A Test of a Counting Model for Chiasma Interference

Eric J. Foss and Franklin W. Stahl

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

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ABSTRACT

According to the model of FOSS, LANDE, STAHL and STEINBERG, chiasma interference is a reflection of the requirement for crossovers to be separated by an organism-specific number of potential conversion events without associated crossovers. This model predicts that tetrads with close double crossovers should be enriched for conversion events that themselves are not associated with crossing over. We tested this prediction in *Saccharomyces cerevisiae* and found it to be unfulfilled.

IN many organisms, meiotic crossovers discourage each other in a distance-dependent manner (STURTEVANT 1915; MULLER 1916; PERKINS 1962; MORTIMER and FOGEL 1974). Because crossing over takes place after premeiotic DNA replication, crossovers could interfere with each other in two ways. Either the interference is limited to the chromatids involved in the crossovers (chromatid interference) or it is not so limited (chiasma interference). Tetrad data from fungi show that chromatid interference is essentially absent (MORTIMER and FOGEL 1974); on the rare occasions that crossovers occur near each other, the choice of chromatids involved in one crossover has little influence on the choice in the other crossover. This shows up as an ~1:2:1 ratio of 2–3–4-strand double crossovers.

Several models have been proposed to explain chiasma interference. MULLER (1916) suggested that interference is a consequence of the stiffness of chromosomes. According to this model, when two chromosomes have crossed over, it is difficult to bend them back on themselves to make a second crossover nearby. FOX (1973) attributed interference to the behavior of a "chiasma-determining mechanism." This hypothetical enzyme moves along the bivalent at a constant speed and periodically "fires" to determine a crossover. After the enzyme has fired, it needs a certain amount of time to "recharge." KING and MORTIMER (1990) suggested that a crossover nucleates the polymerization of an inhibitory substance that spreads along the bivalent, preventing nearby attempts at crossing over.

The physical distances (measured in base pairs) over which interference extends can vary by several orders of magnitude from one organism to another. None of the models mentioned above demands this variation. For example, with no independent measure of chromosome stiffness, the steric model must assume that the stiffness of chromosomes varies over orders of magnitude in the same way as interference. Similarly, the chiasma-determining mechanism and the inhibitory polymer, or the chromosomes over which they act, must have characteristics that vary over several orders of magnitude from one organism to another. If the physical distance were appropriately measured in length of synaptonemal complex [as implied by SYM and ROEDER (1994), and see PETERSON *et al.* (1994)] rather than base pairs, less variation might be demanded of physical models.

HOLLIDAY's model attempts to account for this variation (HOLLIDAY 1977). According to this model, there are factors essential for formation of crossovers evenly distributed along bivalents. A crossover lowers the local concentration of these factors and thereby discourages formation of further crossovers nearby. This model provides unity to the widely differing distances over which interference can extend by positing that approximately equal numbers of these factors are allotted to the chromosomes of all organisms that exhibit interference. However, like the physical models mentioned above, this model fails to make discriminating predictions.

There are two basic types of homologous recombination events: potential conversions with or without associated crossovers. In this paper, we use nomenclature established by MORTIMER and FOGEL (1974). Those events that could show up as conversions with associated crossovers, were there appropriate markers to detect them, are referred to as "C_x events" or simply "crossovers." Those events that could show up as conversions without associated crossovers, were there appropriate markers to detect them, are referred to as "C_o events." Total potential conversion events without regard to the presence of associated crossovers are referred to as "C events."

FOSS *et al.* (1993) attributed interference to the activity of a "machine" that can count. (We refer to this model as the counting model.) According to this model, C events are first distributed at random with

Corresponding author: F. W. Stahl, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229.
E-mail: fstahl@molbio.uoregon.edu

respect to each other, and then the counting machine specifies them such that neighboring C_x events are separated by a specific number (m) of C_o events. m, which can vary from one organism to another, can be estimated in either of two ways, by fitting interference data (McPeek and Speed 1995) or by determining the fraction of conversion events that are associated with crossovers (Foss *et al.* 1993). This model was inspired partially by the observations that C events show no interference and that C_o events do not discourage C_x events (Stadler 1959; Carlson 1971; Mortimer and Fogel 1974). Because the number of C events per physical distance varies over orders of magnitude among organisms, the model accounts in a simple way for interference extending over widely differing physical distances in different organisms. In other words, the model predicts that the genetic map, and not the physical map, is the important variable in determining the distance over which interference extends. Consistent with this prediction, interference, when it occurs, is strong at 5 cM from a crossover and negligible by 40 cM (see Foss *et al.* 1993).

The counting model has quantitative and qualitative precedents in the literature. Both Cobbs (1978) and Stam (1979) published mathematical models that are essentially identical to the counting model. A qualitative precedent for the counting model comes from Mortimer and Fogel (1974). They suggested that C_x and C_o events alternate along the bivalent. However, they failed to follow up on their suggestion and later seem to have abandoned it (King and Mortimer 1990).

