Response to divergent selection on meiotic recombination in *Saccharomyces cerevisiae*

Xavier Raffoux[1], Xanita Saayman[2], Walaa A. Abuelgassim[1], Thomas Maret[1], Anthony Venon[1], Fabrice Dumas[1], Lorenzo Tattini[2], Olivier C Martin[3], Gianni Liti[2], and Matthieu Falque[1]

[1] Université Paris-Saclay, INRAE, AgroParisTech, GQE - Le Moulon, 91190 Gif-sur-Yvette, France. [2] Université Côte d'Azur, CNRS, INSERM, IRCAN, 06107 Nice, France. [3] Université Paris-Saclay, CNRS, INRAE, Université Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190 Gif-sur-Yvette, France.

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Divergent Selection on Recombination in Yeast

Corresponding author:

Matthieu Falque

E-mail: matthieu.falque@inrae.fr

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Abstract

Meiotic recombination is a key driver of evolution in sexually reproducing organisms, reshaping genetic diversity by generating novel allelic combinations. The rate of recombination varies substantially across living organisms depending on *cis*- or *trans*-acting genetic elements, as seen in many species, including the yeast *Saccharomyces cerevisiae*. Here, we report on an experimental evolution-based study to better understand the factors shaping this natural variation. Starting with a genetically diverse population of *S. cerevisiae*, we have carried out recurrent divergent selection on recombination rate using a fluorescence-based sorting approach in four independent lineages. After ten generations, we observed an average response of recombination rate of +28% after positive selection and -24% after negative selection, within the interval used for selection. In the adjacent re-

gion, however, we observed a weaker response in the opposite direction, and no response in four other unlinked genomic regions. Whole-genome sequencing of individuals selected for high recombination revealed mixed outcomes in the four independently evolved lineages. All four lineages showed selection for high recombination locally, with particular haplotypes heavily favored and sequence- or structural variation-based heterozygosity selected against within the selection interval. However, only two of the four lineages showed increases in genome-wide recombination rate. Overall, this experimental evolution approach provides original and useful insights into the evolvability of the meiotic recombination rate and the associated genetic determinants.

Introduction

Meiosis is considered one of the major evolutionary innovations of eukaryotes. The associated genetic recombination is at the heart of many evolutionary processes, both under natural selection (Burt and Bell 1987) and in the genetic improvement of domesticated species (Hill and Robertson 1966; Tourrette et al. 2019). Crossovers (COs) occurring during prophase of the first meiotic division have two major consequences: they generate a physical link between homologous chromosomes, which allows a balanced segregation of homologs at anaphase I, required for the viability of the gametes, and they shuffle the parental haplotypes to produce novel allelic combinations in the recombinant gametes. During meiotic prophase I, a large number of programmed DNA double-strand breaks are produced on the chromatids. They are then repaired by several possible pathways which use the homolog as a template — leading to the formation of a minority of COs, which are reciprocal exchanges of genetic material between homologs, and a majority of non-crossovers, which are non-reciprocal short tract exchanges producing gene conversions (GCs), or which use the sister chromatid as a template with no genetic consequence. Two distinct pathways have been demonstrated to exist in many organisms for CO formation (Higgins et al. 2004; Housworth and Stahl 2003; Falque et al. 2009), one of them being sensitive to the phenomenon of interference that reduces the probability of two COs occurring close to each other (Sturtevant 1915), and the other one being insensitive to interference.

CO number and distribution along chromosomes are strongly regulated (Pan et al. 2011). The number of COs per chromosome usually ranges between one and three, and is similar across species, in spite of very large differences in physical chromosome sizes (Fernandes et al. 2017). The

distribution of COs along chromosomes, often referred to as the CO landscape, is usually uneven, e.g. in yeast (Mancera et al. 2008), which is due to a combination of different factors, including interference (Morgan et al. 2024). Large pericentromeric regions are almost void of COs in many organisms (Haenel et al. 2018), and some very small regions can have high densities of COs (hotspots). CO regulation shows several similar features in various species, see review in (Dluzewska et al. 2018). Among them, (1) CO assurance (Jones 1984) ensures that at least one CO occurs per bivalent during meiosis, allowing faithful chromosome segregation, (2) CO homeostasis (Martini et al. 2006) keeps the rate of COs almost constant even if the number of double-strand breaks is reduced, (3) CO interference (Sturtevant 1915; Morgan et al. 2024) leads to COs being more regularly spaced than if they were placed at random, (4) heterochiasmy (Sardell and Kirkpatrick 2020) leads to different levels and landscapes of recombination in male *vs* female meiosis, and (5) natural genetic variation of CO rate distribution is observed within many species (Kong et al. 2010; Bauer et al. 2013; Brekke et al. 2023; Johnston 2024). Finally, the recombination rate (hereafter noted RR) also shows some phenotypic plasticity, for instance as a response to variation in temperature (Lloyd et al. 2018) or nutritional state (Abdullah and Borts 2001; Novak et al. 2026).

There are two types of genetic determinants of CO formation: *cis* DNA sequences that modulate RR locally in their vicinity and thus change the recombination landscape, and some *trans* factors that affect RR genome-wide. Both contribute to the natural variability of RR, and we estimated their respective contributions to 38% and 17% of the variance of RR in *S. cerevisiae* (Raffoux et al. 2018a). *Cis* effects may involve local heterozygosity (Dluzewska et al. 2018). For significant sequence divergence, recombination decreases as heterozygosity increases (Raffoux et al. 2018a), as expected if strong divergence suppresses COs through the mismatch-repair system (Hunter et al. 1996). Recombination QTLs acting in *trans* have also been detected in several species (Pan et al. 2017; Petit et al. 2017; Ziolkowski et al. 2017). Moreover, RR could be increased in Arabidopsis and in a few crop species by knocking out anti-CO genes (e.g. FANCM, FIGL1 or RECQ4), overexpressing pro-CO genes (e.g. HEI10), or changing the ploidy context in Brassicaceae, see review in Blary and Jenczewski (2019).

Although CO formation is highly regulated, the evolutionary pressures behind such regulations remain unclear. There must be at least one CO per bivalent for gametes to be viable, and there seems also to be a soft upper limit to CO number in most species studied (Fernandes et al. 2017), but the underlying selective pressures remain hypothetical. Recombination is strongly associated with the occurrence of sex, so the fitness cost of recombination itself (crossovers) may not always

be easily distinguished from that of sex (segregation) in experimental evolution approaches, as reviewed in Parée and Teotónio (2025). Sex involves spending both time and energy, and has a 'two-fold cost' because the females transmit their genes at only half the rate of asexual equivalents (Bell 1982). Recombination may also increase or decrease fitness (Charlesworth and Barton 1996): it can break beneficial allelic combinations built up by selection, producing the 'recombination load' (Otto and Lenormand 2002), e.g. in *D. melanogaster* where decreased recombination led to higher female fecundity (Barton 2010). Too many COs may also be mutagenic (Liu et al. 2017) or could be mechanically deleterious for meiosis, although evidence for this is still lacking. Yet despite these costs, sex and recombination are maintained in the great majority of species, which is referred to as the 'paradox of sex' (Otto and Lenormand 2002). On the other hand, shuffling alleles through recombination can be beneficial for adaptation by increasing the genetic variance (Barton 2010). Experimental evolution of yeast populations revealed that sexual reproduction favored adaptation to a new harsh environment, whereas no effect was observed in a new benign environment (Goddard et al. 2005), and that sex both speeds up adaptation and allows natural selection to more efficiently sort beneficial from deleterious mutations (McDonald et al. 2016). Altogether, the relationship between recombination and fitness is difficult to study because it is mostly indirect: COs do not directly change fitness (except a small number of COs required to keep euploidy through meiosis), but they modify the potential to exploit standing genetic variation to produce new haplotypic combinations, which may be beneficial (or deleterious) for fitness. Finally, CO interference can also be an important player for the adaptive consequences of recombination since it lowers the probability that two close COs bring together three favorable alleles linked in repulsion phase (Otto and Payseur 2019).

RR shows intraspecific diversity, and many adaptive processes have been found to be associated with the evolution of recombination modifier genes in theoretical (Gandon and Otto 2007) as well as empirical (Korol and Iliadi 1994; Aggarwal et al. 2015) studies. Thus, recombination seems to be able to respond to indirect selection, and it should also be possible to change RR by applying direct recurrent artificial selection. However, selecting on RR is challenging because of the difficulty to measure recombination phenotypes. COs may be detected by studying recombination between genetic markers in linkage mapping experiments (Bauer et al. 2013) or by direct cytological observations. In the latter case, one can measure CO positions by visualizing late recombination nodules using electron microscopy (Anderson et al. 2003) or by immunofluorescence using antibodies against different proteins that concentrate as foci at the location of COs (Anderson et al. 1999;

128 Chelysheva et al. 2012). In all cases, these methods are destructive, and they are difficult to bring to
129 a high throughput as required for experimental evolution.

130 Nevertheless, direct selection experiments on RR have already been reported a long time ago in
131 Drosophila, based on the segregation of morphological genetic markers. After six generations of
132 positive or negative selection on CO rate in the interval between loci 'dichaète' and 'sepia', no re-
133 sponse to selection was first observed (Gowen 1919), but later on, during 30 generations of selec-
134 tion for low CO rate in the interval between 'white eyes' and 'miniature wings' loci, a continuous re-
135 duction of RR was observed (Detlefsen and Roberts 1921). In the same paper, eight generations of
136 selection for high RR in the same interval also produced a reduction of recombination, which was
137 interpreted by the authors as a positive response to selection producing many double COs. Several
138 similar experiments have been performed in other genomic regions of Drosophila (Parsons 1958;
139 Chinnici 1971a; Kidwell 1972; Abdullah and Charlesworth 1974; Charlesworth and Charlesworth
140 1985a), which confirm that recurrent artificial selection for high or low recombination rate most of-
141 ten results in a genetic response. Further studies on the genetic architecture of the response showed
142 that it is polygenic (Chinnici 1971b; Charlesworth and Charlesworth 1985b) and not due to chromo-
143 somal rearrangements. In the insect *Tribolium castaneum*, significant response to selection for high
144 RR has also been reported during 10 generations, after which a plateau was reached, and RR was
145 stable for five more generations (Dewees 1975). In the same paper, when selecting for low RR,
146 there was no significant decrease of RR along generations, but the average RR over the 15 genera-
147 tions was significantly lower than the initial value, suggesting a response limited to the first genera-
148 tion. In *Schistocerca gregaria*, the response to selection was significant for low RR but not for high
149 RR, although there was a trend suggesting a weak response in the second case (Shaw 1972). In silk-
150 worm, response to selection was obtained for both low and high RR, as cited by Ebinuma and Yo-
151 shitake (1981). Apart from insects, a similar experiment has been achieved in Lima bean (Allard
152 1963), resulting in significant responses to selection for high RR but no response to selection for
153 low RR. In animals and fungi, we did not find any published results on long-term experimental evo-
154 lution carried out with artificial selection on RR.

155 Such studies on the evolvability of CO number can provide useful insights on the evolutionary dy-
156 namics of recombination modifier genes that can play a crucial role in adaptation from standing var-
157 iation, particularly in changing environments (Charlesworth 1993). They can also produce evolved
158 populations with diverged recombination rates, which may then be used for experimental evolution
159 with the aim of studying the impact of recombination rate on the dynamics of adaptation after envi-

160 ronmental changes. Moreover, such evolved populations may be used later on to detect signatures
161 of selection by applying sequencing by the 'evolve and resequence' approach (Long et al. 2015),
162 which could point to recombination QTLs and help identify genes or other genomic elements in-
163 volved in the control of the quantitative variation of CO rate.

164 Here, we carried out experimental evolution on a yeast population with high standing genetic vari-
165 ation by applying ten cycles of sexual reproduction with recurrent divergent selection of spores based
166 on their recombination status in a region between two fluorescent markers. We observed a response
167 to selection in the interval where selection was applied and a corresponding inverse response in one
168 neighboring interval. Selecting for recombination over eight cycles led to an increase in homozy-
169 gosity within the selection interval on both the structural and sequence levels. Genome-wide CO
170 analysis further revealed a global increase in CO rate in two of four lineages without impacting the
171 number of non-crossovers. These results collectively suggest that we successfully selected for high-
172 recombination on both the *cis* and *trans* levels in yeast.

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174

175 Materials and Methods

176 Biological material

177 The original SGRP-4X population (Cubillos et al. 2013) was obtained by inter-crossing for 12
178 generations four representative *S. cerevisiae* founder strains: YPS128 [North American (NA)],
179 DBVPG6044 [West African (WA)], Y12 [Sake (SA)], and DBVPG6765 [Wine/European (WE)].

180 The bi-fluorescent strain Mat-a SK1-VI_C1Y2 ho::hygMX ura3::kanMX used to cross to the
181 SGRP-4X population was constructed as previously described (Raffoux et al. 2018b). The
182 TDH3prom-yECerulean-natMX construct (above named "C1") is inserted on chromosome VI at
183 position 17462 bp (S288c_reference_genome_R57-1-1). The 100 bp surrounding the insertion locus
184 are: GCACGTGAAAACGGTGAACGTGGTTGTATACCTTCGATACGGATTTGCTT[CON-
185 STRUCT]ACTAATTGAGAGCAAAATTAGTAAGCGAAATGTGAAAATTGGCTTACGAA.
186 The TDH3prom-Venus-NatMX construct (above named "Y2") is inserted on chromosome VI at po-
187 sition 87 305 bp (S288c_reference_genome_R57-1-1). The 100 bp surrounding the insertion locus
188 are: CCCAGAGAGAAAAAAGGAAAAATTTAGCTATGAAACCTCAATAAGCTTTT[CON-
189 STRUCT]TAATACACCAAAGATTCAAGATAAGAGCATAGAACGAACTGTAGAATAGT.

190 For recombination phenotyping, we also used the three tri-fluorescent tester strains SK1-

191 I_R2C3Y4, SK1-VI_C1Y2R3, and SK1-VI_R3Y4C5 .

192 Details of constructs, genomic positions, and physical distances between markers for all fluores-
193 cent markers used in this work are given in **Suppl Table S1**.

