Meiotic Chromosome Segregation in Triploid Strains of *Saccharomyces cerevisiae*

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ABSTRACT

Meiosis in triploids results in four highly aneuploid gametes because six copies of each homolog must be segregated into four meiotic products. Using DNA microarrays and other physical approaches, we examined meiotic chromosome segregation in triploid strains of *Saccharomyces cerevisiae*. In most tetrads with four viable spores, two of the spores had two copies of a given homolog and two spores had only one copy. Chromosomes segregated randomly into viable spores without preferences for generating near haploid or near diploid spores. Using single-nucleotide polymorphisms, we showed that, in most tetrads, all three pairs of homologs recombined. Strains derived from some of the aneuploid spore colonies had very high frequencies of mitotic chromosome loss, resulting in genetically diverse populations of cells.

POLYPLOIDY is very common in plants; for example, the frequency of polyploidy in angiosperms is 30–80% (HEGARTY and HISCOCK 2008). Most polyploidy is thought to arise by whole-genome duplication of diploids, producing tetraploids. One mode of producing a triploid is by union of a haploid gamete produced by a "normal" diploid with a diploid gamete produced by a tetraploid. Triploids produced by this mechanism have been detected in animals as well as plants. In a region with both diploid and tetraploid Palearctic green toads, triploid male toads resulting from crosses of diploid females and tetraploid males were observed (STOCK *et al.* 2009). Triploids can also arise from a cross of diploid individuals in which one individual has tetraploid germinal tissue (BRIDGES and ANDERSON 1925). Although the fertility of triploid Drosophila is reduced, viable offspring between diploids and triploids can be readily obtained. In humans, triploidy is responsible for 15–18% of spontaneous abortions, but only 1 in 1200 fetuses live after birth; all of the live-born individuals die within a few months (ILIOPOULOS *et al.* 2005).

Triploid yeast strains have been observed in the wild (EZOV *et al.* 2006) and have been generated in the lab by forced matings between haploids and diploids (POMPER *et al.* 1954). Since diploids of the *MAT***a**/*MAT*α genotype do not mate, it is likely that the forced matings selected for rare diploids that had become homozygous for

*MAT***a** or *MAT*α as a consequence of a mitotic recombination at the mating-type locus on chromosome III. In most recent studies, triploids are generated by mating haploids to diploids that have been constructed to be homozygous at the mating-type locus (PARRY and COX 1970; CAMPBELL *et al.* 1981; CAMPBELL and DOOLITTLE 1987).

The meiotic products derived from sporulating a triploid would be expected to be highly aneuploid, containing chromosome numbers varying between the haploid number of 16 and the diploid number of 32. Since even extra copies of single chromosomes often adversely affect cellular growth rates (TORRES *et al.* 2007), it is unsurprising that the viability of spores derived from triploids is lower than those derived from diploids. There is, however, a wide range of spore viabilities reported in different labs varying between 15 and 18% (PARRY and COX 1970; CAMPBELL *et al.* 1981) to about 75% (POMPER *et al.* 1954).

The first detailed analysis of chromosome segregation in viable spores derived from triploids was carried out by PARRY and COX (1970). The viable spores were crossed with haploids that had multiple auxotrophic markers and the resulting diploids were sporulated. Departures from 2:2 segregation of the markers were used to diagnose aneuploidy. Markers on 14 of the 16 yeast chromosomes were used. Of 34 segregants examined, 3 had one copy of all chromosomes tested, and 2 had two copies of all chromosomes tested. The other 29 segregants had between one and five disomic chromosomes. PARRY and COX (1970) noted that disomes of chromosomes V, VIII, IX, XII, and XIII were more frequently observed in the viable spores than disomes of the other chromosomes. In addition, they suggested that the ability of the triploid-derived spores to tolerate aneu-

ploidy was limited, since most of the spores had less than five disomic chromosomes or were diploid.

A more extensive analysis of viable spores derived from triplods was done by CAMPBELL *et al.* (1981). For chromosome III, they observed that about half of the spores had one chromosome and about half had two. They concluded that during meiotic segregation in the triploid, two spores received two copies of each homolog and two received one. For most chromosomes, the frequency of spores with two copies was significantly less than the frequency with one copy. They attributed this difference to loss of disomic chromosomes during mitotic growth of the spore cultures.

In a subsequent study, CAMPBELL and DOOLITTLE (1987) examined chromosome compositions in triploid-derived tetrads that had two viable spores. If these two viable spores are sister spores either both should have two copies of any given chromosome or both spores should have one copy (Figure 1); below, we use the term "monosomy" to describe spores that have only one copy of a homolog, although this term is also used to describe diploid cells that lack a single chromosome. These two classes of events should be equally frequent. The observed frequencies of tetrads in which both viable spores were disomic for the same chromosome varied between 0 (chromosome VII) and 0.5 (chromosome XIII) with an average of about 0.19 (CAMPBELL and DOOLITTLE 1987). The deviation between the expected (0.5) and observed frequencies of two disomic spores was explained as a consequence of mitotic chromosome loss.

Because of the low spore viability, meiotic recombination in triploid yeast has not been examined previously. However, meiotic recombination in otherwise diploid cells that are trisomic or tetrasomic for a single chromosome has been studied by a number of researchers. SHAFFER *et al.* (1971) suggested two possible mechanisms by which trisomic chromosomes can pair, recombine, and segregate during meiosis. According to the bivalent/univalent-pairing model, two homologs pair, recombine, and segregate to opposite poles while the third homolog randomly segregates to either pole. By the second model (trivalent pairing), all three homologs pair, possibly recombine, and randomly segregate to either pole. A distinguishing feature of the trivalent model is that two chromosomes that have recombined with each other are capable of segregating to the same pole during meiosis I. The majority of studies support the trivalent-pairing model (SHAFFER *et al.* 1971; CULBERTSON and HENRY 1973; RILEY and MANNEY 1978; KOLLER *et al.* 1996). From the genetic data, it is impossible to determine whether all three homologs pair and recombine at the same time or whether there are multiple cycles of pairing and recombination although, by visualizing the synaptonemal complex in triploid strains, LOIDL (1995) showed that simultaneous pairing of all three homologs was common.

Some of the inferences concerning meiotic segregation and recombination in past studies of triploids were indirect for two reasons. First, not all of the meiotic products from a single tetrad were examined. Second, mitotic chromosome loss occurred during the time-consuming assay of disomic chromosomes during growth of the spore cultures or in the diploid strains derived from mating the spore cultures. In the current study, we examine the meiotic segregation of chromosomes in triploid yeast in tetrads with four viable spores using physical methods (microarray analysis and a PCR-based examination of single-nucleotide polymorphisms) that are more rapid and sensitive than the methods used previously. We show that meiotic chromosome segregation in triploids is generally accurate, resulting in two spores with two copies and two spores with one copy of each homolog. The mean number of aneuploid chromosomes for each spore is initially close to 8, but we show directly that mitotic chromosome loss in spore cultures can be very rapid. In addition, we show that triploid chromosomes, like trisomic chromosomes, usually undergo trivalent pairing.

MATERIALS AND METHODS

Strain construction: Our experiments were conducted using two triploid strains: MH10 and JSC2. The three copies of each homolog in MH10 are identical except for alterations introduced by transformation, whereas the three copies of each homolog in JSC2 are derived from three diverged haploid strains. The genotypes of all strains used in our study are given in supporting information, File S1 and Table S1. MH10 was generated by crossing MS71 (*MATα* strain) to JLMy133, an isogenic diploid (*MAT**a**/matα::URA3*). Thus, all three homologs of MH10 have the same DNA sequence except for the mating-type locus on chromosome III. The JSC2 triploid was constructed to contain three diverged homologs of each yeast chromosome. JSC2 was generated by mating the haploid PSL4 to the diploid JSC1. The haploid PSL4 (*MATα* mating type) was derived from the YJM789 genetic background (WEI *et al.* 2007; LEE *et al.* 2009). The JSC1 diploid is a *MAT**a**/matα::NAT* derivative of JAY306, a diploid generated by crossing FY834 (a haploid isogenic with S288c; WINSTON *et al.* 1995) with JAY291 (a recently sequenced haploid derived from an ethanol-producing strain; ARGUESO *et al.* 2009).

Genetic methods and media: We used standard genetic and media protocols (GUTHRIE and FINK 1991). Strains were mated by mixing the parental strains on a plate containing rich growth medium (YPD) and incubating the mixture for 6 hr at 30°. We then picked zygotes and sporulated the resulting triploids. Following tetrad dissection of sporulated triploids, the dissection plates were incubated at 30° for up to 5 days. The entire spore colony of tetrads with four viable spores was transferred as a patch to a YPD plate and grown for 1–2 more days at 30°. Glycerol stocks were then prepared using the entire patch.

Comparative Genome Hybridization (CGH) microarrays: DNA for CGH microarrays was isolated from 5 ml YPD cell cultures grown for 1–2 days at 30°. The isolation procedure was the same as that used for preparing DNA samples for contour-clamped homogeneous electric field (CHEF) gel analysis (NARAYANAN *et al.* 2006). DNA was isolated from the agarose plugs using a QIAgen gel extraction kit. After the plugs were dissolved with buffer QG from the QIAgen kit, the DNA was sonicated. After sonication, DNA was isolated using the standard QIAgen protocol. DNA from spore cultures was

labeled with Cy5–dUTP and the control DNA was labeled with Cy3–dUTP (LEMOINE *et al.* 2005). For the analysis of DNA from MH10-derived spore cultures, MS71 was used as the control DNA; for JSC2-derived spore samples, we used JSC2 DNA for the control. The experimental and control DNAs were competitively hybridized overnight at 65° to microarrays that contained both ORFs and intergenic regions from the yeast genome (ARGUESO *et al.* 2008).

Following hybridization, the slides were scanned using a GenePix 4000B scanner and GenePix Pro 6.0 software. Data files were analyzed using the University of North Carolina Microarray Database (http://genome.unc.edu). The $\log_2$ ratios (hybridization values of experimental and control DNA samples) for each element on the array were first normalized using the $\log_2$ median ratio for the whole data set. If the experimental strain has a mixture of disomic and monosomic chromosomes, following this normalization, the monosomic chromosomes are expected to have an average $\log_2$ ratio of less than zero. Consequently, we determined the average $\log_2$ ratio for all chromosomes with a $\log_2$ ratio less than zero and added this number to the average $\log_2$ ratio for each individual chromosome. If this sum was greater than 0.17, then the chromosome was considered disomic; if it was less than 0.17, then the chromosome was considered monosomic. A $\log_2$ ratio of 0.17 represents disomy of a chromosome in 12.5% of the population. The patterns of hybridization were depicted using CGH Miner (WANG *et al.* 2005).

Diagnostic PCR and restriction digests: The primers listed in Table S2 were used to PCR amplify polymorphic regions near the centromere using genomic DNA from JSC2 spores. By treating the resulting fragments with various restriction enzymes (Table S2), we could determine the source of the homologs (S288c, YJM789, or JAY291) in each spore. The identity of chromosomes XIII and XIV were also diagnosed by amplification of centromeric regions that generated DNA fragments differing by 9–13 bp in size. These PCR products were resolved on a 2% agarose gel. Right and left arm identities were also diagnosed using this method with primers located near the telomeres of each chromosome arm. Primers and restriction enzymes used to diagnose these distal markers are listed in Table S3. We also used PCR methods to specifically amplify the centromere-linked JAY291 SNP on chromosome I and the YJM789-specific polymorphism on chromosome XIII. The names and sequences of the primers used for this analysis were DIST 1.1F (5′ CCACGCCAGGGAATCATCC) and DIST 1.1R (5′ TGCTACAGCATCTCGGCCC) for chromosome I and DIST 13.1F (5′ TTGTCCATAGCAGCAATCATACCAGCACCACC) and DIST 13.2R (5′ AGAAACGCGTCTGGCTTATCTACCGC) for chromosome XIII.

Statistical analysis: The VassarStats Website for Statistical Computation (http://faculty.vassar.edu/lowry/VassarStats.html) was used for the chi-square goodness-of-fit tests and Fisher exact tests. We used the BENJAMINI and HOCHBERG (1995) test to control for the false discovery rate when 12 or more comparisons were made.

RESULTS

Description of experimental methods: We examined meiotic chromosome segregation in two different types of triploids. In the triploid MH10, all three copies of each homolog were identical in DNA sequence, except for a very small number of changes introduced by transformation. In the triploid JSC2, the three copies of each homolog were derived from three different haploid yeast strains, each of which is diverged in DNA sequence from the other two by about 0.1%. As explained below, this sequence divergence allowed a sensitive assay for disomy in spores derived from JSC2 and also allowed detection of meiotic recombination events. Diploids derived from the diverged haploids had good spore viability, indicating that these strains do not have translocations or inversions (data not shown).

