

known to inhibit the yeast enzyme, does not inhibit either liver alcohol dehydrogenase or retinene reductase.

¹¹ Wald, G., *J. Gen. Physiol.*, **19**, 781 (1935-1936); *Biol. Symp.*, **7**, 43 (1942).

¹² Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, **35**, 502 (1941); Handler, P., and Klein, J. R., *J. Biol. Chem.*, **143**, 49 (1942); McIlwain, H., and Rodnight, R., *Biochem. J.*, **44**, 470 (1949).

POLYPLOIDY IN YEAST AND ITS BEARING ON THE OCCURRENCE OF IRREGULAR GENETIC RATIOS*

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The significance of irregular ratios, i.e., phenotypic ratios of 4:0, 3:1, 1:3, and 0:4 in asci obtained from crosses which are expected to give 2:2 segregations and commonly do so, has been the subject of considerable speculation in yeast genetics. Lindegren¹ has cited such irregularities as evidence that a gene can be affected by its allele when the two are present in the same cell. On this interpretation, the 4:0 and 3:1 asci are those in which the dominant gene has converted the recessive allele to the dominant condition; the 1:3 and 0:4 cases are indicative of the degradation of the dominant by the recessive allele.

The gene-conversion hypothesis does not lend itself at the present time to critical test, and therefore depends for its validation on the exclusion of other explanations based on known genetic mechanisms. To this end Lindegren and his colleagues have shown that neither multiple factors nor orthodox mutation can provide a general explanation for irregular ratios. Mundkur² has demonstrated the inadequacy of the Winge and Roberts³ hypothesis that accounts for these ratios in terms of an extra mitosis in the formation of ascospores. Finally, Mundkur⁴ has excluded polyploidy as a factor in this problem on the grounds that it will not account for all of the irregularities that have been found.

The evidence presented below that polyploidy does in fact occur in yeast has prompted us to reappraise its possible role in the interpretation of irregular ratios.

Evidence of Diploid Ascospores.—Among 64 four-spored linear asci whose spores were tested for mating type, galactose fermentation, and growth habit in liquid medium, one ascus was found which exhibited an irregular ratio for each of these characters. The asci were obtained from a cross between two clones of *Saccharomyces* that were presumably haploid and of composition *agf* and *αGF*, respectively.⁵ Clones were grown from the four

spores of the exceptional ascus (No. 55) and were designated A, B, C and D, in the order in which the spores occurred in the linear ascus. Each of the clones gave a negative reaction in mating-type tests with the standard *a* and α strains. Clones A, C and D all fermented galactose within 24 hours after being placed in Durham fermentation tubes. Clone B failed to ferment galactose during the 96 hours of the test. All of the clones exhibited the dominant flaky character in liquid medium.

The failure of the four clones to mate with the standards suggested the possibility that each clone consisted of cells heterozygous for the mating-type alleles. Evidence for this view was obtained when it was found that each of the clones proved to be self-sporulating and produced asci on gypsum slants. Twelve asci, three from each of the four clones, were dissected and tests were made for mating type, galactose fermentation, and

TABLE I
THE RESULTS OF TESTS OF ASCI OBTAINED FROM THE SELF-SPORULATING CLONES 55 A, B, C AND D

CLONE TESTED	ASCUS No.	COMPOSITION OF SPORES			
		aGF	α Gf	aGf	α gf
55A	1	aGF	α Gf	aGf	α gf
	2	aGf	α GF	..	α GF
	3	α gf	aGF	aGf	α GF
55B	1	α GF	α gf	agF	α gf
	2	α GF	α gf	agF	α gf
	3	α GF	α gf	agF	α GF
55C	1	..	α GF	aGf	aGF
	2	α GF	aGf	aGf	α GF
	3	aGf	α GF	aGf	α GF
55D	1	α gf	α GF	aGF	aGf
	2	agF	α Gf	aGF	α gf
	3	aGf	α gf	aGF	α GF

flaky. Ten of the twelve had four viable spores and exhibited a 2:2 segregation for both mating type and flaky. In the remaining two asci, only three of the four spores survived, but in these also a segregation for *a* and α and for *F* and *f* was observed. With respect to galactose fermentation, the asci from Clones A and D segregated for *G* and *g*, the asci from Clone B gave non-fermenters only, and the asci from Clone C gave fermenters only (table 1).

