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Sex-antagonistic selection, deleterious mutations and the evolution of sex chromosomes

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Sex-antagonistic selection, deleterious mutations and the evolution of sex chromosomes

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par

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Sex-antagonistic selection, deleterious mutations and the evolution of sex chromosomes

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Summary

It is generally accepted that sex chromosomes evolved from a pair of autosomes after the appearance of a sex determining gene. Accumulation of sex antagonistic genes in the vicinity of the sex determining gene induces arrest of recombination in this region, leading to the degeneration of sex chromosomes due to the accumulation of deleterious mutations. This model explains the highly differentiated sex chromosomes found in mammals and birds, but it does not account for the sex chromosome homomorphy of most other vertebrates. Occasional recombination in the heterogametic sex and turnover events have been proposed to explain the presence of homomorphic sex chromosomes. However, what determines the path towards one state or the other remains currently unclear. In this thesis we simulate various scenarios to understand the key factors leading to the observed diversity of sexual chromosomes. In particular, we investigate how the sex determination system (which is known to be very different among close related species when those species have homomorphic sex chromosomes) and the mechanism regulating recombination interplay with other factors to lead to this diversity. In chapter one, modeling the occurrence of sex antagonistic alleles and the accumulation of deleterious mutations, we show that a complete arrest of recombination is never beneficial to the heterogametic sex. This finding suggests that if recombination is regulated by a modifier, a rate of recombination different from zero should be maintained. In chapter two, we observe that the position of the recombination modifier relative to the sex chromosome also matters. We indeed find that when the modifier is autosomal, an arrest of recombination is favoured when sex antagonistic selection is strong, while when the modifier is sex-linked, X and Y chromosomes fix different alleles, coding respectively for zero or low recombination rate. In chapter three, we investigated how sex antagonistic selection and recombination regime can influence the transition between different sex determining systems and the maintenance of a polymorphism. Interestingly, a polymorphism between genetic and non-genetic sex determination can be maintained if sex antagonistic selection is strong and unbalanced between the two sexes. Finally, it is known that the high rate of turnover experienced by homomorphic sex chromosomes also contributes to speciation

events. In this context, in chapter four we analyzed the effects of incompatibilities between sex-linked and autosomal mutations in the hybridization process. Different incompatibilities show different patterns of introgression, and in particular we show that the introgression of a dominant allele can happen independently of its benefits.

Résumé

Les chromosomes sexuels ont évolué à partir d'une paire d'autosomes identiques, où un gène déterminant le sexe est apparu. L'accumulation de gènes sexuellement antagonistes proche du gène déterminant le sexe induirait l'arrêt de la recombinaison dans cette région, conduisant à la dégénérescence des chromosomes sexuels par un processus d'accumulation de mutations délétères. Bien que ce modèle explique les chromosomes sexuels hautement différenciés trouvés chez les mammifères et les oiseaux, il n'explique pas l'homomorphie des chromosomes sexuels observée chez la plupart des vertébrés. Une recombinaison occasionnelle dans le sexe hétérogamétique et des événements de turnover ont été proposés pour expliquer ces caractéristiques. Cependant, ce qui détermine le chemin vers l'un ou l'autre état des chromosomes sexuels reste actuellement peu clair. Dans cette thèse, nous simulons différents scénarios pour comprendre le facteur clé menant à la diversité des chromosomes sexuels observés. En particulier, nous étudions comment le système de détermination du sexe (qui est connu pour être très différent parmi les espèces apparentées proches lorsque ces espèces ont des chromosomes sexuels homomorphes) et le mécanisme régulant la recombinaison interagissent avec d'autres facteurs pour mener à cette diversité. Dans le premier chapitre, en modélisant l'apparition d'allèles sexuellement antagonistes et l'accumulation de mutations délétères, nous montrons qu'un arrêt complet de la recombinaison n'est jamais bénéfique au sexe hétérogamétique. Ce résultat suggère que si la recombinaison est régulée par un modificateur, un taux de recombinaison différent de zéro devrait être maintenu. Dans le chapitre deux, nous observons que la position du modificateur de recombinaison par rapport au chromosome sexuel est également importante. Nous trouvons en effet que lorsque le modificateur est autosomique, l'arrêt de la recombinaison est favorisé lorsque la sélection sexuellement antagoniste est forte, tandis que lorsque le modificateur est lié au sexe, les chromosomes X et Y fixent des allèles différents codant pour un taux de recombinaison respectivement nul et faible. Au chapitre trois, nous étudions comment la sélection sexuellement antagoniste et le régime de recombinaison peuvent influencer la transition entre différents systèmes de détermination du sexe et le maintien d'un polymorphisme. Fait

intéressant, un polymorphisme entre la détermination du sexe génétique et non-génétique pourrait être maintenu si la sélection sexuellement antagoniste est forte et déséquilibrée entre les deux sexes. On sait que le taux élevé de turnover des chromosomes sexuels homomorphes contribue également à la spéciation. Dans ce contexte, nous avons analysé dans le chapitre quatre les effets des incompatibilités entre les mutations liées au sexe et les mutations autosomiques dans le processus d'hybridation. Différentes incompatibilités montrent différents modèles d'introgression, et en particulier nous montrons que l'introgression d'un allèle dominant peut se produire indépendamment de ses avantages.

General introduction

Since the discovery of the "X" chromosome, the study of sex chromosomes and their evolution has become a hot topic in evolutionary biology. Experimental and theoretical studies complement each other to unravel some of the not yet understood features of these chromosomes. While some general steps concerned in the evolution of sex chromosomes are well understood, others remain unclear. The study of sex chromosomes finds its origins in 1891, when the biologist Hermann Henking noticed that the sperm cells of the fire bug (*Phyrrhocoris apterus*) had 12 chromosomes while others 11, and that this 12th chromosome looked different from the others. He hypothesized that this extra chromosome plays a role in sex determination, and called it "X" for unknown (Henking 1891). A decade later, the zoologist McClung and his student Walter Sutton further observed (independently) that chromosomes were not equal in number between males and females, and that the "X" element was connected to sex determination (McClung 1902, Sutton 1902). At the same time, the geneticists Nettie Stevens in beetles and E. B. Wilson in multiple insect species observed that in male cells the chromosomes within one pair were different in size: it turned out they were observing the X and Y chromosomes (Stevens 1905, Wilson 1905).

Nowadays, we call the chromosomes carrying the genes that determine sex the "sex chromosomes". Sex chromosomes have evolved independently multiple times in vertebrates (Matsubara et al. 2006, Bellott et al. 2010). Contrary to what was thought in the late 60s, we know today that the sex chromosomes of mammals, birds and snakes have independent origin; moreover, sex chromosomes evolved multiple times even in the same class, like in reptiles and amphibians, and in fish (Bull 1983). Sex chromosomes are found today in a heteromorphic state, like the ones first observed, or in a homomorphic state. Most mammals and birds have heteromorphic sex chromosomes, which are highly differentiated chromosomes (Ohno 1967, Lahn and page 1999, Lahn, Pearson and Jegalian 2001). In these species, one sex is always homogametic (XX females in mammals and ZZ males in birds), while the other is heterogametic (XY males in mammals and ZW females in birds). On the other hand, most cold-blooded vertebrates have homomorphic sex

chromosomes, chromosomes that are cytogenetically identical and indistinguishable (Devlin and Nagahama 2002, Eggert 2004, Schartl 2004, Bellott and Page 2009). Sex chromosomes are subject to different evolutionary forces compared to autosomes, because they spend unequal time in each sex, and they can play an important role in processes such as speciation and adaptation (Rice 1984, Charlesworth et al 1987, Charlesworth and Charlesworth 2000).

Theories on the evolution of sex chromosomes

The canonical model

The most accepted theory on sex chromosomes evolution was proposed by Muller in 1914 (Muller 1914), and it argues that sex chromosomes evolved from a pair of autosomes. A mutation on an autosomal gene into a sex determining gene is the first step into the evolution of a sex chromosome. Individuals carrying this mutation would develop into one sex, and those without into the other sex. This proto-sex chromosome harbours now a hotspot for sex-antagonistic (SA) genes in the vicinity on the sex-determining (SD) gene (Fisher 1931, Rice 1987). Sex-antagonistic genes are genes that are beneficial to one sex, but detrimental to the other. In the guppy *Poecilia reticulata*, for example, being colourful is advantageous for males while costly for females (e.g. Endler 1980). Females are more attracted to colourful males, while males do not have specific female colour preferences, and being colourful increases the predation probability. Colouration genes are in this case sex-antagonistic, conferring a benefit to one sex but being detrimental to the other. When a sex-antagonistic mutation appears close to a male-determining gene, it will be transmitted more often to males than females. In such conditions, this mutation could spread in the population even if its costs in females are higher than the benefits in males. Consequently, reduced or suppressed recombination between the SA mutation locus and the SD locus is favoured, because it will reduce the transmission of male-beneficial mutations to females (Bull 1983, Rice 1987). Another SA mutation occurring near this non-recombining region will repeat the process just described, increasing the size of the non-recombining region. Progressively, the non-recombining region will encompass the whole chromosome, which will degenerate and differentiate (Rice 1996). The arrest of recombination probably happened in multiple steps, as the different evolutionary strata found on the human Y

chromosome suggest. These "strata" are different regions of the sex chromosomes that show different level of divergence between X and Y, suggesting that the arrest of recombination between X and Y happened through multiple inversions involving different part of the chromosome. Lahn and Page (1999) identified four of such strata on the human Y chromosome, the oldest of which contains the master male-determining gene of mammals, and a fifth one was afterwards identified by Ross et al. (2005). A chromosome that does not recombine is subject to the irreversible accumulation of deleterious mutations, a process called Muller's ratchet (Muller 1950), and it will incorporate less beneficial mutations (Ruby in the rubbish, Peck 1994). Background selection and Hill-Robertson interference also contribute to the degeneration of the non-recombining sex chromosomes (Y and W), which experience a smaller effective population size compared to their homologous (X and Z) and to autosomes (Hill and Robertson 1966, Charlesworth 1994). The Y mammalian chromosome and the W avian chromosome are indeed highly degenerated and differentiated from the X and the Z chromosome respectively. For example, the human Y chromosome euchromatic part is around 23 Mb and contains 78 protein-coding genes, while the X one is of 150 Mb with around 800 protein-coding genes (Bachtrog 2013). The heteromorphy of these chromosomes is a consequence of the deletion of segments of non-functional DNA or the accumulation of repetitive DNA, which result respectively in a reduction or increase of the physical size of the Y and the W chromosomes. Both the accumulation of loss-of-function mutations and of repetitive DNA are consequences of the lack of recombination of these chromosomes. Most of the genes that remained on the Y should be male beneficial or "essential" male genes (Lahn et al. 2001), and dosage compensation mechanisms have evolved to counterbalance the lack of genes on the degenerated chromosome (Ohno 1967, Charlesworth 1978, 1996, Jegelian and Page 1998). The degeneration of the Y (or W) chromosome might eventually result in its complete loss, as suggested by the presence of species with an XO (or ZO) system (Makino 1951, Blackmon and Demuth 2014).

And what about homomorphic sex chromosomes?

The model described above leads to highly heteromorphic and degenerated sex chromosomes. Although these chromosomes have been intensely studied and receive the attention of many

researchers, most vertebrates have homomorphic sex chromosomes. Most amphibians, as well as many lineages of fish and non-avian reptiles have chromosomes that have not differentiated and result morphologically identical (e.g. Bellott and Page 2009). Two main hypotheses have found support to explain how these sex chromosomes are maintained homomorphic, namely the fountain of youth hypothesis and the turnover hypothesis (Stein et al. 2002, Schartl 2004, Miura 2007, Volff et al. 2007, Perrin 2009, Guerrero et al. 2012). While the former allows for occasional recombination in the heterogametic sex, the latter considers the re-generation of a sex chromosome by autosomes.

The fountain of youth hypothesis is based on two conditions: firstly, individuals can sex-reverse, secondly, recombination depends on phenotypic rather than on genotypic sex. Both these conditions are met in many cold-blooded vertebrates. In some fishes as well as in some amphibians, sex reversal occurs in natural conditions (Dournon et al. 2003 Baroiller et al. 2009, Rodrigues et al. 2018). Sex reversal means that a genotypic male or female can occasionally develop into the other sex. Additionally, recombination seems to be controlled by phenotypic sex rather than genotypic sex in many cold-blooded vertebrates (e.g. Kondo et al. 2001, Matsuba et al. 2010, Rodrigues et al. 2018). This entails that a genotypic male (XY) developing into a female (sex reversal), has the same recombination rate of an XX female. In the medaka fish *Oryzias latipes*, XY females show the same recombination pattern as XX females (Kondo et al. 2001). In many amphibians like in the common frog *Rana temporaria* or in the European tree frogs (group *Hyla*), we observe the same (Guerrero et al. 2012, Rodrigues et al. 2018). Indications of occasional recombination events are well exemplified by the European tree frogs. Stock et al. (2011) showed that three species in the *Hyla* group have the same pair of sex chromosomes, and males do not recombine. However, the X and Y chromosomes show no signs of divergence, and alleles on the X and Y chromosomes cluster by species and not by gametologs. This basically means that the X and Y chromosomes of each species are more similar between them than to the X and Y chromosomes of the other species, although they share the same X and Y ancestors. The authors concluded that the X and Y homomorphy was due to occasional XY recombination. However, recombination between X and Y can happen only in sex-reversed individuals, because males do not recombine. Sex-reversal is rare but it seems enough to maintain the

sex chromosomes homomorphic. Moreover, rare events of recombination can be sufficient to purge the Y chromosome from its load, as shown in simulation studies. These males might suffer from reshuffling of sex-antagonistic genes, but they will benefit from a lower load of deleterious mutations (Grossen et al. 2012, Cavoto et al. 2018). Because of their rarity, information on the recombination status of sex-reversed individuals is difficult to document. However, recently an XY female of the common frog *R. temporaria* has been found (Rodrigues et al. 2018), and it was shown that the individual recombined as much as an XX female.

The other mechanism suggested for the occurrence of homomorphic sex chromosomes is turnovers. Turnover events occur when an autosome takes over the sex determining function of a sex chromosome and they can involve a *de-novo* mutation or a translocation of the old sex-determining gene to the autosome. The autosome becomes the new chromosome, and the cycle can repeat. Turnovers can be caused by drift, or driven by sex-antagonistic selection, mutation load, or sex-ratio selection (Bull and Charnov 1977, van Doorn and Kirkpatrick 2007, 2010, Blaser et al. 2013). A combination of sex-antagonistic selection and mutation load can lead to infinite cycles of turnovers (Blaser et al. 2013). We can find a fitting example of this process again in amphibians, where closely related species have different pairs of sex chromosomes, or different sex determination systems even co-occur in the same species (Miura et al. 2007). In the Japanese frog *R. rugosa*, both an XY and a WZ system are found. One hypothesis to account for this is the high rate of turnover of sex chromosomes. In some species, indeed, the shift between chromosomes carrying the sex determining gene is quite easy. These transitions might be easier in species where sex determination follows a less strict path.

These two hypotheses do not have to be considered mutually exclusive, but can actually act together, like it has been shown by Dufresnes et al. (2015). Both recombination and turnovers are responsible for the homomorphy of sex chromosomes in the European tree frog *Hyla* (Dufresnes et al. 2015). It is not clear, however, why these two processes do no longer occur in species with highly differentiated sex chromosomes (or have not occurred to prevent their differentiation). It is known

that once two chromosomes evolve too differently, recombination between them is harder, but why in the first place recombination was completely arrested is still an open question. Given the costly consequences of the complete arrest of recombination in heteromorphic sex chromosomes, and the possible alternative evolutionary path shown by the presence of homomorphic sex chromosomes, it is of great interest to understand which mechanism(s) caused the arrest of recombination and in which circumstances. It is nevertheless challenging to disentangle causes from consequences when studying highly degenerated sex chromosomes: for example, a mechanism like an inversion (a rearrangement of the chromosome where a part of it is inverted) can be the cause of the arrest of recombination, but it can also occur after the chromosome stopped recombining. However, investigating the processes going on in recently generated sex chromosomes, such as the neo-Y chromosome of *Drosophila*, could help us to understand what caused the inevitable degeneration of the Y chromosome (Lucchesi 1978, Charlesworth 1996, Steinemann and Steinemann 1998, Bachtrog and Charlesworth 2002). A useful way to study the dynamics of the Y chromosome is to simulate the first step in the evolution of sex chromosomes. Here, one can investigate how the different evolutionary processes (accumulation of sex-antagonistic genes and deleterious mutations) shape the path through a homomorphic or heteromorphic state. In the classical model of the evolution of sex chromosomes, the degeneration of sex chromosomes through the accumulation of deleterious mutations is taken into account only as an inevitable consequence. However, deleterious mutations could also play a role in the maintenance of recombination, counterbalancing the effect of sex-antagonistic selection (Grossen et al. 2012). It is also important to keep in mind the mechanism through which recombination can be regulated. An inversion that occurs on a Y chromosome can indeed be fixed in the population and cause the complete arrest of recombination, while recombination regulated through modifiers could lead to a different destiny of sex chromosomes.

Three important actors in the evolution of sex chromosomes

Sex-antagonistic genes

Sex-antagonistic genes are defined as genes that carry alleles that are beneficial to one sex but detrimental to the other. Sex antagonistic alleles can spread both on autosomes (therefore equally transmitted to males and females) or when sex-linked, depending on the relative benefit and cost to the sexes. An autosomal sex-antagonistic allele can increase in frequency when rare if the benefit to one sex is larger than the cost to the other sex (Rice 1987). However, a sex-antagonistic allele linked to a sex-determining locus can increase in frequency under less stringent conditions. If a male-beneficial mutation, for example, appears close to the male-determining locus, it will be transmitted more often to males than to females; under these circumstances, the male-beneficial mutation will spread even if the benefit to males is smaller than the cost to females. Consequently, the region around the sex-determining loci is a hot spot for the accumulation of sex antagonistic genes. Several coloration genes in guppies are tightly or completely linked to the sex-determining gene, and the same is observed in other species of fish (Aida 1921, Endler 1980, Lindholm and Breden 2002). Sex-antagonistic genes play a prominent role in the theory of sex chromosome evolution, as the process of arrest of recombination that leads to the degeneration of the Y or W chromosome is thought to be initiated by the presence (or the occurrence by mutation) of a sex-antagonistic allele. It is, however, not certain if sex-antagonistic genes occurring in the vicinity of a sex-determining locus started the process. Although physical linkage between a sex-determining and sex-antagonistic gene is observed in some species, sex-antagonistic genes might have accumulated in the vicinity of a sex-determining locus after recombination arrest, benefiting from the linkage (Rice 1987, Beukeboom and Perrin 2014).

Deleterious mutations

Deleterious mutations are mutations that occur throughout the genome and that are detrimental for the individual. In a diploid individual, the strength of deleterious mutations can be characterized by two parameters: the dominance coefficient h and the selection coefficient s. Assuming constant

fitness effects across loci, the decrease in fitness can then be calculated as 1-*hs* for each locus heterozygous for the deleterious allele, and 1-*s* for each locus that is homozygous for the deleterious allele. Using these parameters, and the mutation rate μ at which the mutations occur, the frequency of a deleterious allele in an infinite population at the mutation-selection equilibrium can be calculated. While in an infinite population there will always be some mutation-free individuals, in a finite population, individuals that are mutation-free might be lost by drift, and the individuals with a single deleterious mutation would now represent the least loaded class. Following the same principle, the individuals in the least loaded class might be lost by drift, leading to the accumulation of deleterious mutations over time (assuming no back mutations). This mechanism is called Muller's ratchet (Muller 1950), and it plays a more significant role in small populations. Recombination slows Muller's ratchet, because it can purge the genome from deleterious mutations, and it can "recreate" a gamete with fewer deleterious mutations than in the currently least loaded class). In chromosomes with restricted or no recombination, Muller's ratchet causes the degeneration of the Y and W chromosomes, which are unable to purge the deleterious mutations through recombination, and thus continue to accumulate them. Other processes also can contribute to the accumulation of deleterious mutations. Background selection reduces the effective population size of the Y chromosome, because Y chromosomes that carry strongly deleterious mutations will not contribute to the next generation; this reduction increases the fixation rate of weakly deleterious mutations. In the absence of recombination, the reduction in the effective population size of the Y can be greater (Charlesworth 1996), therefore when recombination is absent deleterious mutations are less efficiently purged. Moreover, selection for strongly beneficial mutations can result in deleterious mutations hitchhiking, or selection against strongly deleterious mutations can cause the elimination of linked beneficial mutations (Bachtrog 2006 for a review of these mechanisms). All these mechanisms can be reunited under the Hill-Robertson effects umbrella. Hill-Robertson effects (or interference) are interactions between linked genes that decrease the effectiveness of selection (Hill and Robertson 1966). When genes are tightly linked, selection at one locus will interfere with selection at another locus, causing an increase in the fixation rate of deleterious mutations and slowing down the fixation of beneficial ones.

Recombination

Recombination is the genetic exchange between chromosomes during meiosis or mitosis, and the way it is achieved or halted is fundamental in the process of sex chromosomes evolution. Recombination can differ in rate and localization between sexes. The sex that recombines more varies among species but also among loci, and recombination varies also among individuals in the same species and population (e.g. Chinnici 1971, Brooks and Marks 1986, Burt et al. 1991, Coimbra et al. 2003, Lenormand 2003, Lenormand and Dutheil 2004, Berset-Brändli et al. 2008). In the genome of several species hotspots and coldspots of recombination have been identified (corresponding to sites with high or low recombination rates). In mammals, the gene Prdm9 has been recently identified as a major determinant of hotspots (Baudat et al 2010, Parvanov et al. 2010). Like Prdm9, genetic modifiers of recombination should be widespread in the genome, and being able to initiate recombination in a specific location in the genome. Moreover, recombination can be halted by structural differences in the chromosomes, like inversions and transpositions (Bergero and Charlesworth 2009). Recombination is generally believed to be advantageous: it can facilitate adaptation, it helps purge the genome from deleterious mutations, it brings together beneficial mutations, it can increase genetic variance when selection would reduce variation. But recombination can also break beneficial associations (recombination load). Recombination arrest is considered the primary cause of sex chromosomes differentiation and degeneration; while the consequences of lack of recombination are well identified, what initially caused it remains uncertain. The mechanism that controls recombination plays an important role in the evolution of sex chromosomes, and it might be at the source of the differences in sex chromosomes that we see today.

Transitions between sex determining systems

While in some classes of vertebrates sex determination is stable and well-defined, in other vertebrates the pattern is much different, with different sex determination systems found in close related species (Ezaz et al. 2006, Mank et al. 2006, 2009), or even in different populations of the same

species (Miura 2007). In these systems, frequent transitions result in sex chromosomes homomorphy (Devlin and Nagahama 2002, Eggert 2004), and often sex determination has both a genetic and an environmental component. In cold-blooded vertebrates, sex determination is much more labile than in warm-blooded vertebrates, and this might explain why various combinations of sex-determining systems are found in this group. Transitions between environmental and genetic sex determination systems have been well investigated (e.g. Ezaz et al. 2009, Muralidhar and Veller 2018). However, some more particular systems remain poorly understood. For example, some species also show a mixture of hermaphroditism and genetic sex determination. While in plants hermaphroditism is considered the ancestral state (Charlesworth and Charlesworth 1978), in fish species where a mixture of hermaphroditism and genetic sex determination is found, the former seems to have evolved after the species evolved genetic sex determination (Mank et al. 2006, 2009). In some amphibians and fish species, a system with multiple chromosomes with different masculinizing strengths is found. In the common frog, differences between populations are found in the amount of XY differentiation; moreover, in some populations males with different Y-haplotypes can be found, as well as XX males (Rodrigues et al. 2017). In guppies different Y-haplotypes are found in populations with high or low predation, which also show different recombination rates (Haskins et al. 1961). In these species sex determination is not a one-way path, but it seems that a dynamism is kept between and within-species.

The role of sex chromosomes in speciation

Sex chromosomes play an important role also in the speciation process. Because of their reduced effective population size and sex-limited transmission, the effects of evolutionary processes on these chromosomes are different than on autosomes. This includes speciation and the process of hybridization at a contact zone. Imagining a speciation process, let us assume that in an ancestral species two alleles, *a* and *b*, are fixed at two loci. After geographical speciation, individuals in one species are *AAbb*, while in the other they are *aaBB*. At a secondary contact, hybrids with the *A* and *B* alleles are produced, but the interaction between the two alleles is unknown (Presgraves 2010). The *A* and *B* alleles might be incompatible, which will depend on their effects and their dominance. These

interactions between genes that did not evolve together are called Dobzhansky-Muller incompatibilities (Dobzhansky 1937, Muller 1940, 1942). Whether these alleles are autosomal or sex-linked also makes a difference in the effects on hybrids. The Haldane's rule indeed states that when two different animal races reproduce and in the first generation one sex is absent, rare or sterile, that sex is the heterogametic sex (Haldane 1922). Assuming an XY system with a degenerated Y, a recessive allele will be protected from selection in females (XX), while it will be exposed to selection in XY males (hemizygous exposure). Moreover, incompatibilities can result from interactions between the alleles on the X and on an autosome, or on the Y and on an autosome. Sex-linked genes can therefore struggle more to overcome hybridization. X-linked alleles (when the Y is degenerated) suffer from hemizygous exposure, and X and Y chromosomes can also be affected by incompatibilities (between them). These dynamics have been largely explored in species with heteromorphic sex chromosomes, while the dynamics for homomorphic sex chromosomes is less known. Some effects should be negligible in homomorphic sex chromosomes, and the hybridization process in species with such chromosomes can help to disentangle sex-linkage effects with hemizygosity due to the degenerated Y (W) chromosome.

Thesis outlook

In this thesis I have explored how sex-antagonistic genes, deleterious mutations and recombination interact and shape the evolution of sex chromosomes. I have used individual-based simulations to simulate the different processes affecting sex chromosome evolution and to understand how they interact.

In the **first chapter**, I have analysed the effect of different level of XY recombination in the dynamics of sex-antagonistic genes and deleterious mutations. The role of sex-antagonistic genes in the classical model on sex chromosome evolution is central (Rice 1984, 1987, Charlesworth and Charlesworth 2000). Sex-antagonistic genes drive the arrest of recombination in the heterogametic sex, unopposed by the accumulation of deleterious mutations, which would select for maintenance of recombination. Hill-Robertson interference might also occur, and the accumulation of deleterious

mutations should impact the fixation of beneficial sex-antagonistic alleles. Moreover, both male and female fitness are impacted by the different recombination rates occurring in males, as the dynamics occurring on the Y chromosomes inevitably affect the dynamics on the X chromosomes. Results from the model show that males never benefit from a complete arrest of recombination, while females do, suggesting that some level of recombination should always be favoured. Moreover, low levels of recombination can generate a recombination load at the sex-antagonistic genes, resulting in the spread of female-beneficial alleles in males. Sex chromosomes might not be the best location for sex-antagonistic genes, which might be autosomal and expressed in a sex-specific manner. In this way the conflict between accumulation of deleterious mutations and sex antagonistic genes would be resolved. However, the mechanism that regulates recombination plays a central role: while a modifier for recombination would allow for a fine control of recombination, chromosome rearrangements like inversions would cause the irreversible arrest of recombination. Moreover, the position of the recombination modifier also affects the equilibrium recombination rate: if autosomal, it will experience selection in males and females at the same rate, and from our results males and females have different optimal fitness for the same recombination in males; however, if recombination in males is controlled by a sex-linked modifier, male benefits will be enhanced.

In the **second chapter** I have investigated how sex chromosome recombination evolves under different strengths of sex antagonistic selection and deleterious mutations. Here I show that recombination is affected by both processes. The complete arrest of recombination predicted by the canonical model is not a certain ending, and the accumulation of deleterious mutations can select for maintenance of some recombination. Moreover, the position of the recombination modifier plays a crucial role in the evolution of recombination: when the modifier is autosomal, selection for an arrest of recombination in males is much stronger than if the modifier is sex-linked. Females can “interfere” with the optimal male recombination rate, and as seen from the previous chapter an arrest of male recombination is beneficial to this sex. When the modifier is sex-linked, however, male recombination is selected to be maintained. Low recombination rates are one of the proposed mechanisms that maintain sex-chromosome homomorphy. In some species, however, homomorphic sex chromosomes

are the result of a high rate of turnover, which can lead to the presence of multiple sex determination system in the same species.

In the **third chapter** I have explored the transition between sex determination systems, firstly inspired by the high number of Y-haplotypes found in the common frog *R. temporaria*. I modelled the invasion of different Y-like alleles to understand if and how an equilibrium among them is possible. Moreover, I investigated the transition between genetic and non-genetic sex determination systems, simulating the birth of a proto-sex chromosome and the transition back to a non-genetic sex determination system. I took into account the strength of sex-antagonistic selection, but also the mode of recombination, contrasting recombination mediated by an inversion, or by the sex of an individual. Recombination in amphibians depends on the phenotypic sex, and it is considered one of the factors that contribute to their homomorphy. An equilibrium between multiple Y-haplotypes was not reached, although implementing deleterious mutations together with sex-antagonistic selection might allow for it. The polymorphism at the sex-antagonistic locus has a fundamental impact on the equilibrium of a new Y mutation, whose extinction rate was correlated to the frequency of the male beneficial allele frequency. Moreover, a polymorphic sex determination system evolved from a genetic one due to sex antagonistic selection, with a stable polymorphism of X, Y and a "random" allele. This result has implications on the multiple haplotypes found in species like *P. reticulata* and *R. temporaria*.

While different haplotypes coexist in this case in the same population, sometimes they evolved independently in populations that have been isolated. These haplotypes might cause hybridization problems and play a major role in contact zones, where two (sub)species enter in contact. In the **fourth chapter** I have considered the hybridization dynamics of two species at a contact zone, with alleles of one species being dominant over the alleles of the other species. The model considered different incompatibility patterns between the sex chromosomes or between each sex chromosome and the autosome. Different relative migration rates of males and females have also been considered. The effects of the incompatibilities have been evaluated through the analysis of the introgression clines, which show the invasion of the allele of one species into the other. Incompatibilities between the sex chromosomes resulted in steep clines, and the cline centres of the two chromosomes always coincided. However, incompatibilities between a sex chromosome and the autosome resulted in the

shift of the cline center into the domain of the species with the recessive allele. Such simulation studies can help to understand and separate the effects of different factors that affect the allele introgression of one species over the other.

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Chapter one

Sex-antagonistic genes, XY recombination and feminized Y chromosomes

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Sex-antagonistic genes, XY recombination and feminized Y chromosomes

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mutational load;
recombination load;
sex-antagonistic genes;
sex-chromosome evolution;
XY recombination.

Abstract

The canonical model of sex-chromosome evolution predicts that sex-antagonistic (SA) genes play an instrumental role in the arrest of XY recombination and ensuing Y chromosome degeneration. Although this model might account for the highly differentiated sex chromosomes of birds and mammals, it does not fit the situation of many lineages of fish, amphibians or nonavian reptiles, where sex chromosomes are maintained homomorphic through occasional XY recombination and/or high turnover rates. Such situations call for alternative explanatory frameworks. A crucial issue at stake is the effect of XY recombination on the dynamics of SA genes and deleterious mutations. Using individual-based simulations, we show that a complete arrest of XY recombination actually benefits females, not males. Male fitness is maximized at different XY recombination rates depending on SA selection, but never at zero XY recombination. This should consistently favour some level of XY recombination, which in turn generates a recombination load at sex-linked SA genes. Hill–Robertson interferences with deleterious mutations also impede the differentiation of sex-linked SA genes, to the point that males may actually fix feminized phenotypes when SA selection and XY recombination are low. We argue that sex chromosomes might not be a good localization for SA genes, and sex conflicts seem better solved through the differential expression of autosomal genes.

Introduction

The evolution of sex chromosomes has attracted much attention over the last century, following the early suggestion by Muller (1914) that they originate from autosomes. Since then, many empirical and theoretical studies, mostly focused on a few model organisms such as *Drosophila* and mice, have contributed to shape a plausible scenario for their evolution (Ohno, 1967; Charlesworth, 1978, 1991; Charlesworth & Charlesworth, 1978, 2000; Bull, 1983; Rice, 1996). As theory goes, the first step in the process is initiated by a mutation of an autosomal gene, such that individuals with the mutation develop into one sex, and those without it into the alternative sex (male heterogamety will be assumed throughout, i.e. XY males and XX females, but all statements below generalize to female heterogamety as well, i.e. ZW females and ZZ males). As a second step, sex-antagonistic (SA) alleles are favoured in the vicinity of this sex-determining (SD) gene: male-beneficial alleles that are physically linked to the male-determining allele are likely to spread even if highly detrimental to females, because linkage disequilibrium makes them more likely to be transmitted to sons than to daughters (Fisher, 1931; Rice, 1987a). This association is then further enhanced through a progressive arrest of recombination in the heterogametic sex, so that sons always inherit male-beneficial genotypes and daughters female-beneficial genotypes (Nei, 1969; Charlesworth & Charlesworth, 1980; Bull, 1983; Rice, 1987a,b). A self-reinforcing loop is thereby initiated: decreased recombination selects not only for stronger SA alleles at SA genes (which in turn select for even less recombination), but also for SA alleles at more

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distant loci (as linkage is strengthened). This chain reaction will induce a progressive extension of the non-recombining region, to finally encompass the whole Y chromosome (Rice, 1996).

As a side effect, however, recombination arrest facilitates the accumulation of deleterious mutations on the Y chromosome through the process of Muller's ratchet (Muller, 1950), amplified by the strong genetic drift stemming from a drastic reduction in their effective population size (Charlesworth & Charlesworth, 2000). As a final step, the degeneration of the Y chromosome will favour the evolution of dosage compensation mechanisms, to cope with gene imbalance between autosomal and X-linked genes (Ohno, 1967; Charlesworth, 1978, 1996; Rice, 1987b; Jegalian & Page, 1998).

This 'canonical' model predicts, therefore, that sex chromosomes are enriched in SA genes and that these genes play a leading role in their evolution. It certainly accounts for a series of empirical results on the dynamics of sex-linked SA genes in *Drosophila* (e.g. Gibson *et al.*, 2002; Dean *et al.*, 2012), as well as several features of the highly degenerated sex chromosomes found in birds or mammals, including evolutionary strata of increasing XY differentiation stemming from stepwise expansions of the nonrecombining segment (e.g. Lahn & Page, 1999; Handley *et al.*, 2004). However, it does not fit the situation found in many lineages of fish, amphibians and nonavian reptiles (i.e. the bulk of vertebrate species), where sex chromosomes do not show many signs of degeneration (Devlin & Nagahama, 2002; Eggert, 2004; Bellott & Page, 2009). Sex-chromosome homomorphy in these lineages likely results from occasional XY recombination and/or frequent turnovers (Schartl, 2004; Miura, 2007; Volff *et al.*, 2007; Cnaani *et al.*, 2008; Ezaz *et al.*, 2009; Stöck *et al.*, 2011; Guerrero *et al.*, 2012; Dufresnes *et al.*, 2014, 2015), possibly the consequences of imperfect genetic control over sex determination (Perrin, 2009; Grossen *et al.*, 2011; Blaser *et al.*, 2014). This lack of fit between theory and data raises a number of questions regarding some assumptions of the canonical model and certainly calls for alternative models of sex-chromosome evolution.

The canonical model relies largely on verbal arguments. The few aspects that received some formalization relate to 1) the dynamics of alleles at sex-linked SA loci (in the absence of deleterious mutations) and 2) the decay of nonrecombining Y chromosomes and ensuing evolution of dosage compensation. Bull (1983) showed that an SA polymorphism is more likely to be maintained if the SA locus is fully sex-linked, than if it is fully unlinked. Rice (1987a, 1996) showed that the equilibrium frequency of an SA allele that is beneficial to males but lethal to females decreases about linearly with its recombinational distance from the sex locus. Charlesworth (1978) and Rice (1987b) showed how deleterious mutations can accumulate on nonrecombining Y chromosomes through Muller's ratchet and/or genetic hitchhiking, and how this can drive the evolution of dosage compensation. All other components of the model (and notably the selective forces acting on and resulting from different levels of XY recombination) are essentially verbal. Auxiliary assumptions have thus to be made explicit, if the plausibility of the predicted scenario has to be evaluated. Among these assumptions, 1) male fitness is supposed to always benefit from a decrease in XY recombination, via the evolution of more male-benefit alleles at sex-linked SA genes, and 2) recombination arrest can go to completion unopposed by other selective forces. Such assumptions might not be met for several reasons. First, a decrease in XY recombination may affect other components of male fitness than just SA selection, notably by favouring the accumulation of deleterious mutations on Y chromosomes (Maynard Smith, 1978; Charlesworth *et al.*, 1993a); the ensuing selective pressure might have the potential to prevent a complete arrest of XY recombination, as shown through individually based simulations (Grossen *et al.*, 2012). Second, low rates of recombination generate strong Hill–Robertson interferences (Hill & Robertson, 1966) such that other selective forces acting on sex chromosomes (notably the purge of deleterious mutations) are likely to also impact the dynamics of SA genes and possibly prevent the fixation of male-beneficial alleles at sex-linked SA genes. Third, changes in XY recombination might also impact fitness components in females (not only in males), notably by affecting the dynamics of female-beneficial alleles and the accumulation of deleterious mutations on X chromosomes, which is expected to interact with the evolution of Y chromosomes.

Hence, the correlates of XY recombination are certainly much more complex than suggested by the mostly verbal arguments at the core of the canonical model. Reduced levels of recombination potentially affect many coevolving genes and impact different components of fitness in sex-specific ways. A proper formalization is needed for an evaluation of the plausibility of different scenarios. Due to this inherent complexity, however, analytical formalization is certainly out of reach. Here, we use individual-based simulations to examine in detail some of the assumptions / predictions of the canonical model of sex-chromosome evolution. More specifically, we delineate the effects of various levels of XY recombination on the evolution of male and female sex phenotypes, while accounting for the dynamic load of deleterious mutations. Does lowered XY recombination indeed favour the spread of male-beneficial alleles on Y chromosomes, and how does it impact the several components of male and female fitness? Our present simulations are not intended to model the evolution of XY recombination (as done by Grossen *et al.*, 2012), but to identify the consequences of reduced XY recombination rates on the sex-specific components of fitness, as a way to characterize the

selective forces at work. Our simulations show that reduced rates of XY recombination a) do have contrasted consequences on fitness components stemming from SA genes and deleterious mutations in both males and females, and b) may actually favour in some cases a reduction in sexual dimorphism, coupled with the evolution of feminized phenotypes in males, owing to Hill–Robertson interferences between SA genes and deleterious mutations. These simulations also show that a complete arrest of XY recombination actually benefits females, but not males, and is thus unlikely to be evolutionarily stable.

Material and Methods

Genetic architecture

We assume sex to be controlled by a sex-determining locus (SD) with two alleles, *x* and *y*. Chromosomes carrying either the *x* or the *y* allele are referred to as X or Y chromosome, respectively (Fig. 1). XX individuals always develop as females and XY individuals as males (male heterogamety). Sex chromosomes also contain a sex-antagonistic locus (SA) encoding a secondary sex character *P* (e.g. a coloration gene). Contrasting with previous analytical approaches (e.g. Rice, 1984, 1987a), which assumed a biallelic SA locus for sake of tractability, we model a more continuous SA phenotype trait, as a way to better quantify the evolution of sexual dimorphism. Allelic values at the 251 possible alleles range from −8.5 to +8.5 and phenotypes from −17 to +17, being determined by the additive effects of alleles. Phenotypic effects were assumed identical in both sexes, because we are specifically interested in the evolution of sexual dimorphisms that build on the sex linkage of genes (i.e. on the fixation of different alleles on X and Y chromosomes), not on the differential expression of genes that might be spread over the whole genome. As our results suggest, the latter option might indeed offer a better solution to sexual conflicts in many instances (see Discussion). SA phenotypes affect in turn the sex-antagonistic component of fitness (W_{SA}) in a sex-specific way: negative trait values are male-detrimental but female-beneficial, whereas the reverse is true for positive trait values (Fig. 2). The relationship has to be sigmoid, because fitness values are constrained between zero and one. Specifically, this fitness component was modelled as a sigmoid function of *P*,

$$W_{SA} = 1 - \frac{\Delta e^{P}}{1 + e^{P}} \text{ for females} \quad (1a)$$

and

$$W_{SA} = 1 - \frac{\Delta e^{-P}}{1 + e^{-P}} \text{ for males} \quad (1b)$$

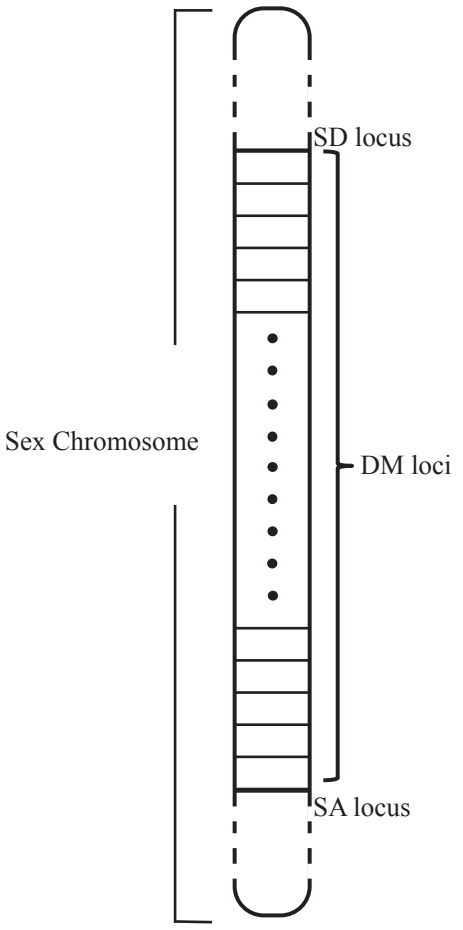

Fig. 1 The sex chromosomes comprise a sex-determining (SD) locus and a sex-antagonistic (SA) locus, separated by 100 loci that can mutate to a deleterious form (DM loci).

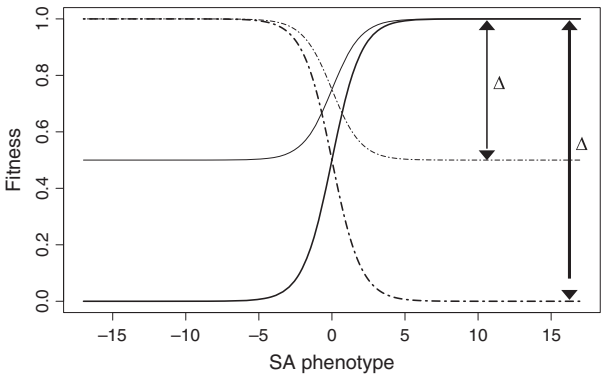

Fig. 2 The sex-antagonistic component of fitness (W_{SA}) is a sigmoid function of the phenotypic trait P, increasing in males (solid lines) and decreasing in females (dashed lines). The strength of sex-antagonistic selection is measured by Δ, the difference between the two asymptotes; two values are illustrated here, corresponding to Δ = 1 (bold lines) and 0.5 (thin lines).

where Δ measures the strength of sex-antagonistic selection (i.e. 1−Δ is the lower asymptote for the fitness function). The initial allele frequency at the SA locus follows a discretized normal distribution with mean 0 and variance 2, truncated at −8.5 and 8.5. Mutations at this locus occur at rate 10^{-4}, with the new allele randomly drawn from a normal distribution centred on its premutation value (also with variance 2 and truncated at −8.5 and 8.5).

