagonistic (male-beneficial) mutation causing female sterility in hermaphrodites could make the population transition from gynodioecy to dioecy [5]. Dioecy is more easily established if the male- and female-determining genes are linked, because recombination would result in the production of **neuters** and hermaphrodites. Thus, the model predicts that recombination suppression evolves to prevent recombination between these sexually antagonistic and sex-determining genes [5,33].

Rice [11] extended this framework by evaluating when recombination suppression around a sex-determining locus is favourable as a function of the level of linkage, magnitude of sexual antagonism, and variation in dominance (Figure 1B). His model was specified for dioecious species with **genetic sex determination** and undifferentiated sex chromosomes, and is applicable to most animals that seem to have evolved genetic sex determination from an ancestral **environmental sex-determination** state [21,34]. The major finding was that the necessary linkage between the sexually antagonistic and sex-determining genes can be surprisingly loose for highly antagonistic mutations [11]. van Doorn and Kirkpatrick [13] showed that sexual antagonism can also drive **sex chromosome turnovers** and select for reduced recombination on the new sex chromosome, when an autosomal sexually antagonistic gene becomes linked to a sex-determining gene (Figure 1C). A similar process has been suggested to occur during **neo-sex chromosome** formation [12], because such fusions may bring sexually antagonistic loci into contact with the sex-determining gene (Figure 1D).

Glossary

Androdioecy: breeding system in which male and hermaphroditic individuals coexist.
Autosome: a chromosome that is not a sex chromosome.
Dioecy: male and female sex organs are in separate individuals, forming two separate sexes. Known as gonochory in animals.
Environmental sex determination: sex determination where sexual development is induced by an environmental cue (e.g., temperature or pH).
Evolutionary stratum: a (relatively large) region on the sex chromosome that has ceased to recombine at the same time and therefore shows similar level of XY (or ZW) divergence. Sex chromosomes typically have several strata that differ from each other in level of divergence. This is thought to indicate progressive block-wise recombination suppression.
Gametologs: homologous nonrecombining genes present on the different sex chromosome copies (e.g., one gametolog on X, and one on Y).
Genetic drift: random change in allele frequencies in a population from one generation to the next.
Genetic sex determination: sex determination by an inherited difference in alleles, genes, or linkage groups.
Gynodioecy: breeding system in which female and hermaphroditic individuals coexist.
Hermaphroditism: when an individual has both male and female sex organs.
Heterochromatinisation: transformation of genetically active euchromatin to inactive heterochromatin.
Heterozygote advantage: when the heterozygote genotype has higher relative fitness than either homozygote.
Meiotic drive: segregation distortion. An alteration of meiosis or gametogenesis caused by selfish genetic elements (e.g., a particular allele or chromosome) that leads to preferential transmission of itself. This may happen when the driver affects a responder locus on another chromosome so that the gamete/chromosome carrying the responder

