BISEXUAL MATING BEHAVIOR IN A DIPLOID OF *SACCHAROMYCES CEREVISIAE*: EVIDENCE FOR GENETICALLY CONTROLLED NON-RANDOM CHROMOSOME LOSS DURING VEGETATIVE GROWTH

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ABSTRACT

A diploid strain of *Saccharomyces cerevisiae* has been isolated which exhabits bisexual mating behavior. The strain mates with either a or α strains with a relative mating efficiency of 1 to 2%. The efficiency of mating is correlated with the frequency with which subclones of this strain revert to a single mating type. Crosses of the bisexual diploid with a/a or α/α diploids yield bisexual segregants with a frequency of approximately 3%. Analysis of the segregation of the mating type alleles and other markers on chromosome III indicates that the primary event which leads to the bisexual phenotype is the loss of one homolog of chromosome III during vegetative growth to produce a monosomic ($2n$–1) diploid. Evidence is presented that the loss of chromosome III and possibly of other chromosomes during vegetative growth is affected by a recessive nuclear gene—*her* (hermaphrodite)—which is not closely linked to the mating type locus.

THE yeast *Saccharomyces cerevisiae* is generally stable as either a haploid or or diploid; however, aneuploid states are also quite stable. Diploids monosomic ($2n$-1) for chromosome I have been studied extensively, especially in terms of gene dosage for ribosomal RNA cistrons (BRUENN and MORTIMER 1970; FINKELSTEIN, BLAMIRE and MARMUR 1972; ØYEN 1973; KABACK, BHARGAVA and HALVORSON 1973) and have been found to remain stable after repeated culturing.

Haploid strains disomic (n+1) for chromosome III have also been studied thoroughly (SHAFFER *et al.* 1971; ROTH and FOGEL 1971; ROTH 1973). As chromosome III contains the mating type locus, it is possible to construct disomic strains which are heterozygous (a/α) for mating type alleles. These strains are non-mating, as are normal (a/α) diploids and capable of undergoing many of the early events of sporulation, including pre-meiotic DNA synthesis and recombination (ROTH and FOGEL 1971; ROTH 1973) as well as sporulation-specific RNA synthesis (KADOWAKI and HALVORSON 1971).

This paper describes the properties of an unusual diploid of *Saccharomyces cerevisiae* which is bisexual, rather than non-mating. Evidence is presented that bisexual behavior is directed by a recessive gene not closely linked to the mating type locus and that bisexual mating behavior reflects mitotic chromosome loss of

one or the other homolog of chromosome III and probably other chromosomes. The bisexual strain can thus be used to generate a number of different aneuploid ($2n$-1) diploid strains for a variety of genetic and biochemical studies.

MATERIALS AND METHODS

Strains

The various strains of *Saccharomyces cerevisiae* used in this study were derived from stocks obtained from the Yeast Stock Center at Berkeley, California, the yeast genetics course at Cold Spring Harbor, New York, or Dr. H. O. HALVORSON. The haploid strains M1 (*α ade1 his1 thr3 met13 ura3 can*^{r}) and M5 (*α ade5 ade6 his1 trp2 thr4 CAN*^{S}) were constructed by a series of matings and dissection of tetrads. Diploid strain J10 was selected as a spontaneous diploid:

a ade1/a ade1.

Diploid J100:

α leu2-1 lys2 his1 ura3 + + + CAN^S/*α leu2-27 + + + ade2 met13 tyr1 CAN*^{S}

was obtained by a forced mating. Diploid J110:

his4 leu2 α + ade1 ade2 trp1 +/his4 leu2 α thr4 + + + lys2

was obtained from two segregants of diploid D649, provided by F. SHERMAN. Other diploid strains were obtained from diploid segregants of tetraploids produced from crosses of the diploids specified above. Diploid J101 *a leu2 ade1 CAN*^{S}/*a leu2 ade1 CAN*^{S} was obtained as a segregant from a cross of J10 and J100. The genotypes of other markers in the cross were not determined.

Media and culture conditions

Cells were normally grown at 30° in liquid or on 2% agar plates in YEPD (1% yeast extract, 2% bacto peptone, 2% dextrose) or minimal medium (0.67% yeast nitrogen base without amino acids and 2% dextrose). Plates containing 2% glycerol instead of dextrose, YEPG, were also used to determine respiratory competence.

Genetic methods

Matings, replica plating, mutagenesis, sporulation and tetrad analysis were carried out as described by MORTIMER and HAWTHORNE (1969) and by SHERMAN, FINK and LUKINS (1970).

Mating tests

Cells were tested for mating type on agar plates by stamping a colony with an overlay of cells of a known mating type and grown for one day on YEPD. These plates were then replica plated to minimal medium to test for complementation by mating.

