

# MITOTIC CHROMOSOME LOSS IN A DISOMIC HAPLOID OF *SACCHAROMYCES CEREVISIAE*

D. A. CAMPBELL, S. FOGEL AND K. LUSNAK

*Department of Genetics, University of California, Berkeley, California 94720*

Manuscript received July 22, 1974

Revised copy received November 6, 1974

## ABSTRACT

Experiments designed to characterize the incidence of mitotic chromosome loss in a yeast disomic haploid were performed. The selective methods employed utilize the non-mating property of strains disomic for linkage group III and heterozygous at the mating type locus. The principal findings are: (1) The frequency of spontaneous chromosome loss in the disome is of the order  $10^{-4}$  per cell; this value approximates the frequency in the same population of spontaneous mitotic exchange resulting in homozygosity at the mating type locus. (2) The recovered diploids are pure clones, and thus represent unique events in the disomic haploid. (3) Of the euploid chromosomes recovered after events leading to chromosome loss, approximately 90% retain the parental marker configuration expected from segregation alone; however, the remainder are recombinant for marker genes, and are the result of mitotic exchanges in the disome, especially in regions near the centromere. The recombinant proportion significantly exceeds that expected if chromosome loss and mitotic exchange in the disome were independent events. The data are consistent with a model proposing mitotic nondisjunction as the event responsible for chromosome loss in the disomic haploid.

IN the heterothallic yeast *Saccharomyces cerevisiae*, aneuploids of composition  $n + 1$  (disomic haploids) may be readily recovered as meiotic segregants of triploids (PARRY and COX 1971). From these, trisomic diploids of composition  $2n + 1$  can be constructed by crosses to normal haploids. Though yeast aneuploids have been employed to advantage in physical characterizations of chromosomal DNA (PETES, NEWLON, BYERS and FANGMAN 1973), in the chromosomal localization of ribosomal RNA cistrons (FINKELSTEIN, BLAMIRE and MARMUR 1972; GOLDBERG, ØYEN, IDRIS and HALVORSON 1972), in genetic mapping and segregational analyses (MORTIMER and HAWTHORNE 1973; SHAFFER, BREARLEY, LITTLEWOOD and FINK 1971; CULBERTSON and HENRY 1973), and in the isolation of mutants affecting meiotic and mitotic gene conversion (RODARTE-RAMON and MORTIMER 1972; ROTH and FOGEL 1971; FOGEL and ROTH 1974), little information exists concerning their relative mitotic stability. This report describes experiments designed to measure the mitotic stability of a yeast disomic haploid.

Strains disomic for linkage group III may be constructed to contain the mating type locus in heterozygous condition ( $\mathbf{a}/\alpha$ ). Such cells cannot mate with normal  $\mathbf{a}$  and  $\alpha$  haploids. Rarely, however, cells capable of mating arise in the  $\mathbf{a}/\alpha$

disomic population, and can be detected and recovered by selective methods (ROTH and FOGEL 1971; FOGEL and ROTH 1974). These cells are of two types: (1) disomic cells that have become homozygous for the mating type locus ( $\mathbf{a/a}$  or  $\alpha/\alpha$ ), primarily by mitotic recombination and rarely by mitotic gene conversion; and (2) normal haploid cells that have lost one of the two chromosomes present in excess in the disome.

We have utilized the non-mating property of  $\mathbf{a}/\alpha$  disomic cells to select for rare cells capable of mating as a means of determining the incidence of chromosome loss in the disome. Sporulation, ascus dissection and tetrad analysis of the resultant diploids has permitted an unambiguous determination of their genetic structure, and has led to inferences concerning the events underlying spontaneous chromosome loss.

#### MATERIALS AND METHODS

*Media:* Ingredients are given in amounts per liter of distilled water. Media were solidified with 15 g/l agar. *YEED:* D-glucose 20 g, Difco Bacto-peptone 20 g, Difco yeast extract 10 g. *Synthetic complete medium (SC):* D-glucose 20 g, Difco yeast nitrogen base without amino acids 6.7 g, autoclaved separately. The following supplements were then added as a filter-sterilized aqueous solution ( $\mu\text{g/ml}$  final concentration): adenine- $\text{SO}_4$  10, L-arginine-HCl 50, L-histidine-HCl 20, L-isoleucine 50, L-leucine 50, L-lysine-HCl 50, L-methionine 50, L-phenylalanine 50, DL-threonine 600, L-tryptophan 50, L-tyrosine 50, uracil 20. *Suboptimal synthetic complete medium (SSC):* Identical to SC except that the adenine- $\text{SO}_4$  concentration was 0.1  $\mu\text{g/ml}$ . *Sporulation medium:* Potassium acetate 20 g, Difco yeast extract 2.2 g, D-glucose 0.5 g, supplemented with 2 times the amounts of nutrilites listed for SC medium. Sporulation media were adjusted to pH 7 by addition of KOH before autoclaving. *Dropout media:* Synthetic complete medium (SC) lacking single nutrilitite supplements. For example, "adenineless" medium is SC without adenine- $\text{SO}_4$ .