In contrast to the other models mentioned above, the counting model makes definite quantitative and qualitative predictions. The quantitative predictions have been examined extensively and provide strong support for the model (Foss *et al.* 1993; Lande and Stahl 1993; McPeek and Speed 1995; Zhao *et al.* 1995). The intensity of interference can be quantified as the coefficient of coincidence, S, the ratio of the observed frequency of double recombinants to the expected frequency of double recombinants obtained by multiplying individual recombinant frequencies. (We use the abbreviation S_4 when four markers determine the two intervals used to calculate S.) To compare quantitative predictions from the model with data, it was first necessary to determine the value of m for the organisms in question. Based on the fraction of conversions that are associated with crossing over in *Drosophila melanogaster* and *Neurospora crassa* (~0.2 and 0.3, respectively) (Stadler 1973; Perkins 1979; Hilliker and Chovnick 1981; Hilliker *et al.* 1991; Perkins *et al.* 1993), the values of m for these organisms were taken as four and two, respectively. The intensity of interference in these organisms as a function of genetic distance between two intervals shows a remarkable match to the predictions of the $m = 4$ and $m = 2$ versions of the model,

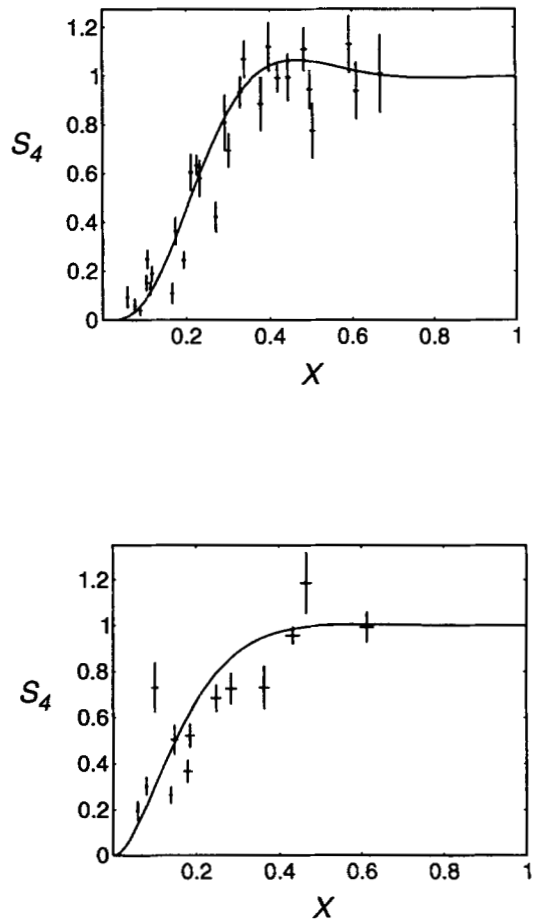

Figure 1.—(Top) Drosophila S_4 data compared with the model with $m = 4$. (Bottom) Neurospora S_4 data compared with the model with $m = 2$. (From Foss *et al.* 1993).

respectively (Figure 1). The $m = 4$ version of the model was also tested for its ability to predict the distribution of crossovers along the linkage map of the X chromosome of Drosophila for single, double and triple exchange tetrads, and the fit is again impressive (Figure 2). This test is, at least to some extent, independent of the previous test.

These quantitative tests provide strong support for the counting model and prompted us to test a qualitative prediction of the model in *Saccharomyces cerevisiae*. The average ratio of C_x:C events in *S. cerevisiae* is ~0.37 (Fogel *et al.* 1983) (but see Discussion), so we assume that yeast is an $m = 2$ organism. Thus, according to the model, simultaneous crossing over in two close intervals is unlikely because two C events are unlikely to occur in the small region separating them. The model predicts that the rare tetrads that do have close double crossovers will be enriched for C_o events at markers between the crossovers when compared either with the general population or, especially, when compared with tetrads with no crossovers in the region under study. This central prediction of the model was not fulfilled by our experiment.

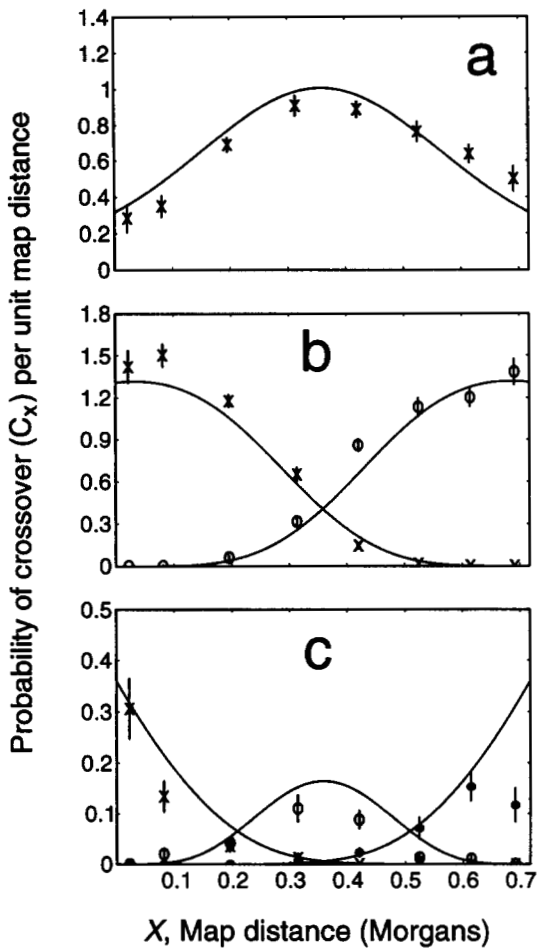

FIGURE 2.—Distributions of exchanges in tetrads of Drosophila with one (a), two (b) or three (c) exchanges, respectively (ranks 1, 2, and 3, respectively), as a function of linkage map position as predicted by the model with $m = 4$. (From LANDE and STAHL 1993).

MATERIALS AND METHODS

Yeast strains and plasmids: We use the following nomenclature to describe the locations of genes: gene A::GENE B indicates that geneA has been disrupted with GENE B. GENE $B_{GENE\ A}$ indicates that GENE B is next to, but not disrupting, GENE A.

