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The effect of aneuploidy on meiotic crossing over and segregation in yeast

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SUMMARY

Meiotic chromosome associations in homothallic strains of *S. cerevisiae* were investigated by analysis of meiotic spore colonies and crossing over in +/+/− trisomics. The segregants of these aneuploids produce phenotypically distinguishable tetrasomic spore colonies. The data indicate that trivalent associations occur with a high frequency in trisomics of chromosome XVII and that the frequency of second division segregation is markedly increased over that found in the normal dipoid.

1. INTRODUCTION

Suitable systems for following the meiotic behaviour of chromosomes are restricted to those experimental systems which are highly developed genetically, cytologically or both. *Saccharomyces cerevisiae* provides an excellent vehicle for studying meiosis genetically. Although this organism is cytologically refractory, the availability of a well delineated genetic map (Mortimer & Hawthorne, 1973) and the ability to recover and genetically characterize all the products of an individual meiosis make it an ideal organism for investigations of meiotic chromosome behaviour. Additionally, homothallic aneuploids are available which have distinct reliable morphological phenotypes either singly or in combinations (James, in prep.). Hence, spore colonies of a single tetrad can be readily classified as to their ploidy for a particular chromosome. This ability to introduce extra homologues into the genome makes it possible to obtain information relevant to the process of exchange pairing and segregation.

The experiments reported here describe a system designed to facilitate studies of recombination in a trisomic genotype.

We present data pertaining to pairing, crossing-over and segregation in chromosome XVII trisomics of *S. cerevisiae* which demonstrate that trivalent meiotic association occurs with a high frequency and that second division segregation frequencies show a marked increase over those expected from bivalent arrangements. These results complement and extend similar observations reported for heterothallic trisomics of chromosomes III and chromosome XI (Shaffer *et al.* 1971; Culbertson & Henry, 1973).

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2. MATERIALS AND METHODS

Homothallic strains of *Saccharomyces cerevisiae* were used which were obtained from a series of crosses between a chromosome XVII aneuploid, derivative of the homothallic B67 strain, and the heterothallic strains X2928-7D, 1893II-6C and P-65 obtained from R. K. Mortimer. The heterothallic strains were the source of the *ade1*, *met14*, *trp1 pha2* and *met4* mutant loci. Test strains were constructed by mass matings utilizing two different auxotrophic markers and subsequent isolation of prototrophs. Spore colonies aneuploid for chromosome XVII were easily identifiable by microscopic examination 24 h after incubation. At this time the colonies contain distinctively enlarged cells. A small number of these cells are dead and are therefore stained bright pink by the addition of 0·025 mg/ml of phloxine B to the plating medium. Details concerning sporulation and the isolation of spore tetrads for dissection have been previously published (James, 1974).

3. RESULTS AND DISCUSSION

The genetic system used in these experiments was developed for two major reasons. First, chromosome XVII trisomics are extremely stable and can be phenotypically classified in spore colonies with ease, and, secondly, recombinational

Table 1. *Segregational analysis of normal homothallic diploid strains of* S. cerevisiae *using trp1 as a centromere marker**

Genotype	Gene pair	Total tetrads	PD	NPD	TT	% second division segregation
ade1/+, *pha2*/+, *trp1*/+	*ade1-trp1*	236	79	116	17	7·4
	ade1-pha2	228	38	23	167	—
	trp1-pha2	246	32	41	173	70·3
ade1/+, *met4*/+, *trp1*/+	*ade1-trp1*	285	135	126	24	8·4
	ade1-met4	285	34	50	201	—
	trp1-met4	284	44	41	199	70·1
pha2/+, *met4*/+, *trp1*/+	*trp1-pha2*	184	28	34	122	66·3
	trp1-met4	182	22	29	131	72·0

* Since *trp*1 is known to be less than 1 centimorgan from its centromere, the frequency of second division segregation (x) can be calculated directly with less than 1% error.

