# Nonhomologous Synapsis and Reduced Crossing Over in a Heterozygous Paracentric Inversion in *Saccharomyces cerevisiae*

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ABSTRACT

Homologous chromosome synapsis ("homosynapsis") and crossing over are well-conserved aspects of meiotic chromosome behavior. The long-standing assumption that these two processes are causally related has been challenged recently by observations in *Saccharomyces cerevisiae* of significant levels of crossing over (1) between small sequences at nonhomologous locations and (2) in mutants where synapsis is abnormal or absent. In order to avoid problems of local sequence effects and of mutation pleiotropy, we have perturbed synapsis by making a set of isogenic strains that are heterozygous and homozygous for a large chromosomal paracentric inversion covering a well marked genetic interval and then measured recombination. We find that reciprocal recombination in the marked interval in heterozygotes is reduced variably across the interval, on average to ~55% of that in the homozygotes, and that positive interference still modulates crossing over. Cytologically, stable synapsis across the interval is apparently heterologous rather than homologous, consistent with the interpretation that stable homosynapsis is required to initiate or consummate a large fraction of the crossing over observed in wild-type strains. When crossing over does occur in heterozygotes, dicentric and acentric chromosomes are formed and can be visualized and quantitated on blots though not demonstrated in viable spores. We find that there is no loss of dicentric chromosomes during the two meiotic divisions and that the acentric chromosome is recovered at only ⅓ to ½ of the expected level.

A MAJOR question of meiosis is the degree to which chromosome synapsis and crossing over influence one another. Typically, it has been assumed that homologous synapsis ("homosynapsis") must precede and restrict crossing over to sequences in similar positions on homologous chromosomes. In theory, such restriction would be required since reciprocal recombination between repeated sequences at ectopic locations, if sufficiently frequent, would result in wholesale rearrangement of the genome. Consistent with this assumption, reductions in crossing over are observed when heterozygosity for chromosome rearrangements interferes mechanically with homosynapsis, for instance in inversions in mouse (MOSES *et al.* 1982) and in maize (RHODES and DEMPSEY 1953), and is similarly seen in Drosophila (STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954; CRAYMER 1981; COYNE *et al.* 1991) where the interference with synapsis is inferred. The cytological consequences of an inversion can be complex, as both failure of synapsis ("asynapsis") and synapsis between heterologous segments of chromosomes ("heterosynapsis") can occur as alternatives to homosynapsis in inversions (MCCLINTOCK 1933; MAGUIRE 1966; NUR 1968) or can even replace homosynapsis during pachytene through a process termed synaptic adjustment (MOSES *et al.* 1982).

An alternative to the simple model outlined above is that the commitment to crossing over precedes, or at least is closely related to, the establishment of homosynapsis. This has found support in careful cytological and genetic examinations in larger eukaryotes [see discussions in ZICKLER *et al.* (1992) and MAGUIRE and RIESS (1994)] as well as in *Saccharomyces cerevisiae* [see discussion in HAWLEY and ARBEL (1993)]. In *S. cerevisiae*, crossing over between relatively short segments of homologous DNA at heterologous locations can occur at significant levels during meiotic prophase, suggesting that stable homosynapsis is not an absolute requirement for crossing over [although a methodical cytological examination has not been carried out (JINKS-ROBERTSON and PETES 1986; LICHTEN *et al.* 1987; ROSS *et al.* 1992)]. In addition, although meiotic crossing over is greatly reduced in certain mutants which appear to lack normal synapsis (HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1990; ENGEBRECHT *et al.* 1990; SYM *et al.* 1993), the residual levels of crossing over seen in meiotic cells are significantly higher than those seen in mitotic cells. These observations support models in which the primary role of synapsis is not to promote crossing over but rather to provide for proper chromosome disjunction (MAGUIRE 1978; ENGEBRECHT *et al.* 1990). However, in *S. cerevisiae*, DNA double-strand breaks appear before synapsis (PADMORE *et al.* 1991) and mature recombinant molecules are not detected until late in meiotic prophase. The mature recombinants are observed at pachytene or following desynapsis (PADMORE *et al.* 1991) and very near the time of the first meiotic division (GOYON and LICHTEN 1993; NAG and PETES 1993). Thus,

**TABLE 1**

**Yeast strains**

| | | | |
|---|---|---|---|
| Haploids | | | |
| MDY431 *MATa ade2 CAN1 cyh2 his7-1 leu1-c lys2-2 met13-c tyr1-2 trp1-63 ura3-1* | | | |
| MDY433 *MATα ade5 can1 CYH2 his7-2 leu1-12 lys2-1 met13-3 trp1-63 tyr1-1 ura3-13* | | | |
| MDY459 *MATα ade2 ade5 can1 CYH2 his7-2 leu1-12 lys2-1 met13-d trp1-63 trp5-d tyr1-1 ura3-13* | | | |
| MDY450 = MDY431 transformed with DNA fragments diagrammed in Figure 1B, to make insertions-only control | | | |
| MDY452 = MDY431 transformed with DNA fragments diagrammed in Figure 1C, to make inversion | | | |
| MDY453 = MDY533 transformed with DNA fragments diagrammed in Figure 1B, to make insertions-only control | | | |
| MDY454 = MDY433 transformed with DNA fragments diagrammed in Figure 1C, to make inversion | | | |
| Orientation in targeted interval in diploids | | | |
| MDY no. | 453 (insertions) | 454 (inversion) | 459 (wild-type) |
| 450 (insertions) | Homozygote | Heterozygote | Homozygote |
| 452 (inversion) | Heterozygote | Homozygote | Heterozygote |
| Testers | | | |
| RMT-X *MATa/α ade1 can1 CYH2 his7-1 leu1-12 lys2-1 met13-d trp5-d tyr1-1 ura3-13 hom3* | | | |
| RMT-Y *MATa/α HO ade1 CAN1 cyh2 his7-1 leu1-c lys2-2 met13-c trp5-c try1-2 ura3-1 hom3* | | | |

MDY strains derived from strains originally constructed in the R. E. ESPOSITO laboratory. RMT testers from R. MALONE.

the temporal order of landmark events of recombination and synapsis leave open the possibility of a direct role for synapsis in regulating crossover numbers and/or their locations.

We have set out to examine the effects of large-scale structural heterozygosity on meiotic recombination and synapsis in yeast, in order to avoid problems of local sequence effects and of mutation pleiotropy. We have constructed yeast chromosomes which carry a relatively large, ~300 kb, inversion and have examined the effects of the inversion when heterozygous and homozygous. We find that in heterozygotes, crossing over within the inverted interval is reduced to approximately 55% of wild-type levels, with reductions apparent even across segments of the interval that are relatively distant (greater than ~50 kb) from the breakpoints. Gene conversion is reduced near one breakpoint but occurs at wild-type levels or higher at a locus nearer the center of the interval. Cytological examination revealed no inversion loops, suggesting that the reduction in recombination could result from the absence of stable homosynapsis (*i.e.*, the absence of homosynapsis that persists until the end of pachytene) because synapsis is heterologous rather than homologous in the inverted region in nuclei where synapsis is complete. Alternatively, the observed effects on synapsis and on crossing over could both result from a perturbation that acts before or during zygotene.

Crossing over in the heterozygous inversion produces a dicentric and an acentric chromosome. We do not see a chromatin bridge in whole cells at the first meiotic division nor do we detect breakage of the dicentric on chromosome blots. However, there is a significant delay between the first and second meiotic divisions and a decrease in spore formation, suggesting that a tenuous bridge forms, at least temporarily. So far, we have been unable to recover the dicentric or acentric chromosomes in viable products of sporulation. Although we expected to recover equal numbers of acentric and dicentric chromosomes on the chromosome gels, the acentric is detected at lower levels than the dicentric.

## MATERIALS AND METHODS

**Strains and media:** Genotypes of the strains used in this study are listed in Table 1. Paracentric inversion and non-inverted insertion-only derivatives were made in haploid strains MDY431 and MDY433, then crossed to make diploids homozygous and heterozygous for orientation of the inverted interval. To construct diploids heterozygous for the *URA3* and *TRP1* markers that mark the ends of the inverted (and non-inverted control) intervals, haploid strain MDY459 was constructed from the same background as used for MDY433. Diploids 450X459, 450X453 and 452X454 constitute the control strains (homozygous for orientation), and diploids 452X459, 450X454 and 452X453 are the experimental strains (heterozygous for orientation). Standard growth media are employed throughout.

Strains to be sporulated in liquid medium were grown from fresh single colonies in acetate-containing rich medium, diluted once and grown to $2 \times 10^7$ cells + buds/ml, washed twice with sterile water, once with sporulation medium (2% potassium acetate, pH 7.0, with supplementing amino acids) and then sporulated at $4 \times 10^7$ cells + buds/ml.

