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Effects of Homology, Size and Exchange on the Meiotic Segregation of Model Chromosomes in *Saccharomyces cerevisiae*

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ABSTRACT

In most eukaryotic organisms, chiasmata, the connections formed between homologous chromosomes as a consequence of crossing over, are important for ensuring that the homologues move away from each other at meiosis I. Some organisms have the capacity to partition the rare homologues that have failed to experience reciprocal recombination. The yeast *Saccharomyces cerevisiae* is able to correctly partition achiasmate homologues with low fidelity by a mechanism that is largely unknown. It is possible to test which parameters affect the ability of achiasmate chromosomes to segregate by constructing strains that will have three achiasmate chromosomes at the time of meiosis. The meiotic partitioning of these chromosomes can be monitored to determine which ones segregate away from each other at meiosis I. This approach was used to test the influence of homologous yeast DNA sequences, recombination intiation sites, chromosome size and crossing over on the meiotic segregation of the model chromosomes. Chromosome size had no effect on achiasmate segregation. The influence of homologous yeast sequences on the segregation of noncrossover model chromosomes was negligible. In meioses in which two of the three model chromosomes experienced a crossover, they nearly always disjoined at meiosis I.

MEIOSIS is the process by which diploid organisms produce haploid cells for the purpose of sexual reproduction. In meiosis I, homologous chromosomes pair and then segregate to opposite poles of the cell (Figure 1a). For many years, crossing over between homologues has been considered to be important for high fidelity chromosome segregation during meiosis I in most organisms (for a discussion of the exceptions see HAWLEY 1988). The evidence for this comes from numerous cytological and genetic studies. For example, in many experimental organisms, it has been found that mutations that eliminate, or greatly reduce, the levels of meiotic recombination also exhibit high levels of missegregation of chromosomes in meiosis I (Figure 1b) (reviewed by HAWLEY 1988). Reciprocal recombination between homologues enhances proper meiotic segregation by contributing to the formation of chiasmata between them. Chiasmata are thought to play a structural role in keeping homologue pairs joined until they migrate away from each other in anaphase of meiosis I.

Crossing over is not *absolutely required* for proper segregation of homologues in meiosis I (reviewed by HAWLEY 1988). Some organisms that use recombination to enhance chromosome segregation fidelity use other systems to partition noncrossover chromosome pairs. The best described example of such an organism is Drosophila (reviewed by HAWLEY and THEURKAUF 1993). In Drosophila females most chromosomes are partitioned by a recombination-dependent system, like that described above, and achiasmate systems, originally collectively referred to as "distributive disjunction", are used to partition the chromosome *4* pair, which is achiasmate in every meiosis, and occasional additional pairs of chromosomes that have failed to experience meiotic crossing over (GRELL 1976; HAWLEY and THEURKAUF 1993).

In Drosophila females, meiotic cells that carry two noncrossover chromosomes will generally partition them to opposite poles at meiosis I even if they are not homologous. One approach that has proven useful in determining how achiasmate chromosomes are partitioned in Drosophila females has been to develop strains that must partition three achiasmate chromosomes during meiosis. In such situations, two of the three achiasmate chromosomes will sometimes preferentially partition to opposite poles. By evaluating the segregation of a number of combinations of three achiasmate chromosomes, it has been possible to determine what features of chromosomal structure are recognized by the achiasmate system. These three-chromosome experiments, and the analysis of Drosophila meiotic mutants, have led to the conclusion that what was previously described as distributive segregation is the manifestation of two achiasmate systems (HAWLEY and THEURKOFF 1993). One of these achiasmate systems utilizes DNA sequence homology, in heterochromatic chromosomal regions, to partition achiasmate homologues (HAWLEY *et al.* 1992). The other achiasmate system is sequence independent. Instead, segregation by this system is influenced by the sizes and shapes of the

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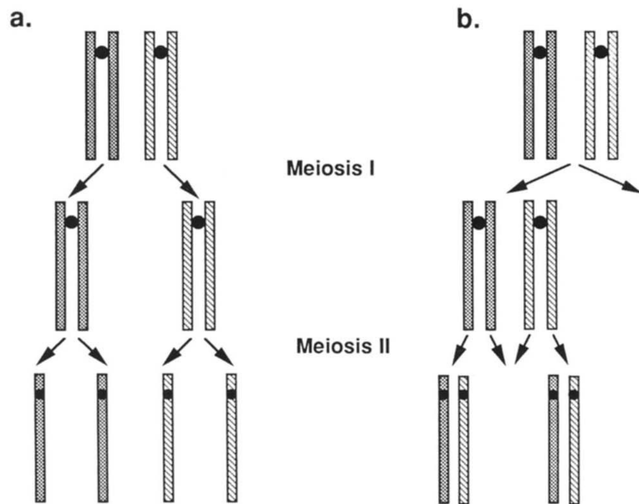


FIGURE 1.—Meiotic chromosome segregation in yeast. (a) Disjunction. (b) Meiosis I nondisjunction.

achiasmate chromosomes (GRELL 1976; HAWLEY and THEURKAUF 1993).

Like Drosophila, the yeast *Saccharomyces cerevisiae* is capable of partitioning nonhomologous achiasmate chromosomes but with much lower fidelity. Yeast can partition noncrossover artificial chromosomes composed primarily of nonyeast DNA sequences (described below) (DAWSON *et al.* 1986; MANN and DAVIS 1986) or noncrossover natural yeast chromosomes (NILSSON-TILLGREN *et al.* 1986; GUACCI and KABACK 1991). There is no evidence that achiasmate meiotic chromosome segregation in Drosophila and yeast occurs via related mechanisms.

Two different three-chromosome experiments have been performed to explore achiasmate segregation in yeast (DAWSON *et al.* 1986; GUACCI and KABACK 1991). One of these provided a test of the effect of chromosome size on the partitioning of three noncrossover nonhomologous chromosomes (GUACCI and KABACK 1991). In this experiment two nonhomologous natural chromosomes and a much smaller yeast centromere plasmid were shown to be treated as equal partners by the achiasmate system; that is, each chromosome exhibited an equal probability of migrating away from the other two at meiosis I. The second experiment tested whether homologous nonyeast DNA could bias achiasmate segregation (DAWSON *et al.* 1986). In this experiment two homologous YLps, composed primarily of bacteriophage lambda DNA, and a similarly-sized mini *III* chromosome were treated as equal partners by the achiasmate system. The homologous bacteriophage lambda DNA sequences did not detectably bias the segregation.

The role of yeast sequence homology in achiasmate segregation has never been tested. The three-chromosome experiments described here are designed to address the role of homologous yeast sequences in the partitioning of achiasmate chromosomes. The experiments were performed with three kinds of model chromosomes all of which carry yeast genes such that their segregation can be followed using tetrad analysis (Figure 2). All of these model chromosomes mimic the meiotic behavior of natural chromosomes and have proven useful tools in characterizing meiotic chromosome behavior because they circumvent two difficulties encountered when examining achiasmate segregation using natural yeast chromosomes (DAWSON *et al.* 1986; GUACCI and KABACK 1991; ROSS *et al.* 1992; SEARS *et al.* 1992). First, natural chromosomes experience crossovers in most meioses, making it difficult to find significant numbers of achiasmate events, whereas the model chromosomes can be constructed such that they are achiasmate in a much higher frequency of meioses. Second, missegregations of natural chromosomes lead to dead cells, whereas missegregations of the model chromosomes are tolerated because they carry no essential genes. Therefore, with the model chromosomes it is possible to recover and analyze all of the products of meiosis as viable cells.

