A single theory for the evolution of sex chromosomes and the two rules of speciation

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Short title: Regulatory evolution can explain both sex chromosome evolution and the two rules of speciation

Abstract

Three major empirical patterns involving sex chromosomes have been observed in higher eukaryotes: Y (or W) chromosomes are often non-recombining and degenerate; when two species hybridize, but one sex is sterile or inviable among hybrid offspring, it is most often the heterogametic sex (XY or ZW)—the so-called Haldane's rule; and the X (or Z) plays a disproportionately large effect on reproductive isolation compared to autosomes—the so-called large X effect. Each observation has generally received its own tailored explanation involving multiple genetic and evolutionary causes[1–3]. Here, we show that these empirical patterns all emerge from a single theory for sex chromosome evolution incorporating the co-evolution of cis and trans-acting regulators of gene expression, and leading to systematic and rapid misexpression of dosage-compensated genes in the heterogametic F1 hybrids, for young or old sex-chromosomes. This theory offers a level of parsimony and generality rarely seen in biology.

Main text

Sex chromosomes play a prominent role in the process of speciation. This is captured in the "two rules of speciation" [4]. The first, 'Haldane's rule' (HR), is named after the British scientist who observed that it was more frequently the heterogametic sex that suffered more in hybrids [5]. Over a century of work has confirmed the generality of this observation in nature [6–10]. Additionally, there is often an asymmetry of the effect observed between reciprocal crosses [11]. The second, the 'Large X effect' (LX), refers to the observation that X chromosomes (and Z chromosomes in ZW species) disproportionately affect hybrid incompatibilities more than autosomes of equivalent size [4,12–15]. These rules, among the few law-like generalizations in biology, have been extensively tested and studied and the subject of intense theoretical investigation to understand their origin [6,8–10,16,17].

Presently, the consensus is that HR and LX are composite phenomena with multiple genetic and evolutionary causes [1–3,6,8–10,16–19]. While some theories have received more support than others, none

offers a general solution—each failing to account for some observations. For example, a prominent explanation for the two rules is the Dominance theory, first suggested by Muller [20] and later formalized [21,18,22], based on the idea that genetic incompatibilities involving at least one gene located on the X chromosome (or Z, in species with female heterogamety) may more strongly affect the fitness of the heterogametic sex if these incompatibilities are on average partially recessive. However, the theory does not explain why incompatibilities should be, on average, recessive [21,23], although fitness landscape models propose possible solutions [24–26]. Furthermore, it does not explain well why HR often involves sterility rather than viability [2,27], why it is observed in groups lacking a hemizygous X [3], or in groups where XX females only express one X, as in marsupials [28] or placentals (although in the latter case, both Xs may be expressed at the level of tissues [18]). Other theories better accounting for the importance of hybrid sterility in HR have their own major limitations. The "faster male theory" explains well why male sterility often occurs in hybrid crosses [2,18,27] and why it may occur in species lacking hemizygosity [3], but critically fails to account for HR in species where females are the heterogametic sex [16–18]. The "meiotic drive theory" explains well why sex chromosomes could play a major role in the sterility of heterogametic hybrids [29–31]. It has received some empirical support [31–33] but does not offer a convincing explanation for HR for viability [12,16]. Lastly, while sex chromosome degeneration is another globally observed and intensely studied phenomenon, none of these theories consider the processes leading to degenerate sex chromosomes and their subsequent evolution to be related to the emergence of either HR or LX (Table 1).

We recently proposed a new theory for the evolution of non-recombining, degenerated, and dosage-compensated sex chromosomes based on XY regulatory divergence and the early emergence of dosage compensation (DC) [34–36]. We show here that the same theory also predicts Haldane's rule and the large X effect. In the present study, we followed the simulated independent evolution of 20 species using this previously described model [34] (Sup. Mat 1, Fig S1). It consists of individual-based stochastic simulations of a population of diploid individuals, with XY males and XX females (all the arguments below also apply to ZZ / ZW systems and large Z effect, but for simplicity we only discuss the XY case), incorporating deleterious mutations occurring at many genes, the evolution of recombination and the evolution of cis and trans regulation of gene expression [34]. We estimated the rate of occurrence and the pattern of hybrid incompatibilities by measuring the fitness of F1 hybrids among the species at different time steps, under scenarios of sex chromosomes at different stages of their evolution (see Methods).

Figure 1 shows the decrease in fitness in male and female F1 hybrids relative to the fitness of male and female offspring from within species crosses. HR is rapidly observed in all cases, with strong asymmetries between the fitness of male and female F1 hybrids. LX also occurs in all cases compared to autosomes of equivalent size (compare Fig 1A to Fig 1B-E). Genes with an effect limited to the heterogametic sex (i.e., involved in fertility rather than viability) also contribute to HR and LX, and in many cases, very strongly (Fig 1E). In Sup. Mat. 4, we show that asymmetries between reciprocal crosses often occur, as observed in "Darwin's corollary" to HR [11] (Fig S2, S3). In this model, autosomes also contribute to hybrid breakdown, but less strongly and without generating an asymmetry between the homo and heterogametic sex (Fig 1A). This outcome results from the coevolution of cis- and trans-regulators of gene expression, and their divergence among species (Fig S4), which has repeatedly been emphasized as a mechanism generating hybrid incompatibilities [37–47].

In this model, regulators on sex chromosomes evolve rapidly due to recombination arrest, chromosomal degeneration and the emergence of dosage compensation [34]. As a consequence, differences in regulatory traits can rapidly evolve between species and cause dysfunctional regulation of dosage compensated genes in hybrids. But why would dysfunctional regulation disproportionately

impact the heterogametic sex (HR) and cause a large X effect? For the latter, the answer is straightforward: if dysfunctional regulation of dosage compensated genes inordinately reduces hybrid fitness in the heterogametic sex, then the X chromosome, which is the only chromosome evolving DC, will necessarily have a disproportionate effect on hybrid fitness compared to autosomes (both in terms of the number and impact of genes involved in hybrid incompatibilities). Regarding HR, the disruption of the regulation of dosage compensated genes can be caused by the portion of the Y that is degenerate and compensated in only one of the two hybridizing species (Fig 1B, 1C) or by the portion of the Y that is degenerate and compensated in both species (Fig 1C, 1D), provided they exhibit some regulatory divergence. We detail each case in turn.

First, consider the case where species *a* evolves a new non-recombining stratum on the Y not present in species *b*. If a gene *g* is degenerate and compensated in species *a* but not in species *b*, then hybrid males will suffer from under-expression if they are $g_a^Y g_b^X$, and overexpression if they are $g_a^X g_b^Y$, assuming codominant effects of the trans-acting factors. In the first case, these hybrids miss half the trans-acting factor required to fully achieve DC with a degenerated g_a^Y copy. In the second case, the hybrids inherit trans-regulators from species *a* increasing expression of gene *g*, despite having two functional copies of the *g* gene. In all cases, however, expression is closer to the optimum in females, regardless of the direction of the cross. This applies to the case where DC evolves by doubling expression of the gene *g* in males through a male-specific trans-acting factor (as with the drosophila DC mechanism, Fig S7a) or where X chromosomes have double expression, one being randomly silenced in females (as in the mammalian DC mechanism, Fig S7b). This argument applies more generally when evaluated quantitatively in a model encompassing these cases and their intermediates (see Sup. Mat. 2). The male/female fitness ratio is decreased proportionally to the number of genes on the stratum, the intensity of stabilizing selection on gene expression, and a constant that depends on the DC mechanism. The effect described here is similar to the verbal argument of Filatov [48], although clarifying the role of regulators.

Consider now the case of two species that inherited from their common ancestor a portion of the Y that is non-recombining, degenerate, and dosage-compensated. In this case, misexpression of dosage compensated genes continues to cause a major fitness decrease in the heterogametic F1. In this scenario, the fitness decrease occurs when the regulatory traits, for a focal dosage compensated gene, have diverged between the two species. In F1 hybrids, these compensations will average out in females (who receive half the autosomal trans-acting factors and half the X cis-acting factors from each species) but not in males, who will have cis-acting factors on the X from a single species, but averaged trans-acting factors on autosomes (Fig S8). Males will consistently strongly under or overexpress compared to females. A quantitative model shows that the reduction in male/female F1 fitness is proportional to the number of genes on the stratum, the intensity of stabilizing selection on expression levels, and the between-species variance in the trait governing DC (see Sup. Mat. 2). This difference in averaging between sexes is similar to the mechanism generating HR in models of quantitative traits coded by multiple loci with additive effects [25,26]. However, while these previous models may be seen as a particular case of the dominance theory (as they assumed a mutation on the X has the same effect when hemizygous in males and when homozygous in females), this is not the case in our model, since a mutation on a cis-regulator on the X does not interact with the same trans-regulators in males and females (see Sup. Mat. 3 and Fig S11).

Sex-limited fertility genes can also contribute to the decrease in hybrid fitness of the heterogametic sex, with some interesting specificities. Female-limited genes on the X retain a standard diploid expression. The coevolution of their cis and trans regulators can slowly diverge between species, but this effect is relatively weak and comparable to what is observed on autosomes (see Sup. Mat. 2). The

effect is much larger for male-limited genes, and even larger than for genes expressed in both sexes (Fig 1E). We also need to distinguish between cases where male-limited genes are present on a recently derived non-recombining portion of the Y in one species or are ancestral to both. In the first case, gene expression divergence between X and Y-linked copies of genes present in the new stratum will generate a fitness cost for male hybrids in a similar way as for the non-sex-limited case (Fig S7a). However, a difference with this non-sex-limited case is that either the X or the Y-linked copy may be silenced (while extinction of the X copy could not occur for genes expressed and required in both sexes). In the second case, male-limited genes may already have been silenced (either on the X or Y chromosome) before the split between the two species, in which case the divergence of their regulatory traits between species will generate a fitness cost for F1 males (Fig S9a). However, genes retaining diploid expression at the time of the split may strongly reduce the fitness of F1 males. Indeed, if the X-linked copy is silenced in one species while the Y-linked copy is silenced in the other, F1 males will either show a complete lack of expression or major overexpression of that gene (Fig S9b). A single essential gene in this situation could therefore cause male sterility in the heterogametic F1.

Hence, HR, its corollary for reciprocal crosses (Sup. Mat. 4), and LX could be caused by misregulation of sex-linked gene expression in hybrids. Although the idea of DC disruption has been repeatedly discussed [4,6,10,19,48–52], and is close to the initial suggestion of Muller [53], it has never been quantitatively formalized. While most authors have been cautious about completely rejecting this hypothesis, it has been usually dismissed as an important explanation [8,9,16] based on various arguments that we list and critically evaluate in Sup. Mat 5. Most of the 13 criticisms we list seemed either questionable or outdated (for instance, the idea that ZZ/ZW systems do not have DC while exhibiting HR) or focused on similar but narrower ideas (e.g. based on the direct disruption of the DC pathway). However, two arguments remain relatively strong and require scrutiny.

The first states that in species with an ancestral 'global' (i.e., chromosome-wide) DC mechanism, there may be little scope for divergence in DC, for any focal gene, in recently diverged species. This would make a theory based on diverging DC less effective, especially in pairs of species where the same portion of the Y is non-recombining and where the cis-regulators involved in global DC do not seem to have diverged. However, DC disruption for a focal gene may still occur if target cis-regulatory sequences do not change but move location on the X (in systems like *Drosophila* with high-affinity sites 'HAS' targeted by the MSL complex [54]) or if some genes escape the global DC mechanism. Current evidence indicates that chromosome-wide DC is the exception, not the norm, and even in these cases, many genes may escape global DC [55–58]. As our theory shows, these genes should be scrutinized for their role in decreased hybrid fitness. Furthermore, global somatic DC is often absent from the germline, where some DC nevertheless occurs for some genes [59–63]. This is observed independently of the mechanism of meiotic sex chromosome inactivation (MSCI), which might have specifically evolved to control sex-chromosome meiotic drive during early meiosis [64,65], and disruption of which has also been suggested to contribute to HR [13,66–68]. This lack of a global DC mechanism for germline-limited genes in the heterogametic sex could explain why male fertility genes are major contributors to HR. The fact that male-limited genes can degenerate on the X and remain on the Y could also be a potent factor preventing somatic DC from being "used" in the germline: upregulating the X is certainly not fitting compensation for Y-linked genes expressed in the male germline. Interestingly, this would predict that HR is most often based on hybrid sterility in groups with global DC, while more often based on viability in groups lacking it — species evolving a global somatic DC mechanism will maintain local DC only for sex-limited fertility genes, while species without global somatic DC will have many viability genes with local DC. Indeed, birds and butterflies lacking global somatic DC [69] show more cases of HR through inviability compared to mammals and Diptera [70].

The second argument concerns species with recombining sex chromosomes. In species without hemizygosity, it has been shown that HR is weaker but present compared to species with a more degenerated Y. This is the case, for example, in *Aedes* compared to *Anopheles* mosquitoes [3]. This example has been used to support the "faster male" theory and to rule out the dominance theory alone could explain HR [3]. The same argument would also argue against the theory described here. However, as recent evidence points out, in *Ae*. *aegyptii* the sex "locus" is a 1.5 Mb region with 30 genes in a 100 Mb non-recombining region encompassing the centromere of chromosome 1 and showing some divergence between males and females [71–73]. Hence, these species may not entirely lack hemizygosity. Like above, genes in this region should be scrutinized for their role in decreased hybrid fitness. The other argument favoring the "faster male" theory is the overrepresentation of male sterility for HR, which is not a pattern directly following the dominance theory [2,27]. However, our results also show that fertility genes can play a disproportionate role on HR, without invoking a "faster male" effect.