Both triploid strains were sporulated and dissected. Spore viability for both strains was about 50% (Table S4). This spore viability is considerably higher than that observed by PARRY and COX (1970) and CAMPBELL *et al.* (1981) (15% and 17.9%, respectively) but less than that observed by POMPER *et al.* (1954) (69–83%). In addition, the distribution of the classes of tetrads (4 live:0 dead, 3 live:1 dead, 2 live:2 dead, 1 live:3 dead, 0 live:4 dead) deviated significantly from that expected by a binomial expansion (Fisher exact test, $P < 0.001$; Table S4). We do not know the reasons for the differences in spore viabilities in different studies. The differences may reflect technical issues (for example, the length of time that asci were treated with glusulase) or variation in the tolerance of aneuploidy in different genetic backgrounds.

About 16% of the tetrads derived from MH10 and JSC2 had four viable spores. All cells in each spore colony were transferred as patches to rich growth solid medium and frozen stocks were prepared. DNA was isolated for microarray analysis by growing large inocula derived from the frozen stocks. This protocol was designed to limit loss or duplication of chromosomes in the mitotic divisions following meiosis. We examined DNA isolated from all four spores for 10 tetrads derived from MH10 and 6 tetrads derived from JSC2. The chromosome compositions of all of these spore cultures were examined by CGH microarrays; we also determined that both of the starting triploids were euploid. In addition, we analyzed the chromosomes in JSC2 spore cultures by examining SNPs as described below.

Analysis of meiotic segregants of the triploid MH10: Figure 2 shows a representative example of analysis of DNA samples from four spores of an MH10 tetrad by CGH microarrays. DNA from MH10 spores and an isogenic haploid reference strain (MS71) were labeled with different fluorescent nucleotides (Cy3 and Cy5) and competitively annealed to a microarray containing all yeast ORFs and intergenic regions. In the depiction of the microarray shown in Figure 2, each of the 16 chromosomes is represented by a line. Along each line are vertical lines whose length and position represents relative hybridization differences between the reference and sample genomic DNA at each element of the microarray. Red and green represent duplications or deletions of the experimental strain with respect to the control strain. For example, the spore MH10-32a (Figure 2A) was disomic for chromosomes X and XIII. In many of the microarrays, chromosomes had significantly elevated hybridization signals, although the hybridization level was not twofold higher than for the

other chromosomes. It is likely that this effect is a consequence of loss of the disomic chromosomes in some fraction of the subcultured cells during vegetative growth.

The spore MH10-32c had the chromosome composition identical to that of MH10-32a (Figure 2A). The other two spores, MH10-32b and MH10-32d, were both disomic for chromosomes I, II, IX, XI, and XIV. MH10-32d was also disomic for chromosomes III, V–VIII, XII, XV, and XVI. The nonsister pairs of spores had nonoverlapping patterns of disomy, and all chromosomes were disomic in at the least one spore except chromosome IV, the largest chromosome. From the pattern of chromosome segregation shown in Figure 1, one would expect that two pairs of spores (the sister spores) should have reciprocal patterns of disomy, and, for each homolog, one pair of sisters would have two copies and one pair would have one copy. Although chromosomes I, II, IX, X, XI, XIII, and XVI met this expectation, the other chromosomes did not. It should be noted that the pairs of spores that had similar patterns of disomic chromosomes had growth properties similar to those of spore colonies on the dissection plates (Figure 2B).

One simple explanation of this discrepancy is that the disomic chromosomes are frequently lost during mitotic growth. For example, the sister spores MH10-32a and MH10-32c initially may have had two copies of chromosomes X and XIII and one copy of all of the other chromosomes. The sister spores MH10-32b and MH10-32d had one copy of chromosomes X and XIII and two copies of all of the other chromosomes. The strain MH10-32b subsequently lost many of the chromosomes originally present in two copies, whereas MH10-32d lost only chromosome IV. It is also possible that sister spores MH10-32a and MH10-32c were originally disomic for chromosome IV, but the extra chromosome was lost in both spore cultures. One alternative explanation of the results is that chromosome loss is very frequent during meiotic segregation in triploids at meiosis I (leading to loss of two copies) or meiosis II (leading to loss of one copy). Another possibility is that there is a high rate of chromosome loss in the triploid prior to meiosis. These issues are discussed in more detail below.

We examined nine other tetrads with four viable spores derived from MH10 by CGH arrays and these data are summarized in Table 1. For the most part, only meiotic sisters were disomic for the same chromosome in any given tetrad. Most tetrads consisted of colonies that were of two different sizes that corresponded with meiotic sisters as determined by disomy of the same chromosome (Table 1).

After we compiled the results of all 10 tetrads analyzed by CGH microarray, it was apparent that spores derived from the triploids are subject to extensive chromosome loss (Table 1). Assuming that there should be six chromosome copies of each homolog per tetrad (following meiotic DNA synthesis), there should be 96 chromo-
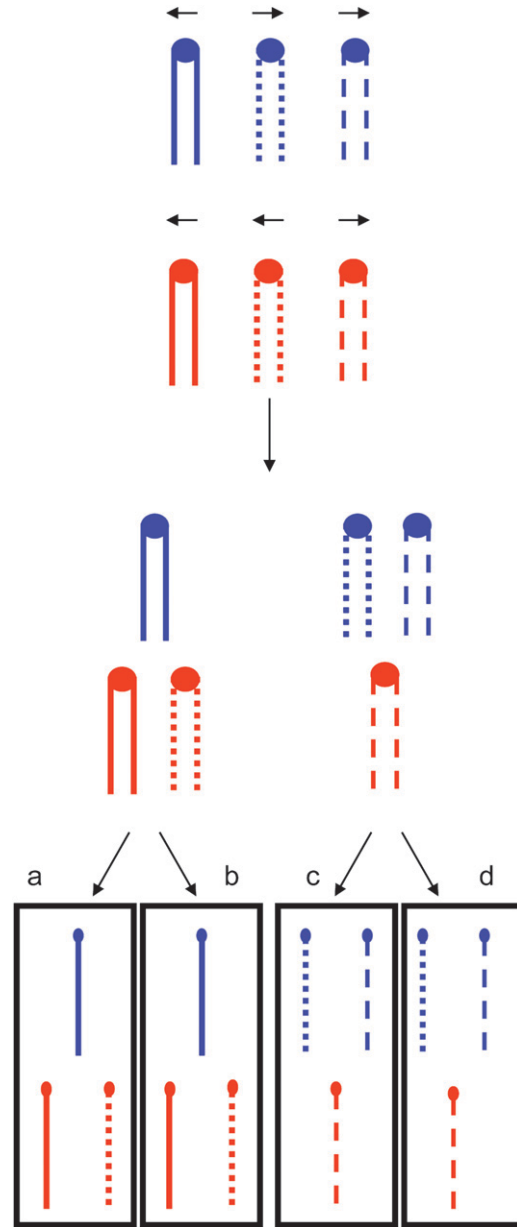
FIGURE 1.—Expected meiotic segregation pattern of chromosomes of a triploid. In this figure, we show the meiotic segregation of two homologs in blue and in red. The three copies of each homolog are shown as lines with long dashes, short dashes, or an uninterrupted straight line. Following the second meiotic division, one would expect to get two disomic spores and two monosomic spores for each chromosome. In this depiction, the sister spores "a" and "b" are disomic for the red homolog and monosomic for the blue homolog, whereas the sister spores "c" and "d" have the reciprocal pattern. With equal frequency, one would observe a pair of doubly disomic sister spores and a pair of spores that are monosomic for both chromosomes. In this figure, we have not shown recombination between the homologs, although such exchanges occur in most tetrads.

somes summed over the four spores of the tetrad. On average, however, each tetrad had about 85 chromosomes, representing a loss of about 11 chromosomes distributed over the four spores. Chromosomes IV, VI,
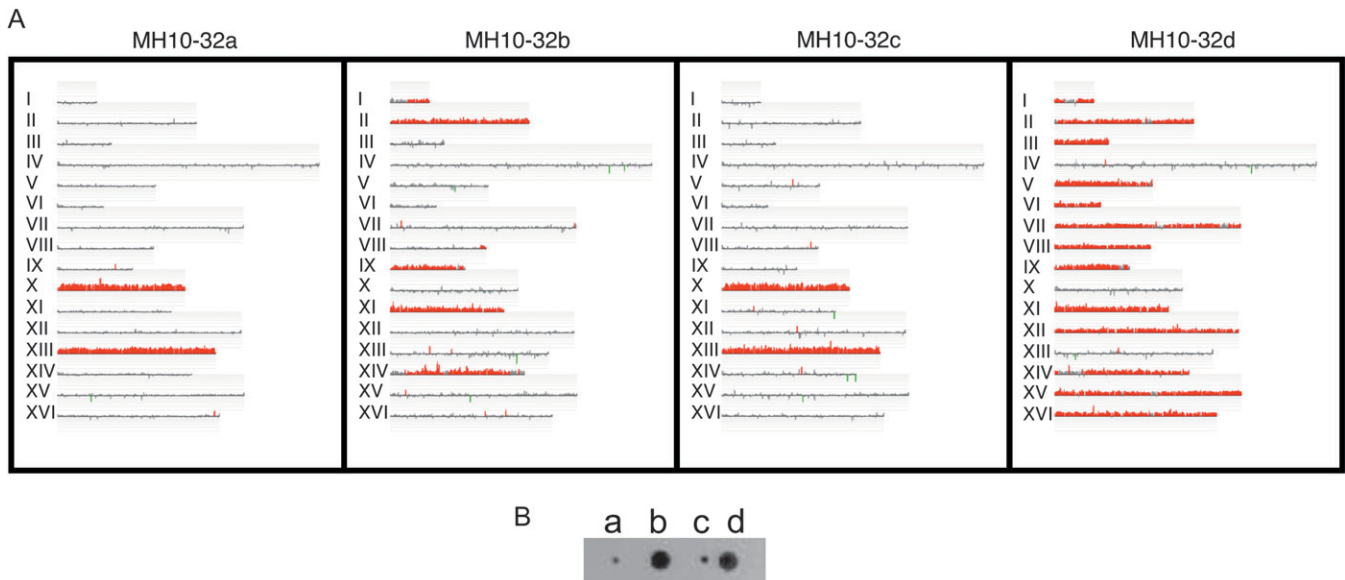
FIGURE 2.—CGH microarray analysis of DNA derived from four spores of a tetrad derived from the triploid MH10. (A) Analysis of the MH10-32 tetrad by CGH microarray. DNA was isolated from individual spore cultures and labeled with Cy5-tagged nucleotides. DNA from the isogenic haploid strain was labeled with Cy3-tagged nucleotides. The individual samples from spore cultures were mixed with the labeled control DNA and the mixture was hybridized to microarrays containing all ORFs and intergenic region. Each chromosome is depicted by a horizontal line whose length is proportional to the length of the chromosome. Red indicates gene amplification relative to the reference strain. The meiotic sister pairs of spores are MH10-32a/MH10-32c and MH10-32b/MH10-32d. Extensive loss of disomic chromosomes is evident in spore MH10-32b. (B) Photograph of MH10-32 spore colonies on the tetrad dissection plate.

and XV were lost at relatively high frequencies and chromosomes I, II, V, VIII, and XI at relatively low frequencies (Table 1).

Previous studies have already shown that spores derived from triploids have unstable karyotypes (PARRY and COX 1970; CAMPBELL *et al.* 1981), although direct measurements of chromosome stability in the spores were not done. In addition, these studies did not determine whether meiotic chromosome segregation was fundamentally accurate in a triploid (PARRY and COX 1970; CAMPBELL *et al.* 1981). To investigate this issue with a more sensitive assay for aneuploidy than CGH arrays, we analyzed sequence polymorphisms that distinguished the three copies of each homolog in the triploid JSC2.

Analysis of meiotic chromosome segregation in a triploid (JSC2): We generated the triploid JSC2 with equal contributions of DNA sequences from three different sequenced backgrounds: S288c, YJM789 (WEI *et al.* 2007), and JAY291 (ARGUESO *et al.* 2009). The details of the construction of the triploid are in MATERIALS AND METHODS. YJM789 and JAY291 have 6.1 and 5.4 single nucleotide polymorphisms (SNPs) per kilobase, respectively, relative to S288c (WEI *et al.* 2007; ARGUESO *et al.* 2009). Although the genome alignments of YJM789 to JAY291 have not been completed, in one 49-kb interval aligned in these strains, there were about 10 SNPs/kb (ARGUESO *et al.* 2009). Using a CGH microarray, we confirmed that JSC2 had three copies of every homolog prior to sporulation.

We were able to distinguish each of the three copies of each homolog for all homologs in JSC2 by PCR amplification of sequences within 5 kb of the centromeres, followed by treatment of the PCR fragment with restriction enzymes that distinguish the amplified regions of each strain. For this analysis, we utilized SNPs that altered restriction enzyme recognition sites. In Figure 3, we illustrate how the three copies of chromosome VI were distinguished by this approach. As shown in Figure 3A, chromosome VI of S288c has a *Bbs*I site near *CEN6* that is not present in JAY291 or YJM789, and JAY291 has a *CEN6*-linked *Eco*RI site that is not present in either S288c or YJM789. Using PCR with primers that flank these heterozygous sites, we generated a DNA fragment of about 530 bp. By examining the sizes of the fragments produced following treatment of the 530-bp fragment with *Eco*RI and *Bbs*I, we can distinguish the three homologs individually (lanes 2–7; Figure 3B) and in pairs (lanes 8–13, Figure 3B). Table S2 lists all the primers and restriction enzymes used to diagnose each chromosome. We refer to this method of analysis as SPA (*s*ingle-nucleotide-polymorphism *P*CR *a*nalysis).

