The recombination landscape of introgression in yeast

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Abstract

Meiotic recombination is an important evolutionary force that acts by breaking up genomic linkage, thereby increasing the efficacy of selection. Meiotic recombination is initiated with a double-strand break which is resolved via a crossover, which involves the reciprocal exchange of genetic material between homologous chromosomes, or a non-crossover, which results in small tracts of non-reciprocal exchange of genetic material. While the meiotic process is largely conserved, crossover and non-crossover rates vary between species, populations, individuals, and across the genome. In recent years, recombination is observed to be positively associated with the distribution of ancestry derived from past interspecific hybridization (introgression) in a variety of species. This trend has been interpreted to signify that introgression carries genetic incompatibilities that are selected against, such that introgression is enriched in regions of high recombination. However, recombination is well known to be suppressed in divergent sequence to prevent non-homologous recombination. Since introgressed DNA is often divergent, we sought to explore this interaction of recombination and introgression by sequencing spores and detecting crossover and non-crossover events from two crosses of the budding yeast *Saccharomyces uvarum*. One cross is between strains isolated from natural environments, and

the other cross is between strains from fermentation environments, in which each strain contains introgression from their sister species, *S. eubayanus*. We find that the recombination landscape is significantly different between *S. uvarum* crosses, and that most of these differences can be explained by the presence of heterozygous introgression in the fermentation cross. Crossovers are significantly reduced and non-crossovers are increased in heterozygous introgression compared to syntenic regions in the natural cross without introgression. This translates to reduced allele shuffling within introgressed regions, and an overall reduction of shuffling on most chromosomes with introgression compared to the syntenic regions and chromosomes without introgression. Our results indicate that recent hybridization can significantly influence the recombination landscape, and suggest that the reduction in allele shuffling contributes to the initial purging of introgressed ancestry in the generations following a hybridization event.

Keywords: introgression, hybridization, recombination, crossover, non-crossover, yeast, Saccharomyces

Introduction

Recombination is the exchange of genetic material between homologous chromosomes during meiosis and is a staple of eukaryotic sexual reproduction. While the processes involved in recombination are largely conserved (Arter & Keeney, 2023), recombination rates vary between sexes, populations, and species (Smukowski & Noor, 2011; Stapley et al., 2017). Recombination rates also vary along the genome, with conflicting patterns of enriched or depleted recombination in promoter regions and punctate or dispersed recombination depending on the species (Auton et al., 2013; Rockman & Kruglyak, 2009; Singhal et al., 2015; Smukowski Heil et al., 2015). These patterns in recombination can affect pairing of alleles after meiosis–in other words, the shuffling of alleles–in a population. Much of the evolutionary advantage of recombination is understood to originate from its role in shuffling alleles, which increases the number of different allele combinations segregating in a population. The increase in allele combinations can reduce selection interference–the effect that genetically linked sites have on the evolutionary fate of either beneficial or deleterious alleles (Felsenstein, 1974; Hill & Robertson, 1966; McDonald et al., 2016; McGaugh et al., 2012).

How much allele decoupling is produced by recombination will depend on the type of recombination event. Each recombination event begins with the severing of both strands of a sister chromatid of one of the homologous chromosomes in what is referred to as a double-strand break (DSB) (Keeney, 2001). The distribution of DSBs is influenced by a variety of factors, many of which are organism-dependent, but often include decreases of DSBs near telomeres and centromeres as well as increases in genomic regions enriched for GC content, CpG sites, and depleted of methylation (CpG islands) and near promoter regions (de Massy, 2013; Lam & Keeney, 2015; Pan et al., 2011; Zelkowski et al., 2019). When a DSB occurs, the homologous strand is recruited to repair the break, and during this process genetic information

is exchanged. The most evident and widely studied resolution of a DSB is a crossover (CO), where all the genetic information on one side of the DSB from one homologous chromosome is spliced with all the genetic information on the other side of the DSB from the other homologous chromosome. DSB resolution can also involve gene conversions–or non-crossovers (NCOs)– which result in one small segment (typically 100-2000 bp) of a homologous chromosome's genetic information being copied onto the other (Chovnick et al., 1971; Hilliker et al., 1994; Jeffreys & May, 2004; Judd & Petes, 1988). DSBs can be resolved through both of these processes in a single instance, and even more than one NCO can occur at a single break point. Each possible resolution can produce a variety of genetic patterns at the site of a DSB. COs generally produce more allele shuffling, and therefore degrade linkage faster than NCOs, because they recombine all loci from one side of the event with all loci on the other side. However, NCOs can occur in regions where COs are typically suppressed, like centromeres and inversions (Korunes & Noor, 2019; Mancera et al., 2008; Miller et al., 2016; Schaeffer & Anderson, 2005; Shi et al., 2010; Talbert & Henikoff, 2010; Wijnker et al., 2013). NCOs are also crucial to reducing linkage within coding regions and, unlike COs, result in 3:1 allele ratio in the meiotic product at heterozygous sites, potentially changing allele frequencies (Korunes & Noor, 2017).

The number of DSBs that occur per meiosis varies by species, as does the number of DSBs that are repaired as COs or NCOs (de Massy, 2013; Korunes & Noor, 2017). In Arabidopsis and maize, several hundred DSBs are resolved into only a handful of COs, whereas in *Saccharomyces cerevisiae* the CO:NCO ratio is close to 2:1 (Choi et al., 2018; He et al., 2017; Mancera et al., 2008). How CO:NCO resolution may be evolving is not well understood, but evidence from *Saccharomyces* suggests that the DSB landscape is well conserved across species, while recombination rates significantly differ between sister species *S. cerevisiae* and *S. paradoxus* (Lam & Keeney, 2015; Liu et al., 2019). This suggests that alternate resolution of

DSBs may be important in altering recombination rates between closely related species. This is further supported by the finding that several genes involved in the CO vs. NCO decision of DSB resolution show evidence of rapid evolution and directional selection in mammals (Dapper & Payseur, 2019), and that some of these genes are linked to variation in recombination rates within and between species (Brand et al., 2018; Kong et al., 2008; Murdoch et al., 2010; Yang et al., 2015).

Variation in the number and distribution of COs and NCOs, and their respective associated effects on linkage, have important implications for molecular evolution. Recombination has long been appreciated to play a role in the distribution of various genomic features including nucleotide diversity. Nucleotide diversity has a positive correlation with recombination rate in a number of species, interpreted to result from selective sweeps and background selection removing genetic variation in regions of low recombination (Begun & Aquadro, 1992; Charlesworth et al., 1993; Smith & Haigh, 1974). Similarly, recombination breaking up genetic associations is particularly notable in the context of interspecific hybridization. In first-generation (F_1) hybrids, each set of homologous chromosomes is composed of sister chromatids carrying genetic information from one of the parents. Since no recombination has physically separated any alleles on the chromosome at the hybrid F_1 stage, each parent's genetic contribution is perfectly linked. If the hybrids then back-cross to one of the parental populations, recombination will produce genomes that are a mosaic of genetic information from the two populations (introgression) (Aguillon et al., 2022). Recombination drives the pattern of fragmentation in the introgressed regions over time, and therefore plays an important role in the distribution of introgressed DNA in a population (Barton & Bengtsson, 1986; Butlin, 2005; Moran et al., 2021; Nachman Michael W. & Payseur Bret A., 2012; Schumer et al., 2018; Veller et al., 2023). When each population has evolved alleles that are deleterious when present in the background of the other population (the Dobzhansky-Muller hybrid incompatibility model) we expect introgressed

131 regions with low rates of recombination to be quickly purged from the population, as the
132 accumulation of incompatible alleles incurs a steep fitness cost. In contrast, when introgressed
133 regions have high recombination rates, the break up of genetic associations will reduce
134 selective interference between the incompatible alleles and their surrounding haplotypes,
135 allowing for neutral and beneficial alleles brought in with the introgression to escape the fate of
136 neighboring incompatibilities (Moran et al., 2021; Schumer et al., 2018). This theory is
137 supported empirically through enrichment of introgressed segments in regions of higher
138 recombination in a number of organisms including Mimulus, maize, butterflies, swordtail fish,
139 stickleback, and humans (Brandvain et al., 2014; Calfee et al., 2021; Edelman et al., 2019;
140 Martin et al., 2019; Ravinet et al., 2018; Schumer et al., 2018).
141
142 This positive correlation between introgressed ancestry and recombination is emerging as a
143 nearly ubiquitous pattern (though see (Dagilis & Matute, 2023; Duranton & Pool, 2022; Pool,
144 2015)), however, it is unclear how these observations relate to the known effect of sequence
145 divergence on DSB resolution. Introgression, particularly between highly diverged species, can
146 have low sequence homology with the genomic region it is replacing. A DSB in a region of low
147 homology will recruit mismatch repair proteins, which ensure COs are occurring between
148 homologous chromosomes and at equivalent positions to prevent ectopic recombination (Harfe
149 & Jinks-Robertson, 2000; Hunter et al., 1996). Mismatch repair proteins reduce the frequency of
150 CO events as sequence divergence increases (Chen & Jinks-Robertson, 1999; Cooper et al.,
151 2021; L. Li et al., 2006; Welz-Voegele & Jinks-Robertson, 2008). Given that heterozygous
152 introgression will have divergent sequences, we expect a decrease in COs, and possibly an
153 increase in NCOs as DSBs fail to be resolved as COs in introgressed regions .
154
155 To help us understand this interaction of introgression and recombination, and identify patterns
156 in CO and NCO in closely related populations, we utilized the budding yeast *Saccharomyces*.

Yeasts provide an excellent opportunity to study DSB resolution, as we can readily isolate and collect all four meiotic products of a given meiosis and detect both CO and more elusive NCO events (Figure 1A) (Brion et al., 2017; Gerton et al., 2000; Liu et al., 2018, 2019; Mancera et al., 2008). Recombination rates vary between strains of *S. cerevisiae* (Cubillos et al., 2011; Raffoux et al., 2018) and between *S. cerevisiae* and its sister species *S. paradoxus* (Liu et al., 2019; Tsai et al., 2010). Strains of different *Saccharomyces* species have often hybridized with other species and carry introgressed DNA from these events (Albertin et al., 2018; Almeida et al., 2014; Bendixsen et al., 2022; D'Angiolo et al., 2020; Langdon et al., 2019; Stelkens & Bendixsen, 2022; Tellini et al., 2023).