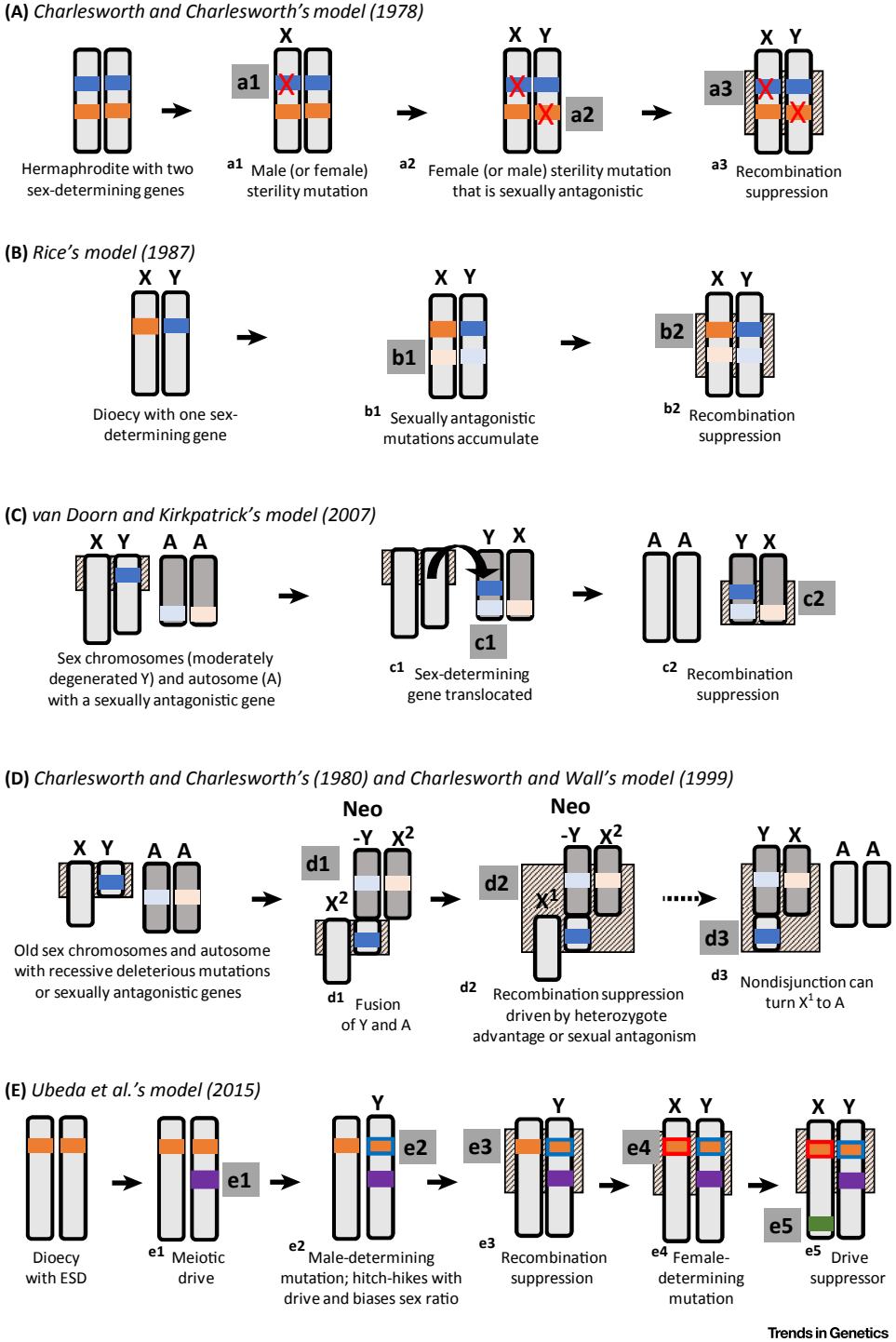

Figure 1. Illustrations of Some Models of Recombination Cessation on Sex Chromosomes. (A) Sex chromosomes evolve *de novo* in a hermaphroditic or monoecious species through a two-mutation process [9]. The first mutation creates females (or males), which is followed by a second, sexually antagonistic, mutation at a linked locus, creating males (or females). Recombination suppression evolves, because otherwise the antagonistic allele would end up in the wrong sex, and neuters and hermaphrodites would be created. (B) Sex chromosomes evolve in dioecious lineages through

(Figure legend continued on the bottom of the next page.)

is impaired. In sex chromosome drive, the sex chromosomes are transmitted unequally in the heterogametic sex.

Methylation: an epigenetic process in which a methyl group is added to the DNA sequence.

Monoecy: separate female and male flowers exist in the same plant individual.

Neo-sex chromosome: a sex chromosome formed by a fusion between the ancestral sex chromosome and an autosome.

Neuter: individual with neither female nor male reproductive organs.

Pseudoautosomal region (PAR): one or both ends of a sex chromosome that still recombine in the heterogametic sex.

Recombination suppression: a process that lowers the level of recombination.

Sex chromosomes: a chromosome pair that harbours sex-determining gene(s).

Sex chromosome turnover: change in which chromosome pair determines sex. Might sometimes lead to transitions in the sex-determination system (i.e., from XY to ZW).

Sex determination: a mechanism that creates the sexual phenotype of an individual.

Sexual antagonism: females and males have different fitness optima, which leads to selection in conflicting directions between the sexes.