194 **Culture Media and Drugs**

195 Solid SPOR medium was prepared with 0.25 % yeast extract, 0.1 % glucose, 1 % potassium ace-
196 tate, and 20 g/L BD Bacto Agar (BD-214030). Nourseothricin was used at a final concentration of
197 30 mg/L, G418 at 100 mg/L, chloramphenicol at 50 µg/mL, and hygromycin B at 200 µg/mL.

198 **Crossing the SGRP population with SK1-VI_C1Y2**

199 To produce the initial population (G0) of our evolution experiment, we crossed spores from the
200 SGRP-4X population with haploid vegetative cells of the SK1-VI_C1Y2 bi-fluorescent strain.

201 First, a vegetative liquid culture of the SGRP-4X population was grown overnight (15 mL YPD in
202 50 mL tubes, 30° C, 200 rpm), then centrifuged 2 min at 2000 rpm. The supernatant was discarded,
203 and cells were resuspended in 800 µL ddH_2O. Then 150 µL was spread on 4 Petri dishes with solid
204 SPOR medium and incubated at 30° C. After ten days of sporulation, cells were picked up from the
205 lawn by scraping one quarter of the Petri dish surface with a bent pipette tip, and resuspended in a
206 1.5 mL Eppendorf tube containing 750 µL ddH_2O with 5 mg/mL 20T zymolyase (Euromedex,
207 Souffelweyersheim, France) and 100 µL glass beads (0.5 mm diameter, Dutscher 67172 Brumath
208 Ref 1606106). To disrupt tetrads, the tubes were vortexed for 30 sec three times, separated by 30
209 min incubations at 30° C, then centrifuged for 1 min at 13000 rpm, and the pellets were resus-
210 pended by vortexing for 2 min in 200 µL ddH_2O. The suspension, containing mostly vegetative
211 cells, was discarded. Spores, which adhere to the tube plastic, were stripped by adding 600 µL
212 ddH_2O with 0.01% NONIDET NP40 (Sigma-Aldrich, Saint Quentin Fallavier, France) and vortex-
213 ing 2 min, as described in (Rockmill et al. 1991).

214 Then, vegetative cells of the haploid Mat-a bi-fluorescent strain SK1-VI_C1Y2 were grown over-
215 night (15 mL YPD liquid culture in a 50 mL tube, 30° C, 200 rpm), centrifuged for 2 min at 2000
216 rpm, and the pellets were resuspended in 600 µL ddH_2O. These vegetative cells and the spores from
217 the SGRP-4X population were crossed: both cell suspensions were mixed, vortexed for 30 sec, and
218 centrifuged for 2 min at 2000 rpm. Pellets were resuspended in 250 µL ddH_2O, and 200 µL were
219 spread on solid YPD Petri dishes and grown overnight at 30 °C. To select only diploids resulting
220 from the desired cross, each of the four quarters of the Petri dish surface was then scraped and re-
221 suspended in 250 µL ddH_2O, and 200 µL was spread on SD medium without uracil and containing

222 nourseothricin. This allowed us to select only diploids originating from crosses between a SGRP-
223 4X spore (Ura+) and a tester cell (natMX), and none of the three other possible diploid genotypes,
224 nor the remaining parental vegetative cells (**Suppl Figure S1**). Selected diploids were stored and
225 used as the generation zero (G0) to start the selection experiments.

226 **Selection of spores depending on their fluorescence status**

227 Isolated spores from the G0 vegetative population were obtained with the same protocol as pre-
228 sented above. The spore suspension was then analyzed with a MoFlo ASTRIOS Fluorescence-Acti-
229 vated Cell Sorter (FACS; Beckman-Coulter, Villepinte, France) and the associated software Sum-
230 mit for manual investigations, or with the CAYSS R package (Raffoux and Falque 2024) for routine
231 analyses. We first selected events corresponding to the size of spores using a gate in the SSC-
232 Height-Log *vs* FSC-Height-Log graph, then we discarded events containing more than one cell us-
233 ing a gate in the SSC-Height-Log *vs* SSC-Area-Log graph, see more details in Raffoux et al.
234 (2018b). Finally, we analyzed the fluorescence intensity of each spore in yECerulean and Venus
235 channels (excitation at 405 and 488 nm, respectively, emission at 448/59 and 526/52 nm, respec-
236 tively).

237 Spores were then selected depending on their fluorescence status and sorted in a Petri dish con-
238 taining solid YPD with chloramphenicol and glass beads (3 mm diameter). At the end of each sort-
239 ing, Petri dishes were gently shaken to spread glass beads and cells on the surface of the medium.
240 Fluorescence intensity classes for spore selection were chosen depending on the direction of the se-
241 lective pressure to be applied: selection of spores recombinant between the fluorescent markers
242 (Sel+; **Figure 1A**), selection of spores non-recombinant between the markers (Sel-; **Figure 1B**), or
243 selection of all events identified as spores, regardless of their recombination status between the
244 markers (Sel=; **Figure 1C**).

245 In the following, we will represent the phenotype of the two fluorescent markers C and Y by
246 "[MN]" where M is "C" if the marker yECerulean is present or "+" if it is not present, and N is "Y"
247 if the marker Venus is present, and "+" if it is not present. So in even generations of Sel+ experi-
248 ments, 150,000 [C+] recombinant spores and 150,000 [+Y] recombinant spores were selected (left
249 side of **Figure 1A**), in odd generations of Sel+ experiments, 150,000 [++] recombinant spores and
250 150,000 [CY] recombinant spores were selected (right side of **Figure 1A**), in Sel- experiments,
251 150,000 [CY] non-recombinant spores and 150,000 [++] non-recombinant spores were selected
252 (**Figure 1B**), and in Sel= experiments, we sorted a total of 300,000 cells from the four fluorescence
253 classes [CY], [++], [C+], [+Y] (**Figure 1C**), ensuring to sort equal numbers of [CY] and [++]

254 spores, and equal numbers of [C+] and [+Y] spores. Using these procedures for each of the three
255 selection modes (Sel+, Sel-, Sel=), we sorted four biological replicates (noted A, B, C, D) from the
256 initial G0 population, leading to 12 independently evolved populations for each generation, hereaf-
257 ter noted Gn Sel+ A, Gn Sel+ B, Gn Sel+ C, Gn Sel+ D, Gn Sel- A, Gn Sel- B, Gn Sel- C,
258 Gn Sel- D, Gn Sel= A, Gn Sel= B, Gn Sel= C, Gn Sel= D, where *n* is the index of the generation.

259 After 2 days of incubation at 30 °C, a quarter of the surface of Petri dishes containing each G1
260 population was scraped using a bent tip, resuspended in 250 µL ddH_2O, vortexed, and spread on Pe-
261 tri dishes containing SPOR medium with chloramphenicol. A second quarter of each Petri dish was
262 also scraped, resuspended in 400 µL liquid YPD and 180 µL 50 % glycerol, vortexed, and stored at
263 -80 °C. After 10 days of incubation at 30 °C, a quarter of each SPOR Petri dish was scraped, and we
264 prepared isolated spores as described above, thus completing the first selection cycle.

265 This recurrent selection was conducted using the same procedure during 10 generations (noted G1
266 to G10). Note that as seen before, in the case of Sel+ experiments, fluorescence classes to be se-
267 lected for recombinant and non-recombinant spores are not the same for even or odd generations.

268 Furthermore, after the first generation, we observed that Venus fluorescence could exhibit three
269 levels of intensity in spores (Raffoux and Falque 2024), depending on the fluorescent marker gene
270 being present or absent in the spore genome, but also depending on the fluorescent protein being
271 present or absent in the cytoplasm of the mother diploid cell. As an example, in Sel- experiments,
272 some [++] spores can descend from a [++/++] diploid vegetative cell and exhibit the lowest Venus
273 fluorescence intensity (only due to autofluorescence), but other [++] spores can also descend from a
274 [C+/Y+] hemizygous diploid vegetative cell and thus exhibit the intermediate fluorescence intensity
275 due to residual cytoplasmic Venus proteins. The highest fluorescence intensity class corresponds to
276 [CY] or [+Y] spores, which do express the Venus protein. In our selection process, we discarded
277 non-fluorescent spores descending from non-fluorescent diploid mother cells because the recombi-
278 nation status of such spores cannot be determined.

279 **Pool-phenotyping recombination in populations**

280 To phenotype recombination at each generation and for each of the 12 populations (replicates A,
281 B, C, D of selection modes Sel+, Sel-, Sel=), 50,000 non-fluorescent spores were sorted in three dif-
282 ferent Petri dishes containing 100 µL of vegetative cell suspension (at plateau phase) of each of the
283 three haploid tester strains SK1-I_R2C3Y4, SK1-VI_C1Y2R3, and SK1-VI_R3Y4C5 (**Suppl Fig-**
284 **ure S2)** .

285 As the Venus (Y) fluorescent marker can exhibit 3 fluorescence levels as mentioned above, de-
286 pending on the selection mode, the positions of the gates used to select the non-fluorescent [++]
287 spores to be crossed with the testers were not always the same: In the case of Sel- (**Figure 1B**) and
288 Sel= (**Figure 1C**) experiments, as well as even generations of Sel+ experiments (lower part of **Fig-**
289 **ure 1A**), we sorted [++] spores belonging to the lowest intensity class of Venus fluorescence. In-
290 deed, these spores were formed by non-fluorescent [++/++] diploids, so the fact that they may or
291 may not have a CO between the marker loci does not affect their probability of being picked up. In
292 the case of odd generations of Sel+ experiments (upper part of **Figure 1A**), all spores come from a
293 diploid hemizygous or homozygous for the presence of the markers, so the Venus intensity levels
294 showed only two peaks, and we had no other choice but to sort [++] spores descending from fluo-
295 rescent diploids. As shown in the top of **Figure 1A**, half of these diploids are hemizygous for both
296 markers [C+/Y+], so [++] spores descending from these diploids must have recombined between
297 the markers. The other diploids, which are homozygous for the presence of one marker and homo-
298 zygous for the absence of the other marker, cannot produce [++] spores.

299 As a consequence, recombination values are comparable only between odd generations or be-
300 tween even generations of Sel+ experiments, but values measured at an odd generation of Sel+ may
301 not be compared to values measured at an even generation. On the other hand, recombination values
302 phenotyped in Sel-, Sel=, and even generations of Sel+ experiments are comparable to each other.
303 Moreover, in odd Sel+ generations (top of **Figure 1A**), non-fluorescent spores must be produced
304 *via* recombination happening in the marker interval during sporulation, whereas in even generations
305 of Sel+ (G0 and bottom of **Figure 1A**) as well as in all generations of Sel- (**Figure 1B**) and Sel=
306 (**Figure 1C**), non-fluorescent spores can also be produced without recombination. So in odd Sel+
307 generations, an extra selection for high recombination is applied during the phenotyping process,
308 whereas this does not happen in other cases.

309 After 2 days incubation at 30 °C, a quarter of each Petri dish was scraped and resuspended in a
310 well of a 96-wells plate containing 500 µL ddH_2O. We then applied to these cell suspensions two
311 consecutive 1/8 dilutions by adding 700 µL ddH_2O and transferring 100 µL to a new plate. 50 µL of
312 these diluted cell suspensions were then deposited on Petri dishes (9 patches per Petri dish) contain-
313 ing solid SPOR medium with hygromycin.

314 After ten days incubation at 30 °C, cells were picked up from the lawn by scraping patches with a
315 bent pipette tip, and suspended in a rack of 96 tubes (Macherey-Nagel Ref 740637) containing
316 375 µL ddH_2O with 5 mg/mL 20T zymolyase (Euromedex, Souffelweyersheim, France) and

317 100 µL glass beads (0.5 mm diameter, Dutscher 67172 Brumath Ref 1606106). To disrupt tetrads,
318 the plates were incubated 1 hour at 30 °C and shaken three times for 1 min at 30-minute intervals at
319 a frequency of 23 Hz in a TissueLyser (Qiagen), then centrifuged for 5 min at 4500 rpm. Superna-
320 tants were discarded by pipetting, and the pellets were resuspended by shaking as previously in
321 200 µL ddH_2O. The suspension, mostly containing vegetative cells, was discarded. Spores, which
322 adhere to the tube plastic, were stripped by vortexing 2 min (23 Hz) the tubes containing 400 µL
323 ddH_2O with 0.01 % NONIDET NP40 (Sigma-Aldrich, Saint Quentin Fallavier, France) following
324 Rockmill et al. (1991). The spore suspensions were then analyzed with a CytoFLEX flow cytome-
325 ter, the associated software CytExpert (Beckman-Coulter, Villepinte, France) for manual analyses,
326 and the dedicated R package CAYSS (Raffoux and Falque 2024), which computes recombination
327 rates in the intervals between markers, and the coefficient of coincidence (CoC) based on the two
328 intervals delimited by the three fluorescent markers in each tester strain. CoC is calculated as the
329 ratio between the observed frequency of double COs (one in each interval) and the expected fre-
330 quency of such double COs in the absence of interference, the latter being estimated as the product
331 of RRs of both intervals (i.e., assuming CO events are independent of each other). So CoC equals
332 one in the absence of interference, and is below one in case of positive CO interference.

333 **Phenotyping recombination of isolated individuals from G8**

334 Glycerol stocks of the G8 populations were spread on solid YPD Petri dishes with chlorampheni-
335 col and incubated for 24 h at 30 °C. Then one quarter was scraped, resuspended in YPD with Glyc-
336 erol, and stored at -80 °C. Another quarter was scraped in 600 µL ddH_2O, and bi-fluorescent [CY]
337 vegetative cells were sorted by FACS using a MoFlo ASTRIOS (Beckman-Coulter, Villepinte,
338 France). During cell sorting, single bi-fluorescent cells were dropped in each well of three 96-well
339 plates on 100 µL solid YPD. For each of the 12 populations, 288 single cells were sorted. The plates
340 were then incubated for 48 hours at 30 °C.

