GENETIC ANALYSIS OF HYBRID STRAINS TRISOMIC FOR THE CHROMOSOME CONTAINING A FATTY ACID SYNTHETASE GENE COMPLEX (*fas*1) IN YEAST

MICHAEL R. CULBERTSON AND SUSAN A. HENRY

Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461

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ABSTRACT

Evidence of spontaneous $n+1$ aneuploidy has been obtained by trisomic segregation analysis of four independently maintained stocks of *Saccharomyces cerevisiae* defective in saturated fatty acid synthesis (*fas* 1). In all cases tested, only the chromosome bearing the mutant fatty acid locus was disomic. Tetrad analysis of trisomic hybrids enabled the identification of chromosome XI as the one bearing the fatty acid locus and the assignment of fragment 5 to chromosome XI. Statistical analysis of tetrad frequencies generated by markers in triplex configuration provided information on the meiotic configuration of pairing of the three homologous chromosomes. The possible relationship between defective nuclear membranes and the disjunction of chromosomes in *fas* 1 strains is discussed.

IN the absence of adequate cytological techniques, the general problem of establishing genetic linkage groups and their relationship to the number of chromosomes in *Saccharomyces cerevisiae* has been approached by a variety of techniques, including the analysis of nonlinear tetrads, random spores, x-ray-induced mitotic recombinants, and spore progeny from aneuploid parents. Especially powerful methods have been perfected for the selection, detection, and use of aneuploidy as a rapid means of determining the locations of genes on chromosomes and the linkage of fragments to their respective centromeres (SHAFFER *et al.* 1971; MORTIMER and HAWTHORNE 1973).

In order to identify chromosomes containing unlocated genes the disome inclusion technique has been generally most useful (MORTIMER and HAWTHORNE 1973). Trisomic hybrids are constructed in the $+/+/-$ configuration by mating haploid strains containing an unlocated mutant allele with either single or multiple disomic strains that carry wild-type alleles of the unlocated gene as well as appropriate markers of known location to monitor the segregation of each chromosome. Departure from $2^+:2^-$ segregation of the unlocated marker in tetrads derived from such hybrids provides evidence for the inclusion of the unlocated marker in one of the chromosomes known to be disomic on the basis of aberrant segregation of monitors. In the case of single chromosome disomes such evidence is sufficient by itself for the identification of the marker location.

By using a modification of the inclusion technique this study provides a quantitative analysis of spontaneous aneuploidy originally detected by the aberrant segregation of complementing markers in numerous independently maintained stocks of *Saccharomyces cerevisiae* defective in saturated fatty acid synthesis (HENRY and FOGEL 1971). Similar mutant stocks have been shown to be defective in components of fatty acid synthetase determined by the *fas*1 locus (SCHWEIZER, BOLLING and CASTROPH 1971). Advantage was taken of the unique and convenient opportunity provided by the knowledge that these strains were minimally disomic for an unknown chromosome bearing one complex locus (*fas*1) conferring fatty acid requirement (HENRY and FOGEL 1971).

In order to identify the unknown chromosome, trisomic hybrids were constructed in the configuration $-/-/+$ through matings between disomic stocks (*fas*1/*fas*1) and haploid testers (*FAS*1) containing numerous centromere-linked and fragment-linked auxotrophic markers as genomic monitors. The chromosomal location of the *fas*1 locus was established by the aberrant segregation of fatty acid markers in conjunction with the aberrant segregation of auxotrophic markers on one chromosome (XI) and one fragment (5).

In addition, trisomic hybrids afforded a valuable opportunity for the analysis of tetrad frequencies generated by various combinations of markers in trisomic configuration. Aberrant segregations due to the presence of the extra chromosomes were analyzed statistically by considering the factors that determine the frequencies of different tetrad arrangements for triplex markers. An attempt was made to distinguish between two different possible pairing relationships between three homologous chromosomes.

Although numerous studies in other organisms have established nondisjunction of chromosomes as a major cause of aneuploidy, little is known about the causes of nondisjunction itself. It was previously suggested that defective nuclear membranes in fatty-acid-deficient strains of yeast might interfere with the normal disjunction of chromosomes (HENRY and FOGEL 1971). Alternatively, selective pressures imposed by the fatty acid requirement, which are currently not understood, might result in the retention of aneuploidy for specific chromosomes. For this reason it was considered important to establish whether aneuploidy in these stocks was generalized or restricted to specific chromosomes. Segregational analysis of the chromosomes in four independently maintained *fas*$^-$ stocks has therefore been accomplished, revealing the likelihood of n + 1 aneuploidy in all four cases.

MATERIALS AND METHODS

Strains

Chromosome marker strains were obtained from the Berkeley collection, as follows:

X2928–4C	*ade1*	*gal1*	*α*	*trp1*	*ura3*	*his2*	*leu1*	*met14*	
	I	II	III	IV	V	VI	VII	XI	
X2928–7D	*ade1*	*gal1*	*α*	*trp1*	*ura3*	*his2*	*leu1*	*met14*	
	I	II	III	IV	V	VI	VII	XI	
X2939–5A	*ade1*	*a*	*trp1*	*ura3*	*his2*	*arg4*	*met14*	*gal2*	*lys7*
	I	III	IV	V	VI	VIII	XI	XII	XIII

X2943–3B	*ade1*	*a*	*his8*	*ade8*	*met2*	*trp3*	*ura1*
	I	III	F1	F2	F3	F5	F5
S856C	*ade1*	*a*	*lys2*				
	I	III	II				
S720B	*trp4*	*a*	*lys1*	*his2*			
	IV	III	IX	VI			
X1012–1D	*lys9*	*a*	*ade2*	*ade1*			
	XIV	III	XV	I			
X1049–9C	*trp1*	*a*	*ura3*	*his8*	*asp5*	*arg8*	
	IV	III	V	F1	XII	–	

Markers listed were tested for 2+:2− segregation in crosses with wild-type strains.

