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RADIOBIOLOGICAL AND GENETIC STUDIES ON A POLYPLOID SERIES (HAPLOID TO HEXAPLOID) OF *SACCHAROMYCES CEREVISIAE*

Robert K. Mortimer

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Robert K. Mortimer

Donner Laboratory of Biophysics and Medical Physics
University of California, Berkeley, California

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ABSTRACT

A related series of Saccharomyces cerevisiae varying in ploidy from haploid to hexaploid was developed by a series of controlled matings.

Measurements of linear dimensions of the various cultures indicated a linear increase of cellular volume with ploidy.

Genetic analysis of the hybrid cultures revealed normal segregations for the diploid, and segregations for the tetraploid that were consistent with previous findings and with theoretical predictions based on the assumption of random bivalent pairing. Low viability of the triploid meiotic products precluded adequate genetic analyses of these cultures. The hexaploid was found to yield an expected low frequency of phenotypically recessive spores and the distributions of segregation ratios in different asci agreed quite well with theoretical predictions for three of the four loci studied. It was necessary to assume the fourth locus to be in duplex constitution in order to account for the results obtained for it.

The radiation studies on the different cultures showed an increase in resistance to x-ray inactivation from haploid to diploid, followed by a progressive decrease in radioresistance with further increases in ploidy from diploid through hexaploid. Considerable variability of radiation resistance of diploid cultures was encountered and the results suggested that this may be at least in part due to variations in degree of genetic homozygosity.

The results of the radiation studies are consistent with the assumption that the majority of inactivations of haploid cultures is brought about by recessive lethals whereas higher-ploidy cultures are inactivated mostly by dominant lethals

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INTRODUCTION

The sporogenous yeast Saccharomyces cerevisiae is a suitable organism for studies relating radiation response to variations of cellular parameters. Detailed studies of the nature of induced radiation damage are facilitated by the availability of relatively stable haploid, diploid, and p yploid cultures to which can be applied suitable genetic tests.

Analyses of the x-ray sensitivity of yeast cells of different ploides have been described in a number of recent reports.[8, 14, 16, 17, 23, 29, 30] Haploid cells generally are found to be inactivated exponentially, while diploid cells exhibit a sigmoid survival curve and are considerably more radioresistant. These findings have been interpreted by some authors as indicating damage that is operationally equivalent to recessive lethals. Such interpretations would predict an increase in resistance to x-ray inactivation with increasing ploidy. Extensions of radiobiological studies on yeast to triploid and tetraploid cells* have failed to demonstrate such a relation of radioresistance to ploidy; tetraploid cells have been shown to be as sensitive as or more sensitive than diploid cells.[14, 17] These latter findings have led to an extension of the interpretations of x-ray-induced lethality in yeast with consideration of an additional form of lethal damage, the frequency of which increases with ploidy. This form of damage was proposed to be in the form of dominant lethals, as suggested previously.[8] Experimental evidence for the presence of dominant lethals in irradiated yeast cells has been presented,[18] and later studies have shown that the frequency of this damage increases with the ploidy of the irradiated cell.[21] These studies would suggest a progressive decrease of radioresistance with further increases in ploidy, and it was of interest to test this on cells of ploidy higher than tetraploid.

A number of recent articles have described the production of triploid and tetraploid hybrids of Saccharomyces.[10, 13, 17, 23, 24] The procedures outlined have usually involved the use of diploid parental cells that are homozygous for mating type (αα or aa).† These cells were derived either from spore cultures producted by chance tetraploid segregations[10, 12] or from partially diploidized

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*In a previous publication (Tobias, C. A., Symposium on Radiobiology (Wiley, N. Y., 1952)) a survival curve ascribed to a "polyploid" yeast culture was presented, which indicated a much higher radioresistance for this culture than for the haploid or diploid cultures used at that time. Further tests have failed to establish the original assumption that this culture was a polyploid strain of Saccharomyces cerevisiae, and for this reason this result is now considered of no significance to the relation between radiation resistance and ploidy in Saccharomyces cerevisiae.