YEF392 is a diploid made from mating YEF349 and YEF388 (Figure 3). The genotype of YEF349 is *MAT***a**, *ura3–52, ade2-ΔEcoRV, his3Δ200, leu2-ΔKpnI, lys2-ΔHpaI, trp1-ΔXbaI, LYS2$_{ARG4}$, leu2-ΔKpnI$_{ARG4}$, ADE2$_{GPA1}$*. The genotype of YEF388 is *MAT*α, *ura-52 HIS3, ade2-ΔEcoRV, his3-Δ200, leu2-ΔKpnI, lys2-ΔHpaI, thr1-ΔEcoRV, arg4-ΔNsphI, lys2-ΔHpaI$_{arg4}$, LEU2$_{arg4}$, URA3$_{ARD1}$*. Strain construction details are given below. All yeast transformations were done with lithium acetate (ITO *et al.* 1983).

The fragment of *ADE2* integrated next to *GPA1* comes from pEF154 cut with *Bam*HI and *Xho*I. pEF154 was made by ligating an *ADE2*-containing *Eco*RI fragment from pEF150 into the *Eco*RI site of pEF83. pEF150 was made by ligating a *Bgl*II fragment from pASZ10 (STOTZ and LINDER 1990) containing *ADE2* to p34H (TSANG *et al.* 1991) cut with *Bam*HI. pEF83 was made by ligating a *Pvu*II, *Xho*I fragment from pMN10 (MIYAJIMA *et al.* 1987) containing the 3′ end of *GPA1* to pRS306 (SIKORSKI and HIETER 1989) cut with *Sma*I and *Xho*I. The presence of the appropriate fragment at *GPA1* was confirmed by Southern analysis.

The fragment of *URA3* integrated at *ARD1* comes from pEF122 cut with *Eco*RI and *Not*I. pEF122 was made by ligating a *Sma*I fragment from pEF45 containing the *URA3* gene into the *Hpa*I site of pEF117. pEF45 was made by ligating a *Hin*dIII fragment from YEp24 containing *URA3* into the *Hin*dIII site in p34H. pEF117 was made by ligating a *Bam*HI fragment from pSPO13–1 (WANG *et al.* 1987) containing DNA 3′ from *ARD1* gene into the *Bam*HI site of pKSII$^+$ (Stratagene). The presence of the appropriate fragment at *ARD1* was confirmed by Southern analysis.

The fragment of *HIS3* integrated at *ura3–52* comes from pEF172 cut with *Eco*RV and *Sma*I. pEF172 was made by ligating a *Stu*I fragment of pEF45 to pJJ215 (JONES and PRAKASH 1990) cut with *Sma*I and *Pvu*II. The presence of the appropriate fragment at *ura3–52* was confirmed by Southern analysis.

The fragment of DNA at *ARG4* containing *LYS2*, *ARG4* and *leu2* comes from pEF146 cut with *Sal*I and *Pst*I. pEF146 was made by ligating an *Xba*I fragment from pEF143 containing *ARG4* into the *Xba*I site of pEF91. pEF143 was made by ligating an *Sna*BI, *Eco*47-III fragment from pMLC28::*ARG4* [*Pst*I fragment of *ARG4* in pMLC28 (LEVINSON *et al.* 1984)] to p34E (TSANG *et al.* 1991) cut with *Sma*I. pEF91 was made by ligating an *Xba*I fragment from pEF76 containing *arg4-ΔBgl*II into the *Xba*I site of pEF37. pEF76 was made by ligating an *Eco*47-III, *Sna*BI fragment of *arg4* with the *Bgl*II site ablated to p34E cut with *Sma*I. pEF37 was made by ligating a *Bam*HI, *Xho*I fragment from pDA6200 (BARNES and THORNER 1986) containing the 5′ end of *LYS2* and a *Xho*I, *Bgl*II fragment from pDA6200 containing the 3′ end of *LYS2* into the *Bgl*II site of pEF29. pEF29 was made by ligating a *Sal*I, *Cla*I fragment of pMJ56 (pBR322 containing *Hin*dIII fragment of *URA3* and *Sal*I, *Xho*I fragment of *leu2-ΔKpn*I) containing *leu2-ΔKpn*I to pEF27 cut with *Sal*I and *Cla*I. pEF27 was made by removing a *Sal*I fragment from YEp13. To target the *Sal*I, *Pst*I fragment of pEF146 to *ARG4*, *ARG4* was first replaced by *LEU2* (Figure 4). This was done by transformation with a *Hin*dIII fragment of pEF97. pEF97 was made by ligating a *Sma*I fragment from pEF87 containing *LEU2* to pNPS425 [same as pMLC28::*ARG4*, except G to C change at translation-initiating ATG and vector is pMLC12 (LEVINSON *et al.* 1984)] cut with *Eco*47-III and *Sna*B1. pEF87 was made by ligating a *Pst*I fragment from YEp13 containing *LEU2* to p34H cut with *Pst*I. The presence of the appropriate fragment of pEF146 at *ARG4* was confirmed by Southern analysis.

The fragment of DNA at *arg4-ΔNsphI* containing *lys2-ΔHpaI*, *arg4-ΔNsphI* and *LEU2* comes from pEF98 cut with *Sal*I and *Pst*I. This fragment was transformed into a strain in which the native copy of *ARG4* had been first replaced with a *Hin*dIII fragment from pEF97, and then replaced with a *Sal*I, *Pst*I fragment from pEF146 (Figure 4). pEF98 was made by ligating an *Xba*I fragment from pEF77 containing *arg4-ΔNsphI* into the *Xba*I site of pEF85. pEF77 was made by ligating an *Eco*47-III, *Sna*BI fragment of pNPS425 containing *arg4-ΔNsphI* to p34E cut with *Sma*I. pEF85 was made by ligating an *Nco*I fragment of pCP7 containing *lys2-ΔHpaI* to pEF79 cut with *Nco*I. pCP7 was made by cutting pCP6 with *Hpa*I and ligating it shut in the presence of *Hin*dIII linkers (New England Biolabs). pCP6 was made by ablation of the *Hin*dIII site in pCP3 with Klenow fragment. pCP3 was made by ligating an *Eco*RI, *Hin*dIII fragment of *LYS2* to YIp5 cut with *Eco*RI and *Hin*dIII. pEF79 was made by ligating an *Xba*I fragment from pEF76 containing *arg4-ΔBglII* into the *Xba*I site of pEF40. pEF40 was made by ligating a *Bam*HI, *Xho*I fragment