Disomic strains:

SAH-124–5D	*a*	*ade1*	*his*	*fas1-SH2/fas1-SH5*
SAH-148–2C	α	*arg4*	*his*	*fas1-SH2/fas1-SH11*
SAH-148–2A	α	*his*	*fas1-SH2/fas1-SH11*	
SAH-148–1C	α	*arg4*	*his*	*fas1-SH2/fas1-SH11*

Haploid strains requiring fatty acid supplementation: A strain containing the allele SH-1 was used in mapping the fatty acid locus with respect to *trp3* and *ura1*.

Trisomic hybrid strains were constructed as follows:

MC-1	(SAH-124–5D × X2928–4C)	*fas1-SH2/fas1-SH5/FAS1*
MC-2	(SAH-148–2C × X2928–7D)	*fas1-SH2/fas1-SH11/FAS1*
MC-3	(SAH-148–2C × X2939–5A)	*fas1-SH2/fas1-SH11/FAS1*
MC-4	(SAH-148–2C × X2943–3B)	*fas1-SH2/fas1-SH11/FAS1*
MC-5	(SAH-148–2A × X2928–7D)	*fas1-SH2/fas1-SH11/FAS1*
MC-6	(SAH-148–2A × X2939–5A)	*fas1-SH2/fas1-SH11/FAS1*
MC-7	(SAH-148–2A × X2943–3B)	*fas1-SH2/fas1-SH11/FAS1*
MC-8	(SAH-148–1C × X2928–7D)	*fas1-SH2/fas1-SH11/FAS1*
MC-9	(SAH-148–1C × X2939–5A)	*fas1-SH2/fas1-SH11/FAS1*
MC-10	(SAH-148–1C × X2943–3B)	*fas1-SH2/fas1-SH11/FAS1*
MRC-1	(MC-1–2B × X2943–3B)	*fas1-SH2/fas1-SH2/FAS1*
MRC-2	(MC-9–11C × X2943–3B)	*fas1-SH2/fas1-SH2/FAS1*

Materials: Tween 80 [polyoxethelene (20) sorbitan monoleate] and Tween 40 [polyoxethelene (20)] sorbitan monopalmitate were purchased from Sigma Chemical Co.

Growth conditions: Incubations and growth trials were performed at 30°.

Construction of trisomic hybrids: Haploid tester stocks containing numerous auxotrophic markers of known and widely distributed locations on the various chromosomes and fragments of the Saccharomyces genome were crossed to disomic stocks of opposite mating type on YEPD (1% yeast extract, 2% bactopeptone, 2% dextrose, 2% agar) supplemented with 1% Tween 80 and 1% Tween 40. Allowing three to five hours for the mating response, cell mixtures were replicated to minimal media to select for the growth of trisomic hybrids.

Sporulation and tetrad analysis: Surviving trisomic hybrid cells were replicated to sporulation media containing 2% potassium acetate (FOGEL and HURST 1967), 0.5% Tween 80 and 0.5% Tween 40. After two to three days of incubation ascospores were liberated by mild digestion with 1:10 dilute glusalase and dissected by micromanipulation. Two days later spore clones were picked from dissection slabs and streaked on YEPD supplemented with 1% Tween 80 and 1% Tween 40. Following growth, tetrads were replicated to various defined media (HAWTHORNE and MORTIMER 1960) and scored for the segregation of nutritional markers. Segregation of fatty acid markers was scored by comparing growth on supplemented and unsupplemented YEPD.

Scoring of auxotrophic markers: Certain difficulties were encountered in obtaining the data of Table 1. The scoring of segregants for auxotrophic markers on media fully supplemented with fatty acids was considered somewhat subjective due to the reduced growth rates of clones that contained alleles conferring fatty acid requirement. In addition there was a tendency toward reversion to wild type on media unsupplemented with fatty acids, as indicated by the emergence

of small papillations after several days of incubation. However, ambiguities in scoring were for the most part alleviated by scoring the segregation of fatty acid markers less than twenty-four hours after replica plating, followed twenty-four hours later by the scoring of auxotrophic markers. Only those asci with four surviving spores which could be unambiguously scored have been included in the data of Table 1.

Linkage analysis: Haploid strains containing the allele *fas1-SH1* (complementary to *fas1-SH2*, *fas1-SH5*, and *fas1-SH11*) were crossed to strain X2943–3B), containing the fragment 5 markers *trp3* and *ura1*. Sporulation and dissection were performed as described above. Linkage values were determined by scoring tetrads as parental ditype (PD); non-parental ditype (NPD), and tetratype (T) and calculating map distance (X) in centimorgans by the relationship $x = 50$ (T + 6NPD/PD + NPD + TT) (PERKINS 1949).

Statistical analysis: Data on the segregation of triplex markers from trisomic hybrids was analyzed by an IBM 370 computer, using FORTRAN IV language.

RESULTS

Preliminary characterization of mutant strains: Previous studies on EMS-induced saturated fatty-acid-requiring mutants of *S. cerevisiae* demonstrated a minimum of three unlinked loci by the criteria of recombination and complementation (HENRY and FOGEL 1971; SCHWEIZER, BOLLING and CASTROPH 1971). Detailed analyses of two complex allelic complementation patterns suggested that two of the three loci represented clusters of genes segregating essentially as single genetic units and recombining freely with their respective centromeres.

During the characterization of the mutants, a gross excess of prototrophic spores was detected in a number of diploid strains heterozygous for complementing fatty acid alleles. This pattern was explained by assuming a spontaneous transition to aneuploid condition (HENRY and FOGEL 1971). Extensive tetrad analysis and allele testing further revealed these strains to be disomic for an unknown chromosome bearing one of the fatty-acid-determining loci. However, it was not clear at this time whether aneuploidy was restricted to one chromosome or whether it was a more generalized phenomenon.