†Here α and a represent complementary alleles of the mating type locus.[12]

haploid cultures.[17, 24] Pomper has described a method of producing higher-ploidy yeast cultures not dependent on the availability of such cells.[22, 23] In his studies, diploid cells heterozygous for mating type (αα) but possessing complementary biochemical requirements were used as parents. Infrequent matings between such cultures were shown to occur and these were isolated prototrophically by plating the mating mixture on a medium minimal for both parents. The success of this method suggests the possibility of producing yeast hybrids of ploidy higher than tetraploid. Auxotrophic triploids of αaa and ααa mating-locus genotypes could possibly be used as parental cells in pentaploid (αaa × αα or ααa × aa) and hexaploid (αaa × ααa) crosses. These cultures, in addition to their aforementioned applicability to radiobiological studies on yeast, would be of value in permitting an extension of previous studies on polyploid segragation in yeast.[10, 25]

This report describes the preparation of a series of cultures of Saccharomyces cerevisiae varying in ploidy from haploid to hexaploid. Analyses of the mode of segregation of genetic characters and studies of the response to x-ray-induced lethality in the different ploidies are presented.

MATERIAL AND METHODS

The cultures used in this study were developed from two respiration-sufficient haploid cultures of Saccharomyces cerevisiae Y02022 and Y02587 described previously,[18] and two additional haploid cultures S163A and S216D obtained as segregants of a cross between Y02022 and Y02587.* The production of the polyploid series from these cultures is described in a following section.

The genetic characters introduced into the crosses by these strains were: Mating type, (α/a); requirement/nonrequirement of tryptophan, (tr/Tr); of methionine, (me/Me); and of uracil, (ur/Ur). These markers correspond to ones described in previous publications[7, 17] and have allelic designations α/a, tr_1, me_1, and ur_1 respectively.

The production of crosses involved either the microisolation and cultivation of a single zygote from a mass-mating mixture of the two parents, or the recovery of a prototroph from this mating mixture.[23] The latter method was applicable only when the parent cultures possessed complementary nutritional requirements. Cultures were maintained on YEPD medium (1% Difco yeast extract, 1% Bacto-peptone, 2% dextrose, and 2% agar).

Sporulation was induced by the following procedure.[1] Cells were grown for 2 days on presporulation medium (2% Difco Nutrient Agar, 1% yeast extract, 5% dextrose, 2% agar) and then were transfered to sporulation medium (0.3% sodium acetate, 0.02% raffinose, 2% agar) and incubated at 25°C. Asci usually appeared within 2 to 4 days. Dissections were not performed until after 4 days, and were limited to asci containing four spores. Segregation data are presented only for asci yielding four viable spores.

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*The cultures used previously under the designations Y02022 and Y02587 have since been found to be cytochrome-deficient variants of the corresponding cultures used in this study. Preliminary results indicate no significant difference between the two types of cultures in the frequency of induced dominant lethals or response to x-ray-induced lethality.

Mating type was scored by examination for the presence or absence of zygotes in a mass-mating mixture of the unknown culture and a tester culture of known mating type.[12] A single-omission series of synthetic media, with complete composition corresponding to Bacto-Yeast Morphology Agar (Difco) (containing methionine and tryptophan) supplemented with uracil (20 mg/l) was used to score for nutritional requirements. A single metabolite (i.e., methionine, tryptophan, or uracil) was omitted in each of the media. Failure of the culture to grow on one of these media indicated a requirement for the metabolite omitted.

The cells used in irradiation experiments were obtained from 4- to 6-day growths on YED medium (0.5% yeast extract, 1% dextrose, 2% agar). Suspensions were made in sterile water. For most of the cultures, the population of cells contained less than 5% budding cells and very few clumps. Suspensions of clumpy strains were passed through cotton filters in order to obtain a population composed mostly (> 98%) of single cells. The starvation technique used previously[3] to reduce bud counts was not employed because it led to considerable loss of viability with many of the strains.

Irradiations were performed with unfiltered x-rays delivered by a Machlett OEG-60 beryllium-window x-ray tube operated at 50 kv peak and 25 ma. The dose rate at the target distance employed (15.6 cm) was 250 roentgens/sec. Appropriate dilutions of cells were spread on the surface of YED medium contained in a 10-cm Petri dish and irradiated directly. The exposed cells were incubated at 30° C for four days, after which time visible colonies were counted. Cell viability was deduced by comparison of these counts--corrected for the dilution factor--with colony counts on the control plates.