For a more quantitative assay, cells of appropriate mating types, each containing at least one complementing auxotrophic marker, were grown to a stationary phase in YEPD. One ml each of two stationary cultures was mixed with 3 ml YEPD and grown for 10 hours at 30° with gentle shaking. The cultures were then serially diluted and plated out on YEPD and minimal medium to measure the total number of cells and the number of zygotic offspring, respectively. The amount of YEPD added to the mating flask is sufficient to support one cell cycle, on the average.

In order to facilitate the mating of the *a/a* strain with M1, an identical set of flasks was made and to each flask approximately 10 units per ml *α* hormone were added.

Preparation of α hormone

Partially purified *α* hormone was obtained according to the methods of DUNTZE *et al.* (1973). Spent medium for *α* mating type cells grown for 48 hours to stationary phase was passed over an Amberlite CG-50 column and the hormone eluted with 0.1 N NCl in 80% ethanol after several previous washings. The eluate was neutralized and the concentration of *α* hormone determined in liquid medium.

Determination of cell volume

Cell volumes were measured using a Coulter Channelyzer (Coulter Electronics) which was standardized using 2μ diameter latex beads.

RESULTS

Isolation of a bisexual strain

During a screening of possible mutants of the mating type locus, diploids wild type except for histidine auxotrophy were isolated from two haploid strains M1 and M5 which had been treated with the mutagen EMS and then grown together to stationary phase in YEPD. The cells were then plated on minimal medium plus histidine to select for α/α as well as a/α diploids. During the testing of non-mating diploids (presumably a/α) which were selected by this treatment, cells were sporulated and random spore segregants were isolated by glusulase treatment, sonication and plating out the separated spores on canavine-containing plates. Among 21 canavanine-resistant colonies which were tested was one which was bisexual—able to mate with both a and α mating type tester strains. Single cell subclones of this strain, designated B1, also exhibited bisexual mating behavior. The genotype of the strain was found to be *ade6*, *his1*, *thr3*, *thr4*, *trp2*, *can*^{r}.

As judged by mating tests, the efficiency of B1 mating with either a or α strains is considerably less than that observed in other matings. Nevertheless, the mating is sufficient to yield a confluent colony after replica plating to medium which selects only the hybrid offspring of the cross.

Demonstration that B1 is diploid

The wild-type cells produced by mating B1 with either a or α lys1 tester strains were able to sporulate well, but tetrads dissected from these crosses yielded very few (about 5%) viable spores. As this lack of viability is characteristic of triploid strains (PARRY and COX 1970), B1 was crossed with two diploids: J100 (*a/a ade1/ade1 leu2/leu2*) and J101 (α/α *leu2/leu2*). The resulting wild-type offspring were sporulated and dissected with the result that greater than 92% viability was obtained. These results suggested that the bisexual strain was diploid and the crosses produced tetraploid cells with good spore viability.

That this strain was in fact diploid was supported by size analysis on the Coulter Channelyzer. Cells were grown to stationary phase, sonicated briefly and measured. B1 was as large as the diploid strains used for reference, with a mean volume of 71μ^3 as opposed to an average volume of 32μ^3 for the haploid strains or 67μ^3 for the diploid tester strains.

The B1 strain, although apparently diploid, is unable to sporulate, either on plates or in liquid media.

Analysis of mating behavior of B1

The nature of the mechanism of bisexual mating was elucidated by several experiments. First, a large number of single colonies were isolated from B1 and tested for mating type. An example of this is given in Figure 1. Of 352 single