In this study, we look at patterns of recombination and introgression at the population level by crossing two pairs of Holarctic *Saccharomyces uvarum* strains. One pair of strains was isolated from natural environments in North America and the other pair was isolated from European fermentation environments (Almeida et al., 2014). The *S. uvarum* strains isolated from European fermentation environments each carry introgression from their sister species, *Saccharomyces eubayanus*, which is approximately 6% divergent from *S. uvarum* (Almeida et al., 2014; Langdon et al., 2020; Nespolo et al., 2020). The diploid F_1 genome of these strains is heterozygous for nine different introgressions which make up approximately 10% of the genome (Figure 1B). The strains from the North American cross do not carry *S. eubayanus* introgression, thus allowing us to assess the impact of introgression on the recombination landscape. We obtained whole genome sequencing data from individual meiotic events from the first offspring generation of each cross and used this data to detect CO and NCO events along the genome (Figure 1A). From these maps, we aim to understand (i) how patterns of CO and NCO differ between closely related strains, (ii) how regions of introgression differ in their CO and NCO patterns, and (iii) how these different patterns affect shuffling of alleles locally and at

the chromosome level. Understanding these objectives will provide us novel insights into how introgression impacts the recombination landscape.

Methods

Strain and library construction

S. uvarum strains (UCD61-137, yHCT78 , GM14, and DBVPG7787) were obtained from the Portuguese Yeast Culture Collection and from Chris Hittinger (Table S1) (Almeida et al., 2014). All four *S. uvarum* strains had their *HO* locus replaced with a kanMX marker using a modified version of the high-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. Briefly, the kanMX marker was amplified from plasmid pCSH2 with homology to genomic DNA flanking the HO ORF with primers CSH239 (GGTGGAAAACCACGAAAAGTTAGAACTACGTTCAGGCAAAgacatggaggcccagaatac) and CSH241 (GTGACCGTATTGGTACTTTTTTTGTTACCTGTTTTAGTAGcagtatagcgaccagcattc). For each strain, overnight cultures were inoculated in 25 mL of YPD at an OD of ~ 0.0005 and incubated at room temperature on a shaker for ~24 hours until the cultures reached an OD between 0.6 and 0.9. Subsequently, 1 ug of the template DNA was transformed with a heat shock temperature of 37°C for 45 minutes. The transformed cells were allowed to recover in liquid YPD for 4 hours before being plated onto G418 selective plates and incubated at room temperature for 2 days.

Single colonies were selected from the transformation plates, restreaked onto G418 plates and allowed to grow at room temperature for 2 days. Single colonies from those plates were then inoculated into 2 mL of YPD + G418 and incubated in a roller drum at room temperature overnight. From those cultures, 250 uL was used to inoculate 2 mL of sporulation media (1%

potassium acetate, 0.1 % yeast extract, 0.05% dextrose) and incubated at room temperature for 3 to 5 days. Strains were confirmed to have the ho::KanMX via tetrad dissection on a Singer SporPlay+ microscope (Singer Instruments). Plates with tetrads were incubated at room temperature for 2 days and then replica plated to test for proper segregation of the kanMX marker and mating type within individual tetrads.

Crosses between strains UCD61-137 and yHCT78 (natural cross), and between strains GM14 and DBVPG7787 (fermentation cross) were set up by micromanipulation of single MATa and MATx cells using a Singer SporPlay+. The plates were incubated at room temperature for 2 days and then replica plated to mating type tester strains to test for potential diploids. Identified diploids were then sporulated by growing a culture of the cross in 2 mL YPD + G418 at room temperature overnight. From those cultures, 250 uL were used to inoculate 2 mL of sporulation media and incubated at room temperature for 3 to 5 days. Sporulated cultures were dissected on 3 YPD plates (24 tetrads per plate) using a Singer SporPlay+. Fifty of the fully viable tetrads were selected and had all their spores inoculated into YPD (200 spores total) and incubated at room temperature. The DNA was extracted from these cultures using a modified version of the Hoffman-Winston DNA Prep (Hoffman & Winston, 1987). The DNA concentration was then measured using SYBR green, and 150 ng of each sample's DNA was used to prepare a sequencing library using an Illumina DNA Prep Kit, modified to use half the normal amounts of reagents. Libraries were pooled and run on an Illumina NovaSeq 500 with 150bp paired end reads.

Calling SNPs

We scored SNPs from parents and offspring using the *S. uvarum* reference genome (Scannell et al., 2011) and custom scripts that invoked bwa (v0.7.17), samtools (v1.12), bcftools (v1.13),

picardtools (v2.25.6), and gatk (v4.2.0.0) (Danecek et al., 2021; H. Li & Durbin, 2009; McKenna et al., 2010). The custom scripts are available in the github repository: *ejschwarzkopf/CO-NCO.* We joint genotyped parents and offspring with default filters for gatk with the exception of the QUAL filter, which was set as < 100 for parents and < 30 for offspring. We further filtered variants by requiring they be fixed differences between the two parental strains. We kept a total of 24,574 markers for the natural cross and 74,619 markers for the fermentation cross. We utilized LUMPY to identify structural variants in the parent strains that were greater than 5000 bp and verified calls using the Integrative Genomics Viewer (Layer et al., 2014; Robinson et al., 2011). We identified three amplifications in strain GM14 (one of the fermentation cross parents) that were absent in other strains (Table S2).

Generating CO/NCO maps

We generated “seg” files by coding tetrad variants by their parental origin. These seg files were the input for CrossOver (v6.3) from the ReCombine suite of programs, which we used to detect COs and NCOs (Anderson et al., 2011). We then filtered to remove non-crossovers with fewer than three associated markers and split the genome into 20kb windows. In each window we counted crossovers, non-crossovers, and markers. We established regions of introgressions through visual inspection of marker density in the fermentation cross (introgressions showed more divergence between fermentation strains) and confirmed them using the findings of Almeida et al. (2014). We found nine heterozygous introgressions on chromosomes 4, 6, 7, 9, 10, 10, 13, 14, and 15 respectively that we included in further analyses (Table S3). We excluded two additional introgressions due to poor mapping (chromosome 13:0-17,000; chromosome 16: 642,000-648,000). To account for the difference in number of markers in introgressed vs non-introgressed windows and their effect on NCO detection, we applied a previously published simulation-based method (Liu et al., 2019; Wijnker et al., 2013). Choosing an average NCO

tract length of 2kb, we simulated 1000 NCO events per window to establish our expected probability of detecting an NCO event in that window. We then divided our observed NCO count by our probability of detecting an NCO event. COs that occurred in large regions devoid of markers would be called in the middle of the empty windows. We decided to deal with this by splitting CO counts in regions with multiple consecutive windows without markers evenly between the empty windows. With these corrected maps, we calculated spearman correlations between crosses using R (v4.1.0, R Core Team 2021). Additionally, we modeled NCO and CO count as a function of introgression, introgression by cross, and GC content using a gaussian generalized linear model in R (v4.1.0, R Core Team 2021).

Homology

We calculated homology between the two fermentation cross strains in 51bp windows with 25bp overlaps. At each nucleotide position in the window, we counted fixed differences as zero homology, invariant sites between strains as full homology (1), and polymorphic sites in either or both strains as half homology (0.5). We then averaged these homology values across the window. This measure represents the probability that both strains will have the same nucleotide base at a given position. We used this measure of fine-scale homology to determine how homology related to NCO counts in introgressed regions. For this, we used Loess regressions and Spearman's correlations on each of the introgressed regions comparing homology to NCO count, both implemented in R (v4.1.0, R Core Team 2021). We then focused on each recombination event (CO or NCO) and compared the homology 100bp up and downstream of CO breakpoints and 100bp up and downstream of NCO tracts. We then used Welch's two sample t-tests to compare CO and NCO homology in each introgression.

$\underline{r}$

We use $\underline{r}$, a measure genetic shuffling defined in Veller et al. (2019) to measure how much shuffling occurs in each chromosome for each cross. Our data provides parental origin for each fixed difference between parental strains. We assume that all loci between pairs of markers that come from the same parent are also from that parent. We also assume that when a pair of successive markers come from different parents, the location of the change from one parental origin to the other happens at the midpoint between our markers. With this in mind, we counted the number of bases that come from one parent and divided by the chromosome size to obtain the proportion of the chromosome that was inherited from said parent (p) and used the formula from Veller et al. (2019): $\underline{r} = 2p(1-p)$. We calculated $\underline{r}$ for each full chromosome and each introgressed regions in every gamete from both crosses. We then averaged across gametes to obtain average $\underline{r}$ values. We then compared average $\underline{r}$ between crosses in each chromosome or introgressed region using Welch two sample t-tests and correcting for multiple tests using a Bonferroni correction in R (v4.1.0, R Core Team 2021).

Data availability

Sequences for the parental strains can be found on NCBI SRA (SRR1119189, SRR1119180 SRR1119199, SRR1119200) (Almeida et al., 2014). Sequencing of the tetrads is deposited at NCBI SRA under Project PRJNA1061120.

Results

The recombination landscape differs dramatically between closely related crosses

We isolated and sequenced products of 48 meioses (192 haploid spores) for two crosses of *S. uvarum*, a cross between strains isolated from North America (natural cross) and a cross between strains isolated from Europe (fermentation cross). We detected COs and NCOs across the 16 nuclear chromosomes of *S. uvarum*. Genomewide, we found significantly more COs on average in the natural cross (82.54 COs/meiosis, SE 1.5; 0.72 cM/kb) than in the fermentation cross (63.66 COs/meiosis, SE 1.9; 0.55 cM/kb) but no significant difference when comparing the average number of NCOs between the natural cross (60.44 NCOs/meiosis, SE 2.7) and the fermentation cross (68.1 NCOs/meiosis, SE 12.47). The number of COs per meiosis in the natural and fermentation crosses are slightly higher than those of *S. paradoxus* (54.8) and *S. cerevisiae* (76.5) respectively, but both crosses have NCO averages higher than those of *S. cerevisiae* (46.4) and *S. paradoxus* (26.9) (Figure 2A; Tables S4 & S5; Liu et al. 2019). The significant difference in COs per meiosis between our fermentation and natural crosses was unexpected, as we predicted that the recombination landscape would be more similar within a species (*S. uvaum*) than between species *S. cerevisiae* and *S. paradoxus*.