The results of these tests show that the spores of Ascus 55 had the following composition: Spore A, $a\alpha Gg Ff$; Spore B, $a\alpha gg Ff$; Spore C, $a\alpha GG Ff$; and Spore D, $a\alpha Gg Ff$. Since the three loci gave no evidence of linkage in these experiments, there is good reason to infer a disomic condition for at least three chromosomes of the complement and it is probable that each of the spores was actually diploid. Thus the irregular

ratios which were exhibited in Ascus 55 can be resolved in terms of an orthodox polyploid explanation.

Discussion.—The information at hand does not permit us to distinguish between two alternatives for the origin of Ascus 55. First, the cell from which the ascus arose may have been tetraploid, of composition $aa\alpha\alpha GGgg FFff$. On this basis, the compositions of the four spores of Ascus 55 would be accounted for on the assumption of a normal meiosis in which a crossover between the locus of G and the centromere had occurred. The manner of origin of the tetraploid cell itself is suggested by the observations of Lindegren¹ that asci are occasionally found within clones of single mating type. These asci have been interpreted to be the result of "illegitimate" hybridization between cells of like mating type. Such fusions would give a certain proportion of aa and $\alpha\alpha$ diploids and the mixing of the two clones by Lindegren's method should produce tetraploids of the type suggested above.

Second, the diploid spores may have been the consequence of an extra division in spore formation. Suppose, for example, that a diploid ascogenous cell had produced four nuclei of composition aGF , αGf , agF and αgf , respectively, and that each nucleus had undergone a mitosis; the result would be eight nuclei, as follows: (1) aGF (2) aGF , (3) αGf , (4) αGf , (5) agF , (6) agF , (7) αgf and (8) αgf . The subsequent nuclear fusion of 1×3 , 2×7 , 4×5 and 6×8 would result in diploid spores of the kind encountered in Ascus 55. The precedent for an extra division is furnished by our own observations and those of Winge and Roberts³ that asci are occasionally found with more than four viable spores. It should be noted that the fusion of nuclei of like mating type following the extra mitosis would provide diploid mating clones from which tetraploid zygotes could be obtained.

The foregoing discussion suggests the possibility that clones derived from haploid spores may consist of both haploid and diploid cells. A cross between two such clones of opposite mating type should produce, in addition to diploid and tetraploid zygotes, a number of triploid zygotes. The relative proportions of the three classes would depend on their relative viabilities and on the time and frequency of diploidization within the parent clones. As indicated above, tetraploid clones could also be the result of the fusion of two diploid spores derived from the extra mitosis and triploid clones would be obtainable from these in further crosses. Spores obtained from the tetraploid and triploid lines should in turn give rise to aneuploid clones of various kinds.

These polyploid situations make possible an array of genetic combinations that can furnish material from which all of the types of irregular ratios can be obtained. Consider, for example, some of the zygote combinations that could be expected from a cross between a galactose-fermenter clone and a non-fermenter. If each of the two clones consists of a mixture of haploid and diploid cells, zygotes would be produced of composition Gg ,

GGg, *GGgg* and *Ggg*. The types of asci which each of these zygotes could produce are given in table 2. Each of the combinations would give asci with a 2:2 segregation for galactose fermentation. In addition, asci derived from *GGg* and *GGgg* cells should exhibit 4:0 and 3:1 ratios, the latter depending on a crossover which places *G* and *g* on the same dyad.

