STUDIES OF POLYPLOID SACCHAROMYCES. I. TETRAPLOID SEGREGATION[1]

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ASCI which exhibit tetraploid segregation are sometimes obtained from crosses between supposedly haploid strains of yeast (ROMAN, HAWTHORNE, and DOUGLAS 1951; LINDEGREN and LINDEGREN 1951). Their occurrence has led to the suggestion that a clone derived from a haploid ascospore may become a mixture of haploid and diploid cells and that crosses between such clones could give rise to triploid and tetraploid zygotes. The fact that diploidization does occur was demonstrated in clones which were originally haploid and which were selected for study because they had become heterogeneous for cell size (ROMAN and SANDS 1953). The diploid cells were found to be of two kinds, differing from each other in mating capacity and sporulating ability. One of these does not mate (except perhaps rarely, POMPER, DANIELS, and MCKEE 1954) with either of the standard mating types, **a** or α. It sporulates readily, however, to give asci in which, as a rule, two of the four spores are **a** and two are α. This diploid is therefore heterozygous for the mating-type alleles a and α; it arises as a result of mutation at this locus in some of the cells of the clone, and subsequent fusion of mutant and non-mutant cells. The diploid of the second kind cannot be induced to sporulate; it is capable of mating, however, and the proof that it is diploid comes from the results of crosses. Asci obtained from crosses with haploids exhibit segregations which are expected of triploids and which indicate that the diploid parent was homozygous at the mating-type locus (these data will be reported elsewhere). Confirmatory evidence is found in the results of crosses between the diploids themselves and is reported below. The types of segregation which are obtained indicate the similarity, with respect to chromosomal pairing and distribution in meiosis, between yeast and other organisms in which polyploidy has been studied.

MATERIALS AND METHODS

The origin of the diploid strains used in this investigation was described by ROMAN and SANDS (1953). The characters employed in the crosses were the following: the mating types **a** and α, rapid vs. slow galactose fermentation, and flaky vs. non-flaky dispersion in liquid medium. The corresponding allelic symbols are a and α, G and g, and F and f. Rapid fermentation and the flaky condition are dominant. Since at least three loci are known to be involved in galactose utilization, it should be mentioned that we are dealing here with the G_2-locus.

The LINDEGREN techniques for mating (1943) and for sporulation (1944) were

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utilized, according to a schedule which ran typically as follows. The diploids were crossed by placing inocula of each parent into 2 cc of a medium containing 1% yeast extract, 2% peptone, and 2% glucose. The next day, either the mating mixture was transferred to presporulation medium or a single zygote was isolated with a micromanipulator and transferred to a nutrient-agar (the above medium plus 1.5% agar) droplet; after two or three days, the colony obtained from the zygote was streaked on a nutrient-agar slant and after two or three days growth on the slant a sample was transferred to presporulation medium. In either case, the cells were grown on the presporulation slant for two or three days and were then placed on an acidified gypsum slant. Sporulation usually occurred within a week.

Only four-spored asci were selected for dissection. Each of the spores was placed on a nutrient-agar droplet. After two or three days, the colony which was produced from the spore was transferred to a nutrient-agar slant and after three or four days of growth on the slant, each segregant was tested for mating type, for its ability to ferment galactose, and for its flocculence in liquid medium. The slants were then refrigerated for later use.

The segregants were tested for mating type by mixing each with known **a** and α strains in 2 cc of the yeast extract-peptone-glucose medium; the mixture was examined the next day for copulation figures.

The ability to utilize galactose was tested by the Durham-tube technique in 8 cc of the above medium, except that 2% galactose was substituted for glucose. Clones which produced enough gas to fill the tubes within 48 hrs were classified as rapid fermenters; the slow fermenters did not produce visible amounts of gas for several days thereafter, if at all during the one to three-week period of test.

The flaky tests were conducted in Difco yeast nitrogen base medium to which 1% glucose was added. The standing tubes, containing 5 cc of this medium, were shaken 24 hrs after inoculation and the flocculence of flaky cultures was at this time in clear contrast to the uniform distribution of cells in nonflaky cultures.

In our interpretation of the results of crosses between the diploid strains, it is assumed that only one sporulation cycle occurred. An extra sporulation cycle could produce new zygotic combinations which could yield asci exhibiting segregations not expected from the original combination. The high proportion of unexpected segregations obtained by LINDEGREN and LINDEGREN (1951) with tetraploid material was attributed to uncontrolled sporulation. In our material, we have never seen asci on the presporulation slants and only very rarely on the nutrient-agar slants if the latter were stored under refrigeration. Sporulation is not negligible, however, if the slants are stored at room temperature.

EXPECTATIONS FROM TETRAPLOIDS

The types of segregation expected in asci from tetraploid cells are shown in figure 1, for a hypothetical locus represented by the alleles $+ + - -$. It is assumed that two of the four homologues go to each pole in meiosis, an assumption which has its exceptions as will be seen later. The chromosomes are diagrammed as bivalents, in chromatid condition; multivalent pairing would result in the same segregational types.