To further explore the differences in recombination landscapes between our crosses, we split the genome into 20kb, non-overlapping windows, and obtained CO, NCO, and marker counts for each region (Figure 2D). We corrected for differences in marker resolution between crosses (see Methods). We found modest, but significant genomewide correlation between our crosses for both COs (Spearman's correlation: 0.273; $p<0.0001$) and NCOs (Spearman's correlation: 0.1456; $p<0.001$). Again, these correlations were unexpectedly lower than those reported by Liu et al. (2019) between *S. cerevisiae* and *S. paradoxus* (0.48 for COs and 0.17 for NCOs).

We hypothesized that the low correlations between crosses might be impacted by the presence of heterozygous introgression from *S. eubayanus* in the fermentation cross. To explore this possibility, we separated the 20kb windows into introgressed and non-introgressed windows (based on whether they overlapped with an introgressed region). We will refer to introgression in

the fermentation cross as “introgression” and use the term “introgressed region” to refer generally to the syntenic region, regardless of which cross we are focusing on. We calculated Spearman’s correlations of COs, NCOs, and marker counts between the two crosses for each chromosome. We find positive correlations between CO counts for all chromosomes when looking at non-introgressed regions (though some were not significant; Table S6). For introgressed regions, we found no significant CO correlations between crosses, which is consistent with the hypothesis that CO landscapes are changed in introgression (Table S7). There was one significant, highly positive correlation among NCOs (chromosome 1), but all other correlations were not significant. This was likely affected by the fact that markers used to detect NCOs are very differently distributed between the crosses, and the small size of NCO tracts means regions with more markers tend to reveal more NCOs.

We find that introgressions tend to have lower CO counts and higher NCO counts in the fermentation cross when compared to syntenic regions in the natural cross. The fermentation cross has fewer COs than the natural cross overall, but the difference is greater in the introgressed regions (Figure 2B-C). To further explore and test possible explanations for the patterns of COs and NCOs in introgressed regions, we constructed linear models for CO and NCO counts. Our model of CO counts showed a significant positive effect of the interactions between natural cross and introgression (whether a genomic window is from the natural cross and whether that window is in an introgressed region), as well as a significant negative effect of introgression on the number of COs, and a significant positive effect of GC content on CO count (Table 3). These results are consistent with increased COs in GC-rich regions and reduced COs in introgressions. Our linear model of NCO counts showed a similar positive effect of GC content on NCO counts, but showed opposite significant coefficients for the other two explanatory variables (Table 4). This indicates that GC content still plays an important role in

localizing NCOs, and supports our findings that patterns of NCOs in introgressed regions are opposite to those of COs.

Reduced sequence homology helps explain non-crossover resolution of DSBs in introgressions

One possible explanation for an increase of NCOs in introgressions is that the reduced homology is biasing DSBs in the region to be resolved as NCOs rather than COs. We would therefore expect to see NCOs to be negatively correlated with homology. To evaluate the relationship of homology to the CO and NCO landscapes in introgressions, we measured homology, NCO depth, and CO count in 101 bp sliding windows along each of the introgressions. Mismatch repair proteins in *Saccharomyces* seem to suppress COs with very little mismatch in small regions (~350 bp), which informed our window size (Chen & Jinks-Robertson, 1999; Datta et al., 1997). We counted the number of NCO tracts that intersect with each window as a measurement of NCO depth, and simply counted the CO events in a given window. We measured homology in the sliding windows by measuring the proportion of bases that are expected to match between the two parent strains (Figure 3). We then ran Spearman's correlations and a loess regression along each introgression and found a weak, but often significant ($p<<0.001$) correlation between NCOs and homology in the introgression (Table 1), suggesting that resolution of double strand breaks is biased towards NCOs when homology is low. From the loess regression, we can observe an increase in NCO as homology reduces until about 0.9-0.8 homology, at which point NCOs level out or reduce. However, this effect is very weak with respect to the NCO counts, and at low levels of homology the uncertainty of the regression line is very large. This is primarily driven by the number of windows with no NCOs (Figure 4).

The low CO count in introgressed regions leaves us unable to investigate effects of homology on CO counts except on chromosomes 7 and 14, where we found significantly higher homology around COs than around NCOs (Table 2). The introgressions on these two chromosomes are

unique in that they contain a highly homologous portion of sequence and therefore contain enough COs for us to have power to detect differences between CO and NCO neighborhoods (Figure 3).

Introgression decreases allele shuffling locally and at the chromosome level

Because NCOs still play a small role in shuffling alleles along the chromosome, we were interested in whether the increase in NCOs of the fermentation cross would supplement the lost shuffling from the suppression of COs in the introgressions. To test this hypothesis, we used the measure $\underline{r}$, which accounts for the number and positioning of recombination events to estimate the probability that a randomly chosen pair of loci shuffles their alleles in a gamete (Veller et al., 2019). We calculated the average $\underline{r}$ per chromosome and for each introgressed region for each of the two crosses. We observed high levels of shuffling at the chromosome level when compared to humans. The intra-chromosomal component of $\underline{r}$ in humans is 0.0135 in females and 0.0177 in males (Veller et al., 2019), while our measurements for chromosomes varied between 0.216 and 0.426. We find that most chromosomes do not have a significantly different amount of allele shuffling between the two crosses, even though the natural cross generally has more COs (Table S8; Bonferroni-adjusted $\alpha = 0.00313$). However, of the six chromosomes with significantly different $\underline{r}$ values, all of them showed more shuffling in the natural cross, and five of the six (chromosomes 4, 9, 10, 14, and 15) contained introgressed regions (Figure 5). Chromosome 12 was the only chromosome without an introgressed region to have significantly different shuffling between crosses, and it also showed more shuffling in the natural cross. All of the introgressed regions showed significantly more shuffling in the natural cross, indicating that the large increase of NCOs in the introgressions does not make up for the loss of shuffling from the depletion of COs (Table S9; Bonferroni-adjusted $\alpha = 0.00556$). This finding indicates that an introgression that is segregating in a population will incur a shuffling cost in heterozygous individuals on top of any other evolutionary effects the introgression may have.

Discussion

Our study is motivated by understanding recombination rate variation within a species and uncovering potential genetic factors underlying this variation. To investigate this question, we crossed two pairs of *S. uvarum* strains, one pair isolated from natural environments and one pair from fermentation environments, and explored the distribution of CO and NCO events from both crosses. We found a similar range of COs per meiosis as previous studies in *S. cerevisiae* and *S. paradoxus* (Liu et al., 2019; Mancera et al., 2008). The number of NCOs detected, however, was considerably higher, despite having less sequence differences between strains in each of our crosses and therefore lower genomic resolution for detection. We also detected more COs and fewer NCOs in our natural cross when compared to our fermentation cross.

We hypothesized that these differences in the recombination landscape were influenced by introgression, given that heterozygous introgression creates sequence divergence, and that COs in regions of lower homology are known to be curtailed (Chen & Jinks-Robertson 1999, Li et al. 2006, Weltz-Voegele & Jinks-Robertson 2008, Cooper et al. 2021). We therefore explored the relationship between introgressions and the differences in CO and NCO counts between crosses. Our correlations between crosses, though mostly not significant, pointed to more CO distribution differences in introgressed regions than in the rest of the genome. When we then modeled CO and NCO locations, correcting for GC content (a well characterized driver of recombination events (Kiktev et al., 2018; Marsolier-Kergoat & Yeramian, 2009)), we found that the distribution of COs and NCOs we observed was well explained by introgressions. While we are limited in our interpretations by only comparing two crosses (one cross with heterozygous introgression and one without introgression), these results are in line with findings in inversions, where heterozygotes show sharp decreases in COs and an increase in NCOs in the inverted

433 region (Crown et al., 2018; Korunes & Noor, 2019). However, unlike heterozygous inversions
434 where an increase in COs is observed on freely recombining chromosomes (the inter-
435 chromosomal effect), we do not see an increase in COs outside of introgression or on
436 chromosomes without introgression.
437
438 One likely effect of this CO reduction is a reduction in shuffling at the regional and chromosomal
439 level. While NCOs can increase local shuffling, they likely have a much weaker effect on the
440 likelihood of two random alleles being shuffled than COs do. We find this is the case for our two
441 crosses, where despite a large number of NCOs in introgressions, the amount of shuffling (as
442 measured by $\underline{r}$) is significantly lower in the fermentation cross. This loss of shuffling translates
443 to frequently lower $\underline{r}$ in the fermentation cross at the chromosome level for chromosomes
444 containing introgressions. Lower $\underline{r}$ is not observed when introgressions are small and near
445 telomeres, while even a small introgression near the center of the chromosome can lead to a
446 large reduction in $\underline{r}$ (as is the case for chromosome 15). This is consistent with the expectation
447 that COs near the center of chromosomes generate much more shuffling of alleles than terminal
448 COs (Veller et al., 2019). Our findings indicate that reducing COs, especially near the center of
449 chromosomes, has a cost to shuffling that is not compensated by the increase of NCOs that we
450 observe. If the benefit of recombination is its ability to generate new combinations of alleles,
451 then the loss of shuffling resulting from being heterozygous for divergent DNA sequences may
452 come at an additional cost beyond the possibilities of genetic incompatibilities between
453 hybridizing species. This cost is likely higher as divergence increases and as the length of
454 divergent sequences is greater, as is the case with early generation hybrids (Dagilis & Matute,
455 2023). Ultimately, if sequence divergence is too high, the resultant failure to recombine can
456 become a postzygotic reproductive barrier (Bozdag et al., 2021; Hunter et al., 1996; Rogers et
457 al., 2018).

458

459 The shuffling cost to introgression that we identify in our crosses may play an important role in
460 the fate of introgression in the generations following hybridization. When heterozygotes for an
461 introgression are formed, the reduction in shuffling inside the introgression will increase the
462 likelihood that the introgression is purged from the population. This is because it will likely be
463 inherited in its entirety and will carry the fitness cost of incompatibilities combined with a cost of
464 shuffling. This cost is incurred because the reduction of COs in the introgression will reduce
465 shuffling of alleles on either side of it and will vary in its intensity depending on the location and
466 size of the introgression. In generations immediately following hybridization, introgressions will
467 be much larger and are therefore expected to be more costly (although this likely depends on a
468 number of factors including time since divergence). These predictions are consistent with
469 modeling and empirical data on the purging of introgression in Drosophila and humans in the
470 first generations following hybridization (Veller et al., 2023).