The three-chromosome experiments described here examine the previously unexplored question of whether homologous yeast DNA sequences bias the segregation of noncrossover chromosomes in yeast. Some of the homlogous sequences tested are those implicated as playing roles in the initiation of meiotic recombination. Additionally, these experiments examine the influence of chromosome size on achiasmate segregation and the ability of crossovers between homologous model chromosomes to bias their segregation in the presence of a third model chromosome.

MATERIALS AND METHODS

Strains, media and genetic methods: Media were prepared as described in Sherman *et al.* (1979). *S. cerevisiae* strains are described in Table 1. Segregation of the model chromosomes in meiosis was assayed using tetrad analysis. One of the characteristics of the model chromosomes is that their copy numbers vary somewhat (most cells have one, a minority have zero or two) in mitotic cells (DAWSON *et al.* 1986; SEARS *et al.* 1992). Thus in the experiments described here, most, but not all, cells entered meiosis with only one copy of each model chromosome. A second characteristic is that these model chromosomes also show higher levels of precocious separation of sister chromatids (PSSC) in meiosis I than wild-type chromosomes (DAWSON *et al.* 1986; SEARS *et al.* 1994, 1995). Both mitotic stability and levels of PSSC vary from one chromosome to another and are dependent upon characteristics such as chromosome length (MURRAY *et al.* 1986) and probably other features as well (for example, size of the centromere region). Strains were propagated under nonselective growth conditions to avoid enrichment for cells that had experienced an event enabling them to stably maintain genes carried on the model chromosomes.

For this analysis, only tetrads in which all chromosomes were in single copy and segregated reductionally at meiosis I were informative, and these are the tetrads we have focused on. Determinations of first and second division segregation were based primarily on the segregation of the chromosomal centromere linked markers *MET14* or *ADE1*. Because *ADE1* is ~5 cM

TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype	Source
DD98	*MATa/MATα trp1-1/trp1-1 ura3-52/ura3-52 his3-11-15/his3-11-15 ade1/+ arg4-17/+ leu2-3-112/leu2-3-112 lys2-Δbgl2/lys2-Δbgl2* Mini *III* 72 kb[*URA3 leu2-3-112 trp1-XbaI* site fill-in] Mini *III* 72 kb [*ura3-NcoI* site fill-in *LEU2 TRP1*] YLp 54 [*leu2-3-112 LYS2, HIS3*]	This study
DSR5	*MATa/Matα trp1-1/trp1-1 ura3-52/ura3-52 his3-11-15/his3-11-15 ade1/+ arg4-17/arg4-17 leu2-3-112/leu2-3-111 lys2-Δbgl2/lys2-Δbgl2 met14/+* YLp 54 [*leu2-3-112 LYS2, HIS3*] Mini *III* 72 kb [*ura3-NcoI* site fill-in *LEU2 TRP1*] HY1 [*TRP1 URA3*]	This study
DM45	*MATa/MATα trp1-1/trp1-1 ura3-52/ura3-52 his3-11-15/his3-11-15 ade1/+ arg4-17/arg4-17 leu2-3-112/leu2-3-112 lys2-Δbgl2/lys2-Δbgl2* Mini *III* 42 kb [*HIS3 TRP1*] Mini *III* 72 kb [*URA3 leu2-3-112 trp1-XbaI* site fill-in] YLp 54 [*LYS2 HIS3*]	This study
DL352 and DL331	*MATa/MATα trp1-1/trp1-1 ura3-52/ura3-52 his3-11-15/his3-11-15 leu2-3-112/leu2-3-112 arg4-ΔHpaI/arg4-ΔHpaI ade1/+* YLp 62 [*leu2-ClaI* site fill-in *TRP1 ura3-NcoI* site fill-in *ARG4 his3-HinDIII* site fill-in] YLp63 [*LEU2 trp1-XbaI* site fill-in *ura3-NcoI* site fill-in *arg4-EcoRV* site fill-in *HIS3*] Mini *III* 72 kb [*URA3 leu2-3-112 trp1-XbaI* site fill-in]	This study

from the centromere, its use leads to a 10% error in making this assignment. In tetrads in which there was no recombination between the model chromosomes, their segregation was used as a second guide to making first and second division assignments. All chromosomes we have tested (DAWSON *et al.* 1986; ROSS *et al.* 1992) show >84% second division segregation of sister chromatids. Therefore, if all three model chromosomes showed a segregation pattern consistent with all sister chromatids exhibiting second division segregation, but in disagreement with the assignment made based on *ADE1* segregation, we assumed that there had been a crossover between *ADE1* and its centromere in this meiosis. Statistical comparisons were performed using the G test (SOKAL and ROHLF 1969).

Mitotic loss analysis to test for crossovers between nondisjoined model chromosomes: Spore colonies that carried nondisjoined homologous model chromosomes that might have experienced a crossover were streaked to single colonies on YPD plates. The model chromosomes are mitotically unstable, being lost in 10^{-3} to 10^{-2} per cell division (DAWSON *et al.* 1986; MURRAY *et al.* 1986). Colonies in which one of the chromosomes has been lost can be identified by replica plating. The markers present on the remaining chromosome were identified and scored for recombination.

Construction of artificial chromosomes and mini chromosomes: The construction and general structures of the YLps and the mini *III*s have been described previously (DAWSON *et al.* 1986; MURRAY *et al.* 1986; ROSS *et al.* 1992). Chromosomal alterations were accomplished using one- and two-step gene replacement techniques (BROWN and SZOSTAK 1983; ROTHSTEIN 1983). The mini *III* chromosomes diagrammed in Figure 2 are the same used in ROSS *et al.* (1992) and have not been modified. HY1 (BURKE *et al.* 1987) was a gift from GEORGES CARLE.

RESULTS

Experimental approach to analyzing the behavior of model chromosomes in three-chromosome experiments: In all of the experiments described here, the same analytical approach was used. Briefly, the experiments were performed in two steps (for a detailed description see DAWSON *et al.* 1986). First, tetrads from a sporulated diploid carrying three model chromosomes were dissected on a rich nonselective medium (YPD). Spore colonies that grew on these plates were replica-plated to media lacking leucine, tryptophan, uracil, arginine, lysine or histidine, adenine and/or methionine. From the growth patterns of the spore colonies on these media, it was possible to deduce the segregation of the different model chromosomes with respect to each other and the centromere-linked marker on a natural chromosome. In some of the experiments described in the following sections, cells contained two model chromosomes that were homologous and could, therefore, experience recombination. In some cases recombination events could be scored simply by observing the growth of the spore colonies on the media described above. However, a second step was performed to determine the genetic maps of chromosomes that had partitioned to the same spore as a result of meiosis I nondisjunction or PSSC. Due to the mitotic instability of the model chromosomes, it was possible to isolate two derivatives from each spore colony carrying two different model chromosomes: cells that had lost one of the model chromosomes and cells that had lost the other. The growth characteristics of these two derivatives were tested, as above, to determine whether the model chromosomes had parental or recombinant configurations of genes.

Because of the mitotic copy number variability of the model chromosomes and their high levels of PSSC (1–14%), many tetrads in these experiments were not informative for analyzing the behavior of homologous chromosomes in meiosis I. The segregation data for noninformative tetrads from these experiments are presented in the figure legends.