341 Ninety-six colonies were picked for each of the four biological replicates of the Sel= populations,
342 and 192 colonies for each of the four biological replicates of the Sel− and Sel+ populations. Each
343 colony was then resuspended in a 96-well plate containing liquid YPD with chloramphenicol and
344 incubated overnight without agitation at 30 °C. Plates were then centrifuged for 6 min at 4500 rpm,
345 supernatant discarded by pipetting, and cells resuspended by adding 50 µL ddH_2O. Then, (1)
346 patches of 10 µL cell suspension were deposited on the surface of a solid rectangular Petri dish con-
347 taining solid SPOR medium with chloramphenicol and after 30 min drying, plates were incubated
348 10 days at 30 °C for sporulation, and (2) 150 µL of liquid YPD with glycerol was distributed in the

349 remaining 40 µL of cells suspension, mixed by pipetting, and stored at -80 °C. After sporulation,
350 cells were picked up from the lawn by scraping patches with a bent pipette tip and suspended in a
351 rack of 96 tubes (Macherey-Nagel Ref 740637) containing 100 µL of ddH_2O with 5 mg/mL 20T
352 zymolyase (Euromedex, Souffelweyersheim, France). To disrupt tetrads, plates were incubated for
353 1 h at 30 °C, and shaken three times for 1 min at 23 Hz in a TissueLyser (Qiagen) separated by 30
354 min incubations at 30 °C, and finally centrifuged 5 min at 4500 rpm. Supernatants were discarded
355 by flipping the plate over, and 100 µL glass beads (0.5 mm diameter, Dutscher 67172 Brumath Ref
356 1606106) were added, as well as 150 µL ddH_2O with 0.01 % NONIDET NP40 (Sigma-Aldrich,
357 Saint Quentin Fallavier, France). Plates were then vortexed 2 min (freq=23Hz) and stored at 4 °C
358 before FACS analysis. Finally, the spore suspensions were analyzed, as for the pool-phenotyping,
359 *via* a CytoFLEX flow cytometer, the associated software CytExpert, and the dedicated R package
360 CAYSS (Raffoux and Falque 2024).

361 Out of 1,920 isolated diploid cells, (768 Sel+, 768 Sel− , 384 Sel=), 267 strains produced fewer
362 than 2,000 spores (117 Sel+, 109 Sel− , 41 Sel=) and were discarded, as well as 571 which pro-
363 duced spores with a single fluorescence intensity peak (no segregation, due to homozygous marker)
364 for one or both markers (217 Sel+, 175 Sel−, 179 Sel=). These observed frequencies of strains with
365 non-segregating markers were compatible (based on exact binomial test) with the expected values
366 (0.33 for Sel+, 0.33 for Sel− , 0.55 for Sel=; see **Suppl Figure S3**) in the case of Sel+ (observed
367 freq=0.33*,* *p*-value=0.87) and Sel= strains (observed freq=0.52*,* *p*-value=0.30), but not for Sel-
368 strains (observed freq=0.26*,* *p*-value=0.0004).

369 We then checked single-locus Mendelian segregation in the 1,082 remaining strains (434 Sel+ ,
370 484 Sel−, 164 Sel=) by comparing the allele frequencies F_C and F_Y of C and Y markers in spores
371 with their expected value of 0.5. Allele frequency thresholds were defined as $Thr = median(X_i) \pm$
372 $3.MAD(X_i)$, where MAD refers to the Median Absolute Deviation, $MAD = median(|X_i -$
373 $median(X)|)$. Using these thresholds, 204 strains (90 Sel+, 82 Sel−, 32 Sel=) were outliers because
374 of non-Mendelian segregation for marker C and 206 (93 Sel+, 80 Sel−, 33 Sel=) for marker Y, so a
375 total of 224 strains (98 Sel+, 88 Sel-, 38 Sel=) were discarded (**Suppl Figure S4**).

376 We also checked for two-loci Mendelian markers segregation in the 858 remaining strains (336
377 Sel+, 396 Sel−, 126 Sel=) by comparing the genotype frequency ratios $F_{[CY]}/\left(F_{[CY]} + F_{[++]}\right)$ and
378 $F_{[C+]}/\left(F_{[C+]} + F_{[+Y]}\right)$ in spores with their expected value of 0.5. Using the same MAD-based geno-
379 type frequency thresholds as above, 35 strains (9 Sel+, 2 Sel−, 24 Sel=) were outliers because of
380 non-Mendelian segregation for genotypes CY *vs* ++, and 22 strains (4 Sel+, 17 Sel−,1 Sel=) for

381 genotypes C+ *vs* +Y, so a total of 57 strains (13 Sel+, 19 Sel-, 25 Sel=) were discarded. We ob-
382 served a surplus of strains producing an excess of CY and C+ spores (**Suppl Figure S5**) as com-
383 pared to other categories of non-Mendelian outliers, and that $F_{[CY]}/\left(F_{[CY]}+F_{[++]}\right)$ ratio was higher
384 in Sel= than in the two other modes of selection (**Suppl Figure S6**).

385 FACS-sorted [CY] vegetative diploid cells from G8 Sel= populations can carry two possible
386 marker haplotypes: CY/++ or C+/+Y, each with a probability of 0.5 (**Suppl Figure S3**). As the cy-
387 tometry analysis software was parameterized to compute RRs with CY/++ marker configurations,
388 we measured in Sel= strains 44 RR values greater than 0.5 corresponding to C+/+Y configurations
389 and 57 values lower than 0.5, corresponding to CY/++ configurations. This proportion of 0.44 was
390 not significantly different from the expected value of 0.5 (exact binomial test, *p*-value = 0.23).

391 However, we also obtained RR values greater than 0.5 for 28 Sel+ strains and for one Sel- strain,
392 which does not match with usual genetic expectations (**Suppl Figure S3**). Moreover, two Sel+
393 strains produced RR values very close to 0.5 (0.488 and 0.491). These 31 strains were discarded
394 from the analysis, and the one with RR=0.491 was selected for whole-genome sequencing (see be-
395 low).

396 **Tetrad dissection and whole-genome sequencing**

397 Individual strains isolated from Sel+ G8 populations and phenotyped (as described above) were
398 then chosen for tetrad sequencing. Overall, we selected Sel+ individuals with the lowest RR (here-
399 after denoted [low]) and Sel+ individuals with the highest RR (hereafter denoted [high], see **Suppl**
400 **Table S2**) of each biological replicate.

401 Each individual strain was inoculated overnight in 10 ml YPD at 30 °C, shaking at 220 rpm. The
402 following day, overnight cultures were centrifuged, washed in 10 ml ddH_2O, and resuspended in
403 2 % KAc at ~1 OD_{600}/ml, and left shaking at 23 °C. After one week, samples that were sporulated
404 were tetrad dissected using standard procedures (see below). Of the 16 individual samples, 2 did not
405 sporulate (one from biological replicates A [high] and one from biological replicates C [low]).

406 For tetrad dissections: 400 µL of the culture was removed and centrifuged (1,000 g, 2 min). Pel-
407 lets were resuspended in 100 µL dissection buffer (1 M sorbitol, 0.2 mM EDTA, 10 mM sodium
408 phosphate) containing 5 µL zymolyase (UZ1000-A, 5 mg/mL), and incubated for 20 minutes at
409 37 °C. An additional 400 µL of dissection buffer was added, and samples were placed on ice. After
410 cooling down, 20 µL was used to perform tetrad dissections with the Singer Tetrad Dissection mi-
411 croscope. Plates were grown at 30 °C for 2 days.

412 A total of 80 segregants from 20 tetrads (9 from [high] strains, 11 from [low] strains) were chosen
413 for whole-genome sequencing. DNA was extracted using the MegaPure kit (MPY80200) using an
414 overnight culture in 5 ml YPD (shaking at 220 rpm, 37 °C) and submitted for whole-genome micro-
415 bial sequencing with Novogene.

416 The sequenced tetrads are named according to the sample from which they were taken. For exam-
417 ple, "high_A_2.1" refers to the first tetrad dissection of the second sample of the high-recombina-
418 tion lineages ([high]) sorted from Sel+, of biological replicate A. Tetrads that independently went
419 through meiosis but started from the same isogenic sample can be identified by the final number,
420 e.g., high_A_2.1 and high_A_2.2.

421 **Recombination analysis from tetrad whole-genome sequencing**

422 To reconstruct segregant genotype compositions, diagnostic polymorphisms were identified by
423 first running whole-genome alignment of subtelomere-masked genomes of all four parental ge-
424 nomes, as well as SK1, using progressiveMauve (released 2015-02-13, default parameters) and then
425 extracting polymorphisms unique to each parent. At this point, WA and SK1 unique polymorphisms
426 were merged into one, as they are genetically highly similar. Short read sequences of each segregant
427 were then mapped to the SGD reference genome (S288C, SGD-R64-1-1) using bwa mem (v.0.7.17-
428 r1198-dirty, default parameters) and variants (skipping indels) were called using bcftools (v.1.20).
429 Variants were then cross-referenced with diagnostic parental polymorphisms to identify regions of
430 the genome that could only belong to a single parental genotype (custom scripts, python v.3.12.4).
431 Contiguous tracts belonging to a single parental genotype were then merged, and transition points
432 between genotypes were further refined. To refine transition points: once two adjacent genotypes
433 were known, a second round of identifying diagnostic polymorphisms between the two adjacent
434 genotypes was performed, followed by cross-referencing with segregant variants. Transition points
435 were then called as the base pair directly following the last known unique polymorphism.

436 To identify structural homology between SK1 and the four parental genomes within the chromo-
437 some VI selection interval, mVista (LAGAN, RankVISTA probability threshold $p = 0.5$) was used
438 to determine conserved regions (CNS) between each pair of genomes. We further filtered conserved
439 regions to require a minimum conservation of 85 % and a minimum region size of 1 kbp, and these
440 were visualized using Python.

441 CO counts were performed manually using genotype patterns, and GC counts were calculated as
442 the number of contiguous blocks that had at least 2 genotypes present but were not 2:2 between
443 chromatids, as quantified using bedtools (v.2.31.1). To adjust for the presence of regions that are

genetically identical between all chromatids (which prevents the detection of COs and GCs), the number, size and location of these blocks were identified for each tetrad using bedtools (v.2.31.1). CO counts were adjusted for any genetically identical blocks on chromosome termini, since CO events within internal genetically identical blocks can be partially inferred by surrounding genotypes. GC counts, on the other hand, were adjusted for all genetically identical blocks.

Whole-genome sequencing of an isolated diploid strain with an outlier RR value

We used Oxford Nanopore Technologies to whole-genome sequence one diploid strain (1A-E8) isolated from the G8 Sel+ population, which had a RR of 0.491 classifying it as an outlier.

The high molecular weight DNA extraction was performed based on the protocol of Oxford Nanopore Technologies for yeast DNA, derived from a pre-existing protocol (Denis et al. 2018). The yeast strain was grown overnight in YPD at 30 °C, diluted to OD600 = 0.2 in fresh YPD, and cultured to OD600 ≥ 0.7. Cells were harvested by centrifugation, washed in PBS 1X, and stored at −80 °C. Pellets were resuspended in sorbitol 1 M, treated with zymolyase (1000 U/mL) for 1 h at 30 °C, and centrifuged. A lysis buffer (TrisHCl 114 mM, EDTA 115 mM, NaCl 571 mM, 1.14 % PVP40) was added, followed by SDS 10 % and RNase A, and samples were incubated for 1 h at 50°C. After the addition of TE 1X and potassium acetate 5 M, lysates were clarified by centrifugation. DNA was precipitated with isopropanol, washed with cold 70 % ethanol, air-dried, and resuspended in TE 1X overnight, then stored at −20 °C.

For high-molecular-weight DNA size selection, following a protocol modified from Jones et al. (2021), the samples (3–10 µg) were diluted in TE and mixed 1:1 with 2× Size Selection Buffer (2.5 % PVP-360k, 1.2 M NaCl, 20 mM Tris-Hcl, pH 8). Samples were centrifuged at 10 000 g for 30 min, and the supernatant was discarded. The DNA pellet was washed twice with 70 % ethanol, recentrifuged at 10 000 g for 3 minutes, air-dried, and resuspended in 50 µL TE 1X at 37–50 °C with gentle mixing. DNA quantity was verified using the Qubit dsDNA BR assay (Invitrogen™, Thermo Fisher Scientific).

After quantification, 1 µg of high molecular weight genomic DNA was sequenced using a Ligation Sequencing Kit (Ligation sequencing DNA V14, SQK-LSK114, 2024, Oxford Nanopore Technologies) and a MinION Flow Cell (FLO-MIN114, 2024, Oxford Nanopore Technologies). The library preparation and the sequencing were performed according to the manufacturer's instructions. The basecalling was done using Guppy software (one strand basecalling, 7.3.9+b2176f600, 2024, Oxford Nanopore Technologies).

475 After that, FASTQ files were concatenated and used to build a BLAST database using
476 makeblastdb. Coding sequences of the two fluorescent markers were used as a query to blastn
477 against this database. The makeblastdb and blastn programs used were both from the BLAST+ suite
478 from NCBI (Camacho et al. 2009). For the yECerulean fluorescent marker, the six best BLAST hits
479 were selected with thresholds of ≥ 99 % identity and ≥ 95 % query coverage, whereas for the Venus
480 fluorescent marker, the five best hits were selected with ≥ 97 % identity and ≥ 95 % query cover-
481 age. ONT reads corresponding to these selected hits were subsequently blasted against the SK1 ge-
482 nome using the same BLAST+ applications.

483 Finally, the whole sequence between the two fluorescent markers in the SK1 tester strain (85 kbp)
484 was extracted and blasted against the database built with the ONT reads. The eight best hits were
485 selected with ≥ 99.3 % identity and ≥ 99 % query coverage. ONT reads corresponding to these se-
486 lected hits were aligned against the chromosome VI of SK1 using BioEdit v7.7.1.0.

487 For comparative analysis, we extracted the sequences between the positions where the two fluo-
488 rescent markers were inserted, from the four other founder parents of our population: WA (85 kb),
489 WE (64 kb), SA (66 kb), and NA (86 kb), and blasted these sequences against the database made
490 with our ONT reads. The selection thresholds were ≥ 95 % identity and ≥ 65 % query coverage.
491 This yielded five best hits for WA, four best hits for WE, and three best hits for SA; and using a
492 ≥ 60 % query coverage, three best hits for NA.

