# TETRAPLOID STRAINS OF *SACCHAROMYCES CEREVISIAE* THAT ARE TRISOMIC FOR CHROMOSOME *III*[1]

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ABSTRACT

Meiotic segregation of several genes has been studied in tetraploid strains that are trisomic for chromosome *III*. The segregation data were compared to a computer simulation that assumes trivalent pairing of homologues involved in exchanges, followed by nonpreferential segregation. Trivalent pairing was characterized by higher frequencies of exchange as compared to bivalent pairing, and by the presence of spores resulting from at least double crossovers involving all three homologues. Trivalent segregation was characterized by a unique recombinant class. The strong interference normally exhibited in diploid meiotic recombination was not evident from the frequency of double crossovers in these strains.

THIS study was undertaken as an approach to the study of mutants that are defective in both mating and sporulating ability (MACKAY and MANNEY 1974a,b). Tetraploids that are trisomic for chromosome *III*, which bears the mating-type locus, *mat1*, are uniquely suited to the study of mating-type and sporulation mutants. Each ascus formed by such strains contains two spores that are disomic for chromosome *III*, and two that are monosomic, facilitating the expression of both mating and sporulation phenotypes in the same ascus.

Many other genetic and biochemical studies in yeast are facilitated by the use of aneuploids. MORTIMER and HAWTHORNE (1966, 1973), for example, have utilized trisomic diploids (+/+/−) to establish linkage of markers to the trisomic chromosome. Markers that give 4:0, 3:1 and 2:2 (+:−) segregation ratios are associated with the trisomic chromosome, while those that give only 2:2 segregation are associated with disomic chromosomes. SHAFFER *et al.* (1971) described a stable disomic strain ($n+1$) and a method for mapping markers with respect to the centromere in trisomic diploids. CULBERTSON and HENRY (1973) used this method to map the *fas1* locus on chromosome *XI*.

SHAFFER *et al.* (1971) considered two possible arrangements of the three homologous chromosomes on the metaphase plate, bivalent-univalent and trivalent. In both cases, however, they assumed that only two of the three homologues take part in crossing over, and that the frequency of recombination

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is unaffected by the arrangement. Consequently, the assumed arrangement influences only the manner in which the chromosomes segregate at Meiosis I. They concluded from their results that the trivalent arrangement occurred at very high frequency. However, their observed frequencies of gene-centromere crossover classes were all systematically higher than expected, at the expense of parental classes. They suggested that this deviation, which reflects a higher than expected frequency of recombination, probably resulted from some type of crossover interference. CULBERTSON and HENRY (1973) observed similar deviations for the *fas1* locus.

While it is true that a higher degree of interference in a bivalent would increase the expected recombination frequency, the participation of all three homologous chromosomes in crossing over is also expected to increase the frequency. Chiasma formation in triploid plants (NEWTON and DARLINGTON 1929) and genetic recombination in triploid Drosophila (BRIDGES and ANDERSON 1925; MATHER 1933) have revealed the involvement of three homologues in exchange. Therefore, it appeared desirable to consider a different model, which tested this possibility, as the basis for calculating expected segregation frequencies. To distinguish between the manner in which three chromosomes associate with respect to exchange and the manner in which the associated chromosomes segregate to opposite poles, the former will be referred to as trisomic (or bivalent-univalent) *pairing*, and the latter as trisomic (or bivalent-univalent) *segregation*.

A computer simulation of meiosis of a trisome, assuming both trivalent pairing and trivalent segregation, provides a convenient way to predict the expected segregation classes and their frequencies. It would be possible for the simulation to accommodate the effects on the frequencies of segregation classes of such genetic phenomena as multiple crossing over, chiasma interference, various kinds of chromatid interference, gene conversion, preferential segregation, and nondisjunction. But gene conversion and nondisjunction appear to occur only at low frequencies in trisomes (SHAFFER *et al.* 1971, CULBERTSON and HENRY 1973) just as in disomes, thereby producing only a small perturbation in segregation frequencies. Accordingly, such refinements have not been included in this computer simulation. Likewise, other atypical events have been deleted from this calculation until deemed necessary. On the other hand, the larger than expected recombination frequencies observed suggest that multiple exchanges might be important. Therefore, the contribution of double crossovers was evaluated. In addition, segregation frequencies for gene-gene intervals, which have previously been ignored due to laborious calculations, were computed.

Since the approach of using a computer simulation of meiosis to predict trisomic segregation frequencies is a departure from conventional genetic analysis, a more detailed discussion dealing with the postulates used is warranted.

## THEORY

*A model for segregation in trisomes*: Meiotic segregation patterns for diploids have been well established (FINCHAM and DAY 1971; HAWTHORNE and MORTIMER 1960).

In the case where three homologous chromosomes are involved in meiosis, pairing and exchange are assumed to occur at the six-strand (chromatid) stage, analogous to the four-strand stage in disomes. These six strands are subsequently segregated into four meiotic products, the ascospores. It is assumed that sister chromatids do not crossover. If crossing over is restricted to two of the three homologues for a given region, bivalent-univalent pairing is said to have occurred. If all three homologues are simultaneously available for crossing over within a region, trivalent pairing is said to have occurred. Trivalent and bivalent-univalent pairing predict the same crossover configurations for single crossovers, but at different frequencies. Trivalent pairing predicts both two- and three-chromosomal double crossovers, while bivalent-univalent pairing restricts crossing over to two chromosomes.

The mean frequency of exchange for a given region in a trisome, due to trivalent pairing, is termed $x$. The corresponding mean frequency of exchange for a short interval in a *disome*, due to bivalent pairing, is twice the conventional map distance. For example, a 2% exchange frequency in 100 diploid cells produces four recombinant spores per 400 spores, and a map distance of 1 cM. If sister-strand exchanges are excluded, there are twelve possible exchanges among the six chromatids in a trisome with trivalent pairing, while only four possible exchanges among the four chromatids in a disome, for the same short interval. If there are no other restrictions on these exchanges, the mean frequency of exchange in a trisome, $x$, is therefore predicted to be three times that found in a disome.

