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Sexually antagonistic coevolution between the sex chromosomes of *Drosophila melanogaster*

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This PDF file includes:
Main Text, Figures 1 to 4, and Table 1

Abstract

Antagonistic interactions between the sexes are important drivers of evolutionary divergence. Interlocus sexual conflict is generally described as a conflict between alleles at two interacting loci whose identity and genomic location are arbitrary. Here we build on previous theory and suggest that when these two loci are located on the sex chromosomes it can lead to cycles of antagonistic coevolution between them, and therefore between the sexes. We tested this hypothesis by performing experimental crosses using *Drosophila melanogaster* in which we reciprocally exchanged the sex chromosomes between five wild-type populations in a round-robin design. Disrupting putatively coevolved sex chromosome pairs resulted in increased male reproductive success in 16 out of 20 experimental populations (10 of which were significant), but also resulted in lower offspring egg-to-adult viability that affected both male and female fitness. After 25 generations of experimental evolution these sexually antagonistic fitness effects appeared to have been resolved. To help formalise our hypothesis, we developed population genetic models of antagonistic coevolution using fitness expressions based on our empirical results. Our models support the conclusion that antagonistic coevolution between the sex chromosomes is plausible under the fitness effects observed in our experiments. Together, our results lend both empirical and theoretical support to the idea that a cycle of antagonistic coevolution can occur between sex chromosomes and illustrates how this process may drive genetic and phenotypic divergence between populations.

Significance

Sex chromosomes are not only involved in genetic sex determination – they are also important factors in sexual conflict and speciation. Here, using a combination of experiments and population genetic models, we show that the sex chromosomes in *Drosophila melanogaster* can coevolve antagonistically. We found that swapping sex chromosomes between five *Drosophila melanogaster* populations increased male fitness at the cost of reduced offspring survival. After 25 generations, the increase had disappeared, consistent with the completion of a cycle of antagonistic coevolution. Using parameter values based on these empirical data, our models show that antagonistic coevolution between the sex chromosomes is a biologically plausible explanation for the results. Thus, our results point to a potentially important path to speciation through sexual conflict.

66 **Introduction**

67 Sex chromosomes have a number of unique properties that distinguish them from
68 autosomes, and one another, including their mode of inheritance, the selection
69 experienced in the two sexes, and gene content (1). These processes are
70 interdependent and strongly influence the genetic variation harboured on each sex
71 chromosome. The Y chromosome is inherited exclusively through males, and
72 therefore only exposed to selection in males. Because the Y chromosome is male-
73 limited, any Y-linked genetic variation that is not male beneficial should experience
74 purifying selection, which is consistent with empirical results (2–4). The X
75 chromosome is inherited through males and females similarly to the autosomes, but
76 experiences very different selection pressures than the autosomes or the Y (1). The X
77 chromosome spends two-thirds of its time in females and is therefore largely exposed
78 to selection in females (1), but since it is hemizygous in males, it is always exposed to
79 selection when present in males (i.e., X-linked genes are unsheltered by dominance
80 effects in males) (5). Thus, genes on the X chromosome are not sex-limited as on the
81 Y chromosome, and whether they are female- or male-beneficial can depend on the
82 dominance coefficient (6). Collectively, these unique properties have made sex
83 chromosomes important factors in two major fields within evolutionary biology:
84 speciation (7) and sexual conflict (1, 8).

85 Here we aimed to investigate whether interactions between sex chromosomes
86 contribute to between-population divergence at the intra-specific level, as has
87 previously been shown in interspecific comparisons (7). To do so, we performed
88 experimental crosses to 'swap' either an X or a Y chromosome between five
89 geographically isolated outbred populations of *D. melanogaster*, allowing us to isolate
90 the effects of novel interactions between the sex chromosomes on male reproductive

91 fitness. As we outline below, we expected to see different patterns of male
92 reproductive success depending on whether population divergence is caused by
93 incipient reproductive isolation, or is instead a result of sexually antagonistic
94 coevolution between the sex chromosomes.

95 The accumulation of incipient 'hybrid incompatibilities' begins in allopatric
96 populations within a species prior to speciation (9), and the X chromosome has been
97 found to have a large effect on hybrid male sterility (10). So, if interactions between
98 the sex chromosomes contribute to divergence between these populations primarily
99 through accumulation of X-linked incompatibilities, we would expect to see a
100 decrease in male fitness in the experimental populations. However, while previous
101 studies have found evidence of early prezygotic isolation between sub-Saharan and
102 cosmopolitan populations of *D. melanogaster* (11, 12), there is limited evidence for
103 postzygotic isolation (13). So, we did not expect to find evidence of incipient
104 reproductive isolation in our population crosses.

105 Sexually antagonistic coevolution between the sex chromosomes could, in
106 principle, provide an alternative mechanism for the sex chromosomes to contribute to
107 evolutionary divergence between populations (14), as suggested by studies of Y-
108 linked regulation of gene expression (15). The theory of sexually antagonistic
109 coevolution is based on the Red Queen-process (16). In short, when males increase
110 their reproductive success through an adaptation that is simultaneously detrimental to
111 females, it creates selection for a counter-adaptation in females to regain their lost
112 fitness. The process may be repeated in multiple cycles over evolutionary time until a
113 resolution is reached or a palliative adaptation ends the conflict (17). Sexually
114 antagonistic coevolution can be considered a form of interlocus sexual conflict (14) if
115 the traits involved are encoded by different genes in males and females (18). As first

116 proposed by Rice & Holland in 1997, when the loci in question are located on the sex
117 chromosomes, this could lead to cycles of sexual antagonistic coevolution between
118 the sex chromosomes (14). If a Y-linked male-beneficial mutation arises that
119 increases male reproductive success but decreases female fitness, it should spread
120 through a population because it is subject to selection in males only. The sexually
121 antagonistic male-beneficial mutation creates selection favouring compensatory
122 mutations that might arise on the X chromosome or autosomes to restore female
123 fitness again. Such mutations will likely differ between allopatric populations, and we
124 would therefore expect to find an increase in male reproductive fitness when a Y
125 chromosome with male beneficial mutations is paired with an X chromosome without
126 the corresponding compensatory mutation(s). According to the sexually antagonistic
127 coevolutionary model, we also predict a decrease in female fitness when mating with
128 males harbouring a Y chromosome paired to a novel X chromosome.

