EVIDENCE OF PREFERENTIAL PAIRING OF CHROMOSOMES AT MEIOSIS IN ANEUPLOID YEAST[1]

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ABSTRACT

Meiotic pairing in homothallic *S. cerevisiae* was studied by tetrad analysis, using strains that were trisomic or tetrasomic for chromosome I. The disomic segregants of these strains produce tetrasomic spore colonies that can be distinguished by their phenotype. Results indicated the existence of preferential pairing and nonrandom assortment of chromosomes at meiosis I. The frequency of crossing over is apparently normal in at least some regions when non-preferred pairing occurs.

INFORMATION about the behavior of yeast chromosomes during meiosis is restricted by inadequate cytological techniques. This restriction can be partially circumvented by use of the classical technique of tetrad analysis. In particular, tetrad analysis provides an efficient method of investigating the behavior of meiotic chromosomes of polyploids and aneuploids. It has been used by ROMAN *et al.* (1955) to study meiotic pairing of chromosomes in tetraploids and, more recently, by SHAFFER *et al.* (1971) and by CULBERTSON and HENRY (1973) to detect the pairing arrangements in strains trisomic for chromosome III and chromosome XI respectively.

We have been studying chromosome behavior in homothallic strains of yeast that are trisomic or tetrasomic for chromosome I. These aneuploids are well adapted for the study of chromosome behavior in meiosis because the colonies produced by disomic spores can be distinguished with ease. Furthermore, the aneuploids are stable. The trisomic in particular is very stable in meiosis; deviations from the expected tetrad ratio of 2 normal:2 disomic have occurred as 1:3 or 3:1 segregations with a frequency of less than one per cent. Chromosome I carries the marker *ade1* and has been further identified as the carrier of a substantial proportion of the cistrons for rRNA (FINKELSTEIN, BLAMIRE and MARMUR 1972; ØYEN 1973). Results indicate the existence of preferential pairing and non-random assortment of chromosomes at meiosis.

MATERIALS AND METHODS

The yeast was *Saccharomyces cerevisiae* and the strains were homothallic. They were derived from a series of crosses between a strain of the homothallic B67 which is tetrasomic for chromosome I (JAMES, INHABER and PREFONTAINE, 1974) and the heterothallic X2928-7D obtained

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from R. K. MORTIMER. The latter strain was the source of the two mutant loci *ade1* located on chromosome I, and *trp1* located close to the centromere on chromosome IV.

Matings were either spore to spore or involved mass matings which utilized the two genetic markers *ade1* and *trp1*, with subsequent isolation of prototrophs.

Disomic segregants were identified by examining spore colonies microscopically after incubation for 24 hours. The cells of these colonies were in fact tetrasomic (2n + 2) because the strains were homothallic. Such tetrasomic colonies are ragged in appearance, many of the cells being enlarged and near the point of death. These distinguishing characteristics were enhanced by the addition of phloxine B to the solid medium at a concentration of .025 mg/ml. Under these conditions the dead cells were stained bright pink. The correspondence between genotype and phenotype in these strains was extremely high if not exact (JAMES, INHABER and PREFONTAINE 1974). Medium containing phloxine B becomes toxic in the presence of visible light, and for this reason incubating cells were exposed to light as little as possible.

Details concerning sporulation and the isolation of spores have already been published (JAMES 1974). Special care was taken to insure that dispersal of spore clusters after glusulase treatment was kept to a minimum. Other techniques were routine.