One hundred loci that can accumulate deleterious mutations (DM loci, Fig. 1) are evenly distributed between the SD and SA loci. The first and last DM loci co-localize with the SD and SA loci, respectively. Mutations affect the deleterious-mutation component of fitness (W_{DM}), which is multiplied by (1-*s*) for each locus homozygous for the deleterious form, and by (1-hs) for each heterozygous locus (where *s* and *h* represent the selection and dominance coefficients, respectively). Hence, this fitness component becomes

$$W_{DM} = (1 - hs)^{het} \cdot (1 - s)^{hom} \quad (2)$$

where *het* and *hom* represent the number of loci that are heterozygous or homozygous for deleterious mutations, respectively. Under full recombination, the frequency of deleterious alleles is expected to stabilize at the mutation–selection equilibrium, given by Burger (1983):

$$\hat{q} = \frac{h(1+\mu)}{2(2h-1)}\left[1 - \sqrt{1 - \frac{4\mu(2h-1)}{(1+\mu)^2 h^2 s}}\right] \quad (3)$$

where μ is the mutation rate towards deleterious alleles. Higher frequencies are obviously expected under restricted recombination. The overall individual fitness *W* is the product of its two components, stemming, respectively, from sexual antagonisms and deleterious mutations:

$$W = W_{SA} \times W_{DM} \quad (4)$$

As the main goal of this study was to investigate the effect of XY recombination on the dynamics of sex-antagonistic genes and deleterious mutations, the level of XY recombination was fixed at specific values (see below), whereas XX recombination was left free to evolve, being controlled by a physically unlinked modifier locus. Allelic values at this locus range 0 to 50 and determine additively the length of recombination maps (in centiMorgans, cM). Mutations occur with the same probability towards any allele (KAM model of mutation), at rate 10^{-3} per generation. Hence, in the absence of selection on recombination rate, the expected average length of sex chromosomes in females is 50 cM. Besides sex chromosomes, individuals are also characterized by a pair of autosomes, which also contains 100 DM loci (same mutation model as for sex chromosomes), with recombination similarly controlled by two additional modifier loci (one for each sex) and the same mutation model (so that autosomal length is also expected to average 50 cM in both sexes at equilibrium).

Simulations

Individual-based simulations were run with a modified version of QUANTINEMO v1.0.3 (Neuenschwander *et al.*, 2008). We simulated a single population with nonoverlapping generations, and a size fixed to 10 000 individuals in order to limit the influence of genetic drift relative to selection. At each generation, gametes formed from individual mothers and fathers were randomly paired to produce 10 000 offspring (i.e. selection was soft), each parent being chosen with a probability proportional to its fitness value (given by eq. 4).

Deleterious mutations occurred at either low or high rate ($\mu = 5 \times 10^{-4}$ and 5×10^{-3} per locus, respectively), with no back mutations. The corresponding chromosomal mutation rate (U) had thus maximal values of 0.1 or 1.0, respectively (reached at the start of simulations, when all alleles were wild-type). Actual U values might lie in between, as suggested by data from *Drosophila* or hominids where genomic mutation rates are estimated between 1.0 and 4.0 (Eyre-Walker & Keightley, 1999; Haag-Liautard *et al.*, 2007; Eöry *et al.*, 2010). From our simulations (see Results), the effects of these two rates do not differ qualitatively, but are only stronger at high mutation rate. Thus, results for the higher mutation rate ($\mu = 5 \times 10^{-3}$) will be presented in the main text and those for the lower mutation rate ($\mu = 5 \times 10^{-4}$) in Supplementary material. Quantitative differences will be spelled out when relevant.

In all simulations, autosomal and XX recombination were allowed to evolve freely (and rapidly reached the expected value of 50 cM). XY recombination rate (R_{XY}) was fixed at different values: cM = {0, 0.5, 2.5, 5, 12, 26, 46, 81}, corresponding approximately to $R_{XY} \sim$ {0, 0.005, 0.024, 0.048, 0.11, 0.2, 0.3, 0.4}. For all R_{XY} values, we tested the effect of SA selection by varying Δ = {0, 0.1, 0.2, 0.3, 0.5, 1}, and the effect of deleterious mutations by varying *s* = {0, 0.05, 0.1, 0.2, 0.5, 1}. At lower mutation rate ($\mu = 5 \times 10^{-4}$), we additionally investigated weaker SA selection (Δ = {0.01, 0.05}) as well as additional low recombination rates (cM = {0.1, 0.2, 1}, corresponding to $R_{XY} \sim$ {0.001, 0.002, 0.01}). In our core simulations, deleterious mutations were highly recessive (*h* = 0.01), and parameters were tested in fully factorial designs, with 100 replicates per parameter set. To test the robustness of some results (see Results), we also performed limited additional simulations with less recessive (*h* = 0.3) and less deleterious mutations (*s* = 0.02).

At the start of simulations, the allelic distribution at the SA locus matched the mutation model (discretized normal distribution with mean 0 and variance 2), and the DM loci were fixed for the nondeleterious alleles (see Table S1 for a summary of parameter values). Alleles encoding the XX and autosomal recombination rates were initially set to 0 (no recombination) but quickly converged towards the mutation model (uniform distribution of alleles from 0 to 50, i.e. average recombination maps of 50 cM). Simulations were run for 10 000 generations, long enough to reach steady states (except for the accumulation of deleterious mutations on the Y in the absence of XY recombination; see Results).

Results

Deleterious load

Deleterious mutations accumulated more slowly at high s values on all chromosomes (Figs 3 and S1), due to stronger purifying selection. On autosomes, their frequencies (q) quickly reached the steady states predicted from eq. 3, for all s values (Figs 3 and S1, crosses). In the absence of XY recombination, accumulation on the Y chromosome exceeded by far that on autosomes for any positive s value, due to the combined effects of recombination arrest and lower effective population size. The frequency of deleterious mutations (q) was much lower on the X chromosome, and actually slightly lower than on autosomes, despite the lower effective population size and lower overall rate of recombination (since it only occurred in females). For all chromosomes, this frequency was unaffected by the strength of sex-antagonistic selection (Δ). At lower mutation rate, the frequency of deleterious alleles was lower for all chromosomes (Fig. S1). For $s = 1$, their frequency was close to 0 for all chromosomes, though still slightly higher for the Y chromosome.

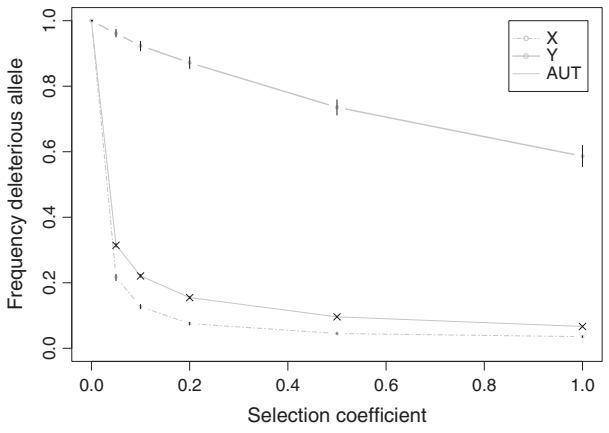

Fig. 3 In the absence of XY recombination, deleterious alleles reach higher frequencies (mean ± 95% CI values at generation 10 000) on Y chromosomes (solid thick line) than on autosomes (solid line) and X chromosomes (dashed line), for any positive s value (x-axis). The black crosses represent the equilibrium frequencies predicted from eq.3. Simulations performed for $\mu = 5 \times 10^{-3}$ and $\Delta = 0.1$ (values for $\mu = 5 \times 10^{-4}$ are provided in Fig S1.)

Implementing some XY recombination had strong effects on the deleterious load, but different ones depending on Δ. A minute amount of XY recombination was enough to induce a drastic drop in the Y chromosome load. With a shift from $R_{XY} = 0.00$ to $R_{XY} = 0.005$, for instance, the frequency of deleterious alleles at generation 10 000 dropped from ~ 0.95 to ~ 0.50 for $\Delta = 1$ and even down to ~ 0.30 for $\Delta = 0.1$ (Fig. 4, right panel). At lower mutation rate, the drop was from ~ 0.5 to ~ 0.25 for $\Delta = 1$ and down to ~ 0.2 for $\Delta = 0.1$ (Fig. S2, right panel). On the X chromosome, in contrast, the frequency of deleterious alleles increased slightly with XY recombination (Figs 4 and S2, left panel). At high XY recombination rates, the load on both sex chromosomes converged towards the equilibrium value expected from eq. 3 (e.g. $q = 0.22$ for $h = 0.01$, $s = 0.1$ and $\mu = 10^{-3}$), similar to the one found on autosomes (Fig. 3).

Sex phenotypes

In the absence of XY recombination, X and Y chromosomes fixed highly divergent SA alleles as soon as Δ values exceeded 0, resulting in strong sexual dimorphism, particularly at large Δ values and small s values (Figs 5 and S3). At high XY recombination rate, sexes evolved instead towards an intermediate, neutral phenotype ($P = 0$), whatever the s values (Figs 6a and S4a). At intermediate XY recombination rates, however, sex phenotypes were strongly affected by the strength of deleterious mutations. In the absence of deleterious mutations ($s = 0.0$), convergence towards neutral phenotypes was monotonic in both sexes (Figs 6a and S4a, upper panels), being roughly linear for $\Delta = 1$, and more logarithmic for smaller Δ values. But interactions with deleterious mutations ($s > 0.0$) induced a drastic and unexpected shift in male phenotypes at low recombination rates, from a strongly masculinized to a strongly feminized appearance (Figs 6a and S4a, lower right panel). This feminized phenotype was most accentuated at low Δ values, low recombination rates and high selection coefficients for deleterious mutations. With increasing R_{XY}, male phenotypes progressively converged towards the trajectory followed in the absence of deleterious effects. Female phenotype was also somewhat affected by deleterious mutations, but to a much lesser extent, leading to more feminized phenotype at low Δ and low R_{XY} values (Figs 6a and S4a, lower left panel).

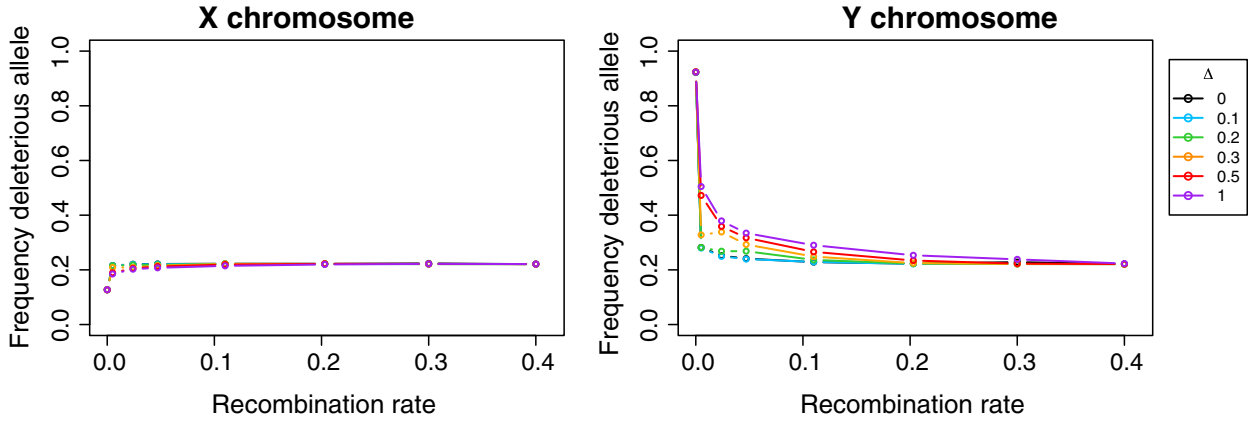

Fig. 4 The mean frequency of deleterious alleles at the end of simulations (generation 10 000) increases with XY recombination (x-axis) for the X chromosome (left), but decreases for the Y chromosome (right). Convergence towards the common equilibrium ($q = 0.22$ at these parameter values, $s = 0.1$, $h = 0.01$ and $\mu = 5 \times 10^{-3}$) is quicker at low Δ values (see colour code). Values for $\mu = 5 \times 10^{-4}$ are provided in Fig. S2.

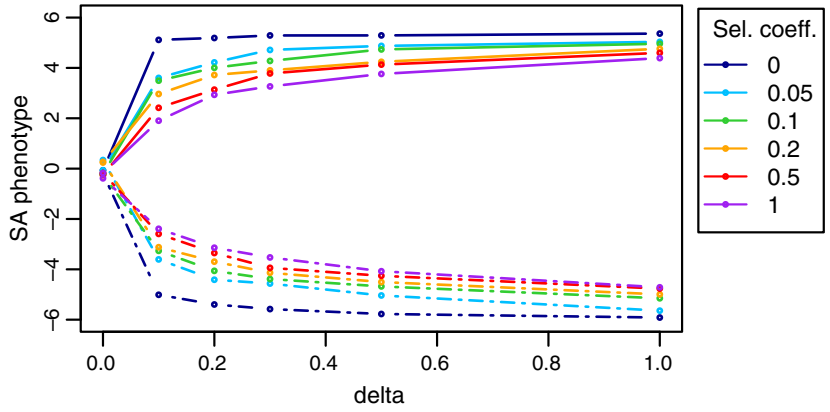

Fig. 5 In the absence of XY recombination, sex phenotypes are strongly differentiated at equilibrium as soon as Δ>0 (generation 10 000), with positive values in males (solid lines) and negative values in females (dashed lines). Differentiation increases with an increase in Δ (x-axis) and a decrease in s (see colour code). Simulations performed for $\mu = 5 \times 10^{-3}$ (values for $\mu = 5 \times 10^{-4}$ are provided in Fig. S3).

Sex-specific effects of XY recombination on fitness

In the absence of recombination ($R_{XY} = 0$), male fitness was strongly affected by the load of deleterious mutations (W_{DM} values being e.g. ~ 0.3 at generation 10 000 for $s = 0.1$ and $\mu = 5 \times 10^{-3}$ and around 0.87 for $\mu = 5 \times 10^{-4}$), but unaffected by Δ. W_{SA} in contrast was affected both by Δ and by s, but only weakly so, being always higher than 0.90 at $\mu = 5 \times 10^{-3}$ and higher than 0.98 at $\mu = 5 \times 10^{-4}$. Both components of fitness were thus negatively affected by deleterious mutations, but the direct effect on W_{DM} was by far the largest. Overall male fitness, therefore, was essentially controlled by deleterious mutations. Patterns in females were somewhat similar, but with much weaker effects of deleterious load overall.

Introducing XY recombination induced a drastic increase in W_{DM} fitness in males and a drastic drop in females (Figs 6b and S4b, middle panels). Changes mostly occurred at low R_{XY} values, followed by a rapid levelling off (with equilibrium value around 0.6 at $\mu = 5 \times 10^{-3}$ and around 0.94 at $\mu = 5 \times 10^{-4}$, Fig. S4b) and were more pronounced at low Δ values. W_{SA} also decreased with XY recombination in females (Figs 6b and S4b, upper left panel), with a decline mostly at high Δ values. A similar monotonic decline occurred in males at high Δ values, but patterns were more complex at low Δ values, with an initial drop followed by a rebound in SA fitness (Figs 6b and S4b, upper right panel). As a result, overall fitness in females was always maximized at $R_{XY} = 0$, followed by a rapid drop at low R_{XY} values, then a more progressive decline with R_{XY} for high Δ values (Figs 6b and S4b, lower left panel). In males, by contrast, fitness was maximized at high XY recombination rates for low Δ values, at low XY recombination for high Δ values (specifically, for $\Delta \geq 0.5$ at

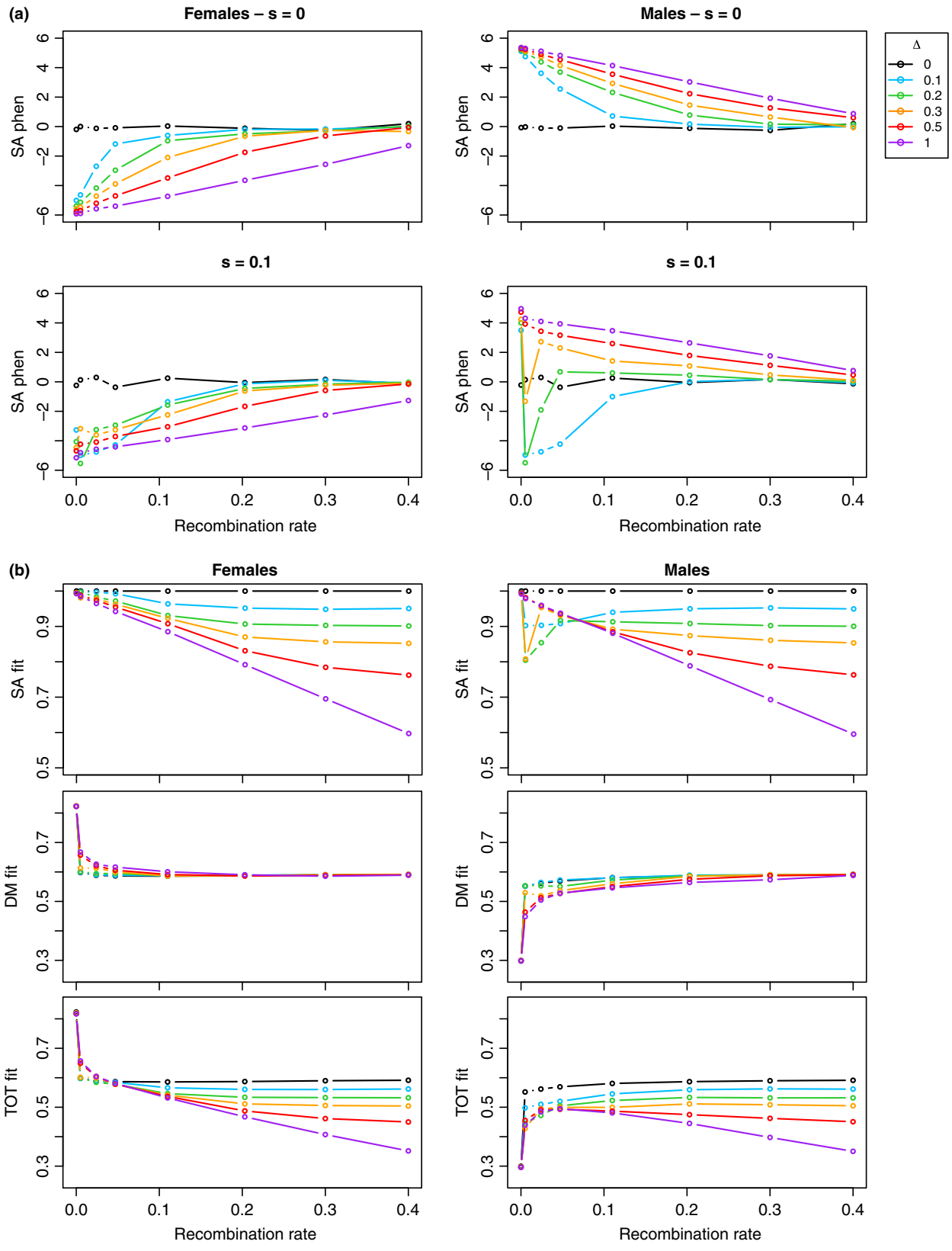
(a)
Females – s = 0
Males – s = 0
s = 0.1
s = 0.1
SA phen
Recombination rate
Δ
0
0.1
0.2
0.3
0.5
1
(b)
Females
Males
SA fit
DM fit
TOT fit
Recombination rate

Fig. 6 (a) in the absence of deleterious effects (s = 0.0, upper two panels), the phenotypes of females (left) and males (right) converge monotonically towards an intermediate neutral phenotype with increasing XY recombination (x-axis). Convergence is more rapid when Δ is weak (see colour code). Average values over 100 replicates. This convergence is not monotonic anymore in the presence of deleterious effects (s = 0.1, lower two panels). Instead, males (right) show a drastic shift towards a strongly feminized phenotype at low XY recombination rate and low Δ values (see colour code). A similar but smaller shift also occurs in females (left) towards more strongly feminized phenotype for the same values of Δ and XY recombination rate. Averages over 100 replicates. (b) W_{SA}, W_{DM} and total fitness as a function of R_{XY} (values for s = 0.1) in females (left) and males (right). Females always benefit from an arrest or XY recombination, whereas male fitness is maximized for either high or low XY recombination depending on the strength of sex-antagonistic selection. Simulations performed for $\mu = 5 \times 10^{-3}$ (values for $\mu = 5 \times 10^{-4}$ are provided in Fig. S4).

$\mu = 5 \times 10^{-3}$ and $\Delta \geq 0.1$ at $\mu = 5 \times 10^{-4}$), but never at zero XY recombination (Figs 6b and S4b, lower right panel).

To better interpret the shift towards feminized phenotypes in males (Figs 6a and S4a, lower right panel), we also computed for both sexes the correlations (within replicates) between the phenotypic trait P and fitness components (plotted for generation 500 as an illustration in Fig. S5). Correlations with W_{SA} (upper panels) were always strongly positive in males (>0.90) and strongly negative in females (<−0.90). However, correlations with W_{DM} (middle panels) were markedly negative in males at low rates of recombination, because feminized Y haplotypes benefited from the low load of deleterious mutations (Fig. S6). As a result, correlations with total fitness were always negative in females (as expected), but very close to neutrality in males at low recombination rates and low Δ values (Fig. S7). Thus, alleles conferring a feminized phenotype were strongly favoured in females overall, but little or not counter-selected in males at these parameter values, allowing their fixation at the population level.

Discussion

The main consequences of a complete arrest of XY recombination are readily explained. On the one hand, deleterious mutations quickly accumulated on Y chromosomes under the action of Muller's ratchet, amplified by the low effective population size (one quarter that of autosomes). In the absence of recombination and back mutations, deleterious mutations are actually expected to reach complete fixation, given enough time (Muller, 1950; Charlesworth & Charlesworth, 1997, 2000). On autosomes, by contrast, deleterious mutations consistently reached the exact equilibrium frequencies expected under complete independence (a result that incidentally validates our individual-based simulations). Interestingly, the X chromosomes were less loaded than autosomes, despite their lower effective population size (three quarters that of autosomes) and lower rate of recombination (which only occurred in females). This alleviated load resulted from stronger purifying selection on the X in males: once a given Y locus has fixed a deleterious allele, X chromosomes with a deleterious allele at the same locus are strongly selected against in XY individuals (a process akin to the enhanced purifying selection on the X chromosomes of hemizygous males in systems with differentiated sex chromosomes; e.g. Rice, 1984; Charlesworth *et al.*, 1987; Vicoso & Charlesworth, 2009).

On the other hand, recombination arrest allowed sex phenotypes to evolve towards highly differentiated sex morphs, unopposed by recombination load. Sexual dimorphism was strongest at high Δ values, due to stronger selection against intermediate, neutral phenotypes (Fig. 2). Sexual dimorphism was also affected by deleterious mutations (Figs 5 and S3): less extreme sex phenotypes were reached when deleterious mutations had strong selection coefficients, as a consequence of Hill–Robertson interferences between SA and DM genes (selection becomes less efficient when interferences are strong; Hill & Robertson, 1966; Felsenstein, 1974; Barton, 1995; Keightley & Otto, 2006; Comeron *et al.*, 2008).

These patterns of sexual dimorphism were drastically affected by XY recombination, due to unexpected interactions with the deleterious-mutation load. In the absence of load, sex phenotypes progressively converged towards intermediate neutral values as XY recombination increased (Figs 6a and S4a, upper panels), due to higher costs of displaying opposite-sex phenotypes (recombination load). In the presence of deleterious load, however, slight increases in XY recombination induced drastic shifts towards a strongly feminized phenotype in males (Fig. 6a, lower right panel). The same feminized phenotype consistently evolved also at lower deleterious-mutation rate ($\mu = 5 \times 10^{-4}$, i.e. with a chromosome-wide mutation rate ≤0.1) and lower Δ values (0.01 and 0.05, Fig. S4a, lower right panel). Fitness analyses of SA phenotypes during the course of simulations (Figs S5 and S7) reveal the underlying selective forces. Through recombination, Y haplotypes gain both a female-beneficial allele at the SA locus ('Y_F' haplotype) and an alleviated deleterious-mutation load (Fig. S6). Provided Δ is low enough (and recombination rare enough), the benefits of a healthy Y_F chromosome roughly compensate the cost of a feminized phenotype. Reciprocally, recombined X haplotypes gain both a male-beneficial allele ('X_M' haplotype) and a heavy load of deleterious mutations; these X_M haplotypes are thus strongly counter-selected in females in terms of both W_{DM} and W_{SA}. Moreover,

recombination in normal males (X_FY_M) also produces low-fitness X_MX_F daughters, which is not the case for feminized X_FY_F males. At the population level, therefore, male-beneficial SA alleles are disfavoured and replaced by female-beneficial alleles in both sexes. Thus, the combination of deleterious mutations and low recombination renders selection at the sex-antagonistic locus in males ineffective, allowing selection to optimize the sex-antagonistic phenotype in females.

This selective process also affected the dynamics of deleterious mutations (Fig. 4). Increasing XY recombination made the deleterious load of sex chromosomes converge towards the predicted equilibrium (eq. 3; Felsenstein, 1974; Maynard Smith, 1978; Charlesworth *et al.*, 1993b), by simultaneously decreasing the load on the Y and increasing it on the X. Interestingly, however, convergence was much quicker at low Δ values, a direct result of the differential fitness of Y haplotypes: recombined (and thus healthy) Y_F haplotypes were more likely to spread if the associated female-beneficial allele at the SA locus had little fitness cost.

Our findings rejoin the interpretation given by Brooks (2000) to the results of sexual-selection experiments in *Poecilia reticulata*. Guppies are characterized by an XY sex-determination system, with series of sex-antagonistic colour genes on or close to the nonrecombining region. Due to occasional XY recombination, some males inherit the female-beneficial dull alleles, which make them less attractive to females. The sons of attractive males have lower survival than those of dull males, apparently due to association with the deleterious mutations that accumulate on nonrecombining Y haplotypes (Brooks, 2000). Even though sex-antagonistic selection is certainly too strong in guppies to allow fixation of the dull phenotype in males, this study suggests that the selective forces identified here can be detected in empirical systems. Fishes might indeed provide ideal model organisms to further test our model, through a unique combination of strong sexual selection and occasional recombination between homomorphic sex chromosomes. We could not find in the literature further empirical support for a feminization of Y chromosomes, which might partly result from an ascertainment bias: in the absence of polymorphism, there is no way of testing whether an allele fixed on the Y is male- or female-beneficial. Furthermore, given the negative side effects documented here (including feminization of the Y), it is to be expected that sexual dimorphisms most often build on the differential expression of autosomal genes, rather than on the fixation of different X and Y alleles at sex-linked genes (see below).

Although the Δ values favouring feminized Y chromosomes were in the lower range of our parameter set, they do not seem unrealistic: Δ values of 0.01 to 0.1, for instance, induce 1% to 10% decrease in male fitness due to having a female-beneficial value for this trait (and reciprocally). We expect shallower slopes of the fitness function (here fixed to +1 and −1 for males and females, respectively; eq. 1) to favour more extreme sex phenotypes, but this would not impede the feminization of males, because a shallower slope would also reduce the fitness costs of displaying opposite-sex phenotypes. It is also worth noting that similar outcomes (fixation of the feminized Y) were observed in additional simulations (not shown) with larger *h* values (0.3) and smaller *s* values (0.02). The same qualitative outcomes also resulted at both high and low rates of deleterious mutations, although the quantitative effects were stronger at the high rate (compare, e.g. Figs 6a and S4a). It is worth recalling in this respect that mutation rates are often markedly larger in males than in females. Implementing such a higher rate in males would only reinforce the effect, because higher mutation loads on the Y relative to the X would further favour XY recombination in males. The same would occur if mutations have a stronger effect in males than in females (Sharp & Agrawal, 2013). Overall, our simulations show that the effects of XY recombination on the evolution of sexual dimorphism can be drastically altered when also accounting for the accumulation of deleterious mutations, an otherwise unavoidable consequence of restricted recombination.

The canonical model of sex-chromosome evolution predicts a complete arrest of recombination in the heterogametic sex, induced by the benefits of genetic linkage between SD and SA alleles (see Introduction): indeed, such an arrest should allow sons to inherit male-beneficial genotypes only, whereas daughters inherit female-beneficial genotypes only. Accordingly, sex-antagonistic selection is assigned an instrumental role in the evolution of nonrecombining and highly differentiated sex chromosomes, such as found in mammals or birds. Our results are challenging this model on several accounts. Under our settings, a complete arrest of XY recombination actually benefits females (through both the evolution of female-beneficial SA alleles and alleviated deleterious-mutation load on X chromosomes), but not males, owing to the accumulated load of deleterious mutations. From our simulations, even minute amounts of XY recombination seem enough for the benefits of purifying selection in males to outweigh the costs of recombining sex phenotypes. As a result, the optimal rate of XY recombination for males is expected to be high when SA selection is weak, low when SA selection is strong, but never zero (Fig. 6b). Maintaining low levels of XY recombination should not only prevent the differentiation of sex chromosomes, but also impact the genetic architecture of sex-antagonistic traits: evolving male or female-beneficial alleles at sex-linked SA genes might not seem the best solution to sexual conflicts, if XY recombination (driven by the load of deleterious mutations) regularly produces feminized male phenotypes of lower fitness. Relying instead on autosomal SA genes, with hormonally controlled sex-specific expression (as do species with

nongenetic sex determination), may constitute a better solution. Accordingly, many lineages of fishes, frogs and nonavian reptiles display not only sex-chromosome homomorphy, but also high rates of sex-chromosome turnovers (Schartl, 2004; Volff *et al.*, 2007), recurrent XY recombination (Stöck *et al.*, 2011; Dufresnes *et al.*, 2015), as well as evidence for fully functional sex-reversed XX males and XY females (Rodrigues *et al.*, 2017; Whiteley *et al.*, 2017). All of this opposes a major role for sex-linked genes in building sexual dimorphisms. It might be argued that several fish studies nevertheless provide a solid support for the sexual conflict model of sex-chromosome evolution (e.g. Lindholm & Breden, 2002; Kitano *et al.*, 2009; Roberts *et al.*, 2009; Wright *et al.*, 2017). This certainly points to strong sexual-selection pressures in these systems: from our simulations, SA alleles conferring a marked sexual dimorphism may still accumulate on sex chromosomes despite rare XY recombination, provided the benefits are large enough (specifically, Δ values >0.5 or >0.1 for high or low rates of deleterious mutations respectively, Figs 6a and S4a). Although sex-linked SA genes in fishes do not contradict our results, the point remains that sex linkage must induce recombination costs, which would be avoided if these SA genes were autosomal. This certainly calls for further investigations regarding what circumstances may pre-empt specific solutions to sexual conflicts (see below).

Also opposing a primary role for sexual conflicts at the origin of sex chromosomes (van Doorn & Kirkpatrick, 2007, 2010) and in subsequent arrest of recombination (Rice, 1984, 1987a), the point must be made that sex chromosomes may originate for reasons other than sexual selection, such as inbreeding avoidance or meiotic drive (Charlesworth & Charlesworth, 1978; Ubeda *et al.*, 2015), and stop recombining for other reasons as well, such as genetic drift. As pointed out by Ironside (2010), an inversion occurring on an autosome is expected to segregate for some time in populations, temporarily preventing recombination in heterozygous individuals, but will ultimately be either fixed or lost by drift, which will restore full recombination; if, by contrast, such an inversion is fixed by drift on the X or on the Y chromosomes, it will definitively stop recombination between sex chromosomes. Recent evidence for evolutionary strata on fungal mating-type chromosomes demonstrates that sex chromosomes may stop recombining and differentiate in the absence of sexual conflict (Branco *et al.*, 2017). When such a drift-induced recombination arrest occurs in species with sexes and sexual conflicts, then SA genes will subsequently accumulate on sex chromosomes, but as a consequence of recombination arrest, and not as a cause.

Our results call for additional simulation studies to integrate male and female fitness components into evolutionary models of XY recombination under the opposing forces of SA selection and deleterious mutations. In this context, the mechanisms controlling recombination arrest deserve particular attention: contrasting with modifier loci that allow fine control over recombination rates, chromosomal inversions induce a complete and definitive arrest of recombination, which not only remove any hindrance to the accumulation of deleterious mutations, but might also pre-empt solutions to sexual conflicts via the fixation of sex-linked SA genes rather than the sex-specific expression of autosomal genes, thereby condemning sex chromosomes to an ineluctable decay.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Table S1 Summary of the parameter values tested in the simulations.

Figure S1 In the absence of XY recombination, the frequencies of deleterious alleles at generation 10 000 (mean ± 95% CI) are higher on Y chromosomes (solid thick line) than on autosomes (solid line) and X chromosomes (dashed line), for any positive s value (x-axis).

Figure S2 The mean frequency of deleterious alleles at the end of simulations increases with XY recombination (x-axis) for the X chromosome, but decreases for the Y chromosome. Different colors represent different Δ values.

Figure S3 Mean sex phenotypes in females (dashed lines) and males (solid lines) at generation 10 000. Sex phenotypes are more differentiated for higher Δ values (x-axis) and lower s values (see color code).

Figure S4 *A*, males and females SA phenotypes in absence ($s = 0$, upper two panels) or in presence ($s = 0.1$, lower two panels) of deleterious effects.

Figure S5 Values of the correlation coefficients (within replicates, generation 500, $s = 0.1$) between the phenotypic trait P and the several fitness components, as a function of XY recombination rate (R_{XY}) and for different Δ values.

Figure S6 Frequency distribution of alleles at the SA and DM loci in females (left) and males (right). This is a snapshot at generation 500 from one replicate, run with parameter values $s = 0.1$, $\Delta = 0.2$ and $R_{XY} = 0.005$.

Figure S7 Boxplots for the correlation values between the SA phenotypic trait P and the overall fitness W ($\Delta = 0.1$, $s = 0.1$, $R_{XY} = 0.005$, $\mu = 5 \times 10^{-3}$, generationtime = 500) for females (left) and males (right).

Data deposited at Dryad: https://doi.org/10.5061/dryad.9vt6v

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Online supplementary material

	SA trait	DM trait	MO trait
Number of loci	1	100	1
Max number of alleles per locus	251	2	51
Allelic effects	[-8.5 – 8.5]	0 (wild type); 1	[0 - 50]
Initial allelic distribution	normal, mean=0, variance=2	wild type fixed	allele 1 fixed (0 cM)
Mutation probability distribution	normal, mean=pre-mutation value, variance=2	from 0 to 1	uniform
Mutation rate per gen. and allele	10^{-4}	$5x10^{-3}$, $5x10^{-4}$	10^{-3}

Table S1. Summary of the parameter values tested in the simulations.

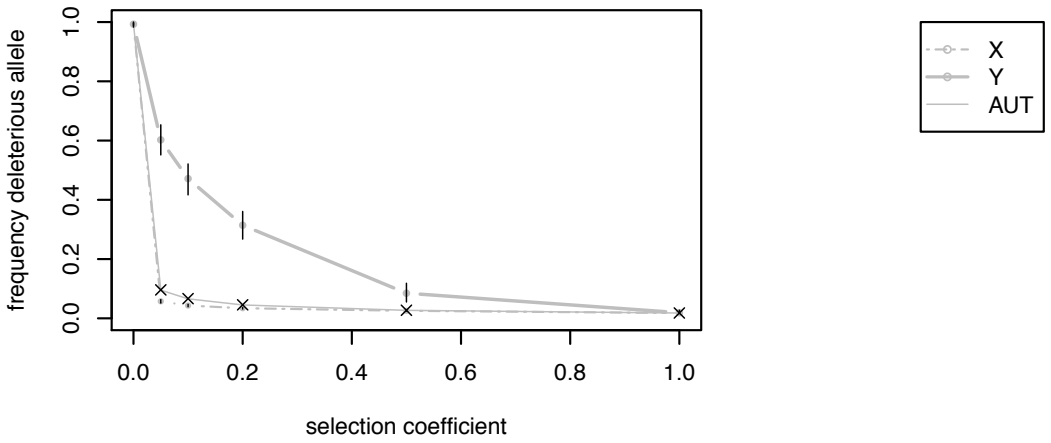


Figure S1. In the absence of XY recombination, the frequencies of deleterious alleles at generation 10,000 (mean ± 95% CI) are higher on Y chromosomes (solid thick line) than on autosomes (solid line) and X chromosomes (dashed line), for any positive *s* value (x-axis). Autosomal values perfectly match the equilibrium frequencies predicted from eq.3 (black crosses). Simulations performed for $\mu = 5 \times 10^{-4}$ and $\Delta = 0.1$.

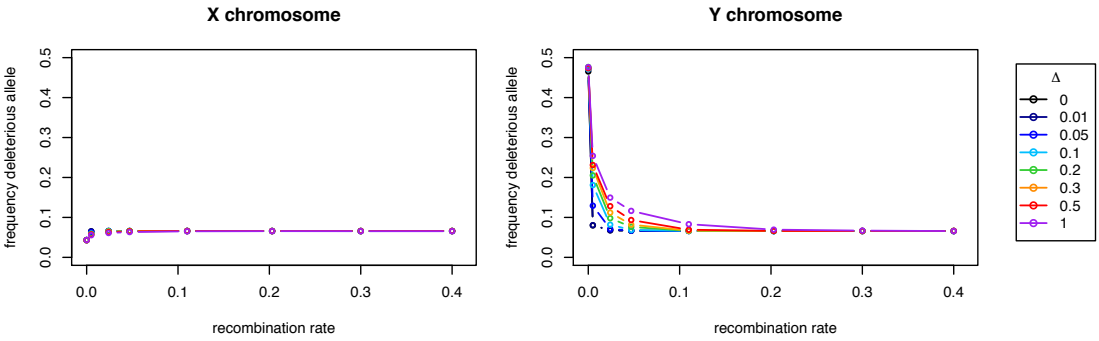


Figure S2. The mean frequency of deleterious alleles at the end of simulations increases with XY recombination (x-axis) for the X chromosome, but decreases for the Y chromosome. Different colors represent different Δ values. Results shown for s = 0.01, h = 0.01 and $\mu = 5 \times 10^{-4}$.

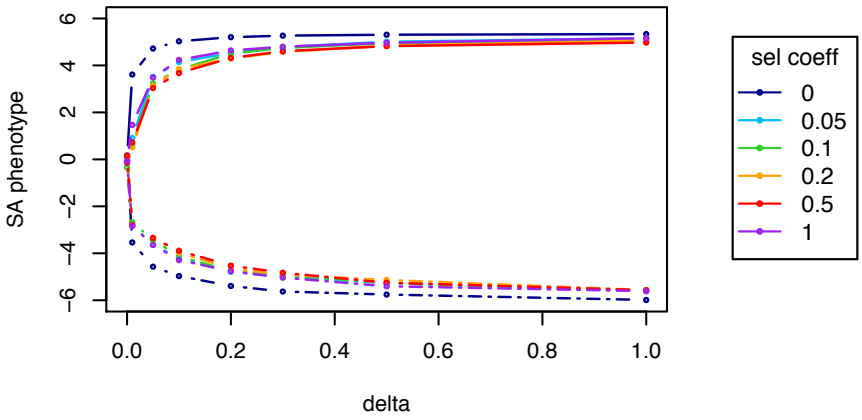
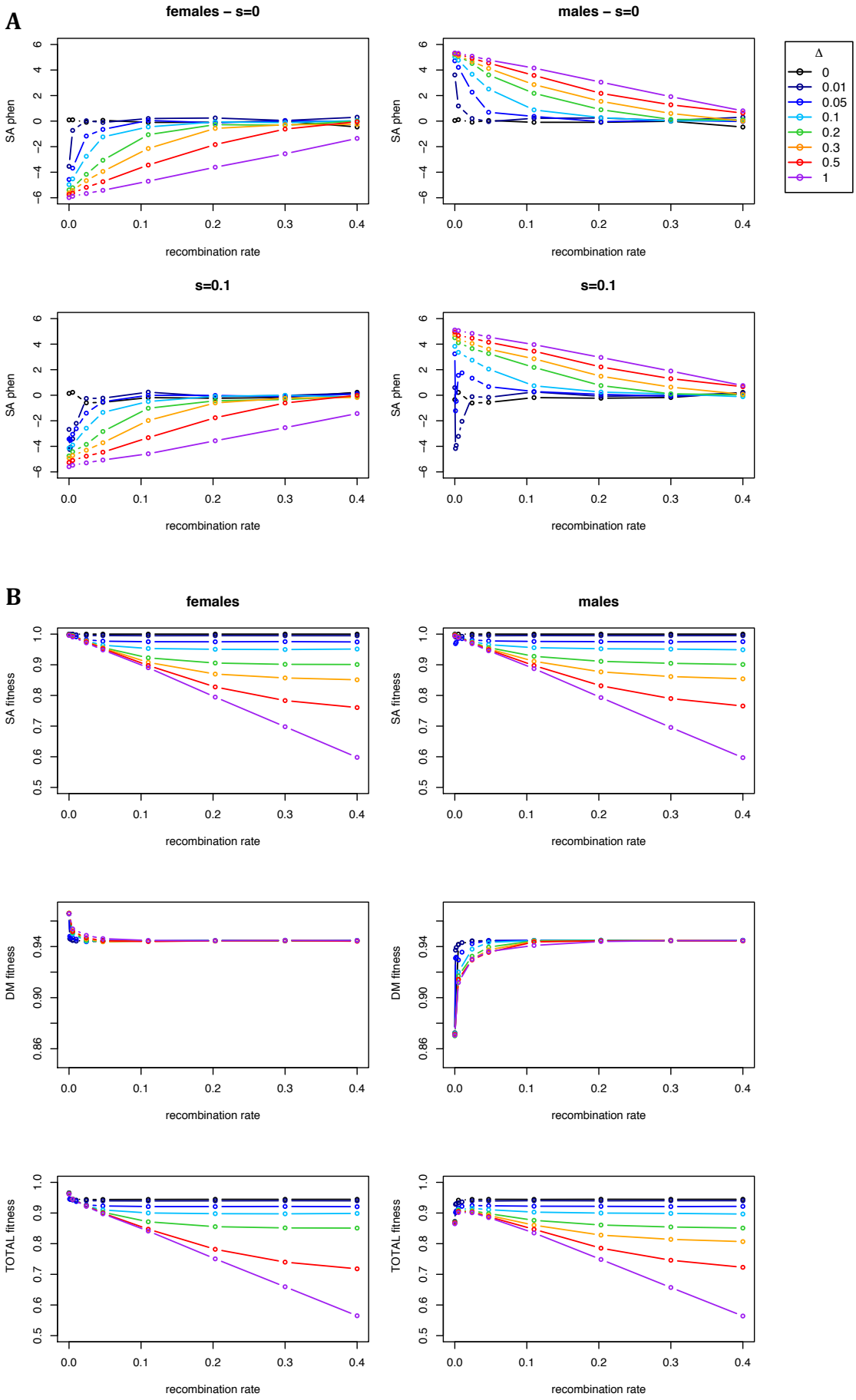


Figure S3. Mean sex phenotypes in females (dashed lines) and males (solid lines) at generation 10,000. Sex phenotypes are more differentiated for higher Δ values (x-axis) and lower *s* values (see color code). Simulations performed for $\mu = 5 \times 10^{-4}$.


A
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males – s=0
s=0.1
s=0.1
SA phen
recombination rate
Δ
0
0.01
0.05
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0.2
0.3
0.5
1
B
females
males
SA fitness
DM fitness
TOTAL fitness
recombination rate

Figure S4. *A*, males and females SA phenotypes in absence (s = 0, upper two panels) or in presence (s = 0.1, lower two panels) of deleterious effects. Average values over 100 replicates at the end of simulations, for different R_{XY} values (x-axis) and Δ (different colors). *B*, W_{SA}, W_{DM} and total fitness as a function of R_{XY}, for different Δ (different colors) and s = 0.1. Simulations performed for μ = 5 x 10^{-4}.