Segregation of three model chromosomes with limited homology: A strain (DSR5) carrying three distinctly genetically marked nonhomologous model chromosomes was constructed. This strain carries HY1

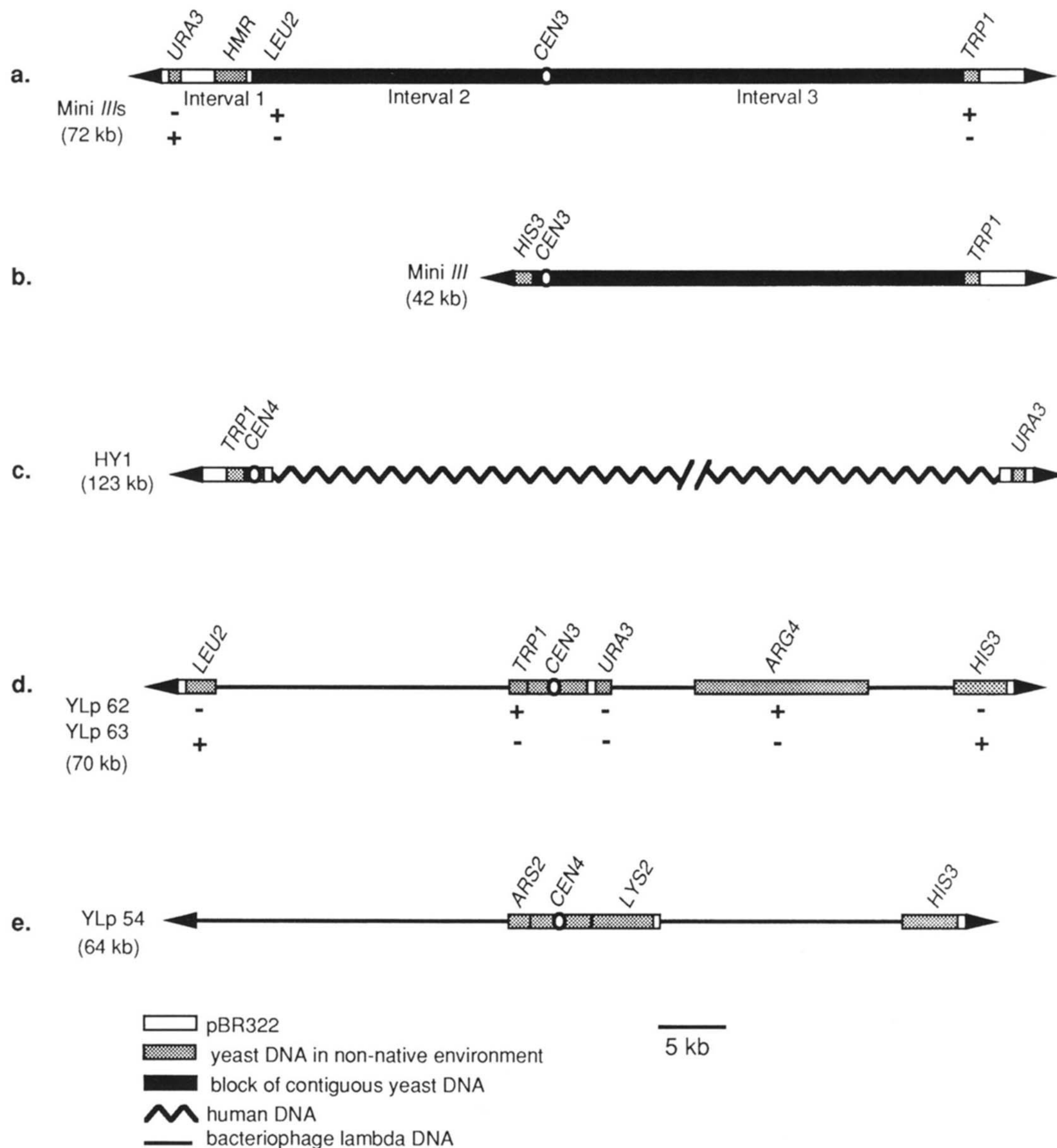


FIGURE 2.—Maps of the model chromosomes used in three-chromosome experiments. (a) The 72-kb mini *III* is composed of a 55-kb fragment of chromosome *III* extending from *LEU2* through the centromere to *PGK1*, flanked by the sequences indicated in the figure. (b) The 42-kb mini *III* is composed of a 31-kb fragment of chromosome *III*, including the centromere, flanked by the *HIS3* and *TRP1* genes. (c) HY1 includes ~114 kb of human DNA flanked by sequences from pYAC2. (d) YLps 62 and 63 are composed of a backbone of bacteriophage lambda DNA, a 5.1-kb *CEN3* fragment, a 12.5-kb region including the *ARG4* locus from chromosome *VIII* and the other indicated yeast genes. The two YLps are identical except for frameshift mutations. (e) YLp 54 is a 64-kb model chromosome with a backbone of bacteriophage lambda DNA, the centromere from chromosome *IV* and the indicated yeast genes.

(Figure 2c), a 123-kb YAC with an insert of human DNA (BURKE *et al.* 1987); YLp 54 (Figure 2e), a 64-kb artificial chromosome composed mostly of bacteriophage lambda DNA (DAWSON *et al.* 1986); and a 72-kb derivative of chromosome *III* (Figure 2a) (MURRAY *et al.* 1986). The maps of the chromosomes indicate the sequences they have in common. The YAC and mini *III* share ~900 bp of *CEN3* sequences, 1.4 kb including *TRP1*, most of pBR322, 700 bp of Tetrahymena telomeric DNA at each end and ~300 bp of yeast telomeres. The YLp and YAC share 2 kb of pBR322, 700 bp of Tetrahymena telomeric DNA at each end, and yeast telomeres. The mini *III* and YLp share ~2000 bp of pBR322 and the Tetrahymena and yeast telomeric homologies.

There are four possible patterns of meiosis I segregation for three achiasmate chromosomes segregating in meiosis I (assuming they do not segregate equationally). These are shown for the three model chromosomes in strain DSR5 in Figure 3a. This experiment is similar to the three-chromosome experiment described by GUACCI and KABACK (1991) in two ways. First, the three chromosomes assayed in both experiments are largely nonhomologous, and second, both experiments

