MEIOTIC DIPLOID PROGENY AND MEIOTIC NONDISJUNCTION IN *SACCHAROMYCES CEREVISIAE*

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Manuscript received September 2, 1980
Revised copy accepted December 24, 1981

ABSTRACT

Abnormalities in chromosome number that occurred during meiosis were evaluated with a specially-constructed diploid strain of *Saccharomyces cerevisiae*. The strain is heterozygous for six markers of the right arm of chromosome *V* and heterozygous for *cyh2* (resistance to cycloheximide) on chromosome *VII*.—Selection of meiotic spores on a medium containing cycloheximide and required nutrilites—except those for the markers of the right arm of chromosome *V*—allows the growth of aberrant clones belonging only to two classes: a) *diploid clones*, caused by failure of the second meiotic division, with a frequency of 0.54×10^{-4} per viable spore; and b) diplo *V*, aneuploids derived from nondisjunctions in meiosis I or meiosis II, with a total spontaneous frequency of 0.95×10^{-4} per viable spore. About two-thirds of the aneuploids originated during meiosis I, the rest during meiosis II. An investigation of these events in control meioses and after treatment with MMS, Benomyl and Amphotericin B suggests that this assay system is suitable for screening environmental mutagens for their effects on meiotic segregation.

THE analysis of chromosome number in gametes is important for a better understanding both of some fundamental aspects of the meiotic process and of the role of aberrant gametes in fertility, zygote viability, and phenotype. One of the better known organisms for the study of nondisjunction is *Aspergillus nidulans* (NORMANSELL *et al.* 1979; MORPURGO *et al.* 1979), in which variations of chromosome number can be studied only in mitosis. Other simple eukaryotes have biological cycles more suitable for the investigation of the meiotic events that give rise to aberrant gametes. In *Sordaria brevicollis*, for example, such phenomena can be studied directly by visual inspection of intact asci (BOND 1976). In *Neurospora crassa* (GRIFFITHS and DELANGE 1977) and *Saccharomyces cerevisiae* (PARRY 1977; PARRY *et al.* 1979), methods have recently been proposed for selecting clones derived from spores that carry abnormal numbers of chromosomes. Such Ascomycetes could permit identification of two types of abnormal meiotic events: nondisjunction of one or a few chromosomes (proper nondisjunction that gives rise to disomic gametes), and failure of complete chromosome set separation (failure of meiosis I or meiosis II, which results in diploid gametes). An ideal system for investigating these phenomena should provide information concerning: a) quantitative relationships between aneuploidy and diploidy of gametes produced by aberrant meioses; b) time of occurrence, *i.e.*,

whether such phenomena occur during meiosis I or meiosis II; c) mechanism of aberrant gamete formation, *i.e.*, whether they derive from a failure of one of the meiotic divisions or from endomitosis before or fusion of nuclei after meiosis; and d) viability and phenotype of zygotes derived from such gametes. Efficient systems have provided considerable information concerning aneuploidy in Drosophila (SOBELS 1979; LEIGH 1979). Aneuploid and diploid gametes are formed with frequencies of 6% and 1.8%, respectively, in man; their effects on zygotes are drastic. In other mammals, *Microtus oeconomus* (TATES *et al.* 1979) and *Mus musculus* (HANSMANN and EL-NAHASS 1979), karyological evidence has been obtained concerning the frequency of aneuploid and diploid gametes, but there is almost no information about whether aberrant gametes, diploid in particular, are really functional.

Among Ascomycetes, *S. cerevisiae* is particularly suitable for detailed genetic investigation of aberrant meiotic products. The organism is stable in both the haploid and diploid states; it has a relatively good tolerance of aneuploidy and polyploidy; and, because of its high frequency of sporulation under proper conditions, events that occur, even at a very low rate, during meiosis can easily be detected.

We have devised a system in *S. cerevisiae* that permits the selection and identification of aneuploid and diploid meiotic products, the purpose of which is to evaluate their relative frequencies and to determine their mechanisms of production. This system is based on selection of clones heterozygous for markers on the right arm of chromosome *V*. A similar system, which involves chromosome *VII* in the same organism, was used by PARRY *et al.* (1979); but no detailed analysis of the selected clones has yet been published. The two systems should be compared to determine whether the frequencies of the observed events depends on the specific chromosome involved in the selection scheme or reflects a general characteristic of *S. cerevisiae*.

MATERIALS AND METHODS

Strains: DIS13 was the major strain we used; its genotype is described in RESULTS. We used 6122/12b (**a** *lys2*) and 6122/3a (α *lys2*) as haploid testers for crosses. All strains were constructed in our laboratory.