Beyond the faster male theory, the most strongly supported explanation for HR is the dominance theory. Support for the dominance theory's predictions comes particularly from experiments in *Drosophila* involving unbalanced females with attached X chromosomes. However, our theory makes similar predictions regarding these crosses (see Sup. Mat. 2.3), meaning they cannot discriminate between theories. How do the dominance and our theories compare beyond this? In marsupials and placentals, dosage compensation works by inactivating one X (the paternal or a random one, respectively). This is a difficulty for the dominance theory, as females are effectively hemizygous (like males) in these cases. This is not a major concern in our theory, as HR is likely to result from divergence in the global DC mechanism or from genes escaping global DC, whose regulation can more easily diverge between species (including genes only expressed in the male germline). In the dominance theory, recessive incompatibilities occurring on those genes escaping global DC may also contribute to HR. However, if such genes are rare, this contribution would be negligible: the vast majority of incompatibilities will concern genes subject to global somatic DC, so the fitness reduction in males and females will tend to be similar. A second point relates to the underlying mechanism. Contrary to the dominance theory, which simply poses that a fraction of genetic incompatibilities are only expressed when one of the underlying loci is homo- or hemizygous (without providing a biological mechanism that would generate this type of interaction), our theory is based on a biological model of cis and trans regulator evolution, with underlying additive traits (see Sup. Mat. 1). Furthermore, direct empirical evidence is accumulating showing DC disruption causes hybrid fitness reduction. For instance, in *Drosophila,* the key elements for DC—the MSL complex and the MSL-binding sites on the X—are fast evolving [50,51,74] as are *cis* and *trans* regulators of X expression [75]. Indeed, Y-degeneration and DC appear sufficiently rapid and species-specific in related species with young gene-rich sex chromosomes for the DC theory to work [48]. Evidence is also accumulating linking the misregulation of sex chromosomes to HR in various hybrids [13,68,76–83].

Overall, and perhaps more importantly, HR has been viewed as a composite phenomenon, produced by different causes [1,2,2–4,6,8–10,16–19], possibly distinct from the large X effect [13,18]. As Coyne once wrote about HR and LX, "biology is not physics" [17], emphasizing that given the complexity of biological systems, unifying theories are unlikely. We suggest this conclusion should perhaps be re-evaluated given the explanatory power of the theory presented here (Table 1). We show that a single model not only explains (1) recombination arrest on sex chromosomes, (2) degeneration of the Y, (3) the evolution of dosage compensation, as we previously showed [34,35], but also why, upon hybridization, (4) the heterogametic sex suffers more than the homogametic one, and (5) the X plays a disproportionate role in speciation. It may also explain why (6) HR is often asymmetrical between reciprocal crosses, (7) HR often involves fertility genes, (8) somatic and germline DC often differ, and why, (9) misregulation

of gene expression on autosomes should follow HR in time and eventually lead to complete reproductive isolation at a later stage of speciation. This degree of generality and parsimony has few equivalents in biology and deserves to be tested empirically.

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Supplementary material.

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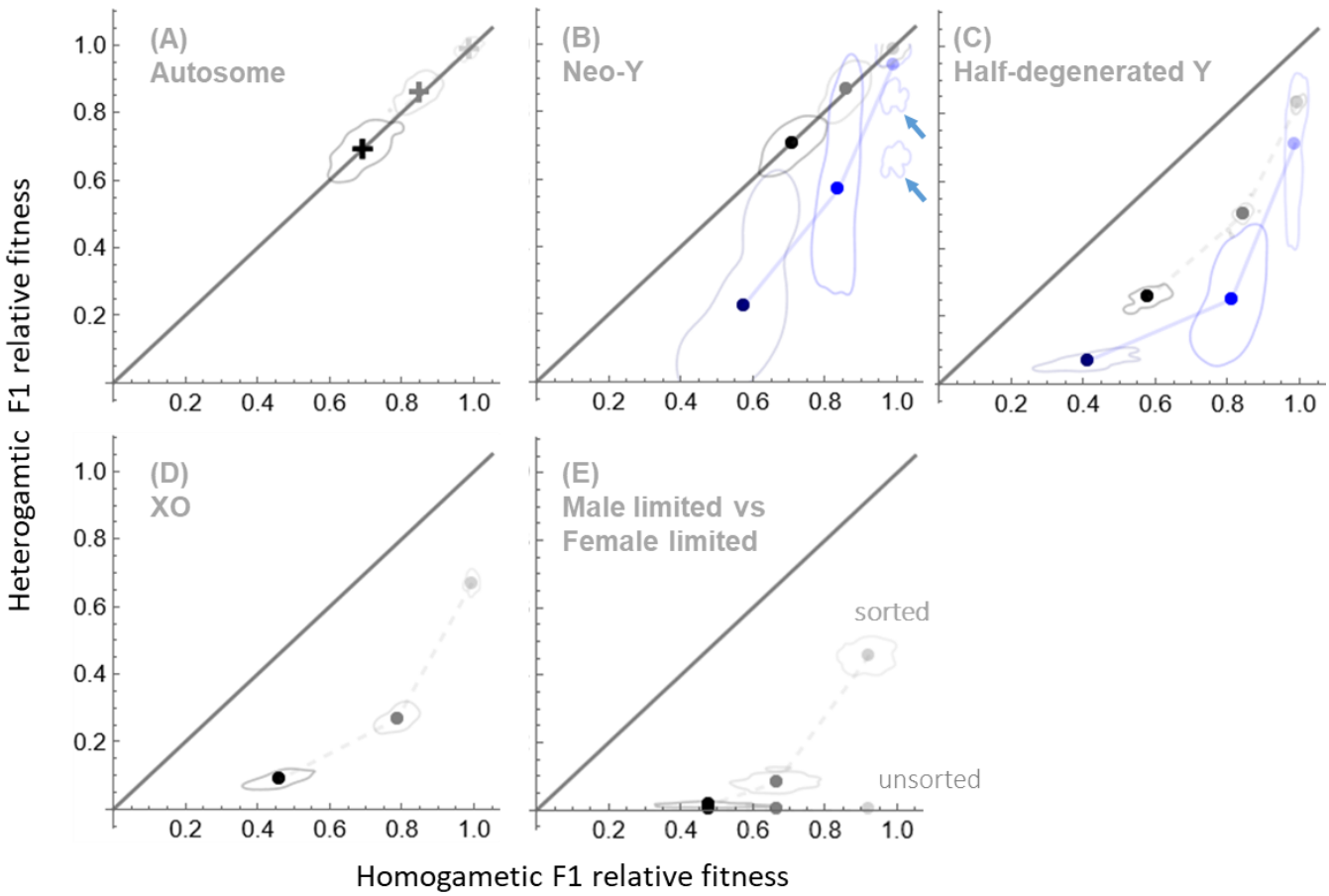


Fig. 1. Hybrid fitness in crosses between Independently evolving species. The figure shows the fitness of homogametic (x-axis) and heterogametic F1 (y-axis), between species that have evolved independently for $2.5x10^5$, 10^6 or $4x10^6$ generations (light, medium, and dark color on each panel, respectively). The data are obtained in each case using 15 independently evolving species and the 105 possible hybrid crosses (averaged in the two directions FxM and MxF for each of them). The fitness of hybrids is computed relative to the average fitness of intraspecific crosses for male and female offspring, respectively. Dots (or crosses for panel A) are mean values for all replicates. Contours represent the areas containing the individual values (the envelope is computed with a smoothed Gaussian kernel, see Fig S5 for an example). The x=y line is added for visualization of the effects. HR corresponds to cases where points fall below this line (the fitness of the heterogametic sex is lower than that of the homogametic sex in F1 hybrids. LX corresponds to a larger effect in panels B-E than the one shown on panel A for autosomes. (**A**) Autosomal case. One fully recombining autosome (with 500 genes, their cis-regulators, and their male and female trans-regulators) evolves in each species (even though all loci are autosomal, the simulation has males and females drawn randomly). (**B**) Neo-Y case. One initially fully recombining XY pair (with 500 genes, their cis-regulators, and their male and female trans-regulators) evolves in each species. In gray: inversions do not occur, so that the XY remains fully recombining throughout. In blue, inversions (and reversions) occur and recombination progressively stops between the X and the Y in each species independently (Fig S6 shows the progression of recombination arrest in the different replicates). After $2.5x10^5$ generations, only two species evolved a non-recombining stratum on the Y, and all hybrids involving those species had a lower fitness in the heterogametic sex (those points form two clouds of points, whose contours are indicated by the blue arrows, see also Fig S5). (**C**) Half degenerate case. As in (B) except that the Y is initially non-recombining, fully degenerate, and dosage compensated on half of its length, with $t_m = 2$ (t_m is the trait value for trans-acting factors expressed in males). Note that regulatory divergence can

accumulate initially for a larger number of dosage-compensated genes (initially 250), explaining why the fitness reduction on hybrids is larger for the same time period than in (B), where the simulations start with no dosage compensated gene; (**D**) As in (B) except that the Y is initially non-recombining, fully degenerate, and dosage compensated on all its length (which is equivalent to a XX/XO system), with t_m = 2. In this case, there are no simulations where inversions evolve since the Y is initially already fully non-recombining. Here too, regulatory divergence can accumulate initially for a larger number of dosage-compensated genes (500) than in (B) and (C), explaining the larger fitness reduction in hybrids. (**E**) As in (D) except that the XY pair contains either only female-limited genes (x-axis) or only male-limited genes (y-axis). These simulations are performed independently and are represented on the same graph for better comparison with other cases (i.e. when a simulation runs with only male-limited genes, the fitness of females stays at 1 throughout, and when a simulation runs with only female-limited genes, the fitness of males stays at 1 throughout, however the effect on females and males can be represented as paired). For male-limited genes, two initial conditions are considered: (1) they are initially non-degenerate and with fair diploid expression. During the course of these simulations, each male-limited gene will degenerate and become silenced on either the X or the Y, independently in different populations. We term this initial condition the "unsorted" case since it is initially not decided which gene will be lost on the Y or on the X. Results show that c.a. 90% of genes end up being degenerate on the Y (and 10% on the X), and the fitness of the heterogametic F1 drops very quickly toward a value close to zero. (2) In the second initial condition we suppose that 50% of the genes are degenerate on the Y (and dosage compensated on the X with t_m = 2) while 50% are degenerate on the X (and dosage compensated on the Y with t_m = 2). We term this initial condition the "sorted case", meaning that it is already decided which genes are degenerate on the X or Y (and it is the same for all replicated populations).

	Domi-nance theory	*Faster male theory*	*Meiotic drive theory*	*Regula-tory theory*
HR in XY species				
HR in ZW species				
Large X/Z effect				
Importance of HR through sterility in several groups				
Importance of HR through inviability in several groups				
Darwin's corollary to HR				
HR through inviability/sterility in species without/with global DC				
HR in species with X inactivation in females				
Theory also explains evolution of sex chromosomes				

Table. 1. Summary of the comparison of main predictions of the different theories for Haldane's rule. In green/orange/red the theory adequately/partially/inadequately predicts the pattern indicated in the first column. See text for details. Regulatory theory refers to the theory presented in this paper.

Supplementary material

1 Methods

We consider a pair of sex chromosomes carrying 500 genes subject to partially recessive deleterious mutations, as observed in many species [84]. Gene expression is controlled by cis-regulatory sequences (affecting expression only on the same chromosome as themselves) interacting with trans regulators that can affect gene copies on both homologs. All these elements can mutate. To allow for dosage compensation on a gene-by-gene basis while keeping the model symmetric for males and females, we assume that each gene is controlled by one male- and one female-expressed trans regulator (we discuss global DC below). We assume that each gene's overall expression level is under stabilizing selection around an optimal level and that the relative expression of the two copies of each gene determines the dominance level of a deleterious mutation occurring in the coding gene [34,35]. For instance, a deleterious mutation occurring in a less expressed gene copy is assumed to be less harmful than one in a more highly expressed copy. We also assume that mutations occur that suppress recombination on a segment of the Y. For simplicity, we refer to these mutations as inversions, although they could correspond to other mechanisms causing recombination arrest. Inversions of any size can occur, but we follow only those on the Y that include the sex-determining locus, which will necessarily be confined to males and cause recombination arrest. We assume that these inversions can add up, such that new inversions can occur on chromosomes carrying a previous inversion and thus extend the nonrecombining part of the Y. Finally, we assume that reversions restoring recombination can occur, and for simplicity, that such reversions cancel only the most recent inversion [34]. We consider that these reversions occur 10 times less frequently than inversions.

We consider three main scenarios. In the first, the sex chromosome pair has just acquired the sex-determination locus. We follow the independent evolution of this recent Y in different replicate species (we call them species for simplicity, but these are, at least initially, only independently evolving populations). After a given number of generations, F1 hybrids are generated between these independently evolved species, in the two directions of the cross (M x F and F x M), and their fitness is compared to that of male and female offspring produced within species. The second scenario corresponds to the case of a partially degenerated Y chromosome: it is equivalent to the first scenario, except that 50% of the Y is already non-recombining, fully degenerated, and fully dosage compensated in the common ancestor of the diverging species. Finally, the third scenario assumes that 100% of the Y is already non-recombining, fully degenerated, and fully dosage compensated in the ancestor (this is equivalent to considering diverging species with a XX / XO sex determination system). These scenarios allow us to compare hybrid fitness between species at different stages of sex chromosome evolution. We also investigate the case of genes with male- or female-limited effects to represent genes involved in fertility rather than viability.

In addition to these three main scenarios, we performed control simulations. We consider simulations with autosomes instead of sex chromosomes (without including new inversions on these autosomes but including the possibility of regulatory evolution, with one male and one female trans-acting factor per gene) and simulations with sex-chromosomes, but without considering new inversions (in the third scenario, this control is irrelevant since the whole Y is already non-recombining).

2 Computing effects

In males, the total expression level Q_i for a gene *i* equals $(c_{X,i} + c_{Y,i})\bar{t}_{m,i}$, where $\bar{t}_{m,i}$is the average strength of the *trans*-regulators expressed in males, while $c_{X,i}$ and $c_{Y,i}$ are the strength of cis regulators associated with the X and Y-linked copies of gene *i*. Symmetrically, it is $(c_{X1,i} + c_{X2,i})\bar{t}_{f,i}$ in females.

Denoting by *I* the intensity of stabilizing selection on the expression level, the fitness resulting from the departure from optimal dosage W_i^Q is

$$W_i^Q = 1 - s_{max}\left(1 - e^{-I\left(\ln Q_i - \ln Q_{opt}\right)^2}\right) \quad (1)$$

See [34] for a justification of this expression. We use Q_{opt}= 2 without loss of generality below. We also denote $\theta = I\ s_{max}$, measuring the overall intensity of stabilizing selection on dosage. We drop below the indices *i* referring to a specific gene, to simplify the notation.