Estimates of $x$ for large intervals are not easily calculated. In diploids the conventional parameter, map distance or recombinants per chromatid, relates to the frequency of recombinant gametes among all gametes. However, since in a trisome some gametes (spores) receive one chromatid while others receive two, this unit is not appropriate and will not be used. Likewise terms such as tetrads no longer convey their classical meaning and will also be avoided.

There are two distinct mechanism by which trivalent pairing could be achieved: (1) All three homologues are simultaneously paired at any given site, and (2) only two of the three homologues are paired at any one site with switching of the particular two homologues paired at different sites within the region of interest. The latter is a routine cytological observation for many plants and also Drosophila (DARLINGTON 1936; MATHER 1938). Assuming that interference cannot be propagated across pairing switches, an increase in exchange frequency would be expected in trisomes. Drosophila data (BRIDGES and ANDERSON 1925; REDFIELD 1930, 1932) indicate, however, that the magnitude of this enhancement is much less than three-fold. The bivalent switching model could still be operational in yeast provided additional mechanisms are also contributing to increasing the trisomic exchange frequency. On the other hand, COMINGS and OKADA (1971) have observed synaptonemal complexes that indicate that "triple" chromosome pairing occurs in chickens. Unfortunately, no cytogenetic data are yet available in trisomic yeast to distinguish between these mechanisms. The observed trisomic exchange frequency in yeast (see RESULTS) approaches three times that in disomes. Thus, regardless of the specific nature of the trivalent

pairing, the resulting exchange frequency is most simply described mathematically as if all three homologues were participating in pairing and exchange at any site. Intragenic segregation patterns should further discriminate between these possibilities.

When combining the various patterns of pairing and exchange with segregation, there are three fundamentally different extreme cases that can be considered: (1) Two of the three homologous chromosomes pair, participate in crossing over, and segregate to opposite poles, while the third one is not involved in exchange, but goes at random to one of the poles (bivalent-univalent pairing and segregation), (2) all three chromosomes associate, but exchanges are limited to two of them, and then the three chromosomes segregate at random to the two poles (bivalent-univalent pairing with trivalent segregation), and (3) all three chromosomes associate, all possible exchanges among them are equally probable, and then they segregate at random to the two poles (trivalent pairing with trivalent segregation). In all three cases, asci containing two monosomic and two disomic spores are produced. The terms in parentheses are included as convenient shorthand notation for these patterns, and do not necessarily imply specific mechanisms or physical interpretations.

SHAFFER *et al.* (1971) and CULBERTSON and HENRY (1973) considered the first two patterns in analyzing their results and concluded that the best fit was obtained by assuming a mixture of the two, with the second predominating. The analysis presented in this paper, however, is based on the third assumption.

In order to incorporate the effect of having all three chromosomes involved in exchange, double crossovers must be considered. The frequency of double crossovers has been approximated by splitting a region with an exchange frequency of $x$ into two equal halves, each with an exchange frequency of $0.5\ x^2$. From the assumptions of trivalent pairing and trivalent segregation, formulas can be derived for the frequency of all possible segregation classes for a gene-centromere interval in the configuration A/A/B. They are given in Table 1. If double crossover terms ($x^2$) are ignored, these frequencies are similar to those given by SHAFFER *et al.* (1971), and used by CULBERTSON and HENRY (1973), derived on the assumption that only two of the three chromosomes take part in recombination. However, their interpretation of $x$ is different. The frequencies of the four segregation classes from a gene-centromere interval for various values of $x$ are listed in Table 2.

"TRISOM" is a computer simulation of three homologues going through meiosis (RILEY 1976). It was used to predict the segregation classes and to calculate their relative frequencies. Any configuration of linked markers on these homologues is allowed and this linkage is expressed in terms of mean frequency of exchange within each region between markers or centromeres (referred to as $x_i$).

Every possible configuration for the entire chromosome is generated by considering all possible combinations of noncrossover and crossover configurations for all the regions. Each configuration is subsequently segregated into an ascus, referred to as a segregation class. A class consists of four ascospores, two of which

TABLE 1

*Expected segregation frequencies for a gene-centromere interval,* x*, *from A/A/B assuming trivalent pairing and trivalent segregation*

| Segregation type | Genotypic class | Phenotypic class a:α:n† | +:—‡ | Frequency |
|---|---|---|---|---|
| Parental I | 2A,2A/B | 2:0:2 | 4:0 | $2/3(1-x/2)^2+[1/6(2/3)+2/3(1/3)](x-x^2/2)+[1/6(7/9)+2/3(2/9)]x^2/4=2/3-x/3+15x^2/216$ |
| Parental II | 2B,2A/A | 2:2:0 | 2:2 | $1/3(1-x/2)^2+1/3(1/3)(x-x^2/2)+1/3(2/9)x^2/4=1/3-2x/9+5x^2/108$ |
| Crossover I | A,B,A/A,B/B | 2:1:1 | $3:1_m$§ | $2/3(2/3)(x-x^2/2)+2/3(7/9)x^2/4=4x/9-5x^2/54$ |
| Crossover II | 2A,A/A,B/B | 3:1:0 | $3:1_d$‖ | $1/6(2/3)(x-x^2/2)+1/6(7/9)x^2/4=x/9-5x^2/216$ |

* $x$ is the mean frequency of exchange between the centromere and the gene assuming trivalent pairing.

† Phenotypic classes for mating-type are given by ratios **a**:α:*n*, where *n* is a non-mating sporulator. A=**a** and B=α.

‡ Phenotypic classes for a nutritional marker are given by the ratios +:—. *A*=+ and *B*=—.

§ Expression of the recessive allele is in a monosome.

‖ Expression of the recessive allele is in a disome.

are monosomic, and two of which are disomic. Identical classes for a given marker or markers are consolidated and their frequencies summed. Multiple crossovers can be eliminated from consideration should their frequencies be smaller than some prescribed limit of accuracy.