Media: YEPD, minimal medium 40 and sporulation medium VB, were prepared as described by MAGNI, PANZERI and SORA (1977). Medium C (complete) is composed of minimal medium supplemented with 2.5 mg/l of adenine, uracil and histidine; 10 mg/l of leucine, isoleucine, methionine and tryptophan; 25 mg/l of threonine; 50 mg/l of ornithine. Medium R (recombination) is composed of medium C lacking histidine and threonine. Medium S (selection) is composed of minimal medium supplemented with 10 mg/l of adenine and uracil and 25 mg/l of leucine. Cycloheximide was added to each medium: 0.4 mg/l in media C and R, and 1 mg/l in medium S. The amount of nutrilites added to each of the media is important to avoid selection of certain phenotypes among the meiotic products of DIS13.

Sporulation and chemical treatments: Log-phase cells of strain DIS13, grown in YEPD, were harvested and transferred in VB medium at a final concentration of 5×10^7 cells/ml. Sporulatiion was carried out on a reciprocating shaker at 28° for nine days to allow maximum sporulation in the presence of chemicals that delay the meiotic process. Inhibitors were added to VB medium at proper concentrations immediately after cell transfer. In control cultures, sporulation

TABLE 1

Fluctuation of spontaneous frequencies

Experiment	Sporulation %	Frequency of *HOM3-HIS1* recombinants per 10^2 viable spores	Total frequency of colonies on S medium per 10^4 viable spores
1	67.0	1.10	1.33
2	79.0	2.50	1.68
3	77.3	—	1.47
4	68.0	—	1.19
5	62.1	2.10	1.50
6	56.2	1.79	1.95
7	68.7	1.88	1.34
Mean	68.3 ± 10.6	1.87 ± 0.32	1.49 ± 0.19

ranged between 56% and 79% (Table 1). Treated samples frequently showed a marked reduction of sporulation (Tables 6, 9 and 13).

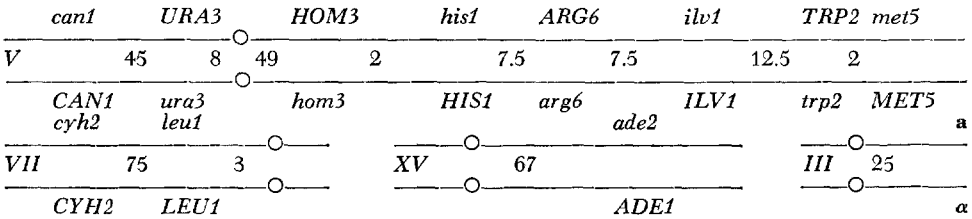
Spore preparation: Asci were counted microscopically, sporulated cultures were centrifuged, washed and ascus walls were lysed in a 1:50 dilution of snail juice (IBF) at 37° for 24 hr. Lysis of most unsporulated cells and separation of spores from each other were carried out by treatment for 3 hr in distilled water with gentle sonication (20–30 sec treatment with a MSE 100 ultrasonic disintegrator set at 6μ amplitude). The resultant suspensions were composed of spores (99% or more in controls and 70% in the worst conditions of sporulation). Between 85% and 95% were single spores; 5% to 15% were still in groups of two to three spores. The few residual unsporulated cells (0.2 to 0.5% in controls and up to 30% in treated samples) were mostly inviable, as demonstrated by micromanipulation of several dozen cells. Survival was approximately 20%.

Selection: Spore suspensions were seeded at proper dilutions on medium C for counting, on medium R for estimation of recombination frequencies (see RESULTS: *genetic system*), and on medium S for selection of aberrant clones. Colonies were counted after 11 days at 28°. Frequencies of recombinants and of aberrant clones were calculated on the basis of the counts on medium C.

RESULTS

The genetic system: The genotype of the strain used, DIS13, is as follows; map distances are expressed in cM (MORTIMER and SCHILD 1980) and chromosomes are designated by Roman numerals.

The first experimental step was to select clones which did not express any of the markers carried in a heterozygous condition on the right arm of chromosome *V*. Spore suspensions were plated on medium S (MATERIALS AND METHODS).



Colonies growing on such medium should fall into one of two classes: a) haploid clones, derived from meiosis in which five crossovers occurred on chromosome *V*, resulting in all of the dominant alleles occurring on the same chromatid; or b) clones carrying the two parental chromosome *V*s. The first event would be expected to occur with a frequency of 2.8×10^{-7} per viable spore, in accordance with the average crossover frequency as reported by MORTIMER and SCHILD (1980).

To confirm the level of expected recombination, particularly after chemical treatments that may increase crossing over, each spore suspension was also plated on medium R. This medium permits detection of *HOM3-HIS1* exchanges, expected to occur with a frequency of 1%. The results of seven independent experiments are reported in Table 1. The data demonstrate that in our strain the recombination frequency between *HOM3* and *HIS1* is as reported by MORTIMER and SCHILD (1980). The average frequency of total colonies on the selective medium is 1.49×10^{-4} per viable spore. This suggests that they all derive from spores carrying two chromosome *V*s and not from multiple crossing over on the right arm of chromosome *V*.