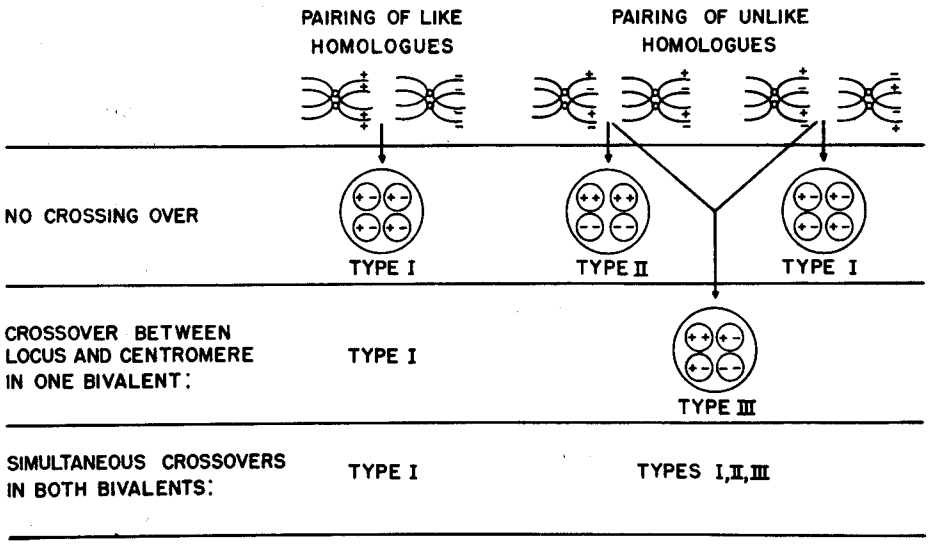

FIGURE 1.—Principal types of asci expected from tetraploid cells of genotype + + − − (see text).

In the absence of crossing over between the centromere and the locus, two types of segregation are expected. In type I, all four spores are heterozygous; in type II, two of the spores are homozygous for one allele and two are homozygous for the other. A crossover in this region between unlike homologues would result in a third type of segregation (III), in which one spore is homozygous for one allele, one is homozygous for the other, and two are heterozygous.

The phenotypic segregation for mating type, since it is possible to identify the heterozygous condition, is 4:0 for type I, 2:2 for type II, and 1:2:1 for type III. Rapid galactose fermentation and the flaky character are both dominant (we could not by our methods distinguish between the heterozygote and the homozygous dominant) and the phenotypic segregation for these two characters is 4:0, 2:2, and 3:1, respectively, for types I, II, and III.

RESULTS OBTAINED FROM TETRAPLOIDS

Table 1 summarizes the segregations obtained from tetraploids of composition *a a α α G G g g F F f f*. The segregations are tabulated separately for each locus. (Trisomic studies now underway indicate that the three loci are on different chromosomes.) The high frequency of type III segregation is evidence of considerable crossing over between each of the loci and its centromere, a point which will be returned to later.

A number of the segregants were subjected to further genetic test for the purpose of affirming their genotypes in the light of the expectations proposed above. The asci from which these segregants were taken, and the pattern of segregation in these asci, are given in table 2. In five of the asci, all four segregants were non-maters. These were induced to sporulate and, as would be expected if they were *a α*, the asci which were obtained exhibited segregation for the two mating types (table 3). The

TABLE 1

The distribution of the three types of asci, classified with respect to segregation for mating type, galactose utilization, and flaky, obtained from a a α α G G g g F F f f *cells*

Cross	Genotypes of diploid parents	Mating type			Galactose utilization			Flaky		
		I	II	III	I	II	III	I	II	III
260L-I × 4L-I	*a a G G f f* × α α *g g F F*	0	1	2	0	0	3	1	0	2
260L-I × 4L-II	*a a G G f f* × α α *g g F F*	12	0	11	12	2	9	10	5	8
260L-I × 4L-III	*a a G G f f* × α α *g g F F*	1	0	4	2	0	3	2	1	2
260L-V × 4L-I	*a a G G f f* × α α *g g F F*	2	0	1	1	1	1	2	0	1
260L-V × 4L-II	*a a G G f f* × α α *g g F F*	2	0	2	1	1	2	3	1	0
260L-V × 4L-III	*a a G G f f* × α α *g g F F*	2	1	1	1	0	3	3	1	0
21L-41 × 1L-II	*a a g g f f* × α α *G G F F*	6	2	2	5	0	5	3	2	5
21L-7 × 1L-II	*a a g g f f* × α α *G G F F*	5	1	3	3	1	5	2	2	5
		30	5	26	25	5	31	26	12	23

TABLE 2

Asci from which segregants were selected for further genetic test. The phenotypes of the segregants are indicated as follows: n = non-mater; a or α = segregant capable of mating and of the corresponding mating type; G or g = rapid or slow galactose utilization; F or f = flaky or non-flaky

Cross	Ascus	Spores				Type of segregation		
		A	B	C	D	MT	G	F
260L-V × 4L-I	304	n G F	n G F	n G f	n G F	I	I	III
	305	n G F	n G F	n G F	n g F	I	III	I
260L-V × 4L-II	307	n G F	n g F	n g F	n G F	I	II	I
260L-V × 4L-III	310	a g F	n G F	α G F	n G F	III	III	I
	317	n G F	n G F	n G F	n G F	I	I	I
	319	α G f	a G F	α g f	a G F	II	III	II
	320	n G F	n g F	n G F	n G F	I	III	I

segregations for galactose fermentation and for flaky were also those expected. In ascus 317, however, two of the segregants (C and D) produced asci in which no more than two of the four spores were viable. This type of segregation would be the consequence if, in meiosis of the tetraploid ascogenous cell from which 317 arose, three of the four homologues were distributed to two of the spores (A and B) and the fourth homologue to the other two (C and D). Segregants C and D would thus be monosomic (for a chromosome not carrying one of the three loci) and would yield n and n-1 spores, the latter two being inviable. Genetic evidence for this type of meiotic distribution will be discussed below.