We analyzed 10 JSC2 tetrads that had four viable spores by SPA, and these data are summarized in Table 2. Additionally, six JSC2 tetrads were also analyzed by CGH microarrays (Table S5). Figure 4 shows a CGH analysis of tetrad 5 of JSC2; the SPA data for this same tetrad are in the first four rows of data of Table 2. By both methods, it is clear that the two pairs of meiotic sister

TABLE 1

CGH analysis of disomy and monosomy in spores derived from the triploid MH10

Spore	Colony size	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
												Chromosomes					
4a	Small	**1**	1	**1**	1	**1**	1	**1**	1	**1**	**1**	**1**	**1**	**1**	1	**1**	**1**
4b	Big	1	2	1	2	1	2	1	2^a	1	1	1	1	1	2	1	1
4c	Small	2	1	2	1	2	2^a	2	2^a	2	2	2	2	2	1	2	2
4d	Big	1	2	1	2	1	2	1	1	1	1	1	1	1	2	1	1
22a	Small	2	1	1	1	2	1	2	2	1	1	1	1	1	1	2	1
22b	Big	1	2	1	1	1	1	1	1	1	1	2	1	1	2	1	1
22c	Small	2	1	1	1	2	1	2	2	1	1	1	1	1	1	2	1
22d	Big	1	**1**	1	1	1	1	1	1	1	1	**1**	1	1	**1**	1	1
24a	Big	1	1	2	1	2	1	1	1	2	1	2	1	1	1	1	1
24b	Big	1	1	2	1	2	1	1	1	2	1	2	1	1	1	1	1
24c	Small	2	2	1	1	1	1	2	2	1	2	1	1	1	**1**	1	1
24d	Small	2	2	1	1	1	1	**1**	2	1	2	1	1	1	2	1	1
25a	Small	**1**	2	1	**1**	1	**1**	**1**	**1**	1	2	2	1	1	1	**1**	1
25b	Big	1	1	2	1	2	1	1	1	2	1	1	2	2	2	1	2
25c	Big	1	1	2	1	2	1	1	1	2	1	1	2	2	2	1	2
25d	Big	2	2	1	2	2^a	2	2	2	1	2	2	1	1	1	2	1
28a	Small	1	2^a	2	2	2	1	1	2	2	2	1	1	2	2	1	2
28b	Big	2	1	1	1	1	1	2	1	1	1	2	**1**	1	1	1	1
28c	Small	1	1	**1**	**1**	2	1	1	2	2	**1**	1	1	**1**	**1**	1	**1**
28d	Big	2	2^a	1	1	1	1	2	1	1	1	2	2	1	1	1	1
29a	Average	1	2	1	1	1	2	2	2	1	2	2	2	2	2	2	2
29b	Average	2	1	1	**1**	2	1	1	1	2	1	1	1	1	1	1	1
29c	Average	2	1	1	2	2	1	1	1	2	1	1	1	1	1	1	1
29d	Average	1	2	1	1	1	2	2	2	1	2	2	2	2	2	2	2
30a	Big	1	1	1	1	2	1	1	2	2	1	1	1	1	1	2	2
30b	Small	2	2	2	2	1	2	2	1	1	2	2	2	2	2	1	1
30c	Big	1	1	1	1	2	1	1	2	2	1	1	1	1	1	2	2
30d	Small	2	2	2	2	1	2	**1**	1	1	2	2	2	2	2	1	1
32a	Small	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1
32b	Big	2	2	**1**	1	**1**	**1**	**1**	**1**	2	1	2	**1**	1	2	**1**	**1**
32c	Small	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1
32d	Big	2	2	2	1	2	2	2	2	1	2	2	1	2	2	2	2
34a	Small	**1**	**1**	1	1	1	1	1	1	1	1	1	2	1	**1**	1	1
34b	Big	1	1	2	1	2	1	1	2	1	2	2	1	1	1	1	2
34c	Small	2	2	1	1	1	1	1	1	1	1	1	**1**	1	2	1	1
34d	Big	1	1	2	1	2	1	1	2	1	**1**	**1**	1	1	1	1	**1**
37a	Average	2	1	1	1	1	2	2	1	2	1	1	2	2	2	1	2
37b	Average	1	2	2	1	2	1	1	2	1	2	2	1	1	1	1	1
37c	Average	1	2	2	1	**1**	1	1	2	1	2	2	1	1	1	1	1
37d	Average	2	1	1	1	1	**1**	**1**	1	**1**	1	1	**1**	**1**	**1**	1	2
Average^b		5.7	5.7	5.3	4.7	5.7	4.9	5.2	5.7	5.4	5.5	5.7	5.1	5.1	5.5	4.9	5.2

Based on CGH microarrays, we determined whether the spore cultures were disomic (indicated by 2 in the table) or monosomic (indicated by 1) for each homolog. Boldface indicates a tetrad in which one sister spore was monosomic, although the other sister was disomic, indicating a chromosome loss event in the monosomic spore.

^{a} In these spores, there was a possible chromosome gain. The extra chromosomes were not counted in the averages at the bottom of the table.

^{b} Average number of individual homologs per tetrad.

spores are JSC2-1-5a and -5d, and JSC2-1-5b and -5c. The two methods of analysis are completely concordant for spores JSC2-1-5b and -5c, both methods showing that these strains are disomic for chromosomes I, III, V, X, XII, and XIII.

The spores JSC2-1-5a and -5d are substantially different when examined by CGH and, in addition, show a number of discrepancies when analyzed by CGH *vs.* SPA. By CGH, JSC2-1-5a is disomic for chromosomes I, II, VII-IX, and XIV-XVI, whereas JSC2-1-5d is only disomic for chromosomes II, IX, and XIV. One obvious explanation for this discrepancy is that many of the disomic chromosomes originally present in the JSC2-1-5d spore were rapidly lost during the mitotic divisions
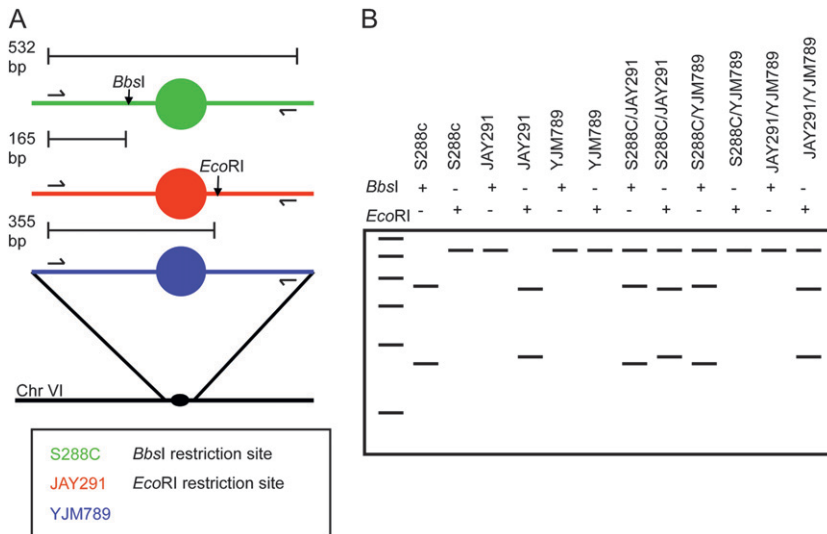
FIGURE 3.—Use of SNPs to determine the number and identity of homologous chromosome homologs within a JSC2-derived spore. For this analysis, we designed primers that could be used to amplify SNP-containing regions located very near (within 10 kb) the centromere of each of the 16 chromosomes (Table S2). By digestion of the resulting PCR products with the relevant restriction enzyme(s), we could diagnose the source of the various copies of each homolog. (A) Depiction of the differential restriction sites present in each strain in the amplified region of chromosome VI. The PCR-amplified regions of S288c, JAY291, and YJM789 are depicted in green, red, and blue, respectively. Positions of PCR primers are indicated by horizontal arrows, and the centromere is indicated by circles. There is a *Bbs*I restriction site on the S288c PCR fragment (shown as a vertical arrow) that is not present in JAY291 or YJM789 and an *Eco*RI restriction site on the JAY291 PCR fragment that is not present in the other two strains. The *Bbs*I and *Eco*RI restriction sites are located at 165 and 355 bp, respectively, from the left end of the PCR fragment. (B) Demonstration of how differential restriction sites can be used to diagnose the source(s) of homologs in spore colonies. Digestion of each possible monosome and heterozygous disome with both *Bbs*I and *Eco*RI produces a unique restriction pattern shown as a schematic gel. The leftmost lane is a 100-bp DNA standard ranging from 100 to 600 bp. The horizontal rows above the gel indicate which lane corresponds to which digest with plus signs indicating treatment with that enzyme.

required to produce the cultures for the CGH analysis. In support of this explanation, when we examined DNA isolated from JSC2-1-5d by SPA, we observed disomy for chromosomes II, IV, VI–IX, XI, and XIV–XVI. This increased level of disomy detected by SPA is the result of two factors. First, it is easier to detect disomy present in a small fraction (less than 12.5%) by SPA than by CGH analysis. Second, we found that some of the spore cultures had two types of cells in approximately equal amounts, one population that had one variant for a particular homolog and one that had a different variant. An example of such a spore culture is shown in Figure 5. We performed SPA on 10 colonies derived from the JSC2-1-8d spore culture; eight had the SNP characteristic of the S288c-derived chromosome II, one had the SNP characteristic of the JAY2981 homolog, and one had both types of homolog, although the S288c-derived homolog predominated. Thus, in this spore colony, although most of cells have only a single copy of chromosome II, the original spore must have had two copies.

As shown in Table 2, in most of the tetrads, most of the homologs segregated to produce two disomic spores and two monosomic spores. This result demonstrates that meiotic chromosome segregation in triploids is accurate, and most of the chromosome loss events detected by CGH arrays represent mitotic chromosome loss. Analyzing 10 MH10 tetrads with CGH arrays, we found 107 chromosome loss events. Analyzing 10 JSC2 tetrads using SPA, we found only 15 chromosome loss events, reflecting the greater sensitivity of SPA in detecting disomy. At least some of these 15 loss events

also represent mitotic loss (as discussed further below), presumably at a very early stage in the growth of the spore colony. Figure 6 shows a comparison of the number of recovered disomes for each method. A line depicts the expected binomial distribution of disomes, assuming that the probabilities of a chromosome being disomic or monosomic are both equal to 0.5. The peaks for the JSC2 spores are shifted to the right relative to the MH10 spores, reflecting the more sensitive nature of SPA compared to CGH. Additionally, the line representing JSC2 spores is symmetrical around eight disomes. Since we examined only complete tetrads, this symmetry is expected.

There were two other observations of interest concerning the JSC2 spore analysis. First, in comparing the CGH and SPA data, we found six examples of apparent chromosome duplication. For example, by CGH, the spores JSC2-1-5a, -5b, and -5c are disomic for chromosome I. By SPA, the spores JSC2-1-5b and -5c both have the S288c-derived and the YJM789-derived copies of chromosome I, but the spore JSC2-1-5a has only the JAY291 homolog. We interpret this result as indicating that a single copy of chromosome I originally present in spore JSC2-1-5a underwent a chromosome gain, either by nondisjunction or by rereplication.

