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1 A single theory for the evolution of sex 2 chromosomes and the two rules of 3 speciation 4

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

15 **Abstract**

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

25 **Main text**

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

35 Presently, the consensus is that HR and LX are composite phenomena with multiple genetic and
36 evolutionary causes (1–3, 6, 8–10, 16–19). While some theories have received more support than
37 others, none offer a general solution—each failing to account for some observations. For example, a
38 prominent explanation for the two rules is the Dominance theory, first suggested by Muller (20) and

39 later formalized (21, 18, 22), based on the idea that genetic incompatibilities involving at least one
40 gene located on the X chromosome (or Z, in species with female heterogamety) may more strongly
41 affect the fitness of the heterogametic sex if these incompatibilities are on average partially recessive.
42 However, the theory does not explain why incompatibilities should be, on average, recessive (21, 23),
43 although fitness landscape models propose possible solutions (24–26). Furthermore, it does not
44 explain well why HR often involves sterility rather than viability (2, 27), why it is observed in groups
45 lacking a hemizygous X (3), or in groups where XX females only express one X, as in marsupials (28) or
46 placentals (although in the latter case, both Xs may be expressed at the level of tissues, 17). Other
47 theories better accounting for the importance of hybrid sterility in HR have their own major limitations.
48 The “faster male theory” explains well why male sterility often occurs in hybrid crosses (2, 18, 27) and
49 why it may occur in species lacking hemizyosity (3), but critically fails to account for HR in species
50 where females are the heterogametic sex (16–18). The “meiotic drive theory” explains well why sex
51 chromosomes could play a major role in the sterility of heterogametic hybrids (29–31). It has received
52 some empirical support (31–33) but does not offer a convincing explanation for HR for viability (12,
53 16). Lastly, while sex chromosome degeneration is another globally observed and intensely studied
54 phenomenon, none of these theories consider the processes leading to degenerate sex chromosomes
55 and their subsequent evolution to be related to the emergence of either HR or LX.

56 We recently proposed a new theory for the evolution of non-recombining, degenerated, and dosage-
57 compensated sex chromosomes based on XY regulatory divergence and the early emergence of dosage
58 compensation (DC) (34–36). We show here that the same theory also predicts Haldane’s rule and the
59 large X effect. In the present study, we followed the simulated independent evolution of 15 species
60 using this previously described model (34). It consists of individual-based stochastic simulations of a
61 population of diploid individuals, with XY males and XX females (all the arguments below also apply to
62 ZZ / ZW systems and large Z effect, but for simplicity we only discuss the XY case), incorporating
63 deleterious mutations occurring at many genes, the evolution of recombination and the evolution of
64 cis and trans regulation of gene expression (34). We estimated the rate of occurrence and the pattern
65 of hybrid incompatibilities by measuring the fitness of F1 hybrids among the species at different time
66 steps, under scenarios of sex chromosomes at different stages of their evolution (see Methods). This
67 work places more than a century of observations previously thought to have compartmentalized and
68 largely separate causes under a single unifying theory.

69 Figure 1 shows the decrease in fitness in male and female F1 hybrids relative to the fitness of male and
70 female offspring from within species crosses. HR is rapidly observed in all cases, with strong
71 asymmetries between the fitness of male and female F1 hybrids. LX also occurs in all cases compared
72 to autosomes of equivalent size (compare Fig 1A to Fig1B-E). Genes with an effect limited to the
73 heterogametic sex (i.e., involved in fertility rather than viability) also contribute to HR and LX, and in
74 many cases, very strongly (Fig 1E). In (37), we show that asymmetries between reciprocal crosses often
75 occur, as observed in “Darwin’s corollary” to HR (11). In this model, autosomes also contribute to
76 hybrid breakdown, but less strongly and without generating an asymmetry between the homo and
77 heterogametic sex (Fig 1A and (37)). This outcome results from the coevolution of cis- and trans-
78 regulators of gene expression, which has repeatedly been emphasized as a mechanism generating
79 hybrid incompatibilities (38–48).

80 In this model, regulators on sex chromosomes evolve rapidly due to recombination arrest,
81 chromosomal degeneration and the emergence of dosage compensation (34). As a consequence,
82 differences in DC can rapidly evolve between species and cause dysfunctional DC in hybrids. But why
83 would dysfunctional DC disproportionately impact the heterogametic sex (HR) and cause a large X
84 effect? For the latter, the answer is straightforward: if dysfunctional DC inordinately reduces hybrid

85 fitness in the heterogametic sex, then the X chromosome, which is the only chromosome evolving DC,
86 will necessarily have a disproportionate effect on hybrid fitness compared to autosomes (both in terms
87 of the number and impact of genes involved in hybrid incompatibilities). Regarding HR, the disruption
88 of DC can be caused by the portion of the Y that is degenerate and compensated in only one of the
89 two hybridizing species (Fig 1B, 1C) or by the portion of the Y that is degenerate and compensated in
90 both species (Fig 1C, 1D), provided they exhibit some divergence in DC. We detail each case in turn.

91 First, consider the case where species a evolves a new non-recombining stratum on the Y not present
92 in species b . If a gene g is degenerate and compensated in species a but not in species b , then hybrid
93 males will suffer from under-expression if they are $g_a^Y g_b^X$, and overexpression if they are $g_a^X g_b^Y$,
94 assuming codominant effects of the trans-acting factors. In the first case, these hybrids miss half the
95 trans-acting factor required to fully achieve DC with a degenerated g_a^Y copy. In the second case, the
96 hybrids inherit trans-regulators from species a increasing expression of gene g , despite having two
97 functional copies of the g gene. In all cases, however, expression is closer to the optimum in females,
98 regardless of the direction of the cross. This applies to the case where DC evolves by doubling
99 expression of the gene g in males through a male-specific trans-acting factor (as with the drosophila
100 DC mechanism, Fig S5a) or where X chromosomes have double expression, one being randomly
101 silenced in females (as in the mammalian DC mechanism, Fig S5b). This argument applies more
102 generally when evaluated quantitatively in a model encompassing these cases and their intermediates
103 (37). The male/female fitness ratio is decreased proportionally to the number of genes on the stratum,
104 the intensity of stabilizing selection on gene expression, and a constant that depends on the DC
105 mechanism. The effect described here is similar to the verbal argument of Filatov (49), although
106 clarifying the role of regulators.

107 Consider the case of two species that inherited from their common ancestor a portion of the Y that is
108 non-recombining, degenerate, and dosage-compensated. In this case, DC disruption continues to
109 cause a major fitness decrease in the heterogametic F1. In this scenario, the fitness decrease occurs
110 when the DC mechanism, for a focal gene, has diverged between the two species. In F1 hybrids, these
111 compensations will average out in females (who receive half the autosomal trans-acting factors and
112 half the X cis-acting factors from each species) but not in males, who will have cis-acting factors on the
113 X from a single species, but averaged trans-acting factors on autosomes (Fig S4). Males will consistently
114 strongly under or overexpress compared to females. A quantitative model shows the reduction in
115 male/female F1 fitness is proportional to the number of genes on the stratum, the intensity of
116 stabilizing selection on expression levels, and the between-species variance in the trait governing DC
117 (37). This difference in averaging between sexes is similar to the mechanism generating HR in models
118 of quantitative traits coded by multiple loci with additive effects (25, 26). However, while these
119 previous models may be seen as a particular case of the dominance theory (as they assumed a
120 mutation on the X has the same effect when hemizygous in males and when homozygous in females),
121 this is not the case in our model, since a mutation on a cis-regulator on the X does not interact with
122 the same trans-regulators in males and females ((37) and Fig S1d).

123 Sex-limited fertility genes can also contribute to the decrease in hybrid fitness of the heterogametic
124 sex, with some interesting specificities. Female-limited genes on the X retain a standard diploid
125 expression. The coevolution of their cis and trans regulators can slowly diverge between species, but
126 this effect is relatively weak and comparable to what is observed on autosomes (37). The effect is much
127 larger for male-limited genes, and even larger than with genes expressed in both sexes (Fig 1E). We
128 also need to distinguish between cases where male-limited genes are present on a recently derived
129 non-recombining portion of the Y in one species or are ancestral to both. In the first case, gene
130 expression divergence between X and Y-linked copies of genes present in the new stratum will

131 generate a fitness cost for male hybrids in a similar way as for the non-sex-limited case (Fig S5a).
132 However, a difference with this non-sex-limited case is that either the X or the Y-linked copy may be
133 silenced (while extinction of the X copy could not occur for genes expressed and required in both
134 sexes). In the second case, male-limited genes may already have been silenced (either on the X or Y
135 chromosome) before the split between the two species, in which case the divergence of DC
136 mechanisms between species will generate a fitness cost for F1 males (Fig S6a). However, genes
137 retaining diploid expression at the time of the split may strongly reduce the fitness of F1 males. Indeed,
138 if the X-linked copy is silenced in one species while the Y-linked copy is silenced in the other, F1 males
139 will either show a complete lack of expression or major overexpression of that gene (Fig S6b). A single
140 essential gene in this situation could therefore cause male sterility in the heterogametic F1.

141 Hence, HR, its corollary for reciprocal crosses (37), and LX could be caused by misregulation of sex-
142 linked gene expression in hybrids. Although this idea has been repeatedly discussed (4, 6, 10, 19, 49–
143 53), and is close to the initial suggestion of Muller (54), it has never been quantitatively formalized.
144 While most authors have been cautious about completely rejecting this hypothesis, it has been usually
145 dismissed as an important explanation (8, 9, 16) based on various arguments that we list and critically
146 evaluate in (37). Most of the 13 criticisms we list seemed either questionable or outdated (for instance,
147 the idea that ZZ/ZW systems do not have DC while exhibiting HR). However, two arguments remain
148 relatively strong and require scrutiny.

149 The first states that in species with an ancestral ‘global’ (i.e., chromosome-wide) DC mechanism, there
150 may be little scope for divergence in DC in recently diverged species. This would make a theory based
151 on diverging DC less effective, especially in pairs of species where the same portion of the Y is non-
152 recombining and where the cis-regulators involved in global DC do not seem to have diverged.
153 However, DC disruption may still occur if target cis-regulatory sequences do not change but move
154 location on the X (in systems like *Drosophila* with high-affinity sites ‘HAS’ targeted by the MSL complex
155 (55)) or if some genes escape the global DC mechanism. Current evidence indicates that chromosome-
156 wide DC is the exception, not the norm, and even in these cases, many genes may escape global DC
157 (56–59). As our theory shows, these genes should be scrutinized for their role in decreased hybrid
158 fitness. Furthermore, global somatic DC is often absent from the germline, where some DC
159 nevertheless occurs for some genes (60–64). This is observed independently of the mechanism of
160 meiotic sex chromosome inactivation (MSCI), which might have specifically evolved to control sex-
161 chromosome meiotic drive during early meiosis (65, 66), and disruption of which has also been
162 suggested to contribute to HR (13, 67–69). This lack of a global DC mechanism for germline-limited
163 genes in the heterogametic sex could explain why male fertility genes are major contributors to HR.
164 The fact that male-limited genes can degenerate on the X and remain on the Y could also be a potent
165 factor preventing somatic DC from being “used” in the germline: upregulating the X is certainly not
166 fitting compensation for Y-linked genes expressed in the male germline. Interestingly, this would
167 predict that HR is most often based on hybrid sterility in groups with global DC, while more often based
168 on viability in groups lacking it — species evolving a global somatic DC mechanism will maintain local
169 DC only for sex-limited fertility genes, while species without global somatic DC will have many viability
170 genes with local DC. Indeed, birds and butterflies lacking global somatic DC (70) show more cases of
171 HR through inviability compared to mammals and Diptera (71).