Many different mechanisms of dosage compensation have been described in a diversity of organisms and may be represented by particular cases of our general model. Indeed, once the Y is fully silenced ($c_Y = 0$), and assuming that the population stays at Q_{opt} in both males and females (which is a good approximation unless stabilizing selection is very weak), we have

$$Q_{opt} = c_X \bar{t}_m = 2c_X \bar{t}_f \quad (2)$$

We therefore have $\bar{t}_m = 2\bar{t}_f$, which defines dosage compensation. Hence the way dosage compensation works (i.e. the triplet $\bar{t}_m, \bar{t}_f, c_X$) can be described by a single parameter (there is only one degree of freedom in the way DC occurs). We can choose e.g. to use $\bar{t}_m$ for this description. Compared to the initial system with $\bar{t}_m = \bar{t}_f = c_X = c_Y = 1$, a final compensation characterized by $\bar{t}_m$ =1 (i.e. $\bar{t}_m = 1, \bar{t}_f = 0.5, c_X = 2, c_Y = 0$) would correspond to the *Caenorhabditis elegans* case, where the X is inherently expressed twice as much ($c_X = 2$) to obtain optimal expression in males (hermaphrodites), while a female-limited *trans*-regulator halves expression ($\bar{t}_f = 0.5$) to recover optimal expression in females. This is also very similar to the mammal case where a female-limited *trans*-regulator halves expression by randomly silencing one X (rather than halving the expression of each X like in *C. elegans*). The case $\bar{t}_m$ = 2 (i.e. $\bar{t}_m = 2, \bar{t}_f = 1, c_X = 1, c_Y = 0$) would correspond to the *Drosophila* case, where a male-limited *trans*-acting factor doubles X expression to obtain optimal expression in males (nothing being changed in females, $\bar{t}_f = 1, c_X = 1$).

2.1 Contribution of autosomes to the fitness reduction of F1 hybrids

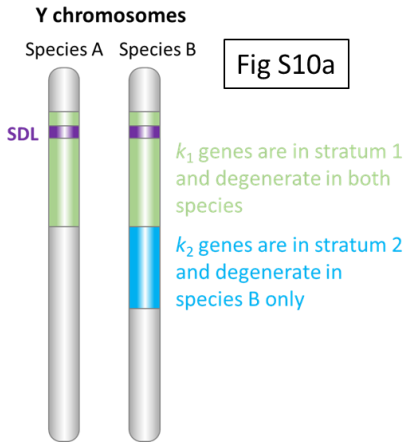
On autosomes, cis and trans regulators can coevolve provided that the overall expression level stays close to its optimum value. This condition implies that, for each gene, t_m and t_f stay both close to 1/*c.* Hence, autosomes can contribute to a reduction in hybrid fitness, but it will be symmetrical for males and females. Noting Δ the difference in t values between the two species for a given gene, and assuming that this difference is weak, we find that the reduction in fitness of F1 hybrids caused by misregulation of this gene is approximately:

$$W_{hybrid} = 1 - \theta(\Delta/2t_m)^4 \quad (3)$$

This effect is modest (note the quartic exponent), but can lead to substantial fitness loss if cumulated over many genes.

2.2 Contribution of the XY to the fitness reduction of F1 hybrids

We can compute the male/female fitness ratio in hybrids, accounting for two types of loci (Fig S10a). We consider first the Y-linked loci that are degenerate and silenced in both species (assume there are k_1 such loci). These loci are for instance located in a non-recombining region that is common to both species (green stratum 1 in Fig S10a, where the sex-detrmining locus, SDL, is located). Second, we consider loci that are degenerate and silenced in only one species (assume there are k_2 such loci). These loci are for instance located in a non-recombining region that only evolved in one species (blue stratum 2 of species B in Fig S10a). We compute the contribution to Haldane's rule of these two types of loci in turn.



2.2.1 Contribution of an ancestral stratum (stratum 1)

For loci that are degenerate in both species, the contribution of a given gene to the male/female fitness can be approximated assuming that the gene is fully silenced on the Y and dosage-compensated in both species, that is, without loss of generality $c_Y = 0$, $c_X = 2/t_m$, $t_f = t_m/2$. Noting Δ the difference in t_m values between the two species for a given gene, and assuming that this difference is weak, we find that the contribution to Haldane's rule of that gene is approximately:

$$\begin{aligned} \bar{W}_{male}/\bar{W}_{female} &= 1 - \theta(\Delta/2t_m)^2 \\ &= 1 - \theta\ \mathrm{var}(\Delta)C_1 \end{aligned} \tag{4}$$

where $\bar{W}_{male}$, $\bar{W}_{female}$ are the mean fitnesses of male and female F1 hybrids, $\mathrm{var}(\Delta)$ a measure of the divergence in the DC mechanism between the hybridizing species and C_1 a positive constant equal to the inverse of $4t_m^2$.

2.2.2 Contribution of a derived stratum (stratum 2)

The male/female ratio contributed by the loci in stratum 2 can be approximated assuming that in species A the Y genes are not degenerate and remain fully expressed (i.e. $\bar{t}_m = \bar{t}_f = c_X = c_Y = 1$), while in species B the genes are degenerate, silenced, and dosage compensated (with a given t_m value, i.e. without loss of generality $c_Y = 0$, $c_X = 2/t_m$, $t_f = t_m/2$). The reciprocal crosses are asymmetrical for males, depending on which Y they inherit. In cross 1 (male B x female A), the Y in hybrid male offspring is carrying degenerated genes, but in cross 2 (male A x female B), it does not.

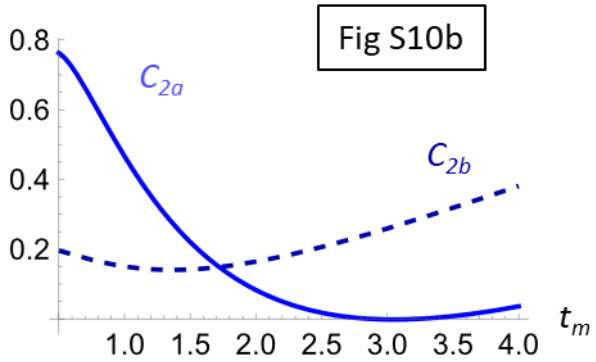
Assuming weak stabilizing selection, we find, for the effect of one gene:

$$\bar{W}_{male}/\bar{W}_{female} = 1 - \theta\bar{C}_2 \tag{5}$$

Where C_2 is a constant equal to C_{2a} in cross 1, and to C_{2b} in cross 2 and to $\bar{C}_2 = (C_{2a} + C_{2b})/2$ on average over the two types of crosses, with:

$$C_{2a} = \mathrm{Log}\left[\frac{1+t_m}{4}\right]^2 - \mathrm{Log}\left[\frac{(2+t_m)^2}{8t_m}\right]^2$$
$$C_{2b} = \mathrm{Log}[\frac{(1+t_m)(2+t_m)}{4t_m}]^2 - \mathrm{Log}[\frac{(2+t_m)^2}{8t_m}]^2 \quad (6)$$

C_{2a} and C_{2b} are positive (except in a very small region around t_m=3 where $C_{2a}$is very close to zero, Fig S10b).



2.2.3 The overall effect of ancestral and derived strata

Hence with weak stabilizing selection, the male/female fitness ratio among F1 offspring, overall, is close to

$$\bar{W}_{male}/\bar{W}_{female} = 1 - \theta[k_1 C_1 \mathrm{var}(\Delta) + k_2 \bar{C}_2] \quad (7)$$

With k_1 genes on the shared ancestral stratum and k_2 genes in the recently evolved stratum in one of the two species, where C_1 and $\bar{C}_2$ are positive constants that depend on the DC mechanism, and var(Δ) is a measure of the divergence in the DC mechanism between the hybridizing species. Hence, HR due to disruption of DC can be caused by the portion of the Y that is degenerate and compensated in both species (provided they exhibit some divergence in DC), and by the portion of the Y that is degenerate and compensated in only one of them.

In a drosophila-like situation where dosage compensation is achieved by overexpressing the X in males (t_m around 2), we have:

$$\text{Cross 1: } \frac{W_{male}}{W_{female}} = 1 - \theta[0.06k_1 var(\Delta) + 0.08k_2] \quad (8a)$$

$$\text{Cross 2: } \frac{W_{male}}{W_{female}} = 1 - \theta[0.06k_1 var(\Delta) + 0.16k_2] \quad (8b)$$

while in a *C. elegans* or mammal case (t_m around 1):

$$\text{Cross1: } \frac{W_{male}}{W_{female}} = 1 - \theta[0.25k_1 var(\Delta) + 0.47k_2] \quad (9a)$$

$$\text{Cross2: } \frac{W_{male}}{W_{female}} = 1 - \theta[0.25k_1 var(\Delta) + 0.15k_2] \quad (9b)$$

In all cases, Haldane's Rule occurs and combines a regulatory effect of the genes that are degenerate in one species and not the other, and a regulatory effect of the genes that are degenerate in both species (but not compensated exactly in the same way in the two species). The two effects combine and contribute to HR.

Loci that are degenerate in one species but not in the other generate an expression mismatch in hybrids, even if trans-acting factors are codominant. In the range of known DC mechanisms, i.e. with t_m values between 1 and 2, hybrid females will tend to show slight overexpression, males from cross 1 will show relatively severe underexpression, and males from cross 2 relatively severe overexpression (Fig S7-S9).

For loci that are degenerate in both species, the effect comes from the fact that genes may exhibit a quantitative difference in the way they are dosage compensated (since achieving optimal DC can be obtained by a different combination of cis and trans effects, i.e. different t_m values). This difference will be however more buffered in females (where cis effects are averaged over the two X) than in males (where cis effects are not averaged and only expressed from a single X, Fig S8). In both males and females, (autosomal) trans-effects are equally averaged. In species pairs sharing an identical and chromosomal-level DC mechanism, this effect is expected to be relatively minor, since genes should not exhibit quantitative differences in the DC mechanism. However, in many species, many genes escape this global DC mechanism and exhibit gene-level expression control [85–91]. These genes, if sufficiently numerous on the X could therefore also contribute to HR. Current evidence indicates that chromosome-wide DC is the exception, rather than the norm [56–58].

Finally, in systems where DC occurs around a Drosophila-like situation (t_m around 2), hybrid males inheriting the most degenerated Y (from cross 1) suffer more than males inheriting the less degenerated Y (from cross 2). The reverse occurs in systems where DC is close to a C. elegans/mammal type (t_m around 1). However, the difference will manifest only in species pairs showing Y with different degrees of degeneration (i.e. only if k_2>>1 and larger than k_1).

2.3 Case of unbalanced females

In several Drosophila experiments, the fitness of F1 females carrying 2 attached X from the same species (XXY females) was investigated to test the dominance theory of Haldane's rule. In these cases, it was expected that homozygous females (carrying two X from the same parental species) would show a large fitness reduction, like hemizygous males (but unlike standard heterozygous XX females). These crosses have revealed that when HR was about F1 fertility, unbalanced females remained fertile (unlike F1 males), while when HR was about F1 viability, unbalanced females were inviable (like F1 males). These results generated considerable discussion [1,2,4,16,17,92–94].

714 Using the same approach as above, we can first compute the expected fitnesses of the different F1s
715 for genes located on stratum 1 (shared between the two hybridizing species), assuming a small
716 divergence in their regulatory traits (small Δ) :

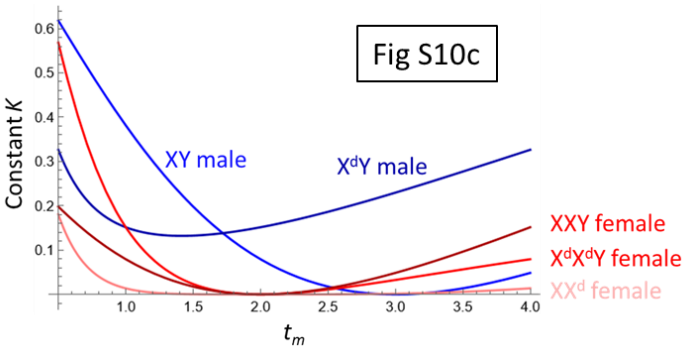
717

718 $$W_{XY} = 1 - \theta(\Delta/2t_m)^2 + O(\Delta)^3 \quad (10a)$$

719 $$W_{XX} = 1 - \theta(\Delta/2t_m)^4 + O(\Delta)^5 \quad (10b)$$

720 $$W_{XXY} = 1 - \theta(\Delta/2t_m)^2 + O(\Delta)^3 \quad (10c)$$

721



722 For genes in that stratum, we find that
723 unbalanced females should present the
724 same fitness reduction as males, much
725 larger than the one seen in regular F1
726 females. For genes located on stratum 2,
727 degenerate and compensated only in
728 species 2, and noting X^d the X from species
729 2, we find that the fitness reduction is
730 proportional to θK, where K is a constant
731 that depends on how X^d is dosage
732 compensated (i.e. it depends on the value of t_m). The different values of K are straightforward to
733 compute and are illustrated in Fig. S10c for the different F1 in the reciprocal crosses. Unbalanced
734 females have a lower fitness than standard females except near t_m =2 where the difference is small.
735 Overall, combining effects in stratum 1 and 2, we can therefore conclude that this model predicts that
736 unbalanced F1 females show a greater fitness reduction compared to standard females. The
737 comparison to males depends on whether genes in stratum 1 or 2 contribute. For genes on stratum 1,
738 the fitness reduction is the same as in males. For genes on stratum 2, it depends on the direction of
739 the cross and t_m values (see Fig. S10c). Overall, these results indicate that unbalanced F1 females
740 should often present a large reduction in fitness, much more similar to that of F1 males than that of
741 F1 females. This result holds for viability. For sterility, the fitness of males and females (balanced or
742 unbalanced) will differ, if genes involved in sterility are expressed in a sex-specific manner (see next
743 section for the effect of such genes). As shown below, male-limited genes will cause a fitness reduction
744 in F1 males, but female-limited genes will not cause a fitness reduction in F1 females. This would
745 therefore explain the contradictory results observed for the *Drosophila* experiments mentioned above
746 where unbalanced females are fertile when HR involves sterility but are inviable when HR involves
747 viability [1,92].