Thus, the sexual antagonism hypothesis is applicable to all major scenarios for the evolution of sex chromosomes, that is, transition from hermaphroditism or monoecy [9], dioecy [11], turnovers [13], and neo-sex chromosome formation [12] (Table 1). That sex-linked antagonistic mutations seem to accumulate frequently provides among the strongest arguments in favour of the sexual antagonism hypothesis [15–20,35], together with recent data in guppies (*Poecilia reticulata*) suggesting that populations with stronger sexually antagonistic selection have larger nonrecombining region and more specialised sex chromosomes [36]. By contrast, the nonrecombining region in both turquoise killifish (*Nothobranchius furzeri*) [22] and Chilean strawberry (*Fragaria chiloensis*) [23] seems to lack sexually antagonistic genes, based on data from functional annotation databases. Likewise, a recent study on fungi mating-type chromosomes showed that recombination cessation had formed **evolutionary strata** comparable to these found on sex chromosomes [37]. As this species has no sex roles or essential differences between mating types, the observed stepwise recombination cessation must have resulted from other processes than sexually antagonistic selection [37].

Meiotic Drive

In hermaphroditic (monecious) or environmental sex-determination systems, an invading sex-determining mutation must overcome the fitness cost of skewing the sex ratio and becoming associated with the most common sex [4]. Linkage between a sex-determining factor and a meiotic drive could, because of the latter's transmission advantage that increases its frequency in the population [24], overcome this cost (induced by the skewed sex ratio) and favour recombination suppression. Sex chromosome drives have been observed in both male and female heterogametic systems [24,38], the former being more common possibly partly because more male heterogametic systems have been studied (e.g., *Drosophila* and mammals [24,38]). Typically the X chromosome drives against the Y, presumably because Y drives easily cause population extinction [24].

Ubeda *et al*. [26] modelled how meiotic drives can give rise to novel sex chromosomes and genetic sex determination in dioecious populations with environmental sex determination, and in outbreeding hermaphrodites (Figure 1E). Their model had three loci: a sex-determining gene, a meiotic driver, and a suppressor. The meiotic driver was associated with deleterious alleles, which agrees with the observation that meiotic drives often carry fitness costs in homozygous form [24]. They showed that a male-determining allele can become established when linked to a driver, which causes male-biased sex ratios. A recessive female-determining allele can then invade. This creates a situation where an unlinked suppressor is favoured in the heterogametic sex, which restores balanced segregation, causes even sex ratios, and gives rise to an XY system (or ZW, if a female-determining allele is linked to the driver). Reduced recombination between the sex-determining gene and the driver is favoured as a means of inhibiting

linkage between sex-determining and sexually antagonistic genes [11]. A sexually antagonistic mutation is established close to a sex-determining gene. Reduced recombination is advantageous because otherwise the antagonistic allele would occur in the wrong sex. (C) A new sex chromosome pair evolves through a turnover [13]. The sex-determining gene is translocated to (or is generated on) an autosome that carries sexually antagonistic genes. Linkage is advantageous and selects for reduced recombination. (D) A neo-sex chromosome is formed when the ancestral sex chromosome fuses with an autosome. Illustrated is a case of Y-to-autosome fusion, where linkage and reduced recombination is favoured to maintain linkage between advantageous combinations of genes (due to sexual antagonism or heterozygote advantage) [12,28]. (E) Sex chromosomes evolve in a dioecious lineage through linkage between a meiotic drive locus and a sex-determining locus. A male-determining mutation is established in linkage with a drive locus, which causes biased sex ratios. This is followed by invasion of a female-determining allele. Recombination is suppressed to avoid homozygosity and expression of recessive deleterious alleles at the drive locus. Suppressors (sex linked or autosomal) may invade to balance the sex ratio [26].