RESULTS AND DISCUSSION

Initial evidence of non-random assortment of chromosomes at meiosis was provided by tetrad analysis of a trisomic of genotype *ADE1 ADE1 trp1/ade1 TRP1*. Both disomy and the tryptophan requirement segregated 2:2 with few exceptions, but the frequencies of 4:0, 2:2, and 3:1 segregations (+:−) for adenine were 158, 41 and 2 (Table 1), deviating significantly ($P < .01$) from the expected 127, 60, and 14, as calculated from the expressions $\frac{1}{3}(2-x)$, $\frac{1}{3}(1-x)$, and $\frac{2}{3}x$, where $x = .1$, the estimated frequency of second division segregations for *ade1* (MORTIMER and HAWTHORNE 1966). These expected frequencies assume that bivalent-univalent arrangements of chromosomes occur at meiosis I, the paired chromosomes migrating to different poles while the univalent migrates to either pole. The data demonstrated a paucity of both 2:2 and 3:1 segregations and, taken at face value, they imply that a "wild-type" chromosome I is more likely to pair with another "wild-type" chromosome I than with a chromosome I

TABLE 1

Tetrad analysis of the trisomic ADE1 ADE1 trp1/ade1 TRP1

Asci	No.	Adenine (+:−)		
		4:0	2:2	3:1
Complete*	201	158	41	2
Gene conversion (*trp1*)	7	5	1	1
Poor colony morphology	7	5	2	
Three colonies only	33	19	7	2
			(1)	(4)†
Two colonies only	5	1	(1)	(3)
		188	53	12
Expected		160	76	17
χ^2		13.3	$P < .01$	

* These data have already been published (JAMES, INHABER and PREFONTAINE 1974).
† Classification of bracketed data was arbitrary. See text.

which is marked by the mutant (*ade1*) locus. There are several alternative explanations, including one based on the common occurrence of trivalent arrangements in which all three chromosomes associate at metaphase I and then assort at random. These explanations, together with that based on preferential pairing, are considered below.

Reduced survival of adenine-requiring segregants: The above frequencies were for complete tetrads only and it is evident that the data might be biased for this reason. Thus, if adenine-requiring segregant spores germinated poorly or were inadequate in subsequent growth many of the potential 2:2 or 3:1 segregations would have been ignored as incomplete tetrads. This possibility could be tested because the segregation for adenine was either evident or could be inferred in much of the excluded data. Fifty-two tetrads had been omitted from the data because they were incomplete or otherwise unsatisfactory. Segregations for adenine could be assigned in 14 of these because exclusion had been based on irregular segregation of tryptophan or on poor colony morphology. In the case of the 38 incomplete tetrads, segregations for adenine could be assigned in 29 instances on the assumption that aneuploid segregants should rarely be adenine requiring. Only two of the remaining nine segregations could be assigned to a 2:2 category. The other seven were arbitrarily classified as 3:1 though some or all of them may have been 4:0. The data, so adjusted, still deviated significantly ($P < .01$) from the expected (see Table 1). It was concluded that the anomalous segregation ratio could not be attributed to the exclusion of incomplete tetrads.

Incorrect estimates of map distance: 3:1 segregations are a consequence of crossing over between *ade1* and its centromere, and the low frequency of these suggested that the map distance between the locus and its centromere is shorter in the homothallic B67 than in the heterothallic strains used by MORTIMER and HAWTHORNE (1966) to calculate the value of x. However, such a circumstance would not provide a satisfactory explanation of the anomalous segregation ratio; reduction in the assumed map distance to accord with the frequency of 3:1 segregations would produce expected relative frequencies of 4:0 and 2:2 segregations even more divergent from the actual data.

Incomplete sporulation: It is usual for a fraction of the asci in a sporulating culture to contain fewer than four spores. Routinely such asci are not genetically analyzed. However, data based only on 4-spored asci would be biased if specific genotypes were at a selective disadvantage during the process of spore formation. This possibility was tested by analyzing a random sample of all asci.

Trisomic strains of genotype *ade1 ade1 TRP1/ADE1 trp1* were used. These are expected to segregate 2:2 for adenine as well as for disomy and for tryptophan, but the expected frequencies of parental ditype, nonparental ditype, and tetratype segregations (PD, NPD, T) for disomy and adenine are equivalent to the frequencies of 2:2, 4:0 and 3:1 ratios for adenine in Table 1.

