Engineering recombination between diverged yeast species reveals genetic incompatibilities

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Abstract

The major cause of the sterility of F1 hybrids formed between *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* is anti-recombination. The failure of homologous chromosomes from the different species to recombine causes them to mis-segregate, resulting in aneuploid gametes, most of which are inviable. These effects of anti-recombination have previously impeded the search for other forms of incompatibility, such as negative genetic interactions (Bateson-Dobzhansky-Muller incompatibilities). By suppressing the meiotic expression of *MSH2* and *SGS1*, we could increase recombination and improve hybrid fertility seventy-fold. This allowed us to recover meiotic tetrads in which all four gametes were viable, ensuring that segregation had occurred properly to produce perfectly haploid, not aneuploid, recombinant hybrid gametes. We sequenced the genomes of 84 such tetrads, and discovered that some combinations of alleles from different species were significantly under-represented, indicating that there are incompatible genes contributing to reproductive isolation.

Introduction

Species are formed and maintained by the restriction of gene flow between diverging populations. Barriers to gene flow can be physical, such as geographic distance, or they can be properties of the species themselves. Here, we focus on one such barrier to gene flow, hybrid sterility. Hybrid sterility is a form of post-zygotic reproductive isolation, meaning that it acts after diverging populations have already mated and produced a hybrid zygote. Hybrid sterility can be caused by a variety of mechanisms that can generally be classified into incompatibilities between diverged chromosomes (such as large-scale chromosomal rearrangements; Rieseberg and Willis, 2007, and anti-recombination) and incompatibilities between individual genes from the diverging populations (Presgraves, 2010). There is particular interest in the latter class of genic incompatibilities, which are often referred to as “Bateson-Dobzhansky-Muller incompatibilities” (BDMIs) or “speciation genes” (Orr, 1996). As we don’t know whether these incompatibilities themselves are the cause of speciation or have developed post-speciation, we will refer to them as BDMIs throughout.

BDMIs represent a case where alleles (at two or more loci) that have evolved to work well together within a species perform poorly when combined in a hybrid individual with alleles from another species, whose alleles have evolved independently (Coyne and Orr, 2004). Since BDMIs offer a universal mechanism for speciation, they have been studied intensely, both theoretically and empirically, yet only a handful have been discovered and characterized at the molecular level (reviewed in Presgraves, 2010; Rieseberg and Blackman, 2010; Maheshwari and Barbash, 2011; Nosil and Schluter, 2011). Understanding the molecular mechanisms underlying additional BDMIs will allow us to address general questions about reproductive isolation, such as the number of BDMIs typically involved and their

effect sizes, whether the same genes or types of genes are involved in different cases, what types or locations of mutations are most likely to cause incompatibility, and whether BDMIs evolve by selection or drift (Nosil and Schluter, 2011).

Yeast are a great system in which to molecularly characterize such interactions because the genomic data, molecular tools and genetic tractability of the model yeast *Saccharomyces cerevisiae* are unsurpassed by any other model eukaryote (Botstein and Fink, 2011). F1 hybrids between *S. cerevisiae* and its closest relative *Saccharomyces paradoxus* have greatly reduced sexual fertility compared to non-hybrids. Haploid gametes from the two different species can fuse to form diploid F1 hybrids that grow normally by mitosis, but only about 1% of the gametes (which are produced as spores) formed via meiosis are viable (able to germinate and grow into colonies) (Hunter et al., 1996). In contrast, nearly all the spores produced by non-hybrids are viable. The two species do not differ by substantial chromosomal rearrangements that might account for this sterility (Fischer et al., 2000; Kellis et al., 2003). Instead, a form of chromosomal incompatibility known as anti-recombination is thought to be the cause. The two species' genomes are so diverged in sequence (about 12% of nucleotide positions differ; Rogers et al., 2018) that homologous recombination is suppressed, and meiotic crossing over is greatly reduced. Because crossovers are important for chromosome segregation during meiosis, efficient segregation is impaired, and gametes are killed because they lack one or more essential chromosomes or, potentially, because they carry extra chromosomes. Consistent with this, the 1% viable gametes produced from hybrid meioses are aneuploid, carrying additional chromosomes, and very few chromosomes are recombinant (Hunter et al., 1996; Kao et al., 2010).

92 In principle, chromosome mis-segregation alone is capable of explaining
 93 yeast hybrid sterility without invoking any role for BDMIs. We recently quantified
 94 the precise rates at which each chromosome segregates in F1 hybrids (Rogers et al.,
 95 2018). The average rate of correct distribution for each chromosome in hybrids
 96 formed between *S. cerevisiae* and *S. paradoxus* is 59.7%, so we expect only 0.03% of
 97 gametes to receive exactly one copy of each chromosome (0.597 for each
 98 chromosome, raised to the power of 16 to account for all sixteen chromosomes).
 99 However, gametes carrying more than one copy of a chromosome can also be viable,
 100 as shown by the high rates of aneuploidy detected in viable hybrid gametes. In the
 101 40.3% of hybrid meioses in which a chromosome does not segregate properly, half
 102 of the resulting spores (20.15%) will receive two copies of the chromosome and
 103 might therefore be viable, whilst the remaining 20.15% will receive no copies and
 104 will certainly be inviable. Therefore 2.7% of gametes (0.597 plus 0.2015 , raised to
 105 the power of 16) will receive *at least* one copy of each essential chromosome, and
 106 could be viable, depending on the effect of the additional chromosomes that they
 107 carry (Boynton et al., 2018). Thus chromosome mis-segregation due to anti-
 108 recombination accounts for at least 97.3%, and potentially all, of the observed
 109 hybrid sterility. However, there is little direct evidence that extra chromosomes
 110 contribute to spore inviability (Rogers et al., 2018), so the smaller figure is more
 111 likely, leaving open the possibility that some hybrid spores are killed because of
 112 incompatible interactions between genes of one species and those of the other
 113 (BDMIs).