Table 1. Models and Support for the Hypotheses of Recombination Suppression in Different Evolutionary Scenarios of Sex Chromosome Evolution (Mating System and Sex Chromosome Origin)[a]

Mating system	Sex chromosome origin	Hypotheses for recombination suppression (RS)			
		Sexual antagonism (SA)	Meiotic drive (MD)	Heterozygote advantage (HA)	Genetic drift
Hermaphroditism or monoecy	*De novo*	Model: SA favours RS between two SDGs [9]. Data: Sex-determining systems in several plant species in line with this scenario (see [9]).	Model: Establishment of linked, novel MD and SDG selects for RS [26]. Data: Not available.	Model: Not modelled. Data: Not available.	Model: Not modelled. Data: Not available.
Dioecy	*De novo* (environmental sex determination)	Model: SA attracts GSD, which favours RS (interpretation of [13,39,66]). SA favours RS between two SDGs [34]. Data: Not available.	Model: Establishment of linked, novel MD and SDG selects for RS [26]. Data: Not available.	Model: Not modelled. Data: Not available.	Model: Not modelled. Data: Not available.
Dioecy	GSD (homomorphic)	Model: RS favoured as SA mutations accumulate around (a novel) SDG [11]. Data: Experiments in *Drosophila* show that SA accumulates close to a novel SDG [15]. Strength of SA correlates with extent of RS in guppy populations [36].	Model: Possible in principle, but not modelled. Data: Linkage between MD and SDG in mosquitoes [43].	Model: Possible in principle, but not modelled. Data: Not available.	Model: Not modelled, but mechanisms causing RS (e.g., inversions) may become established in small populations [29,30]. Data: Not available.
Dioecy	Turnover and/or transition	Model: Linkage between SA genes and SDG drives SC turnover/ transition [13,66]. Data: Sexual conflict over colour genes in cichlids resolved by linkage with novel SDG, causing XY to ZW transition [67].	Model: Linkage between SDG at different chromosome establishes close to MD, which can select for RS (interpreted from [26,39]; see also [25]). Data: Not supported.	Model: Not modelled. Data: Not available.	Model: Drift and sex ratio selection favour novel SDG (without individual selection advantage) [68], but RS not modelled. Data: Not available.
Dioecy	Neo-sex formation	Model: Chromosome fusion linking SDG with SA loci advantageous [12]. Data: Neo-sex chromosome formation in stickleback links male beneficial traits with SDG [35]. *Drosophila* neo-sex chromosomes enriched for SA [20].	Model: Fusion between SC and autosome with MD, which can select for RS (interpreted from [26,39]). Data: Not supported.	Model: HA drives fusion between autosome and SC, and causes RS [28]. Data: Neo-sex chromosomes common in highly inbreeding termite species [44,45].	Model: Drift more easily causes sex-linked than autosomal underdominant chromosomal rearrangements [31]. Data: Not available.

[a]Abbreviations: GSD, genetic sex determination; SC, sex chromosome; SDG, sex-determining gene.

production of meiotic drive homozygotes. This model applies also to sex chromosome turnovers and neo-sex chromosome formation if these events bring the sex-determining gene into the vicinity of the driver [26] (see also [25,39]).

Meiotic drive systems often include multiple genes necessary for expressing the drive (e.g., enhancers, insensitive alleles preventing drive against itself, and suppressors-of-suppressors) [40]. Coadaptation of such gene complexes could be an additional force favouring reduced recombination around meiotic drives [24,41]. Interestingly, inversions are often associated with meiotic drives and may be the mechanism that prevents recombination [24,41].

The meiotic drive hypothesis can be applied to the main evolutionary scenarios (Table 1), and is indirectly supported by the occurrence of sex-linked meiotic drives in some species, for example, *Drosophila simulans* [42], and by tight linkage between a drive and the sex-determining gene in *Aedes* mosquitos [43]. However, for the majority of cases there is no evidence of meiotic drives.

Heterozygote Advantage

Heterozygosity increases fitness by concealing recessive deleterious mutations and causing overdominance at functional loci [28]. Thus, it can be hypothesised that heterozygote advantage around a sex-determining gene in the heterogametic sex can favour recombination suppression (because less recombination means a larger heterozygote region). A challenge is however to explain how the sex-determining gene can be associated with a sufficient amount of inbreeding load (deleterious recessives) for the heterogametic sex to overcome the fitness costs of establishing genetic sex determination in the first place (induced by the skew in sex ratio, mentioned earlier; cf. [27]). A possible scenario is that the sex-determining gene is part of a larger rearrangement (reciprocal translocation or inversion) that captures a suite of loci carrying deleterious mutations, which then becomes fixed for heterozygosity in the heterogametic sex, which therefore experiences higher fitness due to heterozygote advantage. This implies that heterozygote advantage in principle is a possible agent of sex chromosome formation in evolutionary scenarios where inbreeding avoidance is favourable (Table 1). Heterozygote advantage has been suggested to favour the formation of neo-sex chromosomes (Figure 1D) in highly inbreeding species [28], and consistently neo-sex chromosomes have evolved repeatedly in some highly inbreeding species of animals such as termites [44,45].