471

472 As to longer term dynamics of recombination and selection, we predict that the excess NCOs
473 detected in heterozygous introgressions should begin to erode the divergence between the
474 sequences, increasing homology and slowly reducing the cost of the introgression. This
475 hypothesis posits that recombination can act to remove the larger, more deleterious regions of
476 an introgression quickly while whittling away slightly deleterious alleles that may be linked to any
477 beneficial regions of an introgression. However, in our current study, we are limited by only
478 observing one generation of sexual reproduction between two pairs of strains. This means that
479 we don't capture longer term patterns of recombination or the landscape of recombination in
480 introgressions that are segregating in a population. Consequently, our findings do not reflect the
481 effects of long-term selection (and hypothesized degradation of sequence divergence through
482 NCO) that would lead to introgressions preferentially remaining in high-CO regions, as has been
483 observed in other organisms. Furthermore, *Saccharomyces* typically reproduce asexually, with

only infrequent sexual cycles (Magwene et al., 2011; Ruderfer et al., 2006; Zeyl & Otto, 2007). When they do mate, they often mate within a tetrad resulting in increased homozygosity. For example, each diploid progenitor of the parents of our fermentation cross was homozygous for introgression across the genome, meaning that recombination would neither break up nor aid in purging the introgression in isolated populations of each parent. This suggests that the fate of introgressions in this species is perhaps more loosely tied to recombination patterns than it would be in an obligately sexually reproducing species.

Despite some limitations to interpretation, this study provides a unique view of the early dynamics of hybridization and the role of recombination in the presence of introgression. By focusing not only on the distribution of recombination events but on their specific role in shuffling alleles, we can more closely connect the physical process of recombination to its role among other evolutionary forces.

Acknowledgements

We are grateful to members of the Heil lab, Mohamed Noor, Nathan Layman, and Mark Smithson for comments on this manuscript. We thank Chris Hittinger and the Portuguese Yeast Culture Collection for *S. uvarum* strains. This work was supported by NIH R35GM142849 to CSH.

Figures and Tables

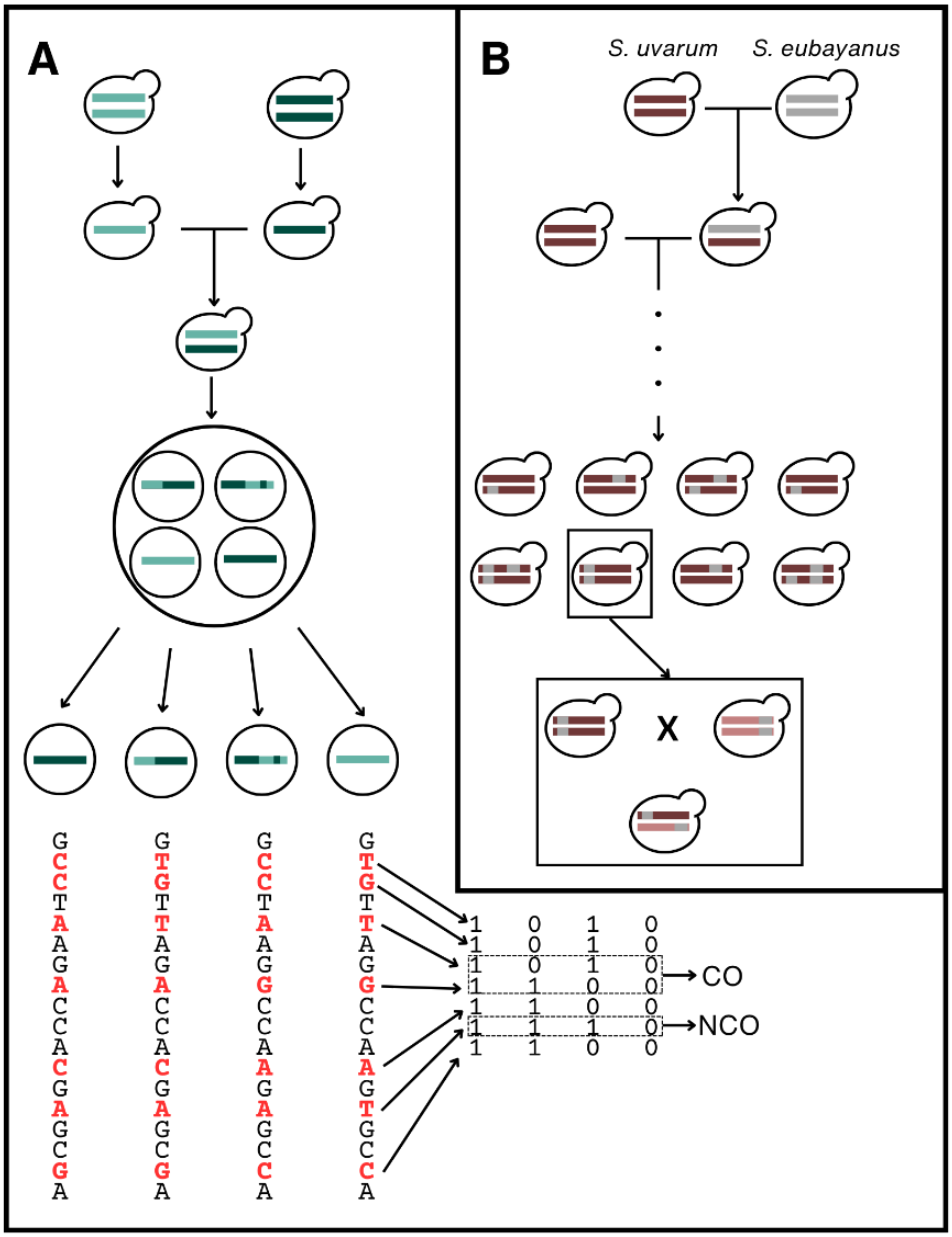


Figure 1: (A) Visual representation of each of our crosses. Yeast from each of the parental strains of a cross are induced to go through meiosis to generate haploids of each mating type. Subsequently, they are mated, and their diploid offspring are induced to enter meiosis. The resulting tetrads are manually dissected, and each haploid meiotic product is grown mitotically

517 *to obtain enough material for DNA extraction and whole genome sequencing. We then call*
518 *SNPs on the resulting sequences and retain loci with fixed differences between parents. These*
519 *loci are then coded as 1 or 0 depending on the parent of origin and the CrossOver software*
520 *detects COs and NCOs. (B) The introgressions we observe in our crosses are due to* S.
521 eubayanus *hybridizing with* S. uvarum*, resulting in F1 hybrids that then potentially crossed with*
522 *other* S. uvarum *individuals for some number of generations. Eventually, the* S. eubayanus
523 *ancestry was degraded in the population of* S. uvarum *until the introgressions we observe today*
524 *remained, potentially segregating in the population. A similar process likely happened in each of*
525 *the parental strains we utilized, but with different introgressions remaining in each strain. We*
526 *then crossed individuals from each strain that were homozygous for the introgression, resulting*
527 *in offspring that were heterozygous for each introgression. It's important to note that due to the*
528 *life cycle of* Saccharomyces*, mitotic recombination likely played an important role in the*
529 *breakdown of introgressions.*
530

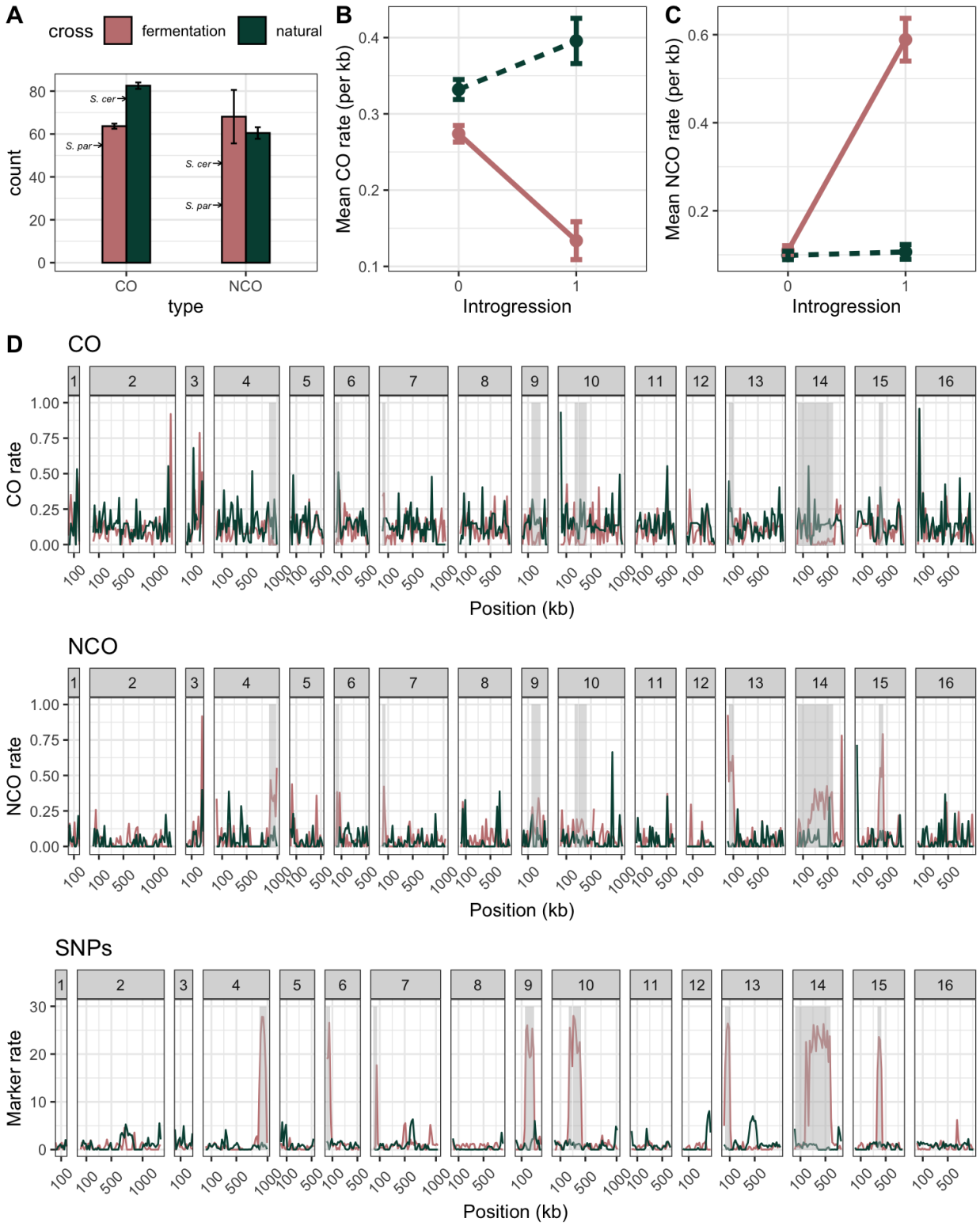


Figure 2: (A) Barplot depicting the number of COs and NCOs detected per meiosis in S. uvarum *crosses (green: natural cross; pink: fermentation cross). The error bars represent the standard*