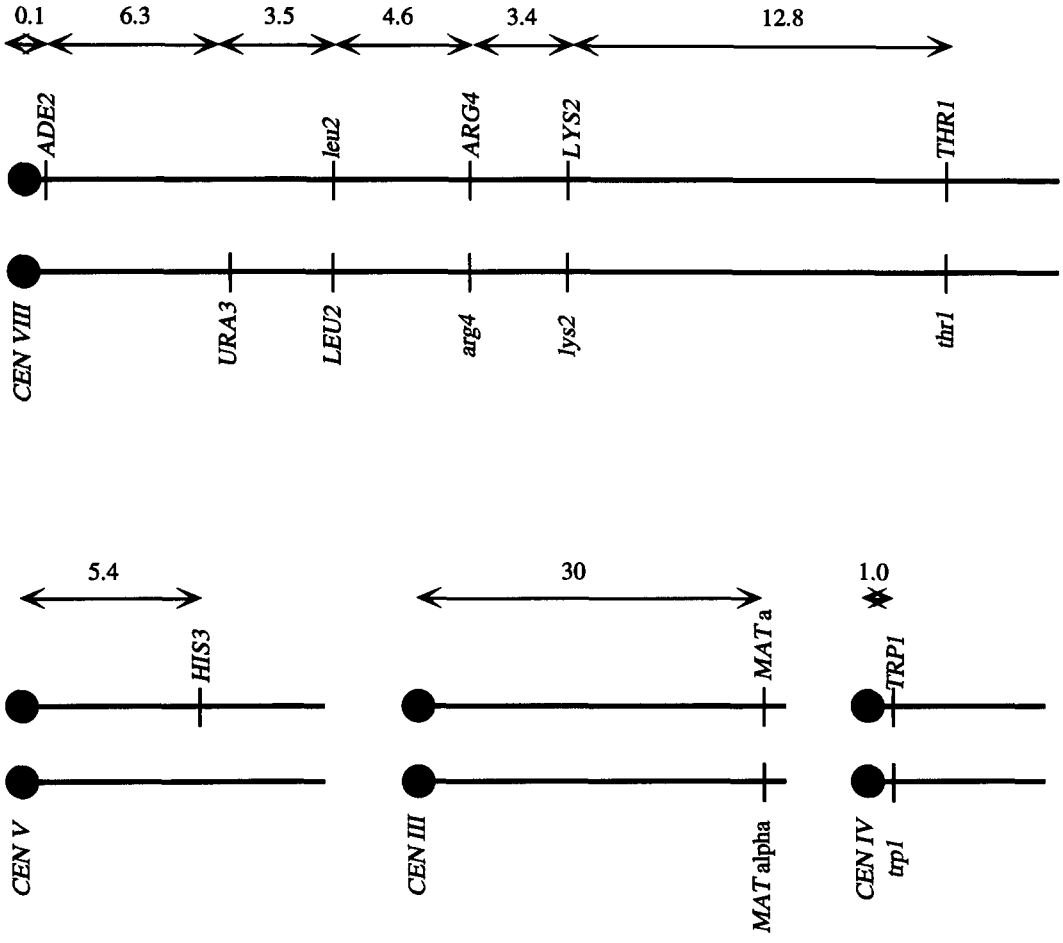

FIGURE 3.—YEF392, the diploid strain used in this experiment. Numbers indicate genetic distances in centimorgans between markers. All genetic distances were calculated from data from YEF392, except *CENIII-MAT*, which was taken from MORTIMER *et al.* (1989).

from pDA6200 containing the 5′ end of *LYS2* and a *Xho*I, *Bgl*II fragment from pDA6200 containing the 3′ end of *LYS2* into the *Bgl*II site of pEF27. The presence of the appropriate fragment of pEF98 at *arg4* was confirmed by Southern analysis.

Sporulation and dissection: YEF392 was patched out on YEPD plates, incubated overnight at 30°, replica-printed to sporulation medium, dissected after 3 days incubation at 30° and scored after 2 more days at 30° (SHERMAN *et al.* 1982). Sporulated cultures were treated for 7 min with 1% Glusulase (DuPont).