DEVELOPMENT OF THE POLYPLOID SERIES

The diploid, triploid, and tetraploid cultures stem from the two haploid cultures Y02022 and Y02587. These two cultures have genotypes α me_1 Tr Ur and $\underline{a}$ Me tr_1 ur_1 respectively. Nonsporulating diploids--presumably completely homozygous--were isolated from each of these haploid cultures by selection of colonies with microscopic morphology characteristic of diploids. These cultures are designated 22D and 87D respectively. They were shown to be phenotypically identical to the haploid cultures from which they arose, and studies described in a later section support the assumption that they are diploid. A third diploid X320 (partially heterozygous) was obtained by a cross of Y02022 and Y02587. Triploid cultures X321 and X322 were produced by the crosses Y02022 × 87D and Y02587 × 22D. The tetraploid culture was derived from the cross 22D × 87D. These crosses were all performed by mass mating followed by isolation of a single zygote. This method for producing higher-ploidy yeast parallels closely that described by Roman, Hawthorne, and Douglas.[24]

The production of pentaploid and hexaploid cultures required a slightly different approach. Triploid cells possessing biochemical requirements were used for one of the parents in the pentaploid cross and for both parents in the hexaploid cross. These auxotrophic triploids were produced by the following crosses: X354 (87D × S216D) and X361 (22D × S163A). The cultures S216D and S163A are haploid segregants of X320 and have genotypes #α Me tr-1 ur-1# and #$\underline{a}$ me-1 Tr Ur# respectively. The triploid X354 thus is expected to have the genotype $\underline{aa}\alpha$ MeMeMe $tr_1tr_1tr_1$ $ur_1ur_1ur_1$, and X361 the genotype #$\underline{a}\alpha\alpha$ $me_1me_1me_1$

TrTrTr UrUrUr. The growth requirements of these cultures are consistent with their assumed genotypes, i.e., X354 was found to require tryptophan and uracil for growth while X361 required methionine.

The pentaploid culture X355 resulted from the cross X354 × 22D, and the hexaploid culture X362 was produced by a cross of X354 and X361. The parent cultures for each of these crosses were inoculated into a culture tube containing liquid YEPD and were incubated together for 24 hours. At this time, the resultant growth was washed by centrifugation and resuspension and then plated onto a synthetic medium lacking methionine, tryptophan, and uracil. About 1000 colonies appeared on the plates, corresponding to an inoculation of about 10^7 cells of the mating mixture. No colonies appeared when a similar number of either of the parent cells, grown in a similar fashion, was plated on the same medium. One of these prototroph colonies was selected for each cross (X355 and X362) and cultured. Genetic analyses to be presented in a following section establish that these prototrophs arose from matings of parental cells and hence are most probably of the assigned ploidy. This is further supported by determinations of segregation ratios for the genetic markers in these polyploid cultures as well as by measurements of average cell volume.

RESULTS AND DISCUSSION

Average Cell Volume of the Cultures in the Polyploid Series

In a variety of biological systems that have been studied, cell size generally has been found to increase with ploidy. Such a relation has been shown to apply to a polyploid series of yeast,[20] and it was of interest to obtain such data for the cultures used in this study.

To determine the average cell volume, measurements were taken of the lengths of the major and minor axes of a number of cells (25 to 40) for each of the cultures under consideration. Forty-eight-hour plate-grown cells were suspended in sterile water and a loopful of the suspension was streaked on the surface of 2% agar and allowed to dry. Measurements were taken of a random sample of cells (25 to 40) by use of a Bausch and Lomb eyepiece micrometer. From the measurements, volumes were computed for each of the cells, assuming an ellipsoid of revolution about the long axis. The average linear dimensions and average volume for the cells of each of the cultures are presented in Table I.

The ratio between average volume and assumed ploidy is also presented, and is seen to have approximately a constant value irrespective of ploidy. This is consistent with the linear relation previously reported between volume and ploidy in yeast.[20]

Genetic Analyses of the Hybrids

A number of asci from each of the hybrid cultures X320, X321, X322, X354, X361, X323, X355, and X362 were dissected and the resultant spore cultures from some of these were tested for phenotypic expression of the characters introduced into the crosses.

TABLE I

Average Linear Dimensions and Volume of Cells of Yeast Cultures of Different Ploidy

Culture	Ploidy	Dimensions (μ) Long axis	Short axis	Volume μ^3	Volume/ploidy
Y02022	1	5.04	4.27	48.5	48.5
Y02587	1	4.72	4.20	45.2	45.2
S163A	1	4.72	4.35	54.8	54.8
S216D	1	4.57	3.91	38.0	38.0
22D	2	6.27	4.77	75.5	37.7
87D	2	6.27	5.37	98.5	49.2
X320	2	5.95	5.04	82.5	41.2
X321	3	7.15	5.69	125	41.6
X322	3	7.17	6.02	141	47.0
X354	3	8.17	6.22	162	54.0
X361	3	8.15	5.95	154	51.3
X323	4	7.97	6.20	165	41.2
X355	5	9.42	6.64	225	45.0
X362	6	10.1	6.80	253	42.2

In a group of eight asci from the diploid cross X320, 2:2 segregation was observed for each of the four characters tested (α/a, me_1, tr_1, and ur_1). This mode of segregation is consistent both with the diploid state of X320 and with the haploid state of the parents Y02022 and Y02587.