The frequency of sporulation exceeded 90 per cent in these strains and spore germination was good. About 25 per cent of the asci were four-spored. A random sample of all asci as well as additional samples of four-spored asci were dissected and classified. The segregations of three-spored asci were unambiguous, though

TABLE 2

Genetic analyses of segregants from cultures of genotype ade1 ade1 TRP1/ADE1 trp1 *using asci containing different numbers of spores*

	4-Spored			3-Spored			2-Spored			1-spored		Total*		
	P	NP	T	P	NP	T	P	NP	T	P	NP	P	NP	T
Disomy-Ade	154	430	22	25	101	9	22	66	7	7	17	201	597	38
Freq.	.25	.71	.04	.18	.75	.07	.23	.70	.07			.24	.71	.05
Disomy-Trp	302	289	15	75	55	5	42	51	2	12	12	419	395	22
Freq.	.50	.48	.02	.55	.41	.04	.44	.54	.02			.50	.47	.03
Ade-Trp	299	274	33	61	62	12	37	51	7	6	18	397	387	52
Freq.	.49	.45	.06	.45	.46	.09	.39	.54	.07			.48	.46	.06

* Omitting one-spored asci.

occasional misclassification may have been caused by gene conversion. In the case of two-spored asci, ambiguity regarding segregation involved only two of the ten possible combinations of any two characters. These were the combinations +−, −+, and ++, −−, which do not distinguish between PD and T in the first instance or between NPD and T in the second. Such segregations were not classified as T on the grounds that they represented the two products of the first meiotic division. Justification of this assumption has been reported (JAMES 1974).

The frequencies of tetrad types were almost identical for the different classes of ascus (Table 2). It was concluded that little or no bias was introduced by restricting data to those of four-spored asci.

Preferential pairing: The accumulated frequencies of tetrad types for disomy and adenine in Table 2, 201, 597, and 38, differed significantly ($P << .01$) from the expected 251, 529, and 56 and the deviations were in a direction similar to those of Table 1, implying an excess of pairing between "like" chromosomes and a consequent reduction in the frequency of recombinations involving adenine. Of the thirty-eight tetratypes, four could be scored as second division segregations of disomy because they were also tetratypes for disomy-tryptophan segregation but were either parental or nonparental for adenine-tryptophan segregation. These might be instances in which crossovers involving both adenine and tryptophan occurred in the same cell, or they might be instances of equational division of the supernumerary chromosome in meiosis I, evidence of which has already been obtained (JAMES, INHABER and PREFONTAINE 1974).

If trisomic chromosomes pair at random to produce bivalent-univalent arrangements in meiotic cells of genotype *ade1 ade1/ADE1*, then like homologs, those