**Approach to constructing the inversion:** We set out to invert a well-marked region that would be as large as possible, potentially to be cytologically detectable, and still have sufficiently long regions outside the inversion to direct homosynapsis of each end of the chromosome in a heterozygote. In addition, we chose to make the inversion paracentric rather than pericentric in order to analyze the behavior of dicentric and acentric chromosome products that result from crossing over in the inversion. Our standard laboratory strains contain a well marked interval on the left arm of chromosome *VII*, one of the larger yeast chromosomes. We targeted the *VIIL* interval for inversion by providing the cells with rearranged linear frag-

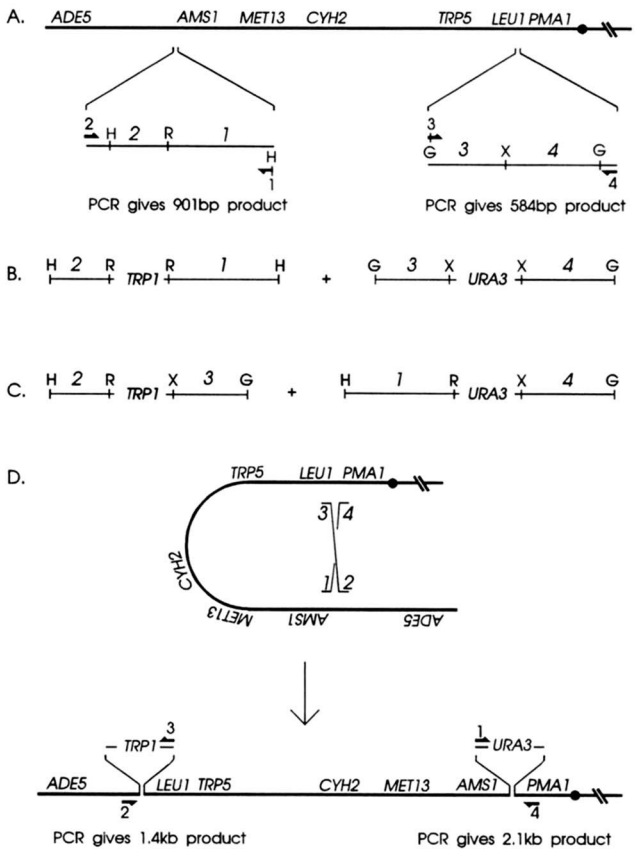


FIGURE 1.—Diagram of the approach used to construct an inversion on the left arm of chromosome *VII*. (A) Diagram of the left arm of chromosome *VII* and of amplification products using primers 1 + 2 and 3 + 4 (half arrows). (B) Diagram of recombinant fragments made from amplification products in A, used to cotransform to make insertions only. (C) Diagram of recombinant fragments made from amplification products in A, used to cotransform to make inversion. (D) Diagram of scheme used to make the inversion by cotransformation of the fragments in C, and to identify the inversion by PCR amplification. G = *Bgl*II, X = *Xba*I, H = *Hin*dIII, R = *Eco*RI.

ments that represent the desired new joins at each end of the interval, as diagrammed in Figure 1.

**Construction of rearranged fragments:** Genomic fragments used to make the rearranged molecules were determined by searching GenBank (Genetics Computer Group, 1991) for sequence information on genes mapped to the appropriate locations on the left arm of chromosome *VII*. Sequences found adjacent to the coding sequences for *AMS1* and *PMA1* were chosen, which by the genetic map lie ~110 cM apart. Data from the physical map (LINDA RILES, unpublished) indicate that interval is ~250–300 kb in length; data from a set of deletions of chromosome *VII* (GIORA SIMCHEN, unpublished) measures the *CUP2-LEU1* interval to be ~325 kb in length, consistent with the upper estimate from the physical map. Primer 1 (5′-CCCAAGCTTCAGGCAAAGAGATTTTCACC) and primer 2 (5′-CTCAATTAAAGGGCAAGATAGC) were used to polymerase chain reaction (PCR)-amplify a 901-bp fragment that contains an *Eco*RI site ~200 bp 5′ to AMS1; primer 1 creates a *Hin*dIII site and primer 2 primes ~42 bp outside an endogenous *Hin*dIII site (Figure 1A). Primer 3 (5′-GCTTAGATCTTTTGAGAAAATTCAGCGGAAACAGCG) and primer 4 (5′-GGCTTTACGTCATAATAGTTCC) were used to PCR-amplify a 584-bp fragment that contains an *Xba*I site ~230 bp 5′ to the *LEU1* coding sequence (3′ to *PMA1* sequence); primer 3 creates a *Bgl*II site and primer 4 primes ~50 bp outside an endogenous *Bgl*II site (Figure 1A). The amplified products were cloned into pCR1000 (InVitrogen) then assembled in various combinations with *URA3* and *TRP1* genes as cloning markers to give appropriate pairs of molecules (Figure 1, B and C). Note that fragments 2 and 4 no longer contain the sites primed by primers 2 and 4, so that integration of these fragments into their respective genomic locations is required for amplification of the proper-sized products from genomic DNA.

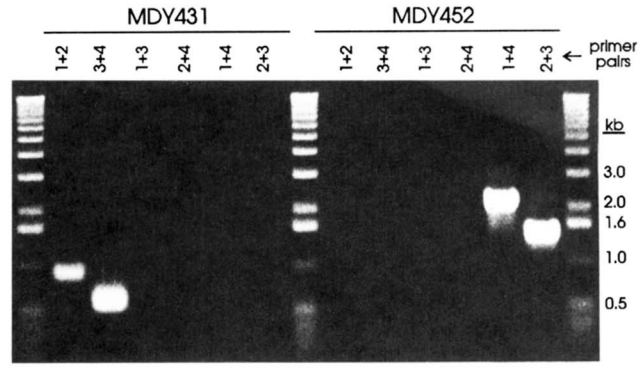


FIGURE 2.—Ethidium bromide-stained agarose gel of amplification products for parent (MDY431) and inversion-containing (MDY452) haploid strains. Primer pair numbers coincide with those in Figure 1.

**Cotransformation and confirmation of new joins in the genome:** Transformants were selected on synthetic media lacking both tryptophan and uracil following cotransformation of appropriate pairs of constructs into yeast strains MDY431 and MDY433 (transformation method of SCHIESTL and GIETZ 1989). Transformations to construct inversions employed ~1 μg of each linear fragment and produced approximately 200 $TRP^+/URA^+$ transformants total. Genomic DNA for PCR amplification was prepared by glass bead lysis (RUNGE and ZAKIAN 1989). Amplification using cruder cell preparations gave less consistent results in these strains. Amplification was carried out in a 50-μl reaction using 3 μl of a 1/10 dilution of the final DNA preparation and each of the four primers in six total pairwise combinations (Figure 2).

**Electrophoretic analysis:** Samples to be run on chromosome separation gels [contour-clamped homogeneous electric field (CHEF) gels, using the Bio-Rad DRII system] were prepared by standard protocols (GERRING *et al.* 1991). For sporulating cultures, samples taken at or beyond 16 hr post shift were processed using double the concentration of Zymolyase as compared with earlier time points. Southern blots from the chromosome gels were hybridized with random-primed, $^{32}P$-labeled probes from gel-purified fragments of the *TRP5* and *CYH2* genes, to detect the normal chromosome *VII* as well as the expected dicentric and acentric products of recombination (see Figure 3), and with an internal fragment of the *TRP1* gene to detect only the normal and acentric chromosomes *VII*. As spore walls form it becomes more difficult to recover intact chromosomes; as a loading and recovery control, blots were also simultaneously or sequentially probed with a fragment of the *LYS2* gene to detect chromosome *II*. Quantitation was carried out by directly assessing the counts per minute per band using an Ambis system. Counts were corrected for "local" background (the dicentric band overlaps that of chromosome *XII* which leads to a higher background in that area of the blots) as well as for loading/recovery (using the *LYS2* probe).

**Cytological analysis:** The extent of sporulation (percent of cells in the population that formed asci) and the number

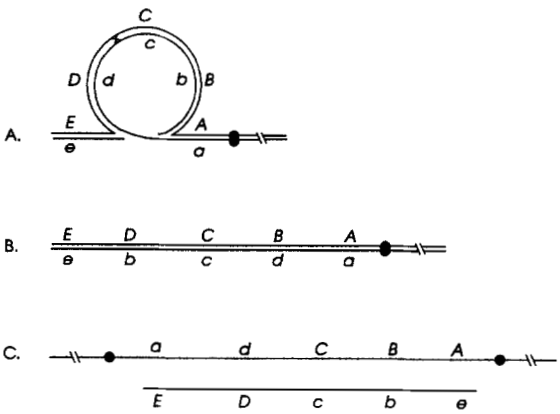


FIGURE 3.—Diagram of possible configurations of synaptonemal complexes and of the products of crossing over in the heterozygous inverted interval. The centromere position is marked by the filled circles. (A) Synaptonemal complex morphology if synapsis is homologous. (B) Synaptonemal complex morphology if synapsis is heterologous. (C) Expected dicentric and acentric chromosomes formed as a result of reciprocal recombination at the small "x" in A.