events occurring with a high frequency in one or both of the chromosome arms can be monitored. The *met4* gene, located on the left arm of chromosome XVII and the *pha2* gene located on the right arm, show virtually random segregation with respect to their centromere, thus resulting in a high frequency of second division segregation tetrads; i.e. tetrads which result as a consequence of a recombinational event occurring between the gene being monitored and its centromere. The theoretical maximum second division frequency (x), assuming no interference, for a

gene which recombined freely with respect to its centromere is 2/3. This value, based on the mapping function of Kosambi (Barratt *et al.* 1954) has been discussed in a similar context by Shaffer *et al.* 1971 and Culbertson & Henry, 1973).

The second division segregation frequencies for *met4* and *pha2* have been reported as 0·70 and 0·62 respectively (Mortimer & Hawthorne, 1966). We have

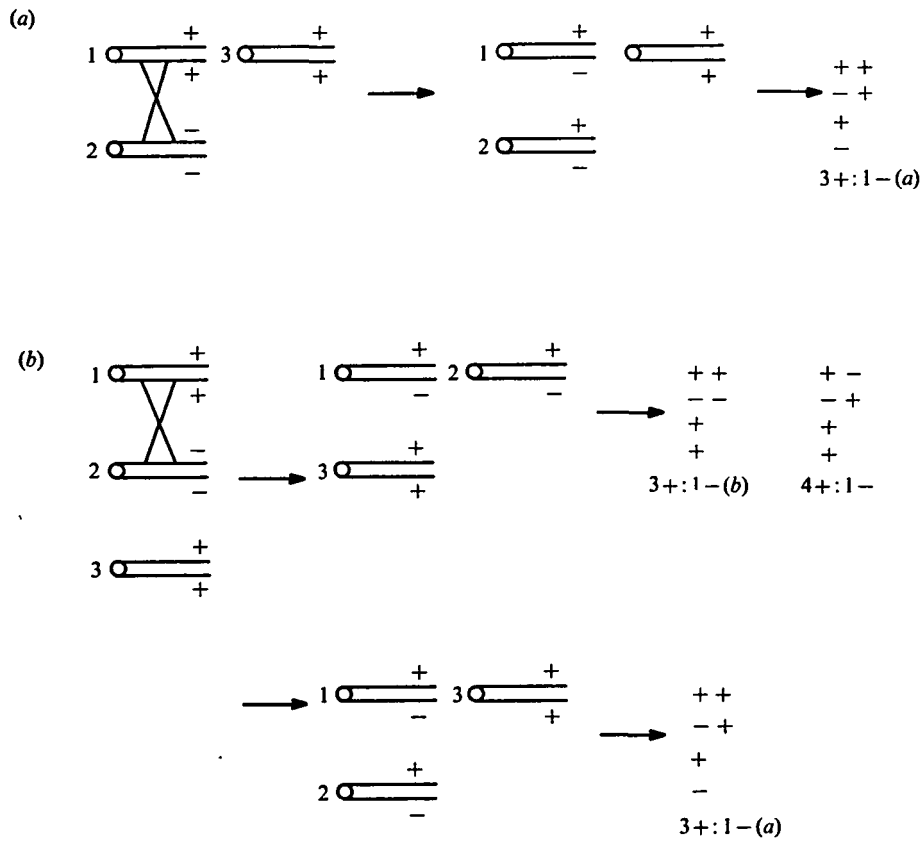

Fig. 1. Consequences of crossing over under conditions of bivalent univalent, or trivalent, chromosome association. (*a*) Bivalent univalent-resulting 3+:1− tetrads have a normal haploid '−' spore [3+:1−(*a*)]. The same result is obtained if centromeres 2 and 3 pair. (*b*) Trivalent–resulting 3+:1− tetrads are obtained in the ratio of four 3+:1− tetrads with a normal haploid '−' spore [3+:1−(*a*)] to one 3+:1− tetrad with a disomic '−' spore [3+:1−(*b*)].

established similar values for our strains using *trp1*, which shows less than 1% second division segregation, as a centromere marker. Our average values, calculated from Table 1, are 0·71 and 0·68 for *met4* and *pha2* respectively. These values do not differ significantly from the 0·67 expected of free recombination.

