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Meiotic Gene Conversion and Crossing Over Between Dispersed Homologous Sequences Occurs Frequently in *Saccharomyces cerevisiae*

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ABSTRACT

We have examined meiotic recombination between two defined *leu2* heteroalleles present at the normal *LEU2* locus and in *leu2*-containing plasmids inserted at four other genomic locations. In diploids where the two *leu2* markers were present at allelic locations on parental homologs, the frequency of Leu2$^+$ spores varied 38-fold, in a location-dependent manner. These results indicate that recombination in a genetic interval can be modulated by sequences at least 2.7 kb outside that interval. Leu2$^+$ meiotic segregants were also recovered from diploids where *LEU2* was marked with one heteroallele, and the other *leu2* heteroallele was inserted at another genomic location. These products of ectopic interactions, between dispersed copies of *leu2* sharing only 2.2 kb of homology, were recovered at a frequency comparable to that observed in corresponding allelic crosses. This high frequency of ectopic meiotic recombination was observed in crosses where both recombining partners could potentially pair with sequences at an allelic position. In addition, a significant fraction (22–50%) of these ectopic recombinants were associated with crossing over of flanking sequences.

MEIOTIC recombination is not restricted to exchange between sequences at the same position on homologous chromosomes, but can also occur between sequences present at different locations in the genome. Evidence for meiotic exchange between repeated genetic elements was first described by STURTEVANT (1925) in studies of the *bar* locus of *Drosophila melanogaster*. Subsequent to this initial observation, numerous workers have reported both direct and indirect evidence of recombination between repeated sequences in metazoan organisms [reviewed in BALTIMORE (1981) and KOURILSKY (1986)]. Gene conversion between dispersed, repeated sequences may be responsible for the maintenance of sequence homogeneity in multigene families (EDELMAN and GALLY 1970; TARTOF 1973; SMITH 1973; HOOD, CAMPBELL and ELGIN 1975). Chromosome rearrangement, which can be produced by crossing over between dispersed, homologous sequences, is often associated with fetal death and human genetic disorders (MCKUSICK 1970); in addition, chromosome rearrangement may play an important role in the evolution of new species (WILSON *et al.* 1975; BUSH *et al.* 1977).

Several examples of meiotic recombination between either naturally occurring or artifically created repeated gene families have been observed in less complex eucaryotic organisms. In *Schizosaccharomyes pombe*, a low level of meiotic recombination occurs between repeated tRNA genes located on nonhomologous chromosomes (AMSTUTZ *et al.* 1985). In *Saccharomyces cerevisiae*, meiotic recombination has been observed between repeated sequences located on the same chromosome (PETES 1980; KLEIN and PETES 1981; BORTS *et al.* 1984; JACKSON and FINK 1985), on homologous chromosomes (KLEIN and PETES 1981; ROEDER 1983; KLEIN 1984; JACKSON and FINK 1985), and on nonhomologous chromosomes (JINKS-ROBERTSON and PETES 1985, 1986).

In a previous examination of the timing of crossing over in *S. cerevisiae* meiosis, we obtained physical evidence for a high level of crossing over between the *LEU2* locus and 2.2-kb *leu2* fragment inserted at the *MAT* locus, on the opposite arm of chromosome *III* (BORTS *et al.* 1984). In this report, we extend this observation to an examination of meiotic recombination between a pair of defined *leu2* heteroalleles inserted at various genomic locations. Both recombination between copies of *leu2* present at the same position on parental homologs (hereafter referred to as *allelic recombination*) and recombination between *leu2* sequences present at different genomic locations (hereafter referred to as *ectopic recombination*) have been examined. We find that ectopic recombination between dispersed copies of a short (2.2 kb) sequence can occur frequently during meiosis, and that these recombination events are often associated with crossing over of flanking sequences to create chromosome rearrangements. Similar conclusions have been reported by JINKS-ROBERTSON and PETES (1986). In addition, we present evidence that the frequency of allelic recombination in a defined interval can be modulated by flanking sequences located at least 2.7 kb from that interval.

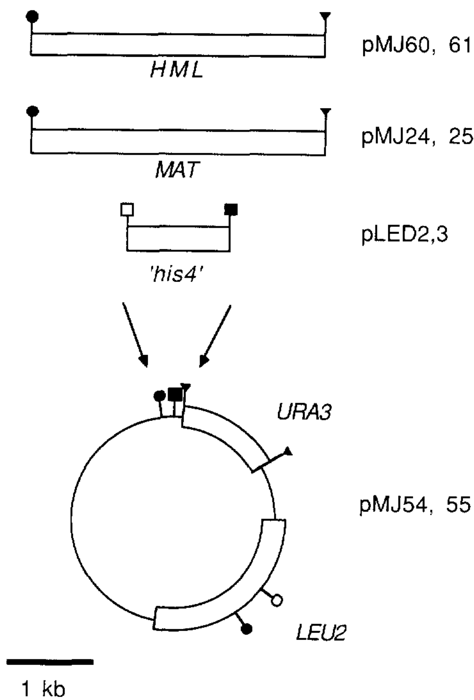


FIGURE 1.—Structure of plasmids. Yeast DNA fragments are indicated by rectangles. Only relevant restriction sites are displayed. (O) *KpnI*; (●) *Eco*RI; (▼) *Hin*dIII; (□) *Pvu*II; (■) *Cla*I.

MATERIALS AND METHODS

Plasmid strains and construction: All plasmids used in this work were derived from plasmids pMJ24 and pMJ25 (alternative names pJH118 and pJH119), which are pBR322 derivatives containing a 3.5-kb *Eco*RI-*Hin*dIII *MAT***a** fragment (ASTELL *et al.* 1981) inserted between the *Eco*RI and *Hin*dIII sites of pBR322, a 1.2-kb *Hin*dIII *URA3* fragment (ROSE, GRISAFI and BOTSTEIN 1984) at the pBR322 *Hin*dIII site, and a 2.2-kb *Xho*I-*Sal*I *LEU2* fragment (ANDREADIS *et al.* 1984) at the pBR322 *Sal*I site. In this plasmid, *URA3* and *LEU2* are oriented with transcription of both genes proceeding clockwise on the standard pBR322 map. pMJ24 and pMJ25 contain, respectively, the *leu2-R* and *leu2-K* mutations, created by ablation of the *Eco*RI or *Kpn*I sites at nucleotides 1295 and 909 in *LEU2* (BORTS, LICHTEN and HABER 1986). pMJ55 (*URA3, leu2-R*) and pMJ54 (*URA3, leu2-K*) were constructed by substituting the 32-bp pBR322 *Eco*RI-*Hin*dIII fragment for the 3.5-kb *Mat***a** *Eco*RI-*Hin*dIII fragment in pMJ24 and pMJ25, respectively. pLED2 (*his4, URA3, leu2-R*) and pLED3 (*his4, URA3, leu2-K*) were constructed by inserting a *Pvu*II-*Cla*I fragment internal to *HIS4* coding sequence [nucleotides 3108 to 1864 in DONAHUE, FARABAUGH and FINK (1982)] between the pBR322 *Eco*RI and *Cla*I sites in pMJ55 and pMJ54, respectively. pMJ60 (*HML, URA3, leu2-R*) and pMJ61 (*HML, URA3, leu2-K*) were constructed by substituting a 3.6-kb *Eco*RI-*Hin*dIII *HML* fragment (ASTELL *et al.* 1981) for the *MAT***a** fragment in pMJ24 and pMJ25. A diagram of these plasmids is presented in Figure 1.

Yeast strains: Genotypes of the diploid yeast strains used in this work are presented in Table 1. All strains used in this work are the products of meiotic segregation or transformation of strains that have been backcrossed four or five times to the Y55 background. Strains containing *leu2-K* or *leu2-R* at *LEU2* were derived from segregants of RHB349 and RHB350 which had undergone ectopic gene conversion between *LEU2* and *MAT::leu2* to convert the wild-type *LEU2* gene to either *leu2-K* or *leu2-R*. Strains containing the *leu2-K,R* double mutation were derived from segregants of RHB422.

Yeast transformants were obtained by site-directed integration (ORR-WEAVER, SZOSTACK and ROTHSTEIN 1983) of the plasmids described above, using either the lithium acetate (ITO *et al.* 1983) or calcium chloride (C. BRUSCHI, personal communication) transformation procedures.