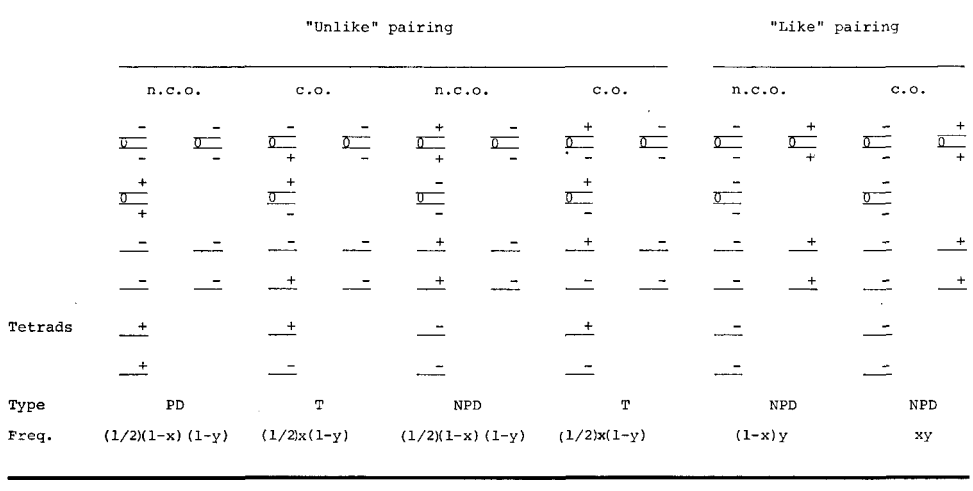

FIGURE 1.—The types and frequencies of asci obtained by sporulation of a trisomic from cross *ade1 ade1* × *ADE1* on the assumption of bivalent-univalent arrangements at meiosis. The locus for adenine is located close to the centromere. x is the frequency of second division segregations and y is the frequency of "like" pairing.

containing the mutant *ade1* locus, will synapse in one third of the instances. Preferential pairing would, in changing this fraction, alter the apparent frequency of recombination between the adenine locus and its centromere as well as the relative frequencies of parental and non-parental ditypes (see Figure 1). The influence of preferential pairing on the distribution of tetrad types can be calculated from the equations:

$$\begin{aligned} \text{PD} &= .5\ (1-y)\ (1-x) \\ \text{NPD} &= y + .5(1-y)\ (1-x) \\ \text{T} &= x(1-y) \end{aligned}$$

where x is the frequency of second division segregations and y is the frequency with which like homologues pair. From these equations it is evident that a unique estimate of y is provided by NPD−PD. Moreover this estimator is unaffected by any value of x. The role of preferential pairing in the data of Table 2 can then be assessed by comparing the actual numbers of non-parental and parental ditypes (597 and 201) with those expected in the absence of preferential pairing. The latter (538 and 260), are calculated from the equations NPD − PD = .333, and NPD + PD = .955 (798/836). The comparison yields a χ^2 of 19.8 ($P << .001$).

The estimate of y was .474. This term and a value (.1) for x were then used to calculate the expected frequencies of PD, NPD, and T. This value of x was preferred to one derived from the present data because it had been calculated from crosses involving normal haploids (MORTIMER and HAWTHORNE 1966). The expected frequencies, so calculated, 198, 594 and 44, were a remarkably close fit to the actual, 201, 597 and 38. Thus, the data for the overall population conformed well to the assumption that like homologs underwent meiotic pairing with a mean frequency that was 42 per cent $\left(\frac{.474 - .333}{.333}\right)$ higher than normal.

The validity of the assumption of preferential pairing was strengthened by the results obtained when similar calculations were applied to the data of Table 1. In this instance the data of those tetrads whose classification had been arbitrary were omitted. The resulting distribution was 188, 51 and 5 for 4:0, 2:2, and 3:1 ratios. The frequency of 4:0 and 2:2 segregations (.770 and .209) yielded a value of .561 for y and expected frequencies of 185, 48 and 11. The difference between these and the actual frequencies was not significant ($P > .1$).

The evidence for nonrandom pairing suggests an innate tendency for like homologues to pair. Alternatively it is possible that such preferential pairing might be a consequence of the manner in which the trisomic strains were constructed. For instance, the two like homologs in the trisomic considered in Table 2 were derived from the same parent cell, a circumstance which might lead to a continuing association of these two chromosomes through subsequent generations and a consequent tendency to pair at meiosis. Evidence against this supposition is presented in Table 3, which contains the data from tetrad analyses of the two strains, *ade1 ade1 TRP1/ADE1 trp1* and *ADE1 ade1 trp1/ade1 TRP1*.

TABLE 3

Tetrad analysis of two trisomics for chromosome I of identical genotype but of different construction

	Disomy-Ade			Disomy-Trp			Ade-Trp		
	PD	NPD	T	PD	NPD	T	PD	NPD	T
a) *ade1 ade1 TRP1/ADE1 trp1**	79	249	20	170	166	12	154	166	28
b) *ADE1 ade1 trp1/ade1 TRP1*	255†	60	13	160	159	9	146	164	18
χ^2 (a vs. b)		3.56			.20			1.59	
P		>.05			>.9			>.3	

* These data are included in those of Table 2.
† PD and NPD are reversed for testing purposes.