One of the major purposes of this research was to detect spontaneous and induced nondisjunctions of chromosome *V*. Strain DIS13 was designed to detect nondisjunctions at both meiosis I and meiosis II. The detection of these events requires, in theory, two quite different situations. Meiosis I nondisjunctants would be selected more easily if the markers on chromosome *V* were quite close to their centromere. For markers not tightly linked to their centromere, any exchange between the centromere and the proximal marker *HOM3* renders some of the recessive alleles homozygous. Such clones would be undetectable in our system. The opposite situation exists for events arising in meiosis II. From the schemes reported in Figure 1, we can estimate the clone loss at 2 of 6 for meiosis I nondisjunctions. For meiosis II, the loss will be 7 of 9 if only one centromere undergoes nondisjunction, 2 of 6 if both centromeres are involved. Furthermore, a fraction of meiosis I and meiosis II events will be missed when exchanges occur in the region *hom3-met5* (31.5 cM). Although this system can provide, at best, only an estimate of meiotic nondisjunction events of chromosome *V*, we believe that it is useful for our main purposes.

The allele *cyh2* (chromosome *VII*), which confers resistance to cycloheximide, was used to select clones derived from spores. The recessive alleles *leu1* (chromosome *VII*) and *ade2* (chromosome *XV*) were used for further genetic analysis.

Discrimination between meiosis I and II division nondisjunction: The presence of *ura3* on the left arm of chromosome *V* (8 cM from its centromere) in heterozygous condition can provide information regarding the relative frequencies of I and II division meiotic nondisjunction. Nondisjunction of chromosome *V*, occurring during the first meiotic division, gives rise to *ura*$^-$ clones when a crossover between *ura3* and its centromere has occurred (Figure 1). In this case, following exchange, 25% of the resulting spores will be homozygous for *ura3*. The maximum level of disomic chromosome *V* clones expressing *ura*$^-$ due to meiosis I nondisjunction is thus 4%, (0.25×0.16).

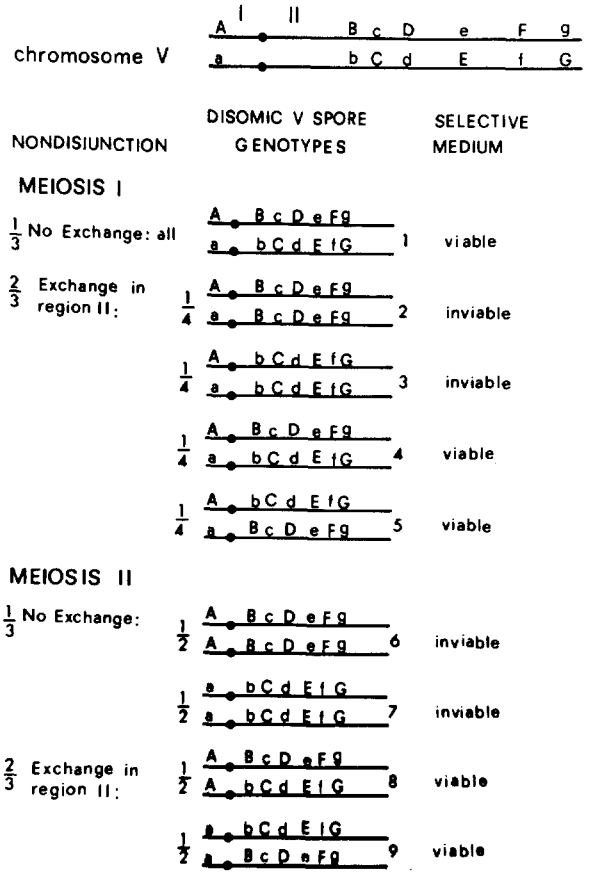


FIGURE 1.—Since the distance *cen5-hom3* (region II) is about 50 cM, it is expected that one-third of tetrads would not have any exchange in the region, and two-thirds would have one exchange. The viable disomics produced by meiosis I nondisjunction (1, 4 and 5) will therefore be $1/3 + (2/4 \times 2/3) = 4/6$. During meiosis II, if nondisjunction affects both centromeres of the same meiosis, the expected frequency of viable disomics is two-thirds (8 and 9). If only one centromere is affected, the produced tetrad will contain : 1 disomic; 2 haploids : 1 nullisomic. Therefore, one-third of the viable spores will be disomic, the overall frequency of disomics is $2/3 \times 1/3 = 2/9$.