*Yeast strains:* Genotypes of the haploid mutant strains of *Saccharomyces cerevisiae* employed in this work are:

5003-21A-1A	$\frac{+ \text{ his4-4 } \text{ leu2-1 } +}{\text{ his4-290 } + \quad + \text{ leu2-27 } \quad \alpha \quad \text{ thr4 } +};$
	<i>ade2; trp1; met2.</i>
5037-5A	<i>his4-290 leu2-27 <math>\alpha</math> mal2; ade8-18; ura3; lys2.</i>
5037-5B	<i>his4-290 leu2-27 <math>\mathbf{a}</math> mal2; ade8-18; ura3; lys2.</i>
S142	<i><math>\alpha</math>; leu1 trp5 met13 tyr3 lys5 ade5,7.</i>
G2-10A	<i><math>\alpha</math>; leu1 trp5 met13 ade5,7.</i>

The heteroalleles at *his4* were kindly provided by DR. G. R. FINK; their map order, independently determined in tetrad analyses leading to the development of these strains, is that reported by FINK and STYLES (1974). The map order of the heteroalleles at *leu2* is not known, and the order shown is arbitrary (FOGEL and ROTH 1974). The *ade8-18* marker is from DR. M. S. ESPOSITO. Strain S142 is a meiotic haploid segregant of diploid strain XS380 (constructed by DR. S. NAKAI) and was obtained through the courtesy of DR. R. E. ESPOSITO. The genetic map of markers on linkage group III is given in Figure 1.

*Preparation of cells:* Cells were streaked from stock cultures onto *YEED* medium and incubated 2 days at 30°. A single colony suspension was then inoculated at about  $10^5$  cells/ml into *YEED* broth supplemented with the same concentrations of nutrilites listed above for SC medium. The broth suspension was incubated in a flask on a rotary shaker for 24 hr at 30°. Cells were then harvested, washed by 3 cycles of centrifugation and resuspension in water, with final resuspension in 0.15 M NaCl (in the case of aggregation experiments) or in water (in all other experiments).

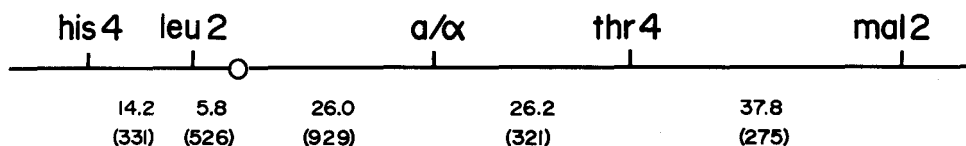


FIGURE 1.—Genetic map of linkage group III. Meiotic map distances are given in cM. The numbers of analyzed 4-spored tetrads from  $2n$  diploids (Table 3) upon which each map distance is based are given in parentheses. The *leu2*-centromere and centromere-mating type locus distances were determined with reference to the centromere marker *trp1* (linkage group IV). The close linkage of *trp1* to its centromere was confirmed by reference to meiotic segregation in trisomic ( $2n + 1$ ) diploids (Table 3), where haploid/disomic disjunction is always in meiosis I. The upper limit of the *trp1*-centromere distance is 0.27 cM (4 tetratype segregations among 747 tetrads). The three intervals in this map to the right of the centromere average 6% larger, and the two intervals to the left of the centromere 38% smaller, than published data (MORTIMER and HAWTHORNE 1973). The discrepancy on the left arm is largely in the *his4*-*leu2* interval (14.2 cM vs. 24.8 cM).

*Mating methods:* (a) *Mating by aggregation.* The mating method based on the vegetative "stickiness" of haploid cells of opposite mating type had been described in detail (CAMPBELL 1973). Aggregation mixtures were prepared as follows: 0.05 ml of the disomic strain, 5003-21A-1A, in 0.15 M NaCl at  $2 \times 10^7$  cells/ml was added to small tubes containing either 0.95 ml of S142 in 0.15 M NaCl at  $2 \times 10^7$  cells/ml [crosses  $a/\alpha (n + 1) \times a (n)$ ], or 0.95 ml of G2-10A in 0.15 M NaCl at  $2 \times 10^7$  cells/ml [crosses  $a/\alpha (n + 1) \times \alpha (n)$ ]. Final input ratios (haploid majority parent to disomic minority parent) were in the range 9-22. The cell mixtures were immediately sedimented by brief (5-min), low-speed centrifugation and incubated at 30°. After 60-min incubation the mixtures were resuspended gently, diluted and plated on SC and on SSC. All platings were by the melted soft agar (0.7%) overlay method, and all plates were incubated at 30°. Independent controls established that  $a/a$  and  $a/\alpha$  disomic haploids participate in aggregation with efficiencies identical to those of normal haploids (>95% of unbudded cells). The haploid testers employed in these experiments (S142 and G2-10A) had the same high efficiency of mating by aggregation in testcrosses with normal haploids of the opposite mating type as did the haploid strains previously described (CAMPBELL 1973). (b) *Mating by replica overlay.* Single clones of the disomic strain, 5003-21A-1A, were isolated on SC medium from platings of broth-growth cells prepared as described above. The clones were individually transferred to YEPD master plates and incubated 2 days at 30°. Matings were made as follows: Cells from each master plate were transferred by replica plating to two YEPD plates. Each of these plates was then stamped with another replicating velvet that had previously been stamped with a YEPD plate containing a confluent lawn of strain 5037-5A ( $\alpha$ ) or strain 5037-5B ( $a$ ). Thus each mating plate contained cells from the original disomic clones covered with a confluent layer of haploid cells. After 1 day's incubation the mating plates were replica-plated to adenineless plates. Since the  $a/\alpha$  disomic haploids carried *ade2* and the overlay haploids contained *ade8*, only diploid cells from successful matings were expected to grow on the adenineless plates. These diploids appeared as small white colonies or papillae against the background of non-growing cells after 4-6 days' incubation. The recovery of adenine-independent diploids within the  $a/\alpha$  clones varied with the relative proportions of the two kinds of cells on the plate. In general, some 5-30 adenine-independent colonies appeared over each original disomic print.