The triploid cultures X321, X322, X354, and X361, were all found to yield a low frequency of viable spores. The ratios of viable spores to total number isolated for each of these cultures were: 12/32, 11/20, 3/24, and 27/68, respectively. Further, the spore cultures classified as viable were quite variable with respect to cell size and growth rate. The majority grew slowly compared with diploid segregants. Low viability of triploid meiotic products is a rather general finding and is considered to arise from genic imbalance associated with their aneuploid state. This reduced viability is in marked contrast to findings for the diploid X320 (the 32 spores isolated all were viable) and for a number of other diploid hybrids recently studied, for which spore viability was found to be in the range 80% to 100%. The lack of any asci of which all four spores survived precluded adequate segregational analyses for these four triploid cultures. The surviving spores of X361 were tested and found in all cases to require only methionine, as expected on the basis of the assumed genotype of this culture.

The pentaploid culture X355 was not studied in detail. A group of five asci were dissected and 15 of the 20 resultant spores were viable. The fractions of these spores that exhibited the dominant phenotype for the nutritional characters me_1, tr_1, and ur_1 were 10/15, 13/15, and 14/15. On the basis of parental genotypes, the methionine locus in this hybrid should be represented by three dominant alleles (triplex), and the other two nutritional markers by two dominant alleles (duplex).

The tetraploid (X323) and hexaploid (X362) hybrids were subjected to more extensive segregational analyses. On the basis of parental genotypes, X323 is expected to be of duplex constitution and X362 of triplex constitution for each of the four loci under consideration. Assuming for the nutritional characters that the normal allele exerts complete dominance over the recessive allele, one expects asci from these hybrids to be of three basic constitutions:[10, 26] Type I, containing four dominant and no recessive spores (4:0), Type II, containing two dominant and two recessive spores (2:2), and Type III, containing dominant and one recessive spore (3:1). Mating reaction as scored is assumed to require homozygosity for α or $\underline{a}$. Hence, the ascus types for this locus would be as follows: Type I (4 nonmaters), Type II (2α:$2\underline{a}$), and Type III (1α:2 nonmaters: $1\underline{a}$).

The relative frequencies of Type I, II, and III asci vary with the distance of the locus under consideration from its centromere, in a manner characteristic for each ploidy. Roman, Phillips, and Sands have computed these frequencies for segregation of tetraploids of genotype AAaa (duplex), assuming both bivalent and tetravalent pairing.[26] The relative frequencies of ascus types found experimentally for three independent loci indicate both types of pairing, with a preference for bivalent pairing. Leupold, in addition, has made both a theoretical and an experimental analysis of tetraploid inheritance in Saccharomyces.[10, 11] The distributions of ascus types reported for three noncentromere-linked markers (adenine, histidine, and galactose) agree more closely with theoretical predictions based on bivalent pairing, although a limited amount of tetravalent pairing is indicated. In the following calculations on the predicted segregation ratios of hexaploids, only bivalent pairing was considered.

For a hexaploid of genotype AAAaaa (triplex), two bivalent pairing patterns can occur: namely AA-AA, AA-aa, aa-aa; and AA-aa, AA-aa, AA-aa. If pairing is nonpreferential, the corresponding probabilities for these patterns are 6/10 and 4/10. In the former, irrespective of exchange between A and the centromere in any or all of the bivalents; or of mode of distribution of centromeres to the poles (assuming one centromere from each bivalent goes to each pole), only Type I asci (4:0) are obtained. For the second mode of pairing and without exchange, random distribution of centromeres yields 1/4 Type II asci (2:2) and 3/4 Type I (4:0) asci. If an exchange occurs in one of the bivalents, a ratio of 1/2 Type I and 1/2 Type III asci is obtained, and similarly for an exchange in two of the bivalents. Exchange between A and the centromere in each of the three bivalents leads to 9/16 (4:0), 6/16 (3:1), and 1/16 (2:2) asci.