The last unusual class of tetrad likely reflects meiotic gene conversion, the nonreciprocal transfer of information from one chromosome to the other. One example of conversion is JSC2-1 tetrad 8. In this tetrad, for chromosome XII, there were two chromosomes with the centromere-linked SNP characteristic of S288c, three chromosomes with the SNP characteristic of the JAY291,

TABLE 2

Analysis of disomy and monosomy in spores derived from the triploid JSC2-1

Spore	Chromosomes															
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
5a	J[a]	J Y	Y	Y[b]	Y	S Y	S Y	J Y	S J	J	S J	Y	S	J Y	S Y	S J
5b	S Y	S	S J	S	S J	J	J	S	Y	S Y	Y	S J	J Y	S	J	Y
5c	S Y	S	S J	S	S J	J	J	S	Y	S Y	Y	S J	J Y	S	J	Y
5d	J	J Y	Y	J Y	Y	S Y	S Y	J Y	S J	J	S J	Y	S	J Y	S Y	S J
6a	J	J	Y	S	S	J[a]	J	S	J Y	J	J Y	Y	Y	S Y	S	S
6b	S Y	S Y	S J	J Y	J Y	S Y	S Y	J Y	S	S Y	S	S J	S J	J	J Y	J Y
6c	J	J	Y	S	S	J	J	S	J Y	J	J Y	Y	Y	S Y	S	S
6d	S Y	S Y	S J	J Y	J Y	S Y	S Y	J Y	S	S Y	S	S J	S J	J	J Y	J Y
8a	S	S J	J Y	J Y	J	J	S	S J	Y	S Y	J	Y[a]	S J	S Y	S	S J
8b	J Y	Y	S	S	S Y	S Y	J Y	Y	S J	J	S Y	S J	Y	J	J Y	Y
8c	J Y	Y	S	S	S Y	S Y	J Y	Y	S J	J	S Y	S J	Y	J	J Y	Y
8d	S	S J	J Y	J Y	J	J	S	S J	Y	S Y	J	J[c]	S J	S Y	S	S J
9a	Y	S J	J	S	S	S Y	S J	J	S Y	Y	S J	S J	S	S J	S Y	J Y
9b	S J	Y	S Y	J Y	J Y	J	Y	S Y	J	S J	Y	Y	J Y	Y	J	S
9c	S J	Y	S Y	J Y	J Y	J	Y	S Y	J	S J	Y	Y	J **Y**	Y	J	S
9d	Y	S J	J	S	S	S Y	S[b]	J	S Y	Y	S J	S J	S	S J	S Y	J Y
10a	J Y	J Y	J Y	J	J Y	S	S Y	Y	J	S Y	Y	S J	S	S J	J	J Y
10b	**J**[d]Y	Y[b]	J[b]	J	J Y	S	S Y	Y	J	S Y	J	S J	S	J[b]	J	Y[b]
10c	S	S	S	S Y	S	J Y	J	S J	S Y	J	S Y	Y[a]	J Y	Y	S Y	S
10d	S	S	S	Y[b]	S	J Y	J	S J	S Y	J	S Y	Y	Y[b]	Y	S Y	S
11a	S Y	Y	Y	J	S Y	S	S J	Y	J	S Y	Y	Y	Y	S Y	S	J Y
11b	J	S J	S J	S Y	J	J Y	Y	S J	S Y	J	S J	S J	S J	J	J Y	S
11c	J	S J	S J	S Y	J	J Y	Y	S J	S Y	J	S J	S J	S J	J	J Y	S
11d	S Y	Y	Y	J	S Y	S	S J	Y	J	S Y	Y	Y	Y	S Y	S	J Y
12a	Y	S J	J	J	J	S Y	Y	S Y	S Y	S	S J	S	J	S Y	S Y	S J
12b	Y	S J	J	J	J	S Y	Y	S[a,b]	S Y	S	S J	S	J	S Y	S Y	S J
12c	S J	Y	S Y	S Y	S Y	J	S[b]	J	J	J Y	Y	J Y	S S[c]	J	J	Y
12d	S **J**	Y	S Y	S Y	S Y	J	S J	J	J	J Y	Y	J Y	S S[c]	J	J	Y
13a	J	S J	S	S J	S Y	Y	J Y	S J	J	S	J	S Y	S	J	S Y	S
13b	S Y	Y	J Y	Y	J	S J	S	Y	S Y	J Y	S Y	J	J Y	S Y	J	J Y
13c	S Y	Y	J Y	Y	J	S J	S	Y	S Y	J Y	S Y	J[a]	J Y	S Y	J	J Y
13d	J	S J	S	S J[e]	S Y	Y	J Y	S J	J	S	J	S Y	S	J	Y[b,e]	S
14a	J	Y	S J	S	Y	J	S	Y	J	J	Y	S J	S	S	S Y	S
14b	J	Y	S J	S	Y	J	S	Y	J	J	Y	S J	S	S	S Y	S
14c	S Y	S J	Y	J Y	S J	S Y	J Y	S J	S Y	S Y	S J	Y	J **Y**	J Y	J	J Y
14d	S Y	S J	Y	J Y	S J	S Y	J Y	S J	S Y	S Y	S J	Y	J Y	J Y	J	J Y
15a	S Y	S J	S J	Y	J	S J	S J	S	J	J	J	J Y	J Y	S Y	Y	J Y
15b	J	Y	Y	S J	S Y	Y	Y	J Y	S Y	S Y	S Y	S	S	J	S J	S
15c	J	Y	Y	S J	S Y	Y	Y	J Y	S Y	S Y	S Y	S	S	J	S J	S
15d	S Y	S J	S J	Y	J	S J	S J	S	J	J	J	J Y	J Y	S Y	Y	J Y
Average[f]	6	5.9	5.9	5.8	6	6	5.8	5.9	6	6	6	6	5.9	5.9	5.9	5.9

Using SPA (described in text), we determined whether spores were monosomic or disomic, and the source of each homolog (S, S288c; J, JAY291; Y, YJM789).

[a] Extra chromosome homolog detected by CGH microarrays. These extra chromosomes were not counted in the averages shown at the bottom of this table.

[b] Chromosome loss.

[c] Putative gene conversion event.

[d] Boldface indicates that homologs were detected using PCR and primers that exclusively amplified only one of the strain-specific SNPs.

[e] Translocation between chromosome IV and XV detected by CGH microarray and gel analysis.

[f] Average number of individual homologs per tetrad.

and only one chromosome with the SNP characteristic of YJM789. This pattern could be explained if there was a gene conversion that substituted the chromosome XII region on one of the YJM789-derived homologs with information derived from the JAY291-derived homologs.

Since the amount of DNA transferred in meiotic gene conversion is usually limited to about 1–3 kb (PETES *et al.* 1991; MANCERA *et al.* 2008), in the spore representing the putative conversion (JSC2-1-8d), we examined (by SPA) a SNP located on chromosome XII on the opposite side
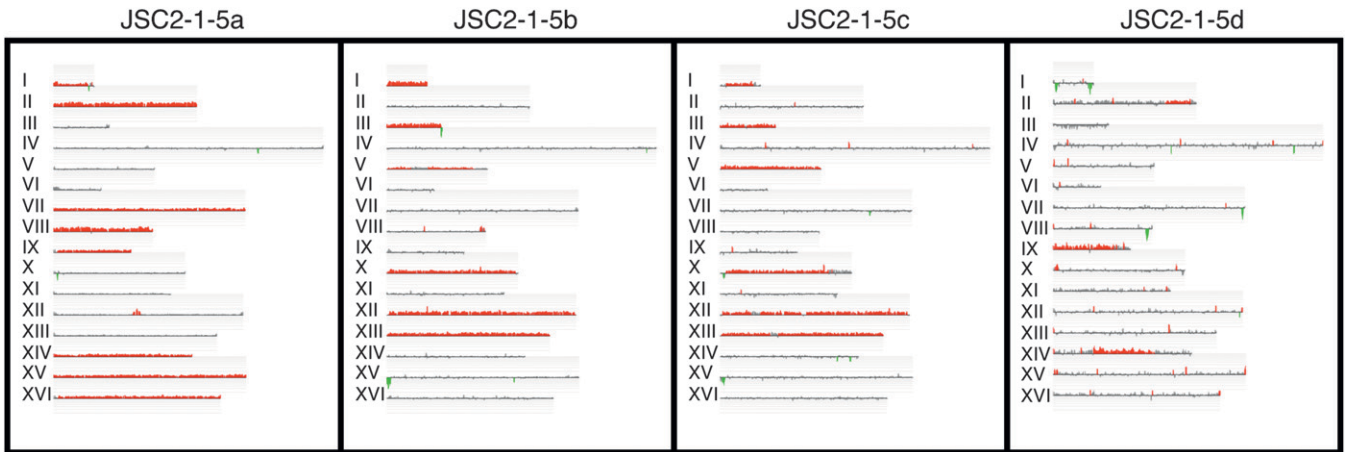
FIGURE 4.—Analysis of a JSC2 tetrad by CGH microarrays. DNA was isolated from spore colonies of a triploid-derived tetrad of JSC2 and examined by the same method described in Figure 2. The meiotic sister pairs are JSC2-1-5a/JSC2-1-5d and JSC2-1-5b/JSC2-1-5c. Extensive loss of disomes from spore JSC2-1-5d is evident.

of the centromere located about 4 kb from the original SNP (primers described in Table S2). As expected, this polymorphism had the SNP characteristic of the YJM789 chromosome. We also observed one tetrad (JSC2-1-12) in which two chromosomes appeared to have undergone gene conversion: four copies of the S288c-associated SNP, no copies of the YJM789-associated SNP, and two copies of the JAY291-associated SNP. Although this tetrad may have undergone a double meiotic event (two conversions of the YJM789 SNP to the S288c SNP), on the basis of the rarity of the single conversion events, it is more likely that the observed pattern reflects a mitotic gene conversion.

Mitotic chromosome loss: Our analysis of JSC2 demonstrates that most tetrads have two spores with two copies and two spores with one copy of each homolog (Table 2).
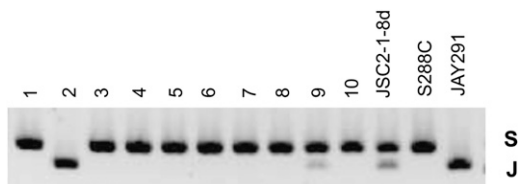

FIGURE 5.—Analysis of SNPs in colonies derived from a single meiotic spore culture (JSC2-1-8d). We isolated DNA from single colonies derived from individual cells of the spore culture JSC2-1-8d and DNA from the original spore culture. By CGH microarrays, this spore had one copy of chromosome II. The region near *CEN2* was amplified in all DNA samples and treated with the restriction enzymes *Dra*I and *Bbs*I, and the resulting fragments were separated by gel electrophoresis. By SPA, the spore colony was disomic for chromosome II with copies derived from S288C and JAY291. In this figure, the *Dra*I digests of the PCR fragment are shown. The digest of DNA isolated from the original spore colony (third lane from the right) indicates that more cells in the culture have the S288c chromosome (uncut band) than the JAY291 chromosome (cut band). This conclusion is confirmed by examining single-colony isolates (labeled 1–10).

However, in some tetrads, one or more chromosomes was lost. As discussed above, such losses have three possible sources: (1) loss of one or more chromosomes in the triploid prior to meiosis, (2) loss of chromosomes during the first or second meiotic divisions, and (3) loss of chromosomes during the mitotic growth of the spores. Several arguments strongly support the third mechanism as the principle factor.

First, we can directly detect chromosome instability in some of the aneuploid spores. We examined the distribution of disomic chromosomes in 10 individual colonies derived from JSC2-1-8c. Our SPA analysis of the chromosome distribution of the original spore colony showed that this spore was disomic for eight chromo-
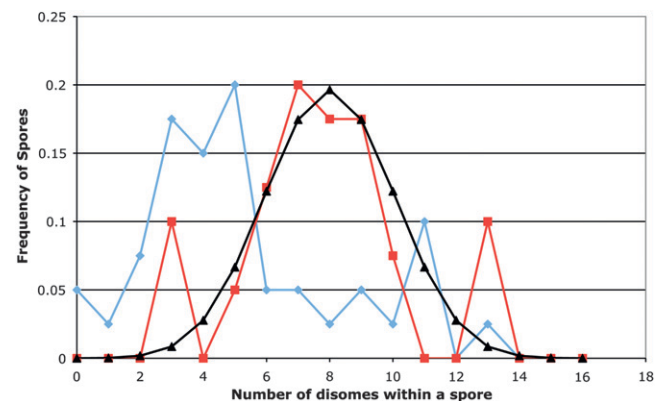