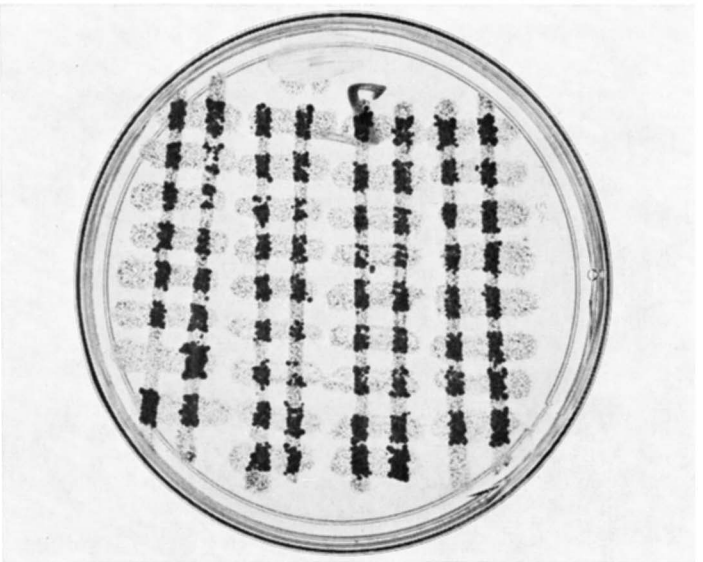

FIGURE 1.—Bisexual mating of B1. Thirty-four single colonies of B1 were arranged on a master plate, grown overnight and replica plated to a YEPD plate which was then stamped with vertical lines of mating type tester strains carrying complementary markers. Of each pair of vertical imprints the α strain (α *ade1*) is on the left and the a tester (a *ade1*) is on the right. The plate was then grown overnight, then replica plated to a minimal medium plate, which would select only for successful matings. The minimal medium plate shown here was grown for 40 hr. With the exception of one colony (lower left), all of the clones exhibited bisexual mating behavior. The remaining colony was typical of revertants to single mating type behavior. In this case B1 has reverted to the α mating type and only mates with the a strain, with an efficiency somewhat greater than that seen for most of the bisexual colonies.

colonies tested, 5 mated only with α strains, 9 mated only with a strains, and the remaining 338 were bisexual maters. These results indicate that bisexual mating reverts to a single mating type with a frequency of about 2.5%. Subclones of the single colonies which mate with only a or only α strains are also able to mate with only one mating type. The fact that B1 can revert from bisexuality to either a or α phenotype may suggest that B1 is an a/α (non-mating) diploid which can mate only after the reversion of the strain to a single mating type.

The frequency of loss of bisexual mating is in close agreement with the relative efficiency of mating of B1 as compared to a/a or α/α diploids. Strains B1, J101, and J10 (a/a *ade1/ade1*) were grown to stationary phase (in each case, about 2×10^8 cells/ml) and then combined pairwise as described in MATERIALS AND METHODS. After mating and growth for approximately one generation, cells were diluted serially and plated on both YEPD and minimal medium to determine the total number of cells and the number of wild-type tetraploids, respectively. As the mating reactions in liquid medium contain significant concentrations of α hormone (DÜNTZE, MACKEY and MANNEY 1970) in the flasks in which α/α cells were added, the relative mating efficiency of B1 with a/a was also measured in the presence of α hormone. The results were given in Table 1.

TABLE 1

Relative efficiency of mating of B1 with a/a *or* α/α *diploid strains*

Cross		α Hormone (Units/ml)	Total cells/ml	Wild-type cells/ml	Fraction wild-type	Relative mating efficiency
J10 × J100	$a/a \times \alpha/\alpha$	0	7.8×10^7	5.7×10^6	7.3×10^{-2}	1
B1 × J100	bisexual × α/α	0	7.5×10^7	6.3×10^4	8.4×10^{-4}	0.012
B1 × J10	bisexual × a/a	0	7.6×10^7	2.5×10^4	3.3×10^{-4}	0.0045
J10 × J100	$a/a \times \alpha/\alpha$	10	7.5×10^7	3.8×10^6	5.1×10^{-2}	0.70
B1 × J100	bisexual × α/α	10	6.5×10^7	4.4×10^4	6.8×10^{-4}	0.009
B1 × J10	bisexual × a/a	10	6.5×10^7	7.2×10^4	1.1×10^{-3}	0.015

Strains B1, J10, and J100 were grown to stationary phase at 30° in YEPD. One ml of these cultures was combined pairwise and an additional 3 ml YEPD added to permit zygote formation and budding. The mating mixtures were incubated for 12 hours with gentle shaking and plated on YEPD and minimal medium to measure total cell number and wild-type cells respectively. Ten units per ml α hormone were added to a parallel set of flasks to facilitate the mating of a/a with the bisexual strain.

The bisexual strain mates with an efficiency relative to the mating of a/a with α/α of approximately 1%. The relative mating efficiency of B1 with the a/a diploid is increased by the presence of partially purified α hormone in the medium. A slightly depressing effect of the hormone on matings of α/α with B1 or of α/α with a/a is also seen. The correspondence between the reversion from bisexual mating and the relative efficiency of mating of this strain support the idea that bisexual mating may require prior reversion from an a/α (non-mating) diploid to either the a or α mating phenotype.

A more detailed genetic analysis of the segregants from matings of B1 was conducted to examine the nature of bisexual mating and the apparent reversion of B1 to a single mating type prior to mating. The results of these dissections are presented in Tables 2 and 3. In crosses of B1 with either a/a or α/α diploids, the segregations of *ade6*, *trp2*, *his1* and canavanine resistance (Table 2) are consistent with the segregation in tetraploid cells of markers not closely linked to centromeres (ROMAN, PHILLIPS and SANDS 1955). In each case, the ratio of

TABLE 2

Segregation of markers in tetrads from crosses of B1 with α/α *and* a/a *diploid strains*

Strain B1 (bisexual diploid, homozygous for *ade6*, *his1*, *thr4*, *thr3*, *trp2* can^r) was crossed with diploid J100 $\frac{\alpha\ leu2\ CAN^S}{\alpha\ leu2\ CAN^S}$ and J101 $\frac{a\ leu2\ ade1\ CAN^S}{a\ leu2\ ade1\ CAN^S}$. The offspring of the crosses were selected, sporulated and dissected.