114
 115 To date, no such BDMIs have been detected in yeast. BDMIs have been
 116 detected between mitochondrial genes from one yeast species and nuclear genes
 117 from another (Lee et al., 2008; Chou et al., 2010; also see Xu and He, 2011), but these

118 act earlier by reducing F1 mitotic viability and preventing F1 meiosis from even
 119 occurring, not by causing inviability of the gametes produced by hybrid meiosis. We
 120 have previously shown that most *S. paradoxus* chromosomes can successfully
 121 replace their homologues in *S. cerevisiae* haploid gametes when substituted one at a
 122 time, indicating that they do not contain always-lethal incompatibilities (Greig,
 123 2007). But this method would not detect weaker BDMLs that kill only sometimes
 124 (incomplete penetrance), or that have a cumulative effect with other BDMLs on
 125 other chromosomes. A possible way to detect such BDMLs is to genotype the
 126 surviving gametes from hybrid meioses and test whether some combinations of
 127 alleles from different species at different loci are statistically under-represented.
 128 The explanation for such under-representation would be that they are incompatible
 129 and cause gamete inviability. This method has been modelled by Li et al. (2013), and
 130 has been implemented by Kao et al. (2010). Whilst the distribution of genotypes
 131 differed significantly from what was expected by chance, the additional aneuploid
 132 chromosomes carried by the genotyped gametes confounded analysis to an extent
 133 that the effective sample size was too low to identify individual pairs of
 134 incompatible loci (Kao et al., 2010).

135

136 In order to identify BDMLs involved in hybrid spore inviability, it is therefore
 137 necessary to overcome the primary effect of anti-recombination, in order to produce
 138 haploid spores without additional aneuploid chromosomes for genotyping. Hunter
 139 et al. (1996) previously showed that knocking out genes involved in monitoring the
 140 fidelity of recombination increases both the rate of recombination and the
 141 proportion of viable gametes produced by hybrid meioses. By deleting the mismatch
 142 repair gene *MSH2*, they increased crossing over in hybrids on average 13-fold,
 143 resulting in a nearly 9-fold increase in hybrid spore viability. Kao et al. (2010)

therefore used *msh2Δ* knock-out mutants in their search for BDIMs, but the improvement in chromosome segregation was insufficient to relieve the extensive aneuploidy of the hybrid gametes. Here we employed two additional tools in order to produce perfectly euploid hybrid gametes for genotyping. First, we repressed the expression of both *MSH2* and a second anti-recombination factor, DNA-helicase *SGS1*, specifically in meiosis, thereby retaining their normal function during mitosis, which reduces the mutagenic effects of knocking them out entirely. Secondly, we dissected hybrid gametes out of their meiotic tetrads and genotyped only those that came from tetrads in which all four spores were viable. Our sample therefore excluded not only those gametes containing lethal combinations of the parent species' alleles, but also aneuploid gametes, since any chromosome mis-segregation will kill some of the gametes in a tetrad.

We sequenced all 336 haploid gametes from 84 F1 hybrid meioses and tested statistically for pairs of alleles for which parental combinations were over-represented. We were able to map four broad pairs of genomic regions that show evidence of incompatibility. Thus, for the first time, we find evidence of naturally-occurring nuclear BDIMs causing sterility of hybrids between two species of yeast.

Results

Restoration of hybrid fertility

We constructed strains of *S. cerevisiae* and *S. paradoxus* in which the native promoters of *MSH2* and *SGS1* were replaced with the *CLB2* promoter, which is specifically repressed during meiosis (Grandin and Reed, 1993; Lee and Amon, 2003) (Table 1, Supplementary File 1, Supplementary File 2). *MSH2* and *SGS1* are both implicated in the anti-recombination process (Chakraborty and Alani, 2016). By maintaining expression of these genes in mitosis, we can

Table 1: List of strains used in this study. For a complete list, see Supplementary File 2.