From these original strains single spore disomic derivatives containing complementing alleles (*fas1-SH2/fas1-SH5* and *fas1-SH2/fas1-SH11*) were selected and cloned for use in the present study. These stocks gave a prototrophic response when grown on media unsupplemented with saturated fatty acids. In addition, the three alleles used here were tested for complementation with similar independently-isolated mutants (SCHWEIZER and BOLLING 1970) known to be defective at one complex locus coding for the enol reductase and dehydratase activities of the fatty acid synthetase (*fas1*) multienzyme complex (KÜHN, CASTROPH and SCHWEIZER 1972). The results indicate that both groups of mutants are defective at the same complex locus.

Chromosomal location of the fatty-acid-determining locus: In order to provide a rigorous analysis of disomy, further characterization was required specifically to identify the chromosome bearing the fatty acid locus, entertaining the possibility that other chromosomes might also be aneuploid. Mapping was accomplished by the use of a rationale for identifying aneuploid chromosomes in trisomic hybrids containing complementary fatty acid markers in −/−/+ configuration.

TABLE 1

Segregation of markers in trisomic hybrids containing complementary fatty acid alleles

Strain designation	Hybrid genotype	Cross	Spore survival	Total asci dissected	Asci with 4 surviving spores	Fatty acid locus				Chromosome XI markers									Marked chromosomes showing regular 2+2- segregation
										met14			*trp3* (frag. 5)			*ura1* (frag. 5)			
						4+0-	2+2-	3+1-	1+1-	4+0-	2+2-	3+1-	4+0-	2+2-	3+1-	4+0-	2+2-	3+1-	
MC-1	*fas1–SH2/ fas1–SH5/FAS1*	124–5D × X2928–4C	100	23	23	2	13	6	2	13	7	1	.	.	.	.	.	.	III, IV, V, VI, VII
MC-2	*SH2/SH11/FAS1*	148–2C × X2928–7D	85	16	11	1	4	5	1	.	.	.	.	.	.	.	.	.	I, III, IV, V, VII
MC-3	*SH2/SH11/FAS1*	148–2C × X2939–5A	95	22	17	2	11	4	0	8	5	0	.	.	.	.	.	.	I, III, IV, V, VI
MC-4	*SH2/SH11/FAS1*	148–2C × X2943–3B	83	16	8	1	5	2	0	.	.	.	2	1	5	2	1	5	I, III, IV, XV, XVII
MC-5	*SH2/SH11/FAS1*	1482A × X2928–7D	85	19	13	0	7	6	0	6	3	0	.	.	.	.	.	.	I, III, IV, V, VI, VII
MC-6	*SH2/SH11/FAS1*	1482A × X2939–5A	97	19	17	1	11	5	0	10	5	1	.	.	.	.	.	.	I, III, IV, V, VI, VIII, XIII
MC-7	*SH2/SH11/FAS1*	1482A × X2943–3B	85	19	13	0	9	2	2	.	.	.	6	2	5	6	2	5	I, III, IV, XV, XVII
MC-8	*SH2/SH11/FAS1*	1481C × X2928–7D	85	19	9	0	5	3	1	.	.	.	.	.	.	.	.	.	I, III, IV, V, VI, VII
MC-9	*SH2/SH11/FAS1*	1481C × X2939–5A	92	21	15	1	8	5	1	8	3	0	.	.	.	.	.	.	I, III, IV, V, VI, XIII
MC-10	*SH2/SH11/FAS1*	1481C × X2943–3B	75	16	4	0	1	2	1	.	.	.	1	2	1	1	2	1	I, III, IV, XV, XVII
Total				190	130	8	74	40	8	45	23	2	9	5	11	9	5	11	
							aberrant			aberrant			aberrant			aberrant			

Total segregations for markers on other chromosomes (2+2- : 3+1-): *ade1*(I): 91:0; *a,α*(III): 128:1; *trp1*(IV): 74:3; *ad8*(IV, fragment 2): 20:0; *ura3*(V): 76:1; *his2*(VI): 73:0; *leu1*(VII): 40:0; *arg4*(VIII): 17:0; *his8*(XV, fragment 1): 21:0; *met2*(XVII), fragment 3): 23:2. (There were no 4+0- or 1+3- segregations for any of these markers.)

The numerous auxotrophic markers of known location included in hybrids were necessarily in +/+/− configuration for those located on trisomic chromosomes and +/− configuration for those on diploid chromosomes. Trisomic hybrids were expected to generate asci containing an excess of prototrophic spores with respect to fatty acid markers as well as any other marker included in triplex configuration (see procedures for the construction of trisomic hybrids in MATERIALS AND METHODS).

Assuming the strains are trisomic for a single chromosome, all markers segregating aberrantly can be unambigously assigned to the same chromosome. The results (Table 1) support the assumption of single chromosome trisomy to the extent that fifteen of the seventeen known chromosomes have been tested. Chromosomes X and XVI were not tested due to the unavailability of suitable markers. Markers segregating on chromosomes II (*lys2*), IX (*lys1*), XIV (*lys9*), and XII (*asp5*) behaved normally, but these data were not included in Table 1.

Inspection of the table reveals the aberrant segregation of fatty acid markers. Aberrant tetrads were detected in three distinct arrangements: 4+:0−, 3+:1−, and 1+:3−. Normal (2+:2−) segregants were also found in appreciable frequency. The significance of the ratios with which these ascal classes appear is considered in a later section.

Regarding auxotrophic markers, aberrant segregations (4+:0− and 3+:1−) were detected with respect to *met14* (chromosome eleven), *trp3* (fragment five), and *ura1* (fragment five). While all other markers segregated normally, several low frequency exceptional asci were detected and are presumably the results of gene conversion. Where necessary, markers conferring identical phenotypes were allele-tested to secure their identity and reveal their segregational pattern.