error around the mean. These values are not corrected by resolution. The counts for S. paradoxus *and* S. cerevisiae *are represented by arrows and taken from Liu et al. (2019). (B) Mean CO/kb and (C) NCO/kb by cross and introgression (0 denotes intervals without introgression; 1 denotes introgression present in the fermentation cross. While the natural cross does not contain introgression, the region where introgression is present in the fermentation cross was compared to its syntenic region in the natural cross). Error bars represent the standard error around the mean. (D)* S. uvarum *chromosomes split into 20kb, non-overlapping windows. CO, NCO, and SNP counts are reported for both crosses (fermentation and natural). Shaded regions denote introgressed regions. CO counts are smoothed when the true location of the CO split could be in one of multiple windows. NCO counts are corrected for marker resolution.*

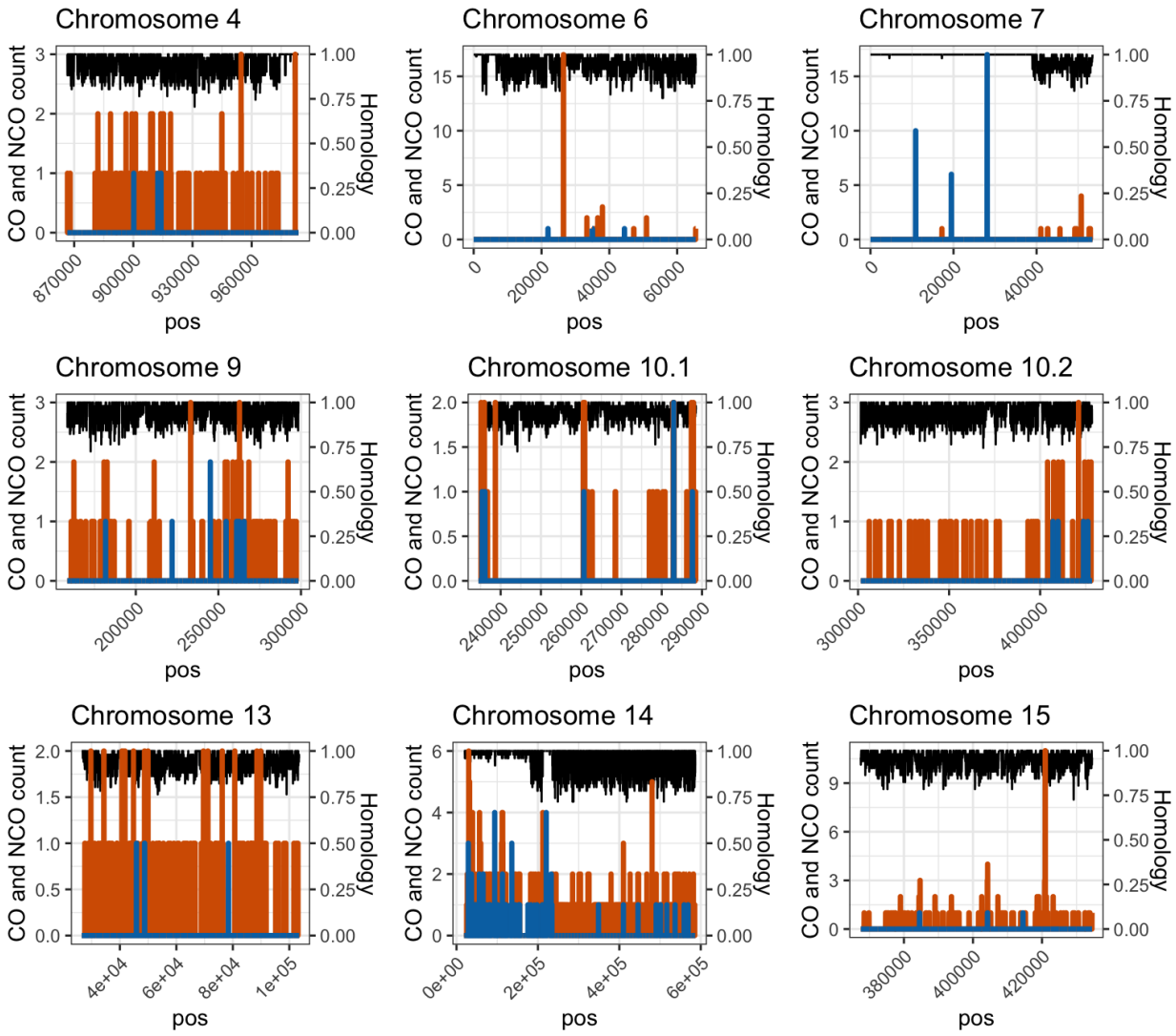
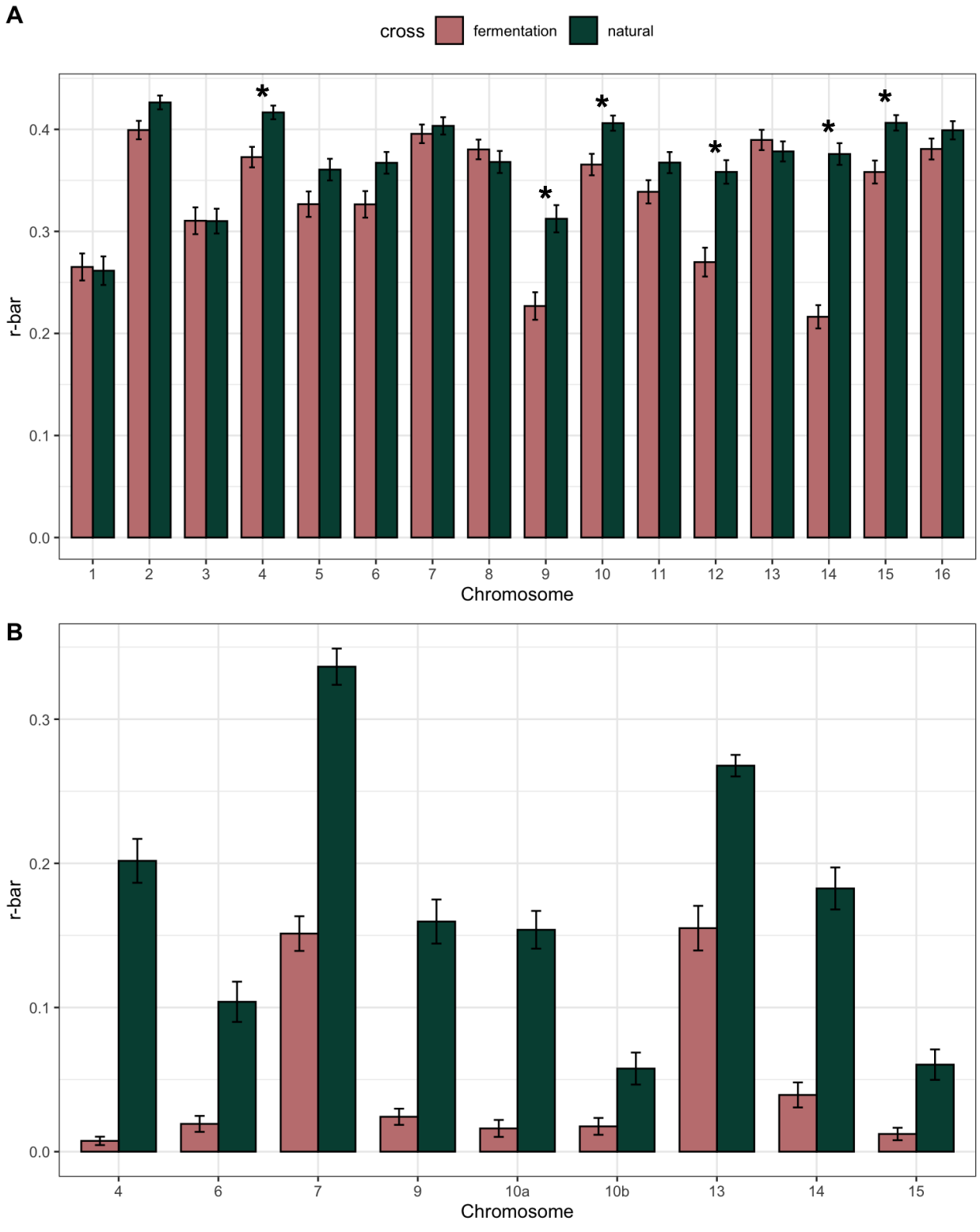


Figure 3: CO, NCO, and homology in 101bp sliding windows of fermentation cross introgressed regions. CO counts are shown in blue, the depth of NCO tracts are shown in orange, and the proportion of expected homologous bases between the two fermentation strains is shown in black.



553

Figure 4: Average $\underline{r}$ for each chromosome (A) and for each introgressed region (B). Asterisks indicate a significant difference in chromosome r-bar between crosses. All introgressed regions had a significant difference in $\underline{r}$. Error bars indicate standard error around the mean.