Tetrad analysis: Tetrads were dissected onto YEPD plates as described (SHERMAN, FINK and HICKS 1982). The *leu2* allele contained in segregants was determined by crossing each segregant with *leu2-K* and *leu2-R* tester strains, and scoring the resulting diploids for Leu$^+$ papillae on synthetic complete plates lacking leucine (SHERMAN, FINK and HICKS 1982).

Random spore analysis: Approximately 10^7 tetrads were resuspended in 50 μl of 0.1× glusulase (DuPont), incubated overnight at 30°, diluted in 5 ml 0.1% (w/v) Tween-80 (National Biochemical Corporation), and sonicated until >90% of spores were present as single spores. Dilutions were plated on YEPD agar plates and on synthetic complete plates lacking either leucine or methionine. A minimum of 200 spore colonies were counted for each of these three plating conditions.

All diploid strains used in this work contained the same pair of *met13* heteroalleles, *met13-2* and *met13-4*. In order to facilitate comparison between crosses, and to correct for any effect that inhomogeneity in genetic background might have on overall levels of meiotic recombination, all frequencies of Leu2$^+$ random spores reported in this work were normalized to the mean frequency of Met13$^+$ recombinants observed in 58 independent crosses, using the formula:

$$\text{Normalized } f(\text{Leu2}^+) = \text{observed } f(\text{Leu2}^+) \times \frac{\text{mean } f(\text{Met13}^+)}{\text{observed } f(\text{Met13}^+)}.$$

There was little variation in the frequency of Met13$^+$ random spores among these experiments. The mean f(Met13$^+$) was 1.5×10^{-2}, with a standard deviation of 5.0×10^{-3}. The majority of these Met13$^+$ recombinants were the products of gene conversion, rather than of exchange in the interval between the two alleles (data not shown).

The relative contribution of mitotic recombination to the yield of Leu2$^+$ spores was determined in a subset of the diploids used in this study (RHB422, MJL189, MJL161, MJL182, MJL227, MJL176, MJL178, RHB404, RHB451, MJL168, MJL169, MJL237 and MJL243). In all of these diploids, the frequency of mitotic Leu2$^+$ recombinants was at least 10-fold lower than the observed frequency of Leu2$^+$ meiotic segregants (data not shown).

Yeast colony hybridization: Colony hybridization was performed essentially as described (SHERMAN, FINK and HICKS 1982), except that Pall Biodyne A filter disks (Pall Ultrafine Filtration Corporation) were used in place of nitrocellulose. DNA from lysed colonies was crosslinked to filters by UV irradiation (CHURCH and GILBERT 1984), and the filters were washed for 1–4 hr at 55° in 0.1 × SSPE (1 × SSPE = 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4) (MANIATIS, FRITSCH and SAMBROOK 1982), 1% (w/v) SDS to remove residual cell debris. The filters were then hybridized with radioactive probe as recommended by the manufacturer.

Restriction endonuclease digestion: Restriction endonucleases were obtained from New England Biolabs and Boehringer Mannheim, and were used as recommended by the supplier.

Gel electrophoresis and Southern hybridization: DNA

TABLE 1

Yeast strains

Diploid name	Relevant genotype
Parent diploids	
RHB349, RHB350	LEU2 MATα-URA3-leu2-R-MATα met13-2 ura3 LEU2 MATa-URA3-leu2-K-MATa met13-4 ura3
Diploids for allelic crosses	
RHB422, RHB506, RHB507, RHB508	leu2-R MATa met13-4 ura3 leu2-K MATα met13-2 ura3
MJL189, MJL190	his4'-URA3-leu2-R-'his4 leu2-K, R MATα met13-2 ura3 his4'-URA3-leu2-K-'his4 leu2-K, R MATa met13-4 ura3
MJL161	leu2-R, K MATα-URA3-leu2-K-MATα met13-2 ura3 leu2-R, K MATa-URA3-leu2-R-MATa met13-4 ura3
MJL182, MJL183	leu2-K, R MATα met13-2 URA3-leu2-K-URA3 leu2-K, R MATa met13-4 URA3-leu2-R-URA3
MJL227, MJL228, MJL229, MJL230	HML-URA3-leu2-R-HML leu2-K, R MATα met13-2 ura3 HML-URA3-leu2-K-HML leu2-K, R MATa met13-4 ura3
Strains for ectopic crosses	
MJL178, MJL179	his4'-URA3-leu2-R-'his4 leu2-K MATα met13-2 ura3 HIS4 leu2-K MATa met13-4 ura3
MJL176, MJL177	his4'-URA3-leu2-K-'his4 leu2-R MATa met13-4 ura3 HIS4 leu2-R MATα met13-2 ura3
RHB404, RHB431	leu2-K MATα-URA3-leu2-R-MATα met13-2 ura3 leu2-K MATa met13-4 ura3
RHB451, RHB452	leu2-R MATα-URA3-leu2-K-MATα met13-2 ura3 leu2-R MATa met13-4 ura3
MJL148	leu2-K MATα-URA3-leu2-R-MATα met13-2 ura3 leu2-K MATa-URA3-leu2-R-MATa met13-4 ura3
MJL141	leu2-R MATα-URA3-leu2-K-MATα met13-4 ura3 leu2-R MATa-URA3-leu2-K-MATa met13-2 ura3
MJL131	leu2-K MATα-URA3-leu2-K-MATα met13-4 ura3 leu2-K MATa-URA3-leu2-R-MATa met13-2 ura3
MJL224	leu2-K MATα met13-2 URA3-leu2-R-URA3 leu2-K MATa met13-4 ura3
MJL224	leu2-R MATa met13-4 URA3-leu2-K-URA3 leu2-R MATα met13-2 ura3
MJL168	leu2-R MATa met13-4 URA3-leu2-K-URA3 leu2-K, R MATα met13-2 ura3
MJL169	leu2-K MATα met13-2 URA3-leu2-R-URA3 leu2-K, R MATa met13-4 ura3
MJL237, MJL238	HML-URA3-leu2-R-HML leu2-K MATα met13-2 ura3 HML leu2-K MATa met13-4 ura3
MJL243, MJL244	HML-URA3-leu2-K-HML leu2-R MATα met13-2 ura3 HML leu2-R METa met13-4 ura3

TABLE 1—*Continued*

Diploid name	Relevant genotype	
Strains for translocation test-crosses		
MJL245	*HML LEU2-URA3 CEN5* *HML leu2-K, R-URA3 CEN5*	*URA3-leu2-K CEN3 MAT***a** *URA3-LEU2 CEN3 MATα*
MJL247	*HML leu2-K, R-URA3 CEN5* *HML leu2-R CEN3 MAT***a**	*URA3-LEU2 CEN3 MATα* *ura3 CEN5*
MJL249	*HML LEU2-URA3 CEN5* *HML leu2-K CEN3 MATα*	*URA3-leu2-K CEN3 MAT***a** *ura3 CEN3*

samples were prepared from overnight cultures grown in 5 ml of YEPD broth, using a scaled-down version of the rapid yeast DNA preparation (SHERMAN, FINK and HICKS 1982). Yeast DNA (0.25–1 μg) was restriction endonuclease digested and displayed on a 0.5% (w/v) agarose gel run in 1× TBE buffer (89 mM TRIS, 89 mM boric acid, 2 mM EDTA, pH 8.0) (MANIATIS, FRITSCH and SAMBROOK 1982). DNA fragments were transferred to Pall Biodyne A filter membranes, using the modification of the technique of SOUTHERN (1975) recommended by the manufacturer. Filters were UV cross-linked, and hybridized with radioactive probe as described above.

Preparation of radioactive probe: ^{32}P-labeled probes were prepared by nick translation (RIGBY *et al.* 1977). Probe specific for the *Sal*I-*Eco*RI region of pBR322 was prepared by nick translation of plasmid pUC9, which contains pBR322 sequences between bp 2067 and 4362 (VIERA and MESSING 1982). Probe specific for the *Hin*dIII-*Sal*I region of pBR322 was prepared from the *Hin*dIII-*Sal*I fragment of pBR322, purified by electroelution from agarose gels (MANIATIS, FRITSCH and SAMBROOK 1982).

Analysis of Leu2$^+$ spores for crossover products

Recombinants between *LEU2* and *HIS4*::*leu2*: Leu2$^+$ spores emerging from MJL176–179 were expected to contain one of the structures diagrammed in Figure 2. These structures were distinguished using the following criteria:

A. Spores which contain this noncrossover structure yield colonies which are His$^+$, Ura$^-$, and fail to hybridize to radioactive pBR322 probe upon colony hybridization.

