GENETIC ANALYSIS OF TRISOMIC TETRAPLOIDS, AND THE EXPRESSION OF CRYPTOPLEURINE, RESISTANCE IN ANEUPLOID, SACCHAROMYCES CEREVISIAE

by

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TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

INTRODUCTION

Genetic analysis of Saccharomyces cerevisiae depends on the mating-type locus, mat1. The two alleles for mat1 not only affect the mating behavior in both haploids and diploids, but also the ability of diploids to undergo meiosis (sporulate). Mutations which affect these mating behaviors and/or the ability to sporulate have been isolated by MACKAY and MANNEY (1974a). An intriguing problem is the genetic analysis of those mating-type mutations which prevent sporulation, and in doing so impede their own genetic analysis.

This thesis deals with the development of a system to analyze such mutants and the consequences thereof. In addition it deals with a problem encountered with cryptopleurine-resistant mutants and the observation of a most unusual genetic phenomenon concerning the expression of cryptopleurine in aneuploid strains upon its analysis using the same system.

Life cycle and function of mating type locus. The life cycle of heterothallic Saccharomyces cerevisiae consists of two stable vegetative (asexual) phases, haplophase and diplophase, and those sexual phases which occur during a transition between them (Figure 1). The two normal alleles of the mating-type locus, mat1, are a and α (LINDEGREN and LINDEGREN 1943b). Mat1 maps twenty centimorgans from the centromere of chromosome III (LINDEGREN and LINDEGREN 1949; HAWTHORNE and MORTIMER 1960). Only in the presence of both of these alleles does a cell undergo meiosis (LINDEGREN and LINDEGREN 1943a; ROMAN and SANDS 1953). Normally only those diploids heterozygous for mating-type (a/α) sporulate, producing an ascus containing

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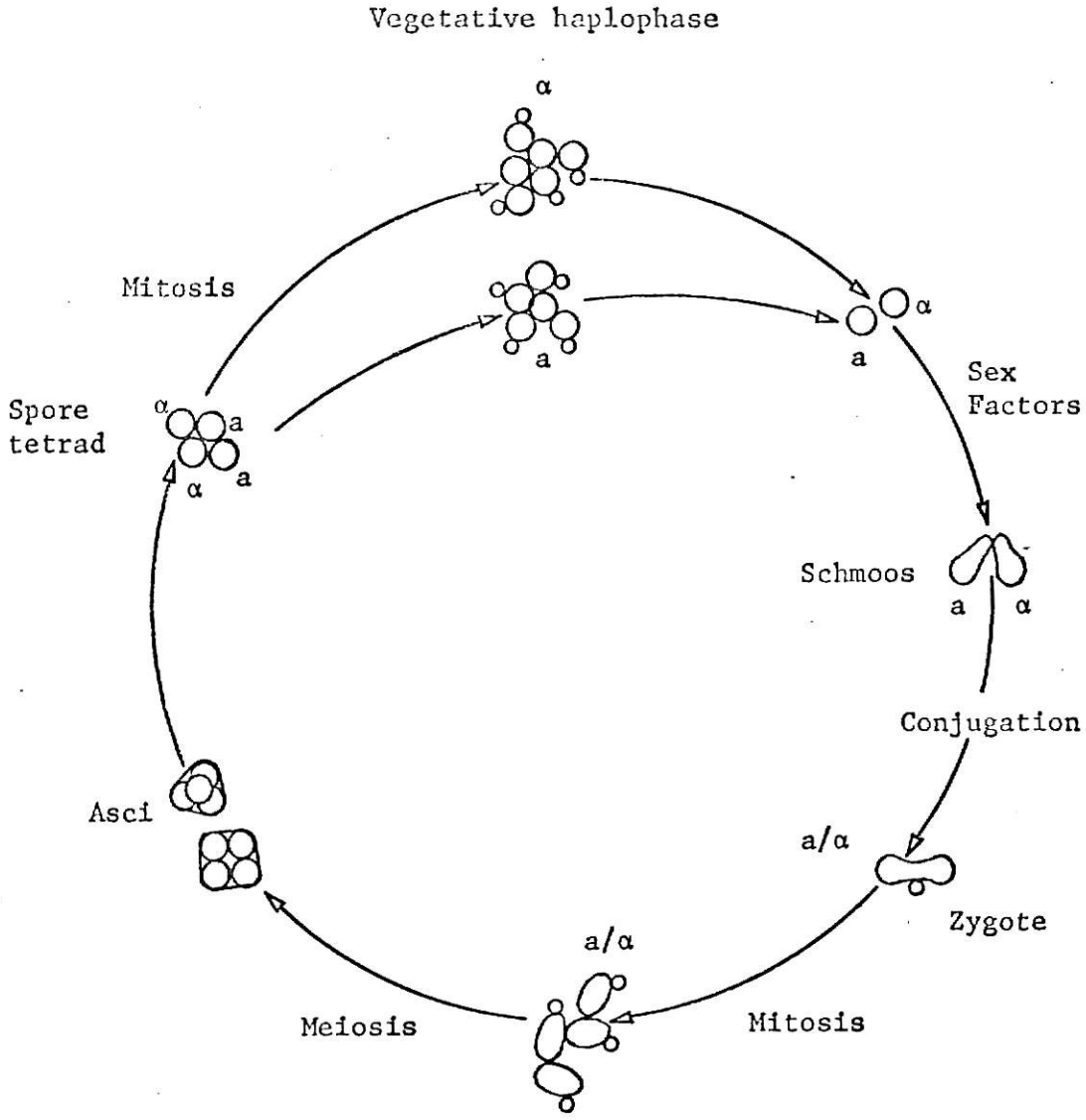
FIGURE 1

The life cycle of Saccharomyces cerevisiae.[a]



[a]Reproduced from MACKAY (1972).

four ascospores, two a mating-type haploids and two α mating-type haploids. When those haploids are isolated from one another they reproduce by budding (mitoticly). The ability of cells to mate depends on the alleles at the mat1. Should two haploid cells of opposite mating-types (one a and one α) become paired close together, mating is allowed. However, mating between two haploids of the same mating type rarely occurs (FRIIS and ROMAN 1968). Diploids homozygous for mating types (a/a or α/α) have the same mating behavior as their corresponding haploids (ROMAN and SANDS 1953; MORTIMER 1958), but diploids heterozygous for mating type (a/α) do not mate. Besides controlling the cell's ability to mate, mat1 controls the production of a mating-type specific sex factor and response to the sex factor of the opposite mating-type in a or α haploids and a/a or α/α diploids, while these functions are absent in a/α diploids (DUNTZE, MACKAY and MANNEY 1970).

Mating is accomplished by cells when schmooing occurs (elongation due to response of a cell to a sex factor), followed by cell fusion and nuclear fusion, whereby a diploid zygote is formed. These zygotes are heterozygous for mating-type and reproduce by budding unless induced to sporulate, whereby the cycle proceeds to repeat itself.

Problems in the Genetic Analysis of Mating-type Mutants. Genetic analysis of yeast mutants normally depends upon their ability to mate and to sporulate. Often mutants are isolated as haploids and characterized. These haploids are then mated to appropriate strains in order that the mutation can be tested for dominance and complementation patterns. The resulting diploids in turn are sporulated to do linkage studies and further characterize inheritance patterns. Mutants from a and α haploids have been isolated which alter the mating ability of these haploids. These mutants are called

steriles and have been given the symbol ste by (MACKAY and MANNEY 1974a). This defect in the life cycle was overcome by the mutants' ability to form rare diploids with mating haploids, isolated by complementation on selective medium.

A number of these rare diploids retain the ability to sporulate and complete the life cycle. A few of these diploids cannot sporulate, but do have the ability to mate. The mating-type of such diploids is the same as that of the mating haploid used. It seems likely that such ste mutations are alleles of mat1 and these rare diploids are presumed to have the genotype, a/ste or α/ste. Mutants derived from a haploids which produced diploids capable of mating but not of sporulating were called Class 9 steriles by (MACKAY and MANNEY 1974a). Similarly, mutants derived from α haploids were called Class 10 steriles. Thus a genetic block in meiosis exists for these mutants and knowledge as to linkage and inheritance patterns is lost.

Use of Trisomic Tetraploids to Analyze Mating-type Mutants. Although yeast normally alternate between haploid and diploid states, occasionally asci which have yielded the segregation patterns expected from a tetraploid have been recovered (ROMAN, et al. 1951; LINDEGREN and LINDEGREN 1951; LEUPOLD and HOTTINGUER 1954). Spore viability of tetraploids is good, as spores are predominately euploid (namely, diploid). Even though some of the diploid spores are non-maters (a/α), other diploid spores will be maters (a/a, α/α), which when mated together, form another tetraploid. Thus, a life cycle between diploid and tetraploid states can be sustained. Since rare diploids heterozygous for Class 9 and Class 10 steriles (a/ste and α/ste) were maters (mating as a and α respectively), they, along with the appropriate diploids homozygous for mating type, can be used a parents in

the construction of sporulating tetraploids (a/a/α/ste and a/α/α/ste). Although these tetraploids segregate appropriate spores necessary for continuation of a diploid-tetraploid cycle, expression of the sterile in diploid spores (ste/ste) is a rare event because this allele is present in only one dose in the tetraploid. (See Tetraploid Analysis). It is also difficult to ascertain the segregation pattern of the sterile, as the genotypes of the diploid spores are difficult to determine. A trisomic segregation pattern (two monosome and two disome spores per ascus) would help overcome these difficulties, as the sterile would often be expressed in the monosome, while at the same time the steriles effect upon mating and sporulation in the heterozygous condition can be determined in disomic segregants where the genotype is known.

Sporulating triploids containing the ste (a/α/ste) can be easily obtained by mating a diploid heterozygous for the ste (a/ste or α/ste) to haploids of the opposite mating type (α, a respectively). However, triploids characteristically have low spore viability, presumably due to the gross imbalance of chromosomes in most spores (resulting from the independent segregation of seventeen pairs of homologues into disomes and monosomes) (MORTIMER and HAWTHORNE 1969). The insensitivity of yeast to some imbalance is noted by the spore viability of aneuploids for one or a few chromosomes (near euploids). Hence the use of tetraploids trisomic for chromosome III emerges as the best candidate for studying these steriles, as it incorporates good viability (spores being diploids or monosomic diploids) with a trisomic segregation pattern for the mating type locus.

In order to use trisomic tetraploids to analyze these steriles, the segregation patterns of a trisomic must be known. Consequently, trisomic

tetraploids were constructed without the sterile, in order to determine their segregation patterns. After suitable strains were derived it was determined that trisomes segregate according to trivalent pairing and trivalent segregation model. By the time a trisomic system was operational, the steriles had lost their original expression in rare diploids selected by complementation. (Table 1). If this has occurred by reversion, the original mutation has been lost; however, if the expression was altered by an intergenic suppressor, the original sterile might be segregated out. Therefore trisomic tetraploids were constructed with these modified steriles to analyze these possibilities and any other irregularities that these mutations might have.

Considerations of the Tetraploid Portion. A trivalent pairing model accounts for the observed mean frequency of exchange in a trisome, which was previously found to be too large when the bivalent pairing model was assumed (SHAFFER, et al. 1971). Therefore, it was of interest at this point to ask if a tetravalent pairing model might fit the tetrasomic data better than the previous models which assume bivalent pairing (ROMAN, PHILLIPS, and SANDS 1954). It was also of interest to check for tetravalent segregation in a tetrasome with only one recessive allele for some chromosome. This model predicts the occasional expression of the recessive allele, previously suggested to occur mainly by non-disjunction (BRUENN and MORTIMER 1970). Hence, a limited analysis of the tetraploid portion was undertaken.

The Problem of Cryptopleurine Resistance in Monosomic Diploids. It is expected that recessive alleles on the monosomic chromosome of a monosomic diploid would express themselves just as haploids containing them (MORTIMER and HAWTHORNE 1969). Such was the case for two auxotrophs on chromosome III --- leu2 and his4. It is reasonable to expect that the mutation rates

TABLE 1

Phenotypes of Steriles						
Mutant type	Parent MT[a]	ste / MT	a × ste / MT	Spor[b]	α × ste / MT	Spor
Class 9	a	n	a	-	α	-
Modified Class 9		α	n	+	α	-
Class 10	α	n	a	-	α	-
Modified Class 10		n	n	-	α	-

[a]Mating-type, n is used for non-mater.

[b]Sporulation ability: + indicates the presence of visible asci and - indicates the lack of visible asci after three day incubation on YEKAC.

induced by ultraviolet irradiation for such alleles on a monosome would be similar to those induced in haploids. (Note: the induced mutation rate for a recessive allele in diploids is approximately the induced haploid rate times the rate of mitotic crossing over). The assay for drug resistant mutants is a convenient and sensitive way to detect induced mutations, unlike the assay used for auxotrophs. Cryptopleurine resistant mutants have been isolated by (SKOGERSON, MCLAUGHIN, WAKATAMA 1973). They have mapped the cryptopleurine resistant locus, cry1, 2.1 centimorgens proximal of mat1 on chromosome III and have shown that the allele conferring resistance, cry1, is recessive. Therefore, cry1 would allow identification of diploids monosomic for chromosome III segregating from trisomic tetraploids by the increased frequency of induced cryptopleurine resistance in monosomic diploids over that of disomic diploids. This would aid in determining the genotype of trisomic segregants and provide an additional means to prove that two monosomes and two disomes segregate from a trisome. Moreover, cryptopleurine resistant mutants can serve as a tag for the modified steriles when incorporated in those strains. Thus, one has a sensitive means to determine if modified steriles are linked to mating type; and, if linked, to follow the segregation of the modified sterile and to determine its genotype.

Cryptopleurine resistant mutants, however, did not behave in these expected fashions. Twenty-two strains tested which were known to be monosomic diploids failed to express cryptopleurine-resistance when irradiated with ultraviolet under the same conditions known to induce many cryptopleurine resistant mutants from haploids. The possibility that monosomic diploids are unstable and become diploid seems unlikely as newly formed monosomes also fail to express cryptopleurine resistance by induction and were later

shown to remain monosomes. The possibility that cryptopleurine resistant mutants were not induced in monosomes seems as remote as the possibility that cryptopleurine resistance cannot be expressed in monosomes. Since monosomic diploids and disomic diploids were determined to segregate from trisomic tetraploids, such trisomes were constructed homozygous for cryl in order to determine if monosomes can express cryptopleurine-resistance.

EXPERIMENTAL DESIGN

Many genetic and biochemical studies in yeast are facilitated by use of aneuploids. MORTIMER and HAWTHORNE (1966) have utilized trisomic diploids to establish linkage of markers to the trisomic chromosome. Trisomic segregation ratios of the constitution +/+/- are 4:0, 3:1, and some 2:2 (+:-), while those of the heterozygous disomes give only the 2:2 ratio. Thus, markers in a trisomic diploid producing ratios other than 2:2 are localized on the trisomic chromosome. A means of mapping markers from the centromere of a trisome was developed by SHAFFER et al. (1971). This was done by predicting the frequencies of segregation classes based upon a bivalent pairing trivalent segregation model. However, the observed gene-centromere map distances were found to be significantly larger than those expected by this model. Their data gave evidence for the occurrence of trivalent segregation, as opposed to a bivalent-univalent segregation. Therefore, trivalent segregation should remain in a model describing trisomic segregation patterns. In examining other aspects which allow more accurate prediction of segregation frequencies, it was noticed that trivalent pairing increases the expected trisomic gene-centromere map distance. Thus it seems likely that a better trisomic model can be developed by assuming trivalent pairing and trivalent segregation.

A computer simulation of meiosis of a trisome according to a trivalent pairing trivalent segregation model, provides a convenient way to predict the expected segregation classes and their frequencies. It also allows the incorporation of effects on the frequencies of segregation classes to be calculated for any combination of genetic phenomena.

Of particular interest would be effects due to multiple crossing-over, chiasma interference, various kinds of chromatid interference, preferential segregation and non-disjunction. Of these only multiple crossing-over was incorporated in the model to obtain reasonable fits to the data. In addition, frequencies concerning gene-gene interval information, which have previously been ignored due to laborious calculations, could be computed.

Since the principles governing trisomic analysis deviate from those commonly used in tetrad analysis, a more detailed discussion is warranted dealing with the postulates used to predict the segregation frequencies and the general manner by which these frequencies were computed according to these postulates. In addition, other related material peculiar to the trisomic tetraploid system and its analysis will be explained.

Construction of trisomic tetraploids. Diploid yeast are frequently constructed by mating two haploid yeasts of differing mating types and isolating a zygote by micromanipulation (WINGE and LAUSTEN 1938) or selecting for prototrophs providing the haploids are complementing auxotrophs (POMPER and BURKHOLDER 1949). It is possible to construct a tetraploid trisomic for chromosome III in a similar fashion, by mating a diploid homozygous for mating-type (a/a, or α/α, hence termed mating diploids) to an appropriate diploid monosomic for chromosome III (a or α). Again, either zygotes can be isolated by micromanipulation or prototrophs selected should the diploids be complementing auxotrophs. However, as mentioned above, most diploids obtained are heterozygous for mating-type (a/α) and will not mate (hence termed non-mating diploids). Thus, specialized procedures must be used to acquire mating diploids and monosomes.

Besides a spontaneous origin of α/α diploids from α haploids, α/α diploids can arise from prototrophic selection of two α haploids which were complementing auxotrophs (MORTIMER and HAWTHORNE 1969; FRIIS and ROMAN 1968). However, a/a diploids by prototrophic selection have not yet been reported. It is known that mitotic crossing-over allows diploids to become homozygous for one arm of a chromosome (STERN 1936; JAMES and LEE WHITING 1955; ROMAN 1956). Thus, a mating diploid can arise by a mitotic crossover proximal to matl from typical non-mating diploids. It is known that mitotic crossing-over is stimulated by ultraviolet radiation (HURST and FOGEL 1964). Mitotic gene conversion is a non-reciprocal event by which a heterozygous diploid becomes homozygous for a single gene (ROMAN 1958; HURST and FOGEL 1964; MORTIMER and HAWTHORNE 1969), and is induced by ultraviolet radiation (JAMES 1955; YAMASAKI et al., 1964; NAKAI and MORTIMER 1967). Mitotic gene conversion is indistinguishable from a mitotic crossover in the formation of mating diploids derived from non-mating diploids unless the diploid configuration was heterozygous for a marker distal to matl in which case it remains heterozygous after a gene conversion event but becomes homozygous for one of the two alleles for a mitotic crossover event. Monosomes on the other hand, often arise by mitotic non-disjunction of typical non-mating diploids (EMEIS 1966; STRÖMNAES 1968; MORTIMER and HAWTHORNE 1969). Non-disjunction has not been reported to be appreciably enhanced by ultraviolet radiation. Strains exist in which the spontaneous level of non-disjunction is greater than the spontaneous level of mitotic crossing-over and gene conversion, but the induced levels for these mechanisms by radiation are reversed. Construction of trisomic tetraploids by prototrophic

selection of a non-mating diploid with another non-mating diploid or a mating diploid, or a monosome (POMPER et al., 1954) most frequently occurs by one of the above mechanisms, as prototrophs formed do not contain the same dosage of alleles as the two parents (GUNGE and NAKATOMI 1972). For example, an a/α crossed to an α by prototrophic selection results in an a/a/α or a/α, not an a/α/α, so that the a/α has become an a/a or a by one of the above mechanisms and subsequently mated, whereby prototrophs were formed. Other mechanisms for obtaining mating diploids or monosomes, such as a mutation of α to a, are negligible when compared to the frequency of the above mechanisms.

In order to determine when non-mating diploids have become maters, the following screening technique was employed. Non-mating diploid clones were replica plated to lawns (a layer of cells completely covering the agar surface of a petri plate) of mating haploids, where mating is allowed. The non-mating diploids and mating haploids are chosen such that each is at least a double auxotroph yet complements the other for at least two markers. The possibility of either strain becoming prototrophic for two markers by reversion is remote. Consequently, only when a non-mating clone becomes a mating diploid or a monosome and mates can a prototroph be formed. Clones are subsequently replica plated to the appropriate selective media to identify which clones have become maters by their ability to form prototrophs. At this point, there is no way of knowing if the mating clone is a mating diploid or a monosome unless the non-mating diploid was constructed heterozygous for two recessive markers in repulsion on the arm opposite matl on chromosome III. In this case, monosomes would express one of the two markers and that mating-type to which that marker

was originally linked. On the other hand, mating diploids would not express either marker as the expression of one of these markers in a mating diploid would involve an additional mitotic crossover. Monosomes and mating diploids of differing mating-types can now be mated in a normal fashion to obtain a trisomic tetraploid. This process for obtaining trisomic tetraploids will be referred to as the indirect selection method.

Instead of using mating haploid lawns to determine when non-mating diploids have become maters, appropriate monosome lawns can be used. The resulting prototrophs are mostly disomic tetraploids and some trisomic tetraploids. Disomes and trisomes are easily distinguished by their segregation patterns after sporulation. If non-mating diploids are irradiated with ultraviolet, mating clones produced are mostly diploids homozygous for mating-type and the subsequent prototrophs are mostly trisomic tetraploids. If mating diploids are used as mating lawns, prototrophs represent mostly trisomic tetraploids, some full tetraploids, and very rarely a disomic tetraploid. Again the subsequent segregation patterns can be used to discern these possibilities. This time when non-mating diploids are irradiated with ultraviolet, prototrophs represent an enhancement of full tetraploids. Thus, once appropriate monosomes or mating diploids have been achieved they can also be used to directly select the trisomic tetraploid. This process of obtaining trisomic tetraploids will be referred to as the direct selection method. Segregants of trisomic tetraploids in turn produce a large supply of monosomes and mating diploids, which can be mated in the usual way.

Trisomic analysis. Meiotic segregation patterns for diploids have been well established (FINCHAM and DAY 1971; HAWTHORNE and MORTIMER 1960).

Trisomes, on the other hand, have not been used as extensively, and their segregation patterns are not as clearly understood. Consequently, it is beneficial to describe the model employed for predicting trisomic segregation patterns.

Trisomes are assumed to undergo a round of DNA replication before meiosis forming two sister chromatids per chromosome, hence called the six strand stage, analogous to the four strand stage in disomes. These six strands are segregated into four meiotic products, called ascospores, after crossing-over occurs. It is assumed that sister chromatids do not crossover. If crossing-over is restricted to two of the three chromosomes for a given region, bivalent pairing is said to have occurred. If all three chromosomes are simultaneously available for crossing-over within a region, trivalent pairing is said to have occurred. Trivalent and bivalent 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 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 $\underline{x}$. The mean frequency of exchange for a short interval in a disome due to bivalent pairing is twice the map distance. Since there are twelve possible exchanges in a trisome assuming trivalent pairing, while only four possible exchanges in the disome assuming bivalent pairing for the same interval, the mean frequencies of exchange in a trisome, $\underline{x}$, is three times that found in disomes. Thus, $\underline{x}$ is six times the disomic map distance, for small map distances. Estimates of $\underline{x}$ for large disomic map distances are not easily calculated.

After crossing-over has occurred, there exists two distinct possible segregation patterns: 1) trivalent segregation which assumes all three homologs are associated during meiosis I, and that any two chromosomes may migrate to one pole while the remaining chromosome migrates to the other pole (segregation occurs at random); and, 2) bivalent-univalent segregation which assumes that those two chromosomes involved in bivalent pairing migrate to opposite poles during meiosis I, while the remaining chromosome, which is unassociated to these, has an equal probability of migrating to either pole. In either case, two monosomic spores and two disomic spores per ascus are produced. Such asci can be classified according to the segregation pattern of a marker. Trivalent segregation has meaning regardless of whether bivalent or trivalent pairing is assumed, while bivalent-univalent segregation is meaningful only when bivalent pairing is assumed. Since trivalent pairing has been assumed, so must trivalent segregation. In addition, trivalent segregation predicts a unique (segregation) class as a result of those two chromosomes involved in an exchange migrating to the same pole. Thus, the allele found in one dose in the trisome is expressed in a disomic spore, and the other allele is expressed in the remaining disomic and two monosomic spores. The detection of this class confirms the hypothesis of trivalent segregation. SHAFFER et al., (1971) have also shown trivalent segregation in trisomic diploids.