How is recombination affected when chromosome XVII is in a trisomic condition rather than a diploid condition? An answer to this question can be obtained from the frequencies of tetrad types expected if crossing-over is not affected by a trisomic configuration. These expectations can be calculated by the equations

shown in Table 2. The formulae show the expected frequencies of ascal types for segregations of a $+/+/-$ trisomic assuming that only two of the three homologues are involved in recombination (Shaffer *et al.* 1971; Culbertson & Henry, 1973). Previous reports have indicated that for chromosome III trisomics, the data are more consistent with higher than expected second division segregation frequencies (Shaffer *et al.* 1971). For chromosome XI trisomics, however, the experimental second division segregation values for three of four genes tested were remarkably close to expected values (Culbertson & Henry, 1973). The fourth gene yielded results that were more consistent with a very slight increase over the normal second division segregation value.

Table 2. *Expected frequencies of tetrad types from a $+/+/-$ trisomic*

Tetrad type	Biv.–univ.	Trivalent	Combined
$4+:0-$	$2/3\text{-}x/3$	$2/3\text{-}x/3$	$2/3\text{-}x/3$
$2+:2-$	$1/3\text{-}x/3$	$1/3\text{-}2x/9$	$1/3\text{-}x/3+xz/9$
$3+:1-(a)$	$2/3x$	$4x/9$	$2x/3\text{-}2xz/9$
$3+:1-(b)$	—	$x/9$	$xz/9$

x, Frequency of second division segregation; z, frequency of trivalent associations.

Table 3. *Segregational analysis of homothallic strains trisomic for chromosome XVII*

Strain no.		... S 467	S 521	S 523	
Genotype		... *pha2*/+/, *ade1*/+, *met14*/+	*met4*/+/+, *ade1*/+	*pha2*/+/+, *met4*/+/+, *ade1*/+	
Gene monitored*		... I, *pha2*	II, *met4*	III, *pha2*	IV, *met4*
Tetrad	A(4+:0−)	84	58	59	56
	B(2+:2−)	16	9	11	10
	C(3+:1−*a*)	130	104	102	105
	D(3+:1−*b*)	37	21	24	25
Totals		267	192	196	196

* Total *ade1* segregations for all crosses resulted in 5·4% second division segregation tetrads for *ade1* and chromosome XVII disomy. Gene conversions for *ade1* were 4%.

The *met14* segregations for S 467 resulted in 0% second division segregation tetrads for *met14* and chromosome XVII disomy. Gene conversions for *met14* were less than 1%.

The $3+:1-$ tetrads produced by a $+/+/-$ trisomic can be divided into two classes: $3+:1-(a)$, in which the '$-$' spore is a normal haploid and $3+:1-(b)$, in which the '$-$' spore is disomic for the chromosome carrying the marker being assayed. The latter class occurs as a consequence of trivalent formation since it implies that both chromosomes involved in an exchange migrated to the same pole at meiosis I (Fig. 1). It can be seen from the Table 2 equations that under conditions of complete trivalent pairing ($z = 1$), the ratio of $3+:1-(a)$ to $3+:1-(b)$ tetrads is 4:1. As the frequency of trivalent pairing decreases, the ratio of $3+:1-(a)$ to $3+:1-(b)$ increases. When only bivalent-univalent pairing occurs ($z = 0$), the $3+:1-(b)$ class is eliminated.

