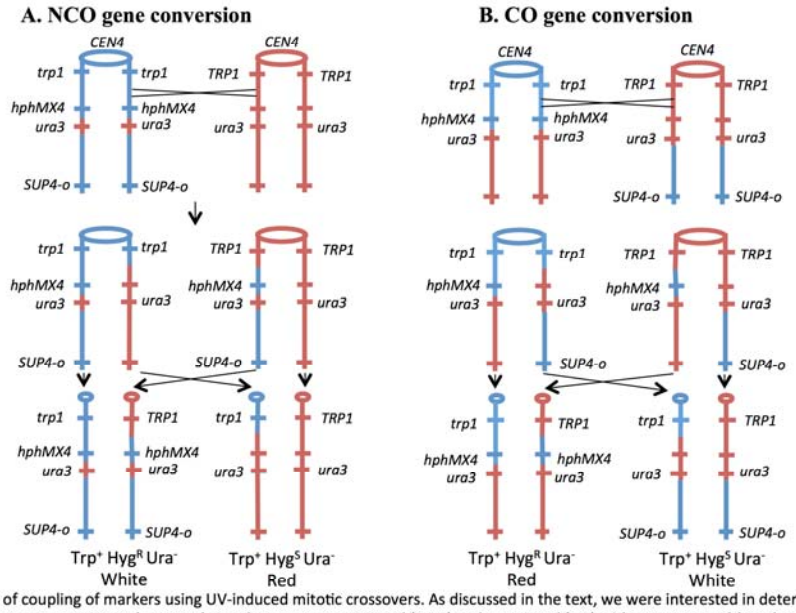

Figure S1 Analysis of coupling of markers using UV-induced mitotic crossovers. As discussed in the text, we were interested in determining the fraction of gene conversion events at the *URA3* locus that were unassociated (NCO) and associated (CO) with crossovers. Although most of our analysis of coupling was done using meiotic analysis (Fig. 6), we found that coupling could also be analyzed by inducing secondary crossover events with UV. More specifically, we determined the coupling of the *hphMX4* and *SUP4-o* markers by identifying red/white sectored colonies in which the *hphMX4* marker was also segregating. Less than 10% of the irradiated colonies formed red/white sectors, as expected from previous studies (Yin and Petes, 2013).

A. Sectoring pattern expected for NCO conversions. If *hphMX4* and *SUP4-o* markers are in the non-recombinant configuration in the diploid before irradiation, then we expect that the red sector will be Hyg^S and the white sector Hyg^R.

B. Sectoring pattern expected for CO conversions. If the *hphMX4* and *SUP4-o* markers are in recombinant configuration in the diploid before irradiation, then we expect that the red sector will be Hyg^R and the white sector Hyg^S

File S1

Supporting text

Details of the microarray analysis: Details of microarray analysis including sample preparation, hybridization conditions, and data analysis were described previously (St. Charles *et al.,* 2012; St. Charles and Petes, 2013). In summary, genomic DNA from strains with a gene conversion event and a control parental strain EY7 were extracted from purified colonies. The extracted DNA was sheared to 200-400 bp fragments. The DNA from the derivatives containing a gene conversion was labeled with the Cy5-dUTP fluorescent dye, while the control DNA was labeled with Cy3-dUTP. Experimental and control samples were mixed and competitively hybridized to SNP probes on a microarray with oligonucleotides derived from chromosome IV. The microarray was then washed and scanned at wavelengths of 635 and 532 nm, specific to Cy5-dUTP and Cy3-dUTP respectively, with a GenePix scanner.

For each probe at each SNP, the relative fluorescence hybridization ratio of experimental to control genomic DNA was measured. A ratio of one indicates heterozygosity (equal representation of both SNP alleles). If the normalized ratio of W303-1A to the control signal is below 0.3, and the ratio of YJM789 to the control is greater than 1.6, we interpret the SNP as homozygous for the YJM789-derived allele. The pattern will be reversed if the SNP is homozygous for W303-1A-derived allele.