In order to incorporate the effect of trivalent pairing, double crossovers must be considered. The frequency of double crossovers have been approximated by splitting the region with an exchange frequency of x into two equal halves, with an exchange frequency of $x/2$. Thus, the

probability of no exchange is $(1-\underline{x}/2)^2$, the probability of a single exchange is $2(1-\underline{x}/2)(\underline{x}/2)$, and the probability of a double crossover is $(\underline{x}/2)^2$. From these assumptions, the trivalent pairing and trivalent segregation, the formulae can be derived for the frequency of possible segregation classes for a gene-centromere interval in the configuration A/A/B. They are given in Table 2. Assuming complete chiasma interference, markers appear to segregate at random at an $\underline{x}$ value of 100% (Appendix 1). If double crossover terms (x^2) are ignored, these frequencies are similar to those of SHAFFER et al., (1971) derived for their bivalent pairing trivalent segregation model, except that $\underline{x}$ has a different meaning. The frequencies of the four segregation classes from a gene-centromere interval for various values of $\underline{x}$ are listed in Table 3.

'TRISOM' is a computer simulation of three homologues going through meiosis. It was used to predict the segregating classes and to calculate their relative frequencies for this process. 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 $\underline{x}_1$). A listing of the program is found in Appendix 2 and the design of the program is described below.

'TRISOM' generates all asci possible from a given trisomic constitution. (Note: When terms such as ascus, chromatids, and homologues are used in the remainder of this section they refer to their representation by the computer, namely character strings). Six chromatids associated to three centromeres will be referred to as a configuration. A parental configuration is derived from a given trisome. As stated earlier, there are two distinct

TABLE 2

Expected Segregation Frequencies for a Gene-Centromere Interval, x[a], from A/A/B Assuming Trivalent Pairing and Trivalent Segregation

Segregation Type	Genotypic Class	Phenotypic Class $\underline{a}:\underline{\alpha}:\underline{n}$[b]	Phenotypic Class $\underline{+}:\underline{-}$[c]	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	$1/3(1-x/2)^2+1/3(1/3)(x-x^2/2)+1/3(2/9)x^2/4=1/3-2x/3+5x^2/108$
Crossover I	A,B,A/A,B/B	2:1:1	$3:1_m$[d]	$(2/3(2/3))(x-x^2/2)+2/3(7/9)x^2/4=4x/9-5x^2/54$
Crossover II	2A,A/A,B/B	3:1:0	$3:1_d$[e]	$(1/6(2/3))(x-x^2/2)+1/6(7/9)x^2/4=x/9-5x^2/216$

[a] x is the mean frequency of exchange between the centromere and A due to trivalent pairing.

[b] Phenotypic classes for mating-type are given by ratios $\underline{a}:\underline{\alpha}:\underline{n}$, where $\underline{n}$ is a non-mater. A=$\underline{a}$ and B=$\underline{\alpha}$.

[c] Phenotypic classes for a nutritional marker are given by the ratios $\underline{+}:\underline{-}$. A=$\underline{+}$ and B=$\underline{-}$.

[d] Expression of the recessive allele is in a monosome.

[e] Expression of the recessive allele is in a disome.

TABLE 3

Expected Frequencies of the Phenotypic Classes for a Gene-Centromere Interval from a/a/α and +/+/- Assuming Trivalent Pairing and Trivalent Segregation for Various Values of x.[a]

	Frequencies in percent							
	Single crossovers[b]				Double crossovers[c]			
a:α:n[d]	2:0:2	2:2:0	2:1:1	3:1:0	2:0:2	2:2:0	2:1:1	3:1:0
+:-[e]	4:0	2:2	$3:1_m$[f]	$3:1_d$[g]	4:0	2:2	$3:1_m$	$3:1_d$
%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

[a] x is the mean frequency of exchange.

[b] Assumes only single crossovers, ignores all second order terms.

[c] Allows double crossovers, and includes second order terms.

[d] Phenotypic classes from a/a/α are given by ratios a:α:n, where n is a non-mating sporulator.

[e] Phenotypic classes from +/+/- are given by ratios +:-.

[f] The expression of the recessive is in the monosome.

[g] The expression of the recessive is in the disome.

processes of meiosis, crossing-over followed by segregation. Crossing-over occurs in each of the regions along the chromosome. A crossover configuration for a region is generated by performing an interchange of distal chromatid material (including the distal marker of the region) between two of the six chromatids from some given configuration. Some crossover configurations are indistinguishable from others (or the initial configuration), and are considered together by summing their frequencies. Every possible configuration for the entire chromosome is generated by considering all possible combinations of non-crossover 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 are monosomes, represented by one chromatid; and two of which are disomes, represented by two chromatids. Similar classes for a certain marker or markers can be consolidated and their frequencies summed.

At the onset of meiosis chromosome replication occurs whereby two identical copies of DNA and other associated chromatin components are attached to the same centromere. These are referred to as sister chromatids. In the program 'TRISOM', sister chromatids 1 and 2 are generated from the first homologue, 3 and 4 from the second, 5 and 6 from the third. These six chromatids serve as the original configuration for the procedure, 'RECOMB' with the probability equal to one.

Crossing-over is assumed to occur in the six strand stage. All crossing-over is generated within the procedure 'RECOMB.' Each time 'RECOMB' is called, it generates both the parental configuration (non-crossover class) and all possible <u>single</u> crossover configurations for a

given region, along with their relative probabilities of occurrence. Each crossover configuration is represented by some recombination of the six chromatids associated to the three centromeres. Each of these configurations and their frequencies in turn serve as the initial chromatids for 'RECOMB' in another region whereby further crossing-over is generated. 'RECOMB' is recalled until all regions are considered, yielding the parental and all possible single, double, and multiple crossover configurations along the entire length of the chromosome. Multiple crossovers can be eliminated from consideration should their frequencies be smaller than some prescribed limit of accuracy. The analysis must begin in the distal region, and work towards the proximal one in order to preserve the occurrence of three strand double crossovers. The parental class for a given region is assigned the frequency of $(1 - \underline{x}_i)$. No interference has been assumed so that adjacent regions produce single crossing-over independently; hence, the frequencies are multiplied together. For example, as the frequency of no exchange in both region 1 and 2 is $(1 - \underline{x}_1)(1 - \underline{x}_2)$.

In generating the single crossover configuration for each region it has been assumed that each of the chromatids has an equal frequency of participating in an exchange, but that sister chromatids do not exchange with each other. This allows twelve equally probable exchanges to occur (1-3, 1-4, 1-5, 1-6, 2-3, 2-4, 2-5, 2-6, 3-5, 3-6, 4-5, 4-6); hence, each is assigned a frequency of $\underline{x}_i/12$. When the same markers are present distal to the exchange region in both exchange chromatids, or when the same markers are present proximal to the exchange region in both exchange chromatids and the sister chromatids of both exchange chromatids are indistinguishable, the crossover configuration remains in the parental configuration. Whenever this happens the frequency of this configuration

is added to the frequency of the parental configuration. Whenever two of the three chromosomes are identical, exchanges between either one and the third, non-identical chromosome generates the same set of crossover classes. For such cases, the frequencies due to one of the identical chromosomes are doubled. Similarly the crossover configuration of identical sister chromatids are indistinguishable, and their frequencies are double.

Since 'TRISOM' generates only single crossovers within any region, it appears that only bivalent pairing has occurred. Trivalent pairing has been allowed for in considering the second crossover, as three and four strand double crossovers involving three chromosomes compete equally with all other double crossovers. Therefore, to simulate trivalent pairing within the region of interest, it must be broken down into two or more adjacent regions such that the sum of $\underline{x}_i$ in these new regions equal that of $\underline{x}_i$ in the original region.

Now that all possible configurations of recombination along the entire length of the chromosome have been generated, each configuration is ready to proceed through segregation. The procedure 'TRISEG' generates all possible segregations of the six strands into four meiotic products, called an ascus. Since segregation is assumed 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. The relative frequency due to segregation is obtained as follows. At meiosis I one of the three chromosomes has an equal frequency of segregating to pole I, while the remaining two segregate to pole II. Trivalent segregation has been assumed such that each of the three chromsomes has an equal frequency of segregating to pole I (1/3) regardless of the nature of the crossing-over. If any of the two chromosomes are identical, the

frequency of one of these identical chromosomes segregating to pole I is doubled. During meiosis II sister chromatids disjoin. Therefore, at pole I two monosomes are produced and at pole II two disomes are produced. In the disomic portion there are two equally frequent ways for sister chromatids to disjoin. Given that homologues 2 and 3 have segregated together at meiosis I, they are chromatids 3 with 5 and 4 with 6, and 3 with 6 and 4 with 5. Should both sister chromatid pairs not be identical ($3 \neq 4$ and $5 \neq 6$), the two are distinguishable and therefore both possibilities are generated with their associated frequencies cut in half.

The six strands of an ascus represent its genotype. From the genotype the phenotype classes of each ascus is determined by the procedure 'PHEN06', for all dominant-recessive markers and for mating-type. The frequencies of each class can be summed up according to similar ascus types between any two markers, or one marker and the centromere (as the centromere has artificially been assigned the phenotype (4:0)). This is done in the procedure 'TOTPROB'. Besides calculating the total frequency of each ascus, 'RECOMB' and 'TRISEG' also keep track of that portion of the total frequency due to zero, one, two, and multiple crossing-over. Thus, it is possible to estimate the total amount of single and double crossovers, from unique double crossover classes.

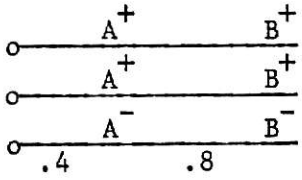
The frequency of the sixteen ascus classes for gene-gene interval can be predicted according to the model. Of particular interest are those classes which only result from double crossovers, (both two and three chromosomal) since such unique classes were not predicted from gene-centromere intervals. Thus, classes from gene-gene intervals provide a way of estimating the total amount 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 of 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 for specific cases in order to approximate the double crossover frequencies. See Tables 4 and 5.

Expected segregants of modified steriles in trisomes. Class 9 and Class 10 steriles presently have a different expression in rare diploids selected by complementation than originally found. Thus they have been called modified steriles. Class 9 steriles have been derived from a haploids while Class 10 steriles are from α haploids. Both classes originally had the ability to mate in rare diploids and did not sporulate. The mating-type of these diploids was the same as that of the complementing haploid used. The modified Class 9 steriles express themselves in these diploids as a normal α allele would and are indistinguishable from an α allele for the purpose of predicting segregation frequencies. The modified Class 10 steriles have retained all the properties expressed by the original Class 10 steriles, except that they no longer mate as an a in the diploids heterozygous for a and ste. Assuming that these modified Class 10 steriles are alleles of mat1, then the segregation frequency of ascospores from the configuration a/α/ste can easily be predicted from a trivalent pairing trivalent segregation model. For the purpose of calculating phenotypic frequencies of segregants the assumption is made that the phenotype of spores heterozygous for the ste have some phenotype

TABLE 4

Expected Frequencies of the Phenotypic Classes for a Gene-Gene Interval Assuming Trivalent Pairing and Trivalent Segregation from:



Segregation Type	Classes[a] A +:-	B +:-	Total	Frequencies in percent Crossovers in A-B[b] Zero	Single	Double
Parental	4:0	4:0	35.5	19.6	13.0	3.0
	2:2	2:2	13.8	9.1	4.0	0.7
	3:1[c]	3:1	15.4	7.3	6.9	1.2
Single Crossover	4:0	4:0	17.9	0.0	12.9	4.9
	2:2	3:1	10.8	0.0	8.0	2.8
	3:1	2:2	0.7	0.0	0.6	0.1
	3:1	4:0	2.2	0.0	1.8	0.4
Double Crossover	4:0	2:2	0.2	0.0	0.0	0.2
	2:2	4:0	0.4	0.0	0.0	0.4
			97.0	36.0	47.2	13.7

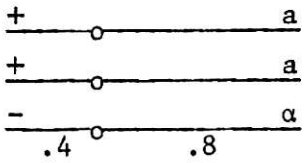
[a]Class from gene-gene interval depend upon phenotypes of both A and B.

[b]That frequency of the total due to crossovers within the A-B interval.

[c]3:1 counts expression of the recessive in both monosomes and disomes.

TABLE 5

Expected Frequencies of Phenotypic Classes for a Gene-Gene Interval Assuming Trivalent Pairing and Trivalent Segregation from:



Segregation Type	Classes +:-	Classes a:α:n[3]	Frequencies in percent Crossover(s)[b] Total	Zero	Single	Double	Triple
Parental	4:0	2:0:2	35.0	14.4	14.4	5.5	0.7
	2:2	2:2:0	13.4	7.2	4.8	1.2	0.1
Single Crossover	2:2	2:1:1	10.4	0.0	6.4	3.6	0.5
	4:0	2:1:1	12.2	0.0	6.4	5.0	0.8
	4:0	3:1	5.6	0.0	3.2	2.1	0.3
	$3:1_m$	2:2:0	4.9	0.0	3.2	1.4	0.2
	$3:1_m$	2:0:2	5.6	0.0	3.2	2.1	0.3
	$3:1_d$	2:0:2	2.8	0.0	1.6	1.1	0.2
Double Crossover	$3:1_m$	2:1:1	5.1	0.0	0.0	4.3	0.8
	$3:1_m$	3:1:0	0.9	0.0	0.0	0.7	0.1
	$3:1_d$	2:1:1	0.9	0.0	0.0	0.7	0.1
	$3:1_d$	3:1:0	0.4	0.0	0.0	0.4	0.1
	4:0	2:2:0	0.2	0.0	0.0	0.2	0.0
	2:2	2:0:2	0.4	0.0	0.0	0.4	0.1
	2:2	3:1:0	0.2	0.0	0.0	0.2	0.0
			98.	21.6	43.2	28.9	4.3

[a]Class from gene-gene interval depend upon phenotypes of both genes.

[b]That frequency of the total due to crossovers within the gene-gene interval.

[c]Mating type n is used to denote a non-mater.

found in rare diploids selected by complementation with modified Class 10 steriles and mating haploids, as listed in Table 1. Table 6 contains phenotypic classes and their frequencies for the trisomes carrying the modified Class 10 steriles. If the modified sterile is not an allele of matl, but still on chromosome III, three additional classes in addition to those found above are also predicted at low frequencies. It has been assumed in these calculations that loosely linked steriles are non-specific, i.e., they give a sterile phenotype in the presence of either a or α haploids. The distribution of frequencies is also different, but because of the large number of classes, a large amount of data is needed to accurately determine these distributions experimentally.

If the modified sterile is recessive and not located on chromosome III in the trisomic tetraploid, the sterile will be in the tetrasomic portion and therefore will seldom express itself. This is because the frequency of obtaining a homozygous ste is low for one dose in a tetraploid. For such cases, occasionally one sterile per ascus will be expressed. If the modified sterile is dominant and in the tetrasome portion, the sterile will express itself in approximately half the spores per ascus.

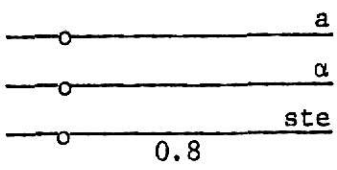
Tetraploid Analysis. In order to determine if the tetraploid portion was behaving properly during meiosis, tetraploid segregation patterns must be known. ROMAN, PHILLIPS, and SANDS (1954) have derived equations for two models. These equations predict the frequencies of all distinct (genotypic) classes segregating from a tetrasome in duplex (i.e., two doses of the allele A and two doses of the allele B; thus, A/A/B/B) for a gene-centromere region. Both models have assumed that there exist two distinct bivalents that pair together during crossing-over and that both

TABLE 6

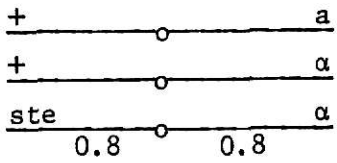
Expected Frequencies of Phenotypic Classes from Trisomes Heterozygous for the Modified [Class 10] Steriles Assuming Trivalent Pairing and Trivalent Segregation

Class a:α:n,s [c]	Linked [a] (percent)	Unlinked [b] (percent)
2:2:0,0	14.4	7.3
0:2:2,0	14.4	15.0
0:0:4,2	16.0	9.3
1:2:1,0	14.5	23.4
1:1:2,0	5.0	6.2
1:1:2,1	15.0	5.3
0:1:3,1	13.2	20.2
2:1:1,0	4.0	2.1
0:2:2,1	-	5.6
1:3:0,0	-	3.0
0:3:1,0	-	0.6

[a] From the configuration:



[b] From the configuration:



[c] n denotes a non-mater. s indicates the number of non-maters that are sporulators.

bivalents participate in a single exchange when considering double crossovers. One model assumed that two bivalents segregate simultaneously (same two bivalents involved in pairing), while the other assumed that tetravalent segregation occurred (whereby two chromosomes are chosen at random to segregate to one pole). Both models predict the same classes, but in differing frequencies. However, neither model satisfactorily explains the entire array of data. Therefore another set of frequencies assuming tetravalent pairing and tetravalent segregation was derived in an attempt to adequately explain the data.

The same classes are again predicted from a tetravalent pairing tetravalent segregation, but their frequencies vary from those previously derived. All sets of equations are found in Table 7. In addition, this model was used to predict the segregation of alleles in triplex (C/C/C/c) and monoplex (C/c/c/c) as no other frequencies have been explicitly derived for these cases by other models (Table 8).

Tetravalent pairing is indistinguishable from bivalent pairing when considering only classes produced by single crossovers (not their frequencies), but tetravalent segregation allows the two chromosomes involved in the crossover to migrate together at meiosis I, making it possible for sister chromatids to disjoin into one spore during meiosis II. Thus, a tetravalent segregation from a tetrasome in triplex C/C/C/c predicts an occasional spore (c/c) from unique segregation class of 3C/C:1c/c. The occasional expression of recessive c spores from triplex data suggests tetravalent segregation.

BRUENN and MORTIMER (1970) have presumed however, that such spores expressing c are composed of monosomes arising primarily by meiotic nondisjunction (and secondarily by mutation and gene conversion). Since it

TABLE 7

Expected Segregation Frequencies for a Gene-Centromere Interval, x, from +/+/-/- for Various Models

Segregation Type	Genotypic Class	Phenotypic Class +/-	Bivalent[2] / Bivalent	Pairing[a] / Segregation: Bivalent[b] / Tetravalent	Tetravalent[c] / Tetravalent
Parental	4 +/-	4:0	$2/3-2y/3+y^2/2$	$2/3+2y/3+7y^2/18$	$1/3-x/3+7x^2/108$
Parental II	2 +/+, 2 -/-	2:2	$1/3-2y/3+y^2/2$	$1/3-4y/9+7y^2/18$	$1/3-2x/9+7x^2/144$
Crossover I	+/+, 2+/-, -/-	3:1	$4y/3-y^2$	$10y/9-7y^2/9$	$5x/9-49x^2/432$

[a] Models are defined according to how tetrasomes pair over how they segregate.

[b] Derive by ROMAN, PHILLIPS and SANDS (1954). y is the second division segregation frequency for bivalent.

[c] x is the mean frequency of exchange due to tetravalent pairing.

TABLE 8

Expected Segregation Frequencies for a Gene-Centromere Interval, $\underline{x}^a$, from +/+/+/- Assuming Tetravalent Pairing and Tetravalent Segregation

Spore Genotype	Spore Phenotype	Frequency[a]
4 +/-	4:0	$1-x/12 + 7x^2/576$
3 +/+, 1 -/-	3:1	$x/12 - 7x^2/576$

[a] $\underline{x}$ = the mean frequency of exchange per strand due to tetravalent pairing.

is not always practical to determine whether the spore is monosomic or disomic for the chromosome in question, the relative frequency of this class might be used to determine which of the mechanisms is most common. The relative frequency should either depend upon the intrinsic frequency of meiotic non-disjunction for a chromosome, or the mean frequency of meiotic exchange in a tetrasome, according to the prevalent mechanism.

Distinguishing disomes, trisomes and tetrasomes for chromosome III. Unique phenotypic classes for mating-type (gene-centromere interval) segregating from heterozygous strains for mating-type can be used to determine the number of homologues present for chromosome III in these strains. Even though trisomes and tetrasomes are capable of producing 2:2:0 (a:α:n, where n is a non-mater, or a/α), only disomes segregate them exclusively.

Trisomes segregate a large frequency of the 2:1:1 recombinant class (with genotypes of a, a/a, α and a/α). Suppose that a tetrasome could segregate a 2:1:1 class. This implies that its genotype is two a/a's, α/α, and a/α. This sums to five a alleles and three α alleles which is an aberrant ascus. The total frequency of aberrant asci was found low in these strains regardless of whether they have resulted from gene conversion or non-disjunction. Hence, the abundance of the 2:1:1 class indicates the segregation of a trisome. Tetrasomes allow the segregation of a 0:0:4 ascus (genotypically 4 a/α's), demanding the presence of four a alleles and four α alleles. This class cannot segregate from a trisome, even in common aberrant asci. Tetrasomes also segregate a 1:1:2 recombinant class which is possible only by gene conversion in trisomes. Therefore a 1:1:2 class is another class by which to identify a tetrasome.

Tetrad Analysis. Sporulating diploids segregating from trisomic tetraploids can be studied by conventional tetrad analysis. This allows the genetic background of the trisome to be studied directly. It was necessary to analyze tetrads which had poor viability and segregation patterns for heterozygous alleles that were significantly different from 2:2. The following are reminders of known examples of skewed segregation patterns and poor viability. If a lethal, such as a deletion is closely linked to another marker, the ratio of viable to inviable spores is 2:2, and the linked allele will seldom be expressed. If there are only four, two and zero viable spores per ascus present, a reciprocal heterozygous translocation is quite possible in the diploid (FINCHAM and DAY 1971; EMERSON 1963; ESSER and KUENEN 1965). If four, three, and two viable spores per ascus are present, two possibilities exist: 1) a non-reciprocal heterozygous translocation and 2) segregation of two genes which act together to yield a lethal.

MATERIALS AND METHODS

Yeast strains and nomenclature. The heterothallic strains of Saccharomyces cerevisiae used in these studies were derived from strains obtained from T. R. Manney (Kansas State University), R. K. Mortimer (University of California at Berkeley), and V. L. MacKay (Rutgers University). Lela Riley derived several of the monosomic diploids and these strains are designated by incorporation of an "L" in the strain symbol. These monosomes originated using screening procedures similar to that described below. Verna Woods constructed and scored phenotypes of all diploids heterozygous for a modified sterile (YT3403 through YT3421). Haploid strains containing the original sterile mutants are those from Class 9 and Class 10 isolated by MACKAY and MANNEY (1974a). Sterile haploid strains used in this study have subsequently altered their phenotypic expression and are called modified steriles.

Symbols representing genetic markers are those given by PILISCHKE, BORSTEL, MORTIMER, and CHAN (1975) (Appendix III). Strains were given a symbol according to their ploidy: X for haploid, Y for diploid, and Z for tetraploid. All aneuploids were for chromosome III, to which mating-type locus (matl) is located. Thus any aneuploid can be recognized from the genotype of matl which is given in parentheses following strain symbol. Independent clones derived by selection, often isogenic, are designated by attaching a small letter or a hyphenated number. Independent zygotes isolated by micromanipulation are also designated in this fashion. (Small a is assumed if not given). For example, ZI3434-27(a/a/α) is a tetraploid trisomic for chromosome III and was the 27th clone selected;

while YI22b(α/α) is clone b of a normal diploid. Spores produced from these strains are indicated by addition of an ascus number and one of the four spore letters (A-D), e.g., XI55-19D(α) is a haploid, spore D of ascus 19, segregating from the diploid YI55a. YI113a-2C(a) is a monosomic diploid, spore C of ascus 2, segregating from the disomic tetraploid ZI113 (a/α). A pedigree representative of strains used in this study is found in Appendix IV and genotypes of all strains are listed in Appendix V.

Media. Recipes for the standard culture media used and their purpose are given below. All ingredients were added to distilled water and sterilized by autoclaving. Media were solidified with 20 grams of agar per liter.

YEPAD Difco yeast extract, 20 grams per liter; Difco Bacto-Pepton, 20 g/1; adenine, 80 mg/1; dextrose, 20 g/1. This is a rich complex medium used for routine culturing and maintenance of stock strains.