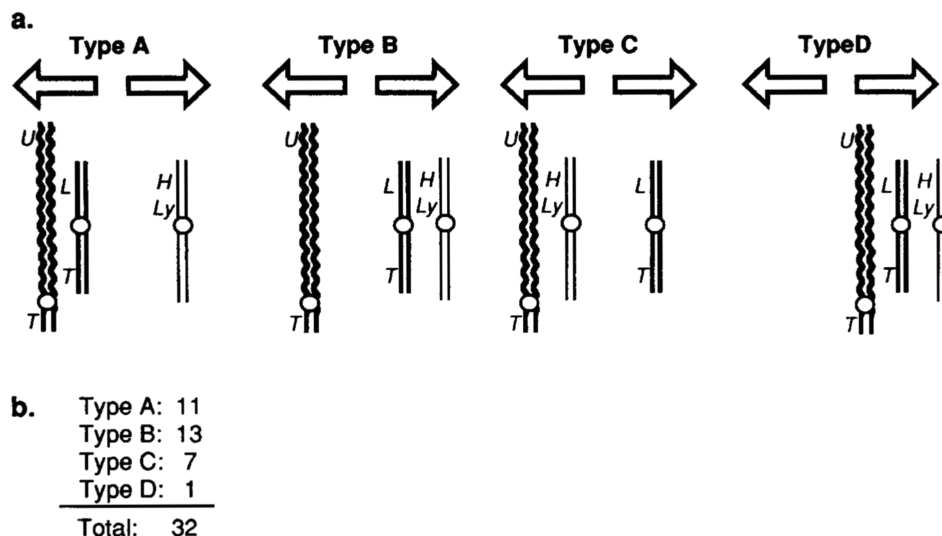


FIGURE 3.—The segregation of three nonhomologous model chromosomes. Tetrad analysis was used to determine the segregation patterns of HY1, YLp 54 and a 72-kb mini *III* in strain DSR5. (a) The four possible patterns of meiosis I segregation of three noncrossover chromosomes. (b) Observed distribution of tetrads from DSR5 among the four types of tetrads. Additional tetrad data: dissection of 168 DSR5 tetrads yielded 124, 29, 11, 1 and 3 tetrads with 4, 3, 2, 1, and 0 viable spores, respectively. Thirty-two of the four viable spore tetrads were informative for examining achiasmate meiosis I segregation of the three model chromosomes. The pool of informative tetrads was limited primarily due to mitotic instability of HY1. HY1 showed high copy number variability, segregating 4:0, 3:1, 2:2, 1:3, 0:4 in 11, 8, 49, 3 and 52 four viable spore tetrads, respectively. Additionally, in four of the four viable spore tetrads the Ura^+ phenotype segregated 2:2, but the Trp^+ phenotype segregated 0:4, suggesting HY1 had lost its *TRP1* gene in a mitotic event. In one tetrad YLp 54 was absent. In four tetrads one of the model chromosomes exhibited PSSC, and in nine tetrads combinations of the above events occurred.

feature two chromosomes of approximately equivalent size and one of a different size. The earlier experiment featured two nonhomologous natural chromosomes and a much smaller centromere plasmid. In contrast, the experiment shown in Figure 3 features two similarly sized model chromosomes and a larger third model chromosome. The results shown in Figure 3b are in agreement with the earlier experiment (GUACCI and KABACK 1991) in that the results fit the model that the three chromosomes are treated as equal partners in meiosis I. This model predicts that three types of tetrads (A–C) will appear with equal frequency (33% each). The failures in this process would lead to nondisjunction of all three model chromosomes (type D). The observed distribution of tetrads in types A–C is indistinguishable from this expected pattern of 33% in each ($G = 0.97$, d.f. = 2, $P > 0.5$). Thus regardless of whether the third chromosome is smaller (GUACCI and KABACK 1991) or larger (Figure 3) than the similarly sized pair, all three seem to act as equal partners.

Segregation of two homologous mini *III*s and a nonhomologous YLp: The three following experiments address the effects that homology might have on the segregation of achiasmate model chromosomes. For the first of these, a strain that carried two homologous mini *III*s and one YLp was constructed (DM45). Two of these model chromosomes, the *URA3*-mini *III* and the YLp 54 (Figure 2, c and e), are approximately the same size, while the third chromosome is a 42-kb mini *III* (Figure 2b). Thus, this experiment allows the evaluation of both size and homology on achiasmate segregation. The mini *III*s share 30 kb of homologous colinear sequences from chromosome *III*. In ~7% of the tetrads the mini *III*s experienced crossovers in their homologous region (Figure 4). The effects of such crossing over on the segregation of the model chromosomes is discussed in the following sections. The four possible outcomes of meiosis I segregation of the noncrossover model chromosomes in this strain are indicated in Figure 4a, along with the observed distribution of tetrad patterns (Figure 4b).

As in the preceding experiment, there is no obvious influence of homology on the three chromosomes, and the observed pattern is not significantly different from that expected if the three model chromosomes segregate as equal partners (33% each of types A–C; $G = 0.8$, d.f. = 2, $P > 0.5$). If the homology shared by the mini *III*s could elevate the level of their disjunction, then significantly >67% of the tetrads would be expected to be of types A and B combined. This is approximately what is observed (69% in types A and B), suggesting that the 25 kb of yeast homology does not strongly bias segregation. This experiment also offers an additional opportunity to test whether chromosome size biases segregation. If chromosomes of equal size were biased to disjoin then significantly, >67% of the tetrads would be in types B and C combined (we observe 70%). Therefore, as in the previous experiment, chromosome size does not seem to bias to segregation of the model chromosomes.

Meiotic segregation of a mini *III* and two homologous

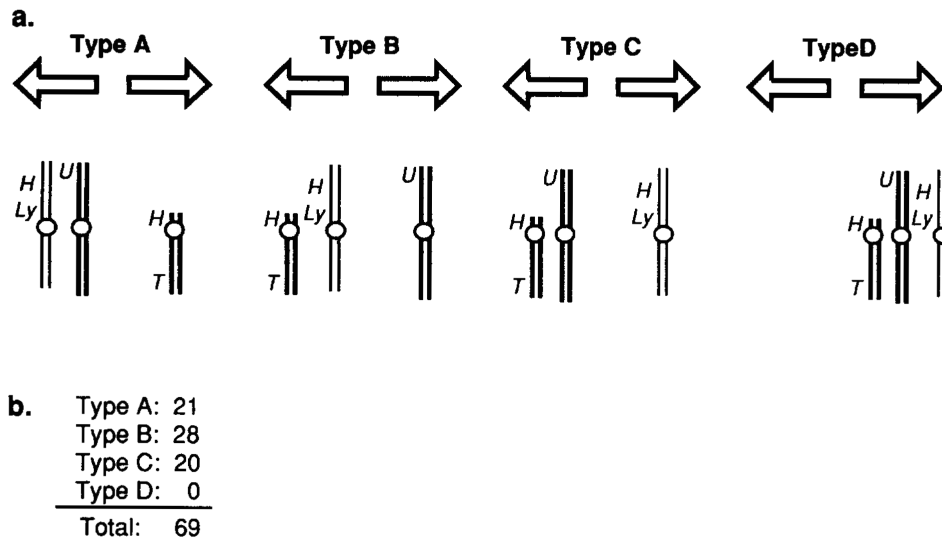


FIGURE 4.—The segregation of homologous mini *III* chromosomes of different sizes in the presence of a YLp equal in size to one mini *III*. Tetrad analysis was used to determine the segregation patterns of the 72- and 42-kb mini *IIIs* and YLp 54 in strain DM45. (a) The four possible patterns of meiosis I segregation of three noncrossover chromosomes. (b) Observed distribution of tetrads from DM45 among the four types of tetrads. Additional tetrad data: dissection of 279 DM45 tetrads yielded 154, 73, 45, 7 and 0 tetrads with 4, 3, 2, 1 and 0 viable spores, respectively. All three model chromosomes exhibited some copy variation, particularly the 42-kb mini *III* as has been described (MURRAY *et al.* 1986), limiting to 98 the population of four spore viable tetrads with one copy of each chromosome (two chromatids). In addition to the categories listed in the figure, six tetrads showed multiple events (PSSC, non-2:2 segregation of one of the model chromosomes, gene conversion, or crossing over), two showed PSSC of one chromosome, 11 showed a single crossover between the mini *IIIs* and 10 showed gene conversion of *TRP1* or *HIS3*.

YLps that bear a meiotic recombination site: Genetic experiments have demonstrated that yeast chromosomes contain regions that act as the sites of initiation of recombination in meiosis (FOGEL *et al.* 1978; NICOLAS *et al.* 1989). The analysis of DNA from specific loci, harvested from synchronous meiotic cells, has demonstrated that these recombination "hotspots" correspond to sites that experience double-stranded breaks in the DNA during meiotic prophase (SUN *et al.* 1989; CAO *et al.* 1990). Mapping of meiotic double-strand break sites on a collection of chromosomes has revealed that the number and distribution of double-strand break sites is consistent with the model that they are the initiation sites for most meiotic crossing over (ZENVIRTH *et al.* 1992). The recombinogenic properties of these regions are conserved when the regions are carried on YLps or mini *III* chromosomes (ROSS *et al.* 1992).