129 Another corollary of the antagonistic coevolutionary model is that the effect of
130 disrupting coevolved sex chromosomes on male and female fitness should decay over
131 subsequent generations as new compensatory mutations arise, or novel combinations
132 of segregating alleles achieve a similar compensatory effect. We tested this prediction
133 by examining male and female fitness in our experimental populations 25 generations
134 of experimental evolution. Crucially, the signature of antagonistic coevolution
135 between the sex chromosomes in terms of male and female fitness differs from that
136 for the accumulation of sex-linked incompatibilities, allowing us to identify whether
137 one or other process is driving evolutionary divergence between populations.

138 To complement our empirical results and help formalize the hypothesis of
139 sexually antagonistic coevolution between the sex chromosomes, we developed
140 population genetic models describing the evolution of two interacting loci located in

141 different genomic regions (i.e., unlinked): a Y-linked locus influencing both adult
142 male fertilization success (i.e., sperm competition) and subsequent offspring survival,
143 and a compensatory locus affecting only offspring survival located on either an
144 autosome or the X chromosome.
145

146 ## Results

147 **Empirical evidence for sexual antagonistic coevolution**

148 To empirically test for evidence of population-specific interactions between the sex
149 chromosomes in *D. melanogaster*, we crossed five outbred laboratory-adapted wild-
150 type populations derived from four continents and three climatic zones in a round
151 robin crossing design (Fig. 1). We created 20 novel populations where either the X
152 chromosome (*novel X* treatment) or the Y chromosome (*novel Y* treatment) from one
153 wild-type population (*wt*) was incorporated into another population (*SI Appendix A*,
154 Fig. S1). We found a significant effect of treatment on male relative reproductive
155 fitness (*SI Appendix A*, Table S1), with males from the *novel X* or *novel Y* treatments
156 having a significantly higher relative fitness than *wt* males (Fig. 2*A*, *SI Appendix A*,
157 Table S2). To exclude interactions between the novel sex chromosome and the
158 autosomes as the cause, we also created a *novel XY* treatment where a pair of sex
159 chromosomes from one *wt* population were introduced into another population (*SI*
160 *Appendix A*, Fig. S2). We found no significant effect of treatment on relative fitness in
161 the assay with *novel XY* treatments (Fig. 2*B*, *SI Appendix A*, Table S1). It therefore
162 seems that introducing an individual novel sex chromosome caused the fitness
163 differences rather than interactions with the autosomal background.

164 To investigate the interaction between the sex chromosomes in more detail, we
165 calculated the difference in fitness between the novel populations and their *wt*

166 counterparts (ΔFitness = $\omega_{\text{novel population}}$ - $\omega_{\text{wild-type}}$). We used bootstrap methods to
167 model the data, and found that 10 of the 20 novel populations were significantly
168 different from zero in a positive direction (Fig. 2*C*, *SI Appendix A,* Table S3). 16 out
169 of the 20 point estimates were also positive, which is significantly different from what
170 would be expected under random deviations (binomial test, $P = 0.01$).
171
172 **Male beneficial traits**
173 To tease apart which fitness components were driving the overall pattern of increased
174 relative fitness in the *novel* populations, we looked at a number of phenotypic traits
175 that are correlated with male fitness. Interestingly, we found that the increase in
176 fitness was related to different traits in the two *novel* sex chromosome treatments. For
177 *novel X* males, we found that the increase in fitness was correlated with an increase in
178 size, as *novel X* males were significantly larger than both *wt* and *novel Y* males (*SI*
179 *Appendix A,* Fig. S3*A*, Table S1 & S2). For *novel Y* males, we found that the increase
180 in fitness was correlated with an increased ability to displace other males' sperm (*SI*
181 *Appendix A*, Fig. S3*B*, Table S1), as *novel Y* males were significantly better at
182 displacing sperm compared to *wt* males (*SI Appendix A,* Table S2).
183
184 **Female harmful traits**
185 If the sex chromosomes coevolved antagonistically, the increase in male fitness
186 should come at a cost to female fitness. We found a significant effect of treatment on
187 total offspring number (*SI Appendix A*, Fig. S4*A*, Table S1), with *novel X* males siring
188 a lower number of live offspring compared to *wt* males (Table S2). However, we did
189 not find any difference in number of eggs laid by females mated with males from the
190 different treatments (*SI Appendix A*, Fig. S4*B*, Table S1). The discrepancy between

number of eggs and live offspring suggests a trade-off between offspring number and offspring quality, which was confirmed by a significant difference in egg-to-adult viability (SI Appendix A, Fig. S4*C*, Table S1). *Novel X* males sired significantly lower numbers of viable offspring than the other two treatments (*SI appendix A*, Table S2). Mating with *novel X* males decrease a female's fitness by reducing the overall number of live offspring she produces. A decrease in offspring survival could be caused by meiotic drives seen in a departure from 50:50 sex ratio (19). We did not find a significant difference in sex ratio between the different treatments (*SI Appendix A*, Fig. S4*D*, Table S1).

We were unable to establish which harmful effects *novel Y* males had on female fitness through the assays performed in this experiment. But we were able to exclude costs to females associated with increased male harassment (e.g. due to larger size), males inducing high fecundity in females, and a reduction in offspring viability.

Counter-adaptation

Any shift towards increased fitness in one sex at the cost of the other sex should lead to counter-adaption, which we expected to see as a reduction in male fitness over time. After 25 generations of experimental evolution (*SI Appendix A,* Fig. S5), we repeated both the male reproductive fitness assay and two of the significant phenotype assays to test if the interactions between the novel pairs of sex chromosomes had changed. As predicted, we no longer found a significant treatment effect on male fitness (Fig. 3*A*, *SI Appendix A,* Table S4). Indeed, we found that ΔFitness (ΔFitness = $\omega_{\text{novel population}} - \omega_{\text{wild-type}}$) between novel populations and their wild-type counterparts had diminished in magnitude and were no longer significantly different from 0 (except for one population, I–O_X) (Fig. 3*B*, *SI Appendix A,* Table S5). We also no

216 longer found an effect of the treatments on sperm competition (*SI Appendix A,* Fig.
217 S6*A*, Table S4) or offspring egg-adult survival (*SI Appendix A,* Fig. S6*B*, Table S4).
218 There was a significant effect of the treatments on sex ratio (*SI Appendix A,* Fig. S6*C*,
219 Table S4) as *novel Y* males produced significantly more male offspring than *wt* males
220 (*SI Appendix A,* Table S6). However, this significant difference was not due to a
221 change in sex ratio in the *novel Y* populations; rather it was due to a (putatively
222 stochastic) change in the sex ratio in the *wt* populations.
223