*Isolation of diploid clones:* (a) Aggregated mating mixtures were plated on SSC medium. The low adenine-SO<sub>4</sub> concentration (0.1  $\mu$ g/ml) permits the resolution of zygotic colonies with the same efficiency as that obtained on SC (10  $\mu$ g/ml adenine-SO<sub>4</sub>) plates, but prevents the adenine-requiring parents from reaching more than microcolony status. The number of disomic cells (red *ade2* microcolony-forming units) was limited to 100-200 per plate. This reduced the chance of inadvertent mating on the plates. After 7 days' incubation, zygotic colonies were

streaked for single clones; the single clones were transferred to master plates (at least 4 per original zygotic colony), and their phenotypes were determined by replica plating to an appropriate series of dropout media. Microcolonies on the SSC plates were counted under a dissection microscope, and from these counts estimates were made of the total numbers of disomic ( $a/\alpha$ ) and  $a$  or  $\alpha$  haploid cells plated. In the three aggregation experiments, microcolonies in 451 fields of 1–2-cm diameter were counted. (b) From each of 132 replica overlay matings of  $a/\alpha$  disomic clones crossed with  $a$  and  $\alpha$  haploids (264 crosses), a single adenine-independent diploid colony was streaked to isolate single clones on adenineless medium. In addition, 16 of the matings exhibited sectoring or patchy areas of adenine-independent growth, and these were also streaked. Four clones from each of 273 streaked isolates were transferred to *YEPD* master plates, and their phenotypes determined by replica plating to appropriate dropout media. In several cases clones were not transferred due to contamination or to obvious abnormal growth on the adenineless plates. Only one transferred clone from each original adenine-independent colony was characterized further as described below.

*Sporulation and ascus dissection:* The isolated diploids were replica plated to sporulation medium, and the sporulation plates were incubated 3 days at 30°. Thereafter, they were maintained in the cold and provided material for ascus dissection with little loss in spore viability over a period of several weeks. Asci were dissected by micromanipulation on the surface of agar slabs by standard methods (JOHNSTON and MORTIMER 1959).

*Genetic testing methods:* Decisions on the genetic structure of recovered diploids were based on tetrad analyses of dissected asci. Trisomic diploids produce, after meiosis, two haploid spores and two disomic spores. The trisomic diploids were identified by (1) the presence of spores unable to mate with  $a$  or  $\alpha$  haploid testers (hence of disomic genotype  $a/a$ ), and (2) the presence of spores which yielded prototrophic intragenic recombinants at the *his4* or *leu2* loci after exposure to sublethal doses (> 95% survival) of ultraviolet (254 nm) light (hence of disomic genotype *his4-4/his4-290* or *leu2-1/leu2-27*). In addition, segregations other than 2+:2m at *thr4* and *mal2* contributed to the final decision in some instances. Table 1 illustrates the reliability of these criteria in identifying the trisomic diploids in terms of the number of tetrads analyzed. Initially, 10–15 asci from each diploid were dissected. As Table 1 shows, a smaller number of asci suffices to characterize the diploids, and in later experiments (for example, experiment 4 in Table 3) fewer tetrads were analyzed with little loss in resolution.

Alleles *his4-4* and *leu2-1* revert to prototrophy in response to ultraviolet light, principally by extragenic suppression, whereas alleles *his4-290* and *leu2-27* do not. The response for both revertible alleles is unambiguously distinguishable from the heteroallelic response. Hence, the genetic constitution at *his4* and *leu2* of haploid spores and of many homoallelic disomic spores could also be determined.

TABLE 1

*Identification of trisomic diploids by sequential tetrad analysis*

Tetrads analyzed per diploid	Cumulative number of diploids identified as trisomic	Fraction of total
1	126	0.93
2	133	0.98
3	135	0.99
4	136	1.00
Total	136	1.00

The number and fraction of trisomic ( $2n + 1$ ) diploids unambiguously identified by tetrad analysis is expressed as a function of the number of tetrads analyzed per diploid. The criteria for identification were (1) the presence of spores showing a heteroallelic response at *his4* or *leu2* (hence of disomic genotype *his4-4/his4-290* or *leu2-1/leu2-27*), and (2) the presence of spores unable to mate with standard  $a$  or  $\alpha$  haploid testers (hence of disomic genotype  $a/a$ ).

## RESULTS

This report describes experiments designed to characterize spontaneous chromosome loss in a yeast disomic haploid. Since mitotic chromosome loss in a disomic haploid restores euploidy, the product of this event is potentially recoverable in a stable genetic configuration. Hence, the experimental design allows genetic events occurring in the disome to be distinguished from events which might be superimposed during subsequent analysis.