When all possible configurations of recombination along the entire length of the chromosome have been generated, each configuration is ready to proceed through segregation. Since segregation is assumed to be independent of crossing over, the relative frequency obtained from segregation is multiplied by the relative frequency of the recombinant configuration to yield the net frequency of an ascus type. The relative frequencies due to segregation are obtained as follows. At Meiosis I, each of the three chromosomes has an equal chance of segregating to pole I, regardless of the nature of the crossover, while the remaining two segregate to pole II. If any two chromosomes are indistinguishable, they are considered together by summing their frequencies. During Meiosis II, sister chromatids disjoin.

The six strands of an ascus represent its genotype. The phenotype class of each ascus is determined from the genotype for all dominant-recessive markers and for mating-type. The frequencies of each class are summed for similar ascus types for pairs of markers, or for one marker and the centromere (as the centromere has arbitrarily been assigned the phenotype 4:0). In addition to the total frequency of each ascus type, that portion of the total frequency due to zero, one, two, and more multiple crossovers is tallied. Thus, it is possible to estimate the total number of single and double crossovers from unique double-crossover classes.

TABLE 2

*Expected frequencies of the phenotypic classes for a gene-centromere interval from* **a**/**a**/$\alpha$ *and* +/+/— *assuming trivalent pairing and trivalent segregation for various values of* x*

| | Frequencies in percent | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Single crossovers† | | | | Double crossovers‡ | | | |
| **a**:$\alpha$:$n$§ +:—‖ | 2:0:2 4:0 | 2:2:0 2:2 | 2:1:1 $3{:}1_m$¶ | 3:1:0 $3{:}1_d$** | 2:0:2 4:0 | 2:2:0 2:2 | 2:1:1 $3{:}1_m$ | 3:1:0 $3{:}1_d$ |
| %$x$ | | | | | | | | |
| 0 | 66.7 | 33.3 | 0 | 0 | 66.7 | 33.3 | 0 | 0 |
| 10 | 63.3 | 31.1 | 4.4 | 1.1 | 63.4 | 31.2 | 4.3 | 1.1 |
| 20 | 60.0 | 28.9 | 8.9 | 2.2 | 60.2 | 29.0 | 8.4 | 2.1 |
| 30 | 56.7 | 26.7 | 13.3 | 3.3 | 57.3 | 28.1 | 12.5 | 3.1 |
| 40 | 53.3 | 24.4 | 17.8 | 4.4 | 54.4 | 25.2 | 16.3 | 4.1 |
| 50 | 50.0 | 22.2 | 22.2 | 5.6 | 51.7 | 23.4 | 19.9 | 5.0 |
| 60 | 46.7 | 20.0 | 26.7 | 6.7 | 49.2 | 21.7 | 23.3 | 5.8 |
| 70 | 43.3 | 17.8 | 31.1 | 7.8 | 46.7 | 20.1 | 26.6 | 6.6 |
| 80 | 40.0 | 15.6 | 35.6 | 8.9 | 44.4 | 18.5 | 29.6 | 7.4 |
| 90 | 36.7 | 13.3 | 40.0 | 10.0 | 42.3 | 17.1 | 32.5 | 8.1 |
| 100 | 33.3 | 11.1 | 44.4 | 11.1 | 40.3 | 15.7 | 35.2 | 8.8 |
| 110 | | | | | 38.4 | 14.5 | 37.7 | 9.4 |
| 120 | | | | | 36.7 | 13.3 | 40.0 | 10.0 |
| 130 | | | | | 35.1 | 12.3 | 42.1 | 10.5 |
| 140 | | | | | 33.6 | 11.3 | 44.1 | 11.0 |
| 150 | | | | | 32.2 | 10.4 | 45.8 | 11.5 |

* $x$ is the mean frequency of exchange between the centromere and gene assuming trivalent pairing.
† Assumes only single crossovers, ignores all second order terms.
‡ Allows double crossovers, and includes second order terms.
§ Phenotypic classes from **a**/**a**/$\alpha$ are given by ratios **a**:$\alpha$:$n$, where $n$ is a nonmating sporulator.
‖ Phenotypic classes from +/+/— are given by ratios +:—.
¶ The expression of the recessive allele is in the monosome.
** The expression of the recessive allele is in the disome.

The frequencies of the sixteen classes of asci for a gene-gene interval can be predicted. Of particular interest are those classes that result only from double crossovers (both two and three chromosomal), since such unique classes were not predicted for gene-centromere intervals. Thus, classes from gene-gene intervals provide a way of estimating the total number of double crossovers. In addition, the presence of that class predicted solely from a three-chromosomal double crossover provides qualitative evidence for trivalent pairing within that gene-gene interval. These sets of probabilities not only depend on the $x$ value between the genes, but also on the $x$ value between the proximal gene and the centromere. Because of the large number of classes and their dependence on two parameters, these sets of probabilities are solved only for specific cases in order to approximate the double crossover frequencies.

## MATERIALS AND METHODS

*Yeast strains*: The strains of *Saccharomyces cerevisiae* used to construct the tetraploids were derived from strains obtained from R. K. MORTIMER (University of California, Berkeley). Their genotypes are given in Table 3.

TABLE 3

*Strains used to construct trisomic tetraploids*

| | |
|---|---|
| Haploids: | |
| XP300–26C | α *thr4 his6 lys1 gal2* |
| XP300–29B | **a** *his6 lys1 trp5 ade2 gal2* |
| XT1177–S24 | **a** *leu1 trp5 can1 gal2* |
| X1069–2D | α *his4 leu2 thr4 trp5 ura1 ade1 met2 gal2* |
| XT1172–S245 | α *leu1 his6 ade6 trp5 met1 can1 gal2* |
| XT1177–S47 | **a** *ade2 his6 lys1 trp5 can1 gal2* |
| Diploids: | |
| XP173 | XP300–26C × XP300–29B |
| XL61 | XT1177–S24 × X1069–2D |
| XL10 | XT1172–S45 × XT1177–S47 |
| XL1011A | cross between two spores from XL10 |
| XL1011A–3(a) | **a** monosomic segregant of XL1011A |
| XL61–1(α) | α monosomic segregant of XL61 |
| Tetraploids*: | |
| XI28b2 and XI29b2 | + *leu2* α *thr4*<br>*his4 leu2* α +<br>*his4* + **a** *thr4* |
| XI76, XI78, XI80 and XI82 | *his4 leu2* α<br>+ + **a**<br>+ + **a** |

* Only the chromosome *III* markers are shown.