The six other segregants which were examined further—310A, 310C, 319A, 319B, 319C, and 319D—were all capable of mating and could not be induced to sporulate, a characteristic of diploids homozygous for mating type in this material. Their genotypes were determined from the results of intercrosses (table 4). The cross 310A × 310C was expected to give the zygotic genotype *a a* α α *F F f f* and either *G G g g* or

TABLE 3

The results of tests of segregants listed in table 2

Segregant	No. of asci	Phenotypic ratio in asci			Genotype of segregant
		a:α	G:g	F:f	
304A	2	2:2	2:2	2:2	*a α G g F f*
B	2	2:2	2:2	4:0	*a α G g F F*
C	2	2:2	2:2	0:4	*a α G g f f*
D	2	2:2	2:2	2:2	*a α G g F f*
305A	2	2:2	4:0	2:2	*a α G G F f*
B	2	2:2	2:2	2:2	*a α G g F f*
C	2	2:2	2:2	2:2	*a α G g F f*
D	2	2:2	0:4	2:2	*a α g g F f*
307A	2	2:2	4:0	2:2	*a α G G F f*
B	2	2:2	0:4	2:2	*a α g g F f*
C	2	2:2	0:4	2:2	*a α g g F f*
D	2	2:2	4:0	2:2	*a α G G F f*
317A	2	2:2	2:2	2:2	*a α G g F f*
B	2	2:2	2:2	2:2	*a α G g F f*
C	7*	1:1	1:1	1:1	*a α G g F f*
D	6†	1:1	0:2	1:1	*a α G g F f*
		1:1	1:1	2:0	
320A	2	2:2	4:0	2:2	*a α G G F f*
B	2	2:2	0:4	2:2	*a α g g F f*
C	3	2:2	2:2	2:2	*a α G g F f*
D	1	2:2	2:2	2:2	*a α G g F f*

* In four of the seven asci dissected, two of the four spores survived to produce clones. The segregation tabulated is for the survivors of one of these asci. Of the remaining three asci, in two only one spore survived, and in the other none survived.

† In five of these asci only two spores survived and in the sixth there was one survivor. The data are given for two asci with two survivors.

TABLE 4

The results of tests of segregants listed in table 2 (continued). The numerals I, II, and III designate, respectively, the 4:0, 2:2, and 3:1 segregations obtained from + + − − cells. Segregations indicating a + − − − (G g g g or F f f f) genotype are given as numerical ratios, 2:2 or 1:3

Crosses of segregants	Segregations from crosses									Genotypes of segregants
	Mating type			Galactose			Flaky			
310A × 310C	8I	2II	12III	8I	4II	10III	13I	3II	6III	310A *a a g g F f* 310C *α α G G F f*
310A × 319A	14I	0II	7III	21	2G:2g		18	2F:2f		319A *α α G g f f*
							3	1F:3f		
319B × 319C	8I	2II	7III*	16	2G:2g		6I	3II	9III	319B *a a G g F F*
				2	1G:3g					319C *α α g g f f*
319D × 319C	7I	2II	11III	7I	2II	10III†	6I	2II	12III	319D *a a G G F F*

* Not including one ascus (408) in which segregation for mating type was 2n:2α.

† Not including one ascus (456) in which segregation for galactose was 1G:3g.

G g g g, depending on whether 310C was *G G* or *G g*. Cells of the first type should yield segregations of types I, II, and III for all three loci; those of the second should give no type I or III for galactose fermentation. The results indicate that 310A was *a a g g F f* and that 310C was *α α G G F f*.

In the cross 310A × 319A, it was again necessary to distinguish between the two possibilities, *G G g g* or *G g g g*, in a genotype which was otherwise expected to be *a a α α F f f f*, depending on whether 319A was *G G* or *G g*. The results show that 319A was *α α G g f f*, since there were no type I or III segregations for galactose utilization in 21 asci dissected. Of special interest, however, is the occurrence of 1:3 ratios for the flaky condition in the asci from this cross. This segregation is expected in + − − − genotypes (HALDANE 1930; ROMAN, HAWTHORNE, and DOUGLAS 1951) from the following sequence of events: crossing over between the locus and its centromere, the distribution of the two + − dyads to the same pole, and a metaphase orientation in the second division to produce one spore carrying both + alleles. The other three spores would then be homozygous for the − allele. The diagnostic test for this type of ascus, therefore, consists of affirming that the dominant segregant is homozygous for the dominant allele. Tests of the 1:3 asci encountered here and in subsequent crosses will be discussed below.

The results of the cross 319B × 319C, the latter presumed to be *α α g g f f*, indicate that 319B was *a a G g F F*, rather than *a a G G F F*, the other possibility. Here again 1:3 ratios were obtained, this time for galactose utilization. There was also one ascus (408) in which the segregation for mating type did not conform to type I, II, or III. This anomaly will be discussed in a separate section.

The identification of the genotypes of 319A and 319B, with respect to galactose utilization, makes it clear that 319D should be *G G* and thus that the cross 319D × 319C should give *a a α α G G g g F F f f* zygotes. The occurrence of segregations of types I, II, and III for each of the three loci agrees with expectation. There was one ascus (456), however, which exhibited a 1:3 segregation for galactose utilization; this case will be discussed with the other anomalies.