Table 1: Spearman's correlations of NCOs to homology in introgressed regions of the fermentation cross.

Chromosome	Start	End	Corr	p<0.001?
4	866500	983774	-0.1562	TRUE
6	1	65500	-0.0409	FALSE
7	1	53500	-0.2102	TRUE
9	158500	298500	-0.1313	TRUE
10	234500	288500	-0.0236	FALSE
10	301500	428500	-0.0990	TRUE
13	26500	103500	-0.1943	TRUE
14	18500	586500	-0.2004	TRUE
15	367500	434500	-0.2076	TRUE

Table 2: Welch two sample t-test results for differences in homology between CO-adjacent regions and NCO-adjacent regions per introgression.

Introgression	CO mean homology	CO SE	NCO mean homology	NCO SE	p-value
Chromosome 4	0.9467	0.0148	0.9248	0.0028	0.2760
Chromosome 6	0.9663	0.0203	0.9784	0.0017	0.5360
Chromosome 7	1.0000	0.0000	0.9838	0.0013	<0.0001
Chromosome 9	0.9650	0.0102	0.9312	0.0036	0.0109
Chromosome 10 (1)	0.9671	0.0092	0.9476	0.0058	0.1008
Chromosome 10 (2)	0.9400	0.0074	0.9276	0.0040	0.1991
Chromosome 13	0.9700	0.0161	0.9304	0.0024	0.1296
Chromosome 14	0.9893	0.0026	0.9415	0.0018	<0.0001
Chromosome 15	0.9317	0.0093	0.9252	0.0029	0.5648

Table 3: Coefficients of gaussian generalized linear model modeling CO counts per 20kb window.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-8.9355	1.4713	-6.0732	1.70e-09
Introgression	-3.6343	0.6293	-5.7755	9.84e-09
GC	37.9733	3.7037	10.2528	< 2e-16
Introgression:crossnatural	5.2378	0.8595	6.0941	1.49e-09

Table 4: Coefficients of gaussian generalized linear model modeling NCO counts per 20kb window.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-1.2261	1.4013	-0.8749	0.3818
Introgression	9.6354	0.5993	16.0770	<2e-16
GC	8.3792	3.5275	2.3754	0.0177
Introgression:crossnatural	-9.6414	0.8186	-11.7780	<2e-16

References

Aguillon, S. M., Dodge, T. O., Preising, G. A., & Schumer, M. (2022). Introgression. *Current Biology*, *32*(16), R865–R868. https://doi.org/10.1016/j.cub.2022.07.004

Albertin, W., Chernova, M., Durrens, P., Guichoux, E., Sherman, D. J., Masneuf-Pomarede, I., & Marullo, P. (2018). Many interspecific chromosomal introgressions are highly prevalent in Holarctic Saccharomyces uvarum strains found in human-related fermentations. *Yeast*, *35*(1), 141–156. https://doi.org/10.1002/yea.3248

Almeida, P., Gonçalves, C., Teixeira, S., Libkind, D., Bontrager, M., Masneuf-Pomarède, I., Albertin, W., Durrens, P., Sherman, D. J., Marullo, P., Todd Hittinger, C., Gonçalves, P., & Sampaio, J. P. (2014). A Gondwanan imprint on global diversity and domestication of wine and cider yeast *Saccharomyces uvarum*. *Nature Communications*, *5*, 4044. https://doi.org/10.1038/ncomms5044

Anderson, C. M., Chen, S. Y., Dimon, M. T., Oke, A., DeRisi, J. L., & Fung, J. C. (2011). ReCombine: A Suite of Programs for Detection and Analysis of Meiotic Recombination in Whole-Genome Datasets. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0025509

Arter, M., & Keeney, S. (2023). Divergence and conservation of the meiotic recombination machinery. *Nature Reviews Genetics*, 1–17. https://doi.org/10.1038/s41576-023-00669-8

Auton, A., Li, Y. R., Kidd, J., Oliveira, K., Nadel, J., Holloway, J. K., Hayward, J. J., Cohen, P. E., Greally, J. M., Wang, J., Bustamante, C. D., & Boyko, A. R. (2013). Genetic Recombination Is Targeted towards Gene Promoter Regions in Dogs. *PLOS Genetics*, *9*(12), e1003984. https://doi.org/10.1371/journal.pgen.1003984

Barton, N., & Bengtsson, B. O. (1986). The barrier to genetic exchange between hybridising populations. *Heredity*, *57*(3), Article 3. https://doi.org/10.1038/hdy.1986.135

618 Begun, D. J., & Aquadro, C. F. (1992). Levels of naturally occurring DNA polymorphism
619 correlate with recombination rates in D. melanogaster. *Nature*, *356*(6369), 519–520.
620 https://doi.org/10.1038/356519a0
621 Bendixsen, D. P., Frazão, J. G., & Stelkens, R. (2022). Saccharomyces yeast hybrids on the
622 rise. *Yeast*, *39*(1–2), 40–54. https://doi.org/10.1002/yea.3684
623 Bozdag, G. O., Ono, J., Denton, J. A., Karakoc, E., Hunter, N., Leu, J.-Y., & Greig, D. (2021).
624 Breaking a species barrier by enabling hybrid recombination. *Current Biology*, *31*(4),
625 R180–R181. https://doi.org/10.1016/j.cub.2020.12.038
626 Brand, C. L., Cattani, M. V., Kingan, S. B., Landeen, E. L., & Presgraves, D. C. (2018).
627 Molecular Evolution at a Meiosis Gene Mediates Species Differences in the Rate and
628 Patterning of Recombination. *Current Biology: CB*, *28*(8), 1289-1295.e4.
629 https://doi.org/10.1016/j.cub.2018.02.056
630 Brandvain, Y., Kenney, A. M., Flagel, L., Coop, G., & Sweigart, A. L. (2014). Speciation and
631 Introgression between Mimulus nasutus and Mimulus guttatus. *PLOS Genetics*, *10*(6),
632 e1004410. https://doi.org/10.1371/journal.pgen.1004410
633 Brion, C., Legrand, S., Peter, J., Caradec, C., Pflieger, D., Hou, J., Friedrich, A., Llorente, B., &
634 Schacherer, J. (2017). Variation of the meiotic recombination landscape and properties
635 over a broad evolutionary distance in yeasts. *PLOS Genetics*, *13*(8), e1006917.
636 https://doi.org/10.1371/journal.pgen.1006917
637 Butlin, R. K. (2005). Recombination and speciation. *Molecular Ecology*, *14*(9), 2621–2635.
638 https://doi.org/10.1111/j.1365-294X.2005.02617.x
639 Calfee, E., Gates, D., Lorant, A., Perkins, M. T., Coop, G., & Ross-Ibarra, J. (2021). Selective
640 sorting of ancestral introgression in maize and teosinte along an elevational cline.
641 *bioRxiv*, 2021.03.05.434040. https://doi.org/10.1101/2021.03.05.434040
642 Charlesworth, B., Morgan, M. T., & Charlesworth, D. (1993). The effect of deleterious mutations
643 on neutral molecular variation. *Genetics*, *134*(4), 1289–1303.

https://doi.org/10.1093/genetics/134.4.1289

Chen, W., & Jinks-Robertson, S. (1999). The role of the mismatch repair machinery in regulating mitotic and meiotic recombination between diverged sequences in yeast. *Genetics*, *151*(4), 1299–1313.

Choi, K., Zhao, X., Tock, A. J., Lambing, C., Underwood, C. J., Hardcastle, T. J., Serra, H., Kim, J., Cho, H. S., Kim, J., Ziolkowski, P. A., Yelina, N. E., Hwang, I., Martienssen, R. A., & Henderson, I. R. (2018). Nucleosomes and DNA methylation shape meiotic DSB frequency in Arabidopsis thaliana transposons and gene regulatory regions. *Genome Research*, *28*(4), 532–546. https://doi.org/10.1101/gr.225599.117

Chovnick, A., Ballantyne, G. H., & Holm, D. G. (1971). Studies on gene conversion and its relationship to linked exchange in Drosophila melanogaster. *Genetics*, *69*(2), 179–209. https://doi.org/10.1093/genetics/69.2.179

Cooper, T. J., Crawford, M. R., Hunt, L. J., Marsolier-Kergoat, M.-C., Llorente, B., & Neale, M. J. (2021). *Mismatch repair disturbs meiotic class I crossover control* (p. 480418). bioRxiv. https://doi.org/10.1101/480418

Crown, K. N., Miller, D. E., Sekelsky, J., & Hawley, R. S. (2018). Local Inversion Heterozygosity Alters Recombination throughout the Genome. *Current Biology: CB*, *28*(18), 2984-2990.e3. https://doi.org/10.1016/j.cub.2018.07.004

Cubillos, F. A., Billi, E., Zörgö, E., Parts, L., Fargier, P., Omholt, S., Blomberg, A., Warringer, J., Louis, E. J., & Liti, G. (2011). Assessing the complex architecture of polygenic traits in diverged yeast populations. *Molecular Ecology*, *20*(7), 1401–1413. https://doi.org/10.1111/j.1365-294X.2011.05005.x

Dagilis, A. J., & Matute, D. R. (2023). The fitness of an introgressing haplotype changes over the course of divergence and depends on its size and genomic location. *PLOS Biology*, *21*(7), e3002185. https://doi.org/10.1371/journal.pbio.3002185

