Mismatch repair impedes meiotic crossover interference

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Summary

Sequence divergence, mediated by the anti-recombinogenic activity of mismatch repair (MMR), forms a potent barrier to meiotic recombination and in turn the formation of viable gametes¹⁻⁵. However, exactly how MMR jeopardizes meiotic success is unclear. Here we utilize a combination of *S. cerevisiae* genetics, genome-wide mapping of recombination and computational modelling to demonstrate that MMR unexpectedly influences the global distribution of recombination through preferential suppression of interfering crossovers (COs) at regions of greater sequence divergence. As a result, inactivation of MMR not only increases the rate of recombination, as previously observed, but also, paradoxically, the strength of CO interference. Our observations reveal a new mechanism by which MMR spatially sculpts the meiotic landscape—linking CO control to the mechanisms that can reproductively isolate a population, and highlighting how genomes may become meiotically incompatible at the molecular level, dependent upon interactions of the primary DNA sequence.

Introduction & Results

Meiosis, a specialized two step nuclear division, is responsible for the generation of genetically diverse, haploid gametes. An integral feature of the meiotic program is the initiation of homologous recombination and the subsequent formation of reciprocal, interhomologue exchanges known as crossovers (COs) that are essential for faithful disjunction of meiotic chromosomes during anaphase I (reviewed in^{6,7}). Failure to form at least one CO per homologue pair risks the formation of aneuploid gametes and thus the process of CO formation is highly regulated. Within many organisms, including *S. cerevisiae*, *M. musculus*, *H. sapiens* and *A. thaliana*, two subclasses of CO co-exist. Interfering ZMM (Zip2-Zip3-Zip4-Spo16, Msh4-Msh5, Mer3)-dependent class I COs, which account for

the majority of COs formed (~70-85% within *S. cerevisiae*), are dispersed evenly along each chromosome by means of CO interference—a process that suppresses the formation of COs in proximity to one another. A subpopulation of recombination events alternatively resolve as non-interfering class II COs dependent upon Mus81-Mms4, Yen1 or Slx1-Slx4. Homologous recombination, the process responsible for CO formation, requires a repair template with near-perfect homology⁸. By contrast, recombination between polymorphic, homoeologous substrates is markedly inefficient, leading to reduced rates of meiotic CO formation, reduced spore viability and increased chromosomal non-disjunction during meiosis I within hybrid strains of *S. cerevisiae*—phenotypes linked to incipient speciation and which are largely reversed within MMR-deficient strains¹⁻⁴. Despite characterization of this anti-recombinogenic activity, a detailed analysis of how MMR alters meiotic recombination on a genome-wide level has not yet been achieved.

In order to investigate the impact sequence divergence has upon CO formation, we mapped recombination (Fig. 1a, Methods) within six wild type and 13 MMR-defective *msh2Δ* meioses—obtained from a cross of two widely utilized laboratory isolates: S288c and SK1^{3,9} (~65,000 SNPs, ~4,000 high confidence INDELs, ~0.57% divergence). Additionally, we re-analyzed datasets comprising 51 wild type^{10,11} and four *msh2Δ* tetrads¹² from a S96 x YJM789 cross of *S. cerevisiae* (~0.6% divergence). On average, we identified 74.3 ±5.4 and 105.9 ±7.8 COs per meiosis within our SK1 x S288c wild type and *msh2Δ* samples respectively, corresponding to a significant 1.4-fold increase in CO frequency ($p < 0.01$; Two-sample T-test) (Fig. 1b). A significant *msh2Δ*-dependent increase (~1.25-fold, $p < 0.01$; Two-sample T-test) is also observed within S96 x YJM789 (Fig. 1b)—collectively reaffirming the known anti-recombinogenic activity of Msh2¹⁻⁵. Notably, CO frequencies are considerably higher within S96 x YJM789 than S288c x SK1 (91.4 vs. 74.3 COs per wild type meiosis)—suggesting that cross-specific differences may exist.

To investigate any possible effect of Msh2 on CO patterning, we calculated the distribution of inter-crossover distances (ICDs)—the separation (in bp) between successive COs along every chromosome. To accommodate comparisons between different sample sizes, ICDs are transformed (Methods and Supplementary Fig. 1) and visualized in rank order as empirical cumulative distribution functions (eCDFs). CO interference—observed as a statistically significant ($p < 0.01$; Two-sample Kolmogorov-Smirnov (KS) test) right-ward skew away from conditions of simulated independence—is present within all mapped strains (Fig. 1c–f) and is stronger within S96 x YJM789 than within S288c x SK1, further suggesting that the meiotic CO landscape is regulated in a cross-specific manner

(Supplementary Fig. 2 and Supplementary Discussion). Unexpectedly, inactivation of Msh2 within both hybrid crosses (Fig. 1c–d) further skews ICD curves to the right ($p < 0.01$; Two-sample KS test)—indicating increased CO interference. Mutation of a second MMR factor, *PMS1*, which acts downstream of Msh2 mismatch binding, caused similar increases in both CO frequency and CO interference relative to wild type (Supplementary Fig. 3a–b), but no additive effect in a double *msh2Δpms1Δ* strain was observed—suggesting that these phenotypes arise as a general consequence of MMR inactivation.

To account for any impact increased CO frequency may have upon CO distribution, we utilized an *ndt80AR* strain where meiotic prophase length is extended via temporary repression of the Ndt80 transcription factor^{13,14}. On average, we identified 94.5 ± 16.3 COs per meiosis within *ndt80AR*—a significant increase relative to wild type ($p < 0.01$; Two-sample T-test) (Fig. 1b). However, no further increase occurs upon inactivation of Msh2 (*msh2Δndt80AR*, 97.5 ± 15.4 COs, $p = 0.87$; Two-sample T-test) (Fig. 1b). Importantly, despite the lack of change in CO frequency, the *msh2Δ*-dependent skew in CO distribution was still observed (Fig. 1e) whereas increased CO frequency alone (*ndt80AR*) does not alter CO distribution compared to wild type (Fig. 1f) ($p = 0.91$; Two-sample KS test). Inactivation of MMR within hybrid *S. cerevisiae* strains therefore gives rise to two distinct phenotypes relative to wild type: (i) increased CO frequency, as previously observed^{1–4} and (ii) a novel, paradoxical increase in CO interference—that can arise independently of changes to CO frequency.