TESTS OF THE SEGREGANTS IN THE 1:3 ASCI

The dominant segregants of five asci which exhibited a 1:3 ratio were subjected to further genetic test. The phenotypic segregations in the five asci are given in table 5 and the results of the genetic tests of the segregants are given in table 6. Segregants 453C, 432C, and 1120A were non-maters and were induced to sporulate. Segregants 425D and 499C were capable of mating and were crossed with the diploid recessive strain 319C. The results indicate that each of the segregants was homozygous for the dominant allele in question, as would be expected from the explanation proposed above for the origin of the 1:3 asci; that is, 425D and 453C were *F F* and 432C, 1120A, and 499C were *G G*. (The anomalous segregation for mating type in ascus 528 will be discussed separately.)

THE EXCEPTIONAL ASCI

The three exceptional asci which were encountered during the course of this investigation will now be discussed. They are 408, 456, and 528; their origin and pattern

TABLE 5

Phenotypic segregations in five asci exhibiting 1:3 segregation for either rapid galactose utilization or flaky

Cross	Ascus	Phenotypes of segregants			
		A	B	C	D
310A × 319A	425	n G f	n g f	α g f	a G F
a a g g F f × α α *G g f f*	453	n g f	n G f	n G F	n g f
319B × 319C	432	n g f	n g F	n G F	n g F
a a G g F F × α α *g g f f*	1120	n G f	n g f	n g F	n g F
425D × 319C	499	α g F	a g F	a G F	α g F
a a G g F F × α α *g g f f*					

TABLE 6

Results of tests of dominant segregants of 1:3 asci. The segregations are listed as I, II, or III if they were derived from + + − − genotypes; otherwise the numerical ratios are given with the dominant phenotype first in order

Segregant	Crossed by	Ascus	Segregation type			Genotype of segregant
			MT	G	F	
425D	319C	499	II	1:3	I	*a a G g F F*
	α α *g g f f*	500	I	2:2	I	
		501	III	2:2	I	
453C	Sporulated directly	527	2:2	2:2	4:0	*a* α *G g F F*
		536	2:2	2:2	4:0	
		537	2:2	2:2	4:0	
432C	Sporulated directly	1136	2:2	4:0	2:2	*a* α *G G F f*
		1137	2:2	4:0	2:2	
		1138	2:2	4:0	2:2	
		1140	2:2	4:0	2:2	
1120A	Sporulated directly	1609	2:2	4:0	0:4	*a* α *G G f f*
		1610	2:2	4:0	0:4	
		1611	2:2	4:0	0:4	
		1613	2:2	4:0	0:4	
		1614	2:2	4:0	0:4	
499C	319C	528	2n:2α	III	2:2	*a a G G F f*
		529	I	III	2:2	
		530	I	I	2:2	
		531	II	II	2:2	
		532	I	III	2:2	
		533	II	I	2:2	

of segregation are given in table 7. In asci 408 and 528, two of the segregants were incapable of mating and the other two were both of mating-type α. This type of segregation would be expected as a consequence of the unequal meiotic distribution of the chromosomes carrying the mating-type locus, so that two of the spores would be trisomic for this chromosome and *a a* α, and two would be monosomic, and α. In ascus 456, only one of the segregants was a rapid fermenter of galactose.

TABLE 7

Phenotypic segregation in exceptional asci 408, 456, 528

Cross	Ascus	Phenotype of segregants			
		A	B	C	D
319B × 319C *aa Gg FF* × *αα gg ff*	408	α G F	n G F	α g F	n g f
319D × 319C *aa GG FF* × *αα gg ff*	456	n g F	n g F	n g F	n G f
499C × 319C *aa GG Ff* × *αα gg ff*	528	α G F	n G f	α g f	n G F

In ascus 528, the genotypes of all four segregants could be established. Segregants 528A and 528C, both of which were capable of mating, were crossed with the diploids 21L-41 (*a a g g f f*) and 319D (*a a G G F F*), respectively, to test the assumption that these segregants carried a single α allele and were therefore monosomic for the mating-type chromosome. Segregants 528B and 528D, which were non-maters and presumably *a a α*, were induced to sporulate directly. The results obtained for the four segregants, and their confirmed genotypes, are given in table 8. The segregations for mating type are those expected from hybrids of genotype *a a α* and indicate that 528A and 528C were monosomic and that 528B and 528D were trisomic for the mating-type chromosome. The segregations for galactose fermentation and flakiness are also those expected.

In ascus 408, the segregants which were incapable of mating, 408B and 408D, could not be induced to sporulate and their genotypes remain unknown. However, 408A and 408C were capable of mating and were crossed, respectively, with the diploids 310A (*a a g g F f*) and 306B (*a a G G F f*); the segregations which were obtained (table 8) indicate that these two segregants, like 528A and 528C, were monosomic for the mating-type chromosome. The failure of 408B and 408D to sporulate suggests the possibility that these two segregants may have been aneuploid to a greater extent than were 528B and 528D.

Ascus 456 requires a more elaborate explanation. The 1:3 segregation for rapid galactose fermentation suggests that the ascogenous cell from which 456 arose had the genotype *G g g g*, rather than the *G G g g* genotype that was expected from the parentage. The change in genotype could have arisen as a result of mutation of *G* to *g*, either in the 319D parent or in the ascogenous cell. It could also have arisen as a consequence of an extra cycle of sporulation; if, for example, the expected tetraploid had sporulated on the presporulation slant to produce *G g* and *g g* cells, the fusion of these would result in a tetraploid of genotype *G g g g*. Our evidence does not permit us to distinguish between these possibilities.