172 The second argument concerns species with recombining sex chromosomes. In species without
173 hemizyosity, it has been shown that HR is weaker but present compared to species with a more
174 degenerated Y. This is the case, for example, in *Aedes* compared to *Anopheles* mosquitoes (3). This
175 example has been used to support the “faster male” theory and to rule out the dominance theory
176 alone could explain HR (3). The same argument would also argue against the theory described here.

177 However, as recent evidence points out, in *Ae. aegyptii* the sex “locus” is a 1.5 Mb region with 30 genes
178 in a 100 Mb non-recombining region encompassing the centromere of chromosome 1 showing some
179 divergence between males and females (72–74). Hence, these species may not entirely lack
180 hemizygosity. Like above, genes in this region should be scrutinized for their role in decreased hybrid
181 fitness. The other argument favoring the “faster male” theory is the overrepresentation of male
182 sterility for HR, which is not a pattern directly following the dominance theory (2, 27). However, our
183 results also show that fertility genes can play a disproportionate role on HR, without invoking a “faster
184 male” effect.

185 Beyond the faster male theory, the most strongly supported explanation for HR is the dominance
186 theory. Support for the dominance theory’s predictions come particularly from experiments in
187 *Drosophila* involving unbalanced females with attached X chromosomes. However, the DC disruption
188 theory makes similar predictions regarding these crosses (37), meaning they cannot discriminate
189 between theories. How do the dominance and DC theories compare beyond this? In marsupials and
190 placentals, dosage compensation works by inactivating one X (the paternal or a random one,
191 respectively). This is a difficulty for the dominance theory, as females are effectively hemizygous (like
192 males) in these cases. This is not a major concern in the DC theory, as HR is likely to result from
193 divergence in the global DC mechanism or from genes escaping global DC, whose regulation can more
194 easily diverge between species (including genes only expressed in the male germline). In the
195 dominance theory, recessive incompatibilities occurring on those genes escaping global DC may also
196 contribute to HR. However, if such genes are rare, this contribution would be negligible: the vast
197 majority of incompatibilities will concern genes subject to global somatic DC, so the fitness reduction
198 in males and females will tend to be similar. A second point relates to the underlying mechanism.
199 Contrary to the dominance theory, which simply poses that a fraction of genetic incompatibilities are
200 only expressed when one of the underlying loci is homo- or hemizygous (without providing a biological
201 mechanism that would generate this type of interaction), our theory is based on a biological model of
202 cis and trans regulator evolution, with underlying additive traits (37). Furthermore, direct empirical
203 evidence is accumulating showing DC disruption causes hybrid fitness reduction. For instance, in
204 *Drosophila*, the key elements for DC—the MSL complex and the MSL-binding sites on the X—are fast
205 evolving (51, 52, 75) as are *cis* and *trans* regulators of X expression (76). Indeed, Y-degeneration and
206 DC appear sufficiently rapid and species-specific in related species with young gene-rich sex
207 chromosomes for the DC theory to work (49). Evidence is also accumulating linking the misregulation
208 of sex chromosomes to HR in various hybrids (13, 69, 77–82).

209 Overall, and perhaps more importantly, HR has been viewed as a composite phenomenon, produced
210 by different causes (1, 2, 2–4, 6, 8–10, 16–19), possibly distinct from the large X effect (13, 18). As
211 Coyne once wrote about HR and LX, “biology is not physics” (17), emphasizing that given the
212 complexity of biological systems, unifying theories are unlikely. We suggest this conclusion should
213 perhaps be re-evaluated, given that a single model not only explains (1) recombination arrest on sex
214 chromosomes, (2) degeneration of the Y, (3) the evolution of dosage compensation, as we previously
215 showed (34, 35), but also why, upon hybridization, (4) the heterogametic sex suffers more than the
216 homogametic one, and (5) the X plays a disproportionate role in speciation. It may also explain why (6)
217 HR is often asymmetrical between reciprocal crosses, (7) HR often involves fertility genes, (8) somatic
218 and germline DC often differ, and why, (9) misregulation of gene expression on autosomes should
219 follow HR in time and eventually lead to complete reproductive isolation at a later stage of speciation.
220 This degree of generality and parsimony has few equivalents in biology and deserves to be tested
221 empirically.

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479

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487 **Supplementary material.**

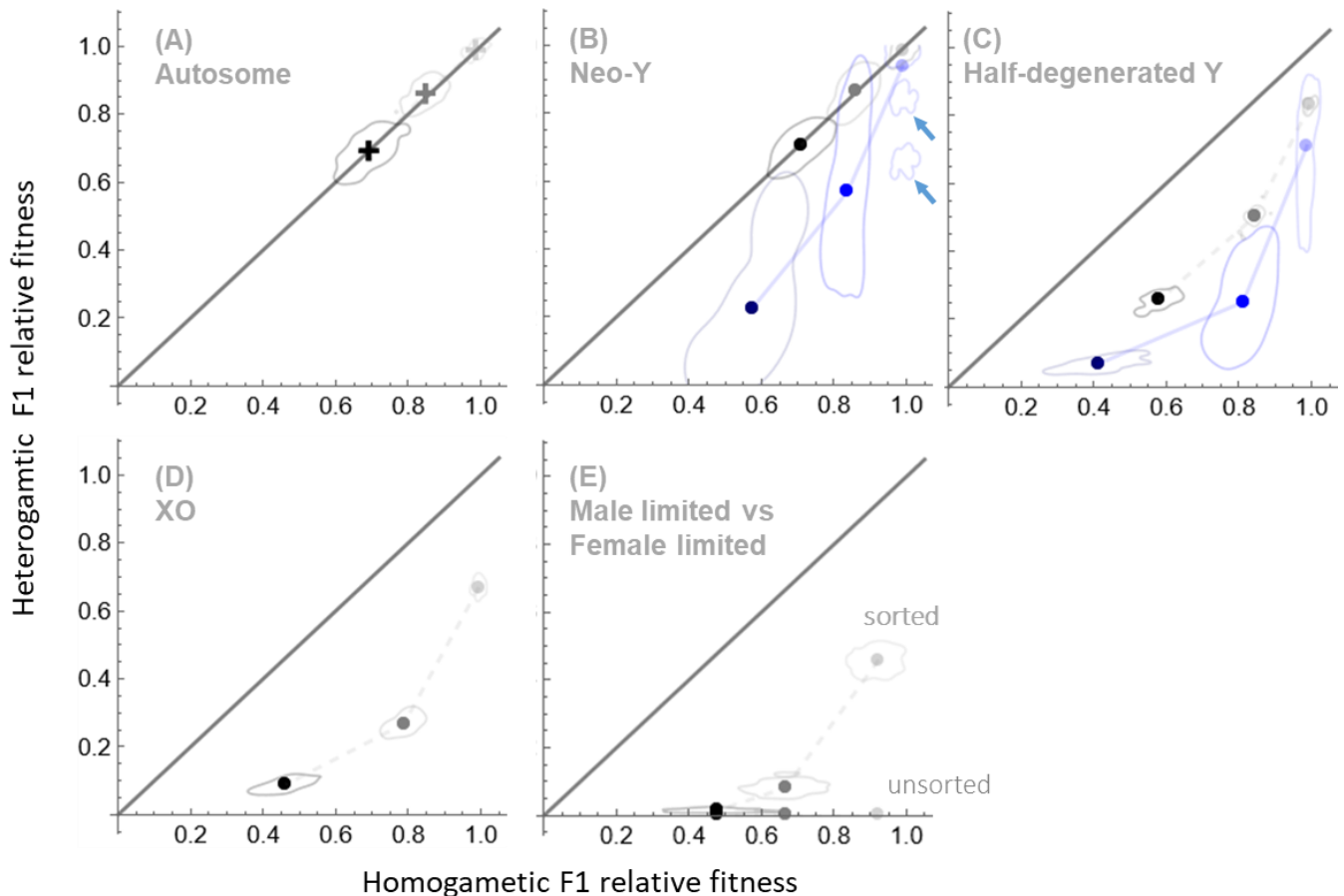
488 Methods

489 Supplementary Text

490 Figs. S1 to S7

491 References 83-111

492



493

494 **Fig. 1. Hybrid fitness in crosses between independently evolving species.** The figure shows the fitness
 495 of homogametic (x-axis) and heterogametic F1 (y-axis), between species that have evolved
 496 independently for 2.5×10^5 , 10^6 or 4×10^6 generations (light, medium, and dark color on each panel,
 497 respectively). The data are obtained in each case using 15 independently evolving species and the 105
 498 possible hybrid crosses (averaged in the two directions FxM and MxF for each of them). The fitness of
 499 hybrids is computed relative to the average fitness of intraspecific crosses for male and female
 500 offspring, respectively. Dots (or crosses for panel A) are mean values for all replicates. Contours
 501 represent the areas containing the individual values (the envelope is computed with a smoothed
 502 Gaussian kernel, see Fig S7 for an example). The $x=y$ line is added for visualization of the effects. HR
 503 corresponds to cases where points fall below this line (the fitness of the heterogametic sex is lower
 504 than that of the homogametic sex in F1 hybrids). LX corresponds to a larger effect in panels B-E than
 505 the one shown on panel A for autosomes. **(A)** Autosomal case. One fully recombining autosome (with
 506 500 genes, their cis-regulators, and their male and female trans-regulators) evolves in each species
 507 (even though all loci are autosomal, the simulation has males and females drawn randomly). **(B)** Neo-
 508 Y case. One initially fully recombining XY pair (with 500 genes, their cis-regulators, and their male and
 509 female trans-regulators) evolves in each species. In gray: inversions do not occur, so that the XY
 510 remains fully recombining throughout. In blue, inversions (and reversions) occur and recombination
 511 progressively stops between the X and the Y in each species independently. After 2.5×10^5 generations,
 512 only two species evolved a non-recombining stratum on the Y, and all hybrids involving those species
 513 had a lower fitness in the heterogametic sex (those points form two clouds of points, whose contours
 514 are indicated by the blue arrows, see also Fig S7). **(C)** Half degenerate case. As in (B) except that the Y
 515 is initially non-recombining, fully degenerate, and dosage compensated on half of its length, with $t_m =$
 516 2 (t_m is the trait value for trans-acting factors expressed in males); **(D)** As in (B) except that the Y is
 517 initially non-recombining, fully degenerate, and dosage compensated on all its length (which is