The $3+:1-(b)$ tetrads could result from bivalent-univalent pairing if the extra chromosome increased the frequency of non-disjunction of meiosis I. If an exchange had occurred prior to non-disjunction, then a $3+:1-(b)$ tetrad could be produced since non-disjunction and trivalent pairing are both mechanisms by which two paired homologues can migrate to the same pole. However, if this was the case, then the production of $3+:1-(b)$ tetrads should be matched by a corresponding production of asci containing two hexasomic and two nullosomic spores as the unpaired homologue can migrate to either pole. This was not the case. Gene conversions associated with bivalent-univalent pairing could only result in production of $3+:1-(b)$ tetrads if this rare event was accompanied by non-disjunction. Furthermore, gene conversions for *met4* and *pha2* in the diploid were less than 3 %. It is reasonable, then to assume that the frequency of $3+:1-(b)$ tetrads provides an acceptable measure of trivalent formation.

The data collected for *met4* and *pha2* genes from segregations of $+/+/-$ trisomics are shown in Table 3. For efficient analysis, these data are arranged in four groups extracted from three different crosses.

Rearranging the equations in Table 2 yields:

$$\begin{aligned} f(4+:0-) &= A = (2-x)/3, \\ f(2+:2-) &= B = 1/3-(3x-xz)/9, \\ f(3+:1-a) &= C = 2(3x-xz)9 = 2/3-2\mathrm{B}, \\ f(3+:1-b) &= D = xz/9. \end{aligned}$$

The above set of equations is subject to two restrictions, viz. $A+B+C+D = 1$ and $2B+C = 2/3$. Two independent equations and two unknowns remain so estimation of x and z should be exact. In practice, we have four sets of observations (of A, B, C and D) which can be shown not to differ ($\chi^2 = 2{\cdot}38$, d.f. $= 9$). Therefore, the four sets can be pooled to give the following tetrad frequencies:

$$A = 0{\cdot}302, \quad B = 0{\cdot}054, \quad C = 0{\cdot}518, \quad D = 0{\cdot}126.$$

However, this gives a value for $2B+C = 0{\cdot}63$ instead of the expected 2/3. Obviously, since the equations were derived on the assumption that only two of the three homologues are involved in any one meiotic exchange event, some deviation is expected. Nevertheless, it is evident that a model system based on the above four equations provides a close, if not exact, correspondence. Hence estimates of x and z can be obtained from the two independent equations as follows:

$$x = 2-3A, \quad z = 3(1-3C/2x).$$

These values are shown in Table 4. The overall values for x and z are 1·09 and 0·86 respectively. Although the equations are only a close approximation to the experimental system, two conclusions can be drawn. First trivalent pairing occurs with a high frequency at meiosis I in trisomics of chromosome XVII and secondly, the presence of an extra chromosome XVII homologue causes a significant increase in the frequency of second division segregations for *pha2* and *met4*, located respectively on the right and left distal regions of that chromosome.

Two explanations are possible for this latter observation. The first is that preferential pairing (James & Inhaber 1975) between a '+' and '−' homologue results in a proportionally increased number of second division segregations (i.e. 3+ : 1− tetrads). However, since there is evidence for almost complete trivalent association, the centromeres must still segregate randomly in order to generate 3+ : 1−(*b*) tetrads. Therefore, if this explanation were correct, preferential pairing would occur for regions on both sides of the centromere and yet still allow random centromere segregation. Studies are in progress using a stable and phenotypically

Table 4. *Estimates of* x *and* z *calculated for groups I–IV data of Table 2.*

Group	*x*	*z*
I	1·06	0·93
II	1·09	0·76
III	1·10	0·87
IV	1·14	0·89
Overall	1·09	0·86

identifiable trisomic of chromosome VII which is extensively marked throughout its entire genetic length to determine if regions close to the centromere will produce frequencies of 3+ : 1−(*b*) tetrads consistent with random segregation from a trivalent complex. The ability to immediately identify the disomic segregants of trisomic parents without the necessity of test crosses, makes it possible to perform large scale experiments of this type with relative ease.

The second possibility is that trivalent associations promote some type of cross-over interference, either chromosome or chromatid, which leads to a decrease in either or both of the two strand and four strand double cross-over events. This would result in an increase in the effective number of single cross-over events and hence a higher frequency of second division segregation tetrads.

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