The further analysis of the segregants of ascus 456 indicated an additional complication, this time in the distribution of the chromosomes carrying the locus for galactose fermentation. Each of the four segregants was a non-mater and was induced to sporulate. Segregants 456A, 456B, and 456C, which were themselves slow fermenters, did not produce rapid fermenters among their progeny (table 9). Segregant

TABLE 8

The results of tests of asci 408 and 528. See table 6 for distinction between segregations expressed by I, II, or III and those expressed as numerical ratios

Segregant	Crossed by	Ascus no.	Segregation type			Genotype of segregant
			MT	G	F	
408A	310A	497	2a:2α	2:2	II	*α G g F f*
	a a g g F f	503	2n:2a	2:2	I	
		504	2n:2a	2:2	I	
		505	2n:2a	2:2	III	
		506	1n:2a:1α	2:2	I	
408C	306B	492	3a:1α	II	II	*α g g F f*
	a a G G F f	493	1n:2a:1α	III	I	
		494	1n:2a:1α	III	I	
		495	1n:2a:1α	I	II	
528A	21L-41	1146	2n:2a	2:2	1:3	*α G g F f*
	a a g g f f	1147	2a:2α	2:2	2:2	
		1148	2n:2a	2:2	2:2	
		1149	1n:2a:1α	1:3	2:2	
		1150	2n:2a	2:2	1:3	
528B	Sporulated directly	1189	1n:2a:1α	4:0	0:4	*a a α G G f f*
		1190	2n:2a	4:0	0:4	
		1191	2n:2a	4:0	0:4	
		1192	1n:2a:1α	4:0	0:4	
528C	319D	1208	3a:1α	III	II	*α g g f f*
	a a G G F F	1209	2n:2a	I	II	
		1210	1n:2a:1α	III	III	
		1211	1n:2a:1α	I	I	
		1212	1n:2a:1α	I	I	
		1213	1n:2a:1α	I	III	
528D	Sporulated directly	1183	2a:2α	2:2	2:2	*a a α G g F f*
		1184	3a:1α	2:2	2:2	
		1185	2n:2a	2:2	2:2	
		1186	2n:2a	2:2	2:2	
		1188	2n:2a	2:2	2:2	

456D, a rapid fermenter, produced both rapid and slow fermenters, and the 3:1 ratios for rapid fermentation suggest that 456D was either *G G g g* or *G G g*. (The unexpected mating-type segregation in ascus 540 was not investigated further.) If the 3:1 segregations (in asci 538, 539, 540) arose from a *G G g g* genotype, two of the three rapid fermenters should be *G g* and the third should be *G G*. If the genotype were *G G g*, the three rapid fermenters should be *G G*, *G g*, and *G*, respectively, or, rarely, *G G*, *G*, *G*. Five of the six rapid fermenters in asci 538 and 539 were tested by crossing each with a rapid fermenter and a slow fermenter, and observing the segregations in the next spore generation. The results may be summarized as follows: 538D and 539A were found to be *G g*; 538B, 539B, and 539C were found to be *G*. Segregant 456D was therefore *G G g*.

Since 456D was trisomic for the chromosome carrying the galactose locus, it follows that one of the other segregants in 456 should also be trisomic and that two

TABLE 9

Results of genetic tests of ascus 456. The four-spored asci obtained from 456B and 456C gave only one or two survivors per ascus

Segregant	Phenotype	Ascus no.	Segregation type			Proposed genotype of segregant
			a:α	G:g	F:f	
456A	n g F	610	2:2	0:4	4:0	*a α g g g F F*
		611	2:2	0:4	4:0	
		612	2:2	0:4	4:0	
		613	2:2	0:4	4:0	
		614	2:2	0:4	4:0	
456B	n g F	617	1:1	0:2	1:1	*a α g F f*
		618	1:1	0:2	1:1	
		629	1:1	0:2	1:1	
		641	1:1	0:2	1:1	
		642	1:0	0:1	1:0	
456C	n g F	627	1:1	0:2	0:2	*a α g F f*
		628	1:1	0:2	1:1	
456D	n G f	538	2:2	3:1	0:4	*a α G G g f f*
		539	2:2	3:1	0:4	
		540	1n:2a:1α	3:1	0:4	
		634	2:2	2:2	0:4	
		635	2:2	2:2	0:4	
		1960	2:2	3:1	0:4	

of the segregants should be monosomic for this chromosome. The evidence presented in table 9 suggests that 456B and 456C were monosomic since each produced asci in which no more than two of the four spores were viable. It is therefore proposed that 456A was trisomic and *g g g*. We can thus account for ascus 456 as the result of the following sequence of events: 1) the production of an ascogenous cell of genotype *G g g g*, either as a consequence of mutation or an extra sporulation cycle; 2) the occurrence of a crossover between *G* and the centromere; and 3) the distribution of three chromosomes to one pole and one to the other.

THE USE OF TETRAPLOID SEGREGATION TO ASCERTAIN GENE-CENTROMERE LINKAGE

Since the type III segregation requires a crossover between a locus and the centromere, it should be possible to ascertain the amount of recombination in this region if the frequency of type III segregation is known. A precise relationship between this frequency and the frequency of recombination is difficult to establish, however, in the absence of cytological information on the way in which the chromosomes associate and disjoin in meiosis. The problem of measuring linkage in tetraploids has been discussed by MATHER (1936).