518 equivalent to a XX/XO system), with $t_m = 2$. In this case, there are no simulations where inversions
519 evolve since the Y is initially already fully non-recombining. (E) As in (D) except that the XY pair contains
520 either only female-limited genes (x-axis) or only male-limited genes (y-axis). These simulations are
521 performed independently and are represented on the same graph for better comparison with other
522 cases (i.e. when a simulation runs with only male-limited genes, the fitness of females stays at 1
523 throughout, and when a simulation runs with only female-limited genes, the fitness of males stays at
524 1 throughout, however the effect on females and males can be represented as paired). For male-
525 limited genes, two initial conditions are considered: (1) they are initially non-degenerate and with fair
526 diploid expression. During the course of these simulations, each male-limited gene will degenerate and
527 become silenced on either the X or the Y, independently in different populations. We term this initial
528 condition the “unsorted” case since it is initially not decided which gene will be lost on the Y or on the
529 X. Results show that c.a. 90% of genes end up being degenerate on the Y (and 10% on the X), and the
530 fitness of the heterogametic F1 drops very quickly toward a value close to zero. (2) In the second initial
531 condition we suppose that 50% of the genes are degenerate on the Y (and dosage compensated on
532 the X with $t_m = 2$) while 50% are degenerate on the X (and dosage compensated on the Y with $t_m = 2$).
533 We term this initial condition the “sorted case”, meaning that it is already decided which genes are
534 degenerate on the X or Y (and it is the same for all replicated populations).

535

536 Supplementary material

537 1 Methods

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

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

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

576 2 Computing effects

577 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
578 strength of the *trans*-regulators expressed in males, while $c_{X,i}$ and $c_{Y,i}$ are the strength of cis regulators
579 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.

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

582

$$583 \quad W_i^Q = 1 - s_{max} \left(1 - e^{-I(\ln Q_i - \ln Q_{opt})^2} \right) \quad (1)$$

584

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

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

592

$$593 \quad Q_{opt} = c_X \bar{t}_m = 2c_X \bar{t}_f \quad (2)$$

594

595 We therefore have $\bar{t}_m = 2\bar{t}_f$, which defines dosage compensation. Hence the way dosage
 596 compensation works (i.e. the triplet $\bar{t}_m, \bar{t}_f, c_X$) can be described by a single parameter (there is only
 597 one degree of freedom in the way DC occurs). We can choose e.g. to use \bar{t}_m for this description.
 598 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
 599 =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
 600 the X is inherently expressed twice as much ($c_X = 2$) to obtain optimal expression in males
 601 (hermaphrodites), while a female-limited *trans*-regulator halves expression ($\bar{t}_f = 0.5$) to recover
 602 optimal expression in females. This is also very similar to the mammal case where a female-limited
 603 *trans*-regulator halves expression by randomly silencing one X (rather than halving the expression of
 604 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
 605 the *Drosophila* case, where a male-limited *trans*-acting factor doubles X expression to obtain optimal
 606 expression in males (nothing being changed in females, $\bar{t}_f = 1, c_X = 1$).

607 **Contribution of autosomes to the fitness reduction of F1 hybrids**

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

614

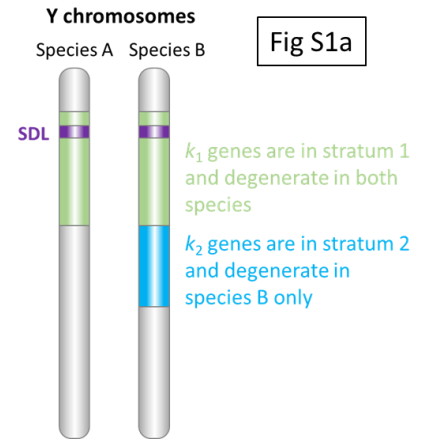
$$615 \quad W_{hybrid} = 1 - \theta(\Delta/2t_m)^4 \quad (3)$$

616

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

619 **Contribution of the XY to the fitness reduction of F1 hybrids**

620 We can compute the male/female fitness ratio in hybrids, accounting
 621 for two types of loci (Fig S1a). We consider first the Y-linked loci that
 622 are degenerate and silenced in both species (assume there are k_1 such
 623 loci). These loci are for instance located in a non-recombining region
 624 that is common to both species (green stratum 1 in Fig S1a, where the
 625 sex-determining locus, SDL, is located). Second, we consider loci that are
 626 degenerate and silenced in only one species (assume there are k_2 such
 627 loci). These loci are for instance located in a non-recombining region
 628 that only evolved in one species (blue stratum 2 of species B in Fig S1a).
 629 We compute the contribution to Haldane's rule of these two types of
 630 loci in turn.



631 **Contribution of an ancestral stratum (stratum 1)**

632 For loci that are degenerate in both species, the contribution of a given gene to the male/female fitness
 633 can be approximated assuming that the gene is fully silenced on the Y and dosage-compensated in
 634 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
 635 in t_m values between the two species for a given gene, and assuming that this difference is weak, we
 636 find that the contribution to Haldane's rule of that gene is approximately:

637

$$638 \quad \begin{aligned} \bar{W}_{male}/\bar{W}_{female} &= 1 - \theta(\Delta/2t_m)^2 \\ &= 1 - \theta \text{var}(\Delta)C_1 \end{aligned} \quad (4)$$

639

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

643 **Contribution of a derived stratum (stratum 2)**

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

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

651

$$652 \quad \bar{W}_{male}/\bar{W}_{female} = 1 - \theta\bar{C}_2 \quad (5)$$

653

654 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
 655 average over the two types of crosses, with:

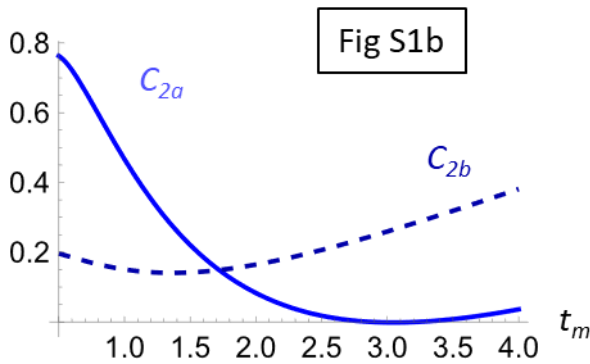
656

$$657 \quad C_{2a} = \text{Log} \left[\frac{(1+t_m)^2}{4} \right] - \text{Log} \left[\frac{(2+t_m)^2}{8t_m} \right] \quad (6)$$

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

658

659 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,
 660 Fig S1b).



661

662

663 The overall effect of strata 1 and 2

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

666

$$667 \quad \bar{W}_{male}/\bar{W}_{female} = 1 - \theta[k_1 C_1 \text{var}(\Delta) + k_2 \bar{C}_2] \quad (7)$$

668

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

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

677

$$678 \quad \text{Cross 1: } \frac{W_{male}}{W_{female}} = 1 - \theta[0.06k_1 \text{var}(\Delta) + 0.08k_2] \quad (8a)$$

$$679 \quad \text{Cross 2: } \frac{W_{male}}{W_{female}} = 1 - \theta[0.06k_1 \text{var}(\Delta) + 0.16k_2] \quad (8b)$$

680

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

682

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

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

685

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

690 Loci that are degenerate in one species but not in the other generate an expression mismatch in
691 hybrids, even if trans-acting factors are codominant. In the range of known DC mechanisms, i.e. with
692 t_m values between 1 and 2, hybrid females will tend to show slight overexpression, males from cross
693 1 will show relatively severe underexpression, and males from cross 2 relatively severe overexpression
694 (Fig S4-S6).

695 For loci that are degenerate in both species, the effect comes from the fact that genes may exhibit a
696 quantitative difference in the way they are dosage compensated (since achieving optimal DC can be
697 obtained by a different combination of cis and trans effects, i.e. different t_m values). This difference
698 will be however more buffered in females (where cis effects are averaged over the two X) than in males
699 (where cis effects are not averaged and only expressed from a single X, Fig S4). In both males and
700 females, (autosomal) trans-effects are equally averaged. In species pairs sharing an identical and
701 chromosomal-level DC mechanism, this effect is expected to be relatively minor, since genes should
702 not exhibit quantitative differences in the DC mechanism. However, in many species, many genes
703 escape this global DC mechanism and exhibit gene-level expression control (85–91). These genes, if
704 sufficiently numerous on the X could therefore also contribute to HR. Current evidence indicates that
705 chromosome-wide DC is the exception, rather than the norm (57–59).

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

711 **Case of unbalanced females**

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

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

722

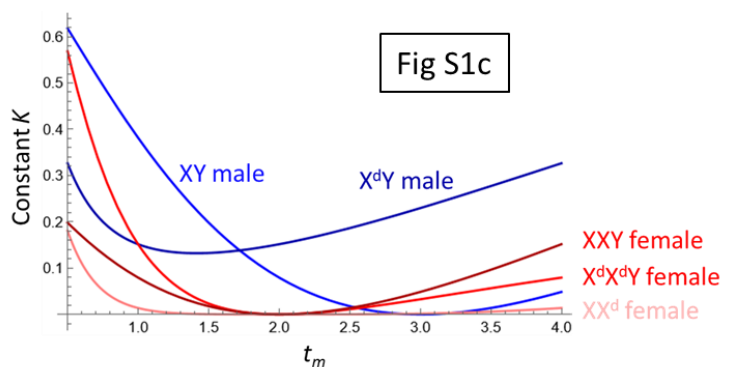
$$723 \quad W_{XY} = 1 - \theta(\Delta/2t_m)^2 + O(\Delta)^3 \quad (10a)$$

$$724 \quad W_{XX} = 1 - \theta(\Delta/2t_m)^4 + O(\Delta)^5 \quad (10b)$$

$$725 \quad W_{XXY} = 1 - \theta(\Delta/2t_m)^2 + O(\Delta)^3 \quad (10c)$$

726

727 For genes in that stratum, we find that
 728 unbalanced females should present the same
 729 fitness reduction as males, much larger than
 730 the one seen in regular F1 females. For genes
 731 located on stratum 2, degenerate and
 732 compensated only in species 2, and noting X^d
 733 the X from species 2, we find that the fitness
 734 reduction is proportional to θK , where K is a
 735 constant that depends on how X^d is dosage
 736 compensated (i.e. it depends on the value of
 737 t_m). The different values of K are straightforward to compute and are illustrated in Fig. S1c for the different F1 in the reciprocal crosses. Unbalanced females have a lower fitness than standard females except near $t_m = 2$ where the difference is small. Overall, combining effects in stratum 1 and 2, we can therefore conclude that this model predicts that unbalanced F1 females show a greater fitness reduction compared to standard females. The comparison to males depends on whether genes in stratum 1 or 2 contribute. For genes on stratum 1, the fitness reduction is the same as in males. For genes on stratum 2, it depends on the direction of the cross and t_m values (see Fig. S1c). Overall, these results indicate that unbalanced F1 females should often present a large reduction in fitness, much more similar to that of F1 males than that of F1 females. This result holds for viability. For sterility, the fitness of males and females (balanced or unbalanced) will differ, if genes involved in sterility are expressed in a sex-specific manner (see next section for the effect of such genes). As shown below, male-limited genes will cause a fitness reduction in F1 males, but female-limited genes will not cause a fitness reduction in F1 females. This would therefore explain the contradictory results observed for the *Drosophila* experiments mentioned above where unbalanced females are fertile when HR involves sterility but are inviable when HR involves viability (1, 92).