YDG	Strain name	Original strain name	
391	NCYC 3708	N17	ho::HYGMX @ ura3::KanMX
542	NCYC 3583	W303	ho::HYGMX a ura3::KanMX ade2-1
832	NHY 2039		ho::hisG a ura3(Δ Sma-Pst) HIS4::LEU2-(BamHI; +ori) leu2::hisG pCLB2-3HA-SGS1::kanMX4
853	YDG391 x YDG542	N17 x W303	ho::HYGMX/ho::HYGMX a/@ ura3::KanMX/ura3::KanMX ade2-1/ADE2
866		W303	ho a ura his3 leu2::NAT trp ade-2 can1r pCLB2-3HA-SGS1::kanMX4
905		N17	ho @ ura3 cyh2r pCLB2-3HA-SGS1::kanMX4
912	YDG866 x YDG905	W303 x N17	ho/ho a/@ ura/ura3 his3/HIS3 leu2::NAT/LEU2 trp/TRP ade-2/ADE can1r/CAN1 CYH2/cyh2r pCLB2-3HA-SGS1::kanMX4/pCLB2-3HA-SGS1::kanMX4
959		N17	ho a lys2 cyh2r pCLB2-3HA-MSH2::kanMX4
960	YSC1059	W303	ho @ ura3-52 his3-11 leu2-3,112 trp1 Δ 2 ade2-1 can1-100 pCLB2-3HA-MSH2::kanMX4
964	YDG959 x YDG960	N17 x W303	ho/ho a/@ URA3/ura3-52 lys2/LYS2 HIS3/his3-11 LEU2/leu2-3,112 TRP1/trp1 Δ 2 ADE2/ade2-1 CAN1/can1-100 cyh2r/CYH2 pCLB2-3HA-MSH2::kanMX4/pCLB2-3HA-MSH2::kanMX4
967		N17	ho a cyh2r pCLB2-3HA-SGS1::kanMX4 pCLB2-3HA-MSH2::kanMX4
968		N17	ho @ cyh2r pCLB2-3HA-SGS1::kanMX4 pCLB2-3HA-MSH2::kanMX4
969		W303	ho a ura3 his3 leu2::NAT trp1 ade-2 can1r pCLB2-3HA-SGS1::kanMX4 pCLB2-3HA-MSH2::kanMX4
970		W303	ho @ ura3 his3 leu2::NAT trp1 ade-2 can1r pCLB2-3HA-SGS1::kanMX4 pCLB2-3HA-MSH2::kanMX4
982	YDG968 x YDG969	N17 x W303	ho/ho @/a URA3/ura3 HIS3/his3 LEU2/leu2::NAT TRP1/trp1 ADE/ade-2 CAN1/can1r cyh2r/CYH2 pCLB2-3HA-SGS1::kanMX4/pCLB2-3HA-SGS1::kanMX4 pCLB2-3HA-MSH2::kanMX4/pCLB2-3HA-MSH2::kanMX4

173 avoid any unwanted effects such as an increased recessive-lethal mutation rate, which would
 174 actually reduce fertility (Hunter et al., 1996). In a previous study, we found that suppressing
 175 meiotic expression of *SGS1* alone improved the rate of correct segregation by almost half in
 176 hybrid meioses (Rogers et al., 2018). Here, we find that spore viability is also dramatically
 177 improved. Suppression of *SGS1* alone increased hybrid spore viability from 0.46% to 20.8%;
 178 and in combination with suppression of *MSH2*, spore viability was further improved to 32.6%
 179 (Figure 1, Source Data 1). Significantly more of the double mutant spores were viable than in
 180 the wild-type hybrid (chi-squared contingency test: $X^2 = 479.91$, $df = 1$, $p\text{-value} < 2.2 \times 10^{-16}$).

Figure 1: Restoration of hybrid fertility by meiotic repression of *MSH2* and *SGS1*. Percentages are spore viabilities of the indicated hybrid strains. In the $P_{CLB2}\text{-}MSH2$ $P_{CLB2}\text{-}SGS1$ strain, a significant 32.14% increase in spore viability was observed (double mutant when compared with the wild type: $X^2 = 479.91$, $df = 1$, $p\text{-value} < 2.2 \times 10^{-16}$). Numbers in parentheses indicate the total number of dissected spores checked for viability. Full data, including other strains, can be found in Source Data 1.

181 The restoration of hybrid fertility vastly increased the production of hybrid tetrads in which
 182 all four spores were viable, which were specifically selected for genotyping and further
 183 analysis. All spores from such tetrads are necessarily euploid, as mis-segregation of even a
 184 single chromosome would result in at least one dead spore (lacking that chromosome). By

185 analyzing only euploid spores, we ensured that recessive BDMIs were not masked by
186 aneuploidy.

187 **Evidence for hybrid incompatibility**

188
189 A fertility-reducing BDMI between a pair of loci would result in fewer gametes
190 containing hybrid combinations of alleles at these loci. Reasoning that we could not
191 map such loci at a resolution higher than the linkage groups produced by the
192 crossovers that occurred within the 84 tetrads in our sample, we divided the
193 chromosomes into 1208 segments defined by all of the recombination breakpoints
194 produced by our genotyping procedure (see Supplementary File 3). Treating each of
195 these segments as a putative incompatibility locus, we tested every segment against
196 every other segment, excluding those on the same chromosome, using two-by-two
197 contingency tables in the manner described by Li et al. (2013). Those on the same
198 chromosome cannot be tested because physical linkage cannot be distinguished
199 from linkage due to interaction. We calculated the odds ratio (OR) for each pair by
200 dividing the product of the numbers of the two parental genotypes observed by that
201 of the two hybrid types:

$$202 \quad \text{OR} = (\text{par1} * \text{par2}) / (\text{hyb1} * \text{hyb2})$$

203 In addition, we calculated the 99% confidence interval (CI) for the odds ratios. An
204 odds ratio of 1 indicated that the parental and hybrid types were present in equal
205 frequencies. An odds ratio greater than 1 would be observed for a bias towards
206 parental types; and an odds ratio less than 1 would indicate that hybrid types were
207 preferentially observed. We found all pairwise comparisons for which the calculated
208 99% CI did not encompass the value of 1 (lower bound of CI > 1 or upper bound of
209 CI < 1). 1.9% of all comparisons (13,082/676,294) had CIs that indicated a hybrid
210 preference and 2.6% (17,492/676,294) had CIs that indicated a parental preference.
211 Parental types were not only over-represented more often, but were also more