748 **2.4 Case of sex-limited genes**

749 We can consider the case of fertility genes. We consider that these genes are expressed in only one
750 sex (they are e.g. expressed in the germline), contrary to viability genes, which in the vast majority of
751 cases, will be expressed in both sexes). We will take male as the heterogametic sex as in XX/XY species,
752 but the same argument can be made by switching sexes for ZZ/ZW species.

753 Female-limited genes can obviously be lost from the Y, as they are not expressed in males. Apart from
754 this, their regulators can diverge between species, like in the case of autosomes, generating a
755 consistent but small decrease in fitness in F1 (as in Eq. 3). Given the lower effective population size on
756 the X (compared to autosomes), and the weaker selection pressure overall (everything else being

equal, sex-limited genes are only selected in one sex), we might nevertheless expect that the regulatory divergence could somehow be faster on the X compared to autosomes (as seen in the results, compare the female fitness drop illustrated on Fig 1E to that on Fig 1A).

Male limited genes can be lost from the Y (like genes expressed in both sexes), but they can also be lost from the X. Indeed, there is no requirement that they remain functional in XX females (contrary to genes expressed in both sexes that can only evolve Y silencing [35]). Diploid expression is unstable after recombination arrest, but silencing can occur both ways. Simulation results confirm this finding. However, despite this apparent symmetry, X and Y silencing is not occurring at an equal rate. Male-limited genes are more likely to be silenced on the Y than on the X. The bias is for instance close to 6:4 in a non-recombining stratum with 50 male-limited genes, but it is 9:1 in a stratum with 500 male-limited genes (in both cases the simulation had a population size of 10 000 individuals, and the X or Y silencing occurred relatively quickly). Presumably, the bias increases with the relative ease at which deleterious mutations initially accumulate on the Y versus X once recombination has stopped (due to selective interference), preferentially pushing the regulatory feedback loop in that direction. In all cases, because one copy is silenced, these male-limited genes will evolve dosage compensation to stay expressed at the right level in males (i.e. their cis-regulators on the X or Y will coevolve with the trans-regulator expressed in males). In an F1 hybrid, when the focal gene has been silenced on the same chromosome in both species, they will therefore cause a reduction in fitness that is similar to the case of a gene expressed in both sexes (i.e. Eq 7 applies). If p is the chance that a gene is silenced on the Y, and $1-p$ on the X, this situation has $p^2+(1-p)^2$ chances to occur. If the focal gene is silenced on the Y in one species, but on the X in the other, male F1 will however suffer from a larger fitness reduction. In one direction of the cross, the F1 male will receive two silenced copies, and its fitness will therefore be reduced by a factor $1-s_{max}$. This has $p(1-p)$ chances to occur. For an essential gene involved in male fertility, a single case like this would be sufficient to cause complete male sterility. In the other direction of the cross, the F1 male will receive two non-silenced copies, which will correspond to severe overexpression. Its fitness will be reduced by a factor $(1-\theta\log(2)^2)$, which is close to $(1-\theta/2)$. This has also $p(1-p)$ chances to occur. Simulation starting with an XY non recombing pair with 500 fully expressed male-limited genes leads to a dramatic reduction of F1 male fitness, due to this sorting effect. Some genes become silenced on the X and some on the Y, but they are not the same in the two diverging species, resulting in many mismatches. A simulation starting with 500 male-limited genes that are already sorted leads to an F1 male fitness reduction closer to that observed for the XO simulation with genes expressed in both cases. The effect is stronger, however (compare figures 1D and 1E), as male-limited genes silenced on the X (and maintained on the Y) will exhibit faster regulatory divergence, due to their lower effective population size (3 times lower than that of genes silenced on the Y and maintained on the X).

Overall, we thus predict a stronger effect on HR of male-limited genes than of genes expressed in both sexes. The argument was made for males but would apply to females in ZZ/ZW species. This finding indicates that our regulatory theory could account for the importance of cases of sterility in HR, without having to suppose a 'faster male' theory. It would also better explain why HR is not weaker in Lepidoptera where males are homogametic [a difficulty of the faster male theory noted by Presgraves [95]].

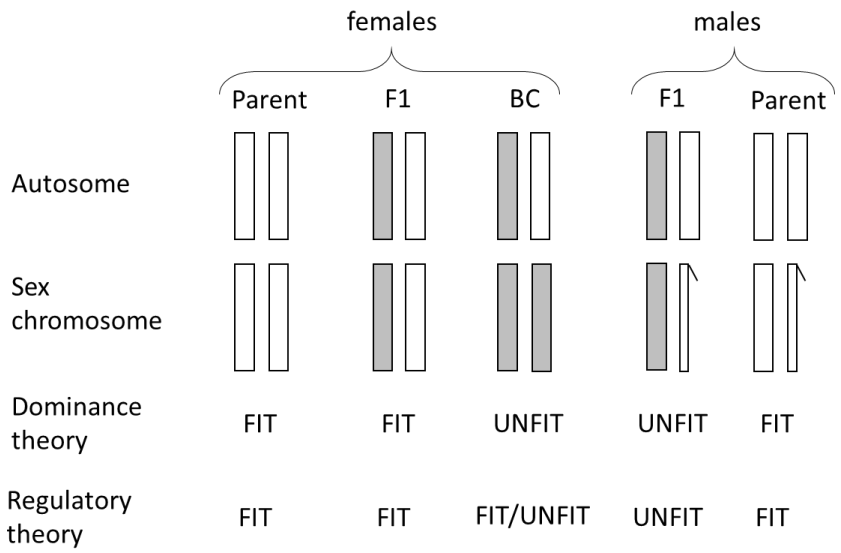
3 Comparison of regulatory theory with the dominance theory

The dominance theory for Haldane's rule proposes that recessive incompatibilities occur, such that, in an hybrid F1 autosomal background, F1 females are fit while F1 males are unfit. The reason why F1 females are fit is that incompatibilities carried on the X are "recessive", meaning that if the X were homozygous (in an F1 hybrid background), these incompatibilities would manifest themselves. If such

803 females could be produced in a backcross or through genetic manipulations (such as the F1 females
804 with attached X in *Drosophila*), they would therefore have low fitness. The theory further considers
805 that hemizygous males express the incompatibilities because, having a single X, these incompatibilities
806 carried on the X cannot be masked. Different models proposed different ways to achieve this pattern.
807 For instance, different beneficial alleles can fix in different populations and some can turn out to be
808 incompatible in the sense just explained (those that turn out to be incompatible and dominant reduce
809 fitness in all F1 hybrids and thus do not create a sex bias) [22,94]. In the model of stabilizing selection
810 proposed by Barton [24], combinations of phenotypically dominant mutations can contribute to bring a
811 population closer to an optimum. When different dominant mutations (on autosomes and on the X)
812 coming from two parental species, who adapted independently, combine in an F1 female, they all add-
813 up to result in a near optimal phenotype (since all the mutations coming from each parental species
814 independently add-up, as they are all dominant and have additive effects between loci). In contrast,
815 F1 males miss the dominant mutations that occurred on the X of one parental species, causing a
816 departure from the optimal phenotype (this model assumes no mutation on the degenerate Y).
817 "Homozygous" F1 females would also miss all the mutations from the X of one of the two parental
818 species (while having the dominant mutations on autosomes from both species), resulting in low
819 fitness. Another model of stabilizing selection has been proposed that includes additive mutations
820 (within and between loci), but assumes that mutations in males have twice the phenotypic effect when
821 hemizygous (as if they were dosage compensated) [25,26]. As in Barton's model, F1 females remain close
822 to the phenotypic optimum because all mutations are additive. F1 males, in contrast, depart from the
823 optimal phenotype because the mutation on their X mismatch with half the additive autosomal allele
824 (coming from the same parental species as the X), while the other half of additive autosomal alleles do
825 not add up with the missing X (from the other parental species). Here too, the "homozygous" females
826 exhibit the same phenotype, and low fitness, as the F1 males. In all cases, Haldane's rule occurs
827 because a female that would be homozygous for a parental X in an otherwise F1 background has a
828 lower fitness than a regular F1 female (implying a dominance effect on the X, i.e., heterozygotes have
829 a higher fitness than the average of the two homozygotes), while the fitness of hemizygous F1 males
830 is assumed to be the same as the fitness of females made homozygous for the X.

831 In our regulatory model, F1 males also show a reduced fitness compared to F1 females (Haldane's
832 rule), but the reason is not a "recessive" effect, in the sense described above. Unlike in the dominance
833 theory, the effect of the F1 genetic background differs between males and females: they express
834 different trans-acting factors, both in the parental species and in the male and female hybrids (Fig S11).
835 Hence, the fitness of hemizygous males is not necessarily the same as the fitness of "homozygous"
836 females; this is particularly clear in the example shown in Figure S7a, where F1 females that would be
837 made homozygous for the X would retain optimal expression, contrarily to F1 males. Generally, the
838 disruption of dosage compensation always impacts more the heterogametic than the homogametic
839 F1, although the effect varies with the type of dosage compensation and the position of the loci
840 involved (on an ancestral or derived stratum, see Eq. 7). The difference between the fitness of F1 males
841 and "homozygous" females also varies with type of dosage compensation and the position of loci,
842 although "homozygous" females tend to have a lower fitness than F1 females (this is the case in the
843 examples shown on Figures S7b and S8).

844



845

846 **Fig. S11**. **Simplified fitness patterns in the dominance versus regulatory theory.** The
847 chromosome with a hook represents the Y. A fundamental difference is that the
848 fitness of "homozygous" females differs between the two theories (indicated by "BC"
849 as these females can be obtained by backcrossing or by creating "attached X" F1
850 females in *Drosophila*). In the dominance theory, "homozygous" females exhibit the
851 same fitness reduction as F1 males. In the regulatory theory, male and female fitness
852 can always be uncoupled since they express different trans-regulators. The sex
853 asymmetry results from lack of averaging of cis-regulator effects in males (see
854 above).

855 ## 4 Darwin's corollary

856 The fitness of F1 hybrids often differs significantly between reciprocal crosses. For instance, Turelli and
857 Orr [94] estimated that 15% of cases of Haldane's rule in *Drosophila* involved a strong asymmetry (the
858 male F1 being sterile or inviable in only one direction of the cross between the two hybridizing species).
859 A similar pattern is seen in *Anopheles* [3] and could be even more prevalent in *Lepidoptera* [95] or *Silene*
860 [11]. Turelli and Moyle termed this pattern "Darwin's corollary", complementing Haldane's rule and the
861 large X effect in the rules of speciation [11]. In our model, fitness asymmetries between reciprocal crosses
862 often arise. The general reason for this asymmetry is that the heterogametic F1 will typically suffer
863 from over-expression in one direction of the cross and from under-expression in the other. This pattern
864 occurs in all cases (see detailed examples in Fig S7-S9). Everything being equal, under-expression is
865 more deleterious than overexpression in our model, as is likely in most biologically plausible situations.
866 In the extreme case, e.g. when a male-limited gene is entirely missing in one direction of the cross,
867 male fitness is much more reduced than when its expression is doubled in the other direction of the
868 cross (Fig S9b). The asymmetry in reciprocal crosses is particularly strong when a discrete event of large
869 effect occurs in one species but not in the other (similarly, Muller noted that asymmetry between
870 reciprocal hybrids must involve loci of large effect [96]). This occurs for instance when a species acquires
871 a new non-recombining stratum and not the other. In this case, the F1 male inheriting the more
872 degenerate Y will suffer more than the F1 male in the reciprocal cross (Fig S7a, S7b). This effect will
873 occur mostly at intermediate steps of Y chromosome degeneration, i.e. when species have different
874 strata, and before F1 male fitness is not too strongly reduced in all crosses (Fig S2). Another case
875 particularly conducive to strong fitness asymmetries in reciprocal crosses is when a male-limited gene
876 is missing in one direction, as explained above. However, if there are many male-limited genes, then
877 different genes will be lost on the X or Y in the different species, and the fitness of all F1 males will be

878 reduced. Darwin's corollary should be strongest when the number of male-limited genes on sex
879 chromosomes is not too large, maximizing the sampling variance of those genes and therefore the
880 fitness asymmetries in the reciprocal crosses (compare Fig S3b and S3c, with 500 and 50 male-limited
881 genes on the chromosome respectively). When the fitness reduction in F1 males results from many
882 small effects, fitness asymmetries in reciprocal crosses tend to be weaker. This is the case for instance
883 when regulatory divergence occurs on a chromosome that is already fully dosage-compensated (see
884 Fig S3a for XO simulations). Overall, our model predicts substantial fitness asymmetries in reciprocal
885 crosses, and at intermediate times of species divergence, which is in line with the available
886 observations.

887 ## 5 Criticisms of the dosage compensation hypothesis for Haldane's rule

888 The list below presents past arguments against a theory of HR based on the disruption of dosage
889 compensation. It has to be taken with precaution for three main reasons.

890 First, a firm theory of Haldane's rule based on the disruption of DC has never been made, so most of
891 these past arguments were made against a verbal and relatively vague theory. For instance, the relative
892 level of expression disruption of autosomal vs. sex-linked genes in hybrids is sometimes mentioned as
893 a decisive observation. Yet, this comparison is mostly uninformative. Autosomal misexpression
894 reduces the fitness of both sexes in F1, and is therefore irrelevant to HR. It does not indicate whether
895 the cause of HR is related to misexpression of sex-linked genes. If for instance DC is controlled by a
896 global mechanism, such as in mammals or *Drosophila*, it might be constrained and slow evolving
897 compared to autosomes. In this case, only few genes escaping this global DC might contribute to HR
898 (including sex-limited genes in the germline not regulated by this somatic global DC). As a result,
899 misregulation may appear larger overall on autosomes than on sex-linked genes, but this observation
900 would not rule out that the cause of HR is misregulation of sex-linked genes.