Mapping conversion tract lengths by an alternative method, SPA (single-nucleotide polymorphism PCR analysis): The microarrays used for mapping do not contain all of the SNPs that distinguish the two homologs. For conversion events that had a breakpoint in a region sparsely represented by oligonucleotides on the microarray, we refined the mapping using a different method (Lee *et al.*, 2009). For this method, we identified SNPs that altered a restriction site in the region of interest. For example, at SGD coordinate 1028509, W303-1A strain has a T and YJM789 strain has a C. This alteration results in a *Ear*I site that is present in the YJM789 homolog that is absent in W303-1A. To monitor LOH at this position, we amplified the region from genomic DNA samples (usually derived from EY7 spore cultures) with primers that produced a fragment of about 580 bp, and treated the resulting product with *Ear*I. The digest was analyzed by electrophoresis to determine whether the strain contained the W303-1A-specific SNP (resulting in one fragment of about 580 bp) or the YJM789-specific SNP (resulting in two fragments of about 440 and 140 bp). In heterozygous diploid EY7 strains, we expect to see three fragments of about 580, 440, and 140 bp. The coordinates of the polymorphisms used in this analysis, the sequence of the primers, and the diagnostic restriction enzyme are in Table S3.

In our analysis, we found that a small fraction of the microarrays (about 10%) had no detectable LOH events. Subsequently, we first used SPA analysis for the polymorphic sites IV1013924 and IV 1011936 before doing microarrays. If both of these sites were heterozygous, we did not examine the samples by microarray. There were five such samples in the total collection of 59 conversion events.

Gene conversion tract measurements

Conversion tract lengths were determined using the transition coordinates summarized in Table S4. The *URA3* gene on chromosome IV was inserted as a 1140 bp fragment between coordinates 1013217 and 1013218. The SNPs that most closely flanked the insertion were at coordinates 1012642 and 1013370. The mutant substitution in *ura3-e* was located at base 170, about 1 kb from 1012642 and about 870 bp from 1013370. In most of the conversion events, both of these markers underwent LOH. In addition, most conversion events had only two transitions: transition "a" marks the centromere-proximal transition between heterozygous (left column) and homozygous (right column) SNPs, and transition "b" marks the centromere-distal transition between homozygous (left column) and heterozygous (right column) SNPs. For these derivatives, the homozygous region duplicates YJM789-derived SNPs. For these classes of conversion, we calculated the tract length by subtracting the mid-point of transition “a” from the mid-point of transition “b”, and adding 1140 bp (the length of the *URA3* insertion).

In conversion events in which one or more of the flanking markers 1012642 and 1013370 did not undergo LOH, the *ura3-e* mutation represented the SNP that had undergone LOH. For example, in EY7-8, neither of the flanking markers had lost heterozygosity. For this event, therefore, we calculated the conversion tract length as distance between the mid-points of the transition between *ura3-e* and 1012642 (transition “a”) and the transition between *ura3-e* and 1013370 (transition “b”).

The conversion events in EY7-63 and EY7-69 had more than two transitions. In EY7-63, there were four transitions: “a” (heterozygous SNPs to SNPs homozygous for YJM789-derived SNPs), “b” (homozygous for YJM789-derived SNPs to heterozygous SNPs), “c” (heterozygous SNPs to homozygous for YJM789-derived SNPs), and “d” (homozygous for YJM789-derived SNPs to heterozygous). The conversion tract length for this event was calculated as the distance from the mid-point of transition “a” to the mid-point between *ura3*-e and coordinate 1013370 (transition “d”). In EY7-69, there were also four transitions: “a” (heterozygous SNPs to SNPs homozygous for W303-1A-derived SNPs), “b” (homozygous for W303-1A-derived SNPs to heterozygous), “c” (heterozygous to homozygous for YJM789-derived SNPs), and “d” (homozygous for YJM789-derived SNPs to heterozygous). For this conversion event, we calculated the distance between the mid-points of transitions “a” and “d”, and added 1140 bp (the length of the *URA3* insertion).