Map distances and coefficients of coincidence: To calculate map distances, we used the formula of PERKINS (1949), with slight variations to allow it to accomodate conversions. We assumed that crossovers happen on the outside of conversion tracts (as is observed), and that those crossovers happen with equal probability on either side of the conversion tract. Rare tetrads showed nonparental ditype (NPD) segregation of markers flanking a converted site. (We use the abbreviation C_{xANPD} to denote such tetrads, where A is the converted marker.) We assume that when two crossovers land in small contiguous intervals, chiasma interference separates them, so that one crossover lands in each interval. With these assumptions, we arrive at the following formula: $X_{A\text{-}B} = (100)\,(T + 6NPD + 0.5C_{xA} + 0.5C_{xB} + C_{xANPD} + C_{xBNPD})/(2)$ (total tetrads) (T = number of tetratype tetrads, C_{xA} = number of tetrads with C_x event at A that does not coconvert the other marker in question, B in this case). For example, $LYS2_{ARG4}$ and *ARG4* segregated in the T configuration 154 times but never in the NPD configuration. There were 21 C_x events at $LYS2_{ARG4}$ that did not coconvert *ARG4*, and 77 C_x events at *ARG4* that did not coconvert $LYS2_{ARG4}$. Two times, flanking markers segregated in the NPD configuration when there was a C event at $LYS2_{ARG4}$ that did not coconvert *ARG4*. Two times, flanking markers segregated in the NPD configuration when there was a C event at *ARG4* that did not coconvert $LYS2_{ARG4}$. Therefore the genetic distance between $LYS2_{ARG4}$ and *ARG4* is $(100)[(154) + (0.5)(21) + (0.5)(77) + (2) + (2)]/(2)(3081) = 3.4$ cM. Conversion events at the terminal marker *THR1* were ignored. This is equivalent to treating the *THR1-*$LYS2_{ARG4}$ interval as "one conversion tract length from *THR1*" to $LYS2_{ARG4}$. The (short) map distances between *TRP1* and *ura3–52::HIS3* and their centromeres were calculated using the formula $X = (100)(T)/(2)$(total tetrads) (SHERMAN and WAKEM 1991). The T in this case means tetratype between the marker in question and the other two markers most tightly linked to nonhomologous centromeres ($ADE2_{GPA1}$ being the third relevant marker).

Coefficients of coincidence for two intervals (1 and 2) were calculated using the formula $S_4 = (0.25T_{12} + 0.5TNPD_{12} + 0.5TNPD_{21})$(total tetrads)$/(0.5T_1 + NPD_1)(0.5T_2 + NPD_2)$. T_{12} stands for number of tetrads that are tetratype in intervals 1 and 2. $TNPD_{12}$ stands for number of tetrads that are tetratype for interval 1 and NPD for interval two. This formula is equivalent to the random spore-based formula in FOSS *et al.* (1993). It is also a logical treatment of tetrad data (WEINSTEIN 1959). Errors were calculated as described in MULLER and JACOBS-MULLER (1925).

Calculations for predicting coefficients of coincidence are described in detail in FOSS *et al.* (1993). When $m = 1$, $S_4 =$

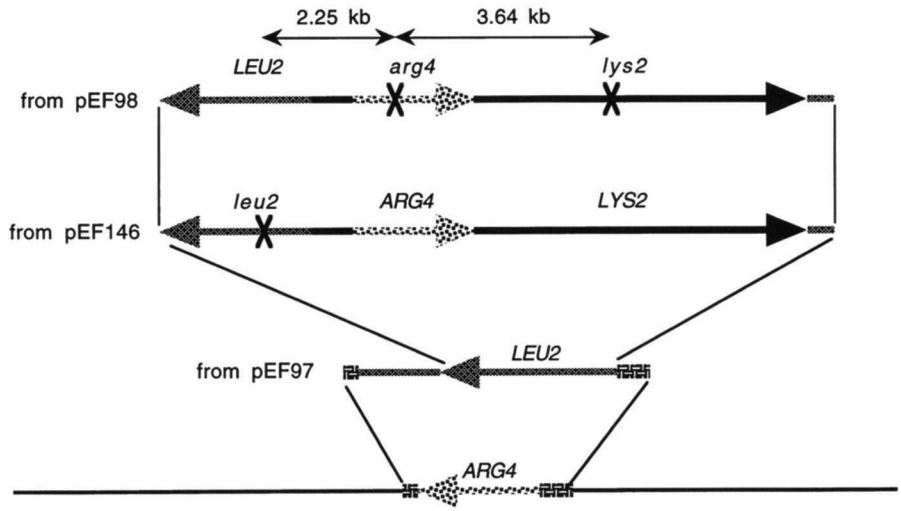

FIGURE 4.—Targeting scheme used to integrate *LYS2*, *LEU2* and *ARG4* at *ARG4*. Numbers indicate kilobases between markers.

$1 - e^{-8X}$. When $m = 2$, $S_4 = 1 - 2e^{-9X} \cos [(\pi/3) - 3\sqrt{3}X]$. In these formulas, X is the genetic distance in Morgans between two intervals of infinitesimal length. In practice, we take X as the distance in Morgans between the midpoints of the two intervals in question. When the distance between the intervals is small, $S_4 \rightarrow S_3$. To assess the maximum error incurred by taking X as the midpoints of the two intervals when calculating S_4, we calculate S_3 as well, using the formula $S_3 = (R_{12} + R_{23} - R_{13})/2R_{12}R_{23}$. R values were calculated following the procedure of FOSS *et al.* (1993) taking the two test intervals as running from the midpoint of the intervening interval to the outermost markers.

RESULTS

According to the model of FOSS *et al.* (1993), crossovers in *S. cerevisiae* are rarely separated by a small genetic distance because it is unlikely that two C_o events will fall in that small region. The model thus predicts that close double crossovers will be enriched for intervening C_o events. To test this prediction, we built a strain, YEF392, containing two close intervals to detect close double crossovers. These intervals are separated by two conversion hot spots to detect intervening C_o events (Figure 3). [The close intervals are *THR1*-$LYS2_{ARG4}$ and $URA3_{ARD1}$-$ADE2_{GPA1}$. Crossovers in these intervals interfere with each other ($S_4 = 0.71 \pm 0.075$). The conversion hotspots are *ARG4* and $LEU2_{arg4}$, which in this context convert at 6.3% and 9.9%, respectively.] It was important to have a control interval on another chromosome, to guard against the possibility that a positive correlation between close double crossovers and intervening C_o events was simply due to a subpopulation of the cells being hyperrecombinagenic. The *CENV* to *ura3–52 HIS3* interval serves this purpose. The presence of two tightly centromere-linked markers on other chromosomes (*TRP1* and $ADE2_{GPA1}$) allowed us to measure this interval. Furthermore, the presence of three markers on chromosomes other than chromosome *VIII* (*ura3–52 HIS3*, *TRP1* and *MAT*) helped guard against false tetrads. Spore viability was high (97%).