When nondisjunction occurs during the second meiotic division, the *ura*$^-$ phenotype will appear in half of the clones (Figure 1). These clones are derived from meiosis in which no exchange has occurred between *ura3* and its centromere. The maximum expected level of *ura*$^-$ disomic chromosome *V* clones from meiosis II is 42% $[0.50 \times (1-0.16)]$. It follows that the percentage of *ura*$^-$ disomic chromosome *V* clones is equal to $p(0.04) + q(0.42)$, where p and q, respectively, are the frequencies of I and II division nondisjunctions; therefore

$$q = \frac{\%ura^- - 0.04}{0.38}$$

Analysis of spontaneous meiotic events: Sporulation, recombination, and the frequency of selected colonies are reported in Table 1. In seven independent experiments fluctuation of of these parameters was very small, suggesting that our strain is reasonably stable.

From each experiment, a number of colonies grown on medium S were randomly isolated and analyzed from their phenotypes relative to nutritional requirements (adenine, leucine and uracil), mating type, and ability to sporulate. The data are reported in Table 2. Colonies were divided into two major classes: *mater* and *nonmater*. The 256 leucine- and/or adenine-dependent mater clones were unable to sporulate, while all of the 57 nonmater clones sporulated. The leucine- and adenine-independent clones were thus also classified into mater and nonmater categories on the basis of their inability or ability to sporulate.

Nonmaters (sporulating): These clones must be heterozygous for the mating type alleles and should therefore contain at least two chromosome *III*s. A number of asci, derived from a sample of nonmater clones, were analyzed for spore survival and phenotype to determine whether these clones were diploid or aneuploid. Table 3 shows that 12 of 13 clones analyzed were diploid; one (c74) was probably an aneuploid 2n-1. The phenotypic analysis of spores (not reported here) revealed the expected 2:2 segregation for markers on the left arm of chromosome *V*.

The possibility that these diploids originated from unsporulated cells that did not start a meiotic process was excluded. If this were the case, they would all require a preliminary somatic recombination for *cyh2* and frequently an additional somatic recombination for *leu1* and/or *ade2*. These events occur spontaneously with frequencies on the order of 10^{-4}. The maximal cell contamination (1%) of our spore suspensions cannot explain the frequencies of nonmater clones we found in our selective conditions (4.5×10^{-5}). We also eliminated the possibility that these clones derived from spore copulation after plating on medium S. Plating an equal number of spores on medium S, either before sonication (95% of clumps containing two to four spores) or after (95% single spores) resulted in almost identical frequencies of nonmater (and total) colonies. Thus, even when conditions favored copulation (clumped spores), haploid spores from DIS13 did not copulate on medium S. Therefore, it can be argued that the nonmaters derived from diploid or partially diploid cells that had accomplished some aspects of meiosis.

Diploid cells can derive from: 1) endomitosis after meiosis I (excluded in our system because the resulting products would be homozygous for all markers); 2) random nuclear fusion at the end of the meiotic process or endomitosis preceding meiosis; 3) a failure of the second meiotic division (*i.e.*, reductional segregation only); 4) a failure of the first meiotic division (no reductional division); or 5) a return by cells to mitotic division upon plating after having started meiosis. The four segregation classes (*a*,*b*,*c* and *d*, see Table 2) should result in different ratios, depending on hypothesis: 25:5:5:1 according to both hypotheses of case two; 5:5:1:1 in case three; and 5:0:1:0 in cases four and five. Due to noncomplete linkage of *leu1* to its centromere (3 cM), slight deviations

TABLE 2

Phenotypic analysis of clones deriving from spontaneous meiotic events

Experiment	Total analyzed colonies	Nonmater (sporulating) *LEU ADE* (a)	*leu ADE* (b)	*LEU ade* (c)	*leu ade* (d)	Total	Mater (nonsporulating) *LEU ADE* (a)	*leu ADE* (b)	*LEU ade* (c)	*leu ade* (d)	Total
1	93	10	10	1	3	24	14	17	23	15	69
2	47	4	7	0	0	11	11	12	5	6	34
3	81	20	6	5	1	32	17	16	9	7	49
4	106	17	11	2	2	32	24	26	10	16	76
5	44	4	3	0	0	7	8	15	8	5	36
6	48	9	2	0	2	13	9	10	10	7	36
7	48	1	2	0	0	3	6	23	8	8	45
Totals	467	65	41	8	8	122	89	119	73	64	345

For nonmater: expected segregation of phenotypic classes a:b:c:d = 5.3:4.7:1.06:0.94, $\chi^2 = 4.21$, $p = 0.25$. For phenotypic classes b:c:d = 4.7:1.06:0.94, $\chi^2 = 0.13$, $p = 0.92$.