212 highly favoured. Parental types were over-represented by 3/4 in 190 cases
 213 (parent/hybrid ratio ≥ 1.75) while hybrid types were over-represented by 3/4 in
 214 only 22 cases (hybrid/parent ratio ≥ 1.75).
 215 Individual significant interactions were determined as described in Li et al.
 216 (2013) and in the Methods. Briefly, a null distribution of top ORs was produced by
 217 randomly re-sampling the observed data 100 times (see Source Code 1). The 5th
 218 largest OR from this set of the top 100 ORs was used as the critical value from which
 219 we judged significance. All observed pairs with a higher OR than the critical value
 220 (3.41) were deemed significant (Supplementary File 4 and Source Data 2). Blocks

221 Figure 2: Four putative BDIMs mapped to six genomic regions. Here, the
 chromosomes are displayed in a circle and each significant pairwise
 interaction is indicated by a line linking the involved segments. The
 interactions were grouped by nearby segments, forming six interacting
 regions and four putative BDIMs. Interactions contributing to different
 putative BDIMs are coloured differently. For the code used to determine
 significance, see Source Code 1. Source Data 2 contains all significant
 interactions.

were then formed from these significant pairs by grouping nearby segments that interacted with other nearby segments (see Methods for details). Of note, region B interacts with a segment adjacent to those interacting with region F (Figure 2). We collapsed both interacting regions into one (region D) as the two interactions may involve a single underlying gene in the broader area. In this way, we found four putative BDIMs involving six regions of the genome: between regions A and E (two significant interactions - highest OR = 4 or 2-fold over-representation of parental combinations), regions B and D (one significant interaction - highest OR = 3.60 or 1.90-fold difference), regions C and E (eight significant interactions - highest OR = 4.71 or 2.17-fold difference) and regions D and F (32 significant interactions - highest OR = 4 or 2-fold difference) (Table 2, Figure 2). All four interactions involve only four chromosomes, with multiple independently significant interactions mapping to the same regions.

Table 2: The six genomic regions involved in putative BDIMs. Region E interacts with both A and C, and region D interacts with both B and F. The ranges and number of genes are based on SGRP sequencing added 10/10/08 (Liti et al., 2009; Bergström et al., 2014) and can be determined using Source Data 2 in conjunction with Supplementary File 3.

Region	Chr	Genomic range (W303)	Number of genes (W303)	Genomic range (N17)	Number of genes (N17)
A	IX	32385-52752	13	11474-28361	11
B	IX	275518-276372	1	248868-249722	1
C	X	25663-113149	55	2529-101136	54
D	XII	138633-196103	30	125307-183140	30
E	XV	16405-26386	5	2188-14304	5
F	XV	63347-279621	121	51621-263564	117

Discussion

Anti-recombination as a barrier between species

240 By reducing the expression of just two genes, *SGS1* and *MSH2*, during meiosis we
 241 were able to rescue the fertility of a sterile hybrid between *S. cerevisiae* and *S.*
 242 *paradoxus*, increasing its ability to produce viable gametes 70-fold, from 0.46% to
 243 32.6%. The fertility of our rescued inter-species hybrid was around one third that of
 244 its non-hybrid parents, which is about the same as intra-species crosses formed
 245 between diverged populations of a single species, *S. paradoxus* (e.g., Greig et al.,
 246 2003; Charron et al., 2014). These anti-recombination genes thus determine most of
 247 the hybrid sterility barrier between *S. cerevisiae* and *S. paradoxus*.

248
 249 The hybrid sterility effects of *SGS1* and *MSH2* are not caused by
 250 incompatibilities between their alleles from the different species, but rather by their
 251 effects on the physical interaction between whole chromosomes from the different
 252 species. In non-hybrids, the Sgs1 and Msh2 proteins act to physically impede the
 253 formation and stabilization of heteroduplex DNA formed during recombination
 254 between mismatched DNA sequences. This activity helps to maintain genome
 255 integrity by permitting recombination at allelic positions between matching
 256 homologous chromosomes, but preventing ectopic (non-allelic) recombination
 257 between non-homologous chromosomes and dispersed repeats, which would cause
 258 rearrangements. However, the genomes of *S. cerevisiae* and *S. paradoxus* differ by
 259 12% at allelic positions across the genome (Rogers et al., 2018), so there are enough
 260 mismatches to globally reduce meiotic recombination between homologous
 261 chromosomes in hybrids. Kao et al. (2010) found that viable hybrid spores had only
 262 2.7 crossovers per meiosis. By inferring crossover rates in dead spores, Rogers et al.
 263 (2018) measured an overall rate of just one crossover per hybrid meiosis, much
 264 lower than the normal rate of about 90 crossovers that occur in a non-hybrid *S.*
 265 *cerevisiae* meiosis (Martini et al., 2006). Thus most of the sixteen pairs of

266 chromosomes in a hybrid lack any meiotic crossovers, leading to aneuploidy and
267 inviability in the spores.