MV Difco yeast nitrogen base without amino acids and ammonium sulfate, 1.45 g/1; ammonium sulfate, 5.22 g/1; dextrose, 20 g/1. This minimal medium was used to select prototrophs without any nutritional requirements.

SC MV containing these supplements: adenine, 30 mg/1; arginine, 30 mg/1; histidine, 20 mg/1; leucine, 40 mg/1; lysine, 40 mg/1; methionine, 20 mg/1; threonine, 150 mg/1; tryptophan, 30 mg/1 (stock solutions of threonine and tryptophane were filtered and the appropriate amounts added aseptically after autoclaving); uracil, 20 mg/1. This synthetic complete medium was used as a control for scoring nutritional phenotypes.

-X SC lacking one or more of its supplements. These omission media were used for scoring nutritional phenotypes and for selecting strains prototrophic for certain nutritional requirements.

CAN SC lacking arginine and containing canavanine (20 mg/l), an analogue of arginine which is lethal to wild-type yeast. CAN is used to score sensitivity or resistance to canavanine.

CRY YEPAD with 1.9 mg/l cryptopleurine, a plant bark alkaloid which inhibits protein synthesis and is therefore lethal to wild-type yeast. CRY is used to score sensitivity or resistance to cryptopleurine.

PET Yeast extract, 10 g/l; Bacto-Peptone, 10 g/l; galactose, 20 g/l; dextrose, .25 g/l; glycerol, 30 mg/l. PET is used to score the respiratory deficient (petite) phenotype.

BTB gal Yeast extract, 10 g/l; Bacto-Peptone, 20 g/l; galactose, 20 g/l; brom thymol blue (1% BTB in ethanol), 33 mg/l; .5N NaOH (pH 8), 5 ml/l. BTB gal is used to score the fermentation of galactose by use of acid color indicator BTB. If galactose is fermented, acids are produced causing BTB to change from blue-green to yellow within 24 hours.

YEKAC Yeast extract, 2.5 g/l; dextrose 1 g/l; potassium acetate, 10 g/l. YEKAC is the sporulation medium.

Incubation. All incubations were at 30°C. When liquid media were used, culture flasks were filled to 20% of capacity and were aerated by shaking at 200 rpm.

Construction of hybrids by mating. Strains from 24 hr. YEPAD agar of the opposite mating-type were mixed together, incubated for three hours. Then zygotes were isolated on agar slabs by micromanipulation and incubated for two to four days until visible colonies were formed (HAWTHORNE and MORTIMER 1960).

Induction of cryptopleurine resistance. After 24 hours growth on YEPAD slants, cells were suspended by adding 2 ml of water and vortexing. Then 0.2 ml of this suspension (approximately 2×10^8 cells/ml) was plated directly

onto a YEPAD plate and irradiated with UV for 17 seconds from a 15 watt GE germicidal lamp at 26 cm. (This dose gave approximately 50% survival in haploid strains and 85% in diploid strains. Plates were not exposed to strong light sources in order to minimize photoreactivation). After 24 hours incubation the plate was replica plated to CRY on which visible colonies form in 2 to 4 days. Haploids typically yielded 20 to 200 cryptopleurine resistant clones per plate.

Construction of trisomic tetraploids. The principles by which monosomic diploids and mating diploids (a/a, α/α) can be obtained from normal non-mating diploids (a/α) were described in Experimental Design. Non-mating diploid strains were grown for 24 hours in liquid YEPAD, diluted in sterile water and plated on YEPAD to approximately 200 cells per plate. If selection of mating diploids was desired, rather than selection of monosomes, mitotic crossing-over and gene conversion were stimulated by 17 seconds irradiation of UV at 26 cm from a 15 watt GE germicidal lamp. These plates were incubated for 2-3 days, whereby visible clones were formed. These master plates were than replica plated to YEPAD lawns of mating strains containing at least two nutritional requirements and complementary to the non-mating clones for at least two nutritional requirements. Uniform lawns were made by plating 0.4 ml of a 1:1 mixture of a 24 hours YEPAD liquid culture of mating strains and water (approximately 10^8 cells/ml) and allowing plates to dry before use. Plates containing replicated clones superimposed upon these lawns were incubated for 24 hours to allow mating to occur and were then replica plated to appropriate selective medium. Formation of prototrophic clones on this selective medium after 24 hours incubation indicates that original clones on the master plate have acquired the ability to mate. If the mating strain used in preparing lawns was a

mating diploid (or a monosomic diploid when clones are irradiated), prototrophic clones are primarily trisomic tetraploids.

Such a trisomic tetraploid growing on the selective media was acquired by direct selection. If the lawns were made with haploids, prototrophs are primarily disomic triploids (or triploids if clones are irradiated). Monosomic diploid or mating diploid clones corresponding to the prototrophic clones can be picked from the master plate. Trisomic tetraploids can be isolated by mating these colonies to the appropriate strains and isolating zygotes in the usual fashion. Such a trisomic tetraploid is formed by indirect selection. Monosomes and mating diploids used in direct selection were usually derived from those acquired by indirect selection. Indirect selection allows the genetic analysis of all strains going into trisomic tetraploids, while direct selection does not. The direct selection procedure is shorter since master plates are not mandatory. In this case, at least 0.2 ml of a mixture of non-mating diploids (10^4 to 10^6 cells per ml, depending on the selection mechanism desired, and 10^2 to 10^3 prototrophs per plate) and mating diploids or monosomes (10^8 cells/ml) from 24 hour liquid cultures are plated on YEPAD, incubated for 24 hours, and replica plated to the selective medium. After incubation for 2 to 4 days visible trisomic tetraploid clones are formed.

Some $\underline{\alpha}/\underline{\alpha}$ diploids were also acquired by prototrophic selection from a mixture of two complementing $\underline{\alpha}$ haploids. The procedure was the same as that for the shortened version of direct selection, except the mixture contained approximately 10^8 cells/ml of each $\underline{\alpha}$ haploid.

Genetic analysis of hybrids. All sporulating strains under genetic analysis by meiosis can be treated without respect to the hybrid nature

of the strain. Thus, the same procedure was used for trisomic tetraploids and conventional diploids. Strains were grown for 24 hours on YEPAD agar for pre-sporulation growth and transferred to YEKAC agar and incubated 3 to 4 days. Good sporulation was generally obtained and these cultures could be stored for two weeks under refrigeration without significant loss of spore viability. If liquid cultures were used in this procedure, the YEKAC broth was innoculated with approximately 10^7 cells/ml. (Cells were washed before innoculation).

Asci from these sporulation cultures were dissected by the method of JOHNSTON and MORTIMER (1956). Cultures were treated with a 1:20 dilution of Glusulase (Endo Labs) for about 15 minutes. This snail enzyme digests the ascus wall without disturbing the four spores. A loopful of the digestion mixture was spread along one edge of a thin YEPAD agar slab. The four spores from an individual ascus were positioned by micromanipulation in a column perpendicular to this edge. Up to 20 asci were dissected on a single slab such that after incubation of the slab (face up on a YEPAD plate for 2 to 4 days) visible spore colonies remained isolated. At this point spore colonies were picked and YEPAD master plates made and incubated 24 hours. YEPAD master plates were replica plated to appropriate omission media to score nutritional phenotype of the spores. Mating-type was scored by complementation, either for nutritional requirements or for utilization of galactose. Each spore culture was cross-streaked with the appropriate a and α tester strains on YEPAD, incubated 24 hours and replica plated to appropriate selective medium or BTB gal. Confluent growth or

yellowish color on BTB gal in the cross-streaked area after 24 hour incubation indicated that the strain mated with the tester. YEKAC replica plates showed that approximately 90% of all non-mating spores (segregating from trisomes without sterile alleles) were sporulators.

Random Spore. Sporulating hybrids were analyzed by the random spore procedure of GILMORE (1967). Liquid sporulation cultures were washed and resuspended in a 1:20 dilution of Glusulase (ENDO Laboratories), for thirty minutes. Cells were then washed, resuspended, and sonicated until the spores were separated. Cells were diluted and plated on YEPAD; or on SC, and appropriate selection media to give about 10^2 colonies per plate. Controls show spore viability on SC was not significantly different than on YEPAD after two to three days incubation. If YEPAD masters were made, they were replica plated to appropriate media for scoring nutritional requirements; otherwise, the percent of spores protorophic for certain nutritional requirements were calculated directly from SC and -X plates. Mating-type was determined by replica plating to YEPAD lawns of tester strains and subsequent selection on MV. Details for this determination are the same as those described for the indirect selection of trisomic tetraploids.

ANALYSIS OF TRISOMIC SEGREGATION

Data from trisomic tetraploids are conveniently separated into two natural analysis areas, namely, trisomic analysis and tetrasomic analysis. The meiotic segregation patterns for trisomes are distinct from those of tetrasomes and as such are best considered separately. Analysis of the cryptopluerine problem also involved special considerations. Therefore, the discussion will immediately follow the results for each distinct area of analysis.

Throughout this study of trisomic tetraploids a low frequency of abberant asci were observed. Most of these asci can be explained equally well by a single gene conversion or non-disjunction at meiosis II. Since characterization of the segregation patterns was of primary interest, asci resulting from such low frequency events were not included in analysis of trisomic tetraploids.

Results

Development of suitable strains. A number of strains were made which were not suitable for trisomic analysis. This section deals with the characterization of this problem in the first set of strains constructed and the manner in which suitable strains were obtained. Trisomic tetraploids shown in Table 9 were constructed by mating an $\underline{\alpha}/\underline{\alpha}$ diploid (obtained by prototrophic selection) to an $\underline{a}$ monosome. They had the following configuration for chromosome III:

TABLE 9

Observed Phenotypic Classes for Gene-Centromere Intervals from the Trisomes

	Strain	Viability[a]	his4[b] 4:0	his4 2:2	his4 3:1	leu2 4:0	leu2 2:2	leu2 3:1	mat1 0:2:2	mat1 2:2:0	mat1 1:2:1	mat1 0:3:1	thr4 4:0	thr4 2:2	thr4 3:1
1)	ZI11a[d]	6/38	6	0	0	6	0	0	0	6	0	0	0	6	0
	ZI13a	21/54	3	3	15	7	7	7	15	1	3	2	17	0	4
	ZI13b	6/20	3	1	2	5	0	1	5	0	1	0	5	0	1
	TOTAL	27/74	6	4	17	12	7	8	20	1	4	2	22	0	5
	%		22.2	14.8	63.0	44.4	25.9	29.6	74.1	3.7	14.8	7.1	81.5	0	18.5
2)	ZI28b2	2/16	-	-	-[e]	0	1	1	0	2	0	0	0	3	0
	ZI29b2	7/16	0	6	0[e]	0	7	1	0	4	4	0	1	4	3

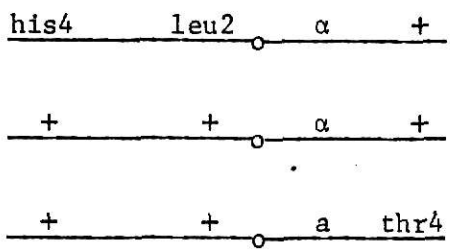
[a] The fraction of asci with four viable spores.

[b] Classes for recessive markers are given by the ratio +:- .

[c] The mating-type classes are given by the ratio a:α:n, where n is a non-mater.

[d] ZI11a was found to be a disomic tetraploid.

[e] His 6 was also segregating, but was not scored for.



It appears from the segregation patterns, however, that ZIIIa (a/α) is a tetraploid disomic for chromosome III. It seems likely that an α monosomic diploid without any auxotrophic markers was selected instead of an α/α diploid heterozygous for his4 and leu2, since both have the same phenotype. Thus ZIIIa appears to have arisen from two monosomic diploids, and will be excluded from the discussion below.

These trisomic tetraploids showed a low frequency of four-viable spores per ascus. In fact none were found in 20 asci of ZI14a and 11 asci of ZI14c. Since they were not analyzed, they are not listed in Table 9. In addition, a skewed distribution of segregation classes with respect to those expected from any trisomic model was observed for ZI13a(a/α/α) and ZI13b(a/α/α). In particular, the 2:2 class for thr4 was absent and the 3:1 class for thr4 along with classes expressing mating-type a (2:2, 1:2:1, 1:3:0) were infrequent. Since thr4 and a alleles are present in only one dose in the trisome, they are expected to express themselves primarily in monosomic spores. Because thr4 and a alleles came into the cross together, it seems possible that a single recessive lethal linked to these loci could account for the diminished number of monosomic spores expressing thr4 and mating-type a. Under such an assumption, the centromere linkage of the lethal can be determined by realizing that asci with four and two-viable spores are parental classes, while those with three-viable spores are the

recombinant class. Since 37%, 11%, and 52% are four-, two- and three-viable spored asci classes respectively, the lethal has a mean frequency of exchange greater than 120%. Thus, the lethal is distal to mating-type, which has a mean probability of exchange of about 120%. It is also closer to thr4 than a, as a is more recombinant with respect to the lethal and thus expressed in 16.4% of the asci and thr4 is recombinant in 12.7%. If the lethal were between these markers, spores expressing a and thr4 would result from a double crossover. (See Figure 2). Similarly, if the lethal is distal to thr4, spores expressing α and thr4 would result from a double crossover. Since there are five a thr4 spores and six α thr4 spores in ZI13a and ZI13b segregants, it is difficult to determine which of these possibilities is occurring when the double crossover class is assumed less frequent than the single crossover class.

However, the chance that this inviability is the result of a single recessive lethal is remote, since the lethal would have to come in on the monosome with a and thr4, whereby the monosomic diploid itself would be lethal. Furthermore if it were a single recessive lethal, only asci with two-viable spores would be expected from sporulating a/α segregants from a trisome and heterozygous for thr4. Only two out of the eight sporulating segregants that were tested yielded solely asci with two-viable spores, while five out of eight gave a mixture of asci with four, three, and two viable spores. (See Table 10). Recombination between mating-type and thr4 appears to be normal in these sporulating segregants, as they produced ten recombinant asci out of the forty-nine (when considering asci with four, three, and two viable spores). In all asci with three viable spores (except one) segregation was 2:1 (+:-) for thr4. In asci with

FIGURE 2

CHANGE IN SPORE VIABILITY WITH POSITION OF LETHAL (lth)

Viable monosomic spore besides α + + after a crossover	in region 1	in region 2	in both regions
Position I a lth thr4 α + + α + + α + + 1 2	a + +	α + thr4	a + thr4
Position II a thr4 lth α + + α + + α + + 1 2	a + +	a + +	α thr4 +

TABLE 10

Viability and Recombinant Asci of Sporulating Diploids Segregating from ZI13a(a/α/α)

Spore	Number of asci	Number of viable spores per ascus 4	3	2	1	0	Number of recombinant asci[a]
5B	8	4	3	1	0	0	3
5C	8	4	3	1	0	0	3
27A	8	0	0	7	1	0	0
27D	8	0	0	7	1	0	0
35A	8	4	2	1	1	1	2
44C	8	1	2	2	1	2	1
35B	8	0	0	0	2	6	-
44D	8	1	3	3	0	1	1
Total	64	14	13	22	6	10	10

[a]Number of recombinants between mating-type and thr4 considering asci with 4, 3, and 2 viable spores.

two-viable spores there was an abundance of 2:0, some 1:1, and no 0:2 (+:-) for thr4. Again the lethality is closely linked to thr4. This time when minimizing the double crossover class the lethality maps distal to thr4 as a thr4 spores occur significantly more frequently than α thr4 spores. (The same relations found in Figure 3 hold for these disomes, if one ignores one of the α + + chromosomes). Thus, another gene or genes not on chromosome III must be interacting with a gene mapping close to thr4 in order to produce lethal spores.

One possibility consistant with diploids giving asci with four, three, and two viable spores is a non-reciprocal translocation of genetic material distal to thr4 onto another chromosome. If such a non-reciprocal translocation were present in only one dose in the trisomic tetraploid, the deletion distal to thr4 is covered half the time, and the 2:2 class for thr4 would be expressed approximately one-half to one-fourth its normal frequency. No 2:2 segregants for thr4 were found, but very few are expected in twenty-seven asci with four-viable spores. Equal frequencies of sporulating diploid segregants from a trisomic tetraploid yield only asci with two viable spores and those which yield asci with four, three, and two viable spores are also predicted from such a translocated trisomic tetraploid. Furthermore, sporulating diploids resulting from the same trisomic ascus have an equal chance of having the same or different sporulation patterns. From the four trisomic asci tested three have diploids with similar sporulation patterns, while the remaining one is undetermined (ascus number 35, Table 10).

Suppose the lethality is the expression of two recessive genes, one linked to thr4 and the other on another chromosome. Whenever sporulating

diploid segregants from a trisomic tetraploid yield only asci with two-viable spores, then these diploids must have segregated heterozygous for one gene and homozygous for the other. In fact, it must be heterozygous for the gene linked to thr4 in order to account for the data. Since two such diploid segregants are found in one trisomic ascus, namely YI13a-27A and YI13a-27D, the trisomic tetraploid must contain at least two doses of the allele conferring lethality in the tetrasomic portion. Such a trisomic tetraploid duplicates the characteristics of the translocation trisome discussed above, i.e., it predicts the same trisomic segregation patterns and the same frequency and sporulation patterns of diploids spores. Hence, it is unresolved as to which model best describes the inviability of ZI13's. When considering the two gene model, the two lethal doses in the tetrasomic portion could not have both come from the disomic portion of the monosomic diploid, as it already carries the allele conferring lethality linked to thr4 in the monosome, and would be lethal if it were also homozygous for the other allele conferring lethality. Thus, at least one of these alleles in the tetraploid portion must come from the α/α diploid which went into these strains. In view of lower spore viability in the ZI14's (0/31) as compared to the ZI13's (27/74), it is reasonable to suspect that there are more or different combinations of lethal causing alleles in the ZI14's than in the ZI13's coming from the α/α portion. Under the translocation model, the lethality seems more likely to be contributed by the monosomic diploid; whereby the viability of the ZI13's and ZI14's are expected to be the same, however they are not. Thus, it was hoped that increased spore viability of trisomic tetraploids could be achieved by using other strains compatible with these monosomes.

In addition to the problem of spore inviability, meiotic non-disjunction is known to occur at high frequency in some tetraploids (MANNEY unpublished). It was thought it might also contribute to the skewed distribution of the trisome. Thus several diploids were constructed and screened for their mitotic stability for chromosome III. The screening technique relied on the ability of an a/α clone to remain a non-mater (HABER 1974). It was intended that such strains be used to construct more suitable trisomic tetraploids, as they might also exhibit less meiotic non-disjunction. One such strain, YI32, was found more stable than the others, but was not in the desired configuration to be used in construction of a trisomic tetraploid. It was sporulated in order that recombinant spores could be used in constructing the desired trisome. However, its viability pattern mimicked those of sporulating diploids segregating from trisomic tetraploid, i.e., asci with four, three, and two viable spores. This confirms the poor spore viability incompatibility in the genetic background of the previous trisomic tetraploids directly in a diploid. The inviability is not due to simply a non-reciprocal translocation, or a two gene interaction, but is more complex as the class of asci with two-viable spores is too infrequent. Diploids were constructed from intercrosses of YI32 spores in hopes of getting rid of this heterogeneity. They were dissected and resulting viabilities are similar to those of YI32 with the exception of ZI55. (See Table 11). A number of monosomic diploids and diploids homozygous for mating-type were acquired from ZI53 through ZI55. Unfortunately, time did not allow for these strains to be incorporated into trisomic tetraploids.

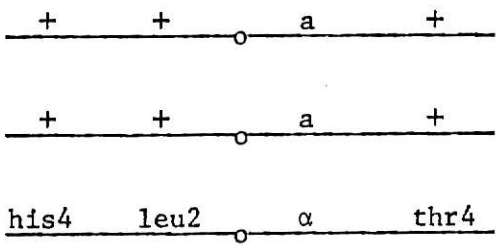
The non-mating diploid YP173 was known to produce good spore viability. It was used with the monosomic diploid YL61-1α to produce

TABLE 11

Viability of Diploids with Similar Genetic Background as Trisomes ZI13a and ZI50's

Strain	Number of asci	Number of viable spores per asci 4	3	2	1
YI32	40	19	17	4	0
YI51	40	26	11	3	0
YI52	40	21	16	3	0
YI55	20	17	1	1	1
YI56	20	13	5	2	0
Total	160	96	50	13	1
%		60.0	31.3	8.1	.6

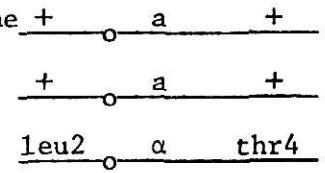
trisomic tetraploids by direct selection. The trisomic portion has this configuration:



These were sporulated and the results of their segregation patterns are given in Table 12. The spore viability is appreciably better. The 2:2 class for thr4 is present, but still far short of the expected frequency. Likewise, the 2:2:0 class for mating-type is also suppressed. When analyzing the asci with three-viable spores, it was observed that there were thirty-seven asci 2:0:1 and twenty asci 2:1:0 for matl (a:α:n). This also indicates a lack of thr4 and α spores. Since there is only one dose of these alleles (thr4 and α) in the trisome, they should primarily be expressed in the monosomic spores. Seeing that the α mating allele is on the same homologue as thr4, it becomes clear that monosomic spores carrying the α and thr4 alleles are lethal. This resembles the problem occurring in ZI13a. Models predicting such patterns are: a) there are two interacting lethal genes--two doses for the lethal in the tetraploid portion and one dose of the lethal linked to thr4 or; b) one dose of the non-reciprocal translocation (chromosome III bearing the deletion, and some other chromosome the addition). Suppose that ZI13a and ZI50's could have been made from various heterozygous non-reciprocal translocations in a monosomic diploid from the same source. The monosome used in ZI13a was a spore from a cross of the monosome used in ZI50's. In order for

TABLE 12

Observed Phenotypic Classes for Gene-Centromere Interval from the Trisome



Strain	Viability[a]	leu2[b] 4:0	2:2	3:1	mat1[c] 2:0:2	0:2:2	2:1:1	3:1:0	thr4 4:0	2:0	3:1
ZI50a	28/56	21	4	3	13	4	8	3	13	1	14
ZI50b	13/16	11	0	2	10	0	2	1	9	0	4
ZI50c	11/16	8	1	2	5	0	4	1	6	0	5
ZI50d	29/56	19	7	3	17	1	8	3	16	2	11
ZI50e	9/16	7	2	0	4	0	4	1	5	0	44
ZI50f	35/56	18	13	4	11	4	20	0	10	2	23
ZI50g	12/16	9	2	1	4	0	4	4	5	1	6
ZI50h	13/16	10	1	2	8	0	3	1	5	1	7
ZI50i	33/56	20	9	4	14	1	15	3	12	1	20
ZI50j	6/8	2	2	2	1	0	5	0	1	0	5
Total	189/312	125	41	23	88	10	73	18	82	8	99
%observed		66.1	21.7	12.2	46.6	5.3	38.6	9.5	43.4	4.2	52.4
%expected[d]		56.3	28.1	15.6	44.4	18.5	29.6	7.4	44.4	18.5	37.0

[a]The fraction of asci with four viable spores

[b]Classes for recessive markers are given by the ratio +:-.

[c]Mating-type classes are given by the ratio a:α:n, where n is a non-mater.

[d]Expected frequencies were calculated for x_1 = 30%, x_2 = 80%, and x_3 = 80%, where x's are the mean frequency of exchange between centromere and leu2, mat1, and thr4 respectively.

this disomic tetraploid cross to have good viability, as ZI67 does, the parental monosomic diploid would usually be assumed homozygous for the non-reciprocal translocation (i.e., two doses of the addition on some disomic chromosome). This would make the monosomic diploid, YL61-1α, incompatible with trisomic segregation patterns of the ZI50's, as only one dose of the non-reciprocal translocation best explains the data (Table 12). Furthermore, when one dose is assumed, the good viability of YI50h-7C (Table 13) demands the rare tetrasomic segregation of translocated additions to the same spore to cover the deletion linked to thr4. Thus, the translocation model is not as favorable as the two interacting gene model.