TABLE 3

Spore survival in spontaneous nonmater clones

Clone	Total asci analyzed	Asci with viable spores/ascus 0	1	2	3	4	Ploidy
p1	10			3	2	5	2n
p4	7	1	2		4		2n
c2	11				6	5	2n
c5	11	1		1	8	1	2n
c75	10			2	4	4	2n
c6	9			1	2	6	2n
MO6	10	1		6	3		2n
c11	11		3	6	3		2n
c74	9		4	5			2n—1
BO10	10	2			2	6	2n
BO8	10	1			4	5	2n
BO7	9		1	2	3	3	2n
BO6	11	1	1		4	5	2n

from these ratios could be expected. The total segregation of 65:41:8:8, reported in Table 2, agrees with hypothesis three. The expected ratio corrected for centromere-*leu1* linkage is 5.3:4.7:1.06:0.94. In these experiments, *ADE LEU* clones appeared to have a slight advantage on medium S. For this reason, in subsequent genetic analysis, we considered only the three classes that revealed a nutritional requirement for leucine and/or adenine.

On the basis of the data; we concluded that most nonmaters originated from a failure of the second meiotic division. The frequency of ura$^-$ clones among the nonmaters also supports the view that these diploids originated from a block in the second meiotic division. The observed frequency was 31.6%, while the expectation was 42% ($\chi^2 = 2.6$, $p = 0.10$).

Maters: A fraction of the mater colonies isolated on medium S may be diploid or partially diploid and homozygous for the mating type alleles (**aa** or $\alpha\alpha$). Since the mating type locus is 25 cM from its centromere, one would expect 50% of clones derived from an almost complete block of the second meiotic division to be maters (**aa** or $\alpha\alpha$); the other 50% should represent nonmaters (**a**α). Among the leucine- and/or adenine-dependent clones analyzed (Table 2) 57 of 313 were **a**/α diploid clones. We therefore expected 57 of 256 mater colonies to be **aa** or $\alpha\alpha$ diploids (22.2%). Tetrad analysis of 32 mater clones was performed after crosses with haploids of the appropriate mating type. Both spore survival and phenotype were determined. The crosses were classified in two groups with respect to spore survival: a) strains yielding more than two surviving spores per ascus, and b) strains yielding two or fewer surviving spores per ascus, as well as a significant number of asci containing four inviable spores. The latter case indicated that the mater clones were either diploid or aneuploid for a large number of chromosomes. The former suggested haploidy.

On the basis of the data in Table 4, clones BO28, MO1, BO22, MO2 and MO24 were classified as diploid (they were the only ones found in the sample of 32

TABLE 4

Spore survival of strains derived from crosses involving spontaneous mater clones and standard haploid partners

Crossed clone	No. asci analyzed	No. asci with viable spores/ascus 0	1	2	3	4	Mater clone ploidy
BO28	8	5	2	1			2n
MO1	17	14	3				2n
MO2	9	7	2				2n
MO24	8	5	3				2n
BO22	11	4	5	2			2n
BO15	16		1	7	3	5	1n
D44	17		1	1	6	9	1n

mater clones), while D44 and BO15 were haploid. The frequency of diploid clones among the maters was 15.6%, not significantly different from the expected 22.2% ($\chi^2 = 0.73$, $p = 0.35$).

The remaining 27 crosses, which involved haploid clones, were analyzed for the segregation of all markers. Two examples of this analysis are reported in Table 5. The diploid derived from D44 exhibited normal 2+:2− segregation for *ade2* and *lys2*. However, the markers on the right arm of chromosome *V* exhibited typical trisomic segregation. The other strain, BO15, appeared trisomic for chromosomes *V* and *II* (*lys2*). [Table 12 summarizes the data from all 27 clones.]

Of 313 *ade*⁻ and *leu*⁻ clones analyzed, 114 (36.4%) were diploid **a**α,**aa** and $\alpha\alpha$ and the remaining 199 (63.6%), haploid diplo V. Among the latter we found 33 ura⁻. The frequency of this phenotype is a good indication that nondisjunction of chromosome *V* can occur during both meiosis I and meiosis II. According to the formula described above, about 33% of spontaneous diplo V aneuploids result from nondisjunction at meiosis II and 67% at meiosis I. Our estimates for the seven independent experiments are: frequency of diploids recovered = $0.54/10^4$ viable spores; and the frequency of nondisjunction of chromosome *V* (diplo V) = $0.95/10^4$ viable spores. We may assume, therefore, that roughly one third of the nondisjunction events seem to happen during meiosis II and two thirds during meiosis I.

Treatments with chemicals: In the following experiments we examined the effects of three drugs known to disturb the division processes: the mutagen methylmethane sulfonate (MMS); Benlate, whose biologically active fraction is Benomyl; and Amphoterycin B.