If it is assumed that the four homologues pair as bivalents, without preference, the frequency of type III asci can be related to the frequency of second-division segregation on the basis of the following considerations. Pairing between genetically unlike homologues should occur twice as often as pairing between like homologues (fig. 1). The latter leads only to type I segregation and crossing over in the region in

question would not be detected. A crossover in one of the bivalents of unlike homologues would result in second-division segregation for the chromatids of this bivalent and, therefore, in a type III segregation. Simultaneous crossovers in both bivalents would lead to segregations of types I, II, and III, in the proportion 1:1:2 respectively. Thus when pairing is between unlike homologues the frequency of type III segregation is related to the frequency x of second-division segregation by the equation:

$$f(\text{III}) = 2x(1 - x) + \tfrac{1}{2}x^2 = 2x - \tfrac{3}{2}x^2$$

This equation is adapted from the equation for segregation in unordered asci obtained from diploids (PERKINS 1949; WHITEHOUSE 1949). Taking the contribution from pairing of like homologues into account, the frequencies of the three ascus types may be calculated from the following:

$$f(\text{I}) = \tfrac{1}{3} + \tfrac{2}{3}[\tfrac{1}{2}(1 - x)^2 + \tfrac{1}{4}x^2] = (4 - 4x + 3x^2)/6$$

$$f(\text{II}) = \tfrac{2}{3}[\tfrac{1}{2}(1 - x)^2 + \tfrac{1}{4}x^2] = (2 - 4x + 3x^2)/6$$

$$f(\text{III}) = \tfrac{2}{3}[2x(1 - x) + \tfrac{1}{2}x^2] = (4x - 3x^2)/3$$

The frequencies are given in table 10 for various values of x. The table includes values of x as high as 100%; however, those in excess of 66.7% are unlikely unless interference extends over relatively long genetic distances in yeast. When x is small enough so that the effects of double crossing over may be disregarded without serious error, the frequency of crossing over is $x/2$. The correction to be applied for higher values of x requires information, at present unavailable for yeast, on the extent of interference (see BARRATT *et al.* 1954).

TABLE 10

Relation of frequency of second-division segregation (x) *to frequencies of segregation types I, II, and III expected from bivalent pairing (second, third, and fourth columns) or from tetravalent pairing followed by random disjunction of centromeres (last three columns)*

x (%)	From bivalent pairing			From tetravalent pairing		
	I	II	III	I	II	III
	%	%	%	%	%	%
0	66.7	33.3	0	66.7	33.3	0
10	60.5	27.2	12.3	60.4	29.3	10.3
20	55.3	22.0	22.7	54.9	26.0	19.1
30	51.1	17.9	31.0	50.2	23.5	26.3
33.3	50.0	16.7	33.3	48.8	22.8	28.4
40	48.0	14.7	37.3	46.2	21.8	32.0
50	45.8	12.5	41.7	43.1	20.8	36.1
60	44.7	11.4	44.0	40.7	20.7	38.7
66.7	44.4	11.1	44.4	39.5	21.0	39.5
70	44.5	11.2	44.3	39.1	21.3	39.7
80	45.3	12.0	42.7	38.2	22.7	39.1
90	47.2	13.9	39.0	38.2	24.8	37.0
100	50.0	16.7	33.3	38.9	27.8	33.3

It is, however, obvious from the results of segregation that pairing is not exclusively bivalent. If it were, we would not expect 1:3 segregations nor unequal distributions of chromosomes as revealed in asci 408, 456, 528, and perhaps in 317. It will be recalled that the 1:3 segregation is the result of a crossover between unlike homologues in + − − − cells, followed by the distribution of these two chromosomes to the same pole. If, after multivalent pairing, which we will assume to be tetravalent, disjunction of the centromeres, two to each pole, is random, the two crossover chromosomes should move to the same pole in one third of the cells in which crossing over has occurred, and the 1:3 segregation should be obtained from only half of these. Thus, no more than one sixth of the asci in which tetravalent pairing has occurred should exhibit a 1:3 segregation for a specific character. The fact that we have observed three 1:3 segregations for galactose utilization in 47 asci and three for the flaky character in 27 asci suggests that tetravalent pairing occurs with substantial frequency.

It would therefore be useful to know what effect tetravalent pairing would have on the frequencies of segregation types I, II, and III. It will be assumed that the centromeres disjoin at random, two to each pole, and that the segment between locus and centromere pairs as a unit. The first assumption may not be valid, for it is possible that if two of the four homologues engage in crossing over in the region between locus and centromere, that these two would have a tendency to disjoin preferentially. If this tendency were pronounced, the expectation from tetravalent pairing would approach that from bivalent pairing. The assumption is made, however, for the purposes of calculating a set of frequencies which is alternative to the set obtained from bivalent pairing, with the understanding that the correct frequencies may fall between the two sets. The second assumption, which presupposes that the chromatids do not exchange pairing partners, may be in error for relatively long distances; we assume that it is likely to hold for shorter distances, i.e., for those in which linkage between gene and centromere is demonstrable. On these assumptions, the frequencies of the segregational types are related to the frequency of second-division segregation as follows:

$$f(\mathrm{I}) = \tfrac{2}{9} + \tfrac{2}{3}[\tfrac{2}{3}(1-x)^2 + \tfrac{1}{6}\{2x(1-x)\} + \tfrac{1}{4}x^2] = (12 - 12x + 7x^2)/18$$

$$f(\mathrm{II}) = \tfrac{1}{9} + \tfrac{2}{3}[\tfrac{1}{3}(1-x)^2 + \tfrac{1}{4}x^2] = (6 - 8x + 7x^2)/18$$

$$f(\mathrm{III}) = \tfrac{2}{3}[\tfrac{5}{6}\{2x(1-x)\} + \tfrac{1}{2}x^2] = (10x - 7x^2)/9$$

The frequencies of the three types of segregation for different values of x are given in table 10. Expectations from the two modes of pairing are quite similar for small values of x. As x increases, the effect of tetravalent pairing becomes apparent in the relatively higher frequencies of the type II segregation, at the expense of types I and III.