Segregation (+:—)	B1 × J100					B1 × J101			
	ade	*trp*	*his*	*leu*	CAN^S	*trp*	*his*	*leu*	CAN^S
4:0	19	26	22	1	22	20	19	0	17
3:1	23	29	21	0	20	15	16	0	16
2:2	7	4	6	46	7	5	5	39	7
1:3	0	0	0	2	0	0	0	1	0

TABLE 3

Segregation of mating types of bisexual strain B1 crossed with a/a *and* α/α *diploids*

Tetrads described in Table 1 were tested for mating phenotype. Only tetrads in which none of the four spores were wild-type were used for mating type analysis.

Tetrad mating types*				B1 × α/α	B1 × a/a
a	a	α	α	6	7
a	α	–	–	1	1
–	–	–	–	0	0
α	α	–	–	20	
a	a	–	–		16
α	α	a	–	8	
a	a	α	–		8
α	α	α	a	3	
a	a	a	α		2
B	α	α	–	3	
B	a	a	–		3
B	B	α	α	2	

* Mating phenotypes for each of the four spores of a tetrad were scored. B indicates bisexual mating with both a and α strains. Non-mating colonies are indicated by –.

tetrads $4^+:0^-$, $3^+:1^-$ and $2^+:2^-$ is approximately 4:4:1. These results support the conclusion that B1 itself is a diploid.

The segregation of *leu2* (Table 2) is, however, not consistent with the expected segregation of a tetraploid. Based on the close linkage (7 cM) of *leu2* to the centromere of chromosome III (MORTIMER and HAWTHORNE 1973) and the assumption that B1 is homozygous wild type for *leu2*, one would expect a distribution of tetrads $4^+:0^-$, $3^+:1^-$ and $2^+:2^-$ in the ratio of 58:17:25. This assumption is based on the fact that the original parental strains M1 and M5 were both wild-type for *leu2*. The observed results are that virtually all of the tetrads show 2:2 segregation. If B1 were heterozygous for *leu2*, then a cross with a *leu2/leu2* diploid would always yield 2:2 segregation. Alternatively, these results would be obtained if B1 were aneuploid for chromosome III, leading to a $-/-/+$ triploid which will also give 2:2 segregation.

More direct information about the nature of bisexuality in B1 can be gained from a study of the segregation of mating type alleles, as seen in Table 3. First, while normal tetraploid segregants are found to give tetrads of the following three phenotypes: ($2a$, 2α), ($1a$, 1α, 2 non-maters), and (4 non-maters), most of the observed segregants fall into entirely different classes. This departure from normal tetrads may be the result of the action of an unspecified gene involved in the bisexual mating type, but is most easily interpreted in terms of the segregation of only three functional alleles: for example, $a/\alpha/\alpha$ if B1 is crossed with α/α strains. An illustration of genotypes and phenotypes of such segregants is presented in Figure 2. A comparison of the expected and observed distribution of tetrads is given in Table 4, assuming either bivalent-univalent pairing or trivalent homolog pairing during meiosis (SHAFFER *et al.* 1971). In either model, it is assumed that exchange takes place between only two homologs, but in triva-

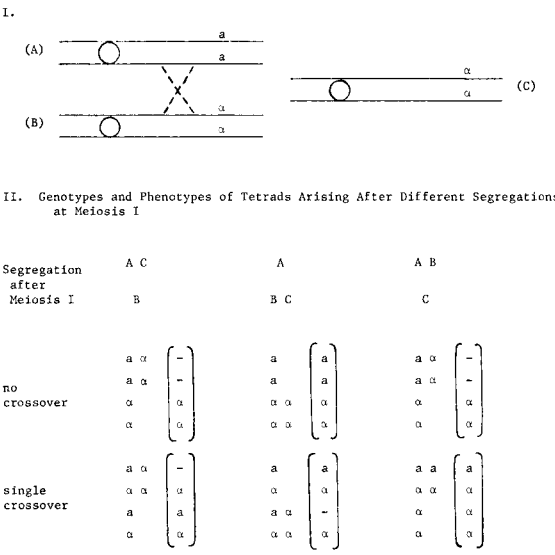

FIGURE 2.—Illustration of possible mating type tetrads which may arise from crossing over and segregation of a triploid during meiosis. It is assumed that crossing over occurs only between two homologs. First division segregation may be of the bivalent-univalent type or may be trivalent. In the latter case chromatids which have undergone exchange may migrate to the same pole during meiosis I. Tetrads which may only arise from trivalent chromosome association are shown on the right part of part II, when the A and B homologs which have exchanged migrate to the same pole. The genotypes and phenotypes of each spore in a tetrad are given. Mating phenotypes are given in brackets. Non-mating (a/α) diploids are indicated by "—".

lent association, both of the exchanged chromosomes may migrate to the same pole. With the exception of a small number of segregants in tetrads of the type ($1a$, 1α, 2 non-maters), the agreement between the observed tetrads and those predicted by triploid segregations of these two types is quite good, especially assuming predominantly trivalent pairing.