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 749 a fitness reduction in F1 females. This would therefore explain the contradictory results observed for
 750 the *Drosophila* experiments mentioned above where unbalanced females are fertile when HR involves
 751 sterility but are inviable when HR involves viability (1, 92).

752 **Case of sex-limited genes**

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

757 Female-limited genes can obviously be lost from the Y, as they are not expressed in males. Apart from
 758 this, their regulators can diverge between species, like in the case of autosomes, generating a
 759 consistent but small decrease in fitness in F1 (as in Eq. 3). Given the lower effective population size on
 760 the X (compared to autosomes), and the weaker selection pressure overall (everything else being
 761 equal, sex-limited genes are only selected in one sex), we might nevertheless expect that the

762 regulatory divergence could somehow be faster on the X compared to autosomes (as seen in the
763 results, compare the female fitness drop illustrated on Fig 1E to that on Fig 1A).

764 Male limited genes can be lost from the Y (like genes expressed in both sexes), but they can also be
765 lost from the X. Indeed, there is no requirement that they remain functional in XX females (contrary to
766 genes expressed in both sexes that can only evolve Y silencing (35)). Diploid expression is unstable
767 after recombination arrest, but silencing can occur both ways. Simulation results confirm this finding.
768 However, despite this apparent symmetry, X and Y silencing is not occurring at an equal rate. Male-
769 limited genes are more likely to be silenced on the Y than on the X. The bias is for instance close to 6:4
770 in a non-recombining stratum with 50 male-limited genes, but it is 9:1 in a stratum with 500 male-
771 limited genes (in both cases the simulation had a population size of 10 000 individuals, and the X or Y
772 silencing occurred relatively quickly). Presumably, the bias increases with the relative ease at which
773 deleterious mutations initially accumulate on the Y versus X once recombination has stopped (due to
774 selective interference), preferentially pushing the regulatory feedback loop in that direction. In all
775 cases, because one copy is silenced, these male-limited genes will evolve dosage compensation to stay
776 expressed at the right level in males (i.e. their cis-regulators on the X or Y will coevolve with the trans-
777 regulator expressed in males). In an F1 hybrid, when the focal gene has been silenced on the same
778 chromosome in both species, they will therefore cause a reduction in fitness that is similar to the case
779 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,
780 and $1 - p$ on the X, this situation has $p^2 + (1 - p)^2$ chances to occur. If the focal gene is silenced on
781 the Y in one species, but on the X in the other, male F1 will however suffer from a larger fitness
782 reduction. In one direction of the cross, the F1 male will receive two silenced copies, and its fitness will
783 therefore be reduced by a factor $1 - s_{max}$. This has $p(1 - p)$ chances to occur. For an essential gene
784 involved in male fertility, a single case like this would be sufficient to cause complete male sterility. In
785 the other direction of the cross, the F1 male will receive two non-silenced copies, which will correspond
786 to severe overexpression. Its fitness will be reduced by a factor $(1 - \theta \log(2)^2)$, which is close to $(1 -$
787 $\theta/2)$. This has also $p(1 - p)$ chances to occur. Simulation starting with an XY non recombining pair with
788 500 fully expressed male-limited genes leads to a dramatic reduction of F1 male fitness, due to this
789 sorting effect. Some genes become silenced on the X and some on the Y, but they are not the same in
790 the two diverging species, resulting in many mismatches. A simulation starting with 500 male-limited
791 genes that are already sorted leads to an F1 male fitness reduction closer to that observed for the XO
792 simulation with genes expressed in both cases. The effect is stronger, however (compare figures 1D
793 and 1E), as male-limited genes silenced on the X (and maintained on the Y) will exhibit faster regulatory
794 divergence, due to their lower effective population size (3 times lower than that of genes silenced on
795 the Y and maintained on the X).

796 Overall, we thus predict a stronger effect on HR of male-limited genes than of genes expressed in both
797 sexes. The argument was made for males but would apply to females in ZZ/ZW species. This finding
798 indicates that our regulatory theory could account for the importance of cases of sterility in HR,
799 without having to suppose a ‘faster male’ theory. It would also better explain why HR is not weaker in
800 Lepidoptera where males are homogametic [a difficulty of the faster male theory noted by Presgraves
801 (95)].

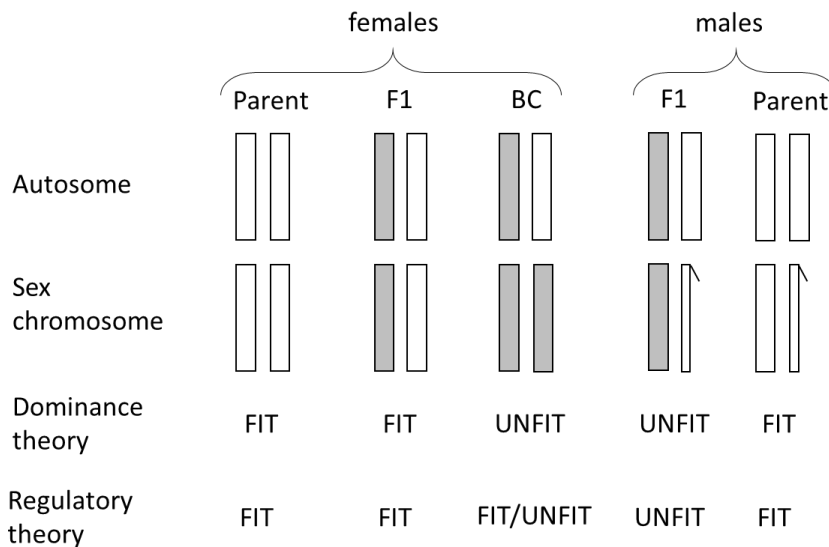
802 [3 Comparison of regulatory theory with the dominance theory](#)

803 The dominance theory for Haldane’s rule proposes that recessive incompatibilities occur, such that, in
804 an hybrid F1 autosomal background, F1 females are fit while F1 males are unfit. The reason why F1
805 females are fit is that incompatibilities carried on the X are “recessive”, meaning that if the X were
806 homozygous (in an F1 hybrid background), these incompatibilities would manifest themselves. If such
807 females could be produced in a backcross or through genetic manipulations (such as the F1 females

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

835 In our regulatory model, F1 males also show a reduced fitness compared to F1 females (Haldane's
836 rule), but the reason is not a “recessive” effect, in the sense described above. Unlike in the dominance
837 theory, the effect of the F1 genetic background differs between males and females: they express
838 different trans-acting factors, both in the parental species and in the male and female hybrids. Hence,
839 the fitness of hemizygous males is not necessarily the same as the fitness of “homozygous” females;
840 this is particularly clear in the example shown in Figure S5a, where F1 females that would be made
841 homozygous for the X would retain optimal expression, contrarily to F1 males. Generally, the
842 disruption of dosage compensation always impacts more the heterogametic than the homogametic
843 F1, although the effect varies with the type of dosage compensation and the position of the loci
844 involved (on an ancestral or derived stratum, see Eq. 7). The difference between the fitness of F1 males
845 and “homozygous” females also varies with type of dosage compensation and the position of loci,
846 although “homozygous” females tend to have a lower fitness than F1 females (this is the case in the
847 examples shown on Figures 4 and S5b).

848



849

850 **Fig. S1d.** Simplified fitness patterns in the dominance versus regulatory theory. The chromosome with
 851 a hook represents the Y. A fundamental difference is that the fitness of “homozygous” females differs
 852 between the two theories (indicated by “BC” as these females can be obtained by backcrossing or by
 853 creating “attached X” F1 females in *Drosophila*). In the dominance theory, “homozygous” females
 854 exhibit the same fitness reduction as F1 males. In the regulatory theory, male and female fitness can
 855 always be uncoupled since they express different trans-regulators. The sex asymmetry results from
 856 lack of averaging of cis-regulator effects in males (see above).

857 4 Darwin’s corollary

858 The fitness of F1 hybrids often differs significantly between reciprocal crosses. For instance, Turelli and
 859 Orr (94) estimated that 15% of cases of Haldane’s rule in *Drosophila* involved a strong asymmetry (the
 860 male F1 being sterile or inviable in only one direction of the cross between the two hybridizing species).
 861 A similar pattern is seen in *Anopheles* (3) and could be even more prevalent in *Lepidoptera* (95) or
 862 *Silene* (11). Turelli and Moyle termed this pattern “Darwin’s corollary”, complementing Haldane’s rule
 863 and the large X effect in the rules of speciation (11). In our model, fitness asymmetries between
 864 reciprocal crosses often arise. The general reason for this asymmetry is that the heterogametic F1 will
 865 typically suffer from over-expression in one direction of the cross and from under-expression in the
 866 other. This pattern occurs in all cases (see detailed examples in Fig S4-S6). Everything being equal,
 867 under-expression is more deleterious than overexpression in our model, as is likely in most biologically
 868 plausible situations. In the extreme case, e.g. when a male-limited gene is entirely missing in one
 869 direction of the cross, male fitness is much more reduced than when its expression is doubled in the
 870 other direction of the cross (Fig S6b). The asymmetry in reciprocal crosses is particularly strong when
 871 a discrete event of large effect occurs in one species but not in the other (similarly Muller noted that
 872 asymmetry between reciprocal hybrids must involve loci of large effect, 96). This occurs for instance
 873 when a species acquires a new non-recombining stratum and not the other. In this case, the F1 male
 874 inheriting the more degenerate Y will suffer more than the F1 male in the reciprocal cross (Fig S5a,
 875 S5b). This effect will occur mostly at intermediate steps of Y chromosome degeneration, i.e. when
 876 species have different strata, and before F1 male fitness is not too strongly reduced in all crosses (Fig
 877 S2). Another case particularly conducive to strong fitness asymmetries in reciprocal crosses is when a
 878 male-limited gene is missing in one direction, as explained above. However, if there are many male-
 879 limited genes, then different genes will be lost on the X or Y in the different species, and the fitness of
 880 all F1 males will be reduced. Darwin’s corollary should be strongest when the number of male-limited
 881 genes on sex chromosomes is not too large, maximizing the sampling variance of those genes and

882 therefore the fitness asymmetries in the reciprocal crosses (compare Fig S3b and S3c, with 500 and 50
883 male-limited genes on the chromosome respectively). When the fitness reduction in F1 males results
884 from many small effects, fitness asymmetries in reciprocal crosses tend to be weaker. This is the case
885 for instance when regulatory divergence occurs on a chromosome that is already fully dosage-
886 compensated (see Fig S3a for XO simulations). Overall, our model predicts substantial fitness
887 asymmetries in reciprocal crosses, and at intermediate times of species divergence, which is in line
888 with the available observations.