B. These noncrossover spores form colonies which are His$^-$, Ura$^+$, and hybridize to radioactive pBR322 probe. They segregate His$^+$, Ura$^-$ papillae by virtue of mitotic crossing over between the duplicated regions of the *his4* gene to yield a wild-type *HIS4* gene and a *URA3*, *leu2* circular plasmid, which is lost. These papillae are uniformly Leu$^+$, and fail to hybridize to radioactive pBR322 probe.

C. These noncrossover spores yield colonies which are His$^-$, Ura$^+$, and hybridize to radioactive pBR322 probe. They segregate His$^+$, Ura$^-$ papillae which are uniformly Leu$^-$, and fail to hybridize to radioactive pBR322 probe.

D. These crossover spores contain a deletion of sequences between *LEU2* and the *leu2* insert at *HIS4*. They form colonies which are His$^-$, Ura$^+$, hybridize to the *Hin*dIII-*Sal*I fragment of pBR322, and fail to hybridize to pUC9. These colonies also fail to segregate His$^+$ papillae.

E. These crossover spores contain a duplication of sequences between *LEU2* and *HIS4*::*leu2*. They form colonies which are His$^-$, Ura$^+$, and hybridize to radioactive pBR322 probe. They segregate His$^+$, Ura$^-$ papillae which are predominantly Leu2$^+$, and hybridize to radioactive pBR322 probe.

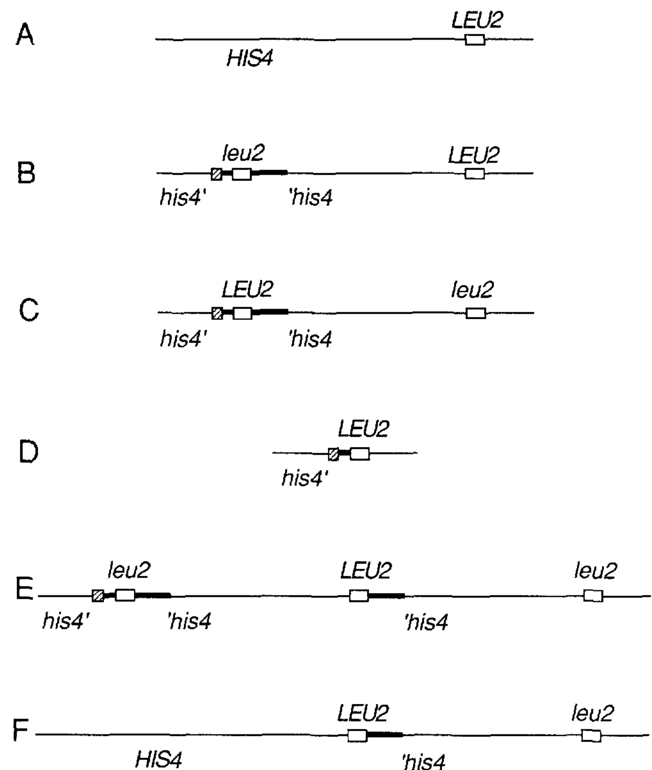


FIGURE 2.—Structures of Leu2$^+$ segregants from MJL 176–179. Open rectangles indicate the 2.2-kb *LEU2 Xho*I-*Sal*I fragment. Cross-hatched rectangles indicate the 1.2-kb *URA3 Hin*dIII fragment. pBR322 sequences are indicated by thick lines. Descriptions of each structure are contained in MATERIAL AND METHODS.

F. These crossover spores contain a duplication of sequences between *LEU2* and *HIS4*::*leu2*. They form colonies which are His$^+$, Ura$^-$, hybridize to radioactive pUC9 probe, and fail to hybridize to the *Hin*dIII-*Sal*I fragment of pBR322.

The structures of segregants which met the criteria outlined in sections C, E and F were confirmed on Southern blots. In addition to the six classes described above, 2 of 474 spore colonies were isolated which were His$^+$, Ura$^+$, and failed to hybridize to radioactive pBR322. Southern analysis revealed that these segregants contained a noncrossover chromosome *III* structure identical to that illustrated in Figure 2A. These segregants are most likely the

products of two independent gene conversion events, in which the mutant allele present at *LEU2* was converted to wild type, and the mutant *ura3* allele present at *URA3* , on chromosome *V*, was also converted wild type.

Recombinants between *LEU2* and *MAT::leu2*: Leu2$^+$ segregants from RHB404, RHB432, RHB451 and RHB452 were analyzed by a "spore-mating test," to determine the linkage of Leu2$^+$ information to *MAT*. Leu2$^+$ segregants were mated to haploid strains containing the auxotrophic markers *leu2 met13 arg6*. The resulting diploids were sporulated, and the spores were replica-plated to YEPD plates and cross-stamped with *MAT***a** *leu2* and *MATα leu2* tester strains. After overnight incubation to allow germination of spores and mating, the spore-mating plates were replica-plated to synthetic minimal plates (SHERMAN, FINK and HICKS 1984). If Leu2$^+$ information is linked to *MAT*, then only Leu2$^+$ spores of a single mating-type will be produced. These will mate with the tester to form prototrophic diploids, and growth will be observed at only one of the two intersections. If, however, Leu2$^+$ is located at *LEU2*, a similar number of *MAT***a** *LEU2* and *MATα LEU2* segregants will be present in spores, and both intersections will yield growth on minimal media. The chromosome *III* structure of a subset of these segregants was also analyzed on Southern blots.

Recombinants between *LEU2* and *URA3::leu2*: Leu2$^+$ segregants from MJL168 and MJL169 formed either Ura$^+$ or Ura$^-$ colonies. Ura$^-$ colonies were assumed to be the products of gene conversion to wild type of the mutant *leu2* allele located on chromosome *III*, followed by cosegregation with a chromosome *V* which did not carry a plasmid insert. These spore colonies failed to hybridize with radioactive pBR322, thus confirming their structure. The structures of all other segregants (which were Ura$^+$ and which hybridized to radioactive pBR322) were analyzed on Southern blots. A *Bgl*II digest was used to identify crossover spores which contained chromosome *III*-chromosome *V* translocations (see RESULTS). A combination of *Hin*dIII/*Asp*718, *Hin*dIII/*Eco*RI, and *Hin*dIII/*Asp*718/*Eco*RI multiple digests was used to determine the location of *LEU2* information in these segregants (*Asp*718 and *Kpn*I have the same 6-bp recognition site).

RESULTS

We have examined allelic and ectopic meiotic recombination between a pair of defined *leu2* heteroalleles, *leu2-K* and *leu2-R*. These mutant alleles were created *in vitro* by enzymatic ablation of a *Kpn*I site and an *Eco*RI site located at nucleotides 909 and 1295, respectively, in the 2.2-kb *LEU2 Xho*I-*Sal*I fragment (ANDREADIS *et al.* 1984). These two *leu2* mutants were introduced at the normal *LEU2* locus, on chromosome *III*. Plasmids carrying these two *leu2* mutants were also inserted by integrative transformation (ORR-WEAVER, SZOSTACK and ROTHSTEIN 1983) at four other locations: at *HML*, about 73 kb centromere-distal to *LEU2* on chromosome *III*; at *HIS4*, about 20 kb centromere-distal to *LEU2* on chromosome *III*; at *MAT*, about 115 kb from *LEU2* on the opposite arm of chromosome *III*; and at *URA3*, on chromosome *V*. The structures on these inserts, and their genomic locations, are illustrated in Figure 3.