Out of 3081 tetrads with four viable spores, there were 1467 tetrads with no crossovers from *THR1* to $ADE2_{GPA1}$. Of these, 171 (11.7%) had a C_o event at at least one of the intervening markers (*ARG4* and $LEU2_{arg4}$). In contrast, among the 64 tetrads with crossovers in each of the outside intervals but without crossovers in the intervening region, only 4 tetrads (6%) had a C_o event at at least one of the two intervening markers. When compared to the "no crossover" group, this double crossover group should have had 38 or 41 C_o events according to the $m = 2$ and $m = 1$ versions of the counting model, respectively (see APPENDIX). This difference is highly significant ($P < 0.0030$ and 0.0026 for $m = 2$ and 1, respectively). The model predicts a smaller difference in the frequencies of C_o events when the double crossover group is compared with the general population. There were 273 C_o events (8.9%) at at least one of the intervening markers among the general population. The counting model predicts 13 C_o events among the double crossover group instead of the 4 observed, regardless of the value of m (see APPENDIX). This difference is significant ($P < 0.038$). The frequencies of crossing over in the control interval (*CENV* to *ura3–52 HIS3*) did not differ significantly between these three groups of tetrads: 8% (5/64) for the double crossover group, 9% (137/1467) for the no crossover group and 11% for the general population.

We are detecting ~30% of the C_o events that occur in the interval $LYS2_{ARG4}$ to $URA3_{ARD1}$. Because we know the number of crossovers in this interval and the number of C_x events at the two markers within this interval, we can calculate that ~30% of C_x events in this interval land on *ARG4* or $LEU2_{arg4}$. Assuming that the lengths of conversion tracts with and without associated crossovers are the same, ~30% of C_o events should also land on

these markers. No special effort was made to detect sectored colonies, which are rare for most yeast markers, and few were detected. We can think of no reason why inefficient detection of 3:5 or 5:3 tetrads should skew our results.

Crossovers in the intervals *THR1* to $LYS2_{ARG4}$ and $LEU2_{arg4}$ to $URA3_{ARD1}$ show strong interference ($S_4 = 0.17 \pm 0.067$). Out of 3081 tetrads, there were only six double crossovers, when 35 were expected based on $S_4 = 1$ (assuming no triple crossovers). One of these tetrads had a C_o event at one of the intervening markers. These numbers are too small to be informative.

When crossovers did occur in both the *THR1* to $LYS2_{ARG4}$ and the $URA3_{ARD1}$ to $ADE2_{GPA1}$ intervals, the choices of chromatids involved in the two exchanges were not demonstrably nonrandom with respect to each other. Out of 64 double crossovers, 20 involved two chromatids, 33 involved three chromatids and 11 involved four chromatids. With no chromatid interference, this distribution would be expected to be 16:32:16. Slight negative chromatid interference has been reported previously (PERKINS 1962; FOGEL *et al.* 1979).

Consistent with what others have seen, conversion events in this strain do not discourage each other. Some 5.6% (173/3081) of tetrads showed a conversion event at *THR1*, 15.5% (478/3081) of tetrads showed a conversion event at $LEU2_{arg4}$ and/or *ARG4* and 0.9% (28/3081) of tetrads showed conversion events at both of these loci, resulting in a "coefficient of coincidence" of 1.0. These are the only loci in this strain that convert at frequencies high enough to address this issue.

DISCUSSION

According to the model of FOSS *et al.* (1993), chiasma interference is a reflection of the requirement for C_x events to be separated by a specific number (m) of C_o events. The model accurately predicts data from Drosophila and Neurospora. The model also accounts for the observation that in different organisms interference extends over widely differing physical distances, but it is consistently strong 5 cM from a crossover and negligible by 40 cM. However, as shown in this paper, a central prediction of the model is not fulfilled in yeast.

The model predicts that tetrads with close double crossovers will be enriched for intervening C_o events when compared with the general population and, especially, when compared with tetrads with no crossovers in the region under study. In contradiction to this prediction, only 6% (4/64) of double crossover tetrads had a C_o event at at least one of the intervening markers, whereas 8.9% of the general population had a C_o event at at least one of the intervening markers and 11.7% (171/1467) of the tetrads with no crossovers in the region had a C_o event at at least one of the intervening markers. The difference between the model's prediction for the comparison of the double crossover group and the general population is significant for any value of m, and the difference for the comparison of the double crossover group and the no crossover group is highly significant for $m = 1$ or 2 (see APPENDIX). Thus, serious doubt is cast on the model. The accuracy of the functions provided by the model remains mysterious but useful (STAHL and LANDE 1995).

Variations on the counting model were presented in FOSS *et al.* (1993) in which the first C event to be resolved would be a C_x, in which C events were resolved starting from the centromere with m C_o events and in which variable numbers of C_o events follow the obligatory m events. The data presented here are inconsistent with these versions of the model as well.

Any model for chiasma interference that takes into account the observations that C events are distributed at random with respect to each other and that C_o events do not discourage C_x events will predict that double crossover tetrads will be enriched for intervening C_o events relative to the general population. This is because a C event at an "intervening" marker will almost always be resolved as a C_o event among double crossover tetrads, whereas resolution of such a C event is not so restrained among the general population. In other words, double crossover tetrads will be enriched for intervening C_o events simply because intervening C events cannot be resolved as C_x events. We fail to see this enrichment. However, our numbers are not large enough to make this failure significant.