Segregation of the bisexual mating phenotype

A second observation from the data in Table 3 is that bisexual behavior is inherited at a relatively low frequency. Of 80 tetrads in Table 3, 8 exhibit one or more bisexual colony. Each bisexual colony can be subcloned to give bisexual single colonies, with a few a or α revertants, as discussed above for B1. Also, these bisexual segregants, when crossed with α/α or a/a diploids, give results of the segregation of mating types analogous to those in Table 3. From the observed distribution of tetrad mating types given in Table 3, one can calculate that approximately 35% of all spores exhibit the a/α (non-mating) phenotype. If bisexuality can be expressed only in an a/α diploid, then the obervation that 3% of all spores are bisexual is most consistent with a segregation of a recessive mutation unlinked to the mating type locus and which is necessary for the expression of bisexuality. The results do not indicate that bisexuality is a property of an allele of the mating type locus.

TABLE 4

Comparison of observed segregation of mating types with distribution predicted for triploids of the type a/a/α *or* a/α/α

The observed mating types of tetrads listed in Table 2 were combined for crosses of B1 with α/α and a/a diploids and grouped to account for equivalent segregation of the two different crosses. Tetrads containing bisexual segregants were omitted from the data and the distribution of the remaining tetrads normalized. The results are compared with the predicted segregation of triploid segregants either in terms of a bivalent-univalent model of pairing at meiosis I or trivalent pairing based on a centromere to mating type distance of 25 cM. Trivalent segregation ratios were calculated by the method of KOSAMBI, as employed by SHAFFER *et al.* (1971).

Tetrad mating types	Observed	Predicted by bivalent-univalent association	Predicted by trivalent association
a a α α	.18	.19	.185
a α – –	.03		
– – – –	.00		
α α – –	.50	.53	.530
a a – –			
α α a –	.22	.28	.240
a a α –			
α α α a	.07		.045
a a a α			

To confirm that the control of bisexuality was nuclear and not mitochondrial, B1 growing in YEPD medium was treated with 25 μg ethidium bromide/ml for five generations and single colonies were isolated. By this treatment (KÜENZI, TINGLE and HALVORSON 1974), more than 99% of the cells become petite, devoid of mitochondrial DNA (GOLDING *et al.* 1970). The *rho*$^-$ B1 cells, unable to grow on YEPG plates, were nevertheless still bisexual.

Several additional observations concerning the segregants summarized in Tables 2 and 3 can be offered to provide more evidence about strain B1 and its bisexual character. In tetrads of the type (2α, 2 non-mating) the non-mating spores can themselves be sporulated and dissected to give viable, apparently haploid spores. The two non-mating colonies from three such tetrads (2α, 2 non-maters) were tested. In one of the six cases, only two of each four spores were viable. In the other cases, however, all four spores of one tetrad were viable, giving the expected 2:2 segregation of heterozygous markers. The viability of spores in these cases ranged from 55% to 80%. In no case was a bisexual spore found.

Loss of alleles on chromosome III

One important additional piece of data concerning the triploid-like segregation of *leu2* and the mating type, both genes on chromosome III, was obtained in a cross using a bisexual segregant of B1, B1/100-11D, which was derived from

TABLE 5

Segregation of two markers on chromosome III

A diploid bisexual segregant (*thr*⁻) from the cross of B1 and J100 was crossed with diploid J110 $\frac{\alpha \ leu2 \ his4}{\alpha \ leu2 \ his4}$ and the resulting wild-type diploid sporulated and dissected.

Growth		
his	*leu*	Number of tetrads
+	+	34
+	+	
—	—	
—	—	
+	+	3
—	+	
—	—	
—	—	
+	+	3
—	+	
+	—	
—	—	
+	+	1
—	—	
—	—	
—	—	

a cross of B1 with J100. This strain (bisexual, *thr*⁻) was crossed with J110 $\left(\frac{\alpha \ leu2 \ his4}{\alpha \ leu2 \ his4}\right)$ and the resulting offspring sporulated. The results, presented in Table 5, show that *his4*, also on chromosome III, segregates $2^+:2^-$ or $1^+:3^-$ in all cases, as does *leu2*. As in the case of *leu2*, B1 was derived from two strains both wild-type for *his4*. The tetrads exhibit a strong linkage between *leu2* and *his4*. The appearance of $1^+:3^-$ segregants is again consistent with trivalent association of homologs. The observed frequency of recombination between *his4* and *leu2* (0.15) is slightly larger than that calculated in a triploid ——/——/++. The predicted frequency for the class of tetrads (*his*:*leu*), ++, —+, ——, ——, is approximately 0.03 (SHAFFER *et al.* 1971) and for all detectable recombinants, about 0.09. These results, including the segregation of mating type, suggest more strongly that one allele for each of these three traits on chromosome III has been inactivated or lost from B1 prior to or during mating or segregation.