224 **Population Genetic Models**

225 Consider two alternative genetic systems involving two unlinked loci: a Y-linked
226 locus (**Y**, with alleles *Y*, and *y*), and a compensatory locus located on either an
227 autosome (**A**, with alleles *A*, and *a*) or the X chromosome (**X**, with alleles *X*, and *x*)
228 (the Autosomal and X-linked models respectively), in a large population with discrete
229 generations. In both models, a mutant *y* chromosome increases male fertilization
230 success by a rate of $1 + s_m$ relative to the wild type (*Y*), but also reduces viability of
231 offspring resulting from matings with females carrying the wild-type allele at the
232 compensatory locus, *A* (or *X*). At the same time, females carrying the mutant *a* (or *x*)
233 compensatory allele may incur a 'cost of compensation' in terms of offspring viability
234 when mating with wild-type (*Y*) males (see Table 1 and Materials and Methods for a
235 summary and description of fitness expressions). These fitness expressions create a
236 scenario of antagonistic coevolution between a potentially male-beneficial Y-linked
237 mutation, and the compensatory locus. Reminiscent of standard theories of
238 compensatory evolution (e.g., 20, 21), each of the mutant alleles (*y* and either *a* or *x*)
239 reduces offspring survival in isolation. However, unlike previous theory, the two loci
240 are located on different chromosomes that do not recombine with one another; if a

241 mutant *y* chromosome spreads to fixation, compensation at the population level
242 requires that the compensatory mutation also fixes (see also recent models of
243 "Father's Curse" for similar scenarios, but without compensatory evolution or
244 parental effects on offspring viability (22); a full description of the models is
245 presented in the Materials and Methods and in *SI Appendix B*). Our theoretical
246 analyses focus on (i) the evolutionary invasion of rare mutants at each locus
247 individually; and (ii) single bouts of coevolution, beginning with invasion of a single-
248 copy mutant *y* chromosome in a population initially fixed for the wild-type *Y*
249 chromosome and for the wild-type *A* (or *X*) allele at the compensatory locus, and
250 completing when both mutant alleles *y* and *a* (or *x*) become fixed (see 31 for a similar
251 approach in the context of mito-nuclear coevolution).

252 Evolutionary invasion analyses of the two models reveal tow key results. First,
253 intuitively, invasion of a rare mutant *y* chromosome into a population initially fixed
254 for the wild-type alleles at both loci requires that the increase in fertilization success
255 for mutant males is greater than the accompanying reduction in offspring survival.
256 Neglecting second order terms, a mutant *y* chromosome can spread when $\delta = s_m - s_o >$
257 0. Second, whether the genomic location of the compensatory locus (either autosomal
258 or X-linked) influences the invasion conditions for the compensatory mutation
259 depends on the relative rate of mutation at the compensatory and Y-linked loci. If
260 compensatory evolution is slow relative to the evolution of the Y-linked locus (e.g.,
261 the compensatory mutation rate is much smaller than the mutation rate to a mutant *y*),
262 the mutant *y* chromosome is most likely to fix before a new compensatory mutation
263 occurs. In this case, the genomic location of the compensatory locus does not
264 influence the invasion conditions for a compensatory mutation. All males carry the
265 mutant *y*, and so compensatory mutations will spread if there is any selection against

266 the wild-type allele at the compensatory locus (i.e., $0 < h_o, s_o < 1$ for both models).
267 Differences between the models emerge when new compensatory mutations can arise
268 while the mutant y chromosome is still segregating in the population. If there is any
269 cost of compensation for females (i.e., $0 < s_c$), compensatory mutations will
270 experience purifying selection while q_y is small because most males carry the wild-
271 type Y chromosome. As q_y increases, more matings involve mutant y males and
272 females homozygous for the wild-type allele at the compensatory locus. When q_y
273 reaches a threshold frequency, $\tilde{q}_y^A$ ($\tilde{q}_y^X$ for the X-linked model), compensatory
274 mutations will be favoured by selection (derivations provided in *SI Appendix B*). As
275 illustrated in Fig. 4*A* for the case of additive compensatory fitness effects ($h = h_o = h_c$
276 $= 1/2$), $\tilde{q}_y^X$ is always less than or equal to $\tilde{q}_y^A$, and the difference between the two
277 thresholds is greatest when $0 < s_o, s_c \ll s_m$. Overall, these results suggest that the
278 genomic location of compensatory mutations (Autosomal or X-linked) will be most
279 important when (i) the mutant y chromosome is strongly beneficial for males and
280 there is little or no cost of compensation for females (i.e., when $0 < s_o, s_c \ll s_m$); and
281 (ii) when compensatory evolution is not limited by mutational variation (i.e., when
282 mutant y chromosomes and compensatory mutations are likely to co-segregate in the
283 population).

284 To complement our analytic results, we performed Wright-Fisher simulations
285 to estimate two other important properties of coevolutionary cycles predicted by our
286 models: (i) the probability of invasion of single-copy autosomal (Π_A) and X-linked
287 (Π_X) compensatory mutations into populations of N individuals that are initially fixed
288 for the mutant y chromosome; and (ii) the total time to complete a single bout of
289 coevolution between the Y-linked and compensatory loci (T_A and T_X, respectively)
290 under recurrent mutation, selection, and drift. Although the invasion conditions for

291 mutant compensatory mutations are the same for both models when the mutant *y*
292 chromosome is initially fixed, the probability of eventual fixation for a single-copy
293 mutation is larger for an X-linked compensatory locus (Π_X) than an autosomal one
294 (Π_A) except for the special case of complete dominance (i.e., when $h_o = 1$) (Fig. 4*B*).
295 The average time to complete a co-evolutionary cycle is also smaller for an X-linked
296 than an autosomal compensatory locus, provided the compensatory mutation rate (μ_a
297 or μ_x) is not dramatically lower than the male-beneficial mutation rate (μ_y) (Fig. 4*C*).
298 When compensatory evolution is strongly limited by mutational variation,
299 compensatory evolution can be faster on the autosomes than the X. This is due to an
300 implicit trade-off between the probability of invasion, which is higher for the X than
301 the autosomes (Fig. 4*B*), and the size of the mutational target, which is lower for the
302 X because males only carry one copy.
303

304 **Discussion**

305 We investigated whether if interactions between the sex chromosomes could
306 contribute to between-population divergence at the intra-specific level. Taken
307 together, our empirical results demonstrated that exchanging a sex chromosome
308 between populations of *D. melanogaster* had an overall positive effect on male
309 fitness. These results were consistent with the expected signature of antagonistic
310 coevolution between the sex chromosomes on male fitness, but not with that of
311 accumulated sex-linked incompatibilities influencing male sterility.