In our previous studies of mitotic gene conversion, we found many other examples of complex conversion events similar to EY7-63 and EY7-69 (St. Charles *et al.*, 2012; St. Charles and Petes, 2013; Yin and Petes, 2013). Conversion events in which a conversion tract is interrupted by a heterozygous region (similar to EY7-63) can be explained by “patchy” repair of mismatches in a heteroduplex. More specifically, if mismatches in a middle region of a heteroduplex are repaired in the restoration mode and mismatches in the flanking region are repaired in the conversion mode, the observed pattern would be detected (Text S1 of St. Charles and Petes, 2013). Similarly, complex events in which LOH regions contributed by two different donating chromosomes (similar to EY7-69) have been observed previously. Although such events can reflect several different mechanisms, one possibility is that they reflect branch migration of the

Holliday junction resulting in symmetric heteroduplexes (Supporting Information in St. Charles *et al.*, 2012). Whatever the mechanisms that give rise to the complex conversion events, in the current study, these events are a small fraction of the total.

LITERATURE CITED

Argueso, J. L., M. F. Carazzolle, P. A. Mieczkowski, F. M. Duarte, O. V. Netto *et al.*, 2009 Genome structure of a *Saccharomyces cerevisiae* strain widely used in bioethanol production. Genome Res. **19:** 2258-2270.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:** 1541-1553.

Lee, P. S., P. W. Greenwell, M. Dominska, M. Gawel, M. Hamilton *et al*, 2009 A fine-structure map of spontaneous mitotic crossovers in the yeast *Saccharomyces cerevisiae*. PLoS Genet. **5:** e1000410.

Pierce, M. K., C. N. Giroux, and B. A. Kunz, 1987 Development of a yeast system to assay mutational specificity. Mutat. Res. **182:** 65-74.

St Charles, J., and T. D. Petes, 2013 High-resolution mapping of spontaneous mitotic recombination hotspots on the 1.1 mb arm of yeast chromosome IV. PLoS Genet. **9:** e1003434.

St Charles, J., E. Hazkani-Covo, Y. Yin, S. L. Andersen, F. S. Dietrich *et al*, 2012 High-resolution genome-wide analysis of irradiated (UV and gamma-rays) diploid yeast cells reveals a high frequency of genomic loss of heterozygosity (LOH) events. Genetics **190:** 1267-1284.

Thomas, B. J., and R. Rothstein, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619-630.

Wei, W., J. H. McCusker, R. W. Hyman, T. Jones, Y. Ning *et al.*, 2007 Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strain YJM789. Proc. Natl. Acad. Sci. U.S.A. **104:** 12825-12830.

Yin, Y., and T. D. Petes, 2013 Genome-wide high-resolution mapping of UV-induced mitotic recombination events in Saccharomyces cerevisiae. PLoS Genet. **9:** e1003894.

Zhao, X., E. G. Muller, and R. Rothstein, 1998 A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. Mol. Cell **2:** 329-340.

Table S1 Strain genotypes and strain construction

Strain name	Genotype	Strain construction or reference
W1588-4C	*MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 RAD5*	Zhao *et al*., 1998
JSC52-1	*MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 RAD5 IV1013217::URA3*	Inserted *URA3* at IV1013217 by transforming W1588-4C with a PCR fragment generated with primers RE HS2 URA3 F and RE HS2 URA3 R using strain JAY291 (Argueso *et al*., 2009) as template.
JSC54-1	*MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 RAD5 IV957578::hphMX4 IV1013217::URA3*	Inserted *hphMX4* at IV957578 by transforming JSC52-1 with a PCR fragment generated with primers RE HS2 HYG F and RE HS2 HYG R using plasmid pAG32 (Goldstein and McCusker, 1999) as template.
EY2-1	*MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 RAD5 IV957578:: hphMX4 IV1013217::URA3 IV1510386::SUP4-o*	Inserted *SUP4-o* at IV1510386 by transforming JSC54-1 with a PCR fragment generated with primers IV 1510386::SUP4-o F and IV 1510386::SUP4-o R using plasmid YCpMP2 (Pierce *et al*., 1987) as template.
PSL4	*MATα ade2-1 ura3-p gal2 ho::hisG*	Lee *et al.,* 2009
EY3-1	*MATα ade2-1 ura3-p gal2 ho::hisG IV1013217::URA3*	Inserted *URA3* at IV1013217 by transforming PSL4 with a PCR fragment generated with primers RE HS2 URA3 F and RE HS2 URA3 R using JAY291 genomic DNA as template.
EY4-2	*MATα ade2-1 ura3-p gal2 ho::hisG IV1013217::ura3-e*	*ura3* mutant obtained as a spontaneous 5-FOA^{R} isolate of EY3-1.
EY6-4	*MAT***a***/MATα leu2-3,112/LEU2 his3-11,15/HIS3 ade2-1/ade2-1 ura3-1/ura3-p trp1-1/TRP1 can1-100/CAN1 GAL2/gal2 ho/ho::hisG IV957578:: hphMX4/IV957578 IV1013217::URA3/IV1013217::ura3-e IV1510386::SUP4-o/IV1510386*	Cross of EY4-2 and EY2-1.