It was desirable to construct trisomic tetraploids retaining the thr4 marker so that more information about trisomic segregation could be acquired. A new source of thr4 was utilized by direct selection of a trisomic tetraploid, ZI64. YP173(a/α) was utilized again; this time selection was for a thr4/a thr4 diploids. Spores from this strain displayed extreme inviability (ZI64a(a/a/α), 0/13; ZI64b(a/a/α), 0/12) and surprisingly expressed thr4 only once. Before discontinuing a search for trisomes retaining the thr4 marker, two more hybrids were constructed, ZI71 and ZI74. These strains were the crosses of ZI50 spores; α/α diploids heterozygous for thr4 were mated to a monosomes. Viability and segregation patterns are similar to those exhibited by the parental strain ZI50. (See Table 14). It seems unlikely that intercrosses, hybrids made from inbreeding of spores from the same hybrid, can be made which contain a thr4 markers, and not those undesirable characteristics linked to thr4 that cause lethality to monosomic thr4 spores. Hence it seems more beneficial to proceed with the construction of intercrosses which do not carry thr4 and those linked to lethal-acting genes, in hopes of finding suitable trisomes. Besides being wild-type for

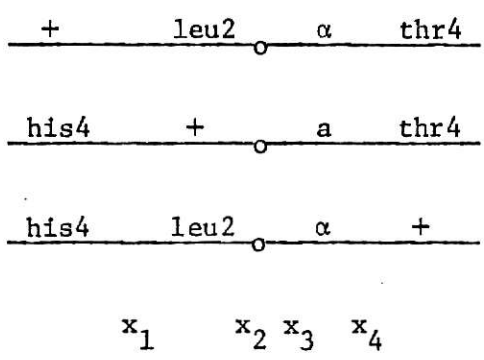
TABLE 13

Viability of Sporulating Diploids Segregating from ZI50's (a/a/α)

Strain	Number of asci	Number of viable spores per ascus				
		4	3	2	1	0
YI50f-4A	4	2	1	1	0	0
YI50f-4B	4	1	2	1	0	0
YI50f-11B	4	0	1	0	1	2
YI50f-14A	4	0	0	2	2	0
YI50h-5A	4	2	1	0	1	0
YI50h-5B	4	1	3	0	0	0
YI50h-7C	4	4	0	0	0	0
YI50-10C	4	0	0	1	0	3
Total	32	10	8	5	4	5

thr4, suspect α monosomic diploids from asci with three-viable spores were chosen for the intercross. Such monosomes have a higher probability of not containing any lethal conferring alleles since the lethal spore is assumed to carry them. Unfortunately, a source of a/a diploids with such properties was not readily available. Therefore, two a/a spores were used from one ascus in which one of them is expected to be homozygous wild-type for thr4, while the other one is heterozygous for thr4. However, nothing could be predicted about the lethal alleles unlinked to thr4. Thus, a series of trisomic tetraploids were made in order to acquire some which do not carry thr4. The trisomic tetraploids ZI75, ZI77, ZI79, and ZI81 were found to have one dose of thr4, coming from a/a diploids heterozygous for thr4. Their segregation patterns and viability patterns are similar to ZI50 and are listed in Table 14. Several trisomic tetraploids which lack the thr4 marker were also found. They are ZI76, ZI78, ZI80, and ZI82. (See Table 15). Spore viability is markedly increased and strains do not exhibit peculiar distributions of segregants. Hence, they are suitable for genetic analysis of the trisome.

Evidence for trivalent pairing. If each of three homologues in the trisome has an equal chance of participating in an exchange, then it is possible for three-chromosomal double-crossovers to occur. Such three-chromosomal exchanges indicate trivalent pairing for a given region. These trisomic strains were constructed such that each homologue was uniquely marked in order to detect such three-chromosomal exchanges. The following configuration was used in ZI28b2 and ZI29b2 to test trivalent pairing within the his4-thr4 region.



Thus a monosomic spores wild-type for these three nutritional requirements (prototrophic for chromosome III) most frequently occur when a single strand of a homologue is involved in a double crossover, one exchange being with each of the other two homologues. (See Figure 3). Most disomic spores predicted from double crossovers involving three chromosomes often yield segregants indistinguishable from those predicted from a single exchange and from other two-chromosomal double exchanges. The segregation frequency of a/a disomes is much less than the predicted frequency of α/α disomes, so that spores expressing mating-type a are more likely to be monosomes than spores expressing mating-type α. In addition, a/a prototrophic spores for chromosome III most likely arise from three chromosomal double crossovers (x_3 and x_4). These prototrophic spores of mating-type a become a sensitive measure of three chromosomal exchanges. Therefore a random spore analysis was performed on ZI28b2 and ZI29b2. Total amount of sporulation was 84% and 87% respectively. Spores were germinated on synthetic complete (SC) minus histidine, leucine and threonine, so that only prototrophic clones for chromosome III would grow. Controls were diluted and plated on SC. Spore viabilities on SC approximated that of YEPAD. This gave 11.5% wild-type clones of chromosome III for ZI28b2 and ZI29b2 respectively. These clones (approximately 100 per plate) were then tested for their ability to mate as a. The fraction of prototrophic clones for chromosome III

TABLE 14

Observed Phenotypic Classes of Gene-Centromere Interval from the Trisomes

1) leu2 — o — a — + / + — o — α — thr4 / + — o — α — + ; x_1 x_2 x_3

2) + — o — a — + / + — o — a — thr4 / leu2 — o — α — + ; x_1 x_2 x_3

Strain	Viability[a]	leu2[b] 4:0	leu2[b] 2:2	leu2[b] 3:1	mat1[c] 2:0:2	mat1[c] 2:2:0	mat1[c] 2:1:1	mat1[c] 3:1:0	thr4 4:0	thr4 2:2	thr4 3:1
1)											
ZI71	6/14	-	-	-[d]	2	1	3	0	3	0	3
ZI74	12/20	-	-	-[d]	3	2	5	2	6	1	5
2)											
ZI75	12/16	9	1	2	5	0	7	0	6	0	6
ZI77	12/20	8	2	2	7	2	1	2	7	1	4
ZI79	14/20	8	4	2	6	2	5	0	4	2	8
ZI81	16/20	11	3	2	6	0	10	0	7	3	6
Total of 1 and 2											
	72/110	36	10	8	29	7	31	4	33	7	32
% observed		66.7	18.5	14.8	40.8	9.9	43.7	5.6	45.8	9.7	44.4
% best fit[e]		57.3	28.5	15.6	44.4	18.5	29.6	7.4	42.3	17.1	40.6

[a] The fraction of asci with four viable spores.

[b] Classes for recessive markers are given by the ratio +:-.

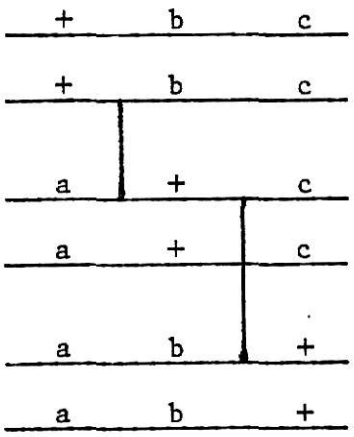
[c] Mating-type classes are given by the ratio a:α:n, where n is a non-mater.

[d] Leu1 was also segregating, but was not scored for.

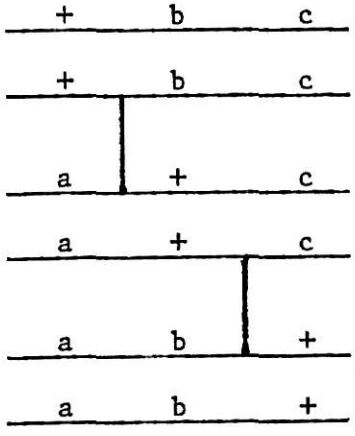
[e] Best fit frequencies are calculated for x_1=30%, x_2=80%, and x_3=10%.

FIGURE 3

Types of Three Chromosomal Double Crossovers



single strand involved



both sister strands involved

mating as a was 25% for ZI28b2 and 7% for ZI29b2 (somewhat low due to crowded plate condition). Hence, the frequency of prototrophic a spores is 2.9% for ZI28b2 and 1% for ZI29b2. This observed frequency of a clones is not expected to be significantly larger than the expected frequency of prototrophic a monosomes and a/a disomes resulting from double crossovers which is $F_p = (2/4)(1/3)(2(x_1/6)(x_3/6))+(1/4)(1/6)((x_2/3)(x_3/3))=(x_1x_2)/108+(x_2x_3)/216$. For $x_1=x_2=x_3=.8$, $F_p = .9\%$. Both values are in reasonable agreement. Asci of these strains were also dissected; poor viability (3/16 and 8/16 four spored viable asci) and skewed distribution of segregants were found. (See Table 9b). Certainly this contributed to the fluctuation of observed frequency from expected for this class. An explanation for this abnormal behavior is similar to that given later for other related strains. In addition there are several aberrant asci possibly arising from non-disjunction or conversion.

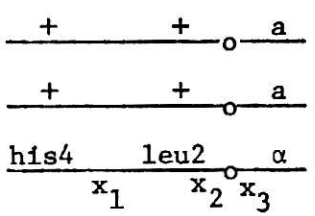
Certainly the presence of prototrophic spores from a limited sample is qualitative evidence for trivalent pairing in the his4-thr4 interval, as all three homologues must be paired together within this region for the occurrence of double crossovers involving each homologue. The high frequency of exchange needed to predict the observed frequency of prototrophs is also characteristic of trivalent pairing and provides quantitative evidence for trivalent pairing.

The genetic analysis of trisomes.

Meiosis of a trisome can now be analyzed by strains which demonstrate good spore viability (Table 15). These strains were constructed duplex at several loci by mating a/a diploids and α monosomes in the usual fashion. Crossovers proximal to loci in duplex are readily identifiable by the 3:1 phenotypic classes (+:-) (Table 2). Although the expression of a recessive allele was sometimes observed in a/α disomic segregants (in $3:1_d$), it was not always possible to determine whether this expression occurred in a disome or a monosome. Hence, the two crossover classes, $3:1_m$ and $3:1_d$, are considered together in the 3:1 class when accumulating and analyzing data. Tetrad data from diploid spores and other intercrosses of monosomic spores, which were a/α disomes and heterozygous for leu2 and his4, are listed in Table 16. Tetrad analysis of such disomes allows mapping distances to be determined in the same genetic background. The map distances in centimorgans (cM) between two linked genes can be calculated from this formula: 100(T+6NPD)/2(PD+NPD+T) (PERKINS 1949). Since such a small sample of tetrads exist with tightly linked centromere markers, 6cM was assumed to be the leu2 - centromere distance as ascertained by the published values listed in Figure 4. From this assumption, his4 is calculated as 26.8cM and mat1 as 35.9cM from the centromere. Thus, the markers leu2, his4, and α are in order of increasing distance from the centromere, and are in general agreement with the published values. The best fit for the values of x, the mean frequency of exchange for the gene-centromere interval assuming trivalent pairing and trivalent segregation, also increases with increasing gene-centromere distance in trisomic data (Table 15).

TABLE 15

Observed Phenotypic Classes for Gene-Centromere Interval from the Trisome



Strain	Viability[a]	his4[b] 4:0	his4[b] 2:2	his4[b] 3:1	leu2 4:0	leu2 2:1	leu2 3:1	MT[c] 2:0:2	MT[c] 2:2:0	MT[c] 2:1:1	MT[c] 3:1:0
ZI76	114/131	47	21	46	59	33	22	32	14	62	6
ZI78	17/20	4	2	11	8	5	4	9	3	5	0
ZI80	83/101	45	13	25	49	19	15	34	14	31	4
ZI82	17/20	6	3	8	12	4	1	7	1	7	2
Total	231/272	102	39	90	128	61	42	82	32	105	12
%		44.2	16.9	39.0	55.4	26.4	18.2	35.5	13.9	45.5	5.2
Best fit[d]		44.4	18.5	37.0	55.6	25.9	18.5	36.7	13.3	40.0	10.0

[a]The fraction of asci with four viable spores.

[b]Classes for recessive markers are given by the ratio +:-.

[c]Mating-type classes are given by the ratio a:α:n, where n is a non-mater.

[d]Best fit valueswere found for $x_1+x_2=80\%$, $x_2=36\%$, and $x_3=120\%$.

TABLE 16

Observed Tetrad Ratios (PD:NPD/T) for Strains Derived from ZI76 and ZI80

Strain	Viability	his4-leu2	leu2-mat1	his4-mat1	leu2-leu1
YI76-5B	3/10	3:0 / 0	0:0 / 3	0:0 / 3	1:2 / 0
YI76-5C	7/10	4:0 / 3	3:0 / 4	3:0 / 4	4:3 / 0
YI76-15A	2/10	2:0 / 0	0:0 / 2	0:0 / 2	-
YI76-15C	19/20	-	9:0 / 9	-	-
YI80-12B	19/20	12:0 / 5	8:2 / 8	6:1 / 11	-
YI80-12C	9/10	6:0 / 3	5:0 / 4	3:0 / 6	-
YI80-35B	8/10	-	1:0 / 2	-	-
YI80-53A	15/20	7:0 / 4	6:1 / 5	4:2 / 8	6:5 / 1
YI80-53B	18/20	7:0 / 6	3:1 / 12	1:3 / 11	6:3 / 1
ZI113(a/α)	19/20	8:0 / 10	7:1 / 10	5:0 / 14	-
ZI114(a/α)	9/10	4:0 / 4	3:0 / 5	1:0 / 8	-
ZI115(a/α)	8/10	2:0 / 3	1:1 / 5	1:0 / 5	-
ZI116(a/α)	8/10	4:0 / 4	5:0 / 3	3:0 / 5	-
Total	144/180	59:0 / 42	51:6 / 72	27:6 / 77	17:13 / 2
Map Distance		20.8cM	41.9cM	51.4cM	3.1cM

FIGURE 4

Genetic Map of Chromosome III

	(Map distances in centimorgens)		
	his4	leu2 o	mat1
MORTIMER and HAWTHORNE (1966)[a]	17.6	6.5	28.0
SHAFFER, BREARLEY, LITTLEWOOD, and FINK (1971)	15.0	5.0	40.0
MORTIMER and HAWTHORNE (1973)	24.8	6.5	25.0
CAMPBELL, FOGEL, and LUSNAK (1975)	14.2	5.8	26.0

[a]Calculated from their tetrad data given leu2 is 12.9% SDS. These values are different from those determined solely by SDS.

The relative distribution of the phenotypic classes for leu2 (gene-centromere interval) are in excellent agreement with those predicted from the trivalent pairing trivalent segregation model, considering only up to 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, i.e., 2:1:1 class is high and 3:1:0 class is low. It would appear that a slight preferential segregation was occurring, i.e., that chromosomes participating in an exchange do not segregate together to the same spore (3:1:0). Computations which considered multiple exchanges of higher order than two do not predict a distribution which demonstrated this effect. If these two classes are considered together, as was done for the two auxotrophic markers, good agreement is established. A random spore analysis presented in the cryptopleurine section yields a value which is much higher for the 3:1:0 class (18%). Thus, the data are best explained by statistical fluctuation.

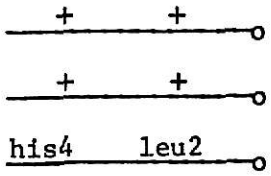
The mean frequency of exchange, x, is expected to be six times the diploid map distance. If the map distance is assumed to be 6cM for the leu2-centromere interval, then x is estimated at approximately 36%. This is in good agreement with the best fit value found for this interval (Table 3, Table 15). Since the frequency of producing double crossovers and the efficiencies for detecting them are different in disomes and trisomes for a given interval, the estimates of x from the map distance are valid only for small intervals, for which the effects of multiple crossovers are negligible. Thus, similar estimates for his4-centromere and mat1-centromere intervals are inaccurate, as they are much larger (160% to 215%) than the

best fit values for these intervals (approximately 80% and 120%, respectively).

These large observed values for the mean frequency of exchange indicated that multiple crossing over is important. If positive chiasma interference is as strong in trisomes as it is in disomes, a reduction in frequency of double crossovers is expected to be significant by the x values of 100% (Appendix I). This reduced frequency of double crossovers should be lower than that predicted from a non-interference model from which the best fit values were calculated. Gene-gene intervals can be used to detect double crossovers; the smallest one available is leu2-his4. When one dose of each of these alleles come in together, the two unique phenotypic classes of asci arising from at least a second order exchange are: 4:0 for his4 while 2:2 for leu2; and 2:2 for his4 while 4:0 for leu2. These two classes comprise approximately 1/23 of all the double crossovers and should be present in .33% of all asci according to the non-interference model for x values given in Table 17. From tetrad data (Table 16) no double crossover data (NPD) were observed in disomes, which is consistant with the MORTIMER and HAWTHORNE'S data (1966), only 3 NPD out of 521 asci. Strains listed in Table 17 produced 3.4% of their asci in these classes, an order of magnitude higher than expected. Likewise, a double crossover consisting of a single crossover in the his4-leu2 region and a crossover in the proximal region (e.g., 4:0 for his4 while 3:1 for leu2; and 2:2 for his4 while 3:1 for leu2) are also more frequent than predicted. Results for leu2-mat1 interval also are greater than those expected for the major classes, but lower for the minor classes (Table 18). Although these x values are a crude fit of the gene-gene interval data, these results do indicate that positive interference is not a significant factor in trisomes.

TABLE 17

Observed Phenotypic Classes for Gene-Gene Interval from the Trisome



Strain	his4[a] 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
ZI76	114[b]	42	19	17	15	14	5	0	2	0
ZI78	17	3	2	2	5	4	1	0	0	0
ZI80	83	37	11	9	12	4	4	2	0	4
ZI82	17	6	2	1	4	1	0	1	2	0
Total	231	88	34	29	36	23	10	3	4	4
%observed		38.1	14.7	12.6	15.6	10.0	4.3	1.3	1.7	1.7
%expected		39.7	16.2	16.7	14.1	8.6	1.8	.6	.1	.2

[a]Classes for gene-gene interval depends upon both markers, given by the ratios +:-, +:-.

[b]The column lists the total number of asci tested with four viable spores.

[c]Expected frequencies were calculated for x_1=60%, x_2=40%.

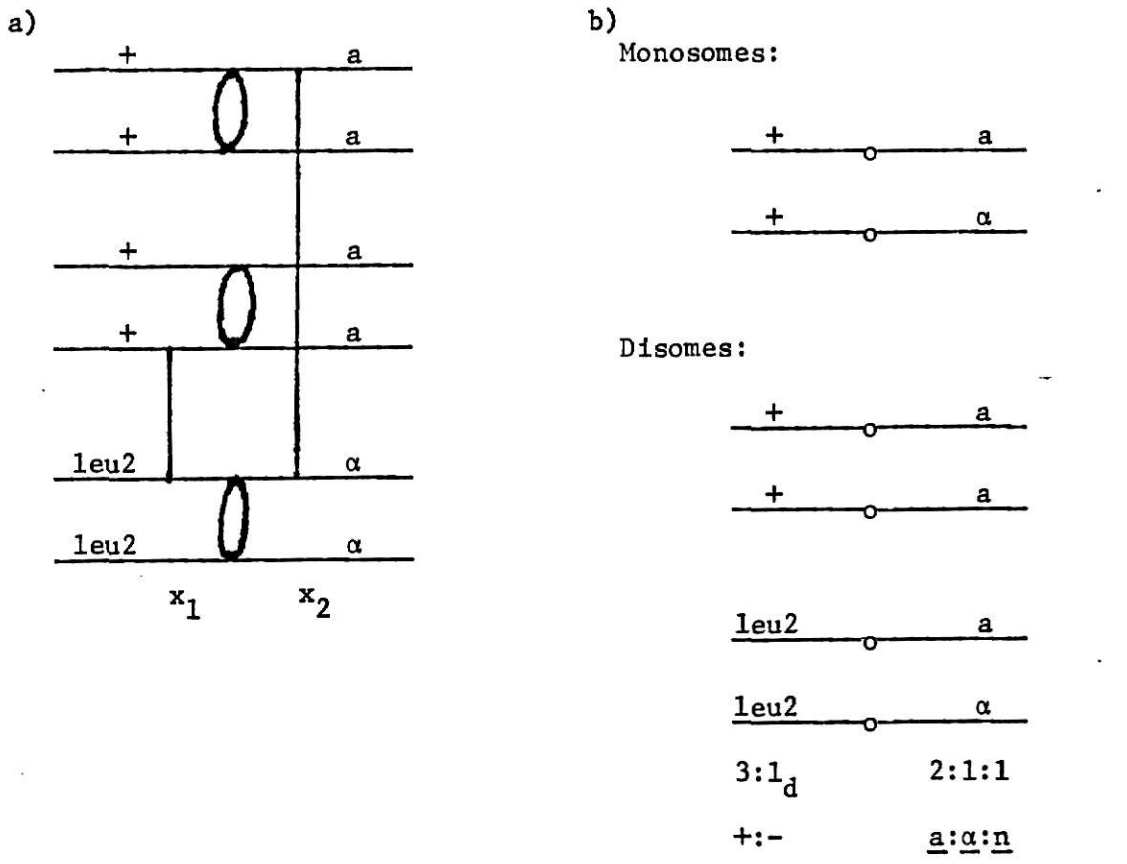
It is of no surprise that the observed frequencies of a prototrophic spores resulting from three chromosomal double crossovers in random spore analysis (presented in the previous section) were also high according to the predicted frequencies of a non-interference model. Since double crossovers are frequent enough to detect easily, perhaps a few asci resulting solely from three chromosomal exchanges might also be found. Thus, the trivalent pairing model can be confirmed by detecting particular double crossover classes in a gene-gene interval. Although some strains give poor viability and peculiar segregation patterns, their asci can still be used to show the existence of the three-chromosomal double crossover. The first two such asci were found in ZI13, one in the leu2-mat1 interval and the other in the his4-mat1 interval. The gene-gene class was 3:1 for auxotrophs and 2:1:1 for mat1, which results from a three-chromosomal exchange of second order or higher (Figure 5).

There were an appreciable number of asci resulting from double crossovers within the leu2-mat1 interval in trisomic strains containing thr4 (10.2% in ZI50's, 9.3% in ZI75, 77, 79, 81). Although three chromosomal exchanges were expected to be a major contributor in a number of double crossover classes, only one class consists exclusively of them. None of these asci in the above strains belong to this class. When trisomic strains with good viability were used, however, two such asci were found (See Table 18). Thus, three-chromosomal crossovers have been detected, showing the occurrence of trivalent pairing. Unfortunately, the leu2-his4 interval cannot be used to analyze three-chromosomal exchanges, as no crossover class is composed exclusively of them.

FIGURE 5

The Class Used to Detect Three Chromosomal Exchange

in leu2-mat1 Interval



[a]Crossing over in regions one and two involving all three homologues.

[b]The unique class segregating from such a double crossover.

TABLE 18

Observed Frequencies of Double Crossover Classes for α-leu2 Interval

Strain	Number of asci	leu2[a] 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
ZI76	114		13	0	1	2	0	0	0
ZI78	17		0	0	0	0	0	0	0
ZI80	83		6	0	1	0	0	0	0
ZI82	17		0	1	2	0	0	0	0
Total	231		79	1	4	2	0	0	0
%observed			8.2	.4	1.7	.9	0	0	0
%expected[b]			5.1	.42	.85	.85	.43	.22	.22

[a]Classes for gene-gene interval depends upon both markers, given by the ratio +:- for leu2 and by the ratio a:α:n for mating-type, where n is a non-mater.

[b]Expected frequencies were calculated for leu2-centromere = 40% and mat1-centromere = 80%.

Discussion

Analysis of trisomic segregation. Several trisomic strains exhibit meiotic segregation patterns that can be characterized by a trivalent pairing trivalent segregation model. For gene-centromere interval the four phenotypic classes of asci predicted by this model are observed for each of the three markers used. A bivalent-univalent model does not predict the $3:1_d$ class. The two crossover classes of an auxotroph, $3:1_m$ and $3:1_d$, have been lumped into the 3:1 class for analysis, as a few spores could not be diagnosed as either monosomic or disomic by their mating-type segregation patterns. The expression of cryptopleurine resistance only in disomic spores from a duplex trisome (+/+/cryl) provides additional evidence that trivalent segregation is occurring.