312 Mitochondria-nuclear interactions have previously been shown to affect male
313 fertility in *D. melanogaster* (23), and both *novel X* and *novel Y* chromosomes were
314 expressed with novel mitochondria. However, the *novel XY* chromosomes also were
315 expressed with novel mitochondria and we found no effect on male fitness in these

316 populations, suggesting that the magnitude of any mito-nuclear interactions was
317 minor in these populations compared to the effect of interactions between the sex
318 chromosomes.

319 We further wanted to know which specific phenotypic traits facilitated the
320 increase in male reproductive fitness. We found that *novel X* males seemed to gain a
321 mating advantage from an increase in size, as body size is an important factor in male
322 fitness (24), and larger males have been shown to have a mating advantage (25). For
323 *novel Y* males it seemed that they gained the advantage through being better at sperm
324 displacement. Thus, the results suggest that different phenotypic and genetic
325 mechanisms may be responsible for the increased male fitness observed in the two
326 *novel* sex chromosome treatments.

327 Since the reproductive fitness results were consistent with antagonistic
328 coevolution, we expected to find a corresponding decrease in female fitness. We
329 found that offspring sired by *novel X* males had a significant lower egg-to-adult
330 survival rate. We did not find the decrease in offspring survival to be caused by a
331 distortion in sex ratio, which suggests the effect is not due to meiotic drive or sexually
332 antagonistic zygotic drive (i.e. mortality resulting from competition between opposite-
333 sex siblings (26). In addition, there are no known sex ratio meiotic drivers in *D.*
334 *melanogaster*. Thus, the decrease in offspring survival was most likely caused by the
335 father's genotype. Reproduction is costly for females (27), so any eggs which do not
336 produce live offspring are a expense and will over time reduce the reproductive
337 fitness of the females. We were not able to identify which traits in females were
338 negatively affected when mating with *novel Y* males. A possible cause to investigate
339 in the future is the reduction of female receptivity after mating with males, which
340 could lead to a reduction in female lifetime reproductive fitness.

341 As a final test of the hypothesis of antagonistic coevolution, we ran an
342 evolution experiment for 25 generations to see if we would observe any counter-
343 adaptation. After 25 generations, we found a reduction in fitness indicating strong
344 selection pressure for compensatory evolution, which is supported by the simulation
345 results. In principle, this subsequent reduction in male fitness could be due to new X-
346 linked compensatory mutations; however, given the time-scale of the experiment it is
347 more likely that any compensatory evolution by females must utilize standing genetic
348 variation in the stock populations, with novel allelic combinations achieving a
349 compensatory effect.

350 Collectively, our empirical results are consistent with predictions for
351 antagonistic coevolution between the sex chromosomes. To further validate that we
352 found evidence of antagonistic coevolution, we built a theoretical model based on our
353 empirical evidence. Our theoretical results support the conclusion that antagonistic
354 coevolution is (i) plausible under the fitness effects observed in our empirical
355 experiments; and (ii) more likely to involve both sex chromosomes than the Y and an
356 autosome, especially when compensatory dynamics are not slow relative to those of
357 male-beneficial Y-linked mutations. An interesting corollary to these results is that
358 antagonistic coevolution should drive more rapid among-population divergence on the
359 X chromosome than the autosomes.

360

361 In conclusion, we empirically examined the role of the sex chromosomes in
362 evolutionary divergence among allopatric populations of *D. melanogaster*. We found
363 evidence of intragenomic conflict between the sex chromosomes that was independent
364 of any interaction with the autosomes or mitochondria. Disrupting coevolved sex
365 chromosomes resulted in an overall increase in reproductive fitness for *novel* males,

through increased body size (for *novel X* males) and sperm displacement (for *novel Y* males), but also decreased offspring egg-to-adult viability. The accompanying reduction in offspring viability created indirect selection on females to mitigate the loss in fitness resulting from mating with a *novel* male. After 25 generations of experimental evolution *novel* males no longer enjoyed higher reproductive fitness and there were no differences in offspring survival, indicating that the sexually antagonistic effects of disrupting coevolved sex chromosomes had been resolved, probably by standing genetic variation for compensatory alleles in the experimental populations. Overall, our empirical results were consistent with a sexually antagonistic coevolutionary model of sex chromosome evolution in allopatric populations. Analysis of our theoretical models supports the plausibility of antagonistic coevolution between the sex chromosomes under the fitness effects observed in the experiments and predicts that such coadaptation is more likely to involve sex chromosomes than autosomes provided that compensatory evolution is not limited by mutational variation. These new insights into the interactions between the sex chromosomes can help further our understanding of early speciation events. Antagonistic coevolutionary cycles between the sex chromosomes will most likely follow different trajectories in different populations due to random mutations and the interaction between the environment and sexual conflict (28), resulting in genetic and phenotypic divergence between allopatric populations. Thus, over evolutionary time, antagonistic coevolutionary cycles could lead to hybrid incompatibility, and thereby contribute to speciation.

389 # Materials and Methods

390 ## Empirical methods

391 ***Drosophila* husbandry and fly stocks.** We used five outbred laboratory-adapted
392 wild-type populations: (1) *Dahomey*, Africa, tropical (25); (2) *Innisfail*, Oceania,
393 tropical; (3) LH_M, North America, mediterranean; (4) *Odder*, Europa, temperate (29);
394 (5) *Tasmania*, Oceania, temperate. We maintained all wild-type populations under the
395 standard LH_M culturing protocol (25°C, 12-12 light-dark cycle, 60% relative
396 humidity, cornmeal-molasses-yeast medium, *SI Appendix A,* 30) for at least two
397 generations before the cross. For the fitness assays we used an outbred LH_M
398 population homozygous for the visible brown eye (*bw*) genetic marker (LH_M-*bw*).
399