FIGURE 6.—Comparison of the detection of disomes in spores derived from the triploids MH10 and JSC2. The graph shows the number of disomic chromosomes per spore as a function of the frequency of spores with that chromosome composition. The blue line with diamonds represents the data from the MH10 triploid (based on CGH), and the red line with squares shows the data from the JSC2 triploid (based on SPA). The black line with triangles is the curve predicted by the binomial expansion with the assumption that there is the same probability (0.5) that a spore will be monosomic or disomic.
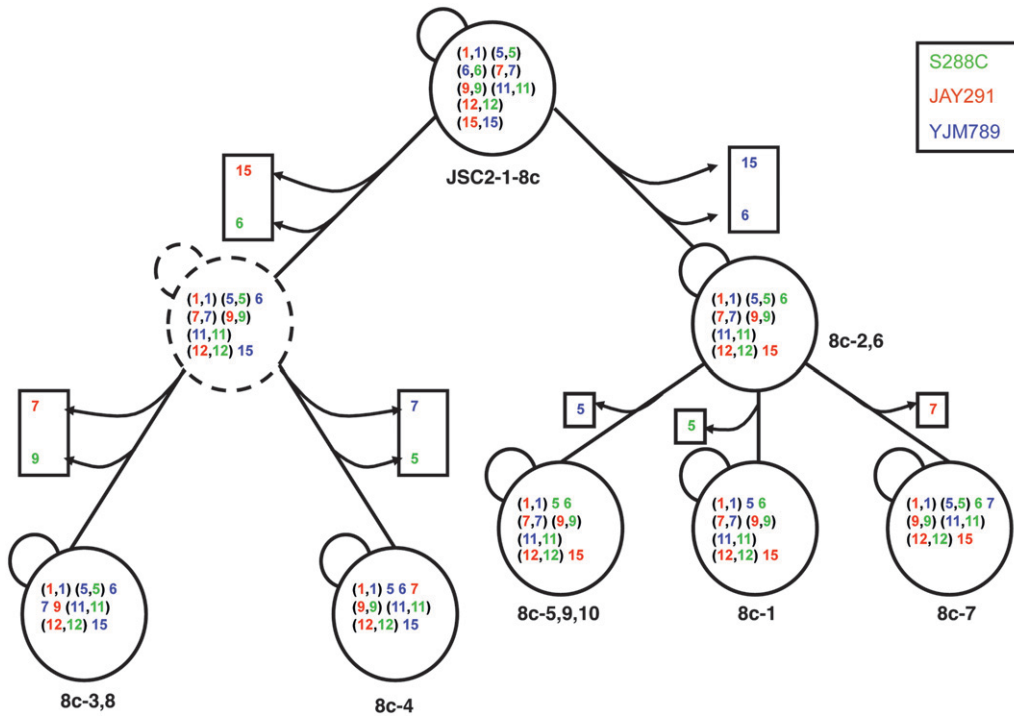
Figure 7.—Mitotic loss of disomic chromosomes from spore JSC2-1-8c. The spore JSC2-1-8c was originally disomic for eight chromosomes (I, V-VII, IX, XI, XII, and XV). We examined 10 single-colony isolates of JSC2-1-8c (labeled 8c-1-10) by SPA, and found six genotypes (shown in solid-line depictions of yeast cells). In this figure, we show a pathway of chromosome loss consistent with these genotypes. The silhouette of a yeast cell with dashed lines is a hypothetical intermediate that was not detected. The numbers within each circle are the chromosome numbers, and the colors indicate the haploid from which the chromosome was derived.
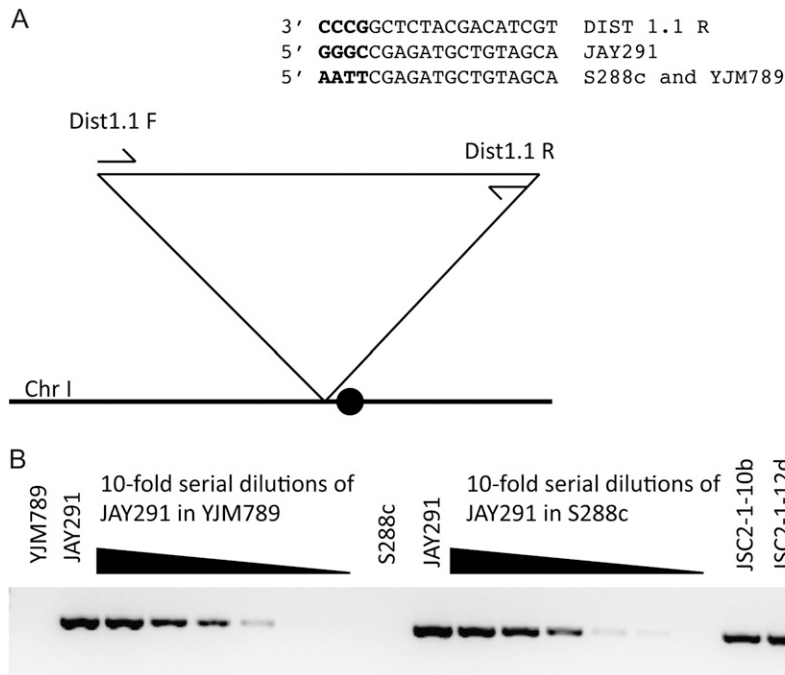
somes (I, V–VII, IX, XI, XII, and XV). As shown in Figure 7, none of the 10 colonies (labeled 8c-1 to 8c-10) examined retained disomy for all eight chromosomes. All disomic chromosomes, except I, XI, and XII, were lost in at least 1 of the 10 colonies. In Figure 7, we show a pathway by which the chromosomes may have been lost; although other pathways could be depicted, the one shown requires the fewest steps.

Although it is difficult to calculate a rate of chromosome loss from this analysis, we estimated the rate of loss of chromosome V in JSC2-8c by another method. The initial spore isolate had two copies of chromosome V, one with the wild-type *URA3* allele and one with the mutant allele. Thus, the spore was initially Ura$^+$. Since the *URA3* gene is centromere linked, the rate of sectored Ura$^+$/Ura$^-$ colonies observed when JSC2-8c colonies cultured on rich growth medium are replica plated to medium lacking uracil is an estimate of the rate of loss of the chromosome with the wild-type allele. Of 8200 colonies examined, 223 were sectored, a rate of about 0.027. This rate of loss is much higher than the rate of chromosome V loss in a wild-type diploid (10^{-5}; Hartwell and Smith 1985). The frequency of chromosome loss was somewhat spore specific, and lower rates of loss were observed in JSC2-1-5b (3.5×10^{-3}) and JSC2-1-6d (no sectored colonies observed).

Since the triploid JSC2 had one copy of chromosome V with a wild-type *URA3* gene and two copies of chromosome V with mutant *ura3* genes, we also examined sectored colonies in this strain. No sectored Ura$^+$/Ura$^-$ colonies were observed in 24,000 examined, suggesting a low rate of chromosome loss ($<4 \times 10^{-5}$). This

result is consistent with the measurement of chromosome loss rate of 1.4×10^{-5} obtained by Mayer and Aguilera (1990). Thus, mitotic loss of chromosomes in the JSC2 triploid prior to sporulation is unlikely to be a substantial contributor to the observed loss in the spores derived from JSC2.

A second argument for a high rate of chromosome loss during mitotic growth of spores of the triploid is that we find a lower rate of chromosome loss when we examine the spores with a method of detection that is more sensitive (SPA *vs.* CGH). For two JSC2 spores, we extended our analysis using a method that was even more sensitive than SPA. By our initial SPA analysis, spore cultures of JSC2-1-10b and JSC2-1-12d were monosomic for chromosome I, although their meiotic sisters were disomic. By SPA, JSC2-1-10b had only the "Y" homolog whereas the sister spore had both the Y and J homologs; for JSC2-1-12d, only the S form was detectable by SPA, although the sister spore had both the S and J homologs. To determine whether cells with the J homolog of chromosome I were present in very low amounts in spore cultures of JSC2-1-10b and JSC2-1-12d, we designed a pair of primers that would specifically amplify a centromere-linked sequence from the J homolog (primer sequence in materials and methods). As shown in Figure 8, by PCR, we found that the J homolog was present in both spore cultures. A similar assay was used to detect missing chromosome XIII in spores JSC2-1-9c and JSC2-1-14c (data not shown). In summary, taking into account these results, there are maximally 11 meiotic chromosome loss events in JSC2 of a total of 960 total chromosomes, establishing an upper limit for the