Previous studies have demonstrated that conversion events in yeast do not show interference and that chromatid interference is essentially absent. If interference in our strain failed to demonstrate these qualities, the results of our experiment would be suspect. However, our strain behaved normally in these regards.

Conversion events in yeast generally show parity. Conversion events at *THR1* and at *LEU2*, however, did not. Both markers converted significantly more often to wild type than to mutant ($0.05 > P > 0.01$ and $P < 0.001$, respectively). This disparity could reflect poor detection of 3:5 tetrads. Our strain contains three copies of *leu2-ΔKpnI* (one at *ARG4* and two at the native loci) and one copy of *LEU2* (at *arg4*). If a large fraction of the conversion events at *LEU2* involved an interaction between *LEU2* and a copy of *leu2* at its native location, this might somehow skew our results. However, our results with conversions at *ARG4*, where ectopic interactions are not possible, are also inconsistent with the counting model. Among double crossover tetrads, there were 3 C_o events at *ARG4*. There should have been 15 or 16 C_o events if the counting model were correct with $m = 1$ or 2, respectively, when double crossover tetrads are compared with no crossover tetrads. There should have been six C_o events if the counting model were

correct regardless of the value of m, when double crossover tetrads are compared with the general population. If, on the other hand, C_o events occurred at *ARG4* at the same frequency among double crossover tetrads as among no crossover tetrads or as among the general population, there should have been three or two C_o events at *ARG4*, respectively.

The counting model is inconsistent in its ability to predict the coefficients of coincidence observed in this experiment. For the outside intervals ($THR1\text{-}LYS2_{ARG4}$ and $URA3_{ARD1}\text{-}ADE2_{GPA1}$, $X = 0.211$), the model's predictions match the data. The model predicts a coefficient of coincidence $S_4 = 0.70$ when $m = 2$ ($S_3 = 0.50$), and the observed coefficient of coincidence is 0.71 ± 0.075. However, for the other pair of intervals mentioned above ($THR1\text{-}LYS2_{ARG4}$ and $LEU2_{arg4}\text{-}URA3_{ARD1}$, $X = 0.162$), the model predicts $S_4 = 0.53$ when $m = 2$ ($S_3 = 0.39$), whereas the data show $S_4 = 0.17 \pm 0.067$. The model's predictions at $m = 1$ show worse fits to the data. These calculations are described in MATERIALS AND METHODS.

Estimates of the ratio of C_x:C events vary widely in *S. cerevisiae*. The average C_x:C ratio of 0.37 for yeast mentioned above comes from five estimates that range from 0.18 to 0.66 (FOGEL *et al.* 1981). When these numbers are corrected for incidental exchanges within the context of the counting model, the average C_x:C ratio becomes 0.33, with a range from 0.16 to 0.60 (STAHL and LANDE 1995). (For both these data and the Neurospora data mentioned below, we do not know how much of this variation is due to sampling error.) This suggests values for m that range from 0.67 to 5.3 . Such variation is seen even within a single region of the genome (FOGEL *et al.* 1979). Variation in estimates of the C_x:C ratio is seen in Neurospora also, although to a lesser degree. When corrected within the context of the counting model, the values for this ratio range from 0.18 to 0.38 in nine estimates, with an average value of 0.30. In the absence of knowledge regarding sampling variation, this could mean that m varies from 1.6 to 4.6. If the counting model were modified to account for such possible variation, its delightful simplicity would be sacrificed.

The counting model was promising because its mathematical formulation describes data from Drosophila and Neurospora with noteworthy accuracy (and, of course, the model may apply to those creatures). These quantitative predictions were based on the assumption that C events are Poisson distributed. To be Poisson distributed, a given "trial" must be "successful" with low probability. The "hotter" a recombination hotspot is, the less it satisfies this requirement. ENGELS (personal communication) brought this point to our attention and addressed it quantitatively. He showed that hotspots in yeast are not hot enough to cause significant deviations from the model's predictions.

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APPENDIX

Russell Lande

Department of Biology, University of Oregon, Eugene, Oregon 97403

Statistical analysis of experimental conversion frequencies: We perform statistical tests of an hypothesis generated by the theory of chiasma interference derived by FOSS *et al.* (1993). The hypothesis concerns chromosomes with three adjacent marked intervals within a moderately short distance (Figure A1). Tetrads with a double crossover, one in each of the flanking intervals, will be enriched for gene conversions in the middle interval, in comparison either with all tetrads or, even more so, to tetrads with no crossover. To compare experimental results with predictions from the general theory, we need to derive a specific prediction of the magnitude of the enrichment expected for this experiment, as well as the standard error of the appropriate test statistic for the experimental observations.

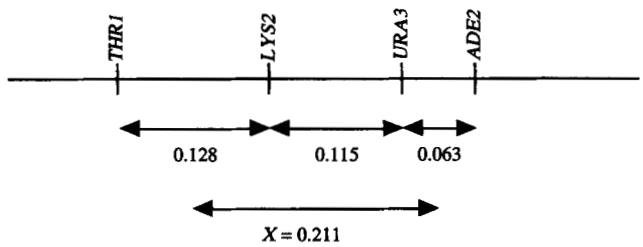

FIGURE A1.—Map distances (in Morgans) between markers in YEF392. Distances are based on the tetrads dissected for these experiments as described in MATERIALS AND METHODS.

Theoretical prediction: In the general theory, conversion events, C, have a Poisson distribution as a function of linkage map distance along the chromosome. Two adjacent conversions each with a crossover, C_x, must be separated by m conversions without a crossover, C_o. In an interval of map length X Morgans, the mean number of C events per tetrad is $2(m + 1)X$.

Among all tetrads examined, the probability of a C_o in a short interval dX is $Q_1 = 2m dX$. In tetrads with a double crossover (C_x in each flanking interval), the probability of a C_o in a short interval dX is approximately $Q_2 \cong m dX/X$, assuming that tetrads with three or more crossovers occur with negligible frequencies.