Methylmethane sulfonate: Several doses of MMS were used; they caused inhibition of sporulation varying from 0 to 90%. The overall frequency of clones growing on medium S increased from 2- to about 13-fold (Table 6). The recombination frequency in the *hom3-his1* region was slightly affected by the mutagen, but the maximal 2-fold increase did not significantly modify the estimate of nondisjunction. Table 7 shows the phenotypic analysis of randomly chosen clones at different doses. The distribution of the phenotypic classess b, c and d, among

TABLE 5

Meiotic segregation of markers in tetrads from crosses involving presumptive disomic V clones

Strain	Locus	Tetrad type (+:−) 4:0	3:1	2:2	1:3	0:4
D44	MAT			9		
	ade2			9		
	leu1	9				
	lys2			9		
	ura3			8	1	
	hom3		8	1		
	his1	3	6			
	arg6		9			
	ilv1	5	3	1		
	met5	5	4			
	trp2	2	6	1		
BO15	MAT			5		
	ade2			5		
	leu1			5		
	lys2	2	1	2		
	ura3	3	2			
	hom3	3	2			
	his1	2	2	1		
	arg6	3	2			
	ilv1	2	2	1		
	met5	2	2	1		
	trp2	3	2			

The genotypes of the clones are: D44 α *ade ura*: BO15 a *ade leu*.

TABLE 6

Results of treatment with methylmethane sulfonate

Dose (mM)	% Sporulation inhibition	Frequency of *HOM3-HIS1* recombinants per 10^2 viable spores	Total frequency of colonies on S medium per 10^4 viable spores
0	0	1.87	1.49
0.6	0	2.88	3.17
1.2	25.6	2.28	5.54
1.8	57.3	3.65	5.70
2.4	67.5	3.50	19.24
3.0	90.4	4.05	20.70

The means from Table 1 are used as control values.

nonmater clones, agreed with the expected ratio of 4.7:1.06:0.94 at every dosage. Spore survival and tetrad analysis of nine of these clones confirmed that MMS-induced nonmaters were complete or almost complete diploids (Table 12). The frequency of ura$^-$ clones among nonmaters (Table 8) was consistent with the expectation (42%) of a blockage of the second meiotic division.

TABLE 7

Phenotypic analysis of clones derived from methylmethane sulfonate-induced meiotic events

Dose (mM)	Total colonies analyzed	Nonmater						Mater			
		leu ADE (d)	*LEU ade* (c)	*leu ade* (d)	Total	χ^2	p	*leu ADE*	*LEU ade*	*leu ade*	Total
0.6	32	3	1	0	4	—	—	11	13	4	28
1.2	89	13	3	6	22	3.19	0.23	28	27	12	67
1.8	90	12	4	3	19	0.50	0.80	27	21	23	71
2.4	163	45	10	13	68	1.46	0.50	50	25	20	95
3.0	172	46	6	8	60	1.67	0.45	50	38	24	112

χ^2 refers to the hypothesis of nonmater phenotypic distribution b:c:d = 4.7:1.06:0.94.

TABLE 8

Frequencies of different MMS-induced meiotic events

Dose (mM)	% 2n	% n+1	Events per 10^4 viable spore 2n	n+1	% *ura* among 2n	n+1	% meiosis II nondisjunction
0	36.4	63.6	0.54	0.95	31.6	16.6	0.33
0.6	25.0	75.0	0.79	2.37	—	20.8	0.44
1.2	49.4	50.6	2.74	2.80	36.3	28.9	0.65
1.8	42.2	57.8	2.40	3.29	57.9	3.8	0
2.4	83.4	16.6	16.04	3.19	38.2	1.4	0
3.0	69.8	30.2	14.45	6.25	31.7	23.0	0.50

Tetrad analysis was performed on 33 mater clones taken at random from the highest dose (3 mM) after crossing with the appropriate haploid strain. At the two highest doses, 128 of 335 nonmater diploid clones were found, among the 207 mater clones one would expect an equal number of mater diploid clones: 128 of 207, or 61.83%. Among 33 crosses, 23 exhibited spore survival typical of a triploid. The observed frequency of diploids **aa** and $\alpha\alpha$ was thus 69.7%, in agreement with the expectation of 61.8% ($\chi^2 = 0.87$, $p = 0.33$). The remaining ten clones were disomic for chromosome *V* and one of them was also disomic for chromosome *II*.

One may conclude from these results that MMS can induce both disomy and formation of diploid gametes. Diploids are due predominantly to an almost complete failure of the second meiotic division. The marked fluctuation of the frequency of ura$^-$ clones among disomics did permit an evaluation of MMS's effect on meiosis I and II nondisjunctions.