A

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3' CCCGGCTCTACGACATCGT   DIST 1.1 R
5' GGGCCGAGATGCTGTAGCA   JAY291
5' AATTCGAGATGCTGTAGCA   S288c and YJM789
```

Dist1.1 F

Dist1.1 R

Chr I

B

10-fold serial dilutions of JAY291 in YJM789

10-fold serial dilutions of JAY291 in S288c

YJM789
JAY291
S288c
JAY291
JSC2-1-10b
JSC2-1-12d

FIGURE 8.—PCR method of detecting very small amounts of JAY291-derived chromosome I in strains with S288c- or YJM789-derived chromosomes. Although the sister spores of JSC2-1-10b and JSC2-1-12d were disomic for chromosome I and contained the JAY291-derived homolog, we could not detect this homolog using SPA. Consequently, to detect very small amounts of this homolog, we designed primers to specifically amplify DNA with this polymorphism. (A) Primer combinations DIST1.1F/DIST1.1R amplify a region ~12 kb from the centromere of chromosome I. The 3′-end of DIST1.1R anneals to a 4-bp mismatch region that is present only in the JAY291 strain. (B) 1% agarose gel of a PCR products generated with the DIST1.1F/DIST1.1R primer pair. Genomic DNA from JAY291 was diluted into YJM789 genomic DNA in 10-fold serial dilutions from 10^{-1} to 10^{-6} in lanes 3–8. The same analysis is shown with JAY291 genomic DNA diluted into S288c in lanes 11–16. Lanes 17 and 18 contain the products of the same PCR reaction with genomic DNA from spores JSC2-1-10b and JSC2-1-12d, showing that these samples have the JAY291-specific sequence.

frequency of meiotic chromosome loss events in triploids as 0.01.

Chromosome pairing during meiosis: As discussed in the INTRODUCTION, in near-diploid strains with one trisomic chromosome, all three copies of the trisomic chromosome recombine (SHAFFER *et al.* 1971). To determine whether the three copies of each homolog in triploid also engage in trivalent pairing, we examined meiotic recombination for four chromosomes (IV, V, IX, and XIII) using the same centromere-linked SNPs as in our previous analysis and SNPs located near (within 50 kb) of the left and right telomeres of each chromosome. Table S3 lists the primers and restriction enzymes used for the telomere-linked SNPs, and Table S6 summarizes the results. The evidence for trivalent pairing is that at least one spore contains a chromosome in which there has been a crossover between the centromere-linked markers for the S, J, and Y SNPs and the telomere-linked SNPs. One example of this analysis (for chromosome IV of JSC2-1-8) is shown in Table S6 and Figure 9. In spores with a single copy of chromosome IV (spores -8b and -8c), the coupling of the chromosome IV markers is unambiguous. For spores that are disomic for IV, the coupling relationships of the markers are unclear. For some spores of this type, we screened multiple colonies derived from the original spore colony to identify derivatives that had lost one of the two disomic chromosomes. Analysis of the SNPs in these derivatives clarified the coupling relationships. In this tetrad, there is redundant information demonstrating trivalent pairing. For example, the existence of a single copy of chromosome IV in JSC2-1-8a-2 (a colony derived from the -8a spore) with the centromere-linked Y marker, the left telomere J marker, and the right telomere S marker

(Table S6) is sufficient to argue for trivalent pairing in this meiosis.

Chromosomes IV and V had trivalent pairing in all 10 tetrads. Six of 10 tetrads had trivalent pairing for chromosome IX. Four tetrads (JSC2-1-5, -6, -12, and -13) could not be completely diagnosed because we were unable to isolate derivatives of disomic spores that had only copy of IX; in such spores, we could not determine the coupling arrangements of the markers. Chromosome XIII underwent trivalent pairing in 9 of 10 tetrads, but, in 1 tetrad (JSC2-1-5), the coupling arrangements of the markers on chromosome XIII also could not be determined. In summary, of 35 events in which we could unambiguously determine the recombination patterns, the chromosomes showed trivalent pairing in all cases.

DISCUSSION

In this study, we showed that meiotic segregation of chromosomes in triploids is usually accurate with two spores receiving one copy and two spores receiving two copies of each homolog. As expected from previous studies, spore viability was low (about 50%) compared to the viability of spores derived from diploids. No particular combinations of two or three disomic chromosomes were favored in the viable spores. The aneuploid strains derived from the spores often had very high rates of mitotic chromosome loss. In most meioses, all three copies of each homolog pair and recombine.

Spore viability: We studied meiotic chromosome segregation in two very different triploids. MH10 has three copies of a haploid set derived from MS71 (STRAND *et al.* 1995) and JSC2 is a triploid derived from
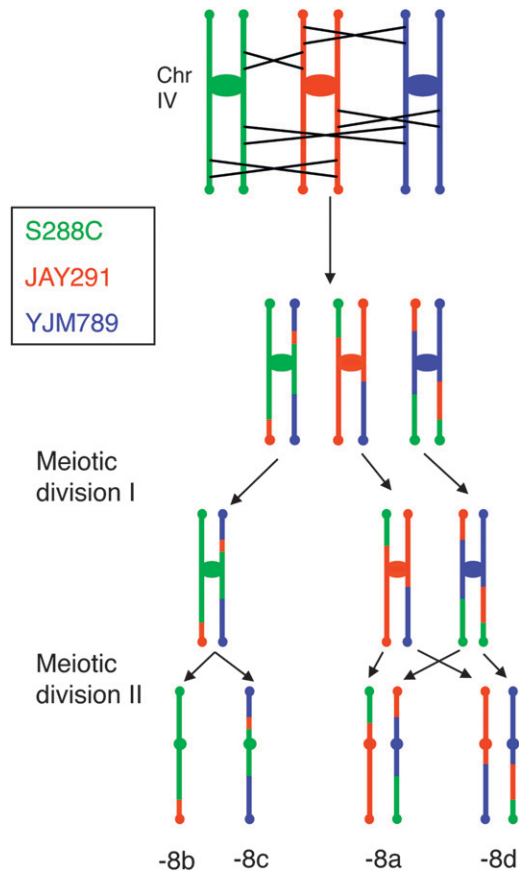
FIGURE 9.—Chromosome pairing and recombination during meiosis in a triploid. As discussed in the text, using SNPs located near the centromere and telomeres, we mapped crossovers on four chromosomes in 10 tetrads (data in Table S6). This figure shows patterns of crossovers consistent with the recombination events detected in tetrad JSC2-1-8 on chromosome IV. The centromeres are shown as circles and ovals at the telomeres represent SNPs; the left and right telomeres are at the top and bottom of the chromosome. The individual spore numbers corresponding to those in Table S6 are shown at the bottom of the figure. In this tetrad, every chromatid engaged in at least one exchange. The pattern of exchanges is shown in this figure is not the only possible pathway for generating the recombinant chromosomes at the bottom of the picture.

three different haploid strains with diverged sequences: S288c, YJM789, and JAY291. Despite this difference, both triploids had similar spore viabilities which were greater than those observed in most past triploid studies (PARRY and COX 1970; CAMPBELL *et al.* 1981; CAMPBELL and DOOLITTLE 1987; LOIDL 1995). The similar spore viability between our strains indicates that the 0.4–1% sequence divergence between homologs in JSC2 does not affect spore viability in triploids.

We examined patterns of chromosome segregation to determine whether certain combination of disomic chromosomes led to spore inviability. All 120 two-chromosome disomy combinations and all 560 three-chromosome disomy combinations within the MH10 and/or JSC2 tetrads were observed in viable spores (Tables 1 and Tables

2). No combination of two or three coincident disomies were significantly overrepresented or underrepresented in the viable spores (analysis described in File S1), consistent with previous studies (CAMPBELL *et al.* 1981).

PARRY and COX (1970) observed that most triploid-derived spores were aneuploid for five chromosomes or less, with an average of 2.6 disomes per spore. They suggested that either triploid segregation produced two near-haploid and two near-diploid spores or that aneuploid chromosomes were rapidly eliminated during vegetative growth of the spore cultures. Our results demonstrate that the second explanation is correct, although the strains in our study seem more tolerant of high levels of disomy (Figure 6) than those examined by PARRY and COX (1970).

Patterns of chromosome loss: Complete or partial loss of disomic chromosomes during the vegetative growth of spore cultures was very common in our study. Two types of observations demonstrate a high rate of chromosome loss. First, the loss events (chromosomes with less than six copies in the tetrad) were much more common when we analyzed the spore cultures by CGH than when we analyzed them by the more sensitive SPA procedure; for six tetrads of JSC2, both methods were used on the same DNA samples. Second, we directly showed that subcultures of some of the disomic spores had unstable karyotypes (Figure 5 and 7).

In MH10-derived spores, we frequently observed that one sister spore was disomic for a particular chromosome and the other was not, suggesting a chromosome loss event. There were 53 such loss events (indicated in boldface in Table 1). These loss events were nonrandomly distributed among the spores; 70% of the losses were in 5 of the 40 spores. In addition, the two sister spores often had very different loss rates. One extreme example is the sister spores MH10-4a and MH10-4c. The MH10-4a spore lost 11 chromosomes (becoming a euploid haploid strain), whereas the MH10-4c spore did not lose any chromosomes.

Two different statistical tests were applied to these data (Table S7). First, for each of the 40 spores, we determined whether there was a significantly elevated rate of chromosome loss compared to the expected rate of loss per disomic chromosome for all 40 spores (0.2 loss events/disomic chromosome). By the chi-square goodness-of-fit test, spores MH10-4a, -22d, -25a, -28b, -32b, and -37c had significantly elevated rates. Second, we used the Fisher exact test to compare chromosome loss events in pairs of sister spores. By this test, five pairs of sister spores had significantly different numbers of loss events (Table S7). Thus, multiple pairs of sister spores in MH10, which presumably initially had identical karyotypes and genotypes, had different rates of chromosome loss. We suggest two explanations of this interesting observation. One possibility is that certain spores receive a partially defective spindle pole body and this defect persists for a number of generations following spore germination,

leading to an elevated rate of chromosome loss. An alternative possibility is that a stochastic loss of one of the disomic chromosomes from a spore results in a much elevated rate of loss of other chromosomes.

Several more points concerning these observations should be made. First, in analyzing the MH10 data, we did not consider chromosome loss events in which all four spores were monosomic, since we could not determine which of the four spores had lost chromosomes. Second, the observation that certain spores have very high rates of chromosome loss in MH10 was considerably less obvious in the JSC2 data (Table S8). Of the 24 JSC2 spore cultures examined by microarrays, we detected 33 examples of loss in one sister but not the other (total of 160 events). Five spores had lost five or more chromosomes, whereas the other 19 spores had lost two or fewer chromosomes. Despite this asymmetry, none of the spores had a significantly elevated rate of chromosome loss compared to the average loss rate for the six tetrads (0.21 events/disomic chromosome). Since the sister spores of JSC2 are not isogenic (unlike those of MH10), it is possible that genotype-dependent mechanisms of chromosome loss prevent the detection of genotype-independent mechanisms of loss.

Another important point to emphasize is that we do not know the mechanism of chromosome loss. If the loss of the disomic chromosome is a consequence of nondisjunction, then we should observe trisomic derivatives at the same frequency as monosomic derivatives. Trisomic strains were not observed, although it is possible that such strains would have a severe growth disadvantage. Although we found a few examples of monosomic chromosomes becoming disomic consistent with chromosome nondisjunction events (Tables 1 and Tables 2), such events were much less frequent than chromosome losses.

An alternative mechanism for chromosome loss is failure to replicate one or both of the disomic chromosomes before cell division. Although we have no direct evidence in favor of this mechanism, the pattern of chromosome loss diagrammed in Figure 7 has several branch points in which symmetrical chromosome loss (consistent with a defect in chromosome replication) is postulated. For example, we show a symmetric loss of chromosomes VI and XV at the first branch point. This pattern is consistent with failure to replicate VI and XV and segregation of the unreplicated homologs to the two daughter cells. To prove this mechanism, however, one would need to perform pedigree analysis on the aneuploid strains.

TORRES *et al.* (2007) constructed 13 yeast strains, each disomic for a different yeast chromosome. All disomic strains had proliferative defects, increased glucose uptake, and were sensitive to cycloheximide, a protein synthesis inhibitor. Strains disomic for chromosomes IV and XV were among those with the most profound proliferative defect, and these chromosomes were among those most frequently lost from the MH10 tetrads. TORRES *et al.*