The experiments described in this and the next section test whether yeast sequences that contribute to homologous recombination (and possibly pairing) might also contribute to partner choice in achiasmate situations. The first test of this was performed with a strain with one mini *III* (Figure 2a) and two homologous YLps, YLp 62 and YLp 63, (Figure 2d) that each carry a 12.5-kb insert of yeast DNA from chromosome *VIII*. This region has been shown to be the site of high levels of meiotic crossing over and gene conversion in its normal position on chromosome *VIII*. Three double-strand break sites also map to this region. When this 12.5-kb region is transferred to a YLp, its recombinogenic properties are not detectably changed (ROSS *et al.* 1992) and its double strand break sites are functional (L. ROSS and D. DAWSON, unpublished data). YLps that carry the 12.5-kb insert frequently experience meiotic recombination in meiosis, and the YLps are marked with yeast genes that make it possible to score crossing over along the whole length of the YLps and gene conversion at the *ARG4* gene in the 12.5-kb insert.

The presence of the 12.5-kb region on the YLps and homologies between the mini *IIIs* and chromosome *III* raise the possibility of interactions between the model chromosomes and natural chromosomes that might complicate the interpretation of these experiments. It has been previously demonstrated that chromosome *III* fragments (mini chromosomes) and plasmids carrying double-strand break sites from chromosome *III* can interact with the natural chromosome *IIIs* in diploid cells at low levels during meiosis (SUROSKY and TYE 1988; GOLDWAY *et al.* 1993a,b). Recombination between the extrachromosomal sequences and chromosome *III* result in nondisjunction of the natural chromosomes. The ability of the extrachromosomal sequences to recombine with the natural chromosome seems to correlate with the number and activity of the double-strand breaks sites in the region of shared homology (GOLDWAY *et al.* 1993a). A 115-kb chromosome *III* fragment carrying four double-strand break sites, including the very active double-strand break site that maps near *THR4*, caused ~4% nondisjunction of chromosome *III*. The region of chromosome *III* carried on the 72-kb mini *III* chromosome used in these experiments showed two double-strand break sites in the double-strand

break site mapping experiments performed by ZENVIRTH *et al.* (1993). The 12.5-kb insert of the YLps contains three moderately active double-strand break sites but a 2-kb region including one of these sites (and the *ARG4* gene) has been deleted from both copies of chromosome *VIII*. To test whether the YLps and/or the mini *III* are interacting with chromosome *VIII* and *III*, respectively, we compared spore viabilities of tetrads obtained by dissecting DL325 and a derivative of DL325 that had lost all three model chromosomes. Interactions between the model and natural chromosomes would be expected to lead to elevated levels of tetrads with two and three viable cells, and in the case of chromosome *III* missegregation, many of these tetrads would be predicted to contain both **a** and α mating type information and therefore to test as nonmaters. The spore viabilities of the two strains were indistinguishable (86% four viable spore tetrads, $n = 119$, one nonmating spore colony with model chromosomes; 83% four viable spore tetrads, $n = 120$, no nonmating colonies without the model chromosomes). The results demonstrate that if recombinations between the model chromosomes and chromosome *VIII* or *III* are occurring, it is at a level that would not significantly alter our findings.

The arrangement of markers on the YLps made it possible to access the disjunctional fates of the model chromosomes in cases where the YLps had, or had not, experienced a crossover. The methods used to determine whether nondisjoined YLps had experienced recombination are discussed in MATERIALS AND METHODS. The four possible meiosis I segregation patterns for the three model chromosomes when they have not recombined are indicated in Figure 5a. Note that in type A and B, the YLps have disjoined, and in type C they have nondisjoined. Among tetrads in which the YLps appeared to be noncrossover (Figure 5b), the distribution of the three model chromosomes was statistically indistinguishable from that predicted if they were partitioned as equal partners; that is 33% each of types A–C ($G = 1.2$, d.f. $= 2$, $P > 0.5$), consistent with the model that the shared homology of the 12.5-kb insert does not increase disjunction of noncrossover model chromosomes.

Forty-five tetrads in which the chromosomes had experienced a single crossover were identified (Figure 5c). The majority of crossovers were in the right arm, as predicted from earlier experiments (ROSS *et al.* 1992). Pairs of YLps that experienced a single crossover showed significantly lower levels of nondisjunction than those that had not, 7 *vs.* 33% (Figure 5c) ($G = 15.33$, d.f. $= 1$, $P < 0.005$). Therefore, crossovers stimulated by the presence of the 12.5-kb insert greatly bias segregation.

Crossing over and segregation of a mini *III* chromosome pair in the presence of a nonhomologous YLp: To test whether the extensive stretches of native yeast sequences could bias the segregation of achiasmate model chromosomes, a diploid strain carrying the two 72-kb mini *III*s (Figure 2a) and a 64-kb YLp (Figure 2e) was subjected to tetrad analysis. The homologous mini *III* chromosomes used in this experiment carry a central region of 55 kb of contiguous sequences from chromosome *III* including the centromere. The recombination characteristics of this 55-kb region when it is carried on a pair of mini *III*s is indistinguishable from its behavior in its natural context on chromosome *III* (ROSS *et al.* 1992). As with the experiment described in the previous section, we analyzed the behavior of the model chromosomes in tetrads in which they were nonrecombinant and in tetrads in which the mini *III*s had crossed over.

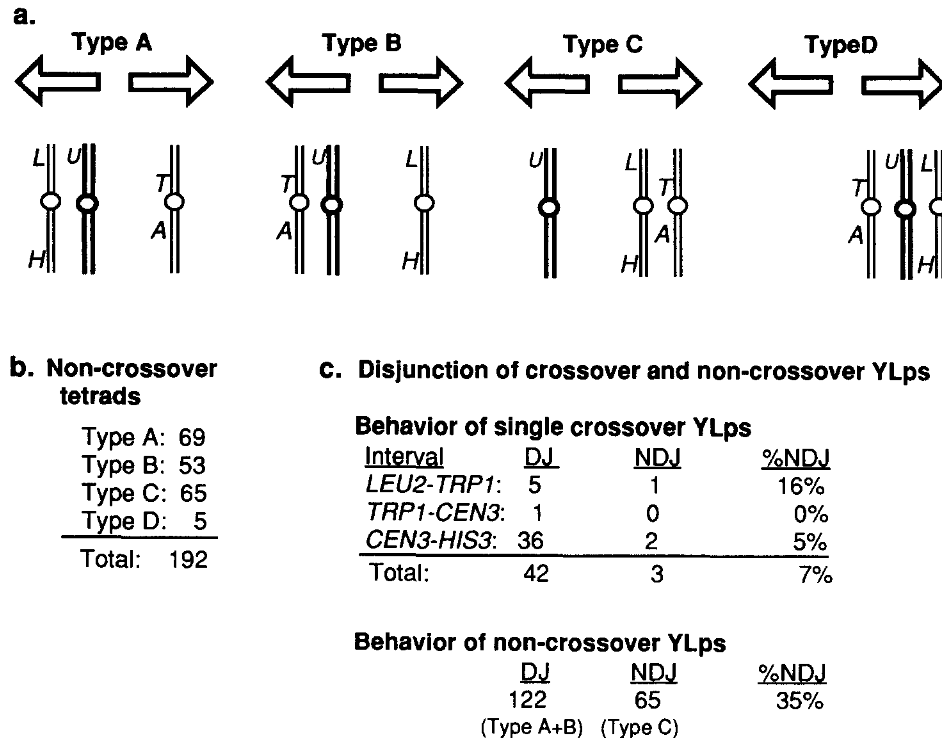
The four possible types of achiasmate segregation of the model chromosomes are shown in Figure 6a. In this experiment the pattern of segregation deviated from that expected if the three chromosomes are treated as equal partners. Instead, the group of tetrads in which the mini *III*s had nondisjoined (type C) was modestly underrepresented. The pattern of distribution among types A–C deviates slightly but significantly from the expected 33% in each category ($G = 6.0$, d.f. $= 2$, $P < 0.05$). One interpretation of this result is that the homology shared by the mini *III*s makes them marginally favored partners in the presence of a third nonhomologous model chromosome, even in cases in which no evidence of crossing over was detected.