268

269 Manipulating the expression of *SGS1* has previously been shown to greatly
270 improve meiotic segregation of chromosomes from different species, both in partial
271 hybrids, in which only one chromosome comes from another species, and in full
272 hybrids as we used here. Amin et al. (2010) found that meiotic non-disjunction of a
273 single chromosome III from *S. paradoxus* in an otherwise *S. cerevisiae* background
274 fell 2.5-fold, from 11.5% to 4.6% per meiosis, when *SGS1* was repressed during
275 meiosis using the *CLB2* promotor. Rogers et al. (2018) found that the non-
276 disjunction rate in full hybrids was much higher than in partial hybrids, averaging
277 40.3% per chromosome per meiosis. Nevertheless, repressing *SGS1* expression also
278 improved segregation in the full hybrids by between 2-fold and 3.2-fold, depending
279 on the chromosome. Here we showed that this improvement in segregation is
280 sufficient to greatly improve fertility, confirming that anti-recombination comprises
281 the major component of the species barrier.

282

283 The much larger effect of repressing *SGS1* expression on hybrid viability,
284 relative to that of repressing *MSH2*, could be explained by Sgs1 having several
285 effects on homolog interactions and meiotic recombination. First, Sgs1 is assumed to
286 act downstream of mismatch recognition by Msh2 to unwind strand-exchange
287 intermediates containing a high density of mismatches (Golfarb and Alani, 2005;
288 Sugawara et al., 2004; Spell and Jinks-Robertson, 2004; Chakraborty and Alani,
289 2016). It is also possible that Sgs1 possesses Msh2-independent anti-recombination
290 activity. Second, *SGS1* mutants have an increased number of cytologically visible
291 connections between homologs, which could help to stabilize interactions between

292 diverged chromosomes (Rockmill et al. 2003). Finally, Sgs1 also limits crossing over
 293 by facilitating recombinational repair without an associated exchange of
 294 chromosome arms (non-crossovers outcome; Bizard and Hickson, 2014). When
 295 *SGS1* expression is suppressed during meiosis, recombination intermediates are
 296 processed by structure-selective endonucleases to yield higher levels of crossovers
 297 (Oh et al, 2007; Zakaryevich et al. 2012; De Muyt et al. 2012; Rockmill et al. 2003).
 298 All of these factors may contribute to the increased rate of crossing over observed in
 299 the *SGS1* repression mutants.

300 301 **Genetic incompatibility as a barrier between species**

302
303 In other organisms, hybrid sterility is shown to be caused by incompatibility
 304 between allele(s) from one species at one or more loci and allele(s) from the other
 305 species at one or more distinct loci (ex: Long et al., 2008; Mihola et al., 2009; Ting et
 306 al., 1998). BDMIs are expected to evolve quite readily when populations are isolated
 307 because new alleles, compatible with the genomes they evolve in, can spread by
 308 natural selection within their population, and are only costly if hybrids are formed
 309 with another isolated diverging population, combining new alleles that have not
 310 been together before. When experimental *S. cerevisiae* populations are evolved in
 311 divergent laboratory environments, hybrids between them have lower mitotic
 312 fitness in either environment (Dettman et al., 2007). Similar results are found when
 313 natural *S. cerevisiae* isolates and crosses between them are grown in a range of
 314 different laboratory conditions (Hou et al., 2015). These results suggest that genetic
 315 incompatibilities affecting F1 hybrid mitotic fitness occur readily within a species.
 316 However F1 hybrids between *S. paradoxus* and *S. cerevisiae* do not show such
 317 incompatibilities in growth. On the contrary, they tend to show enhanced viability
 318 or “hybrid vigour” (Bernardes et al., 2017), so BDMIs for mitotic growth do not

319 appear to be a major part of the species barrier between these well-established
 320 species. What of BDMIs affecting meiosis? Dettman et al. (2007) also report that
 321 their experimental hybrids show a relative reduction in “meiotic efficiency”, that is
 322 the proportion of diploids that enter meiosis when starved, but this is more a
 323 change of life history strategy than an intrinsically deleterious trait as presumably
 324 both unsporulated and sporulated cells remained viable. Well-defined mitochondrial-
 325 nuclear incompatibilities among *S. cerevisiae*, *S. paradoxus*, and *S. bayanus* can cause
 326 hybrids to lose the ability to respire, preventing entry into meiosis altogether (Lee
 327 et al., 2008; Chou et al., 2010). Such mito-nuclear incompatibilities may well reflect
 328 divergent adaptation of these different species (also see Xu and He, 2011).

329

330 Given the apparent ease with which incompatibilities affecting other parts of
 331 the yeast life cycle can evolve, it is surprising that BDMIs causing hybrid gamete
 332 inviability have not been detected to date (Xu and He, 2011; Kao et al., 2010). The
 333 largest and most direct attempt to identify BDMIs between nuclear genomes in
 334 yeast was conducted by Kao et al. (2010). They concluded that there were no
 335 “simple” BDMIs between the nuclear genomes of *S. cerevisiae* and *S. paradoxus*,
 336 where a simple BDMI is one that kills a certain hybrid genotype. They found several
 337 pairs of segments with distributions that were statistically significantly different
 338 than what would be expected by chance, but they attributed these to more complex
 339 interactions, likely involving multiple loci with weak effects. They found some
 340 evidence of three-way interactions, but lacked confidence due to limited statistical
 341 power. Li et al.’s simulation study explicitly investigated whether previous attempts
 342 at mapping BDMIs in yeast were adequate to conclude that they did not exist (Li et
 343 al., 2013). They found that BDMIs with incomplete penetrance (those do not kill all
 344 gametes of the incompatible genotype) would not be detected in Kao et al. (2010)’s

study due to the limited sample size. Moreover, higher order interactions (involving three or more loci) would behave the same as incompletely penetrant two-way interactions and thus, given a sufficient sample size, could be detected statistically in the same way. They recommend using OR instead of ChiSq because it has the advantage of differentiating between differences due to over-representation and under-representation of a genotype relative to expectations.

