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The tolerance of aneuploidy in yeast

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SUMMARY

Single ascospore cultures from triploids were screened as a potential source of disomic tester stocks for the purpose of genetical mapping of chromosomes in yeast. A high tolerance of aneuploidy was found, and strains disomic for only one chromosome were rare. There was a high occurrence of strains disomic at specific chromosomes and in certain multiple combinations of disomic chromosomes.

1. INTRODUCTION

It is not known how many chromosomes there are in cells of the bakers' yeast *Saccharomyces cerevisiae*. The most recent cytological investigations by Tamaki (1965) provide evidence for eighteen bivalents. Genetical chromosome counting by enumerating centromeres has provided evidence for fourteen linkage groups based upon independently segregating centromere-linked markers, as well as eight linkage groups not yet associated with a centromere (Mortimer & Hawthorne, 1966; Hwang, Lindegren & Lindegren, 1963, 1964). Together, these studies suggest that yeast has a large number of genetically long chromosomes. Both of these factors contribute to the apparent scarcity of cases of linkage. Combined with their small size, which makes reliable cytological studies difficult, this has left the question of the number of yeast chromosomes unsettled.

In addition to standard tetrad analysis, a technique involving the use of disomic tester stocks has been used in yeast (Mortimer & Hawthorne, 1966). This method can provide unambiguous evidence for the linkage of markers that recombine too freely to be identified as linked by conventional tetrad analysis. The method involves a great economy in ascus dissection and tetrad analysis, since the analysis of only a few asci will establish whether or not any association exists between an unlinked marker and the disomic chromosome. If the hybrid of the mutant and disomic tester stock is of the constitution A/A/a, then asci with the aberrant segregation ratios 4A:0a and 3A:1a will predominate for that chromosome, while heterozygous markers on the other chromosomes of constitution of the type B/b will segregate 2B:2b as usual. Thus, for any unmapped gene included in this system, the observation of many 4:0 and 3:1 segregation ratios should identify it with the disomic chromosome.

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Ascus formation in triploids is expected to give a high proportion of inviable spores with grossly unbalanced chromosome numbers; but among the survivors there may be many disomics. In this study the surviving ascospore cultures from triploid parents were screened for possible disomics in an attempt to produce a disomic tester stock collection.

2. MATERIALS AND METHODS

Media and genetic techniques

Three basic media were used throughout: a complete medium, a minimal medium and a sporulation medium. The complete medium was that described by Cox & Bevan (1962); the minimal medium used was Difco Yeast Nitrogen Base without amino acids (Difco Manual 1969). The sporulation medium was that developed by Hurst & Fogel (1964).

Matings were performed by a mass mating technique; zygotes were isolated by micromanipulation.

After sporulation of diploid or triploid cultures, ascus dissections were performed by micromanipulation on complete plates, the ascus walls having been digested before dissection with an extract of the fruiting bodies of the common cultivated mushroom (Bevan & Costello, 1964).

A replica plating technique was used to test cultures for the various markers; the series of minimal medium plates being suitably supplemented with different combinations of growth factors. In cases where more than one gene determining a particular auxotrophic requirement were segregating, these were distinguished by complementation tests. Only complete tetrads were included in the analyses.

Yeast strains

Triploids were isolated by the prototroph recovery method of Pomper, Daniels & McKee (1954); they were:

141/3b × (21 × 21): *a/a/α, leu1,trp5/+ +/+ +, met2/+/+, +/ade2-1/ade2-1,*
141/3b × (20 × 20): *a/a/α, leu1,trp5/+ +/+ +, met2/+/+, +/ade2-0/ade2-0,*
141/8b × (20 × 20): *α/a/α, leu1, trp5/+ +/+ +, +/ade2-0/ade2-0.**

One other triploid was a cross between a self-diploidized haploid and another haploid:

141/12d × 20: *a/α/α, leu1/leu1/+, +/+/ade2-0.*

The tester strains used in the test crosses for the screening of the triploid segregants for disomics were kindly supplied by Dr R. K. Mortimer:

X1271–7D: *α his6 trp5 leu1 trp1 lys7 thr5 arg8 thr4 met4 ade1 his2 arg9 ura2*
X901–35C: *a thr2 tyr4 trp5 leu1 ade6 lys1 his6 ura1 arg4-1 thr1.*

* The symbols used for determinants of mating-type and auxotrophic requirements are those agreed at the Tokyo Yeast Genetics Conference, 1969 (*Microbial Genetics Bull.*).