901 Second, the idea that HR may be caused by DC disruption, made in the past, is much narrower than
902 our regulatory theory. The idea usually considered is that the mechanism of dosage compensation is
903 disrupted in hybrids, due to a divergence in the genes *directly* involved in the DC pathway. This is much
904 narrower than in our regulatory theory, where the reduction of hybrid fitness is caused by the
905 misregulation of gene expression *for all genes that are dosage compensated*, not only for genes directly
906 involved in DC regulation. Our theory potentially includes most genes on the non-recombining part of
907 the X or Z, not just the genes directly identified in the pathways controlling DC (the latter can
908 nevertheless play a role in the misexpression of dosage compensated genes). If for instance, again, DC
909 is controlled by a global mechanism, such as in mammals or *Drosophila*, only genes escaping this global
910 DC might contribute to HR, including sex-limited genes in the germline not regulated by this somatic
911 global DC. Their misexpression would play a key role in the fitness reduction of heterogametic F1, but
912 they would be entirely unaffected by the global DC mechanism, and entirely unrelated to the control
913 of this DC regulation. Note also that defining dosage compensation for sex-limited genes can only be
914 made here relative to the ancestral expression level (before the evolution of recombination
915 suppression). Since such genes are only expressed in one sex, male-female comparisons cannot be
916 made, as usually done, to assess DC. In these cases, the comparison of expression levels in parental
917 species and the hybrids would be the important comparison to make.

918 Third, the genetics of post-zygotic isolation is a broader topic than the genetic cause of HR. Many
919 incompatibilities can impact hybrid fitness, in addition to those causing HR (i.e. not specifically reducing
920 the fitness of the heterogametic sex). Importantly, such incompatibilites can be caused by many other
921 mechanisms than expression disruption. Even in the context of HR, exceptions to the rule are known
922 and relatively well understood (in particular nucleao-cytoplasmic incompatibilities[70]). Hence, assessing
923 the merit of the different theories of HR based on a specific gene disrupting hybrid fitness in a specific

924 cross must be done carefully. Documenting broad patterns such as those mentioned on Table 1 could
925 be a more informative and more robust way of comparing the different theories.

926 With these caveats in mind, here are 13 arguments made in the literature against a (relatively narrow)
927 theory based on the idea that the cause of HR is due to the direct disruption of the dosage
928 compensation pathway in hybrids.

929 The 1st major critique of the DC hypothesis is that the rule was observed in groups in which DC was
930 allegedly absent (such as in birds and lepidopterans [4,6]). However, DC has since been documented in
931 these groups[56,58], although global DC is perhaps less frequent in ZW species[97].

932 The 2nd argument is based on an experiment with *Drosophila* [49]. However, this experiment was based
933 on crosses with mutants in which DC was supposedly fully functional, but without showing that this
934 was the case. Hence, the results presented could not really discard DC disruption as a cause of HR.
935 Specifically, it could not discard the possibility that hybrid male sterility was caused by a failure in DC
936 downstream of *Sxl* regulation (which is the sex-determining switch in Drosophila).

937 The 3rd argument is that DC evolves very slowly, being an essential function under strong constraints
938 [50]. However, as our results show, divergence in DC can readily occur, even with substantial stabilizing
939 selection on expression levels. Note that DC evolution could be triggered by different phenomena,
940 including coevolution with cytoplasmic bacteria targeting DC pathways to achieve male killing [98–100].

941 The 4th argument proposes that if Haldane's rule was caused by DC disruption, cis and trans regulators
942 involved in DC should exhibit signs of divergence between species exhibiting Haldane's rule. Yet, Jaffe
943 and Laird [101] reported unpublished data where the X-linked *D. pseudoobscura Hsp82* gene remained
944 dosage compensated when transformed into various autosomal sites in *D. melanogaster,* suggesting
945 conservation of the cis-regulatory elements involved in DC for ~20 million years. Indeed, if cis-
946 regulators involved in DC are highly conserved, they could not cause Haldane's rule [4,49]. However,
947 further evidence showed that this conclusion, besides applying only to *Drosophila*, was premature.
948 More recent investigations revealed that both cis and trans regulators involved in DC were actually
949 fast evolving in *Drosophila melanogaster*. This is the case with *msl*, *mof,* and *mle* trans-acting genes
950 [50,74] as well as cis-acting binding sequences on the X [51]. The case of male lethality in *D. melanogaster* x
951 *D. simulans* hybrids is not clear-cut. Some studies support the role of DC in male lethality [102,103] while
952 others challenge this interpretation [104]. In this cross, both males and females show a reduction in
953 viability, but to a different degree depending on temperature. However, the effect of mutants is not
954 always evaluated in this GxE context. Barbash shows that deletion of mel-specific DC genes—ie, forcing
955 Xmel/Ysim hybrid males to develop using only *simulans* DC complex components—does not
956 exacerbate F1 male lethality when this lethality was first rescued by lhr mutation. The test is based on
957 the premise that the lhr rescue occurs and that playing with DC genes should show an independent
958 effect when manipulated. However, lhr might be acting by interacting with these DC genes, so there
959 may be no clear 'independent' manipulation of the DC phenotype in the first place in the experiment.
960 Barbash also argues that DC disruption is unlikely because a lower-than-expected number of genes
961 downregulated in lethal F1 males are located on the X chromosome. However, only the misregulation
962 associated with sex chromosomes will have a differential fitness effect on males vs females. HR
963 depends on the presence of the latter, not on the proportion of misregulation of X versus autosomes.
964 Whether there is more or less than "expected" misregulation on the X says nothing about the cause of
965 HR. What can be noticed, however, although it could be a coincidence, is that major genes involved in
966 these hybrid incompatibilities interact with the dosage compensation complex. For instance, *Lhr*
967 interacts with HP1, a chromodomain-containing protein that localizes to heterochromatic regions of

968 chromosomes [105] and is also involved in DC [106]. Nuclear pore complex proteins also cause hybrid male
969 lethality [107,108], and are also involved in DC [109].

970 The 5th argument is a refinement of the fourth. After the accumulating evidence demonstrating fast
971 DC evolution in *D. melanogaster*, Tang and Presgraves [108] suggested that this phenomenon was not
972 general and limited to that species. They concluded that the limited evolution of the MSL complex in
973 *D. simulans* could not well explain the rapid evolution of Nup160 and the other Nup107 subcomplex
974 genes in that species (these autosomal simulans alleles being involved in male hybrid lethality through
975 incompatibility with the melanogaster X). This may be the correct interpretation, and this specific case
976 may be regarded as mere exception to the general case. However, this argument can be nuanced by
977 several points, that might warrant further investigation. First, the *mof* gene involved in the DC complex
978 does show evidence of rapid adaptive evolution in *simulans* [74]. Second, Nup160 and the other Nup107
979 subcomplex genes are known to be involved in DC in *Drosophila* [109]. Third, DC disruption is not
980 necessarily limited to the coevolution of the MSL complex and its cis-binding sites. For instance, MSL
981 cis-binding sites could change location on the X, thereby changing the pattern of DC, as shown by the
982 rapid and extensive turnover of individual binding sites of roX lncRNAs, which are essential for
983 *Drosophila* DC [54]. Fourth, some genes are dosage-compensated but non-MSL-binding, suggesting that
984 MSL is not the only mechanism for achieving DC [86]. This is also particularly the case for genes expressed
985 in the germline, impacting male fertility in *Drosophila* [63].

986 The 6th argument, also by Tang and Presgraves [108] and also concerning Nup160 in *D. melanogaster*,
987 note that Nup160sim kills both Xmel/Ysim hybrid males and Xmel ·Xmel/Ysim hybrid females, and in a
988 similar way, suggesting a common cause. This would exclude the role of DC, on the premise that DC
989 only concerns males in *Drosophila*. Like above, this may be the correct interpretation, and this case
990 may well be an exception (there are several other known exceptions to HR in other groups, involving
991 specific Y effects or incompatibilities with mitochondria, etc.). However, interestingly, our results show
992 that, especially for ancient Y, unbalanced females should show an equal decrease in fitness to males,
993 especially for viability (Eq. 10). (For hybrid sterility, it is easy to uncouple the fitness effect seen in
994 males and unbalanced females: it is sufficient to have male-limited genes, as discussed above). This
995 result relies on a quantitative DC trait divergence between species. In our model, the *Drosophila* DC
996 system corresponds to a particular case where the strength of X cis-regulators does not change and
997 where male trans regulators double X expression in males (case $t_m = 2$ in our notation). Any quantitative
998 departure from this point (where the strength of X cis regulator changes) involves a correction in
999 females. In a quantitative model, DC is a phenomenon that always involves both sexes, as long as t_m is
1000 not exactly 2. Hence, anytime males will show DC disruption (because of t_m divergence), so will
1001 unbalanced females.

1002 The 7th argument has not directly been made against the DC hypothesis but against the dominance
1003 theory. However, this issue also concerns the DC disruption hypothesis. Haldane's rule is observed in
1004 species lacking heteromorphic sex chromosomes. In particular, in the genus *Aedes*, Haldane's rule
1005 applies to many interspecific crosses [3]. Yet, *Aedes* have homomorphic sex chromosomes with a sex
1006 locus. A priori this observation would rule out theories based on hemizygosity (dominance theory) or
1007 DC. However, recent genomic data have revealed that in *Ae. aegyptii*, the sex "locus" is a 1.5 Mb region
1008 with 30 genes in a 100 Mb non-recombining region encompassing the centromere of chromosome 1,
1009 that diverged between males and females. Hence, the premise that these species have a sex
1010 chromosome with just a sex-determining locus seems erroneous. More work is required to evaluate
1011 the extent of hemizygosity and the occurrence of (local) DC in these species, but the conclusion
1012 favoring the "faster male" hypothesis based on these *Aedes* data certainly requires re-evaluation.

1013 The 8th argument is that one might expect a breakdown in dosage compensation to affect the
1014 homogametic more than the heterogametic sex in cases where DC involves an active mechanism in
1015 the homogametic sex, such as in mammals or *C. elegans* [6]. This argument does not hold up
1016 theoretically. The larger impact of DC disruption in the heterogametic sex holds up irrespectively of
1017 the type of DC (Eq. 7). Despite showing extensive misregulation in *Caenorhabditis* hybrids, especially
1018 involving males, and involving X-autosome and cis-trans coevolution, a hallmark of DC disruption,
1019 Sánchez-Ramirez et al. [46] excluded DC as an explanation based on the observation that expression
1020 levels were not strongly disrupted in females. This reasoning is based on the idea that DC is a female
1021 phenomenon, as it works by halving X expression in females. This argument (similar to that of Laurie)
1022 does not hold, since it is in fact both a male and a female phenomenon (the halving in females corrects
1023 X overexpression evolving in both males and females). Even with a *C. elegans*-like system, DC
1024 disruption will be larger in males than in females (Eq. 7).

1025 The 9th argument is that DC could play a role, but only in the case of species with fast and ongoing Y
1026 degeneration, dramatically reducing the scope for the general application of the DC hypothesis [48].
1027 Indeed, the effect of DC disruption has been understood as resulting solely from the mismatch of
1028 having non-functional (but dosage-compensated) genes on the Y in one species but not in the other [48].
1029 However, this is an incomplete picture, and overly restrictive, as DC disruption may also occur for genes
1030 that are degenerate in both species (Eq. 4, Fig 11).

1031 The 10th argument is that the DC hypothesis is supposed to entail that "*any* anomaly (not just sterility
1032 and inviability) appearing in hybrids results from a disturbance in dosage compensation" [4]. Since
1033 morphological anomalies in hybrids do not seem to be more severe in *Drosophila* hybrid males, it
1034 would then indicate that DC disturbance is not occurring [4]. This argument seems greatly exaggerated,
1035 in the sense that there is no reason to suppose that all hybrid problems are DC-related. For many traits,
1036 the genes involved may not be sex-linked, so there is no reason to expect that they all show an HR
1037 pattern.

1038 The 11th argument is that DC disruption could hardly predict partial hybrid sterility or inviability [4]. This
1039 would be true if DC was an all-or-nothing phenomenon, but, as our results show without ambiguity, a
1040 partial fitness reduction is very easy to obtain after short divergence. As is now better understood, DC
1041 occurs often on a gene-by-gene basis, even when a global DC system is in place [56,110]. This observation
1042 was not available at the time this critique was formulated, and is thus, now, less convincing than it
1043 was.

1044 The 12th argument is that "it is difficult to envision how the failure of dosage compensation could
1045 explain the sterility of hybrids that are viable and morphologically normal" [4]. In this view, disruption of
1046 dosage compensation should affect hybrid viability more than fertility [17]. This argument is close to the
1047 previous one, in the sense that DC is viewed as a global chromosomal level process. However, it would
1048 be fairly easy to observe sterile but viable hybrids: it only requires that DC disruption only involves a
1049 few sterility genes in recently derived species. The same explanation applies (and was applied) to the
1050 dominance theory: it would only involve the occurrence of recessive incompatibilities on sterility genes
1051 in recently derived species [94]. We also directly show how genes only expressed in the heterogametic
1052 sex (like genes involved in fertility) can produce a stronger fitness reduction in heterogametic F1,
1053 compared to genes expressed in both sexes.

1054 The 13th argument is that, in *Drosophila*, the absence of dosage compensation in the male germline
1055 excludes its disruption as a cause of hybrid male sterility [60]. This argument does not take into account
1056 that some form of dosage compensation may be occurring in the germline, even if does not involve
1057 the global somatic DC mechanism. Some regulation seems to take place on both the X and Y in the

1058 germline, beyond the effect of MSCI [63,111]. If this regulation is local (on a gene-by-gene basis) instead
1059 of following the global somatic mechanism, it would rather facilitate the evolution of regulatory
1060 divergence between species on those genes. We showed that male-limited genes could play a
1061 disproportionate effect on HR. This observation would tend to reinforce this conclusion. If DC
1062 divergence of viability genes is limited (because of the global somatic DC mechanism that remains
1063 constant), it would exacerbate the role of HR of genes expressed in the germline that escape this global
1064 regulation.