We were able to build on the work of Kao et al. (2010) and Li et al. (2013) to successfully detect pairwise BDIMs. Using our *P_{CLB2}-MSH2 P_{CLB2}-SGS1* double mutant strains, we were able to restore recombination to an average of 37.9 cross-overs per meiosis (or 18.9 per spore). This was an improvement over Kao et al.'s deletion mutant of *MSH2*, which had an average of 17.8 recombination events per strain (Kao et al., 2010). They were also forced to exclude aneuploid chromosomes from most of their analysis, thus greatly decreasing the effective sample size (Kao et al., 2010). By obtaining complete tetrads, we avoided this problem. Moreover, we also reduced potential genotyping errors because each recombination event is supported in two separate, reciprocal samples. As well as these improvements, by using OR instead of ChiSq, as recommended by Li et al. (2013), we could focus solely on the case in which there is a depletion of hybrid types (OR higher than expected).

Using these improved methods, we found six major regions of the genome that appear to define four putative two-locus BDIMs (Figure 2). These regions were found on only four chromosomes (chr IX, X, XII and XV). Many genes map to these regions, and fine-scale mapping will be necessary to determine the causative loci. Among the known interacting genes in BioGRID, there are none identified between genes found in regions A and E or B and D (Oughtred et al., 2018). Among the genes

in regions C and E, there is one known interaction; a negative genetic interaction between *IMA2* (an isomaltase) and *CDC6* (an essential protein required for DNA replication), which was found in a large-scale genetic interaction study (Costanzo et al., 2016). Regions D and F harbour many known interacting genes, but this is unsurprising because together they encompass the largest number of genes. Despite no known interactions between regions A and E or regions B and D, there are some good candidates based on similar proteins. For example, in region E, gene *YOL159C-A*, encoding a protein of unknown function, interacts positively with *COA4*, encoding a protein involved in the organization of cytochrome c oxidase (Cherry et al., 2012; Costanzo et al., 2016). *COA4* is not found in region A but *COA1*, which is also required for assembly of the cytochrome c oxidase complex, is. Additionally, *CSS3*, another protein of unknown function in region E, interacts negatively with *MAL12*, a maltase that hydrolyzes sucrose (Cherry et al., 2012; Costanzo et al., 2016). *MAL12* is not found in region A but *SUC2*, a sucrose hydrolyzing enzyme, is. In region B, there is only one gene, *CBR1*, a cytochrome b reductase. It has physical interactions with *GSC2*, a synthase involved in the formation of the inner layer of the spore wall (Cherry et al., 2012; Krogan et al., 2006) similar to *SP075* found in region D, which is required for spore wall formation. It also interacts physically with *ORC1*, the largest subunit of the origin recognition complex (Cherry et al., 2012; Müller et al., 2010), and *ORC3*, another subunit of the origin recognition complex, is found in region D. *CBR1* also interacts negatively genetically with both *MDM36*, a mitochondrial protein which is proposed to have involvement in the formation of Dnm1p-containing cortical anchor complexes that promote mitochondrial fission, where *DNM1* is found in region D, and *WHI2*, a binding partner of Psr2p required for full activation of STRE-mediated gene expression, where *PSR2* is found in region D (Cherry et al., 2012; Costanzo et al., 2016). Whilst these incompatible regions

397 contain interesting candidate genes, finer scale recombination mapping followed by
 398 candidate allele replacement and sensitive fertility assays will be necessary to
 399 determine the underlying molecular genetics of these BDMIs.

400

401 **Conclusion**

402

403 Suppressing the expression of just two genes, *SGS1* and *MSH2*, rescues the fertility of
 404 normally sterile yeast hybrids and allows the recovery of recombinant euploid
 405 hybrid gametes, permitting the detection of nuclear-nuclear BDMIs in yeast for the
 406 first time. Whilst these incompatibilities comprise only a small part of the
 407 reproductive barrier between the parent species, with the vast majority coming
 408 from anti-recombination between the diverged genomes, they may have been
 409 important in the early stages of speciation. These results not only bring the power of
 410 yeast genetics to bear on the genetics of post zygotic reproductive isolation, they
 411 also enable crossing between highly diverged species, potentially allowing other
 412 interesting traits to be mapped or producing recombinant hybrids with novel
 413 commercial or research uses.