*Media and methods of genetic analysis*: The media, growth conditions, and the specific methods employed in constructing hybrids and for genetic analysis of meiosis have been described previously (MACKAY and MANNEY 1974a). Spores from trisomes which failed to mate were generally shown to sporulate. Random spore samples were obtained by the procedure of GILMORE (1967).

*Construction of trisomic tetraploids*: Two general methods were used to construct trisomic tetraploids. In one case zygotes were isolated by micromanipulation from an appropriate mixture of two mating diploids; in the other a mating diploid was mixed with a nonmating one, and the tetraploids, which arise at low frequencies, were isolated by prototroph selection (POMPER and BURKHOLDER 1949). The mating diploids were either homozygous at *mat1* or monosomic for chromosome *III* as evidenced by the segregation patterns for several markers on chromosome *III* from subsequent hybrids (see RESULTS). Nonmating diploids were heterozygous at *mat1*.

Several methods were used to obtain mating diploids. Homozygous α strains are readily isolated by prototroph selection from a mixture of two nutritionally complementing α haploids. Homozygous **a** strains, however, have not been isolated by this approach. A variety of mating diploids arise from heterozygous ones and can be identified by replica-plating colonies to a lawn of an appropriate tester strain on complete medium and subsequently replica-plating this replica to selective medium. (1) Mating mitotic segregants that are homozygous at *mat1* can originate from heterozygotes by mitotic crossing over (STERN 1936; JAMES and LEE-WHITING 1955; ROMAN 1956) or by mitotic gene conversion (ROMAN 1958). Both of these events are stimulated by ultraviolet radiation (HURST and FOGEL 1964; JAMES and LEE-WHITING 1955; YAMASAKI, ITO and MATSUDAIRA 1964; NAKAI and MORTIMER 1967). (2) Mating segregants that are monosomic for chromosome *III* can be derived from heterozygotes by mitotic nondisjunction (EMEIS 1966; STROMNAES 1968; MORTIMER and HAWTHORNE 1969). Nondisjunction has not yet been

reported to be appreciably enhanced by ultraviolet radiation; however, strains exist in which the spontaneous level of nondisjunction is greater than the spontaneous level of mitotic crossing over and gene conversion (HABER 1974). This was found to be true for XL61 and XL10, but not for XP173.

The tetraploids strains XI28b2 and XI29b2 were constructed by mating a homozygous $\alpha$ diploid with a monosomic **a** diploid. The $\alpha$ strain was isolated from a mixture of two complementing $\alpha$ spores from XL61. The monosomic strains were spores from a disomic tetraploid produced by crossing XL1011A-3(**a**) with XL61-1($\alpha$). XI28b2 and XI29b2, which were **a**$\alpha\alpha$, sporulated well, but produced few asci with four viable spores. The basis of this poor viability was not specifically identified, but it did show linkage to the *thr4* allele in X1069-2D. Accordingly, subsequent strains were constructed without this marker.

A series of trisomic tetraploids with good spore viability were isolated by a slightly different approach. Tetraploids were selected from a mixture of the monosomic diploid XL61-1($\alpha$) and the UV-irradiated nonmating diploid XP173. Most of these tetraploids apparently resulted from mating of the monosomic strain with homozygous **a** mitotic segregants in the XP173 culture. Upon sporulation, these tetraploids produced a variety of monosomic and homozygous spores in both mating types, with a variety of marker combinations. XI76, XI78, XI80, and XI82 are trisomic tetraploids constructed by mating some of these spores.

## RESULTS

*Evidence for trivalent pairing*: If each of three homologues in a trisomic tetraploid has an equal chance of participating in an exchange, then it is possible for three-chromosomal double-crossovers to occur. The existence of such exchanges provides evidence for trivalent pairing in a given region. Trisomic strains were constructed such that each homologue was uniquely marked in order to detect such three-chromosomal exchanges. The configuration of markers in XI28b2 and XI29b2 (Table 3) was used to examine trivalent pairing within the *his4-thr4* region.

A monosomic spore of mating-type **a** carrying the wild-type alleles of these three nutritional markers would arise most frequently when one of the **a** chromatids is involved in a double crossover, one exchange being with each of the other two homologues as shown in Figure 1a. A mating-type **a** disomic spore having the same phenotype (prototrophy) would be expected at approximately equal frequency when one or both of the **a** chromatids are involved in a double crossover, as shown in Figure 1b. Therefore, spores of this phenotype provide a sensitive measure of three-chromosomal exchanges.

A random-spore analysis was performed on XI28b2 and XI29b2. The sporulated cultures contained 84% and 87% spores respectively. Free spores were plated on SC minus histidine, leucine, and threonine to isolate prototrophs for the chromosome *III* markers, and on SC to count the number of viable spores (most of the vegetative cells are killed by the procedure). The colonies on the selective medium were scored for mating type. The results are given in Table 4. The frequency ($f_p$) of prototrophic **a** spores, 2.3% for XI28b2 and 0.97% for XI29b2, may be compared with the expected frequency of prototrophic **a** monosomes and **a**/**a** disomes resulting from double crossovers (Figure 1), which is $f_p = (1/4)(1/3)\,[2(x_1/6)(x_4/6)] + (1/4)(1/6)(x_3/3)(x_4/3) = (x_1x_4)/216 + (x_3x_4)/216$. Using the values $x_1 = 0.51$, $x_2 = 0.36$, $x_3 = 1.24$ from Table 5 and