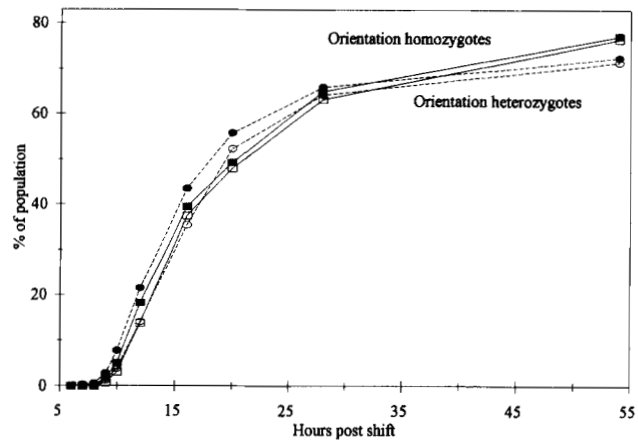


FIGURE 4.—Plot of the kinetics of meiosis in inversion heterozygotes and homozygotes. Liquid cultures were shifted into meiosis and scored for progress into or beyond the first meiotic division (filled markers) or into or beyond the second meiotic division (open markers) assayed by DAPI-staining and fluorescence microscopy. Each point represents the average of four isolates of the inversion heterozygotes (ovals, two from 450X454 and two from 452X453) or four isolates of the orientation homozygotes (boxes, two from 450X453 and two from 452X454). Standard deviations for the points at 10 hr post shift and above range from 1.5 to 7.3, with no obvious pattern to the distribution of the larger and smaller values.

of spores formed in each ascus (from cultures sporulated on solid medium) were analyzed for eight independent isolates of each cross, using differential interference contrast optics at 2,000× final magnification and counting 100 cells per isolate; sporulation conditions were the same as used for the tetrad analyses.

The kinetics of sporulation in liquid medium was assayed using a rapid 4,6-diamidino-2-phenylindole (DAPI)-staining method, where 10 μl of culture and stain (1 μg/ml DAPI in 95% ethanol) are mixed and viewed immediately using fluorescence microscopy to visualize the stained nuclei. Cells designated as "into or beyond the first division" include cells that are in the first division, binucleate, in the second meiotic division, tetranucleate, or by phase microscopy (at the latest stages of sporulation) containing formed spore walls; cells designated as "into or beyond the second division" are in the second meiotic division or beyond (Figure 4). Our use of the terms "binucleate" and "tetranucleate" are not strictly correct, as karyokinesis is incomplete until spore wall formation (MOENS and RAPPORT 1971) and indicate only that, in whole cells stained with DAPI, there are two or four distinct chromatin masses.Nuclei for electron microscopic analysis of synapsis were prepared in three separate experiments from liquid sporulation cultures of MDY452X459, using the methods of DRESSER and GIROUX (1988). Several hundred nuclei, representing all stages of meiotic prophase, were examined at the electron microscope.

**Genetic analysis:** Tetrad analysis was performed by cross-stamping fresh isolates of the haploids onto rich medium to allow mating, transferring to sporulation medium after 1 day at 30°, then dissecting tetrads after 3 days at 30°. Spore clones were allowed to grow into large colonies on the dissection plates, were scored for viability visually and microscopically, then were replica-plated to test for sectoring for cycloheximide resistance or for red/white color (*ADE5* segregating in an *ade2* background), results expected if unstable dicentric or acentric products, respectively, are recovered in live spores. The colonies were then patch-mastered on rich medium and tested for auxotrophic markers and mating type. Allelism testing was carried out by mating spore clones to RMT-X and RMT-Y, sporulating the resulting diploids, then scoring for papillation on medium lacking one of the required nutrients; prototrophy results largely from gene conversion to wild type during sporulation.

Map distances based on the genetic data were calculated using the PERKINS (1949) equation. Tetrad rank for the inverted interval (bounded by *URA3* and *TRP1*) was also used to estimate the level of crossing over, according to the KING and MORTIMER (1991) model, using a computer program provided by KING. Derivations of the KING and MORTIMER model, used to estimate levels of crossing over in the inversion heterozygotes, were calculated using MathCad (MATHSoft).

Levels of gene conversion at *HIS7*, *LEU1*, *LYS2*, *MET13* and *TYR1* were estimated for sporulated cultures of six independent isolates each of 450X459 and 452X459 by counting the number of colonies when plated on medium lacking the appropriate amino acid and dividing by the number of colonies on complete synthetic medium. Parallel platings of the cultures from vegetative growth medium, before transfer to sporulative medium, were performed for each isolate to rule out jackpots.

**Correction for losses in spore viability:** Spore viability is corrected for the losses due to dicentric/acentric formation by the following. When random spore death alone accounts for observed levels of spore viability, spore viability by tetrad can be modeled by the binomial expansion $(v + i)^4 = v^4 + 4v^3i + 6v^2i^2 + 4vi^3 + i^4$, where $v$ = frequency of viable spores given "random" spore death, $i$ = frequency of inviable spores, and each term in the expansion represents the frequency of 4–0 spores-viable classes, respectively. Setting $v$ = (live spores/total spores dissected) the proportions of the classes seen in the control strains show a reasonable fit with this simple model but the inversion heterozygotes do not, as expected (using the data in Table 3, results not shown). Similarly, given that two spores at a time are eliminated by formation of dicentric/acentric products, the model for the 2–0 spores-viable tetrad classes that result from one such event is the expansion of $(v + i)^2$. Finally, the 0 spores-viable tetrad class that results from two dicentrics/acentrics makes up the remaining fraction of tetrads. Overall spore viability in the inversion heterozygote

population then reflects three subpopulations whose relative proportions depend on the frequency with which 0, 1 or 2 dicentrics/acentrics are formed during meiosis. The model for spore viability then becomes the sum of the models for the three subpopulations, each of which is present at some frequency, $f_n$, where $n$ is the number of dicentric/acentric products. Thus, $[f_0(v + i)^4 + f_1(v + i)^2 + f_2] = [f_0(v^4)] + [f_0(4v^3i)] + [f_0(6v^2i^2) + f_1(v^2)] + [f_0(4vi^3) + f_1(2vi)] + [f_0i^4 + f_1i^2 + f_2]$, where each bracketed term represents the 4–0 spores-viable classes, respectively.

## RESULTS

**Cotransformation with rearranged fragments leads to formation of the predicted inversion:** PCR amplification employing primers that flank the breakpoints generates products of the sizes predicted for the new DNA joins (Figures 1 and 2). The appearance in CHEF gel blots of two new chromosome-sized bands generated by crossing over within the inverted segment in a heterozygote demonstrates physically the presence of the inversion (Figures 3 and 5A). These bands represent the dicentric (~1,580 kb) and acentric (~690 kb) chromosomes that are formed when a single chromatid is involved in an odd number of crossovers in the inverted interval. The sizes sum to ~2,270 kb, which is twice the length of a normal chromosome *VII* (~1,110 kb in these strains), the expected result.

**The insertions of *URA3* and *TRP1*, and inversion of the included interval, have no obvious effect on spore germination or vegetative growth:** Haploid strains that carry the inversion and the control insertions produce colonies that grow at the same rate as the parental strains (data not shown). The diploid inversion heterozygotes also show no reduction in growth rates by comparison with the homozygous diploids. Viable spores form visible colonies at the same rates whether they contain the wild-type, inversion or insertion chromosomes.

**The kinetics of the meiotic divisions are affected by inversion heterozygosity:** Given that there is essentially no genetic length between the centromere and the inversion, in virtually all cases the two centromeres of a dicentric formed by crossing over are expected to segregate at the first rather than the second division, thus giving rise to a chromosome bridge. So far, we have not seen a clear bridge, *i.e.*, a persistent DAPI-fluorescent strand, between the separating chromosome masses at meiosis I. However, the two divisions are more distinct temporally in the heterozygotes than in the controls (Figure 4), and when the data for the heterozygotes *vs.* the homozygotes are pooled, the numbers of "binucleates" are significantly higher in the heterozygotes at 10, 12, 16 and 20 hr ($G$ test with $P < 0.05$, SOKAL and ROLHF 1969), indicating a delay. Thus, while there is not a complete block in the meiosis I or binucleate stages, a chromosome bridge, however tenuous or quickly resolved (perhaps via one centromere detaching from the spindle in later anaphase), may form and cause the observed delay. Bridge formation could also account for the failure of some dicentrics to be incorporated into spores as well as for the failure of spore walls to form around 2 of the 4 nuclei following the two meiotic divisions (see below). The heterozygotes also seem to enter meiosis I earlier than the homozygotes; the reason for this is not clear.

**TABLE 2**

**Final sporulation results**

| Strain | No. of spores per ascus (%) 0 (veg) | 1 | 2 | 3 | 4 | Final percent sporulation |
|---|---|---|---|---|---|---|
| Orientation homozygotes | | | | | | |
| 450X459 | 6 | 3 | 15 | 34 | 44 | 94 |
| 450X453 | 9 | 1 | 25 | 36 | 29 | 91 |
| 452X454 | 11 | 2 | 22 | 31 | 34 | 89 |
| Average | 9 | 2 | 21 | 34 | 35 | 91 |
| Orientation heterozygotes | | | | | | |
| 452X459 | 12 | 10 | 32 | 20 | 26 | 87 |
| 450X454 | 20 | 2 | 38 | 25 | 15 | 80 |
| 452X453 | 29 | 4 | 37 | 17 | 13 | 71 |
| Average | 20 | 5 | 36 | 21 | 18 | 79 |

Average of eight independent isolates for each diploid. Values for the homozygotes are similar to values observed for related homothallic strains (data not shown).