A global increase in CO interference, as observed within *msh2Δ*, may arise when the balance between class I and class II CO formation is altered. CO distributions are typically modelled by the gamma (γ) distribution^{15,16}, where $\gamma(\alpha)$ values > 1.0 indicate increasing strength of interference. However, we note that models based on a single (γ) distribution deviate substantially from experimental data, which contains significantly more ICDs < 50 kb than expected—indicative of a randomly distributed subpopulation of COs (i.e. class II) (Fig. 2a). To solve and investigate this problem, we developed computational methods based on (γ)-mixture modelling (Fig. 2b), to statistically deconvolute ICD data—obtaining estimates of the class I:class II ratio (Methods and Supplementary Fig. 4). A non-random ($\gamma(\alpha) > 1.5$), and a random ($\gamma(\alpha) \sim 1.0$) component was successfully identified for all genotypes. Importantly, simulations based on the obtained ratios improve model fit and eliminate the observed ICD deviations below 50 kb (Fig. 2c). Consistent with prior estimates¹⁷, *MSH2* wild type class I:class II ratios are estimated at ~ 2.0 and ~ 3.0 within S288c x SK1 and S96 x YJM789 respectively (Fig. 2d). By contrast, deletion of *MSH2* increases ratio estimates to ~ 5 – 6 (Fig. 2d), suggesting a ~ 1.7 -fold increase in class I CO formation in the

absence of Msh2 (Fig. 2e). Corresponding *msh2Δ*-dependent decreases in class II CO formation are also predicted (~0.5-0.7-fold) (Fig. 2f). Our modelling results therefore suggest that the formation of ZMM-dependent class I COs is preferentially favored within Msh2-deficient cells and that the vast majority of additional COs observed within *msh2Δ* relative to wild type are class I.

Zip3 foci specifically mark the sites of interfering, class I COs¹⁸. In order to verify the results of our modeling, we counted Zip3 foci within spread, pachytene-arrested hybrid cells (Fig. 3a–b). On average, we identified 34.1–34.7 and 51.7–58.7 foci per meiosis (7–8h timepoints) within *ndt80AR* and *msh2Δndt80AR* respectively, corresponding to a significant 1.5-fold increase in class I CO formation upon inactivation of MMR ($p < 0.01$; Two-sample T-test). Such an increase in Zip3 foci count is in agreement with the predictions of our model—strongly implicating Msh2 as a regulator of the class I: class II decision within *S. cerevisiae*.

MMR machinery forms a potent barrier to homoeologous recombination^{1–5}. To understand the interplay between DNA mismatches and CO formation, we calculated polymorphism densities (SNPs, INDELs) ± 500 bp around every mapped CO (Fig. 4a) and compared between genotypes. To generate a comparative reference, the expected environment for meiotic recombination, as defined by the polymorphism density surrounding ~3600 recombination hotspot midpoints¹⁹, was also calculated. Polymorphism density surrounding COs within *MSH2* wild type strains (S288c x SK1: wild type, *ndt80AR*) is significantly different to expected in both distribution ($p < 0.01$; Two-sample KS test) and in mean variant density (5.32 vs. 6.18, $p < 0.01$; Two-sample T-test)—characterized by a skew towards COs arising within regions of lower genetic divergence (Fig. 4b). By contrast, *msh2Δ* COs display statistical similarity to expected ($p > 0.25$; Two-sample KS-test) (6.26 vs. 6.18, $p = 0.52$; Two-sample T-test) (Fig. 4b), and such a disparity between wild type and *msh2Δ* is recaptured within the independent S96 x YJM789 cross (Fig. 4c). A similar pattern was observed when considering polymorphism density arising ± 1000 bp around each CO but was diminished with increasing distance (± 2000 bp)—revealing DNA mismatches to exert a local effect on CO formation (Supplementary Fig. 5a–b and Supplementary Discussion).

Inactivation of *MSH2* increases CO interference by altering the class I: class II balance (Fig. 2, Fig. 3). To determine whether the role that Msh2 plays in regulating CO outcome is related to its role in suppressing COs at sites where mismatches will arise during strand invasion, we further calculated polymorphism densities within mutants that disrupt class I (*zip3Δ*, *msh4Δ*) or class II (*mms4Δ*) CO formation¹². Strikingly, mutants devoid of class I COs

phenocopy *msh2Δ*—that is, COs within these mutants are no longer skewed away from regions of higher polymorphism density despite the presence of Msh2 and share mean densities that are not statistically dissimilar to expected ($p > 0.5$; Two-sample T-test) (Fig. 4b–c). Moreover, the impact of *zip3Δ* and *msh2Δ* appears to be epistatic rather than additive (Fig. 4b), indicating a shared pathway. By contrast, removal of class II formation (*mms4Δ*) has no impact on the interplay between CO formation and polymorphism density (Fig. 4c)—collectively suggesting that mismatch-dependent repression of CO formation is class I specific.

Discussion

Sequence divergence suppresses recombination within a wide range of eukaryotes including *M. musculus* and *H. sapiens*^{20–22}. Findings presented here expand upon these observations and suggest that the anti-recombinogenic activity of Msh2, exerted at homoeologous sites, does not mediate an indiscriminate suppression of COs but rather acts preferentially at sites of ZMM-dependent, interfering COs—thereby altering the spatial distribution of recombination across the genome by modulating the class I:class II balance (Fig. 4d) and highlighting how even low rates of divergence, present within intra-specific hybrids of *S. cerevisiae*, can profoundly impact upon meiosis. Nevertheless, COs frequently encounter heterologous regions (Fig. 4b–c) and yet, despite this, class I COs still form at a high rate in wild type (67%–75%) (Fig. 2d). Mismatches are therefore unlikely to form an absolute barrier to interfering COs, but rather influence the *probability* of formation. In our envisioned model (Fig. 4d), rejected class I events are redirected toward the NCO pathway, via synthesis-dependent strand annealing (SDSA)^{9,23} and double Holliday-junction (dHJ) dissolution^{9,24}, or toward inter-sister recombination, which remains invisible in our assay.