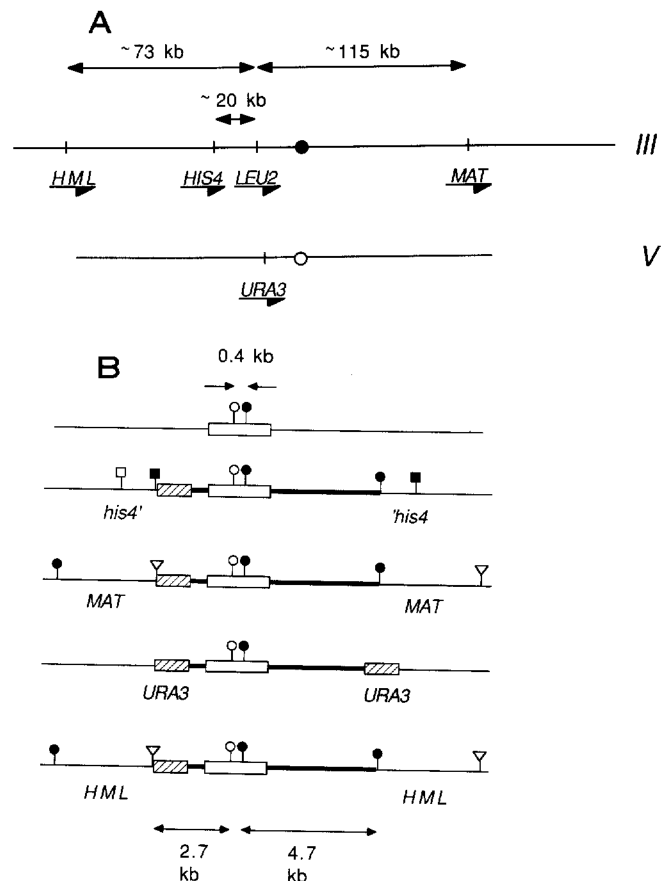


FIGURE 3.—Structure of *leu2* plasmid inserts. A, Genomic location of the inserts used in this study. Physical distances on chromosome *III* are approximate (NEWLON *et al.* 1985). Half arrows indicate the direction of transcription of the *LEU2* gene at each location. B, Physical structure of *leu2* plasmid inserts, showing the 0.4-kb *leu2-K-leu2-R* interval, and the degree of homology shared by insert structures. Open rectangles indicate the 2.2-kb *LEU2 Xho*I-*Sal*I fragment. Cross-hatched rectangles indicate the 1.2-kb *URA3 Hin*dIII fragment. pBR322 sequences are indicated by thick lines. Restriction enzyme recognition sites are indicated as follows: Only relevant restriction sites are displayed. (○) *Kpn*I; (●) *Eco*RI; (▼) *Hin*dIII; (□) *Pvu*II; (■) *Cla*I. The four *leu2* inserts (at *HIS4*, *MAT*, *URA3*, and *HML*) share 2.7 kb of homology to the left of the *leu2-K-leu2-R* interval, and 4.7 kb of homology to the right. The plasmids used to insert *leu2* at *HIS4* contained a 1.2 kb *Pvu*II-*Cla*I fragment internal to the *HIS4* gene; therefore, integration of this plasmid creates two terminally-deleted copies of *HIS4* (SHORTLE, HABER and BOTSTEIN 1982). These are designated *his4'* and *'his4* in the figure.

The nature of meiotic recombination events which generated *LEU2* prototrophs was determined by analysis of tetrads from crosses where both *leu2-K* and *leu2-R* were located at the normal *LEU2* locus (Table 2). More than 90% (46 of 51) of tetrads which contained Leu2$^+$ spores were the products of gene conversion, rather than of crossing over between the two mutant alleles. About 80% (40 of 51) were the products of events in which the *leu2-K* allele was converted to wild type. It should be noted that events which yielded a *LEU2* prototroph constituted only one third of the total identifiable interactions between the two mutants *leu2* genes. In addition to the 28 of 628

TABLE 2

Tetrad analysis of gene conversion at *LEU2*

Tetrad genotype a	b	c	d	Presumed conversion event	Unselected tetrads[a]	Selected tetrads[b]
1. Tetrads containing a Leu2$^+$ spore						
+	K	R	R	K → +	22	40
K	K	+	R	R → +	1	4
+	K	K, R	R	Crossover	4	5
+	+	R	R	Multiple event		1
+	K, R	R	R	Multiple event		1
2. Other tetrads						
K	K	K, R	R	+ → K	18	
K	K, R	R	R	+ → R	9	
K	R	R	R	K + → + R	18	
K	K	K	R	+ R → K +	21	
K	K, R	R	K, R	Multiple event	1	
K	K	R	R	Parental	534	
Total examined					628	51

[a] Six hundred and twenty eight tetrads from RHB422 were dissected. *LEU2* genotypes of spores were determined as described in MATERIALS AND METHODS.

[b] In addition to the 27 tetrads containing a Leu2$^+$ spore reported in the previous column, 24 tetrads containing a Leu2$^+$ spore from additional dissections of RHB422, RHB506, RHB507 and RHB508 were analyzed.

TABLE 3

Allelic recombination between *leu2-K* and *leu2-R* inserted at various chromosomal locations

Diploid name	Location of mutant alleles	$\frac{\text{Leu2}^+ \text{ spores}}{\text{Total spores}} \times 10^3$ [a]
RHB422	*LEU2*	15
RHB422		11
RHB422		12
RHB506		13
RHB507		12
RHB508		13
		13 ± 1.4
MJL189	*HIS4*	5.3
MJL190		4.6
		5.0 ± 0.5
MJL161	*MAT*	1.7
MJL182	*URA3*	0.67
MJL183		0.85
		0.76 ± 0.12
MJL227	*HML*	0.39
MJL228		0.34
MJL229		0.26
MJL230		0.35
		0.34 ± 0.05

Diploids contained *leu2-K* and *leu2-R* at the indicated location on parental homologs. In crosses involving inserted copies of *leu2* (*HIS4, MAT, URA3* and *HML*), the normal *LEU2* locus in both parents contained a *leu2-K, R* double mutation, and therefore could not contribute to the yield of Leu2$^+$ recombinants.

[a] Frequencies of Leu2$^+$ random spores were normalized as described in MATERIALS AND METHODS. When multiple determinations were made, the mean frequency of Leu2$^+$ and the standard deviation of the mean are reported.

unselected tetrads which contained a Leu2$^+$ spore, another 66 tetrads contained the products of gene conversion events which did not yield a Leu2$^+$ segregant.

Location affects the frequency of allelic meiotic recombination: To assess the effect of flanking sequences on allelic meiotic recombination between *leu2-K* and *leu2-R*, we measured the frequency of Leu2$^+$ random spores emerging from diploids where both mutant *leu2* alleles were present at *LEU2, HIS4, MAT, URA3* or *HML*. The results of these crosses are presented in Table 3. The frequency of Leu2$^+$ random spores recovered varied almost 40-fold (from 3.4×10^{-4} to 1.3×10^{-2}), with the highest frequency of *LEU2* prototrophs recovered from diploids where both mutant alleles were present at *LEU2*. In the four crosses involving inserted copies of *leu2*, the frequency of Leu2$^+$ recombinants varied almost 15-fold, from 3.4×10^{-4} to 5.0×10^{-3}. It should be noted that in these latter four diploids, the two mutant *leu2* alleles were present in the same 7.8-kb structure, with identical sequences flanking the *leu2-K-leu2-R* interval for at least 2.7 kb on either side (Figure 3B).

Meiotic recombination between dispersed copies of *LEU2* occurs frequently: The frequency of recombination between dispersed copies of the *LEU2* gene was determined using a series of diploid strains, in which *LEU2* was marked with either *leu2-K* or *leu2-R*, and the other *leu2* heteroallele was inserted at *HIS4*, *MAT, URA3* or *HML*. In these strains, the two dispersed copies of *leu2* shared 2.2 kb of homology. The structure of the diploids used, and the frequency of Leu2$^+$ spores recovered, is presented in Table 4. In most cases, Leu2$^+$ spores were recovered at frequencies comparable to those observed in the corresponding allelic crosses, where *leu2-K* and *leu2-R* were present in the context of contiguous and continuous flanking homology. It should be noted that, in the crosses presented in Table 4, and most notably in the crosses in sections B, C and D, Leu2$^+$ recombinants accompanied by crossing over of flanking sequences can produce haploid-inviable chromosome rearrangements, which are not recovered among spores (see below). Therefore, the frequencies presented are a minimum measure of the total ectopic recombination events.