Both sets of data for disomy-adenine segregations differ from those expected of random pairing, but the difference between the two is small and even if real, is in the direction opposite to that expected of the assumption under test.

Is the production of aberrant tetrad ratios an invariant characteristic of the trisomic strains under consideration here? An analysis designed to answer this question provided no statistical evidence that the segregational pattern of these strains becomes more normal with continued culture. Segregational data could be separated into two categories, one containing data in which zygotic cultures were sporulated as soon as was technically feasible, the other containing data in which sporulation was carried out after the strains had been cultured for two weeks. The two sets of data (see Table 4) differed significantly ($P < .05$) but this difference could not be attributed to the relative frequencies of parental and non-parental ditypes ($P > .1$), an indication that the tendency for preferential pairing did not decrease significantly with time. The estimated frequency with which like homologs paired (γ) was .508 for the combined data. Again, the expected ratios, based on this degree of preferential pairing, were in close accord with the actual data (Table 4).

TABLE 4

*The effect of continued culture on tetrad ratios (Disomy-*Ade)* produced by trisomic strains of genotype* ADE1 ade1 ade1

	Immediate spor.					Delayed spor.		
	PD	NPD	T			PD	NPD	T
No.	105	387	15			156	465	36
Freq.	.207	.763	.030			.237	.708	.055
χ^2 (PD, NPD, T; imm. vs. del.)			6.528		P < .05			
χ^2 (PD, NPD; imm. vs. del.)			1.98		P > .1			
γ^2 (NPD–PD)				.508				
				PD	NPD	T		
Exp. (adjusted for γ)				258	849	57		
χ^2 (Exp. vs. Act.)					.67	P > .7		

* These data include those of Table 2.

Tetrasomics: Can evidence for preferential pairing be found by examining tetrads of tetrasomics? Affirmative evidence was obtained using two tetrasomics of identical genotype but different construction, *ADE1 ADE1/ade1 ade1*, and *ADE1 ade1/ADE1 ade1*. Additionally, the effect of a delay of two weeks in sporulation was determined. Data are summarized in Table 5. The frequencies of tetrad types differed significantly ($P << .01$) from those expected under the assumption of random pairing and bivalent formation (ROMAN, PHILLIPS and SANDS 1955). The degree of preferential pairing was estimated from the equations:

$$\begin{aligned} 4{:}0 &= y + (1-y)\{\tfrac{1}{2}(1-x)^2 + \tfrac{1}{4}x^2\} \\ 2{:}2 &= (1-y)\{\tfrac{1}{2}(1-x)^2 + \tfrac{1}{4}x^2\} \\ 3{:}1 &= (1-y)\{2x(1-x) + \tfrac{1}{2}x^2\} \end{aligned}$$

Using the value $y = 4{:}0 - 2{:}2$, there was no evidence that the amount of preferential pairing was affected either by the manner in which the strains were constructed or by an extension of the interval between zygote formation and sporulation. The expected frequencies of 4:0, 2:2, and 3:1 segregations for adenine, where $y = .541$ and $x = .1$, were 168, 43, and 20, in good agreement with the actual frequencies of 170, 45, and 16. The data thus suggested that like chromosomes paired with a frequency of 55 per cent rather than with the expected frequency of 33 per cent.