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X1271–7D is the most completely marked strain available having chromosomes I, III, IV, V, VI, VII, IX, XII and XIII marked. X901–35C has markers on chromosomes III, VII, VIII and IX. Both strains also carry markers on chromosome fragments and a few unmapped markers. The location of the various determinants on chromosomes and fragments is included in Table 1.

Disomic screening

The surviving ascospore cultures from the triploid parents were crossed to one of the heavily marked strains. For the majority of the markers in the tester stock, their constitution in the hybrid would therefore be either of the type $+/+/-$ (trisomic) or $+/-$ (eusomic). Tetrad analyses were performed on these hybrids and tetrad ratios were recorded for all the segregating markers. No analysis was based on less than nine complete tetrads. Irregular ratios in three or more tetrads were accepted as evidence of disomy of a marked chromosome. Where a disomic chromosome was marked with two or more genes, it was found that, as expected, all segregated irregularly.

3. RESULTS

The results of the analyses of thirty-four segregants from the four triploids have been summarized in Tables 1–4. Nearly all of the chromosomes tested showed some aberrant tetrad ratios in some crosses, and most of the segregants showed some aberrant tetrad ratios. Only three of the segregants showed no aberrant tetrad ratios, and in four others there was insufficient evidence of disomy, there being too few 4:0 and 3:1 tetrad ratios present. Certain chromosomes appeared as disomics more frequently than others, and also gave better indications of disomy. These are chromosomes V, VIII, IX, XII and XIII and the unlinked gene *tyr4*. The overall distributions of 4:0, 3:1 and 2:2 tetrad ratios for any disomic chromosome was not generally as expected; there were usually too many in the 2:2 class. As is discussed below, this may be the result of the loss of the extra chromosome in some cells of the parental culture.

Since the incidence of disomy was high among the chromosomes tested, it is highly probable that some of the untested chromosomes were also disomic. It cannot therefore be claimed that any segregant is disomic for only one chromosome.

The coincidence of the occurrence of disomy between chromosomes for the segregants is shown in Tables 3 and 4. There is a significant coincidence of disomy between chromosomes VIII, IX and the unlinked marker *tyr4* (Table 4), and also between chromosomes V and XII (Table 3). Coincident disomy was also found between chromosomes V and IX, V and XIII, IX and XII, IX and XIII and between XII and XIII. Multiple disomy was also found (Table 2). In these cases, the coincidence is close to what might be expected from the random occurrence of disomy.

It was not possible to assign any one unlinked gene or fragment segregating in

Table 1. *Incidence of disomy in the single ascospore segregants from four triploids*

Chromosomes and genetic markers represented		Number of segregants showing evidence of disomy					
		Five segregants from the triploid 141/8b × (20 × 20) crossed with X1271–7D	Eight segregants from the triploid 141/3b × (20 × 20) crossed with X901–35C	Ten segregants from the triploid 141/3b × (21 × 21)		Eleven segregants from the triploid 141/12d × 20α	
Chromosome	Marker			Five crossed with X1271–7D	Five crossed with X901–35C	Six crossed with X1271–7D	Five crossed with X901–35C
Chromosome							
I	*ade1*	2	.	0	.	1	.
III	*a/α thr4*	0	0	2	0	1	0
IV	*trp1*	0	.	0	.	1	.
V	*arg9*	3	.	5	.	3	.
VI	*his2*	1	.	1	.	3	.
VII	*ade6, leu1, trp5*	0	1	1	0	1	0
VIII	*arg4-1 thr1*	.	6	.	3	.	2
IX	*his6 lys1*	4	7	2	3	2	1
XII	*thr5*	3	.	3	.	3	.
XIII	*lys7*	5	.	2	.	2	.
Fragment							
1	*ade2-0*	0	.	2	.	1	.
2	*thr2*	.	1	.	0	.	1
3	*met2*	.	1	.	.	.	.
5	*ura1*	.	0	.	0	.	1
Unlinked							
	tyr4	.	6	.	3	.	3
	arg8	0	.	0	.	0	.
	met4	0	.	1	.	1	.
	metx	.	0	.	1	.	1
	ura2	0	.	0	.	0	.

these test crosses to any one chromosome. Disomy for the *tyr4* gene was usually associated with disomy of chromosomes VIII or IX or both, but not invariably.