To evaluate whether the mini *III*s were interacting with the natural chromosome *III*s, mating type was scored for the first set of 162 tetrads dissected from this experiment. None of the spore colonies were nonmaters, even in tetrads with fewer than four viable spores, demonstrating that recombination between the mini *III*s and the natural chromosome *III*s occurs at low levels in these strains.

In tetrads in which the mini *III*s had experienced a crossover, the mini *III*s exhibited low levels of nondisjunction when compared to the nondisjunction frequency of noncrossover mini *III*s, 3.1 *vs.* 26% (Figure 6b) ($G = 40.03$, d.f. $= 1$, $P < 0.005$), demonstrating that crossovers between the mini *III*s strongly biases their disjunction.

DISCUSSION

The experiments described in this work address the roles played by size, homology and exchange in biasing partner choice in three-chromosome experiments. These experiments have their origins in experiments done to characterize what historically has been described as distributive segregation in Drosophila females. The behavior of assorted chromosomal combinations in both wild-type and mutant Drosophila have led to the recent development of the model that there are two distinguishable achiasmate segregation systems in this organism. The behavior of achiasmate chromo-



b. Non-crossover tetrads

Type	Count
Type A:	69
Type B:	53
Type C:	65
Type D:	5
Total:	192

c. Disjunction of crossover and non-crossover YLps

Behavior of single crossover YLps

Interval	DJ	NDJ	%NDJ
LEU2-TRP1:	5	1	16%
TRP1-CEN3:	1	0	0%
CEN3-HIS3:	36	2	5%
Total:	42	3	7%

Behavior of non-crossover YLps

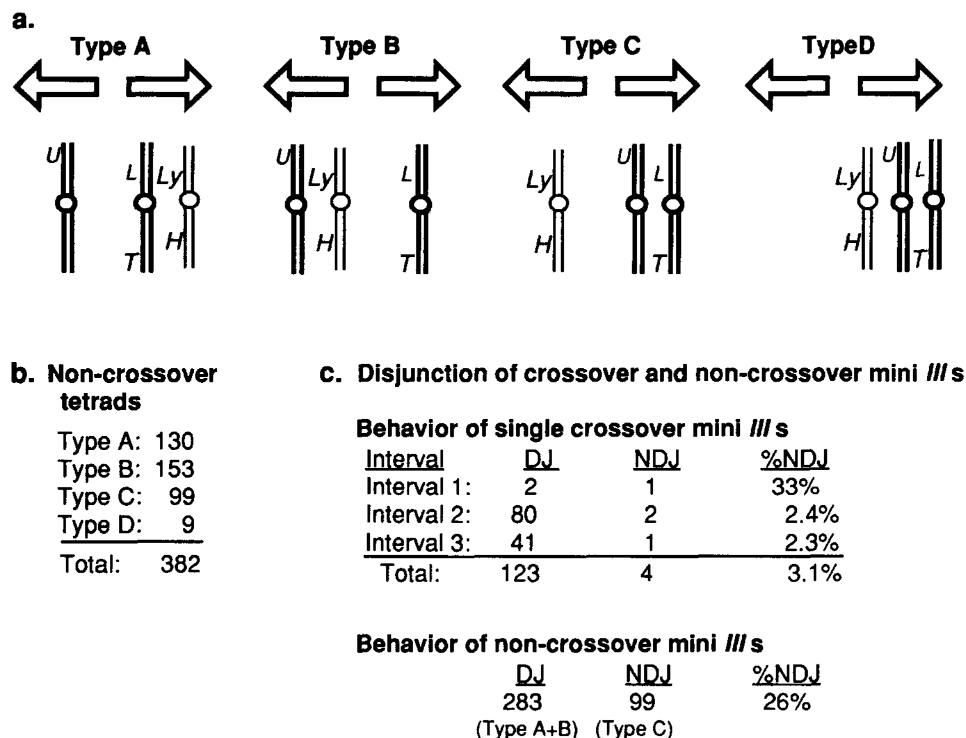
DJ	NDJ	%NDJ
122	65	35%
(Type A+B)	(Type C)	

FIGURE 5.—The segregation of YLps bearing a 12.5-kb insert from chromosome *VIII* in the presence of similarly-sized mini *III*. The data shown in this figure were pooled from the dissection of two congenic strains with identical model chromosomes, DL325 and DL331. The distribution of tetrads among types A–C was statistically indistinguishable in the two strains (G = 3.3, d.f. = 2, $P > 0.1$). (a) The four possible patterns of meiosis I segregation of three noncrossover chromosomes. (b) Distribution of tetrads from DL325 and DL331 among the four types of tetrads. (c) Behavior of YLps that have experienced no crossovers or single crossovers. Additional tetrad data: dissection of 962 tetrads yielded 556 four-spore viable tetrads. Of these four-spore viable tetrads 398 had one copy of each model chromosome (144 had lost one of the model chromosomes and 14 carried extra copies). Ten tetrads had segregation patterns consistent with the modification of one of the model chromosomes during mitotic growth. In seven tetrads the model chromosomes had experienced multiple recombination events. In 144 tetrads one or more model chromosomes showed PSSC. In 103 of these tetrads, the model chromosomes appeared nonrecombinant and a single chromosome exhibited PSSC (19 mini *III* and 84 YLp). In 16 of the remaining PSSC tetrads, the YLps had experienced gene conversion (eight tetrads) or crossing over (eight tetrads). The final 21 PSSC tetrads showed PSSC of multiple model chromosomes.

somes in wild-type Drosophila is the manifestation of the combined influences of these systems on meiosis I segregation. Three-chromosome experiments were used to demonstrate that one of the Drosophila systems utilizes homology, in the form of heterochromatic regions on the *X* and fourth chromosomes, to bias segregation at meiosis I. That is, in a three-chromosome experiment, two chromosomes that share the heterochromatic sequences will be more likely (as much as three times) to disjoin at meiosis I than two chromosomes that do not share this homology (HAWLEY *et al.* 1992). The second system is prone to bias partner choice by features such as chromosome size and shape. The exact ways that size and shape direct achiasmate segregation are poorly defined. Generally, in experiments with three achiasmate metacentric chromosomes, if two of the three chromosomes are of similar size, they are more likely to disjoin (GRELL 1976; O'TOUSA 1982; HAWLEY *et al.* 1992). In three-chromosome experiments with both meta- and acrocentric chromosomes, shape also plays a role (BRIDGES 1916; ZHANG and HAWLEY 1991).

The ability of chromosome size and shape to bias achiasmate chromosome segregation in yeast has been examined three experiments; two presented here and one done previously (GUACCI and KABACK 1991). The first tested the behavior of two nonhomologous natural chromosomes and a centromere plasmid (GUACCI and KABACK 1991); the others examined the behaviors of a YLp, a similarly sized mini *III* and a larger acrocentric YAC (Figure 3); and a mini *III*, a similarly sized YLp and a smaller mini *III* (Figure 4). In all three experiments the three chromosomes showed patterns of segregation indistinguishable from that expected if they were treated as equal partners in meiosis I. Thus if chromosome size or shape influences the segregation of achiasmate segregation in yeast, the effects are slight.