**The final products of sporulation are affected by inversion heterozygosity:** On solid sporulation medium, the inversion heterozygotes reproducibly sporulate to slightly lower levels than the controls (Table 2), consistent with the slight decrease seen in liquid sporulation medium (Figure 4). In addition, the number of spores formed per ascus is lower in the heterozygotes, with a reduction in the number of 4-spored asci and an increase in the number of 2-spored asci (Table 2). This may be related to the delay in the divisions, either because a bridge forms and prevents 2 spore walls from forming or because the delay itself occurs at a sensitive time in development (DAVIDOW *et al.* 1980). In either case, we would expect the 2 spores to contain non-sister products of the reductional division, which is observed by dissection of 2-spored asci where 21/23 "dyads" from 452X459 give 1 ura$^-$ and 1 URA$^+$ spore colony (using the URA3 adjacent to *LEU1* as a centromere-linked marker). As the same bias is observed for "dyads" from the control 450X459, where 21/28 contain non-sister products, the role of the putative bridge is not clear. There is also a clear decrease in spore viability in spores dissected from tetrads (by definition, from 4-spored asci) seen mainly in the increase in the numbers of 2 spores-viable asci, and the decrease is distinguishable from "random spore inviability" (Table 3) in that it acts on 2 spores at a time rather than on each of the 4 spores independently (see MATERIALS AND METHODS). This too could result, at least in part, from bridge formation and consequent exclusion of the dicentric (and perhaps the acentric) from incorporation into a spore.

Overall losses before tetrad analysis can be compared as follows: In the orientation homozygote 450X459,

**TABLE 3**

**Spore viability in tetrads**

| Strain | No. of tetrads | Viability | $v$ | $f_0, f_1, f_2$ | | Tetrad class, percent of total 4 | 3 | 2 | 1 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|
| Orientation homozygotes | | | | | | | | | | |
| 450X459 | 194 | 0.93 | | | Obs | 79 | 12 | 7 | 1 | 0 |
| | | | 0.96 | 0.93,0.07,0.00 | Calc | 79 | 13 | 6 | 1 | 0 |
| 450X453 | 183 | 0.85 | | | Obs | 60 | 26 | 9 | 3 | 1 |
| | | | 0.89 | 0.95,0.04,0.01 | Calc | 60 | 29 | 9 | 1 | 1 |
| 452X454 | 122 | 0.95 | | | Obs | 82 | 16 | 1 | 0 | 2 |
| | | | 0.96 | 0.97,0.00,0.03 | Calc | 82 | 14 | 1 | 0 | 3 |
| Orientation heterozygotes | | | | | | | | | | |
| 452X459 | 391 | 0.69 | | | Obs | 38 | 9 | 43 | 8 | 3 |
| | | | 0.94 | 0.49,0.48,0.03 | Calc | 38 | 10 | 43 | 5 | 3 |
| 450X454 | 261 | 0.74 | | | Obs | 50 | 6 | 38 | 4 | 2 |
| | | | 0.97 | 0.57,0.41,0.02 | Calc | 50 | 6 | 39 | 2 | 2 |
| 452X453 | 256 | 0.68 | | | Obs | 40 | 10 | 36 | 9 | 5 |
| | | | 0.93 | 0.53,0.42,0.05 | Calc | 40 | 12 | 38 | 6 | 5 |

The upper three strains are homozygous for orientation in the inversion interval; the lower three are heterozygous. Tetrad class is number of viable spores/tetrad, and viability is the simple ratio of live/total spores dissected. $v$ = spore viability calculated assuming "random" spore death, $f_0$–$f_2$ = frequency of subpopulations containing 0–2 dicentric (and acentric) chromosomes. For details, see MATERIALS AND METHODS.

~44% of the starting cells form 4-spored asci, ~79% of which have all 4 spores viable and are analyzed as tetrads; in the orientation heterozygote 452X459, the values are ~26% and ~38%, respectively. Thus, of the cells shifted into meiosis, ~35% of the homozygote and ~10% of the heterozygote strains are analyzed as 4 spores-viable tetrads.

**The products of reciprocal recombination in the inversion interval appear well before the first meiotic division and remain intact as sporulation progresses:** Chromosomes from inversion heterozygotes were separated in CHEF gels, blotted and probed with *TRP5* to detect the normal and dicentric and acentric derivatives of chromosome *VII* and with *LYS2* (for chromosome *II*) as a loading/recovery control. An autoradiogram of samples from meiotic time points and plots of the corrected counts over the bands from two of the inversion heterozygotes are shown in Figure 5. The dicentric and acentric chromosomes appear well before the first meiotic division and show no evidence of smearing or loss throughout sporulation (as would be expected, *e.g.*, if the dicentric were being broken). Using the times at which cells at or beyond metaphase I have reached 50% of the maximum, and at which the dicentric and acentric chromosomes (summed) reach 50% of the maximum amounts, products of reciprocal recombination apparently are formed well before the first meiotic division, roughly 1.7 and 4.0 hr for the two experiments reported in Figure 5, B and C, respectively.

**The dicentric, acentric and normal chromosomes *VII* are differentially recovered from sporulating cells:** A reciprocal exchange within the inversion generates one dicentric and one acentric chromosome, each of which carries a single copy of *TRP5*. Thus, the label over the two bands should be equal in amount at each time point. However, on average there are ~2-fold as many counts in the dicentric as in the acentric bands when labeled with *TRP5* probe. A similar difference (~3-fold) is seen with *CYH2* probe. Re-probing the blots with *TRP1* sequence, which should be present in two, one and zero copies on the acentric, normal and dicentric chromosomes, respectively (as for the marker at *E(e)* in Figure 3C), doubles the acentric/normal *VII* ratio and reveals no signal over the dicentric band or elsewhere (the normal copy of *TRP1* on chromosome *IV* has been deleted from these strains).

Control experiments exclude the hypothesis that the larger size of the dicentric leads to its preferential extraction. In a diploid strain with single copies of *TRP1* on chromosomes *V* (~610 kb) and *VII* (and deleted from *IV*), Southern blot analysis shows slightly more signal over the *shorter* chromosome (*i.e.*, a difference in the opposite direction from that seen for the acentric and dicentric) at all time points. Thus, the difference in recovery of acentric and dicentric chromosomes does not result from a simple technical artifact based on length. Also, recovery of the dicentric and acentric is less affected by the events of late sporulation than is the normal *VII*. This is most evident when comparing the 28-hr with the 54-hr time points in Figure 5, B and C. The difference may result from the acentric and dicentric chromosomes being packaged into spores less frequently and thus being more easily extracted (GAME *et al.* 1989; NAG and PETES 1993). In order to test the possibility that the dicentric is preferentially excluded from spores, we isolated spores by mild Zymolyase digestion and vigorous vortexing prior to preparing CHEF gel plugs. In one experiment, this pre-treatment reduced the amount of the normal, dicentric and acentric chromosomes recovered to 77, 58 and 70%, respectively, of the un-pretreated samples. These results suggest that the dicentric chromosome is more susceptible to the

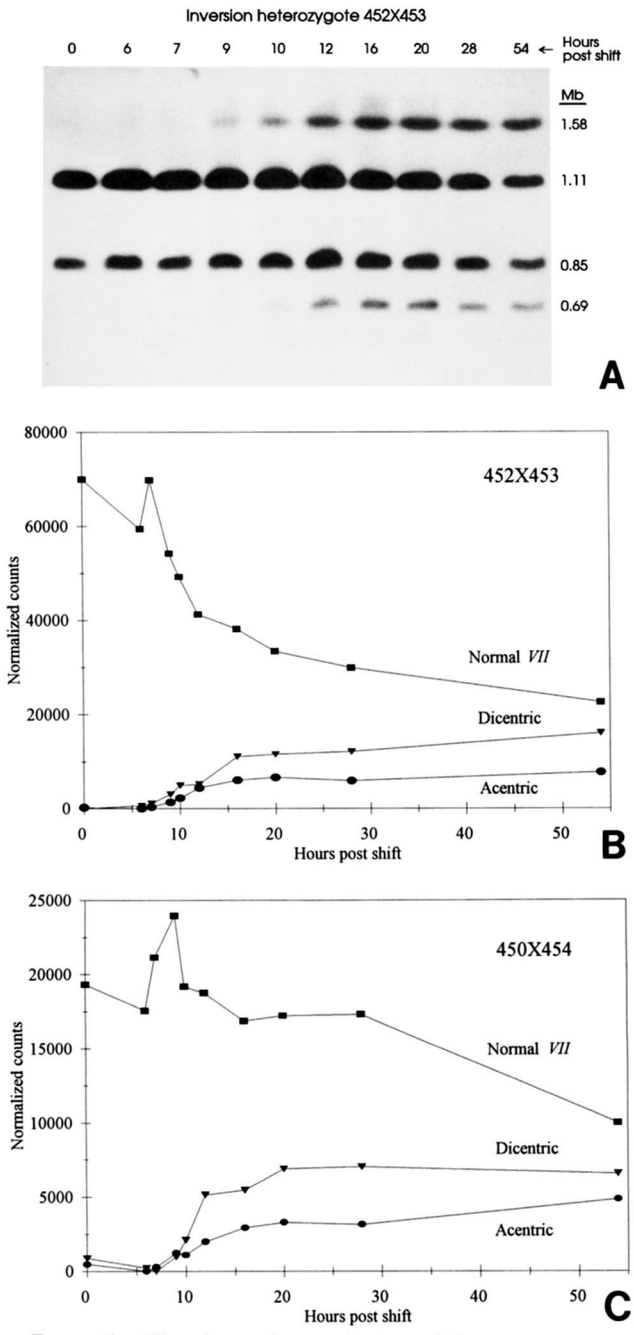


FIGURE 5.—Kinetics and quantitation of the appearance of dicentric and acentric chromosome products of crossing over in the heterozygous inversions. (A) Southern blot of CHEF gel run on samples of MDY452X453 taken at different times following the shift into meiosis, probed with *TRP5* to label the dicentric (1.58 Mb), normal *VII* (1.11 Mb), and acentric (0.69 Mb) chromosomes and with *LYS2* to label chromosome *II* (0.85 Mb) as a loading/recovery control. (B) Plot for the blot in A. Counts per minute for each band at each time point were determined by direct counting using an Ambis system. Corrections for background and loading (see MATERIALS AND METHODS) lead to the apparent disparities between the blot and plot (for example at 12 hr post shift). (C) Plot as in B, on samples of MDY450X454.

pre-treatment, presumably because it more often lies outside the spores (although, from these data, it would seem to be contained within a spore wall in a majority of the asci). However, its greater length would also be expected to make it more susceptible to the shearing pre-treatment. At this time, the reason for the differential recoveries, in particular the reduced amount of acentric compared to dicentric, remains unexplained.