The percentage spore germination in the triploid parents was around 15%. In all of the segregant crosses the percentage spore germination was greater than 50%, demonstrating that, in spite of the indications of multiple disomy in many cases, it is unlikely that any of the segregant crosses are triploid.

Table 2. *A list of the thirty-four segregants tested showing the chromosomes, fragments or unlinked markers for which they were disomic*

Number of disomic chromosomes detected	Chromosomes, fragments or unlinked markers involved	Number of segregants
None	—	3
One	VI	1
	IX	1
	XIII	2
	Tyr4	1
Two	V XII	1
	VIII *Tyr4*	1
	IX F1	1
	IX *Tyr4*	1
Three	III V XIII	1
	III VI F1	1
	V VI XII	1
	V IX XII	2
	VI IX XIII	1
	VIII IX F3	1
	VIII IX *Tyr4*	5
	IX *Tyr4 Metx*	1
Four	III VIII IX *Tyr4*	1
	V IX XII XIII	1
Five	I V IX XII XIII	2
	V VII XII XIII *Met4*	1
	VIII IX F2 F3 *Tyr4*	1
	VIII F2 F3 F5 *Metx*	1
Diploid	All	2

4. DISCUSSION

It has been demonstrated that there is in general a high degree of tolerance of aneuploid chromosome numbers in yeast. Although, possibly, some of the aberrant 3:1 tetrad ratios could equally well be the result of gene conversion (Lindegren, 1953; Roman, 1963), in general the frequency with which they occurred was too high for this to be taken as a reasonable explanation. Certain chromosomes, notably chromosomes V, VIII, IX, XII, XIII and that bearing the *tyr4* gene, were tolerated more readily in the disomic condition. In a recent report, Rodarte, Fogel & Mortimer (1968) describe the use of a yeast strain disomic for chromosome VIII which was recovered as a spontaneous meiotic segregant. Stocks disomic for

chromosomes V, VII, VIII, IX and XII have also been described by Takahashi & D. C. Hawthorne (unpublished) and for chromosome I by Cox & Bevan (1962).

The distribution of disomy among the identifiable chromosomes is worth comment. If any one chromosome is considered, then, on a random basis, half the segregants from a triploid should be disomic. This condition is only approached by six of the nineteen chromosomes and fragments marked. On the other hand, all but three appear as disomics at least once. This suggests that none is intrinsically detrimental in a disomic condition.

Tables 3 and 4. *The coincidence of disomy among the chromosomes tested by crosses with X901–35C (Table 3) and X1271-7D (Table 4)*

(Column 'n' represents the number of times the chromosome was testable; column 'd' the number of times it was disomic. The rest of the figures show the number of times both chromosomes at the intersect were disomic in the same segregant.)

Table 3

Markers	Chromosome	Chromosome							Markers			n	d
		VII	VIII	IX	F1	F2	F3	F5	*tyr4*	*metx*	None		
a/α, thr4	III	.	1	1	.	.	.	.	1	.	.	18	1
ade6, leu1, trp5	VII	.	.	.	.	.	.	.	.	.	.	18	0
arg4, thr1	VIII	.	.	8	.	2	3	1	8	1	.	18	10
his6, lys1	IX	.	.	.	.	1	2	.	9	1	1	18	11
ade2	F1	.	.	.	.	.	.	.	.	.	.	7	0
thr2	F2	.	.	.	.	.	2	1	1	1	.	18	2
met2	F3	.	.	.	.	.	.	1	1	1	.	18	3
ura1	F5	.	.	.	.	.	.	.	.	1	.	18	1
tyr4	.	.	.	.	.	.	.	.	.	1	1	18	11
metx	.	.	.	.	.	.	.	.	.	.	.	18	2
	None	.	.	.	.	.	.	.	.	.	3	.	.