Thus, if $f_3(I)$, $f_3(II)$, and $f_3(III)$ are the frequencies of Types I, II, and III asci respectively, and x the probability of exchange between the locus and its centromere, then the relations are:

$$f_3(I) = 6/10 + 1/10[3 \cdot \tfrac{1}{2} \cdot x^2(1-x) + 9/16\, x^3] + 3/10\, [(1-x)^3 + 3 \cdot 2/3 \cdot x\,(1-x)^2 + \tfrac{1}{2} \cdot x^2\,(1-x) + 9/16\, x^3] = 1/10\,(9 - 3x + 3x^2 - 3/4\, x^3)$$

$$f_3(II) = 1/10\,[(1-x)^3 + 1/16\, x^3] + 3/10 \cdot 1/16\, x^3 = 1/10\,(1-3x + 3x^2 - 3/4\, x^3)$$

$$f_3(III) = 1/10\,[3 \cdot 1 \cdot x\,(1-x)^2 + 3 \cdot \tfrac{1}{2} \cdot x^2\,(1-x) + 1 \cdot 6/16 \cdot x^3] + 3/10\,[3 \cdot 1/3 \cdot x(1-x)^2 + 3 \cdot \tfrac{1}{2} \cdot x^2\,(1-x) + 1 \cdot 6/16 \cdot x^3] = 3/5\,(x - x^2 + x/4^3).$$

Values of f_3(I), f_3(II), and f_3(III) for different values of x were computed by use of these formulae and are presented in Table II.

TABLE II

Relative frequencies (%) of Segregation Types I, II, and III as a function of the frequency of second-division segregation (x) for hexaploid cells of duplex and triplex constitution.

x%	Triplex (AAAaaa)			Duplex (AAaaaa)		
	f_3 (I) (4:0)	f_3 (II) (2:2)	f_3 (III) (3:1)	f_2 (I) (4:0)	f_2 (II) (2:2)	f_2 (III) (3:1)
0	90.0	10.0	0	60.0	40.0	0
5	88.5	8.6	2.9	56.0	36.0	8.0
10	86.8	7.3	5.9	52.8	32.5	14.4
20	85.2	5.1	9.7	47.4	25.8	26.8
30	83.4	3.5	13.1	43.6	20.0	36.4
40	82.3	2.3	15.4	41.6	15.2	43.2
50	81.5	1.6	16.9	41.2	11.3	47.5
60	81.2	1.2	17.6	42.6	8.2	49.6
66.7	81.1	1.1	17.8	44.4	6.7	49.3

Experimental results obtained for one of the loci necessitated consideration of the theoretical segregation ratios expected for a hexaploid in duplex constitution. By use of an approach similar to that described above, the corresponding relations for f_2 (I), f_2(II), and f_2 (III) were derived; they are as follows:

$$f_2\ (I) = 1/5(3 - 4x - 17/4\ x^2),$$

$$f_2\ (II) = 2/5(1 - 2x - 9/8\ x^2),$$

$$f_2\ (III) = 4/5(2x - 13/8\ x^2).$$

Values of f_2(I), f_2(II), and f_2(III) for different values of x also were computed by use of these relations and are presented in Table II.

A number of asci from the two cultures X323 (4n) and X362 (6n) were dissected and the segregants analyzed to determine the experimental distribution of ascus types for each of the four loci. These loci, mating type (α/a), me_1, ur_1 and tr_1, have been found previously in diploid segregations to have second-division segregation frequencies x of approximately 40, 67, 67, and 5% respectively.[7, 10, 12] It is assumed that these values also apply to the polyploid segregations although this may not be correct.

The experimental distributions of ascus types obtained for these two cultures are presented in Table III. A higher frequency of Type I (4:0) asci is expected from the hexaploid, and this is confirmed by the results. The distributions of ascus types expected for each locus in both ploidies, as computed on the basis of the values presented by Roman et al. for tetraploids[26] and those in Table II for hexaploids, are included for comparison. The χ^2 and P values relating experimental and theoretical distributions are included in the corresponding spaces.

TABLE III

Distribution of Ascus Types for the Tetraploid and Hexaploid Cultures

Cross	Ploidy	No of Asci Dis-sected	Asci with 4 viable spores		Distributions of Ascus Types											
					a			me_1			ur_1			tr_1		
					I	II	III	I	II	III	I	II	III	I	II	III
X323	4	21	21	Exptl.	10	1	10	9	4	8	9	4	8	14	5	2
				Theor. (duplex)	10.1	3.1	7.8	9.3	2.3	9.3	9.3	2.3	9.3	13.3	6.3	1.4
				χ^2		2.03			0.95			0.95			0.56	
				P		0.35			0.62			0.62			0.76	
X362	6	81	53	Exptl.	15	2	6[a]	41	2,	10	37	2	14	28	24	1
				Theor. (triplex)	18.9	0.8	3.5	43	0.5	9.5	43	0.5	9.5	47	[illegible].5	1.5
				χ^2		4.39			4.6			7.45				
				P		0.11			0.10			0.04			0.001	
				(Duplex)		---			---			---		29.7	19.1	4.2
				2											2.8	
				P											0.25	

[a]Only 23 of the asci from X362 were tested for mating type.