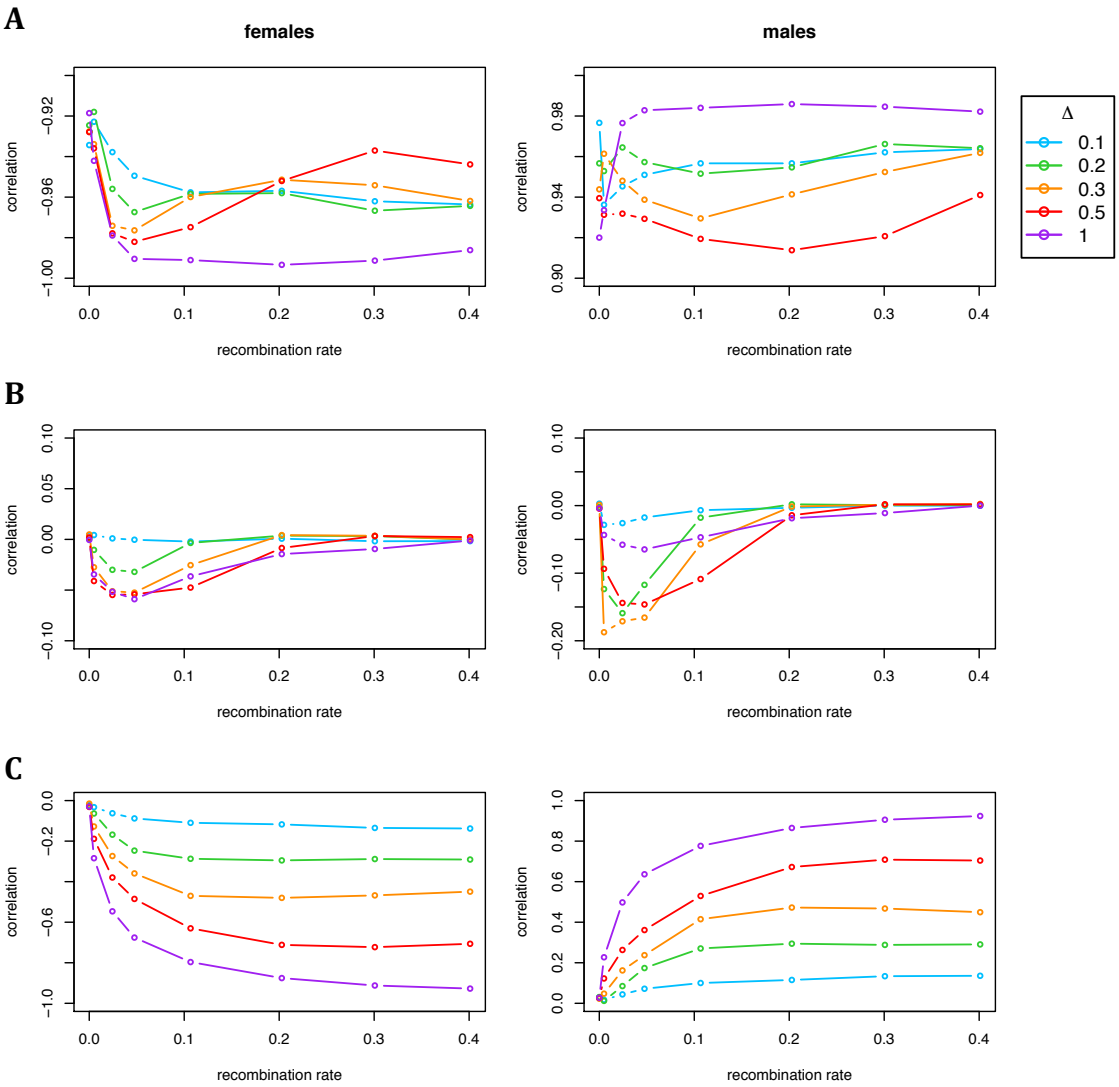

Figure S5. Values of the correlation coefficients (within replicates, generation 500, s = 0.1) between the phenotypic trait P and the several fitness components, as a function of XY recombination rate (R_{XY}) and for different Δ values. *A*, the sex-antagonistic component of fitness (W_{SA}) is always strongly correlated with the SA phenotype, negatively in females (left) and positively in males (right). *B*, the deleterious-mutation component of fitness (W_{DM}) shows negative correlations with the SA phenotype under a large set of parameter values, and mostly so in males at low recombination rate (right). *C*, the overall fitness W correlates negatively with the SA phenotype in females (left) and in general positively in males (right), but at low recombination rate, the selective pressure for smaller SA values in females exceeds that for larger SA values in males (see fig. S7).

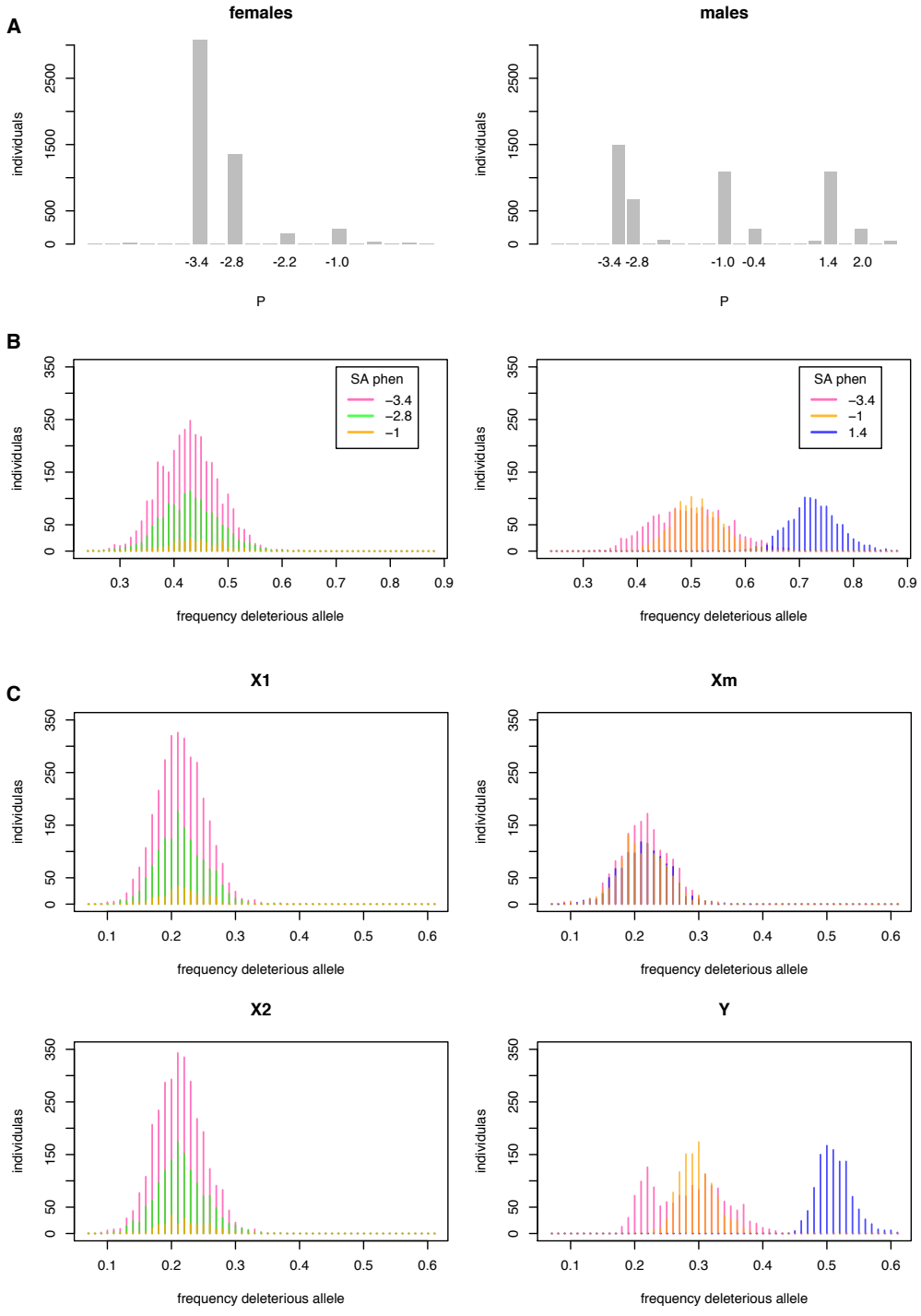

Figure S6. Frequency distribution of alleles at the SA and DM loci in females (left) and males (right). This is a snapshot at generation 500 from one replicate, run with parameter values s = 0.1, Δ = 0.2 and R_{XY}=0.005. Note that at the end of the simulation (generation 10,000) all males will have fixed a feminized SA phenotype. *A*, barplot of SA phenotypes showing that some males (right) already

present female-beneficial phenotypes. *B*, frequency distribution of deleterious alleles for the 3 most frequent SA phenotypes in females (left) and males (right), showing that males with a male-beneficial allele also suffer from a higher load (blue bars, right panel). *C*, frequency distribution of deleterious alleles per SA phenotype and per chromosome (X1-X2 for females, left, Xm-Y for males, right), showing that the load is associated with the male-beneficial allele on the Y chromosome.

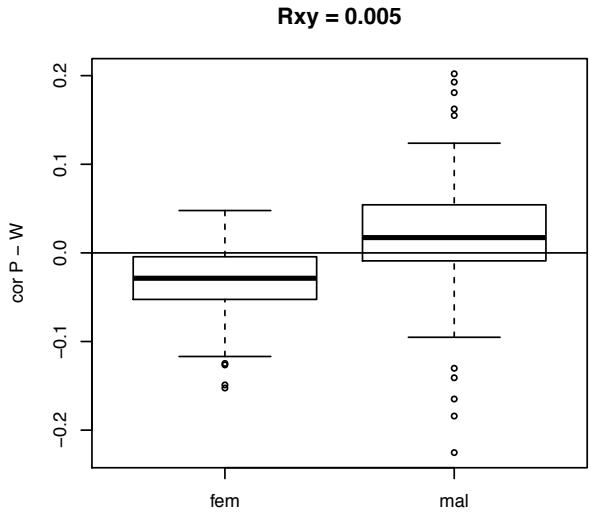

Figure S7. Boxplots for the correlation values between the SA phenotypic trait P and the overall fitness W ($\Delta = 0.1$, $s = 0.1$, $R_{XY} = 0.005$, $\mu = 5 \times 10^{-3}$, generation time=500) for females (left) and males (right). The selection for smaller trait values in females exceeds that for larger values in males.

Chapter two

Sexual conflicts over XY recombination:

When should male or female interests prevail?

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Keywords: recombination modifiers, evolution of recombination, sexually antagonistic selection, deleterious mutations, sex chromosomes

Abstract

The canonical model of sex-chromosome evolution holds that an arrest of XY recombination benefits males, by generating a strict linkage between the sex-determining locus and male-beneficial alleles at sex-linked sexually antagonistic genes. It has been argued, however, that male benefits are more than offset by the load of deleterious mutations accumulating in non-recombining genomic regions and that the arrest of XY recombination actually benefits females, through both the fixation of female-beneficial alleles and the purging of deleterious mutations on X chromosomes. Using individual-based simulations, we show that this sexual conflict over XY recombination is solved mostly to the benefit of males when the modifier of XY recombination is sex linked: X chromosomes fix alleles for no-recombination, while Y chromosomes fix alleles for some recombination, resulting in a low but non-zero equilibrium of XY recombination. When the modifier is autosomal, in contrast, this sexual conflict cannot be solved through a balanced polymorphism, and female interests prevail, favoring a complete arrest of XY recombination. Hence the mechanisms underlying XY recombination, in particular the genomic localization of the modifier, may strongly affect solutions to this sexual conflict and thereby the evolutionary trajectories of sex chromosomes.

Introduction

A typical hallmark of sex-chromosome evolution, observed from a series of lineages that include birds and mammals, is the striking differentiation between a large, gene-rich X or Z chromosome, and a small, gene-poor Y or W chromosome (Muller 1914; Ohno 1967; Charlesworth 1991). Sexually antagonistic (SA) selection has been proposed to play a crucial role in this differentiation (Nei 1969, Rice 1984, 1987a; Charlesworth and Charlesworth 2000). As theory goes, male-beneficial mutations that occur on the Y chromosome close to the sex locus should be favored, even if strongly detrimental to females, because they are preferentially transmitted to sons. In turn, these male-beneficial mutations should select for a complete arrest of XY recombination in males, as a way to reinforce linkage with the sex locus. As a consequence of recombination arrest, however, deleterious mutations will also accumulate on Y chromosomes (respectively W chromosomes in female-heterogametic systems) due to a variety of Hill-Robertson interferences (including Muller's ratchet) and ultimately provoke their degeneration (Charlesworth and Charlesworth 2000).

Contrasting with predictions from this "canonical" model of sex-chromosome evolution, however, many fishes, amphibians, and non-avian reptiles possess homomorphic sex chromosomes, where the Y or W chromosomes lack any visible sign of degeneration. One potential proximate cause for homomorphy resides in occasional XY recombination, originating from either low rates of XY recombination in males (e.g. Stöck et al. 2013), or occasional events of sex reversal (e.g. Rodrigues et al. 2018): whenever the patterns of recombination are controlled by phenotypic sex (rather than by genotypic sex), then X and Y chromosomes will recombine in occasional XY females, preventing their long-term differentiation (the fountain-of-youth model; Perrin 2009). But the ultimate causes for why such lineages depart from the canonical model remain unclear. This canonical model actually relies mostly on verbal arguments. Among the few arguments that received some formalization are the dynamics of alleles at sex-linked SA loci in the absence of deleterious mutations (e.g. Bull 1983; Rice 1987a, 1996) and the decay of non-recombining Y chromosomes with ensuing evolution of dosage compensation (e.g. Charlesworth 1978; Rice 1987b). Other components of the model (in

particular the selective forces acting on and resulting from an arrest of XY recombination) remain essentially verbal. Theoretical models have been developed to investigate the evolution and role of recombination modifiers in the interaction of sex-determining genes with sex-antagonistic genes, or in the presence of beneficial or deleterious mutations (e.g. Nei 1969, Lenormand 2003, Barton 1995, Otto and Barton 1997, Otto 2014). However, a model considering the coevolution and the interaction among the sex-determining, sex-antagonistic and deleterious loci have not been developed. Auxiliary assumptions have thus to be made explicit, if the plausibility of the predicted scenario has to be evaluated. However, analytical formalization of the full model is certainly out of reach, due to the multiple evolutionary forces involved: reduced levels of recombination potentially affect many coevolving genes, and impact different components of fitness in sex-specific ways.

Cavoto et al. (2018) performed individual-based simulations aimed at investigating some aspects of this model, in particular the effects of different rates of XY recombination on sex-specific components of fitness, accounting for interactions between SA genes and deleterious mutations. From their results, the benefits brought by the fixation of male-beneficial mutations on the Y are more than offset by the costs of accumulating deleterious mutations, so that male fitness is not typically maximized by a complete arrest of XY recombination. Such an arrest, however, benefits females, through both the fixation of female-beneficial alleles on the X, and the purging of deleterious mutations on the X via hemizygous exposure in XY males. Simulations furthermore predicted a feminization of Y chromosomes at fixed low recombination rates and low SA selection: the rare recombined Y haplotypes benefit from a reduced load of deleterious mutations, which more than compensates for the transmission of female-beneficial SA alleles. In contrast, the rare recombined X haplotypes suffer from both the male beneficial alleles at the SA locus and the heavier load of deleterious mutations. As a result, female-beneficial alleles in the simulations went to fixation on both X and Y chromosomes at the population level. This study, however, did not formally investigate the evolution of XY recombination.

The evolution of XY recombination was actually addressed by Grossen et al. (2012), also through individual-based simulations. XY recombination in this study was mediated by sex reversal, inspired by the empirical patterns documented in frogs (the fountain-of-youth model). Specifically,

the sex chromosomes harbored a sex locus encoding a sex factor (e.g. a male hormone). Juveniles developed as male (in which sex chromosomes do not recombine) when the sex-factor production exceeded a given threshold, and otherwise as female (in which sex chromosomes recombine). The production of the sex factor by XY individuals would typically exceed the threshold on average, but due to the variance stemming from developmental noise, some XY individuals could occasionally develop as females. These simulations showed that the accumulation of deleterious mutation on little-recombining sex chromosomes indeed selected for a decrease in the average production of the sex factor by the Y allele, resulting in occasional sex reversals and XY recombination, thereby preventing XY differentiation and Y degeneration over evolutionary times.

The mechanisms controlling XY recombination are expected to matter: if recombination is directly controlled by the sex locus (as in Grossen et al. 2012), then sexual conflicts over XY recombination might be partly solved through the fixation of different X and Y alleles. We thus expect an arrest of XY recombination to be favored by the X alleles (which spend 2/3 of their time in females) but opposed by the Y alleles (which only occur in males), resulting in low but non-zero equilibrium rates of recombination (as obtained by Grossen et al. 2012). If, however, XY recombination is controlled by an autosomal locus (with equal time spent in males and females), then sexual conflicts cannot be solved via fixation of distinct alleles. The female interests are actually expected to prevail in this case: the arrest of XY recombination is strongly favored in females (which then benefit from both the fixation of female-beneficial alleles at sex-linked SA genes and an alleviated load of deleterious mutations) but only mildly opposed in males (which suffer then from the heavier load of deleterious mutations but benefit from the fixation of male-beneficial alleles at sex-linked SA genes). We might therefore expect in this case the fixation of an arrest of XY recombination.

Here we explore this hypothesis through individual-based simulations, by contrasting situations where XY recombination is controlled by an autosomal locus on the one hand or by a strictly sex-linked locus on the other hand. In line with our expectations, simulations show that in the former case the combination of selective forces favor a complete arrest of XY recombination (though the actual outcome also depends on mutations occurring at the modifier locus), while in the latter

case, X and Y gametologs indeed fix different alleles, resulting in a low but non-zero XY recombination at equilibrium. We conclude that the genomic locations of the genes that control XY recombination might strongly constrain the evolutionary trajectories of sex chromosomes.

Material and methods

Model structure and parameter values tested were all identical to Cavoto et al. (2017), in order to have comparable results. The main differences from this previous study are that XY recombination was allowed to evolve and controlled by a modifier that was either strictly sex-linked, or strictly unlinked, depending on simulations.

Genetic architecture

We simulated the evolution of a population where individuals carry a pair of sex chromosomes and a pair of autosomes. The sex chromosomes are characterized by a sex-determining (SD) locus, a sex-antagonistic (SA) locus and one hundred loci that can accumulate deleterious mutations (Fig. 1). Sex is controlled by two alleles (*x* and *y*) at the SD locus. Chromosomes carrying the *x* or the *y* alleles are called X and Y respectively. We will assume male heterogamety throughout (XX females and XY males) but without loss of generality (i.e. our conclusions also apply to female heterogametic systems, *mutatis mutandis*). The SA locus encodes a secondary sex character *P* (e.g. a coloration trait), the value of which results from the additive effects of alleles at the locus (251 possible alleles, with allelic values between -8.5 and 8.5). This character *P* affects the SA component of fitness (W_{SA}) in a sex-specific way, with negative *P* values being male-detrimental but female-beneficial, and the reverse for positive *P* values. Specifically, the fitness value is a sigmoidal function of *P*, being

$$W_{SA} = 1 - \frac{\Delta e^{P}}{1 + e^{P}} \qquad \text{for females} \qquad \text{(eq. 1a)}$$

and

$$W_{SA} = 1 - \frac{\Delta e^{-P}}{1 + e^{-P}} \qquad \text{for males} \qquad \text{(eq. 1b)}$$

where Δ measures the strength of sex-antagonistic selection (i.e., 1-Δ is the lower asymptote for the fitness function). One hundred loci, evenly distributed between the SD and SA loci, can accumulate deleterious mutations (DM loci), which affect the deleterious-mutation component of fitness (W_{DM}) given by:

$$W_{DM} = (1\text{-}hs)^{het} (1\text{-}s)^{hom} \qquad \text{(eq. 2)}$$

where *het* and *hom* represent the number of loci that are respectively heterozygous or homozygous for the deleterious allele, h is the dominance coefficient, and s is the selection coefficient of deleterious mutations. For simplicity, h and s are assumed constant across the loci. The overall individual fitness results from the product of W_{SA} and W_{DM} :

$$W = W_{SA} \text{ x } W_{DM} \qquad \text{(eq. 3)}$$

The recombination rate of sex chromosomes is controlled by two recombination modifiers (one for females and one for males), which, depending on simulations, are either strictly linked to the SD locus (co-localizing with this locus), or strictly unlinked (i.e., on an autosome). The recombination modifiers are modeled with 51 alleles, with allelic values ranging from 0 to 50 (steps of 1). The allelic values determine additively the distance d in cM between the SD and the SA loci, corresponding to a recombination rate R of:

$$R = \frac{1 - e^{-2d/100}}{2} \qquad \text{(eq. 4)}$$

In addition to sex chromosomes, each individual carries a pair of autosomes, characterized by 100 DM loci with the same properties as the sex-linked ones. Autosomal recombination is controlled by two pairs of unlinked modifiers (one for recombination in females and one for recombination in males), modeled in the same way as for sex chromosomes (Fig. 1).

Simulations

We ran individual-based simulations with a modified version of the program Quantinemo v1.0.3 (Neuenschwander et al., 2008). A population of 10,000 individuals follows a simple life cycle with

non-overlapping generations. 10,000 offspring are produced at each generation (i.e., selection is soft) by pairing gametes from mothers and fathers randomly chosen with probability proportional to their fitness (eq. 3). At the start of simulations, allele frequencies at the SA locus follow a discretized normal distribution, centered at 0 and with variance 2, and truncated at -8.5 and 8.5. The DM loci are fixed for the wild type allele (non-deleterious), and the recombination modifiers are fixed for the allele with 0 recombination. At each generation, mutations can occur at the SA locus at rate 10^{-4}, and the probability to mutate to any allele follows a discretized normal distribution centered on the pre-mutation value, with variance 2 and truncated at -8.5 and 8.5. Depending on the simulations, alleles at the DM loci can mutate at either a high or low rate ($\mu_{DM} = 5 \times 10^{-3}$ or 5×10^{-4} per locus respectively), and mutations only occur from the wild type to the deleterious allele (no back mutation). The corresponding chromosomal mutation rate (U) had thus maximal values of 1.0 or 0.1 respectively (reached at the start of simulations, when all alleles were wild-type). Actual U values might lie in between, as suggested by data from *Drosophila* or hominids where genomic mutation rates are estimated between 1.0 and 4.0 (Eyre-Walker & Keightley, 1999; Haag-Liautard et al., 2007; Eöry et al., 2010). From our simulations (see Results), the effects of these two rates do not differ qualitatively, but are stronger at the higher mutation rate. Thus, results for the higher mutation rate ($\mu = 5 \times 10^{-3}$) will be presented in the main text, and those for the lower mutation rate ($\mu = 5 \times 10^{-4}$) in Supplementary material. Quantitative differences will be spelled out when relevant. The alleles at the recombination modifiers mutate with a rate of 10^{-3}, with the same probability of mutating to any other allele.

We implemented different strengths of SA selection by varying Δ (with values of {0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 1}), and different effects of deleterious mutations by varying s (set to {0, 0.1, 0.15, 0.2, 0.5, 1}). Parameters were tested in a fully factorial design, with 100 replicates per parameter combination. Most simulations were ran with highly recessive deleterious mutations (h = 0.01), but we also ran a subset of simulations with codominant mutations (h = 0.5).

Simulation extensions

XY-recombination rates reached very low equilibrium values when SA selection was strong and the modifier unlinked to sex (see Results), which might result either from selection for a low but non-zero recombination rate or from recurrent mutations reintroducing recombination at the modifier loci. In order to disentangle the effects of selection and mutation on these equilibrium recombination rates, we ran additional simulations for another 10'000 generations, using the equilibrium values at the SA and DM loci as our new initial conditions, but limiting the modifier to two alleles: one (a_0) for non-recombination and the other (a_1) for rare recombination (1 cM). These two alleles had initial frequencies of 0.5, with no mutation (while SA and DM loci were still allowed to mutate).

Results

Recombination rates

Under all settings, XX- and autosomal recombination rates quickly evolved from the initial value of 0 towards 50 cM on average, with a uniform distribution of alleles from 0 to 50, as expected from the mutation model (Fig. 2, dotted lines). In contrast, XY recombination evolved different equilibrium values depending on SA selection (Δ), deleterious mutation load (s and μ), as well as on the localization of the modifier.

Consider first results with the modifier unlinked to the sex locus (i.e., autosomal): for Δ values below 0.1, equilibrium XY recombination rates were similar to XX- and autosomal values (i.e., 50 cM), whatever the load of deleterious mutations (Fig. 2a). This rate rapidly dropped with increasing Δ to reach ~ 2 cM at $\Delta = 0.3$, then further declined progressively down to ~ 0.5 cM at $\Delta = 1$. The rapid drop in the interval $\Delta = \{0.12, 0.3\}$ (grey shaded area) occurred at different rates depending on s values, so that genetic map lengths were larger at higher s values in this interval. Closer inspection of simulation results over this interval unveils a bimodal distribution: within a set of simulations with identical Δ and s values, replicates reached either high or low equilibrium recombination rates (Fig. S1a). The decline with Δ actually resulted from a decrease in the proportion

of simulations ending with high recombination. This suggests a bistable equilibrium, with a random component in the probability of reaching one or the other equilibrium value. A lower rate of deleterious mutations (μ_{DM} = 5 x 10^{-4}) resulted in qualitatively similar results, except that the rapid drop occurred at lower Δ values and over a much reduced interval, namely Δ = {0.1; 0.15}, also leaving less scope for differential recombination rate between different *s* values (Fig. S2).

Results differed markedly in the case of a strictly sex-linked modifier (Fig. 2a). In the absence of deleterious mutations (*s* = 0), the rapid drop in XY recombination occurred as soon as Δ values departed from 0, then equilibrium value slowly declined down to ~ 0.5 cM at Δ = 1. For *s* > 0, the drop only started at Δ = 0.15, then equilibrium recombination stabilized at ~ 2.0 cM for Δ values 0.5 and above. Results for different *s* values mostly differ over a range of Δ values spanning 0.15 to 0.5 (grey shaded area), with higher recombination rates reached at higher *s* values. Interestingly, inspection of equilibrium allelic distributions at high Δ values (average length 2 cM) shows different allelic compositions between the X and the Y chromosomes: alleles for no recombination segregate at much higher frequencies on the X than on the Y chromosome (Fig. S3).

Sex phenotypes

Equilibrium XY-recombination rates strongly affected male and female SA phenotypes, with additional effects of Δ and *s*. The general trend was for an increase in sexual dimorphism with increasing Δ values, but the exact form of this increase also depended on the localization of the modifier of XY recombination.

Consider first results of simulations with the modifier unlinked to the sex chromosomes (Fig. 3a; see Fig. S4 for simulations with a lower μ_{DM}). In the absence of deleterious mutations (*s* = 0), no sexual dimorphism occurred below Δ = 0.12 (in accordance with the high rate of XY recombination; Fig. 2a). Then sexual dimorphism suddenly increased, and quickly reached high values, with strongly positive SA trait values in males, and strongly negative ones in females. For positive *s* values, the sudden increase occurred at larger Δ values (~ 0.15), and sexual dimorphism reached less extreme values. There was furthermore a significant effect of *s*: weaker sexual dimorphism was reached at higher *s* values. Interestingly, we note a sex asymmetry over the Δ interval during which

recombination rate declines (grey shaded area): at high s values, males are relatively less masculinized than females are feminized. We also note a bimodal distribution of phenotypes in both males and females in this shaded area: in simulations where different equilibriums of recombination were reached under the same parameter setting, males and females reached divergent SA phenotypes in replicates with low recombination, but not in replicates with high recombination (Fig. S1b).

Simulations with a sex-linked modifier resulted in largely similar patterns, with the difference that sex phenotypes were more differentiated at $s = 0$ (and in this case for any positive Δ value), and less differentiated at $s > 0$ (Fig. 3b). Sex asymmetry also occurred over the range of Δ values during which recombination drops (grey shaded area): over this range, male phenotype was less masculinized than the female was feminized, and mostly so at high s values.

Deleterious mutation load

As expected from the mutation-selection balance, the equilibrium loads of deleterious mutations were smaller at higher s values (Fig. S5). Furthermore, the drop in XY recombination with increasing Δ values translated into parallel changes in the load (see Fig. S5 for the case of an autosomal modifier; Fig. S6 for the lower μ_{DM} value): at low Δ values, where X and Y fully recombine, the equilibrium load on sex chromosomes matches that on autosomes (as well as theoretical expectations; Burger 1983). It then increases in males over the Δ interval corresponding to a drop in XY recombination (grey shaded area). In parallel, it decreases in females, due to stronger purifying selection in XY males (when a Y locus has fixed a deleterious mutation, a deleterious mutation occurring at the homologous locus on the X is strongly counter-selected in XY males).

To test the robustness of our conclusions regarding the effect of localization of the modifier, we also performed some simulations with co-dominant deleterious alleles ($h = 0.5$). When the modifier was autosomal, XY recombination reached the same very low values as for $h = 0.01$ under strong SA selection (~0.5 cM at $\Delta = 1$, Fig. 2a). When the modifier was sex linked, however, the asymptotic XY recombination largely exceeded the one reached for highly recessive mutations, approaching 5 cM at $\Delta = 1$ (as opposed to 2 cM for $h = 0.01$, Fig. 2b), also resulting in a lower sex dimorphism (Fig. 3) and a reduced load of mutations on the Y (Fig. S7).

Disentangling selection and mutation effects on XY recombination

Equilibrium levels of XY recombination were very low at large Δ values, particularly so when the modifier was autosomal (~0.5 cM as compared to ~2 cM in the case of a sex-linked modifier; Fig. 2). The question arises whether such low values were maintained by positive selection for low levels of recombination (which allow a purge of deleterious mutations) or by mutations at the modifier locus, which consistently generate some level of recombination even in cases where selection would otherwise favor a complete arrest. In order to disentangle these effects, we ran an additional series of simulations for another 10,000 generations, using as new initial conditions the SA and DM allelic values reached at the end of the first series, but limiting the modifier to two alleles: one (a_0) for non-recombination and the other (a_1) for rare recombination (1 cM), assigned randomly with initial frequencies 0.5 and no mutation (while SA and DM loci were still allowed to mutate). Hence, a_0a_0, a_0a_1 and a_1a_1 individuals had recombination maps of 0.0, 1.0 and 2.0 cM respectively.

Consider first simulations with an autosomal modifier (Fig. S8). In the absence of deleterious mutations ($s = 0$), both alleles were still segregating in most replicates at the end of simulations at $\Delta = 0$; in the few cases where fixation had occurred, a_0 and a_1 were fixed with the same probability, pointing to a neutral situation. With $\Delta > 0$, however, a_0 was rapidly fixed in all replicates, clearly indicating selection for recombination arrest. Outcomes slightly differed in the presence of deleterious mutations ($s = 0.1$ and 0.2): first, a_0 was more likely to be fixed than a_1 even without SA selection ($\Delta = 0$), likely a result of our multiplicative model for DM fitness (average progeny fitness is higher when deleterious mutations concentrate in sons rather than being redistributed across all offspring via XY recombination); second this probability increased only progressively with Δ (with long fixation times at intermediate Δ values), which we interpret as a result of the opposite effects of SA selection and deleterious mutations (higher Δ values are required for the rapid fixation of a_0). However, allele a_0 was always more likely to be fixed than the alternative allele a_1, clearly pointing to selection for a recombination arrest.

For a sex-linked modifier (Fig 4), outcomes were similar in the absence of deleterious mutations ($s = 0$): very few replicates at $\Delta = 0$ had fixed one or the other allele after 10,000

generations, and the fixation of a_0 and a_1 on X or Y was random, testifying to purely neutral dynamics. With positive Δ values, similarly, a_0 was also rapidly fixed on both X and Y, indicating strong selection for recombination arrest in both sexes. However, in the presence of deleterious mutations (s = 0.1, 0.2), X and Y chromosomes rapidly fixed distinct alleles for any Δ value in the majority of simulations. At weak SA selection, the Y chromosome fixed the recombination allele (a_1) in 75-80% of simulations, while the X fixed the non-recombination allele (a_0) in ~90% of simulations, suggesting selection for XY recombination on the Y, but for an arrest of recombination on the X. Inspection of the dynamics of fixation (Fig. S9) shows a pattern of rapid fixation (mostly of a_1) on the Y (on the order of 100 generations), followed by a more delayed fixation (mostly of a_0) on the X (on the order of 1,000 generations). As Δ increased, the probability of fixation of a_0 increased on both X and Y chromosomes, ending up with 100% fixation of a_0 on the X and 50% fixation of a_1 on the Y at Δ = 1.

Discussion

The dynamics of XY recombination in our simulations depended on four main distinct evolutionary forces, stemming from both neutral and selective processes. Besides genetic drift, neutral forces included mutations at the modifier locus, with the potential to prevent a complete arrest of recombination. Selective forces included first SA selection, with convergent interests in both sexes: an arrest of XY recombination benefited both males and females, via the fixation of male- and female beneficial mutations on Y and X respectively, thereby partially solving sexual conflicts at the SA locus. The other selective force was the load of deleterious mutations, with divergent fitness consequences on the two sexes: an arrest of XY recombination decreased the DM component of fitness in males (and Y chromosomes) but increased it in females (and X chromosomes). The overall effects of varying Δ values (Fig. 2) have to be interpreted in the light of the relative contributions of these several forces. Of special interest for our present work are the drastic differences in the interplay between these forces (and outcomes of simulations) depending on whether the modifier was autosomal or sex linked.

Consider first the case of autosomal control (Fig. 2a). Under weak SA selection pressure ($\Delta < 0.1$), the evolution of XY recombination mostly depends on neutral processes (mutation and drift); the genetic map of sex chromosomes equilibrates at 50 cM in males (i.e., same as for females and autosomes), with a uniform distribution of alleles between 0 and 50, matching the mutation model. Due to this high rate of XY recombination, sexual dimorphism cannot evolve (Fig. 3a), and the load of deleterious mutations on X and Y reaches the same value as on autosomes. At intermediate Δ values (0.1 – 0.3), SA selection progressively takes on a more significant role, resulting in a rapid drop in XY recombination over a relatively limited range of Δ values. Closer inspection of individual simulations (Fig. S1) points to a bistable equilibrium, with a random component (stemming from genetic drift and mutations) in the probability to reach either the high or the low equilibrium XY recombination value. The selective coefficient of deleterious mutations also matters: higher *s* values exert stronger selection in favor of XY recombination, so that stronger SA selection is also required to switch to the lower equilibrium. This drop in XY recombination induces both a heavier load of deleterious mutations on the Y (respectively alleviated load on the X; Fig. S5) and the progressive buildup of sexual dimorphism. The slight sex asymmetry (males are less masculinized than females are feminized) results from the differential effects of recombination in males and females: rare recombining Y chromosomes inherit a maladaptive feminized SA allele but also an alleviated load of deleterious mutations and can thus spread in the population, while rare recombining X chromosomes (which inherit both the maladaptive masculinized SA allele and a heavier load of deleterious mutations) are quickly counter-selected (Cavoto et al. 2017). At high Δ values finally, all simulations converge towards the same low level of XY recombination (~ 0.5 cM at $\Delta = 1$), independent of *s* values. Accordingly, sexual dimorphism is strong (Fig. 3a), with however a clear effect of *s* resulting from Hill-Robertson interactions between SA and DM genes: strongly deleterious mutations impede the fixation of highly beneficial SA alleles. Importantly, our additional sets of simulations show that the low level of XY recombination is only maintained by recurrent mutations at the modifier locus. The combined effects of SA selection and DM load in both sexes actually favors an arrest of XY recombination (Fig. S8): the twofold benefits to females (stemming from the fixation of female beneficial alleles and the purge of deleterious alleles on the X) more than offset the costs incurred by

males from the accumulation of deleterious mutations on the Y (i.e., female interests prevail). These results are in line with the findings of previous theoretical work done by Lenormand (2003), where decreased recombination evolved under the assumption of loosely linked modifier in presence of alleles with different effect on male and female's fitness.

Consider now the case of a sex-linked modifier. In the absence of deleterious mutations (s = 0), recombination arrest benefits both sexes via the buildup of sexual dimorphism (Fig. 3b), so that XY recombination rapidly drops below 50 cM as soon as Δ departs from 0 (Fig. 2b). At $\Delta = 1$, a low level of XY recombination (0.5 cM) is maintained by the constant input of new mutations. In the presence of deleterious mutations ($s > 0$), the drop in XY recombination only occurs for Δ values over the range {0.15-0.5}, and more rapidly so at low s values. This drop is also accompanied by a progressive phenotypic differentiation of sexes, still with the same sex asymmetry (weaker masculinization of males), stemming from the same causes (rare recombined Y haplotypes with a feminized SA allele also benefit from the lower load of deleterious mutations). At larger Δ values, all simulations converge towards a rate of XY recombination that is distinctly larger than for an autosomal modifier (2.0 cM versus 0.5 cM). Importantly, alleles for no XY recombination are then maintained at much higher frequencies on the X than on the Y chromosomes (Fig. S3). Our additional sets of simulations furthermore confirm that, in the absence of mutations at the modifier locus, the X gametolog tends to fix the non-recombination allele (a_0), while the Y gametolog tends to fix the recombination allele (a_1) (Fig. 4). This striking difference between situations where the modifier is either sex linked or autosomal seems quite robust with respect to the rate and dominance coefficient of deleterious mutations (Fig. 2a,b): selection always favored an arrest of XY recombination when the modifier was autosomal, but maintained some recombination at the Y allele when the modifier was sex linked. The contrast was even stronger with codominant mutations (h = 0.5), where even higher equilibrium XY-recombination rates were reached at high Δ values with a sex-linked modifier (5 cM), likely because codominance weakened the selection on females for an arrest of XY recombination.

Thus, our analyses indeed show that males and females (respectively Y and X chromosomes) have divergent interests regarding XY recombination; under the joint action of SA selection and deleterious mutations, an arrest of XY recombination is strongly favored in females (and X

chromosomes), but slightly disfavored in males (and Y chromosomes). If the modifier of recombination is sex linked, this sexual conflict over XY recombination can be partially solved via the fixation of different alleles on the X and the Y gametologs, Or, putting it differently: a strictly Y-linked allele that increases XY recombination is likely to be fixed because Y-linkage ensures that it will gain from the purging of deleterious mutations. As a result, low levels of XY recombination are selectively maintained at equilibrium. If, in contrast, the modifier is autosomal, there is no way of solving the sexual conflict via the differential fixation of male- and female beneficial alleles. In this case the female interests prevail, because the benefits to females more than offset the costs to males. These results seem at first in contrast with the finding of Otto (2014), where increased recombination could evolve for unlinked modifiers, but not for sex-linked ones. In this paper, the author shows how in case of over-dominance in males increased recombination evolves when modifiers for recombination are loosely linked, because of the short-term advantage of recombination. We observe the opposite because under our settings there is a long-term advantage of recombination, but a short-term disadvantage.

Our results echo those of Grossen et al. (2012), despite distinctly different settings. In this former study, XY recombination was mediated by sex reversal, assuming that sex chromosomes recombine in phenotypic females, but not in phenotypic males (the fountain-of-youth model). Sex reversal was controlled by the sex locus: juveniles developed as males if production of a sex factor by this locus exceeded a given threshold, and as females otherwise (threshold model of sex determination). In these simulations, the Y allele evolved towards lower production of the sex factor for *s* values generating a high load of deleterious mutations, generating sex-reversed XY females in which X and Y recombined (thereby alleviating the load of deleterious mutations on the Y). Interestingly, the X allele evolved in the meantime towards a higher production of the sex factor, counteracting the Y evolution and reducing the occurrence of sex-reversed XY females. The overall outcome was a low but non-zero rate of XY recombination, similar to our present results for a sex-linked modifier, and for the same reasons.

Thus, the evolution of XY recombination is expected to depend on the underlying mechanisms, in particular on the localization of the modifier, which might potentially account for

some of the differences in the evolutionary trajectories of sex chromosomes documented among lineages of vertebrates (see Introduction). Unfortunately, these questions have been little investigated empirically. Sex-reversal experiments (e.g. Kondo et al. 2001) as well as field evidence (Rodrigues et al. 2018) support the idea that XY recombination in several lineages of fishes and amphibians is mediated by rare events of XY sex reversal, occurring at a frequency controlled by the sex locus (see formalization in Grossen et al. 2012). This would explain the low but non-zero rate of XY recombination and ensuing lack of sex-chromosomes differentiation in these lineages. For lineages with highly differentiated sex chromosomes (mammals, birds, *Drosophila*), inversions have often been invoked as the main mechanism underlying the arrest of recombination (with limited empirical support, however). Inversions also show strict association with the sex locus, but obviously differ from modifiers in preventing any fine-tuning of the recombination rate: an inversion on the X or on the Y entirely and definitively stops XY recombination. Further formalization of the consequences of inversions as an alternative mechanism would provide interesting extensions of the present work. A potential outcome of such formalization, given our present results, is that inversions occurring on X chromosomes are favored (because females benefit from the arrest of XY recombination), while inversions occurring on Y chromosome are counter-selected (because male fitness is reduced by an arrest of XY recombination).

More generally, our present results oppose some of the assumptions underlying the canonical model of sex-chromosome evolution (notably by showing that an arrest of XY recombination actually benefits females, and not males), and support the idea that the mechanisms underlying XY recombination (notably the genomic localization of the modifier of recombination) may preempt specific solutions to sexual conflicts over XY recombination or other phenotypic traits and thereby drastically affect the evolutionary trajectories of sex chromosomes and the evolution of sexual dimorphism.

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Figures

Figure 1. Structure of sex chromosome and autosome with unlinked modifiers. The sex chromosome contains one sex determining (SD) locus, one sexually antagonistic (SA) locus, and one hundred functional loci that may accumulate deleterious mutations (DM). Sex chromosome recombination in males is controlled by one unlinked modifier, and by another unlinked modifier in females. The same applies for the autosomal pair, which however lacks any SD or SA locus.