A notable feature of the results presented in Table 4 is the effect that the configuration of markers had on the frequency of Leu2$^+$ spores recovered. In general, diploids where *leu2-K* was present at *LEU2* yielded more (by a factor of 1.3–4.7) Leu2$^+$ recombinants than did diploids where *leu2-K* was present at

TABLE 4

Recombination between *leu2* alleles inserted at different chromosomal locations

Diploid structure	Diploid name	$\frac{\text{Leu2}^+ \text{ spores}}{\text{Total spores}} \times 10^{3a}$
A. *HIS4*		
-his4-URA3-leu2-R-his4 —— *leu2-K* ——	MJL178	8.3
—— *HIS4* —— *leu2-K* ——	MJL179	9.3
-his4-URA3-leu2-K-his4 —— *leu2-R* ——	MJL176	7.0
—— *HIS4* —— *leu2-R* ——	MJL177	6.1
B. *MAT*		
—— *leu2-K* —— *MATα-URA3-leu2-R-MATα* ——	RHB404	10
—— *leu2-K* —— *MAT***a** ——	RHB431	8.4
—— *leu2-R* —— *MATα-URA3-leu2-K-MATα* ——	RHB451	2.7
—— *leu2-R* —— *MAT***a** ——	RHB452	1.8
C. *URA3*		
—— *leu2-K* —— *-URA3-leu2-R-URA3* ——	MJL225	2.2
—— *leu2-K* —— —— *ura3* ——	MJL226	2.0
—— *leu2-R* —— *-URA3-leu2-K-URA3* ——	MJL224	0.45
—— *leu2-R* —— —— *ura3* ——		
D. *HML*		
-HML-URA3-leu2-R-HML —— *leu2-K* ——	MJL237	4.0
—— *HML* —— *leu2-K* ——	MJL238	5.5
-HML-URA3-leu2-K-HML —— *leu2-R* ——	MJL243	1.4
—— *HML* —— *leu2-R* ——	MJL244	2.1

[a] Frequencies of Leu2$^+$ random spores were normalized as described in MATERIALS AND METHODS.

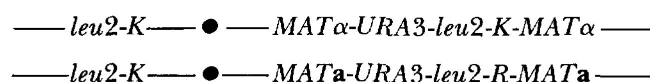
an insert position. To further examine this phenomenon, we determined the location of the wild-type copy of *LEU2* in Leu2$^+$ spores which contained a parental chromosome configuration. These segregants are most likely the products of ectopic recombination events which involved conversion of a mutant *leu2* allele to wild type without crossing over of flanking sequences. These results are presented in Table 5.

In all cases, the absolute frequency of conversion to wild type of a given *leu2* mutant allele in diploids where that allele was located at *LEU2* was higher (by a factor of 1.4–50) than the frequency of conversion to wild type of the same allele in a corresponding cross, where the allele was present at an insert position. In addition, the frequency of conversion to Leu2$^+$ of a given locus in diploids which contained *leu2-K* at that locus was higher (by a factor of 2.3–26) than in corresponding diploids, where *leu2-R* was present at the same locus. Thus, ectopic gene conversion displays the same marker preference (for a given locus, *leu2-K* is more frequently converted to wild type than is *leu2-R*) and locus dependence (the normal *LEU2* locus is more frequently converted to wild type than are insert loci) observed in allelic crosses.

Competition between allelic and ectopic recombination: In the diploids presented in Table 4, the mutant copy of *leu2* inserted at *HIS4*, *MAT*, *HML* or *URA3* was present in only one parent, and could not pair with homologous sequences at the same position on the other parental homolog. To determine whether the inability to pair at a homologous position was responsible for the high frequency of ectopic recombination observed, we constructed a pair of diploid strains where either *leu2-K* or *leu2-R* was homozygous at *LEU2*, and the other mutant allele was present in homozygous plasmid inserts at *MAT*. The results of these crosses are presented in Table 6.

In these diploids, a mutant copy of *leu2* inserted at *MAT* can interact either with *leu2* sequences bearing the same mutation at *MAT*, or with a copy of *leu2* bearing the other mutation at *LEU2*. Only these latter ectopic interactions can yield Leu2$^+$ recombinants. In both cases, the frequency of Leu2$^+$ spores recovered was comparable to that observed in diploids which contained a single *leu2* insert at *MAT* (1.3×10^{-2} vs. 9.2×10^{-3}, 1.7×10^{-3} vs. 2.3×10^{-3}). These results indicate that pairing interactions between sequences at allelic positions only modestly competes with ectopic interactions between homologous sequences at different chromosomal locations.

This observation is further illustrated in the diploid diagrammed below:



Leu2$^+$ recombinants can be produced either by allelic exchange between copies of *leu2-R* and *leu2-K* in-

TABLE 5

Position and allele effects

Diploid structure	Diploid name	Average frequency of noncrossover segregants, × 10^3 [a]	No. of segregants containing Leu2$^+$ at: *LEU2*	Insert[b]
A. *HIS4*				
-his4-URA3-leu2-R-his4 —— *leu2-K* —— —— *HIS4* —— *leu2-K* ——	MJL178 MJL179	4.4	105	13
-his4-URA3-leu2-K-his4 —— *leu2-R* —— —— *HIS4* —— *leu2-R* ——	MJL176 MJL177	3.9	44	99
B. *MAT*				
—— *leu2-K* —— *MATα-URA3-leu2-R-MATα-* —— *leu2-K* —— *MAT***a** ——	RHB404 RHB431	8.8	356	2
—— *leu2-R* —— *MATα-URA3-leu2-K-MATα-* —— *leu2-R* —— *MAT***a** ——	RHB451 RHB452	2.3	79	111
C. *URA3*				
—*leu2-K*—— *-URA3-leu2-R-URA3* —— —*leu2-K, R*— —— *ura3* ——	MJL169	1.4	111	1
—*leu2-R*—— *-URA3-leu2-K-URA3* —— —*leu2-K, R*— —— *ura3* ——	MJL168	0.16	90	19

[a] Only Leu2$^+$ segregants which contained parental chromosome configurations were analyzed. The frequencies presented in this column were obtained by correcting the mean total frequency of Leu2$^+$ spores to reflect the fraction of spores analyzed which contained parental chromosome configurations (*cf.* Tables 7 and 8). Overall frequencies of Leu2$^+$ random spores from diploids in sections A and B are presented in Table 4. The total frequency of Leu2$^+$ random spores in MJL169 was 1.5×10^{-3}; in MJL168, 1.8×10^{-4}.

[b] The location of the mutant *leu2* copy which had been converted to Leu2$^+$ was determined as described in MATERIALS AND METHODS.

TABLE 6

Effect of allelic pairing on ectopic recombination

Diploid structure	Diploid name	$\frac{\text{Leu2}^+ \text{ spores}}{\text{Total spores}} \times 10^3$ [a]
—— *leu2-K* —— *MATα-URA3-leu2-R-MATα-* —— *leu2-K* —— *MAT***a***-URA3-leu2-R-MAT***a***-*	MJL148	13
—— *leu2-K* —— *MATα-URA3-leu2-R-MATα-* —— *leu2-K* —— *MAT***a** ——	RHB404 RHB431	10 8.4
—— *leu2-R* —— *MATα-URA3-leu2-K-MATα-* —— *leu2-R* —— *MAT***a***-URA3-leu2-K-MAT***a***-*	MJL141	1.7
—— *leu2-R* —— *MATα-URA3-leu2-K-MATα-* —— *leu2-R* —— *MAT***a** ——	RHB451 RHB452	2.7 1.8

[a] Frequencies of Leu2$^+$ random spores were normalized as described in MATERIALS AND METHODS.

serted at *MAT* (*MAT::leu2-R* and *MAT::leu2-K*), or by ectopic gene conversion events involving *MAT::leu2-R* and one of the copies of *leu2-K* at *LEU2*. Leu2$^+$ spores were recovered at a frequency of 6.5×10^{-3}. About 60% of these (117 of 198) were conversions of *leu2-K* at *LEU2*, an event that could only occur via ectopic interactions (without associated crossing over). These results again indicate that ectopic exchange events can occur frequently, even under circumstances where both interacting copies have the opportunity to pair with sequences at a homologous position.

Ectopic meiotic recombination is associated with crossing over: Meiotic gene conversion events involving sequences at homologous chromosomal locations are frequently accompanied by crossing over of flanking sequences (FOGEL, MORTIMER and LUSNAK 1981). To determine whether ectopic recombination between dispersed copies of *leu2* was also accompanied by crossing over, we used a combination of genetic and Southern analyses to estimate the fraction of Leu2$^+$ recombinant spores containing crossover products.