The experiments involve the selection of rare, mating-competent cells present in the  $\mathbf{a}/\alpha$  disomic population by exposure to standard  $\mathbf{a}$  and  $\alpha$  haploids. The selected mating-competent cells can be either disomic and homozygous for mating type, or haploid. This latter class represents our principal concern, since it defines cells in which one of the two chromosomes originally present in duplicate in the disome has been lost.

Two methods for detecting mating were employed. They differ with respect to both the physiological state of the tested disomic populations, and the efficiency of recovering mating-competent cells. Though the two approaches yield different data, the results display common qualitative features that permit general statements to be made independent of particular methodologies.

*Mating by aggregation*

The "aggregation" mating method has been described (CAMPBELL 1973). The method involves close packing by centrifugation of an unequal mixture of cells of opposite mating type under non-nutrient conditions. The resulting aggregates are weakly bound together, and gentle handling procedures are required to preserve them intact. When plated, they are resolved as zygotic colonies consisting wholly of diploid cells. At input ratios greater than about five, each zygotic colony arises from an aggregate containing a single minority cell and one or more majority cells. Aggregation is restricted to the unbudded cells in the mating mixture.

Table 2 shows the results of aggregation experiments in which the  $\mathbf{a}/\alpha$  disomic haploid was the minority parent. The mean frequency of mating cells in the disomic population is  $2.58 \pm 0.58 \times 10^{-4}$ . This estimate is independent of which mating type is selected. As noted above, two types of diploids may be expected: trisomic diploids, representing matings with homozygous segregants of a mitotic exchange in the disome; and normal diploids, representing matings with cells that have lost one chromosomal element of the disomic pair. Nine of the ten diploids recovered in crosses with  $\mathbf{a}$  sporulated, and could be characterized by tetrad analysis. One was trisomic for linkage group III and eight were normal diploids. Among the ten diploids recovered in crosses with  $\alpha$ , nine were trisomic for linkage group III and one was a normal diploid.

Despite the limited sample presented in Table 2, the data taken together suggest that the recovery frequency of  $2n$  diploids, representing chromosome loss in the disome, approximates that of  $2n + 1$  diploids, representing mitotic exchange in the disome. The results are asymmetric: in crosses with  $\mathbf{a}$ , eight of

TABLE 2

*Recovery of mating-competent cells from the a/α disomic haploid by aggregation*

Experiment (Cross)	Unbudded cell fraction		Total disomic cells (microcolonies counted)	Mating opportunities	Recovered diploids	
	Minority	Majority			Number	Frequency (10 <sup>-4</sup> )
1 (a/α × a)	0.825	0.490	5.48 × 10 <sup>4</sup> (553)	2.22 × 10 <sup>4</sup>	4	1.80
2 (a/α × a)	0.824	0.759	2.19 × 10 <sup>4</sup> (1341)	1.81 × 10 <sup>4</sup>	6	3.32
3 (a/α × α)	0.798	0.868	4.67 × 10 <sup>4</sup> (1801)	3.73 × 10 <sup>4</sup>	10	2.68
Total			12.34 × 10 <sup>4</sup> (3695)	7.76 × 10 <sup>4</sup>	20	2.58

Recovery of mating-competent cells in the a/α disome. Aggregation crosses were performed as described in MATERIALS AND METHODS. Diploids were detected as zygotic colonies on SSC medium. Estimates of the numbers of disomic cells plated were made from counts of red (*ade2*) microcolonies in calibrated fields under a dissection microscope. (The number of cells in the disomic population that engaged in mating and produced zygotic colonies is negligible compared to total disomic cells plated.) Estimates of the numbers of mating opportunities were made by applying a correction based on the observation that budded cells do not aggregate (e.g. Expt. 1  $5.48 \times 10^4 \times .825$ ) (CAMPBELL 1973). An additional correction was applied to Experiment 1 to compensate for the reduced efficiency of aggregation due to the large proportion of budded cells in the haploid majority parent population (e.g.  $5.48 \times 10^4 \times .825 \times .5$ ). The latter correction was based on simultaneous control matings between the a haploid majority parent and a normal α haploid minority parent. Crosses 1 and 2: 5003-21A-1A (a/α disomic haploid) × S142 (a haploid); Cross 3: 5003-21A-1A (a/α disomic haploid) × G2-10A (α haploid).

the nine diploids represent cases of chromosome loss in the disome; in crosses with α, only one of the ten diploids represents chromosome loss. The asymmetry in recovery may reflect an underlying genetic asymmetry, or it may be a methodological or sampling artifact. For example, the clonal relatedness of diploids recovered within the two classes cannot be ascertained with certainty; i.e. "jackpots", in which a significant proportion of the detected events are mitotic descendants of a single, early event, are not excluded. Though the data do not distinguish among these alternatives, the following considerations may be of significance.

The aggregation mating method depends on the expression of cell surface properties unique to each mating type. Only those cells in which a mating phenotype is present will be detected. In the disomic strain it is likely that a delay exists between segregation of a mating genotype and its phenotypic expression (BALLOU and RASCHKE 1974). The duration of this phenotypic lag could differ depending on whether the cell is disomic (and homozygous for a mating type allele) or haploid, especially if the mating type alleles are not dosage-compensated. Also, the phenotypic lag may vary depending on which mating type allele is expressed.