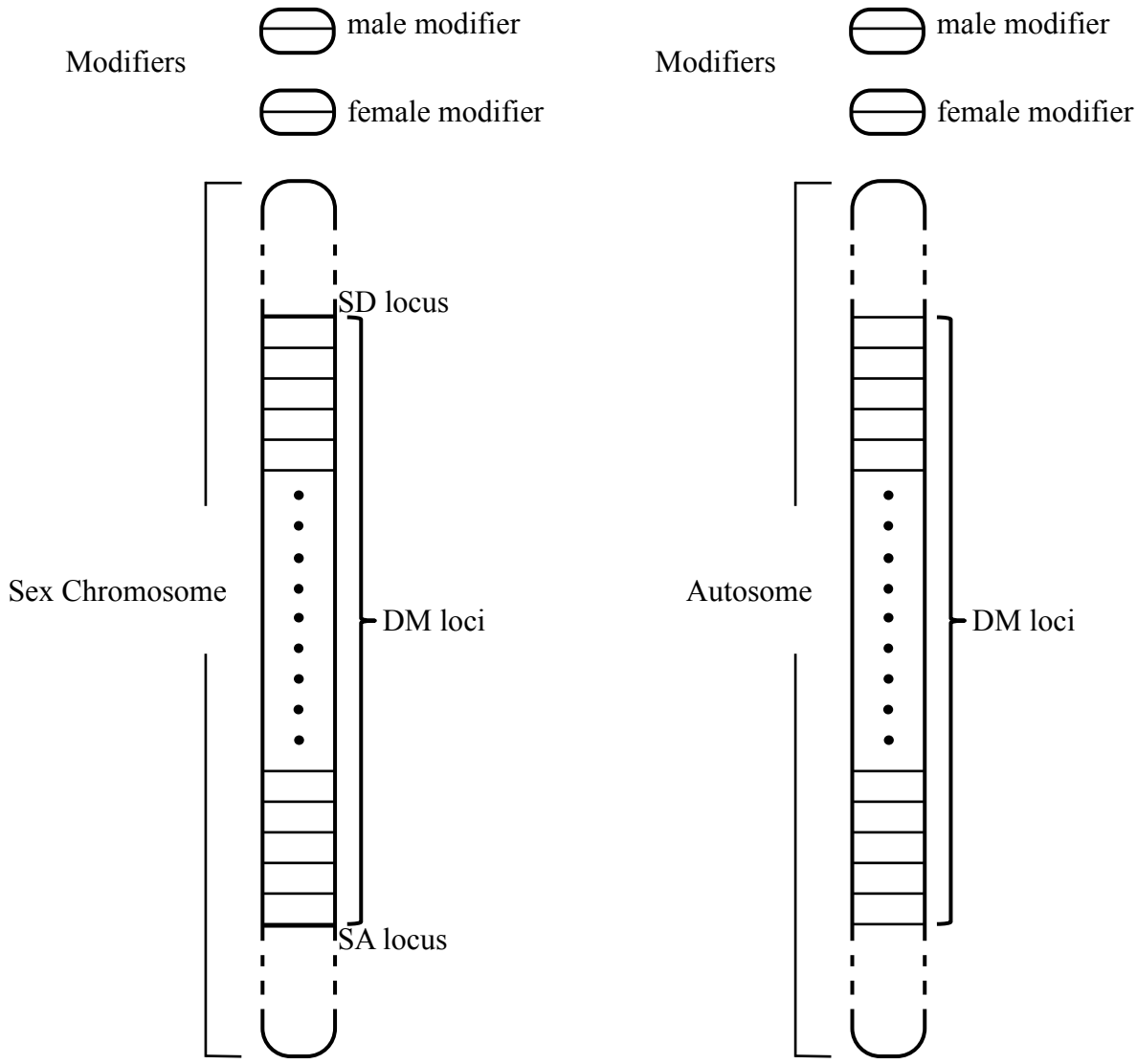
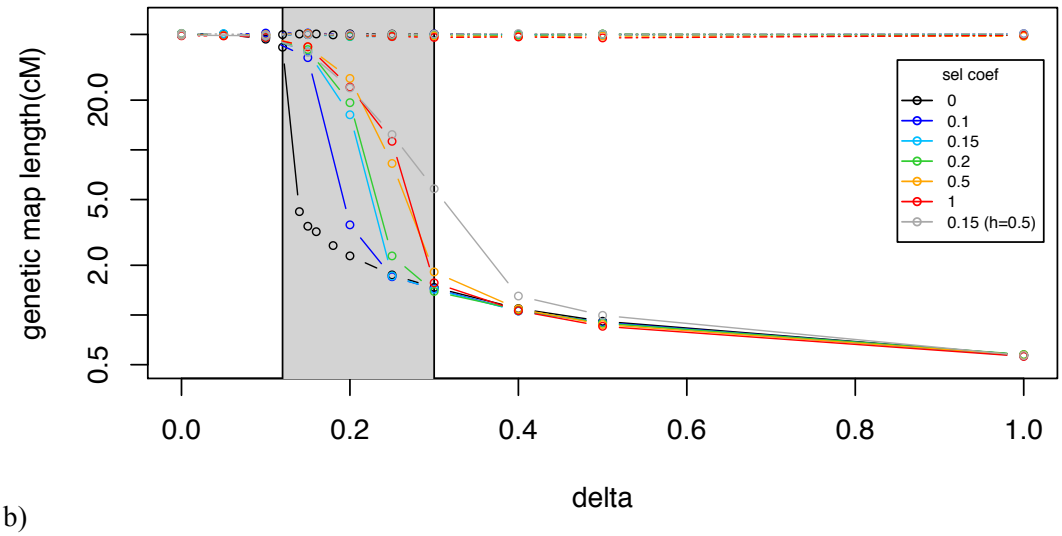


Figure 2. Average genetic map length in cM of males (solid lines) and females (dotted lines), for different Δ values (x-axis) and different strengths of deleterious mutations (*s*; see color code). Each point is the average end result of 100 replicates, with $\mu_{DM} = 5 \times 10^{-3}$. The modifier was either autosomal (a) or sex-linked (b). Drops in recombination occurred at intermediate Δ values (grey area).

a)



b)

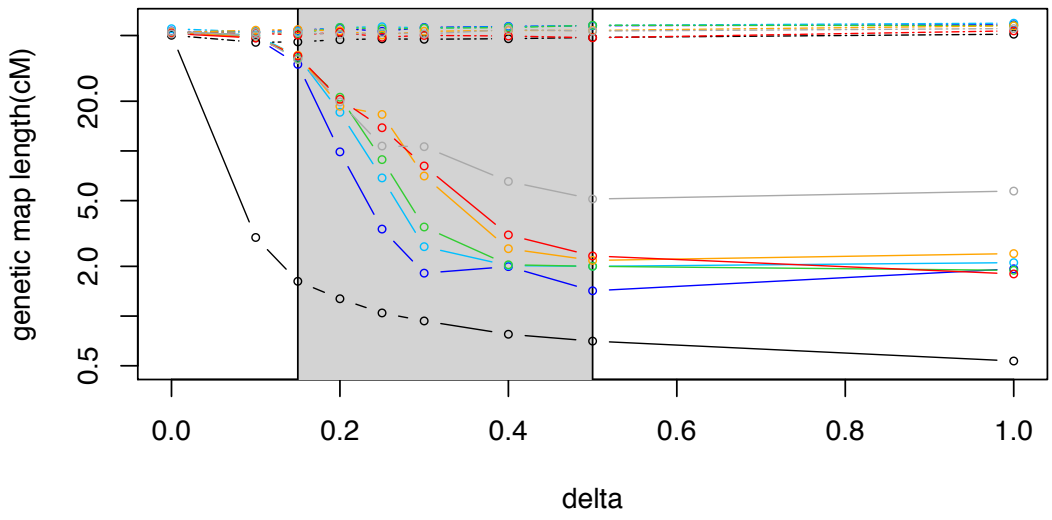
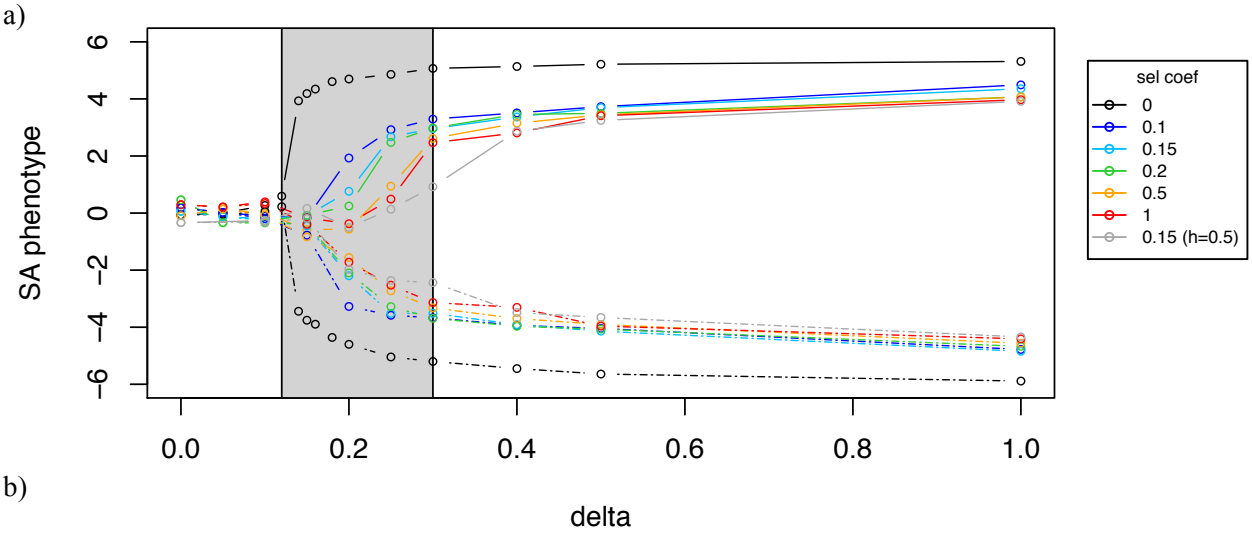


Figure 3. Mean SA phenotype of males (solid lines) and females (dotted lines) for different Δ values (x-axis) and different strengths of deleterious mutations (*s*; see color code). Each point is the average end result of 100 replicates, with μ_{DM} = 5 x 10^{-3}. The modifier was either autosomal (a) or b) sex linked (b). The build up of sexual dimorphism occurred at intermediate Δ values (grey area).

a)



b)

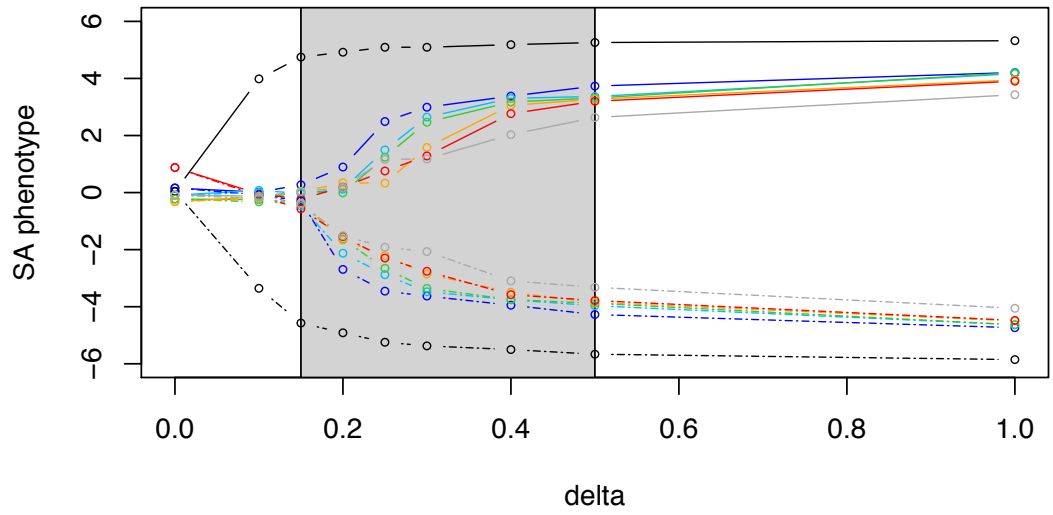
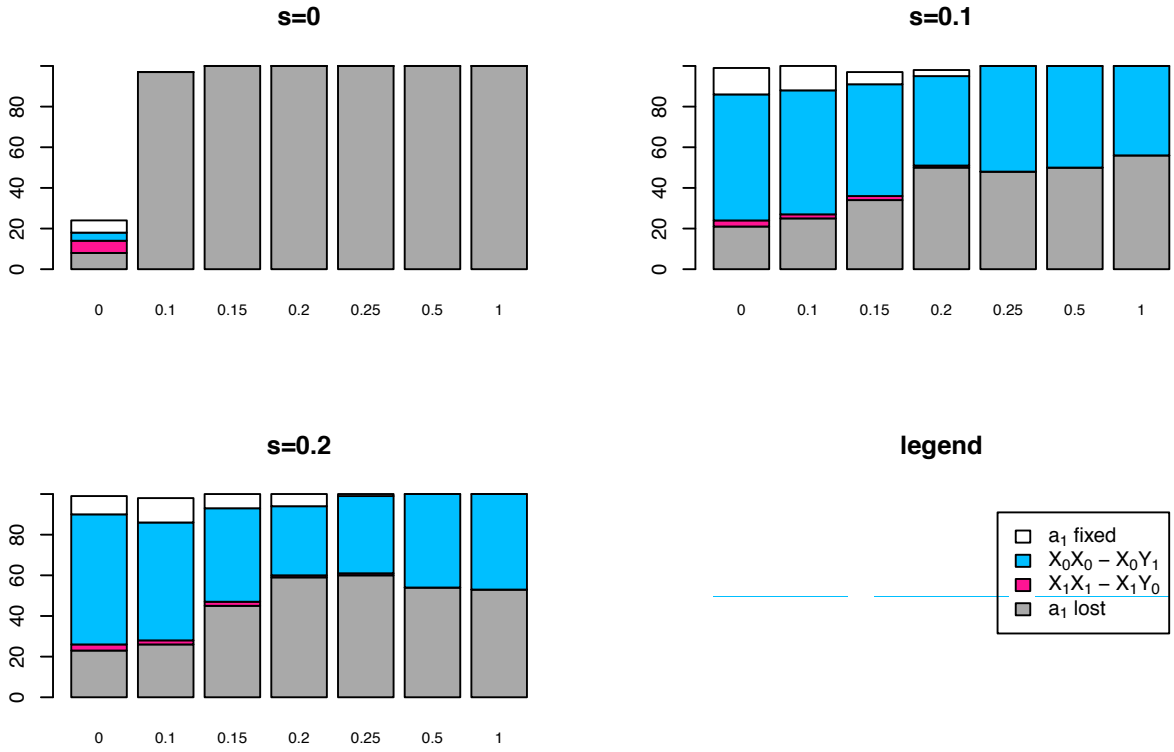


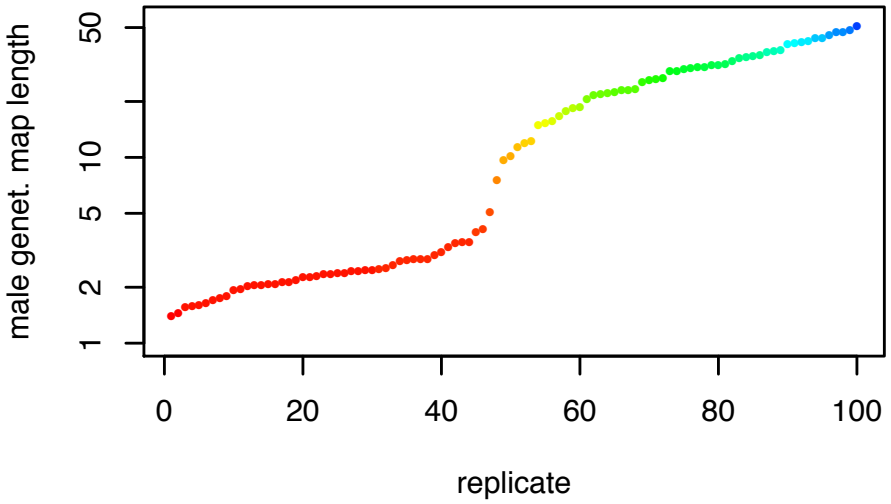
Figure 4. Fixation frequency on X and Y chromosomes of a_0 and a_1 alleles at a sex-linked modifier of XY recombination, for different values of *s* (panels) and Δ (bars). The X chromosome tends to fix the allele for no recombination (grey and blue) while the Y chromosome tends to fix the allele for recombination (white and blue).



Supplementary figures

Figure S1. For identical parameter values (here s = 0.15, Δ = 0.15, μ_{DM} = 5 x 10^{-3}) the modifier locus may fix alleles for very different XY recombination values, with a seemingly bimodal distribution (a). Simulations resulting in low XY recombination (red dots) associate with a significant sex dimorphism at the SA locus, while those with high XY recombination (yellow to blue) associate with little or no sex dimorphism (b).

a)



b)

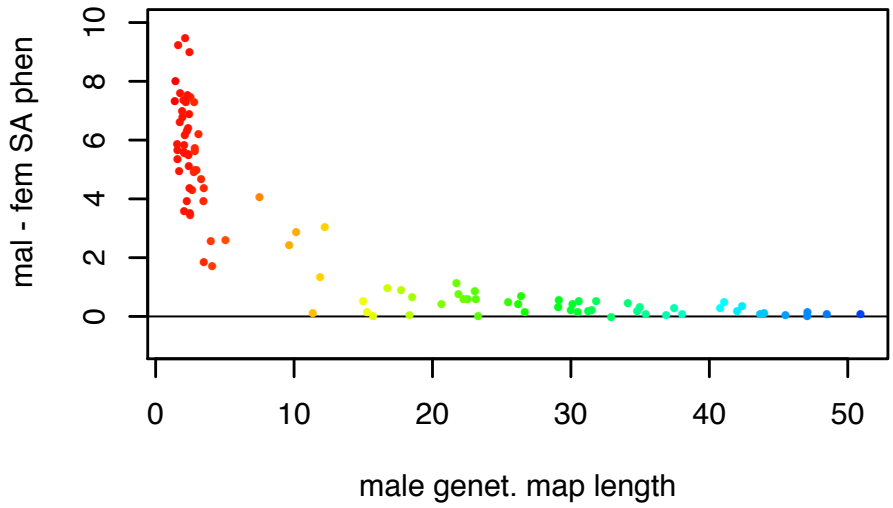


Figure S2. Average genetic map length in cM of males (solid lines) and females (dotted lines), for different Δ values (x-axis) and at different strengths of deleterious mutations (see color code). Each point is the average of 100 replicates, after the simulations ran for 10'000 generations. Deleterious mutations occurred at a rate μ_{DM}= 5 x 10^{-4} and the modifier was unlinked to the sex chromosome. The drop in recombination occurred over a very small interval of Δ values (0.1-0.15; grey area).

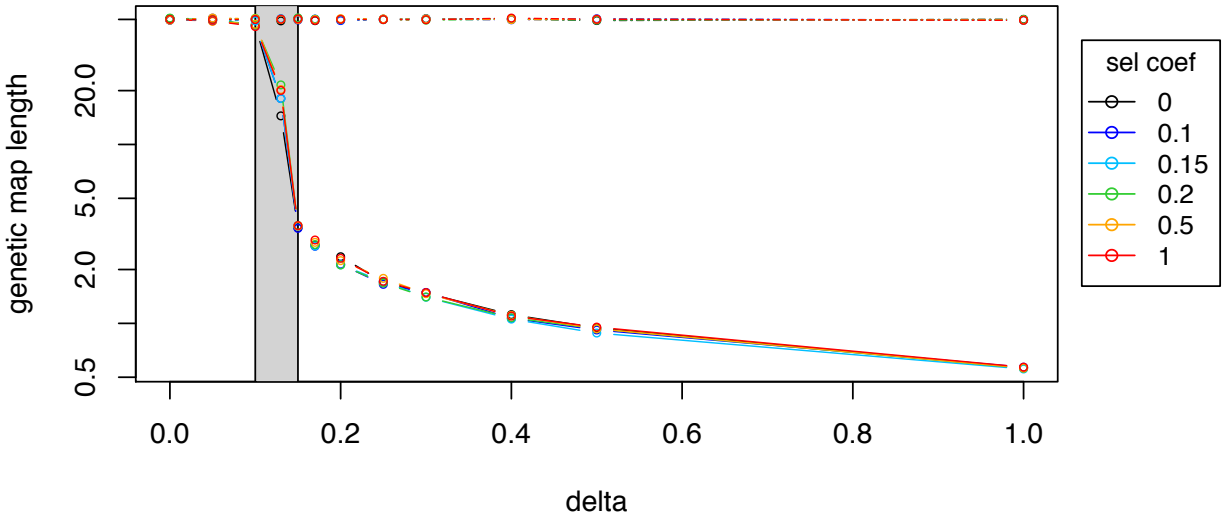


Figure S3. Mean equilibrium frequency distributions of alleles (range 0 to 50) at the modifier locus, for several Δ values (color code). When the modifier is sex linked, the allele for no recombination (first left) segregates at very high frequency (>90%) on the X (triangles), but at much lower values (~11%) on the Y (circles). For comparison, this allele reaches near fixation when the modifier is autosomal (black dots; Δ=1). Simulations with s = 0.1 and $\mu_{DM} = 5 \times 10^{-3}$.

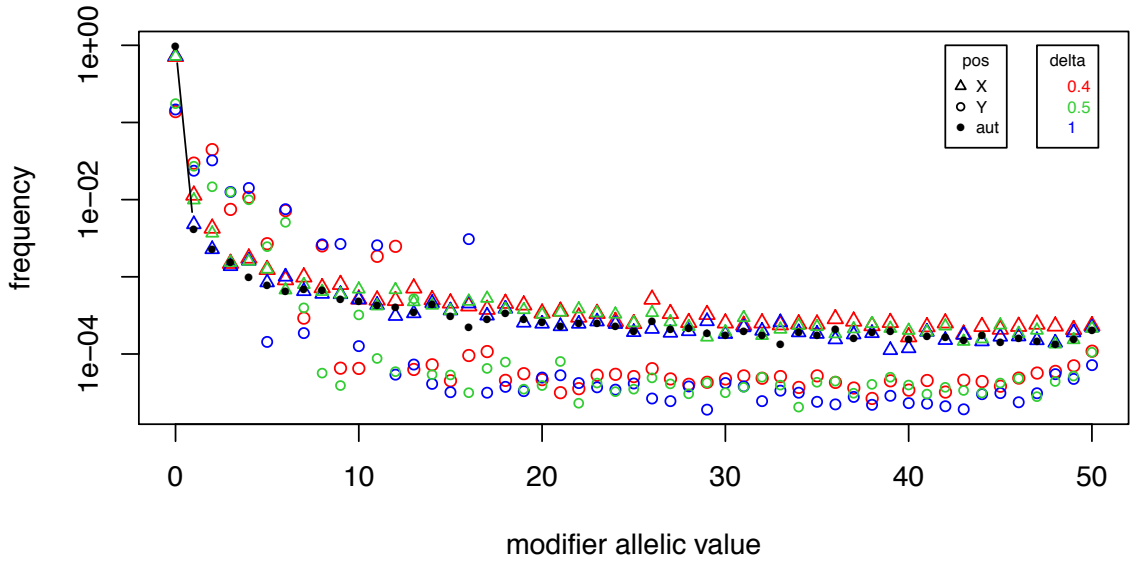


Figure S4. Mean SA phenotype of males (solid lines) and females (dotted lines) for different Δ values (x-axis) and different strengths of deleterious mutations (*s*; see color code). Each point is the average end value of 100 replicates, with μ_{DM} = 5 x 10^{-4} and autosomal modifier. The build up of sexual dimorphism occurred over a very short range of Δ values (grey area).

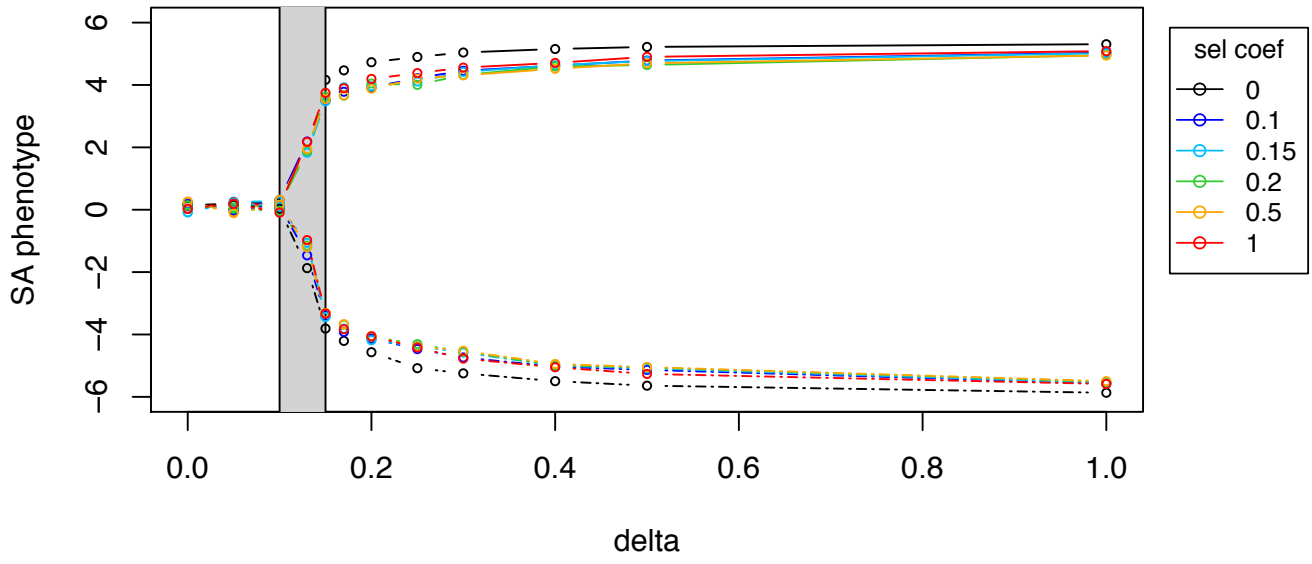


Figure S5. Amount of deleterious mutations in males (upper panel) and females (lower panel). Each point represents the average end value over 100 replicates, for different *s* values (see color code), with μ_{DM} = 5 x 10^{-3} and an autosomal modifier. The grey area corresponds to the Δ interval over which XY-recombination rate dropped.

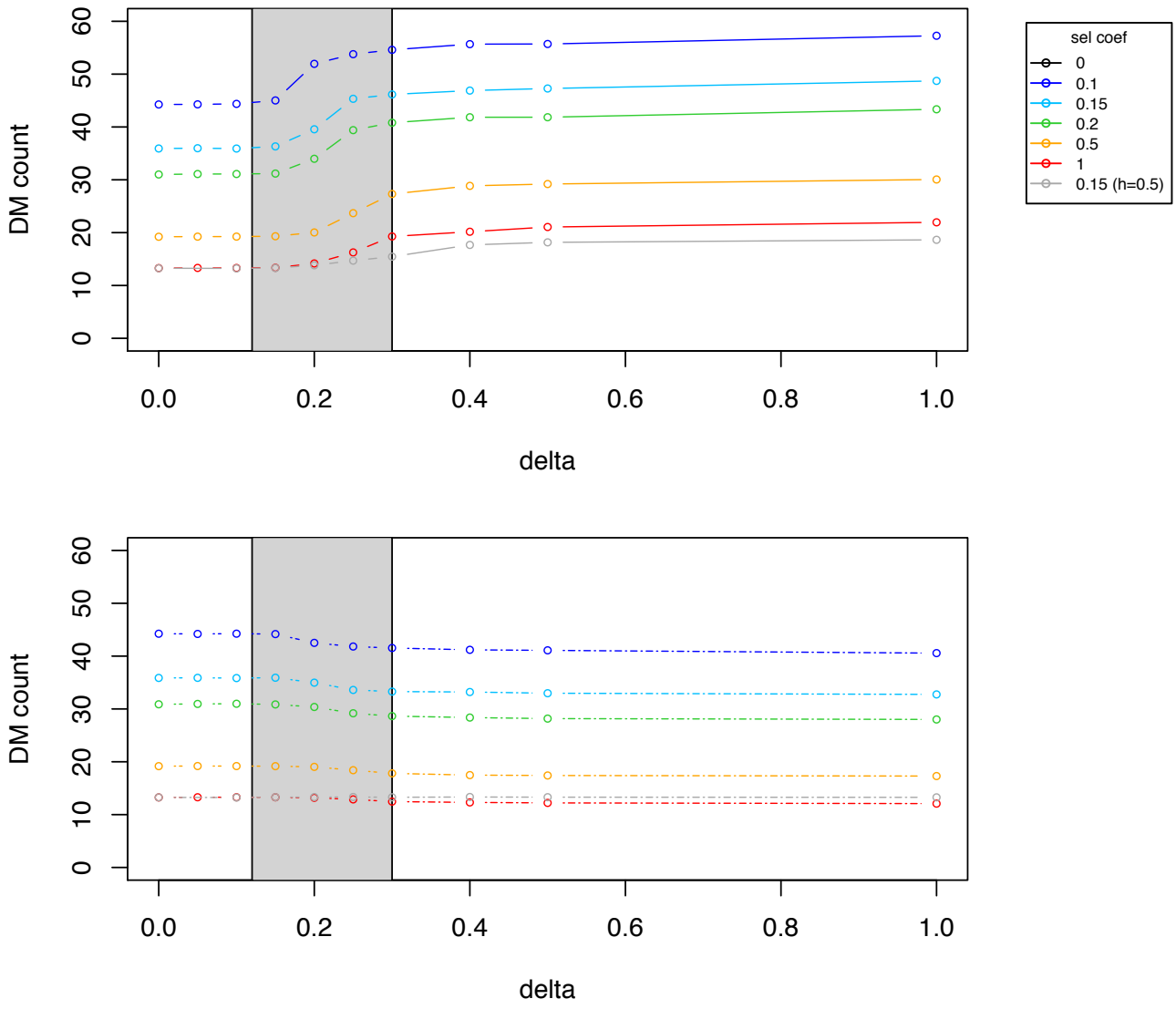


Figure S6. Amount of deleterious mutations in males (upper panel) and females (lower panel). Each point represents the average end value over 100 replicates, for a different *s* values (see color code), with μ_{DM} = 5 x 10^{-4} and an autosomal modifier. The grey area corresponds to the Δ interval over which XY recombination rate dropped..

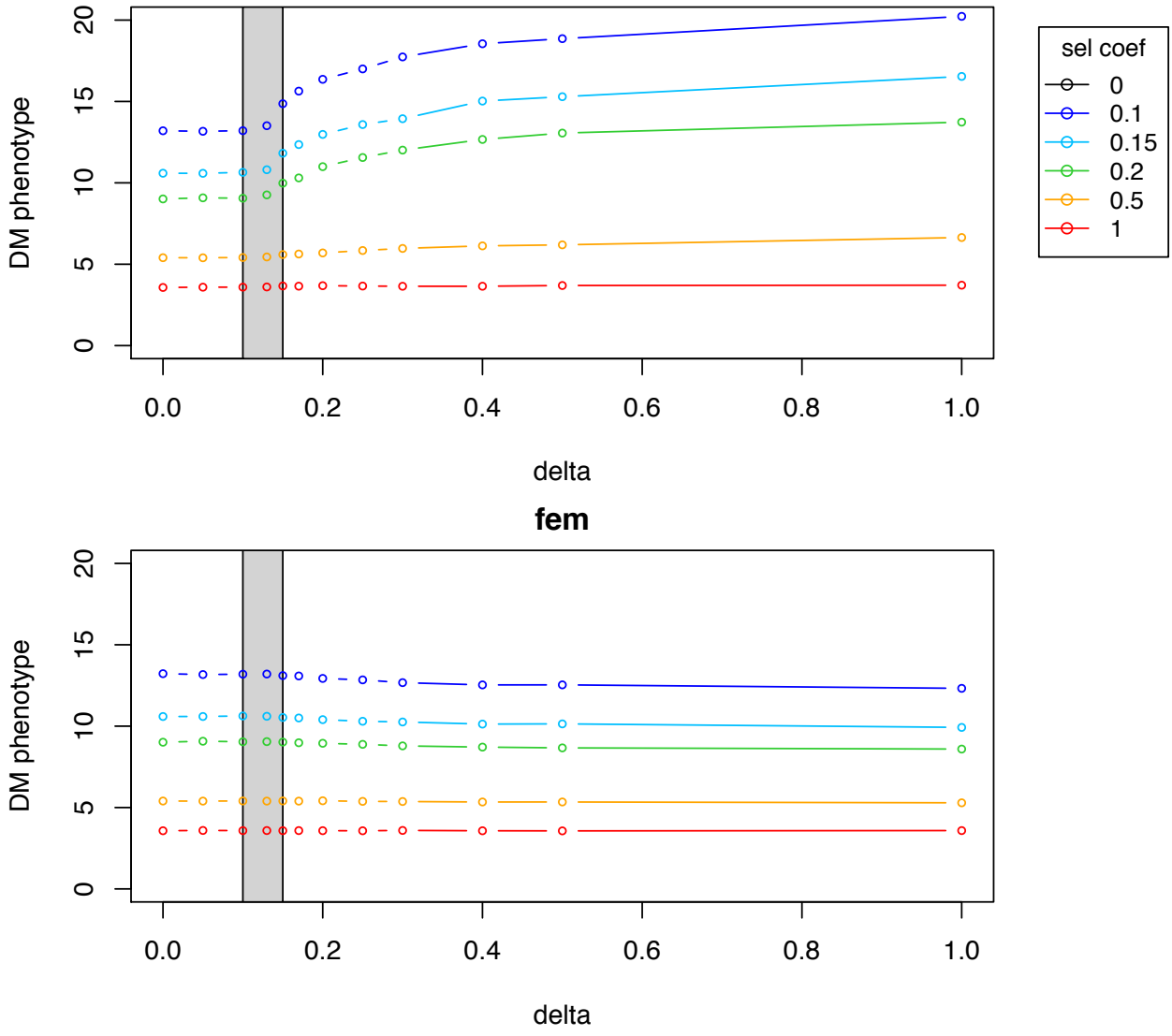


Figure S7. a) Amount of deleterious mutations in males (upper panel) and females (lower panel). Each point represents the average end value over 100 replicates, for different *s* values (color code), with $\mu_{DM} = 5 \times 10^{-3}$ and a sex-linked modifier. The grey area corresponds to the Δ interval over which recombination rate dropped. b) Boxplots of the amount of deleterious mutations on the X (left panel) and on the Y (right panel), for simulations with autosomal and sex-linked modifier, $\mu_{DM} = 5 \times 10^{-3}$, $h = 0.5$, $s = 0.15$, and $\Delta = 1$.

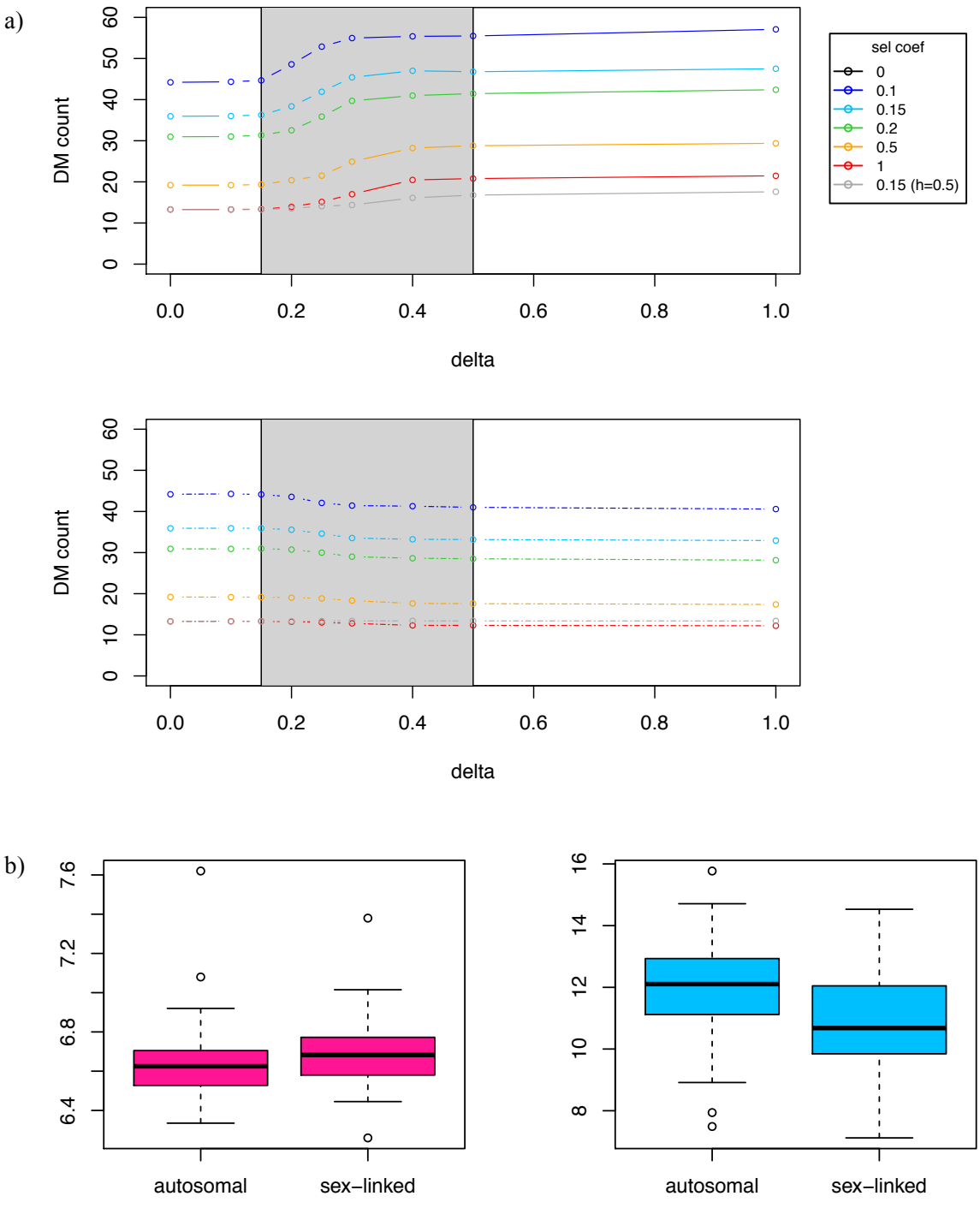


Figure S8. Fixation frequency of a_0 and a_1 at the population level, for different *s* values (different panels) and different Δ values (different bars). Simulations with a bi-allelic autosomal modifier, where a_0 codes for 0 recombination and a_1 for low recombination (1 cM). Loss of a_1 is represented in dark-grey, fixation of a_1 in white, while replicates that have not fixed one of the two alleles are represented in light grey.

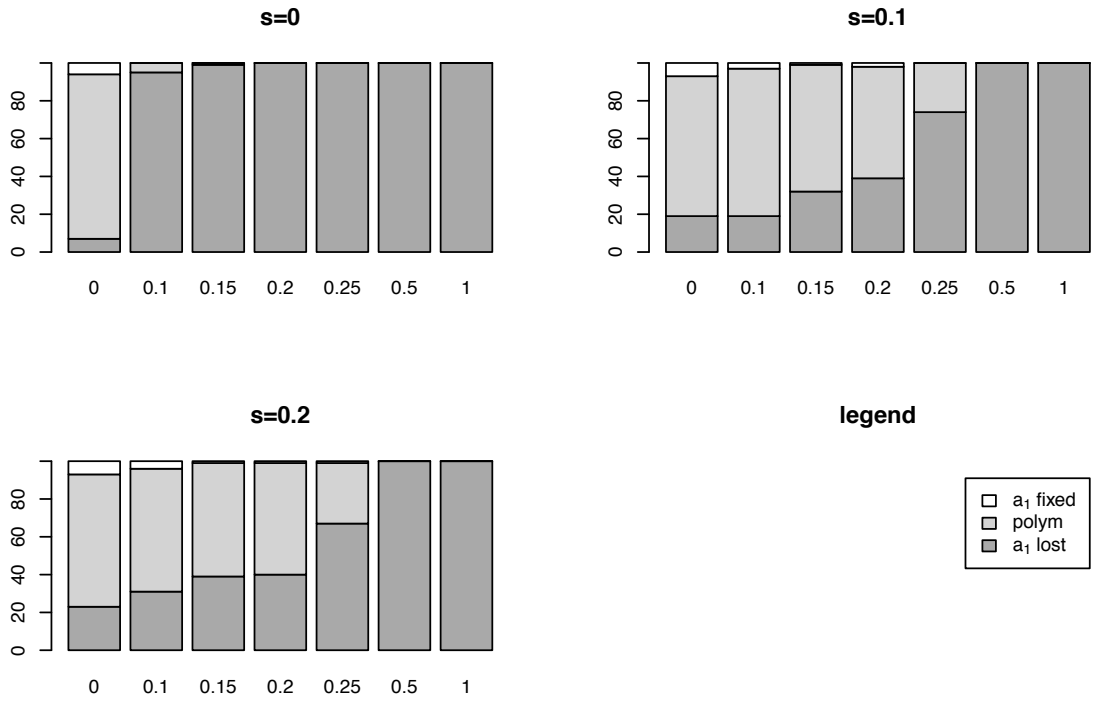
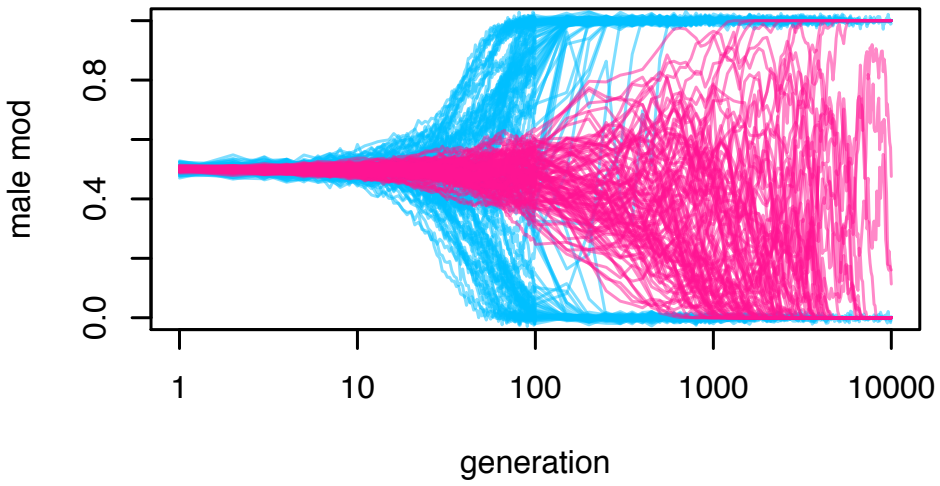


Figure S9. Dynamics of fixation of a_0 and a_1 on X (red) and Y (blue) chromosomes at a sex-linked modifier of XY recombination. A value of 0 means that allele a_1 is lost, while a value of 1 means that a_1 is fixed on the chromosome.



Chapter three

Polymorphism in the penetrance of sex-determining alleles maintained by sexually antagonistic selection

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Status: in preparation

Contributions: All authors contributed to the conceptual design and the writing of the manuscript. EC performed the simulations and analysed the data.

Keywords: sex-determining system, recombination regime, sex chromosomes

Abstract

Recent investigations in common frogs have revealed a polymorphism in the level of penetrance of sex-determining alleles, resulting in sex-determination systems that seemingly range from strictly genetic (GSD) to strictly random (RSD). Such non-genetic components of sex determination allow the long-term maintenance of homomorphic sex chromosomes (provided XY recombination depends on phenotypic sex, not on genotypic sex), but suffer a priori from a weakened association between the sex-determining locus and sexually antagonistic (SA) genes. Using individual-based simulations, we investigate the conditions favoring random or 'leaky' genetic sex determination (LSD) over strictly genetic sex determination, under different SA selection regimes and modes of XY recombination. As expected, LSD or GSD can invade RSD under several SA-selection regimes. A polymorphism can nevertheless be maintained when SA selection is asymmetric (with males suffering more from female-beneficial alleles than the reverse) and recombination depends on genotypic sex. However, this cannot account for the situation of common frogs, in which recombination only depends on phenotypic sex. Hence, other components (including the load of deleterious mutations accumulating on non-recombining chromosomes) are also likely to play a role in maintaining the polymorphism documented in frogs.

Introduction

Sexually antagonistic genes are thought to play a central role in the evolution of sex chromosomes (Rice 1984, Charlesworth and Charleswroth 2000). As theory goes, male beneficial mutations occurring close to the sex locus on the Y chromosome should spread, even if highly detrimental to females, because sex linkage makes them more likely to be transmitted to sons than to daughters (Fisher 1931, Rice 1987). Their fixation should in turn favor an arrest of XY recombination, as a way to further enforce linkage between the sex-determining locus and the sexually antagonistic locus (Charlesworth and Charlesworth 1980, Bull 1983). As a side effect, however, the arrest of XY recombination will favor the accumulation of deleterious and loss-of-function mutations on the non-recombining segment of the Y chromosome, leading to its progressive degeneration (Charlesworth and Charlesworth 2000). Such a process has been invoked to account for the highly differentiated sex chromosomes found e.g. in mammals, birds, and *Drosophila* (Bergero and Charlesworth 2009).

However, many lineages of fishes, frogs and non-avian reptiles lack any visible differentiation of sex chromosomes (Schartl 2004), which poses a challenge for current theories of sex-chromosome evolution. Sex-chromosome homomorphy has been proposed to result from a lack of a strict genetic control over sex determination (Perrin 2009). In the common frog *Rana temporaria*, for instance, sex determination normally associates with chromosome 1, with male heterogamety. However, variation is found among and within populations at the levels of both sex-chromosome differentiation and genetic contribution to sex determination. In some populations (living in cold climates), all males harbor differentiated X and Y chromosomes, and all females are XX. In other populations (from milder climates), all individuals are XX, with no genetic differentiation between phenotypic males and females. In still other populations (found at intermediate climatic conditions), XY males with different levels of X-Y differentiation co-occur with XX males as well as rare XY females (e.g. Rodrigues et al. 2017).

The most parsimonious hypothesis to account for these empirical patterns is that different alleles at the sex-determining locus have different levels of penetrance, resulting in different

probabilities of developing into males or females. As recombination in frogs only depends on phenotypic sex (Rodrigues et al. 2018), X and Y show differentiation in populations where the local Y haplotypes have high penetrance, because XY individuals always develop as males, which only recombine at chromosome tips. We will refer to this as 'strict' genetic sex determination (GSD). In populations where Y haplotypes have lower penetrance, by contrast, recurrent XY recombination in sex-reversed XY females prevents the long-term differentiation of Y chromosomes. We will refer to this as 'leaky' genetic sex determination (LSD). At the extreme, all individuals are thought to be genetically identical at the sex locus, and sex determination to be purely random (Perrin 2016). We will refer to this as random sex determination (RSD). This situation echoes the populations of *R. temporaria* from milder climates, where all individuals are XX with no genetic differentiation between phenotypic males and females.

This situation raises the important evolutionary question of what ultimate forces may favor one or the other mechanism of sex determination in some populations and maintain a polymorphism in others. In the present paper, we use individual-based simulations to test whether several sex determiners with different levels of penetrance can be stably maintained in a single population. More specifically, we investigate the role of (i) sexually antagonistic selection, a force expected to favor transitions between sex chromosomes (van Doorn and Kirkpatrick 2007, 2010) as well as transitions from non-genetic to genetic sex determination (Muralidhar and Veller et al. 2018), and (ii) the mechanisms underlying the sex-specificity of recombination (namely, phenotype- vs genotype dependent), on the fate of rare mutant sex determiners with a level of penetrance differing from the common one.