EY7-3	*MAT**a**/MATα::natMX4 leu2-3,112/LEU2 his3-11,15/HIS3 ade2-1/ade2-1 ura3-1/ura3-p trp1-1/TRP1 can1-100/CAN1 GAL2/gal2 ho/ho::hisG IV957578:: hphMX4/IV957578 IV1013217::URA3/IV1013217::ura3-e IV1510386::SUP4-o/IV1510386*	Deletion of *MATα* from EY6-4 by transforming with the PCR product generated with primers MAT ALPHA NAT F and MAT ALPHA NAT R using the plasmid pAG25 (Goldstein and McCusker, 1999) as a template.

Haploids W188-4C, JSC52-1, JSC54-1, and EY2-1 are isogenic with W303-1A (Thomas and Rothstein, 1989), except for changes introduced by transformation. Haploids PSL4, EY3-1, and EY4-2 are isogenic with YJM789 (Wei *et al*., 2007) except for changes introduced by transformation.

Table S2 Primers used in strain construction and DNA sequence analysis

Primer name	SGD coordinates for primer sequence	Primer sequence (5' to 3')	Purpose
RE HS2 HYG F	IV957528-957578	CTTCCGTTATGACCCCCTCATCCGTTTAATGTTATTTGTT TTTATGGTATTCGTACGCTGCAGGTCGAC	Strain construction
RE HS2 HYG R	IV957629-957579	AGGGGTTACAGGGATCAACAAAAAAAAGCAAGAAAA AAAATGAATAAAGGGATCGATGAATTCGAGCTCG	Strain construction
IV 1510386::SUP4-o F	IV1510336	CATACGTTATGCACTTCATTCTTCTTGTCGGTTTGATAA CAGCAGAATCTAGGATCCGGGACCGGATAAT	Strain construction
IV 1510386::SUP4-o R	IV1510436	GCGTTTTCGAGGTATGGCTTCTGCCGGGCTAACGTTCA AATTAAAGGAACTGGATCCGGAATTCTTGAAAG	Strain construction
RE HS2 URA3 F	IV1013167-1013217	TTTATATAGATAAACAAACTTGCAGGACAGATAGTTAA GCGTCTATATCATAATGTGGCTGTGGTTTCAGG	Strain construction
RE HS2 URA3 R	IV1013268-1013218	TCTTTTTTGCCTTTTATCATTTTTGTACTTTTTTCTTCGCT TAAAATACACAGATTCCCGGGTAATAACTG	Strain construction
MAT ALPHA NAT F	III293135-293178	ATATATATATATATATTCTACACAGATATATACATATTT GTTTTTCGGGCCGTACGCTGAAGGTCGAC	Strain construction
MAT ALPHA NAT R	III294497-294545	TGAACAACATTCAGTACTCGAAAGATAAACAACCTCCG CCACGACCACACTCATCGATGAATTCGAGCTCG	Strain construction
URA3-ey F	IV 1013093-1013112	GCCACCCATCTGATAAAAGG	DNA sequencing
URA3-ey R	IV 1013321-1013339	CTCCCCCGCTGTTAATTTT	DNA sequencing
URA3-1 F	V116039-116058	AACGAAGGAAGGAGCACAGA	DNA sequencing
URA3-1 R	V117042-117061	GACCGAGATTCCCGGGTAAT	DNA sequencing

The SGD coordinates are for the 2014 version of the sequence.