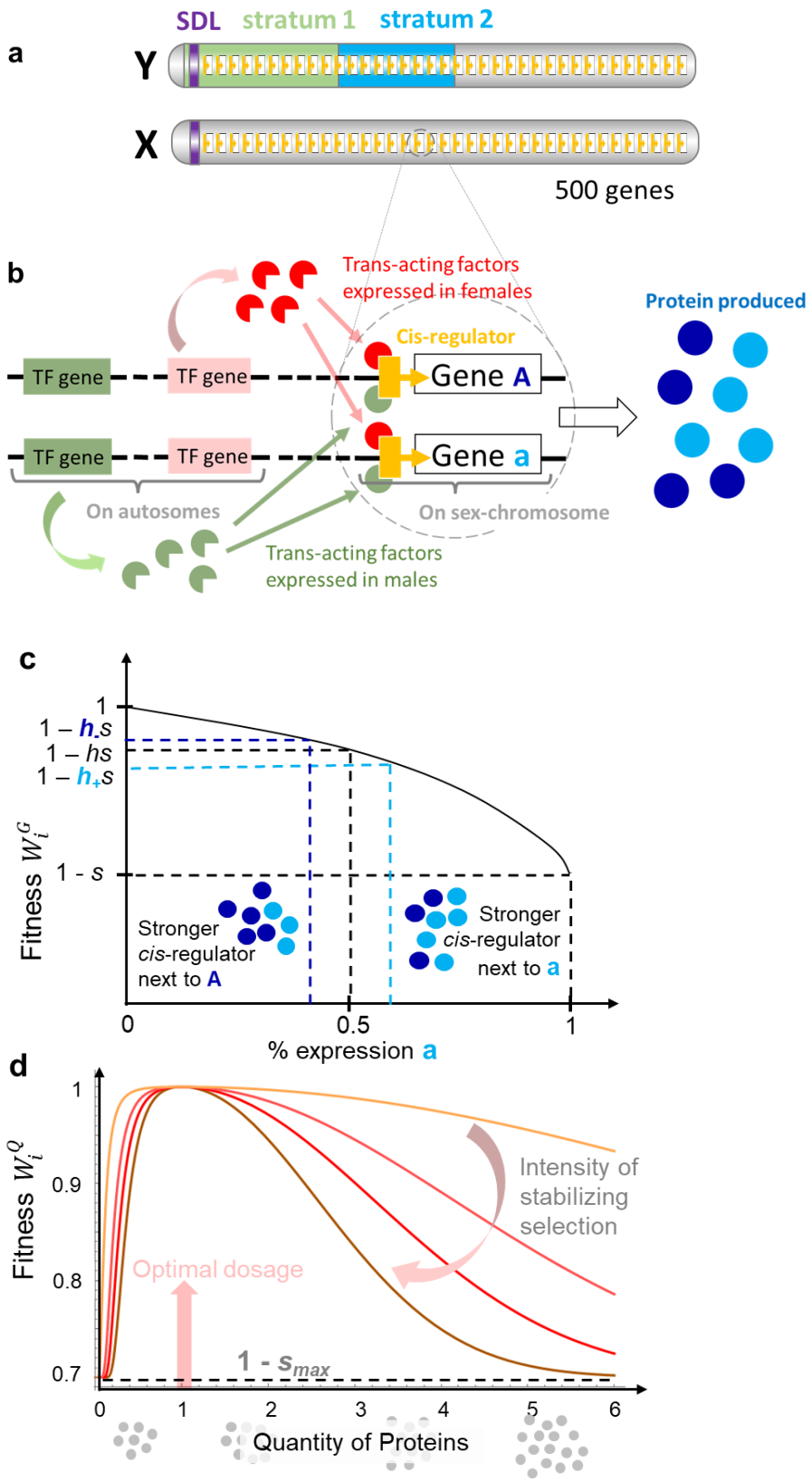


Fig. S1. Illustration of the model. (a) The model considers X and Y sex chromosomes with a sex determining locus (SDL) located at one end of the chromosome. The Y can evolves non-recombining strata (the figure illustrates a Y with two strata like on Figs S10a, S7-S9, in green and blue). 500 coding genes, each with its own cis-regulator are present on the Y and X. **(b)** The expression of each gene is symmetrically controlled by two autosomal trans-regulators, one expressed in males, one in females,

allowing for free evolution of dosage compensation. **(c)** Fitness effect of deleterious mutations: each allele of the coding gene expresses a given amount of protein determined by the trait values corresponding to its cis and trans-regulators (see methods). The relative proportion of protein produced from the two alleles modulates the dominance level of the deleterious alleles that could occur on the coding sequence as shown by the curve. **(d)** Fitness effect of expression level: The total amount of protein produced for a gene (as determined by trait values corresponding to its cis and trans-regulators) is under stabilizing selection around an optimal dosage, for each gene. Zero expression has a fitness effect of s_{max}, corresponding to the maximal fitness effect of deleterious mutations that can accumulate on that gene (such that knocking out a gene by accumulating deleterious mutations has the same effect as reducing its expression to zero). This s_{max} value is equal to 0.3 in all simulations.

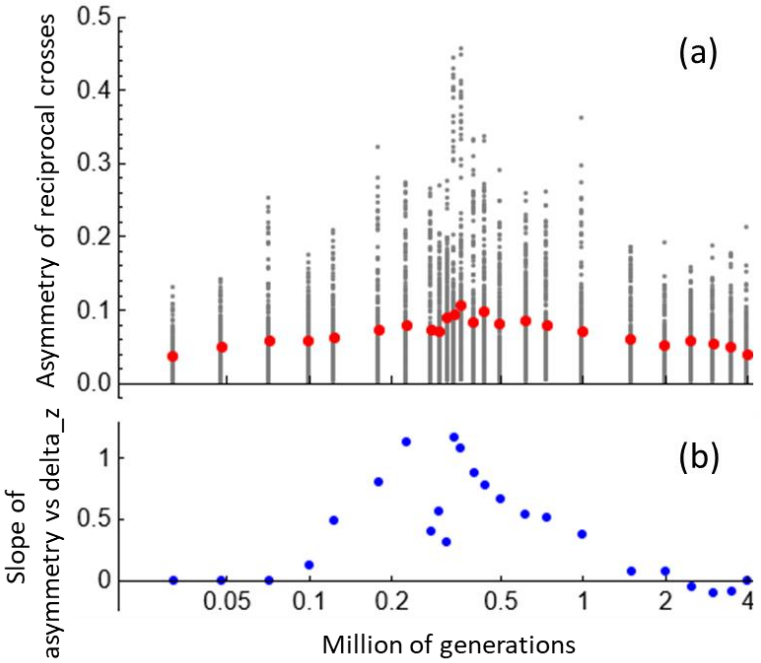


Fig S2. Darwin's corollary in the neo-Y scenario. (**a**) Crosses asymmetries in neo-Y simulations (same simulations as the one illustrated in Fig1B). The axes show the absolute value of the difference in the fitness of males produced in reciprocal crosses (y-axis) against time (x-axis, in millions of generations, in log-scale). A larger value means that the male fitness reduction is larger in one direction of the cross. Gray dots are the individual values obtained from all hybrids among independently evolving populations; red dots indicate mean values. (**b**) The graph shows the slope of the regression of the (signed) fitness difference between males produced in reciprocal crosses against the (signed) difference in the size of the non-recombining region (*z*) between the Ys in the two populations (y-axis). A positive slope indicates that males suffer more in the direction of the cross where the father carries a Y with a larger non-recombing region (i.e. a larger z value).

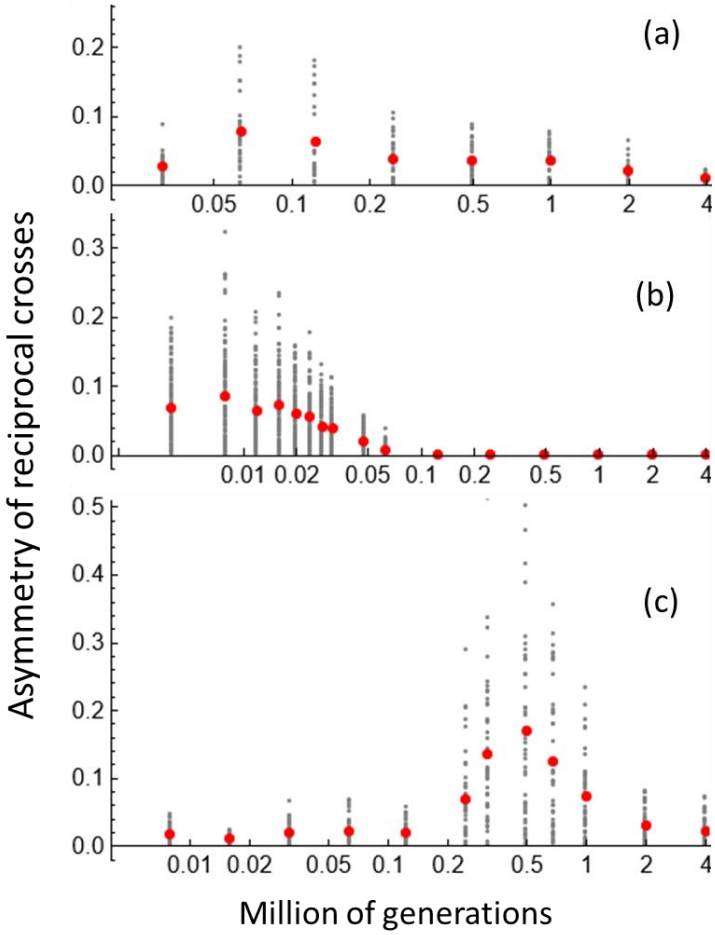


Fig S3. Darwin's corollary in other scenarios. (a) Crosses asymmetries in XO simulations (same simulations as the one illustrated in Fig1D). The graphs show the absolute value of the difference in the fitness of males produced in reciprocal crosses (y-axes) against time (x-axis, in millions of generations, in log-scale). A larger value means that the male fitness reduction is larger in one direction of the cross. Gray dots are the individual values obtained from all hybrids among independently evolving populations; red dots indicate mean values. **(b)** Same as in (a), but for male-limited simulations with 500 genes (same simulations as the unsorted case illustrated in Fig1E). (**c**): Same as in (a), but for male-limited simulations with 50 genes. The x-axis does not represent the exact same range of values in the three panels to better emphasize the period during which asymmetries are maximized.

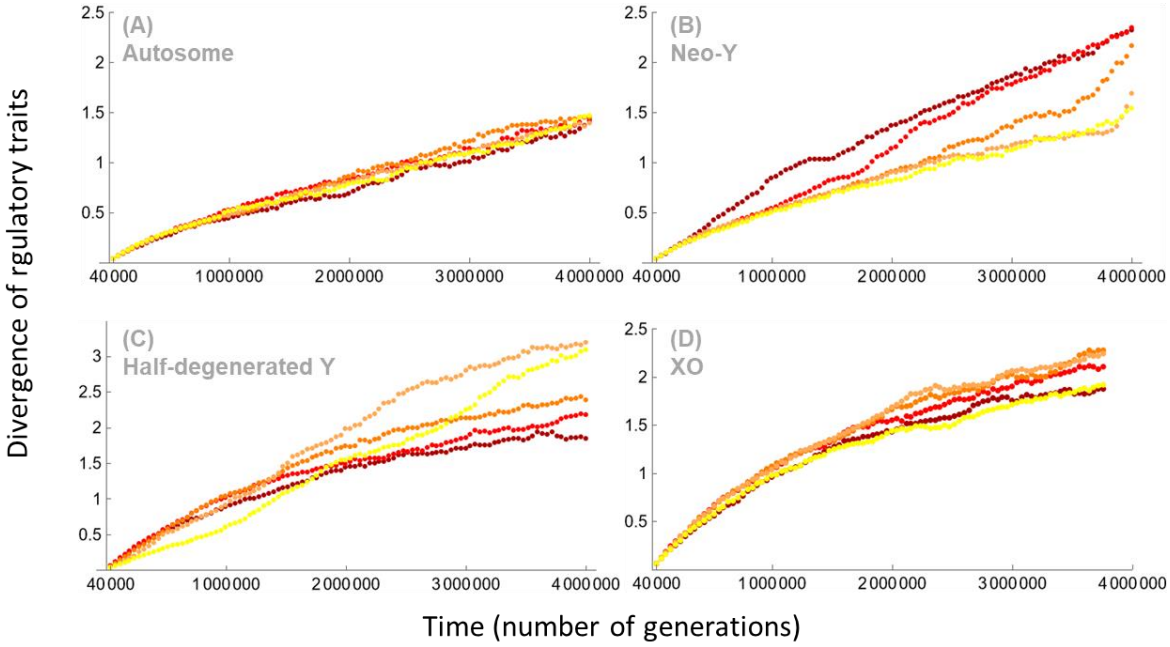


Fig. S4. Divergence of regulatory traits through time. The x-axis is time (number of generations). The y-axis is a measure of the divergence of regulatory traits through time in independently evolving species. To compute it, the chromosome is divided in five equally sized regions: region 1 (brown) includes genes 1 to 100, region 2 (red) genes 101 to 200, region 3 (orange) genes 201 to 300, region 4 (light orange) genes 301 to 400, region 5 (yellow) genes 401 to 500. The regulatory system for a given species and a given gene can be characterized by the vector $\mathbf{r} = (t_m, t_f, c_X)$, where t_m, t_f, c_X are the trait values for the male trans-regulator, the female trans-regulator and the cis-regulator on the X. On the Y, the cis-regulatory trait tends towards zero after recombination suppression and thus do not contribute to regulatory variation among independently evolving species. At a given time and for a given gene, we can therefore measure the regulatory divergence among independently evolving species as the mean over replicates *i* of $(\mathbf{r}_i - \bar{\mathbf{r}}).(\mathbf{r}_i - \bar{\mathbf{r}})$. This divergence is averaged every 40 000 generations for the 100 genes in the different regions mentioned above, and this is the value reported on the y-axis. The simulations are the same as those represented on Fig. 1: **(a)** Simulations with an autosome, **(b)** starting with a neo-Y, **(c)** starting with a half-degenerated Y, **(d)** with a fully degenerated Y. When recombination suppression evolves in some replicates, but not all, it causes a slight acceleration of regulatory divergence, as expected. This effect can be seen on simulations starting with a neo-Y (panel b). Recombination suppression occurs first in some replicates in the first region (brown), then in the second (red) etc. It can also be seen on simulations starting with a half-degenerated Y (panel c). Recombination suppression occurs first in some replicates in half of the third region for genes 250 to 300 (orange), then in the fourth (light orange) and the fifth (yellow).