Methods

Model assumptions

We performed individual-based simulations in which each individual carries a pair of chromosomes with a sex-determining (SD) locus and a sexually antagonistic (SA) locus. The *genotypic sex value* (G_{sex}) of an individual is determined additively by the values of alleles at the SD locus. Its *phenotypic*

sex value (P_{sex}) is sampled from a normal distribution with mean G_{sex} and variance 1.5. Individuals with $P_{sex} > 0$ develop as males, while individuals with $P_{sex} < 0$ develop as females (fig. 1). We tested combination of alleles at the SD locus with possible allelic values {-6 ; 0 ; 8 ; 12} which will be referred to respectively as X, R (for "random"),Y_L (for "leaky Y") and Y_S (for "strong Y") in line with their effect of genotypic sex (see table 1). Different combinations of alleles allow simulating different sex determination systems: (i) standard "strong" genetic sex determination (GSD, with full penetrance of the Y chromosome), is obtained with the X and Y_S alleles, which will generate XX females and XY_S males; (ii) "leaky" genetic sex determination (LSD, with incomplete penetrance) is obtained with X and Y_L alleles, which mostly generate XY_L males and XX females, but also some sex-reversed XY_L females and Y_LY_L males; (iii) random sex determination (RSD) is obtained with the R allele, in which both males and females are RR.

The individual *phenotypic value* (P_{SA}) is determined additively by the two allelic copies at the SA locus. Allele a_1 contributes negatively to P_{SA}, while allele a_2 contributes positively. Positive P_{SA} values (associated with genotype a_2a_2) are beneficial to males but detrimental to females, and negative P_{SA} values (a_1a_1) have the opposite effect. Heterozygotes of a given sex have a fitness exactly intermediate between the two homozygotes of the same sex (fig.2). We simulated sex antagonistic selection as i) symmetrical (with identical detrimental effects of a_1 and a_2 to males and females respectively), ii) weakly asymmetrical (a_1 being slightly more deleterious to males than a_2 to females), or iii) strongly asymmetrical (a_1 being much more deleterious to males than a_2 to females). We also ran simulations without any SA selection (i.e. a_1 and a_2 having no effect on fitness of either males or females), to estimate the influence of neutral drift alone on the establishment of novel SD mutations, as well as some where SA selection was stronger in females (i.e., a_2 more detrimental to females than a_1 to males). The fitness of the two sexes under the different SA regimes is summarized in Table 2.

Recombination between the SD and SA loci depended on either phenotypic or genotypic sex. First, in line with the situation found in *Rana temporaria* (in which recombination depends on phenotypic sex and males only recombine at the tips of chromosomes), recombination distance between SD and SA was fixed to 0.0 cM in phenotypic males and 50 cM (~0.4 recombination rate) in phenotypic

females. Second, in line with the common assumption that, in lineages with differentiated sex chromosomes, recombination arrest is mediated by inversions, we added a modifier of recombination fully linked with the SD locus, with different alleles associated with the X, R, Y_S and Y_L alleles. Recombination between SD and SA allele only occurred in individuals homozygous at the recombination locus (with a 50 cM recombination map), and was entirely stopped in individuals heterozygous at this locus.

Simulations

We used a modified version of quantiNemo v1.0.3 (Neuenschwander et al., 2008) to simulate all possible combinations of sex-determination systems (three levels: GSD, LSD or RSD), SA selection regimes (four levels: absent, symmetrical, weakly asymmetrical, strongly asymmetrical) and recombination mechanisms (two levels: dependent on either phenotypic or genotypic sex). The population size was fixed to N=1,000 with non-overlapping generations. At each generation 1,000 gametes were randomly sampled from males and females with a probability proportional to their fitness, and paired to form 1,000 new diploid individuals. Each population was initiated with the relevant alleles in balanced frequencies at the SD and SA loci and allowed to reach an equilibrium during a burn-in phase of 10,000 generations (equilibrium checked by plotting allele frequencies over time). Then a new sex-determination system was introduced by mutating 1% of the SD alleles at generation 10,000, and the system was allowed to reach a new equilibrium for another 10,000 generations (see figure 3).

In simulations initiated with GSD (X and Y_S alleles), the invasion of LSD was tested by randomly mutating 1% of the Y_S alleles into Y_L, and the invasion of RSD by randomly mutating 1% of the Y_S allele or 1% of the X alleles into R (fig. 3, green arrow from RSD to GSD, and light-blue arrow from LSD to GSD). In simulations initiated with LSD (X and Y_L alleles), the invasion of GSD was tested by randomly mutating 1% of the Y_L alleles into Y_S, and the invasion of RSD by randomly mutating 1% of the Y_S alleles or 1% of the X alleles into R (fig. 3, green arrow from RSD to LSD, and dark-blue arrow from GSD to LSD). In simulations initiated with RSD (R allele only), the

invasion of GSD or LSD was tested by randomly mutating 1% of the R alleles into either X, Y_S or Y_L (fig.3 red and blue arrows from GSD/LSD to RSD). As a full transition to GSD or LSD necessitates two mutations (one masculinizing, one feminizing), the required complementary mutation was introduced after an additional 1,000 generations (if the first mutation was still present).

We ran 100 replicates for each combination of parameters, and assessed the state of the SD and SA loci at equilibrium (end of the burn-in phase) by measuring the frequency of the different alleles at these two loci. This was repeated 10,000 generations after the introduction of the mutant SD allele to assess whether it led to a transition in sex determination or not.

Results

Equilibrium state at the end of the burn-in phase

At the end of the 10,000 generation burn-in phase, the three different sex-determining systems converged to different equilibria, which under RSD and GSD were largely independent of recombination mechanisms. Under RSD, in the absence of SA selection, one of the two SA alleles always ended up drifting to fixation, with a 0.50 probability each, as expected under neutrality. With symmetrical SA selection, the two alleles were always kept at a frequency close to 0.50, pointing to balancing selection. With weakly asymmetrical SA selection, the male beneficial allele (a_2) went to fixation in 50-60% of the replicates and was maintained at frequency ~ 0.76 in the remaining replicates . With stronger SA asymmetry, the a_2 allele always went to fixation (fig. S1).

Under GSD, the X and Y alleles rapidly reached the frequencies required for a balanced sex ratio (0.75 and 0.25 respectively, figs. S7, S10). In the absence of SA selection, the two SA alleles were fixed with equal probabilities on X and Y, as expected for a neutral locus. With symmetrical or weakly asymmetrical SA selection, the male beneficial allele (a_2) always went to fixation on the Y_S, and the female beneficial allele (a_1) on the X. Under strongly asymmetrical SA selection, a_2 also went to fixation on the Y_S, but the X chromosome retained polymorphism in most replicates (82%), because the benefits for males of having two a_2 copies exceeded the cost for females of being

heterozygous at the SA locus. Genetic drift made a_1 or a_2 fixed on the X in 6% and 12% of replicates, respectively.

Under LSD, the X and Y alleles stabilized at frequencies ~ 0.73 and ~ 0.27 respectively, resulting in a population sex ratio of 50%. With phenotypic-sex dependent recombination (fig. S13), one of the two alleles at the SA locus went to fixation in the absence of SA selection, as under RSD. This loss of polymorphism was due to the occasional XY recombination in sex-reversed XY_L females, allowing fixation of the same (neutral) SA allele on the X and the Y. With symmetrical and weakly asymmetrical SA selection, a_2 went to fixation on the Y_L chromosome (similar to GSD), but recombination in sex-reversed XY_L females allowed maintenance of a_2 on the X, so that its overall frequency exceeded that of the Y_L allele (respectively 0.35 with symmetrical SA selection, and 0.37 with weakly asymmetrical SA selection). Under strongly asymmetrical SA selection, a_2 went to fixation on both chromosomes (similar to RSD). Outcomes differed markedly when recombination depended on genotypic sex (fig. S16). In the absence of SA selection, X and Y_L randomly fixed one of the two SA alleles with the same probability (similar to GSD). With symmetrical and weakly asymmetrical SA selection, the Y_L and X chromosomes always fixed a_2 and a_1 respectively. Under strongly asymmetrical SA selection, finally, the Y_L chromosome always fixed a_2, while the X chromosome retained polymorphism in most replicates, with an average equilibrium frequency of a_2 around 0.65).

Invasion dynamics

Invasion of RSD by GSD or LSD only occurred if the initial mutation was masculinizing (i.e., mutation from R to Y_S or Y_L, not to X), and only when SA selection was symmetrical (figs. 4 and 5, arrows towards RSD). Thanks to their linkage with the male-beneficial a_2 allele (figs S2, S3), Y_S or Y_L could be maintained at low frequencies in some simulations (figs. 4 and 5, arrows towards RSD, dark- and light-blue squares), despite generating an excess of males at the population level. In such cases, GSD or LSD could invade and replace RSD after introduction of the feminizing mutation (figs. 4 and 5, arrows towards RSD, dark- and light-blue squares, black part of the barplot). In contrast, X

was always quickly eliminated by sex-ratio selection when introduced first. In the simulations where a genetic sex determination could invade (15% and 6% of replicates for Y_S and Y_L respectively), the male-beneficial a_2 allele always went to fixation on Y_S, but on Y_L only when recombination depended on genotypic sex (figs. S2, S3). The Y_L and Y_S mutant never invaded under weakly or strongly asymmetrical SA selection (figs. 4 and 5), because a_2 was then maintained at frequency high enough that most or all of RR males were homozygous for a_2, so that RY_L or RY_S males had no fitness advantage and were more easily eliminated by sex-ratio selection. GSD and LSD also invaded more easily when SA selection was stronger in females (i.e., a_2 more detrimental to females than a_1 to males). The female beneficial allele a_1 then reached higher frequencies during the burn-in period, so that most RR males were homozygous a_1a_1 and thus easily displaced by Y_L or Y_S males having fixed the a_2 allele (data not shown). Thus, standing variation at the SA locus is required for the replacement of RSD by a GSD or LSD system; the more frequent the female-beneficial alleles, the easier it is for a masculinizing Y_L or Y_G allele to invade, thanks to the fixation of the male-beneficial SA allele..

Complete replacement of GSD or LSD by RSD never occurred under symmetric or weakly asymmetric SA selection (figs. 4 and 5, arrows towards GSD), because Y_S or Y_L males benefitted then from their association with a_2. It occurred at rare occasions in the absence of SA selection, or conversely when SA selection was strongly asymmetrical, because a_2 was then maintained at high enough frequencies that RR males were mostly homozygous for a_2, hence suffering no fitness disadvantage over Y_S or Y_L males. Interestingly, a stable polymorphism could evolve in the latter case (strongly asymmetric SA selection) when recombination was controlled by genotypic sex (fig. 5). In simulations where R succeeded in invading GSD or LSD, it stabilized at frequency ~ 0.73, vs ~ 0.11 for Y and ~ 0.16 for X (fig. S11). This only occurred when the R alleles derived from existing Y alleles because they were then associated with the male beneficial a_2 allele.

Invasion and replacement of GSD by LSD only took place in the absence of SA selection when recombination was phenotypic-sex dependent, but under all SA selection scenarios when recombination was genotypic-sex dependent, and at a rate similar to their introduction frequency, suggesting a predominant role for genetic drift rather than SA selection (fig. 5). Conversely, invasion

and replacement of LSD by GSD occurred more often, and under a large range of SA scenarios (figs. 4 and 5, arrow from GSD to LSD). Under symmetrical or weakly asymmetrical SA selection, Y_S had a roughly 50% chance to displace Y_L when recombination depended on phenotypic sex, and 15% when recombination depended on genotypic sex, pointing in both cases to selection favoring GSD. This selection was stronger when recombination was controlled by phenotypic sex, because recombination in sex-reversed XY_L females prevented a complete linkage between Y_L and a_2, while XY_S were never sex-reversed, preserving a strict linkage. Under strongly asymmetric SA selection, invasion by GSD occurred more randomly, because a_2 was kept at frequencies high enough (1.0 and 0.65 respectively) to ensure its high occurrence on Y_L despite rare recombination.

Discussion

A first result from our simulations is that a genetic system of sex determination, whether strict (GSD) or leaky (LSD), can invade a random mechanism (RSD), provided SA selection maintains the female-beneficial alleles at significant frequencies. This occurred in our simulations when SA selection was symmetrical (in which case a_1 segregated at frequency 0.50), or, more likely, under stronger selection against a_2 in females (so that a_1 segregated at still higher frequencies). Invasion is made possible in such cases because, despite introducing an initial bias in sex ratios, the Y chromosomes benefit from their association with the male-beneficial allele a_2. This explanation is consistent with previous theoretical model that explored the dynamics and the stability of sex determination systems. Rice (1986) explains how polygenic sex determination is unstable when the "Y gene" increases fitness or is tightly linked to sexually antagonistic alleles. This allows the Y chromosome to be maintained by balancing selection at a low frequency, until a feminizing mutation comes in and drives GSD or LSD to fixation. This is in line with previous models on evolution of genetic sex determination from hermaphrodites, where two mutations are needed to evolve from hermaphroditism to dioecy (Charlesworth and Charlesworth 1978). A high-penetrance allele (Y_S) is more likely to invade (due to stricter association with a_2), and, for a low penetrance allele (Y_L), invasion is more likely if

recombination depends on genotypic sex (which induces a stronger association between SD and SA alleles).

This process also requires that the masculinizing mutation (Y) occurs before the feminizing one (X). If X occurs first, it will not benefit from the association with a_1 (due to the high recombination rate in females). As this asymmetry stems from the pattern of heterochiasmy assumed throughout, it is expected that X (and not Y) should be able to invade first if recombination takes place in males, not in females. In a ZW system, reciprocally, W (and not Z) should also be able to settle first in the absence of female recombination (as occurs e.g. in Lepidoptera) and 'wait' for the occurrence of Z to displace an established RSD system. Hence, pre-existing patterns of heterochiasmy might somewhat pre-empt the systems of heterogamety (i.e., XY might be more likely to evolve if males recombine little, and ZW if females recombine little). The prevalence of XY systems in some lineages (such as frogs) might thus simply reflect intrinsic differences in the patterns of heterochiasmy (males recombine intrinsically less than females in frogs).

Conversely, a random system of sex determination had low probabilities of invasion under our settings, because male- and female-beneficial alleles at the SA locus cannot be transmitted preferentially to sons and daughters under random allocation. Interestingly, however, a balanced polymorphism of genetic and non-genetic sex determination was maintained when SA selection was asymmetric, with a_1 much more deleterious to males than a_2 to females, provided recombination was controlled by genotypic sex (and R evolved from Y, not from X). This likely occurred because a_2 segregated then at high frequency on the R, so that RR individuals were selected when developing as males (being mostly homozygous a_2a_2, while many XY males were heterozygous a_1a_2 with lower fitness) but counter-selected when developing as females. Previous theoretical work has investigated the dynamics of sexually antagonistic genes on sex chromosomes, considering or not recombination (e.g. Kidwell et al. 1977, Rice 1987). Here we expanded this investigation including recombination that depends on the phenotypic sex and the interaction between different sex-determining systems.

Thus, our simulations unveil a range of parameter values that favor the kind of polymorphism documented in common frogs (see Introduction). Our results, however, can actually not account for

the common-frog situation, because recombination in frogs clearly depends on phenotypic sex only, not on genotypic sex (Rodrigues et al. 2018), which in all our simulations prevented the evolution of such a polymorphism. This implies that other factors not considered here must affect the evolution of sex-determination systems in frogs. A strong candidate is obviously the deleterious mutations that necessarily accumulate on non-recombining Y chromosomes (Cavoto et al. 2018, in prep.). These should provide clear additional benefits to LSD (and RSD) over GSD, because recombination in sex-reversed XY_L females should allow regular purging of the deleterious mutation load. Additional simulations along this line should help elucidate the interactions between SA selection and deleterious mutations on the evolutionary dynamics of SD alleles with different degrees of penetrance.

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Figures

Figure 1. Distributions of P_{sex} values for different sex genotypes (XX in red, RR in green, XY_L in pale blue, XY_S in dark blue). Each distribution centers on its specific G_{sex} value, with variance 1.5. Individuals with $P_{sex} < 0$ (dotted line) develop as females, while individuals with $P_{sex} > 0$ develop as males.

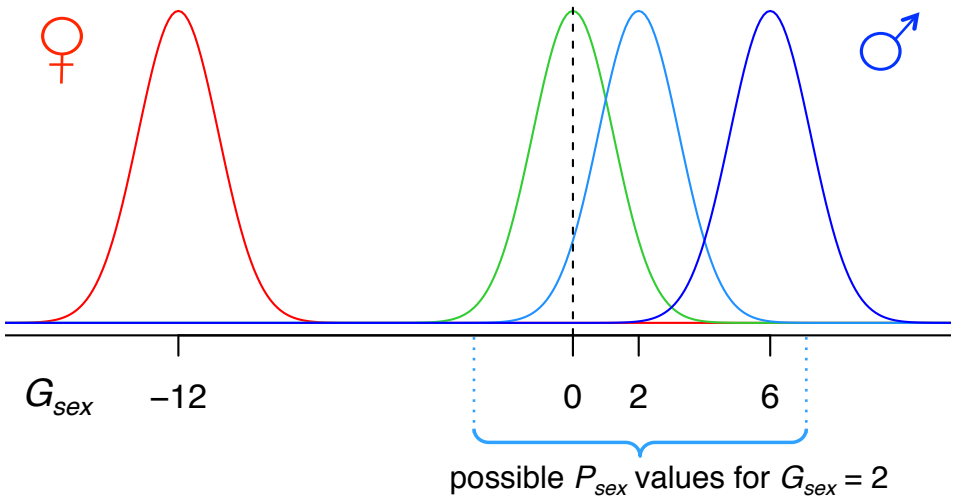


Table 1. Genotypic values (G_{sex}) and phenotypic sex for different sex genotypes (i.e., combinations of alleles at the sex locus (for allelic values X=-6, R=0, Y_L=8, Y_S=12).

Sex genotype	G_{sex} *value*	Phenotypic sex
XX	-12	Female
XY_L	2	0.95 Male / 0.05 Female
XY_S	6	Male
XR	-6	Female
Y_SY_S	24	Male
Y_SY_L	20	Male
Y_SR	12	Male
Y_LY_L	16	Male
Y_LR	12	Male
RR	0	0.5 Male/0.5 Female

Figure 2. a) Table showing the different fitness values for the three patterns of SA selection. **b)** Plot showing how SA selection is modeled. Fitness values are different for females (pink line) and males (blue lines), and depend on P_{SA}. Three possible values of P_{SA} are considered in this model (dotted black lines). SA selection can be symmetrical (solid pink and blue lines) or asymmetrical (when weakly asymmetrical, the fitness of males follows the dotted blue line).

a)

	symmetrical		**weakly asymmetrical**		**strongly asymmetrical**	
sex	Female	Male	Female	Male	Female	Male
a_1a_1	1	0.8	1	0.78	1	0.6
a_1a_2	0.9	0.9	0.9	0.89	0.9	0.8
a_2a_2	0.8	1	0.8	1	0.8	1

b)

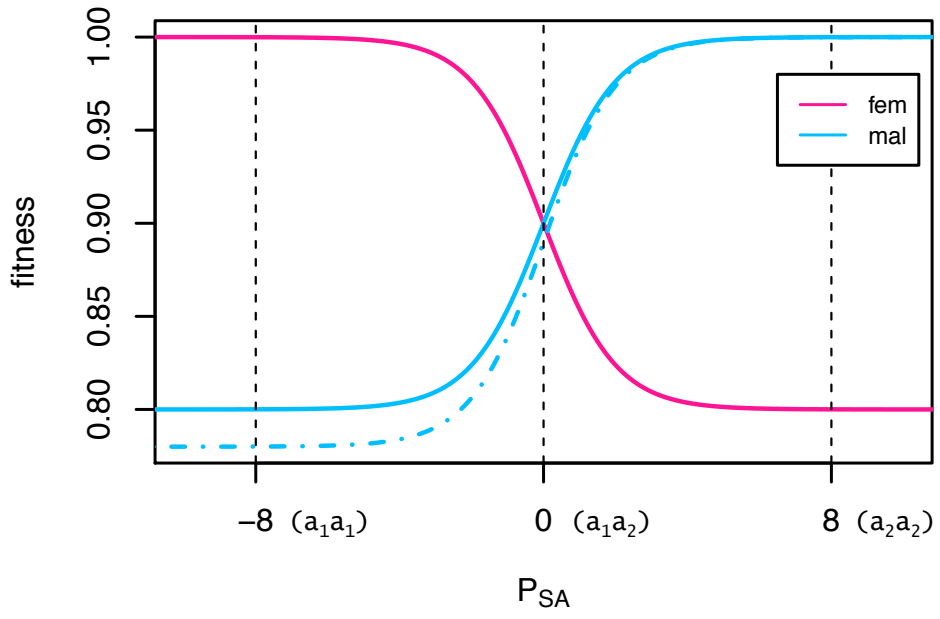


Figure 3. Representation of the different SD alleles introduced in each initial SD system, after the burn-in phase. Different alleles are represented in different colors (see color code). The arrows point in the direction of the invasion. For example, the green arrow from RSD to GSD indicates the introduction of the R allele in the simulations with initial GSD system.

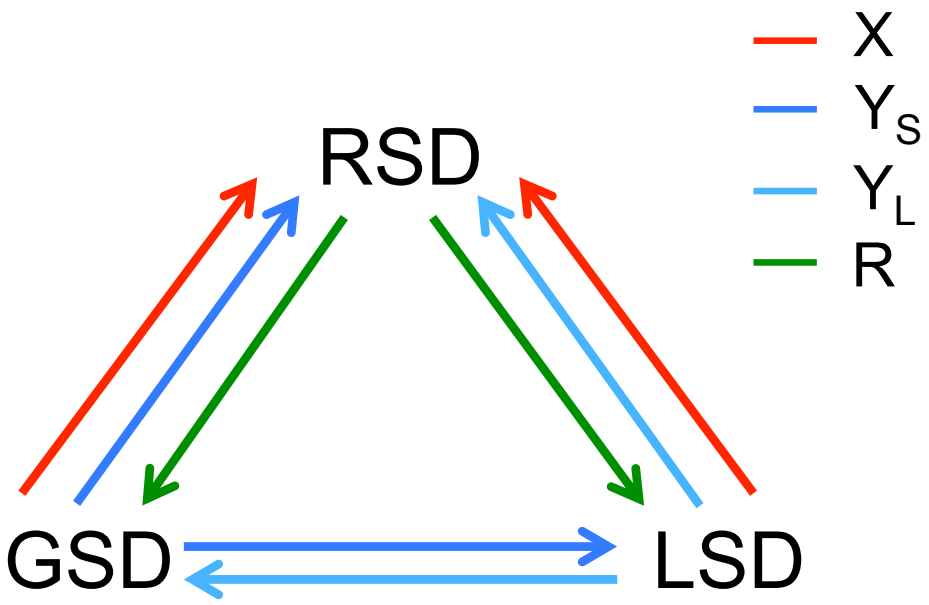


Figure 4. Recombination depends on the phenotypic sex. Barplots representing the amount of replicates (out of 100) where an invasion occurs (black), a polymorphism between the mutation and the initial SD allele(s) is maintained (grey), or the mutation does not invade (white). Each bar is for a different setting of SA selection (from left to right: absent, symmetrical, weakly asymmetrical, strongly asymmetrical).

The arrows point in the direction of the invasion. For example, the arrow from RSD to GSD indicates the introduction of the R allele in the simulations with initial GSD system. The color of the box around the barplots indicates if the mutation is on/with the X (red) or on the Y (dark- light-blue).

When a second invasion is simulated, the bar is divided in two, showing the situation before and after the introduction of the second mutation.

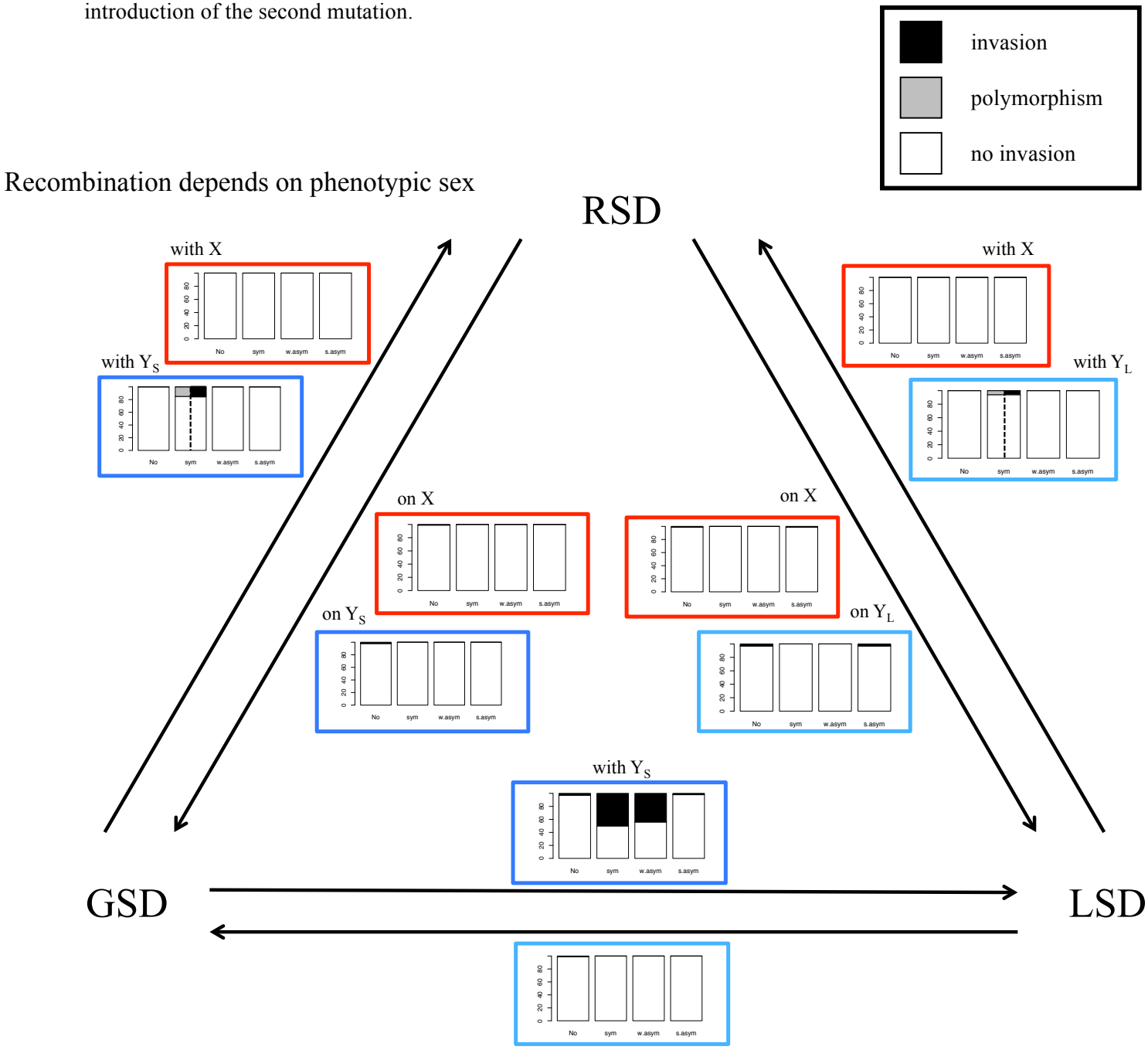
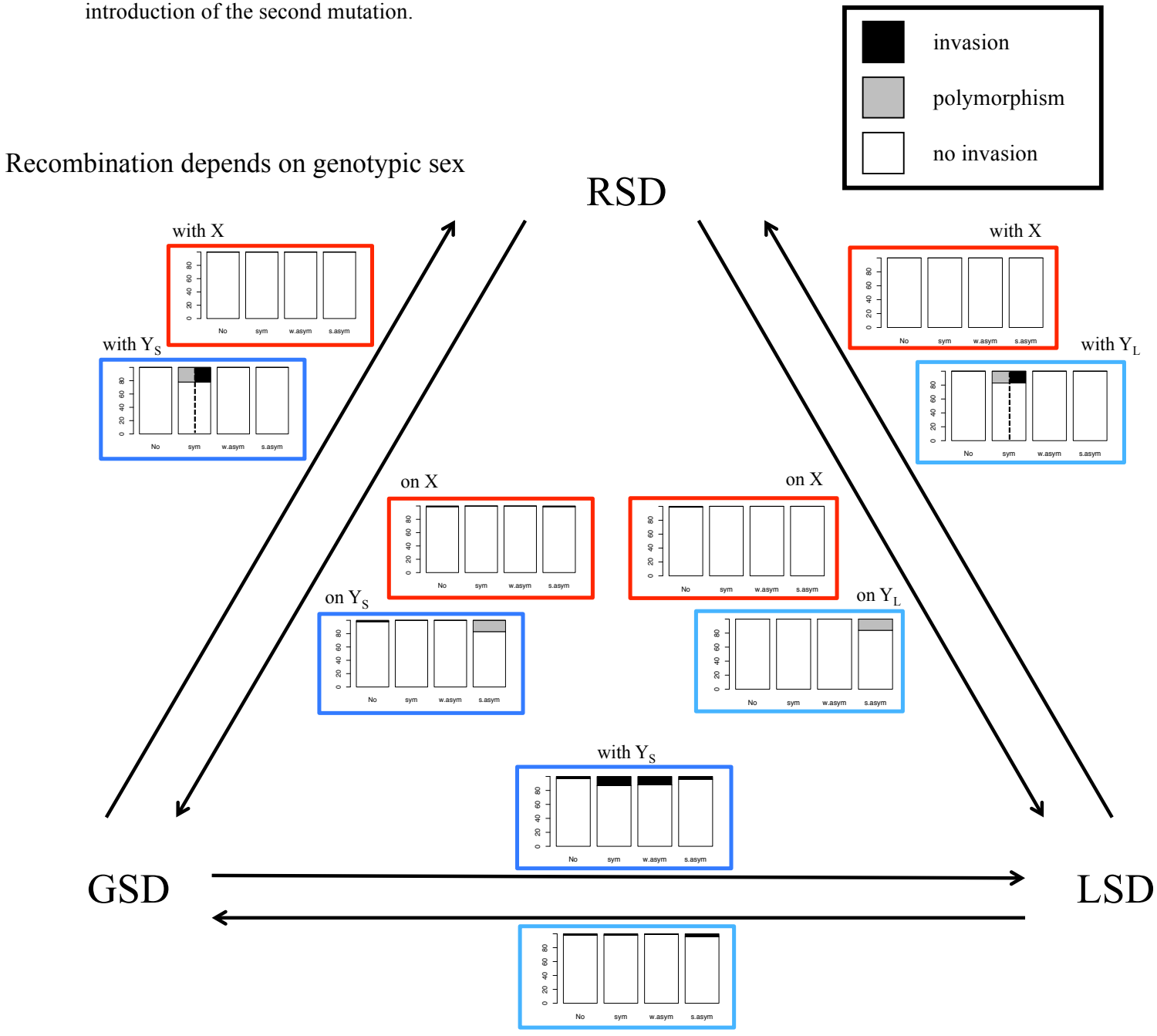


Figure 5. Recombination depends on the genotypic sex. Barplots representing the amount of replicates (out of 100) where an invasion occurs (black), a polymorphism between the mutation and the initial SD allele(s) is maintained (grey), or the mutation does not invade (white). Each bar is for a different setting of SA selection (from left to right: absent, symmetrical, weakly asymmetrical, strongly asymmetrical).

The arrows point in the direction of the invasion. For example, the arrow from RSD to GSD indicates the introduction of the R allele in the simulations with initial GSD system. The color of the box around the barplots indicates if the mutation is on/with the X (red) or on the Y (dark- light-blue).

When a second invasion is simulated, the bar is divided in two, showing the situation before and after the introduction of the second mutation.



Supplementary table and figures

Table S1. Table summarizing the equilibria at the SA locus after the burn-in phase.

Column 1: Rec : recombination regime: "phen" if depending on phenotypic sex, "gen if depending on genotypic sex

Column 2: System : the 3 different sex-determination systems

Column 3 to 6: the 4 different SA selection regimes (no SA selection, symmetrical, weakly asymmetrical, strongly asymmetrical SA selection)

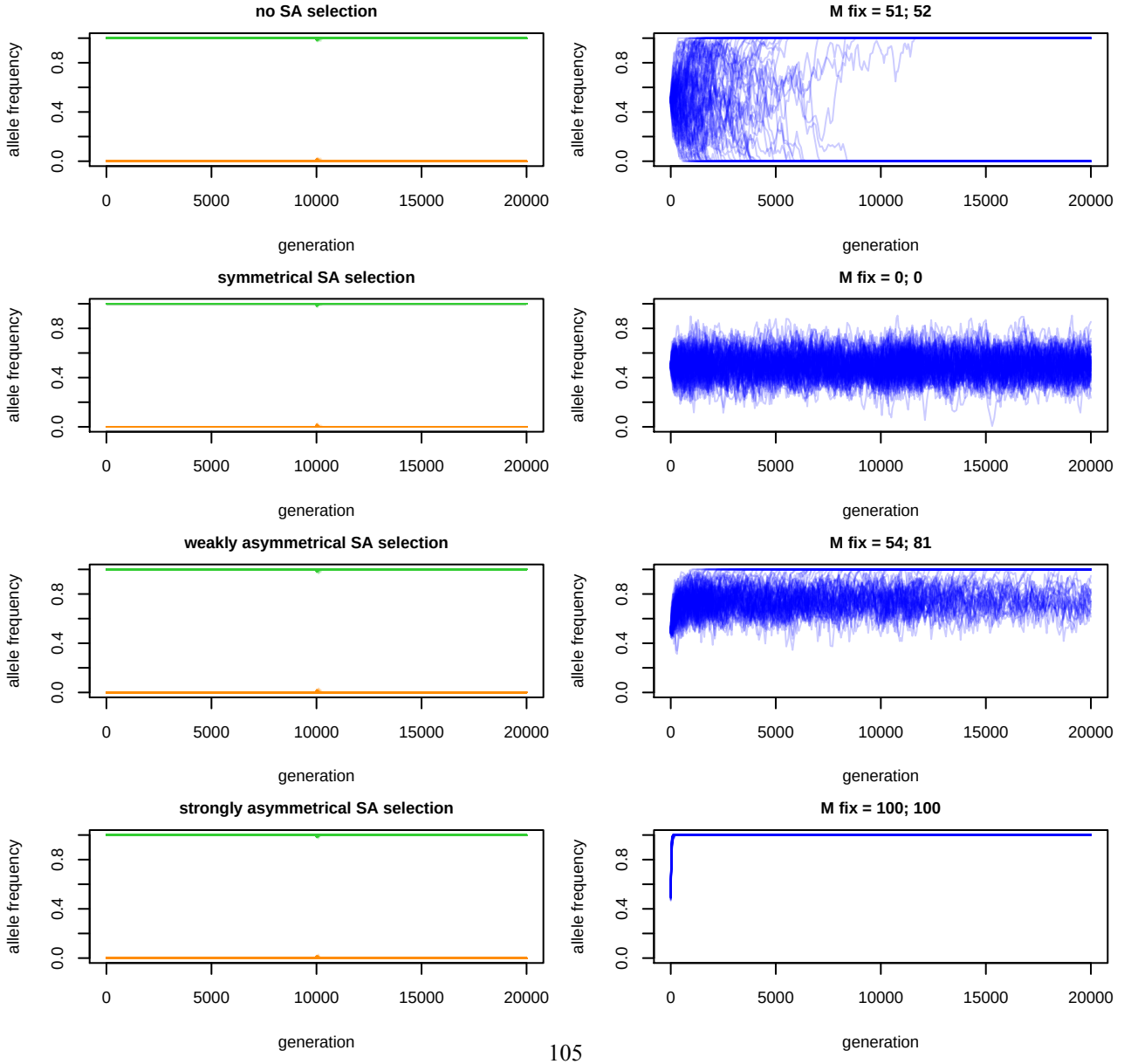
a_1 is the female beneficial allele, while a_2 is the male beneficial allele

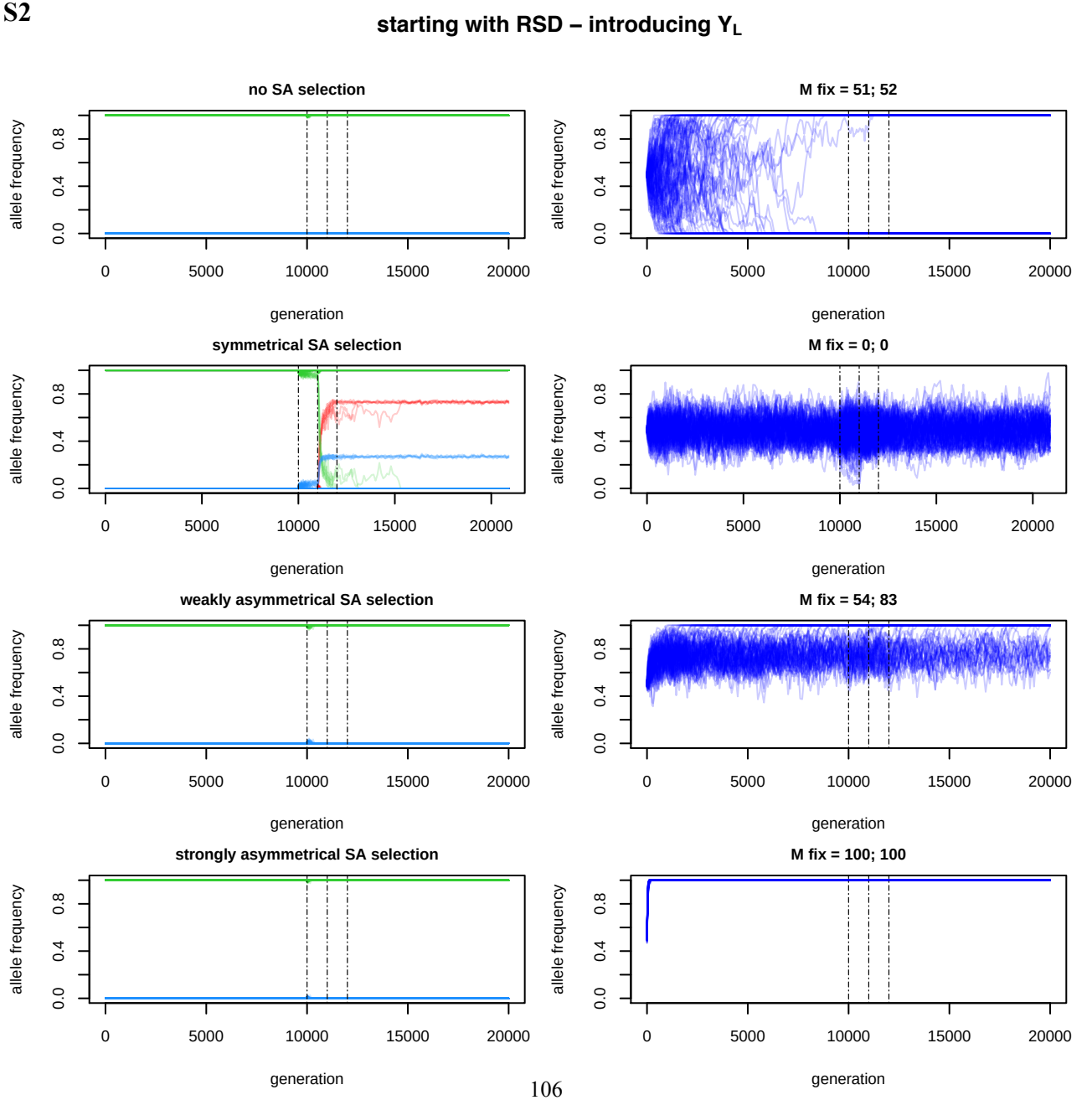
Rec	System	No SA	sym	w.asym	s.asym
phen/gen	RSD	a_1 or a_2 fixed	$a_1 \sim a_2 \sim 0.5$	a_2 fixed (54%/60%); 0.76	a_2 fixed
phen/gen	GSD	a_1 or a_2 fixed on X or Y	a_2=0.25 (on Y)	a_2=0.25 (on Y)	a_2 fixed (12%) a_2=0.25 (on Y, 6%) a_2=0.63 (82%)
phen	LSD	a_1 or a_2 fixed	a_2=0.35 (on Y and some X)	a_2=0.37 (on Y and some X)	a_2 fixed
gen	LSD	a_1 or a_2 fixed on X or Y	a_2= 0.27 (on Y)	a2=0.27 (on Y)	a_2 fixed (7%) a_2=0.27 (on Y, 4%) a_2=0.65 (89%)

Figures S1 to S18. Each page reports results of one invasion pattern (for example: RSD is invaded by GSD with strong Y). Each row is for a different SA scenario. The left column reports the dynamic at the SD locus, the right column reports the dynamic at the male-beneficial SA allele. The values on top of each righ-side plot report the amount of replicates in which a_2 is fixed at the end of the burn-in phase, or at the end of simulations (20'000 generations).