These results establish the location of the fatty acid locus on fragment five, in agreement with results obtained by different methods (BÜRKL, CASTROPH and SCHWEIZER 1972). In addition the association of chromosome eleven and fragment five is confirmed (MORTIMER and HAWTHORNE 1973). A genetic map is provided (Figure 1).

Further confirmation of chromosomal location: These results were also substantiated by selecting disomic spore clones homozygous for *fas1–SH2* and

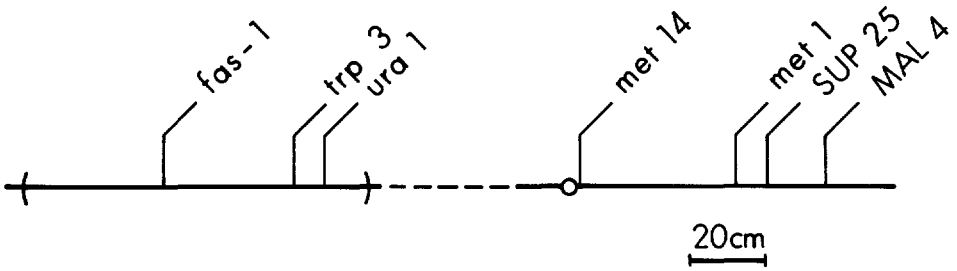

FIGURE 1.—Genetic map of chromosome eleven. Order of genes within parentheses has not been established in relation to the centromere. The fragment five markers (*trp3*, *ura1*, *met1*, *SUP25*, and *MAL4*) were recently located on chromosome eleven by MORTIMER and HAWTHORNE (1973).

TABLE 2

Segregation of markers in fas1–SH2/fas1–SH2/FAS1

Strain	*fas1* genotype	Cross	Percent spore survival	Total asci	Complete tetrads	Fatty acid segregation 2+2-	Fatty acid segregation 1+3-	4+0-	*trp3* 3+1-	2+2-	4+0-	*ura1* 3+1-	2+2-	Other markers segregating 2+2-
MRC-1	*fas1–SH2/fas1–SH2/FAS1*	MC-1–2b × X2943–3B	97	46	42	39	3	14	16	12	14	16	12	*arg4*, *his8*, *met2*
MRC-2	*fas1–SH2/fas1–SH2/FAS1*	MC-9–11c × X2943–3B	80	35	12	11	1	5	4	3	5	4	3	*arg4*, *lys7*, *his8*, *met2*
Total						50	4	19	20	15	19	20	15	

mating them to the same haploid tester stocks as used previously. The resulting trisomic hybrids contained fatty acid alleles in the configuration −/−/+, but in the absence of complementary alleles a different pattern of segregation was detected for fatty acid markers (Table 2). The two classes of tetrads expected from these hybrids, (2+:2− and 1+:3−), were observed, and the auxotrophic markers *trp3* and *ura1* continued to segregate aberrantly.

Linkage studies: Haploid strains containing the allele *fas1–SH1* were crossed to tester strains containing *trp3* and *ura1* as well as other markers. The *fas1–SH1* allele is ochre-suppressible and maps at the same locus as *fas1–SH2*, *fas1–SH5* and *fas1–SH11* (HENRY and FOGEL 1971). In addition, *fas1–SH1* has never been associated with aneuploidy for this chromosome. Linkage was estimated by a deficiency of non-parental ditype asci with respect to parental ditype and distances were calculated by the equation given in MATERIALS AND METHODS (PERKINS 1949). These results indicate loose linkage between the fatty acid gene cluster and the fragment 5 markers *trp3* and *ura1* (Table 3), in close agreement with the results of BÜRKL, CASTROPH and SCHWEIZER (1972).

Bivalent-Univalent and Trivalent Pairing in Trisomic Hybrids:

Two different modes of pairing between three homologous chromosomes can be considered: bivalent-univalent and trivalent pairing (SHAFFER *et al.* 1971). Whole tetrad data, as presented in Table 1, may be employed to obtain information on the relative frequencies of bivalent-univalent and trivalent associations at meiosis.

Bivalent-univalent pairing assumes the random probability of any pairwise combination of three homologous chromosomes followed by the random movement of the unpaired extra chromosome to either pole and the restricted movement of the paired chromosomes to opposite poles. Trivalent pairing assumes the simultaneous association of all three homologous chromosomes in the first meiotic prophase, followed by independent assortment to each pole. Multiple recombination between trivalently paired chromosomes is restricted, for the sake of mathematical simplicity, to the involvement of only two of the three chromosomes. The major distinction between the bivalent-univalent and trivalent alternatives is the possibility in a trivalent complex for the movement of both chromosomes involved in genetic exchange to the same pole.

TABLE 3

Linkage of fas-1 *to fragment 5 markers**

Cross	Total tetrads	PD	NPD	TT	x(cM)†	x(cM)‡
fas1–SH-1 × *ura1*	159	36	3	120	43.4	..
fas1–SH-1 × *trp3*	169	56	3	110	37.9	..
ura1 × *trp3*	156	138	0	18	5.7	5.6

* Fourth spore deduced where required.
† Data from this study.
‡ Data from MORTIMER and HAWTHORNE (1966).

In trisomic hybrids containing the complementing alleles *fas1–SH2* and *fas1–SH11* in the 2/11/+ configuration (or *fas1–SH2* and *fas1–SH5* in the 2/5/+ configuration) the bivalent-univalent model predicts an additional ascal class, namely the 1+3− arrangement (Table 5). Thus the detection of eight 1+3− tetrads (Table 1) constitutes evidence that trivalent pairing occurs at some frequency greater than zero. This conclusion must be qualified, however, because even though the bivalent-univalent model does not predict the generation of 1+3− tetrads directly, such asci might nevertheless be generated by one or the other of two possible mechanisms: gene conversion or nondisjunction at meiosis II.