Inhibition of homoeologous recombination, by means of heteroduplex rejection, relies upon Msh2 and the RecQ-family helicase, Sgs1^{5,25,26}. An *sgs1Δ* mutant may therefore be expected to phenocopy *msh2Δ* if suppression of class I COs occurs via this mechanism. However, the strength of CO interference is reduced in *sgs1Δ* relative to wild type (Supplementary Fig. 3c) and the increase in CO interference, observed upon the inactivation of Msh2, requires Sgs1 (Supplementary Fig. 3d)—suggesting that Msh2 and Sgs1 are not epistatic, but rather antagonistic in the formation of class I COs. Thus, unexpectedly, Msh2 appears to mediate suppression of class I COs in a pathway different to that of Sgs1-mediated heteroduplex rejection, instead relying upon the downstream properties or factors of MMR, including Pms1 (Supplementary Fig. 3a), to achieve its effect.

Exactly how MMR-specificity for class I COs arises remains unclear. *In vitro* data suggests that Mlh1-Mlh3—essential class I CO factors—facilitate binding of Msh2 to heteroduplex DNA arising at sites where mismatches exist between parental strains and are in turn stimulated by Msh2²⁷. The distinct genetics of each CO subclass may therefore be responsible for their differential sensitivity to sequence mismatch, through preferential recruitment or activation of Msh2 and other MMR components at class I sites. Interestingly, large-scale regions of heterozygosity promote CO formation within *A. thaliana*²⁸—proposed to arise from either increased recombination due to mismatch-dependent delay in CO maturation, or increased sensitivity of mismatched events to crossover designation²⁸. Given the evolutionary conservation of MMR and class I CO formation^{6,29}, our results in *S. cerevisiae* may distinguish between these mechanisms, suggesting the former is more likely.

Understanding the molecular mechanisms that contribute to speciation is fundamental to our understanding of biological diversity and evolution. As class I CO formation is specifically required for correct meiosis I disjunction⁶, our observations provide a mechanistic insight into how hybrid sterility may arise. Specifically, the activity of Msh2 has the potential to jeopardize chromosome segregation, risking the formation of aneuploid gametes and rendering hybrids derived from distantly related individuals sub- or infertile—contributing to the sexual isolation of a population. MMR may therefore not only influence the rates of evolution, favoring more gradual changes to the gene pool by limiting the amount of genetic exchange that may occur between divergent homologues, but also serve to promote speciation over evolutionary time.

Contributions

T.J.C, M.C. and M.J.N. conceived of the project. T.J.C. analyzed and interpreted the data, performed in-silico simulations and designed the mixture modelling algorithm. M.C. performed all genome-wide mapping and event calling. L.J.H. performed all microscopy and foci analysis. M.M.K. and B.L. provided scripts, protocols and additional samples. T.J.C. and M.J.N. wrote the manuscript.

Competing interests

The authors declare no competing financial interests.

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Methods

Yeast Strains

All *Saccharomyces cerevisiae* strains used in this study are derivatives of SK1³⁰ and S288c³¹. Hybrid strains, utilised in genome-wide mapping, were derived from a cross of haploid SK1 and S288c. Strain genotypes are detailed in (Supplementary Table 1). Knockouts were performed and tested by standard transformation and PCR techniques³². *msh2Δ::kanMX6* and *zip3Δ::HphMX* were generated by PCR mediated gene replacement using a pFA6a-*kanMX6* or pFA6-*hphMX* plasmid³³. The *P_{GAL}-NDT80::TRP1* allele has the natural *NDT80* promoter replaced by the *GAL1-10* promoter, and strains include a *GAL4::ER* chimeric transactivator for β-estradiol-induced expression¹³. S288c x SK1 hybrids create viable spores (91.98% WT, 72.99% *msh2Δ* spore viability, data not shown), eliminating any observational bias that may arise from assaying a limited, surviving population [Crawford et al. 2018, DOI pending].

Meiotic Timecourse (*ndt80AR* strains)

Diploid strains were incubated at 30°C on YPD plates for 48h. For SK1 diploids, a single colony is inoculated into 4 mL YPD (1% yeast extract, 2% peptone, 2% glucose) and incubated at 30°C at 250 rpm for 24 h. For hybrid crosses, haploid parental isolates are mated in 1 mL YPD for 8 h. An additional 3 mL of YPD is subsequently added and the cells are grown for 16 h. Cells are inoculated to a density of (OD₆₀₀) 0.2 into 30 mL YPA (1% yeast extract, 2% peptone, 1% K-acetate) and incubated at 250 rpm at 30°C for 14h. Cells are collected by centrifugation, washed in H₂O, and resuspended in 30mL pre-warmed sporulation media (2% potassium acetate, 5 µg/mL Adenine, 5 µg/mL Arginine, 5 µg/mL Histidine, 15 µg/mL Leucine, 5 µg/mL Tryptophan, 5 µg/mL Uracil). The culture is then incubated at 30°C at 250 rpm for the duration of the time course. After 8h, 2mL of the synchronised cultures were split and exposed to β-estradiol to a final concentration of 2mM, which induces the transcription of *NDT80* and thus sporulation. Cultures were then incubated to a total of 48 h at 30°C prior to dissection.

Tetrad Dissection

In order to produce hybrid spores for sequencing, SK1 x S288c haploid parents were mated for 8-14 h on YPD plates, with the exception of *ndt80AR* strains, which are mated and grown in liquid YPD for 24 h (see above). Haploids are mated freshly on each occasion and not propagated as diploids, in order to reduce mitotic recombination. Sporulation is induced, and tetrads were dissected after 72 h in 2% potassium acetate. For octads,

spores are additionally grown for 4-8 h on YPD plates until a single mitotic division is completed, after which the mother-daughter pair are separated. Colonies were grown for 16 h within liquid YPD for genomic DNA extraction. Only tetrads and octads producing four or eight viable spores/colonies, respectively, are considered for genotyping by NGS.