889 5 Criticisms of the dosage compensation hypothesis for Haldane's rule

890 The 1st major critique of the DC hypothesis is that the rule was observed in groups in which DC was
891 allegedly absent (such as in birds and lepidopterans, 4, 6). However, DC has since been documented in
892 these groups (57, 59, although global DC is perhaps less frequent in ZW species, 97).

893 The 2nd argument is based on an experiment with *Drosophila* (50). However, this experiment was based
894 on crosses with mutants in which DC was supposedly fully functional, but without showing that this
895 was the case. Hence, the results presented could not really discard DC disruption as a cause of HR.
896 Specifically, it could not discard the possibility that hybrid male sterility was caused by a failure in DC
897 downstream of *Sxl* regulation (which is the sex-determining switch in *Drosophila*).

898 The 3rd argument is that DC evolves very slowly, being an essential function under strong constraints
899 (51). However, as our results show, divergence in DC can readily occur, even with substantial stabilizing
900 selection on expression levels. Note that DC evolution could be triggered by different phenomena,
901 including coevolution with cytoplasmic bacteria targeting DC pathways to achieve male killing (98–
902 100).

903 The 4th argument proposes that if Haldane's rule was caused by DC disruption, cis and trans regulators
904 involved in DC should exhibit signs of divergence between species exhibiting Haldane's rule. Yet, Jaffe
905 and Laird (101) reported unpublished data where the X-linked *D. pseudoobscura Hsp82* gene remained
906 dosage compensated when transformed into various autosomal sites in *D. melanogaster*, suggesting
907 conservation of the cis-regulatory elements involved in DC for ~20 million years. Indeed, if cis-
908 regulators involved in DC are highly conserved, they could not cause Haldane's rule (4, 50). However,
909 further evidence showed that this conclusion, besides applying only to *Drosophila*, was premature.
910 More recent investigations revealed that both cis and trans regulators involved in DC were actually
911 fast evolving in *Drosophila melanogaster*. This is the case with *msl*, *mof*, and *mle* trans-acting genes
912 (51, 75) as well as cis-acting binding sequences on the X (52). The case of male lethality in *D.*
913 *melanogaster* x *D. simulans* hybrids is not clear-cut. Some studies support the role of DC in male
914 lethality (102, 103) while others challenge this interpretation (104). In this cross, both males and
915 females show a reduction in viability, but to a different degree depending on temperature. However,
916 the effect of mutants is not always evaluated in this GxE context. Barbash shows that deletion of mel-
917 specific DC genes—ie, forcing X_{mel}/Y_{sim} hybrid males to develop using only *simulans* DC complex
918 components—does not exacerbate F1 male lethality when this lethality was first rescued by *lhr*
919 mutation. The test is based on the premise that the *lhr* rescue occurs and that playing with DC genes
920 should show an independent effect when manipulated. However, *lhr* might be acting by interacting
921 with these DC genes, so there may be no clear 'independent' manipulation of the DC phenotype in the
922 first place in the experiment. Barbash also argues that DC disruption is unlikely because a lower-than-
923 expected number of genes downregulated in lethal F1 males are located on the X chromosome.
924 However, only the misregulation associated with sex chromosomes will have a differential fitness
925 effect on males vs females. HR depends on the presence of the latter, not on the proportion of
926 misregulation of X versus autosomes. Whether there is more or less than "expected" misregulation on
927 the X says nothing about the cause of HR. What can be noticed, however, although it could be a

928 coincidence, is that major genes involved in these hybrid incompatibilities interact with the dosage
929 compensation complex. For instance, *Lhr* interacts with HP1, a chromodomain-containing protein that
930 localizes to heterochromatic regions of chromosomes (105) and is also involved in DC (106). Nuclear
931 pore complex proteins also cause hybrid male lethality (107, 108), and are also involved in DC (109).

932 The 5th argument is a refinement of the fourth. After the accumulating evidence demonstrating fast
933 DC evolution in *D. melanogaster*, Tang and Presgraves (108) suggested that this phenomenon was not
934 general and limited to that species. They concluded that the limited evolution of the MSL complex in
935 *D. simulans* could not well explain the rapid evolution of Nup160 and the other Nup107 subcomplex
936 genes in that species (these autosomal simulans alleles being involved in male hybrid lethality through
937 incompatibility with the melanogaster X). This may be the correct interpretation, and this specific case
938 may be regarded as mere exception to the general case. However, this argument can be nuanced by
939 several points, that might warrant further investigation. First, the *mof* gene involved in the DC complex
940 does show evidence of rapid adaptive evolution in *simulans* (75). Second, Nup160 and the other
941 Nup107 subcomplex genes are known to be involved in DC in *Drosophila* (109). Third, DC disruption is
942 not necessarily limited to the coevolution of the MSL complex and its cis-binding sites. For instance,
943 MSL cis-binding sites could change location on the X, thereby changing the pattern of DC, as shown by
944 the rapid and extensive turnover of individual binding sites of roX lncRNAs, which are essential for
945 *Drosophila* DC (55). Fourth, some genes are dosage-compensated but non-MSL-binding, suggesting
946 that MSL is not the only mechanism for achieving DC (86). This is also particularly the case for genes
947 expressed in the germline, impacting male fertility in *Drosophila* (64).

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

964 The 7th argument has not directly been made against the DC hypothesis but against the dominance
965 theory. However, this issue also concerns the DC disruption hypothesis. Haldane's rule is observed in
966 species lacking heteromorphic sex chromosomes. In particular, in the genus *Aedes*, Haldane's rule
967 applies to many interspecific crosses (3). Yet, *Aedes* have homomorphic sex chromosomes with a sex
968 locus. A priori this observation would rule out theories based on hemizygoty (dominance theory) or
969 DC. However, recent genomic data have revealed that in *Ae. aegyptii*, the sex "locus" is a 1.5 Mb region
970 with 30 genes in a 100 Mb non-recombining region encompassing the centromere of chromosome 1,
971 that diverged between males and females. Hence, the premise that these species have a sex
972 chromosome with just a sex-determining locus seems erroneous. More work is required to evaluate

973 the extent of hemizygoty and the occurrence of (local) DC in these species, but the conclusion
974 favoring the “faster male” hypothesis based on these *Aedes* data certainly requires re-evaluation.

975 The 8th argument is that one might expect a breakdown in dosage compensation to affect the
976 homogametic more than the heterogametic sex in cases where DC involves an active mechanism in
977 the homogametic sex, such as in mammals or *C. elegans* (6). This argument does not hold up
978 theoretically. The larger impact of DC disruption in the heterogametic sex holds up irrespectively of
979 the type of DC (Eq. 7). Despite showing extensive misregulation in *Caenorhabditis* hybrids, especially
980 involving males, and involving X-autosome and cis-trans coevolution, a hallmark of DC disruption,
981 Sánchez-Ramírez et al. (47) excluded DC as an explanation based on the observation that expression
982 levels were not strongly disrupted in females. This reasoning is based on the idea that DC is a female
983 phenomenon, as it works by halving X expression in females. This argument (similar to that of Laurie)
984 does not hold, since it is in fact both a male and a female phenomenon (the halving in females corrects
985 X overexpression evolving in both males and females). Even with a *C. elegans*-like system, DC
986 disruption will be larger in males than in females (Eq. 7).

987 The 9th argument is that DC could play a role, but only in the case of species with fast and ongoing Y
988 degeneration, dramatically reducing the scope for the general application of the DC hypothesis (49).
989 Indeed, the effect of DC disruption has been understood as resulting solely from the mismatch of
990 having non-functional (but dosage-compensated) genes on the Y in one species but not in the other
991 (49). However, this is an incomplete picture, and overly restrictive, as DC disruption may also occur for
992 genes that are degenerate in both species (Eq. 4, Fig 1D).

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

1000 The 11th argument is that DC disruption could hardly predict partial hybrid sterility or inviability (4).
1001 This would be true if DC was an all-or-nothing phenomenon, but, as our results show without
1002 ambiguity, a partial fitness reduction is very easy to obtain after short divergence. As is now better
1003 understood, DC occurs often on a gene-by-gene basis, even when a global DC system is in place (57,
1004 110). This observation was not available at the time this critique was formulated, and is thus, now, less
1005 convincing than it was.

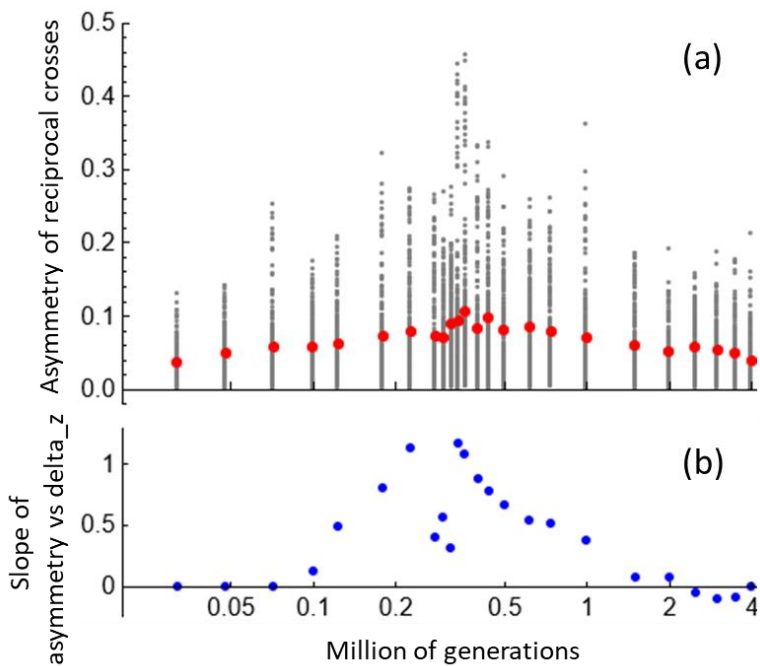
1006 The 12th argument is that “it is difficult to envision how the failure of dosage compensation could
1007 explain the sterility of hybrids that are viable and morphologically normal” (4). In this view, disruption
1008 of dosage compensation should affect hybrid viability more than fertility (17). This argument is close
1009 to the previous one, in the sense that DC is viewed as a global chromosomal level process. However, it
1010 would be fairly easy to observe sterile but viable hybrids: it only requires that DC disruption only
1011 involves a few sterility genes in recently derived species. The same explanation applies (and was
1012 applied) to the dominance theory: it would only involve the occurrence of recessive incompatibilities
1013 on sterility genes in recently derived species (94). We also directly show how genes only expressed in
1014 the heterogametic sex (like genes involved in fertility) can produce a stronger fitness reduction in
1015 heterogametic F1, compared to genes expressed in both sexes.