The possibility that bivalent-univalent pairing might generate 1+:3− tetrads through gene conversion can be eliminated on theoretical grounds. The generation of a 1+:3− tetrad by bivalent-univalent pairing and gene conversion requires a situation in which one of the two *FAS1* sister chromatids is converted to one of the other input alleles (*fas1–SH2* or *fas1–SH11*). Such a conversion must be followed by appropriate segregations at meiosis I and II that will yield a tetrad consisting of one prototrophic, disomic spore that is heterozygous (*FAS1/fas1*) and three *fas1* spores of which one is disomic. The only appropriate segregations at meiosis I and II that could generate this kind of ascus are those that would result if the chromosome carrying the converted *FAS1* allele is unpaired at meiosis I. If, however, this allele is unpaired at meiosis I, it cannot at the same time be converted, since pairing is assumed to be prerequisite for gene conversion. Thus the generation of a 1+3− tetrad by a combination of bivalent-univalent pairing and gene conversion is unlikely.

The possibility that bivalent-univalent pairing might generate 1+:3− tetrads through nondisjunction at meiosis II can be tested experimentally. The critical evidence is provided in Table 4 by the results of progeny testing the spores of a 1+3− tetrad derived from a *fas1–SH2/fas1–SH11/FAS1* trisomic parent. Trivalent pairing can be distinguished from bivalent-univalent pairing in conjunction with nondisjunction at meiosis II, because the former predicts that the *FAS1* spore will be *FAS1/FAS1* genotypically, whereas the latter situation predicts that the *FAS1* spore will be trisomic (*fas1/FAS1/FAS1*). The data in Table 4 demonstrates that the single prototrophic spore in the tested 1+3− ascus had the genotype *FAS1/FAS1* and that one fatty-acid-requiring spore was also disomic. Further confirmation of these results was obtained by crossing progeny spores derived from the crosses in Table 4 to *trp3*, *ura1* and *met14* markers. The predicted segregation of the disomic chromosomes was observed and trisomy for the single prototrophic spore was excluded.

The complete genotypic analysis of one ascus from each of the four ascal types (1+:3−, 2+:2−, 3+:1− and 4+:0−) is provided, showing that in each case the genotype of each spore can be unambiguously assigned on the basis of progeny testing (Table 4) and allelic complementation. All input alleles in the tested asci were recovered with complete fidelity in a manner consistent with trisomic segregation in the absence of gene conversion or nondisjunction.

While the existence of the tested 1+3− ascus demonstrates that trivalent pairing must occur at some frequency greater than zero, it is important to establish

TABLE 4

Complete genotypic analysis of ascal types

Asci tested	Ascal type	Spore phenotypes	Progeny testing 4+0-	1+3-	2+2-	3+1-	Proposed spore genotype
		+	32	0	0	0	*FAS1*
1	4+0-	+	32	0	0	0	*FAS1*
		+	2	2	17	14	*fas1-SH2/fas1-SH11*
		+	3	1	16	13	*fas1-SH2/fas1-SH11*
		+	32	0	0	0	*FAS1/FAS1*
1	1+3-	—	0	3	25	0	*fas1-SH2/fas1-SH2*
		—	0	0	32	0	*fas1-SH11*
		—	0	0	29	0	*fas1-SH11*
		+	25	0	0	0	*FAS1*
1	2+2-	+	19	0	0	0	*FAS1*
		—	0	2	30	0	*fas1-SH2/fas1-SH2*
		—	0	1	23	0	*fas1-SH11/fas1-SH11*
		+	35	0	0	0	*FAS1*
1	3+1-	+	1	1	14	11	*fas1-SH2/fas1-SH11*
		+	9	0	5	10	*fas1-SH11/FAS1*
		—	0	0	31	0	*fas1-SH2*

Four tetrads, representing each of the four classes of asci detected and reported in Table 1, were thoroughly tested by individually mating all four ascospores of each tetrad with a wild-type strain (*FAS1*). Second-generation asci derived from these crosses were scored for the segregation of *fas1* alleles. These asci have been classified according to ascal type and are listed in appropriate columns under "Progeny testing". A sample of progeny spores was crossed to fragment 5 markers *trp3* and *ura1* to confirm the segregation of the disome. By supplementing these results with complementation data it was possible to unambiguously deduce the genotypes of all of the original sixteen ascospores. In no case was it necessary to assume that either gene conversion or nondisjunction had taken place.

the relative frequencies of the two modes of pairing. This is by nature a highly theoretical problem whose solution depends on the use of an empirical mapping function. Nonetheless an attempt to obtain some estimate of relative frequency is of considerable practical relevance. Since the quantitative analysis of whole tetrad data that follows hinges critically upon the proper diagnosis of ascal types and, in theory, does not take account of gene conversion or nondisjunction events, an assessment of the potential contribution of gene conversion and nondisjunction to the misclassification of ascal types is required.

In separate crosses involving *fas1-SH2* and *fas1-SH11*, gene conversion was detected at a level of less than one percent (one 3+:1— converted tetrad was detected among 142 tetrads derived from a *fas1-SH2/FAS1* parent; no conversion events were detected among 98 tetrads derived from a *fas1-SH11/FAS1* parent). Thus in the sample of 130 tetrads reported here (Table 1), not more than one or two undetected conversion events are expected. Furthermore, conversion events that give rise to diagnostic 1+:3— tetrads can do so only in associ-

ation with trivalent pairing, as previously discussed, and thus cannot obscure the origin of these tetrads with respect to mode of pairing.