NGS Library Preparation

Genomic DNA is purified from overnight, saturated YPD cultures using standard phenol-chloroform extraction techniques. Samples of genomic DNA are diluted to 0.2-0.3 ng/μL. DNA concentration is measured using the Qubit High Sensitivity dsDNA Assay. Genomic DNA is fragmented, indexed and amplified via the Nextera XT DNA library Prep Kit according to the best practices recommended by Illumina. In order to check fragment length distribution and concentration of purified libraries, 1 μL of undiluted library is run on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip. To pool samples for sequencing, 5 μL of each sample is combined into a 1.5 mL tube and mixed. 24 μL of the mix is transferred to a tube containing 570 μL hybridisation buffer. The mix is boiled at 96°C for 2 minutes and placed in ice water for 5 minutes. 6 μL of denatured PhiX control (prepared according to Illumina protocol, final concentration 1%) is added to the library, mixed well and then loaded into a MiSeq reagent cartridge. Sequencing was performed in-house using Illumina MiSeq instruments.

Alignment, SNP and indel Detection

Individual spores were sequenced to an average read-depth of ~45x. Initially, paired-end read FASTQ files are aligned, via Bowtie2³⁴, to the SacCer3 reference genome (v. R64-2-1)³⁵ using the parameters: -X 1000 —local —mp 5,1 -D 20 -R 3 -N 1 -L 20 -i S,1,0.50. In order to create a custom SK1 genome to facilitate more accurate genotype-calling, SNP and indel polymorphisms were detected using the GATK (GenomeAnalysisToolKit) function *HaplotypeCaller*³⁶. An in-house script (*VariantCaller.pl*) subsequently parses the resulting VCF files from 72 spores to calculate: (i) the call frequency (% of spores any given allele is present within), (ii) the cumulative allelic read depth (% of reads that contain a specific allele at a specific loci), and (iii) the cumulative total read depth. To identify legitimate SNPs and indels, variants were filtered for a call-frequency between 44-55%, a total read depth of >250 and an allelic read depth of 95%. Variants within repeat regions, long terminal repeats, retrotransposons and telomeres were also discarded—yielding a final, robust list of 64,591 SNPs and 3972 indels amounting to ~0.57% divergence. A custom SK1 genome (SK1_Mod) is then generated by modifying SacCer3 (v. R64-2-1) to include all filtered/called SNPs and indels.

Genotype-Calling

Spore data from individual samples was aligned to the both the custom SK1_Mod genome and the SacCer3 reference (see below). Alignment produces a SAM file, which is converted into a sorted BAM file using the Samtools function, *view*³⁷, for downstream processing. The PySamStats (v. 1.0.1, Miles & Mattioni) module, *variation*, is used to process the sorted BAM file for each sequenced spore, producing a list of the number of reads containing A/C/T/G, insertion or deletion for each genomic position specified in the S288c and SK1 references. Variant reads are isolated and genotyped using in-house, custom scripts as follows. Genotypes are assigned according to the rules: (i) A minimum read-depth of 5; (ii) A SNP is called as having the variant genotype if $\geq 70\%$ of the reads at that position match the called variant, or as reference if $\geq 90\%$ of the reads match the reference; (iii) If the variant and reference reads are above 90% of all reads and within 70% of each other, the position is called as heteroduplex; (iv) indels are called as having the variant genotype if $\geq 30\%$ of the reads at that position match the variant. Such a low threshold is utilised because alignment of indel sequences is biased towards the reference, which means that they are unlikely to be erroneously called as matching the variant genotype. For an indel to be called as the reference genotype, $\geq 95\%$ of the reads must match the reference sequence and there must be fewer than 2 reads matching the variant call. Any variants that fall below these thresholds are discarded. Genotype calls are converted into a binary signal, either 1 for S288c or 0 for SK1.

Event Calling

Using the binarised input, chromosomes are split into segments with the same segregation pattern using scripts available in^{3,9}. Segment types (i.e. 1:7, 2:6, 2:6, 3:5, 4:4, 4:4*, 5:3, 6:2, 6:2* or 7:1 as described in⁹ are also recorded. Recombination events are subsequently called as being a set of segments located between two 4:4 segments longer than 1.5 kb⁹. A 4:4 segment corresponds to a Mendelian segregation profile, 5:3 and 3:5 segments to half-conversion tracts and 6:2 and 2:6 segments to full conversion tracts. Each recombination event can contain between 0-2 COs or NCOs. Events are additionally classified by the number of chromatids involved (i.e. 1, 2, either sister or non-sister, 3, 4).

Event Position & Inter-Crossover Distances (ICDs)

Crossover position, or “midpoint”, is defined as the distance between the mid-points of the first and last SNP/indel markers—an estimate of true event tract length. Inter-crossover distances (ICDs) are then calculated as the distance (in bp) between successive CO midpoints.

Simulating Meiosis

Randomised or mixed (class I + class II) ICD simulations were performed using a novel simulation platform (*RecombineSim*) built in MATLAB 2018a. A typical simulation run is depicted in (Supplementary Fig. 4a). In brief, virtual chromosomes are constructed as binned, numerical arrays at a 100 bp resolution adjusted to reflect the limit of experimental detection governed by the leftmost and rightmost genetic markers (SNPs/indels). Any given 100 bp bin possesses a numerical recombination potential ($\text{recom}(P)$), which governs the ability of an interfering CO to successfully form at that site. Class I COs impose a zone of “interference”, by altering $\text{recom}(P)$ values in adjacent bins in a distance-dependent manner—a similar principle to the beam-film model of CO interference³⁸. The exact shape and width of interference imposed is determined by the best fit (γ) parameters (α, β) for the genotype currently being simulated—and applied as a hazard function (EQN 1.1):

$$h(x) = \frac{PDF(x)}{1 - CDF(x)} \quad (1.1)$$

A hazard function describes the probability that, given a pre-existing CO at position $x(0)$, another CO will form at any given distance (x) away¹⁵—and thus is a natural representation of interference. A fractional amount of class II COs that remain insensitive to $\text{recom}(P)$ are introduced via the C_{PROB} parameter where necessary. All simulations ($N = 10,000$ cells) precisely match the experimentally observed event count for any given genotype or cell. Virtual event merging, at the 1.5 kb threshold, is included in order to closely match the in vivo data.