Benlate: The effect of Benomyl on the two phenomena revealed by our method are reported in Tables 9 and 10. The frequency of clones growing on medium S increased up to 12-fold over the control frequency at the maximal dose. In the *hom3-his1* region there was a very limited increase, if any, in the recombination frequency. In spite of the rather small number of nonmater clones analyzed, the segregation ratio of the three phenotypic classes b, c and d, agreed with the ex-

TABLE 9

Treatment with Benlate

Dose (μg/ml)	% Sporulation inhibition	Frequency of *HOM3-HIS1* recombinants per 10^2 viable spores	Total frequency of colonies on medium S per 10^4 viable spores
0	0	1.87	1.49
15	9.1	2.30	2.70
20	20.1	2.15	4.25
30	57.5	4.10	18.58
40	75.0	3.10	13.63

The means from Table 1 are used as control values.

TABLE 10

Phenotypic analysis of clones deriving from Benlate-induced meiotic events

Dose (μg/ml)	Total colonies analyzed	Nonmater						Mater			
		leu ADE (b)	*LEU ade* (c)	*leu ade* (d)	Total	χ^2	p	*leu ADE*	*LEU ade*	*leu ade*	Total
15	96	8	4	2	14	1.76	0.43	41	20	21	82
20	98	13	1	4	18	—	—	39	23	18	80
30	134	10	0	4	14	—	—	46	48	26	120
40	191	18	4	4	26	0.04	0.98	63	54	48	165

χ^2 refers to the hypothesis of nonmater phenotypic distribution b:c:d = 4.7:1.06:0.94.

pected ratio of 4.7:1:06:0.94. Spore survival was determined for 12 randomly chosen nonmater clones; 11 turned out to be complete diploids and 1 to be $2n$-1 (Table 12). The frequency of ura$^-$ nonmater clones was close to the expected 42% (Table 11). Diploid recovery induced by Benlate was caused by a failure of the second meiotic division, as observed in untreated samples. Genetic analysis of 36 mater clones after crossing with the appropriate haploid, gave the following results: a) eight clones were diploid **aa** or $\alpha\alpha$. Their frequency (22.2% was not significantly different from that calculated on the basis of nonmater clones (72 of 447 = 16.1%; $\chi^2 = 0.99$, $p = 0.30$). b) The remaining 28 clones were disomic for chromosome *V*. Two were also disomic for either chromosome *VII* or *II*

TABLE 11

Frequencies of different Benlate-induced meiotic events

Dose (μg/ml)	% $2n$	% $n+1$	Events per 10^4 viable spores		% *ura* among		% meiosis II nondisjunction
			$2n$	$n+1$	$2n$	$n+1$	
0	36.4	63.6	0.54	0.95	31.6	16.6	0.33
15	29.1	70.9	0.78	1.92	50.0	2.9	0
20	36.7	63.3	1.56	2.69	50.0	16.1	0.32
30	20.9	79.1	3.88	14.69	35.7	22.6	0.48
40	27.2	72.8	3.70	9.92	31.6	25.1	0.55

TABLE 12

*Pattern of ploidy of spontaneous and induced clones**

Origin	Diagnosis based on spore survival			Diagnosis based on Meiotic segregation			
	Total clones analyzed	$2n$	$2n$-1	Total clones analyzed	Spore survival $2n$	$n+V$	$n+V+II$ or VII
Untreated	13	12	1	32	5	25	2
Treated with MMS	9	4	5	33	23	9	1
Treated with Benlate	12	11	1	36	8	25	3

* As indicated by tetrad analysis.

TABLE 13

Amphotericin B treatment

Dose μg/ml	Sporulation inhibition	Frequency of *HOM3-HIS1* recombinants per 10^2 viable spores	Total frequency of colonies on medium S per 10^4 viable spores
0	0	1.87	1.49
0.05	13.0	2.54	1.52
0.08	18.0	2.50	1.19
0.11	55.6	2.13	1.61
0.30	91.8	2.66	2.82
1.00	96.1	2.05	1.79

The means from Table 1 are used as control values.

(Table 12). Diploid recovery and nondisjunction of chromosome *V*, both increased with amount of dose. The induction of nondisjunction seemed slightly more efficient. The proportion of meiosis II nondisjunction also increased with dosage.

Amphoterycin B: The data reported in Table 13 show quite clearly that the strong inhibition of sporulation due to amphoterycin B is not associated with the formation of diploid gametes or aneuploids. Because the data correspond completely to the control, this chemical can be considered inactive. Therefore we have not reported the subsequent analyses carried out on mater and nonmater clones.

DISCUSSION

We have devised a genetic system that permits the detection of two aberrant phenomena: diploid recovery following meiosis, and nondisjunction of one or a few chromosomes. The availability of a simple experimental procedure to distinguish between these phenomena could be useful not only to strengthen our understanding of the mechanisms causing genomic variation, but also to facilitate the identification of chemicals or environmental conditions with different specific effects on cell division.