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A.,

670 Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools
671 and BCFtools. *GigaScience*, *10*(2), giab008. https://doi.org/10.1093/gigascience/giab008
672 D'Angiolo, M., De Chiara, M., Yue, J.-X., Irizar, A., Stenberg, S., Persson, K., Llored, A., Barré,
673 B., Schacherer, J., Marangoni, R., Gilson, E., Warringer, J., & Liti, G. (2020). A yeast
674 living ancestor reveals the origin of genomic introgressions. *Nature*, *587*(7834), Article
675 7834. https://doi.org/10.1038/s41586-020-2889-1
676 Dapper, A. L., & Payseur, B. A. (2019). Molecular evolution of the meiotic recombination
677 pathway in mammals. *Evolution*, *73*(12). https://doi.org/10.1111/evo.13850
678 Datta, A., Hendrix, M., Lipsitch, M., & Jinks-Robertson, S. (1997). Dual roles for DNA sequence
679 identity and the mismatch repair system in the regulation of mitotic crossing-over
680 in yeast. *Proceedings of the National Academy of Sciences of the United States of*
681 *America*, *94*(18), 9757–9762.
682 de Massy, B. (2013). Initiation of Meiotic Recombination: How and Where? Conservation and
683 Specificities Among Eukaryotes. *Annual Review of Genetics*, *47*(1), 563–599.
684 https://doi.org/10.1146/annurev-genet-110711-155423
685 Duranton, M., & Pool, J. E. (2022). Interactions Between Natural Selection and Recombination
686 Shape the Genomic Landscape of Introgression. *Molecular Biology and Evolution*, *39*(7),
687 msac122. https://doi.org/10.1093/molbev/msac122
688 Edelman, N. B., Frandsen, P. B., Miyagi, M., Clavijo, B., Davey, J., Dikow, R. B., García-
689 Accinelli, G., Van Belleghem, S. M., Patterson, N., Neafsey, D. E., Challis, R., Kumar,
690 S., Moreira, G. R. P., Salazar, C., Chouteau, M., Counterman, B. A., Papa, R., Blaxter,
691 M., Reed, R. D., … Mallet, J. (2019). Genomic architecture and introgression shape a
692 butterfly radiation. *Science*, *366*(6465), 594–599.
693 https://doi.org/10.1126/science.aaw2090
694 Felsenstein, J. (1974). The evolutionary advantage of recombination. *Genetics*, *78*(2), 737–756.
695 Gerton, J. L., DeRisi, J., Shroff, R., Lichten, M., Brown, P. O., & Petes, T. D. (2000). Global

696 mapping of meiotic recombination hotspots and coldspots in the yeast Saccharomyces
697 cerevisiae. *Proceedings of the National Academy of Sciences*, *97*(21), 11383–11390.
698 https://doi.org/10.1073/pnas.97.21.11383
699 Harfe, B. D., & Jinks-Robertson, S. (2000). Dna Mismatch Repair and Genetic Instability.
700 *Annual Review of Genetics*, *34*(1), 359–399.
701 https://doi.org/10.1146/annurev.genet.34.1.359
702 He, Y., Wang, M., Dukowic-Schulze, S., Zhou, A., Tiang, C.-L., Shilo, S., Sidhu, G. K., Eichten,
703 S., Bradbury, P., Springer, N. M., Buckler, E. S., Levy, A. A., Sun, Q., Pillardy, J.,
704 Kianian, P. M. A., Kianian, S. F., Chen, C., & Pawlowski, W. P. (2017). Genomic
705 features shaping the landscape of meiotic double-strand-break hotspots in maize.
706 *Proceedings of the National Academy of Sciences*, *114*(46), 12231–12236.
707 https://doi.org/10.1073/pnas.1713225114
708 Hill, W. G., & Robertson, A. (1966). The effect of linkage on limits to artificial selection.
709 *Genetical Research*, *8*(3), 269–294.
710 Hilliker, A. J., Harauz, G., Reaume, A. G., Gray, M., Clark, S. H., & Chovnick, A. (1994). Meiotic
711 gene conversion tract length distribution within the rosy locus of Drosophila
712 melanogaster. *Genetics*, *137*(4), 1019–1026.
713 https://doi.org/10.1093/genetics/137.4.1019
714 Hoffman, C. S., & Winston, F. (1987). A ten-minute DNA preparation from yeast efficiently
715 releases autonomous plasmids for transformation of Escherichia coli. *Gene*, *57*(2–3),
716 267–272. https://doi.org/10.1016/0378-1119(87)90131-4
717 Hunter, N., Chambers, S. R., Louis, E. J., & Borts, R. H. (1996). The mismatch repair system
718 contributes to meiotic sterility in an interspecific yeast hybrid. *The EMBO Journal*, *15*(7),
719 1726–1733. https://doi.org/10.1002/j.1460-2075.1996.tb00518.x
720 Jeffreys, A. J., & May, C. A. (2004). Intense and highly localized gene conversion activity in
721 human meiotic crossover hot spots. *Nature Genetics*, *36*(2), Article 2.

https://doi.org/10.1038/ng1287

Judd, S. R., & Petes, T. D. (1988). Physical Lengths of Meiotic and Mitotic Gene Conversion Tracts in Saccharomyces Cerevisiae. *Genetics*, *118*(3), 401. https://doi.org/10.1093/genetics/118.3.401

Keeney, S. (2001). Mechanism and control of meiotic recombination initiation. *Current Topics in Developmental Biology*, *52*, 1–53. https://doi.org/10.1016/s0070-2153(01)52008-6

Kiktev, D. A., Sheng, Z., Lobachev, K. S., & Petes, T. D. (2018). GC content elevates mutation and recombination rates in the yeast *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*, *115*(30), E7109–E7118. https://doi.org/10.1073/pnas.1807334115

Kong, A., Thorleifsson, G., Stefansson, H., Masson, G., Helgason, A., Gudbjartsson, D. F., Jonsdottir, G. M., Gudjonsson, S. A., Sverrisson, S., Thorlacius, T., Jonasdottir, A., Hardarson, G. A., Palsson, S. T., Frigge, M. L., Gulcher, J. R., Thorsteinsdottir, U., & Stefansson, K. (2008). Sequence Variants in the RNF212 Gene Associate with Genome-Wide Recombination Rate. *Science*, *319*(5868), 1398–1401. https://doi.org/10.1126/science.1152422

Korunes, K. L., & Noor, M. A. F. (2017). Gene conversion and linkage: Effects on genome evolution and speciation. *Molecular Ecology*, *26*(1), 351–364. https://doi.org/10.1111/mec.13736

Korunes, K. L., & Noor, M. A. F. (2019). Pervasive gene conversion in chromosomal inversion heterozygotes. *Molecular Ecology*, *28*(6), 1302–1315. https://doi.org/10.1111/mec.14921

Lam, I., & Keeney, S. (2015). Nonparadoxical evolutionary stability of the recombination initiation landscape in yeast. *Science*, *350*(6263), 932–937. https://doi.org/10.1126/science.aad0814

Langdon, Q. K., Peris, D., Baker, E. P., Opulente, D. A., Nguyen, H.-V., Bond, U., Gonçalves, P., Sampaio, J. P., Libkind, D., & Hittinger, C. T. (2019). Fermentation innovation

through complex hybridization of wild and domesticated yeasts. *Nature Ecology & Evolution*, 1–11. https://doi.org/10.1038/s41559-019-0998-8

Langdon, Q. K., Peris, D., Eizaguirre, J. I., Opulente, D. A., Buh, K. V., Sylvester, K., Jarzyna, M., Rodríguez, M. E., Lopes, C. A., Libkind, D., & Hittinger, C. T. (2020). Postglacial migration shaped the genomic diversity and global distribution of the wild ancestor of lager-brewing hybrids. *PLOS Genetics*, *16*(4), e1008680. https://doi.org/10.1371/journal.pgen.1008680

Layer, R. M., Chiang, C., Quinlan, A. R., & Hall, I. M. (2014). LUMPY: A probabilistic framework for structural variant discovery. *Genome Biology*, *15*(6), R84. https://doi.org/10.1186/gb-2014-15-6-r84

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, L., Jean, M., & Belzile, F. (2006). The impact of sequence divergence and DNA mismatch repair on homeologous recombination in Arabidopsis. *The Plant Journal: For Cell and Molecular Biology*, *45*(6), 908–916. https://doi.org/10.1111/j.1365-313X.2006.02657.x

Liu, H., Huang, J., Sun, X., Li, J., Hu, Y., Yu, L., Liti, G., Tian, D., Hurst, L. D., & Yang, S. (2018). Tetrad analysis in plants and fungi finds large differences in gene conversion rates but no GC bias. *Nature Ecology & Evolution*, *2*(1), Article 1. https://doi.org/10.1038/s41559-017-0372-7

Liu, H., Maclean, C. J., & Zhang, J. (2019). Evolution of the Yeast Recombination Landscape. *Molecular Biology and Evolution*, *36*(2), 412–422. https://doi.org/10.1093/molbev/msy233

Magwene, P. M., Kayıkçı, Ö., Granek, J. A., Reininga, J. M., Scholl, Z., & Murray, D. (2011). Outcrossing, mitotic recombination, and life-history trade-offs shape genome evolution in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences*, *108*(5),

1987–1992. https://doi.org/10.1073/pnas.1012544108

Mancera, E., Bourgon, R., Brozzi, A., Huber, W., & Steinmetz, L. M. (2008). High-resolution mapping of meiotic crossovers and non-crossovers in yeast. *Nature*, *454*(7203), 479–485. https://doi.org/10.1038/nature07135

Marsolier-Kergoat, M.-C., & Yeramian, E. (2009). GC Content and Recombination: Reassessing the Causal Effects for the Saccharomyces cerevisiae Genome. *Genetics*, *183*(1), 31–38. https://doi.org/10.1534/genetics.109.105049

Martin, S. H., Davey, J. W., Salazar, C., & Jiggins, C. D. (2019). Recombination rate variation shapes barriers to introgression across butterfly genomes. *PLOS Biology*, *17*(2), e2006288. https://doi.org/10.1371/journal.pbio.2006288

McDonald, M. J., Rice, D. P., & Desai, M. M. (2016). Sex speeds adaptation by altering the dynamics of molecular evolution. *Nature*, *531*(7593), 233–236. https://doi.org/10.1038/nature17143

McGaugh, S. E., Heil, C. S. S., Manzano-Winkler, B., Loewe, L., Goldstein, S., Himmel, T. L., & Noor, M. A. F. (2012). Recombination Modulates How Selection Affects Linked Sites in Drosophila. *PLOS Biology*, *10*(11), e1001422. https://doi.org/10.1371/journal.pbio.1001422

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

Miller, D. E., Smith, C. B., Kazemi, N. Y., Cockrell, A. J., Arvanitakis, A. V., Blumenstiel, J. P., Jaspersen, S. L., & Hawley, R. S. (2016). Whole-Genome Analysis of Individual Meiotic Events in Drosophila melanogaster Reveals That Noncrossover Gene Conversions Are Insensitive to Interference and the Centromere Effect. *Genetics*, *203*(1), 159–171. https://doi.org/10.1534/genetics.115.186486