These considerations suggest that the diploid recovery frequency recorded in Table 2, though representing an efficient estimate of cells expressing a mating phenotype, may significantly under-estimate the mating genotype frequency.

The same considerations also suggest that the method itself may be partially responsible for the observed asymmetrical recovery of normal and trisomic diploids.

#### *Mating by replica overlay*

In these experiments single clones of the  $\mathbf{a}/\alpha$  disome were crossed individually to  $\mathbf{a}$  and  $\alpha$  haploids by replica plating under growing conditions. Diploids from successful matings appear as small colonies or papillae on appropriate selective medium. A single diploid colony was taken randomly from each cross, purified and then characterized by ascus dissection and tetrad analysis. This design assures that all recovered diploids are entirely independent of each other. The replica overlay procedure allows mating to occur under conditions of active cell growth. This means that the opportunity for expression of a mating genotype is improved. At the same time, however, the overlay method permits only an approximate estimate of the chromosome loss frequency to be made.

Table 3 shows the results obtained by the replica overlay method. Listed for each experiment are the numbers of diploids recovered in the four genetic categories. Several conclusions are apparent. First as suggested by the aggregation experiments, the relative recovery of  $2n$  diploids, representing chromosome loss in the disome, approximates that of  $2n + 1$  diploids, representing mitotic exchange in the disome. Second, the asymmetry in recovery of the two diploid types in reciprocal crosses is less pronounced than in the aggregation experiments, and is reversed from that previously found. The asymmetry is preserved in all subsets of the experiment, however, implying that it is not an artifact of experimental variance alone. Though we tentatively assign the asymmetry to aspects of the methods employed, further data are clearly desirable and necessary to resolve this finding.

Though only a single diploid colony was isolated from each cross, the secondary diploid clones derived from these isolates did not always comprise a homogeneous set. The question arose whether such heterogeneity reflected undetected conflu-

TABLE 3

*Recovery of mating-competent cells from the  $\mathbf{a}/\alpha$  disomic haploid by replica overlay*

Cross: Experimental subset	$\mathbf{a}/\alpha$	$\frac{\mathbf{a}/\alpha \times \alpha}{\mathbf{aa}/\alpha}$	Total	$\alpha/\mathbf{a}$	$\frac{\mathbf{a}/\alpha \times \mathbf{a}}{\alpha\alpha/\mathbf{a}}$	Total
1	23	8	31	13	19	32
2	20	14	34	13	20	33
3	19	13	32	13	21	34
4	21	12	33	12	19	31
Totals	83	47	130	51	79	130

Recovery of mating-competent cells in the  $\mathbf{a}/\alpha$  disome. Replica overlay crosses were performed as described in MATERIALS AND METHODS. The data are categorized in terms of the genetic constitutions of diploids recovered in the two crosses ( $\mathbf{a}/\alpha \times \mathbf{a}$  and  $\mathbf{a}/\alpha \times \alpha$ ). The total number of asci in which all 4 spores germinated, and upon which the genetic characterizations were based, was 1918 (of 2506 asci dissected). In the first 3 experimental subsets, 8-9 asci per diploid were tested; in the fourth subset, 4-5 asci per diploid were tested.

ence of two or more independently arising diploids, or whether the individual papillae were mixed clones. A control experiment was performed, in which only well-separated diploid papillae were chosen. From each of 50 such primary isolates at least 8 secondary diploid clones were derived and characterized phenotypically. Forty-nine of the 50 isolates yielded homogeneous sets of secondary clones. Only one papilla proved to be heterogeneous. It probably represented superimposed mitotic event(s) subsequent to mating. Clearly, the major conclusion is that each diploid colony is the zygotic product of a *single* mitotic event in the disomic haploid.

#### *Correlation between mitotic recombination and chromosome loss*

An unexpected result was revealed by genotype analyses of the  $2n$  diploids recovered in the replica overlay experiments. Though a majority possessed the genotypes expected from disomic segregations alone, a significant minority were recombinant with respect to the predicted configurations. The data are presented in Tables 4 and 5 for exceptional  $2n$  and  $2n + 1$  diploids, respectively. As shown in Table 4,  $2n$  diploids recovered in crosses  $\mathbf{a}/\alpha \times \alpha$  are expected to be heteroallelic at *his4* and *leu2*;  $2n$  diploids recovered in crosses  $\mathbf{a}/\alpha \times \mathbf{a}$  are expected to be homoallelic at *his4* and *leu2*. Fifteen of the 134 diploids (11.2%) do not fit these expectations. In the case of crosses  $\mathbf{a}/\alpha \times \alpha$  the possibility that the exceptional diploids ( $4/83 = 4.8\%$ ) arose by mitotic events at the level of the diploid is not excluded. In the case of crosses  $\mathbf{a}/\alpha \times \mathbf{a}$ , however, the exceptional diploids ( $11/51 = 20.4\%$ ) *must* be the consequence of recombinational events occurring at the level of the disome.