Nondisjunction at meiosis II of the type that produces an ascus containing one trisomic spore and three haploid spores could also result in misclassification. While rates of nondisjunction have not been measured as extensively as rates of gene conversion in these strains, it was found that among forty tetrads derived from trisomic hybrids and thoroughly analyzed by progeny testing and allelic complementation, only one qualified as being the potential result of a nondisjunctional event at meiosis II (HENRY and FOGEL 1971). Since a nondisjunctional event of this type could theoretically result in the misclassification of asci, any estimate of the frequency of trivalent pairing must be regarded as an approximation.

Bearing these potential difficulties in mind, equations that predict the frequencies of aberrant tetrad arrangements from trisomic hybrids have been derived on the basis of assumptions inherent to each pairing model taken separately (Table 5). These equations are designed to take account of the effects of complementation, the triplex configuration of markers, and the second division segregation frequency. The separate equations derived on the basis of each pairing model have been combined into equations (see Table 5) that reflect the frequency of trivalent pairing in a population of meiotic cells.

The probability of obtaining a particular tetrad arrangement thus depends on a variable Y, defined as the frequency of trivalent pairing, and a variable X, defined as the probability of segregation in the second meiotic division. The value of X for a given locus a certain distance from the centromere is a complex variable defined here and elsewhere (SHAFFER *et al.* 1971) by the single strand mapping function of KOSAMBI (BARRATT *et al.* 1954), where

$$X = 2r - 4/3r^2$$

and r is the frequency of single strand recombination. $4/3r^2$ represents a correction factor for the effects of undetectable double crossing over on the probability of second division segregation. A theoretical maximum of $X = 2/3$ is obtained for a given gene that recombines freely with its centromere, assuming no chiasma interference.

For trisomic hybrids in the configuration $-/-/+$ a value of $X = 2/3$ can theoretically be assigned to the fatty acid locus, since it is known to recombine freely with its centromere (HENRY and FOGEL 1971). The task remains of defining a value for the frequency of trivalent pairing (Y) which produces expected frequencies that best fit the observed frequencies. This has been accomplished in a rigorous manner by a lengthy computerized analysis designed to calculate chi-square values and probabilities of all combinations of X and Y taken at arbitrary 0.02 increments for $1 \geq X \geq 0.02$ and $1 \geq Y \geq 0.02$. Assuming the theoretical $X = 2/3$, the value of Y which provides the best statistical fit with observed data is 0.82. The corresponding probability is not significant at the 2.5% level (Table 6). Calculation of homogeneity chi-square (Table 6) indicates that the pooling of data from individual crosses (Table 1) for purposes of this analysis is justified.

TABLE 5

*Prediction of ascal frequencies based on pairing models**

Allelic configuration	Expected ascal class	Meiotic products† A	B	C	D	Probability assuming 100% bivalent-monvalent pairing	Probability assuming 100% trivalent pairing	Probability combined
	4+0-	2/11	2/11	+	+	1/3–1/3*X*	1/3–5/18*X*	1/3–1/3*X*+1/18*XY*
		2/+	2/+	11	11			
fas1–SH2/fas1–	2+2-	11/+	11/+	2	2	2/3–1/3*X*	2/3–5/18*X*	2/3–1/3*X*+1/18*XY*
SH11/FAS1		2/+	11/+	2	11			
		2/2	11/11	+	+			
	3+1-	2/11	+/11	2	+	2/3*X*	4/9*X*	2/3*X*–2/9*XY*
		2/11	+/2	11	+			
	1+3-	2/2	+/+	11	11	0	1/9*X*	1/9*XY*
		11/11	+/+	2	2			
	4+0-	—/+	—/+	+	+	2/3–1/3*X*	2/3–4/9*X*	2/3–1/3*X*–1/9*XY*
+/+/— for an	2+2-	+/+	+/+	—	—	1/3–1/3*X*	1/3–1/9*X*	1/3–1/3*X*+2/9*XY*
auxotrophic marker	3+1-	+/+	—/+	—	+	2/3*X*	5/9*X*	2/3*X*–1/9*XY*
		+/+	—/—	+	+			
		2/+	2/+	2	2	1	1–1/9*X*	1–1/9*XY*
fas1–SH2/fas1–	2+2-	2/2	2/2	+	+			
SH2/FAS1		2/2	2/+	2	+			
	1+3-	2/2	+/+	2	2	0	1/9*X*	1/9*XY*

* *X* equals the probability of second division segregation.
Y equals the frequency of trivalent pairing.
† + equals wild type.
2 equals *fas1–SH2*.
11 equals *fas1–SH11*.

TABLE 6

Statistical parameters for trisomic hybrids

Trisomic hybrids	X 2nd division segregation	Y Frequency of trivalent pairing	Sample size	Ascal class frequencies 4+:0- obs	exp	2+:2- obs	exp	3+:1- obs	exp	1+:3- obs	exp	Test for homogeneity chi-square	Hp	Test for fitness chi-square	p
1* *fas1–SH2/fas1–SH11/FAS1*	.70	.86	130	8	17.35	74	60.68	40	43.28	8	8.70	15.95 (df=27)	.975>p>.950	8.26 (df=3)	.05>p>.025
+/+/*met14*	.022‡	.86	70	45	46.00	23	23.12	2	.880	–	——	5.85 (df=8)	.75>p>.50	1.56 (df=2)	.50>p>.25
+/+/*trp3*	.70	.86	25	9	9.16	5	5.85	11	9.99	–	——	3.64 (df=4)	.50>p>.25	0.59 (df=2)	.75>p>.50
+/+/*ura1*	.70	.86	25	9	9.16	5	5.85	11	9.99	–	——	3.64 (df=4)	.50>p>.25	0.59 (df=2)	.75>p>.50
2† *fas1–SH2/fas1–SH2/FAS1*	.70	.86	54	–	——	50	50.39	–	——	4	3.61	.0199 (df=1)	.90>p>.75	.0071 (df=2)	.95>p>.90
+/+/*trp3*	.70	.86	54	19	19.79	15	12.62	20	21.59	–	——	.2771 (df=2)	.90>p>.75	.1954 (df=2)	.95>p>.90
+/+/*ura1*	.70	.86	54	19	19.79	15	12.62	20	21.59	–	——	.2771 (df=2)	.90>p>.75	.1954 (df=2)	.95>p>.90

* Data is summarized from Table 1.
† Data is summarized from Table 2.
‡ MORTIMER and HAWTHORNE (1973).