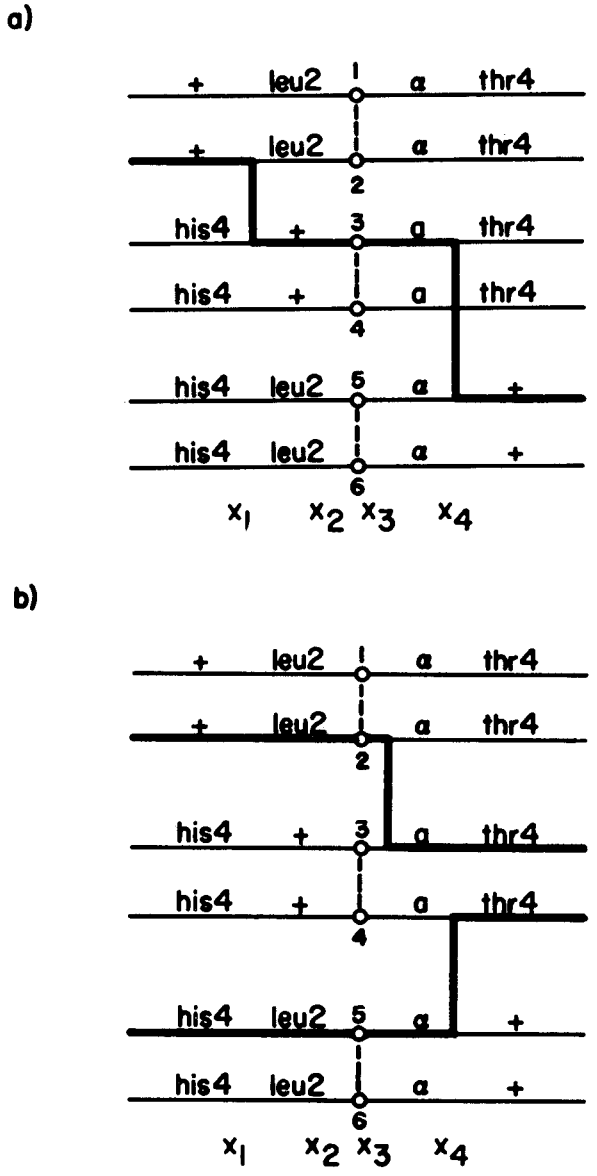


FIGURE 1.—Trivalent associations at metaphase I that can yield unique three-chromosomal exchange products. (a) If a double exchange occurs as shown, and (chromatid) 3 segregates into a monosomic spore, it will have the phenotype + + a +. (b) If a double exchange occurs as shown and (chromatids) 2 and 4 segregate into the same disomic spore, it will have the phenotype + + a +.

estimating $x_4 = 1.0$ from the disomic map, the value $f_p = 1.2\%$ is obtained, which is in reasonable agreement with both experimental values. Asci from these strains were also dissected; poor spore viability (3/16 and 8/16 four-viable-spored asci) and apparently skewed distribution of segregants were found. This probably con-

TABLE 4

*Frequencies of spores observed involving three-chromosomal exchange*

| Strain | Spores tested | *HIS*+ *LEU*+ *THR*+ No. | Percent | a *HIS*+ *LEU*+ *THR*+ No. | Percent |
|---|---|---|---|---|---|
| XI28b2 | $1.1 \times 10^4$ | $1.1 \times 10^3$ | 10.0 | 253 | 2.3 |
| XI29b2 | $9.5 \times 10^3$ | $1.4 \times 10^3$ | 15.0 | 92 | 0.97 |
| Total | $2.05 \times 10^4$ | $2.5 \times 10^3$ | 12.2 | 345 | 1.7 |

tributed to the deviations of observed frequencies from the expected for this class.

However, the presence of prototrophic spores in this limited sample is qualitative evidence for three-chromosome exchange, which requires trivalent pairing in the *his4-thr4* interval. The high frequency of exchange needed to account for the observed frequency of prototrophs is also characteristic of trivalent pairing.

*Exchanges in gene-centromere intervals*: The trisomic tetraploids XI76, XI78, XI80, and XI82 produced spores with good viability (Table 5). These strains were constructed (+/+/−) at several loci by mating **a/a** diploids with $\alpha$ monosomic diploids. Crossovers proximal to loci in duplex are readily identifiable by the 3:1 phenotypic classes (+:−) (Table 1). Although the expression of a recessive allele was sometimes observed in nonmating disomic segregants in $3{:}1_d$ asci, it was not always possible to determine whether the recessive-bearing spore in a 3:1 ascus was monosomic or disomic in mating segregants. Hence, the two crossover classes, $3{:}1_m$ and $3{:}1_d$, are tabulated together as the 3:1 class when accumulating and analyzing data. A few percent of aberrant asci have been dismissed, since characterization of normal trisomic meiosis was of primary interest.

The observed distributions of phenotypic classes for three markers are shown in Table 5. They are compared with the distributions calculated from the trivalent pairing, trivalent segregation model giving the closest agreement (see Table 2).

The relative distribution of the phenotypic classes for *leu2* (gene-centromere interval) are in excellent agreement with those predicted, considering no higher than second-order exchanges. Similarly, distributions for *his4* are also in good agreement, but the two crossover classes for mating type are somewhat distorted from those predicted from the best fit values of $x$, specifically the 2:1:1 class is high and the 3:1:0 class is low. It would appear that a slight preferential segregation was occurring, *i.e.*, that chromosomes participating in an exchange have a slightly reduced probability of segregating to the same spore (3:1:0), either at Meiosis I or at Meiosis II. Computations that considered multiple exchanges of higher order than two do not predict a distribution demonstrating this effect. Furthermore, if these two classes are considered together, as was done for the two auxotrophic markers, good agreement is established.