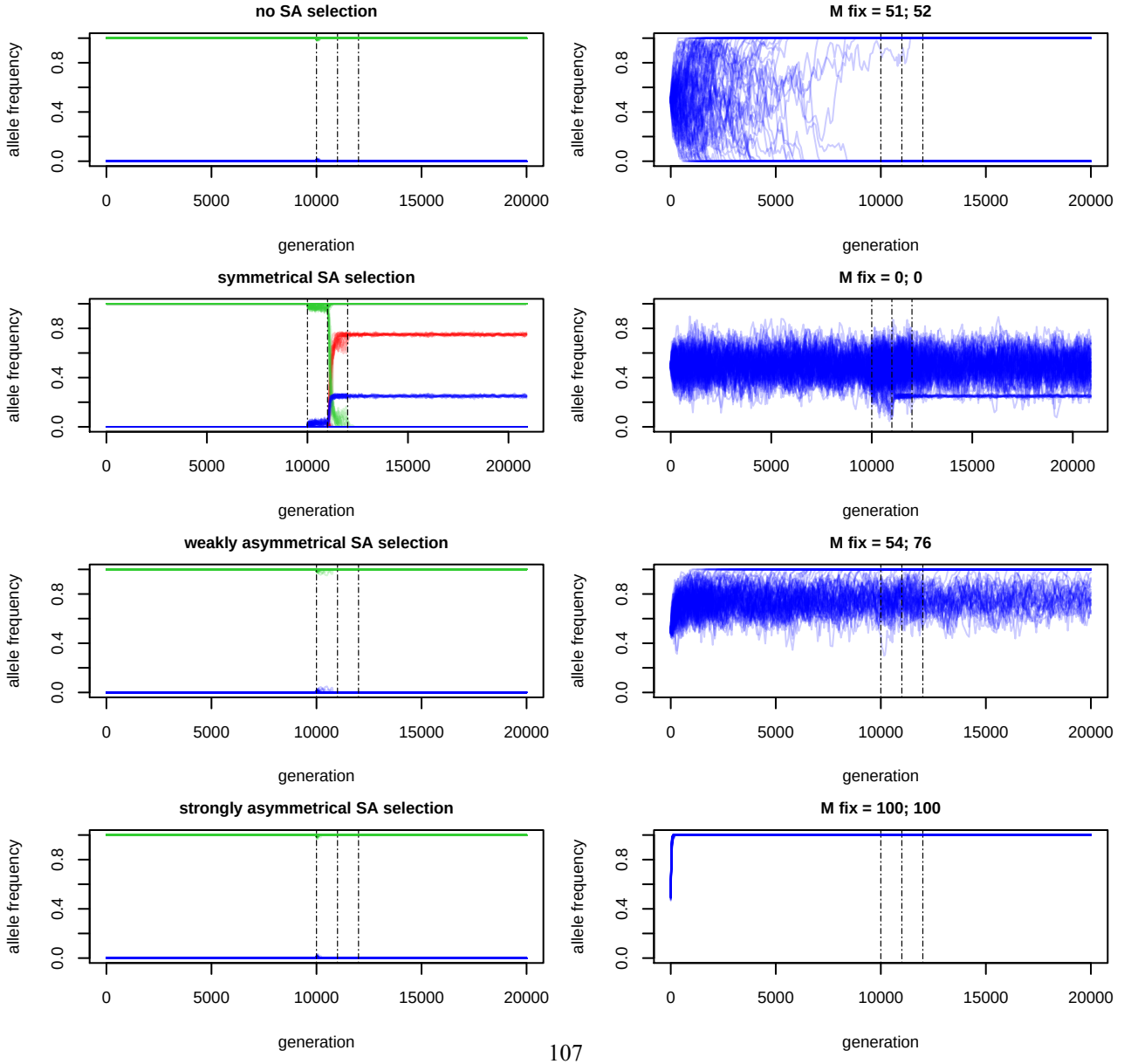
S1

starting with RSD – introducing X – rec. depends on phenotypic sex

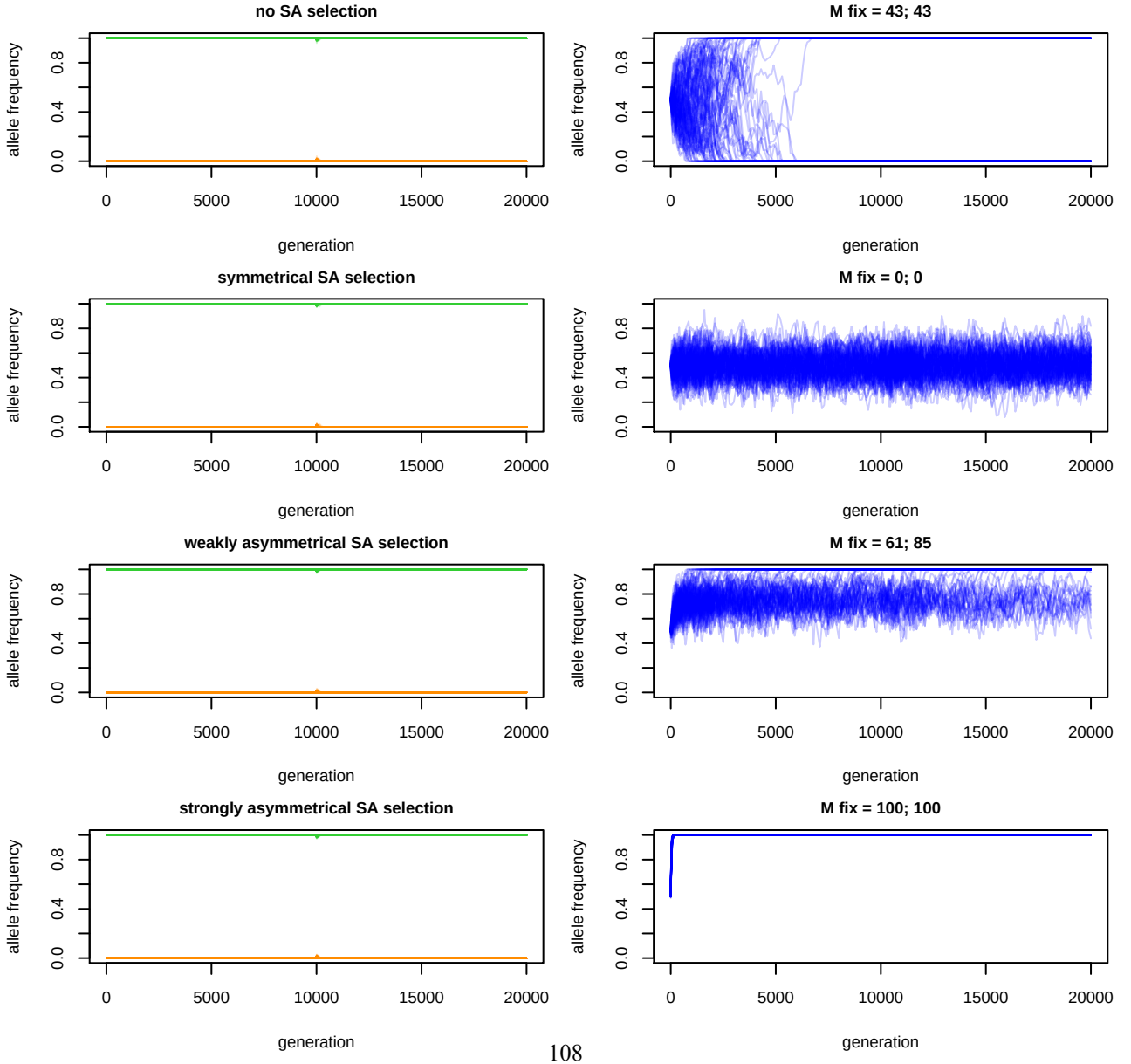



starting with RSD – introducing Y_L
no SA selection
M fix = 51; 52
symmetrical SA selection
M fix = 0; 0
weakly asymmetrical SA selection
M fix = 54; 83
strongly asymmetrical SA selection
M fix = 100; 100
allele frequency
0.0
0.4
0.8
0
5000
10000
15000
20000
generation

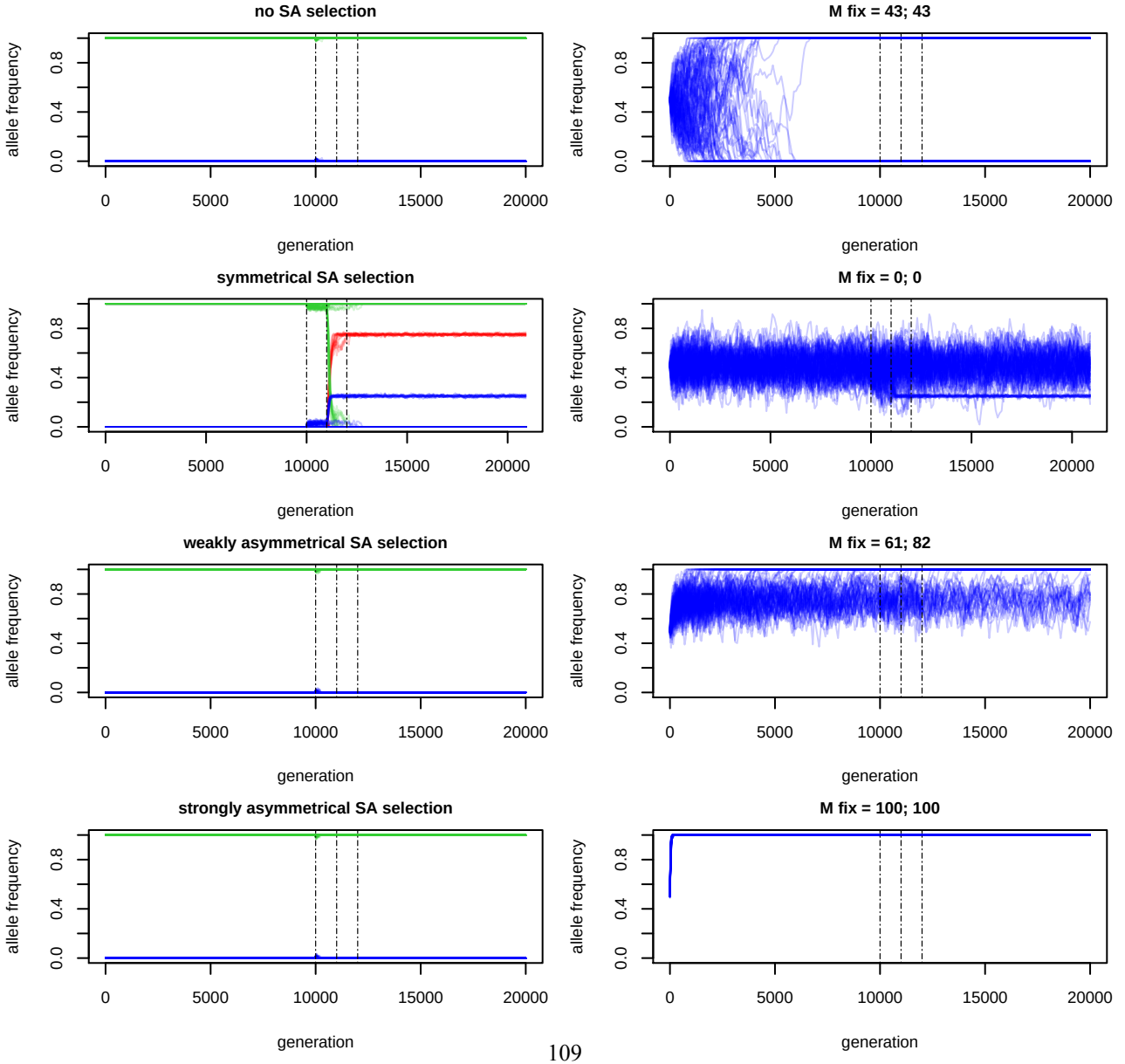
starting with RSD – introducing Y_S



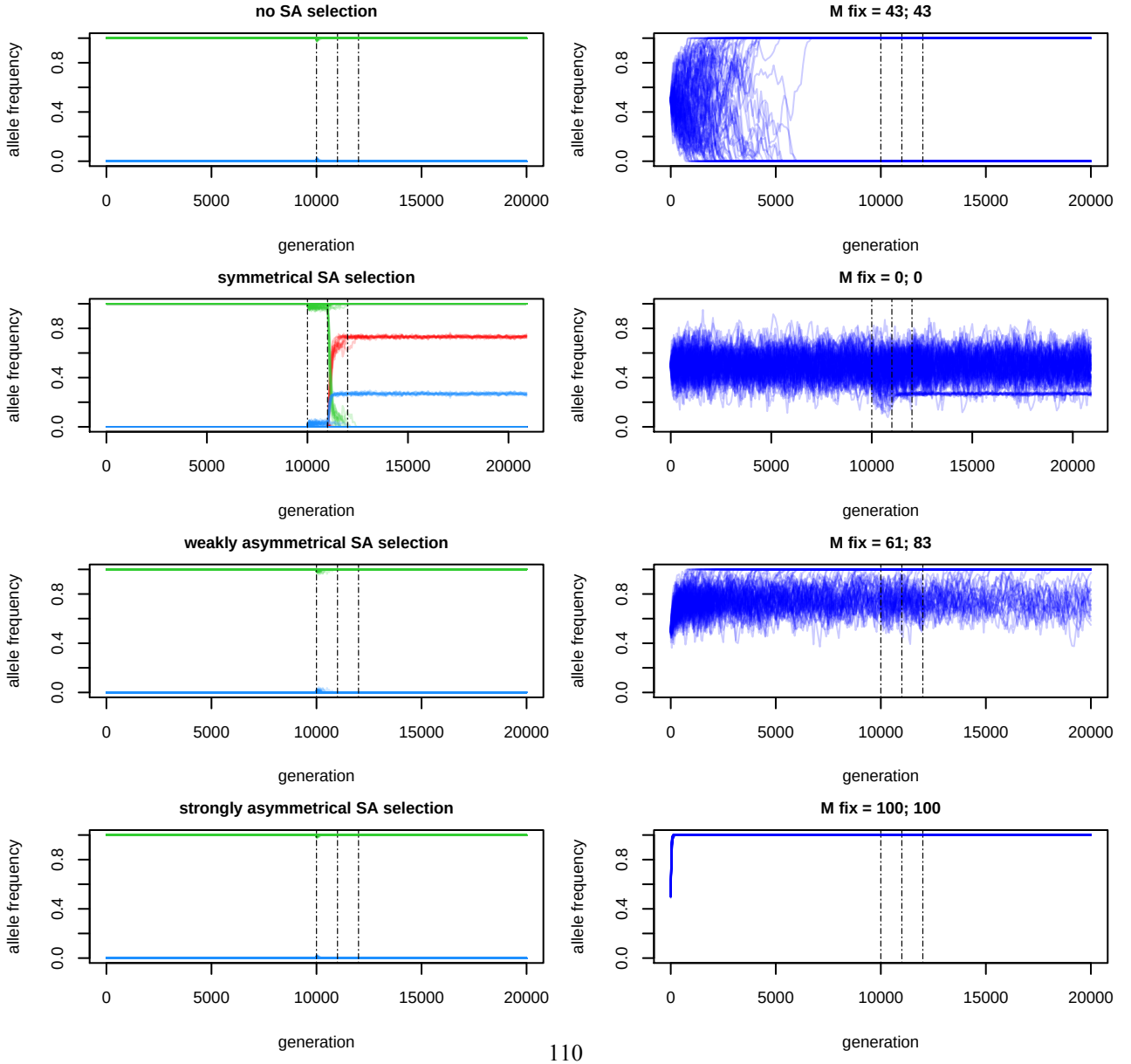
starting with RSD − introducing X − rec. depends on genotypic sex



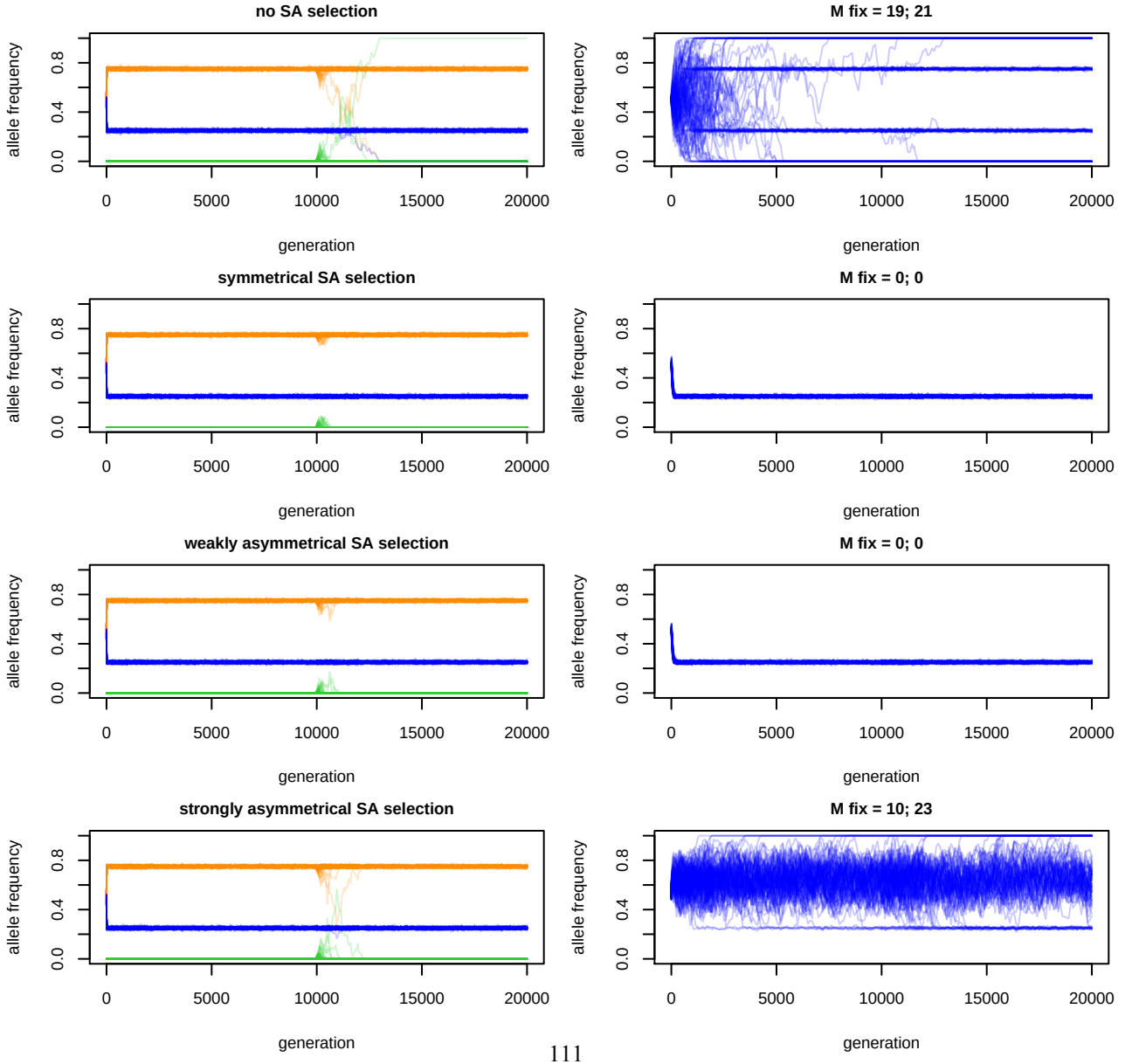
starting with RSD – introducing Y_S



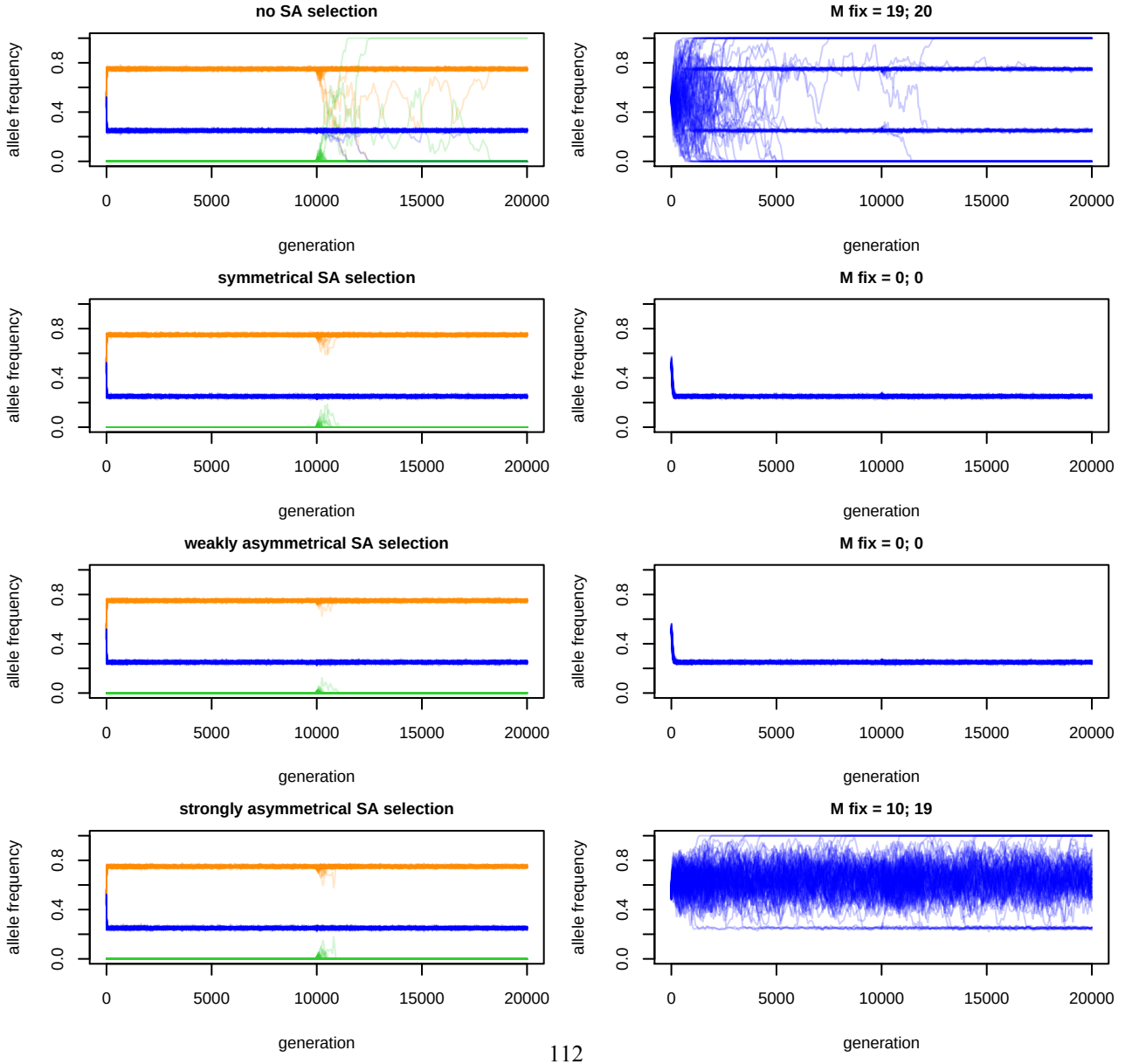
starting with RSD – introducing Y_L



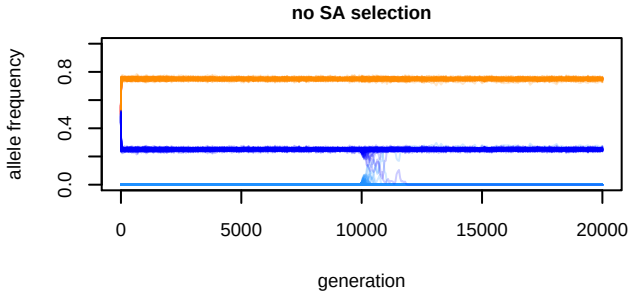
starting with GSD − introducing R (on X) - rec. depends on phenotypic sex

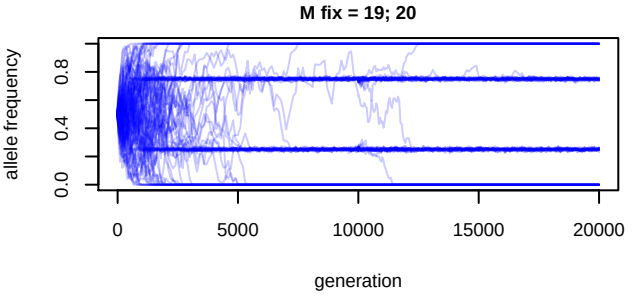


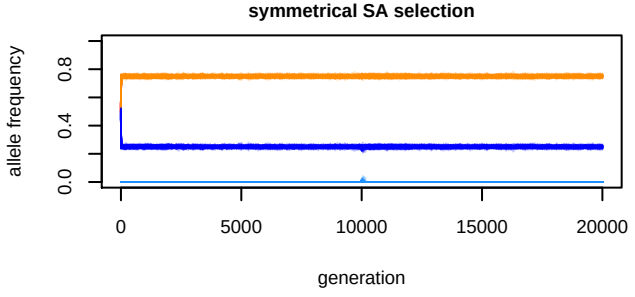
starting with GSD − introducing R (on Y)

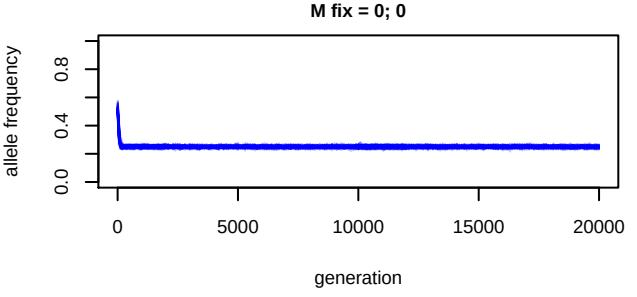


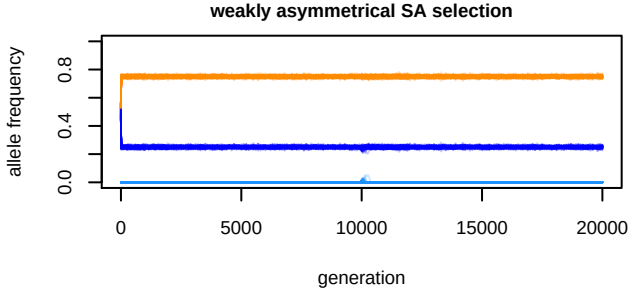
starting with GSD – introducing Y_L

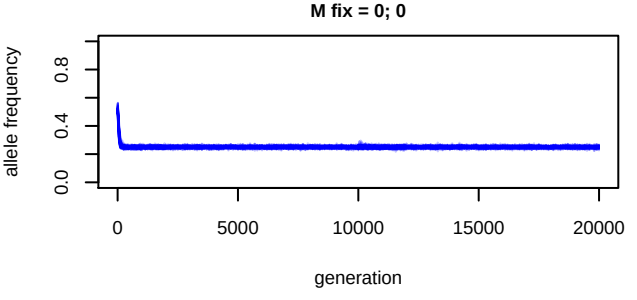


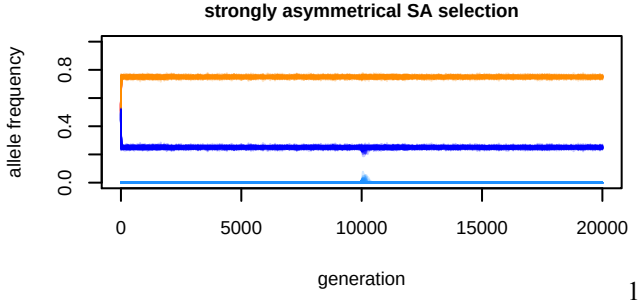


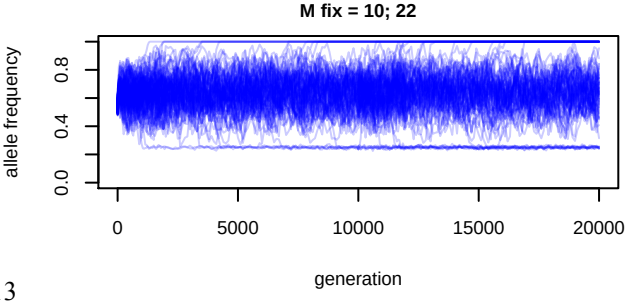




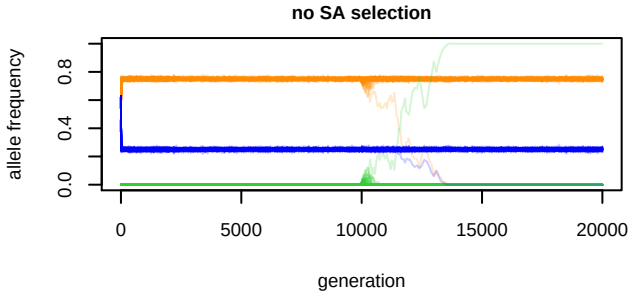


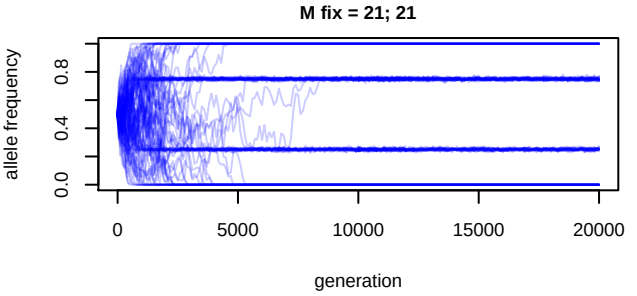


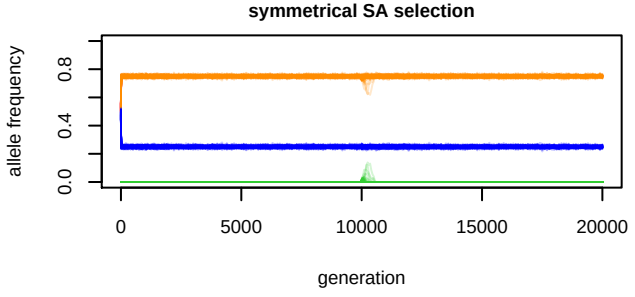


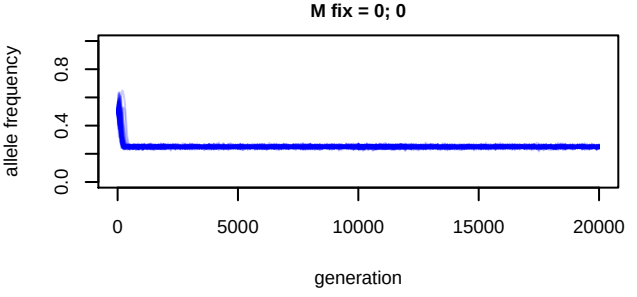


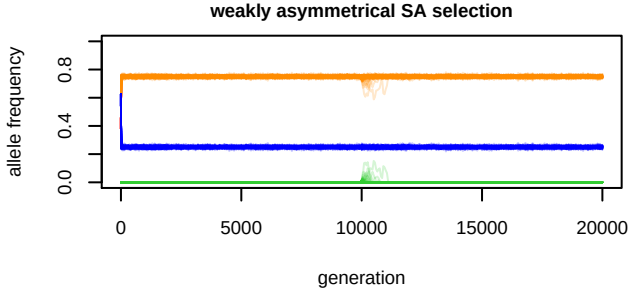
starting with GSD − introducing R (on X) − rec. depends on genotypic sex

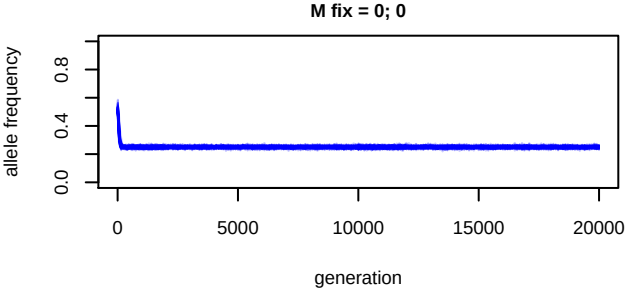


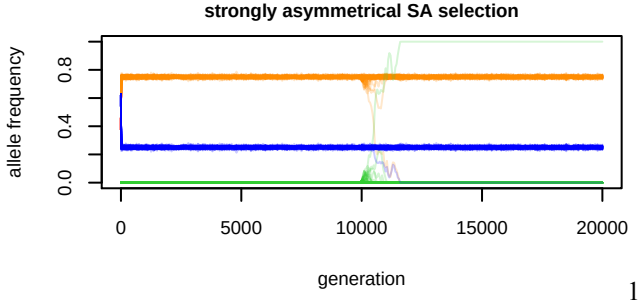


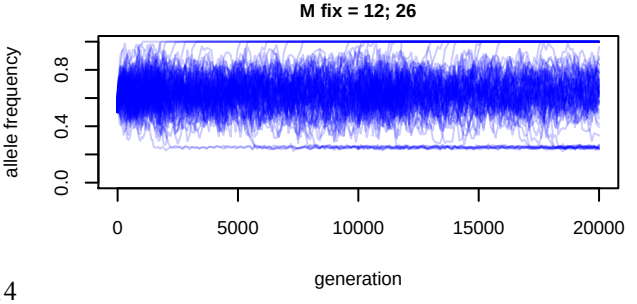




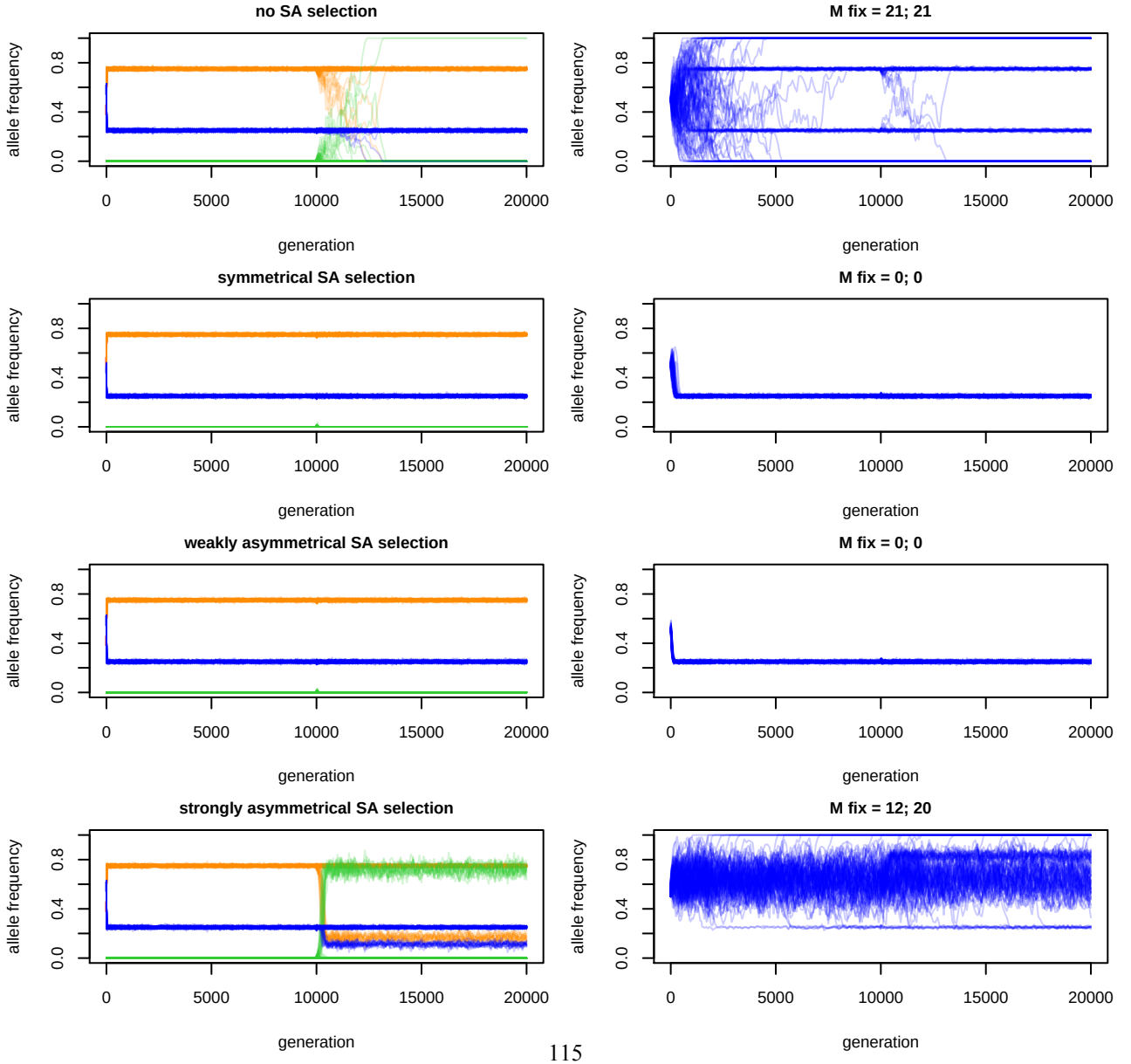




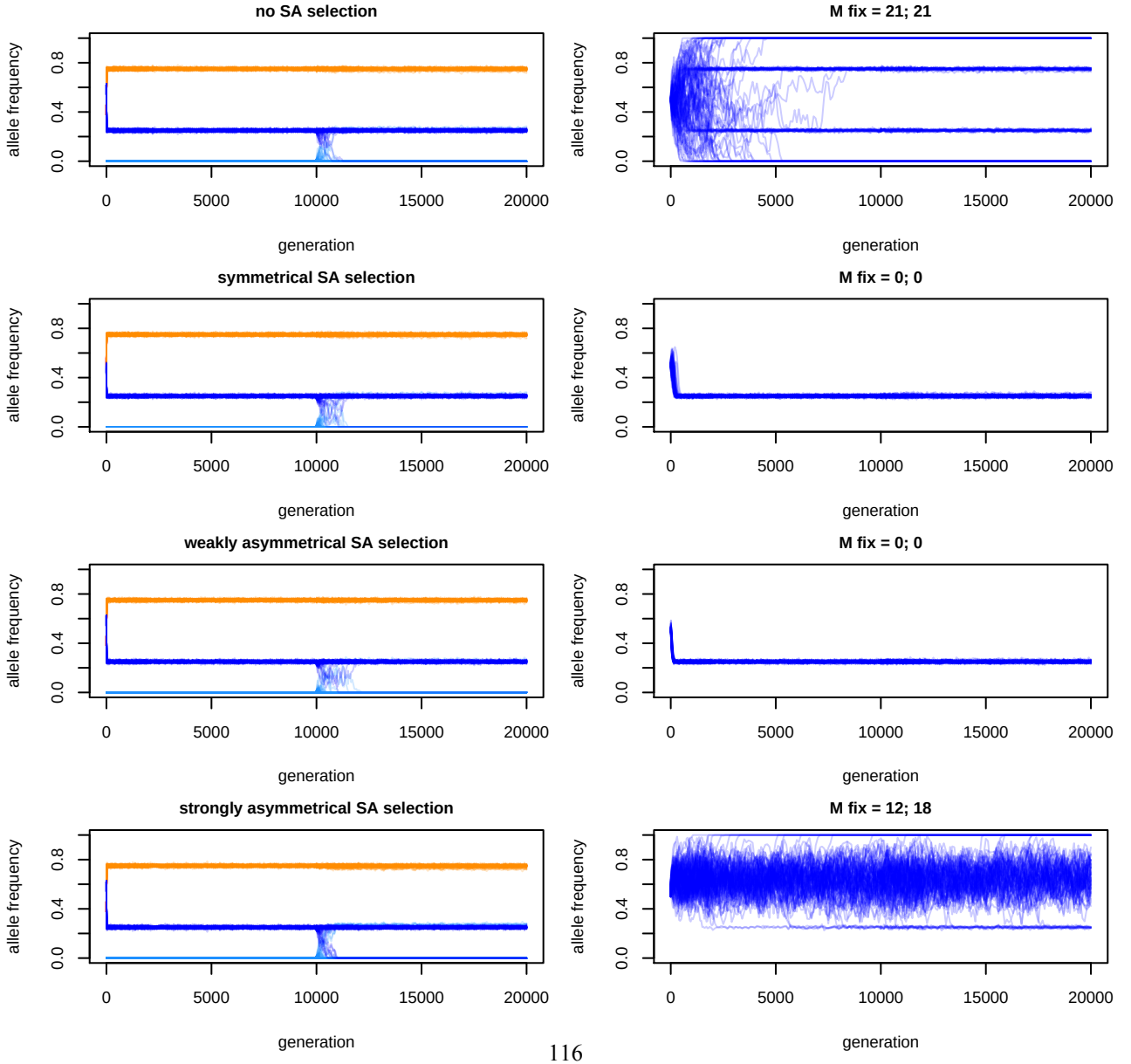




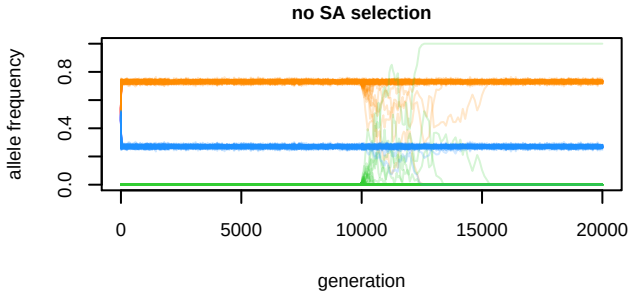
starting with GSD − introducing R (on Y)

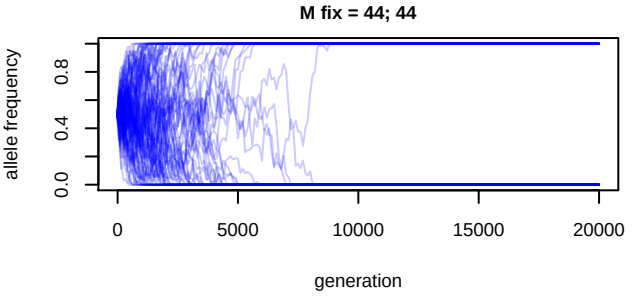


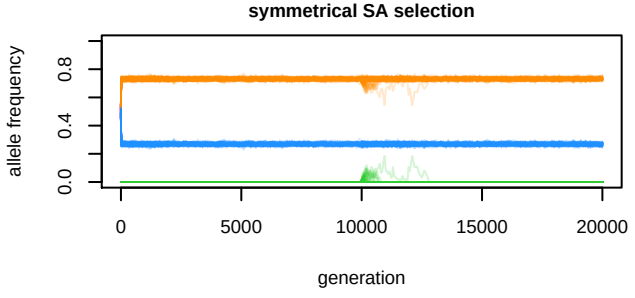
starting with GSD – introducing Y_L

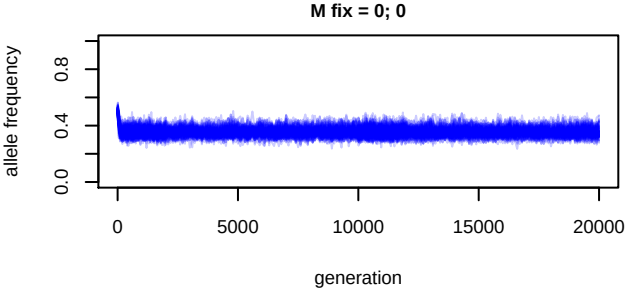


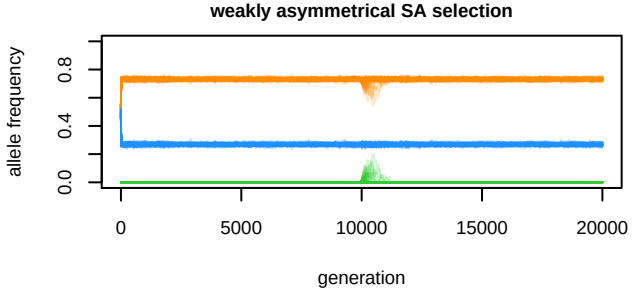
starting with LSD − introducing R (on X) - rec. depends on phenotypic sex

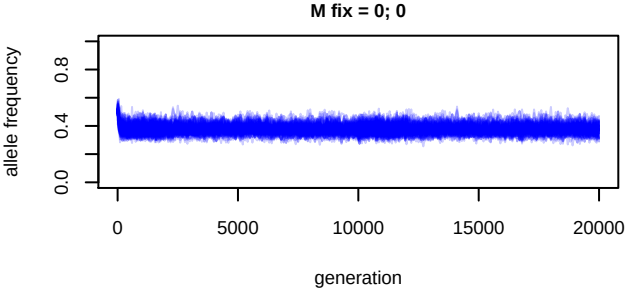


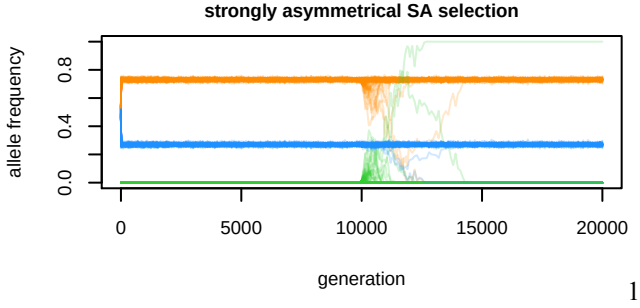


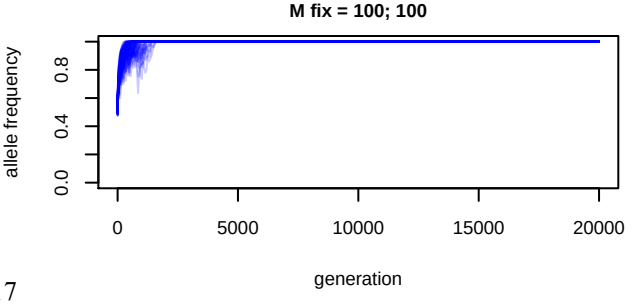




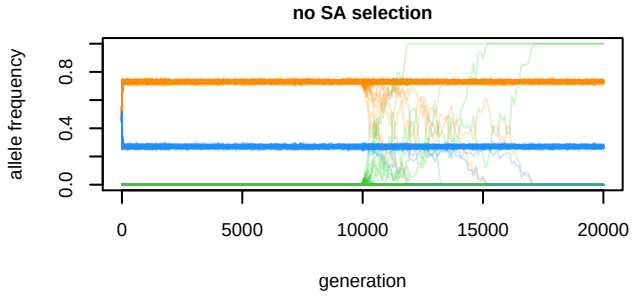


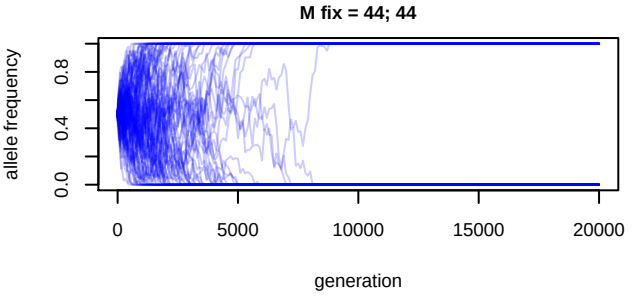


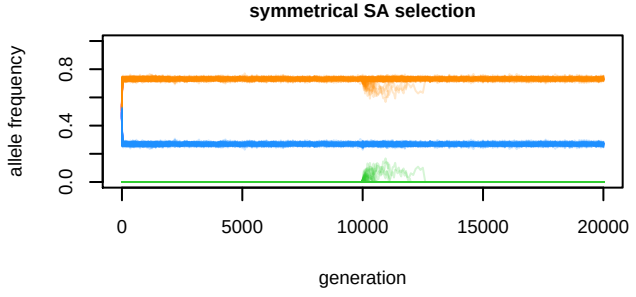


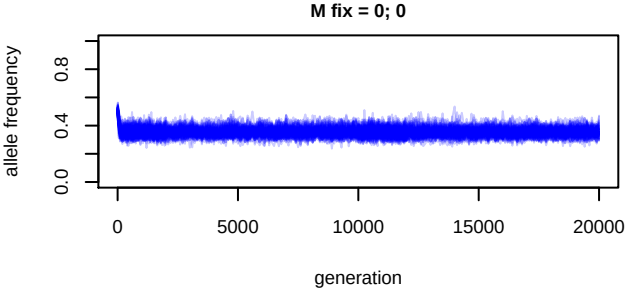


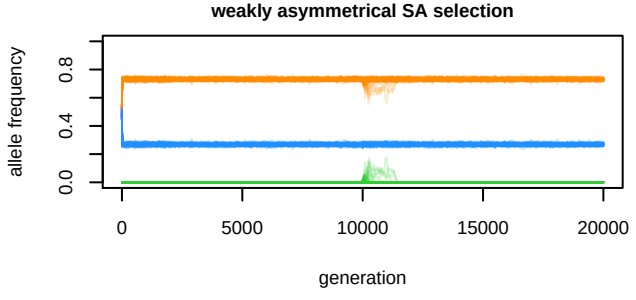
starting with LSD − introducing R (on Y)