Preferential pairing would seem to be a consequence of differences in degree of chromosome homology and it is appropriate to consider the nature of these differences. Among the chromosomes of *S. cerevisiae*, chromosome I has the shortest genetic length (5 cM), and only three genes, including *ade1*, have so far been assigned locations on it (MORTIMER and HAWTHORNE 1973). It would be surprising if the degree of preferential pairing suggested by these studies were attributable to the single locus for adenine. Furthermore, two previous publications have included data from tetrad analyses of trisomics for chromosome I (HAWTHORNE and MORTIMER 1960; Cox and BEVAN 1962) and neither set of data provided any indication of preferential pairing. This investigation, on the other hand, was carried out with homothallic rather than with heterothallic strains. Diploidy in a homothallic strain is normally initiated when two haploid mitotic progeny of a single spore fuse soon after spore germination. Thus, the cells are by nature completely homozygous except at the locus for sex. In fact, it seems likely that this high degree of homozygosity is one of the reasons why the phenotypic consequences of aneuploidy can be so easily detected in the segregants of a homothallic strain. In this study, however, the chromosome which contained the mutant adenine allele was introduced from an unrelated heterothallic strain. A considerable degree of non-homology was thus possible for chromosome I. In aneuploids, such non-homology can be maintained even after fusion of identical mitotic progeny from a single spore. There is good evidence that chromosome I contains a substantial proportion of the cistrons for ribosomal RNA (FINKELSTEIN, BLAMIRE and MARMUR 1972; ØYEN 1973), and it may be that the lack of homology reflects differences in the region containing these genes.

TABLE 5

Genetic analyses of tetrasomics for chromosome I

Strain		Sporulation	*Ade+*:*Ade−* 4:0	2:2	3:1	
1	*ADE 1 ADE 1/ade 1 ade 1*	immediate	62	15	6	
		delayed	42	8	3	
						χ^2 (PD, NPD; imm. vs. del.) = .16 P > .5
						χ^2 (PD, NPD; str. 1 vs. str. 2) = 1.11 P > .2
2	*ADE 1 ade 1/ADE 1 ade 1*	immediate	32	12	1	
		delayed	34	10	6	
	Total		170	45	16	
	Expected ($\gamma = .333$)		140	63	28	χ^2 (exp. vs. act.) = 16.72 P << .01
	Expected ($\gamma = .541$)		168	43	20	χ^2 (exp. vs. act.) = .16 P > .5

Trivalent arrangements of meiosis: The data of this study are consistent with the assumption that bivalent-univalent arrangements of chromosome I are common in yeast. In this respect they are in contrast with those of SHAFFER *et al* (1971) and CULBERTSON and HENRY (1973) which provided very good evidence that trivalent arrangements with random assortment of chromosomes occur with a high frequency in trisomics of chromosome III and XI. This evidence was provided by the existence of a category of segregations that could not easily be explained otherwise than by the migration of both chromosomes involved in a crossover event to the same pole at meiosis I. In some other respects those data did not provide particularly close fits to that expected of trivalent arrangements. The present data are not very informative with regard to the presence of the category mentioned above because the clonal products of so-called irregular segregations of the adenine locus were not further analyzed. Of eighteen irregular segregations produced by 4-spored asci of Table 2, all could be explained in terms of single gene-conversion events. However, ten of them could also be explained in terms of trivalent formation and random assortment of chromosomes. This number is, in fact, that expected of trivalent arrangements, being one quarter the frequency of tetratypes. Otherwise, using the formulas of SHAFFER *et al.* (1971), the data of this study are discordant with the supposition that trivalent arrangements are common.

It is possible that the non-random assortment of chromosomes indicated by this study occurs in the absence of preferential pairing. However, we have considered it more likely that non-random assortment is a consequence of preferential pairing. In turn, preferential pairing suggests the occurrence of bivalent-univalent arrangements. It may be that both trivalent and bivalent-univalent arrangements can occur in the same strain. If so, it seems likely that any tendency toward preferential pairing would increase the frequency of bivalent-univalent arrangements. The relative frequency of these two arrangements would then vary depending upon the strain or chromosomes under consideration. The results of this study are not inconsistent with the existence of such variation.