The data do not reveal the nature of these recombinational events. Specifically, the exceptional recombinant strands could be explained by variously located reciprocal mitotic exchanges, or by mitotic gene conversion at the *leu2* or *his4* loci. It is likely that both phenomena contribute to these results. For example, 14 of the 15 recombinant exceptions can be accounted for by mitotic crossing over in the centromere-*leu2* interval alone (diploid classes 2 and 4), or by mitotic gene conversion at the *leu2* locus (diploid class 3). The central point is that genetic exchange in the disome, whether reciprocal or non-reciprocal, is rare. The reciprocal mitotic exchange frequency in the intervals considered here is much less than  $10^{-3}$  (Table 2); the frequencies of spontaneous mitotic single-site conversions at *leu2* and *his4* in the  $\mathbf{a}/\alpha$  disome, as determined by prototroph selection, are  $4 \times 10^{-6}$  and  $4 \times 10^{-5}$ , respectively. At minimum, 11 of the 134 (8.2%)  $2n$  diploids represent instances of chromosome loss associated with genetic exchange in the disome, when less than a single ( $< 0.1$ ) such association was expected. This is a strong positive correlation, exceeding by more than 100-fold that expected if chromosome loss and mitotic exchange were independent events.

Recombinant exceptions among  $2n + 1$  diploids are listed in Table 5. Nearly all (7 of 9) can be accounted for by mitotic gene conversion at the mating type locus alone. One diploid (class 4) represents an unambiguous reciprocal exchange at the level of the trisomic diploid. Significantly, no diploid is recombinant at *leu2*, and only one is recombinant at *his4*.



TABLE 4

*Genetic structure of exceptional 2n diploids*

1. Cross: $a/\alpha \times \alpha$								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	+	<i>mal2</i>
Expected genotype:								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	+	<i>mal2</i>
Diploid class	Recombinant genotype							Number found
1	<i>290</i> •	<i>1</i> +	+	○	<b>a</b>	+	<i>mal2</i>	1
	<i>290</i> +	+ <i>27</i>	+	○	$\alpha$	+	<i>mal2</i>	
2	<i>290</i> •	• <i>27</i>	+	○	<b>a</b>	+	<i>mal2</i>	3
	<i>290</i> +	+ <i>27</i>	+	○	$\alpha$	+	<i>mal2</i>	
2. Cross: $a/\alpha \times a$								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	<b>a</b>	+	<i>mal2</i>
Expected genotype:								
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	<b>a</b>	+	<i>mal2</i>
Diploid class	Recombinant genotype							Number found
3	<i>290</i> +	<i>1</i> +	+	○	$\alpha$	<i>thr4</i>	+	7
	<i>290</i> +	+ <i>27</i>	+	○	<b>a</b>	+	<i>mal2</i>	
4	+ <i>4</i>	<i>1</i> +	+	○	$\alpha$	<i>thr4</i>	+	4
	<i>290</i> +	+ <i>27</i>	+	○	<b>a</b>	+	<i>mal2</i>	

Genetic structure of  $2n$  diploids recovered in replica overlay matings of the  $a/\alpha$  disome. For each hybrid, the diploid genotype expected from segregation alone, and the recombinant exceptions, are listed. There were no recombinant exceptions among  $2n$  diploids for markers to the right of the centromere on linkage group III. Diploid genotypes at *his4* and *leu2* were deduced from phenotype tests of the diploids, and tests of haploid spore clones recovered from dissected asci. In cross 1 ( $a/\alpha \times \alpha$ ) homoallelism for *leu2-27* and *his4-290* obscures detection of the reversion response at *leu2-1* and *his4-4* (see MATERIALS AND METHODS). The resultant indeterminacy is indicated by the dot (•). In cross 2 ( $a/\alpha \times a$ ) the determinations are unambiguous: a heteroallelic response in the diploid, and 2:2 segregation of the reversion response in tetrads.

TABLE 5

*Genetic structure of exceptional 2n + 1 diploids*

1. Cross: $a/\alpha \times \alpha$								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	+	<i>mal2</i>
Expected genotype:								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	+	<i>mal2</i>
Diploid class								
Recombinant genotype								
Number found								
1	+	4	1	+	○	<b>a</b>	+	<i>mal2</i>
	290	+	+	27	○	<b>a</b>	<i>thr4</i>	+
	290	+	+	27	○	$\alpha$	+	<i>mal2</i>
2	+	4	1	+	○	<b>a</b>	+	+
	290	+	+	27	○	<b>a</b>	<i>thr4</i>	+
	290	+	+	27	○	$\alpha$	+	<i>mal2</i>
2. Cross: $a/\alpha \times a$								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	<b>a</b>	+	<i>mal2</i>
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	<b>a</b>	+	<i>mal2</i>
Expected genotype:								
	+	<i>his4-4</i>	<i>leu2-1</i>	+	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	$\alpha$	<i>thr4</i>	+
	<i>his4-290</i>	+	+	<i>leu2-27</i>	○	<b>a</b>	+	<i>mal2</i>
Diploid class								
Recombinant genotype								
Number found								
3	+	4	1	+	○	$\alpha$	+	<i>mal2</i>
	290	+	+	27	○	$\alpha$	<i>thr4</i>	+
	290	+	+	27	○	<b>a</b>	+	<i>mal2</i>
4	290	•	1	+	○	<b>a</b>	+	+
	290	+	+	27	○	<b>a</b>	+	<i>mal2</i>
	290	+	+	27	○	$\alpha$	<i>thr4</i>	<i>mal2</i>

Genetic structure of  $2n + 1$  diploids recovered in replica overlay matings of the  $a/\alpha$  disome. For each hybrid, the diploid genotype expected from segregation alone and the recombinant exceptions are listed. Diploid genotypes were deduced from phenotype tests of the diploids, and from tests of haploid and disomic spore clones recovered from dissected asci.