Moran, B. M., Payne, C., Langdon, Q., Powell, D. L., Brandvain, Y., & Schumer, M. (2021). The genomic consequences of hybridization. *eLife*, *10*, e69016. https://doi.org/10.7554/eLife.69016

Murdoch, B., Owen, N., Shirley, S., Crumb, S., Broman, K. W., & Hassold, T. (2010). Multiple loci contribute to genome-wide recombination levels in male mice. *Mammalian Genome*, *21*(11), 550–555. https://doi.org/10.1007/s00335-010-9303-5

Nachman Michael W. & Payseur Bret A. (2012). Recombination rate variation and speciation: Theoretical predictions and empirical results from rabbits and mice. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1587), 409–421. https://doi.org/10.1098/rstb.2011.0249

Nespolo, R. F., Villarroel, C. A., Oporto, C. I., Tapia, S. M., Vega-Macaya, F., Urbina, K., Chiara, M. D., Mozzachiodi, S., Mikhalev, E., Thompson, D., Larrondo, L. F., Saenz-Agudelo, P., Liti, G., & Cubillos, F. A. (2020). An Out-of-Patagonia migration explains the worldwide diversity and distribution of Saccharomyces eubayanus lineages. *PLOS Genetics*, *16*(5), e1008777. https://doi.org/10.1371/journal.pgen.1008777

Pan, J., Sasaki, M., Kniewel, R., Murakami, H., Blitzblau, H. G., Tischfield, S. E., Zhu, X., Neale, M. J., Jasin, M., Socci, N. D., Hochwagen, A., & Keeney, S. (2011). A Hierarchical Combination of Factors Shapes the Genome-wide Topography of Yeast Meiotic Recombination Initiation. *Cell*, *144*(5), 719–731. https://doi.org/10.1016/j.cell.2011.02.009

Pool, J. E. (2015). The Mosaic Ancestry of the Drosophila Genetic Reference Panel and the D. melanogaster Reference Genome Reveals a Network of Epistatic Fitness Interactions. *Molecular Biology and Evolution*, *32*(12), 3236–3251. https://doi.org/10.1093/molbev/msv194

Raffoux, X., Bourge, M., Dumas, F., Martin, O. C., & Falque, M. (2018). Role of Cis, Trans, and Inbreeding Effects on Meiotic Recombination in Saccharomyces cerevisiae. *Genetics*,

826 *210*(4), 1213–1226. https://doi.org/10.1534/genetics.118.301644

827 Ravinet, M., Yoshida, K., Shigenobu, S., Toyoda, A., Fujiyama, A., & Kitano, J. (2018). The
828 genomic landscape at a late stage of stickleback speciation: High genomic divergence
829 interspersed by small localized regions of introgression. *PLOS Genetics*, *14*(5),
830 e1007358. https://doi.org/10.1371/journal.pgen.1007358

831 Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., &
832 Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26.
833 https://doi.org/10.1038/nbt.1754

834 Rockman, M. V., & Kruglyak, L. (2009). Recombinational Landscape and Population Genomics
835 of Caenorhabditis elegans. *PLOS Genetics*, *5*(3), e1000419.
836 https://doi.org/10.1371/journal.pgen.1000419

837 Rogers, D. W., McConnell, E., Ono, J., & Greig, D. (2018). Spore-autonomous fluorescent
838 protein expression identifies meiotic chromosome mis-segregation as the principal cause
839 of hybrid sterility in yeast. *PLOS Biology*, *16*(11), e2005066.
840 https://doi.org/10.1371/journal.pbio.2005066

841 Ruderfer, D. M., Pratt, S. C., Seidel, H. S., & Kruglyak, L. (2006). Population genomic analysis
842 of outcrossing and recombination in yeast. *Nature Genetics*, *38*(9), Article 9.
843 https://doi.org/10.1038/ng1859

844 Scannell, D. R., Zill, O. A., Rokas, A., Payen, C., Dunham, M. J., Eisen, M. B., Rine, J.,
845 Johnston, M., & Hittinger, C. T. (2011). The Awesome Power of Yeast Evolutionary
846 Genetics: New Genome Sequences and Strain Resources for the Saccharomyces sensu
847 stricto Genus. *G3: Genes, Genomes, Genetics*, *1*(1), 11–25.
848 https://doi.org/10.1534/g3.111.000273

849 Schaeffer, S. W., & Anderson, W. W. (2005). Mechanisms of Genetic Exchange Within the
850 Chromosomal Inversions of Drosophila pseudoobscura. *Genetics*, *171*(4), 1729–1739.
851 https://doi.org/10.1534/genetics.105.041947

852 Schumer, M., Xu, C., Powell, D. L., Durvasula, A., Skov, L., Holland, C., Blazier, J. C.,
853 Sankararaman, S., Andolfatto, P., Rosenthal, G. G., & Przeworski, M. (2018). Natural
854 selection interacts with recombination to shape the evolution of hybrid genomes.
855 *Science*, *360*(6389), 656–660. https://doi.org/10.1126/science.aar3684
856 Shi, J., Wolf, S. E., Burke, J. M., Presting, G. G., Ross-Ibarra, J., & Dawe, R. K. (2010).
857 Widespread Gene Conversion in Centromere Cores. *PLoS Biology*, *8*(3), e1000327.
858 https://doi.org/10.1371/journal.pbio.1000327
859 Singhal, S., Leffler, E. M., Sannareddy, K., Turner, I., Venn, O., Hooper, D. M., Strand, A. I., Li,
860 Q., Raney, B., Balakrishnan, C. N., Griffith, S. C., McVean, G., & Przeworski, M. (2015).
861 Stable recombination hotspots in birds. *Science (New York, N.Y.)*, *350*(6263), 928–932.
862 https://doi.org/10.1126/science.aad0843
863 Smith, J. M., & Haigh, J. (1974). The hitch-hiking effect of a favourable gene. *Genetical*
864 *Research*, *23*(1), 23–35.
865 Smukowski, C. S., & Noor, M. a. F. (2011). Recombination rate variation in closely related
866 species. *Heredity*, *107*(6), 496–508. https://doi.org/10.1038/hdy.2011.44
867 Smukowski Heil, C. S., Ellison, C., Dubin, M., & Noor, M. A. F. (2015). Recombining without
868 Hotspots: A Comprehensive Evolutionary Portrait of Recombination in Two Closely
869 Related Species of Drosophila. *Genome Biology and Evolution*, *7*(10), 2829–2842.
870 https://doi.org/10.1093/gbe/evv182
871 Stapley, J., Feulner, P. G. D., Johnston, S. E., Santure, A. W., & Smadja, C. M. (2017).
872 Variation in recombination frequency and distribution across eukaryotes: Patterns and
873 processes. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
874 *372*(1736), 20160455. https://doi.org/10.1098/rstb.2016.0455
875 Stelkens, R., & Bendixsen, D. P. (2022). The evolutionary and ecological potential of yeast
876 hybrids. *Current Opinion in Genetics & Development*, *76*, 101958.
877 https://doi.org/10.1016/j.gde.2022.101958

878 Talbert, P. B., & Henikoff, S. (2010). Centromeres Convert but Don't Cross. *PLOS Biology*, *8*(3),
879 e1000326. https://doi.org/10.1371/journal.pbio.1000326
880 Tellini, N., De Chiara, M., Mozzachiodi, S., Tattini, L., Vischioni, C., Naumova, E., Warringer, J.,
881 Bergström, A., & Liti, G. (2023). *Ancient and recent origins of shared polymorphisms in*
882 *yeas*. https://doi.org/10.21203/rs.3.rs-2573222/v1
883 Tsai, I. J., Burt, A., & Koufopanou, V. (2010). Conservation of recombination hotspots in yeast.
884 *Proceedings of the National Academy of Sciences*.
885 https://doi.org/10.1073/pnas.0908774107
886 Veller, C., Edelman, N. B., Muralidhar, P., & Nowak, M. A. (2023). Recombination and selection
887 against introgressed DNA. *Evolution*, *77*(4), 1131–1144.
888 https://doi.org/10.1093/evolut/qpad021
889 Veller, C., Kleckner, N., & Nowak, M. A. (2019). A rigorous measure of genome-wide genetic
890 shuffling that takes into account crossover positions and Mendel's second law.
891 *Proceedings of the National Academy of Sciences of the United States of America*,
892 *116*(5), 1659–1668. https://doi.org/10.1073/pnas.1817482116
893 Welz-Voegele, C., & Jinks-Robertson, S. (2008). Sequence Divergence Impedes Crossover
894 More Than Noncrossover Events During Mitotic Gap Repair in Yeast. *Genetics*, *179*(3),
895 1251–1262. https://doi.org/10.1534/genetics.108.090233
896 Wijnker, E., Velikkakam James, G., Ding, J., Becker, F., Klasen, J. R., Rawat, V., Rowan, B. A.,
897 de Jong, D. F., de Snoo, C. B., Zapata, L., Huettel, B., de Jong, H., Ossowski, S.,
898 Weigel, D., Koornneef, M., Keurentjes, J. J., & Schneeberger, K. (2013). The genomic
899 landscape of meiotic crossovers and gene conversions in Arabidopsis thaliana. *eLife*, *2*,
900 e01426. https://doi.org/10.7554/eLife.01426
901 Yang, F., Silber, S., Leu, N. A., Oates, R. D., Marszalek, J. D., Skaletsky, H., Brown, L. G.,
902 Rozen, S., Page, D. C., & Wang, P. J. (2015). TEX11 is mutated in infertile men with
903 azoospermia and regulates genome-wide recombination rates in mouse. *EMBO*

Molecular Medicine, *7*(9), 1198–1210. https://doi.org/10.15252/emmm.201404967

Zelkowski, M., Olson, M. A., Wang, M., & Pawlowski, W. (2019). Diversity and Determinants of Meiotic Recombination Landscapes. *Trends in Genetics*, *35*(5), 359–370. https://doi.org/10.1016/j.tig.2019.02.002

Zeyl, C. W., & Otto, S. P. (2007). A short history of recombination in yeast. *Trends in Ecology & Evolution*, *22*(5), 223–225. https://doi.org/10.1016/j.tree.2007.02.005