Since the 20 kb interval between *HIS4* and *LEU2* contains no essential sequences (ROEDER 1983) recombinants between *HIS4::leu2* and *LEU2* which contain either deletions or duplications of this region are haploid-viable (Figure 4). Thus, the majority of crossovers between these dispersed copies of *leu2* can be recovered. Leu2$^+$ spores which contain these crossover structures can be identified either by analysis of

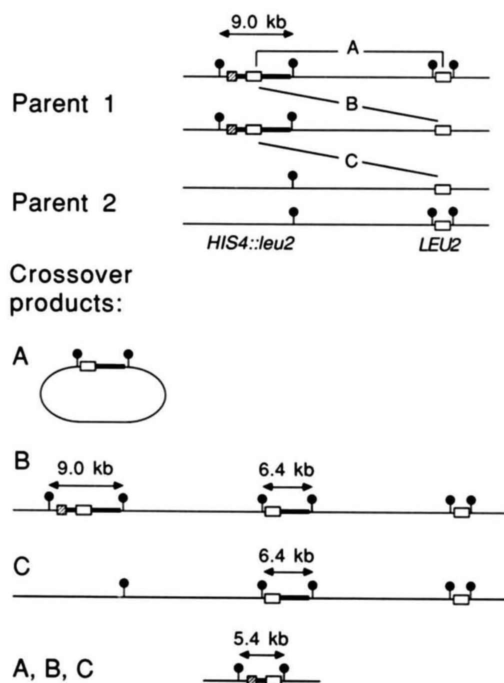


FIGURE 4.—Structures produced by crossing over between *LEU2* and *HIS4::leu2*. The parental diploid at the four-strand stage of meiosis is illustrated in the top half of the figure. Crossover products are illustrated below. Open rectangles indicate the 2.2-kb *LEU2 XhoI-SalI* fragment. Cross-hatched rectangles indicate the 1.2-kb *URA3 HindIII* fragment. pBR322 sequences are indicated by thick lines. Relevant *BglII* restriction sites are indicated by filled circles. Sizes of *BglII* fragments hybridizing to pBR322 are indicated. The insert-bearing parental chromosome contains a 9.0-kb *BglII* fragment which hybridizes to pBR322. A, Crossing over between *leu2* sequences on the same chromatid yields an acentric circle containing a single copy of *LEU2* and chromosome *III* sequences between *HIS4* and *LEU2*, and a chromosome *III* which contains a deletion of these intervening sequences (illustrated at the bottom of the figure). The circle produced does not contain sequences necessary for autonomous replication (NEWLON *et al.* 1985) and will be lost. The deletion-bearing chromosome contains a 5.4 kb *BglII* fragment which hybridizes to pBR322. B, Crossing over between dispersed *LEU2* sequences on sister chromatids will yield a chromosome which contains a duplication of sequences between *HIS4* and *LEU2*, and a chromosome which contains a deletion of these intervening sequences. The duplication chromosome contains two *BglII* fragments (9.0 and 6.4 kb) which hybridize to pBR322. C, Crossing over between dispersed *LEU2* sequences on non-sister homologs will yield a chromosome which contains a duplication of sequences between *HIS4* and *LEU2*, and a chromosome which contains a deletion of these intervening sequences. The duplication chromosome contains a single 6.4-kb *BglII* fragment which hybridizes to pBR322.

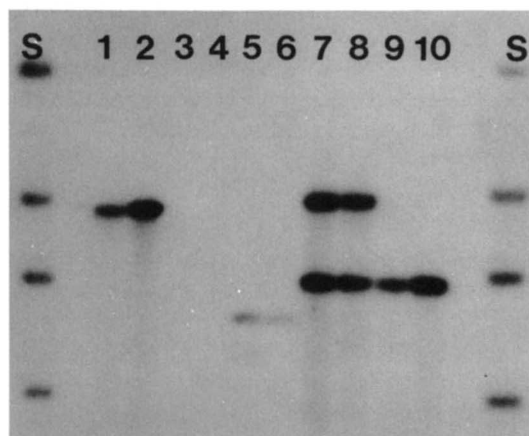


FIGURE 5.—Southern analysis of Leu2$^+$ segregants from MJL176–MJL179. DNA samples were prepared from representative strains identified by genetic analysis (see MATERIALS AND METHODS) as containing each of the chromosome *III* structures diagrammed in Figure 2. Samples were digested with *BglII*, displayed on a 0.5% agarose gel, transferred to filter membranes, and hybridized with radioactive pBR322. Lanes marked "S" contain *HindIII* digests of λ DNA as molecular weight standards (from top to bottom: 23, 9.4, 6.6 and 4.4 kb). Lanes 1 and 2, Noncrossover recombinant of the Parent 1 configuration. Lanes 3 and 4, Noncrossover recombinant of the Parent 2 configuration. Lanes 5 and 6, Deletion crossover structure produced by all three ectopic crossover classes (Figure 4, classes A, B and C). Lanes 7 and 8, Duplication crossover structure produced by ectopic sister-chromatid exchange (Figure 4, class B). Lanes 9 and 10, Duplication crossover structure produced by ectopic exchange between homologs (Figure 4, class C).

Southern blots (Figure 5) or by a combination of genetic and colony hybridization analyses (see MATERIALS AND METHODS).

About half (215 of 474) of the Leu2$^+$ spores emerging from the diploids presented in Table 4a contained structures expected from a crossover between *HIS4::leu2* and the normal *leu2* locus (Table 7). A marked asymmetry in the recovery of deletions and duplications of the *HIS4-LEU2* region was observed. In diploids containing *HIS4::leu2-K* and *leu2-R*, the majority of Leu2$^+$ crossover products (92 of 96) were duplications of the *HIS4-LEU2* interval. When the alleles were reversed (*i.e.*, *HIS4::leu2-R* and *leu2-K*), most Leu2$^+$ crossover products (100 of 119) contained deletions of the *HIS4-LEU2* interval. This asymmetry in the direction of crossovers recovered is similar to that observed in studies of allelic meiotic recombination between heteroalleles (FOGEL and HURST 1967), and is consistent with the suggestion that crossover-associated intragenic recombinants are produced primarily by events that terminate in the interval between the two markers.

Ectopic recombination between *LEU2* and *MAT::leu2*: In a previous examination of DNA extracted from cells undergoing meiosis, we obtained physical evidence of a high frequency (about 0.5% of haploid genomes) of crossing over between the normal *LEU2* locus and *leu2* sequences inserted at *MAT* (BORTS *et al.* 1984). These events yield haploid-inviable acentric, dicentric, and deficiency circle chromosomes (Figure 6) and would not be expected to be present among the Leu2$^+$ spores recovered from the crosses diagrammed in Table 4B. This expectation was confirmed by Southern blot analysis of DNA extracted from Leu2$^+$ segregants from these crosses. The vast majority (149 of 150) contained a single copy of chromosome *III* in either of the two parental configurations. A single Leu2$^+$ spore (which was other-

TABLE 7

Crossing over associated with ectopic recombination

Parental diploid structure	Structure of Leu2$^+$ segregants[a]		
		Crossover	
	Parental	Deletion	Duplication
MJL176, MJL177			
-his4-URA3-leu2-K-his4 ——— *leu2-R* —— / —— *HIS4* ——— *leu2-R* ——	141/237	4/237	92/237
MJL178, MJL179			
-his4-URA3-leu2-R-his4 ——— *leu2-K* —— / —— *HIS4* ——— *leu2-K* ——	118/237	100/237	19/237

[a] Chromosome *III* structures were determined as described in Experimental Procedures. Segregants with deletion crossover structures contained a deletion of sequences between *LEU2* and the *leu2* insert at *HIS4*. Segregants with duplication crossover structures contained a duplication of sequences between *LEU2* and the *leu2* insert at *HIS4* (see Figures 4 and 5 for details).

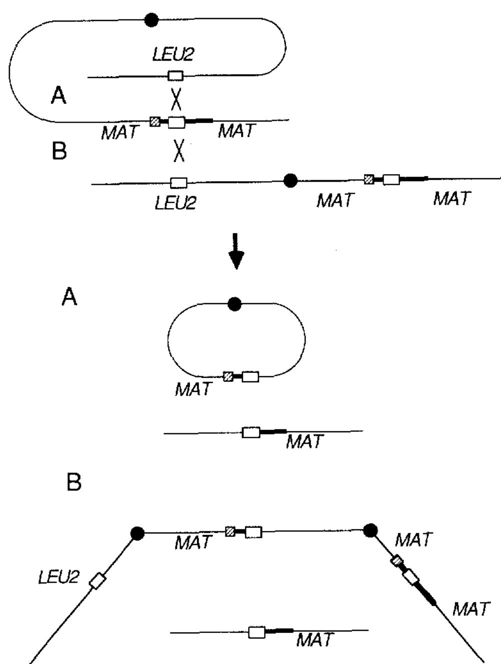


FIGURE 6.—Crossing over between *LEU2* and *MAT::leu2* yields haploid-inviable chromosomes. Open rectangles indicate the 2.2-kb *LEU2 XhoI-SalI* fragment. Cross-hatched rectangles indicate the 1.2-kb *URA3 HindIII* fragment. pBR322 sequences are indicated by thick lines. A, Crossing over between dispersed *LEU2* sequences on the same chromatid will yield an acentric linear and a deficiency-circle chromosome *III*. B, Crossing over between dispersed *LEU2* sequences on different copies of chromosome *III* will yield an acentric linear fragment and a dicentric linear chromosome *III*. Such dicentric chromosomes are mitotically unstable, and will break to form haploid-inviable products (HABER, THORBURN and RODGERS 1984).