In conclusion, though revealed as a minority class among recovered diploids, chromosome loss in the disome is positively associated with genetic exchange in the disome, especially in regions near the centromere. The meaning of this correlation in terms of models of somatic chromosome loss is considered in the DISCUSSION.

#### DISCUSSION

Haploid yeast cells disomic for single linkage groups give rise spontaneously to mitotic segregants that have lost one of the two chromosomes present in excess in the disome. We have investigated this phenomenon by selective methods based on the non-mating property of haploid strains disomic for linkage group III and heterozygous at the mating type locus. The principal results of this work are: (1) The frequency of spontaneous chromosome loss in the disomic population is of the order  $10^{-4}$  per cell; this value approximates the frequency in the same population of spontaneous mitotic exchange resulting in homozygosity at the mating type locus. (2) The recovered diploids are pure clones, and thus represent unique events in the disomic haploid. (3) Of haploid chromosomes recovered after events leading to chromosome loss, approximately 90% retain the parental marker configuration expected from segregation alone, while the remainder are recombined for marker genes. (4) Among the latter, chromosome loss is correlated with mitotic exchange in the disome, especially in regions near the centromere. In the following discussion we consider these findings in relation to possible mechanisms of chromosome segregation and recombination.

A significant and well-documented source of chromosome loss or gain is nondisjunction—the failure of normal modes of chromosome segregation. The present results may be explicable in terms of this mechanism. Alternatively, mechanisms invoking anomalies of chromosome replication followed by normal disjunction may also account for the data.

We consider significant the finding of a marked correlation between chromosome loss and genetic exchange in the disome. On the assumption that the disomic population is uniform, this association is observed as an increased probability of exchange among disomic cells undergoing chromosome loss, when compared to the population as a whole. Alternatively, the disomic population could consist of two subpopulations, one in which the correlation between chromosome loss and mitotic exchange is obligatory, and one in which chromosome loss and recombination are not correlated. The data do not allow us to distinguish between these alternatives, nor to determine when chromosome loss relative to exchange occurs, since within the disomic population recombinant lines can arise in any cell generation. The association of genetic recombination and chromosome loss suggests that genetic exchange may signal the nondisjunctional event, and it implies that the two processes may be closely spaced in time, perhaps in the same cell generation.

One speculation that may account for this correlation is that exchange itself might potentiate sister centromeres to separate prematurely. In normal mitotic division, sister centromeres remain conjoined through metaphase, and only then separate and disjoin to opposite poles. If genetic exchange in the disome, includ-

ing converted segments, were to alter the associative binding of sister centromeres, or to hasten their separation, the probability of abnormal segregations leading to chromosome loss might be enhanced. In this regard, DAVIS (1971) has described a meiotic mutant of *Drosophila melanogaster* which displays markedly enhanced frequencies of equational nondisjunction and somatic mosaicism. The effect of the mutation is to cause premature sister-centromere separation, generally after anaphase I and before metaphase II; equational nondisjunction is independent of exchange in the first meiotic division. These findings are not inconsistent with our speculation, since a low level of separation of sister centromeres as a secondary consequence of exchange might not be seen in the mutant.

Failure of the disomic chromosomes to replicate in preparation for nuclear division could also result in mitotic segregation of normal haploid daughter cells. In this case the association of mitotic exchange with chromosome loss requires that exchange at a four-stranded stage would have to occur at least one cell division before the replicative failure, or else at a two-stranded stage in the same division. In this regard, WILDENBERG (1970) has suggested that at least some mitotic exchanges are best accounted for by events occurring at a two-stranded stage.

The relative ease of detecting mitotic chromosome loss by the methods described here presents several unique experimental opportunities. The isolation of mutants affecting the frequency of spontaneous chromosome loss, analogous to those currently under study in *Drosophila* (SANDLER, *et al* 1968; ROBBINS 1971; DAVIS 1971; BAKER and CARPENTER 1972; PARRY 1973; CARPENTER and SANDLER 1974) may be facilitated by these methods. Such mutants could provide information on centromere structure and function in yeast, and on the genetic control of chromosome segregation (BYERS and GOETSCH 1973; MOENS and RAPPORT 1971; MOENS, ESPOSITO and ESPOSITO 1974). The known recombinogenic effects of radiations and radiomimetic chemicals suggest parallel investigations of their effects on chromosome loss; such studies might provide tests of the models described above.

Finally, the finding that mitotic chromosome loss in the disome occurs at a frequency approximating that of mitotic exchange suggests that chromosome loss could function alongside mitotic exchange as a significant mechanism for revealing, or allowing expression of, new gene combinations in single linkage groups. Yeast monosomic ( $2n - 1$ ) diploids arising spontaneously in laboratory populations, for example, exhibit normal diploid vegetative viability, though their stability depends upon which specific chromosome is monosomic (BRUENN and MORTIMER 1970). This suggests that mitotic chromosome loss could influence differentially the genetic variance of linked gene combinations, with consequent implications for population studies in eukaryotic microorganisms (ADAMS and HANSCH 1974).