The segregations from + + − − hybrids are summarized in table 11, for each of the three loci. Theoretical distributions, expected from bivalent and tetravalent pairing, are also given; these are calculated from the frequencies in table 10, for selected values of x. The G_2 and F loci do not exhibit linkage with the centromere in diploid segregation (HAWTHORNE 1955, and personal communication) and the value

TABLE 11

Summary of crosses giving type I, II, or III segregations. The theoretical distributions, for bivalent and tetravalent pairing, are given for selected values of x (*see text*). *Below each theoretical distribution is given, in parentheses, the corresponding chi-square value and probability*

Cross	Genotypes	Segregation types								
		MT			G			F		
		I	II	III	I	II	III	I	II	III
260L × 4L	*a a G G f f* × *α α g g F F*	19	2	21	17	4	21	21	8	13
310A × 310C	*a a g g F f* × *α α G G F f*	8	2	12	8	4	10	13	3	6
319D × 319C	*a a G G F F* × *α α g g f f*	7	2	11	7	2	10	6	2	12
21L × 1L	*a a g g f f* × *α α G G F F*	11	3	5	8	1	10	5	4	10
260L × 13L	*a a G G f f* × *α α g g f f*	4	1	5	7	2	1	—	—	—
425D × 319C	*a a G g F F* × *α α g g f f*	1	1	1	—	—	—	3	0	0
499C × 319C	*a a G G F f* × *α α g g f f*	3	2	0	2	1	3	—	—	—
319B × 319C	*a a G g F F* × *α α g g f f*	8	2	7	—	—	—	6	3	9
319D × 528C	*a a G G F F* × *α g g f f*	—	—	—	4	0	2	2	2	2
310A × 408A	*a a g g F f* × *α G g F f*	—	—	—	—	—	—	3	1	1
21L × 13L	*a a g g f f* × *α α g g f f*	6	1	2	—	—	—	—	—	—
310A × 319A	*a a g g F f* × *α α G g f f*	14	0	7	—	—	—	—	—	—
		81	16	71	53	14	57	59	23	53
Frequencies (%)		48.2	9.5	42.3	42.7	11.3	46.0	43.7	17.0	39.3
Theoretical distributions:										
For bivalent pairing	x = 50%	77	21	70						
		(1.4; P = .5)								
	x = 66.7%				55	14	55	60	15	60
					(.15; P = .9)			(5.1; P = .05—.1)		
For tetravalent pairing	x = 50%	72	35	61						
		(13.1; P = <.01)								
	x = 66.7%				49	26	49	53	28	53
					(7.2; P = <.05)			(1.6; P = .3 — .5)		

of x for these is taken as 66.7%. For the mating-type locus, the value of 50% is chosen since LINDEGREN (1949) and HAWTHORNE (1955) give frequencies of second-division segregation of 47% and 53%, respectively, for this locus. The chi-square values indicate that the results obtained for the G_2 and mating-type loci are in good agreement with the hypothesis of bivalent pairing, and are not expected from tetravalent pairing, whereas the reverse is true for the F locus. We interpret this to mean that while both types of pairing occur, bivalent pairing is more frequent for the chromosomes carrying the G_2 and mating-type loci and less frequent for the chromosome of the F locus. Excellent agreement is obtained with the observed results for the F locus if each of the two types of pairing is assumed to occur half the time.

POLYPLOIDY AND THE PROBLEM OF THE OCCURRENCE OF IRREGULAR RATIOS

When a cross is made between two clones, one carrying the gene + and the other the recessive allele −, and both clones have been derived from haploid ascospores, it is expected that the four-spored asci which are produced will segregate 2+:2−. However, "irregular" ratios—4:0, 3:1, 1:3, and 0:4—have also been obtained from such crosses and with frequencies which would seem to preclude conventional gene mutation as a likely explanation. Other mechanisms have therefore been suggested. LINDEGREN (1949, 1953) has proposed gene conversion, a special mutation hypothesis which assumes the alteration of a gene by its allele, in the heterozygous condition. WINGE and ROBERTS (1950), having observed that asci with more than four spores sometimes occur, have inferred from this that in some asci the four spores may be the survivors of an originally larger number and thus that some of the meiotic products have been lost. In some aberrant asci, it has been found that one of the spores is diploid whereas the other three are haploid. This has led to the hypothesis of "post-meiotic nuclear fusion" (ROMAN and SANDS 1953) which presupposes an extra mitotic division in the ascus with the subsequent fusion of haploid nuclei. WINGE and ROBERTS (1954) have obtained similar genetic evidence in homothallic as well as in heterothallic yeast, and cytological evidence of supernumerary mitoses and binucleate spores. Finally, polyploidy has been shown to contribute to the production of asci with irregular ratios (LINDEGREN and LINDEGREN 1951; ROMAN, DOUGLAS, and HAWTHORNE 1951; ROMAN and SANDS 1953; LEUPOLD and HOTTINGUER 1954). It thus seems clear that there are a number of reasons for irregular ratios when 2:2 segregation is expected.