The effect of yeast sequence homology on achiasmate segregation has been examined in three experiments presented here. A previous study explored the ability of homologous nonyeast DNA sequences to influence segregation. In that work, homologous YLps composed primarily of bacteriophage lambda DNA, one with



b. Non-crossover tetrads

Type A:	130
Type B:	153
Type C:	99
Type D:	9
Total:	382

c. Disjunction of crossover and non-crossover mini *III* s

Behavior of single crossover mini *III* s

Interval	DJ	NDJ	%NDJ
Interval 1:	2	1	33%
Interval 2:	80	2	2.4%
Interval 3:	41	1	2.3%
Total:	123	4	3.1%

Behavior of non-crossover mini *III* s

DJ	NDJ	%NDJ
283	99	26%
(Type A+B)	(Type C)	

FIGURE 6.—The segregation of two mini *III*s and a similarly sized YLp. Tetrad analysis was used to determine the segregation patterns of the two 72-kb mini *III*s and YLp 54 in strain DD98. (a) The four possible patterns of meiosis I segregation of three noncrossover chromosomes. (b) Observed distribution of noncrossover and single crossover tetrads from DD98. Additional tetrad data: this experiment was initiated with a pilot dissection of 162 tetrads of DD98, yielding 108, 35, 16, 3, and 0 tetrads with 4, 3, 2, 1, and 0 viable spores. These tetrads were scored for all markers on the model chromosomes, *ADE1* segregation and mating type. Approximately 1600 additional tetrads were then dissected, but only those with four viable spores were further analyzed. A total of 972 four-spore viable tetrads were examined. Of these, 761 showed 2:2 segregation for all three-model chromosomes. In addition to the tetrads described in the figure, 181 showed PSSC for one of the model chromosomes (138 for the YLp and 43 for the mini *III*s), 19 showed both PSSC and recombination of the mini *III*s, and 45 showed 1:3 or 3:1 segregation of markers on the mini *III*s; most of these were 1:3 for the Ura^+ phenotype. Note that 1:3 segregation of the Ura^+ phenotype could indicate gene conversion or chromosome loss or nondisjunction of the *URA3* mini *III*. Finally, in seven tetrads alteration of one of the model chromosomes appeared to have occurred in mitotic growth.

CEN3 the other one with *CEN4*, and a mini *III* were tested (DAWSON *et al.* 1986). The three chromosomes acted as equal partners, demonstrating that a limited set of yeast sequences and 50 kb of bacteriophage DNA cannot bias achiasmate segregation in yeast. This experiment would likely not have detected a homology-dependent achiasmate segregation system if it operated through certain yeast sequences analogous to the heterochromatic DNA on the *X* and fourth chromosomes in Drosophila females. Similarly, if a homology-dependent process required extensive tracts of homologous yeast sequences, as would be found on a pair of achiasmate homologues in a natural situation, then this experiment would not have detected the ability of homology to bias partner choice.

The early stages of chromosome synapsis in yeast meiosis are approximately coincident with the appearance of double-strand breaks in the DNA, an observation that has contributed to the model that these sites might participate in chromosome-pairing processes (ROEDER 1990). We have tested whether a 12.5-kb piece of yeast DNA that contains three double-strand break sites when it is carried in its natural location has features that allow it to influence the segregation behavior of achiasmate chromosomes. Although this 12.5-kb region is the site of high levels of meiotic recombination and presumably allows intimate interaction of the DNA of the YLps, in cases where there was no crossover it had no detectable effect on segregation of the YLps (Figure 5). The YLps carried heteroalleles at the *ARG4* gene on the 12.5-kb insert (one was *ARG4* the other *arg4-RV*) so it was possible to monitor gene conversion at this locus (note that the chromosomal *ARG4* loci are deleted). Among the noncrossover tetrads in this experiment (Figure 5b) were seven in which there was a gene conversion at *ARG4*. Five of these were in tetrads in which the YLps disjoined (types A and B), while two were in tetrads in which they nondisjoined (type C). Although these numbers are small, they demonstrate that interactions that can result in gene conversion are insufficient to ensure disjunction of the noncrossover YLps.

Experiments with more extensive homologies were performed with mini *III* pairs in the presence of a YLp. A mini *III* pair, unmatched in size but with 30 kb of

contiguous homologous sequences, showed no segregation bias in the presence of a YLp (Figure 4). In contrast, a mini *III* pair with 72 kb of contiguous homologous sequences showed a slight segregation bias in the presence of a nonhomologous YLp in cases where the mini *IIIs* had not crossed over. Instead of showing 67% disjunction (type A plus type B) and 33% nondisjunction, the noncrossover mini *III* pair showed 74% disjunction and 26% nondisjunction. One possible reason for the underrepresentation of type C tetrads is that two strand double crossovers between the *URA3* and *TRP1* genes of the mini *IIIs* would not be detected but would greatly bias the segregation of the mini *IIIs* such that these tetrads would be scored as types A and B. However, the number of two strand double crossovers should equal the number of four strand double crossovers, and only one four strand double crossover was detected in our experiment. An alternate explanation for this result is that the mini *IIIs* are interacting with the natural copies of chromosome *III* in a way that causes the mini *IIIs* to migrate away from each other slightly more often than would be expected if the YLp was an equal partner. While we can detect no significant disruption of chromosome *III* segregation by the mini *IIIs* (and would expect at most a few percent chromosome *III* nondisjunction based on experiments performed by others), we cannot eliminate the possibility that the bias in segregation of the 72-kb mini *IIIs* is attributable to interactions with the natural copies of chromosome *III* that do not disrupt chromosome *III* disjunction. The most important conclusion from this experiment is that homology provided at most a very small bias to the segregation of the mini *IIIs* compared to the bias seen in the homologous system of Drosophila.

Factors such as size or homology may be important for segregation of achiasmate natural homologues in yeast but have gone undetected in our experimental system. Yeast has no extensive heterochromatic sequences like those observed in Drosophila, but it is conceivable that sequences we have not yet identified play an analogous role in yeast. These concerns could be addressed by evaluating the segregation of achiasmate natural homologues in yeast, but this is a difficult task given the high frequency of meiotic crossovers in yeast and the low incidence with which any one of the yeast chromosomes is achaismate (KABACK *et al.* 1992). The segregation of homeologous achiasmate chromosome *Vs*, one from *S. cerevisiae* and one from *S. carlsbergensis,* suggests that factors not yet described may be important for achiasmate segregation in yeast. These homologues share similar genetic maps but rarely experience crossovers and are partitioned with slightly better fidelity in the achiasmate system than the model molecules described here (NILSSON-TILLGREN *et al.* 1986; R. MAXFIELD and D. DAWSON, unpublished data).

The effects of crossing over between model chromosomes (Figures 5 and 6) suggest that the achiasmate process is not interfering with the ability of the model chromosomes to recombine and be partitioned by a recombination-dependent process. The recombination frequencies of two homologous chromosomes in the presence of a third are similar to frequencies observed when only two model chromosomes are present (ROSS *et al.* 1992). These results are consistent with the model that the achiasmate process either temporally follows the recombination-dependent process, or if it occurs simultaneously, does not interfere with it.