493 **Checking for possible effects of fluorescent markers on fitness**

494 To assess the extent to which the presence and/or expression of fluorescent marker genes had an
495 effect on cell fitness, G0 cells were sporulated and spores were isolated from vegetative cells as de-
496 scribed above. Then, a total of 2,000,000 spores were sorted by FACS (see below) based on their
497 fluorescence status: non-fluorescent [++], yECerulean only [C+], Venus only [+Y], or with both
498 markers [CY]. Sorted spores from each category were plated on solid YPD supplemented with 50
499 mg/L chloramphenicol and incubated at 30 °C for 48 h to allow germination and random mating.
500 Diploid cells were then selected in SD medium lacking uracil and lysine. After overnight growth
501 (10 mL SD–ura–lys in 50 mL tubes, 30 °C, 220 rpm), cultures were centrifuged for 5 min at
502 3 000 rpm, the supernatant was removed, and cells were resuspended in 1 mL ddH_2O. Fitness meas-
503 urements were then carried out on these diploids by measuring growth rate, sporulation efficiency,
504 and spore viability as explained as follows:

505 **Vegetative growth kinetics** were monitored in 200 µL of cell suspension diluted to an OD_{600} of
506 0.01 and distributed across the wells of a 96-well microplate to account for possible temperature

507 variation within the plate. The remaining wells were supplemented with sterile YPD as negative
508 controls to control potential contamination. In total, five microplates were prepared. Additionally,
509 300 µL of each suspension was spread onto four SPOR plates, incubated at 30 °C for ten days for
510 subsequent sporulation analysis. Growth kinetics were studied by measuring turbidity every 10 min
511 for 48 h using five custom MiniRead 96-well plate readers (Falque et al. 2024). Growth rates were
512 then computed from the growth curves as the maximum growth rate, using the MiniRead R package
513 and calibration procedures associated with the devices.

514 **Sporulation rates** were measured after 10 days of sporulation as before, by quantifying the pro-
515 portions of tetrads and vegetative cells under the microscope, after scraping cells from the solid me-
516 dium and resuspending them in 10 µL ddH_2O on a microscope slide.

517 **Spore viability** was assessed by sorting single spores by FACS after sporulation of diploids and
518 spore isolation (see before). A total of 768 spores from sporulated G0 diploid cells were sorted and
519 deposited on solid YPD in eight one-well rectangular plates (96 spores per plate) for each spore cat-
520 egory. Plates were then incubated at 30 °C for 2 days and colonies were counted.

521 **Fitness assessments of evolved populations at generation eight (G8)**

522 To assess fitness in evolved populations, growth kinetics, sporulation rate, and spore viability
523 were measured as explained before for G0, but from diploid G8 cells coming from each of the three
524 selection modes (Sel+, Sel-, Sel=). For spore viability assessment, a total of 2304 [++] non-fluores-
525 cent spores (to limit confounding effects) were sorted from spore preparations of sporulated G8 dip-
526 loid cells for each of the three selection modes (Sel+, Sel-, Sel=) and deposited onto 24 rectangular
527 YPD plates (96 spores per plate). FACS sorting parameters were set to select spores and discard
528 vegetative cells and debris based on FSC/SSC. Sorted spores from each category (Sel+, Sel-, Sel=)
529 were plated on solid YPD supplemented with 50 mg/L chloramphenicol and incubated at 30 °C for
530 48 h to allow germination and random mating. Fitness measurements were then carried out on these
531 diploids by measuring growth rate, sporulation efficiency, and spore viability as described before.

532 **Statistical analyses**

533 All data analyses and statistical tests were carried out using R v4.5.0 or python v.3.12.4. For pair-
534 wise comparisons of means, independent *t*-tests were calculated using the stats package from scipy.
535 Multiple comparisons of means were performed by ANOVA using the *lm()* function of R after
536 checking that variances were homogeneous across treatments and residuals were close to normality,
537 otherwise specific non-parametric tests were used as specified in the text. Contrasts were addressed

538 using post-hoc Tukey tests. In the case of proportions, we used the *glm()* function with parameter
539 family=*binomial()*. All multiple tests were corrected using Bonferroni procedure.

540 To quantitatively characterize the distributions of RR among individuals isolated from the evolved
541 populations, symmetry of distributions was tested with the function *symmetry.test()* from the R
542 package "lawstat" with option="MGG" (Miao et al. 2006), side="both", boot=TRUE, B=1000, and
543 q=8/9. The degree of skewness of distributions was quantified using Pearson's third-order moment
544 coefficient of skewness γ_1 (Joanes and Gill 1998).

545

546

547 # Results

548 ## 1. Recombination rate responds to divergent selection

549 In our divergent selection experiment, we started with an advanced intercross yeast population
550 known as SGRP-4X. SGRP-4X was originally created by recurrently mating four genetically diver-
551 gent wild founders from the Sake (SA), North America (NA), West African (WA), and Wine/Euro-
552 pean (WE) clades of *S. cerevisiae*. This highly admixed population results in a representative pool
553 of genetic diversity, which can be used as a powerful tool for mapping quantitative traits (Cubillos
554 et al. 2013).

555 To artificially select for low- or high-recombination, we bulk-mated SGRP-4X with a tester strain
556 in the SK1 background, which contains two fluorescent markers (VI_C1 and VI_Y2) positioned on
557 chromosome VI (**Suppl Figure S2**). We then recurrently selected for (Sel+) or against (Sel-) spores
558 recombinant within that selection interval VI_C1Y2, based on the segregation of fluorescent signals
559 (**Figure 1**). At each generation of selection, the recombination rate (RR) was phenotyped by sorting
560 non-fluorescent spores obtained from the diploid population, mating back to fluorescent tester haploid
561 strains, and quantifying RR within each interval (**Suppl Figure S2**) on a population level from the
562 segregation of fluorescent signals in spores. It should be noted that because of this selection for non-
563 fluorescent haploids, only RR values measured in even generations are comparable between Sel+,
564 Sel-, and Sel= experiments (see **Figure 1**, fully explained in Materials and Methods).

565 We performed ten sexual generations of selection in four independent lineages (A, B, C, D) using
566 VI_C1Y2 as the selection interval. When phenotyping RR within this same interval, we observed a
567 clear response to directional selection (**Figure 2A**). The four populations G0, G10 Sel+, G10 Sel=,

568 and G10 Sel- had a significant effect on RR (p-value=2.5×10^{-4}) with all pairwise comparisons
569 significant (p-values<0.016) except G0 *vs* G10 Sel= (p-value=0.998). When looking at the evolution
570 of RR across generations (**Figure 2A**), the curves and their confidence intervals suggest that RR
571 diverged between G0 and G5 or G6. From G6 or G7 on, RR values seem to reach a plateau during
572 which the values of Sel+ and Sel- relative to Sel= were on average +27.6% and -24.5%, respectively.

573 We observed higher RR values measured in odd than in even generations before G8 (**Figure 2A, B**),
574 consistent with the additional selection applied to odd generations because of our phenotyping method
575 based on sorting non-fluorescent spores (see Materials and Methods).

576 While RR changes were largely consistent amongst the four biological replicates (**Figure 2B**), one
577 exception can be seen at G5 and G6 for biological replicate C, Sel+. This was expected since there
578 was a failure of the FACS when sorting spores produced by G4 Sel+ C diploids, leading to no selec-
579 tion on recombination. This affected selection both from G4 Sel+ C to G5 Sel+ C but also from
580 G5 Sel+ C to G6 Sel+ C (see **Figure 1A**). As a result, further generations (G7 to G10) of replicate
581 C missed two generations of efficient selection, and thus, replicate C was removed from G5 to G10
582 in the calculated means (**Figure 2A, C**).

583 When looking at RRs in the immediately adjacent interval Y2R3 on chromosome VI (**Figure 2C**),
584 RR differed significantly among G0, G10 Sel+, G10 Sel=, and G10 Sel- populations (p-
585 value=3.0×10^{-5}), with all pairwise comparisons significant (p-values<0.011) except G0 *vs* G10 Sel+
586 (p-value=0.34) and G0 *vs* G10 Sel= (p-value=0.080). However, the responses were in opposite di-
587 rections as compared to what happened in the VI_C1Y2 interval, where selection was applied. Spe-
588 cifically, when looking at the evolution of RR across generations (**Figure 2C**), the curves and their
589 confidence intervals suggest that between G0 and G4 or G5, RR in Sel+ became lower than in Sel=,
590 and RR in Sel- became higher than in Sel=. In further generations, RR seemed to reach a plateau
591 during which the values of Sel+ and Sel- relative to Sel= were approximately -12% and +13%, re-
592 spectively. RR data for all individual replicates are available as **Figure 2D**, and do not show any
593 particular difference across biological replicates of a given selection mode.

594 CO interference estimated *via* the coefficient of coincidence (CoC) did not show any pairwise sig-
595 nificant difference among G10 Sel+, G10 Sel-, and G10 Sel= populations (p-values>0.16) in the re-
596 gion including the selected interval (**Suppl Figure S7A, B**) and the immediately adjacent one
597 (**Suppl Figure S7C, D**).

598

599 **2. Divergent selection reshapes the distributions of RR values**

600 While the selection process seemed to successfully generate high- and low-recombining popula-
601 tions on average, it was unclear how penetrant these traits were on a population level. Therefore, to
602 gain insights into the distributions of RR values in the evolved populations, we FACS-sorted 1,920
603 isolated bi-fluorescent diploid cells from generation G8 and phenotyped each of the derived line-
604 ages for recombination. Because the FACS cannot distinguish between diploid cells homozygous or
605 hemizygous for the fluorescent markers, only part of these strains were hemizygous, and thus suita-
606 ble for RR measurement (see Materials and Methods and **Suppl Figure S3**). Among them, we
607 measured RR in 293 Sel+, 101 Sel=, and 376 Sel- strains.

608 Average RR values for each selection mode were 0.29 for Sel+, 0.26 for Sel=, and 0.19 for Sel-
609 (**Figure 3A**), and these three values were significantly different pairwise (Bonferroni-corrected Wil-
610 coxon rank sum tests, *p*-values<0.00018). Average RR values were not significantly different across
611 biological replicates for Sel= strains (adjusted *p*-value=1). However, in Sel+ strains, average RR
612 values differed significantly (*p*-value=0.047) between replicate C (RR=0.27) and replicates A
613 (RR=0.30), which may be related to the different selection regimes applied to replicate C at genera-
614 tions G5 and G6 (**Suppl Table S3; Suppl Figure S8**).

615 **Figure 3B** shows the smoothed distributions of RR values for each of the three selection modes.
616 The Sel= distribution was approximately symmetrical (skewness γ_1=0.144, MGG symmetry test, *p*-
617 value=0.512), whereas the Sel+ distribution was significantly negatively skewed (γ_1= - 0.317, *p*-
618 value<2.2×10^{-16}) and the Sel- distribution was significantly positively skewed (γ_1=0.751, *p*-
619 value<2.2×10^{-16}). The variance of RR in Sel- strains was significantly lower (Fligner-Killeen test)
620 than in Sel= strains (*p*-value=0.0008) and also lower than in Sel+ strains, although less clearly so
621 (*p*-value=0.047). The variance of Sel+ strains showed a tendency to be lower than in Sel= strains,
622 though not significantly (*p*-value=0.052). The overall *shapes* of these distributions were signifi-
623 cantly different for all pairs (KS tests, *p*-values<0.00085). This suggests that selection successfully
624 enriched the initial population for individuals carrying high- and low-recombining genotypes.

625 When isolating bi-fluorescent diploid cells from G8 Sel+ populations, we found two individuals
626 with outlier RR close to 0.5. Such apparent independent segregation of two linked markers may be
627 due to either a drastic increase in RR, or a change in the chromosomal configuration of the fluores-
628 cent markers, for instance, a translocation or duplication of one marker, or a drastic change in the
629 physical length of the interval between the markers. To investigate these possibilities, we whole-
630 genome sequenced one of these two diploid strains. The results showed that the two markers are

631 still at the prediction positions on chromosome VI and neither of the markers are duplicated at an-
632 other genomic location (**Suppl Figure S9**). Moreover, Mendelian segregation of both markers con-
633 firmed that they are each present at a single locus. Finally, the sequence between the two markers
634 (85 kbp) in the sequenced strain was homozygous and identical to the SK1 sequence, ruling out the
635 idea that some large DNA fragment could have been inserted between the markers. We therefore
636 conclude that the individuals have truly attained a high RR.

637

638 **3. Selection for high recombination acts on both local (*cis*) and genome-wide**
639 **(*trans*) levels**

640 Following ten generations of divergent selection, RR responded to selection within the selected
641 interval and in opposite directions in the immediately adjacent one (**Figure 2A, 2C**). However,
642 there appeared to be no differences in RR between Sel+, Sel- and Sel= in other probed regions of
643 chromosomes I and VI (**Suppl Figure S10, Suppl Figure S11**). There was also no significant CoC
644 differences among selection modes at G10 in other probed regions of chromosomes I and VI (**Suppl**
645 **Figure S7**), but CoC values at G0 were significantly higher than at G10 in both chromosome VI re-
646 gions (p-value<0.0044).

647 Collectively, these results suggest that the observed response to selection involved primarily local
648 mechanisms. However, the fluorescent markers used in this phenotyping only probe a very limited
649 proportion of the genome and therefore do not accurately reflect genome-wide recombination pat-
650 terns, nor do they provide insight into features that may have been selected on the local (*cis*) level.

651 To gain a more complete view of genome-wide RRs, as well as local (*cis*) factors that may have
652 been selected for, segregants of individual meioses were whole-genome sequenced to quantify
653 recombination rates genome-wide. Given the asymmetric distribution of recombination rates within
654 the Sel+ experiments (see before, and **Figure 3B**), we reasoned that this population may contain
655 lineages with truly high recombination rates as well as some passenger lineages with near wild-type
656 recombination rates. We therefore sequenced the eleven highest-recombining [high] and the eleven
657 lowest-recombining [low] individuals, all isolated from the Sel+ populations at generation G8 (two
658 to three individuals from each biological replicate A, B, C, and D).