On the assumption of trivalent pairing, the mean frequency of exchange in a trisome, $x$, is expected to be three times the diploid exchange frequency, which is equivalent to second division segregation (SDS) frequency for small intervals where multiple exchanges are not important. For longer intervals the calculated

TABLE 5

*Observed phenotypic classes for gene-centromere intervals*

| Strain | Viability* | *his4*† 4:0 | 2:2 | 3:1 | *leu2*† 4:0 | 2:1 | 3:1 | *mat1*‡ 2:0:2 | 2:2:0 | 2:1:1 | 3:1:0 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XI76 | 114/131 | 47 | 21 | 46 | 59 | 33 | 22 | 32 | 14 | 62 | 6 | |
| XI78 | 17/20 | 4 | 2 | 11 | 8 | 5 | 4 | 9 | 3 | 5 | 0 | |
| XI80 | 83/101 | 45 | 13 | 25 | 49 | 19 | 15 | 34 | 14 | 31 | 4 | |
| XI82 | 17/20 | 6 | 3 | 8 | 12 | 4 | 1 | 7 | 1 | 7 | 2 | |
| Total: Obs. | 231/272 | 102 | 39 | 90 | 128 | 61 | 42 | 82 | 32 | 105 | 12 | (117)‖ |
| Best Fit§ | 231 | 99.6 | 40.8 | 90.6 | 129 | 60.3 | 41.6 | 83.2 | 29.8 | 94.4 | 23.6 | (118) |
| Percent: Obs. | | 44.2 | 16.9 | 39.0 | 55.4 | 26.4 | 18.2 | 35.5 | 13.9 | 45.5 | 5.2 | (50.7) |
| Best Fit§ | | 43.1 | 17.6 | 39.2 | 55.9 | 26.1 | 18.0 | 36.0 | 12.9 | 40.9 | 10.1 | (51.1) |
| $\chi^2$ | | | 0.136 | | | 0.018 | | | | 7.07 | | (0.139) |
| $P$ | | | $>90\%$ | | | $>99\%$ | | | | $>5\%$ | | ($>90\%$) |

* The fraction of asci with four viable spores.
† Classes for recessive markers are given by the ratio +:—.
‡ Mating-type classes are given by the ratio **a**:**a**:*n*, where *n* is a nonmating sporulator.
§ Best fit values were found for $x_1 + x_2 = 86\%$, $x_2 = 35\%$, and $x_3 = 124\%$.
‖ Values in parentheses are for the sum of the last two columns (see text).

TABLE 6

*Comparison of disomic and trisomic exchange frequencies*

| | *his4* | *leu2* | *mat1* |
|---|---|---|---|
| Trisomic tetraploid: | | | |
| Mean frequency of exchange ($x$) | 86 | 35 | 124 |
| Diploid: | | | |
| Frequency of SDS* | 41.6 | 12.9 | 41.0 |
| 2(Map distance) | 48.2 | 13.0 | 56.0 |
| Ratios: | | | |
| $x$/SDS | 2.1 | 2.7 | 3.0 |
| $x$/2(Map distance) | 1.8 | 2.7 | 2.2 |

* MORTIMER and HAWTHORNE 1966.

trisomic exchange frequency takes into account both single and double exchanges, whereas SDS frequency does not, and therefore underestimates exchange frequency. However, an estimate of the exchange frequency in diploids, corrected for multiple exchanges, can be obtained by doubling the map distance, in centimorgans (cM). In Table 6 the best-fit values of $x$ are compared with values of SDS frequencies and map distances for diploids, published by MORTIMER and HAWTHORNE (1966). Although somewhat different map distances have been reported by other workers (SHAFFER *et al.* 1971; CAMPBELL, FOGEL and LUSNAK 1975), only MORTIMER and HAWTHORNE published actual segregation data from which SDS frequencies and map distances are calculated. For the centromere-*leu2* interval, which is short enough that the effect of multiple crossovers is negligible, the ratio of exchange frequencies approaches the predicted value of three. For the longer intervals, where multiple exchanges are important, the ratio, when both values have been corrected, is only approximately two.

*Exchanges in gene-gene intervals*: These results indicate that multiple crossing over is important. The best-fit values for gene-centromere intervals are the result of assuming that double crossovers occur without interference. These data do not, however, give direct information about multiple exchanges. To detect double crossovers it is necessary to analyze an interval bounded by two genes. This has been done for the *his4-leu2* interval (Table 7), and the *leu2-mat1* interval (Table 8). When one dose of each of the recessive alleles, *his4* and *leu2*, are coupled in monoplex, there are two unique phenotypic classes of asci that require at least a double exchange. They are 4:0 for *his4* while 2:2 for *leu2*; and 2:2 for *his4* while 4:0 for *leu2* (last two columns, Table 7). These two classes represent approximately 4.4% of all the double crossovers. On the assumption of no interference for the values of $x$ given in Table 7, they should be present in only 0.33% of all asci. But the strains listed in Table 7 produced 2.6% of their asci in these classes. Likewise, other double crossover classes consisting of a single crossover in the *his4-leu2* region and a crossover in the proximal region (columns 6 and 7, Table 7) are also more frequent than predicted.

TABLE 7

*Observed phenotypic classes for* his4–leu2 *interval*

| Strain | Number of asci | *his4**: *leu2* | 4:0 : 4:0 | 2:2 2:2 | 3:1 3:1 | 3:1 4:0 | 3:1 2:2 | 4:0 3:1 | 2:2 3:1 | 2:2 4:0 | 4:0 2:2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| XI76 | 114 | | 42 | 19 | 17 | 15 | 14 | 5 | 0 | 2 | 0 |
| XI78 | 17 | | 3 | 2 | 2 | 5 | 4 | 1 | 0 | 0 | 0 |
| XI80 | 83 | | 37 | 11 | 9 | 12 | 4 | 4 | 2 | 0 | 4 |
| XI82 | 17 | | 6 | 2 | 1 | 4 | 1 | 0 | 1 | 0 | 0 |
| Total: Observed | 231 | | 88 | 34 | 29 | 36 | 23 | 10 | 3 | 2 | 4 |
| Best Fit† | | | 98.9 | 41.8 | 36.3 | 28.4 | 17.5 | 3.2 | 1.1 | 0.2 | 0.4 |
| Percent: Observed | | | 38.4 | 14.7 | 12.6 | 15.6 | 10.0 | 4.3 | 1.3 | 0.85 | 1.7 |
| Best Fit† | | | 42.8 | 18.1 | 15.7 | 12.3 | 7.6 | 1.4 | 0.48 | 0.08 | 0.16 |

* Classes for the gene-gene interval are represented by the +:— ratios for both markers.
† Best fit frequencies were calculated for $x_1$ (*his4–leu2*) = 50%, $x_2$ (*leu2*-centromere) = 36%.