Genetic Drift

Chromosomal rearrangements and mutations that prevent recombination on sex chromosomes may increase in frequency just by chance. For example, one can imagine an inversion that affects the expression of a gene involved in the sex-determining pathway and at the same time causes recombination suppression over the involved region. However, inversions are often associated with heterozygous disadvantage (or underdominance), generated by structural abnormalities during meiosis, and are therefore often negatively selected when in minority [29,30]. Thus, they are more likely to become established in small and isolated populations where genetic drift may increase their frequency [29,30].

When the nonrecombining region of sex chromosomes has already been formed, the spread of recombination suppression may continue without selection, either through drift-induced inversions or accumulated dissimilarities [21]. In this way, heteromorphic sex chromosomes with low recombination around the PAR may diverge gradually through neutral mutations, which leads to further spread of recombination suppression [21]. Likewise, drift may cause recombination suppression in neo-sex chromosomes [31], in particular if the neo-sex chromosome is translocated to the nonrecombining part of the ancestral sex chromosome, instead of the PAR [5].

The genetic drift hypothesis can be applied to some of the evolutionary scenarios listed in Table 1, but requires small population sizes and has limited support [29,30].

Why the Hypotheses Lack Strong Support

A main challenge for understanding why sex chromosomes evolve recombination suppression is that experiments and direct tests are difficult to construct for most of the hypotheses. Even the well-designed experiments in *Drosophila* (e.g., [15]) may not be conclusive regarding the sexual antagonism hypothesis, because (as mentioned earlier) mutations may have accumulated after recombination had already ceased. Moreover, sexual antagonism or inbreeding depression might be expressed only under natural conditions, implying that greenhouse experiments might bias the conclusions about the relative importance of the different hypotheses. In the absence of strong experimental support, we are left with interpretations of indirect evidence, which has several drawbacks.

First, evolutionary conditions favouring different hypotheses may overlap, which means that we are not searching for a single hypothesis. For example, the sexual antagonism hypothesis is obviously relevant in species with strong sexual conflict [37,46], because more sexually antagonistic genes increase the likelihood of linkage between such genes and the sex-determining gene. However, species with strong sexual conflict may also distinguish themselves in other ways. For instance, they may have a polygynous mating system, which deceases the effective population size and increases genetic drift, or may experience sex-specific mortality, which skews the sex ratio and thus favours meiotic drives. Moreover, single evolutionary conditions (e.g., small population size) are sometimes associated with several processes (e.g., drift and heterozygote advantage). Similarly, populations fluctuate spatially and temporally, and populations that are large and thriving today may have had a history of reduced population size and inbreeding that may go unnoticed. Potential effects related to complex demographic processes or fluctuating selection regimes are difficult to measure and are thus often neglected in studies of sex chromosome evolution [8,47].

Second, processes may be difficult to verify even when present. Putative sexually antagonistic traits can be rather easy to pinpoint [11], but it is often difficult to determine the traits' specific genetic basis and at what life stage(s) antagonistic selection actually occurs [48]. Moreover, in situations where antagonism is partially resolved, small effect sizes would require large sample sizes to be detected. A different problem relates to using gene annotation databases, which are mainly based on functional studies in laboratory environments, for drawing conclusions about the function of specific genes in natural situations. For example, even when a small number of genes are pinpointed as candidates for driving recombination suppression (e.g., [22,23]), their functions and importance may be difficult to interpret due to nonrelevant annotations (e.g., they may be sexually antagonistic but not annotated as such).