1016 The 13th argument is that, in *Drosophila*, the absence of dosage compensation in the male germline
1017 excludes its disruption as a cause of hybrid male sterility (61). This argument does not take into account

1018 that some form of dosage compensation may be occurring in the germline, even if does not involve
1019 the global somatic DC mechanism. Some regulation seems to take place on both the X and Y in the
1020 germline, beyond the effect of MSCI (64, 111). If this regulation is local (on a gene-by-gene basis)
1021 instead of following the global somatic mechanism, it would rather facilitate the evolution of
1022 regulatory divergence between species on those genes. We showed that male-limited genes could play
1023 a disproportionate effect on HR. This observation would tend to reinforce this conclusion. If DC
1024 divergence of viability genes is limited (because of the global somatic DC mechanism that remains
1025 constant), it would exacerbate the role of HR of genes expressed in the germline that escape this global
1026 regulation.

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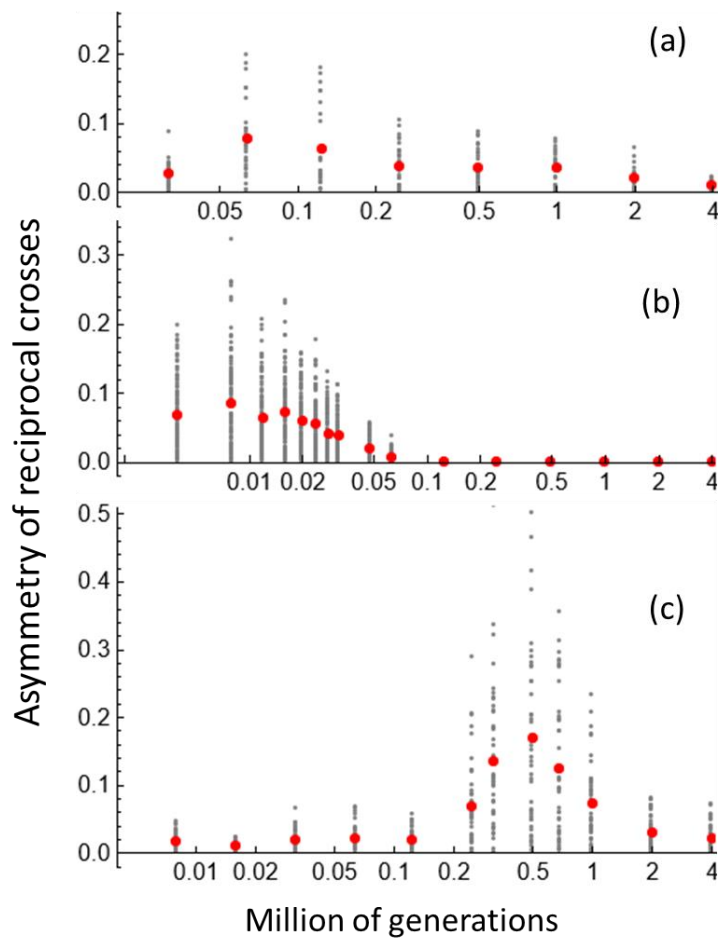
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1030 **Fig S2. Darwin's corollary in the neo-Y scenario.** (a) Crosses asymmetries in neo-Y simulations (same
1031 simulations as the one illustrated in Fig1B). The axes show the absolute value of the difference in the
1032 fitness of males produced in reciprocal crosses (y-axis) against time (x-axis, in millions of generations,
1033 in log-scale). A larger value means that the male fitness reduction is larger in one direction of the cross.
1034 Gray dots are the individual values obtained from all hybrids among independently evolving
1035 populations; red dots indicate mean values. (b) The graph shows the slope of the regression of the
1036 (signed) fitness difference between males produced in reciprocal crosses against the (signed)
1037 difference in the size of the non-recombining region (z) between the Ys in the two populations (y-axis).
1038 A positive slope indicates that males suffer more in the direction of the cross where the father carries
1039 a Y with a larger non-recombining region (i.e. a larger z value).

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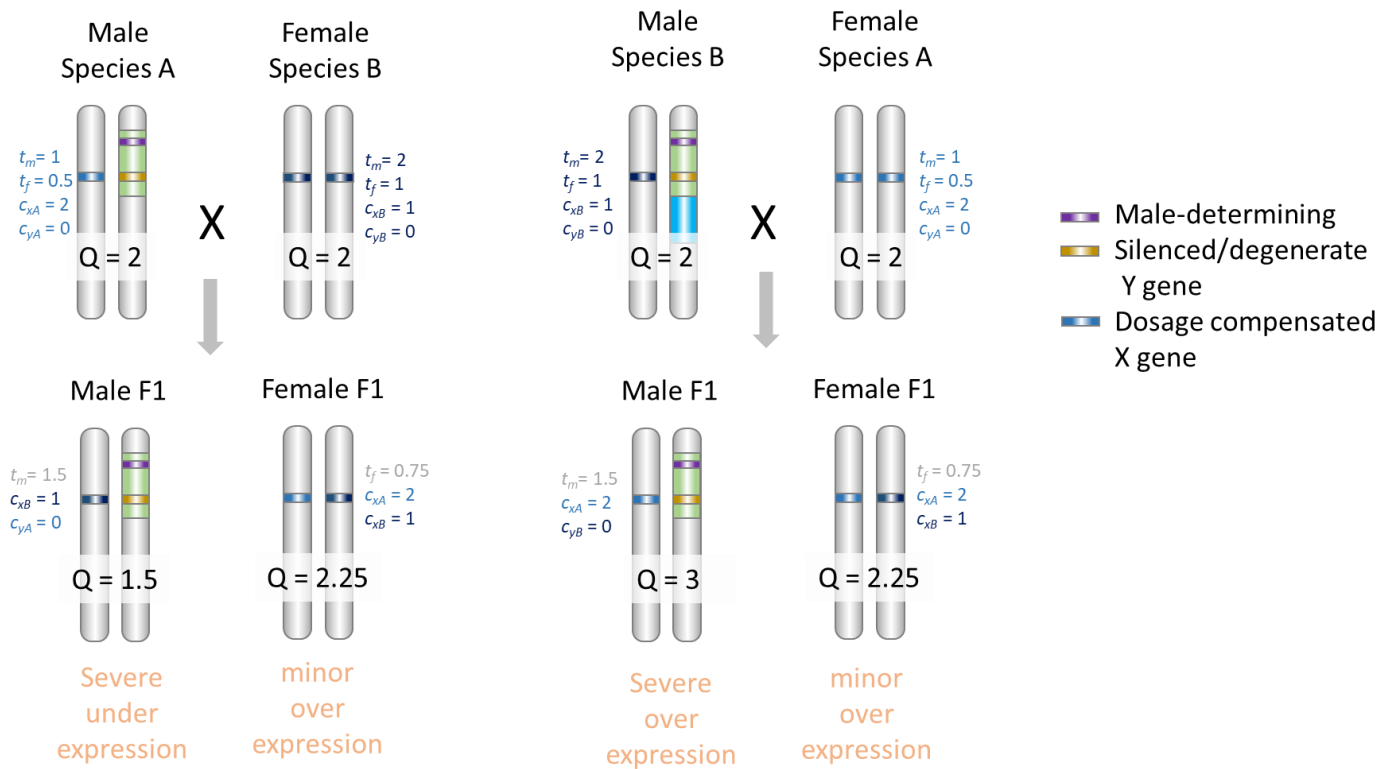


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1042 **Fig S3. Darwin's corollary in other scenarios. (a)** Crosses asymmetries in XO simulations (same
1043 simulations as the one illustrated in Fig1D). The graphs show the absolute value of the difference in
1044 the fitness of males produced in reciprocal crosses (y-axes) against time (x-axis, in millions of
1045 generations, in log-scale). A larger value means that the male fitness reduction is larger in one direction
1046 of the cross. Gray dots are the individual values obtained from all hybrids among independently
1047 evolving populations; red dots indicate mean values. **(b)** Same as in (a), but for male-limited
1048 simulations with 500 genes (same simulations as the unsorted case illustrated in Fig1E). **(c):** Same as in
1049 (a), but for male-limited simulations with 50 genes. The x-axis does not represent the exact same range
1050 of values in the three panels to better emphasize the period during which asymmetries are maximized.

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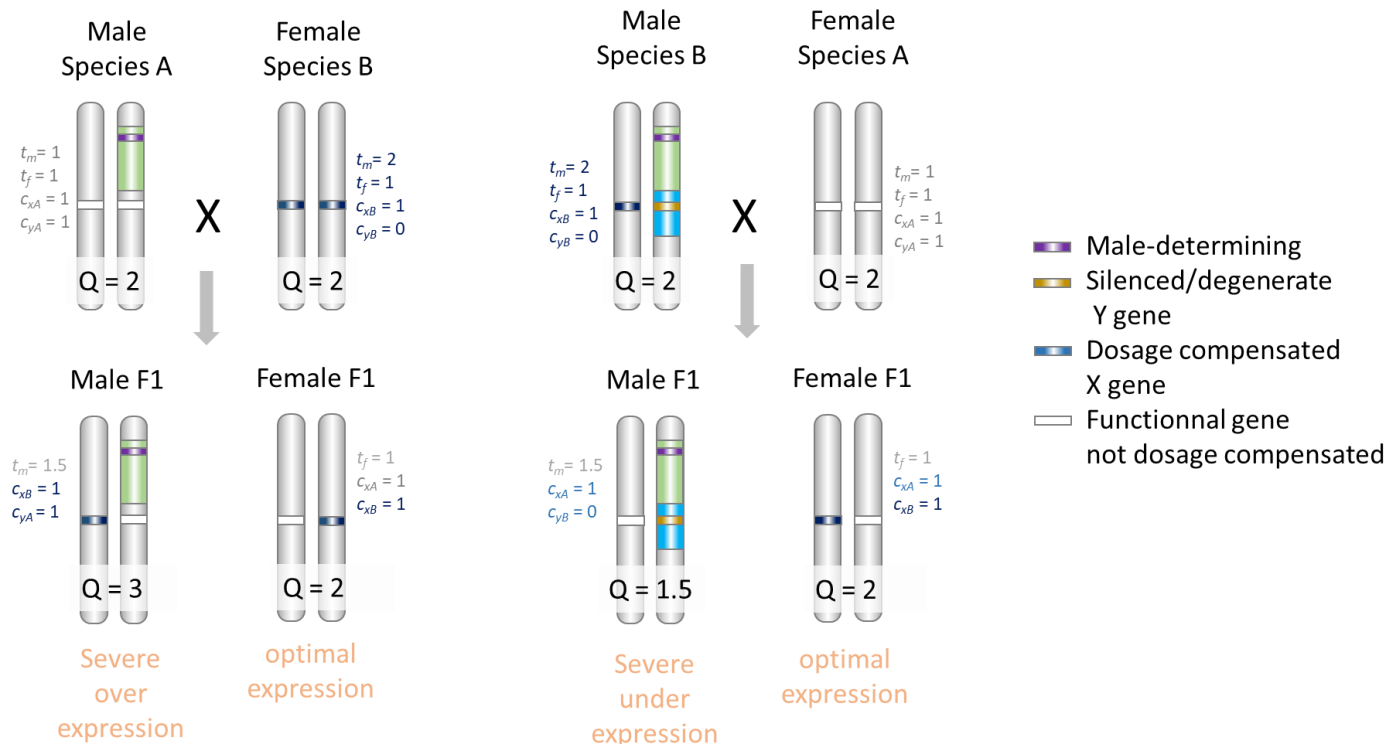