The 1:3 and 0:4 ratios are to be expected from the *Ggg* clone. The 1:3 ratio would be obtained as a consequence of the movement of two *G-g* dyads to the same pole following crossing over. To account for the 0:4 ratio it is necessary only to assume that in yeast, as in many other organisms that have been investigated, chromosomal loss in meiosis is the con-

TABLE 2

TYPES OF ASCI EXPECTED FROM A CROSS BETWEEN A GALACTOSE-FERMENTER CLONE AND A NON-FERMENTER ON THE ASSUMPTION THAT EACH CLONE CONTAINS BOTH HAPLOID AND DIPLOID CELLS. THE EFFECT OF CHROMOSOMAL LOSS IS SHOWN ONLY FOR THE *Ggg* GENOTYPE

ZYGOTIC GENOTYPE	TYPES OF ASCI				PHENO- TYPIC RATIO WITHIN ASCUS	REMARKS
	<i>G</i>	<i>Gg</i>	<i>g</i>	<i>g</i>		
<i>Gg</i>	<i>G</i>	<i>G</i>	<i>g</i>	<i>g</i>	2:2	
<i>GGgg</i>	<i>Gg</i>	<i>Gg</i>	<i>Gg</i>	<i>Gg</i>	4:0	
	<i>GG</i>	<i>GG</i>	<i>gg</i>	<i>gg</i>	2:2	
	<i>GG</i>	<i>Gg</i>	<i>Gg</i>	<i>gg</i>	3:1	Crossover
<i>GGg</i>	<i>Gg</i>	<i>Gg</i>	<i>G</i>	<i>G</i>	4:0	
	<i>GG</i>	<i>GG</i>	<i>g</i>	<i>g</i>	2:2	
	<i>GG</i>	<i>Gg</i>	<i>G</i>	<i>g</i>	3:1	Crossover
	<i>GG</i>	<i>G</i>	<i>G</i>	<i>gg</i>	3:1	Crossover
<i>Ggg</i>	<i>Gg</i>	<i>Gg</i>	<i>g</i>	<i>g</i>	2:2	
	<i>G</i>	<i>G</i>	<i>gg</i>	<i>gg</i>	2:2	
	<i>Gg</i>	<i>G</i>	<i>gg</i>	<i>g</i>	2:2	Crossover
	<i>GG</i>	<i>gg</i>	<i>g</i>	<i>g</i>	1:3	Crossover
	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>	0:4	Loss of chromosome carrying <i>G</i>

comitant of the polyploid condition. The loss of the chromosome carrying *G* would result in an ascus in which all four spores would produce non-fermenter clones.

A polyploidy hypothesis can thus provide a formal basis for the interpretation of all of the irregular ratios that have been encountered in crosses. Moreover, the hypothesis is subject to test since it predicts a number of types of identifiable asci, some of which are given in table 2. The tests must be conducted under conditions which will permit the detection of illegitimate hybridization if it occurs. Unfortunately, the most direct evidence, from chromosomal counts, cannot as yet be obtained for lack of suitable cytological techniques.

There are, however, two aspects of the already published data that bear on this question. First, there is the evidence that an irregular ratio for one character is usually accompanied by an irregular ratio for another within the same ascus. Thus among 48 four-spored asci in which irregular ratios occurred,^{1, 4} 35 exhibited an irregularity for more than one character. It is perhaps also significant that the 48 asci were produced from crosses in which one parent was either a clone (CIA), obtained from an ascospore of *Saccharomyces carlsbergensis*, or a descendant of this clone. An interpretation of this information in terms of the gene-conversion hypothesis requires the somewhat difficult assumption that the capacity for conversion can affect several loci simultaneously in one pedigree but not in another; the clone CIA is regarded as a "converter" parent which transmits the capacity for conversion to its progeny.