Table S3 Primers used in analysis of polymorphic markers

SGD coordinates for polymorphic site[1]	Primer name	Primer sequence (5' to 3')	Diagnostic restriction enzyme[2]
IV 1002467	IV 1002467 F1	TGCAGGGATATGTATAACGAGGT	***HpyCH4*III**
	IV 1002467 R2	CAACGTTGTGAAGTGGTTGG	
IV 1011936	IV 1011936 F4	TCCTACGATCGTACAATCCCG	***Mnl*I**
	IV 1011936 R1	CCTCGTACATGTTTTCTTGCC	
IV 1013924	IV 1013924 F3	GCGACTGGTGGTAAGAAAAGG	***Mlu*CI**
	IV 1013924 R1	AAACCCGAGACAGCGAGAGG	
IV 1022417	IV1022417 F2	CAAGTTTGGTATGGCAGTTGAC	*Mly*I
	IV 1022417 R1	GACTCGTCTCTTGTATGGCG	
IV 1028509	IV 1028509 F2	CCGAACGGTTATGGTATCTCC	*Ear*I
	IV 1028509 R3	GCAACAGCGCGTATTTGG	
IV 1037347	IV 1037347 F1	GCCAAGATCGTTAAAGAGAATCC	*HpyCH4*V
	IV 1037347 R1	AGCAATCTTTGAACCATCGC	
IV 1044571	IV 1044571 F3	GGCTACTATTGTGGCTGTTGG	*Sau3A*I
	IV 1044571 R1	ACTACGTCGATATTCTTCAGGG	
IV 1051452	IV 1051452 F1	TCATGCCAAAGTAATAAGCAGC	*Msp*I
	IV 1051452 R1	TGAGCGCTAAAATGTATGCCC	
IV 1058335	IV 1058335 F1	TGCCTGGATACACGAACATATATATG	*Afe*I
	IV 1058335 R1	CGGTGAGATTGTTCGGTGTT	

[1]Current (2014) SGD coordinates.

[2]Bold-faced type indicates that the SNP in the W303-1A genetic background had the indicated restriction site. Plain-faced type indicates that the SNP in the YJM789 background had the site.

Table S4 Coordinates of gene conversion events and conversion tract size

Available for download as an Excel file at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.167395/-/DC1

Table S5 Meiotic analysis of coupling[1]