400 **Male reproductive fitness.** We estimated male reproductive fitness as the proportion
401 of live offspring sired by males of the target treatment. Five adult target males were
402 placed in a vial for two days with ten competitor LH_M-*bw* males and 15 virgin LH_M-
403 *bw* females. The females were transferred into a single test tube to oviposit for 18
404 hours after which the females were discarded, and the test tubes left under standard
405 LH_M conditions for 12 days. After which we counted the adult offspring and recorded
406 their eye-colour to assess paternity. The *bw* genetic marker is recessive to the wild-
407 type red eye-colour allele, so all red-eyed offspring can be assigned to red-eyed target
408 males. We calculated relative fitness of the target males by dividing the fitness for
409 each replicate by the maximum fitness across all replicates. We measured male fitness
410 for three different assays: at generation 0 (n = 2 blocks x 7 experimental replicates x
411 25 populations; fitness estimates for 70 individuals per population), at generation 25
412 (n = 2 blocks x 3 experimental replicates x 2 replicate populations x 14 populations;
413 fitness estimates for 60 individuals per population) and for the sex chromosome-

414 autosome interactions (*n* = 10 experimental replicates x 10 populations; fitness
415 estimates for 50 individuals per population).
416
417 Construction of the *novel* populations, the evolution experiment, male thorax size,
418 sperm competition assay, male effect on female fecundity, and offspring egg-to-adult
419 viability assay are described in *SI Appendix A*.
420
421 **Statistical procedures**. All the statistical analyses were conducted in R version 3.4.4
422 (31). We fit linear models with treatment as a fixed factor to test each of the
423 dependent variables: thorax size and male effect on female fecundity, and tested for
424 significant effects using ANOVA. We performed post-hoc Tukey HSD comparisons
425 for all analyses with a significant treatment effect. We used linear mixed models
426 (**lme4** 32) with treatment as a fixed factor and experimental block as a random factor
427 to test the dependent variables: male reproductive fitness, egg-to-adult offspring
428 viability, sex ratio, sperm competition, and total offspring number, and again tested
429 for significant effects using ANOVA. We performed post-hoc Tukey HSD
430 (**multcomp** 33) comparisons for all analyses with a significant treatment effect. We
431 calculated bootstrap 95% confidence intervals around ΔFitness (ΔFitness = ω_{novel}
432 $_{\text{population}}$ - $\omega_{\text{wild-type}}$) randomly resampling 13 out of 14 data points and recalculating
433 ΔFitness 10,000 times. We used the Exact Binomial Test to test if there were more
434 positive ΔFitness estimates than expected by chance. For statistical analysis after the
435 25 generations of experimental evolution we fit linear mixed models with treatment
436 nested within replicate populations to test the dependent variables: male reproductive
437 fitness, egg-to-adult offspring survival, sperm competition and sex ratio. For assays
438 done in experimental blocks, we added experimental block as a random factor, tested

439 for significant effects using ANOVA, and performed post-hoc Tukey HSD tests for
440 analyses with significant treatment effect. We calculated bootstrap 95% confidence
441 intervals around treatment means for ΔFitness by randomly resampling 11 out of 12
442 data points and recalculating ΔFitness 10,000 times.
443
444 **Theoretical models**
445 We developed two population genetic models, identified by the location of the
446 compensatory locus: the Autosomal and X-linked models respectively. We assumed
447 discrete generations, and a life-cycle that proceeds as follows: (i) birth, (ii) selection
448 on offspring survival, (iii) meiosis and mutation, (iv) selection on male mating
449 success. Both loci in each model are biallelic. The Y-linked locus, **Y**, has wild-type *Y*
450 and mutant *y* alleles with frequencies q_Y and $q_y = 1 - q_Y$. The compensatory locus is
451 denoted **A** (with alleles *A* and *a* and frequencies q_A and $q_a = 1 - q_A$) for the autosomal
452 model, and **X** (with alleles *X* and *x* and frequencies q_X and $q_x = 1 - q_X$) for the X-
453 linked model (wild-type alleles are indicated by capital letters, mutant alleles in
454 lowercase). Following standard population genetics theory, **Y** is effectively haploid
455 with strictly paternal inheritance, while the autosomal or X-linked compensatory loci
456 (**A** or **X**) are diploid with bi-parental inheritance.
457 We based the fitness expressions used in our models loosely on our
458 experimental results. The mutant *y* chromosome increases male mating success by a
459 factor of $1 + s_m$ relative to the ancestral *Y* chromosome. Offspring survival depends on
460 the paternal genotype at **Y** and the maternal genotype at the compensatory locus (**A** or
461 **X**), as sires effects offspring viability (34) and any such negative effects should create
462 selection for females to compensate by maternal effects. Offspring sired by mutant *y*
463 males may experience reduced survival, depending on their mother's genotype at the

464 compensatory locus: matings involving parental genotypes [y : AA], [y : Aa], and [y :
465 aa] result in offspring relative fitness of $1 - s_o$, $1 - h_o s_o$, and 1 respectively. To make
466 the models more general, and analytically tractable, we allowed females carrying the
467 mutant a allele to incur a 'cost of compensation' when mating with wild-type (Y)
468 males: matings involving parental genotypes [Y : AA], [Y : Aa], and [Y : aa] result in
469 relative offspring fitnesses of 1, $1 - h_c s_c$, and $1 - s_c$ respectively (Table 1). Similar to
470 standard theories of compensatory evolution (e.g. 20, 21) each of the mutant alleles (y
471 and either a or x) is deleterious in isolation. In our models, however, compensation
472 requires that both parents have the appropriate mutant genotype at the other locus.

473 We model evolutionary invasion and single co-evolutionary cycles between
474 the male-beneficial Y-linked locus and the compensatory locus (see 35 for a similar
475 approach in the context of mito-nuclear coevolution). A bout of coevolution begins
476 with the invasion of a single-copy mutant y chromosome in a population initially
477 fixed for the wild-type A (or X) allele at the compensatory locus. The mutant y
478 evolves under net positive selection if the increase in male mating success outweighs
479 the reduction in offspring survival (i.e., if $\delta = (s_m - s_o) > 0$) until it becomes fixed (q_y
480 $= 1$) or is lost from the population ($q_y = 0$). The compensatory locus (**A** or **X**) evolves
481 under recurrent mutation and selection, with the population initially fixed for the
482 wild-type A allele ($q_a = 1$ for the autosomal model) or X ($q_x = 1$ for the X-linked
483 model). For simplicity we assume one-way mutation from $A \rightarrow a$ at a rate u_a, and X
484 $\rightarrow x$ at a rate u_x per meiosis. If females experience a 'cost of compensation' (i.e.,
485 when $s_c > 0$), the mutant compensatory locus will evolve under purifying selection
486 until the mutant y chromosome reaches a threshold frequency, denoted $\tilde{q}_y^A$ and $\tilde{q}_y^X$, at
487 which y becomes selectively favoured. A co-evolutionary cycle completes when the
488 mutant allele becomes fixed at both loci (i.e., $q_y = q_a = 1$ or $q_y = q_x = 1$).