659 Briefly, tetrads from each of the 20 individuals were dissected to acquire four monosporic segre-
660 gants, which were then sequenced using Illumina short read sequencing. Segregants were sequenced
661 with a minimum read depth of 39X and a mean read depth of 87X (**Suppl Figure S12A**). No chro-

662 mosomal aneuploidies or large copy number variations were apparent among segregants (visual in-
663 spection; **Suppl Figure S13**). Using a custom pipeline (further described in Materials & Methods),
664 the genotype compositions of each segregant were reconstructed. It should be noted that the geno-
665 type compositions can only be assigned to one of the four parental genotypes within syntenic re-
666 gions, so this analysis did not reconstruct genotypes within subtelomeric regions. In addition, the
667 WA and the SK1 tester strain genotypes are so genetically close that they were merged into a single
668 genotype for this analysis.

669 We first wanted to determine whether there had been any selection for pro-recombinergic features
670 on the local (*cis)* level. Strikingly, the WA and SK1 genotypes were heavily enriched within the
671 Chromosome VI selection interval in [high], but not in [low], segregants (**Figure 4A;** individual tet-
672 rads shown in **Suppl Figure S12B)**. This may be because relative to the SK1 tester strain, the pa-
673 rental strain WE has a truncated chromosome VI arm, and the parental strain SA has a deletion
674 within the selection interval (**Suppl Figure S12C**). Together, these results suggest that there has in-
675 deed been a selection for pro-recombinergic genotype compositions on the *cis* level. Namely, by en-
676 riching for the WA/SK1 genotype within the selection interval of Sel+ populations, the [high]
677 evolved lines have both maximized structural similarity and minimized nucleotide heterozygosity to
678 the SK1 tester strain.

679 The reconstructed genotypes were next used to determine genome-wide recombination rates.
680 Crossover (CO) events were manually called based on genotype patterns, whereas gene conversion
681 (GC) events were calculated by counting the number of contiguous genome regions containing 3:1
682 marker patterns (examples shown in **Figure 4B**).

683 When quantifying the number of recombination events, it should be noted that because there are
684 'patches' where all four chromatids are genetically identical, the precise location or the precise
685 number of COs within these regions cannot be determined, and GCs cannot be detected at all.
686 Therefore, while some COs occurring within these regions can be inferred based on the surrounding
687 genotype pattern, the total CO number will almost always be under-estimated. This is unlikely to
688 confound our analysis, as the number and cumulative size of such genetically identical blocks are
689 very similar between [low] and [high] populations (**Suppl Figure S12D**). [high] samples had a
690 slight but non-significant (p-value=0.052) increase in the cumulative size of blocks, meaning that
691 CO and GC estimates in [high] samples may be more under-represented than in [low] samples,
692 which is conservative. To reduce the impact of these genetically identical patches as a potential con-
693 founder, CO and GC rate estimates were adjusted for each sample according to the size and location

694 of the genetically identical patches.

695 Genome-wide, CO rates, but not GC rates, were significantly higher in [high] individuals than in
696 [low] individuals, regardless of whether variations in the genetically identical patches were adjusted
697 for (**Figure 4C**). Interestingly, different biological replicates seemed to have different genome-wide
698 responses to selection (**Suppl Figure S12E**). Biological replicates A and B showed a more con-
699 sistent increase in global COs following selection, while biological replicates C and D showed ei-
700 ther no response (C) or a milder response (D) to selection. The increase in CO rates was also not
701 uniformly spread through the genome: there were increases (non-adjusted p-values<0.05) in chro-
702 mosomal CO rates on chromosomes III, IX, and IV, slight but non-significant increases on chromo-
703 somes II, V, XII and XIII, slight but non-significant decreases in CO rates on chromosomes XI and
704 XIV, and the remaining chromosomes remained unchanged (**Figure 4D**). Surprisingly, there was no
705 apparent increase in the total number of COs on chromosome VI, which contains the selection inter-
706 val, perhaps due to CO interference. Importantly, the increase in CO rates could be observed on
707 chromosomes besides chromosome VI, suggesting that in addition to selecting for pro-recombiner-
708 gic *cis* elements, at least some [high] lineages have evolved *trans* effectors increasing CO rate re-
709 motely. As we see no concomitant increase in GC rates, we propose that this may be through
710 CO:NCO choice pathways.

711

712 **4. Single-generation response of RR when relaxing and re-applying selection**

713 Due to a FACS failure, the biological replicate C of Sel+ selection was not properly sorted from
714 G4 to G5, where actually Sel= was applied instead of Sel+. **Figure 2B** shows that RR dropped
715 down to the Sel= level in that replicate C during the single G4 to G5 transition. Proper Sel+ was
716 then applied from G5 to G6, but given the fact that all G4 spores were kept for G5 in the replicate
717 C, the next spore selection from G5 to G6 (i.e., selecting bi-fluorescent and non-fluorescent spores,
718 see **Figure 1A, C**) did not, in fact, particularly select recombinant spores. Thus, as expected, RR in
719 G6 Sel+ C stayed at the same level as in G5 Sel+ C. Selection was efficient at the next generation
720 when sorting from G6 to G7 Sel+ C. Consistently, RR went up from G6 to G7 during that single
721 generation interestingly up to a level higher than in G4.

722 To try to reproduce such a rapid change in RR and further investigate possible different evolution-
723 ary trajectories among biological replicates, we took aliquots of the four biological replicates of
724 G8+, and for each of them, we carried out one generation of Sel= followed by one generation of
725 Sel+ (black lines in **Figure 2B**). For replicates A and B, RR did not change much during those two

726 new generations, whereas for replicates C and D, RR values tended to decrease. These replicates C
727 and D thus partially reproduced the rapid drop in RR that was observed in G5 Sel+ C and G6 Sel+
728 C, although RR values did not reach the level of Sel= populations as in G5 Sel+ C and G6 Sel+ C.

729 To investigate whether some non-genetic mechanisms might have played a role in the quick re-
730 sponses observed previously when relaxing and re-applying positive selection in replicate Sel+ C,
731 we carried out two generations of Sel+ and Sel= as before, but instead of a genetically diverse G0
732 population, we started from a diploid SK1 strain without any genetic diversity except the hemizy-
733 gosity of the two fluorescent markers in the same configuration as in the previous G0. From a strict
734 genetic point of view, no response to selection should be expected then. And in fact, we did not ob-
735 serve any difference between Sel= and Sel+ evolutions for RR, either in the interval selected (**Suppl**
736 **Figure S14A**), the immediately adjacent interval (**Suppl Figure S14B**), or the other regions on
737 chromosomes I and VI (**Suppl Figure S14D, E, G, H**). Similarly, CoC did not differ between Sel=
738 and Sel+ in all three regions (**Suppl Figure S14C, F, I**).

739

740 **5. Fitness effects of divergent selection on RR**

741 To investigate possible variations of fitness as an associated response to divergent selection on re-
742 combination, we first assessed the extent to which the presence and/or the expression of fluorescent
743 markers may introduce confounding effects on fitness. To do so, we sorted spores from G0 having
744 four different configurations of fluorescent markers [++], [+Y], [C+], and [CY], and measured fit-
745 ness components on the resulting diploids after panmixis within each category. We observed no sig-
746 nificant effect of the fluorescent marker status on growth kinetics parameters maximum rate (*p*-
747 value=0.93; **Suppl Figure S15A**) and plateau (*p*-value=0.56; **Suppl Figure S15B**). However, a sig-
748 nificant effect was detected on sporulation rate (*p*-value=0.035) with [+Y] diploids sporulating sig-
749 nificantly better than [C+] ones (*p*-value=0.033; **Suppl Figure S15C**), and on spores viability (*p*-
750 value=0.0040) with [+Y] spores being significantly more viable than [C+] ones (*p*-value=0.0021,
751 **Suppl Figure S15D**). Two-factors analysis of variance indicated that the presence of the Y marker
752 insertion had a significant positive effect on spore viability (*p*-value=0,022) and that the presence of
753 the C marker insertion had a significant negative effect (*p*-value=0,0047), but that there was no sig-
754 nificant interaction between their effects, so the positive effect of Y might be counteracted by the
755 negative effect of C in [CY] cells.

756 Then, to assess the fitness of evolved populations, we sorted non-fluorescent spores from popula-
757 tions G0, G8 Sel+, G8 Sel-, and G8 Sel=, obtained diploids from each category by panmixis, and

758 measured fitness components as before. We observed no significant difference between the four
759 populations for growth kinetics parameters maximum rate and plateau (**Figure 5A, B**), as well as
760 for sporulation rate (**Figure 5C**). However, spore viability in G8 Sel+ and in G8 Sel- were signifi-
761 cantly higher than in G8 Sel= and GO populations (**Figure 5D**).

762

763

764 Discussion

765 The present divergent selection experiment showed a response of local recombination rate after
766 ten generations of direct selection of recombinant or non-recombinant spores in a region of chromo-
767 some VI. The experiment was designed to investigate evolutionary mechanisms during a limited
768 number of generations. The response to selection is thus most likely due to selection and recombi-
769 nation among allelic variants present in the starting population, rather than accumulated novel muta-
770 tions. To further limit the occurrence of mutations, we kept the number of mitotic cell divisions low
771 (approximately 10 mitotic cycles per sexual generation). On the other hand, to maximize the stand-
772 ing genetic variation, four of the five founder parents used to build the initial SGRP-4X population
773 were chosen to capture as much as possible of the genetic diversity of the *S. cerevisiae* species (Cu-
774 billos et al. 2013). Moreover, the genomic interval chosen for selecting recombinant spores was pre-
775 viously found to show a high level of variation in recombination rate across these five founders
776 (Raffoux et al. 2018a). Indeed, we observed a plateau in the response to selection after about eight
777 generations (**Figure 2A**), suggesting that we've reached the limits allowed by the initial genetic var-
778 iation and thus that the impact of novel mutations on that response was probably limited.

779 The experimental protocol used in this work was designed to sort 300,000 cells at each generation.
780 The main bottleneck in our experiments is due to FACS, so population size should never be less
781 than 300,000 throughout all steps of all sexual generations. Genetic differentiation between G0 and
782 Sel= populations is thus expected to be mainly due to unwanted selection (e.g. on growth, sporula-
783 tion, viability) rather than genetic drift. Therefore, using Sel= populations as a control should allow
784 us to investigate the effect of selection on recombination without confounding effects of drift or un-
785 wanted selection on other traits.

786 We do not know of any similar experiment performed previously in *S. cerevisiae*. In *S. pombe*, an
787 experiment of evolution under selection for recombinant or non-recombinant spores was reported in
788 a recent PhD dissertation (Berenguer Millanes 2025). The author carried out a similar approach

789 based on FACS-sorting spores expressing fluorescent proteins, but the initial diploid was obtained
790 by crossing two quasi-isogenic variants (differing only at a few marker loci) from a single Leupold
791 lab strain, so the main source of genetic variance allowing for evolution was novel mutation. After
792 36 meiotic generations, the author observed a significant positive response when selecting double-
793 recombinant spores in two adjacent intervals on Chromosome I, but unclear results in single inter-
794 vals. It should be noted that (1) in such selection for double recombinants, the effective selection
795 pressure applied on RR is much stronger than in our case (single interval) because it requires two
796 different COs, and (2) the absence of crossover interference in *S. pombe* also makes simultaneous
797 selection on two close intervals more effective than in *S. cerevisiae*. More generally, quantitative
798 genetics studies within and between species suggest that RR is expected to display a weak response
799 to short-term selection (Payseur 2025). Our results are consistent with previous works showing that
800 RR is able to evolve under selective pressure, e.g., in Drosophila (Detlefsen and Roberts 1921;
801 Chinnici 1971a; Chinnici 1971b; Charlesworth and Charlesworth 1985a) and other species, see
802 large review in (Stapley et al. 2017).

803 In our experiment, selection was stronger in Sel+ than in Sel- since spores selected in Sel- may
804 result from hidden recombination between the markers (**Figure 1A**). Quantitatively, the expected
805 frequency of spores produced with a (hidden) recombination event between the marker loci, among
806 all spores kept by the FACS in Sel- regime, ranges between zero and 1/3 as RR ranges between 0
807 and 0.5, so one could expect selection to be more efficient in Sel+. However, we observed no clear
808 difference in the absolute values of slopes of the responses between Sel+ and Sel- (**Figure 2A**).

809 We observed an inverted response to divergent selection in the interval next to the one where se-
810 lection was applied. This may be due to CO interference: if local determinants drive double-strand
811 breaks to be preferentially repaired into COs in one region, then interference is expected to commit
812 double-strand breaks surrounding that region to the NCO pathway, thus depleting COs in adjacent
813 intervals. When we assessed the strength of interference along generations, no significant changes
814 were observed between Sel+, Sel-, and Sel= experiments, indicating that changes in interference
815 strength were not the main mechanism that led to modified CO frequency as a response to selection.
816 In the interval VI_R3Y4C5, we measured surprisingly high values of CoC (greater than one in the
817 first three generations) whereas CoC values became stable and lower than 1 in further generations.
818 Values of CoC greater than one are supposed to indicate negative interference, which is not in ac-
819 cordance with our previous measurements of positive interference (corresponding to gamma param-
820 eter nu>1) with the same method in the founder strains of the SGRP-4x population (Raffoux et al.

821 2018a). Given the high variance associated with the data, we suggest that negative interference
822 measured in generations G0 to G2 should be taken with care. Nevertheless, in other regions too, we
823 observed a similar tendency of interference to increase (*i.e.,* of CoC to decrease) across generations,
824 whatever the mode of selection, Sel+, Sel-, or Sel=. If this increase is related to genetic changes in
825 the population, it cannot be due to our selection on recombination, but might be a consequence of
826 unwanted selection on some other traits, which is unavoidable in experimental evolution, particu-
827 larly when sporulation is involved in the selection cycle.