Third, different processes are expected to leave similar chromosomal signatures. Rearrangements such as inversions reduce recombination and cause evolutionary strata [21,49], but it is difficult to know whether the rearrangement was established by sexual antagonism, meiotic drive, heterozygote advantage, or even drift. Other chromosomal mechanisms of recombination suppression, including transposable elements and **heterochromatinisation**, have also been linked to several of the hypotheses (Box 1).

Finally, processes may continue after sex chromosome formation, or be transient, which poses a particular problem when interpreting old heteromorphic sex chromosomes. Both sexually antagonistic genes and meiotic drives are expected to accumulate on established sex

Box 1. Mechanisms of Recombination Suppression

Recombination can be supressed either though chromosome rearrangements (e.g., inversions or translocations) or through gradual reduction in crossover frequencies due to, for example, heterochromatinisation [21]. As sex-limited chromosomes are typically enriched for rearrangements as well as heterochromatin, research on newly formed sex chromosome systems is needed to understand how the initial recombination suppression was established.

Rearrangements within a sex-determining region can effectively reduce recombination rates. Inversions have been suggested to be especially important reducers of recombination, as they have been found to span across the sex-determining region in many taxa and may increase linkage even between relatively distant alleles [49,65,69]. Inversions and translocations could also lower recombination rates by physically moving sex-determining genes to already recombination-poor regions. Rearrangements are undoubtedly important mechanisms for reducing recombination rates between sex chromosomes. However, they are facilitated by low recombination rates, meaning that the presence of rearrangements may be an effect rather than the cause [7,70].

Studies of sex chromosomes without rearrangements reveal gradual development of recombination cessation as an alternative mechanism [71,72]. Transposable elements are central for the degeneration process of the sex-limited chromosome, and frequently accumulate in sex-determining regions [69,73–75]. By causing insertions and duplications in genic regions, they can cause gene silencing or alternations in gene function and expression [76,77]. Transposable elements have therefore been hypothesised to be able to not only cause recombination suppression but also simultaneously create the sex-determining genes [70,73].

Transposable elements can also trigger heterochromatinisation, due to selection for removing invasive DNA through epigenetic silencing mechanisms such as **methylation** [78,79]. This process has been proposed as an alternative mechanism for recombination suppression between sex chromosomes when methylation leads to sex-linked heritable methylation patterns [80]. Heterochromatinisation and the accumulation of repeat elements are however also facilitated by low recombination rates, potentially formed through a rearrangement [5,81]. Finally, transposable elements can lead to recombination suppression by facilitating chromosomal rearrangements [82].

chromosomes due to adaptations to sex-specific environments, which makes it difficult to tell whether any of them was present also during the evolution of recombination cessation (cf. [39]). Meiotic drives are additionally thought to often be transient [24,40], which further complicates verifying their role in sex chromosome evolution. Furthermore, mechanisms causing recombination suppression (e.g., inversions) may also be involved in subsequent differentiation once recombination has halted.

Distinguishing the Hypotheses

Sex chromosome research has a strong modelling history and these efforts continue to generate important insights. Ubeda *et al.*'s [26] recent meiotic drive model is a good example. Others include demonstrations that recombination suppression between the sex-determining gene and linked loci under selection may evolve even when the direction of selection is the same between sexes (no antagonism) but differs in strength [50], and that recombination suppression may be selected against under certain conditions, counteracting the evolution of classical nonrecombining sex chromosomes [51]. The effects of ecology on sex chromosome evolution are challenging to study empirically, and models may bring clarity to the role of demography (e.g., range shifts) and environment (e.g., local selection pressures) in the context of sex chromosome evolution [47,52,53]. Models generating predictions to distinguish the different hypotheses that can be tested empirically are urgently needed.

Population genomics and improved sequencing methods offer other avenues for evaluating hypotheses. This is becoming increasingly feasible as long-read sequencing technologies facilitate assembling complex genomic regions such as sex chromosomes [54], and thereby resolving their gene content as well as rearrangements and repeat acquisition. Characterising the sex-linked region is a first step towards making conclusions regarding this region in light of the hypotheses. Analyses of signs of selection in sequence data of **gametologs** within and

Box 2. Recent Results from Young Sex Chromosome Systems

Characterisation of young sex chromosomes uncovers a surprising heterogeneity regarding the early stages of sex chromosome evolution. This heterogeneity is potentially a strength that allows study of many aspects of sex chromosome evolution, but also implies that we need to be cautious with expecting general patterns.