Our analytic results all assume large population sizes (negligible drift), and an equal sex ratio. To identify the conditions under which rare mutant alleles at each locus can spread during key points of an coevolutionary cycle, we performed a linear stability analysis for each model under three different scenarios: (i) invasion of mutant a y chromosome into populations initially fixed for the wild-type allele at both loci (initial frequencies of $q_y = 0$, and $q_i = 0$; where $i \in \{a, x\}$); (ii) invasion of a rare mutant compensatory allele into a population fixed for y ($q_y = 1$, and $q_i = 0$); and (iii) invasion of a mutant compensatory allele into a population with an arbitrary initial frequency of y ($q_y = q_y$, $q_i = 0$). Mutant alleles can invade (i.e., the initial equilibrium is unstable) when the leading eigenvalue of the Jacobian of the system of recursions is greater than one ($\lambda_L > 1$; 43). When the initial frequency of y is arbitrary, solving the expression $\lambda_L > 1$ for q_y yields the threshold frequency at which compensatory mutations become selectively favoured, ($\tilde{q}_y^A$ or $\tilde{q}_y^X$). A full derivation of all models and analytic results is provided in *SI Appendix B*.

We complemented our analytic results with stochastic Wright-Fisher simulations for the Autosomal and X-linked models with population size N and an equal sex ratio. We estimated two important properties of co-evolutionary cycles from the simulations: (i) the probability of invasion of single copy autosomal (Π_A) and X-linked (Π_X) compensatory mutations into populations initially fixed for the mutant y chromosome; and (ii) the total time required to complete a single bout of coevolution between the Y-linked and compensatory loci (T_A and T_X for the autosomal and X-linked models respectively) under recurrent mutation, selection, and drift. Additional details and R code for the simulations can be found in *SI Appendix B*, and online at https://github.com/colin-olito/sexChromCoAdapt.

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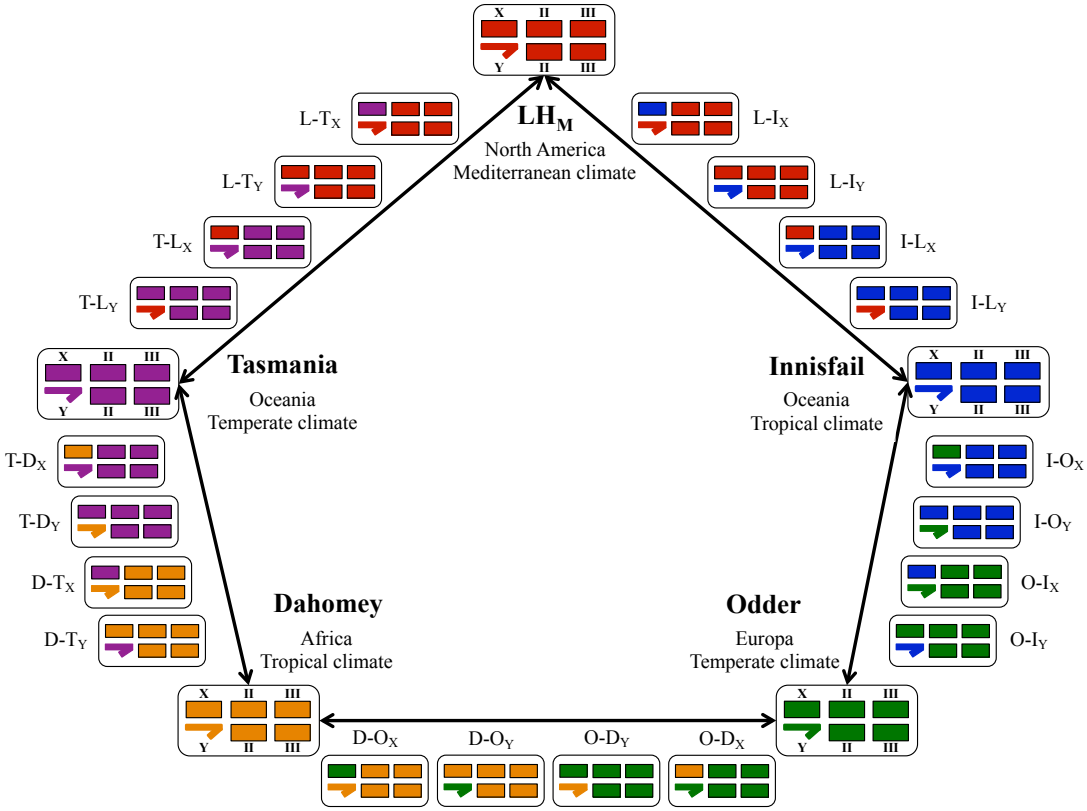

Fig. 1: Schematic of the round robin experimental cross design. Each of the five wild-type populations is color-coded: LH_M (L, red), *Innisfail* (I, blue), *Odder* (O, green), *Dahomey* (D, orange), and *Tasmania* (T, purple). We crossed each wild-type population (large boxes) with two others from either a different continent or climate. This procedure generated four novel genotypes from each cross, so that every combination of the novel sex chromosome pairs experienced a different autosomal genetic background. In total, 10 *Novel X* and 10 *Novel Y* genotypes were generated (small boxes). Coloured bars represent the two major chromosomes (II and III) and chromosome X, with chromosome Y depicted by a half arrow. *Novel* genotypes are annotated as 'genetic background – origin of sex chromosome', with the novel sex chromosome indicated by an X or Y subscript.