In what follows we will restrict ourselves to a discussion of the consequences of polyploidy, which has been a recurring factor in the production of unexpected asci in the heterothallic material at our disposal. When a colony is obtained from a haploid spore on an agar droplet and is transferred either to 5 cc of broth or to a nutrient-agar slant, the resulting crop as a rule consists chiefly of haploid cells. If the clone is then subcultured in serial transfer, it frequently happens that after a number of transfers a subclone is obtained which consists largely or wholly of diploid cells. A clone which was originally haploid may thus be converted to a clone which is diploid; the diploid cells may be heterozygous or homozygous for the mating-type alleles, or both may be present in the same clone.

Suppose, therefore, that a cross is made between two clones originally of genotype $a+$ and $\alpha-$, respectively. In addition to the $a\ \alpha + -$ zygotes which are expected, polyploid zygotes may also occur and the types and frequencies of these would depend on the extent to which each of the parental clones consists of diploid cells homozygous for the mating-type alleles. Diploidization in only the a parent would lead to triploids of genotype $a\ a\ \alpha + + -$; if it occurred only in the α parent, $a\ \alpha\ \alpha + - -$ triploids would be expected. If diploid homozygotes were present in both parents, the tetraploid $a\ a\ \alpha\ \alpha + + - -$ could also be obtained.

Mating-type segregation is an excellent indicator for the detection of polyploid segregation. As table 11 shows, only about one out of 10 asci from tetraploid cells exhibits the 2a:2α ratio which could be confused with diploid segregation. Charac-

teristic mating-type ratios serve to identify the triploid condition as well (see table 8) and here again the **2a**:2α ascus is in the decided minority. The tetraploid condition and the triploid + + − can be identified also by the 4:0 and 3:1 segregations for the + phenotype. The frequency of 2:2 segregation is not expected to exceed $\frac{1}{3}$ and would be less than this if crossing over occurs between the marker locus and the centromere, an event which is also necessary for the production of 3:1 segregation. The opportunity for detection of polyploid segregation depends on the frequency with which asci of polyploid origin arise in the cross and, in an individual ascus, on the number of segregating markers.

If, however, mating-type segregation is ignored as a criterion and triploid cells of genotype + − − are obtained in a cross, the detection of polyploidy is more difficult to achieve since this type of triploid is expected to yield chiefly asci with a 2:2 segregation. The 1:3 ratio is diagnostic; as can be seen from the results of segregation obtained from the comparable tetraploid condition, + − − −, which gave three 1:3 segregations for galactose utilization in 47 asci and three for the flaky character in 27 asci, the opportunity for the detection of triploids of this type would not be great unless a relatively large number of asci were dissected. Thus 2:2 segregation cannot in all circumstances be taken as evidence of diploid segregation unless triploidy has been excluded as a factor: this can be accomplished if several markers are used and the cross is made so that dominants are contributed by each of the two parents.

If segregants obtained from polyploids are used in crosses, either unwittingly or for further genetic test, the fact that aneuploid combinations may arise must also be taken into account in the interpretation of the results of crosses. Aneuploid spores are produced by tetraploids and, more commonly, by triploids (unpublished evidence). A cross between a haploid and an aneuploid of composition $2n$-x would, for example, yield diploid segregation ratios for the markers located on the chromosomes present in disomic condition in the zygote and triploid ratios for the others. Moreover, an aneuploid clone can become a mixture of aneuploid and euploid cells, possibly as a consequence of mitotic nondisjunction and the competitive advantage enjoyed by the euploid; evidence is at hand that this happens in clones which are disomic for one chromosome and otherwise haploid. For example, in clones disomic for the chromosome carrying the G_2 locus and of genotype G_2 g_2, haploid cells which have lost either G_2 or g_2 can be identified genetically. Such clones, although heterogeneous, are classified as fermenters; in crosses with nonfermenter haploids, they yield asci exhibiting 2:2, 1:3, and 0:4 ratios for galactose fermentation. The relative frequencies of the three types of asci depend of course on the balance existing between the three types of cells in the heterogeneous culture.

Thus polyploidy and its consequences may lead to the production of each of the four types of irregular asci. If polyploidy is excluded as a factor, either by appropriate genetic test of the segregants or by segregational evidence exhibited by the aberrant ascus itself, other mechanisms may be invoked. Some of these have been mentioned above; others may come to light as further information is obtained on the genetics of yeast.

SUMMARY

Evidence is presented to show that tetraploid segregation in yeast follows the pattern which could be expected of an orthodox meiosis. Tetraploids of genotype + + − − yield asci which exhibit phenotypic ratios of 4:0, 3:1, and 2:2; those of genotype + − − − yield 2:2 and 1:3 asci. The effect of multivalent pairing is seen in the occasional production of exceptional asci, which contain aneuploid spores.

Calculations are made relating the frequency of second-divison segregation to the frequencies of the three types of asci obtained from the + + − − tetraploid, thus providing a means of determining gene-centromere linkage. Two sets of calculations are given, one based on bivalent pairing, the other on tetravalent pairing. The observed frequencies of segregation for three loci suggest that both types of pairing occur.

The problem of the occurrence of irregular asci is discussed in terms of polyploidy and its consequences.

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