In plants, the Chilean strawberry (*Fragaria chiloensis*) has a 280-kb ZW sex-determining region with lower maternal than paternal recombination rate. The ZW divergence is elevated within the sex-determining region, but only a single polymorphism is in linkage disequilibrium with sex [23]. Spinach (*Spinacia oleracea*) has moderately heteromorphic sex chromosomes and the Y locus is located in the centromeric region, which suggests that it has been hitch-hiking with a region of already suppressed recombination [83]. A second locus, 'monoecious', determining dioecy versus monoecy, is located on the same chromosome as Y but is not tightly linked (13 cM). Papaya (*Carica papaya*) has two different Y chromosomes controlling the development of males and hermaphrodites, respectively. The hermaphrodite Y carries two large inversions, the first of which likely causes recombination suppression between X and Y, supporting the role of inversions in recombination suppression [69].

In fish, the medaka (*Oryzias latipes*) sex-determining gene (*Dmy*) has been formed through duplication of the male-related autosomal *Dmrt1* [84], and might explain recombination suppression [85]. In the platyfish (*Xiphophorus maculatus*), expansion of a repetitive element (XIR) near the male-determining factor may be one of the first molecular events of the evolution of recombination suppression [86]. The turquoise killifish (*Nothobranchius furzeri*) shows a between-population Y polymorphism with one population having a very small sex-determining region (196 kb) [22]. This region contains a gene (*gdf6*) that likely is sex determining. There are neither inversions nor potentially sexually antagonistic genes in this region, but a 241-bp deletion may tentatively be the primary cause of recombination suppression. Moreover, major lifespan loci are located close to the sex-determining region. This raises the question of whether sex-determination and lifespan genes have coevolved to couple strategies of fast reproduction and overall fitness? Alternatively, suppressed recombination may have allowed lifespan traits to hitch-hike with sex determination, without any direct fitness benefit *per se* [87]. Recently, Wright *et al*. [36] showed that guppy (*Poecilia reticulata*) populations with higher levels of sexually antagonistic selection had a more extended nonrecombining region, supporting the role of sexual conflict in driving recombination cessation.

between species (tested with, for example, Tajima's D, dN/dS, and F_{ST}) may support the idea that selection drives sex chromosome evolution (and thus exclude genetic drift), but do not necessarily reveal which type of selection has been operating [18–20,55]. However, results of such genome scans can be coupled with gene annotations of increasing quality and relevance, and functional verification (e.g., with recent gene editing tools; cf. [56]), to distinguish among different selection-based hypotheses. Species with young sex chromosomes, restricted non-recombining regions, and few sex-linked genes (e.g., many plants and fishes; Box 2) would be particularly suitable for such tests. Moreover, improved sequencing and assembly methods may facilitate characterising the sex-determining genes and how they are linked and organised on the chromosomes. This in turn would be informative for distinguishing models predicting at least two linked sex-determining loci [9] from those based on a single sex-determining locus with linked sex-specific alleles [11,26].

Sequence-based methods may also improve our understanding of meiotic drives, which are often ephemeral and difficult to study [26]. Traditionally, meiotic drives have been detected with transmission distorter analysis [41]. More and more sex-linked meiotic drives are being molecularly functionally characterised [40]. For example, protein-induced heterochromatinisation coded by the X-linked gene *HP1D2* has been suggested to be involved in female-bias meiotic drive in *D. simulans* by preventing the segregation of Y chromatids during meiosis II [42] (for other examples see [57,58]). If distinct genomic signatures can be attributed to meiotic drives (and suppressors) [24,40], unknown drives may be detected in available assemblies, which in turn may help testing their prevalence on sex chromosomes. Such tests are preferably conducted in species with known but previously uncharacterised meiotic drives (e.g., wood lemming *Myopus schisticolor* [59]) and in species with skewed sex ratio (e.g., the plant *Silene diclinis* [60]).