**The dicentric and acentric chromosomes have not been detected in viable spore colonies:** In 452X459, spore clone colonies are red due to the *ade2* mutation, unless they also carry the *ade5* mutation which lies distal to the inversion, in which case they are white. Viable spore colonies where the *ade5* mutation is on the normal chromosome *VII* and *ADE5* (1 or 2 copies) is on the acentric would be red with white sectors if the acentric were moderately unstable. Similarly, spore clone colonies that carry the *cyh2* mutation on the normal chromosome *VII* will be resistant to cycloheximide unless they carry a dicentric or acentric that has the *CYH2* wild-type gene, in which case they should sector for cycloheximide sensitivity if the dicentric or acentric is moderately unstable. If either dicentric or acentric were present and stable, spore colonies should be recovered that are heterozygous for the *leu2* and *met13* alleles. No sectoring or heterozygosity has been detected. Since the majority of the dicentric and acentric molecules are no more susceptible to shearing than the normal chromosome *VII*, we presume that usually the dicentric and acentric are incorporated into spores. These results suggest that the dicentric and acentric are not incorporated into the same spores as the normal *VII*, unless the dicentric and acentric are so highly unstable as to be lost within the first few divisions after germination.

If both the dicentric and acentric were incorporated into the same spore (without a normal *VII*), that spore would be genetically $n + 1$ for chromosome *VII* and presumably would germinate. Observations made by phase contrast light microscopy demonstrate that the inviable spores in tetrads with only two viable spores show no evidence of attempting to germinate. Thus, it seems likely that the acentric and dicentric are not segregated to the same spore, and it remains possible that all four chromosome *VII* products regularly segregate apart, in spite of the absence of a centromere on the acentric.

**Recombination in the inversion heterozygotes is decreased inside but not outside the inversion:** Map distances for intervals on chromosome *II* and on the distal part of chromosome *VIIL* are similar in all the strains (Table 4; the increases between *TRP1* and *ADE5* in the inversion heterozygotes are not statistically significant). The orientation heterozygotes exhibit a marked reduction in crossing over within the inversion. Since all rank 1 tetrads (single crossovers) and the majority of the tetrads of rank 2 or above will have only 2 or 0 spores viable (see Table 6), this measured reduction in crossing over within the inversion in *4 spores-viable* tetrads is inevitable. However, the fraction of 4 spores-viable tetrads

**TABLE 4**

**Map distances in centiMorgans**

| Strain | Tetrads scored | Chromosome *VII* | | | | | Chromosome *II* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *URA3/ LEU1* | *CYH2* | *MET13* | *TRP1/ LEU1* | *ADE5* | *LYS2* | *TYR1* | *HIS7* |
| Orientation homozygotes | | | | | | | | | |
| 450X459[a] | 150 | 59.4 | 12.1 | 21.1 | 53.9 | | 50.7 | 74.6 | |
| 450X453 | 107 | 77.3 | 17.3 | | | | 71.6 | 67.4 | |
| 452X454 | 100 | 49.7 | 13.9 | | 50.3 | | 40.5 | 51.3 | |
| Orientation heterozygotes | | | | | | | | | |
| 452X459[b] | 145 | 10.1 | 6.3 | 6.6 | 69.0 | | 49.2 | 44.1 | |
| 450X454 | 130 | 6.6 | 5.9 | 5.4 | 57.2 | | 47.5 | 52.1 | |
| 452X453 | 99 | 18.0 | 10.5 | 17.0 | 69.2 | | 44.1 | 59.8 | |

Map distances calculated using PERKINS' (1949) equation, corrected when >35cM (MA and MORTIMER 1983).
[a] *LEU1-TRP5* and *TRP5-CYH2* intervals in 450X459–17.8 cM and 49.3 cM, respectively, if measured separately.
[b] *LEU1-TRP5* and *TRP5-CYH2* intervals in 452X459–1.0 cM and 11.4 cM, respectively, if measured separately.

**TABLE 5**

**Recombination in the inversion interval**

| | No. of crossovers: | No. of crossovers (rank) and no. of strands involved | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ? | 0 | 1 | 2 | | | 3 | | | 4 | | |
| | No. of strands: | | 0 | 2 | 2 | 3 | 4 | 2 | 3 | 4 | 2 | 3 | 4 |
| 4 spores-viable: | 450X459 (hom)[a] | 2 | 6 | 51 | 17 | 30 | 16 | 1 | 7 | 17 | 0 | 0 | 3 |
| | 452X459 (het) | 0 | 111 | 0 | 32 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | 450X454 (het) | 1 | 107 | 0 | 20 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | 452X453 (het) | 4 | 58 | 0 | 28 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 1 |

| | | Type of tetrad[b] | | | |
|---|---|---|---|---|---|
| | | ? | 2nn | 2nd | 2dd |
| 2 spores-viable: | 452X459 (het) | 1 | 100 | 11 | 6 |
| | 450X454 (het) | 7 | 70 | 12 | 7 |
| | 452X453 (het) | 4 | 52 | 9 | 21 |

[a] (hom), (het) = homozygous, heterozygous for orientation of interval.
[b] See Table 6 for description of types.

from the inversion heterozygotes that are rank 0 suggests that there *is* a reduction in crossing over. For example, there are no crossovers in the *URA3-TRP1* interval in 3.1% (6/194 tetrads) of the control 450X459 compared with no crossovers in 28.4% (111/391 tetrads) of the inversion heterozygote 452X459 (Table 5). As discussed above, there is only a ~3-fold difference in the numbers of 4 spores-viable tetrads formed (35 *vs.* 10%), too little to account for the ~9-fold difference in the numbers of rank 0 tetrads.

**The ratio of rank 0 to rank 2 tetrads provides a sensitive method for estimating the levels of crossing over in the heterozygous inversion interval, where the level is reduced to ~55% of that of the control:** As there is clearly a loss of tetrads where dicentrics/acentrics are formed, estimation of crossover frequency based solely on cells where these are not formed should be the most accurate. In practice this ensues almost entirely from those cells that give rise to rank 0 or to rank 2, 2-strand (2-strand double crossover) tetrads. Pairwise comparisons between the control and the inversion heterozygotes (Table 5) demonstrate that the differences are significant with $P < 0.005$ for each ($G$ values that range from 12.4 to 30.6; SOKAL and ROLHF 1969). In order to estimate the degree of the effect of inversion heterozygosity, it is necessary to derive a model that incorporates the inviability caused by each dicentric and acentric product, and that predicts the outcomes at different levels of crossing over within the inversion. The theoretical predictions for spore viability and tetrad types, given the losses outlined, are listed in Table 6. The final distributions then depend on the frequencies of each rank. The model developed by KING and MORTIMER (1991) for determining map distance assigns values to two variables, $p$, which measures the probability of there being a crossover in the interval, and $k$, which measures crossover interference. Their model provides a useful set of equations for calculating the distribution of tetrads of rank 0 to 4. By combining the KING and MORTIMER equations with the predictions in Table 6, it is possible to plot expectations of overall spore viability (used below) and of the fractions of rank 0 and rank 2 tetrads in the 4-spores-

**TABLE 6**

**Theoretical frequencies of viability and tetrad types given crossing over in the heterozygous inversion**

| Viability | Type[a] | Tetrad rank 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| 4 spores-viable | All | 1 | 0 | 1/4 | 1/8 | 3/16 |
| 2 spores-viable | 2nn | 0 | 1 | 0 | 1/16 | 0 |
| | 2nd | 0 | 0 | 1/2 | 1/4 | 3/32 |
| | 2dd | 0 | 0 | 0 | 7/16 | 3/8 |
| | 2nq | 0 | 0 | 0 | 0 | 1/16 |
| | 2dq | 0 | 0 | 0 | 0 | 3/32 |
| 0 spores-viable | All | 0 | 0 | 1/4 | 1/8 | 3/16 |
| Chromatids lost to dicentric | | 0 | 1/2 | 1/2 | 1/2 | 1/2 |
| Crossover breakpoints lost to dicentric | | | 1 | 1/2 | 1/2 | 1/2 |

[a] 2nn, no crossovers in either spore; 2nd, no crossovers in one spore, two crossovers in the other; 2dd, two crossovers in each spore; 2nq, no crossovers in one spore, four crossovers in the other; and 2dq, two crossovers in one spore, four crossovers in the other.