Is there truly an independent achiasmate segregation process in yeast as there is in Drosophila females? There is clearly a smaller need for it in yeast. In yeast, the backup system might be needed to partition achiasmate pairs in occasional meioses (DAWSON *et al.* 1986; KABACK *et al.* 1989) or may also be used to partition homologues whose chiasmata have failed (L. ROSS, R. MAXFIELD and D. DAWSON, unpublished). With a low fidelity achiasmate meiotic segregation like that seen in yeast, it is easy to imagine that achiasmate disjunction is a consequence of features of the normal recombination-dependent pathway, such as spindle shape or components of the synaptonemal complex, that slightly biases the segregation of the achiasmate chromosomes in meiosis I. Cytological examination of achiasmate yeast chromosomes in pachytene have shown that pairing may be important for achiasmate segregation (LOIDL *et al.* 1994). These results are consistant with the model that nonhomologous pairing functions might weakly mimic the role of chiasmata in contributing to the positioning or orienting of achiasmate pairs on the spindle in a fashion that improves the likelihood they will disjoin.

The best evidence for an independent achiasmate system with dedicated components would be the identification of proteins that are specifically required for achiasmate segregation, as have been found in Drosophila. These have not been identified in yeast. However, the observation that expression of certain suppressor tRNAs in yeast can dramatically and specifically disrupt the segregation of noncrossover chromosomes (LOUIS and HABER 1989) suggests that such gene products might exist.

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LITERATURE CITED

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1:** 1–52.

BROWN, P. A., and J. W. SZOSTAK, 1983 Yeast vectors with negative selection, pp. 278–290 in *Methods in Enzymology,* edited by R. WU, L. GROSSMAN and K. MODDAVE. Academic Press, New York.

BURKE, D. T., G. F. CARLE and M. V. OLSON, 1987 Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science **236:** 806–812.

CAO, L., E. ALANI and N. KLECKNER, 1990 A pathway for generation

and processing of double-strand breaks during meiotic recombination in *Saccharomyces cerevisiae*. Cell **61:** 1089–1101.

DAWSON, D. S., A. W. MURRAY and J. W. SZOSTAK, 1986 An alternate pathway for meiotic chromosome segregation in yeast. Science **234:** 713–717.

FOGEL, S., R. K. MORTIMER, K. LUSNAK and F. TAVARES, 1978 Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

GOLDWAY, M., T. ARBEL and G. SIMCHEN, 1993a Meiotic nondisjunction and recombination of chromosome *III* and homologous fragments in *Saccharomyces cerevisiae*. Genetics **133:** 149–158.

GOLDWAY, M., A. SHERMAN, D. ZENVIRTH, T. ARBEL and G. SIMCHEN, 1993b A short chromosomal region with major roles in yeast chromosome *III* meiotic disjunction, recombination and double strand breaks. Genetics **133:** 159–169.

GRELL, R. F., 1976 Distributive pairing, pp. 435–486 in *Genetics and Biology of Drosophila melanogaster*, Vol. 1A, edited by M. A. ASHBURNER and E. NOVITSKI. Academic Press, New York.

GUACCI, V., and D. B. KABACK, 1991 Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. Genetics **127:** 475–488.

HAWLEY, R. S., H. IRICK, A. E. ZITRON, D. A. HADDOX, A. LOHE *et al.*, 1993 There are two mechanisms of achiasmate segregation in Drosophila, one which requires heterochromatic homology. Dev. Genet. **13:** 440–467.

HAWLEY, S. R., 1988 Exchange and chromosomal segregation in eukaryotes, pp. 497–527 in *Genetic Recombination*, edited by R. KUCHERLAPATI and G. R. SMITH. American Society for Microbiology, Washington DC.

HAWLEY, S. R., and W. E. THEURKAUF, 1993 Requiem for distributive segregation: achiasmate segregation in Drosophila females. Trends Genet. **9:** 310–317.

KABACK, D., V. GUACCI, D. BARBER and J. M. MAHON, 1992 Chromosome size-dependent control of meiotic recombination. Science **256:** 228–232.

LOIDL, J., H. SCHERTHAN and D. KABACK, 1994 Physical association between nonhomologous chromosomes precedes distributive disjunction in yeast. Proc. Natl. Acad. Sci. USA **91:** 331–334.

LOUIS, E. J., and J. E. HABER, 1989 Nonrecombinant meiosis I nondisjunction in *Saccharomyces cerevisiae* induced by tRNA ochre suppressors. Genetics **123:** 81–95.

MANN, C., and R. W. DAVIS, 1986 Meiotic disjunction of circular minichromosomes in yeast does not require DNA homology. Proc. Natl. Acad. Sci. USA **83:** 6017–6019.

MURRAY, A. W., and J. W. SZOSTAK, 1983 Construction of artificial chromosomes in yeast. Cell **34:** 961–970.

MURRAY, A. W., N. P. SCHULTES and J. W. SZOSTAK, 1986 Chromosome length controls mitotic chromosome segregation in yeast. Cell **45:** 529–536.

NICOLAS, A., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature **338:** 35–39.

NILSSON-TILLGREN, T., C. GJERMANSEN, S. HOLMBERG, J. G. LITSKE PETERSON and M. C. KIELLAND-BRANDT, 1986 Analysis of chromosome V and the *ILV1* gene from *Saccharomyces carlsbergensis*. Carls. Res. Comm. **51:** 309–326.

O'TOUSA, J., 1982 Meiotic chromosome behavior influenced by mutation-altered disjunction in *Drosophila melanogaster* females. Genetics **102:** 503–524.

ROEDER, G. S., 1990 Chromosome synapsis and genetic recombination: their roles in meiotic chromosome segregation. Trends Genet. **6:** 285–289.

ROSS, L. O., D. TRECO, A. NICOLAS, J. W. SZOSTAK and D. S. DAWSON, 1992 Meiotic recombination on artificial chromosomes in yeast. Genetics **131:** 541–550.

ROTHSTEIN, R. J., 1983 One step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SEARS D. D., J. H. HEGEMANN and P. HEITER, 1992 Meiotic recombination and segregation of human derived artificial chromosomes in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:** 5296–5300.

SEARS, D. D., P. HEITER and G. SIMCHEN, 1994 An implanted recombination hotspot stimulates recombination and enhances sister chromatid cohesion of heterologous YACs during yeast meiosis. Genetics **138:** 1055–1065.

SEARS, D. D., J. H. HEGEMAN, J. H. SHERO and P. HEITER, 1995 Cis-acting determinants affecting centromere function, sister chromatid cohesion and reciprocal recombination during meiosis in *Saccharomyces cerevisiae*. Genetics **139:** 1159–1173.

SHERMAN, F., G. R. FINK and C. LAWRENCE, 1979 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SOKAL, R. R., and F. J. ROHLF, 1969 *Biometry*. Freeman, San Francisco.

SUN, H., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 Double strand breaks at an initiation site for meiotic gene conversion. Nature **339:** 87–90.

SUROSKY, R. T., and B.-K. TYE, 1988 Meiotic disjunction of homologs in *Saccharomyces cerevisiae* is directed by pairing and recombination of chromosome arms but not by pairing of the centromeres. Genetics **119:** 273–287.

ZENVIRTH D., T. ARBEL, A. SHERMAN, M. GOLDWAY, S. KLEIN *et al.* 1992 Multiple sites for double-strand breaks in whole meiotic chromosomes of Saccharomyces cerevisiae. EMBO J. **11:** 3441–3447.

ZHANG, P., and R. S. HAWLEY, 1990 The genetic analysis of distributive segregation in Drosophila melanogaster. II. Further genetic analysis of the *nod* locus. Genetics **125:** 115–127.

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