828 Phenotyping individuals isolated from the evolved populations revealed that eight generations of
829 directional selection reduced the variance of RR values in the selected interval for Sel+ and Sel- ex-
830 periments, but not in the Sel= control. This may be explained by the fact that strong selection gener-
831 ally reduces genetic diversity, which in turn results in reduced phenotypic variance. Still, in both
832 Sel+ and Sel- evolved populations, we observed continuous distributions of RR (**Figure 3B**) with
833 gradual changes in the mean across generations as compared to Sel= (**Figure 2A**), pointing to the
834 fact that we've successfully evolved population-level changes in the recombination rates, without
835 only selecting for some strong recombination variant. However, the shapes of the distributions were
836 also modified, with a negatively skewed distribution in G8 Sel+ and a positively skewed distribu-
837 tion in G8 Sel-, while the distribution remained symmetrical in G8 Sel= (**Figure 3B**). These two
838 asymmetric distributions might be explained by considering the hypothesis of a rather strong QTL
839 affecting RR. The Sel+ population would mainly contain individuals carrying the 'high RR' allele
840 at this QTL, visualized by the highest peak on the right side of the red curve in **Figure 3B**, and a
841 minority of individuals with the 'low RR' allele revealed by the shoulder on the left side of the red
842 curve. Symmetrically, the Sel- population would mainly contain individuals carrying the 'low RR'
843 allele at this QTL, visualized by the highest peak on the left side of the blue curve in **Figure 3B**,
844 and a minority of individuals with the 'high RR' allele revealed by the shoulder on the right side of
845 the blue curve. In Sel=, both alleles would have similar frequencies, which would result in the ob-
846 served symmetrical distribution. Moreover, the Sel= curve shows three peaks, and the shapes of
847 Sel+ and Sel- curves also suggest a possible third bump between the two main parts of the peaks,
848 which might correspond to the three genotypes of a codominant QTL. Elsewhere, in Sel-, the peak
849 is higher than in Sel+, indicating a greater loss of variance. This may suggest that selection was
850 stronger in Sel- than in Sel+ experiments, which is not expected given our experimental design al-
851 lowing some hidden recombinants to be selected in Sel-. Another hypothesis is that higher recombi-
852 nation in Sel+ increased the genetic variance by creating new allele combinations, thus reducing the

853 loss of variance due to selection.

854 Following selection within the chromosome VI_C1Y2 interval, the responses in RR in the five
855 other intervals carrying fluorescent markers suggest a *cis*-only change in RR. However, the regions
856 probed by fluorescent markers are very limited as compared to the whole genome, leading us to se-
857 quence individual [high] and [low] segregants from G8 Sel+ to obtain a genome-wide and higher-
858 resolution picture of recombination landscapes. [high] samples almost universally showed an in-
859 crease in homozygosity to the chromosome VI SK1 tester strain (both for sequence and at the struc-
860 tural level), suggesting a strong selection on the *cis* level, which is in accordance with previous ob-
861 servations in *S. cerevisiae* (Raffoux et al. 2018a). In addition, we observed a significantly higher
862 genome-wide CO number in [high] compared to [low] tetrads. Given that COs are relatively rare on
863 a per-chromosome level, the data are noisy and statistical power is low, but we also observed a
864 (non-significant) trend to increase on chromosomes not containing the selection interval. Together,
865 these suggest an addition selection on the *trans* level.

866 Intriguingly, the tetrads sequenced from the four G8 Sel+ evolution lines (*i.e.,* biological repli-
867 cates A, B, C, and D) appeared to increase the chromosome VI recombination rates through differ-
868 ent mechanisms that we were able to discern by sequencing individual segregants from each tetrad:
869 In tetrads from G8 Sel+ A [high] and G8 Sel+ B [high], we observed consistently high genome-
870 wide recombination rates (80, 89, 81, 87, and 87 COs per tetrad), suggesting successful selection for
871 genome-wide increases in recombination rates, as intended. Conversely, in G8 Sel+ C [high] and
872 G8 Sel+ D [high], we did not observe high genome-wide recombination rates (68, 69, 73 COs per
873 tetrad), and we observed no striking abnormalities on the *cis* level. It is therefore unclear why the
874 tetrads sequenced from G8 Sel+ C [high] and G8 Sel+ D [high] display high recombination rates
875 within the chromosome VI selection interval. These different behaviors between replicates A and B
876 on one side, and C and D on the other side may be related to the RR changes observed when we re-
877 laxed selection for two generations in the four G8 Sel+ biological replicates (black lines in **Fig-**
878 **ure 2B**). Such variation in evolutionary outcomes of technically identical experimental setups high-
879 lights the power of this approach for uncovering biological novelty.

880 In the biological replicate C of Sel+ experiment (**Figure 2B**), when the selection pressure was re-
881 laxed (all spores were kept instead of only recombinant ones) between G4 and G5, RR went back to
882 its initial level within only one generation, and recovered its G4 value as soon as recombinant
883 spores were again specifically selected, which occurred only at G7 (see Results, and **Figure 1A**).
884 When we tried to reproduce the same situation by relaxing spore selection starting from each of the

885 four biological replicates of the G8 Sel+ population, a similar response was observed for replicates
886 A and B, but replicates C and D were not affected by the change in the selection mode (black lines
887 in **Figure 2B**). These results raise the question of which kind of genetic element or factor affecting
888 recombination could have its frequency massively altered in a single generation without selection,
889 and massively recovered in a single generation when selecting again recombinant spores. Because
890 the action of this element was observed at the local level only, one could think of a sequence motif
891 with major local effect, such as a hotspot, and/or a particular diploid genotype with an inhibitory
892 effect on COs, due, for instance, to heterozygosity of a structural variant such as an inversion or a
893 deletion. Although we have not identified the real mechanism at work in our experiment, it seems to
894 be able to play an important role in the response to directional selection.

895 Our results show that eight generations of divergent selection on recombination did not change
896 growth kinetics (**Figure 5A, B**) or sporulation rate (**Figure 5C**). Given the experimental design in-
897 volving mitotic growth and sporulation steps, it is likely that some unwanted selective pressure was
898 applied on these traits, so an increase of fitness may have been expected between G0 and G8 gener-
899 ations. However, the SGRP-4X population had already undergone 12 generations of sexual repro-
900 duction before we started our experiments (Cubillos et al. 2013), so major alleles controlling these
901 fitness components may have already been more or less fixed. On the other hand, spore viability in
902 G8 Sel+ and in G8 Sel- were significantly higher than in G8 Sel= and GO populations (**Figure 5D**).
903 Because it increased CO rates to some extent genome-wide, the Sel+ treatment may have reduced
904 the frequency of achiasmatic bivalents leading to aneuploidy, thus increasing the ability of spores to
905 germinate as compared to the Sel= case. However, this does not explain why Sel- populations also
906 gained better spore viability than Sel= ones. A hypothesis might be that this is due to fitness effects
907 of the fluorescent markers inserted in the genome. Indeed, [C+] spores were less viable — but [+Y]
908 spores more viable — than [++] spores (**Suppl Figure S15**), both effects being more or less addi-
909 tive (see Results). The advantage of being [+Y] might be explained by the fact that severely non-
910 viable spores, which could not express fluorescent proteins, cannot be present in the [+Y] class but
911 may be present in the [++] class, introducing some sampling bias. Besides that, [C+] spores might
912 be less viable than [++] ones because of some deleterious effect of C marker insertion: even though
913 we checked that the insertions did not disrupt any known coding sequence, it might alter some fit-
914 ness-related regulatory element. Altogether, the gain in viability of Sel- spores as compared to Sel=
915 spores may not be clearly attributed to confounding effects of the fluorescent markers.

916

In conclusion, the recurrent divergent selection approach used in this work proved to be an efficient way to investigate the ability of the recombination rate to respond to directional selection. A clear response was observed rather symmetrically in both directions in the selected region. *Cis* effects were observed at the sequence level, but *trans* effects were also detected, providing quite direct evidence that various types of recombination modifiers are at work to control the evolution of recombination through multiple paths. It also suggests that the ones at work in our experiments involve some QTLs with quite strong effects contributing to the response.

Data availability statement

Yeast strains produced in this work are available upon reasonable request. All data underlying this article will be publicly available in the french government data warehouse 'Recherche Data Gouv' (https://recherche.data.gouv.fr/en) under the persistent DOI: https://doi.org/10.57745/YTFYTT. A provisional confidential access is possible for reviewing purposes at: https://moulon.inrae.fr/DATA_GENETICS-2026-309198/ (User name: GENETICS, Password: ahd6yuVu). R and Python codes used to analyze the data and produce graphs and conclusions are publicly available in the french government software forge 'ForgeMIA' at https://forge.inrae.fr/gqe-base/evolrec_wp1.

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Conflict of interest

The authors declare no conflict of interest

Figure Legends

Figure 1. Details of the three modes of selection applied to spores. In Sel+ experiments, only recombinant spores are selected by FACS-sorting **(A)**, in Sel-, only non-recombinant spores are selected by FACS-sorting **(B)**, and in Sel= controls, all spores are kept **(C)**. Note that in Sel+, two selection cycles are represented on the sketch: odd generations on the top side of the circle, and even generations on the bottom side of the circle. In contrast, for Sel- and Sel=, one single cycle of selection is represented in the sketch.

Figure 2. Response of recombination to divergent selection. Recombination rate in the interval VI_C1Y2, where selection was applied **(A, B)** and in the adjacent interval VI_Y2R3 **(C, D)**. Left panels **(A, C)**: averages and 95% confidence intervals of RR based on four biological replicates. Right panels **(B, D)**: values of each individual replicate (named with colored letters A, B, C, and D on the plots). Black triangles represent the mean of four technical replicates (black circles) of phenotyping the G0 population. Dashed red lines indicate odd generations of Sel+ experiments. Red, green, and blue solid lines indicate Sel+ (even generations), Sel=, and Sel- experiments, respectively. Black lines in panels **B** and **D** correspond to aliquots of generation G8 Sel+ which were submitted to one generation of relaxed selection as for Sel=, and then again to the Sel+ regime. The chromosome sketch on the left side of the figure indicates the positions of the two intervals considered on chromosome VI.

Figure 3. Recombination rate of individuals isolated from G8 populations. Individual values of recombination rate in the selected interval VI_C1Y2 **(A)** and their smoothed distributions **(B)** measured from 323 Sel+, 377 Sel-, and 101 Sel= isolated diploid individuals from populations at generation G8, after pooling biological replicates A, B, C, D. Dashed lines indicate the means of distributions.

Figure 4. Genome-wide analysis of recombination at G8. (A): Meta-analysis across chrVI of pa-

977 rental allele frequency in segregants derived from 9 highest-recombining ([high]) tetrads and 11
978 lowest-recombining tetrads ([low]) at generation G8 of Sel+ selection. The selection interval used
979 for recombination phenotyping, as well as tetrad selection, is indicated in grey. **(B):** Examples of
980 crossover (CO) and gene conversion (GC) calling in a [high] and [low] tetrad. Given the presence
981 of genetically identical patches within each tetrad, both COs and GCs are under-represented, and
982 precise locations cannot always be determined. **(C):** Genome-wide quantification of COs and GCs
983 in [low] and [high] segregants, both with and without adjusting for genetically identical patches.
984 **(D):** CO quantification by chromosome, with chromosomes ordered by increasing size. *p*-value in-
985 dicates the non-adjusted result of each independent *t*-test.

986 **Figure 5. Effect of selection for recombination on fitness.** Maximum growth rate **(A)**, concentra-
987 tion at the plateau **(B),** sporulation rate **(C)**, and spore viability **(D)** of populations at generation G8
988 for Sel+, Sel=, and Sel- selection modes, and of the initial population G0. The four biological repli-
989 cates were pooled. Boxplots were made from a minimum of 30 independent measurements for A
990 and B, and of 6 (C) or 8 (D) independent countings of 96 spores. Pairwise statistical comparisons
991 shown on the graph correspond to Bonferroni-adjusted *p*-values. * <0.05, ** <0.01, *** <0.001.

992

993

994 Supplementary Figure Legends

995 **Figure S1. Procedure used to build the population G0 from the SGRP-4X population.** Mixed
996 spores from SGRP-4X (Mat-a or Mat-α ho::hygMX ura3::kanMX, lys2::URA3 or LYS2) were
997 mated with a fluorescent SK1-VI_C1Y2 tester (Mat-a ho::hygMX ura3::kanMX VI-C1:TDH3prom-
998 yECerulean-NatMX VI-Y2:TDH3prom-Venus-NatMX). Diploids hemizygous for the fluorescent mark-
999 ers were then selected based on their ability to grow on medium without Uracil containing nourseo-
1000 thricin.

1001 **Figure S2. Marker positions in the testers used to probe recombination rate.** These testers are
1002 SK1 strains containing three fluorescent markers, used to cross to non-fluorescent cells to measure
1003 recombination rate in six different intervals: two on chromosome I and four on chromosome VI
1004 (among which the VI_C1Y2 interval where selection was applied). C, Y, R refer to markers yECe-
1005 rulean, Venus, and mRFP, respectively.

1006 **Figure S3. Phenotyping recombination rate of individuals isolated from G8 populations**. Experi-

1007 mental design and expected diploid frequencies after FACS-sorting bi-fluorescent diploid cells in
1008 the case of Sel+ and Sel- (upper panel) or Sel= (lower panel) experiments.

1009 **Figure S4. Distribution of fluorescent marker frequency in spores from individuals isolated**
1010 **from G8 populations. (A):** Marker yECerulean. **(B):** Marker Venus. X-axis: marker frequency. Y-
1011 axis: number of isolated individuals. Red lines: lower and upper boundaries defined as median +/- 3
1012 times Median Absolute Deviation. Green dashed lines correspond to a frequency value of 0.5.

1013 **Figure S5. Distribution of haplotype frequency ratios in spores from individuals isolated from**
1014 **G8 populations.** X-axis: haplotype frequency ratios $F_{[CY]}/\left(F_{[CY]}+F_{[++]}\right)$ **(A)** and
1015 $F_{[C+]}/\left(F_{[C+]}+F_{[+Y]}\right)$ **(B)**. Y-axis: number of isolated individuals. Red lines: lower and upper
1016 boundaries defined as median +/- 3 times Median Absolute Deviation.

1017 **Figure S6. Distribution of haplotype frequency ratios in spores from individuals isolated from**
1018 **G8 populations for each selection mode.** X-axis: selection mode: Sel+ (red), Sel= (green), and
1019 Sel- (blue). Y-axis: haplotype frequency ratios $F_{[CY]}/\left(F_{[CY]}+F_{[++]}\right)$ **(A)** and $F_{[C+]}/\left(F_{[C+]}+F_{[+Y]}\right)$
1020 **(B)**. Red dashed lines: lower and upper boundaries defined as median +/- 3 times Median Absolute
1021 Deviation.