TABLE 8

*Observed frequencies of double crossover classes for* mat1–leu2 *interval*

| Strain | Number of asci | *leu2**: *mat1* : | $3{:}1_m$ 2:1:1 | $3{:}1_d$ 3:1:0 | $3{:}1_m$ 3:1:0 | $3{:}1_d$ 2:1:1 | 2:2 2:0:2 | 4:0 2:2:0 | 2:2 3:1:0 |
|---|---|---|---|---|---|---|---|---|---|
| XI76 | 114 | | 13 | 0 | 1 | 2 | 0 | 0 | 0 |
| XI78 | 17 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| XI80 | 83 | | 6 | 0 | 1 | 0 | 0 | 0 | 0 |
| XI82 | 17 | | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| Total: Observed | 231 | 26 | 19 | 1 | 4 | 2 | 0 | 0 | 0 |
| Best Fit† | | 26 | 14.8 | 1.2 | 2.8 | 2.8 | 2.6 | 1.5 | 1.3 |
| Percent: Observed | | | 8.2 | 0.4 | 1.7 | 0.9 | 0 | 0 | 0 |
| Best Fit† | | | 6.4 | 0.51 | 1.2 | 1.2 | 1.13 | 0.63 | 0.55 |

* Classes for the gene-gene interval are represented by the +:— ratio for *leu2* and the **a**:$\alpha$:*n* ratio for mating type (*n* = nonmating sporulator).
† Expected frequencies were calculated for *leu2*-centromere = 36% and *mat1*-centromere = 124%.

TABLE 9

*Observed tetrad ratios for strains derived from XI76 and XI80*

| | *his4–leu2* | *leu2–mat1* | *his4–mat1* |
|---|---|---|---|
| Diploids: | | | |
| PD:NPD:T | 40:1:21 | 35:4:49 | 17:6:45 |
| Map distance (cM)* | 16.9 | 41.5 | 59.6 |
| Disomic tetraploids: | | | |
| PD:NPD:T | 18:0:21 | 16:2:23 | 10:0:23 |
| Map distance (cM)* | 26.9 | 42.7 | 38.1 |

* cM = [(T+6NPD) × 100]/[2(PD+NPD+T)] (PERKINS 1949).

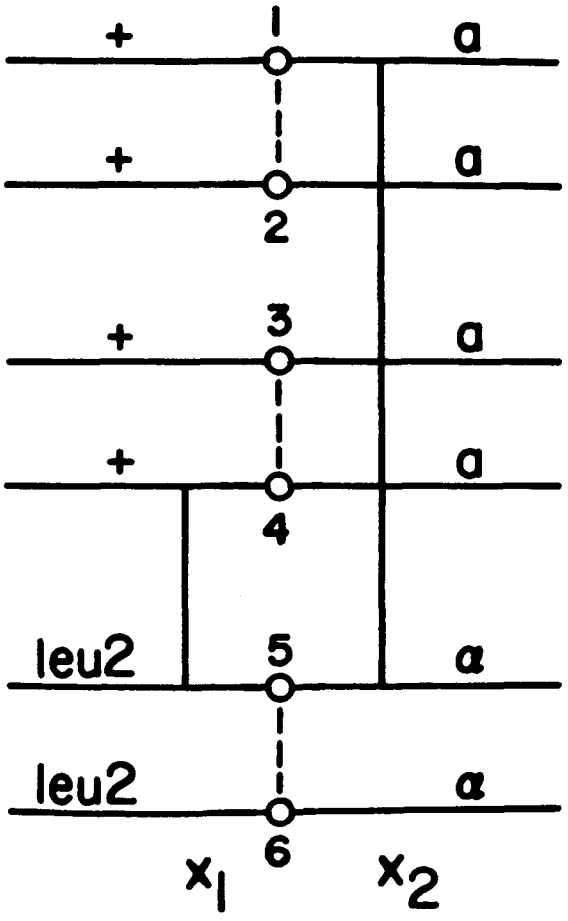


FIGURE 2.—Trivalent association at metaphase I that can yield a unique type of ascus. When a double exchange occurs as shown, and chromatids 4 and 6 segregate into the same disomic spore, the resulting ascus will be $3{:}1_d$ for *leu2*, while 2:1:1 for *mat1*.

Table 8 lists the frequencies of double crossover classes for the *leu2-mat1* interval, which spans the centromere. The overall frequency of these classes and their relative distribution are in good agreement with the best fit values for this limited sample. The observed total frequency and their relative distribution are both in close agreement with the best fit values, which assume no interference.

Table 9 summarized the tetrad ratios observed in nine diploids and four disomic (III) tetraploids derived from spores of XI76 and XI80. These values are in substantial agreement with published results (MORTIMER and HAWTHORNE 1966; SHAFFER *et al.* 1971; CAMPBELL, FOGEL and LUSNAK 1975). This agreement minimizes any concern about the observed high frequencies of exchange and of double crossovers being the result of abnormal linkages in these strains.

*Further evidence for three-chromosomal exchanges*: The double crossovers reported in Table 8 provide additional and independent evidence for the involve-

ment of all three homologues in some double crossovers. Figure 2 illustrates the manner in which a three-chromosome double crossover is necessary to generate a unique type of ascus, being $3:1_d$ for *leu2* and 2:1:1 for *mat1*. Two such asci were found, in excellent agreement with the number expected in the sample. These three-chromosomal crossover asci further demonstrate the occurrence of trivalent pairing, that is, with involvement of all three chromosomes in an exchange.