(2007) were unable to isolate a strain that was disomic only for chromosome VI and suggested that such strains could not be recovered because of the imbalance between levels of β-tubulin (located on chromosome VI) and α-tubulin (located on chromosome XIII). In support of this suggestion, they were able to obtain strains disomic for both VI and XIII. Similarly, in our experiments, chromosome VI was frequently lost from MH10 tetrads (Table 1), although we were able to recover strains that were disomic for VI and monosomic for XIII.

The rate of chromosome loss that we observed in the spore cultures is clearly much greater than that of wild-type diploid cells. The rate of chromosome V loss in a wild-type diploid is about 10^{-5}/cell division (HARTWELL and SMITH 1985). Since many of the disomic chromosomes were lost in less than 50 cell divisions, the lost rate is likely to be considerably greater than 10^{-2}/division. This high loss rate probably reflects defective structures involved in segregation or replication of the chromosomes resulting from nonstochiometric amounts of the proteins required to form these structures. In addition, since most disomic yeast strains grow more slowly than wild type (TORRES *et al.* 2007), those strains that lose chromosomes have a selective advantage. In our study, two of the haploid strains derived by sporulating MH10 (MH10-4a and MH10-22d) lost all disomic chromosomes to revert to haploidy (Table 1).

There is also evidence that chromosome segregation may be inherently less accurate in triploids and tetraploids compared to diploids. GERSTEIN *et al.* (2008) observed that subcultured triploid and tetraploid strains underwent a reduction in chromosome number to diploidy. Since this reduction occurred quickly, they suggested that there was a mechanism of concerted chromosome loss. MAYER and AGUILERA (1990) showed that triploids had chromosome loss rates elevated about 30-fold compared to a diploid (MAYER and AGUILERA 1990; ANDALIS *et al.* 2004). In addition, Mayer and Aguilera found that loss of one chromosome from the triploid or tetraploid increased the likelihood of loss of a second chromosome. STORCHOVA *et al.* (2006) showed that tetraploids have an elevated level of monopolar kinetochore attachments and suggested that tetraploids had partially defective spindle pole bodies, spindles, and/or kinetochores as a consequence of defective "scaling" of complex structures in yeast cells with high ploidies.

Chromosome pairing and recombination: We first examined whether there was any evidence of preferential segregation of homologs derived from the three strains used to construct JSC2. More specifically, using the centromere-linked polymorphism data (Table 2), we tested each of the sixteen homologs for whether the disomic spores had a significant excess of the chromosomes derived from any of the three possible pairs of S288c, YJM789, or JAY291. After correcting for the rate of false discoveries with the BENJAMINI and HOCHBERG (1995) test, no significant strain-specific selection for any

pairing combination was observed (detailed discussion of the statistical analysis in File S1).

Although meiotic recombination in triploids has not been examined in any detail, several studies have shown that all three copies of a trisomic chromosome in a near-diploid strain frequently engage in recombination in a single meiosis (Shaffer *et al.* 1971; Culbertson and Henry 1973; Riley and Manney 1978; Koller *et al.* 1996). In our study, we examined meiotic recombination on chromosomes IV, V, IX, and XIII. As discussed above, in all of the 35 events in which we could unambiguously diagnose meiotic exchanges, all three copies of these homologs recombined. Our results are consistent with the cytological studies of Loidl (1995) in which he observed that all three homologs in triploids were involved in synaptonemal complex formation.

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GENETICS

Meiotic Chromosome Segregation in Triploid
Strains of *Saccharomyces cerevisiae*

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FILE S1

Supporting Materials and Methods

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Statistical analysis of preferential co-segregation of two different homologues into sister spores

Following a triploid meiosis, there are only two ways to distribute the twelve copies of two different homologous chromosomes within a single tetrad: 1) double disomes (two sister spores disomic for both chromosomes and the remaining two sister spores monosomic for both chromosomes) or 2) mixed disomes (two sister spores disomic for one homologue and monosomic for the other with the other sister pair receiving the reciprocal arrangement of chromosomes). If segregation of the chromosomes into four-viable spore tetrads is random, these two events should occur at equal frequencies. Among the MH10/JSC2 data set, for all 120 two-chromosome combinations, we found some tetrads with double-disomes (indicating no double-disome combination is lethal). In addition, since we also recovered some tetrads with mixed disomes for all two-chromosome combinations, there is no situation in which disomy for one type of homologue requires disomy for another type of homologue for viability.

We calculated whether the numbers of double disomes and mixed disomes were significantly different for each two-chromosome combination by comparing the number of tetrads in each class for each homologue with the expectation of equality by using the chi-square "Goodness of Fit" test on the Vassar Stat Website (http://faculty.vassar.edu/lowry/VassarStats.html). After correcting for the false-discovery rate (BENJAMINI and HOCHBERG 1995), no significant departures from equality were observed. We point out, however, that because of the relatively small number of tetrads examined and the large number of comparisons examined, this statistical test is not particularly sensitive.

Statistical analysis of preferential co-segregation of three different homologues into sister spores

There are 560 combinations of three-chromosome disomes. Among the tetrads of MH10 and JSC2, we found all 560 of these combinations, demonstrating that no combination of three-chromosome disomes is lethal.

There are four possible ways to distribute 18 copies of three homologues within a tetrad as shown below:

	Chromosome 1	Chromosome 2	Chromosome 3
Meiotic pair 1	monosome	monosome	monosome
Meiotic pair 2	disome	disome	disome
Meiotic pair 1	monosome	disome	disome
Meiotic pair 2	disome	monosome	monosome
Meiotic pair 1	monosome	disome	monosome
Meiotic pair 2	disome	monosome	disome
Meiotic pair 1	monosome	monosome	disome
Meiotic pair 2	disome	disome	monosome

As with the two-chromosome combinations, each of these situations should occur at equal frequencies if presence of a disome is independent of all other disomes. Because there were so many missing homologues in the MH10 experiment, we tested this expectation with only the JSC2 data. Using the chi-square "Goodness of Fit" test and the BENJAMINI-HOCHBERG correction as described above, we did not detect any significant deviation from random. As discussed above, because of the relatively small numbers of tetrads examined, this test is not sensitive.

Statistical analysis of the non-random segregation of disomic chromosomes in JSC2 based on whether the chromosomes are derived from S288c, JAY291, or YJM789

We also examined whether disomes had a non-random pattern with respect to which genetic background they were derived from. For each spore with a disomic chromosome, there are three possible patterns: 1) one chromosome derived from S288c and one derived from JAY291, 2) one chromosome derived from S288c and one derived from YJM789, and 3) one chromosome derived from JAY291 and one derived from YJM789. In the absence of preferential segregation, these three patterns should be found with equal frequencies. We examined the JSC2 tetrad data for any deviation from random pairing for all 16 chromosomes with both the chi-square "Goodness of Fit" test and the BENJAMINI-HOCHBERG method as described above. No evidence for preferential segregation was obtained.

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that are isogenic to S288C. Yeast **11:** 53-55.

TABLE S1

Strain genotypes and constructions

Strain name	Genotype	Strain construction or reference
MS71	*MATα ade-1 his7-2 leu2-3 LEU2 trp1-289 ura3-52*	(STRAND *et al.* 1995)
JLMy100	*MAT**a**/MATα ade5-1/ade5-1 his7-2/his7-2 leu2-3/leu2-3 LEU2/LEU2 trp1-289/trp1-289 ura3-52/ura3-52 + pSAD3-3b/MEC1*	(J. MCCULLEY, Duke University)
JLMy133	*MAT**a**/matα::URA3 ade5-1/ade5-1 his7-2/his7-2 leu2-3/leu2-3 LEU2/LEU2 trp1-289/trp1-289 ura3-52/ura3-52*	Transformation of JLMy100 with PCR fragment with *matα::URA3*. The template was genomic DNA from a *URA3* yeast strain and primers were Malpha2/URA3F and Malpha1/URA3R (BARBERA and PETES 2006)
MH10	*MAT**a**/MATα/matα::URA3 ade5-1/ade5-1/ade5-1 his7-2/his7-2/his7-2 leu2-3/leu2-3/leu2-3 trp1-289/trp1-289/trp1-289 ura3-52/ura3-52/ura3-52 LEU2/LEU2/LEU2 CAN1/CAN1/CAN1*	Cross of JLMy133 and MS71
JAY291	*MAT**a***	(ARGUESO *et al.* 2009)
FY834	*MATα ura3-52 leu-2Δ1 trp1-Δ63 his3-Δ200 lys2-Δ202*	(WINSTON *et al.* 1995)
JAY306	*MATα/MAT**a** URA3/ura3-52 LEU2/leu2-Δ1 TRP1/trp1-Δ63 HIS3/his3-Δ200 LYS2/lys2-Δ20*	Cross between JAY291 and FY834
PSL4	*MATα ade2-1 ura3 gal2 ho::hisG*	(LEE *et al.* 2009)
JSC1	*matα::NAT/MAT**a** URA3/ura3-52 LEU2/leu2-Δ1 TRP1/trp1-Δ63 HIS3/his3-Δ200 LYS2/lys2-Δ20*	Transformation of JAY306 with a PCR fragment (*matα::NAT^R*). The PCR template was pAG25 (GOLDSTEIN and MCCUSKER 1999). The primers were MatAlpha Nat F and MatAlpha Nat R (LEE *et al.* 2009).
JSC2	*MAT**a**/MATα/matα::NAT URA3/ura3-52/ura3 LEU2/LEU2/leu2-Δ1 TRP1/TRP1/trp1-Δ63 HIS3/HIS3/his3-Δ200 LYS2/LYS2/lys2-Δ20 ADE2/ADE2/ade2-1 GAL2/GAL2/gal2*	Cross between JSC1 and PSL4

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TABLE S2

Primers and restriction enzymes used to distinguish centromeric sequences derived from strains S288c, JAY291, and YJM789[a]

SGD coordinates for forward primer[b]	Primer name[c]	Primer sequence (5' to 3')	Restriction Enzyme	Diagnostic restriction enzymes and fragment sizes following digestion of PCR product		
				Size of PCR fragment after treatment with restriction enzyme for each strain (bp)		
				S288C	**JAY291**	**YJM789**
149166	CEN1 PM F	TCTTTCAGGATGTACCTTTGGATAGC	*Xcm*I	549	150, 399	150, 399
	CEN1 PM R	TACAACGGACAGCGATGGAC	*Hpa*I	256, 293	256, 293	549
239655	CEN2 PM F	CCAAAATACTCCTTAATGACGAAAG	*Bsr*I	158, 324	482	158, 324
	CEN2 PM R	AGATCTCAGCCACCATTCCTAAC	*Dra*I	482	89, 393	89, 393
115864	CEN3 PM F	TCCCAACTCCTTCGGCTAATATG	*Bsl*I	143, 359	502	502
	CEN3 PM R	ATGCGCAGGTGAGCTGATTG	*Pst*I	502	502	131, 371
451010	CEN4 PM F	GGCACATACGCCCTCAAACC	*Bst*XI	543	56, 487	543
	CEN4 PM R	TGGTTGGGACTCCTATATGATGG	*Bsm*I	119, 424	543	543
151849	CEN5 PM F	TTGGTAAACAAAGGGCCAAGC	*Taq*$^{\alpha}$I	447	100, 347	447
	CEN5 PM R	ATGTGCGGCTTTGTCAGCAG	*Cac*8I	132, 315	132, 315	447
148329	CEN6 PM F	AATTTGGGCGATGGAAGAGG	*Bbs*I	165, 367	532	532
	CEN6 PM R	TTCTGCTTTCCTTCCCAAACAG	*Eco*RI	532	177, 355	532

499780	CEN7 PM F	CTTTGCATGCCCGTCACTTG	*Mwo*I	341	141, 200	341
	CEN7 PM R	TCAGATTTGAGTTAGCCCGTTTAGAG	*Dra*III	341	121, 220	121, 220
104245	CEN8 PM F	GGAAATAAACGCGCAAATAAACC	*Nco*I	469	178, 291	178, 291
	CEN8 PM R	TTTGGCAATTGAGGAGGTATGG	*Hph*I	469	190, 279	469
355523	CEN9 PM F	CGGATGAATATTGCCAATGACTG	*Dra*I	173, 173	173, 173	346
	CEN9 PM R	CAGCTGAAGCTTGCCTCTGTATG	*Ase*I	154, 192	346	346
434671	CEN10 PM F	TGCGTCTTGTTCCGAGCTAAAG	*Nsi*I	368	161, 207	161, 207
	CEN10 PM R	CGGATTCCGTTGCGAGTG	*Mfe*I	171, 197	368	171, 197
437899	CEN11 PM F	TTTGATGGGTCAACGACAGG	*Acl*I	274	45, 229	274
	CEN11 PM R	CAAAGTGTTCAACCGAGCTTGC	*Hinf*I	78, 196	274	274
147404	CEN12 PM F	CAGAGGAAGGCGCAATAGAAGC	*Bcg*I	30, 137, 436	604	30, 137, 436
	CEN12 PM R	CCGGAGGATTGTGACCCAAC	*Pst*I	75, 529	75, 529	604
152181	CEN12.2 PM F[c]	GGATGGATAACTCCCTGGTTGA	*Apo*I	68, 129	68,129	196
	CEN12.2 PM R	TTCGAAGACCTTATTTATTCCAACA				
267890	CEN13 PM F	AGGGATGTGATGGCCCTTTC	*Mfe*I	363	78, 285	363
	CEN13 PM R	TCAACTCTAGCCAATTTATCCTGTCG				

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267890	CEN13 PM F	AGGGATGTGATGGCCCTTTC	PCR[d]	258	249	249
	CEN13.2 PM R	TTTAAAGTTCGGAACGCATACAC				
628382	CEN14.1 PM F	GGCTGAGTATGGCGCAATTAG	*Bsr*I	299	299	129, 170
	CEN14.1 PM R	TCCGATTTATTATAGGGTTGCAAAT				
627401	CEN14.4 PM F	TTCGTTGCTGCATCGTTGTC	PCR[d]	151	164	164
	CEN14.5 PM R	AACAGTAAACAGATCTTCCTTGTCCTC				
326813	CEN15.1 PM F	AAGCACCCAATAAGTGCGTTATG	*Bbv*I	87, 230	317	87, 230
	CEN15.1 PM R	CAGCTTATCCAGCGAATGGTCTG				
328652	CEN15.2 PM F	AGTCTGCCTTAGCAAACTTGGAG	*Sau*3AI	137, 173	310	310
	CEN15.2 PM R	TCGCTAGATGATGGGTCGAATC				
555679	CEN16 PM F	GATGATTATAGGCGCATTATAGAGTCG	*Bsm*AI	46, 149	46, 149	195
	CEN16.2 PM R	CGACAACATAACGGCTTCAACC	*Dra*I	195	81, 114	81, 114