1022 **Figure S7. Crossover interference across generations of selection.** Coefficient of coincidence
1023 (CoC) based on two adjacent intervals delimited by three fluorescent markers, in two regions of
1024 chromosome VI **(A, B, C, D)**, and in one region of chromosome I **(E, F)**. CoC=1 corresponds to no
1025 interference, whereas CoC<1 corresponds to positive interference. **(A, C, E):** Mean CoC values and
1026 95% confidence intervals based on four biological replicates. **(B, D, F):** CoC values of each individ-
1027 ual biological replicate. Dashed red lines indicate even generations of Sel+ experiments. Red, green,
1028 and blue solid lines indicate Sel+ (odd generations), Sel=, and Sel- experiments, respectively. The
1029 chromosome sketches on the left side of the figure indicate the positions of the pairs of intervals
1030 considered.

1031 **Figure S8**. **Recombination rates of individuals isolated from G8 populations per selection mode**
1032 **and per replicate.** Individual values of recombination rate in the selected interval VI_C1Y2 for
1033 each biological replicate A, B, C, D, and each selection mode Sel+ (red), Sel= (green), and Sel-
1034 (blue).

1035 **Figure S9**. **ChrVI sequence analysis of an outlier hyper-recombinant strain.** Alignment against
1036 the region of the SK1 chromosome VI reference sequence (bottom) between the fluorescent mark-
1037 ers used for selection. All sequences above the reference are 16 ONT long reads obtained from a

1038 strain having an outlier recombination rate equal to 0.491. This strain was isolated from the G8 Sel+
1039 population. Grey color indicates a match with the reference sequence (including shared gaps), red
1040 color indicates mismatches, and black color indicates gaps.

1041 **Figure S10. Response to selection in two other genomic regions on chromosome VI.** Recombina-
1042 tion rate in the two regions VI_R3Y4 **(A, B)** and VI_Y4C5 **(C, D)**. **(A, C):** Means and 95% confi-
1043 dence intervals based on four biological replicates (named with colored letters A, B, C, and D on
1044 the plots). **(B, D):** Individual values of each biological replicate. Dashed red lines indicate even gen-
1045 erations of Sel+ experiments. Red, green, and blue solid lines indicate Sel+ (odd generations), Sel=,
1046 and Sel- experiments, respectively. Black lines in panels **B** and **D** correspond to aliquots of genera-
1047 tion G8 Sel+ which were submitted to one generation of relaxed selection as for Sel=, and then
1048 again to the Sel+ regime. The chromosome sketch on the left side of the figure indicates the posi-
1049 tions of the two intervals considered on chromosome VI.

1050 **Figure S11. Response to selection in two genomic regions on chromosome I.** Same as Figure S10
1051 but in two regions of chromosome I: I_R2C3 **(A, B)** and I_C3Y4 **(C, D)**.

1052 **Figure S12 Genome-wide analysis of recombination following eight generations of selection. (A):**
1053 Mean read depth for each of the 80 sequenced sergregants. **(B):** Individual genotype compositions
1054 across chromosome VI for the 9 high-recombining ([high]) tetrads and 11 low-recombining tetrads
1055 ([low]) at G8 Sel+. **(C)**: Homology plot for chromosome VI between the 4 parental genotypes (SA,
1056 NA, WA, WE) as well as the SK1 tester strain. Each grey connection bar represents a section of ho-
1057 mology detected by mVISTA (probability threshold 0.5) with an additional filter of minimum con-
1058 servation 85% and minimum block size 1 kbp. **(D):** Quantification of the number (left) and cumula-
1059 tive size (right) of the four-way genetically identical patches in which GCs cannot be detected, and
1060 COs cannot be precisely located. **(E):** Breakdown of the CO rate shown in Figure 4C by biological
1061 replicate. *p*-value indicates the non-adjusted result of each independent *t*-test.

1062 **Figure S13 CNV analysis of all sequenced segregants**. Genome-wide read counts of 80 sequenced
1063 segregants to ensure that there are no chromosomal aneuploidies or major CNVs. Each data point
1064 represents an average read count across a non-overlapping 5-kbp window.

1065 **Figure S14. Response to selection without initial genetic diversity.** Recombination rate (RR) and
1066 coefficient of coincidence (CoC) during two generations of selection for recombinant spores (Sel+,
1067 red lines) or without selection (Sel=, green lines) from a SK1 VI_C1Y2 diploid strain hemizygous
1068 for the two fluorescent markers C (yECerulean) and Y (Venus) on chromosome VI. Values of RR at
1069 each generation in intervals VI_C1Y2 where selection was applied **(A),** VI_Y2R3 **(B)**, VI_R3Y4

1070 **(D)**, and VI_Y4C5 **(E)** of chromosome VI, and I_R2C3 **(G)** and I_C3Y4 **(H)** of chromosome I, and
1071 coefficients of coincidence (CoC) in double intervals VI_C1Y2R3 **(C)** and VI_R3Y4C5 **(F)** of
1072 chromosome VI, and I_R2C3Y4 **(I)** of chromosome I.

1073 **Figure S15. Effect of fluorescent markers on growth kinetics.** Maximum growth rate **(A)**, concen-
1074 tration at the plateau **(B)**, sporulation rate **(C)**, and spore viability **(D)** of sub-samples (N>50,000) of
1075 population G0 sorted according to the presence of fluorescent markers. X-axis: ++, +Y, C+, and CY
1076 indicate the presence of fluorescent markers: no marker, Venus only, yECerulean only, and yECeru-
1077 lean and Venus, respectively. Boxplots were made from a minimum of 110 independent measure-
1078 ments for (**A**) and (**B**), and of 4 (**C**) or 8 (**D**) independent countings of 96 spores. Pairwise statistical
1079 comparisons shown on the graph correspond to Bonferroni-adjusted *p*-values. * <0.05, ** <0.01.

1080

1081

1082 Supplementary Tables

1083 **Table S1**. Details of all fluorescent marker constructions inserted in the genome of the testers

1084 **Table S2.** Selection of diploid strains isolated from G8 Sel+ populations for tetrad sequencing

1085 **Table S3.** Effect of selection mode and biological replicate on RR measured in 770 individuals iso-
1086 lated from the populations at generation G8. Bonferroni-adjusted *p*-values from pairwise Wilcoxon
1087 Test.

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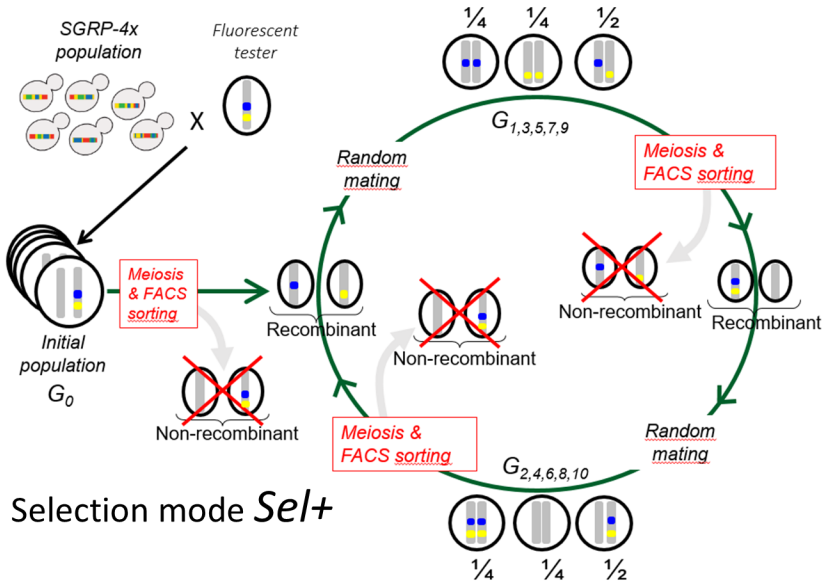
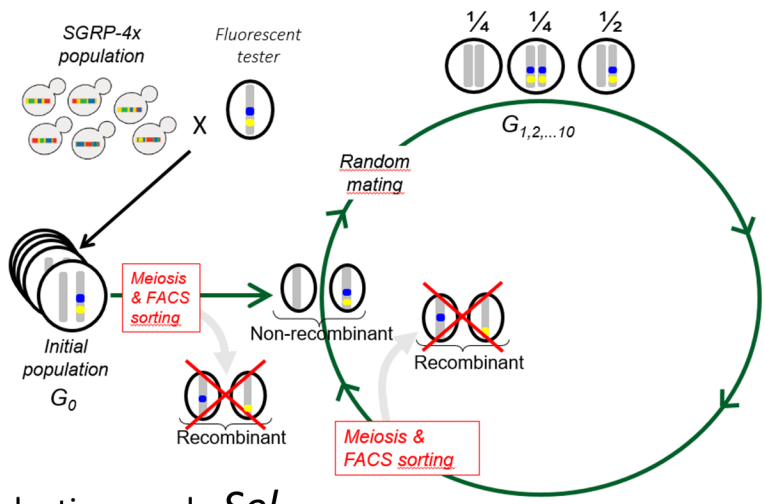
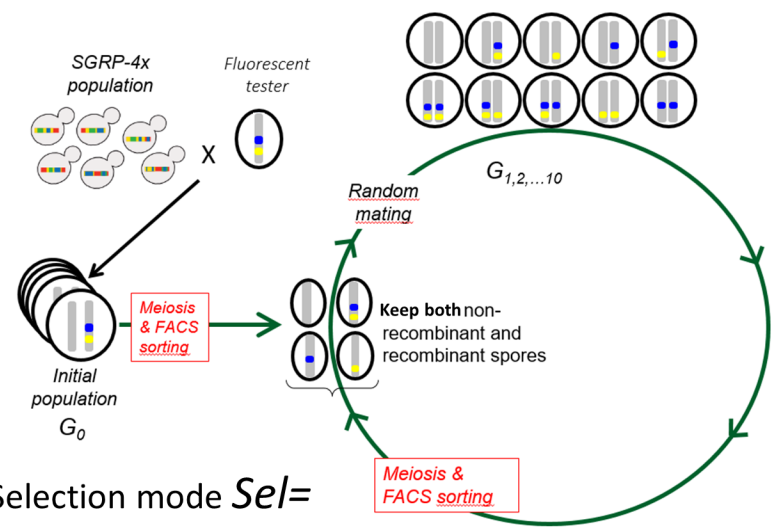
1264