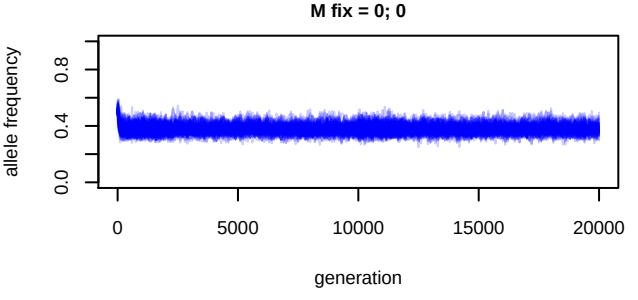


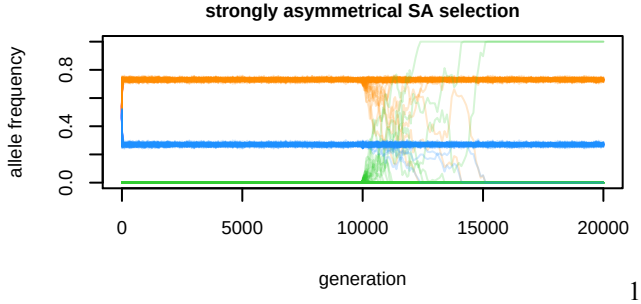


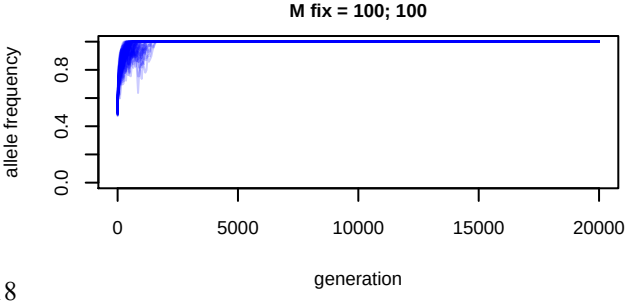




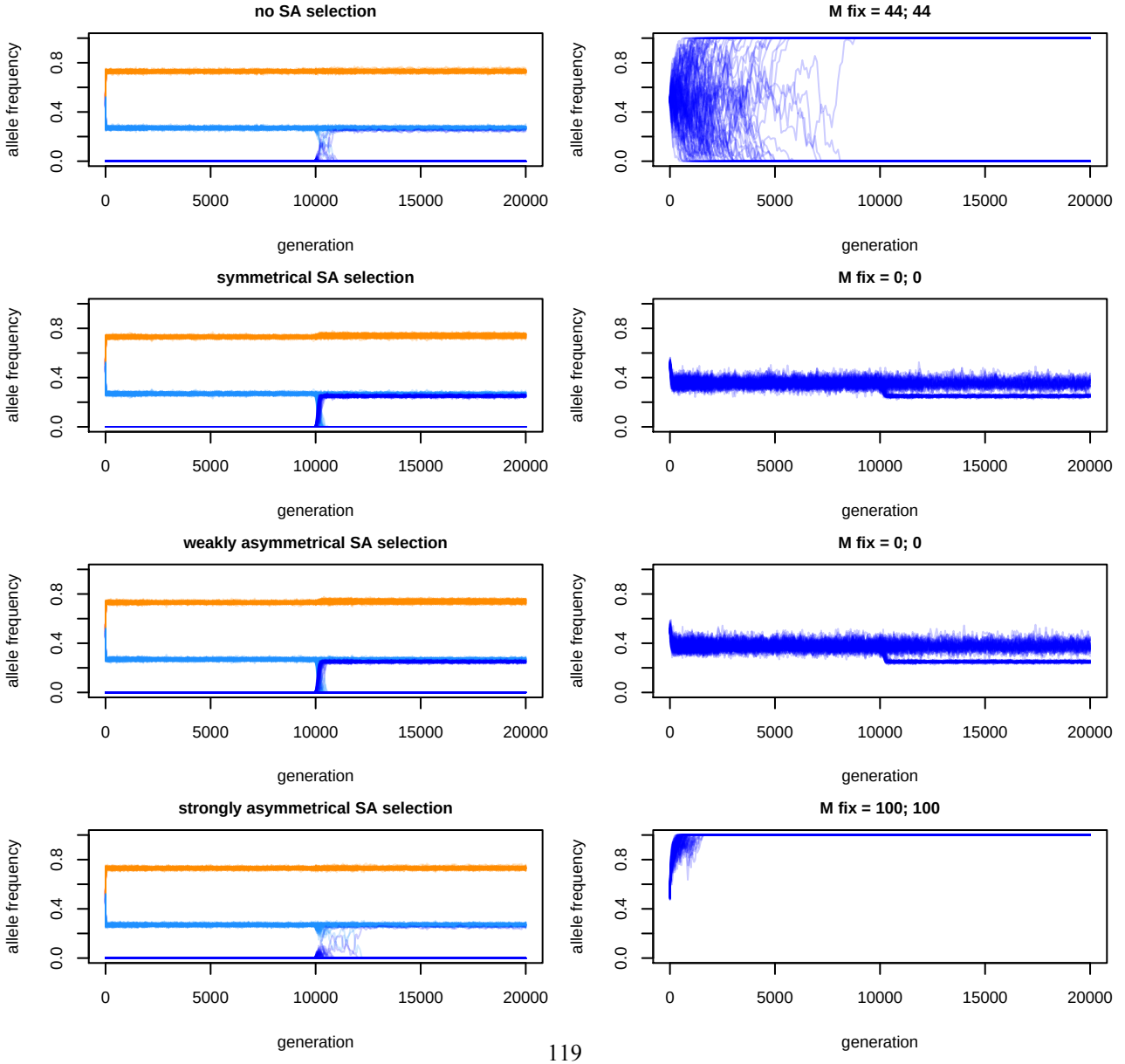




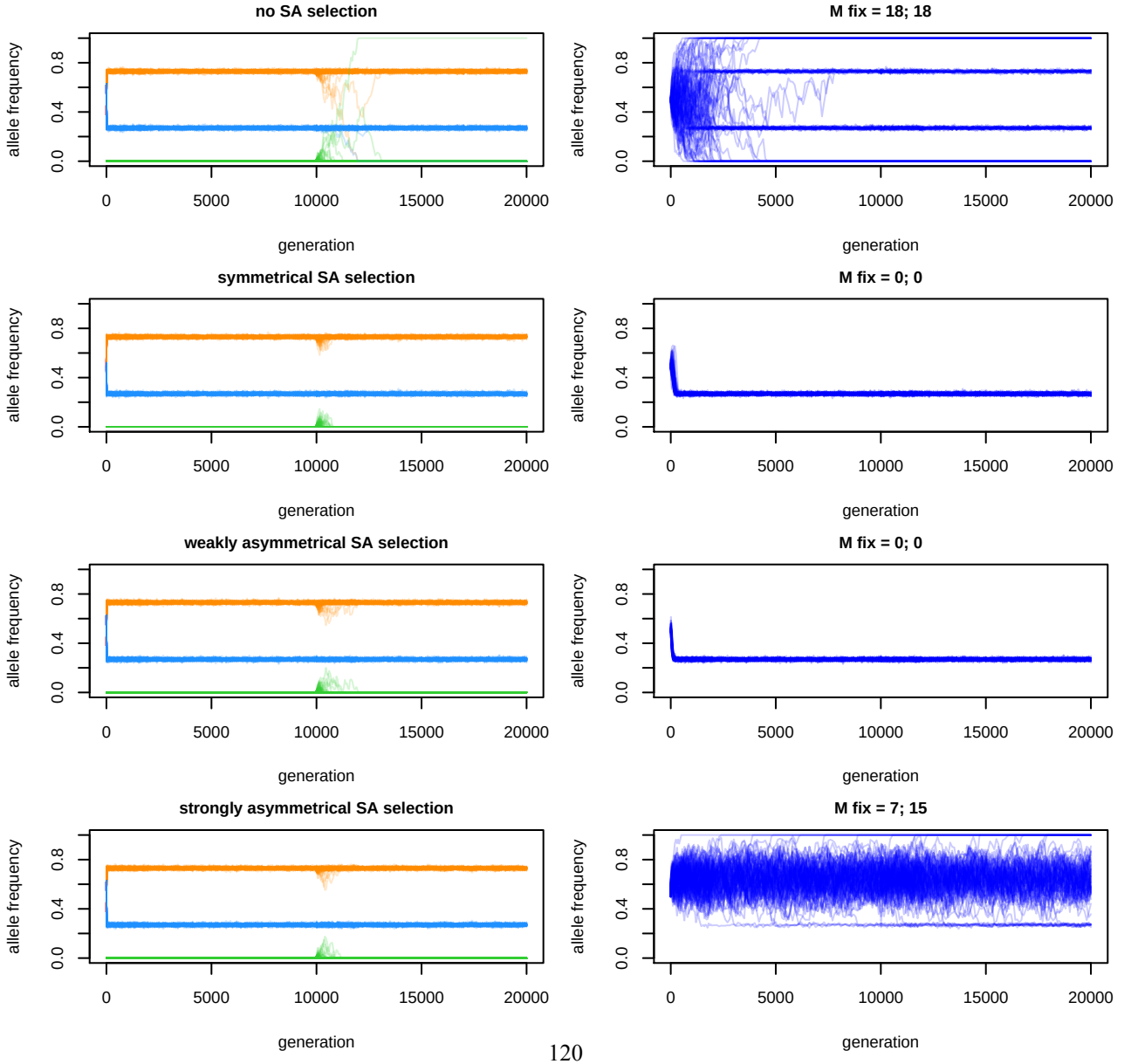




starting with LSD – introducing Y_S



starting with LSD − introducing R (on X) − rec. depends on genotypic sex



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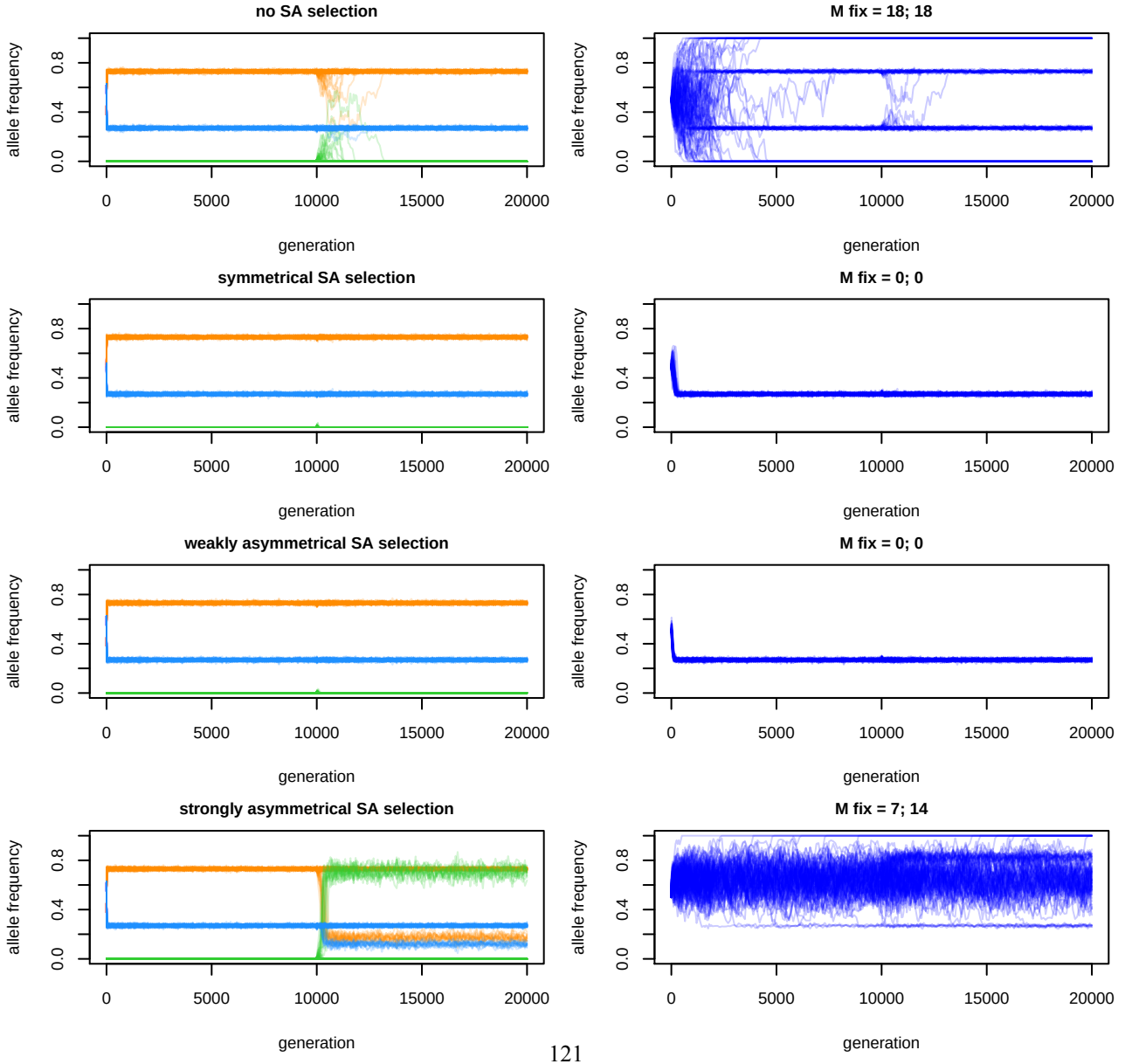
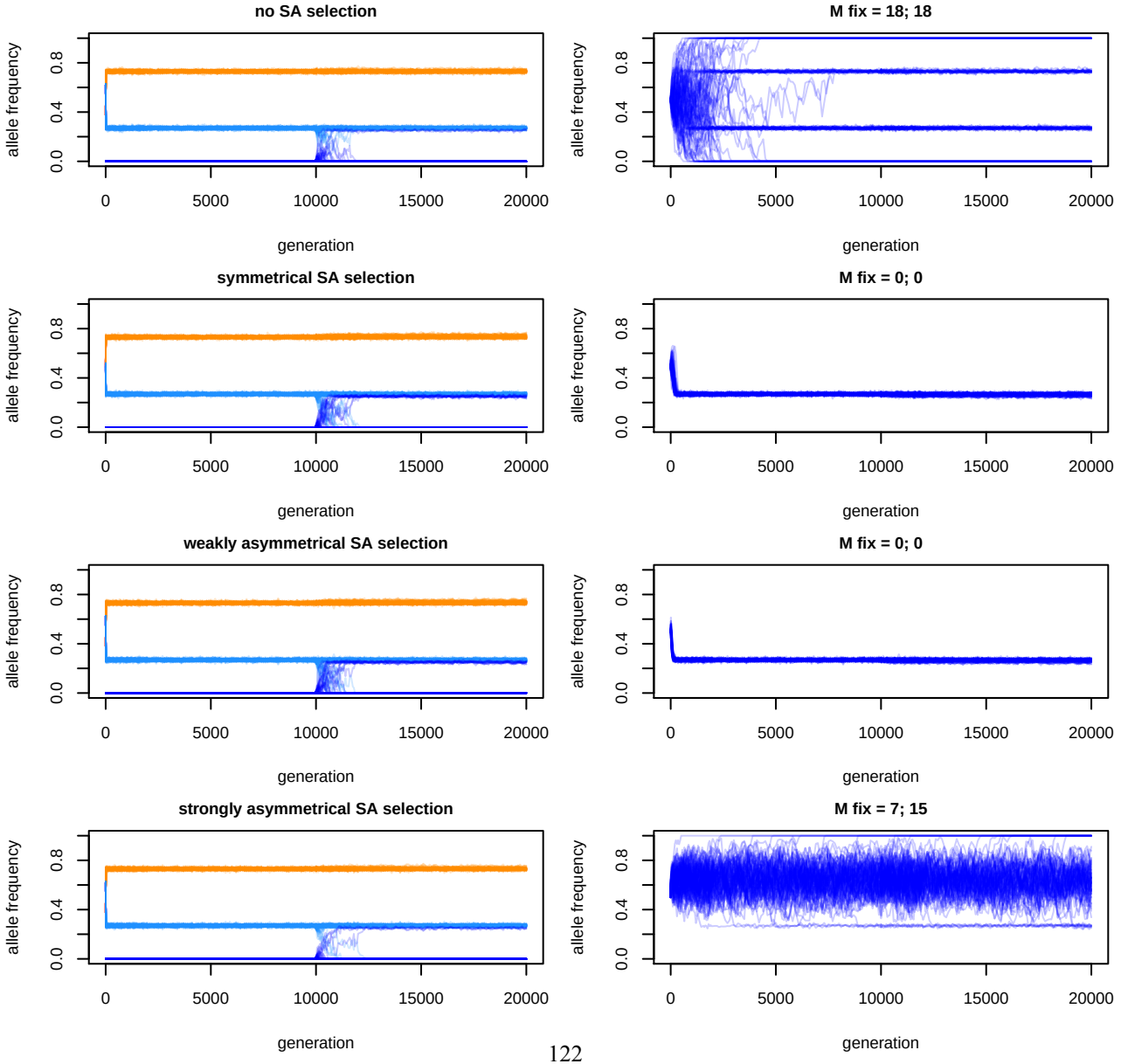


starting with LSD – introducing Y_S



Chapter four

Dobzhansky-Muller incompatibilities, dominance drive, and sex-chromosome introgression at secondary contact zones: a simulation study

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Contributions: All authors designed the study. LS, CD, EC and AB conducted the analyses. NP, LS and CD drafted the manuscript, subsequently improved by EC and AB.

Summary

Dobzhansky-Muller (DM) incompatibilities involving sex chromosomes have been proposed to account for Haldane's rule (lowered fitness among hybrid offspring of the heterogametic sex) as well as Darwin's corollary (asymmetric fitness costs with respect to the direction of the cross). We performed simulation studies of a hybrid zone to investigate the effects of different types of DM incompatibilities on cline widths and positions of sex-linked markers. From our simulations, X-Y incompatibilities generate steep clines for both X-linked and Y-linked markers; random effects may produce strong noise in cline center positions when migration is high relative to fitness costs, but X- and Y-centers always coincide strictly. X-autosome and Y-autosome incompatibilities also generate steep clines, but systematic shifts in cline centers occur when migration is high relative to selection, as a result of a dominance drive linked to Darwin's corollary. Interestingly, sex-linked genes always show farther introgression than the associated autosomal genes. We discuss ways of disentangling the potentially confounding effects of sex biases in migration, we compare our results to those of a few documented contact zones, and we stress the need to study independent replicates of the same contact zone.

Introduction

The build up of reproductive isolation during speciation processes may follow several pathways, from the progressive accumulation of divergently selected mutations each with little effects on hybrid fitness (Feder *et al.* 2014; Flaxman *et al.* 2014), to the disruption of co-adapted gene networks: alleles that are fit in one genetic background may reveal unfit in others. Negative epistatic interactions between genes with different evolutionary histories are referred to as Dobzhansky - Muller (DM) incompatibilities, following the suggestion by Dobzhansky (1937) and Muller (1940, 1942) that hybrid sterility or unviability stem from incompatible interactions between alleles from two or more genes. To illustrate this process, one may consider two loci with alleles *a* and *b* fixed in an ancestral species (such that all individuals are *aabb*), and assume that allopatric speciation makes one descendant population fix allele *A* at the first locus, while another population fixes allele *B* at the second locus. Upon secondary contact, crosses between *AAbb* and *aaBB* individuals produce *AaBb* hybrids, inducing interactions between alleles *A* and *B* that have never been tested previously in the same genome, and might thus reveal incompatible.

Actual expression of *AB* incompatibilities also depends on the patterns of dominance: problems will only appear if both *A* and *B* are expressed, but not if one or the other is recessive. Different modes of interactions and patterns of dominance will thus differently affect the fitness of F_1 and F_2 hybrids, and thereby the potential for long-term maintenance of species integrity (e.g. Tulchinsky *et al.* 2014; Lindtke & Buerkle 2015). Survival of F_1 hybrids, for instance, is required for recombination and gene flow to erode species barriers. As shown by Turelli & Orr (1995), the 'dominance model' of DM incompatibilities might also account for Haldane's rule, which states that, "when in the F_1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous [heterogametic] sex" (Haldane 1922). Indeed, assuming one of the genes involved in a DM incompatibility to be located on the X chromosome, while its Y gametolog is degenerated or silenced, then recessive alleles on the X will be protected from selection in XX females, but not in XY males due to hemizygous exposure. This model furthermore accounts for frequent asymmetries in

hybrid sterility (sometimes referred to as 'Darwin's corollary' to Haldane's rule; Turelli & Moyle 2007): if the autosomal allele *A* is dominant over *a*, then *AaB* hybrid males from a cross between an *aaBB* mother and an *AAb* father will show reduced fertility or viability, but not *Aab* hybrid males from a reverse cross between an *AAbb* mother and an *aaB* father.

Haldane's rule and Darwin's corollary may also result from incompatibilities between Y-linked and autosomal genes: these should affect only males, and in an asymmetric way, depending on the patterns of dominance of the autosomal gene (if *A* is dominant over *a*, sterility is expected in *AaB* hybrid males, but not in the *Aab* hybrid males from the reverse cross). Similarly, Haldane's rule might also result from incompatibilities between X- and Y-linked (or Z- and W-linked) genes (the 'fast heterogametic sex' model; Tao & Hartl 2003). Such incompatibilities might stem from a history of genetic conflict, such as X-linked meiotic drive repressed by Y elements (Frank 1991; Hurst & Pomiankowski 1991); alternatively, epistatic interactions between X- and Y-linked genes might affect the fitness of the heterogametic sex or be required for its proper differentiation (e.g. Chippindale & Rice 2001; Jiang *et al*. 2010; Kamiya *et al*. 2012). Whatever their causes, however, X-Y incompatibilities are not affected by dominance relationships (because both X- and Y-linked genes are hemizygous in males) so that interactions are expected to be symmetrical (i.e., Darwin's corollary does not apply in this case).

This reduction in hybrid fitness stemming from endogenous barriers has the potential to build up barriers to gene flow at secondary contacts (e.g. Gavrilets 1997). Hence, the consequences of DM incompatibilities on speciation processes can be investigated empirically via cline analyses of species-specific alleles across hybrid zones. In such zones, the balance between migration and selection against hybrids is expected to induce narrow clines in allele frequencies, referred to as 'tension zones' (Barton & Hewitt 1985). According to cline theory, the width *w* of a cline (calculated as the inverse of maximal slope, measured at the inflection point) is expected to increase proportionally to the effective rate of dispersal (measured for autosomes as the standard deviation of distance between parents and offspring, s), and to decrease proportionally to the square root of the selection coefficient against hybrids (√s) (Haldane 1948; Slatkin 1973):

$$w \propto \sigma / \sqrt{s} \qquad (1)$$

where s_i measures the effective dispersal rate of marker *i*. Note that the effective dispersal rate of sex-linked markers differ from that of autosomal markers due both to different occurrences in males and females, and to sex biases in migration (see Methods). Genomic regions harboring so-called 'speciation genes' are thus predicted to display steep clines (Barton & Hewitt 1985; Payseur 2010). In line with their predicted involvement in Haldane's rule, sex chromosomes have repeatedly been shown to display reduced introgression (i.e., steep clines) across contact zones in mammals, birds and insects (e.g. Vanlerberghe *et al.* 1986; Tucker *et al.* 1992; Saetre *et al.* 2003; Geraldes *et al.* 2008; Carling & Brumfield 2008; Presgraves 2008), testifying to their important role in speciation. Analytical and simulation studies by Muirhead & Presgraves (2016) have confirmed that neutral markers genetically linked to incompatibility genes are expected to display lower permeability (which should induce steeper clines) when located on X chromosomes rather than on autosomes. Permeability was lowest when incompatibility alleles had strong negative effect on hybrid fitness and were highly recessive, in link with hemizygous exposure in XY males (i.e., Haldane's rule).

Genomic divergences and barriers to introgression at secondary contact zones may also result from exogenous factors, whereby one species has fixed adaptations that make it more fit in environment 1, and the other species more fit in environment 2. Local adaptation pressure might also induce directional introgression (shifts in cline centers) for genes under selection, whenever the environmental transition does not coincide with the initial contact zone. Furthermore, endogenous and exogenous barriers may display some geographical coupling: while tension zones are expected to initially move more or less randomly according to local population densities and dispersal patterns, they should stabilize when reaching exogenous barriers, with which they might ultimately coincide (Barton 1979; Barton & Hewitt 1985; Hewitt 1988; Bierne *et al*. 2011, 2013). This coupling of barriers has the potential to reinforce speciation by contributing to the progressive build up of genomic islands of divergence, until a threshold is reached at which nonlinear transitions occur and reproductive isolation dramatically increases, in spite of gene flow (Gompert *et al.* 2012; Feder *et al.* 2014; Flaxman *et al*. 2014).

The present study is not intended to investigate the genomics of speciation or the maintenance of species integrity at secondary contact zones, but specifically focuses on the patterns of introgression at sex-linked markers between two otherwise weakly differentiated genomes. Such markers are of interest first because, as already mentioned, sex chromosomes are *a priori* expected to be involved in DMIs affecting hybrid fitness (in line with Haldane's rule and Darwin's corollary). Second, empirical studies of introgression patterns are consistently documenting not only sharp clines for sex-linked loci (in line with expectations from their involvement in Haldane's rule), but also shifts in cline centers relative to the bulk of genomic clines (see Discussion). Hence, we are asking whether the position of cline centers for sex-linked markers, in addition to their width, might depend on the specific underlying DM incompatibilities. In the neutral case, and assuming homogeneous environment, cline centers should on average lie wherever the two divergent populations first met (Barton & Hewitt 1985). For genes affecting local fitness (e.g. conferring local adaptation), centers might be shifted to coincide with exogenous barriers, with advantageous alleles spreading into the domain of less fit ones (Barton & Hewitt 1985). As a matter of fact, empirical evidence for such shifts has often been interpreted within this adaptationist framework (see Discussion). Our point, however, is that shifts might sometimes also occur in the absence of exogenous selective forces or any form of coupling (Buggs 2007). Simulations of a hybrid zone for a single-locus, two-alleles warning-color gene, for instance, has shown that the dominant allele may expand into the domain of the recessive one, even if the two phenotypes are equally fit (Mallet 1986). We reasoned that a similar 'dominance drive' might also affect cline centers in the case of DMI incompatibilities involving sex chromosomes. The rationale was that specific patterns of dominance for autosomal and sex-linked genes involved in DM incompatibilities and responsible for Darwin's corollary might generate asymmetries in introgression, shifting cline centers for the genomic regions involved, even in the absence of any coupling with exogenous barriers. From the arguments made above, such asymmetric introgressions would be expected from X-A or Y-A incompatibilities, but not from X-Y incompatibilities. The latter should affect cline width for both X and Y-linked markers, but not cline positions, due to the absence of dominance interactions.

Introgression patterns of sex-linked markers are also expected to depend on demographic parameters, notably the effective rates of dispersal, defined as the proportion of immigrant copies per generation. Under balanced male and female migration (and in the absence of DM incompatibilities), cline width for Y chromosomes should be one third that of X chromosomes and one quarter that of autosomes (i.e., proportional to their effective dispersal; eq. 1). Male- or female biases in migration will affect these values: under male-only migration, for instance, Y chromosomes should have the same cline width as X chromosomes, being half that of autosomes, while under female-only migration, cline width should be zero for Y chromosomes and identical for X chromosomes and autosomes. This raises the empirical problem of disentangling the potentially confounding effects of sex-specific demographic traits and asymmetric reproductive isolation on the differential introgression of sex-linked markers across hybrid zones.

In the present study, we performed individual-based simulations under stable demographic settings to characterize the effect of different types of DM incompatibilities (X-A, Y-A and X-Y) on the introgression clines of sex-linked and autosomal markers, while controlling for the rate and sex biases in migration. As our simulations show, different DM incompatibilities indeed translate into different patterns of cline widths and center shifts. In particular, we show that the patterns of dominance underlying Darwin's corollary have the potential to generate strong shifts in the position of cline centers, independent of any intrinsic or extrinsic benefit of the invading allele.

Methods

Individual-based simulations were run with QuantiNemo (Neuenschwander *et al.* 2008). The demographic settings consisted in a one-dimensional stepping-stone model with twelve demes, numbered #1 to #12, each with a fixed population size of $N = 100$. Generations were non-overlapping and selection was soft, meaning that enough offspring were produced each generation to fill every patch. For each offspring, one father and one mother were chosen randomly with replacement (mimicking a promiscuous mating system) with a probability set by their relative fitness values (see

below). Offspring sex was assigned randomly from a binomial distribution with expectation 0.5 (so that sex-ratios were balanced throughout simulations). Reproduction was followed by juvenile migration, which occurred to each of the two neighboring demes with the same probability. Individual migration rates were set to m = 0.01, 0.02 or 0.05 (proportion of migrating offspring), corresponding to autosomal s values of 0.10, 0.14 and 0.218, respectively (standard deviation of distances between parents and offspring). For each of these three values, the percentage of male migration (r) was set to 0% (pure female), 50% (mixed), or 100% (pure male). Hence, the effective dispersal rates of autosomal and sex-linked markers scaled as $s_A = s$, $s_Y = sr/2$ and $s_X = s(1-r/2)$. The two end demes (#1 and #12) did not receive any migrant, behaving effectively as genetic reservoirs for the two species. Thus, demes #1-2 and #11-12 only sent migrants in one direction (so that emigration rate was only half that from other patches).

Individuals were characterized by one sex-linked locus with species-specific alleles (such that males and females were *xy* and *xx* in species 1, respectively *XY* and *XX* in species 2) and two independent autosomal loci, also with species-specific alleles. One autosomal locus involved in either X-A or Y-A incompatibilities was initially fixed for allele *a* in species 1 and *A* in species 2. Another autosomal locus not involved in incompatibilities, and unlinked to the first one, was initially fixed for allele *n* in species 1 and *N* in species 2. Hybrid fitness was determined by epistatic interactions between genes on different chromosomes, according to four scenarios. In the first scenario (*no incompatibility*), hybrids did not suffer from any fitness loss. In the second scenario (*X-Y incompatibility*), fitness was depressed in all hybrid males (either *xY* or *Xy*), due to incompatibilities between the X-linked allele from one species and the Y-linked allele from the other. In the third scenario (*X-A incompatibility*), hybrid fitness was depressed by incompatibilities between autosomal and X-linked alleles. The homologous Y-linked allele was assumed suppressed or silenced, resulting in asymmetric sex-specific effects (Haldane's rule and Darwin's corollary). Species-2 alleles were assumed dominant over those of species 1, so that the *xaA* hybrid males (stemming from a cross between a species-1 mother and a species-2 father) had a depressed fitness, but not the *XaA* hybrid males from a reverse cross. In the fourth scenario (*Y-A incompatibility*), hybrid fitness was also affected by the patterns of dominance; *A* was assumed dominant over *a*, so that the *yaA* hybrid males

from a cross between a species-2 mother and a species-1 father suffered from depressed fitness, but not the *YaA* hybrid males from a reverse cross. The coefficients of selection against incompatible hybrids were set to s = 0.1, 0.25 and 0.5 (resulting in relative hybrid fitness of W_H = 0.9, 0.75 and 0.5 respectively). Due to complete dominance of *A* over *a* and *X* over *x*, fitness values for all genotypes were fully specified by the single parameter s (see Table S1 for genotype-specific fitness values under all DMI scenarios). Ancestral states and a possible evolutionary scenario leading to the DMI situations analyzed here are provided in Fig. 1.

Parameters were varied in a fully factorial design for all of the four scenarios, with 100 replicates for each parameter set (summing to 9,000 simulation runs altogether). At the start of each simulation, patches #1 to #6 were occupied by species 1 only, and patches #7 to #12 by species 2 only. The system was then allowed to evolve for 10'000 generations (far enough to reach the equilibrium under all scenarios, as shown by preliminary tests), after which allele frequencies at each locus were recorded for every deme. Clines were then fitted to allele frequencies for each replicate independently, using the HZAR package (Derryberry 2013) in R (R development core team 2008). We applied a sigmoid model with two parameters: cline center position (c) and cline width (w, defined as the inverse of the maximal slope; Payseur 2010). The sigmoid function incorporates these parameters as $y = \frac{1}{2}\left[1 - \tanh\left(2\frac{x-c}{w}\right)\right]$. Both parameters were estimated with 95% confidence intervals using a Markov Chain Monte Carlo method and a Metropolis-Hasting algorithm, with chains of 2000 iterations preceded by a burn-in period of 500 iterations. Parental allele frequencies were left with default values (1 and 0), which is appropriate since parental source populations did not receive migrants. Examples of fitted functions with parameter values are provided in Fig. S2. The effect of model parameters s, m and r on cline centers and cline widths under the four scenarios were analyzed with multivariate linear models with the *lm* function of the R package *stats*. Variables were used untransformed and interactions were not considered. Analyses thus included 27 combinations of s, m and r for each DMI scenario. We applied a backward selection procedure to progressively remove non-significant variables from the models. In the final models, significance was tested by 1'000

bootstrap replicates. As p-values have limited meaning in case of simulation studies (where power can be controlled by increasing replicate numbers), we also provide effect sizes via R^2 values, and do not interpret effects accounting for less than 1% of total variance.

Results

Cline widths and centers under balanced dispersal are provided in Fig. 2 for all markers and DMI scenarios investigated. In the absence of incompatibilities (Fig. 2a), cline widths (vertical axis) depend on the effective rate of dispersal, but with a large random variance. As expected, *w* increases with *m* for all markers (from left to right; R^2 = 0.06 to 0.12 depending on marker, Table 1). Furthermore, at any given *m* value, clines are widest for the neutral autosomal marker N (black circles), steepest for Y (pale blue triangles), and intermediate for X (pale red crosses), in line with differences in effective dispersal. Cline centers (horizontal axis) correspond to the initial contact zone on average, but also with a large random variance. The effects of sex biases in migration (Fig. S1a) further illustrate the role of effective dispersal rate: as the proportion of male migration increases, cline width increases strongly for Y chromosomes (R^2 = 0.41) and decreases slightly for X chromosomes (R^2 = 0.01), while autosomes remain unaffected. As expected, cline widths for X chromosomes reach the same value as for autosomes under female-only migration, and the same value as Y chromosomes under male-only migration. Regarding centers, the position of the Y is strictly constrained to the original contact zone in the case of female-only migration (left panel), but expands to the same wide distribution as other markers as soon as males show some migration.

Under X-Y incompatibilities (Fig. 2b), neutral autosomal markers (black circles) behave as in the absence of incompatibility (Fig. 2a). In contrast, X and Y markers (bright blue triangles and red crosses) show very steep clines with little noise (vertical axis). As a result, migration rate *m* explains a large part of the variance in cline width (16% and 36% for Y and X respectively, Table 1), as does the selection coefficient *s* (8% and 32% respectively, Table 1). For both markers, cline width increases with $m/\sqrt{s}$ (from left to right), as expected. Cline centers (horizontal axis) always lie on the initial contact zone on average, but with a strong effect of $m/\sqrt{s}$ on random noise, which is very small at low

migration and strong selection, but increases drastically at large migration and weak selection. Despite this noise, however, X and Y centers show strong coincidence over all simulations (R^2 = 96%). The effect of sex-biases in migration (Fig S1b) also illustrates differences in the effective rate of dispersal. An increase in the proportion of male migration results in shallower clines for Y but steeper clines for X (R^2 = 40% and 4% respectively, Table 1), and drastically increases the variance in cline positions for both markers, by releasing the constraint on the Y center imposed by the absence of male migration.

Under X-A incompatibilities (Fig. 2c), N and Y markers (black circles and pale blue triangles) behave as in the absence of incompatibilities (Fig. 2a). In contrast, X and A markers (bright red crosses and green squares) show very steep clines with little noise (vertical axis), especially for the X. As a result, migration rate *m* explains a large part of the variance in cline width (18% and 34% for X and A respectively, Table 1), as does the selection coefficient *s* (18% and 15% respectively, Table 1). For both markers, cline width increases with $m/\sqrt{s}$ (from left to right), as expected. Unexpectedly, however, cline centers for these two loci (horizontal axis), which lie on the initial contact zone at low $m/\sqrt{s}$ values, display a strong shift towards the domain of recessive alleles at high $m/\sqrt{s}$ values. These shifts are stronger for the X-linked locus than for the autosomal one (significantly so for 25/27 parameter sets). As noise is small overall, *m* and *s* account for large parts of the variance in cline centers (respectively 41% and 23% for X, 39% and 21% for A; Table 1). Sex biases in migration affect cline width for the X chromosome but not the A marker, in accordance with their effective dispersal rates (Fig S1c). In contrast, clines centers are significantly affected for both markers (R^2 = 2% in both cases, Table 1), especially at intermediate $m/\sqrt{s}$ values (Fig. S1). Shifts in X and A centers occur at female-biased migration but much less at male-biased migration (due to stronger effective dispersal rate of X chromosomes under female-biased migration). Overall, cline centers for X and A markers correlate strongly across all simulations (R^2 = 99%).

Patterns under Y-A incompatibilities (Fig 2d) are qualitatively similar to the X-A situation (Fig. 2c), *mutatis mutandis*, though with different quantitative effects on the proportion of variance explained. N and X markers (black circles and pale red crosses) behave as in the absence of incompatibilities (Fig. 2a), while Y and A markers (bright blue triangles and green squares) show

very steep clines with little noise, especially for the Y. As a result, migration rate *m* explains a large part of the variance in cline width (10% and 35% for Y and A respectively, Table 1), as does the selection coefficient *s* (9% and 19% respectively, Table 1). For both markers, cline width increases with $m/\sqrt{s}$ (from left to right), as expected. Similar to the previous case, cline centers for these two loci lie on the initial contact zone at low $m/\sqrt{s}$ values, but display a strong shift towards the domain of recessive alleles at high $m/\sqrt{s}$ values. Here again, the shift is stronger for the Y-marker (significantly so for 26/27 parameter sets). As noise is small overall, *m* and *s* account for consistent parts of the variance in cline centers (respectively 8% and 4% for Y, 7% and 2% for A). Sex biases in migration have a relatively large effect on cline width for the Y chromosome (26%) but not for the A marker, in accordance with their effective dispersal rates (Fig S1d). In contrast, these biases drastically affect clines positions for both markers (R^2 = 58% and 59% respectively, Table 1), mostly due to the fact that the shift, which occurs under strong $m/\sqrt{s}$ values when males contribute to migration, is totally prevented under female-only migration. Overall, cline centers for Y and A markers correlate strongly across all simulations (R^2 = 0.98).

Fig. 3 plots cline widths (w_i) as a function of $s_i/\sqrt{s}$ for the markers involved in DM incompatibilities under all three scenarios. A linear regression over the complete set of data (including three DM scenarios and three values each for hybrid fitness *s*, migration rate *m* and sex bias *r*) accounts for 62% of the variance ($p = 2.2\ e^{-16}$), with regression line $w = 4.84\ s/\sqrt{s} + 0.035$. The weak but positive intercept stems from the fact that *w*, contrasting with theoretical expectations (eq.1), is constrained under our settings by the asymptotic value imposed by the maximal length of our linear stepping-stone population model, so that the relation is expected to saturate at large $s/\sqrt{s}$ values.

Discussion

From our simulations, the several DM incompatibilities considered here affect differently the relative cline widths and localization of centers for the genomic regions involved in reproductive isolation. In the absence of incompatibilities, cline widths are often large, and increase markedly with the effective rate of dispersal, with however a large stochastic variance across replicates. Cline centers coincide on

average with the original contact zone, but genetic drift may strongly affect the precise location for individual simulations, so that independent markers may differ largely in cline center (as would also the same marker across independent replicates of the same contact zone). In contrast, DM incompatibilities generate much steeper clines, a strong coincidence between the cline centers of genes involved in incompatibilities, and in some cases systematic shifts in cline centers relative to neutral markers.

Estimated cline widths for loci involved in DM incompatibilities are in good accordance with expectations from cline theory (Haldane 1948; Slatkin 1973; Barton & Hewitt 1985; Payseur 2010), being proportional to the effective rate of dispersal (accounting for the differential effects of sex-biased migration on sex-linked makers), and inversely proportional to the intensity of selection against hybrids (eq. 1). Steep X-chromosome clines are also in line with analytical and simulation studies predicting lower permeability for incompatibility genes located on X chromosomes rather than on autosomes, due to hemizygous exposure in XY males (Muirhead & Presgraves 2016). In our case, the mechanisms generating sharp clines and cline-center coincidences are pretty clear: epistatic interactions between genes involved in incompatibilities induce positive feedback loops between conspecific alleles (and mutual antagonisms between allospecific ones), resulting in bistable equilibriums and threshold effects. Under X-A incompatibilities, for instance, selection favors *A* over *a* above a threshold frequency of *X*, but *a* over *A* below this threshold (and vice versa). As a consequence, one pair of the conspecific alleles involved in incompatibilities eliminates the alternative combination. Which pair takes over mostly depends on initial conditions, immigrant inflow, and genetic drift. The latter factor actually accounts for the large variance in the positions of cline centers among simulations, when migration is high and selection against hybrids is weak. These several effects fully account for the patterns of X- and Y-clines in case of X-Y incompatibilities, which are much steeper than those of autosomal markers (Fig. 2b); X- and Y centers may largely vary when migration is high and selection weak, but do not show systematic biases, and strictly coincide within the same simulation. Note that steep clines for both X and Y chromosomes may also result from independent X-A and Y-A incompatibilities (see below), but cline centers are then only

expected to coincide if the patterns of dominance, as well as the ratios of migration to selection, are similar.

More unexpectedly, unidirectional shifts in cline centers occurred in our simulations when interactions involved autosomal genes (i.e. X-A or Y-A incompatibilities; Figs. 2c-d), under precise conditions for migration, hybrid fitness, and dominance relationships between alleles involved in antagonisms. Specifically, dominant alleles invaded the domain of recessive alleles when migration was high relative to selection. Note however that substantial levels of gene flow (relative to selection) are needed to properly reveal the patterns of shifted clines with X-A and Y-A DMIs (Fig. 2). This one-way introgression directly arises from the dominance model of DM incompatibilities, via the asymmetric selection against hybrid males (i.e., Darwin's corollary). Under Y-A incompatibilities, for instance, *y* alleles are selected against in *yaA* hybrid males born to a cross between a species-1 father and a species-2 mother (because *A* is dominant over *a*), whereas *YaA* males born to a reverse cross are perfectly fertile. Hence *Y* will spread at the expense of *y* at the contact zone. This spread will in turn facilitate that of *A* among backcrosses, through selection against *aa* homozygous males. Thus, the positive feedback loop between *Y* and *A*, together with the asymmetry in hybrid fitness stemming from the dominance patterns at the autosomal gene (Darwin's corollary), favors their conjugate invasion into the domain of species 1. Interestingly, *Y* precedes *A* at the forefront of invasion (Fig. 2d). This discrepancy also stems from the dominance patterns: the selection against *a* is less strong than that against *y*, because it is effectively neutral in *aA* heterozygous males, while *y* is not. *Mutatis mutandis*, the same situation arises for X-A incompatibilities (Fig. 2c), and for the same reasons. The *X* chromosome from the species with a dominant autosomal allele invades the domain of species 1 when selection is weak enough relative to effective dispersal. The spread of *X* favors in turn invasion by the functionally linked autosomal allele (*A*), and *X* also shows farther introgression than *A*.