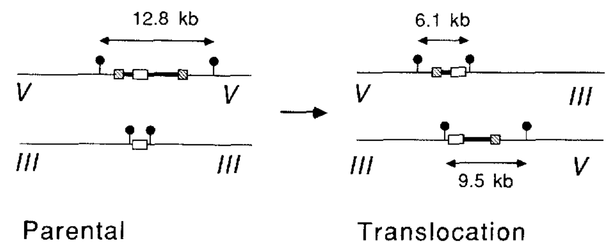


FIGURE 7.—Crossing over between *LEU2* and *URA3::leu2* yields a reciprocal translocation between chromosomes *III* and *V*. Open rectangles indicate the 2.2-kb *LEU2 XhoI-SalI* fragment. Cross-hatched rectangles indicate the 1.2-kb *URA3 HindIII* fragment. pBR322 sequences are indicated by thick lines. Filled circles indicate *BglII* restriction sites. Sizes of *BglII* fragments containing pBR322 sequences are indicated.

wise haploid) contained, in addition to a parental chromosome *III*, a circular, Leu2$^+$ deficiency chromosome of the type illustrated in Figure 6 (data not shown). This spore was most likely the product of a crossover between the dispersed copies of *leu2*, combined with a subsequent chromosome *III* meiotic nondisjunction.

Although such crossover products are rarely found among viable haploid spores, they were recovered at higher levels among "meiototic" Leu2$^+$ segregants isolated by meiotic reversal. In this procedure, diploids undergoing meiosis are returned to vegetative growth by plating on nitrogen-rich media. Cells which have already become committed to meiotic recombination, but which have not yet become committed to haploidization, are recovered as diploids which display meiotic levels of recombination (ESPOSITO and ESPOSITO 1974). About 10% (4 of 37) of diploid Leu2$^+$ segregants obtained by meiotic reversal of RHB404, after 6 hr of sporulation, contained products of crossing over between *leu2* and *MAT::leu2* (M. LICHTEN, R. H. BORTS and J. E. HABER, unpublished observations).

Reciprocal translocations produced by crossing over between *LEU2* and *URA3::leu2*: Ectopic recombination between *leu2* sequences inserted at *URA3* and the normal *LEU2* locus is also associated with crossing over. Crossovers between these dispersed copies of *leu2* yield a reciprocal translocation between chromosomes *III* and *V* (Figure 7), and can be distinguished from noncrossover Leu2$^+$ spores on Southern blots (Figure 8). Reciprocal translocations were recovered in about 7% (16 of 240) of all Leu2$^+$ spores

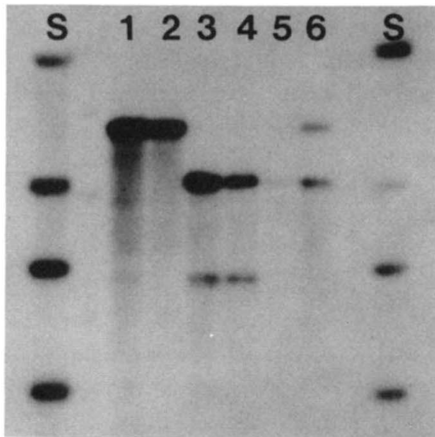


FIGURE 8.—Southern analysis of Leu2+ segregants from MJL 168 and 169. DNA samples from representative Leu2+, Ura3+ segregants were digested with *Bgl*II, displayed on a 0.5% agarose gel, transferred to filter membranes, and hybridized with radioactive pBR322. Lanes marked "S" contain *Hin*dIII digests of λ DNA as molecular weight standards (from top to bottom: 23, 9.4, 6.6 and 4.4 kb). Lanes 1 and 2, Non crossover segregants. A single 12.8 kb pBR322-hybridizing fragment is detected. Lanes 3 and 4, Crossover segregants which contain a reciprocal translocation between chromosomes *III* and *V*. Two pBR322-hybridizing fragments (6.1 and 9.5 kb) are detected. Lanes 5 and 6, Crossover segregants which contain only one of the two products of a reciprocal translocation. Both of these segregants contained the 9.5-kb chromosome *III-LEU2*-pBR-*URA3*-chromosome *V* crossover product (see Figure 7). In addition, both segregants contained a normal complement of chromosomes *III* and *V*. In one case (lane 5), the normal chromosome *V* was derived from the parent which lacked a *LEU2* insert at *URA3*; in the other case (lane 6), the normal chromosome *V* was derived from the insert-containing parent.

(Table 8). Both translocation products are expected to cosegregate (and thus produce a viable haploid spore) in only one quarter of all crossover tetrads. We therefore used the following formula to estimate the actual fraction of Leu2+ meiotic recombinants associated with crossing over:

$$\text{Actual fraction crossovers} = \frac{n + 3n}{m + 3n}$$

where n is the number of Leu2+ spores which contain reciprocal translocation products, $3n$ is the number of inviable Leu2+ spores which contain only one translocation chromosome, and m is the total number of Leu2+ spores recovered. Applying this formula, we estimate that 22% of exchanges between *leu2* sequences on chromosomes *III* and *V* are associated with crossing over of flanking sequences.

The identification of these segregants as reciprocal translocations was confirmed by genetic analysis. Two diploids heterozygous for the putative translocation were constructed by mating translocation-containing segregants to normal haploid strains; in addition, a diploid homozygous for the putative reciprocal translocation was created by mating two translocation-

TABLE 8

Crossing over associated with ectopic recombination

Diploid structure	Structure of Leu2+ segregants[a] Parental	Reciprocal translocation	Other
MJL 168 III ——*leu2-K, R*—— ——*leu2-R*—— V -*URA3-leu2-K-URA3*—— ——*ura3*——	110/120	9/120	1/120
MJL 169 III ——*leu2-K, R*—— ——*leu2-K*—— V -*URA3-leu2-R-URA3*—— ——*ura3*——	112/120	7/120	1/120

[a] Chromosome *III* and *V* structures were determined as described in MATERIALS AND METHODS. Segregants with a reciprocal translocation crossover structures contained both products of a crossover between *leu2* sequences located on chromosomes *III* and *V*. Crossover segregants marked "other" contained only one of the two expected crossover products, and, in addition, parental copies of chromosomes *III* and *V*.

TABLE 9

Tetrad analysis of reciprocal translocations

Strain name	Putative chromosome structure	Spore viability (live:dead) 4:0	3:1	2:2	1:3	0:4
RHB422	Normal	226	38	6	2	0
MJL245	Translocation homozygote	36	10	3	0	0
MJL247	Translocation heterozygote	20	4	31	6	11
MJL249	Translocation heterozygote	19	2	28	6	16

containing segregants. Asci from these diploids were dissected and examined for spore viability (Table 9). The vast majority of tetrads from both a normal diploid strain and from the translocation homozygote contained four viable spores. In contrast, tetrads from the two putative translocation heterozygotes exhibited a trimodal pattern of spore viability, and predominantly displayed live:dead tetrad types of 4:0, 2:2, and 0:4. This pattern of spore lethality is characteristic of translocation heterozygotes (PERKINS and BARRY 1977).

In addition to the 16 Leu2+ spores which contained both translocation products, two Leu2+ haploid spores were recovered which were partially disomic for chromosome *V*. Both contained one parental copy of chromosome *V*, while the other copy of chromosome *V* had a structure consistent with one of the two expected products of crossing over between *LEU2* and *URA3:leu2* (Figure 8, lanes 5 and 6). These segregants are most likely the products of a crossing over between the dispersed copies of *leu2* combined with a chromosome *V* meiotic nondisjunction.