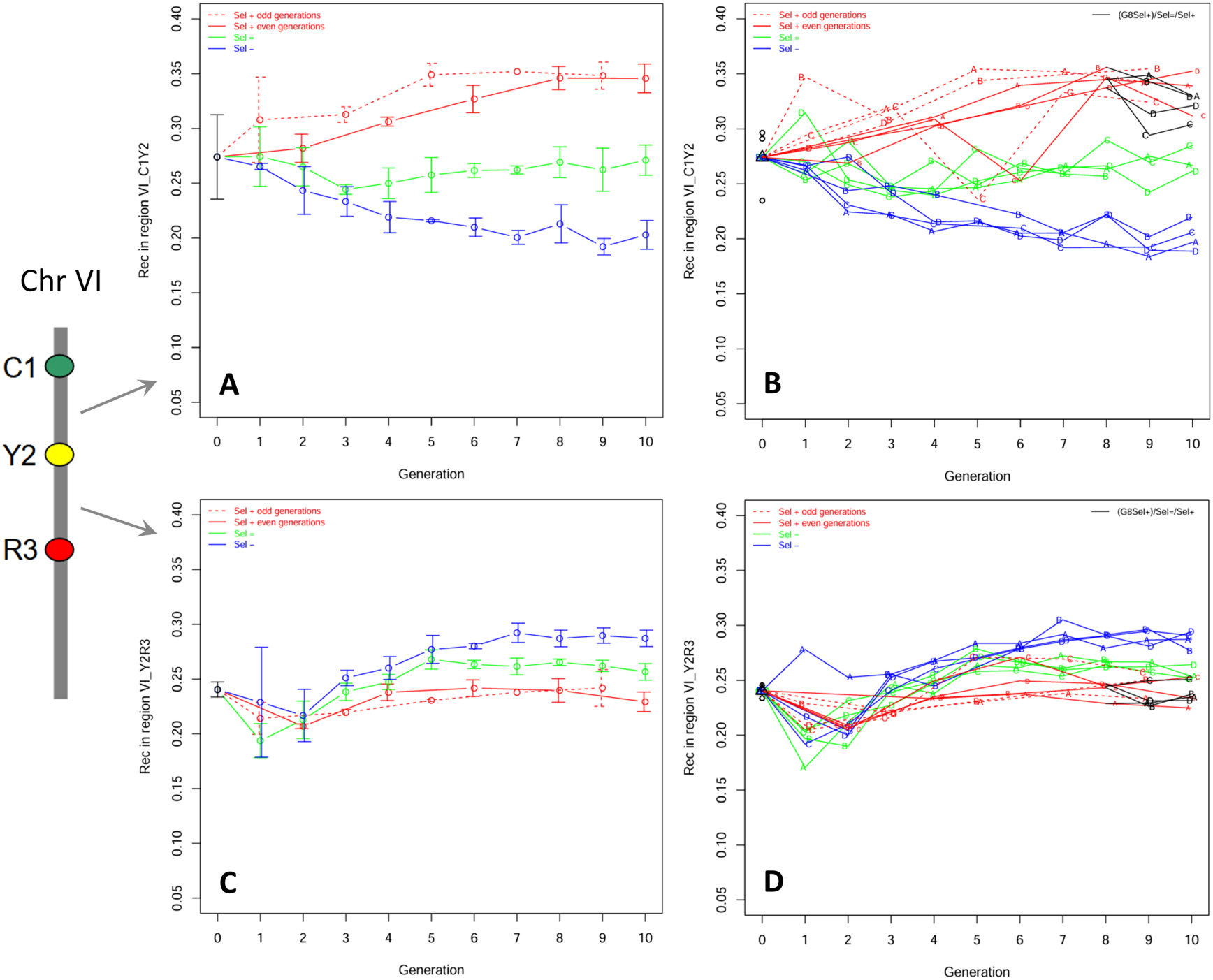
A Selection mode *Sel+*

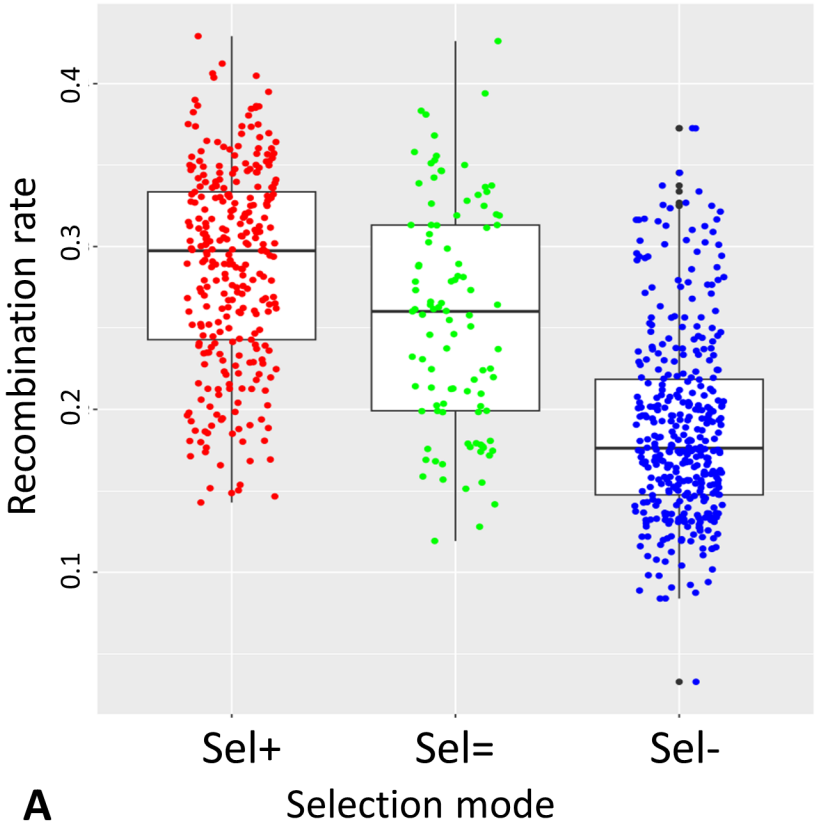


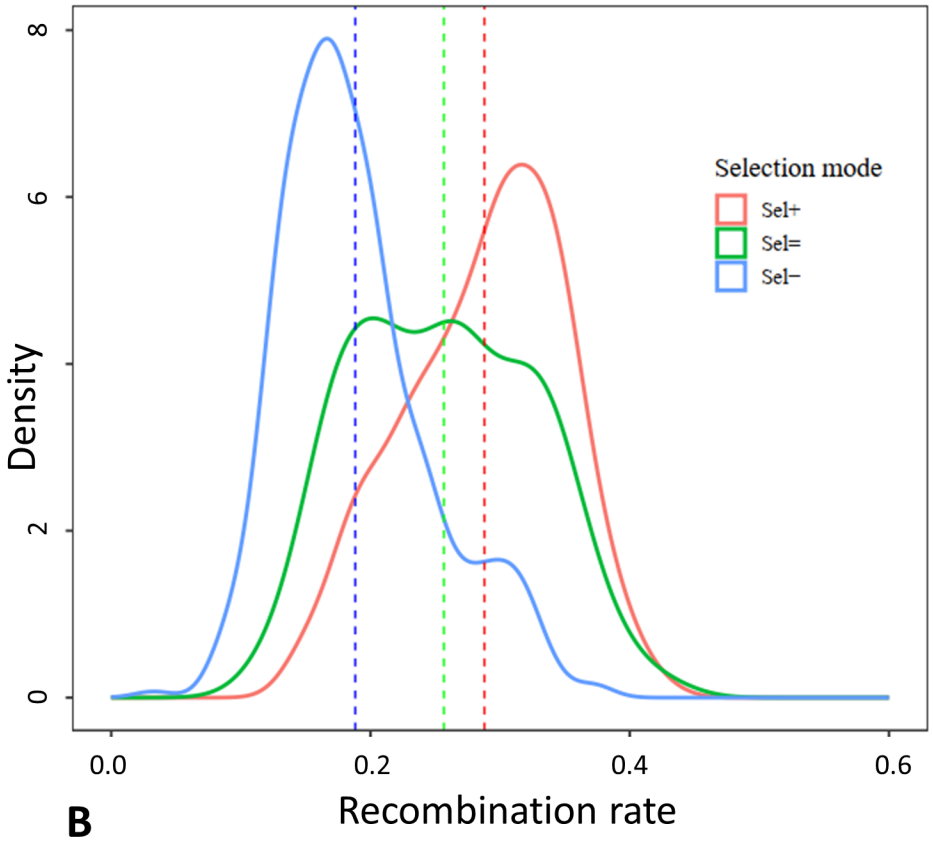
B Selection mode *Sel-*

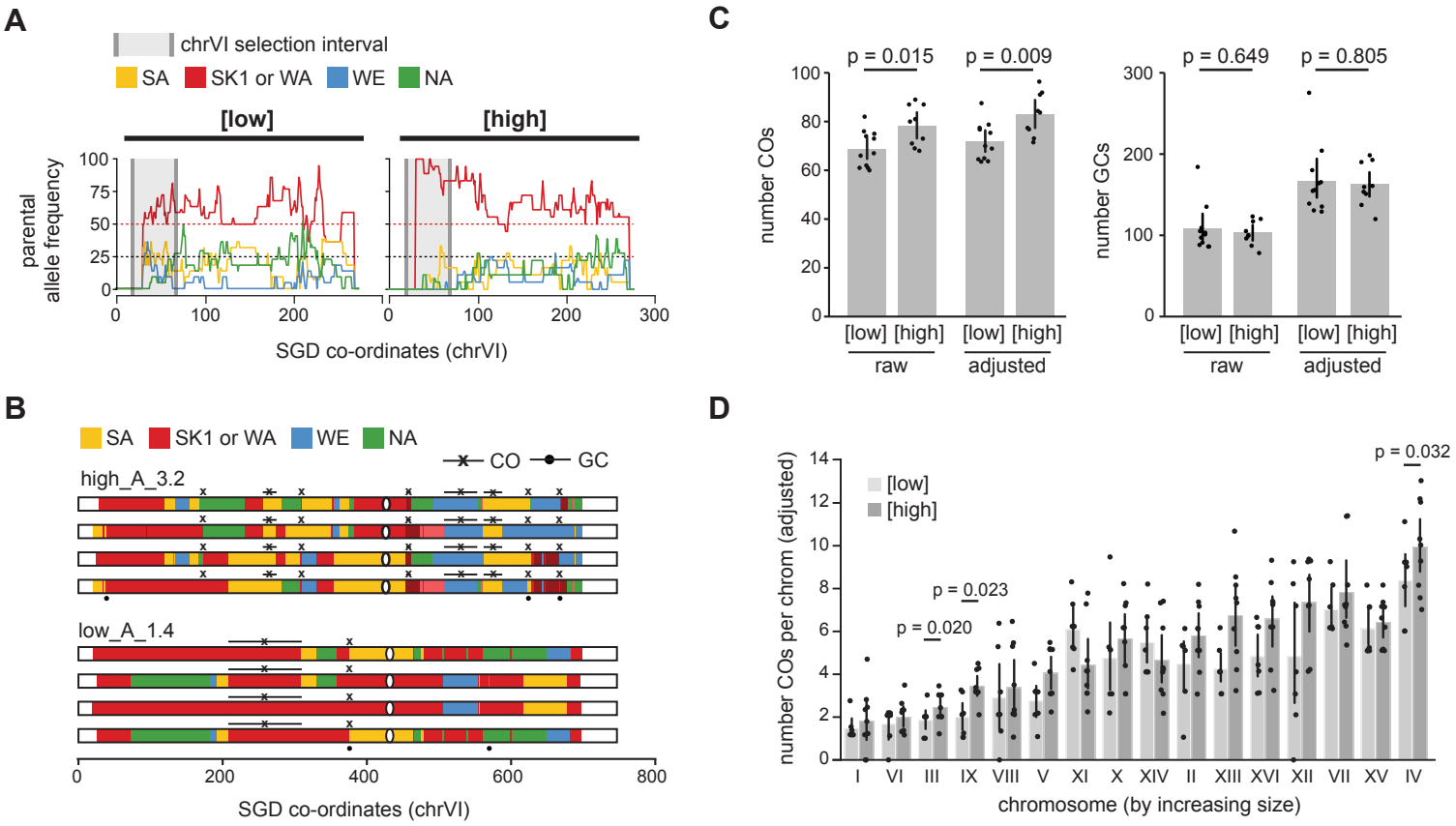


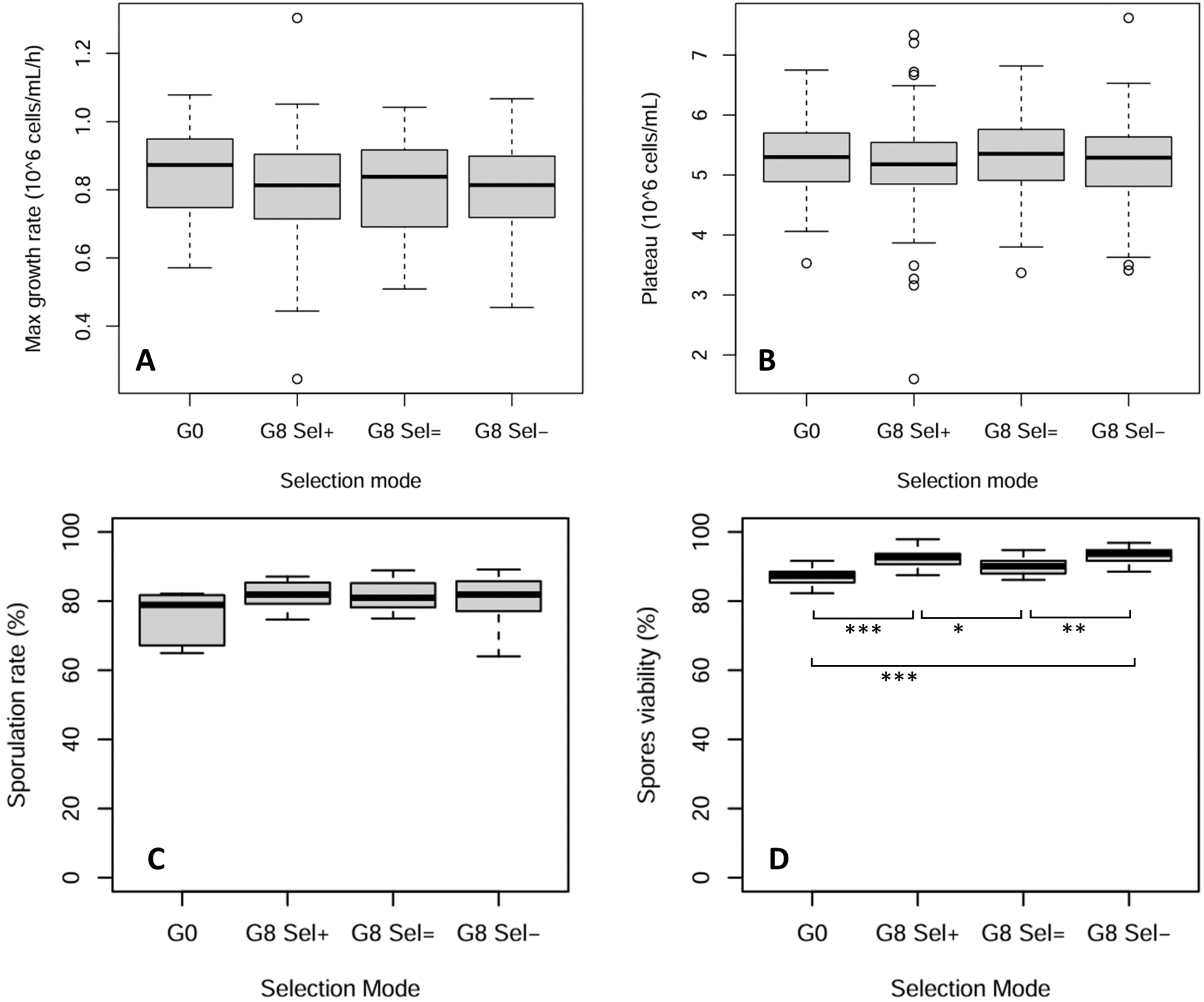
C Selection mode *Sel=*


Chr VI
C1
Y2
R3
Sel + odd generations
Sel + even generations
Sel =
Sel –
(G8Sel+)/Sel=/Sel+
Rec in region VI_C1Y2
Rec in region VI_Y2R3
Generation
A
B
C
D


Recombination rate
0.1
0.2
0.3
0.4
Sel+
Sel=
Sel-
Selection mode
A


Density
0
2
6
8
0.0
0.2
0.4
0.6
Recombination rate
Selection mode
Sel+
Sel=
Sel−
B


A
chrVI selection interval
SA
SK1 or WA
WE
NA
[low]
[high]
parental allele frequency
SGD co-ordinates (chrVI)
B
SA
SK1 or WA
WE
NA
CO
GC
high_A_3.2
low_A_1.4
SGD co-ordinates (chrVI)
C
p = 0.015
p = 0.009
p = 0.649
p = 0.805
number COs
number GCs
[low] [high]
raw
adjusted
D
[low]
[high]
p = 0.032
p = 0.023
p = 0.020
number COs per chrom (adjusted)
I VI III IX VIII V XI X XIV II XIII XVI XII VII XV IV
chromosome (by increasing size)


A
Max growth rate (10^6 cells/mL/h)
0.4
0.6
0.8
1.0
1.2
G0
G8 Sel+
G8 Sel=
G8 Sel−
Selection mode
B
Plateau (10^6 cells/mL)
2
3
4
5
6
7
G0
G8 Sel+
G8 Sel=
G8 Sel−
Selection mode
C
Sporulation rate (%)
0
20
40
60
80
100
G0
G8 Sel+
G8 Sel=
G8 Sel−
Selection Mode
D
Spores viability (%)
0
20
40
60
80
100
***
*
**
***
G0
G8 Sel+
G8 Sel=
G8 Sel−
Selection Mode