The distribution of these classes depends on x, the mean frequency of exchange in an interval due to trivalent pairing. Values of x were found such that the distribution of classes predicted by x matched closely those observed. The large amount of recombination in a trisome is reflected in the high values of x, as compared to recombination values for the same interval in a disome. In fact, for leu2-centromere interval, x was about six times the map distances in disomes, very close to that estimated for trivalent pairing for short intervals. A bivalent pairing model for trisomes does not adequately explain such high recombinant values. It is evident that trivalent pairing in trisomes produces an intrinsically higher amount of recombination than does bivalent pairing in disomes for the same

interval. Trivalent pairing is occurring over larger intervals also, as observed in three chromosome exchanges in asci and in prototrophic spores from random spore analysis discussed below.

For such large frequencies of exchange, effects of multiple crossovers are expected to become important even though they were negligible for some interval in a disome. Considerations of double crossovers were necessary to derive such closely matched distributions for the gene-centromere intervals above (even for short intervals). Gene-gene intervals were utilized to identify double crossovers. Certainly the detection of at least as many, if not more, of the expected number of double crossovers assuming no interference justifies their consideration in computing the best $\underline{x}$ value for the gene-centromere intervals mentioned above. These observed frequencies for double exchanges indicate no appreciable positive interference.

Trisomes were constructed such that involvement of all three chromosomes in crossing-over could be detected in prototrophic spores. The occurrence of such spores from a random spore analysis strongly supports the trivalent pairing model. A bivalent pairing model does not explain double crossovers involving all three homologues. Since trivalent pairing displays a large amount of recombination, a sizeable fraction of double crossovers for gene-gene interval was observed. This made possible the detection of a few asci which were the result of three chromosomal exchanges. Thus trivalent pairing is supported by the participation of all three homologues during crossing-over and by the high frequency of exchange demonstrated by both single and double crossover classes.

The distribution predicted by a trivalent pairing trivalent segregation model have been approximated by not including multiple crossovers of higher order than two. The effect of doubles is also approximated by splitting a given region into halves in which only single crossovers are allowed. This does not generate a Poisson distribution for exchanges advocated by BARRATT *et al.* (1954) for disomes. These effects are no longer small for *mat1*-centromere interval with an exchange value of 120%. These effects and others due to interference makes it difficult to determine the exact relationship between the linkage parameter *x* and the more conventional parameter, map distance. However, *x* does increase with an increase in map distance for the intervals tested. Thus, trisomic segregation patterns are characterized well enough to allow analysis of other trisomes.

GENETIC ANALYSIS OF STERILES BY THE USE OF TRISOMIC TETRAPLOIDS

Results

Certain steriles which prevented their own genetic analysis by conventional tetrad analysis, namely Class 9 and Class 10 steriles, can now be analyzed by the trisomic system as discussed in the previous section. These steriles were presumed to be alleles of matl (MACKAY and MANNEY 1974b). Before these steriles could be incorporated into trisomic tetraploids, they altered their expression as discussed in Experimental Design (Table 1). Thus these modified steriles were incorporated into trisomes and analyzed. The original steriles were not recovered from these segregants of these hybrids as no asci were found which would have resulted solely from the expression expected of the original sterile. The modified steriles were also presumed to be alleles of matl for initial analysis.

The sterile phenotype is indicated by the lack of mating ability and by the lack of sporulation. The non-maters (>95%) in the previous section were found to be sporulators, hence, their genotype of the non-maters was a/α. In order to distinguish steriles from a/α's, the number of sporulators, s, will be included in the phenotypic ratios for matl, a:α:n,s, in this section. Thus a 1:1:2,1 denotes two non-maters, one of which is a sporulator and therefore a/α; the other is a non-sporulator and therefore is a sterile. Sporulation was scored by visible asci after three day incubation on YEKAC.

Results of the phenotypic classes for several trisomic strains are found in Tables 19 and 20. Those strains whose numbers are in the 3400's are found to have reduced spore viability and a skewed distribution of segregation classes. A similar phenomenon was exhibited by the first set of strains. Those strains numbered in the 4300's have better viability; however, too few asci were dissected to accurately establish a distribution of classes. Consequently the data for all crosses has been considered together for qualitative analysis. For the purpose of providing a rough quantitative comparison, expected frequencies found in Table 20 are for a mean frequency of exchange value (x) of 80%. This value underestimates that value predicted from the total frequency of these recombinant asci assuming double crossovers, approximately 110%.

Trisomes containing the Modified Class 9 steriles (Ref. Table 2), a/α/'a ste', segregate as if they were a/α/α/, with one important difference: not all non-maters are sporulators (Table 19). Hence, these steriles behave for the most part as α alleles, as expected (Table 1). If these non-sporulating non-maters represent the sterile in monosomes, many more phenotypic classes would be predicted. Thus this sterile phenotype must be the result of expression of some disomic segregants. Diploids heterozygous for the sterile and a, described in Table 1, were non-maters, however they are also sporulators. Suppose that the sterile phenotype was produced by two loci: one linked to matl conferring α mating ability, but not having α sporulation function, called α'; the other unlinked to matl, which allows α' to sporulate in its presence. Notice that this unlinked gene must be dominant for sporulation.

TABLE 19

Observed Phenotypic Classes of the Gene-Centromere Interval from a/α/'a ste'[a]

Strains	Viability	0:2:2			2:2	a:α:nb / a:α:n,s 1:2:1		1:3:0	2:1:1
		0:2:2,2	0:2:2,1	0:2:2,0	2:2:0,0	1:2:1,1	1:2:1,0	1:3:0,0	2:1:1,0
ZI3403	13/20	0	1	1	6	0	1	0	4
ZI3404	5/16	1	0	0	1	3	0	0	0
ZI3405	11/16	0	1	1	3	4	2	0	0
ZI3406	11/16	2	1	0	3	2	1	2	0
ZI3407	11/16	0	2	2	1	2	2	2	0
ZI4305	8/10	2	1	1	1	2	0	1	0
ZI4306	10/10	0	3	0	1	5	0	1	0
	69/104	5	9	5	16	18	6	6	4
%		7.2	13.0	7.2	23.2	26.1	8.7	8.7	7.2
%expected[c]		40.3	-	-	15.7	35.2	-	8.8	-

[a]The 'a ste' is a modified Class 9 sterile.

[b]The mating-type classes are given by the ratios a:α:n,s, where n is the number of non-maters, and s is the number of sporulating non-maters.

[c]From Table 3, form matl-centromere interval - 100%.

This dominant gene is likely to be present in only one dose in the trisomic tetraploid (coming from the sterile haploid). Thus it would allow only half of the a/α' segregants to sporulate, regardless of whether the unlinked dominant was in the trisomic or tetrasomic portion. This hypothesis adequately explains the unexpected lack of sporulation as observed in the data. Strain ZI3404, a/α/'a ste', produced four 2:1:1,0 asci which cannot be explained.

Classes segregating from trisomes containing modified Class 10 steriles (Table 20) are essentially those expected if modified steriles express themselves in heterozygous diploids as listed in Table 1 (Ref. Table 6, linked case). Thus the peculiar expression for these steriles in disomic segregants is preserved, and these steriles appear to be alleles of matl. Seven asci from two classes (0:0:4,1 and 0:1:3,0) are not predicted by this simple one gene model. They appear to result from the inability of normal a/α disomic segregants to sporulate. The segregation of an additional dominant gene unlinked to matl which inhibits sporulation arising from the haploid sterile does not adequately explain this result; nor do other such simple Mendelian segregation patterns. Although sporulation frequency of non-maters is lower (81%) than the average (approximately 90%) it is not much different from that worst observed frequency (82% for XI76). Thus this lack of sporulation appears to be due to a number of unidentifiable genes inherent in the genetic background. Note that the sporulation frequency of non-maters from trisomes containing modified Class 9 steriles is 65%, much lower than can reasonably be expected from non-specific inhibition of sporulators suggested above.

TABLE 20

Observed Phenotypic Classes of the Gene-Centromere Interval from a/α/'α ste'[a]

		a:α:n,s[b]									
				0:0:4		1:1:2			0:1:3		
Strain	Viability	2:2:0,0	0:2:2,0	0:0:4,2	0:0:4,1	1:1:2,0	1:1:2,1	1:2:1,0	0:1:3,1	0:1:3,0	2:1:1,0
ZI3418	9/16	0	0	0	0	3	0	3	3	0	0
ZI3419	8/16	0	0	1	1	1	1	2	1	1	0
ZI3420	10/16	1	0	2	2	1	1	2	1	0	0
ZI3421	11/16	1	0	1	2	1	2	2	1	0	1
ZI4320	7/10	1	2	0	0	1	0	1	2	0	0
ZI4321	8/10	0	0	1	0	1	2	2	0	1	1
Total	53/84	3	2	5	5	8	6	12	8	2	2
%		5.7	3.8	9.4	9.4	15.1	11.3	22.6	15.1	3.8	3.8
%expected[c]		14.4	14.4	16.0	-	5.0	15.0	14.5	13.2	-	4.0

[a]The 'α ste' is a modified Class 10 sterile.

[b]The mating-type classes are given by the ratio a:α:n,s where n is the number of non-maters and s is the number of sporulating non-maters.

[c]From Table 6 (linked), for matl-centromere interval = 80%.

Discussion

The complex nature of the number and kinds of mutations affecting the mating-type expression makes analysis of modified steriles difficult, especially without prior analysis of the original steriles. Assuming the lack of sporulation is not entirely due to non-specific inhibition, the Class 9 modified steriles appear to consist of two loci, one linked or allelic to matl, and the other unlinked to matl which affects sporulation. Further construction of hybrids from these spores and their analysis is needed to confirm such a hypothesis. Since recovery and analysis of the original steriles was desired, such further analyses was not pursued. It may prove beneficial to pursue such an analysis for the acquisition of mutants directly affecting sporulation.

The modified Class 10 phenotype appears to be allelic to matl. They fail to mate as haploid and to sporulate in diploids heterozygous for either normal alleles. They also inhibit mating in a/ste diploids, just as listed in Table 1. It seems highly probable that the original steriles from both classes were alleles of matl from the linkage studies of modified steriles derived from them.

Once the nature of steriles allelic to matl are known, they provide a unique opportunity to further investigate trisomes. Trisomes can be constructed for three distinguishable alleles at mating-type, namely a/α/ste. Segregation from such a trisome yields up to three distinguishable parental classes, six distinguishable single crossover classes, and three distinguishable double crossover classes for the gene-centromere interval. Such a variety of classes lends itself to detailed investigation of trivalent pairing, chiasma interference and chromatid interference, and

of trivalent segregation, preferential segregation and non-disjunction without reference to a second gene. Thus not only can steriles be analyzed by trisomic analysis, but certain steriles can provide more information about the basic process of trisomic segregation.

ANALYSIS OF TETRASOMIC SEGREGATION

Results

Segregants from trisomic tetraploids were also scored for nutritional markers in the tetrasomic portion. The genotype of some tetraploid hybrids was not always known, because the genotypes of the parental diploids could not always be assessed from their phenotypes. The data for markers in duplex (+/+/-/-) is listed in Table 21.

Segregation distribution for met2 is lacking the 2:2 parental class. If these strains were actually triplex (+/+/+/-) for met2 the frequency of the 3:1 class would be more than seven times the frequency observed for strains known to be triple for met2. The nature of this skewed distribution is unresolved and omitted from further discussion.

Of the remaining markers, lys1 is linked closest to the centromere (approximately 32cM) (MORTIMER and HAWTHORNE 1966), while the remaining markers are unlinked to the centromere (ade2 = 66.7cM, can1 and ura1 > 66cM) from the diploid data. The observed distributions for lys1 and ade2 in Table 21 are not significantly different. The bivalent pairing bivalent segregation model (BPBS) (Ref. Table 7) is the one which gives the most reasonable fit for these distributions. Since BPBS model predicts essentially the same distribution for SDS (y) values of 55% and 80%, lys1 might correspond to the 55% value and ade2 to the 80% value. There exists an appreciable amount of fluctuation in the distributions from that of ade2 for the remaining markers. The BPBS model cannot be used to fit these distributions satisfactorily. The other model presented by ROMAN, PHILLIPS and SANDS (1955), the bivalent pairing tetravalent segregation

TABLE 21

Observed Frequencies for Gene-Centromere Interval for Tetrasome Duplex (+/+/-/-) at these Loci

	ade2			can1			met2			lys1			ura1		
Strain	4:0	2:2	3:1	0:4	2:2	1:3	4:0	2:2	3:1	4:0	2:2	3:1	4:0	2:2	3:1
ZI13a	8	2	9				13	0	6				15	0	4
ZI50a-j										74	25	82			
ZI75	7	2	3	9	1	2	9	0	3	4	5	2	6	1	5
ZI76	47	22	49	23	16	42				57	14	49	37	14	58
ZI77	2	2	7							6	1	4			
ZI78	3	2	12							8	5	4	2	4	11
ZI79	8	3	3												
ZI80	49	3	36	28	8	20									
ZI81										13	0	3	7	1	8
ZI82	8	2	7												
Total	132	36	126	60	25	64	22	0	9	162	50	144	67	20	86
%	44.9	12.2	42.9	40.3	16.8	43.0	70.9	0	29.0	45.5	14.1	40.4	38.7	11.6	49.7

model, was not found to predict any of the observed data, as the frequency of the 2:2 class was much higher for all values of SDS. However, the mean frequency of exchange value of 95% in the tetravalent pairing tetravalent segregation model (TPTS) yields distributions closely fitting the canl distribution. The ural distribution is at best poorly fitted with the TPTS model at 120%. This model nearly characterizes the lysl data as well as does the BPBS model, but does fit the ade2 data. Thus, a number of models with a number of different linkage values are needed to describe the data for different markers unlinked to the centromere.

Table 22 gives the observed distribution for markers in a monoplex (+/-/-/-) and triplex (+/+/+/-). These data have been considered together since the nature and frequency of the distinguishable crossover classes for a marker in a monoplex (1:3) is the same as that for the same marker in triplex (3:1). With the exception of canl and trp5 data, the frequency of crossover classes (3.4 to 5.2%) are not statistically different from one another, because very few events were observed. These linkage values fall within a factor of two of those expected from a TPTS model on the assumption that the mean frequency of exchange (x) is no greater than 120% for noncentromere linked genes. A bivalent segregation model does not predict the 3:1 class, hence tetravalent segregation must be involved. The frequency of the crossover class of canl is greater than three times that for the other markers. It is thought to be due to expression of leaky canl mutants on media containing a low concentration of canavanine (20 mg/l), and can probably be alleviated by using 60 mg/l of canavanine. The frequency of trp5 is expected to be somewhat lower than others as it is closer to centromere than the other markers (17cM).

TABLE 22

Observed Frequencies of Gene-Centromere Interval for Tetrasome Triples (+/+/+/-) and Monoplex (+/-/-/-) at These Loci

	ade2		can1		met2		lys1		trp5		ura1	
Classes from +/+/+/-	4:0	3:1	0:4	1:3	4:0	3:1	4:0	3:1			4:0	3:1
Classes from +/-/-/-			2:2	3:1			2:2	3:1	2:2	1:3		
ZI50a-j	164	9	157	24	174	7			183	1	176	5
ZI76					120	4						
ZI77											10	1
ZI79							14	0				
ZI80					83	4	84	3			83	4
ZI81			13	3								
ZI82			15	2			16	1			15	2
Total	164	9	192	38	377	15	114	4	183	1	286	12
%	94.8	5.2	83.5	16.5	96.2	3.8	96.6	3.4	99.5	.5	96.0	4.0

BRUENN and MORTIMER (1970) have suggested that the spores from triplex strains (+/+/+/-) expressing the recessive phenotype (as in the 3:1 class above) are monosomes. If these suspected monosomes (not for chromosome III) are sporulated, they are expected to produce only two viable spores per ascus. Unfortunately the frequency of a/α spores is low, and only one spore expressing met2 was recovered in an a/α (YI50-35B). It produced 7/10 asci with four viable spores, implying that it was indeed the result of tetravalent segregation.

ZI50a-j (a/a/α) was monoplex (+/-/-/-) for trp5. Seven asci out of one hundred and ninety-one were found to be 3:1. This can perhaps be explained by gene conversion. Other loci, such as can1, also exhibit this phenomenon, but to a lesser extent.

Discussion

The number of models which partially characterize tetrasome segregation data suggest that either a number of distinct processes are occurring during meiosis of a tetrasome or that an accurate model has yet to be devised. The latter seems more likely. In order that meiosis of a tetrasome might be properly investigated, markers which are closely linked to the centromere need to be analyzed. This alleviates any interference problem and other inaccuracies of models due to approximations presently encountered by use of markers unlinked to the centromeres. It is observed that the mean frequency of exchange in trisomes is much greater than that of disomes. Therefore, tightly linked markers may be most desireable if the exchange frequency for tetrasomes in also much

higher than the frequency for disomes.

The frequency of recessive spores from strains triplex at several loci (13) would be greater than 6.4%, according to data given by BRUENN and MORTIMER (1970). Certainly a value computed from this frequency (4(6.4%/13)) is compatible with the observed frequency of asci exhibiting the recessive phenotype for one locus from .5% to 5.2%. If these spores are presumed to be monosomes as suggested by BRUENN and MORTIMER, meiotic non-disjunction is quite frequent. Since three viable spored asci are not usually analyzed, non-disjunction rates may be overlooked in diploids, whereas in tetrasomes non-disjunction at meiosis II may still produce four viable spored asci and is likely to be detected. Even if non-disjunction is not high in diploids, tetraploids may have their own intrinsically high frequency. However, from these spores tested by BRUENN and MORTIMER, less than .5% of all spores were found to be stable monosomes. Hence, non-disjunction does not account for a large fraction of spores expressing the recessive phenotype. If tetravalent segregation is occurring, such spores are occasionally segregated. Although too few asci were dissected to accurately determine linkage values, they appear to fall within the range predicted by a tetravalent pairing tetravalent segregation model. It seems likely that tetravalent segregation is also responsible for a sizeable fraction of recessive spores. Since cryptopleurine resistance can be used to distinguish monosomic diploids from full diploids, the frequency of non-disjunction for chromosome III can be determined from tetrasomes homozygous for cryl. Similarly, the frequency of recessive spores due to tetravalent segregation at a given exchange value can be determined from tetrasome

triplex for cryl. A comparison of these two frequencies would provide an estimate of relative contribution these two phenomena have in the production of recessive spores.

EXPRESSION OF CRYPTOPLEURINE RESISTANCE IN ANEUPLOIDS

During the course of this study, a peculiar observation concerning cryptopleurine resistance was made which warranted further investigation. In this section, this observation is described along with the experiments revealing the nature of this phenomenon.

Results

Cryptopleurine resistance is expressed by recessive alleles of cryl, which maps 2.1 centromorgens proximal of matl on chromosome III. Recessive alleles for other markers on chromosome III, his4 and leu2 are expressed in monosomic diploids, independent of whether these monosomes were derived by indirect selection or from spores of disomic tetraploids. Trisomic tetraploids segregate two monosomic and two disomic spores per ascus. When present in one dose in a trisome, leu2 and his4 can be expressed in either monosomic or homozygous disomic spores, e.g., leu2/leu2. In order to establish trisomic segregation patterns, these two possibilities need to be distinguished. Matl can be used to differentiate monosomes from disomes (for chromosome III) in an ascus provided no crossing-over occurs in the centromere-matl interval. However, there exist a considerable amount of recombination in this region. An alternate means to distinguish monosomes from disomes (for chromosome III) is by induction of cryptopleurine resistance by UV. Monosomic spores from trisomes homozygous for cryptopleurine sensitivity (+/+/+) are expected to mutate to cryptopleurine resistance at approximately the same rate as wild-type (+) haploids (10^{-6}), while disomes should have an induction rate close to that for diploids (10^{-8},

which is essentially undetectable). Thus, induction of cryptopleurine resistance was to be used as a genetic tool.

Fourteen diploids monosomic for chromosome III derived by indirect selection failed to be induced, just as if they had become homozygous disomes. Eight newly formed clones from monosomic spores were also tested for induction and showed no resistant clones for cryptopleurine. Four of these spore cultures were subsequently intercrossed and gave disomic tetraploid segregation patterns (ZI113(a/α) through ZI116(a/α), Table 16) showing they are indeed monosomes. Obviously, induction of cryptopleurine resistance could not be used as a genetic tool to distinguish monosomes from disomes.

The failure of cryptopleurine resistance to be induced in diploids monosomic for chromosome III is perplexing. It was conjectured that this phenomenon might be explained by failure to induce mutation in monosomes or by failure of induced mutations at cryl to be expressed in a monosome. A trisome lends itself to investigation of the expression hypotheses. Since a trisome segregates two monosomes and two disomes per ascus, a trisome constructed homozygous for cryl will yield only two resistant to two sensitive spores per ascus, if cryl cannot be expressed in monosomes. On the other hand all four spores will be resistant if cryl can be expressed in monosomes.

Since cryl is closely linked to matl, no new selection methods were necessary to construct trisomes homozygous for cryl. Diploids homozygous for cryl (e.g., YT3427-1 (cryl a/cryl a), expressing resistance) were isolated by indirect selection of irradiated diploids constructed heterozygous for cryl (e.g., YT3427 (cryl a/+ α), not expressing resistance).

Various tetraploids (ZI3434-2) were recovered by direct selection of non-mating diploids homozygous for cryl (e.g., YT3434 (cryl a/cryl α), expressing resistance) with complementing mating diploids isolated above.

One hundred and twenty of these clones were isolated and all but one, number 111, were resistant to cryptopleurine, and one of these, number 7, seems to grow appreciable better on CRY medium than any of the others. Three typical clones and these two exceptional ones were sporulated and dissected (Table 23). The three typical tetraploids showed trisomic segregation patterns for matl. When their spores were tested on CRY medium, two spores per ascus were sensitive and two were resistant. (Only two asci from clone number 27 produced four resistant spores and one of these asci gave tetrasomic segregation for matl. This trisomic clone was assumed to be mixed with a few tetrasomic tetraploids.) Those spores which were determined to be monosomes by phenotypic classes of matl, were found to be sensitive; conversely, known disomes were found to be resistant. Such a 2:2 segregation pattern is predicted by a trisome having one dose of the sensitive allele. However, it is improbable that the sensitive allele always segregates into the monosome. Since only one ascus deviated from this pattern (i.e., that monosomes are sensitive and disomes are resistant), it provides evidence that these trisomes were constructed homozygous for cryptopleurine resistance, just as designed. Thus, this 2:2 segregation pattern for cryptopleurine from trisomes homozygous resistant at cryl validates the supposition that cryptopleurine resistant alleles are not expressed in monosomic diploids.

TABLE 23

Observed Phenotypic Classes for Gene-Centromere Interval from Strains Homozygous for Cryptopleurine Resistance

Strain[a]	Viability[b]	mat1[c]							cryl[d]	
		2:0:2	2:2:0	2:1:1	3:1:0	0:0:4	1:1:2	4:0	2:2	0:4
ZI3434-1(a/a/α)	5/10	2	0	2	1	0	0	0	5	0
ZI3434-2(a/a/α)	7/10	2	1	2	2	0	0	0	7	0
ZI3434-27(a/a/α)	8/10	0	1	4	2	0	1	2	6	0
Subtotal	20/30	4	2	8	5	0	1	2	18	0
ZI3434-111(a/α)	6/9	0	6	0	0	0	0	0	0	6
ZI3434-7(a/a/α/α)	10/10	0	0	1	0	2	6	10	0	0

[a]Strains are all tetraploids derived by direct selection from one experiment.

[b]The fraction of asci with four viable spores.

[c]Mating-type classes are given by the ratio a:α:n, where n is a non-mater.

[d]Classes for cryl are given by the ratio +:- (resistance: sensitivity).