## DISCUSSION

We have developed a relatively simple model that predicts meiotic segregation frequencies of centromere-linked markers in trisomic tetraploids. We have used this model in a computer simulation to analyze observed meiotic segregations of monosomic and disomic spores from trisomic tetraploids of *Saccharomyces cerevisiae*. We have been able to fit the experimental results with a model incorporating the following assumptions: (1) all three homologous chromosomes pair for exchange as a trivalent, (2) crossing over occurs at the same intrinsic frequency (see below) as in disomes (*e.g.*, diploids), (3) all pairs of chromatids, except sister strands, have equal probabilities of exchanging, (4) double crossovers may involve all three chromosomes and encounter no interference, and (5) after exchanges have occurred, the three chromosomes segregate nonpreferentially into two disomic and two monosomic nuclei.

Three-chromosome exchanges are revealed qualitatively by the recovery of unique double crossover classes that must involve all three homologs (see Tables 4 and 8). That these exchanges are produced by trivalent pairing is quantitatively supported by the agreement between the predicted and observed recombination values, in gene-centromere as well as gene-gene intervals. The alternative hypothesis of univalent-bivalent pairing of entire chromosomes cannot account for these unique classes and predicts significantly lower frequencies of recombinant classes. Furthermore, the trivalent pairing model provides a better quantitative fit to the observed frequencies by correctly predicting the frequencies of recombinant classes. The predictions of the two models are compared with the observed values in Table 10. The best-fit expected values for trivalent pairing with trivalent segregation have been calculated for each set of observed frequencies. The values expected for the bivalent-univalent pairing model were calculated by SHAFFER *et al.* (1971) from gene-centromere map distances computed from their own data for disomic diploids.

The high recombination frequencies cannot be attributed to the specific genetic background of these trisomic tetraploids. The data in Table 9 demonstrate that the recombination frequencies in closely related disomic tetraploids from the same genetic background are not significantly different from those found in diploids. Furthermore, the higher frequency of exchange in trisomes is expected because of the greater opportunity for exchange that exists for three pairs of chromatids compared with only two pairs in disomes. For the *leu2*-centromere interval, in which multiple exchanges are negligible, the mean frequency of exchange, $x$, in the trisomic tetraploid is 2.7 times the second division segregation frequency

TABLE 10

*Comparison of observed and expected recombination frequencies*

| Genotype | Phenotype of recombinant class | Percent expected: Univalent-bivalent pairing with trivalent segregation* | Percent expected: Trivalent pairing with trivalent segregation | | Percent observed: Trisomic tetraploids | Percent observed: Trisomic diploids* |
|---|---|---|---|---|---|---|
| | | | *4n*† | *2n*‡ | | |
| +/+/*leu2* | 3+:1— | 5 | 18 | 10 | 18 | 10 |
| +/+/*his4* | 3+:1— | 19 | 39 | 32 | 39 | 32 |
| **a**/**a**/$\alpha$ | 2**a**:1$\alpha$:1*n* | 24 | 41 | 46 | 46 | 43 |
| | 3**a**:1$\alpha$:0*n* | 6 | 10 | 10 | 5 | 10 |

* SHAFFER *et al.* 1971.
† Best-fit values for trisomic tetraploid data.
‡ Best-fit values for trisomic diploid data.

found in diploids. Since there are 12 possible ways for chromatid-chromatid synapsis to occur among six strands (excluding sister strands) and only four ways among four strands, the probability of exchange between synapsed chromatids appears to be about the same in both cases.

The best fits to the double crossover data were obtained assuming no interference (Tables 7 and 8). It would seem reasonable that double exchanges involving all three homologous chromosomes might not experience much interference. Furthermore, if this were indeed the case, while doubles involving only two chromosomes suffer interference as in diploids, then there would be an enrichment for the three-chromosomal double crossover classes. The majority of segregation classes produced by three chromosomal double crossovers are not generally distinguishable from two chromosomal ones, hence this limited sample does not resolve this possibility.

The model used in this analysis assumed that the segregation of chromosomes to spindle poles (Meiosis I), and subsequent disjunction (Meiosis II) following exchange was nonpreferential. However, in Table 5 there is evidence for a slight preferential segregation to opposite poles either at Meiosis I or Meiosis II by chromosomes that participate in an exchange. The 3**a**:1$\alpha$ class (last column) results from incorporation of the reciprocal products into the same spore, while the 2**a**:1$\alpha$:1*n* (nonmater) class results from the same exchange, but with reciprocal products being incorporated into different spores (next to last column). The frequency of the former class is substantially lower than predicted, but the frequency of the two classes taken together is in good agreement with the model. Calculated for the two classes separated, the best fit gives a large $\chi^2$ value (corresponding to $5\% < P < 10\%$), but with the combined values the fit is quite good ($P > 95\%$). If the segregation were always preferential at Meiosis I, the pattern of classes would be indistinguishable from the univalent-bivalent case, which does not give as satisfactory a fit to the results. Such data might also be explained by preferential nondisjunction of reciprocal products at Meiosis II to yield **a** and **a**/$\alpha$/$\alpha$ spores. However, subsequent tetrad analysis of several nonmating spores

(eight strains totaled in Table 9, and 13 others not reported) failed to produce trisomic segregation ratios. Nondisjunction at Meiosis II is also discounted as a frequent event by CULBERTSON and HENRY (1973).

Gene conversion could also account for some aberrant classes, but it is difficult to identify such events in this system.

The use of a computer simulation of meiosis to predict phenotypic segregation classes represents an unconventional, if not novel, approach to genetic analysis. But in the case of complicated genomes, such as polyploids, where large numbers of probable segregation classes and uncertainties about pairing and segregation rules make conventional analysis impractical, it provides an alternative. It is a practical way to predict the outcomes of specific crosses. A conspicuous disadvantage of this approach is that the answers are not readily expressed in conventional map units. However, the mean frequency of exchange, $x$, can be calculated for both gene-centromere and gene-gene intervals and provides a usable measure of linkage, which may prove to be approximately three times the map distance, for short intervals.

Although the close fits to the data do not prove the validity of all the specific assumptions of the model, they provide confidence that these assumptions are quite adequate for practical applications. The qualitative results, however, provide a strong indication that three-chromosome double exchanges are a regular feature of meiosis in trisomes.

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