Table 4

Markers	Chromosome	Chromosome								Markers				n	d
		IV	V	VI	VII	IX	XII	XIII	F1	*arg8*	*met4*	*ura2*	None		
ade1	I	.	2	.	.	2	2	2	.	.	.	.	.	16	2
a/α, thr4	III	1	1	1	.	1	1	1	1	.	.	.	.	16	3
trp1	IV	.	.	.	.	.	.	.	.	.	.	.	.	16	0
arg9	V	.	.	1	1	5	8	5	.	.	1	.	.	16	9
his2	VI	.	.	.	.	1	1	1	1	.	.	.	1	16	4
ade6, leu1, trp5	VII	.	.	.	.	.	1	1	.	.	1	.	.	16	1
his6, lys1	IX	.	.	.	.	.	5	4	1	.	.	.	.	16	7
thr5	XII	.	.	.	.	.	.	4	.	.	1	.	.	16	8
lys7	XIII	.	.	.	.	.	.	.	.	.	1	.	2	16	8
ade2	F1	.	.	.	.	.	.	.	.	.	.	.	.	13	2
arg8	.	.	.	.	.	.	.	.	.	.	.	.	.	16	0
met4	.	.	.	.	.	.	.	.	.	.	.	.	.	16	1
ura2	.	.	.	.	.	.	.	.	.	.	.	.	.	16	10
	None	.	.	.	.	.	.	.	0	.	.	.	.	.	.

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Thus the situation is that neither of the two extreme situations; completely random aneuploidy nor total euploidy is the rule. The best way of explaining these findings is to say that yeast will tolerate aneuploidy at up to about four chromosomes but not more, those six chromosomes that are each disomic in about half the segregants being exceptions to this rule.

However, the germination of spores from triploids (25 %) is too high to allow much restriction in the occurrence of aneuploids if chromosome segregation is random. The situation could be accounted for in one of two ways: (1) *preferential segregation*, so that the rule, obeyed to a greater or lesser extent, is for meiosis to produce two $2n$ and two n spores; (2) random meiotic segregation, but post-meiotic *mitotic elimination* of aneuploid chromosomes, comparable to the events in the haploidization of diploid *Aspergillus* (Käfer, 1961). Pomper *et al.* (1954) have reported an example of the first situation. However, it is clear that the triploids described here behave in no way as regularly as theirs and we prefer the second explanation. It accounts for the rather non-random tetrad ratios commonly found when these aneuploid segregants were mated and analysed. Disomic yeast has previously been shown to be unstable (Cox & Bevan, 1962). The rate of elimination need not necessarily be related to the number of disomic chromosomes, but it may be. It may also depend on the chromosome involved.

It was also shown that disomy at some chromosomes was frequently coincident with that at others. The chromosomes involved were among those that occurred most frequently as disomics. The coincidence of disomy of specific chromosomes suggests that the tolerance of aneuploidy in these cases is controlled by the informational content of the chromosomes themselves. The problems of genetic imbalance imposed upon the cell by aneuploidy may be less for certain chromosomes and further lessened by certain multiple combinations of disomic chromosomes. That is, there is a selective pressure for double disomics maintained by their improved genetic balance. Such balancing may not always involve one specific pair, but different chromosomes may substitute, e.g. chromosomes VIII, IX and *tyr4* in any paired assortment were common.

If such a system of keeping a genetic balance in aneuploids exists, it has implications for the use of disomics in linkage studies. In the first place there may be few chromosomes which can exist alone as disomics in an otherwise haploid cell, so a comprehensive set of disomic stocks may not be obtainable. Secondly, if chromosome compensation is non-random, as these results suggest it may be, it makes nonsense of allocating non-linked markers to any known linkage group by this method. The existence of such restrictions could be tested by repeated back-crosses of aneuploid strains to haploids.

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