This result may be taken as evidence that trivalent pairing is preferred in the majority of meioses in a population of trisomic cells. However, deviations from expectation are apparent even in the case of calculated expectations that give the best statistical fit (where $X = 2/3$). Table 6 reveals that these deviations consist of a deficiency of 4+:0− tetrads with a corresponding excess of 2+:2− tetrads. Similar deviations from expectation, which currently remain unexplained, have been found in studies of strains aneuploid for chromosome III (SHAFFER *et al.* 1971). However, it should be emphasized that such discrepancies were observed only for the fatty acid locus. All other auxotrophic markers segregating on the same chromosome show a high degree of correspondence with expected values (Table 6).

While changes in the value of Y from 0.82 to any other value do not improve the statistical fit for the fatty acid locus as long as X is held constant at 0.67, the fit is somewhat improved by a modest increase in the value of X to 0.70, at which point, the value of Y giving the best statistical fit is 0.86. Figure 2 provides a graphical representation of the probability curve generated when $Y = 0.86$, where reciprocal chi-square is plotted against second division segregation frequency. The maximum point on this curve at $X = 0.70$ represents maximum probability. Curves generated by any other value of Y have lower peaks and are less probable. On the basis of this curve, the maximum estimate of the frequency of trivalent pairing is 86%.

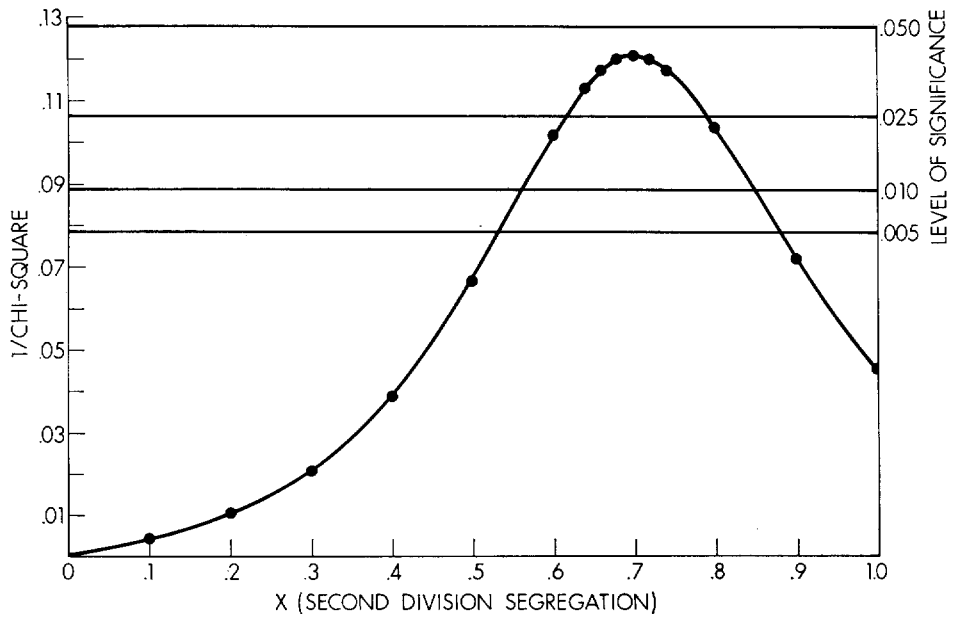

FIGURE 2.—Probability curve generated for $Y = 0.86$ (frequency of trivalent pairing). Reciprocal chi-square is plotted against the probability of second division segregation. Maximum statistical fit is obtained at the top of the peak, where the second division segregation frequency equals 0.70. Similar curves generated by any other value of Y produce less probable values for second division segregation frequency.

One basis for the observed increase in second division segregation frequency above the theoretical maximum of $X = 2/3$ is that the factor $4/3r^2$ fails in this case to accurately correct for the effects of undetectable double crossing over. Since this factor is based on the assumption of no chromatid interference, chromatid interference may be operative and may partially account for the deviations. In the absence of such interference two-strand, three-strand and four-strand double crossovers are expected to occur in the relative ratio 1:2:1. Distortion of this ratio by chromatid interference may account for the increased value of X. Taking possible interference into account, it is apparent that the probability generated by the best statistical fit, where $X = 0.70$ and $Y = 0.86$, is still only marginally acceptable (Table 6). Several explanations are possible and will be discussed in a later section.

DISCUSSION

Analysis of trisomic hybrids: The conclusions that emerge from these studies are (1) that the fatty acid locus is located on fragment five in loose association with the markers *trp3* and *ura1*, confirming the results of BÜRKL, CASTROPH and SCHWEIZER (1972); (2) fragment five is linked to chromosome eleven, confirming the results of MORTIMER and HAWTHORNE (1973); (3) the segregational behavior of markers in these strains indicates $n+1$ aneuploidy for chromosome eleven; (4) trivalent pairing of three homologous chromosomes does occur in these trisomic hybrids to some extent and possibly as frequently as 86%; (5) certain deviations from expectation found for complementary markers in $-/-/+$ configuration can be explained in part by assuming chromatid interference.