Alternatively, it is possible that non-random segregation is associated with trivalent arrangements under some circumstances. Thus, a sophisticated analysis of preferential segregation in Drosophila (STURTEVANT 1936) indicated that preferential segregation in triplo-IV females is due to non-random orientation of the hexad on the first meiotic spindle. At any rate, the occurrence of preferential pairing is not incompatible with trivalent arrangements if this pairing is confined to particular chromosome regions. Thus, it seems likely that if two chromosomes of a trivalent engage in recombinational pairing near the centromere they would tend to migrate to different poles. If so, preferential pairing in a region not far removed from the centromere would result in a non-random assortment indicative of a bivalent-univalent arrangement. In regard to such a possibility it is noteworthy that the segregational data obtained by SHAFFER *et al.* are consistent with the supposition of bivalent-univalent arrangements in trisomics for chromosome III if the data for the exceptional category of segregants noted above are excluded. Thus, assuming a rather high degree of preferential pairing of *unlike* chromo-

somes ($y = .183$), the segregations for *leu2*, *his4*, and the sex locus (their Table 3), show an extraordinarily close fit of actual to expected. However, the data of CULBERTSON and HENRY (1973) are not so easily explained in terms of preferential pairing. For instance, their data (4:0, 2:2, and 3:1 ratios only) for the fatty acid locus, which is far removed from its centromere ($x = \frac{2}{3}$), are consistent with the assumption of bivalent-univalent arrangements and preferential pairing ($y = .541$) of mutant alleles. But their data for loci closer to the centromere are, as the authors show, entirely consistent with the assumption of trivalent arrangements and random assortment.

The good agreement between expected and actual frequencies of tetrads recombinant for adenine in this investigation indicates an amount of crossing over consistent with the location ascribed to the adenine locus. Thus, although these data imply a reduction in the probability that unlike chromosomes will pair, they also imply that the amount of crossing over between unlike chromosomes is normal, at least in the region between *ade1* and its centromere, when these chromosomes do synapse.

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LITERATURE CITED

COX, B. S. and E. A. BEVAN, 1962 Aneuploidy in yeast. New Phylologist **61**: 342–355.

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.

FINKELSTEIN, D. B., J. BLAMIRE and J. MARMUR, 1972 Location of ribosomal RNA cistrons in yeast. Nature New Biology **240**: 279–281.

HAWTHORNE, D. G. and R. K. MORTIMER, 1960 Chromosome mapping in Saccharomyces: centromere-linked genes. Genetics **45**: 1085–1110.

JAMES, A. P., 1974 A new method of detecting centromere linkage in homothallic yeast. Genetical Res. **23**: 201–206.

JAMES, A. P., E. R. INHABER and G. J. PREFONTAINE, 1974 Lethal sectoring and the delayed induction of aneuploidy in yeast. Genetics **77**: 1–9.

MORTIMER, R. K. and D. C. HAWTHORNE, 1966 Genetic mapping in Saccharomyces. Genetics **53**: 165–173. ——, 1973 Genetic mapping in Saccharomyces. IV. Mapping of temperature sensitive genes and use of disomic strains in localizing genes. Genetics **74**: 33–54.

ØYEN, T. B., 1973 Chromosome I as a possible site for some rRNA cistrons in *Saccharomyces cerevisiae*. FEBS Letters **30**: 53–56.

ROMAN, H., M. M. PHILIPS and S. M. SANDS, 1955 Studies of polyploid Saccharomyces I. Tetraploid segregation. Genetics **40**: 546–561.

SHAFFER, B., I. BREARLEY, R. LITTLEWOOD and G. R. FINK, 1971 A stable aneuploid of *Saccharomyces cerevisiae*. Genetics **67**: 483–495.

STURTEVANT, A. M., 1936 Preferential segregation in triplo-IV females of *Drosophila melanogaster*. Genetics **21**: 444–466.

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