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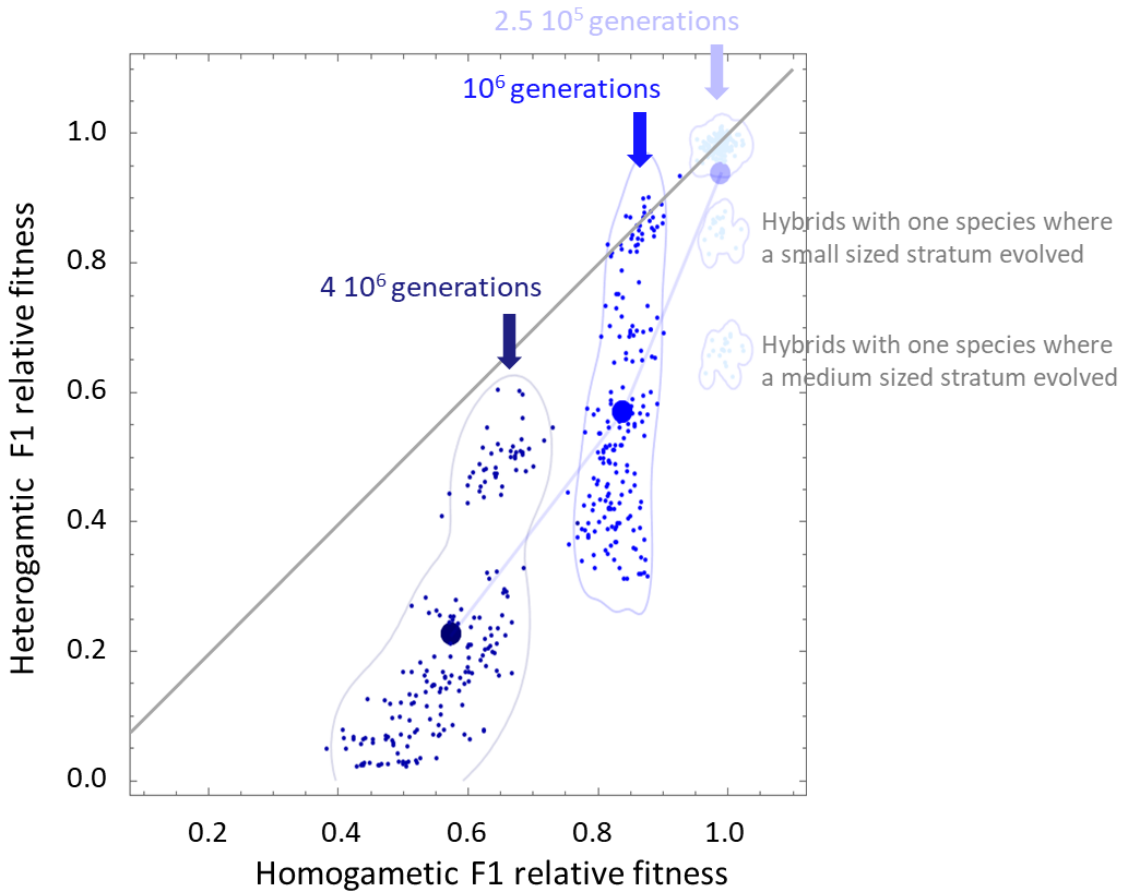

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1133 **Fig. S5. Detail of Fig 1B where a neo-Y evolves.** The figure shows the individual points and the
1134 corresponding contours.

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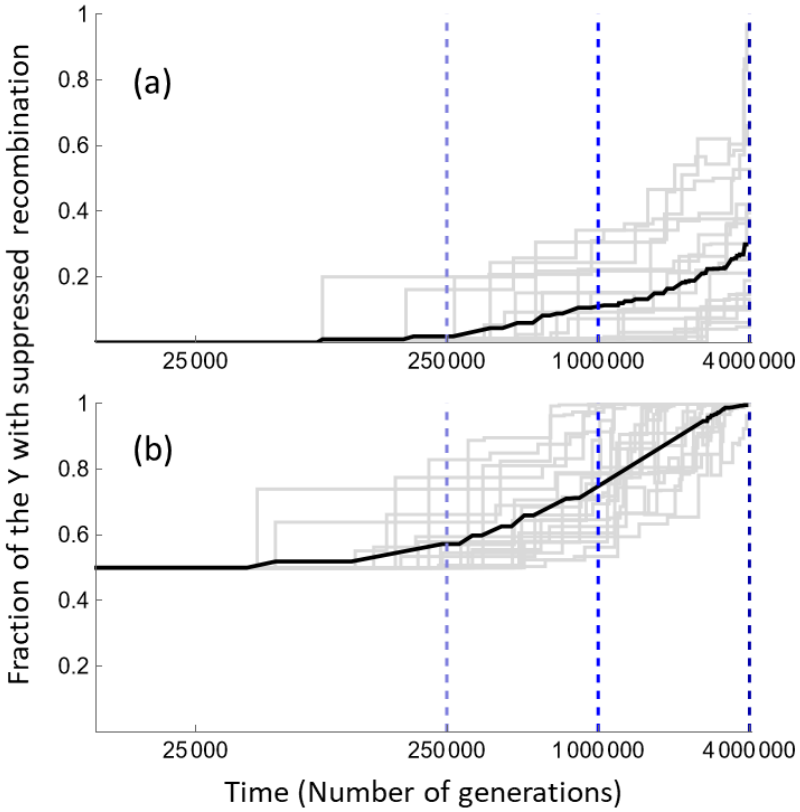

Fig S6. Evolution of recombination suppression in neo-Y and half-degenerated Y scenarios. (a) Evolution of recombination suppression in the neo-Y simulations corresponding to Fig 1B. The x-axis is time in number of generations, in log-scale, with the three time points illustrated on Fig 1 shown by the vertical dashed curves. The y-axis is the fraction of the Y with suppressed recombination corresponding to the accumulation of several strata. The gray lines represent individual replicates (F1 hybrids are generated by crosses among these replicates) and the black curve the mean across replicates. **(b)** Same as in (a), but for the simulations starting with a 'half-degenerated' Y, corresponding to Fig 1C.

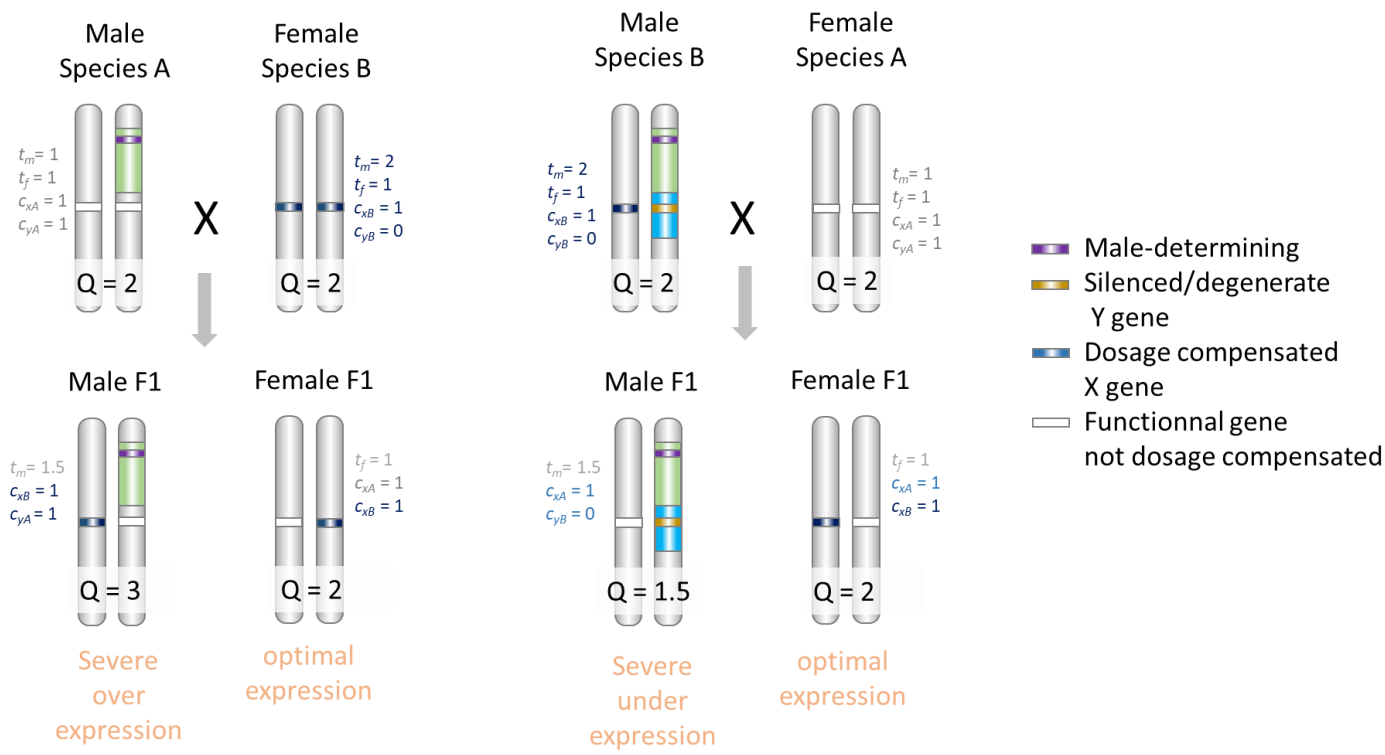

Fig S7a. Example of the effect of a gene located in a derived stratum present in only one species, with a drosophila-like DC mechanism. The figure shows species A and B, where the Y has a non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-recombining stratum only in species B (in blue), as in Fig S10a. The example focuses on a gene in the derived (blue) stratum. In species A, DC does not evolve (since the gene is located in a recombining portion of the Y), while in species B, DC is achieved for that gene, since it is located in the non-recombining region. In this example, DC in species B is achieved by having male overexpression of the X, like in *Drosophila* (i.e. by evolving a male-specific trans-acting factor increasing X expression in males, $t_m = 2$ in species B). Male and female expression levels is indicated by the value of Q. $Q = 2$ is the optimal expression level (Q is computed as $(c_{X,i} + c_{Y,i})\bar{t}_{m,i}$ and $(c_{X1,i} + c_{X2,i})\bar{t}_{f,i}$ in males and females, respectively, see methods). F1 males show a departure from optimal expression, while F1 females maintain optimal expression, in both directions of the cross. Note that the reduction in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over or underexpression.

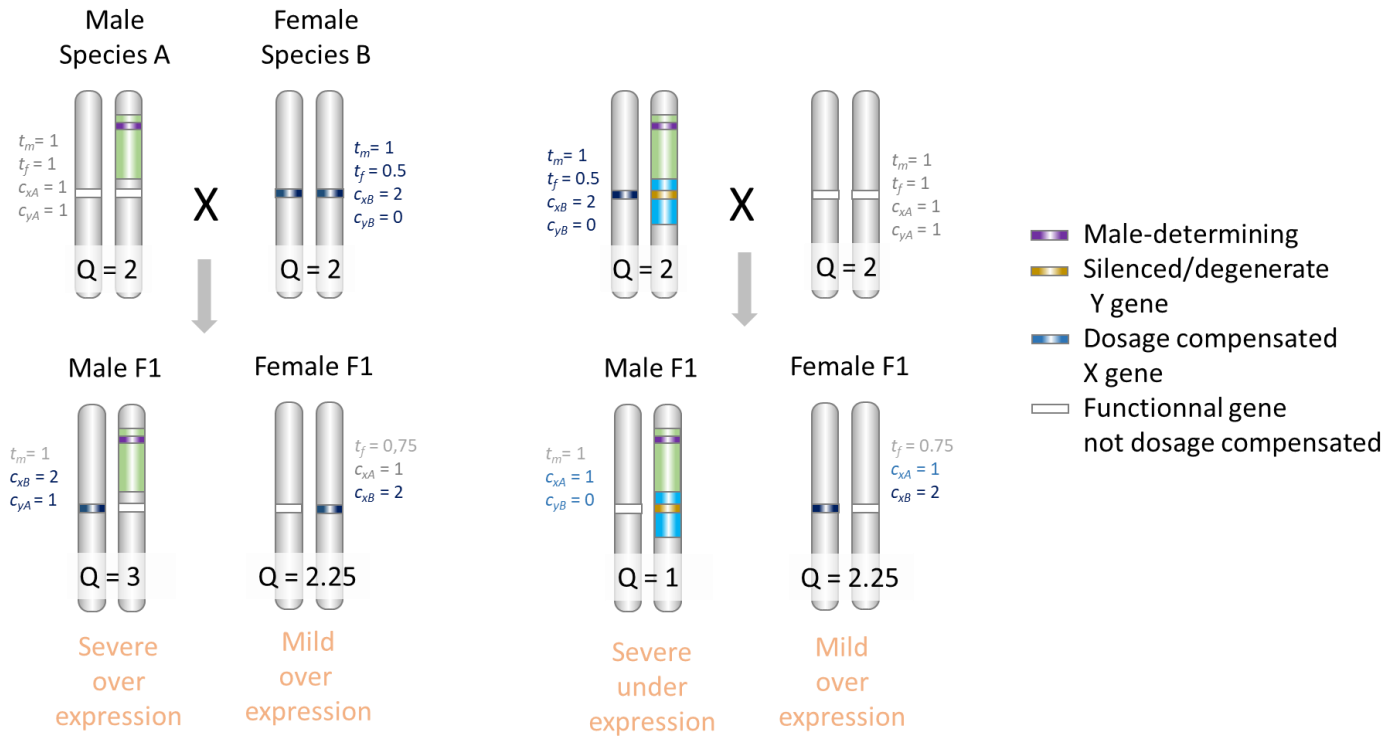

Fig S7b. Example of the effect of a gene located in a derived stratum present in only one species, with a mammal-like DC mechanism. The figure shows species A and B, where the Y has a non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-recombining stratum only in species B (in blue), as in Fig S10a. The example focuses on a gene in the derived (blue) stratum (like in Fig S7a). In species A, DC does not evolve (since the gene is located in a recombining portion of the Y), while in species B, DC is achieved for that gene, since it is located in the non-recombining region. The example shows a mammal-like pattern of DC for species B: DC in species B is achieved by having overall X overexpression (by evolving stronger X cis-regulatory elements, c_{xB} = 2 in species B) which is corrected in females to avoid overshooting (by evolving a female-specific trans-acting factor decreasing X expression in females, t_f = 0.5 in species B). Male and female expression levels is indicated by the value of Q. Q = 2 is the optimal expression level (Q is computed as $(c_{X,i} + c_{Y,i})\bar{t}_{m,i}$ and $(c_{X1,i} + c_{X2,i})\bar{t}_{f,i}$ in males and females, respectively, see methods). F1 males show a greater departure from optimal expression than F1 females. Note that the reduction in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over or underexpression.