414

415

416 **Materials and Methods**

417

418 **Strains**

419

420 We used as a template a previously constructed *S. cerevisiae* strain NHY 2039, in
 421 which the promotor of *SGS1* had been replaced by the *CLB2* promotor (Oh et al.,
 422 2008) using the pFA6a-KANMX6-pCLB2-3HA created by Lee and Amon (2003). We
 423 amplified the *CLB2* promotor and the *KANMX4* drug resistance marker out of
 424 NHY2039 (i.e. YDG832) using primer pairs (see Supplementary File 5) that allowed
 425 us to transform it in place of the natural promoters of *MSH2* and *SGS1* in both *S.*
 426 *cerevisiae* (W303 background) and *S. paradoxus* (N17 background). The resulting *S.*

427 *cerevisiae* and *S. paradoxus* haploid strains YDG968 and YDG969 (see Table 1,
428 Supplementary File 1 and Supplementary File 2 for details) were crossed together
429 producing an F1 hybrid diploid YDG982 in which both homologous copies of both
430 *SGS1* and *MSH2* were under the control of the *CLB2* promotor, suppressing the
431 expression of these genes during meiosis. To obtain a non-hybrid, double-mutant
432 (i.e. *P_{CLB2}-MSH2*, *P_{CLB2}-SGS1*) control strain under the *S. paradoxus* background, we
433 crossed haploid strains YDG967 and YDG968. Next, we crossed YDG969 and
434 YDG970 strains to obtain a similar non-hybrid, double mutant (i.e. *P_{CLB2}-MSH2*, *P_{CLB2}-*
435 *SGS1*) control strain for the *S. cerevisiae* background. Finally, to obtain a wild-type
436 hybrid control strain (i.e. without CLB2 promoter replacement), we crossed haploid
437 strains YDG391 (*S. paradoxus*) and YDG542 (*S. cerevisiae*), and selected for diploid
438 clones (to form YDG853).

439 **Fertility**

440 We induced meiosis and sporulation by incubating the hybrid diploid (YDG982) in 3
441 ml KAc (2% potassium acetate sporulation media) for four days in room
442 temperature with vigorous shaking. To digest the ascus walls of the hybrid
443 ascospores, we incubated them in 1 unit (per 10 µl) zymolyase (Zymo Research EU,
444 Freiburg, Germany) for 30 minutes. After enzymatic digestion of the ascus walls, we
445 placed the four spores of each tetrad onto YEPD (2% glucose, 1% yeast extract, 2%
446 peptone, 2% agar) plates using an MSM400 tetrad dissection microscope (Singer
447 Instruments, Watchet, UK). Plates containing dissected tetrads were incubated at 30
448 °C before examining them for visible colonies founded by germinating spores.

451
452 We defined fertility as the proportion of viable gametes, i.e. the number of
453 spores that germinated and formed colonies visible to the naked eye after 2 days,

454 divided by the total number of spores that were dissected. For the hybrid crosses,
455 we dissected a large number of spores (≥ 880 , see Source Data 1). This was
456 necessary for the hybrid crosses because they were known to have low gamete
457 viability. For the non-hybrid crosses, we only dissected 384-400 spores (Source
458 Data 1). Because the non-hybrid crosses had much higher rates of gamete viability
459 than the hybrid crosses, dissecting a lower number of spores was sufficient to obtain
460 a good estimate of their true fertility. Only technical replicates (repeated meioses of
461 the same original diploid strain) were performed and were all considered to be part
462 of a single sample.

463 464 **Sequencing and genotyping** 465

466 To ensure that the hybrid gametes we sequenced were euploid, we only genotyped
467 gametes from tetrads that contained four viable spores. Even with the observed 70-
468 fold increase in hybrid gamete viability, only 5% of the tetrads contained four viable
469 spores. In order to maximize useable data from a single lane of sequencing, we
470 limited our sample size to 94 tetrads. Again, repeated meioses of a single diploid
471 strain were performed and were all considered to be part of a single sample. We
472 extracted DNA from all 376 colonies from 94 tetrads (in addition to two non-hybrid
473 control tetrads) using MasterPure™ Yeast DNA Purification Kit (Epicentre, Biozyme
474 Biotech, Oldendorf, Germany). To prepare the samples for sequencing, we used
475 double digestion based RAD-tag library preparation method (Etter et al., 2011;
476 Hohenlohe et al., 2010; Peterson et al., 2012). We digested 50 ng of DNA from each
477 colony using restriction enzymes *Csp6I* and *PstI* and ligated adapters
478 (adapterX_TagY_fq and adapterX_TagY_rv) in the same reaction at 37 °C for two
479 hours. We cleaned up the excess adapters, enzymes, and fragments smaller than
480 300bp by using Ampure beads at a 1:1 ratio. Next we mixed Phusion Hot Start II

481 High-Fidelity DNA Polymerase (2U/μl), adding P5 and P7 primers at 10 mM
 482 concentration, dNTPs (2mM per dNTP), and 5X Phusion HF Buffer to amplify the
 483 target regions (Acinas et al., 2005; Etter et al., 2011). 30 μl PCR mixtures were
 484 amplified as an initial 98 °C incubation for 30s, followed by 25 cycles of 98 °C for
 485 10s, 68 °C for 15s, 72 °C for 30s, and then a final extension at 72 °C for 5 mins. To
 486 sequence the tagged samples, we mixed all tagged samples in one pool. All samples
 487 were multiplexed using combinations of 24 unique barcodes therefore reads from a
 488 single sequencing reaction would have unique reverse and forward tags that will
 489 help us to distinguish all samples after obtaining the pool of MiSeq reads. We used
 490 MiSeq platform to obtain 300 bp paired-end reads. Raw sequence data is available
 491 from Dryad (Bozdag et al., 2019).