**TABLE 7**

**Interference in the inversion interval**

| Strain | No. of tetrads[a] | | *TRP5-CYH2* | and | *CYH2-MET13* | and | *MET13-TRP1* | and | *TRP5-CYH2* |
|---|---|---|---|---|---|---|---|---|---|
| 450X459 (hom) | 52 | Coincident crossovers[b] | 9/10.2 | | 0/6 | | 21/22 | | |
| | | Interference | 0.11 | | 1.00 | | 0.04 | | |
| 452X459 (het) | 32 | Coincident crossovers | 11/13.5 | | 4/9.5 | | 15/16.0 | | |
| | | Interference | 0.18 | | 0.58 | | 0.06 | | |

[a] Includes only data from rank 2 tetrads with no conversion in the interval, see text. The 452X459 data include results from 8 2 spores-viable tetrads, see Table 5. Note that the final column is for noncontiguous intervals.
[b] No. observed/no. expected.

viable tetrads as a function of $p$ (derivations available on request). Calculation of the *URA3-TRP1* map distance from the ranked tetrad data for the control strain 450X459 (Table 5) gives a map distance of 90.1 ± 5.3 cM. This value is in good agreement with the sum of map distances of the smaller intervals, 92.6 cM, calculated using the PERKINS (1949) equation (data in Table 4) and assigns values of $P = 0.51 \pm 0.03$ and $k = 0.84 \pm 0.05$. The predictions made at $k = 0.84$ are plotted in Figure 6. As calculated graphically (and marked in Figure 6), the $p$ value for the control is in reasonable agreement with that calculated using all the data. The inversion heterozygote values are 49, 55 and 66% of the control, the most direct comparison providing 55%.

This model assumes that the potential for crossing over is normally distributed in each strain. In addition, the calculations above assume that interference, described by $k$, is similar across the diploids. To test this assumption, we have compared interference in 450X459 and 452X459, the only strains where both ends of the inversion are marked. In the orientation heterozygotes, tetrads with one crossover within the inversion are lost to analysis, causing an underestimate in the frequency of crossing over. Thus, when interference is calculated as ((1 − (observed coincident crossovers)/(expected coincident crossovers)), using all the tetrad data, there is an apparent excess of coincident crossovers and interference is calculated, incorrectly, to be negative. Almost all of the information for the inversion heterozygote is in the rank 2 (2-strand) tetrads, and we can calculate interference based on these tetrads alone (although it changes the interpretation of the absolute values of $k$). In order to make the direct comparison, we have used a limited data set, consisting of only those tetrads with 2 crossovers (and no ambiguities due to gene conversions) between *URA3* and *TRP1* for the control as well as for the inversion heterozygote (Table 7). Very few crossovers are observed between *URA3* and *TRP5* in 452X459 (so it is omitted from the table) but among all the tetrads scored (*i.e.*, including ranks 3 and 4), the 6 crossovers in this interval coincide with crossovers in the *TRP5-CYH2*, *CYH2-MET13* and *MET13-TRP1* intervals 5, 2 and 2 times, respectively, *i.e.*, roughly in proportion to the lengths of those intervals. The values in Table 7 are similar for the two strains except for *CYH2-MET13* and *MET13-TRP1*, where positive interference is "complete" in the control and high in the inversion heterozygote. Using all the tetrad data for 450X459, interference between these two intervals measures to be 0.47 (8 coincident crossovers observed/15.1 expected), and presumably the measured interference of "1.00" in Table 7 is spuriously high due to the small data set. It is interesting that interference between the noncontiguous intervals, *MET13-TRP1* and *TRP5-*

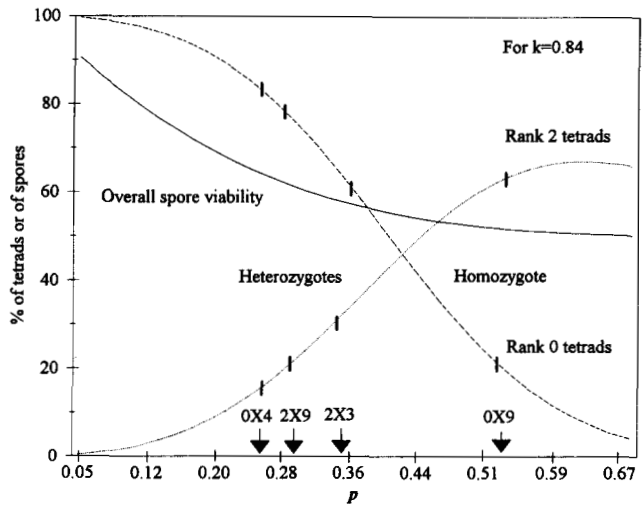


FIGURE 6.—Plots of the expected fraction of the 4 spores-viable tetrads that are rank 0 and rank 2 tetrads (dashed lines) and of the spores that are viable (solid line), at different levels of crossing over (measured by $p$). At higher values of $p$, increasing numbers of tetrads of higher ranks (not shown here) would be expected, so that the sum of the rank 0 and 2 tetrads does not equal 100%. "$n \times n$" refers to the last digit of the haploids used to make the diploids that provided the indicated fractions of rank 0 and 2 tetrads, and the estimated values of $p$.

*CYH2*, is quite low and similar in both strains (see DISCUSSION). In any case, apparently there is positive crossover interference between segments in the inversion. Using the model outlined above, the relative fractions of rank 0 and 2 tetrads for $P < \sim 0.4$ are largely insensitive to variations in $k$ from 0.1 to 1.05 (from stringent to slightly negative interference in this model), so that the values calculated above for $p$ should be reasonably accurate.

**The amount of the acentric and dicentric chromosomes recovered in the CHEF gel blots provides an independent measure of the level of crossing over in the inverted interval:** The fraction of signal expected in the dicentric + acentric bands relative to the total for chromosome *VII* (dicentric + acentric + normal *VII*) in the CHEF blots is the inverse of the fraction of expected overall spore viability, plotted in Figure 6. This measurement is inherently less sensitive than the genetic measurement, given that the ratio generally varies less dramatically with $p$ than do the fractions of rank 0 and 2 tetrads. In addition, the differential recoveries of the acentric and dicentric in CHEF blots introduces a degree of uncertainty. This measure is nevertheless useful, if only because it presumably is independent of the genetic measure. Instead of calculating the ratio (acentric + dicentric signal)/(total *VII* signal), we can substitute (2 × acentric signal) and (2 × dicentric signal) for the numerator to set lower and upper bounds, respectively. Using the counts for the 20-hr time points (and assuming 100% of the cells enter meiosis), we get ratios of 0.30 and 0.41 and thus $p$ values of 0.19 and 0.34 for 452X453 (Figure 5B) and ratios of 0.28 and 0.44 and $p$ values of 0.18 and 0.39 for 450X454 (Figure 5C). These calculated values of $p$ flank the genetic estimates in each case, and at their largest still indicate a decrease in crossing over as compared to the orientation homozygotes. Conversely, assuming the genetic measurements to be unbiased, these results confirm the relative under- and overrepresentation in CHEF gel blots of the acentric and dicentric, respectively.

**The distribution of crossovers is altered within the inverted interval:** By comparing the positions of crossovers in the smaller intervals in rank 2 tetrads only from 450X459 and 452X459 (Table 8), it is apparent that crossing over is most reduced in the *URA3-TRP5* interval, less so in the *TRP5-CYH2* and *MET13-TRP1* intervals, and little reduced in the *CYH2-MET13* interval (given that all the inversion heterozygote values should be reduced by $\sim 0.29/0.53 = 0.55$, the ratio of the $p$ values). These differences in the distributions of the crossovers are, using the raw numbers of crossovers observed in each interval and 3 d.f., significant at $P < 0.01$ ($G = 12.7$, SOKAL and ROHLF 1969).

**Gene conversion in the inversion interval is not reduced at *MET13* but is reduced at *LEU1* in the inversion heterozygote:** In the inversion heterozygote 452X459, levels of conversion at *HIS7*, *LYS2* and *TYR1*, which are not on chromosome *VII*, are measured at 0.66, 0.70 and 0.62 of the control 450X459 (Table 9). Given that spore viability is predicted to be ~0.62 of the control (Figure 6), these numbers are very near what would be predicted, *i.e.*, are unchanged from the control. The raw frequency of surviving convertants at *MET13* is 0.43 and at *LEU1* is 0.10 that of the control (Table 9).

Given that crossing over accompanies conversion in some fraction of the events, selection against convertants for markers in the inverted interval has to be taken into account in calculating the rate of gene conversion; conversions associated with crossing over will be lost from all rank 1 tetrads and from ½ of tetrads of greater rank (from the fraction of breakpoints associated with dicentric/acentric formation, bottom of Table 6). At the estimated level of crossing over, tetrads of rank 1, 2 and 3 occur at 47, 26 and 5%, respectively. Losses to dicentric/acentric formation leave 0, 13 and 3%, so that only (13 + 3)/(47 + 26 + 5), or ~21% of conversions associated with crossing over will be present in live spores. Detection of conversions not associated with crossing over depends only on overall spore survival (~62%). Assuming that 0.18 to 0.66 of the conversions at *MET13* are associated with crossing over (FOGEL *et al.* 1981) and that the rate of conversion is the same as in the control, convertants should be recovered at a frequency of 41–55% that of the control, in reasonable agreement with the measured 43% (Table 9). The data from 4 spores-viable tetrads, where in 150 tetrads from the control 450X459, 23 *MET13* conversion events (of all potential types, Table 10) were recovered and in 145

**TABLE 8**

**Crossover positions in rank 2 tetrads**

| Strain | No. of tetrads | Crossovers[a] | Percent of total | | | | |
|---|---|---|---|---|---|---|---|
| | | | *URA3/LEU1* | *TRP5* | CYH2 | *MET13* | *TRP1/LEU1* |
| 450X459 (hom) | 63 | 113 | 19 | 46 | 11 | 24 | |
| 452X459 (het) | 32 | 55 | 4 | 47 | 24 | 25 | |

Almost identical results are obtained combining data from all ranks.
[a] Positions of the remainder of crossovers equivocal due to gene conversion.