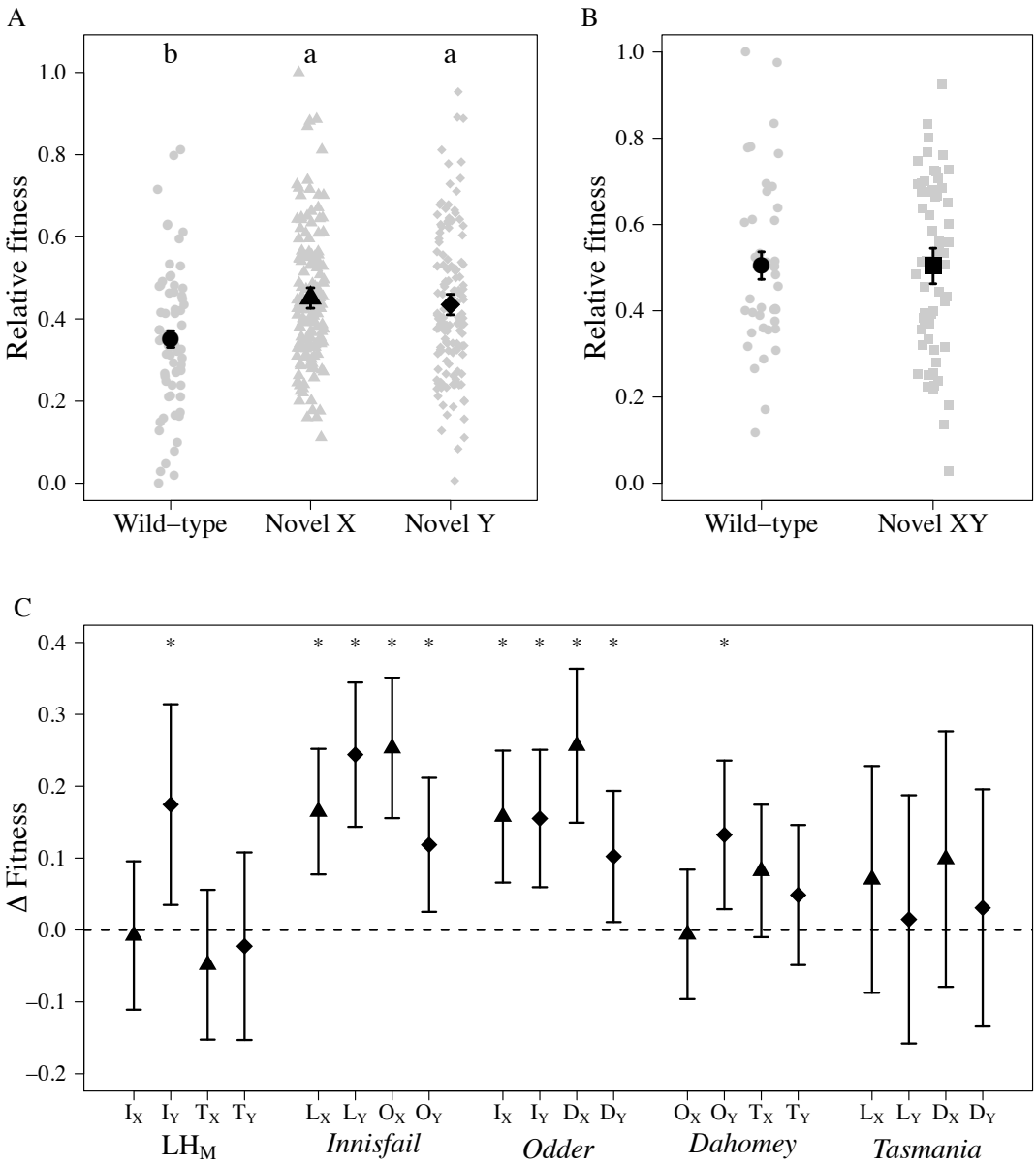

Fig. 2: Male reproductive fitness assays at generation 0. (A) Relative reproductive fitness for wt (circles), novel X (triangle), and novel Y (diamonds) males. We detected statistically significant (P = 2.45e-04) increases in reproductive fitness for both novel X and novel Y males relative compared to the wild-type populations (Tukey HSD; P < 0.05). Mean (±SE) of fitted values from the linear model. The raw data is plotted as grey points. (B) Relative reproductive fitness for wt (circles) and novel XY (squares) treatment groups. There was no significant difference between wt and novel XY. Mean (±SE) of fitted values from the linear model. The raw data is plotted as grey points. (C) Change in relative fitness between the wt and the novel populations. ΔFitness = ωnovel population - ωwild-type with bars indicating bootstrap 95% confidence (see Materials and Methods). 10 out of 20 novel

633 populations (Novel X: triangle, novel Y: diamond) had significantly higher fitness
634 than their wt counterpart (indicate by asterisks), and 16 populations had changed in a
635 positive direction, which was more than expected by chance (P = 0.01). Each of the
636 five wild-types are indicated underneath their four novel populations. Capital letters
637 indicate which population the novel sex chromosome originates from (D: Dahomey, I:
638 Innisfail, L: LH_M, O: Odder, and T: Tasmania) and the novel sex chromosome is
639 denoted by X or Y subscripts.