The other tetraploids isolated by this selection procedure are consistant with the above data. Clone number III, sensitive to cryptopleurine, segregated only 2:2 (a:α) asci and was therefore a disomic tetraploid. It is reasonable to expect that a hybrid composed of two monosomic diploids unable to express its resistant alleles would also be unable to express resistance. Since all the spores from such a disomic tetraploid (cry1/cry1) are monosomic diploids, it was reasonable to find that all these spores were sensitive. Clone number 7 shows tetrasomic segregation patterns for mat1 and only 4:0 (resistant:sensitive) asci for cry1. Such a result is expected from a tetrasome homozygous for cry1, since all of its spores are disomes.

Another parallel set of tetraploids (ZI3434-201 to ZI3434-320) selected for the a/α/α/ genotype was isolated. Unfortunately, these strains gave very few asci with four viable spores, and for this reason could not be analyzed. Since thr4 was segregating in these trisomes, the nature of this inviability was attributed to be the same as that exhibited by the first set of hybrids. Again only one of these tetraploids was sensitive to cryptopleurine and showed segregation of a disome for chromosome III.

Additional evidence that a monosomic diploid carries cry1 without expressing resistance also exists. A hybrid, ZI125, was made with an α/α diploid homozygous sensitive, and with an a monosomic diploid isolated by indirect selection and suspected of carrying the cry1 allele, although it does not express it. If the hypothesis is correct, expression of resistance is only expected in a/a disomic spores since cry1 is tightly linked to a. Only two of the fifteen asci were 1:3:0 (a:α:n) for mat1, and both a/a spores were cryptopleurine resistant. No other spores were resistant, even though many monosomes were known to be a (and presumable cry1).

Use of cryl to map matl. Random spore procedure was done with ZI125 described above in order that a more accurate estimation of the frequency of the 1:3:0 class (a:α:n) for matl be made. Twenty-one resistant clones were found out of four hundred and sixty spores tested. This implies about 18% of the asci were 1:3:0. This is much higher than the 10% value predicted from a mean frequency of exchange value of 120%, used for the centromere to matl interval. This high value offsets the low value of 5.2% observed earlier.

Discussion

The phenotypic expression of recessive alleles for cryptopleurine resistance is not observed in monosomic diploids, as is normally found for other recessive alleles in other genes. Failure to express resistance in other aneuploids homozygous for cryl depends on the relative dose of cryl to that of the loci on other chromosomes. Disomic tetraploids with a relative dose of 1/2 (same as monosomic diploids) also fail to express resistance, while trisomic tetraploids with a relative dose of 3/4 express resistance. Full tetraploids (relative dose of 1) appeared to be somewhat more resistant than trisomes. Although the mechanism for this variable expression is unknown, these results are suggestive of a competition reaction of gene products of cryl and of another locus on a different chromosome. GRANT, SANCHEZ, and JIMENEX (1974) have attributed cryptopleurine resistance in yeast to an alteration of its 40S ribosomal subunit, produced by cryl. They also observed that resistant ribosomes and their activity in the presence of cryptopleurine is also partially reduced. Being consistant with these phenomena, suppose that the resistant

cryl subunit and a similar subunit produced by another unlinked locus require the same factor necessary for function (possibly the 60S subunit). The level of activity by the resistant ribosomal complex is determined by competition for this factor, presumably controlled by the dosage of loci for the two 40S ribosomal subunits. Should the activity of the resistant complex fall below some threshhold level in the presence of cryptopleurine, the cell becomes inviable; while the amount of activity above this level would allow increased resistance. If the ribosomal complex to which the cryl locus contributes is specialized such that only certain proteins are synthesized, then the competition may be at the protein level.

This expression of cryl is more than just a puzzle for the molecular biologist. Its ability to distinguish monosomic diploids from full diploids makes it invaluable as a genetic marker. A study of chromosome loss such as that by CAMPBELL, FOGEL, and LUSNAK (1975) can be done with much greater ease, since mating monosomes, derived from non-disjunction, can be easily identified from mating diploids derived from mitotic crossing-over or gene conversion. Studies of chromosomal gain, previously impractical to study because homozygous disomes resulting from non-disjunction would appear just as their monosomic predecessors, are now feasible. Cryl can further be used in trisomes to verify segregation patterns. Likewise, they can be used in tetraploids to study tetravalent segregation and meiotic non-disjunction rates. Besides the variety of ways in which to use this unique aspect of cryl, its linkage to matl and its drug resistance makes it suitable for a number of genetic studies and selection schemes. Therefore, cryl is an exceptionally useful genetic tool, as well as a means for further understanding of the biochemistry of ribosomes.

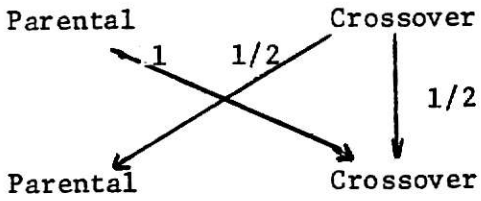
Appendix I

THE MAXIMUM VALUE FOR THE RECOMBINANT CLASS

The maximum value for the recombinant class for the gene-centromere interval can be determined once segregation patterns are known and the equilibrium of chromatid configuration is established for multiple changes. In the disomic case the parental configuration always gives a crossover configuration with an additional exchange. On the other hand, the crossover configuration gives one-half crossover and one-half parental configurations with additional exchange (See Figure 6). When the fraction of parental configurations becoming crossover configurations equals the fraction of crossover configurations becoming parental configurations, equilibrium between the two configurations is achieved (See Equation in Figure 1). The fraction of the crossover configurations, f_d, necessary to sustain the equilibrium is 2/3. Since a recombinant class is segregated by every crossover configuration, 2/3 is the maximum fraction that the recombinant class (often referred to as SDS, second segregation) can have in a disome. Thus it is said the markers go at random (i.e., the markers appear to be at very large distances from the centromere) when SDS equals 2/3, assuming no interference. If all crossover configurations are assumed to be produced by single crossovers (complete interference), then as the map distance becomes 33.3cM as SDS becomes 66.7%. Since strong interference is occurring in disomes, SDS approaches 2/3 much faster than expected assuming no interference.

FIGURE 6

EFFECT OF ADDITIONAL EXCHANGES IN DISOMES

Configuration	Parental	Crossover
Representation	+ / +	+ / -
	- / -	+ / -
Fraction	(1 - fd)	(fd)
Result of one addition exchange		
Equilibrium Equation	1(1 - fd) = 1/2 fd	
Solution	fd = 2/3	

The presence of interference is observed well before a map distance of 33.3cM.

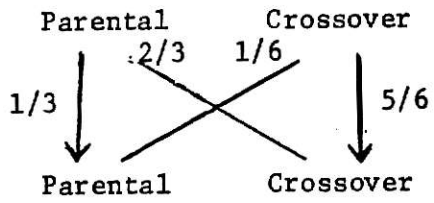
Similarly, the maximum fraction of the recombinant class in trisomes ($3{:}1_m$ and $3{:}1_d$ for auxotrophs in one dose) can be determined. For an additional exchange in a trisome, only 2/3 of the parental configurations become crossover configurations and 1/6 for the converse. (See Figure 7). The fraction of the crossover configurations, f_t, achieving the equilibrium is 4/5. Since 5/6 of the crossover configurations segregate into the 3:1 (where 3:1 refers to $3{:}1_m$ and $3{:}1_d$) recombinant class (assuming trivalent segregation), the markers in a trisome go at random when the 3:1 class equals to 2/3. Should all the crossover configurations be obtained by single crossovers (complete interference), and keeping in mind that only 2/3 of the single crossovers produced are in the crossover configuration, a mean frequency of exchange $\underline{x}$ equal to 120% would be required to yield a 66.7% value for the 3:1 class. However, the exchange frequency for singles can be no greater than 100%; 5/8 would be the maximum fraction for the 3:1 class. Thus if interference is operating in trisomes, deviation in observed $\underline{x}$ values is expected to be seen well before the $\underline{x}$ value of 100% predicted from a non-interference model.

FIGURE 7

EFFECT OF ADDITIONAL EXCHANGES IN TRISOMES

Configuration	Parental	Crossover
Representation	+ / +	+ / -
	+ / +	+ / -
	- / -	+ / +
Fraction	$(1 - f_t)$	f_t

Result of one additional exchange



Equilibrium Eq. Solution

$$2/3(1 - f_t) = 1/6(f_t)$$

$$f_t = 4/5$$

Appendix II

SAMPLE OF INPUT DATA FOR AND LISTING 'TRISOM'

Input data for 'TRISOM'

A 1) Define these variables

LOCI, the number of loci.

CENTPOS, the centromere position.

MTPOS1, the mating-type position for one dose of ALPHA.

MTPOS2, the mating-type position for two doses of ALPHA.

2) These variables may be also defined (but have default values),

SLIMIT (.001), the probability limit below which further crossing over is no longer considered.

MAXCOM (0), the number of intervals to be tabulated.

LM (5), the length of marker.

MTPHENO ('_ _ A _ _'), the mating-type symbol (i.e. another representation of A is +–).

PRINT ('ON'), the printing of every possible configuration and classes of asci (tabulated results are always printed).

WT ('_ _ + _ _') the wild-type symbol.

MULTCO (3), the number of multiple crossovers to be tabulated.

3) Follow these data with a semicolon.

e.g. LOCI = 2, CENTPOS = 2, MTPOS2 = 3, MAXCOM = 3, PRINT = 'OFF';

there are two intervals, one on each side of centromere.

B List the intervals to be tabulated according to the two marker positions (consider the centromere as a marker).

e.g. 1,3 2,3 1,2

C List the parental configuration of the trisome without the centromere.

e.g. '__ __ + __ __ __ __ A __ __', 'LEU2__ALPHA', '__ __ + __ __ ALPHA'

D List the frequency of exchange in order of occurrence of the intervals.