For a tetraploid of duplex constitution, markers not showing centromere linkage are expected to produce approximately equal frequencies of (4:0) and (3:1) asci, i.e., Type I : Type II : Type III = 4/9 : 1/9 : 4/9. Centromere-linked markers are expected to produce few (3:1) asci, since these arise only when there is an exchange between the locus and its centromere. The segregation data for the tetraploid X323 agree very well with theoretical predictions and with previous studies for all four loci.[7, 10, 26] This agreement is evidence in support of the genotypes assumed for the homozygous diploids 22D and 87D, the cultures used to produce X323.

The distributions of ascus types obtained for X362, however, do not all correspond with theoretical predictions as do those of X323. The distributions observed for the two markers me_1 and ur_1 agree quite well with predictions for a hexaploid of triplex constitution, as does that of the centromere-linked marker, mating type (α/a). The marker tr_1, however, failed to give such agreement. Although a deficiency of Type III (3:1) asci is observed, consistent with expectations for centromere-linked markers, the percentage of Type II (2:2) asci was far in excess of theoretical predictions for a hexaploid triplex constitution. It is possible that X362 is of duplex constitution for Tr_1 (i.e., $Tr_1Tr_1\ tr_1tr_1tr_1tr_1$). The expected distributions of ascus types in a hexaploid for a locus in duplex constitution and with second-division segregation frequency of 5% (Table II) is: Type I, 56%; Type II, 36%; Type III, 8%. The 53 asci studied would distribute on the basis of these ratios as follows: Type I, 29.7; Type II, 19.1; Type III, 4.2. The observed values of 28, 24, and 1 agree quite well ($\chi^2 = 2.8$; $P = 0.25$) with this theoretical distribution. The triploid parent of this cross X361 was tested genetically and found to yield no tryptophan-requiring spores, and on this basis should have contributed three dominant alleles of Tr_1 to X362. However, since X362 was derived from a single mating, it would be possible to obtain the observed results if some of the cells of X361 were duplex (i.e., $Tr_1Tr_1tr_1$). It is also possible that one of the chromosomes containing a Tr_1 allele was lost from X362.

A more complete analysis of X362 would depend on the determination of the genotype of its meiotic products. The spore isolates of a hexaploid are expected to be triploid, and the problem of low spore viability associated with triploid cultures makes a thorough analysis difficult. For instance, the four cultures derived from a single ascus of X362 were sporulated and the resultant asci dissected. The spore viabilities of these cultures were 16/20, 3/20, 1/20 and 4/11. These low viabilities are consistent with the assumption that these cultures are triploid but at the same time precludes adequate genetic analysis.

The results of above theoretical analysis of polyploid segregations could be altered considerably, depending on the reliability of a number of assumptions which were made. These include the assumptions of (1) nonpreferential bivalent pairing, (2) equal and nonpreferential distribution of centromeres to the poles, and (3) equal crossing-over frequency in diploids and polyploids.

Radiation Studies

In the preceding sections are described the production and subsequent genetic analysis of a related series of *Saccharomyces cerevisiae*, varying in ploidy from haploid to hexaploid. The availability of this genetically defined polyploid series permits an extension of previous studies of the relation between radiation resistance and ploidy in this organism.

X-ray-survival curves were obtained for most of the cultures described (above) and these are presented in Figures 1A, 1B, 1C. In these graphs, the percentage of cells capable of forming visible colonies is plotted as a function of the x-ray dose.

The survival curves of the haploid cultures Y02022 and Y02587 (Fig. 1A) are approximately linear on the semilogarithmic plots indicating exponential inactivation. The tail apparent on these survival curves most probably is due to the presence of budding cells in the irradiated population.[3] The homozygous diploids 22D and 87D derived from these haploids exhibit sigmoid survival curves and are more radioresistant.[*] Similar findings for haploid and diploid yeast cultures have been reported previously on a number of occasions.[8, 16, 29, 30]

In Figure 1B are presented survival curves for the diploid (X320), tetraploid (X323), and hexaploid (X362) cultures. A number of features are of interest in relation to the survival curves of these hybrid cultures: (a) All three cultures exhibit sigmoid survival curves. (b) The diploid culture X320 is considerably more radioresistant than the homozygous diploids 22D and 87D. This difference is discussed in a later section.