Derivatives of EY7	Markers examined	# tetrads analyzed	# PD-1 or PD-2	# NPD-1 or NPD-2	# TT	Association with crossover
1	*hphMX4*; IV 1028509	5	2 PD-1	0	3	NCO
2	*hphMX4*; IV 1058335	3	2 PD-1	0	1	NCO
4	*hphMX4*; IV 1028509	5	2 PD-1	0	3	NCO
5	IV 1002467; IV 1028509	3	2 PD-1	0	1	NCO
6	*hphMX4*; IV 1044571	6	3 PD-1	0	3	NCO
7	*hphMX4*; IV 1028509	4	4 PD-1	0	0	NCO
8	*hphMX4*; IV 1028509	4	3 PD-1	0	1	NCO
10	*hphMX4*; IV 1037347	3	3 PD-2	0	0	CO
13	*hphMX4*; IV 1028509	4	3 PD-1	0	1	NCO
14	*hphMX4*; IV 1028509	4	4 PD-1	0	0	NCO
16	*hphMX4*; IV 1028509	5	3 PD-1	0	2	NCO
17	*hphMX4*; IV 1028509	3	3 PD-1	0	0	NCO
18	*hphMX4*; IV 1028509	5	4 PD-1	0	1	NCO
26	*hphMX4*; IV 1037347	2	2 PD-2	0	0	CO
27	*hphMX4*; IV 1044571	5	2 PD-1	0	3	NCO
29	*hphMX4*; IV 1028509	5	4 PD-1	0	1	NCO
30	*hphMX4*; IV 1028509	4	2 PD-2	0	2	CO
31	*hphMX4*; IV 1028509	3	2 PD-1	0	1	NCO
33	*hphMX4*; IV 1097831	6	2 PD-1	0	4	NCO
35	*hphMX4*; IV 1044571	3	3 PD-1	0	0	NCO
36	*hphMX4*; IV 1028509	4	4 PD-1	0	0	NCO
37	*hphMX4*; IV 1028509	7	3 PD-1	0	4	NCO
38	*hphMX4*; IV 1028509	5	3 PD-2	0	2	CO
39	*hphMX4*; IV 1037347	3	2 PD-2	0	1	CO
40	*hphMX4*; IV 1028509	5	5 PD-2	0	0	CO
41	*hphMX4*; IV 1028509	6	3 PD-2	0	3	CO
42	*hphMX4*; IV 1028509	5	3 PD-2	0	2	CO
43	*hphMX4*; IV 1028509	4	4 PD-1	0	0	NCO
44	*hphMX4*; IV 1028509	2	2 PD-2	0	0	CO
45	*hphMX4*; IV 1028509	2	2 PD-1	0	0	NCO
46	*hphMX4*; IV 1028509	3	3 PD-1	0	0	NCO
47	*hphMX4*; IV 1028509	3	3 PD-1	0	0	NCO
48	*hphMX4*; IV 1028509	3	3 PD-2	0	0	CO
49	*hphMX4*; IV 1028509	2	2 PD-1	0	0	NCO

50	*hphMX4*; IV 1028509	3	2 PD-1	0	1	NCO
51	*hphMX4*; IV 1028509	3	2 PD-1	0	1	NCO
52	*hphMX4*; IV 1051452	4	3 PD-1	0	1	NCO
53	*hphMX4*; IV 1028509	3	2 PD-2	0	1	CO
54	*hphMX4*; IV 1028509	3	2 PD-2	0	1	CO
55	*hphMX4*; IV 1028509	4	2 PD-1	0	2	NCO
56	*hphMX4*; IV 1028509	3	2 PD-1	0	1	NCO
57	*hphMX4*; IV 1028509	3	3 PD-1	0	0	NCO
58	*hphMX4*; IV 1028509	3	3 PD-1	0	0	NCO
59	*hphMX4*; IV 1028509	4	4 PD-2	0	0	CO
60	*hphMX4*; IV 1028509	2	2 PD-1	0	0	NCO
61	*hphMX4*; IV 1028509	3	2 PD-2	0	1	CO
62	*hphMX4*; IV 1028509	6	3 PD-1	0	3	NCO
63	*hphMX4*; IV 1028509	4	2 PD-1	0	2	NCO
64	*hphMX4*; IV 1037347	3	2 PD-1	0	1	NCO
65	*hphMX4*; IV 1028509	3	2 PD-1	0	1	NCO
66	*hphMX4*; IV 1028509	3	2 PD-1	0	1	NCO
69	*hphMX4*; IV 1044571	8	4 PD-2	0	4	CO
70	*hphMX4*; IV 1028509	6	4 PD-1	0	2	NCO
71	*hphMX4*; IV 1028509	4	3 PD-1	0	1	NCO

[1]Tetrads were dissected and examined for markers flanking the conversion event. For most events, the *hphMX4* marker was the centromere-proximal marker. The centromere-distal marker was examined by SPA (described in text). The primers and restriction enzymes used for this analysis are given in Table S3. PD-1 tetrads are those with two Hyg^R SNP^W to two Hyg^S SNP^Y spores; SNP^W and SNP^Y are defined as in Figure 6. PD-2 tetrads are those with two Hyg^R SNP^Y to two Hyg^S SNP^W spores. Based on this analysis, we classified the events as crossover-associated (CO) or unassociated (NCO).