Apparent loss of markers on other chromosomes

The loss of chromosome III from the bisexual diploid occurs with a frequency considerably greater than the loss of markers on other chromsomes. The reversion of heterozygous wild-type markers to auxotrophy was examined in a bisexual segregant of B1, B1/100-11D. In the tetrad containing this bisexual segregant, all four spores of the tetrad were wild-type for adenine and tryptophan

requirements, while in other tetrads both *ade6* and *trp2* segregated $4^+:0^-$, $3^+:1^-$, and $2^+:2^-$ in approximately a 4:4:1 ratio (see Table 2). It was concluded that in B1/100-11D both *ade6* and *trp2* were heterozygous. While reversion to *a* or α occurred with a frequency of 0.015, reversion of *ade6*/+ (chromosome VII) to adenine auxotrophy occurred with a frequency of 0.002, while reversion of *trp2* (chromosome V) heterozygotes to auxotrophy was not observed in any of 772 single colonies tested. Thus, for these two markers on chromosomes other than chromosome III, the frequency of loss of one homolog is at least one order of magnitude less.

Reversion of *ade6*/+ to adenine requirement did not result in a loss of bisexuality, as the adenine-requiring colonies were still bisexual. Conversely, adenine auxotophs could also be found among colonies which had reverted to single mating type.

DISCUSSION

Strain B1 is apparently a diploid cell with bisexual mating behavior. The relative efficiency of B1 mating with either *a* or α strains is about 1–2%, approximately the same frequency with which B1 reverts to a single mating type. An explanation of these results is that B1 is predominately a normal a/α (non-mating) diploid in which one or the other mating type allele may be either inactivated or lost. Cells of B1 which are able to mate can do so only with one mating type, depending on which allele remains expressed.

The nature of reversion of B1 to either *a* or α mating phenotype is made more clear by the observed segregation of other markers on chromosome III, which contains the a/α locus. In tetrad analysis of B1 crossed with a/a or α/α strains, the segregation of *leu2* and *his4* is consistent with triploid segregation, while the segregations of markers on other chromosomes are all consistent with tetraploid genotype. It seems, then, that the reversion of B1 to single mating type is attributable to the loss of an entire homolog of chromosome III.

The frequency of reversion of heterozygous markers on different chromosomes is not uniform. The reversion of B1 to a single mating type occurs with a frequency at least one order of magnitude greater than that observed for *ade6* (chromosome VII) or *trp2* (chromosome V). It should be understood, however, that the frequency of adenine-requiring single colonies in a bisexual strain heterozygous for *ade6* is approximately 0.002, more than 1000 times greater than that observed by spontaneous mitotic recombination (THORNTON and JOHNSTON 1971).

Because B1 and its bisexual segregants do not sporulate, analysis of segregation has been restricted to tetraploids. Considerable detail is lost in working at higher levels of ploidy, but an understanding of the control of bisexuality can nevertheless be achieved.

A model for bisexual behavior and chromosome loss

The data presented here can most readily be explained in terms of the effects of a single, nuclear recessive mutant. It is proposed that bisexual mating behavior

arises by the action of this recessive gene, *her* (hermaphrodite), which is not linked to the mating type locus on chromosome III. Bisexual mating will only be observed in strains with the genotype a/α *her/her*.

During vegetative growth and mitosis, cells of this genotype may lose one homolog of chromosome III, leading to a small fraction of cells within a colony which are no longer non-mating (a/α) true diploids but rather aneuploids ($2n-1$) with either a or α mating type. Only the aneuploid cells can mate with tester strains; consequently the relative efficiency of mating is low and reflects the frequency of formation of monosomic chromosome III offspring.

Under the influence of *her*, other chromosomes may also be lost at differing frequencies or not at all.

Evidence that chromosome loss is directed by a single recessive locus

The frequency of bisexual segregants obtained from dissection of tetraploid asci of a cross between B1 and a/a or α/α diploids can be compared with the model of bisexual control presented above. The predicted frequency of bisexual segregants depends first on the proportion of a/α segregants from a triploid ($a/a/\alpha$ or $a/\alpha/\alpha$) chromosome III. Based on the predicted frequencies of tetrad mating type using the trivalent model of meiotic chromosome association (Table 4), one finds that 33% of the spores should be a/α. The independent segregation of the chromosome loss mutant (+/+/*her/her*) will yield homozygous recessive genotype *her/her* 16.5% of the time. Thus the proportion of segregants with the genotype a/α *her/her* is $0.33 \times .165$—approximately 5.5% of the total offspring should be bisexual. From the data given in Table 3 one finds that 10% of the tetrads contained at least one bisexual spore. The actual proportion of bisexual spores was 3.1%.