e.g. .36, 1.2

where .36 is for the leu2-centromere interval and 1.2 is for the centromere-mat1 interval.

```
TRISOM:  PROC OPTIONS(MAIN);                                                                    1   1
         DCL(NSTR,PLOIDY,LOCI,LM,LT,CENTPOS,CENTRO,ILIMIT)BIN FIXED(15);                        1   2
         DCL SLIMIT DEC FLOAT(6);                                                               1   3
         DCL CTRMERF CHAR(5)INIT('__.__');                                                      1   4
         DCL MULTCO INIT(4)BIN FIXED(15);                                                       1   5
         DCL MAXCOM BIN FIXED(15);                                                              1   6
         DCL(POS1(20),POS2(20))BIN FIXED(15);                                                   1   7
         DCL(MTPOS1,MTPOS2)BIN FIXED(15);                                                       1   8
         DCL PRINT INIT('ON')CHAR(3);                                                           1   9
         DCL MTPHENO INIT('  A  ')CHAR(5);                                                     1  10
         DCL WT INIT('  +  ')CHAR(5);                                                         1  11
         ON ENDFILE(SYSIN)                                                                      1  12
         GO TO SEND;                                                                            1  13
NEXT:    NSTR=6;                                                                                1  14
         PLOIDY=3;                                                                              1  15
         MAXCOM=0;                                                                              1  16
         POS1(*),POS2(*)=0;                                                                     1  17
         LM=5;                                                                                  1  18
         SLIMIT=.01;                                                                            1  19
         MTPOS1=0;                                                                              1  20
         MTPOS2=0;                                                                              1  21
         GET FILE(SYSIN)DATA(LOCI,CENTPOS,MTPOS1,MTPOS2,SLIMIT,MAXCOM,LM,MTPHENO,PRINT,WT,MULTCO);  1  22
            DO J=1 TO MAXCOM;                                                                   2  23
            GET FILE(SYSIN)LIST(POS1(J),POS2(J));                                               2  24
            END;                                                                                2  25
         ALIMIT=SLIMIT;                                                                         1  26
         ILIMIT=0;                                                                              1  27
ILIM:       IF ALIMIT<1 THEN                                                                    2  28
               DO;                                                                              3  29
               ALIMIT=10.*ALIMIT;                                                               3  30
               ILIMIT=ILIMIT+1;                                                                 3  31
               GO TO ILIM;                                                                      3  32
               END;                                                                             3  33
         CENTRO=CENTPOS;                                                                        1  34
         CENTPOS=CENTPOS*LM;                                                                    1  35
         LT=LM*(LOCI+1);                                                                        1  36
MAIN1:      BEGIN;                                                                              2  37
            DCL PROBINT(LOCI)INIT((LOCI)(0.0))DEC FLOAT(6);                                     2  38
            DCL PARENT(PLOIDY)CHAR(LT);                                                         2  39
            DCL TID(NSTR)CHAR(LT);                                                              2  40
            DCL ICOPOS CHAR(LT)INIT('                                                  ');      2  41
            DCL PROBICO(MULTCO)INIT((MULTCO)(0.0))DEC FLOAT(6);                                 2  42
            DCL PROBIREG(MAXCOM,MULTCO)INIT((MAXCOM*MULTCO)(0.0))DEC FLOAT(6);                  2  43
            DCL PHENO(LOCI+1)CHAR(LM)INIT((LOCI+1)'  +  ');                                     2  44
            DCL PROBASC(MAXCOM,4,4)INIT((16*MAXCOM)(0.0))DEC FLOAT(6);                          2  45
            DCL PROBXASC(MAXCOM,4,4,MULTCO)INIT((16*MAXCOM*MULTCO)(0.0))DEC FLOAT(6);           2  46
            DCL PROBXREG(MAXCOM,4,4,MULTCO)INIT((16*MAXCOM*MULTCO)(0.0))DEC FLOAT(6);           2  47
            DCL SUMPROB(MAXCOM)INIT((MAXCOM)(0.0))DEC FLOAT(6);                                 2  48
            DCL SUMXPROB(MAXCOM,MULTCO)INIT((MAXCOM*MULTCO)(0.0))DEC FLOAT(6);                  2  49
            DCL SUMXREG(MAXCOM,MULTCO)INIT((MAXCOM*MULTCO)(0.0))DEC FLOAT(6);                   2  50
            DCL OTHRPROB(MAXCOM)INIT((MAXCOM)(0.0))DEC FLOAT(6);                                2  51
            DCL ASCTYPE(MAXCOM,4,4,2)CHAR(5);                                                   2  52
            DCL HEADING(MAXCOM)CHAR(2*LM);                                                      2  53
```

```
                DCL(IPOS1(MAXCCM),IPOS2(MAXCCM))BIN FIXED(15);                                                    2  54
                   IF MTPOS1>0 THEN                                                                               3  55
                   PHENO(MTPOS1)=MTPHENO;                                                                         3  56
                   IF MTPOS2>0 THEN                                                                               3  57
                   PHENO(MTPOS2)=MTPHENO;                                                                         3  58
                GET FILE(SYSIN)LIST((PARENT(J)DO J=1 TO PLOIDY));                                                 2  59
                GET FILE(SYSIN)LIST((PROBINT(J)DO J=1 TO LOCI));                                                  2  60
                   DO J=1 TO PLOIDY;                                                                              3  61
                   TID(2*J-1),TID(2*J)=SUBSTR(PARENT(J),1,CENTPOS-LM)||CTRMERE||SUBSTR(PARENT(J),CENTPOS-LM+1);   3  62
                   END;                                                                                           3  63
                CALL ASCINIT;                                                                                     2  64
                PROB1=1.;                                                                                         2  65
                PROBICO(1)=1.;                                                                                    2  66
                PROBIREG(*,1)=1.;                                                                                 2  67
                LN=LM;                                                                                            2  68
                   IF LN=CENTPOS THEN                                                                             3  69
                   LN=LT-LM;                                                                                      3  70
                CALL RECOMB(TID,LN,PROB1,ICOPOS,PROBICO,PROBIREG);                                                2  71
RECOMB:            PROC(TID,POS,PROB,COPOS,PROBXCO,REGX)RECURSIVE;                                                3  72
                   DCL TID(*)CHAR(*);                                                                             3  73
                   DCL COPOS CHAR(*);                                                                             3  74
                   DCL(POS,I,ND,NPOS)BIN FIXED(15);                                                               3  75
                   DCL PROB DEC FLOAT(6);                                                                         3  76
                   DCL PROBXCO(*)DEC FLOAT(6);                                                                    3  77
                   DCL REGX(*,*)DEC FLOAT(6);                                                                     3  78
                      IF PROB<SLIMIT THEN                                                                         4  79
                      RETURN;                                                                                     4  80
                      IF POS=LT THEN                                                                              4  81
                         DO;                                                                                      5  82
                            IF PRINT='ON' THEN                                                                    6  83
                            CALL TABCO(TID,COPOS,PROB,PROBXCO);                                                   6  84
                         CALL TRISEG(TID,PROB,PHENO,PROBXCO,REGX);                                                5  85
                         RETURN;                                                                                  5  86
                         END;                                                                                     5  87
                      IF POS<CENTPOS THEN                                                                         4  88
                         DO;                                                                                      5  89
                         NPOS=POS+LM;                                                                             5  90
                            IF NPOS=CENTPOS THEN                                                                  6  91
                               IF CENTPOS<LT THEN                                                                 7  92
                               NPOS=LT-LM;                                                                        7  93
                               ELSE                                                                               7  94
                               NPOS=LT;                                                                           7  94
                         END;                                                                                     5  95
                      ELSE                                                                                        4  96
                         DO;                                                                                      5  96
                         NPOS=POS-LM;                                                                             5  97
                            IF POS=CENTPOS THEN                                                                   6  98
                            NPOS=LT;                                                                              6  99
                         END;                                                                                     5 100
                   ISUB=POS/LM;                                                                                   3 101
                   PCO=PROBINT(ISUB);                                                                             3 102
                      DO J=1 TO MAXCCM;                                                                           4 103
                         IF POS1(J)<POS2(J)THEN                                                                   5 104
```

```
                DO;                                                                                                  6  105
                IPOS1(J)=POS1(J)*LM;                                                                                 6  106
                IPOS2(J)=(POS2(J)-1)*LM;                                                                             6  107
                END;                                                                                                 6  108
             ELSE                                                                                                    5  109
                DO;                                                                                                  6  109
                IPOS1(J)=POS2(J)*LM;                                                                                 6  110
                IPOS2(J)=(POS1(J)-1)*LM;                                                                             6  111
                END;                                                                                                 6  112
          END;                                                                                                       4  113
       ND=1;                                                                                                         3  114
       NDES=13;                                                                                                      3  115
STR6:  BEGIN;                                                                                                        4  116
          DCL NEWTID(NSTR,NDES)CHAR(LT);                                                                             4  117
          DCL PROBCO(NDES)DEC FLOAT(6);                                                                              4  118
          DCL NPROBXCO(NDES,MULTCO)INIT((MULTCO*NDES)(0.0))DEC FLOAT(6);                                             4  119
          DCL NREGX(NDES,MAXCOM,MULTCO)INIT((NDES*MAXCOM*MULTCO)(0.0))DEC FLOAT(6);                                  4  120
          DCL IEXCH(MAXCOM)INIT((MAXCOM)(0))BIN FIXED(15);                                                           4  121
          DCL NCOPOS(NDES)CHAR(LT);                                                                                  4  122
          NEWTID(*,ND)=TID(*);                                                                                       4  123
          NCOPOS(ND)=COPOS;                                                                                          4  124
          PROBCO(ND)=PROB*(1.-PCO);                                                                                  4  125
             DO J=1 TO MULTCO;                                                                                       5  126
             NPROBXCO(ND,J)=PROBXCO(J)*(1.-PCO);                                                                     5  127
             END;                                                                                                    5  128
             DO J=1 TO MAXCOM;                                                                                       5  129
                IF POS>=IPOS1(J)&POS<=IPOS2(J)THEN                                                                   6  130
                IEXCH(J)=1;                                                                                          6  131
                DO JJ=1 TO MULTCO;                                                                                   6  132
                NREGX(1,J,JJ)=NREGX(1,J,JJ)+REGX(J,JJ)*(1.-PCO);                                                     6  133
                END;                                                                                                 6  134
             END;                                                                                                    5  135
          PCO=PCO/12.;                                                                                               4  136
          CALL SETEQ(TID,ISETEQ);                                                                                    4  137
COSTR1:      DO JCO1=1,3;                                                                                            5  138
             NEQPAR1=0;                                                                                              5  139
             ICO1=JCO1;                                                                                              5  140
             NDPAR1=ND+1;                                                                                            5  141
             INTRA1=1;                                                                                               5  142
COSTR2:         DO JCO2=JCO1+2 TO 5 BY 2;                                                                            6  143
                NDPAR2=ND+1;                                                                                         6  144
                ICO2=JCO2;                                                                                           6  145
                INTRA2=1;                                                                                            6  146
TESTPAR:        NEQPAR2=0;                                                                                           6  147
                LCO1=JCO1;                                                                                           6  148
                   IF ICO1=JCO1 THEN                                                                                 7  149
                   LCO1=JCO1+1;                                                                                      7  150
                LCO2=JCO2;                                                                                           6  151
                   IF ICO2=JCO2 THEN                                                                                 7  152
                   LCO2=JCO2+1;                                                                                      7  153
                   IF TID(LCO1)=TID(LCO2)THEN                                                                        7  154
                   GO TO PAR;                                                                                        7  155
                   IF POS<CENTPOS THEN                                                                               7  156
```

```
                    IF SUBSTR(TID(ICO1),1,POS)=SUBSTR(TID(ICO2),1,POS)THEN                  8  157
                    GO TO PAR;                                                              8  158
                    ELSE                                                                    8  159
                    GO TO GENCO;                                                            8  159
                 IF SUBSTR(TID(ICO1),POS+1)=SUBSTR(TID(ICO2),POS+1)THEN                     7  160
                    DO;                                                                     8  161
PAR:                PROBCO(1)=PROBCO(1)+PROB*PCO;                                           8  162
                       DO J=2 TO MULTCO;                                                    9  163
                          DO JJ=1 TO MAXCOM;                                               10  164
                             IF IEXCH(JJ)=1 THEN                                           11  165
                             NREGX(1,JJ,J)=NREGX(1,JJ,J)+REGX(JJ,J-1)*PCO;                 11  166
                             ELSE                                                          11  167
                             NREGX(1,JJ,J-1)=NREGX(1,JJ,J-1)+REGX(JJ,J-1)*PCO;             11  167
                          END;                                                             10  168
                       NPROBXCO(1,J)=NPROBXCO(1,J)+PROBXCO(J-1)*PCO;                        9  169
                       END;                                                                 9  170
                    NEQPAR1=NEQPAR1+1;                                                      8  171
                    NEQPAR2=NEQPAR2+1;                                                      8  172
                    GO TO TRA2;                                                             8  173
                    END;                                                                    8  174
GENCO:           DO;                                                                        7  175
                 ND=ND+1;                                                                   7  176
                 NEWTID(*,ND)=TID(*);                                                       7  177
                 KCO1=ICO1;                                                                 7  178
                 KCO2=ICO2;                                                                 7  179
                    IF POS<CENTPOS THEN                                                     8  180
                       DO;                                                                  9  181
                       KCO1=ICO2;                                                           9  182
                       KCO2=ICO1;                                                           9  183
                       END;                                                                 9  184
                 NEWTID(ICO1,ND)=SUBSTR(TID(KCO1),1,POS)||SUBSTR(TID(KCO2),POS+1);          7  185
                 NEWTID(ICO2,ND)=SUBSTR(TID(KCO2),1,POS)||SUBSTR(TID(KCO1),POS+1);          7  186
                 PROBCO(ND)=PROB*PCO;                                                       7  187
                    DO J=2 TO MULTCO;                                                       8  188
                       DO JJ=1 TO MAXCOM;                                                   9  189
                          IF IEXCH(JJ)=1 THEN                                              10  190
                          NREGX(ND,JJ,J)=NREGX(ND,JJ,J)+REGX(JJ,J-1)*PCO;                  10  191
                          ELSE                                                             10  192
                          NREGX(ND,JJ,J-1)=NREGX(ND,JJ,J-1)+REGX(JJ,J-1)*PCO;              10  192
                       END;                                                                 9  193
                    NPROBXCO(ND,J)=NPROBXCO(ND,J)+PROBXCO(J-1)*PCO;                         8  194
                    END;                                                                    8  195
                 NCOPOS(ND)=COPOS;                                                          7  196
                 SUBSTR(NCOPOS(ND),POS,1)='*';                                              7  197
                 END GENCO;                                                                 7  198
                 IF INTRA2=1 THEN                                                           7  199
TRA2:               DO;                                                                     8  200
TESTRA2:            INTRA2=2;                                                               8  201
                       IF TID(ICO2)=TID(ICO2+1)&NEQPAR2¬=1 THEN                             9  202
                          DO;                                                              10  203
                          PROBCO(ND)=2.*PROBCO(ND);                                        10  204
                          NPROBXCO(ND,*)=2.*NPROBXCO(ND,*);                                10  205
                             DO JJ=1 TO MAXCOM;                                            11  206
```

```
                        NREGX(ND,JJ,*)=2.*NREGX(ND,JJ,*);                                  11  207
                        ENC;                                                               11  208
                     END;                                                                  10  209
                  ELSE                                                                      9  210
                     DO;                                                                   10  210
                     ICO2=ICO2+1;                                                          10  211
                     GO TO TESTPAR;                                                        10  212
                     END;                                                                  10  213
               END TESTRA2;                                                                 8  214
            IF ISETEQ=1&JCO2=5 THEN                                                         7  215
            GO TO EQSET;                                                                    7  216
            ELSE                                                                            7  217
               IF ISETEQ=2&JCO2=3 THEN                                                      8  217
               GO TO EQSET;                                                                 8  218
               ELSE                                                                         8  219
                  IF ISETEQ=3&JCO2=3 THEN                                                   9  219
EQSET:               DO;                                                                  10  220
                     PROBCO(1)=PROBCO(1)+NEQPAR2*PROB*PCO;                                 10  221
                        DO J=2 TO MULTCO;                                                  11  222
                           DO JJ=1 TO MAXCOM;                                              12  223
                              IF IEXCH(JJ)=1 THEN                                          13  224
                              NREGX(1,JJ,J)=NREGX(1,JJ,J)+REGX(JJ,J-1)*PCO*NEQPAR2;        13  225
                              ELSE                                                         13  226
                              NREGX(1,JJ,J-1)=NREGX(1,JJ,J-1)+REGX(JJ,J-1)*PCO*NEQPAR2;    13  226
                           END;                                                            12  227
                        NPROBXCO(1,J)=NPROBXCO(1,J)+PROBXCO(J-1)*PCO*NEQPAR2;              11  228
                        END;                                                               11  229
                        DO JS=NDPAR2 TO ND;                                                11  230
                        PROBCO(JS)=2.*PROBCO(JS);                                          11  231
                           DO JJ=1 TO MAXCOM;                                              12  232
                           NREGX(JS,JJ,*)=NREGX(JS,JJ,*)*2.;                               12  233
                           END;                                                            12  234
                        NPROBXCO(JS,*)=2.*NPROBXCO(JS,*);                                  11  235
                        END;                                                               11  236
                     END EQSET;                                                            10  237
               IF ISETEQ=3 THEN                                                             7  238
               GO TO TRA1;                                                                  7  239
         END COSTR2;                                                                        6  240
TRA1:    IF INTRA1=1 THEN                                                                   6  241
TESTRA1:    DO;                                                                             7  242
            INTRA1=2;                                                                       7  243
               IF TID(ICO1)¬=TID(ICO1+1)THEN                                                8  244
                  DO;                                                                       9  245
                  ICO1=ICO1+1;                                                              9  246
                  GO TO COSTR2;                                                             9  247
                  END;                                                                      9  248
               ELSE                                                                         8  249
                  DO;                                                                       9  249
                  PROBCO(1)=PROBCO(1)+NEQPAR1*PROB*PCO;                                     9  250
                     DO J=2 TO MULTCO;                                                     10  251
                        DO JJ=1 TO MAXCOM;                                                 11  252
                           IF IEXCH(JJ)=1 THEN                                             12  253
                           NREGX(1,JJ,J)=NREGX(1,JJ,J)+REGX(JJ,J-1)*PCO*NEQPAR1;           12  254
```

```
                              ELSE                                                                                    12  255
                                 NREGX(1,JJ,J-1)=NREGX(1,JJ,J-1)+REGX(JJ,J-1)*PCO*NEQPAR1;                            12  255
                              END;                                                                                    11  256
                           NPROBXCO(1,J)=NPROBXCO(1,J)+PROBXCO(J-1)*PCO*NEQPAR1;                                      10  257
                           END;                                                                                       10  258
                           DO JS=NDPAR1 TO ND;                                                                        10  259
                           PROBCO(JS)=2.*PROBCO(JS);                                                                  10  260
                              DO JJ=1 TO MAXCOM;                                                                      11  261
                              NREGX(JS,JJ,*)=NREGX(JS,JJ,*)*2.;                                                       11  262
                              END;                                                                                    11  263
                           NPROBXCC(JS,*)=2.*NPROBXCO(JS,*);                                                          10  264
                           END;                                                                                       10  265
                        END;                                                                                           9  266
                     END TESTRA1;                                                                                      7  267
                  IF ISETEQ=1|ISETEQ=2 THEN                                                                            6  268
                  GO TO DONE;                                                                                          6  269
               END COSTR1;                                                                                             5  270
DONE:          DO I=1 TO ND;                                                                                           5  271
               CALL RECCMB(NEWTID(*,I),NPOS,PRCBCO(I),NCOPOS(I),NPROBXCO(I,*),NREGX(I,*,*));                           5  272
               END;                                                                                                    5  273
            END STR6;                                                                                                  4  274
         END RECOMB;                                                                                                   3  275
TRISEG:  PROC(TID,PROBIN,PHENO,XCO,REGX);                                                                              3  276
         DCL TID(*)CHAR(*);                                                                                            3  277
         DCL PROBIN DEC FLOAT(6);                                                                                      3  278
         DCL PHENO(*)CHAR(*);                                                                                          3  279
         DCL XCO(*)DEC FLOAT(6);                                                                                       3  280
         DCL REGX(*,*)DEC FLOAT(6);                                                                                    3  281
         DCL POS BIN FIXED(15);                                                                                        3  282
         NDES=6;                                                                                                       3  283
Z:          BEGIN;                                                                                                     4  284
            DCL RATIC(LOCI+1)CHAR(5);                                                                                  4  285
            DCL SEGTID(NSTR,NDES)CHAR(LT);                                                                             4  286
            DCL TEMPTID CHAR(LT);                                                                                      4  287
            DCL SEGPRCB(NDES)DEC FLOAT(6);                                                                             4  288
            DCL DUEXCO(NDES,MULTCO)DEC FLOAT(6);                                                                       4  289
            DCL NREGX(NDES,MAXCOM,MULTCC)INIT((NDES*MAXCOM*MULTCO)(0.0))DEC FLOAT(6);                                  4  290
            ND=1;                                                                                                      4  291
            MI=1;                                                                                                      4  292
            NDESC=3;                                                                                                   4  293
            PROBIN=PROBIN/3.;                                                                                          4  294
            XCO(*)=XCC(*)/3.;                                                                                          4  295
            REGX(*,*)=REGX(*,*)/3.;                                                                                    4  296
            MIMAX=3;                                                                                                   4  297
               DO II=1 TC MIMAX;                                                                                       5  298
               SEGPROB(II)=PROBIN;                                                                                     5  299
               DUEXCO(II,*)=XCO(*);                                                                                    5  300
               NREGX(II,*,*)=REGX(*,*);                                                                                5  301
               END;                                                                                                    5  302
            CALL SETEQ(TID,ISETEQ);                                                                                    4  303
               IF ISETEQ=0 THEN                                                                                        5  304
               GO TO SEGMII;                                                                                           5  305
            NDESC=2;                                                                                                   4  306
```

```
                  MIMAX=2;                                                                  4  307
                     IF ISETEQ=1|ISETEQ=2 THEN                                              5  308
                        DO;                                                                 6  309
                        SEGPROB(ND)=2.*PROBIN;                                              6  310
                        DUEXCO(ND,*)=2.*XCO(*);                                             6  311
                        NREGX(ND,*,*)=2.*REGX(*,*);                                         6  312
                        END;                                                                6  313
                     ELSE                                                                   5  314
                        DO;                                                                 6  314
                        SEGPROB(ND+1)=2.*PROBIN;                                            6  315
                        DUEXCO(ND+1,*)=2.*XCC(*);                                           6  316
                        NREGX(ND+1,*,*)=2.*REGX(*,*);                                       6  317
                        END;                                                                6  318
SEGMI1:           NROLL=0;                                                                  4  319
SEGMI:               DO NS=1 TO NSTR;                                                       5  320
                     NR=NS+NROLL;                                                           5  321
                        IF NR>NSTR THEN                                                     6  322
                        NR=NR-NSTR;                                                         6  323
                     SEGTID(NS,ND)=TID(NR);                                                 5  324
                     END;                                                                   5  325
                  NROLL=2*MI;                                                               4  326
                     IF ISETEQ=1 THEN                                                       5  327
                     NROLL=4;                                                               5  328
                  IX=4;                                                                     4  329
                  IY=5;                                                                     4  330
                  MII=1;                                                                    4  331
                     IF SEGTID(3,ND)¬=SEGTID(4,ND)&SEGTID(5,ND)¬=SEGTID(6,ND)THEN           5  332
                        DO;                                                                 6  333
MII2P:                  MII=2;                                                              6  334
                        NDESC=NDESC+1;                                                      6  335
                           IF ND+1<NDESC THEN                                               7  336
                              DO ND1=NDESC TO ND+2 BY-1;                                    8  337
BUMPMII:                      SEGPROB(ND1)=SEGPROB(ND1-1);                                  8  338
                              DUEXCO(ND1,*)=DUEXCO(ND1-1,*);                                8  339
                              NREGX(ND1,*,*)=NREGX(ND1-1,*,*);                              8  340
                              END BUMPMII;                                                  8  341
                        SEGPROB(ND),SEGPROB(ND+1)=SEGPROB(ND)/2.;                           6  342
                        DUEXCO(ND,*),DUEXCO(ND+1,*)=DUEXCO(ND,*)/2.;                        6  343
                        NREGX(ND,*,*),NREGX(ND+1,*,*)=NREGX(ND,*,*)/2.;                     6  344
                        END MII2P;                                                          6  345
                  TEMPTID=SEGTID(IX,ND);                                                    4  346
SEGMII:           SEGTID(IX,ND)=SEGTID(IY,ND);                                              4  347
                  SEGTID(IY,ND)=TEMPTID;                                                    4  348
                  CALL PHENO6(SEGTID(*,ND),RATIO,PHENO);                                    4  349
                  CALL TCTPROB(RATIO,SEGPROB(ND),DUEXCO(ND,*),NREGX(ND,*,*));               4  350
                     IF PRINT='ON' THEN                                                     5  351
                     CALL TABSEG(SEGTID(*,ND),RATIO,SEGPROB(ND),DUEXCO(ND,*));              5  352
                     IF MII=2 THEN                                                          5  353
                        DO;                                                                 6  354
MII2:                   SEGTID(*,ND+1)=SEGTID(*,ND);                                        6  355
                        ND=ND+1;                                                            6  356
                        IY=6;                                                               6  357
                        MII=3;                                                              6  358
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```
                        GO TO SEGMII;                                    6  359
                        END MII2;                                        6  360
                     ND=ND+1;                                            4  361
                     MI=MI+1;                                            4  362
                        IF MI<=MIMAX THEN                                5  363
                        GO TO SEGMI;                                     5  364
                     END Z;                                              4  365
                  END TRISEG;                                            3  366
SETEQ:            PROC(TID,ISETEQ);                                      3  367
                  DCL TID(*)CHAR(*);                                     3  368
                  DCL ISETEQ BIN FIXED(15);                              3  369
                  ISETEQ=0;                                              3  370
                     DO IE1=1 TO 2;                                      4  371
E1:                     DO IE2=IE1+1 TO 3;                               5  372
E2:                     IS1=2*IE1-1;                                     5  373
                        IS2=2*IE2-1;                                     5  374
                           IF TID(IS1)¬=TID(IS2)THEN                     6  375
                           GO TO OTHPOS;                                 6  376
                           ELSE                                          6  377
                              IF TID(IS1+1)¬=TID(IS2+1)THEN              7  377
                              GO TO FAIL;                                7  378
                              ELSE                                       7  379
                              GO TO EQUV;                                7  379
OTHPOS:                    IF TID(IS1)¬=TID(IS2+1)THEN                   6  380
                           GO TO FAIL;                                   6  381
                           ELSE                                          6  382
                              IF TID(IS1+1)¬=TID(IS2)THEN                7  382
                              GO TO FAIL;                                7  383
EQUV:                   ISETEQ=IE1+IE2-2;                                5  384
                        GO TO QUIT;                                      5  385
FAIL:                   END E2;                                          5  386
                     END E1;                                             4  387
QUIT:             END SETEQ;                                             3  388
PHENO6:           PROC(TID,RATIO,PHENO);                                 3  389
                  DCL TID(*)CHAR(*);                                     3  390
                  DCL(RATIO(*),PHENO(*))CHAR(*);                         3  391
                  DCL POS BIN FIXED(15);                                 3  392
                  DCL PLUS INIT('+')CHAR(1);                             3  393
                  DCL(LIFE,LETHAL,IPHENO1,IPHENO2,IPHENO3)DEC FIXED(1,0);3  394
                  DCL(CLF,CLH,CPHEN1,CPHEN2,CPHEN3,STAR)CHAR(1);         3  395
ALLRATIOS:           DO J=1 TO LOCI+1;                                   4  396
                     RATIO(J)='     ';                                   4  397
                        IF J=CENTRO THEN                                 5  398
                           DO;                                           6  399
                           ;                                             6  400
                           RATIO(J)='4:0  ';                             6  401
                           GO TO DONE;                                   6  402
                           END;                                          6  403
                     POS=(J-1)*LM+1;                                     4  404
                     IPHENO1=0;                                          4  405
                     IPHENO2=0;                                          4  406
                     IPHENO3=0;                                          4  407
                        DO I1=1 TO 2;                                    5  408
```

```
                  IF SUBSTR(TID(I1),POS,LM)=PHENO(J)THEN                    6  409
                  IPHENC1=IPHENC1+1;                                        6  410
                  ELSE                                                      6  411
                  IPHENO2=IPHENO2+1;                                        6  411
               END;                                                         5  412
            STAR=' ';                                                       4  413
DISOM:         DO I2=3,5;                                                   5  414
                  IF SUBSTR(TID(I2),POS,LM)=PHENO(J)THEN                    6  415
                  GO TO OTHTID;                                             6  416
                  ELSE                                                      6  417
                     IF SUBSTR(TID(I2+1),POS,LM)¬=PHENO(J)THEN              7  417
                        DO;                                                 8  418
                        IPHENC2=IPHENC2+1;                                  8  419
                        STAR='*';                                           8  420
                        END;                                                8  421
                     ELSE                                                   7  422
                     IPHENO3=IPHENO3+1;                                     7  422
                  GO TO DI;                                                 5  423
OTHTID:           IF SUBSTR(TID(I2+1),POS,LM)=PHENO(J)THEN                  6  424
                  IPHENC1=IPHENO1+1;                                        6  425
                  ELSE                                                      6  426
                  IPHENO3=IPHENO3+1;                                        6  426
DI:            END DISOM;                                                   5  427
               IF(IPHENO1+IPHENO2+IPHENO3)¬=4 THEN                          5  428
               PUT EDIT('ERROR IN IPHENOS')(A);                             5  429
            LIFE=0;                                                         4  430
            LETHAL=0;                                                       4  431
R:             DO IPOS=1 TO LM;                                             5  432
                  IF SUBSTR(PHENO(J),IPOS,1)=PLUS THEN                      6  433
PLUSDOM:             DO;                                                    7  434
                     LIFE=IPHENC1+IPHENO3;                                  7  435
                     LETHAL=IPHENO2;                                        7  436
                     PUT STRING(CLF)EDIT(LIFE)(F(1));                       7  437
                     PUT STRING(CLH)EDIT(LETHAL)(F(1));                     7  438
                     RATIO(J)=CLF||':'||CLH||STAR;                          7  439
                     GO TO DONE;                                            7  440
                     END PLUSDOM;                                           7  441
               END R;                                                       5  442
            PUT STRING(CPHEN1)EDIT(IPHENO1)(F(1));                          4  443
            PUT STRING(CPHEN2)EDIT(IPHENO2)(F(1));                          4  444
            PUT STRING(CPHEN3)EDIT(IPHENO3)(F(1));                          4  445
            RATIO(J)=CPHEN1||':'||CPHEN2||':'||CPHEN3;                      4  446
DONE:       END ALLRATIOS;                                                  4  447
         END PHENO6;                                                        3  448
ASCINIT: PROC;                                                              3  449
         DCL ASCPLUS(4)INIT('4:0  ','2:2  ','3:1  ','3:1* ')CHAR(5);        3  450
         DCL ASCMT1(4)INIT('2:0:2','2:2:0','2:1:1','3:1:0')CHAR(5);         3  451
         DCL ASCMT2(4)INIT('0:2:2','2:2:0','1:2:1','1:3:0')CHAR(5);         3  452
         DCL(ASCTEMP1(4),ASCTEMP2(4))CHAR(5);                               3  453
         DCL(HEAD1,HEAD2)CHAR(LM);                                          3  454
         ASCTEMP1(*),ASCTEMP2(*)=ASCPLUS(*);                                3  455
            DO J=1 TO MAXCOM;                                               4  456
               IF POS1(J)=MTPOS1 THEN                                       5  457
```

```
                ASCTEMP1(*)=ASCMT1(*);                                    5  458
                IF POS1(J)=MTPOS2 THEN                                    5  459
                ASCTEMP1(*)=ASCMT2(*);                                    5  460
                IF POS2(J)=MTPOS1 THEN                                    5  461
                ASCTEMP2(*)=ASCMT1(*);                                    5  462
                IF POS2(J)=MTPOS2 THEN                                    5  463
                ASCTEMP2(*)=ASCMT2(*);                                    5  464
                DO J1=1 TO 4;                                             5  465
                   DO J2=1 TO 4;                                          6  466
                   ASCTYPE(J,J1,J2,1)=ASCTEMP1(J1);                       6  467
                   ASCTYPE(J,J1,J2,2)=ASCTEMP2(J2);                       6  468
                   END;                                                   6  469
                END;                                                      5  470
             JPOS1=(POS1(J)-1)*LM+1;                                      4  471
             JPOS2=(POS2(J)-1)*LM+1;                                      4  472
                DO JJ=1 TO 5 BY 2;                                        5  473
                HEAD1=SUBSTR(TID(JJ),JPOS1,LM);                           5  474
                   IF HEAD1¬=PHENO(POS1(J))THEN                           6  475
                   GO TO CN1;                                             6  476
                END;                                                      5  477
CN1:            DO JJ=1 TO 5 BY 2;                                        5  478
                HEAD2=SUBSTR(TID(JJ),JPOS2,LM);                           5  479
                   IF HEAD2¬=PHENO(POS2(J))THEN                           6  480
                   GO TO CN2;                                             6  481
                END;                                                      5  482
ON2:         HEADING(J)=HEAD1||HEAD2;                                     4  483
          END ASCINIT;                                                    3  484
TOTPROB:  PROC(RATIO,PROB,PRCBS,PRCBR);                                   3  486
          DCL RATIO(*)CHAR(*);                                            3  487
          DCL(PROB,PRCBS(*),PROBR(*,*))DEC FLOAT(6);                      3  488
             DO J=1 TO MAXCOM;                                            4  489
                DO J1=1 TO 4;                                             5  490
                   IF RATIO(POS1(J))=ASCTYPE(J,J1,1,1)THEN                6  491
                      DO;                                                 7  492
                      P1=J1;                                              7  493
                      GO TO ON1;                                          7  494
                      END;                                                7  495
                END;                                                      5  496
             CTHRPROB(J)=CTHRPROB(J)+PRCB;                                4  497
             RETURN;                                                      4  498
CN1:            DO J2=1 TO 4;                                             5  499
                   IF RATIO(POS2(J))=ASCTYPE(J,1,J2,2)THEN                6  500
                      DO;                                                 7  501
                      P2=J2;                                              7  502
                      GO TO ON2;                                          7  503
                      END;                                                7  504
                END;                                                      5  505
             OTHRPROB(J)=CTHRPROB(J)+PROB;                                4  506
             RETURN;                                                      4  507
CN2:         PROBASC(J,P1,P2)=PROBASC(J,P1,P2)+PROB;                      4  508
             PROBXASC(J,P1,P2,*)=PROBXASC(J,P1,P2,*)+PROBS(*);            4  509
             PROBXREG(J,P1,P2,*)=PROBXREG(J,P1,P2,*)+PROBR(J,*);          4  510
             SUMPROB(J)=SUMPROB(J)+PROB;                                  4  511
```

```
                  SUMXPROB(J,*)=SUMXPROB(J,*)+PROBS(*);                                                    4  512
                  SUMXREG(J,*)=SUMXREG(J,*)+PROBR(J,*);                                                    4  513
               END TOTPROB;                                                                                3  514
TABCC:         PROC(TID,COPOS,PROB,PROBS);                                                                 3  516
               DCL TID(*)CHAR(*);                                                                          3  517
               DCL COPOS CHAR(*);                                                                          3  518
               DCL PROB DEC FLOAT(6);                                                                      3  519
               DCL PROBS(*)DEC FLOAT(6);                                                                   3  520
               L=ILIMIT+3;                                                                                 3  521
               K=ILIMIT+1;                                                                                 3  522
               PUT FILE(SYSPRINT)EDIT(TID(*),PROB,PROBS(*),COPOS,' ')(SKIP,2(2(A,SKIP),SKIP),A,SKIP,A,5(X(5  3  523
               ),F(L,K)),SKIP,A,SKIP(3),A);                                                                3  523
               END TABCO;                                                                                  3  524
TABSEG:        PROC(TID,RATIO,PROB,PROBS);                                                                 3  525
               DCL TID(*)CHAR(*);                                                                          3  526
               DCL RATIO(*)CHAR(*);                                                                        3  527
               DCL PROB DEC FLOAT(6);                                                                      3  528
               DCL PROBS(*)DEC FLOAT(6);                                                                   3  529
               ICL=25;                                                                                     3  530
               L=ILIMIT+3;                                                                                 3  531
               K=ILIMIT+1;                                                                                 3  532
               PUT FILE(SYSPRINT)EDIT(TID(*),PROB,PROBS(*),RATIO(*),' ')(SKIP,2(COL(ICL),A,SKIP(2)),2(COL(I  3  533
               CL),A,SKIP),SKIP,COL(ICL),A,SKIP,COL(ICL),A,5(X(5),F(L,K)),SKIP,COL(ICL),(LOCI+1)(A),SKIP(2)  3  533
               ,A);                                                                                        3  533
               END TABSEG;                                                                                 3  534
TABTOT:        DO J=1 TO MAXCOM;                                                                           3  535
               PUT FILE(SYSPRINT)PAGE EDIT(HEADING(J),'SUBTOTAL     ZERO  ONE   TWO   THREE ')(A(10),X(5),A  3  536
               (36));                                                                                      3  536
                  DO J1=1 TO 4;                                                                            4  537
                     DO J2=1 TO 4;                                                                         5  538
                        IF PROBASC(J,J1,J2)>0.0 THEN                                                       6  539
                        PUT FILE(SYSPRINT)EDIT(ASCTYPE(J,J1,J2,1),ASCTYPE(J,J1,J2,2),100.*PROBASC(J,J1,J2),  6  540
                        100.*PROBXASC(J,J1,J2,*),100.*PROBXREG(J,J1,J2,*))(SKIP(2),A(5),A(5),X(6),F(5,2),2(  6  540
                        X(7),(MULTCO)(F(5,2),X(1))));                                                      6  540
                     END;                                                                                  5  541
                  END;                                                                                     4  542
               PUT FILE(SYSPRINT)EDIT('TOTAL %',100.*SUMPROB(J),100.*SUMXPROB(J,*),100.*SUMXREG(J,*))(SKIP(  3  543
               2),A(7),X(8),F(6,2),2(X(7),(MULTCO)(F(6,2))));                                              3  543
               PUT FILE(SYSPRINT)EDIT('OTHER ',100.*OTHRPROB(J))(SKIP(2),A(6),X(9),F(6,2));                3  544
               PUT FILE(SYSPRINT)EDIT(PARENT,PROBINT)(SKIP(5),(3)(SKIP(2),A),SKIP(2),X(2),(LOCI)(F(5,2)));  3  545
               END;                                                                                        3  546
            END MAIN1;                                                                                     2  547
         PUT PAGE;                                                                                         1  548
         GO TO NEXT;                                                                                       1  549
SEND:    PUT PAGE;                                                                                         1  550
         END TRISCM;                                                                                       1  551
```