Empirical studies are regularly unveiling steeper clines (or more differentiation between allopatric populations) for sex-linked markers than for autosomal ones, as documented e.g. in mammals (Geraldes *et al.* 2008; Carneiro *et al.* 2013; Tucker *et al.* 1992; Macholan *et al.* 2007; Janousek *et al.* 2012), birds (Carling & Brumfield 2008, Storchova *et al.* 2010; Elgvin *et al.* 2011; Taylor et al. 2014; Walsh *et al.* 2016) and insects (Hagen & Scriber 1989; Sperling & Spence 1991;

Ferris *et al.* 1993; Herrig *et al.* 2014; Maroja *et al.* 2015). Interestingly, the trend seems also to hold for homomorphic sex chromosomes, as recently documented in tree frogs (Dufresnes *et al.* 2016), possibly indicating a role for direct XY interactions (fast-heterogametic sex) rather than dominance effects involving autosomal genes, which are only expected to occur when males are hemizygous for X-specific genes. There are few exceptions, however, such as in poplars, where the interbreeding species *Populus alba* and *P. tremula* show less differentiation at sex-linked than at autosomal markers (Stölting *et al*. 2013).

Of particular interest in the context of our study are empirical evidences for shifted clines of X or Y markers, indicating introgression of X or Y chromosome from one species into the range of the other. Examples include e.g. spotted eagles (where the Z chromosome from *Aquila clanga* is introgressed into the range of *A pomarina*; Backström & Väli 2011), *Chorthippus* grasshoppers (where the X chromosome of *C. parallelus* is introgressed into the range of *C. erythropus*; Ferris et al. 1993), *Microtus* rodents (where the Y chromosomes of the Lund race of *M. agrestis* are introgressed into the Standard race domain; Jaarola et al. 1997) or *Canis* species (where dog Y chromosomes are introgressed into coyote populations; Wheeldon et al. 2013). Cline shifts might possibly also account for the patterns found in poplars, if the lack of differentiation at sex-linked markers results from the massive introgression of *P. alba* sex chromosomes into the genomic background of *P. tremula* (Stölting *et al.* 2013). Such differential introgression, with unidirectional spread of genes from one species well into the range of another, has sometimes been interpreted as evidence for positive selection stemming from intrinsic benefits of the invading alleles (e.g. Payseur *et al.* 2004; Jones *et al.* 2010; Staubach *et al.* 2012), asymmetric hybridization (Backström & Väli 2011), or sex-ratio selection (Macholan *et al.* 2008). As our simulations show, such patterns might also stem from the dominant model of Dobzhansky - Muller incompatibilities, more precisely from the same forces that underlie Darwin's corollary to Haldane's rule. The preferential introgression of Y chromosomes from dog to coyote (Wheeldon *et al.* 2013), for instance, might be driven by a Y-A incompatibility, assuming that the dog allele for the autosomal locus involved in the incompatibility is dominant over the coyote allele, and that male migration is strong relative to selection.

The best-studied mammalian contact zone so far is that between two subspecies of mice, *Mus m. musculus* and *Mus m. domesticus*, which spreads from Scandinavia to the Balkans. Both X- and Y linked markers (as well as a few autosomal markers) consistently show steep clines along this contact zone (Vanlerberghe *et al.* 1986; Tucker *et al*. 1992; Payseur *et al.* 2004; Macholán *et al.* 2007; Teeter *et al.* 2008; Janoušek *et al.* 2012; Campbell & Nachmann 2014). These clines are not always coincident, however, suggesting independent X-A and Y-A incompatibilities with different autosomal genes involved. In particular, extensive introgression of the *musculus* Y into the *domesticus* domain has been documented at several locations along the contact zone (e.g. Macholan *et al.* 2008; Jones *et al.* 2010, Ďureje *et al.* 2012). Possible causes include selective advantage of the *musculus* Y, due e.g. to intrinsic benefit (Jones *et al.* 2010) or sex-ratio selection (Macholan *et al.* 2008). From our simulations, this asymmetric introgression might also stem from Y-A interactions, with a *musculus* autosomal A_m dominant over the *domesticus* allele A_d. Supporting this interpretation, laboratory crosses between the two subspecies have revealed asymmetries in hybrid male sterility: hybrid males from a cross between a male *domesticus* and a female *musculus* ($X_mY_dA_mA_d$) are sterile or subfertile, whereas hybrid males from the reverse cross with a *M. m. musculus* Y ($X_dY_mA_dA_m$) are usually reproductively normal (Good *et al.* 2008; Campbell *et al.* 2013). Conversely, some X-markers locally show asymmetric introgression of *domesticus* alleles into the *musculus* range (Payseur *et al.* 2004), which has been suggested to result from adaptive introgression, with an X_d allele more fit in a foreign genomic context. From our simulations, such patterns might also signal X-A incompatibilities where the *domesticus* autosomal allele A_d is dominant over the A_m allele.

This large variance among replicates of the same contact zone, resulting from local differences in introgression patterns, is itself an interesting result. It argues against intrinsic benefits to X or Y chromosomes in a foreign genome: intrinsically favorable alleles should be quickly fixed on both sides of the hybrid zone, as the flow of an advantageous allele is not expected to be much delayed, even in the presence of strong barriers (Pialek & Barton 1997). Variance in introgression shifts might also result from local adaptation, given that environmental transitions are not expected to strictly coincide with the initial contact zone (see Introduction). However, shifts triggered by local adaptation would generate significant genetic-environment associations, which is not the case for

shifts triggered by DMIs. Variance among replicates has also been suggested to reflect local differences in the genetic architecture underlying incompatibilities (Macholan *et al.* 2011). From our simulations, it may also result from differences in the amount or sex-specificity of migration relative to selection: shifts are only expected when local conditions favor migration (Fig. 2), and may depend on local sex biases in migration (Fig. S1). This point illustrates the need for independent replicates of the same contact zone, as also underlined by the large stochastic component in cline steepness and center shifts documented throughout all our simulations.

Another new prediction stemming from our simulations, which is worth testing empirically, is that, whenever clines are shifted as a result of interactions between X (or Y) chromosomes and autosomal loci, the shifts are more pronounced for the sex-linked than for the autosomal genes involved. No such pattern has apparently been reported so far, which is not surprising however: the effect is subtle, and thus likely to be hidden by the large stochastic components mentioned. Furthermore, empirical evidence would require specific documentation of the precise genomic regions involved in the interaction. However, it offers the potential to test for a prediction uniquely stemming from specific DM interactions (X-A and Y-A) and under precise conditions (migration strong relative to selection). Although other patterns emerging from our simulations might not present unique signatures of specific DMIs (e.g. steep clines with shifted centers might also stem from other causes as mentioned above), we propose that consistently steep clines, associated with a strong coincidence between sex-linked and autosomal markers over multiple replicates, together with the absence of genetic-environment associations, should be considered as likely hallmarks of DM incompatibilities involving sex chromosomes.

Sex biases in migration may of course also affect the patterns of introgression, with potentially confounding effects. These might be disentangled along different lines. The absence of male migration obviously generates steep clines for Y-linked markers, which are however strictly localized at the initial contact zone, not at the forefront of invasions (Fig. S1). Conversely, a steep cline for mitochondrial markers at the initial contact zone would be expected in the absence of female migration. Purely neutral models can possibly be rejected in such situations using independent information on sex biases in migration. The Y chromosomes at the contact zone between subspecies

of rabbits in Spain, for instance, show a very sharp cline, despite evidence for male-biased migration (Geraldes *et al.* 2008). This suggests not only a role for Y-A incompatibilities, but also that selection is strong relative to migration, as no shift is observed in cline centers relative to the bulk of autosomal markers. Similarly, Y markers show a steeper cline than mtDNA markers at contact zones between subspecies of *Microtus arvalis,* despite evidence for a male-biased migration (Beysard *et al.* 2012, Sutter *et al.* 2013; Beysard & Heckel 2014). Along the same line, the occurrence of sharp clines for both X and Y chromosomes relative to autosomes (such as found in rabbits and mice) cannot result from sex differences in migration only, but rather point to DM incompatibilities involving both X and Y chromosomes.

Finally, systematic shifts in cline centers only occurred in our simulations under DM incompatibilities involving autosomal alleles, and were never generated by mere sex differences in migration. The caveat applies, however, that our simulations assumed symmetric demographic parameters for the two interacting populations, including the same sex-specific patterns of migration. Outcomes might differ if the interacting species also differ in sex-specific patterns of migration. Furthermore, our simulations assumed the same constant population size for the two populations, in which case the only consequences of sex biases in migration will be a slightly steeper X cline (in case of male-only migration), or strongly steeper Y cline (in case of female-only migration). Outcomes are also expected to differ under disequilibrium dynamics, where migration rates interact with sex-specific effective population size and demography to generate more complex and sometimes counterintuitive patterns. Asymmetries are particularly expected if contact zones result from a recent range expansion, since markers associated with the least migrating sex are expected to display more introgression from the local species into the genome of the colonizing species (Petit & Excoffier 2009). Further simulations would be required to clarify these effects, and outline ways of empirically disentangling the selective pressures linked to gene incompatibilities from neutral demographic effects.

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Figures and table

Table 1: Effects of the parameters *m* (migration), *r* (sex bias in migration) and *s* (coefficient of selection against hybrids with DMI) on cline widths and centers for autosomal and sex-linked markers under the four DMI scenarios, measured with Generalized Linear Mixed Models (GLMM). *P*: P-value; R^2 proportion of variance explained.

No incompatibility		**m**		**r**	
		p	**R^2**	***p***	**R^2**
N	width	**<0.001**	0.12	0.92	-
	center	0.47	-	0.37	-
X	width	**<0.001**	0.12	**0.004**	0.01
	center	0.97	-	0.69	-
Y	width	**<0.001**	0.06	**<0.001**	0.41
	center	0.14	-	0.75	-

X-Y incompatibility		**m**		**r**		**s**	
		p	**R^2**	***p***	**R^2**	***P***	**R^2**
N	width	**<0.001**	0.14	0.93	-	**0.02**	<0.01
	center	0.64	-	0.48	-	0.55	-
X	width	**<0.001**	0.36	**<0.001**	0.04	**<0.001**	0.32
	center	0.24	-	0.98	-	0.87	-
Y	width	**<0.001**	0.16	**<0.001**	0.4	**<0.001**	0.08
	center	0.34	-	0.96	-	0.91	-

X-A incompatibility		**m**		**r**		**s**	
		p	**R^2**	***p***	**R^2**	***P***	**R^2**
N	width	**<0.001**	0.19	0.15	-	0.07	-
	center	**<0.001**	<0.01	**0.008**	0.004	**<0.001**	<0.01
X	width	**<0.001**	0.18	**<0.001**	0.04	**<0.001**	0.18
	center	**<0.001**	0.41	**<0.001**	0.02	**<0.001**	0.23
Y	width	**<0.001**	0.07	**<0.001**	0.4	0.11	-
	center	**<0.001**	<0.01	**0.002**	<0.01	**<0.001**	<0.01
A	width	**<0.001**	0.34	0.55	-	**<0.001**	0.15
	center	**<0.001**	0.39	**<0.001**	0.02	**<0.001**	0.21

Y-A incompatibility		**m**		**r**		**s**	
		p	**R^2**	***p***	**R^2**	***P***	**R^2**
N	width	**<0.001**	0.14	0.28	-	0.27	-
	center	0.32	-	**0.0023**	<0.01	0.6	-
X	width	**<0.001**	0.14	**<0.001**	0.02	0.31	-
	center	0.17	-	0.43	-	0.61	-
Y	width	**<0.001**	0.1	**<0.001**	0.26	**<0.001**	0.09
	center	**<0.001**	0.08	**<0.001**	0.58	**<0.001**	0.04
A	width	**<0.001**	0.35	**0.01**	<0.01	**<0.001**	0.19
	center	**<0.001**	0.07	**<0.001**	0.59	**<0.001**	0.02

Figure 1. Possible scenario leading to the different DM incompatibilities modeled here. From an xxaa/xyaa ancestor (bold symbols), X-Y incompatibilities might arise if distinct X-linked meiotic drives invade the daughter lineages (respectively *x* and *X*; step 1), to be then repressed by lineage-specific Y elements (respectively *y* and *Y*, step 2). This will lead to a fitness drop in hybrid males if *Xy* or *xY* combinations reveal incompatible. X-A and Y-A incompatibilities may result if autosomal genes interacting with X or Y-linked genes fix different alleles in the two lineages (step 3), such that *Xa*, *xA*, *Ya* or *yA* combinations reveal incompatible. Note that incompatibilities involving Y occur only in males, while *Xa* and *xA* incompatibilities may also occur in females (see Table S1).

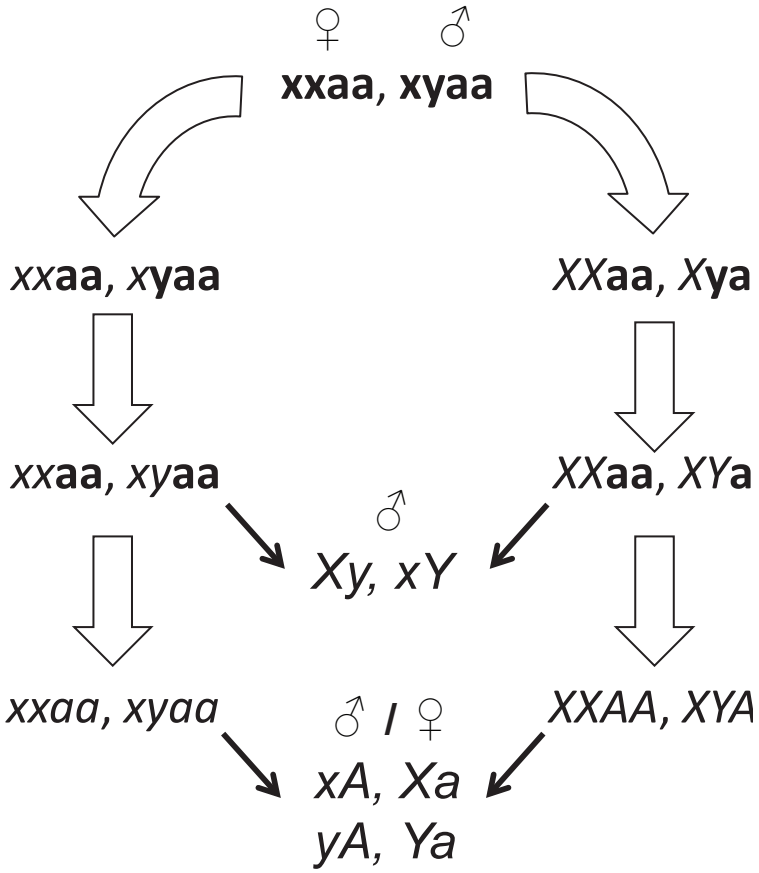


Figure 2: Fitted cline parameters under balance dispersal. Cline widths (y-axis) are plotted as a function of cline centers (x-axis) for different DMI scenarios (rows) and ratios of migration-to-selection (columns, increasing from right to left). Each symbol represents one simulation, with black circles for neutral autosomal markers, green squares for DMI-autosomal markers, red crosses for X markers and blue triangles for Y markers. Symbols for sex-linked markers are pale when neutral, and bright when involved in a DMI. The dotted vertical line marks the initial contact zone.

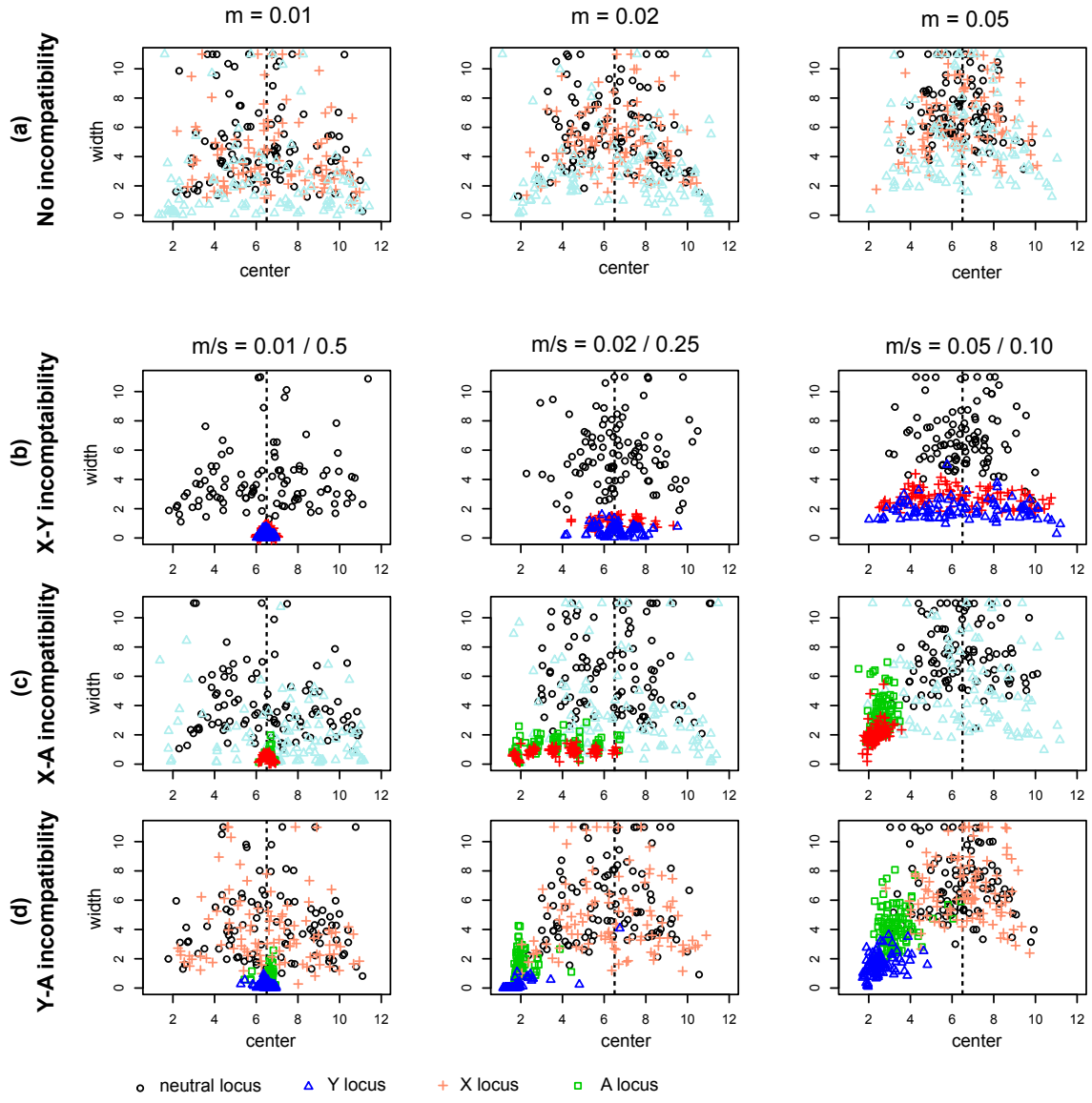
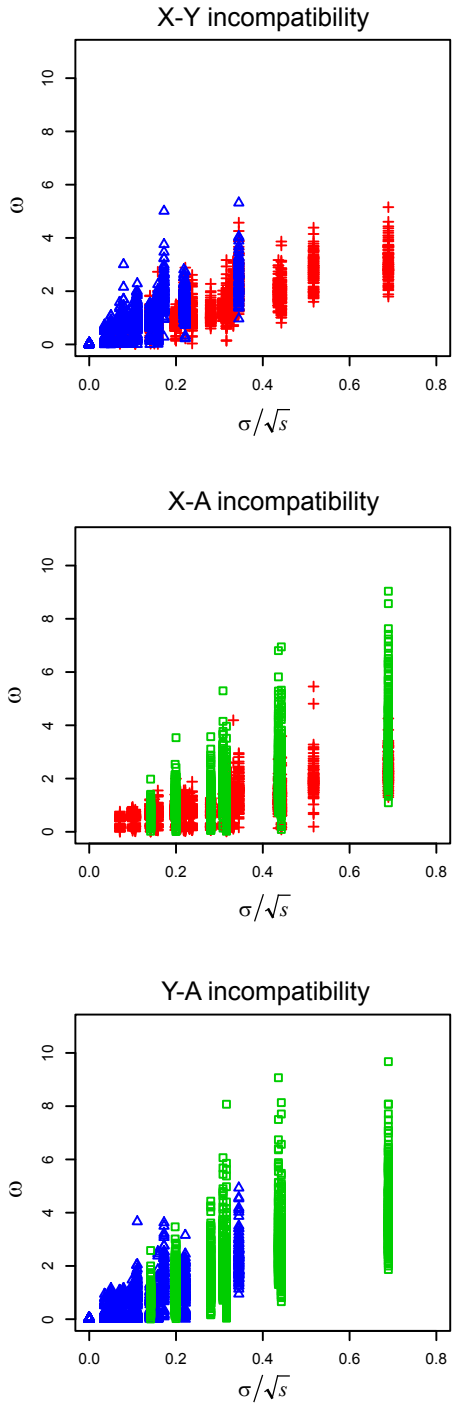


Figure 3: Cline width as a function of dispersal-to-selection ratio. As expected from eq. 1, cline width w increases proportionally to $s_i/\sqrt{s}$, where s_i measures the effective dispersal rate of marker i (accounting for the effect of sex-biased migration on sex-linked markers) and s the selection coefficient against hybrids. Green squares are for autosomal markers, red crosses for X markers and blue triangles for Y markers.



Supplementary Material

Fig S1: Effect of sex-biased dispersal on cline widths and centers. Values are only presented for intermediate values of dispersal and selection (m = 0.02, s = 0.25). Cline widths (y-axis) are plotted as a function of cline centers (x-axis) for the DMI scenarios (rows) and three proportions of male dispersal (columns). Each symbol represents one simulation, with black circles for neutral autosomal markers, green squares for DMI-autosomal markers, red crosses for X markers and blue triangles for Y markers. Symbols for sex-linked markers are pale when neutral, and bright when involved in a DMI.

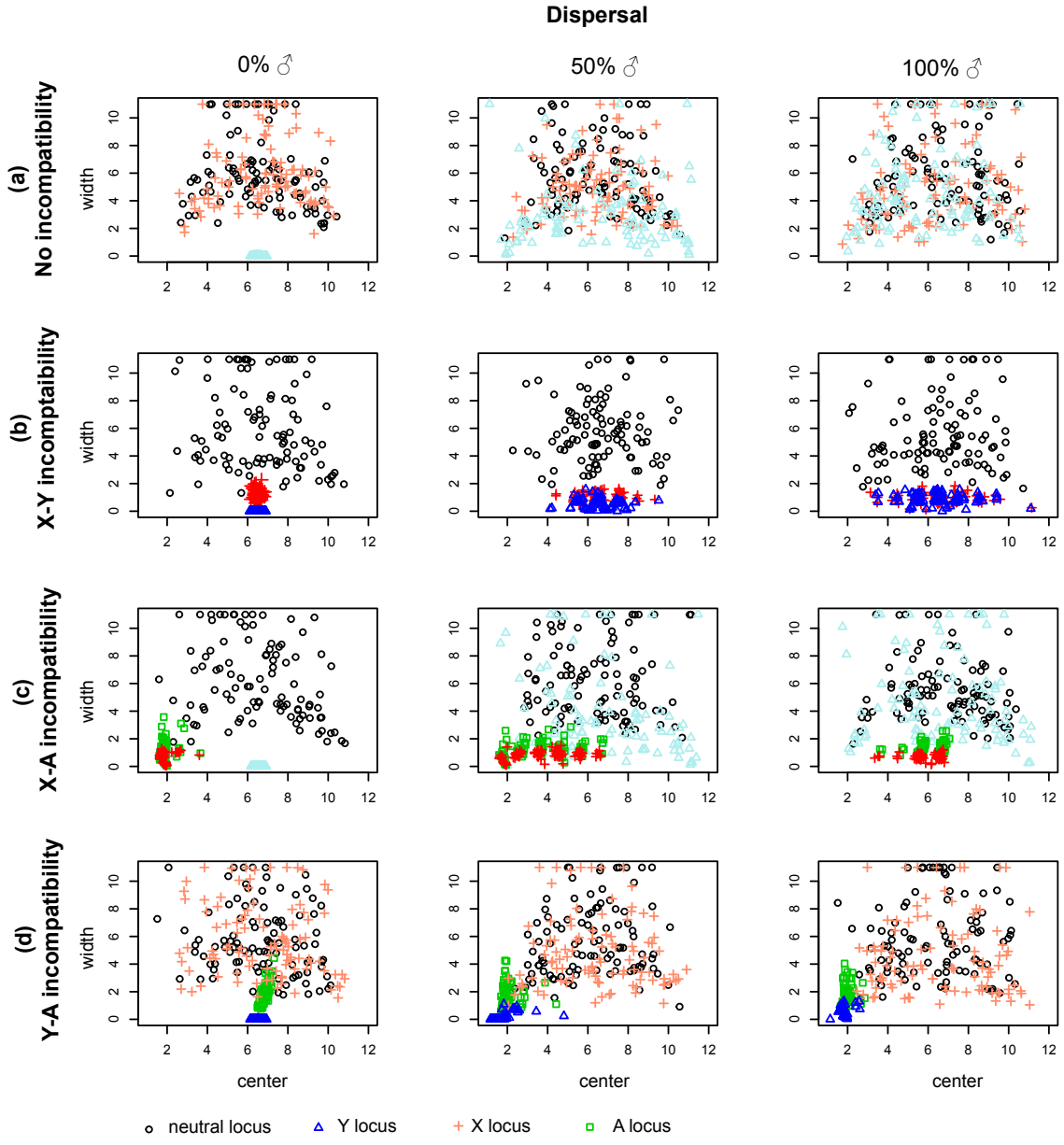


Fig. S2: Fitted clines for the different types of loci under balanced migration and representative parameter combination (corresponding to Figure 2); m = migration, s = selection. Grey: neutral locus; blue: Y locus; red: X locus; green: autosomal locus involved in DMI

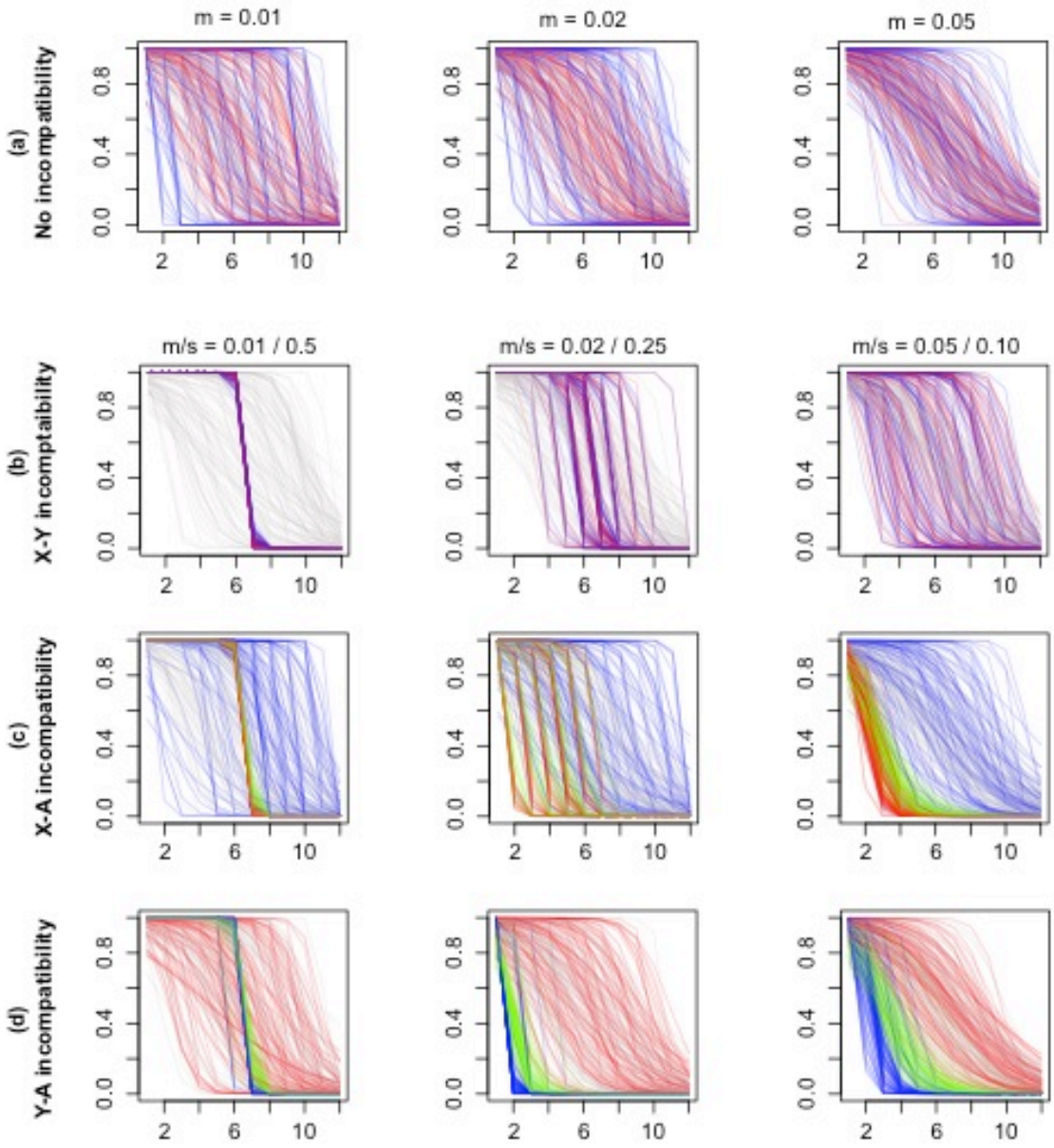


Table S1: Fitness values for all genotypes under the three DMI scenarios envisaged.

	X-Y	X-A	Y-A
xyaa	1	1	1
xyaA	1	1-s	1-s
xyAA	1	1-s	1-s
xYaa	1-s	1	1-s
xYaA	1-s	1-s	1
xYAA	1-s	1-s	1
Xyaa	1-s	1-s	1
XyaA	1-s	1	1-s
XyAA	1-s	1	1-s
XYaa	1	1-s	1-s
XYaA	1	1	1
XYAA	1	1	1
xxaa	1	1	1
xxaA	1	1-s	1
xxAA	1	1-s	1
xXaa	1	1-s	1
xXaA	1	1	1
xXAA	1	1	1
XXaa	1	1-s	1
XXaA	1	1	1
XXAA	1	1	1

General discussion

When sex chromosomes were identified as responsible for the determination of sex (in some species), hypotheses over their evolution started to be formulated. Heteromorphic sex chromosomes were the first to be identified, and therefore the first theories on sex chromosome evolution tended to explain such a pattern. The first time that homomorphic sex chromosomes were discussed was apparently in 1949, by Robert Matthey in his PhD thesis (Matthey 1949), also from the University of Lausanne. The first theories to explain the maintenance of homomorphic sex chromosomes are quite recent (Volff 2007, Perrin 2009), and they are supported by a growing amount of empirical evidence.

In my thesis I investigated the interaction among some of the main actors that play a role in the evolution of sex chromosomes. Sex-antagonistic genes have been assigned a major role since the first theories on the evolution of sex chromosomes, together with the recombination arrest in the heteromorphic sex, which causes the accumulation of deleterious mutations and the degeneration of the Y (or W) chromosome. Here I have considered the joint effect of sex-antagonistic genes, deleterious mutations and recombination in the evolution of sex chromosomes.

The evolution of sex chromosomes

In the first chapter of my thesis I analysed the evolution of sex-antagonistic genes and the accumulation of deleterious mutations under different fixed recombination rates in males. This allowed me to understand under which conditions an arrest of recombination would be selected. Recombination can be regulated by a modifier of recombination or halted by an inversion. While the first mechanism would allow a fine control of recombination rate, the second would cause an irreversible arrest of recombination. I found that when the accumulation of both deleterious mutations and sex-antagonistic alleles are considered, a complete arrest of recombination is never beneficial to males. This corresponds to the fixation of an inversion on the Y chromosome. Here, females always had higher fitness if male recombination was completely arrested, rather than present. However, when recombination was rare in males and sex-antagonistic selection weak, recombining males were

selected for, despite carrying the female beneficial sex-antagonistic allele. Purging deleterious mutations was as beneficial as carrying the male-beneficial allele in males, while females benefitted in this case of rare male recombination.

These results thus highlight the fact that in order to fully understand the evolution of sex chromosomes, the accumulation of deleterious mutations needs to be considered as part of the play, not just as a silent spectator. An inversion occurring on a chromosome with a male determining gene could increase in frequency if including a male-beneficial sex-antagonistic allele. However, deleterious mutation will start accumulating and the inversion might be counter-selected for and be lost in the population. Under these circumstances, sexual conflict would be better resolved with sex-biased gene expression of sex-antagonistic genes.

After having investigated the coevolution of sex-antagonistic genes and deleterious mutations under different rate of recombination, the logical next step was to allow for recombination to evolve. This was accomplished in the second chapter, where I explored the dynamics of sex-antagonistic genes and deleterious mutations with an evolving recombination rate, as well as the effects of the position of a recombination modifier. First of all, I show that both deleterious mutations and sex-antagonistic selection affect the evolution of recombination rate. Stronger sex-antagonistic selection selects for lower recombination rates in males, although this is never optimal for males, as shown in the first chapter. When sex-antagonistic selection was weak, different level of male recombination evolved, depending on the effect of deleterious mutations, confirming their importance in the process of sex-chromosome evolution. Why then an arrest of recombination evolved in males, despite the fact that it causes a reduction in male fitness? We hypothesized that selection in females might be the cause. As found in the first chapter, females always benefit from an arrest of recombination in males. When recombination was regulated by autosomal modifiers, the male and female modifiers spent the same amount of time in the two sexes. Because the benefits to females from an arrest of XY recombination are higher than the costs to males, an arrest of recombination was favoured.

This becomes clear when linking the recombination modifiers to the sex-determining gene. In this scenario, male recombination evolved to higher rates than when the modifier was unlinked. A

detailed analysis of the genotypes at the male recombination modifier revealed a very interesting pattern. On the Y chromosome there was strong selection for alleles for recombination, while on the X chromosome alleles for zero or very low recombination were selected for. It is important to remember that in this model the modifier can mutate, therefore there cannot be fixation of one allele. However, the allele distribution highly diverged between simulations with an autosomal or a sex-linked modifier. In the former, the allele for no recombination was the most frequent, and some small alleles were at a low frequency due to the mutation model. This was further confirmed by the fixation of the allele for no recombination when mutation was not allowed. When the modifier was sex linked, however, there was clearly selection for alleles for recombination on the Y, while the pattern on the X was similar to the unlinked modifier. Recombination in males behaves like a sex-antagonistic trait, because it is beneficial to males but detrimental to females.

These results match the finding of Grossen et al. (2012), where recombination was allowed through sex-reversal. In their paper, the authors allowed the amount of sex reversal to evolve, and this was controlled by the sex locus. Despite the differences in the two models, a control over XY recombination mediated by a sex-linked factor leads to a low but non-zero XY recombination rate in both cases. Our results partially match those of analytical models, with XY recombination evolving towards zero in presence of only sexually antagonistic selection. However, when deleterious mutations are also modelled, we found selection for maintenance of recombination, and more strongly so if the modifier was sex-linked. Analytical models have shown that an increase in recombination is never favoured when modifiers of recombination are closely linked to the sex-determining region (Otto 2014, Scott and Otto 2017), but in those models deleterious mutations were not considered. Our results show that in presence of both sexually antagonistic selection and deleterious mutations, a recombination modifier that is sex-linked can evolve to higher recombination rates in males. However, our result finds support in the one of Otto and Barton (1997), who showed that the effect of a modifier for recombination increasing the probability of fixation of favourable alleles is maximal when it is linked to the loci under selection. In our case we found that a modifier that increases recombination, when tightly linked, fixes more often because it remains linked to the Y long enough to gain the purging benefits.

To summarize, we found that under similar selective forces, a sex-linked modifier can (selectively) maintain non-zero recombination rates in the heterogametic sex, while an autosomal modifier would always converge to zero recombination.

The strong difference observed in the evolved recombination rate in males when a modifier is autosomal or sex-linked begs the question of how recombination is regulated in the genome. Recombination modifiers are widespread in the genome. In mammals, recombination hotspots are determined by the protein PRDM9. PRDM9 binds to specific sequences in the genome, and this starts recombination at a specific location. Although recombination hotspots are nonrandomly distributed in mammals (Kauppi et al. 2004), the target sequences of PRDM9 have high mutation rates and can evolve rapidly. Moreover, the activity of a hotspot (and therefore the recombination rate of a region of the genome) can be controlled by distant regions of the genome, as found by Shiroshi et al. (1991) in mice. Contrasting with recombination modifiers, an inversion fixing on a sex chromosome would inevitably cause the arrest of recombination in that region of the genome in the heterogametic sex. However, for such inversions to be fixed there is no need of sex-antagonistic genes, as inversions can be fixed by genetic drift alone (Ironside 2010). This weakens the importance of sex-antagonistic genes in causing the arrest of recombination and the consequent degeneration of sex chromosomes. It would be interesting then to model the invasion of an inversion occurring on sex chromosomes and calculate its fixation rate when "competing" with a recombination modifier that can reduce recombination in the heterogametic sex. Implementing such a model under different selective pressures might help to understand if some conditions might favour an inversion over a reduced but non-zero recombination rate.

Sex determination transitions

The results from these first two models focused on the effect of recombination on the maintenance of homomorphic sex chromosomes. In some species, the presence of such chromosomes is due to the high rate of turnovers, while in others by a combination of rare recombination and turnover events (Kitano and Peichel 2012, Dufresnes et al., 2015). High rates of turnovers have been

documented in fishes and in amphibians, where closely related species have adopted different chromosomes as sex chromosomes (Miura 2007, Tanaka et al. 2007).

Turnovers can result in a transition to a different sex determining system. In the frog *Rana rugosa*, both XY and ZW systems are found (Miura 2007). In some fish species, both hermaphroditism and genetic sex-determination can occur in the same species (Mank et al. 2006, Mank and Avise 2009). Interestingly, in this case the genetic sex determination system seems to be the ancestral state, from which hermaphroditism evolved, as it seems to have happened in plants (Charlesworth and Charlesworth 1978). In the common frog *R. temporaria* a very particular situation has been documented. In Swiss lowland populations, individuals have only one type of sex chromosome. They are all "XX" and generating both males and females. Populations at higher altitudes show a mixture of "XX" individuals and different Y haplotypes. It is still not known if the "X" chromosome producing both males and females is the same in lowland and high-altitude populations. Moreover, the different Y haplotypes are present in a different proportion of males, suggesting different levels of masculinization of those. This raised the question about the equilibrium of these multiple haplotypes and sex-determination systems, and which selective processes act to maintain it. Orzack et al. (1980) and Blaser et al. (2011) have shown that a stable polymorphism between multiple sex-determining gene (X, Y, W) can be maintained under certain strength of sex-antagonistic selection and when recombination is low or absent. In order to expand the understanding of polymorphism in amphibians (and apply the results to a "frog-like" population), in the third chapter different levels of genetic sex-determination, non-genetic sex determination, and different ways of controlling recombination were implemented. With this model it was shown that when an allele for non-genetic sex determination is introduced in a population with an XY system, a polymorphism can be maintained between the three haplotypes. This stable equilibrium is maintained when there is strong sex-antagonistic selection in males, and when the Y chromosome is associated with an inversion. This polymorphism reminds us of the situation found in fish, and to some extent to the situation found in the common frog (Mank et al. 2006, Mank and Avise 2009, Rodrigues et al. 2017, 2018). Although it has been shown that sex-antagonistic variation can promote turnover events

(homogametic or heterogametic, van Doorn and Kirkpatrick 2007, 2010), an equilibrium with a non-genetic sex-determination system was never found.

In chapter three, a multiple Y-haplotype equilibrium was not found, but instead one of the two Y alleles was always lost. Moreover, a completely-dominant Y was able to invade a system with a non-completely dominant Y at a higher rate than the reverse. This does not reflect the situation found in the common frog, where multiple Y-haplotypes coexist in the same population. However, in the model we considered sex-antagonistic variation as a driving force. Implementing deleterious mutations would likely shift this equilibrium, because the non-completely dominant Y would produce males with a lower deleterious load. It would therefore be interesting to further investigate this, in a model that considers both sex-antagonistic genes and deleterious mutations. Investigating sex chromosomes turnovers, Blaser et al (2013) found that mutational load can be a driving force of those events, showing also that the neo-sex chromosome continued to recombine after its establishment. In the model implemented in the third chapter of this thesis, recombination in males was restricted, but allowed in sex-reversed females (when recombination depended on the phenotypic sex). Therefore, allowing for the accumulation of deleterious mutation, there would be an advantage for the non-completely dominant Y. Whether the two Y haplotypes would coexist in equilibrium or one would establish over the other, will probably depend on the different forces at stake. In this context, it is meaningful to recall the results from Grossen et al. (2012). In their model, recombination depended on phenotypic sex (with recombination not possible in males). The "amount of sex reversal" was controlled by the sex locus, and it could mutate, and both deleterious mutations and sex antagonistic selection were considered. Interestingly, they found evolution on the Y chromosome towards alleles allowing for more sex reversal (and therefore more XY recombination) in the presence of mildly deleterious mutations. The increased rate of XY recombination allowed the purging of deleterious mutations on the Y. However, certain conditions might favour recombining and non recombining Y at a similar level. Investigating whether multiple Y haplotypes could be maintained at the same time was not the purpose of the study. Introducing deleterious mutations in our model could then help to verify if multiple Y-haplotypes with different masculinizing effects could be maintained at the equilibrium.

The role of sex chromosomes in speciation

Finally, I have investigated the effect of different Dobzhansky-Muller incompatibilities on the introgression pattern of sex-linked and autosomal alleles. Sex chromosomes play an important role in the speciation process, as allowing for lower permeability when they carry incompatibility genes. Different Dobzhansky-Muller incompatibilities generate different widths of the cline of introgression and different positions of the cline center, and this can depend on the demographic pattern of invasion or on the type of incompatibility itself (X-autosomal, Y-autosomal or X-Y). Interestingly, X-autosomal and Y-autosomal incompatibilities result in the invasion of the dominant allele into the domain of the recessive allele, when dispersal is high relative to selection. This pattern is due to the dominance model of the incompatibility, and not to the benefits carried by the invading allele. Therefore, observed patterns of introgression of X or Y of one species into another might stem from the dominance of one allele over another. These results can help understand pattern of invasion at a contact zone. For example, at the contact zone between two subspecies of rabbits in Spain, the Y chromosome shows a very sharp cline despite male-biased dispersal, and both X and Y chromosomes have sharper clines than autosomes (Geraldes et al. 2008). These patterns suggest that both X and Y are involved in Dobzhansky-Muller incompatibilities and that selection is strong relative to dispersal. Simulation studies can help to disentangle between different factors that shape the genes introgression at contact zones. Moreover, simulations can reveal effects that might not be easily acquired empirically. For example, incompatibility between a sex-linked and an autosomal gene resulting in a unidirectional invasion also show a more pronounced shift for the sex chromosome than for the autosome. This result has never been documented so far, but this might be due to the difficulty of isolating it from the stochastic noise. Being aware of such patterns might still be useful, however, to understand the dynamics of introgression under different Dobzhansky-Muller incompatibilities. The model of this study considered the same population size between the two populations, as well as the same pattern of dispersal. An expansion of the model, including such variations, will improve our understanding of the introgression dynamic and further clarify which selective pressures are in act in natural populations at secondary contact.

In summary, during my PhD I have investigated the effects of XY recombination on the equilibrium frequencies of sex-antagonistic alleles and deleterious mutations. The accumulation of both has an impact on the fitness of males and females, suggesting that a complete arrest of male recombination is rarely beneficial. Furthermore, I have modelled the evolution of a recombination modifier, when linked or unlinked to the sex chromosomes. The striking difference between recombination rates reached in males when the modifier was linked or unlinked suggests strong effect of females on male recombination. Moreover, I found that a polymorphism between a genetic and a non-genetic sex determining system can be stable under strong sex-antagonistic selection. Additional simulation studies that take into account the accumulation of deleterious mutations on non-recombining sex chromosomes might help to understand the occurrence of multiple Y-haplotypes with different masculinizing effects. Finally, I showed how patterns of Dobzhansky-Muller incompatibilities and demography can result in different introgression dynamics of sex-linked and autosomal genes. These results can help to interpret empirical data on introgression at the contact zone between (sub)species.

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