A second set of tetrads analyzed for mating type is given in Table 6. In this case, B1 was crossed with J110 and tetrads not containing a wild-type spore (47% of the tetrads) were tabulated. Analysis of all of the spores as single, random spores was also done. Here again the proportion of bisexual offspring is approximately 5% to 3%. These proportions are in good agreement with the predicted segregation of a single unlinked recessive gene. If two unlinked genes were directly responsible for the chromosome loss in B1, the proportion of spores which would be bisexual would be less than 1%. The possibility of several recessive genes partially linked cannot be ruled out, but the data are quite consistent with a single-gene model.

Segregation of the bisexual phenotype is not consistent with a close linkage between the mating type locus and *her*; but it is still possible that *her* is linked to chromosome III. If B1 had the genotype $\begin{matrix} a & her \\ \alpha & her \end{matrix}$ the initial reversion of the strain to a single mating type would leave *her* hemizygous as well. In a cross of an a monosome of B1 with an α/α diploid, the segregation of *her* would also be trisomic (*her*/+/+). Such a cross would yield a significant number of segregants which have the genotype for bisexuality illustrated above. The frequency of dif-

TABLE 6

Mating type segregation of diploid spores from a cross of B1 and J110

Asci from the cross B1 and J100 were dissected and tested for mating type. The data are separated into (A) those tetrads which have no wild-type spores (wild-type spores are not easily tested for mating type), and (B) the individual mating types of all non-wild-type spores from all 45 tetrads dissected. Non-mating spores are indicated by "–" and bisexual colonies by "B".

A. Tetrads without a wild-type spore:

Tetrad type	Number	Frequency	Proportion of total spores which are bisexual
a a α α	4	.19	0.000
α α – –	8	.38	0.000
α α *a* –	6	.28	0.000
B α α –	2	.10	0.025
B B α α	1	.05	0.025
	21		0.05

B. Non-wild-type spores from all 45 tetrads:

Mating type	Number	Frequency
a	27	.18
α	70	.46
–	50	.33
B	5	.03

ferent tetrad types can be calculated from equations devised by SHAFER *et al.* (1971).

Assuming that *her* freely recombines with the mating type locus and that trivalent association of homolog is predominant (see below), tetrads of the type (+/+, +, +, *her/her*) would be expected 7.4% of the time; and the proportion of spores homozygous for *her* would then be about 1.8%. Of these spores, approximately one-half would also have an *a*/α genotype (see Figure 3). Thus, bisexual segregants would be expected in a frequency of 0.9%. This proportion is substantially below the observed frequency of 3–5% and suggests that the control of the loss of chromosome III is more likely affected by a recessive gene on another linkage group.

Evidence that trisomic meiotic association is trivalent

The data presented for the segregation of mating type and for other markers on chromosome III support the observation of SHAFFER *et al.* (1971) and of CULBERTSON and HENRY (1973), that trisomic chromosome association during meiosis I must be predominantly trivalent, rather than bivalent-univalent. In trivalent association it is assumed that recombination may occur between any two homologs but that subsequent disjunction occurs at random, so that the recombined chromosomes will migrate to the same pole approximately one-third of the time. This pattern is seen not only for the segregation of individual markers but for the segregation of linked markers on chromosome III (Table 5) as well. In this case, $3^-:1^+$ are predicted on the basis of a bivalent-univalent segregation model in which chromatids undergoing recombination do not migrate to the same

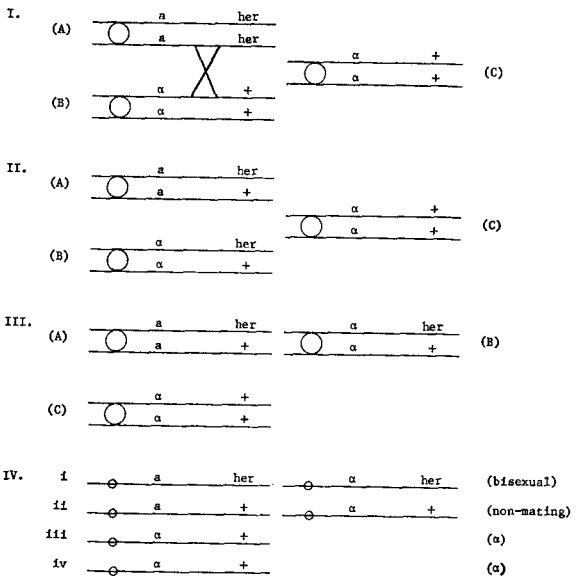

FIGURE 3.—Illustration of the appearance of bisexuality in diploid spores of a tetraploid trisomic for chromosome III. It is assumed that a recessive allele, *her*, directs the loss of chromosome III to yield a bisexual phenotype which is linked to chromosome III but which freely recombines with the mating type locus. One particular chromosome association is shown, in which after a single crossing over event (I, II) the two homologs which have exchanged migrate to the same pole (III). The genotypes and phenotypes of the four spores formed after meiosis II are shown in part IV.