In tetrads with no crossover, the probability of a C_o in a short interval dX can be constructed from Table A1 by summing the products of the last two columns. For $m = 1$ this is $Q_3 = 2e^{-4X}dX$, and for $m = 2$ it is $Q_3 = 4(1 + 3X)e^{-6X}dX$.

We test the following two hypotheses. (1) The predicted magnitude of enrichment of C_os in the middle interval in tetrads with double crossovers (one in each flanking interval) compared with the entire sample is $Q_2/Q_1 \cong 1/(2X)$, which is independent of m. (2) The predicted magnitude of enrichment of C_os in the

TABLE A1

Constructing the probability of C_o in tetrads without crossover in an interval of X Morgans

C_o/C_x ratio	Tetrad type	Probability of tetrad	Probability of C_o in dX of tetrad
$m = 1$	—	e^{-4X}	0
	C_o	$(1/2)(4X)e^{-4X}$	dX/X
$m = 2$	—	e^{-6X}	0
	C_o	$(2/3)(6X)e^{-6X}$	dX/X
	C_o C_o	$(1/3)(6X)^2e^{-6X}/2$	$2dX/X$

TABLE A2

Comparison of theoretical predictions and experimental observations on chiasma interference, for an interval of length $X = 0.211$ Morgans

Quantity tested	C_o/C_x ratio	Predicted enrichment	Observed enrichment	Standard error	Significance level[a]
Q_2/Q_1	Any	2.37	0.684	±0.329	$P < 0.038$
Q_2/Q_3	$m = 1$	5.51			$P < 0.0026$
			0.520	±0.255	
	$m = 2$	5.15			$P < 0.0030$

[a] From Chebychev inequality.

middle interval in tetrads with double crossovers (one in each flanking interval) compared with tetrads with no crossovers is $Q_2/Q_3 \cong e^{4X}/(2X)$ for $m = 1$ and $Q_2/Q_3 \cong e^{6X}/[(2X)(1 + 3X)]$ for $m = 2$.

The experimental observations are as follows. From a total of 3081 tetrads examined, three involved triple crossovers, which satisfies the assumption that tetrads with three or more crossovers are very rare. There were 273 tetrads with C_o at markers in the middle interval, regardless of the state of the flanking intervals. There were 66 double crossover tetrads (with a C_x in each flanking interval), and of these only four had C_o at markers in the middle interval. There were 1467 tetrads with no crossover, and of these 171 had C_o at markers in the middle interval. We take the length of the interval in the theoretical formulas above to be the distance between the midpoints of the two flanking intervals, that is $X = 0.211$ Morgans. These data can be used to evaluate the quantities Q_2/Q_1 and Q_2/Q_3 and to compare them to the theoretical predictions derived above.

Table A2 shows that instead of the predicted enrichment of C_os in the middle interval, the data manifest a (nonsignificant) depletion. The discrepancies between the theory and observation are significant in the first test and highly significant in the second test, regardless of whether $m = 1$ or 2. Formulas for approximate standard errors and significance levels used in the statistical tests are given below.

Standard errors: Taylor series expansion was used to obtain approximate sampling variance of the test statistics, assuming that mutually exclusive categories of tetrads followed a multinomial sampling distribution.

The ratio Q_2/Q_1 can be estimated from the data as

$$r = \left(\frac{n_1}{n_1 + n_2}\right)\left(\frac{N}{n_1 + n_3}\right),$$

where n_1 = number of tetrads with C_x in each flanking interval with C_o in middle interval, n_2 = number of tetrads with C_x in each flanking interval without C_o in middle interval, n_3 = number of tetrads with C_o in middle interval (regardless of flanking intervals) $- n_1$ and N = total number of tetrads.

The ratio Q_2/Q_3 can be estimated from the data as

$$r = \left(\frac{n_1}{n_1 + n_2}\right)\left(\frac{n_3 + n_4}{n_3}\right),$$

where n_1 and n_2 are as defined above, and now n_3 = number of noncrossover tetrads with C_o in middle interval and n_4 = number of noncrossover tetrads without C_o in middle interval.

In both cases, the sampling variance of the estimate can be approximated as

$$\sigma_r^2 \cong r^2 \sum_{i=1}^{4}\sum_{j=1}^{4} a_i a_j(\delta_{ij}p_i - p_i p_j)N,$$

where $\delta_{ij} = 1$ if $i = j$ and 0 otherwise, and $p_i = n_i/N$. The standard error is σ_r.

For the first test estimating Q_2/Q_1, $a_2 = -1/(n_1 + n_2)$, $a_3 = -1/(n_1 + n_3)$, $a_1 = a_2 + a_3 + 1/n_1$, $a_4 = 0$, and we assume that $n_1 + n_2$ and $n_1 + n_3$ are both large ($\geqslant 10$).

For the second test estimating Q_2/Q_3, $a_1 = n_2/[n_1(n_1 + n_2)]$, $a_2 = -1/(n_1 + n_2)$, $a_3 = -n_4/[n_3(n_3 + n_4)]$, $a_4 = 1/(n_3 + n_4)$ and we assume that $n_1 + n_2$ and n_3 are both large ($\geqslant 10$).

Significance levels: Because the statistics involve ratios of random variables, one of which is small, the observed number of double crossover tetrads (with C_x in both flanking intervals) = 4, the test statistics are likely to be far from normally distributed. Conservative significance levels were obtained using Chebychev's inequality, which for the random variable x states that $P\{(|x - E[x]|)/\sigma_x \geq \lambda\} \leq 1/\lambda^2$ (KENDALL and STUART 1977).