Diploid progeny: The classification of colonies as **a**/α diploids is based on their inability to mate and their ability to sporulate. Spore survival and genetic segregation of such clones are typical of diploid or near diploid strains. As expected, diploids **aa** or $\alpha\alpha$ were also among the mater clones. These exhibited spore survival typical of triploid strains when they were mated to appropriate haploids.

The origin of diploid meiotic products appears to be a failure of the second meiotic division. This conclusion is based on three findings: a) The distribution of the phenotoypes *leu ADE*, *LEU ade* and *leu ade* is always in agreement with the ratio 4.7:1.06:0.94, which is expected if they derive from an absence of second meiotic division. This was observed with regard to spontaneous diploids and those induced by MMS and Benlate. b) The frequency of diploids homozygous for mating-type alleles is expected to be identical to that of heterozygous **a**/α only if diploids originate during meiosis II. This is due to the distance of the mating-type locus from its centromere (25 cM). Our data agree with this expectation. c) The frequencies of nonmaters homozygous for *ura3* suggest that most occur because of failures in meiosis II. None of the three types of observations may be sufficient, *per se*, to prove that diploid recovery is due only to a failure of the second meiotic division. However, since all three support this hypothesis it is probable that the diploid meiotic products originated mainly from this source. Our system can detect only about 10% of the diploid clones. The inability to detect the majority stems partially from the selection for cycloheximide resistance (we miss all tetratypes and one-half of PD and NPD between *cyh2* and its centromere), partially from crossover between *hom3* and its centromere, and partially from crossover between *hom3* and *met5*. The frequency of spontaneous diploid clones was $0.54/10^4$ viable spores. We can therefore estimate that, in our strain, spontaneous diploid recovery following meiosis occurs with a frequency of about $5/10^3$ spores.

Disomy: The data demonstrated the occurrence of disomic diplo V clones. The observed overall frequency ($0.95/10^4$ viable spores) can be differentiated into meiosis I nondisjunction and meiosis II nondisjunction, with frequencies of 0.64×10^{-4} and 0.31×10^{-4} per viable spore.

This genetic system cannot detect all the events of nondisjunction because of the crossovers on chromosome *V* (Figure 1). A reliable estimate of the frequency of spontaneous nondisjunction of chromosome *V* would be about 2×10^{-4} per viable spore. Should this frequency be applicable without variation to the other chromosomes of cerevisiae, one could theoretically expect the frequency of meiotic nondisjunction to be on the order of $3–4/10^3$ viable spores. Our data indicate that about 10% of cells disomic for chromosome *V* also carry two *II* or *VII* chromosomes. As these are the only two we can recognize, by extension to the the other chromosomes one can deduce that a nondisjunction event restricted to a single chromosome is rare.

Induced genomic variations: The three chemicals used were chosen from among those able to inhibit sporulation in cerevisiae. MMS is a powerful mutagen that is also able to induce somatic nondisjunction in cerevisiae (PARRY 1977) and in Aspergillus (GUALANDI *et al.* 1979). It is also active during meiosis in Microtus, inducing formation of both aneuploid and diploid gametes (ZIMMERMANN, DE SERRES and SHELBY 1979). Benomyl is very active on somatic nondisjunction in *Aspergillus nidulans* (MORPURGO *et al.* 1979). It probably interferes with spindle formation (MORRIS 1980). One of its derivatives, methylbenzimidazole carbamate (MBC), causes meiotic nondisjunction in Microtus (ZIMMERMANN, DE SERRES and SHELBY 1979). Amphoterycin B induces somatic nondisjunction in Aspergillus (MORPURGO *et al.* 1979) and acts on the plasma membrane (KERRIDGE 1980).

In our system the three compounds inhibit sporulation proportional to dose. They differ from each other with respect to diploid recovery and induction of aneuploidy. Amphoterycin B appears to be the most efficient inhibitor of sporulation, but it does not induce the genomic abnormalities detected by our system. The inhibition must be due to causes other than interference in chromosome separation during meiosis. MMS and Benlate increase both diploid recovery and nondisjunction. MMS seems more efficient in increasing diploid recovery than aneuploidy (with maximal increases exceeding spontaneous frequencies of 26-fold and 7-fold respectively). Benlate affects both phenomena equally.

This genetic system; therefore, seems useful not only for theoretical purposes, but also for applied environmental mutagenesis. The evidence so far collected seems to render it unnecessary to carry out the complex analysis described above on each compound. Practically speaking, whatever the treatment, the only two phenomena detected are: complete or almost complete diploid clones and nondisjunction of chromosome *V*, accompanied or not by simultaneous nondisjunction of chromosome *II* or *VII*.

This investigation was supported by a grant of CNR (Roma), Progetto finalizzato Promozione della Qualità dell'Ambiente, and by grant ENV/345/1 of ECC (Bruxelles).

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