(γ)-Mixture Modelling

Distributional analysis of CO distributions is complicated by the existence of non-interfering, Mus81-Mms4 class II COs—indistinguishable from interfering ZMM-dependent class I COs in our assay. In essence, meiotic ICDs represent a heterogeneous, mixed system (Fig. 2b) with unknown quantities of each subclass. Latent variables (e.g. class II CO %) may, however, be inferred through probabilistic and statistical methods. Expanding upon the use of the gamma (γ) distribution to model meiotic ICDs^{15,16}, experimental data was deconvoluted by fitting two (γ) distributions—one for each subclass of CO—via an expectation maximisation (EM) algorithm (MATLAB 2018a). EM is a commonly applied method for iterative clustering and parameter estimation in mixed models³⁹. Briefly, any given ICD is assigned a probability reflective of how likely it is to belong to one of the two sub-distributions. Subsequently, sub-distributions are iteratively shifted and data point identity is reassigned until a maximum likelihood (ML) solution is converged upon. One (γ) distribution is expected to yield a final (γ)(α) value of ~ 1.0 (class II, random), while the other is expected to produce a (γ)(α) value of > 1.5 (class I, non-random), with their relative contributions to the overall mixture (i.e. the class I:class II ratio) is dependent upon genotype. In order to validate

this approach, simulated ICD datasets of two component mixtures with known parameters, at variable sample sizes (S), were generated using *RecombineSim* and deconvoluted (Supplementary Fig. 4b). As a measure of accuracy, the average % difference ($N(\%\Delta)$) between estimated and actual parameters was calculated. Accuracy is dependent upon sample size (S) and to a lesser extent on the relative proportions of each subpopulation—and thus how likely a subpopulation is to be readily observed within the mixed population. For example, (γ) mixtures containing 10 or 25% class II COs exhibit average errors of 10.0% and 9.1% at (S) = 500 and 4.53% and 3.73% at (S) = 2000 respectively (Supplementary Fig. 4c). Experimental datasets range from (S) values of 354 to 3365, therefore reasonable error rates of $\sim <10\%$ were expected. The ability to mathematically separate CO subclasses may be of great use to assess, in more detail, the mixed composition of meiotic CO formation present in both published and future datasets of this type—enabling further identification of those components that directly influence the CO interference mechanism.

ICD Transformation

The formation of a variable number of events (N) within a finite space (*lim*) (i.e. a chromosome or genome length) skews CDFs i.e. a higher CO frequency causes a downward shift in ICD size. An ICD distribution produced under identical spatial rules but with a different event count would therefore generate significantly different CDFs—failing or biasing statistical testing and undermining the ability to assess distributional agreement. This skew can be readily observed using simulated data (Supplementary Fig. 1a). Notably, higher values of (N) cause a leftward skew. The relationship between (N) and ICD size for a given *lim* is, however, linear⁴⁰. Consequently, in order to isolate the distributional identity of any given sample (i.e. isolate $\gamma(\alpha)$ from $\gamma(\beta)$), ICD data can be transformed by calculating the product of ICD size (ICD x event count). Data transformation results in perfectly aligned CDFs despite varying (N), validating this approach (Supplementary Fig. 1b).

Statistical Analyses

Kolmogorov-Smirnov Test

A *Kolmogorov-Smirnov* goodness-of-fit (GoF) test is a non-parametric test used to compare continuous probability distributions in order to assess the null hypothesis that both samples derive from identical populations, based on their maximal difference (D_{KS})^{41,42}. (P) values of the KS-test effectively describe the probability that, if the null hypothesis is true, the observed CDFs would be as far apart as observed. (P) values may therefore constitute

an indirect measure of distributional agreement, as employed throughout this paper. KS-tests were performed using the MATLAB 2018a packages: *kstest* and *kstest2*.

Two-sample T-tests

A two-sample T-test is utilised to determine whether a difference in mean value is significant or has arisen by chance. Two-sample T-Tests were performed using the MATLAB 2018a package: *ttest2*.

Microscopy & Cytological Analysis

4.5 mL of meiotic culture is spun down on a bench centrifuge and resuspended to 500 μ L with 1M pH 7.0 D-Sorbitol. 12 μ L of 1.0 M DTT and 7 μ L of 10 mg/mL Zymolyase in 10% glucose solution is added and cells are spheroplasted by incubation at 37°C for 35-50m with agitation. Spheroplasting success is determined by taking 2-3 μ L of the aforementioned solution and adding an equivalent volume of 1.0% (w/v) Sodium N-Lauroylsarcosine while under microscopic observation. Cells should immediately lyse as the exposed membrane is disrupted by the detergent. 3.5 mL of Stop Solution (0.1M MES, 1 mM EDTA, 0.5 mM MgCl₂, 1M D-Sorbitol, pH 6.4) is subsequently added and the cells are spun down to be resuspended in 100 μ L Spread Solution (0.1M MES, 1 mM EDTA, 0.5 mM MgCl₂, pH 6.4) and distributed between 4 slides, which are soaked in 70% EtOH overnight and wiped clean. To each slide, fixative (4.0% (w/v) Formaldehyde, 3.8% (w/v) Sucrose, pH7.5) is added dropwise, followed by detergent (1% Lipsol, 0.1% Bibby Sterilin) to a ratio of 1:3:6 (suspension : fixative : detergent) before lightly mixing and incubating for 1m at room temperature (RT). Further fixative is added dropwise to a final ratio of 1:9:6 and the mixture spread across the slide. Each spread is subsequently incubated at RT for 30m in damp conditions, then allowed to air-dry at RT overnight. Once dry, slides are sequentially washed in 0.2% (v/v) PhotoFlo Wetting Agent (Kodak) and dH₂O, and stored at 4°C.