The same evidence can be explained readily on the basis of polyploidy. The simultaneous occurrence of irregular ratios for two or more characters in an ascus would be expected if the zygote were polyploid. For example, zygotes of composition $GGg FFf$ should produce a large proportion of asci with 4:0 ratios for both galactose fermentation and flaky. Moreover, the selection for lines which give a high frequency of irregularity could be interpreted as a selection for the polyploid condition. It would follow from this that the CIA clone and some of its descendants were probably diploid, to take the simplest view.

Second, the most striking and direct support for the polyploidy hypothesis comes from the observations that crosses which yield irregular ratios for other characters also produce mating type disturbances. By analogy with the segregations for G and g in table 2, we should expect asci in which one, two, or four spores are of composition $a\alpha$. These spores, according to our experience with Ascus 55, should give rise to clones that will not mate with either the a or α standards. Five asci have been reported^{1, 4, 6} in which all of the spores gave "sterile" clones. It is noteworthy that each of the five asci, like Ascus 55, exhibited irregular ratios for other characters as well. Mundkur⁴ also makes reference to asci in which two of the four spores gave sterile clones. We ourselves have found an ascus in which only one of the spores was of this type. Tests of the clone derived from this spore have shown that the spore was $a\alpha$. The occurrence of sterile clones and their correlation with the $a\alpha$ genotype is convincing evidence of the role of polyploidy in the production of irregular ratios.

The foregoing discussion indicates that the polyploidy hypothesis offers a basis for an interpretation of irregular ratios on conventional grounds and moreover that it is supported by the evidence at hand. Further investigation is required to determine the extent to which other explanations may be necessary.

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¹ Lindegren, C. C., *The Yeast Cell*, Educational Publishers Inc., St. Louis, 1949.

² Mundkur, B. D., *Current Science*, **19**, 84-85 (1950).

³ Winge, O., and Roberts, C., *Nature*, **165**, 157-158 (1950).

⁴ Mundkur, B. D., *Ann. Missouri Bot. Gard.*, **36**, 259-280 (1949).

⁵ All of the crosses reported in this paper were between clones derived from single ascospores. We are indebted to Dr. C. C. Lindegren for the parent clones. The symbols refer to genes affecting the following characters: a and α , mating type; G and g , galactose fermentation and non-fermentation (within 96 hours), respectively; F and f , flaky and free dispersion, respectively, in liquid medium. The flaky character was particularly clear-cut in DIFCO Yeast Nitrogen Base medium with 1% glucose added.

⁶ Mundkur, B. D., and Lindegren, C. C., *Am. J. Bot.*, **36**: 722-727 (1949).

ON THE FUNCTIONAL EQUATIONS OF THE DIRICHLET SERIES DERIVED FROM SIEGEL MODULAR FORMS

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The striking analogy between the formulas

$$\int_0^\infty e^{-tx} x^{s-1} dx = \Gamma(s)t^{-s}, \quad t > 0, \operatorname{Re}(s) > 0, \quad (1a)$$

and

$$\int_{X>0} e^{-\operatorname{tr}(TX)} |X|^s - \left(\frac{n+1}{2}\right) dX = \pi^{n(n-1)/4} \Gamma(s) \Gamma\left(s - \frac{1}{2}\right) \dots \times \\ \Gamma\left(s - \frac{n-1}{2}\right) |T|^{-s}, \quad \operatorname{Re}(s) > \frac{n-1}{2}, \quad (1b)$$

where, in the second formula, the integration is over the space of positive definite $n \times n$ matrices, $|X|$ represents the determinant of X , and T is a positive definite matrix, leads one to conjecture that there should be a theory connecting Siegel modular forms and Dirichlet series possessing certain types of functional equations, similar to that created by Hecke connecting Dirichlet series and modular forms of one variable.

The first step in the construction of such a theory has been taken by Maass,¹ who showed that from modular forms of the second degree one can derive an infinite set of Dirichlet series possessing functional equations. Contrary to what occurs in the Hecke theory, the derivation of the functional equation is no longer a simple preliminary step, but requires some detailed analysis. Since the proof given by Maass is rather complicated,