We are indebted to PATRICIA LESLIE for skillful and conscientious technical assistance, and to L. B. ROWE for fruitful discussions and suggestions concerning the nondisjunction models discussed herein. This work was supported by NIH Grants GM00367 and GM17317.

## LITERATURE CITED

- ADAMS, J. and P. E. HANSCH, 1974 Population studies in microorganisms. I. Evolution of diploidy in *Saccharomyces cerevisiae*. *Genetics* **76**: 327-338.
- BAKER, B. S. and A. T. C. CARPENTER, 1972 Genetic analysis of sex-chromosomal meiotic mutants in *Drosophila melanogaster*. *Genetics* **71**: 255-286.
- BALLOU, C. E. and W. C. RASCHKE, 1974 Polymorphism of the somatic antigen of yeast. *Science* **184**: 127-134.
- BRUENN, J. and R. K. MORTIMER, 1970 Isolation of monosomics of yeast. *J. Bacteriol.* **102**: 548-551.
- BYERS, B. and L. GOETSCH, 1973 Duplication of spindle plaques and integration of the yeast cell cycle. *Cold Spring Harbor Symp. Quant. Biol.* **38**: 123-131.
- CAMPBELL, D. A., 1973 Kinetics of the mating-specific aggregation in *Saccharomyces cerevisiae*. *J. Bacteriol.* **116**: 323-330.
- CARPENTER, A. T. C. and L. SANDLER, 1974 On recombination-defective meiotic mutants in *Drosophila melanogaster*. *Genetics* **76**: 453-475.
- CULBERTSON, M. R. and S. A. HENRY, 1973 Genetic analysis of hybrid strains trisomic for the chromosome containing a fatty acid synthetase gene complex (*fas1*) in yeast. *Genetics* **75**: 441-458.
- DAVIS, B. K., 1971 Genetic analysis of a meiotic mutant resulting in precocious sister-centromere separation in *Drosophila melanogaster*. *Molec. Gen. Genetics* **113**: 251-272.
- FINK, G. R. and C. A. STYLES, 1974 Gene conversion of deletions in the *HIS4* region of yeast. *Genetics* **77**: 231-244.
- FINKELSTEIN, D. B., J. BLAMIRE and J. MARMUR, 1972 Location of ribosomal RNA cistrons in yeast. *Nature New Biol.* **240**: 279-281.
- FOGEL, S. and R. ROTH, 1974 Mutations affecting meiotic gene conversion in yeast. *Molec. Gen. Genetics* **130**: 189-201.
- GOLDBERG, S., T. ØYEN, J. M. IDRIS and H. O. HALVORSON, 1972 Use of disomic strains to study the arrangement of ribosomal cistrons in *Saccharomyces cerevisiae*. *Molec. Gen. Genetics* **116**: 139-157.
- JOHNSTON, J. R. and R. K. MORTIMER, 1959 Use of snail digestive juice in isolation of yeast spore tetrads. *J. Bacteriol.* **78**: 272.
- MOENS, P. B. and E. RAPPORT, 1971 Spindles, spindle plaques, and meiosis in the yeast *Saccharomyces cerevisiae* (Hansen). *J. Cell Biol.* **50**: 344-361.
- MOENS, P. B., R. E. ESPOSITO and M. S. ESPOSITO, 1974 Aberrant nuclear behavior at meiosis and anucleate spore formation by sporulation-deficient (*SPO*) mutants of *Saccharomyces cerevisiae*. *Exptl. Cell Res.* **83**: 166-174.
- MORTIMER, R. K. and D. C. HAWTHORNE, 1973 Genetic mapping in *Saccharomyces* IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. *Genetics* **74**: 33-54.
- PARRY, D. M., 1973 A meiotic mutant affecting recombination in female *Drosophila melanogaster*. *Genetics* **73**: 465-486.
- PARRY, E. M. and B. S. COX, 1971 The tolerance of aneuploidy in yeast. *Genet. Res.* **16**: 333-340.
- PETES, T. D., C. S. NEWLON, B. BYERS and W. L. FANGMAN, 1973 Yeast chromosomal DNA: Size, structure and replication. *Cold Spring Harbor Symp. Quant. Biol.* **38**: 9-16.
- ROBBINS, L. G., 1971 Nonexchange alignment: a meiotic process revealed by a synthetic meiotic mutant of *Drosophila melanogaster*. *Molec. Gen. Genetics* **110**: 144-166.

- RODARTE-RAMON, U. S. and R. K. MORTIMER, 1972 Radiation-induced recombination in *Saccharomyces*: Isolation and genetic study of recombination-deficient mutants. *Radiation Res.* **49**: 133-147.
- ROTH, R. and S. FOGEL, 1971 A system selective for yeast mutants deficient in meiotic recombination. *Molec. Gen. Genetics* **112**: 295-305.
- SANDLER, L., D. L. LINDSLEY, B. NICOLETTI and G. TRIPPA, 1968 Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. *Genetics* **60**: 525-558.
- SHAFFER, B., I. BREARLEY, R. LITTLEWOOD and G. R. FINK, 1971 A stable aneuploid of *Saccharomyces cerevisiae*. *Genetics* **67**: 483-495.
- WILDENBERG, J., 1970 The relation of mitotic recombination to DNA replication in yeast pedigrees. *Genetics* **66**: 291-304.

Corresponding editor: R. E. ESPOSITO