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1054 **Fig S4. Example of the effect of a gene located in an ancestral stratum, with different DC**
 1055 **mechanisms in the diverging species.** The figure shows species A and B, where the Y has a non-
 1056 non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-
 1057 non-recombining stratum only in species B (in blue), as in Fig S1a. The example focuses on a gene in the
 1058 ancestral (green) stratum. In species A, DC is achieved, for the focal gene, by having overall X
 1059 overexpression (by evolving a stronger X cis-regulatory element, $c_{xA} = 2$ in species A) which is
 1060 corrected in females to avoid overshooting (by evolving a female-specific trans-acting factor
 1061 decreasing X expression in females, $t_f = 0.5$ in species A). In species B, DC is achieved, for the same
 1062 gene, by having male overexpression of the X (i.e. by evolving a male-specific trans-acting factor
 1063 increasing X expression in males, $t_m = 2$ in species B). Male and female expression levels is indicated
 1064 by the value of Q . $Q = 2$ is the optimal expression level (Q is computed as $(c_{X1,i} + c_{Y,i})\bar{t}_{m,i}$ and
 1065 $(c_{X1,i} + c_{X2,i})\bar{t}_{f,i}$ in males and females, respectively, see methods). Male F1 show a greater
 1066 departure from optimal expression than female F1, in both directions of the cross. Note that the
 1067 reduction in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting
 1068 from either over or underexpression.

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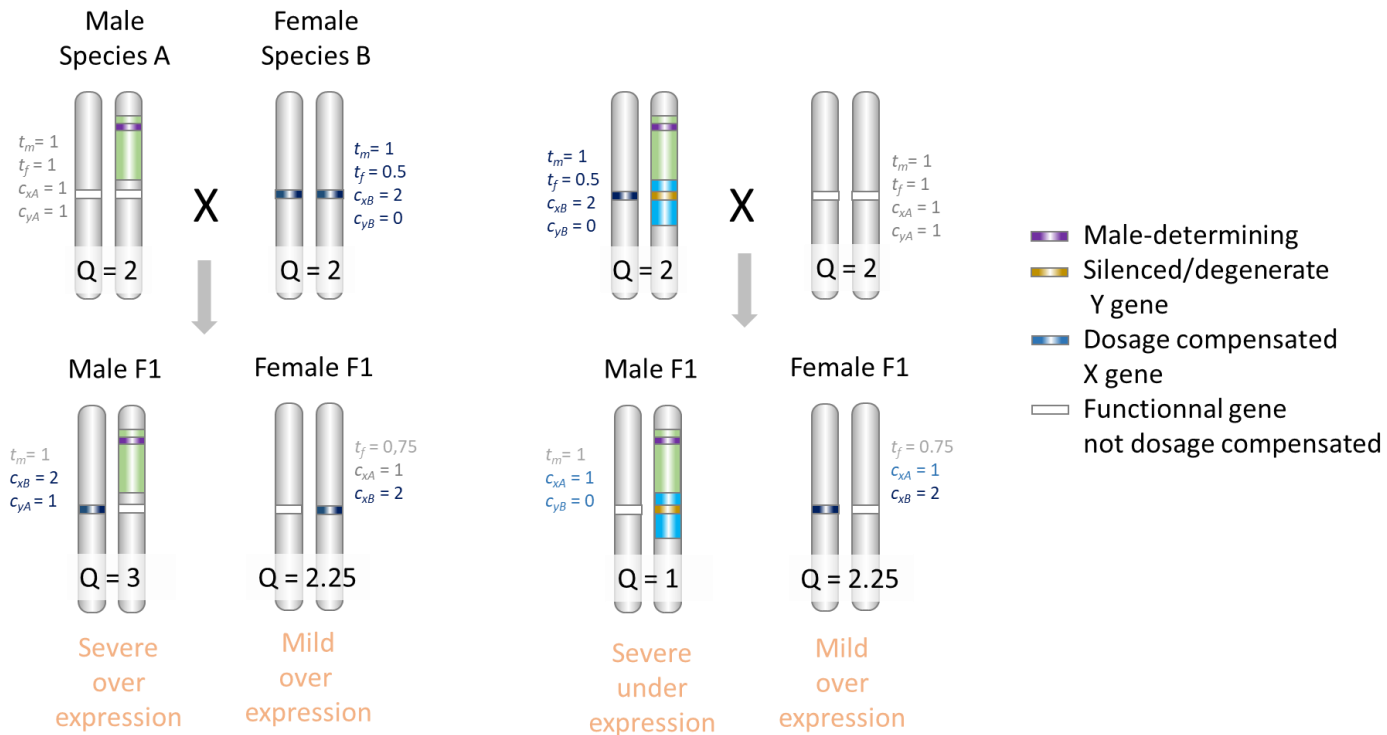


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1072 **Fig S5a. Example of the effect of a gene located in a derived stratum present in only one species,**
 1073 **with a drosophila-like DC mechanism.** The figure shows species A and B, where the Y has a non-
 1074 non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-
 1075 non-recombining stratum only in species B (in blue), as in Fig S1a. The example focuses on a gene in the
 1076 derived (blue) stratum. In species A, DC does not evolve (since the gene is located in a recombining
 1077 portion of the Y), while in species B, DC is achieved for that gene, since it is located in the non-
 1078 non-recombining region. In this example, DC in species B is achieved by having male overexpression of
 1079 the X, like in *Drosophila* (i.e. by evolving a male-specific trans-acting factor increasing X expression
 1080 in males, $t_m = 2$ in species B). Male and female expression levels is indicated by the value of Q . $Q = 2$ is
 1081 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
 1082 females, respectively, see methods). F1 males show a departure from optimal expression, while F1
 1083 females maintain optimal expression, in both directions of the cross. Note that the reduction in F1
 1084 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over or
 1085 underexpression.

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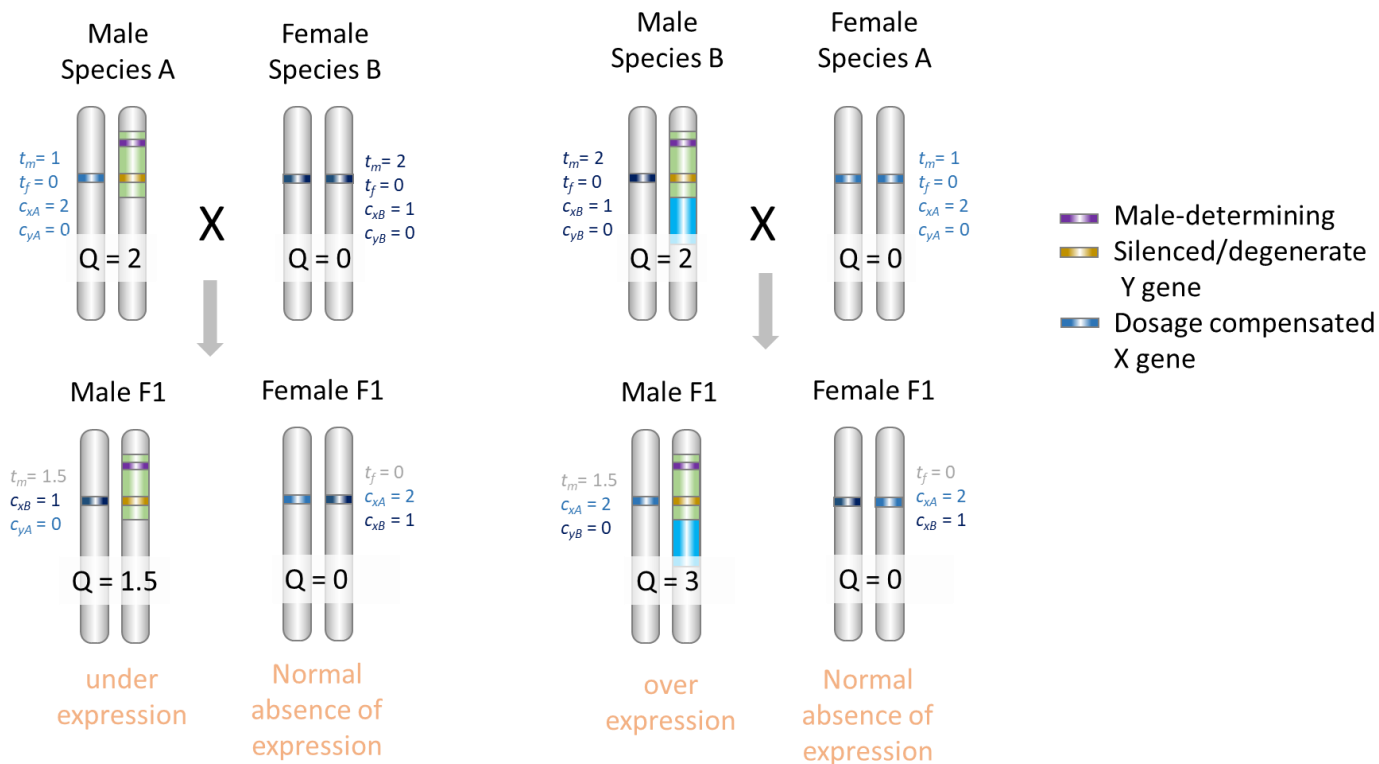


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1089 **Fig S5b. Example of the effect of a gene located in a derived stratum present in only one species,**
 1090 **with a mammal-like DC mechanism.** The figure shows species A and B, where the Y has a non-
 1091 recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-
 1092 recombining stratum only in species B (in blue), as in Fig S1a. The example focuses on a gene in the
 1093 derived (blue) stratum (like in Fig S5a). In species A, DC does not evolve (since the gene is located in a
 1094 recombining portion of the Y), while in species B, DC is achieved for that gene, since it is located in
 1095 the non-recombining region. The example shows a mammal-like pattern of DC for species B: DC in
 1096 species B is achieved by having overall X overexpression (by evolving stronger X cis-regulatory
 1097 elements, $c_{xB} = 2$ in species B) which is corrected in females to avoid overshooting (by evolving a
 1098 female-specific trans-acting factor decreasing X expression in females, $t_f = 0.5$ in species B). Male and
 1099 female expression levels is indicated by the value of Q . $Q = 2$ is the optimal expression level (Q is
 1100 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).
 1101 F1 males show a greater departure from optimal expression than F1 females. Note that the reduction
 1102 in F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either
 1103 over or underexpression.