(c) Finally, and most significant, there is a progressive decrease in radioresistance (LD_{10}) with increasing ploidy. The LD_{10} values for the diploid, tetraploid, and hexaploid cultures are 47.5, 30, and 16.8 krad,[**] and these values are related to one another approximately as the inverse of the respective genome number.

The survival curves of the four triploid cultures X321, X322, X354, and X361 and the pentaploid culture X355 are presented in Figure 1C. The triploid cultures have LD_{10} values of 36, 41, 37.5, and 29 krad, and the pentaploid culture a value of 17.5 krad. The LD_{10} values of the triploid cultures fall between those of the diploid X320 and tetraploid X323 hybrids. The survival curve of the pentaploid culture X355 is approximately the same as that of the hexaploid X362.

DISCUSSION

It is apparent from these studies that resistance to cellular inactivation in yeast does not increase continuously with ploidy, as might be expected on the basis of recessive lethal damage alone. From haploid to diploid, radioresistance was found to increase considerably along with a change from an exponential to a sigmoid inactivation curve. With further increases in ploidy to hexaploid, the radioresistance was found to decrease continuously. The LD_{10} values of the hybrid cultures (diploid to hexaploid) are plotted against ploidy in Figure 1D, and approximate agreement is apparent between these points and the curve (drawn proportional to the inverse of ploidy). Dominant lethality has been suggested as a possible form of lethal damage in irradiated yeast,[8] and again was implicated to account for unexpected sensitivity of tetraploid cells.[17] Later studies provided evidence for this form of damage in irradiated yeast cells.[18] In addition, it was

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[*] The dose necessary to reduce viability to 10% (LD_{10}) is used as a somewhat arbitrary unit of radiation resistance.

[**] The rad as a unit of radiation dose corresponds to an energy dissipation of 100 ergs/g. One roentgen of X-radiation delivers approximately 0.93 rad to a tissue sample.

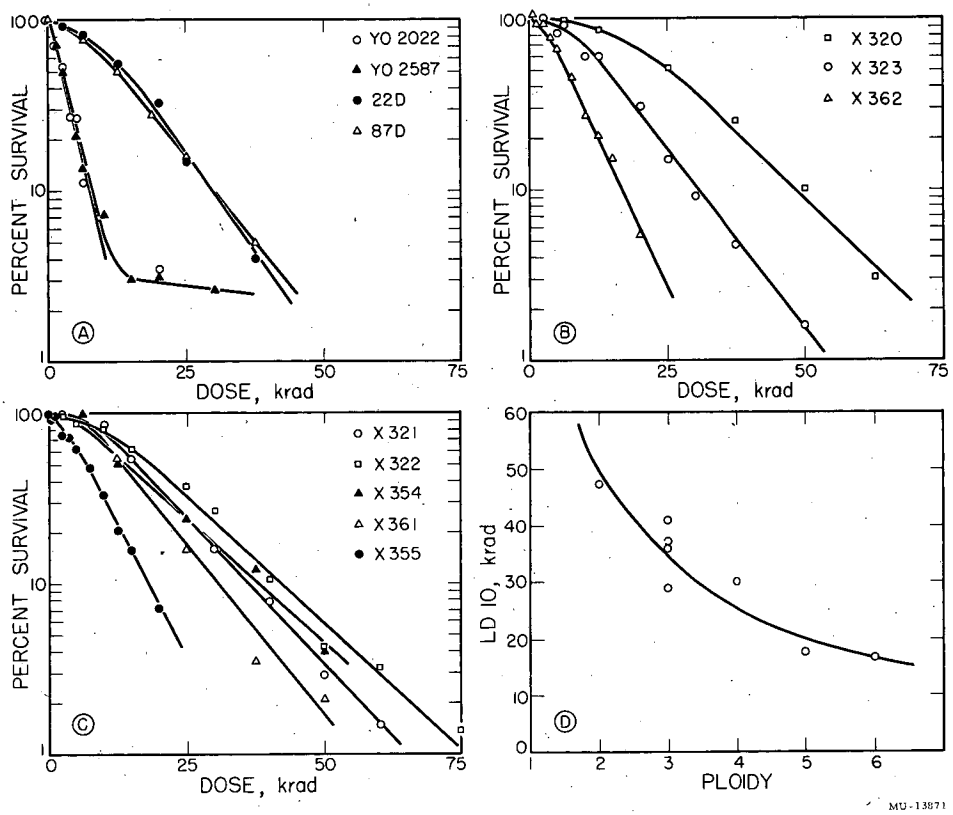

Fig. 1. A. X-ray survival curves of the haploid cultures Y02022 and Y02587 and of the homozygous diploid cultures 22D and 87D. B. X-ray survival curves of the diploid (X320), tetraploid (X323), and hexaploid (X362) hybrid cultures. C. X-ray survival curves of the triploid (X321, X322, X354, X361) and pentaploid (X355) hybrid cultures. D. Plot of the dose necessary to reduce viability to 10% (LD_{10}) vs the ploidy for the hybrid cultures.