492
 493 To map the reads, we assembled two simplified co-linear reference genomes
 494 consisting of the coding DNA only from the set of open reading frames shared
 495 between *S. cerevisiae* and *S. paradoxus*, removing open reading frames that were
 496 present in one species but not in another or which were not co-linear (based on
 497 SGRP sequencing added 10/10/08; Liti et al., 2009; Bergström et al., 2014). We
 498 mapped reads to these reference genomes using NGK. At this point, we excluded 10
 499 tetrads due to poor sequencing coverage and quality, leaving us with 336 samples
 500 from 84 tetrads. We assigned ORFs to one or other species using two simplifying
 501 assumptions: that no non-Mendelian segregation occurred and that recombination
 502 occurred only in intergenic regions. Thus, if reads in all four spores of a tetrad
 503 contained reads mapping to a given ORF of one or the other or both species, the two
 504 spores with the highest proportion of reads mapping to one species ORF would have
 505 it assigned to that species and the other two would have the ORF assigned to the
 506 other species. If the four copies of an ORF within a tetrad did not all contain reads

mapping to either or both species then the ORF would be assigned to the same species as the neighbouring ORF. These genotyping rules produced a recombination map (see Supplementary File 6 for an example, full data available in Bozdag et al., 2019) of the four spores within each tetrad at ORF-level resolution, with no gain or loss of genetic material (i.e. no gene-conversion).

Analysis

We divided the chromosomes into 1208 segments defined by the recombination breakpoints observed in all 84 tetrads (Supplementary File 3), reasoning that we could not resolve a locus smaller than the closest crossovers flanking it. Each segment was tested against each other segment, excluding pairs found on the same chromosome, similar to the method described by Li et al. (2013) (exact method can be found in Source Code 1). Within chromosome pairs were not tested because physical linkage would skew the numbers towards parental combinations, the same effect that we expect to see due to incompatibility between loci, thus making the results difficult to interpret. Following the procedure of Li et al. (2013), the odds ratio (OR) was calculated for each pair of segments by dividing the product of the numbers of the two parental genotypes observed by that of the two hybrid types:

$$OR = (par1 * par2) / (hyb1 * hyb2)$$

Pairs of segments on the same chromosome were excluded because physical linkage would skew the numbers towards parental combinations, the same effect that we expect to see due to incompatibility between loci, thus making the results difficult to interpret.

To control for type I error, we produced a null distribution against which to test our observed ORs (Source Code 1). First, a new set of 84 tetrads was simulated

534 by pulling each chromosome for each tetrad from the pool of chromosomes of that
 535 type (with replacement). This is similar to, but not exactly the same as, the method
 536 used by Li et al. (2013). They, in contrast, shuffle the chromosomes without
 537 replacement instead of sampling with replacement, and they do so among random
 538 spores instead of whole tetrads. ORs for the segment pairs were then calculated as
 539 before but on the simulated set of chromosomes. This process was repeated 99
 540 more times, and the top OR from each set of simulated tetrads was recorded. The
 541 5th largest OR from this set of 100 top ORs was chosen as the critical value from
 542 which to judge significance. All observed pairs with a higher OR than this critical
 543 value (3.4131) were deemed statistically significant (Supplementary File 4 and
 544 Source Data 2). For our sample size of 84 tetrads, this represents a 1.85-fold over-
 545 representation of parental combinations (218 parental combinations vs. 118 hybrid
 546 combinations for that pair of alleles).

547
 548 The significant pairs of segments were then grouped into blocks comprising
 549 neighbouring pairs. Instead of looking at blocks of seven markers, as was done in Li
 550 et al. (2013), we decided that if two adjacent segments both had a significant
 551 interaction with the same segment in another chromosome, they were part of the
 552 same block. In one case, one significant interaction was between a non-adjacent
 553 segment and a segment that interacted with many other nearby segments. Because
 554 this interaction was so close to the others (within nine segments), we arbitrarily
 555 decided to treat it as part of the same interaction (Source Data 2, row 37 as
 556 compared to surrounding rows). Similarly, region B was found to interact with a
 557 segment adjacent to those interacting with region F. In this case, we collapsed both
 558 interacting regions into one (region D), as we consider it most likely that the two
 559 regions are interacting with a single gene in the region.

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Supplementary Material

- Supplementary File 1: A schematic of how the strains used in this study were constructed.
- Supplementary File 2: Complete list of strains used in this study.
- Supplementary File 3: Segments used for mapping BDMIs. Each segment is defined by observed recombination between genes and all start and end locations are based on the sequence of W303.
- Supplementary File 4: All pairwise combinations of segments, the observed number of parental and hybrid combinations and the summary statistics.
- Supplementary File 5: Primers used in the study.
- Supplementary File 6: An example recombination map of a single tetrad. Gametes were genotyped by ORF into one of the two species, ensuring a 2:2 segregation of species identity at each ORF.
- Source Data 1: Raw counts of dissected and viable spores for each strain assayed in this study.
- Source Data 2: Significant pairwise combinations of segments grouped by interacting regions.

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Source Code 1: Code used to perform statistical analysis on gene interaction data, along with necessary input files.

Species:

S. paradoxus

S. cerevisiae

Hybrid

a. Transformation

b. Mating & segregation

c. Mating