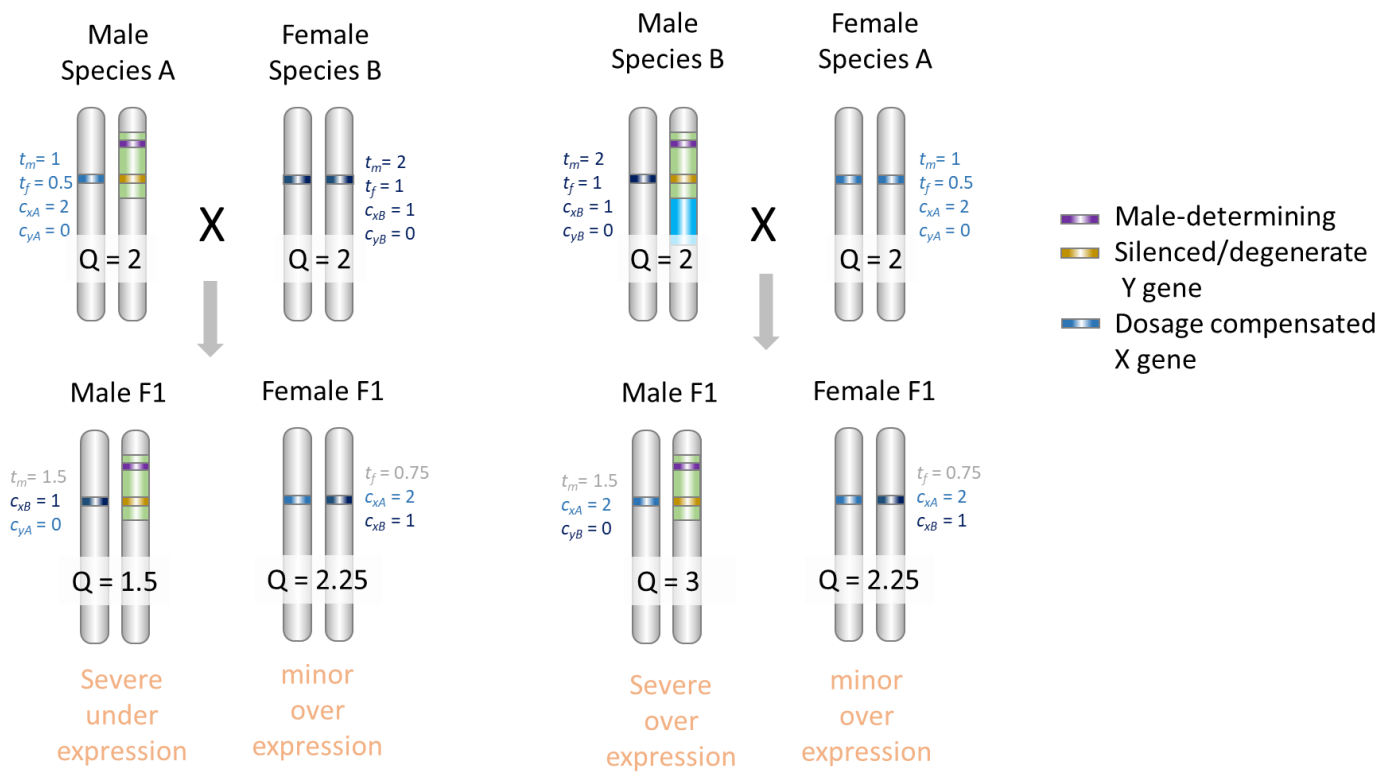

Fig S8. Example of the effect of a gene located in an ancestral stratum, with different DC mechanisms in the diverging species. The figure shows species A and B, where the Y has a non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-recombining stratum only in species B (in blue), as in Fig S10a. The example focuses on a gene in the ancestral (green) stratum. In species A, DC is achieved, for the focal gene, by having overall X overexpression (by evolving a stronger X cis-regulatory element, c_{xA} = 2 in species A) which is corrected in females to avoid overshooting (by evolving a female-specific trans-acting factor decreasing X expression in females, t_f = 0.5 in species A). In species B, DC is achieved, for the same gene, by having male overexpression of the X (i.e. by evolving a male-specific trans-acting factor increasing X expression in males, t_m = 2 in species B). Male and female expression levels is indicated by the value of Q. Q = 2 is the optimal expression level (Q is computed as $(c_{X,i} + c_{Y,i})\bar{t}_{m,i}$ and $(c_{X1,i} + c_{X2,i})\bar{t}_{f,i}$ in males and females, respectively, see methods). Male F1 show a greater departure from optimal expression than female F1, in both directions of the cross. Note that the reduction in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over or underexpression.

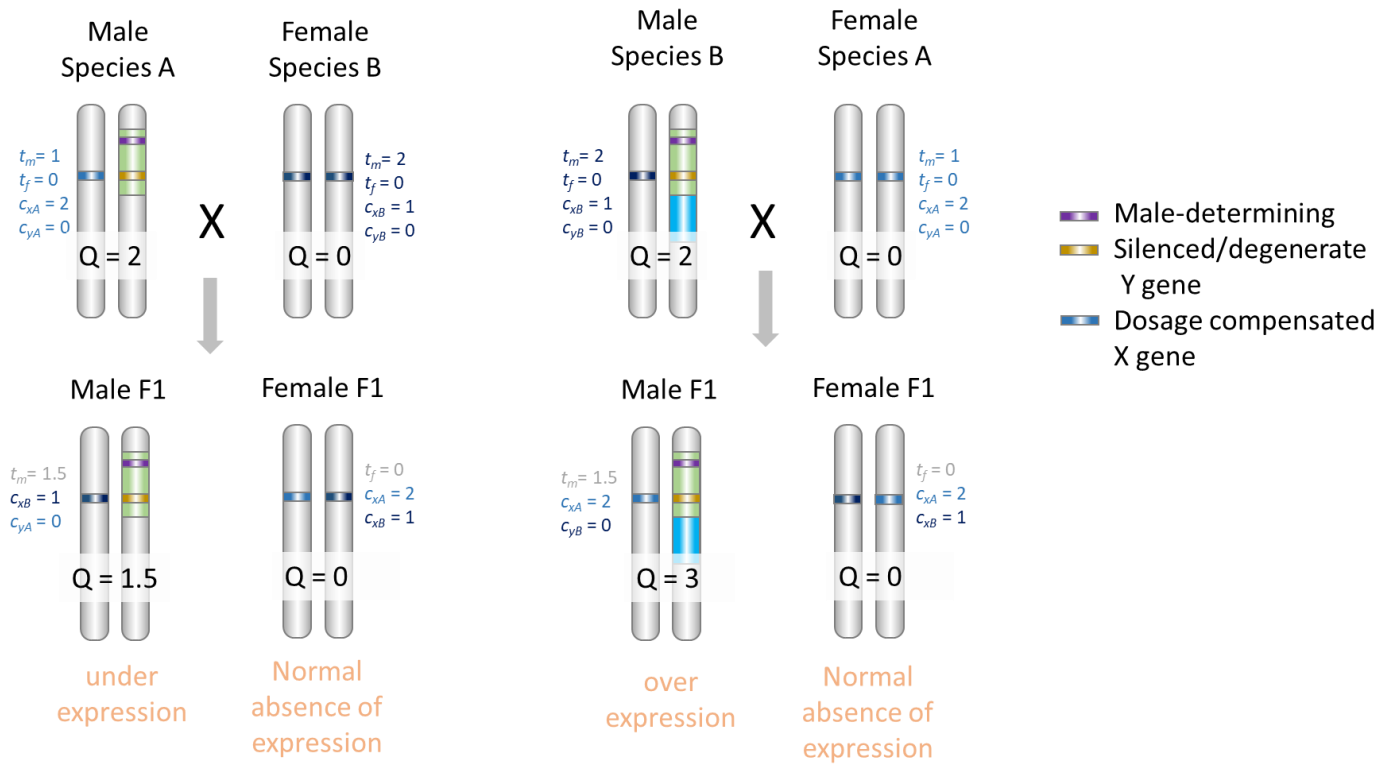

Fig S9a. Example of the effect of a male-limited gene located in an ancestral stratum, with different DC mechanisms in the diverging species. The figure shows species A and B, where the Y has a non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-recombining stratum only in species B (in blue), as in Fig S10a. The example focuses on a male-limited gene in the ancestral (green) stratum. The gene is not expressed in females. In species A, DC is achieved, for the focal gene, by having overall X overexpression (by evolving stronger X cis-regulatory elements, c_{xA} = 2 in species A) which is corrected in females to avoid overshooting (by evolving a female-specific trans-acting factor decreasing X expression in females, t_f = 0.5 in species A). In species B, DC is achieved, for the same gene, by having male overexpression of the X (i.e. by evolving a male-specific trans-acting factor increasing X expression in males, t_m = 2 in species B). Male and female expression levels is indicated by the value of Q. Q = 2 is the optimal expression level (Q is computed as $(c_{X,i} + c_{Y,i})\bar{t}_{m,i}$ and $(c_{X1,i} + c_{X2,i})\bar{t}_{f,i}$ in males and females, respectively, see methods). Male F1 show a departure from optimal expression in both directions of the cross. Note that the reduction in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over or underexpression. Note also that this situation is identical to Fig S7, ignoring females.

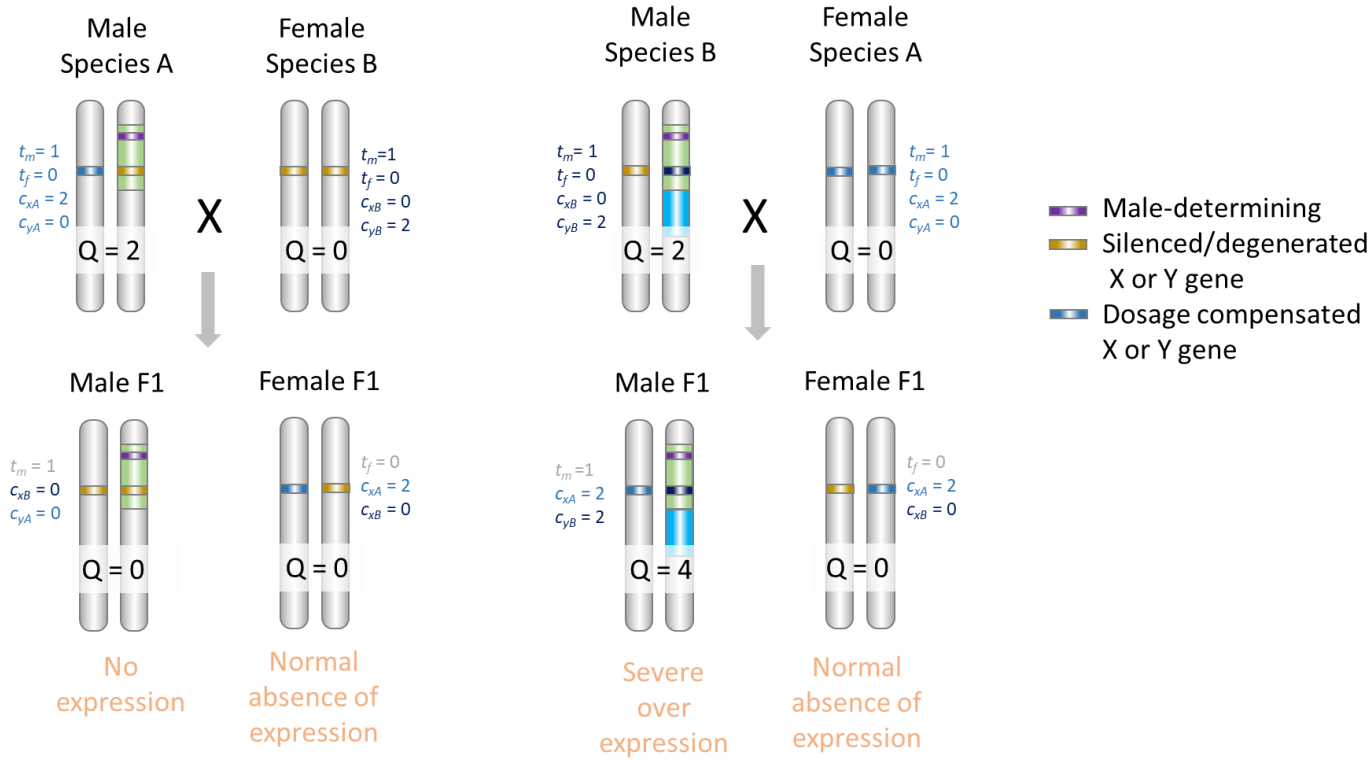

Fig S9b. Example of the effect of a male-limited gene located in an ancestral stratum, with alternate X or Y degeneration in the diverging species. The figure shows species A and B, where the Y has a non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-recombining stratum only in species B (in blue), as in Fig S10a. The example focuses on a male-limited gene in the ancestral (green) stratum which is silenced on the Y in species A (c_{yA} = 0), but silenced on the X in species B (c_{xB} = 0). Indeed, male-limited genes can degenerate both ways, which differs from non-sex-limited cases shown in Fig S7 and S8 where degeneration can only occur on the Y. The gene is not expressed in females. In both species, DC is achieved by having male overexpression of either the X or Y, through the evolution of stronger cis-regulators (c_{xA} = c_{yB} = 2). Male F1 show a strong departure from optimal expression in both directions of the cross, notably in one direction where the gene is completely silenced. Note that the reduction in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over or underexpression.