Slides are washed once in 0.025% Triton X-100 for 10m at RT and twice in PBS for 5m at RT. Slides are blocked in 5% skimmed milk with PBS for 3h at 37°C. Excess liquid is removed and slides laid horizontally in damp conditions. 40 μ L of primary antibody (anti-Zip3⁴³ from rat at 1:200 and anti-Red1 (Genecust, affinity purified, raised against aa(426-827)) from rabbit at 1:200) in 1% skimmed milk with PBS is added under coverslips. Slides are incubated at 4°C overnight (15.5h) and washed three times in PBS for 5m at RT. Excess liquid is then removed and slides are returned to damp conditions. 40 μ L of secondary antibody (anti-rat AlexaFluor555 at 1:200 and anti-rabbit AlexaFluor488 at 1:500) in 1% skimmed milk with PBS is added under coverslips. Slides are incubated

at RT for 2.5h and then washed three times with PBS for 5m at RT. Cover slips are affixed using Vectashield mounting medium with DAPI, sealed with clear varnish and imaged on an Olympus IX71 ($z = 0.2\mu\text{M}$, Exposure times: TRITC-mCherry = 0.2s, eGFP = 1.0s, DAPI = 0.1s). Images were randomised, deconvoluted via Huygens (software) and foci were automatically counted using an in-house plugin for ImageJ (FindFoci) as previously described⁴⁴, with an appropriate mask to discard signals outside of nuclei.

Data Availability

Raw sequence data is deposited in the NIH Sequence Read Archive (SRA) under accession numbers SRP152953 (*zip3Δ*), SRP151982 (wild type, *msh2Δ*, *ndt80AR*) and SRP111430 (*msh2Δ*). Scripts, tools, software and additional data are available upon request or at [GitHub repository link].

Supplementary Discussion

The SK1-ML3 allele has a reduced capacity to generate CO interference

As previously noted, wild type CO frequencies are higher within S96 x YJM789 (74.3 vs 97.3 COs/meiosis) than S288c x SK1 (Fig. 1b). Moreover, wild type CO interference is significantly stronger within S96 x YJM789 ($p < 0.01$; Two-sample KS test) (Supplementary Fig. 2a) and (y)-mixture modelling results (Fig. 2d) suggest that class I CO fraction is higher in S96 x YJM789 (75% vs. 67%). Molecular incompatibilities between certain alleles of the CO formation or CO interference machinery may account for these cross-specific differences. To investigate this hypothesis further, we analysed the frequency and distribution of COs within a *mlh3Δ* S288c x YJM789 background containing a functional copy of the SK1 *MLH3* allele (*SK1-MLH3*)⁴⁵. Surprisingly, introduction of the SK1 *MLH3* allele is sufficient to reduce the global strength of CO interference and produce a CO distribution identical to that of S288c x SK1 wild type ($p = 0.91$; Two-sample KS Test) (Supplementary Fig. 2b). *SK1-MLH3* also results in a modest but significant ($p < 0.01$; Two-sample T-test) reduction in CO frequency (97.2 vs. 90.9 COs per meiosis) (Supplementary Fig. 2c). Collectively, these results suggest that, in some manner, the SK1 *MLH3* allele is deficient in the generation of CO interference and/or CO-formation—however, the reduction in CO frequency observed within S288c x SK1 cannot be fully ascribed to SK1 *MLH3* alone. In the context of this study, such a defect improves the visibility of the *msh2Δ* phenotype in S288c x SK1 (Fig. 1c–d).

Localised impact of polymorphism density upon CO formation

S288c x SK1 variants have an average density of 1 per 175 bp and a median inter-variant distance of 81 bp (93.12% of inter-variant distances are <500bp) and are therefore evenly spaced and present at high density across each chromosome. Maps of polymorphism density are shown in (Supplementary Fig. 5c–d) for two example chromosomes. In general, S288c x SK1 chromosomes are organised into local peaks and troughs of variant density while maintaining overall uniformity. Therefore, it seems unlikely that inactivation of Msh2 would result in a gross-redistribution of CO formation toward any particular region of the chromosome as it may do within organisms with less uniform SNP/indel density, such as *A. thaliana*²⁸. To further investigate the way in which polymorphisms sculpt the meiotic landscape, we repeated the analysis shown in (Fig. 4b–c) using expanded ± 1000 bp and ± 2000 bp windows (Supplementary Fig. 5a–b). A Msh2-dependent and statistically significant skew toward regions of lower sequence divergence ($p < 0.01$; Two-sample KS test) is retained at ± 1000 bp but is significantly diminished at ± 2000 bp ($p = 0.51$; Two-sample KS test), suggesting that mismatched sequences have the greatest impact when present within the recombination intermediate structures.

Figure Legends

Fig. 1 | Inactivation of Msh2 increases CO frequency and the global strength of CO interference

a, Genome-wide mapping of recombination. Meiosis is induced within hybrid S288c x SK1 *S. cerevisiae* diploid cells and genomic material is prepped from individual, isolated spores for paired-end Illumina sequencing in order to genotype SNP/INDEL patterns and therefore determine the parental origin of any given loci (Methods). Only a single chromosome is shown for clarity. Inter-crossover distances (ICDs), a measure of CO interference, are calculated as the distance (in bp) between successive COs along a given chromosome. **b**, Average number of COs per meiosis for each genotype. The number of individual meioses sequenced per genotype is indicated. Error bars: 95% confidence intervals (CI). *P* values: Two-sample *T*-test. **c–f**, Empirical cumulative distribution functions (eCDFs) showing the fraction of ICDs at or below a given size. The total number of experimental ICDs is indicated in brackets. ICDs are transformed as per the protocol described to correct for skews generated by differing CO frequencies (Methods). Untransformed ICD plots are available in (Supplementary Fig. 1c–h). Randomized datasets were generated via simulation to represent a state of no interference (Methods). Pairwise goodness-of-fit tests were performed between genotypes as indicated (triangular legend). *P* values: Two-sample KS-test.