**TABLE 9**

**Levels of meiotic conversion to prototrophy (×100,000)**

| | 450X459 (hom) | | 452X459 (het) | | |
|---|---|---|---|---|---|
| | Avg. | SD | Avg. | SD | Ratio[a] |
| Outside inversion: | | | | | |
| *his7-1/his7-2* | 202 | 9 | 134 | 31 | 0.66 |
| *tyr1-1/tyr1-2* | 85 | 10 | 52 | 17 | 0.62 |
| *lys2-1/lys2-2* | 39 | 5 | 27 | 6 | 0.70 |
| Inside inversion: | | | | | |
| *met13-c/met13-d* | 7560 | 597 | 3220 | 546 | 0.43 |
| *leu1-c/leu1-12* | 2980 | 285 | 312 | 52 | 0.10 |

Values based on six isolates of each strain, expressed as the number of colonies formed on selective medium divided by the number on complete synthetic medium. Pre-sporulative values (for vegetative events) were also measured for each isolate to rule out jackpots.
[a] Ratio of 452X459/450X459 values.

tetrads from the inversion heterozygote 452X459, 24 *MET13* conversion events were recovered, are consistent with there being no reduction in conversion at *MET13*, an observation that holds for all six diploids.

By the same arguments used above, conversion at *LEU1* in the inversion heterozygote *is* reduced to ~25% of the control (Table 9), and the tetrad data are consistent with there being a reduction (Table 10). Conversion at *LEU1* may be influenced by proximity to the breakpoint heterozygosity, which lies only ~250 bp from the first codon of *LEU1*. As heterozygosity occurs at the same point in the control, the reduction would seem related to the inversion *per se*.

**Simple, straight heterosynapsis only is seen in pachytene nuclei from the inversion heterozygote:** No inversion loop (diagram in Figure 3A) has been seen in the several hundred meiotic prophase nuclei analyzed to date using electron microscopy (Figure 7; a smaller inversion is similarly undetected in serial section reconstruction; MOENS and ASHTON 1985). During zygotene it is generally difficult to assess completely the synaptic configurations of all the bivalents, but by pachytene, when the synaptonemal complexes (SC) are completely formed, there is no evidence for homosynapsis in the inverted interval. Given homosynapsis, we would expect ~27% (300 kb/1110 kb) of the second or third longest continuous SC to form a reverse-pairing loop centered ~0.3 of the SC length from one end [(690/(690 + 1580)]. Given a genome (minus rDNA) size of 12,910 kb and SC complement lengths of 21.2–35.7 μm (DRESSER and GIROUX 1988), we would expect a loop (or other perturbation of synapsis) of ~0.5–0.8 μm in an SC ~1.8–3.1 μm in length. Instead, all bivalents form simple, straight SCs, indicating that synapsis in the inverted interval is heterologous. Whether homosynapsis to form a loop or partial loop at earlier stages is adjusted to give rise to the observed heterosynapsis cannot be ascertained clearly from these preparations. However, it is clear that in yeast, as in mouse (MOSES *et al.* 1982) but not in maize (MAGUIRE and RIESS 1994), crossing over neither requires nor leads to stable homosynapsis.

## DISCUSSION

**Cotransformation with rearranged genomic fragments provides a general method for making genetically marked chromosome rearrangements:** The inversions were made by cotransforming genetically marked DNA fragments that mimic the desired new joins at the breakpoints (Figures 1 and 2). This approach has the major advantage that it places genetic markers precisely at the breakpoints, allowing measurement of crossing over across the entire interval. [In an inversion where products with uneven numbers of crossovers are inviable, the ends are perfectly linked and one marked end suffices.] Cotransformation with the markers inserted into unrearranged DNA fragments provides controls by interrupting the sequences at the breakpoints without otherwise rearranging the genome.

**Heterozygosity for the inversion reduces crossing over and gene conversion non-uniformly in the targeted interval:** The reduction in crossing over in the heterozygotes, to ~55% of the controls, reflects the average effect across the interval, the smaller intervals showing effects that range from more pronounced to no decrease at all (Tables 4 and 8). Rates of conversion are similarly non-uniformly affected, the *leu1* heteroalleles showing a ~4-fold reduction and the *met13* heteroalleles showing little or no change from the control (Tables 9 and 10, and RESULTS). The level of conversion at *MET13* is high, with prototrophs formed in ~8% of the wild-type 450X459 cells (Table 9); when conversion of all kinds is analyzed in tetrads the number rises to ~16% of asci. This suggests proximity to a hot-spot for recombination and the same is true for *LEU1*, which has ~40% the level of conversion of *MET13* in 450X459 and even higher levels in 450X453 and 452X454 (Table 10). *LEU1* is

**TABLE 10**

**Conversion events of all types, in tetrads**

| Strain | Tetrads | No. of events | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *LEU1* | *CYH2* | *MET13* | *ADE5* | *LYS2* | *TYR1* | *HIS7* |
| Orientation homozygotes | | | | | | | | |
| 450X459 | 150 | 9 | 4 | 23 | 7 | 1 | 3 | 2 |
| 450X453 | 107 | 36 | 1 | 24 | 4 | 1 | 2 | 3 |
| 452X454 | 100 | 16 | 0 | 11 | 4 | 2 | 1 | 0 |
| Orientation heterozygotes | | | | | | | | |
| 452X459 | 145 | 1 | 1 | 24 | 9 | 0 | 0 | 3 |
| 450X454 | 130 | 4 | 2 | 16 | 4 | 0 | 2 | 2 |
| 452X453 | 99 | 4 | 2 | 27 | 3 | 2 | 1 | 7 |

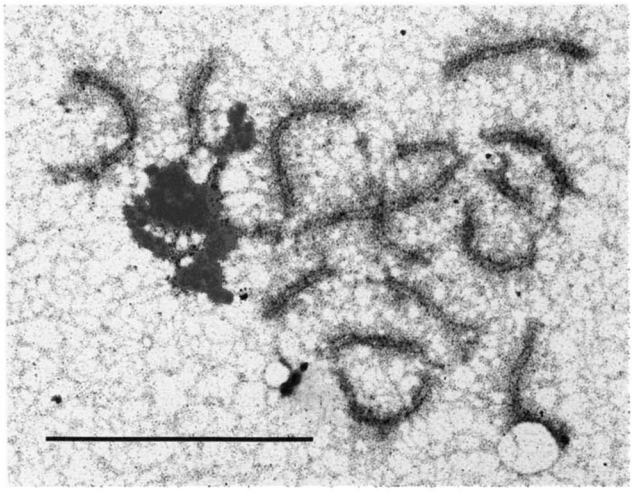

FIGURE 7.—Electron micrograph of a silver-stained pachytene nucleus of inversion heterozygote MDY452X459. No inversion loop, as diagrammed in Figure 3A, is evident, indicating the absence of stable homosynapsis of the inverted region. Magnification bar represents 5 μm.

quite close to the proximal breakpoint, which may account for the greater reduction in conversion in *LEU1* and in crossing over nearby. However, breakpoint-associated local affects cannot account for the entire ~45 cM decrease. The large *TRP5-CYH2* interval, marked in the crosses involving MDY459, is well removed from the nearest breakpoint (18 cM or 50–60 kb) and, even after correcting for losses, shows a ~2-fold reduction in map length. Insofar as the decreases in recombination in some intervals result from a more global reduction in the whole inversion, the remaining recombination may represent in part "local" sequence behaviors along the interval. This conclusion complements that of ROSS *et al.* (1992), who suggest that "the recombination behavior of the sequences in a particular small interval is largely determined by the sequences in that interval." The effect of chromosome size on levels of recombination in yeast is another demonstration of global regulation (KABACK *et al.* 1992), as is positive crossover interference generally. Thus, the final patterns and levels of crossing over across the inversion are likely to result from the interaction of different processes that act to initiate or regulate crossing over, not all of which are perturbed by the inversion.

**Stable homosynapsis may be required for normal levels of crossing over:** Several observations support this interpretation. First, in a diploid that is homozygous for the inversion the sequences are colinear and levels of crossing over are largely restored (452X454 in Table 4), thus the observed reduction in crossing over is not simply the result of rearranging chromosome *VII* organization. Second, ectopic crossing over between sequences at nonhomologous locations, which are unlikely to form stable synaptic associations, typically is lower than allelic crossing over (PETES *et al.* 1991). Third, recombination is reduced in mutants with abnormal synapsis (PETES *et al.* 1991; BISHOP *et al.* 1992; SYM *et al.* 1993); in *red1* this effect varies at different locations in the genome (ROCKMILL and ROEDER 1990). Fourth, we have applied the model developed here to data for crossing over on chromosome *III* when it is heterozygous for a circular derivative (HABER *et al.* 1984) and have calculated a similar reduction in crossing over. Most of chromosome *III* is included on the circular derivative (sufficient for survival of haploids), making it similar in physical length to the inverted interval analyzed here, although ~50% greater in genetic length. There is no reason to expect heterosynapsis to occur between the circular and linear chromosomes *III*, but synapsis could be somewhat unstable, perhaps because of the small size of the circle and the apparent stiffness of axial/lateral elements. Mechanical difficulties in forming the sharp turns required to bring homologous regions into register may also account for the failure of homosynapsis in the inversion heterozygote. Heterosynapsis within the inversion may then form by default, as is seen in haploid yeast meiosis (LOIDL *et al.* 1991).