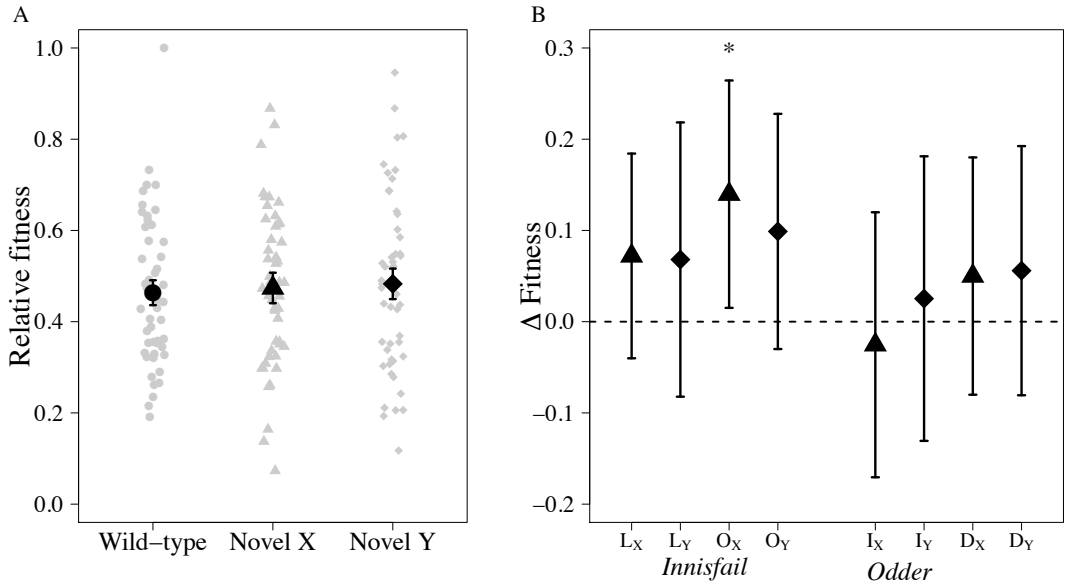

Fig. 3: Reproductive fitness assays at generation 25. We selected 8 of the 10 significant populations for the evolution experiment. (A) Relative reproductive fitness for wt (black circle), novel X (triangle), and novel Y (diamonds) males. Novel treatment males no longer had a higher fitness relative to wt males after 25 generations of experimental evolution. Mean (±SE) of fitted values from the linear model. The raw data is plotted as grey points. (B) Change in relative fitness between wt and novel X (triangles) and novel Y (diamonds) populations (ΔFitness = ωnovel population - ωwild-type), with bootstrap 95% confidence intervals (see Materials and Methods). The asterisk indicates whether ΔFitness was significantly greater than zero (Tukey HSD; $P < 0.05$). Each of the wt populations are indicated underneath their four novel populations. Capital letters indicate which population the novel sex chromosome originates from (D: Dahomey, I: Innisfail, L: LHM, O: Odder) and the novel sex chromosome is denoted by X or Y subscripts.

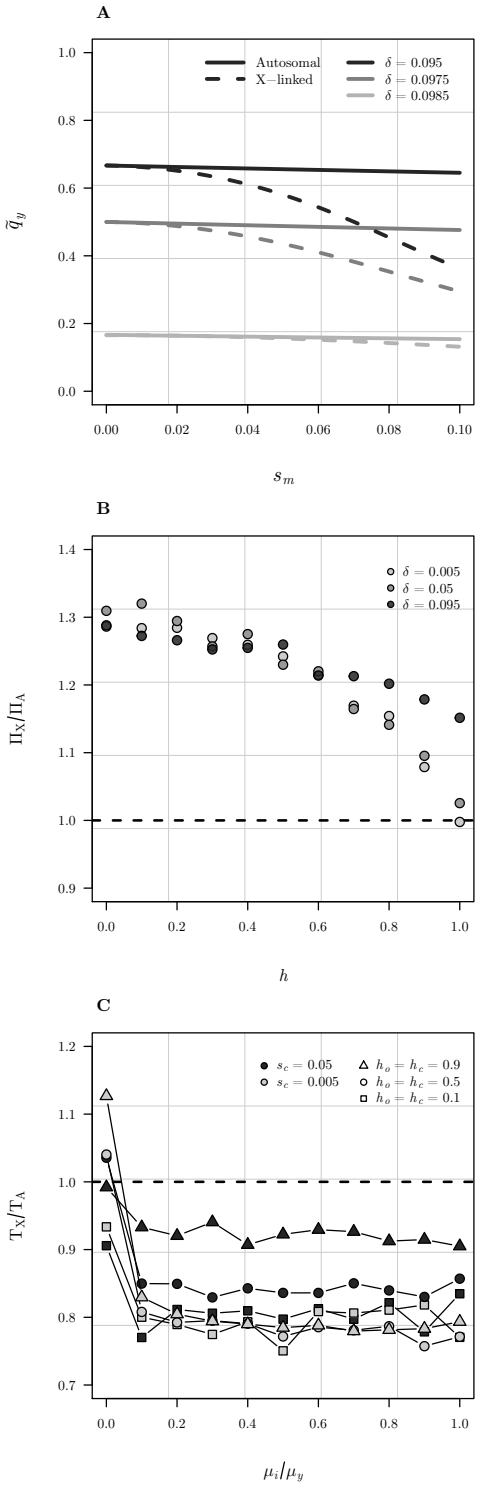

Fig. 4: Summary of theoretical results. (A) Threshold frequencies of the mutant *y* chromosome at which a new compensatory mutation will experience positive selection for the Autosomal ($\tilde{\boldsymbol{q}}_{\boldsymbol{y}}^{\boldsymbol{A}}$, solid lines) and X-linked ($\tilde{\boldsymbol{q}}_{\boldsymbol{y}}^{\boldsymbol{X}}$, dashed lines) models respectively). As the mutant *y* chromosome sweeps to fixation, X-linked compensatory mutations will become selectively favoured earlier than autosomal

660 ones. Results are shown for the case of additive fitness ($h_o = h_c = 1/2$), $s_m = 0.1$, and s_c
661 $= 0.005$. (B) The relative probability of invasion for new Autosomal and X-linked
662 compensatory mutations into populations initially fixed for the mutant *y* chromosome
663 is always greater than or equal to 1, suggesting that when compensatory evolution is
664 limited by mutational variation, the probability of invasion will be higher for X-linked
665 compensatory mutations except under complete dominance. For simplicity we
666 assumed equal dominance for compensatory fitness effects (i.e., $h = h_o = h_c$). Results
667 are shown for three different values of δ (recall that $\delta = s_m - s_o$), where $N = 1{,}000$, s_m
668 $= 0.1$, and $s_c = 0.005$. Each point indicates the mean of 10^6 replicate Wright-Fisher
669 simulations for $\delta = 0.005$ and $\delta = 0.05$, but 5.0×10^6 for $\delta = 0.095$. (C) The relative
670 time to complete a co-evolutionary cycle for the Autosomal vs. X-linked models
671 plotted as a function of the relative mutation rates at the compensatory and Y-linked
672 loci. To explore the consequences of slow vs. fast compensatory dynamics, μ_Y was
673 held constant at 10^{-3}, while μ_i (where $i \in \{\boldsymbol{A}, \boldsymbol{X}\}$) was incremented from 10^{-7} to μ_Y. At
674 low values of μ_i/μ_Y, the rate of compensatory evolution is limited by mutational
675 variation, but at intermediate to high values, the mutant *y* chromosome and
676 compensatory mutations are more likely to segregate simultaneously. Results are
677 shown for two different 'costs of compensation' (s_c) and three different dominance
678 scenarios (we again assume equal dominance, $h_o = h_c$), and population size $N = 1{,}000$.

Table 1: Fitness expressions for the Y-linked locus (**Y**) influencing male siring success, and the viability of offspring resulting from all possible combinations of parental genotypes at **Y** and the compensatory locus **A** (or **X**).

Male Fitness

Male **Y** genotype	Mating success
Y	1
y	$1 + s_m$

Offspring viability

	Mother's genotype		
Father's **Y** genotype	AA, XX	Aa, Xx	aa, xx
Y	1	$1 - h_c s_c$	$1 - s_c$
y	$1 - s_o$	$1 - h_o s_o$	1