Fig. 2 | Computational modelling predicts a Msh2-dependent shift in the class I:class II CO ratio

a, Ratio of experimentally observed ICD sizes (OBS) versus the theoretical expectation based on a single, best-fit gamma (γ)-distribution (EXP). Ratio values were calculated at 5kb intervals. **b**, Example (γ) mixture model ($\alpha 1.0 - \beta 1.0 + \alpha 3.0 \beta 5.0$). I = class I. II = class II. Meiotic datasets, owing to the existence of two CO subclasses, are a heterogeneous population of three ICD types (as shown). **c**, As in (a) but based on a mixed (γ)-model (no. of distributions fitted = 2). **d**, Best-fit (γ) mixture modelling results. N = sample size (no. of ICDs). α = Single-fit $\gamma(\alpha)$ value. P_{γ} = Fit quality of a single (γ)-distribution (one-sample KS-test). α /II = Mixed model $\gamma(\alpha)$ values. Class I/II = estimated fraction of each CO subclass. Ratio = class I:class II. P_{γ} = Fit quality of a mixed (γ)-mode (Two-sample KS-test) **e–f**, Estimated class I and class II CO counts respectively. Estimates were obtained using the best-fit class I:class II ratios. Total CO frequencies are overlaid (grey bar). Error bars: 95% confidence intervals (CI). The number of individual meioses sequenced per genotype is indicated.

Fig. 3 | Zip3 foci counts are significantly elevated within Msh2-deficient cells

a, Box-and-whisker plot showing Zip3 foci counts from spread, pachytene arrested, Red1-positive S288c x SK1 cells. Midlines denote median values. *P* values: Two-sample *T*-test. **b**, Representative example for each genotype

(7h timepoint). Cells are fluorescently labelled for the meiosis-specific axis protein Red1 (green), the interfering CO marker, Zip3 (red) and DNA (blue). Only cells with sufficient Red1 signal, as determined by the relative intensity of Red1 foci above the background, were included in the analysis. The total number of nuclei counted is indicated in brackets.

Fig. 4 | Suppression of class I COs occurs at regions of higher sequence divergence

a, SNP/INDEL count is assayed using a ± 500 bp window centered on CO or DSB hotspot midpoints. All contained SNP/INDELs are then subsequently tallied, with equal weight. **b–c**, Empirical cumulative distribution functions (eCDFs) showing the fraction of COs that reside within a region of a given SNP/INDEL count for S288c x SK1 and S96 x YJM789 respectively. Expected is calculated using DSB hotspot midpoints¹⁹. Pairwise goodness-of-fit tests were performed between pooled *msh2* Δ and *MSH2*⁺ datasets as indicated (triangular legend). P values: Two-sample KS-test. **d**, Model summarizing the impact Msh2-dependent regulation has upon CO formation. In the presence of Msh2 or a functional MMR pathway, regions of higher sequence divergence redirect repair toward the NCO or inter-sister pathways. Inactivation of MMR alleviates this repression, increasing the frequency of class I CO formation and thus the global strength of CO interference.

Supplementary Fig. 1 | CO interference is present to varying degrees within all mapped strains

a, Empirical cumulative distribution function (eCDF) showing ICD data derived from interfering simulations ($\gamma(\alpha) = 3.0$) at varying CO per cell frequencies (N). **b**, As in **(a)** but ICDs are transformed as per the protocol described to correct for skews generated by differing CO frequencies (Methods). **c–h**, eCDFs showing the fraction of ICDs at or below a given size. The total number of experimental ICDs is indicated in brackets. Randomized datasets were generated via simulation to represent a state of no interference (Methods). Pairwise goodness-of-fit tests were performed between genotypes as indicated (triangular legend). P values: Two-sample KS-test.

Supplementary Fig. 2 | Cross-specific differences—the SK1-MLH3 allele has reduced capacity to mediate CO interference

a–b, Empirical cumulative distribution functions (eCDFs) showing the fraction of ICDs at or below a given size. The total number of experimental ICDs is indicated in brackets. ICDs are transformed as per the protocol described to correct for skews generated by differing CO frequencies (Methods). Randomized datasets were generated via simulation to represent a state of no interference (Methods). Pairwise goodness-of-fit tests were

performed between genotypes as indicated (triangular legend). A schematic of the *SK1-MLH3* strain analyzed is shown. P values: Two-sample KS-test. **c**, Average number of COs per meiosis for each genotype. P values: Two-sample T-test.

Supplementary Fig. 3 | Mechanistic details of MMR-dependent suppression of interfering COs

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Supplementary Fig. 4 | Modelling CO distributions.

a, *RecombineSim* overview. Virtual chromosomes are constructed at a 100bp resolution as binned, numerical arrays upon which meiotic CO formation is simulated (Methods). Any given 100bp contains a value in the range of [0.0-1.0], designating its recombination potential (Rec(P)). Prior to CO formation, bins are initially populated with [1.0]—denoting an equal probability of class I CO formation. During the formation of an interfering CO, *RecombineSim* imposes CO interference as a distance-dependent zone of repression by modifying Rec(P) values—influencing the position of all subsequent interfering COs. Non-interfering, class II COs are distributed randomly independently of Rec(P) and do not impose CO interference. Successive events falling within a set threshold of one another (e.g. 1.5kb) are merged into a single event residing at the midpoint position. These processes repeat until a pre-determined number of simulated ICDs are obtained. **b**, Gamma (γ) mixture modelling was utilised to resolve and estimate individual components of simulated two component mixtures with known parameters (α, β), at known weights (W)—generated via *RecombineSim*. A set of representative examples are shown. Percentage differences between actual and estimated parameters are calculated and averaged to estimate error rate ($N(\% \Delta)$) and algorithm accuracy. S = number of ICDs. **c**, Error rate ($N(\% \Delta)$) values for three (γ) mixtures calculated at varying sample size (S).

Supplementary Fig. 5 | Localised impact of polymorphism density upon CO formation.

a—b, Empirical cumulative distribution functions (eCDFs) showing the fraction of COs that reside within a region ($\pm 1000\text{bp}$ and $\pm 2000\text{bp}$ respectively) of a given SNP/INDEL count (S288c x SK1 only). Expected is calculated using DSB hotspot midpoints¹⁹. Pairwise goodness-of-fit tests were performed between pooled *msh2Δ* and *MSH2*⁺ datasets as indicated (triangular legend). P values: Two-sample KS-test. **c—d**, Smoothed SNP/INDEL density maps for S288c x SK1 ChrVI and ChrVII respectively.