Aneuploid mapping procedures: The ease with which mapping of the fatty acid locus has been accomplished should be stressed. Previous studies designed to answer various questions regarding the overall Saccharomyces map have in general utilized crosses involving multiple disomes (generated by sporulating triploids) and haploid stocks carrying unlocated genes. In practice the stability of single chromosome aneuploids has been disappointing (MORTIMER and HAWTHORNE 1973). Since our complementary markers were introduced in duplex configuration by exceptionally stable, singly disomic parents, only a single basic cross was required to identify the trisomic chromosome, thus greatly minimizing the time and effort involved.

Although the spontaneous origin of $n+1$ aneuploidy in our strains eliminated the necessity of producing aneuploids containing the marked fatty acid locus, procedures have been reported for the selection of aneuploid cells from diploid strains (SHAFFER *et al.* 1971). Thus in theory any new mutant locus for which complementary markers have been isolated can be mapped efficiently by first selecting aneuploids from diploid parents containing complementary markers. One must anticipate, however, that the problem of stability may in some cases necessitate the more laborious use of multiple aneuploids for the purposes of mapping.

Unexplained deviations from expected tetrad frequencies in trisomic hybrids: Several explanations for the observed deviations in frequencies of $4^+:0^-$ and $2^+:2^-$ segregations for fatty acid requirement are possible—namely that in fact the hybrid strains used in this study are not trisomic, but some higher aneuploid multiple, that the strains are composed of mixed populations of different aneuploid multiples, that the observations are distorted by sampling error, or that some unknown factor is responsible, which was not considered in the above theoretical derivations. The first and second possibilities are extremely unlikely on the basis of extensive allele testing and analysis of strain stability (HENRY and FOGEL 1971) and the degree of conformity of other markers on the same chromosome to the expected tetrad frequencies. The third possibility is also unlikely, since the calculation of homogeneity chi-square (Table 6) indicates that samples taken from individual crosses (Table 1) are homogeneous.

In order for a factor such as gene conversion to account for the deviations it would have to have occurred at a level of about 11%. This is unreasonable because gene conversion was shown to occur at a level of less than 1% for these *fas1* alleles. Nondisjunction at meiosis II of the kind that would result in a tetrad consisting of one trisomic spore and three haploid spores cannot be invoked to explain the deviations, because it is believed on the basis of limited tetrad data to occur at a level of about 2.5% in these strains. Thus even if gene conversion and nondisjunction at meiosis II had occurred at their maximum observed rates and were restricted entirely to the production of $2^+:2^-$ tetrads at the expense of 4^+0^- tetrads, the major proportion of deviations would still remain unexplained.

Deviations from the expected frequencies of tetrad arrangements for complementing *fas1* alleles predicted on the basis of the best statistical values for second division segregating frequency (.70) and trivalent pairing frequency (.86) were *not* observed for markers at other loci (*trp3*, *ura1*, *met14*) even though these other markers were segregating on the same chromosomes in the same crosses (see Table 1 and 6). In addition such deviations were not observed in other crosses involving noncomplementing *fas1* alleles (see Tables 2 and 6). Any attempt to explain the deviations for complementing *fas1* alleles must also explain these apparent differences in behavior. At present, these deviations remain unexplained.

Pattern of spontaneous aneuploidy in fatty-acid-synthetase-defective strains: Although two chromosomes were not tested, aneuploidy in the strains analyzed in this study appears to occur in a specific, non-random pattern involving only the chromosome containing the locus that confers fatty acid requirement. However, in at least one of the strains originally described, chromosome III aneuploidy was detected as well (HENRY and FOGEL 1971). Assuming a direct relationship between membrane defects and the aberrant disjunction of chromosomes, one would expect a random pattern of nondisjunction for the various chromosomes. Ultrastructural studies (MOENS 1971) have shown that meiotic spindle fibers are intimately associated with the nuclear membrane in Saccharomyces. Thus defects in nuclear membranes could explain, at least qualitatively, an apparent high rate of nondisjunction. However, this theory cannot readily account for the

specific aneuploid pattern observed, unless selective pressures ensuring the survival of specific nondisjunctional events are assumed. Since normal rates of nondisjunction in yeast are difficult to measure, it is not clear whether nondisjunction in these strains is actually elevated. Thus, the possible relationship between membrane defects and the aberrant disjunction of chromosomes remains obscure.

Although the time of origin and the conditions under which aneuploidy arises in these strains is uncertain, it clearly occurred subsequent to the induction of the mutations with EMS and is not related to the mutagen itself (HENRY and FOGEL 1971). In addition, the parent strain used in mutagenesis, X2180, obtained from the Berkeley collection, has been thoroughly tested. Aneuploidy is not a property of this strain. Studies currently in progress have been undertaken to determine both the genetic and environmental conditions that lead to the production of aneuploidy in hopes of establishing the specific role of postulated selective pressures that may determine the pattern of aneuploidy observed.

One pertinent observation has been made regarding disomic clones identical with respect to fatty acid markers (*fas1–SH2/fas1–SH11*) grown on both supplemented and unsupplemented YEPD. Clones grown and stored for lengthy periods of time on unsupplemented media often become morphologically distinguished by extensive colony sectoring, which has been shown to be symptomatic of extensive aneuploidy (STRÖMNAES 1968). Identical clones grown and stored on media supplemented with fatty acids have remained normal and uniform in appearance. Thus while these $n+1$ aneuploids remain remarkably stable upon indefinite storage on media supplemented with saturated fatty acids, their stability under starvation conditions appears to be reduced even in the presence of markers that complement. Genetic analyses designed to reveal the extent of any changes in aneuploid condition in these sectored colonies is currently in progress. Preliminary evidence from tetrad analysis indicates that aneuploidy of additional chromosomes develops in stocks maintained in unsupplemented media, while supplemented stocks are completely stable.

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Corresponding editor: R. E. ESPOSITO