pole. The observed frequency of $3^-:1^+$ segregants is somewhat higher than that predicted by trivalent association of homologs assuming no change in recombination frequency over normal bivalent exchange. For (*his4/his4/+*) the expected frequency of $3^-:1^+$ meiotic segregants is 4%. In this study 10% of 41 tetrads were $3^-:1^+$.

Indeed, if one assumes that the tetrads containing bisexual spores in Table 6 are derived from tetrads of mating type $\alpha\alpha$——, then the proportion of tetrad mating types ($a\ a\ \alpha\ \alpha$), ($\alpha\ \alpha$——), and ($\alpha\ \alpha\ a$—) are in the ratio 0.52:0.29:0.19, which are extremely similar to the ratios predicted by trivalent segregation of a triploid shown in Table 4.

Relation of B1 behavior to chromosome loss in other organisms

The mitotic loss observed by B1 appears quite similar in many respects to mutants in *Drosophila melanogaster* which exhibit mosaics caused by high frequency chromosome loss during mitosis. In Drosophila three related instances of such mitotic instability have been reported: claret nondisjunction (ca^{nd}) (LEWIS and GENCARELLA 1952; DAVIS 1969); paternal loss (*pal*) (SANDLER 1971); and mitotic loss inducer (*mit*) (GELBART 1974). Each of these mutants is recessive and acts parentally, producing gynadromorphs and haplo-4 mosaics among the progeny of female homozygous recessive for one of these mutants. Of

the three mutants, *mit* is most like the behavior of B1 in that either maternally or paternally derived chromosomes may be lost. Further, loss of chromosomes is on average later during early embryogenesis than for ca^{nd}, *pal*, or ring *X* chromosomes (GELBART 1974), which are usually lost during the first nuclear division. The frequency of *X* chromosome loss with these mutants ranges from about 1% to 5%. The frequency of chromosome *4* loss is also greater than 1%. One striking feature of the chromosome loss in these *Drosophila* is that it may occur in flies which themselves are not homozygous recessive for the loss mutations.

Among the fungi, chromosome loss has been observed in both *Aspergillus* (KÄFER 1961; UPSHALL 1971) and *Neurospora* (PITTENGER 1954; SMITH 1974), but in these cases loss is associated with unstable aneuploidy. In yeast, aneuploids appear to be far more stable (SHAFFER *et al.* 1971; PARRY and COX 1970; BRUENN and MORTIMER 1970). In the specific case of B1, an explanation of loss induced by severe aneuploid effects seems unlikely, as sporulating (non-mating) diploids taken from tetrads ($\alpha\alpha$——) of a cross of B1 with an α/α diploid are not themselves aneuploid. Spore viability of tetrads from these diploids is much greater than the 2:2 (viable to inviable) segregants which would be observed for monosomic diploids (BRUENN and MORTIMER 1970). Aneuploids of types such as $(2n+1)$ cannot be ruled out.

Related mutants in Saccharomyces cerevisiae

Recently, CULBERTSON and HENRY (1973) have described a fatty acid-requiring mutant in *Saccharomyces cerevisiae* which exhibits a high frequency of spontaneous aneuploidy $(n+1)$ for the chromosome on which *fas1* has been mapped. The basis of chromosome duplication in this strain is unknown. In one case a fatty acid-requiring strain also led to aneuploidy $(n+1)$ chromosome III (HENRY and FOGEL 1971); however the effect appears to be non-random and almost exclusively associated with the chromosome on which the gene is mapped. There is no clear relation between the effects of severe auxotophy observed in fatty acid-requiring strains and the formation of monosomes in B1. It is possible that *her*, if linked to chromosome III, might lead to the overproduction of some metabolite, and produce conditions which favor dosage compensation by chromosome loss. Alternatively, *her* might exert a direct effect on chromosome structure or organization such that nondisjunction or altered chromosome replication might occur.

In understanding the mechanism of chromosome loss it is also important to recall that none of the bisexual colonies tested have been successfully sporulated. Preliminary evidence does suggest that premeiotic DNA synthesis and recombination do occur (HABER, unpublished results), so that the block in sporulation may occur at a stage later in meiosis or during subsequent spore formation. Further studies along these lines are currently in progress.

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