shown that the doses required to produce the same percentage of dominant-lethal inactivation decreased inversely with the ploidy of the irradiated cell.[21] The above results, when considered in the light of these dominant-lethal studies, would be consistent with the assumption that the majority of cellular inactivation in the hybrid cultures arises through induced dominant lethals.

The high sensitivity of the haploid cultures has been explained on the basis of their obligate susceptibility to recessive lethal damage. This is consistent with the demonstration of the ability of a majority of haploid cells, previously exposed to a lethal dose of x-rays, to form viable zygotic combinations with unirradiated cells of opposite mating type.[18] In addition, recessive lethals have been demonstrated in irradiated diploid cells.[16, 19] The observed frequency of such damage, however, is considerably lower than would be expected on the assumption that the majority of haploid inactivation arises through induced recessive lethals. Magni has concluded that "only a small fraction of the haploid cells is inactivated by recessive lethals; i.e., no more than 25% of those killed."[16] This estimation could be in error if the assumption of equal sensitivity of haploid and diploid chromosomes to recessive lethal damage were incorrect or if recessive lethals were eliminated by diploid cells before assay by a mechanism such as mitotic crossing-over. Until further experimental results are available, it would be difficult to make a quantitative estimate of the role of recessive lethal damage in inactivation of yeast cells of different ploidy. Most probably it is of minor importance in cultures of ploidy higher than diploid and possibly also in diploid hybrids.

That the homozygous diploids 22D and 87D are more sensitive than the diploid hybrid X320 (as well as a number of other diploid hybrids studied recently) might indicate a greater susceptibility to recessive lethal inactivation in these cultures than in the hybrids. This could arise if chromosomal regions sensitive to recessive lethal damage were localized and in different locations in the two haploid cultures Y02022 and Y02587. Under these conditions, a hybrid between these two cultures would be less susceptible to recessive lethal inactivation than would be homozygous diploids derived from either of them. In this respect, it is interesting that diploid cultures derived from spore isolates of a related tetraploid vary widely in their susceptibility to x-ray-induced lethality.[1] Homozygosity for particular chromosome regions that would occur in tetraploid segregants might provide one explanation for this variability.

The progressive decrease in resistance to x-ray inactivation with increasing ploidy of the hybrid cultures is somewhat analogous to findings in bacteria[15] or in viruses[9] in which radiosensitive volume was found to increase with the volume of the irradiated cell or particle. Studies on other biological systems have varied as to the relation reported between degree of radiobiological effect and ploidy. Polyploid cereal seeds have been shown in most cases to be more resistant to inhibition of germination than diploid seeds.[27] Clark has studied the relative degree of inhibition of eclosion in haploid and diploid Habrobracon embryos, and except for very early stages, the diploids were found to be less sensitive.[5] At cleavage stage, the diploids were more sensitive than the haploids.

The nature of the dominant lethal damage in x-irradiated yeast cells is not definitely indicated by the studies described here. The size of the cells makes it difficult to associate the dominant lethals with cytological disturbances.

In Habrobracon dominant lethality has been associated with swollen Feulgen negative nuclei and chromosome bridges and fragments,[2] and in a number of other systems is often attributed to mechanical disturbances resulting from anaphase bridges.[5, 9] The frequency of chromosomal abnormalities (including bridges) has been shown to increase in proportion to ploidy in a number of biological systems,[4, 6, 27, 28] and the results obtained in this study could be accounted for by assuming that lethality in the higher ploidies was associated mostly with chromosomal disturbances.

Except for the haploid cultures, no indication of an integral relationship between ploidy and zero-dose extrapolate of the survival curves, as reported previously for yeast,[14] is indicated by the results of this study. The use of radiobiological survival curves as a tool in ploidy analysis[16] would seem, on the basis of the results presented in the earlier sections, to be reliable only in distinguishing between haploid and higher-ploidy cultures. This limitation, however, does not detract greatly from the method as a useful taxonomic tool.

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