DISCUSSION

In the experiments reported above, we have examined both allelic and ectopic meiotic recombination between copies of the *LEU2* gene present at various locations in the *S. cerevisiae* genome. In allelic crosses, where both *leu2-K* and *leu2-R* were at the same position on homologous chromosomes, the frequency of Leu2$^+$ spores recovered varied widely. In particular, a 15-fold variation in the frequency of allelic recombination was observed when *leu2* sequences were inserted at four different chromosomal locations, in the context of 7.8 kb of sequence identity. Such position effects have important implications for the study of DNA sequences that stimulate or repress meiotic recombination. Our findings indicate that at least one class of such elements can exert their effect over distances of at least 2.7 kb. We suggest that such long distance effects may be due to the action of modulating elements distinct from those thought to be responsible for the gradients of levels of gene-conversion often observed within genes. The effects of these latter elements appear to dissipate over a distance of 1–2 kB (FOGEL *et al.* 1978).

The position effects we observed for the same alleles inserted at different loci are, in general, consistent with the level of gene conversion observed in normal genes at these locations. For example, specific alleles of *MAT* and *URA3* gene-convert four to six times less often than do specific alleles of *LEU2* and *HIS4* (FOGEL *et al.* 1978; M. LICHTEN, R. H. BORTS and J. E. HABER, unpublished observations). However, it is apparent that measurements of the frequency of gene conversion at a given locus can be strongly influenced by the nature and position of alleles chosen (FOGEL *et al.* 1978). By examining recombination between the same two alleles inserted at different genomic locations, we have been able to avoid such specific marker effects.

Ectopic recombination occurs frequently during meiosis: We have measured the frequency of meiotic recombination to form Leu2$^+$ spores in diploids where one of two defined *leu2* mutations (*leu2-K* or *leu2-R*) was present at *LEU2*, and the other allele was present in a 2.2-kb *leu2* fragment inserted at *HIS4*, *MAT*, *URA3* or *HML*. A remarkably high level of recombination between these dispersed homologous sequences was observed. In many cases, the frequency of ectopic recombination to form Leu2$^+$ spores was comparable to that observed in allelic crosses. No obvious correlation between the linkage relationship of the four loci on chromosome *III* where *leu2* alleles were integrated and the frequency of ectopic recombination could be drawn, and only a modest reduction in the frequency of ectopic recombination was observed when the two loci were on different chromosomes. A high frequency of ectopic recombination has also been observed between dispersed copies of *HIS3* or *URA3* located on nonhomologous chromosomes (JINKS-ROBERTSON and PETES 1985, 1986). We suggest that the overall frequency at which two copies of a dispersed sequence recombine may primarily be a function of the intrinsic level at which the loci in question participate in meiotic exchange.

In *S. cerevisiae*, as in other eucaryotes, meiosis proceeds through a stage of pairing of homologous chromosomes and formation of synaptonemal complex (BYERS and GOETSCH 1975; ZICKLER and OLSON 1975), which is thought to play an important role in the orderly pairing and disjunction of homologs in meiosis. Although pairing of nonhomologous chromosomes by synaptonemal complex has been observed in other organisms (reviewed in VON WETTSTEIN, RASMUSSEN and HOLM 1984), such ectopic pairings have not yet been detected in yeast (BYERS and GOETSCH 1975). We observed a high frequency of meiotic recombination between dispersed copies of *leu2* in crosses where both recombining partners could potentially pair with sequences at an allelic position. These results indicate either that pairing of homologs by synaptonemal complex does not preclude recombination between dispersed regions of homology, or that ectopic exchange does not occur at this stage of meiosis. It should be noted that, in experiments which examined the timing of recombination in meiosis, the DNA products of allelic crossing over at *MAT* and those characteristic of ectopic crossing over between *LEU2* and *MAT::leu2* appeared simultaneously (BORTS *et al.* 1984).

Marker and position effects in ectopic meiotic recombination: A striking effect of the configuration of markers on both the frequency and location of Leu2$^+$ recombinants was observed in crosses where ectopic recombination was examined. Diploids containing *leu2-K* at *LEU2* yielded a high frequency of Leu2$^+$ gene convertants, and most of these were the products of events in which the *leu2-K* allele at *LEU2* was converted to wild type. Diploids bearing the opposite marker configuration exhibited a somewhat lower frequency of Leu2$^+$ recombinants, and a greater symmetry in the distribution of Leu2$^+$ gene convertants was observed. One way to account for these allele and position effects is to suggest that the frequency of ectopic recombination to form Leu2$^+$ products in such crosses was, to a large extent, determined both by the relative frequency of conversion to wild type of each mutant allele during pair-wise interactions, and by the intrinsic ability of each of the two particular loci to serve as recipients in gene conversion events. Our results are consistent with this suggestion. Tetrad analysis of allelic crosses (Table 2) indicates that Leu2$^+$ recombinants are about 10 times more likely to derive from conversion of *leu2-K* to wild type than from conversion of *leu2-R*, and results of allelic

crosses (Table 3) identify *LEU2* as the locus with the highest intrinsic frequency of meiotic exchange.

If the above interpretation is correct, then we would predict that ectopic recombination between any pair of mutant allele inserts should display an allele configuration effect similar to that observed in the crosses reported in this work. Preliminary results of experiments examining ectopic recombination between *MAT*::*leu2* and *URA3*::*leu2* are consistent with this expectation (M. LICHTEN, R. H. BORTS and J. E. HABER, unpublished observations).

Ectopic recombination is associated with crossing over: Allelic meiotic gene conversion is frequently associated with crossing over of flanking sequences, with the fraction of conversions associated with crossing over ranging from 18 to 66% (FOGEL, MORTIMER and LUSNAK 1981). In the experiments reported in this paper, a significant fraction of ectopic recombinants displayed associated crossing over of flanking sequences. Forty-six percent of recombinants between *LEU2* and *HIS4*::*leu2* were associated with crossing over; in crosses involving recombination between *LEU2* and *URA3*::*leu2*, we estimate that 22% of ectopic recombinants were associated with crossing over to form a reciprocal translocation between chromosomes *III* and *V*. JINKS-ROBERTSON and PETES (1986) have estimated that about half of the meiotic recombinants between *URA3* and a 5.5-kb *URA3* fragment inserted at *HIS3* are associated with crossing over. All of these values lie well within the range of conversion-associated crossing over cited above. Taken together, these results indicate that pairing of extensive regions of homology is not required for meiotic recombination events to be resolved as crossovers. Our experiments place a lower limit of 2.2 kb on the extent of homology necessary for such resolution events to occur.

This conclusion contains implications regarding the meiotic behavior of the 6-kb Ty element, a retrotransposon present at about 30–35 copies per haploid yeast genome (CAMERON, LOH and DAVIS 1979). Ty elements frequently participate in ectopic mitotic gene conversion (SCHERER and DAVIS 1980; RODER and FINK 1982; ROEDER, SMITH and LAMBIE 1984). Although a high frequency of meiotic crossing over between a specific pair of Ty elements (0.5% of all chromatids) has been observed (ROEDER 1983), the meiotic behavior of the ensemble of Ty elements has not been determined. We can calculate the expected frequency of crossing over between dispersed copies of Ty, based on the assumption that Ty elements may recombine at frequencies similar to those observed between dispersed copies of *LEU2*. In a previous physical study of meiotic recombination (BORTS *et al.* 1984) we found that crossing over between *leu2* and *MAT*::*leu2* occurred in approximately 0.5% of all chromatids, in agreement with the frequency of crossing over between a specific pair of Ty elements cited above. We assume that a Ty element would be as likely to interact with a dispersed copy of Ty as with its allelic partner, so that nearly all Ty-Ty recombination events would be ectopic. Thus, 30 ectopic pairs of Ty elements would be expected to create chromosome rearrangements in approximately 15% of all chromatids, or 30% of all tetrads. This value is inconsistent with both the frequency and pattern of spore inviability observed in wild-type diploids used in this study (Table 8). We observed only 5% spore inviability; in addition, most inviable spores were present in tetrads containing only one inviable spore, while translocations created by ectopic recombination should generate tetrads containing two or more inviable spores.

It is possible, therefore, that meiotic recombination between dispersed copies of Ty is suppressed. This suppression could simply be a consequence of the sequence heterology observed between Ty elements, or could be mediated by a system which specifically suppresses recombination between Ty elements (ROTHSTEIN 1984). Alternatively, it is possible that selective pressure for spore viability (either natural or imposed by investigators) maintains the distribution of Ty elements in the genome in such a way that relatively few are located in regions which undergo a high level of meiotic recombination. Further experimentation will be required to determine which of these alternatives is correct.

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