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a, Genome-wide mapping of recombination. Meiosis is induced within hybrid S288c x SK1 *S. cerevisiae* diploid cells and genomic material is prepped from individual, isolated spores for paired-end Illumina sequencing in order to genotype SNP/INDEL patterns and therefore determine the parental origin of any given loci (Methods). Only a single chromosome is shown for clarity. Inter-crossover distances (ICDs), a measure of CO interference, are calculated as the distance (in bp) between successive COs along a given chromosome. **b**, Average number of COs per meioses for each genotype. The number of individual meioses sequenced per genotype is indicated. Error bars: 95% confidence intervals (CI). *P* values: Two-sample *T*-test. **c–f**, Empirical cumulative distribution functions (eCDFs) showing the fraction of ICDs at or below a given size. The total number of experimental ICDs is indicated in brackets. ICDs are transformed as per the protocol described to correct for skews generated by differing CO frequencies (Methods). Untransformed ICD plots are available in (Supplementary Fig. 1c–h). Randomized datasets were generated via simulation to represent a state of no interference (Methods). Pairwise goodness-of-fit tests were performed between genotypes as indicated (triangular legend). *P* values: Two-sample *KS*-test.

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FIGURE 2

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a, Empirical cumulative distribution function (eCDF) showing IGD data derived from interfering simulations ($\gamma(\alpha) = 3.0$) at varying CO per cell frequencies (N). **b**, As in (a) but IGDs are transformed as per the protocol described to correct for skews generated by differing CO frequencies (Methods). **c–h**, eCDFs showing the fraction of IGDs at or below a given size. The total number of experimental IGDs is indicated in brackets. Randomized datasets were generated via simulation to represent a state of no interference (Methods). Pairwise goodness-of-fit tests were performed between genotypes as indicated (triangular legend). P values: Two-sample KS-test.

FIGURE S1

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FIGURE S2

Supplementary Fig. 3 | Mechanistic details of MMR-dependent suppression of interfering COs

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a, *RecombineSim* overview. Virtual chromosomes are constructed at a 100bp resolution as binned, numerical arrays upon which meiotic CO formation is simulated (Methods). Any given 100bp contains a value in the range of [0.0-1.0], designating its recombination potential (Rec(P)). Prior to CO formation, bins are initially populated with [1.0]—denoting an equal probability of class I CO formation. During the formation of an interfering CO, *RecombineSim* imposes CO interference as a distance-dependent zone of repression by modifying Rec(P) values—influencing the position of all subsequent interfering COs. Non-interfering, class II COs are distributed randomly independently of Rec(P) and do not impose CO interference. Successive events falling within a set threshold of one another (e.g. 1.5kb) are merged into a single event residing at the midpoint position. These processes repeat until a pre-determined number of simulated ICDs are obtained. **b**, Gamma (γ) mixture modelling was utilised to resolve and estimate individual components of simulated two component mixtures with known parameters (α, β), at known weights (W)—generated via *RecombineSim*. A set of representative examples are shown. Percentage differences between actual and estimated parameters are calculated and averaged to estimate error rate (N(% Δ)) and algorithm accuracy. S = number of ICDs. **c**, Error rate (N(% Δ)) values for three (γ) mixtures calculated at varying sample size (S).

FIGURE S4

Supplementary Fig. 5 | Localised impact of polymorphism density upon CO formation.

a—b, Empirical cumulative distribution functions (eCDFs) showing the fraction of COs that reside within a region (± 1000 bp and ± 2000 bp respectively) of a given SNP/INDEL count (S288c x SK1 only). Expected is calculated using DSB hotspot midpoints¹⁸. Pairwise goodness-of-fit tests were performed between pooled *msh2 Δ* and *MSH2⁺* datasets as indicated (triangular legend). P values: Two-sample KS-test. **c—d**, Smoothed SNP/INDEL density maps for S288c x SK1 ChrVI and ChrVII respectively.

FIGURE S5

Genotype	Strain	Background	Mat	Genotype
WT	MJ513	SK1	a	<i>ho::LYS2 lys2Δ leu2Δ arg4Δ</i>
	MJ600	S288c	α	<i>ade8Δ</i>
<i>msh2Δ</i>	MC26	SK1	α	<i>ho::LYS2 lys2Δ ura3Δ arg4 leu2 msh2Δ::Kan</i>
	MC49	S288c	a	<i>ade8Δ msh2Δ::Kan</i>
<i>ndt80AR</i>	MJ43	SK1	α	<i>ho::LYS2 lys2Δ arg4Δ leu2Δ::hisG trp1Δ::hisG his4XΔ::LEU2 nuc1Δ::LEU2 PGAL1-NDT80::TRP1 ura3::pGPD1-GAL4(848)-ER::URA3</i>
	MC42	S288c	a	<i>ade8Δ ndt80Δ::Kan</i>
<i>msh2Δndt80AR</i>	MC298	SK1	a	<i>ho::LYS2 lys2Δ ura3Δ arg4 leu2 trp1Δ::hisG ura3Δ::PGPD1-GAL4(848)-ER::URA3 PGAL1-NDT80::TRP1 msh2Δ::Kan</i>
	MC300	S288c	α	<i>ade8Δ ndt80Δ::Kan msh2Δ::Kan</i>
<i>zip3Δ</i>	MC322	SK1	α	<i>ho::LYS2 lys2Δ ura3Δ arg4 leu2 zip3Δ::HphMX4</i>
	MC313	S288c	a	<i>ade8Δ zip3Δ::HphMX4</i>
<i>msh2Δzip3Δ</i>	MC326	SK1	α	<i>ho::LYS2 lys2Δ ura3Δ arg4 leu2 msh2Δ::Kan zip3Δ::HphMX4</i>
	MC317	S288c	a	<i>ade8Δ msh2Δ::Kan zip3Δ::HphMX4</i>

Supplementary Table 1 | Strain Table (S288c x SK1).