Appendix III

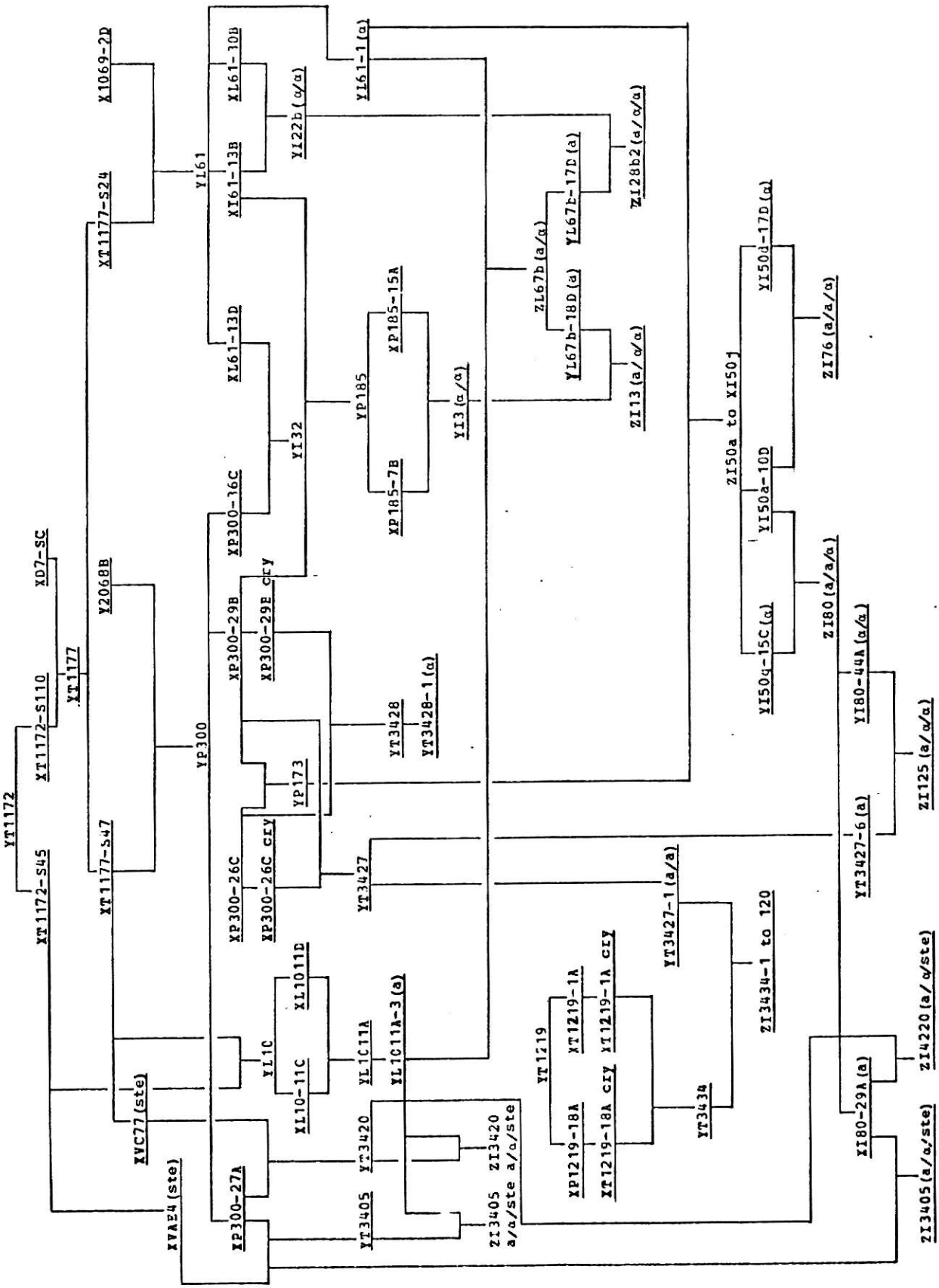
NOMENCLATURE FOR GENETIC MARKERS

Nutritional Requirement	Symbol
Adenine	ade
Histidine	his
Leucine	leu
Lysine	lys
Methionine	met
Threonine	thr
Tryptophan	trp
Uracil	ura

Others	
Canavanine (resistance)	can
Cryptopleurine (resistance)	cry
Galactose (loss of fermentation ability)	gal
Mating-type	a,α
Sterile	ste

APPENDIX IV

PEDIGREE OF SOME STRAINS USED



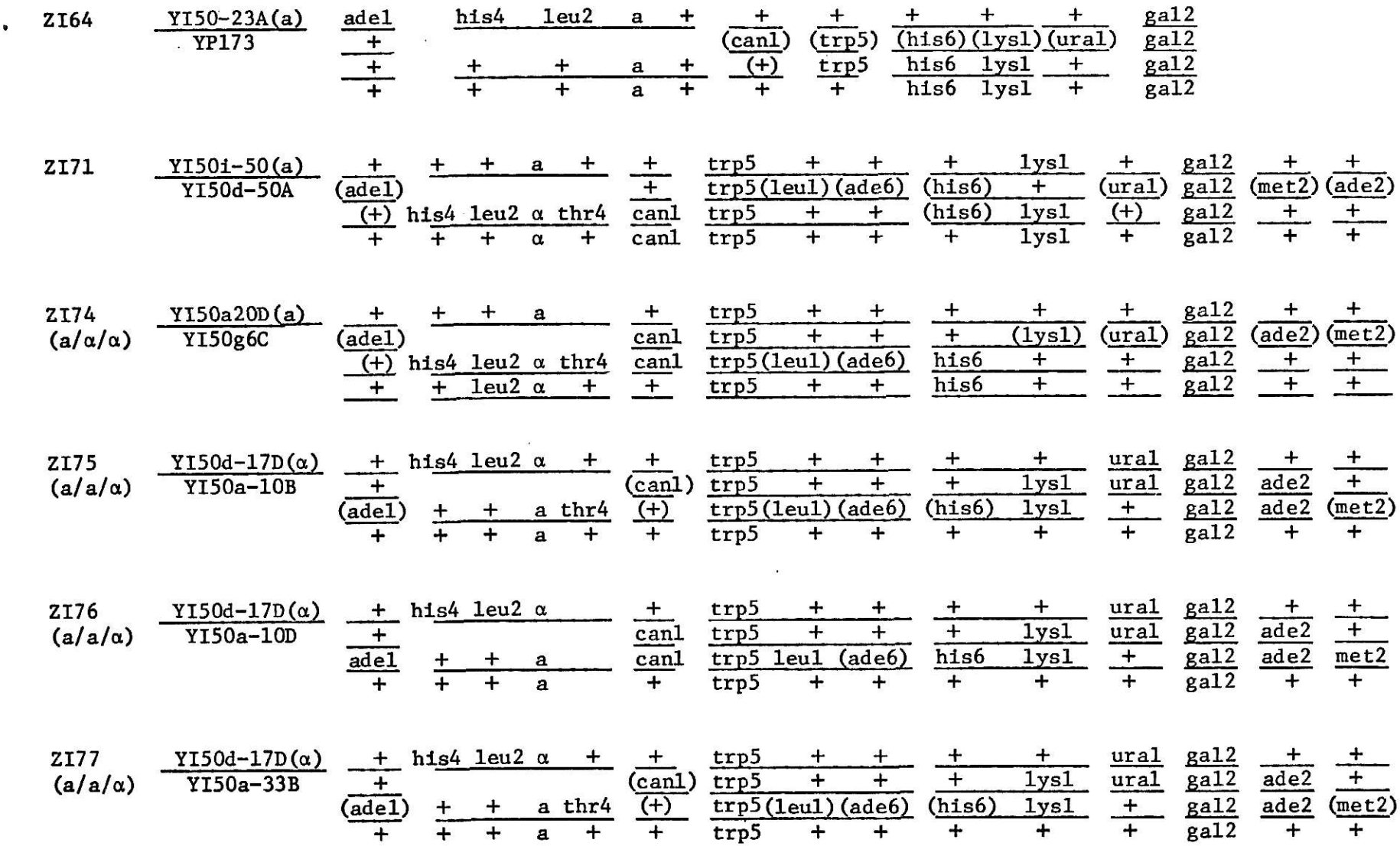
APPENDIX V

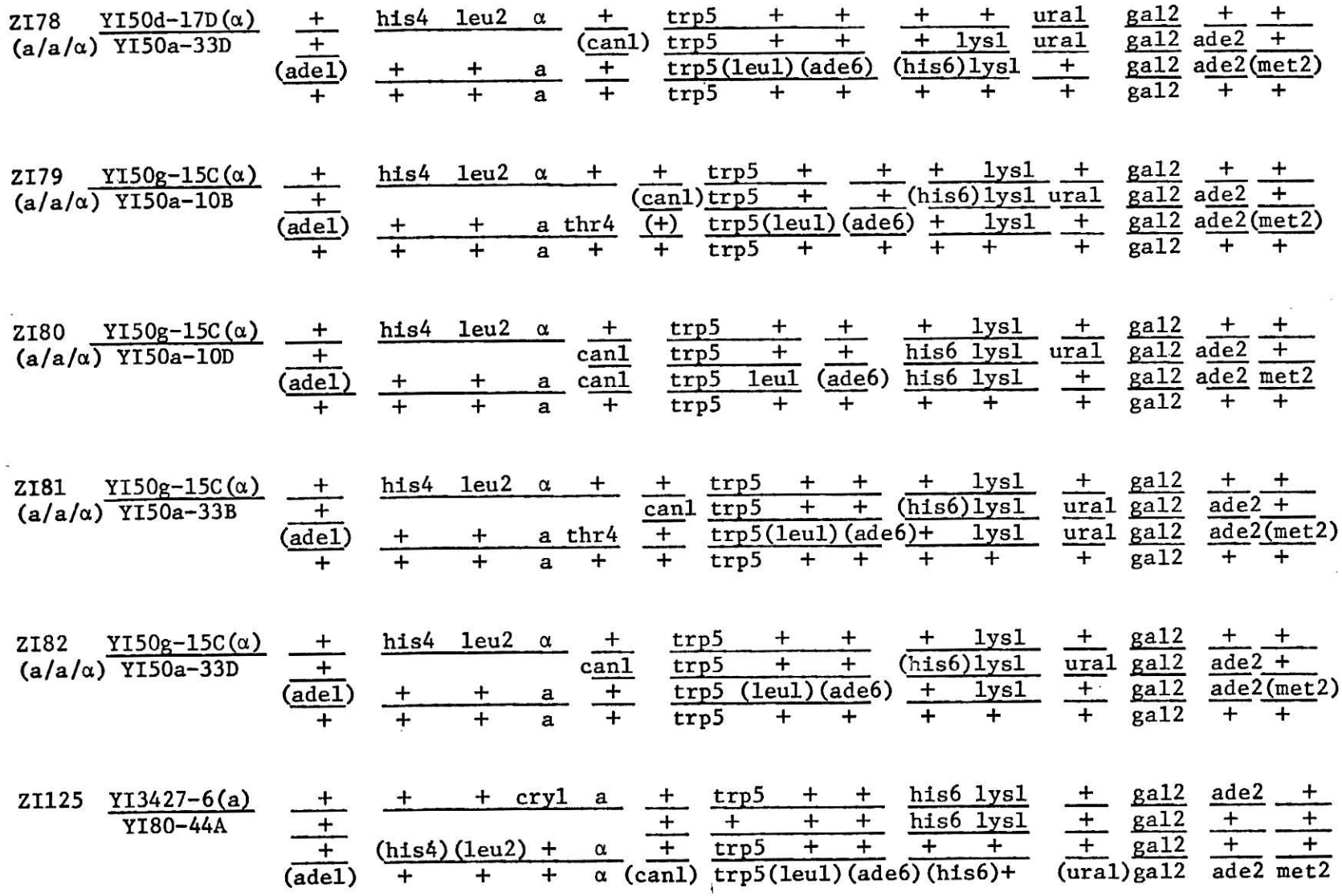
GENOTYPES OF YEAST STRAINS

Note: markers (followed by *) may be part of the genotype since uncertainties exist due to insufficient data, particularly for markers which are triplex (+/+/+/-). If strains are aneuploid for chromosome III, the genotype for matl follows in parenthesis.

ZI11	YI1 (α)		can1	trp5	+	ura1	gal2	ade2	+
(a/α)	YL67-18D (a)	+ + α +	can1	trp5	lys1	+	gal2	+	(+)
		+ + a thr4	can1	trp5	+	ura1	gal2	(ade2)	met2
			+	trp5	+	+	gal2	+	+
ZI13	YI3	his4 leu2 α +	can1	trp5	+	gal2	ade2	+	
(a/α/α)	YL67-18D (a)	+ + α +	can1	trp5	ura1	gal2	ade2	met2	
		+ + a thr4	can1	trp5	ura1	gal2	+	met2	
			+	trp5	+	gal2	+	+	
ZI14	YI4	his4 leu2 α +	can1	trp5	+	ura1	gal2	ade2	+
(a/α/α)	YL67-18D (a)	+ + α +	can1	trp5	lys1	ura1	gal2	+	+
		+ + a thr4	can1	trp5	+	ura1	gal2	+	met2
			+	trp5	+	+	gal2	+	+
YI28b2	YI22b	+ leu2 α thr4	ade1	+	trp5	ura1	gal2	+	
	YI67b-17D (a)	his4 leu2 α +	+	can1	trp5	ura1	gal2	met2	
		his4 + a thr4	+	can1	trp5	+	gal2	+	
			+	+	trp5	ura1	gal2	met2	

YI29b2	YI23b	+	leu2	α	thr4	ade1	can1	trp5	+	gal2	met2		
	YI67b-17D(a)	his4	leu2	α	+	+	can1	trp5	ura1	gal2	met2		
		his4	+	a	thr4	+	can1	trp5	ura1	gal2	met2		
						+	+	trp5	+	gal2	+		
YI32	XP300-36C	+	+	a	+	ade1	+	trp5	gal2	+			
	XL61-13D	his4	leu2	α	thr4	+	can1	trp5	gal2	met2			
ZI50a	XL61-1(α)	his4	leu2	α	thr4	ade1	+	trp5	+	+	+	ura1	gal2
-ZI50j	YP173(a/a)					+	can1	trp5	leu1	+	+	+	gal2
(a/a/α)		+	+	a	+	+	+	trp5	+	his6	lys1	+	gal2
		+	+	a	+	+	+	+	+	his6	lys1	+	gal2
		+	met2										
		+	+										
		ade2	+										
		+	+										
YI53	XI32-7C	+	leu2	α	thr4	+	+	trp5	lys1	gal2	met2		
	XI32-33D	his4	+	a	thr4	ade1	can1	trp5	lys1	gal2	met2		
YI54	XI32-7C	+	leu2	α	thr4	trp5	lys1	gal2	met2				
	XI32-28C	his4	+	a	thr4	trp5	lys1	gal2	met2				
YI55	XI32-7D	his4	+	α	thr4	ade1	can1	trp5	+	gal2	+		
	XI32-34C	+	leu2	a	+	ade1	can1	trp5	lys1	gal2	met2		
YI56	XI32-7D	his4	+	α	thr4	ade1	can1	trp5	+				
	XI32-3D	+	leu2	a	+	ade1	+	trp5	lys1				


ZI64
YI50-23A(a)
YP173
ade1 + + +
his4 leu2 a +
+ + a +
+ + a +
+ + + + +
(can1) (trp5) (his6)(lys1)(ura1)
(+) trp5 his6 lys1 + gal2
+ + his6 lys1 + gal2
ZI71
YI50i-50(a)
YI50d-50A
+ + + a + + trp5 + + + lys1 + gal2 + +
(ade1) + trp5(leu1)(ade6) (his6) + (ura1) gal2 (met2) (ade2)
(+) his4 leu2 α thr4 can1 trp5 + + (his6) lys1 (+) gal2 + +
+ + + α + can1 trp5 + + + lys1 + gal2 + +
ZI74
(a/α/α)
YI50a20D(a)
YI50g6C
+ + + a + trp5 + + + + + gal2 + +
(ade1) can1 trp5 + + + (lys1) (ura1) gal2 (ade2) (met2)
(+) his4 leu2 α thr4 can1 trp5(leu1)(ade6) his6 + + gal2 + +
+ + leu2 α + + trp5 + + his6 + + gal2 + +
ZI75
(a/a/α)
YI50d-17D(α)
YI50a-10B
+ his4 leu2 α + + trp5 + + + + ura1 gal2 + +
+ (can1) trp5 + + + lys1 ura1 gal2 ade2 +
(ade1) + + a thr4 (+) trp5(leu1)(ade6) (his6) lys1 + gal2 ade2 (met2)
+ + + a + + trp5 + + + + + gal2 + +
ZI76
(a/a/α)
YI50d-17D(α)
YI50a-10D
+ his4 leu2 α + trp5 + + + + ura1 gal2 + +
+ can1 trp5 + + + lys1 ura1 gal2 ade2 +
ade1 + + a can1 trp5 leu1 (ade6) his6 lys1 + gal2 ade2 met2
+ + + a + trp5 + + + + + gal2 + +
ZI77
(a/a/α)
YI50d-17D(α)
YI50a-33B
+ his4 leu2 α + + trp5 + + + + ura1 gal2 + +
+ (can1) trp5 + + + lys1 ura1 gal2 ade2 +
(ade1) + + a thr4 (+) trp5(leu1)(ade6) (his6) lys1 + gal2 ade2 (met2)
+ + + a + + trp5 + + + + + gal2 + +


ZI78 YI50d-17D(α) / (a/a/α) YI50a-33D
+ / + / (ade1) / +
his4 leu2 α / + + a
+ / (can1) / +
trp5 + + / trp5 + + / trp5(leu1)(ade6) / trp5 + +
+ + ura1 / + lys1 ura1 / (his6)lys1 + / + + +
gal2 + + / gal2 ade2 + / gal2 ade2(met2) / gal2 + +
ZI79 YI50g-15C(α) / (a/a/α) YI50a-10B
+ / + / (ade1) / +
his4 leu2 α + / + + a thr4
+ / (can1) / (+)
trp5 + / trp5 + / trp5(leu1) / trp5 +
+ + lys1 / + (his6)lys1 ura1 / (ade6) + lys1 / + + +
gal2 + + / gal2 ade2 + / gal2 ade2(met2) / gal2 + +
ZI80 YI50g-15C(α) / (a/a/α) YI50a-10D
+ / + / (ade1) / +
his4 leu2 α / + + a
+ / can1 / can1
trp5 + / trp5 + / trp5 leu1 / trp5 +
+ + lys1 / + his6 lys1 ura1 / (ade6) his6 lys1 / + + +
gal2 + + / gal2 ade2 + / gal2 ade2 met2 / gal2 + +
ZI81 YI50g-15C(α) / (a/a/α) YI50a-33B
+ / + / (ade1) / +
his4 leu2 α + / + + a thr4
+ / can1 / +
trp5 + + / trp5 + + / trp5(leu1)(ade6) / trp5 +
+ + lys1 / (his6)lys1 ura1 / + lys1 ura1 / + + +
gal2 + + / gal2 ade2 + / gal2 ade2(met2) / gal2 + +
ZI82 YI50g-15C(α) / (a/a/α) YI50a-33D
+ / + / (ade1) / +
his4 leu2 α / + + a
+ / can1 / +
trp5 + + / trp5 + + / trp5 (leu1)(ade6) / trp5
+ lys1 / (his6)lys1 ura1 / + lys1 / + + +
gal2 + + / gal2 ade2 + / gal2 ade2(met2) / gal2 + +
ZI125 YI3427-6(a) / YI80-44A
+ / + / + / (ade1)
+ + cry1 a / (his4)(leu2) + α
+ / + / + / (can1)
trp5 + + / + + + / trp5 + + / trp5(leu1)(ade6)
his6 lys1 / his6 lys1 / + + / (his6)+
+ / + / + / (ura1)
gal2 ade2 + / gal2 + + / gal2 + + / gal2 ade2 met2

ZI3403	YT3403	α thr4	trp1	+	+ + +	his6 +	+	gal2	+
-ZI3407	-YT3407	'ste' +	+	can1	+ + trp5	his6 lys1	+	gal2	ade2
a/α/(ste)	YL1011A-3(a)	a +	+	+	ade6 leu1 trp5	his6 +	+	gal2	ade2
			+	can1	+ + trp5	his6 +	met1	gal2	+

ZI3418	YT3418	a thr4	trp1	+	+ + +	his6	+	gal2	+
-ZI3421	-YI3421	'ste' +	+	can1	ade6 leu1 trp5	his6	met1	gal2	+
	YL1011A-3(a)	a +	+	+	ade6 leu1 trp5	his6	+	gal2	ade2
			+	can1	+ + trp5	his6	met1	gal2	+

ZI3434-1	YT3434(a/α)	ade1	gal1	cry1 α	trp1	his2	+	+ +	+	+
-ZI3434-120	YT3427-1(a/a)	ade1	gal1		trp1	his2	+	+ +	+	+
		+	+	cry1 a	+	+	trp5	his6 lys1	gal2	ade2
		+	+	cry1 a	+	+	+	his6 lys1	gal2	+

ZI3434-201	YT3434(a/α)	ade1	gal1	cry1 a +	trp1	his2	+	+ +	+	+
-ZI3434-220	YT3428-1(α/α)	ade1	gal1		trp1	his2	+	+ +	+	+
		+	+	cry1 α thr4	+	+	trp5	his6 lys1	gal2	ade2
		+	+	cry1 α thr4	+	+	+	his6 lys1	gal2	+

ZI4305	YT3405	+	α thr4	trp1	+	+ his6 +	+	gal2	+	+
-ZI4306	-YT3406	+	'ste' +	+	can1	trp5 his6 lys1	+	gal2	ade2	+
	ZI80a-29A(a)	+	a +	+	+	trp5 his6 lys1	+	gal2	ade2	+
		(ade1)		+	can1	trp5 his6 lys1	ura1	gal2	ade2	(met2)

ZI4320	YT3420	+	α thr4	trp1	+	+ + +	his6 +	+ +	gal2	+	+
-ZI4306	YT3421	+	'ste' +	+	can1	ade6 leu1 trp5	his6 +	met1 +	gal2	+	+
	XI80a-29A(a)	+	a +	+	+	+ + trp5	his6 lys1	+ +	gal2	ade2	+
		(ade1)		+	can1	+ + trp5	his6 lys1	+ ura1	gal2	ade2	(met2)

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GENETIC ANALYSIS OF TRISOMIC TETRAPLOIDS AND THE EXPRESSION OF CRYPTOPLEURINE RESISTANCE IN ANEUPLOID SACCHAROMYCES CEREVISIAE

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Tetraploid strains of Saccharomyces cerevisiae, trisomic for chromosome III, have been constructed by direct and indirect selection. Meiotic segregation frequencies of these strains have been investigated. The trisomic chromosome was observed to segregate according to a trivalent pairing, trivalent segregation model. Trivalent pairing was characterized by its higher frequency of exchange as compared to bivalent pairing and by the presence of spores resulting from double crossovers involving all three homologues. Trivalent segregation was characterized by the presence of a unique recombinant class. Strong interference, exhibited in diploids, was not observed in these trisomes. The mating-type locus is mapped on chromosome III, thus trisomes for chromosome III allow the genetic analysis of several steriles (mutants which alter the mating response in haploids) which also impaired sporulation when heterozygous in diploids.

The cryptopleurine resistance locus (cryl) is located on chromosome III. Induction of cryptopleurine resistance by UV radiation in diploids monosomic for chromosome III was not observed under the same conditions that induce such resistance in haploids. Thus it seems probable that cryptopleurine resistant alleles cannot express themselves in monosomes. This was verified by observing that the monosomic diploids (2n - 1) segregating from trisomic tetraploids, constructed homozygous for cryptopleurine resistance were not resistant, while disomic (2n) segregants were. Expression of cryptopleurine resistance in other aneuploids is also reported.