[a]As described in the text, we could diagnose strain-specific SNPs for the S288c-, JAY298-, and YJM789-derived chromosomes by PCR amplification of the chromosomal region containing the SNP, followed by restriction digests of the resulting fragments.

[b]The Saccharomyces Genome Database (SGD) coordinate is for the first base of the forward primer.

[c]As discussed in the text, this primer pair was used to characterize a meiotic gene conversion in tetrad 8 of JSC2.

[d]For these PCR products, we diagnosed the source of the homologue by measuring the size of the PCR product rather than by restriction enzyme digestion of the product.

TABLE S3

Primers and restriction enzymes used to distinguish telomeric sequences of chromosomes IV, V, IX, and XIII derived from strains S288c, JAY291, and YJM789[a]

SGD coordinates for forward primer	Primer Name	Primer sequence (5' to 3')	Restriction enzymes used for diagnosis and strains diagnosed			
			Restriction Enzyme	Size of PCR fragment after treatment with restriction enzyme for each strain (bp)		
				S288c	JAY291	YJM789
48202	LA 4.1 F	TTCTGCAAACCTCATTTGAGTAGC	*Hph*I	70, 83, 134	287	287
	LA 4.1 R	AAACCTATGGTTTACGAAATGATCC				
49577	LA 4.2 F	TGATAAAGGAACCATGTGATCTTACG	*Dde*I	169	169	69, 100
	LA 4.2 R	GTGCGTAGAATAACGAACATTCATAGC				
1515307	RA 4 F	AAAGTGGGATTCTGCCTGTGG	*Pvu*I	143, 242	385	385
	RA 4 R	GCGACCTGGGTTTACATTTCC	*Bsa*I	140, 245	140, 245	385
24783	LA 5 F	CACTTGAGGCCACGCATACTG	*Hin*dIII	402	402	120, 282
	LA 5 R	GCAACGTTTGGGAAGAAAACG	*Eco*RV	136, 266	402	136, 266
560490	RA 5 F	TTCTCAGCCGTACAATCATGC	*Taq*[α]I	491	55, 436	491
	RA 5 R	AAACTCCTTCCAAAGGGTCTGG	*Eco*RI	491	491	221, 270

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27145	LA 9 F	TTGCCTTTCGTGTCTATTTGC	*Hph*I	380	69, 311	69, 311
	LA 9 R	TGCCTCGATATACAAATCGTACTGC	*Ahd*I	157, 223	157, 223	380
393735	RA 9 F	CGAGGGATAATAACTGATGAATAGGG	*Taq^a*I	111, 207	318	318
	RA 9 R	TGGGATTCAAGGCATCATCG	*Bsm*AI	318	84, 234	318
13879	LA 13.1 F	TGTGTGTGTGAACTATGGCTACTGG	*Bam*HI	105, 114	105, 114	219
	LA 13.1 R	GCAATGAAGTCACTTTCGTGATGG				
13088	LA 13.2 F	TTTGGTCGGTGGCGATGTAG	*Bsr*I	56, 155	211	56, 155
	LA 13.2 R	TTGTTCAATCATCCTTGAAGCTAAG				
905692	RA 13.1 F	GGAAGAACTCGGGTGTCAGAGC	*Mwo*I	77, 148	225	77, 148
	RA 13.2 R	TCAACGCAGGAAGGGAACG				
904665	RA 13.3 F	TGTTGTAGGCTTGGCCATCG	*Hin*dIII	122, 197	319	319
	RA 13.3 R	CCATCCTCGCCGTTTATCTCG				

[a]As in Table S2, we show the primers and restriction enzymes required to detect sequence-specfic SNPs from the three haploid strains used to construct JSC2. LA and RA indicate the SNPs located near the left and right telomeres, respectively. The chromosome number is indicated in the primer name.

TABLE S4

Patterns of spore viability in the triploid strains JSC2 and MH10[a]

Strain name	Total tetrads dissected	4 live:0 dead	3 live: 1 dead	2 live: 2 dead	1 live: 3 dead	0 live: 4 dead	Spore viability
JSC2-1	77	13	22	22	12	8	0.56
JSC2-2	79	11	15	28	19	6	0.52
MH10	135	24	17	58	19	17	0.52
Totals	291	48	54	108	50	31	0.53
Observed frequencies		0.16	0.19	0.37	0.17	0.11	
Expected frequencies[b]		0.08	0.28	0.37	0.22	0.05	

[a]Tetrads of JSC2 and MH10 were dissected onto solid rich growth medium and the patterns of spore viability monitored by colony growth. JSC2-1 and JSC2-2 are isogenic and were generated by mating the same two parental strains, and picking two different zygotes.

[b]Expected frequencies were calculated by a binomial expansion based on the observed spore viability frequency of 0.53.

TABLE S5

CGH analysis of disomy and monosomy in spores derived six tetrads of the triploid JSC2[a]

Spore	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
									Chromosomes							
5a	2[b]	2	1	1	1	1	2	2	2	1	**1**	1	1	2	2	2
5b	2	1	2	1	2	1	1	1	1	2	1	2	2	1	1	1
5c	2	1	2	1	2	1	1	1	1	2	1	2	2	1	1	1
5d	1	2	1	1	1	1	**1**	**1**	2	1	2	1	1	2	**1**	**1**
6a	1	1	1	1	1	2[b]	1	1	2	1	**1**	1	1	**1**	1	1
6b	2	**1**	2	2	2	2	**1**	2	1	**1**	1	**1**	**1**	1	1	1
6c	1	1	1	1	1	1	1	1	2	1	2	1	1	2	1	1
6d	2	2	2	2	2	2	2	2	1	2	1	2	2	1	1	1
8a	1	2	2	2	1	1	1	2	1	1	1	2[b]	2	**1**	1	2
8b	2	1	1	1	2	2	**1**	1	2	1	**1**	2	1	1	1	1
8c	2	1	1	1	2	2	2	1	2	1	2	2	1	1	1	1
8d	1	**1**	**1**	**1**	1	1	1	2	1	1	1	1	**1**	2	1	**1**
10a	2	2	2	1	2	1	2	1	1	2	1	2	1	2	1	1
10b	**1**	**1**	**1**	1	2	1	**1**	1	1	2	1	2	1	**1**	1	1
10c	1	1	1	2	1	2	1	2	2	1	2	2[b]	2	1	2	1
10d	1	1	1	**1**	1	**1**	1	2	2	1	**1**	1	**1**	1	2	1

12a	1	2	1	1	1	2	1	2	2	1	2	1	1	2	2	2
12b	1	2	1	1	1	2	1	2[b]	2	1	2	1	1	2	2	2
12c	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1
12d	**1**	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1
13a	1	2	1	**1**	2	1	**1**	2	1	1	1	2	1	1	1	1
13b	2	1	2	1	1	1	1	1	2	2	2	1	2	2	1	2
13c	2	1	2	1	1	1	1	1	2	2	**1**	2[b]	2	2	1	2
13d	1	2	1	2	2	1	2	2	1	1	1	1	1	1	1	1
Ave.	**5.7**	**5.5**	**5.7**	**4.8**	**5.7**	**5.0**	**4.8**	**5.8**	**6.0**	**5.2**	**5.2**	**5.5**	**5.2**	**5.5**	**4.8**	**5.0**

[a]Based on CGH microarrays, we determined whether the spore cultures were disomic (indicated by 2 in the table) or monosomic (indicated by 1) for each homologue. Boldface indicates a tetrad in which one sister spore was monosomic, although the other sister was disomic, indicating a chromosome loss event in the monosomic spore.
[b]In these spores, there was a possible chromosome gain. The extra chromosomes were not counted in the averages at the bottom of the table.

TABLE S6

Patterns of marker exchange on chromosomes IV, V, IX, and XIII in spores derived from the triploid JSC2[a]

	Chromosomes											
	IV			V			IX			XIII		
Spore	LT	CEN	RT	LT	CEN	RT	LT	CEN	RT	LT	CEN	RT
5a	S	Y	S	J	Y	J[b]	J Y	S J	S J	S	S	S
5b	J	S	J	S J	S J	J J	S	Y	Y	J Y	J Y	J Y
5c	S	S	S	S Y	S J	S Y	S	Y	Y	J Y	J Y	S Y
5d	J Y	J Y	Y Y	Y	Y	S	J Y	S J	S J	S	S	J
5d-7[c]	J	J	Y									
6a	Y	S	S[b]	S	S	S	S J	J Y	S J	S	Y	S
6b	S J	J Y	S Y	J J	J Y	S J	S	S	S	J Y	S J	S Y
6c	J	S	Y	Y	S	Y	J Y	J Y	J Y	S	Y	J
6d	S Y	J Y	S J	S Y	J Y	J Y	Y	S	Y	J Y	S J	J Y
8a	S J	J Y	S J	J	J	Y	J	Y	Y	S Y[b]	S J	S Y
8a-2	J	Y	S									
8b	S	S	J	S Y	S Y	J Y	J Y	S J	S J	J	Y	J
8c	Y	S	Y	J Y	S Y	S J	S S	S J	S J	Y	Y	J
8d	J Y	J Y	S Y	S	J	S	Y	Y	Y	J J	S J	S Y
8d-1	J	J	Y									
8d-2	Y	Y	S									
9a	S	S	S	S	S	J	S S	S Y	S Y	J	S	S
9b	S Y	J Y	S J	Y Y	J Y	S Y	J	J	J	S J	J Y	S J

9c	J Y	J Y	Y Y	J J	J Y	S Y	Y	J	J	Y	J	Y
9d	J	S	J	S	S	J	J Y	S Y	S Y	S	S	J
10a	J	J	Y	J J	J Y	J Y	S	J	Y	S^b	S	Y
10b	S	J	S	S Y	J Y	J Y	Y	J	J	Y	S	J
10c	S Y	S Y	J Y	S	S	S	S Y	S Y	S Y	J Y	J Y	S J
10d	J	Y	S	Y	S	S	J J	S Y	S J	Y	Y	Y
11a	Y	J	J	S Y	S Y	S Y	J	J	S	J	Y	J
11b	J J	S Y	S Y	J	J	Y	S J	S Y	J Y	S Y	S J	S S
11c	S S	S Y	S J	S	J	J	Y Y	S Y	S Y	S J	S J	J Y
11d	Y	J	Y	J Y	S Y	S J	S	J	J	Y	Y	Y
12a	J	J	J	Y	J	S	J Y	S Y	S Y	Y	J^c	S
12b	S	J	Y	S	J	Y	S Y	S Y	S Y	J	J	J
12c	S J	S Y	S J	J Y	S Y	J Y	J	J	J	J Y	S S	J Y
12d	Y Y	S Y	S Y	S J	S Y	S J	S	J	J	S S	S S	S Y
13a	Y Y	S J	Y Y	S Y	S Y	S J	S	J	J	J	S	S^b
13a-1				S	Y	J						
13a-2				Y	S	S						
13b	J	Y	J	J	J	Y	J Y	S Y	S Y	S J	J Y	J Y
13c	S	Y	J	J	J	J	J Y	S Y	S Y	Y Y	J Y	Y Y
13d	S J	S J	S S	S Y	S Y	S Y	S	J	J	S	S	S
14a	Y	S	J	J	Y	J^b	S	J	J	J	S	S
14b	J	S	S	J	Y	S	J	J	S	J	S	S

14c	J Y	J Y	S Y		Y Y	S J	J Y		S J	S Y	S Y		S ?[d]	J Y	Y ?[d]
14d	S S	J Y	J Y		S S	S J	S J		Y Y	S Y	J Y		S Y	J Y	J Y
15a	Y	Y	S		J	J	J		S	J	J		S J	J Y	S Y
15b	S J	S J	J Y		S Y	S Y	S Y		J Y	S Y	S Y		Y	S	S
15c	S J	S J	J Y		S Y	S Y	J Y		J Y	S Y	S J		S	S	J
15c-1	J	S	J		Y	S	J								
15c-2	S	J	Y		S	J	Y								
15d	Y	Y	S		J	J	S		S	J	Y		J Y	J Y	J Y

[a]As described in the text, we examined the patterns of marker segregation using SNPs located near the centromere (CEN), the left telomere (LT), and the right telomere (RT). As in other tables, chromosomes that are derived from S288c, JAY291, and YJM789 are indicated by S, J, and Y, respectively. The data from the original spore cultures are indicated by a number and a letter (for example, 15c). Strain names that contain an additional hyphen and a number are single colony isolates derived from the original spore culture (for example, 15c-1).

[b] This superscript indicates a tetrad with a meiotic gene conversion event. For example, in tetrad 5, for the SNP at the right end of chromosome V, the Y-associated SNP was converted to a J-associated SNP.

[c]In this tetrad, the CEN-linked SNP for chromosome XIII underwent a double meiotic conversion or a mitotic gene conversion.

[d]The spore culture JSC2-1-14c had much more of the J-derived chromosome than the Y-derived chromosome; the YJM789-derived chromosome could only be detected with primers designed to specifically amplify the YJM789 SNP. Consequently, we detected only one allele for the left and right telomere SNPs JSC2-1-14c.

TABLE S7

Statistical analysis of loss of disomic chromosomes in spores derived from MH10[a]

Spore name[b]	Initial # disomic chrom.[c]	# chrom. lost[d]	Freq. of loss[e]	Exp. # of lost chrom.[f]	(χ^2)[g]	p value for χ^2	p value for sister spore test[h]
4a	11	11	1.0	2.2	39	<0.0001[i]	**<0.0001[i]**
4c	11	0	0	2.2	1.6	0.2	
4b	4	0	0	0.8	0.1	0.7	1
4d	4	0	0	0.8	0.1	0.7	
22a	5	0	0	1	0.3	0.6	**1**
22c	5	0	0	1	0.3	0.6	
22b	3	0	0	0.6	0.02	0.9	0.1
22d	3	3	1.0	0.6	7.5	0.006[i]	
24a	4	0	0	0.8	0.1	0.7	**1**
24b	4	0	0	0.8	0.1	0.7	
24c	6	1	0.2	1.2	0	1	1
24d	6	1	0.2	1.2	0	1	
25a	9	6	0.7	1.8	9.5	0.002[i]	**0.009[i]**
25d	9	0	0	1.8	1.2	0.3	
25b	7	0	0	1.4	0.7	0.4	1
25c	7	0	0	1.4	0.7	0.4	
28a	9	0	0	1.8	1.2	0.3	**0.009[i]**
28c	9	6	0.7	1.8	9.5	0.002[i]	
28b	4	1	0.3	0.8	0	1	1
28d	4	0	0	0.8	0.1	0.8	
29a	11	0	0	2.2	1.6	0.2	**1**
29d	11	0	0	2.2	1.6	0.2	
29b	4	1	0.3	0.8	0	1	1
29c	4	0	0	0.8	0.1	0.8	
30a	5	0	0	1	0.3	0.6	**1**
30c	5	0	0	1	0.3	0.6	
30b	11	0	0	2.2	1.6	0.2	1
30d	11	1	0.1	2.2	0.3	0.6	

32a	2	0	0	0.4	0	1	**1**
32c	2	0	0	0.4	0	1	
32b	13	8	0.6	2.6	11.5	0.0007^i	0.002^i
32d	13	0	0	2.6	2.1	0.1	
34a	4	3	0.8	0.8	4.5	0.03	**0.5**
34c	4	1	0.3	0.8	0	1	
34b	6	0	0	1.2	0.5	0.48	0.2
34d	6	3	0.5	1.2	1.8	0.2	
37a	8	0	0	1.6	1	0.3	**0.007^i**
37d	8	6	0.8	1.6	11.9	0.0006^i	
37b	6	0	0	1.2	0.5	0.5	1
37c	6	1	0.17	1.2	0	1	
Total	**264**	**53**					

[a]This analysis is based on using CGH microarrays to examine disomy and monosomy in spores derived from the MH10 triploid. For this analysis, we count only those chromosomes that were disomic in one spore of the meiotic sister pair and monosomic in the other. This condition allows us to diagnose chromosome loss unambiguously. If a homologue is present in one copy in all spores, we are unable to determine which spores lost the chromosomes.

[b]One pair of sister spores is shown in boldface and the other shown without boldface.

[c]The total number of homologues that are present in two copies in at least one of the two sister spores.

[d]The number of homologues present in one copy in the spore that were present in two copies in the sister spore.

[e]The number of chromosomes lost divided by the initial number of disomic chromosomes.

[f]The expected number of chromosomes lost was calculated by multiplying the initial number of disomic chromosomes by 0.20. The value of 0.20 is the number of chromosomes lost for the whole data set (53) divided by the number of disomic chromosomes for the whole data set (264).

[g]The "goodness of fit" chi-square value was calculated from the observed numbers of chromosomes lost and retained, and the expected numbers of chromosomes lost and retained.

[h]These p values were calculated by comparing the number of lost and retained chromosomes in each member of the pair of meiotic sisters by the Fisher exact test. The boldface numbers represent the calculation for the meiotic sisters shown in boldface in the leftmost column.

[i]Significant difference at a p value of <0.05 after application of the BENJAMINI-HOCHBERG correction (1995).

TABLE S8

Statistical analysis of loss of disomic chromosomes in spores derived from JSC2[a]

Spore name	Initial # disomic chrom.	# chrom. lost	Freq. of loss	Exp. # of lost chrom.	χ^2	p value for χ^2	p value for sister spore test
5a	8	1	0.1	1.7	0.02	0.9	
5d	8	4	0.5	1.7	2.6	0.1	**0.3**
5b	6	0	0	1.2	0.6	0.5	
5c	6	0	0	1.2	0.6	0.5	1
6a	3	2	0.7	0.6	1.6	0.2	
6c	3	0	0	0.6	0.03	0.9	**0.4**
6b	11	5	0.5	2.3	2.7	0.1	
6d	11	0	0	2.3	1.8	0.2	0.04
8a	7	1	0.1	1.4	0	1	
8d	7	5	0.7	1.4	8.1	0.004	**0.1**
8b	7	2	0.3	1.4	0	1	
8c	7	0	0	1.4	0.8	0.4	0.5
10a	8	0	0	1.7	1	0.3	
10b	8	5	0.6	1.7	6.1	0.01	**0.03**
10c	7	0	0	1.4	0.8	0.4	
10d	7	4	0.6	1.4	3.7	0.06	0.07
12a	8	0	0	1.7	1	0.3	
12b	8	0	0	1.7	1	0.3	**1**
12c	2	0	0	0.4	0	1	
12d	2	1	0.5	0.4	0.02	0.9	1
13a	5	2	0.4	1	0.3	0.6	
13d	5	0	0	1	0.3	0.6	**0.4**
13b	8	0	0	1.7	1	0.3	
13c	8	1	0.1	1.7	0.01	0.9	1
Total	**160**	**33**					

[a]This analysis is based on using CGH microarrays to examine disomy and monosomy in spores derived from the JSC2 triploid; to make the comparison similar to that used for MH10, we did not incorporated our SPA data. The methods and descriptions of each column are identical to those previously described for Table S7.