It seems reasonable to conclude that some fraction of crossing over takes place independent of stable synapsis, while the remainder of crossing over is somehow initiated or fostered by stable homosynapsis during a period that extends into pachytene. The simplest mechanism consistent with this possibility is that a single class of events initiates crossovers and that these events are less efficient in the absence of stable homosynapsis. If the

role of homosynapsis is to bring or keep sequences with the potential for crossing over in close proximity, then sequences near the middle of the inversion might show wild-type levels of crossing over or even higher levels if there is a reduction in nearby, interfering, crossovers. Crossing over in the *CYH2-MET13* interval may reflect this effect. If so, moving the breakpoints of the inversion should affect the pattern of crossing over in the different included intervals; sequences near the physical middle of the inverted interval would show the least reduction, or even an increase, in crossing over. Alternatively, there may be distinct classes of initiating events or more than one mechanism for processing the same initiations, in each case distinguished by being either independent or dependent on synapsis. If so, it may be possible to detect qualitative differences between the two fractions by measuring, for example, the frequency with which gene conversion is associated with crossing over or the relative levels of intrachromosomal and homolog exchange, in the presence *vs.* the absence of stable homosynapsis.

It remains possible, however, that the numbers and positions of crossovers are established before synapsis occurs or becomes stable [see discussions in ZICKLER *et al.* (1992) and MAGUIRE and RIESS (1994)]. According to this scenario, the decrease in crossing over associated with inversion heterozygosity could result from an earlier perturbation in chomosome pairing (NUR 1968), perhaps via a decrease in the number or stability of pairing interactions on which crossing over may depend (WEINER and KLECKNER 1994; and N. KLECKNER, personal communication). If so, we may be able to detect a reduction in the levels of presumed early recombination intermediates, *e.g.*, double-strand breaks or joint molecules (SCHWACHA and KLECKNER 1994; COLLINS and NEWLON 1994), formed in the interval in inversion heterozygotes.

**Positive crossover interference is observed in the inversion:** Even though crossing over is reduced and synapsis in pachytene is heterologous in the heterozygous inversion, crossing over apparently is still subject to positive interference (Table 7). If the SC, or some part of it, functions to produce interference [see discussion in KING and MORTIMER (1990)], then crossing over in the inversion produces an interesting situation. If each chromosome is arranged linearly along its axial/lateral element (and we have no reason to suspect otherwise), then heterosynapsis brings the *URA3* of one chromosome *VII* into register with *TRP1* of the other chromosome *VII* (as for markers B and D in Figure 3B). By this arrangement, crossing over near the ends of the inversion occurs between sequences located at *both* ends of the heterosynapsed inversion.

If positive interference is a function of stable synapsis, as might be expected if transduction occurs via the formed central region of the SC during pachytene, then crossing over in intervals at one end of the inversion should interfere with crossing over in intervals at the other end. The noncontiguous intervals listed in Table 7 are somewhat offset (*MET13-TRP1* adjacent to one end and *TRP5-CYH2* ~50–60 kb from the other end), and as a result are not the perfect test pair, but we see very little interference between them. The *URA3-TRP5* and *MET13-TRP1* are a better test pair and even though we have scored very few crossovers in the *URA3-TRP5* interval in 452X459 we nevertheless do see coincident crossovers (see RESULTS). These results suggest that interference is not mediated by the formed SC during pachytene, at least not when it is heterosynapsed. If crossover sites are established earlier (see above), then interference would also have to act before pachytene, perhaps via short homosynaptic stretches of SC. If so, then crossing over involving sequences near either end of the inversion presumably would not interfere with crossing over outside the inversion, which remains to be measured.

It is possible that the axial/lateral elements alone bring about the required transduction of information along the chromosomes, in which case interference should remain largely unaffected, as is observed (Table 7). For example, axial element formation or modification may be initiated at DNA double-strand breaks and prevent further breaks, thus acting early in meiotic prophase. Alternatively, some recombination intermediate may initiate structural changes that propagate along the formed lateral elements during pachytene [such as those described in the Chinese hamster (DRESSER and MOSES 1980)] and prevent crossing over at relatively distant sites, thus acting later in prophase. In either case, synapsis would not necessarily be required and crossing over in the inversion should interfere with crossing over outside the inversion.

**The dicentric products of crossing over remain intact and recoverable but a large fraction of the acentric products fails to appear in chromosome gels:** In vegetative divisions in yeast, dicentric chromosomes give rise to breaks and chromosome instability (HABER *et al.* 1984; HILL and BLOOM 1989), so that *a priori* one might expect loss of the dicentric and not of the acentric chromosomes. However, the dicentric apparently goes unbroken during the meiotic divisions and spore formation (Figure 5), suggesting that spindle forces are not sufficient to break the dicentric bridge. This is consistent with and extends the genetic (HABER *et al.* 1984) and physical (GAME *et al.* 1989) observations from the circle/linear *III* heterozygote. Karyokinesis is incomplete until spore wall formation (MOENS and RAPPORT 1971) and there is an increase in the numbers of asci in the inversion heterozygotes where only 2 spores are formed (36% in the heterozygotes *vs.* 21% in the homozygotes, Table 2). Thus, some fraction of the dicentrics may escape breakage via interference with spore wall formation, consistent with the relative overabundance of dicentric

chromosomes in CHEF gel blots. However, this cannot account for all of the dicentrics remaining intact, since at $P = 0.29$, the model predicts 24% rank 0, 47% rank 1, 25% rank 2 and 4% rank 3 cells which should give rise to ~62% cells having one dicentric (0 + 47 + 12 + 3, respectively, using the predictions in Table 6), far in excess of the 36% 2-spored asci observed. Also, many of the dicentrics are presumably incorporated into spores rather than simply being omitted, given that ~58% of the starting amount of dicentrics are still recovered in samples prepared selectively from spores (*i.e.*, cells pretreated with Zymolyase and vortexing).

It remains surprising that the acentric DNA is recovered 2–3-fold less efficiently than the dicentric (Figure 5, B and C, and RESULTS). Several possibilities could account for this result: (1) fewer acentric chromosomes are formed (*i.e.*, heretically, that many of the crossover events are not reciprocal), (2) some acentric chromosomes are quickly degraded, (3) the acentric chromosomes are more difficult to extract for electrophoresis or (4) the acentric chromosomes enter the gel less efficiently. The last of these seems most likely and experiments are underway to explore this possibility. Also, it will be interesting to see if any of the products of crossing over in a *peri*centric inversion, all of which still carry a centromere, are also recovered differentially.

The acentric also has not been detected in viable spores, and it seems either to segregate away from the other chromosome *VII* products (mechanism unknown) or to segregate with the normal chromosomes *VII* and be very unstable in the mitotic divisions (perhaps hitchhiking via entanglements). *In situ* hybridization on cells in the divisions should be particularly informative concerning the presence and disposition of a bridge and the segregation of the dicentric and acentric chromosomes. For example, by using a probe against the inversion, the bridge should be particularly obvious and by using differently colored probes against chromosome *VIIR* and distal *VIIL* in the same preparations, the acentric, normal and dicentric chromosomes can be distinguished.

**Heterozygosity for the inversion provides a physical assay for crossing over along a large chromosomal region:** Assays using restriction enzyme polymorphisms (BORTS *et al.* 1984; PADMORE *et al.* 1991; NAG and PETES 1993; SYM *et al.* 1993), which monitor regions that are ~20-fold shorter than the inversion and are more prone to local sequence effects, are likely to be less sensitive than appearance of the dicentric and acentric chromosomes. The assay based on heterozygosity for the circular chromosome *III* (GAME *et al.* 1989; GAME 1992) has the advantage that it detects a variety of events–single and double crossovers, sister chromatid exchanges and double strand breaks–and the disadvantage that interactions between crossing over and sister chromatid exchange make it more difficult to derive directly a value for $p$. In addition, using the method presented here any chromosome segment can be inverted, marked and analyzed genetically as well as physically. However, given the differential recoveries of the chomosomes and the relative insensitivity of the present assay by comparison with the genetic measures at large values of $p$ (Figure 6), the physical quantitation of crossing over to detect differences of 2-fold or less will be difficult. Introduction of mutations in late sporulation genes required for spore formation may alleviate the differential recoveries of the normal and dicentric (and perhaps the acentric) chromosomes and allow a more accurate measure based on the ratio of their amounts. Alternatively, pericentric inversions or other rearrangements may not have the same problems of recovery. Also, measurements using shorter inversions would place the amount of recombination in the more sensitive range of the assay, and would allow an assessment of the congruity of the genetic and physical measures. A very important aspect of the assay is that it offers a particularly sensitive means of monitoring the time of appearance of recombinant products. In the experiments reported here, the products appear well in advance of the first meiotic division. Whether these products appear earlier than those detected by other assays will require a direct comparison.

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