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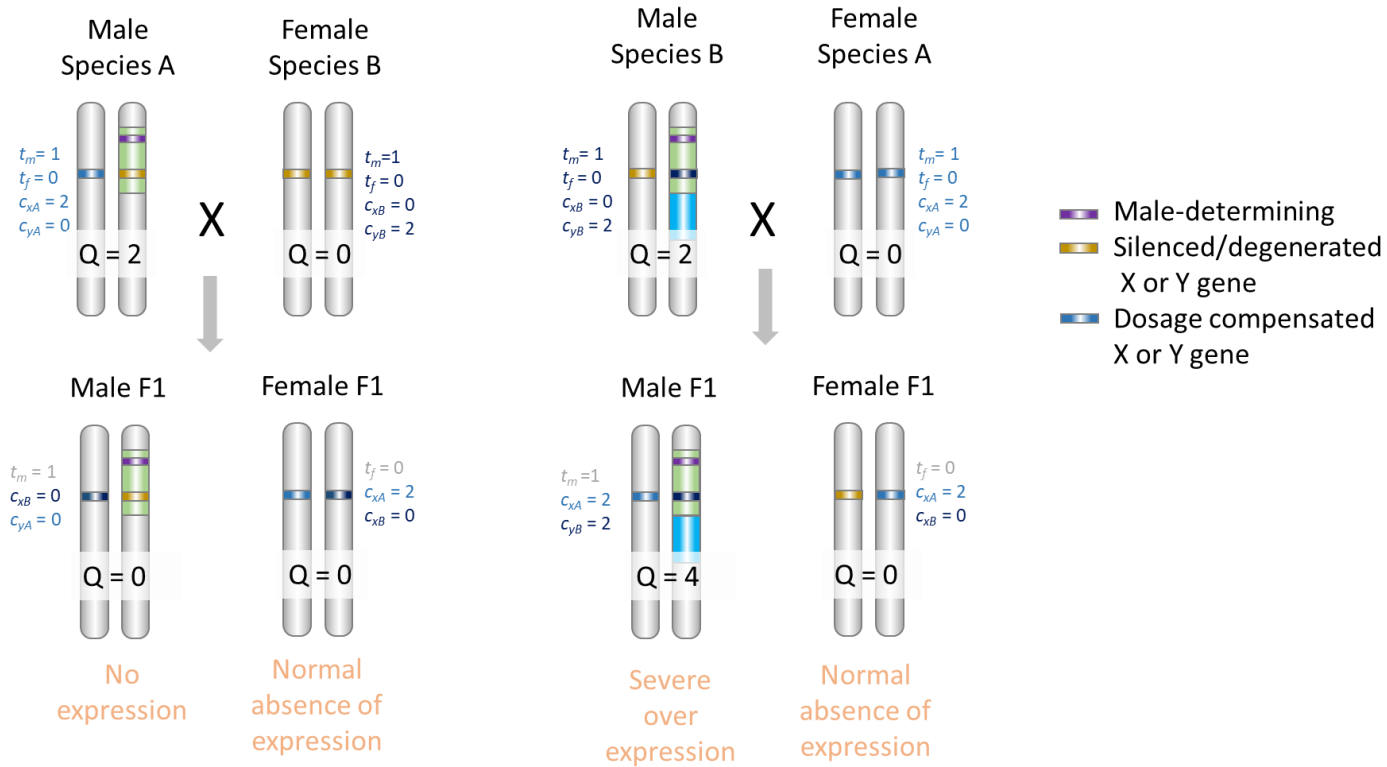


1106

1107 **Fig S6a. Example of the effect of a male-limited gene located in an ancestral stratum, with different**
 1108 **DC mechanisms in the diverging species.** The figure shows species A and B, where the Y has a non-
 1109 non-recombining stratum (in green) inherited from the common ancestor, and a newly evolved non-
 1110 recombining stratum only in species B (in blue), as in Fig S1a. The example focuses on a male-limited
 1111 gene in the ancestral (green) stratum. The gene is not expressed in females. In species A, DC is
 1112 achieved, for the focal gene, by having overall X overexpression (by evolving stronger X cis-regulatory
 1113 elements, $c_{xA} = 2$ in species A) which is corrected in females to avoid overshooting (by evolving a
 1114 female-specific trans-acting factor decreasing X expression in females, $t_f = 0.5$ in species A). In species
 1115 B, DC is achieved, for the same gene, by having male overexpression of the X (i.e. by evolving a male-
 1116 specific trans-acting factor increasing X expression in males, $t_m = 2$ in species B). Male and female
 1117 expression levels is indicated by the value of Q . $Q = 2$ is the optimal expression level (Q is computed
 1118 as $(c_{X,i} + c_{Y,i})\bar{E}_{m,i}$ and $(c_{X1,i} + c_{X2,i})\bar{E}_{f,i}$ in males and females, respectively, see methods). Male F1
 1119 show a departure from optimal expression in both directions of the cross. Note that the reduction in
 1120 F1 male fitness is potentially asymmetric in the two directions of the cross, resulting from either over
 1121 or underexpression. Note also that this situation is identical to Fig S4, ignoring females.

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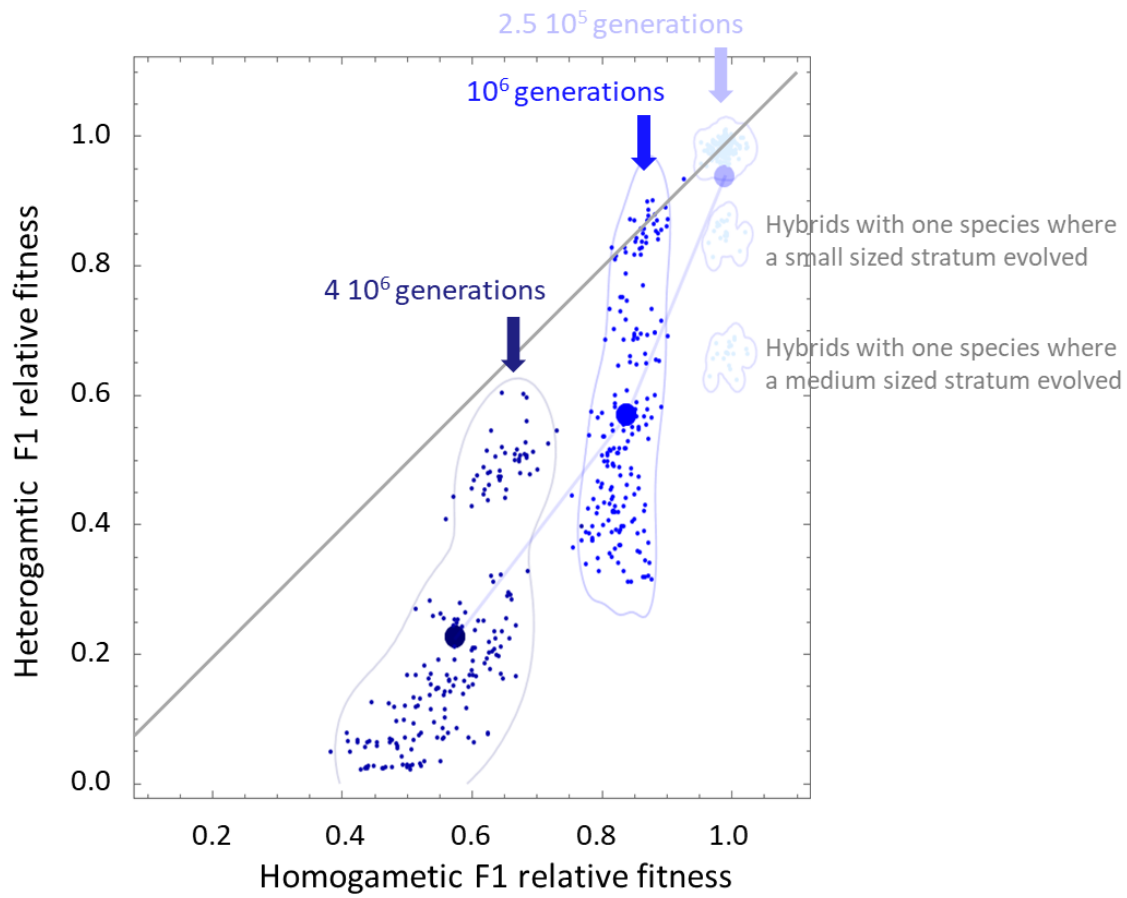
1124

1125 **Fig S6b. Example of the effect of a male-limited gene located in an ancestral stratum, with**
 1126 **alternate X or Y degeneration in the diverging species.** The figure shows species A and B, where the
 1127 Y has a non-recombining stratum (in green) inherited from the common ancestor, and a newly
 1128 evolved non-recombining stratum only in species B (in blue), as in Fig S1a. The example focuses on a
 1129 male-limited gene in the ancestral (green) stratum which is silenced on the Y in species A ($c_{yA} = 0$),
 1130 but silenced on the X in species B ($c_{xB} = 0$). Indeed, male-limited genes can degenerate both ways,
 1131 which differs from non-sex-limited cases shown in Fig S4 and S5 where degeneration can only occur
 1132 on the Y. The gene is not expressed in females. In both species, DC is achieved by having male
 1133 overexpression of either the X or Y, through the evolution of stronger cis-regulators ($c_{xA} = c_{yB} = 2$).
 1134 Male F1 show a strong departure from optimal expression in both directions of the cross, notably in
 1135 one direction where the gene is completely silenced. Note that the reduction in F1 male fitness is
 1136 potentially asymmetric in the two directions of the cross, resulting from either over or
 1137 underexpression.

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1142 Fig. S7. Detail of Fig 1B where a neo-Y evolves. The figure shows the individual points and the
1143 corresponding contours.