Sex chromosome recombination patterns and degree of degradation differ between taxa [2], and by using comparative approaches one can make use of this heterogeneity. Comparative phylogenetics in lineages with variation in sex determination, sex chromosome systems, degree of sexual selection, and/or mating system may reveal evolutionary prerequisites during sex chromosome formation. Poeciliidae fishes may be suitable for such tests [36,61]. For instance, the sexual antagonism hypothesis suggests an association between sexual selection and sex chromosome evolution. Furthermore, divergence times and historical population sizes can be modelled using coalescence approaches to understand demographic effects and influence of effective population size during sex chromosome evolution, thereby potentially providing insights into the importance of heterozygote advantage and genetic drift. Comparing broadly taxonomically diverged taxa with evolutionarily young sexual systems is a promising avenue for understanding under which ecological and evolutionary circumstances nonrecombining sex chromosomes are formed and thus distinguishing the relative importance of the different hypotheses.

Finally, experiments testing each hypothesis separately are requested. Experiments with sex-specific evolution have provided some of the strongest support for the accumulation of sexual antagonism on sex chromosomes [15]. Repeating these experiments in species with homomorphic sex chromosomes (with little differentiation and large PARs, such as guppies that harbour potentially sexually antagonistic colour genes outside the nonrecombining region [61]) would be valuable because this would allow testing the prediction from the sexual antagonism hypothesis that sexually antagonistic mutations accumulate in the recombining region of the sex chromosomes, prior to the evolution of recombination suppression [6,10]. Experiments for the other hypotheses are probably more difficult to conduct since they would require manipulating meiotic drives, population sizes, or mating systems. In plants with young sex chromosomes, experimentally created homozygotes for the nonrecombining sex chromosome, YY or WW individuals, may be possible [62], and could reveal the presence of deleterious recessive mutations during controlled conditions, and thus support the heterozygote advantage hypothesis. Likewise, experimental crosses between individuals from populations with different meiotic drive and suppressor genes (such as in some insects and plants [63,64]) may make it possible to manipulate the dynamics of sex chromosome drives, to gain insights into the importance of the meiotic drive hypothesis.

Concluding Remarks

Theory outlines several alternative routes and mechanisms for the evolution of recombination cessation on sex chromosomes, which may also apply to its spread once recombination suppression is initiated [6]. We believe that hypotheses based on selection rather than drift are most likely – because sex chromosomes have evolved in parallel in separate lineages [1–4,65] – and among these, sexual antagonism and meiotic drive are the strongest candidates. The heterozygote advantage hypothesis requires significant inbreeding load around the sex-determining gene, and thus applies during restricted circumstances. We agree that sexual antagonism is a particularly likely hypothesis since it is supported by most theories and some data [15–20,36], but cases failing to find support, or at least without clear support, are accumulating [22,23,37]. Modelling work outlining testable predictions, population genomics providing chromosomal characterisation and functional understanding, comparative phylogenetics revealing evolutionary patterns, and experiments distinguishing between hypotheses will be necessary to understand the evolution of nonrecombining heteromorphic sex chromosomes from their ancestral autosomal state (see Outstanding Questions).

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Outstanding Questions

Is sexual antagonism the main driver of sex chromosome formation? The sexual antagonism hypothesis is by far the most well-cited hypothesis for the evolution of recombination suppression. This hypothesis needs substantially stronger support before other hypotheses are routinely neglected.

How frequently does meiotic drive cause recombination cessation on sex chromosomes? Recent models have suggested that meiotic drives may be more important for sex chromosome formation than previously suggested. More work is needed, but the ephemeral nature of meiotic drives makes them difficult to study.

How far can new sequencing technologies take us to understanding sex chromosome evolution? Future research will benefit from long-read sequencing technologies by making it possible to assemble complex regions such as the sex chromosomes. However, sequencing data are good at describing patterns, but the underlying evolutionary processes are difficult to infer.

How do we distinguish among hypotheses for recombination suppression on sex chromosomes? Young sex chromosome systems are being explored at increasing rates and will continue to provide important insights into recombination suppression. However, given that the processes are complex, often overlapping, and sometimes ephemeral, it is likely that a combination of approaches, including evolutionary modelling, comparative approaches, population genomics and functional verification, and experiments, will be necessary to understand sex chromosome evolution.

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