The General Properties of a Strain of Yeast with Less DNA than Normal

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SUMMARY

A strain of yeast called 2nL is described which behaved genetically as a diploid, but had approximately 18% less DNA than a normal diploid. It produced apparently normal haploid ascospore cultures, which had approximately 20% less DNA than normal haploids. The growth of 2nL from low yeast concentrations was normal, but at high concentrations it was abnormal; viability was not affected. The timing of DNA synthesis in a synchronously dividing culture was in the usual position in the growth cycle for yeast. DNA was estimated by the diphenylamine reaction; there was no special effect on this reaction, peculiar to 2nL and its ascospore cultures. DNA values were confirmed by A_{280} measurements. The yeast 2nL was more sensitive to both gamma and u.v. radiations than were normal diploids and there was some indication that defective repair processes were involved.

INTRODUCTION

It was first pointed out by Boivin, Vendrely & Vendrely (1948) that while the mean amount of DNA in the nucleus varies quite widely from species to species it is apparently constant for the nuclei of the different somatic tissues of a given species. They also noted that the DNA in the haploid sperm nuclei is approximately half of that found in the somatic cell nuclei of the same species. These observations have been confirmed by Mirsky & Ris (1949, 1951) and subsequently by many workers.

Data from chemical analyses of nuclei in bulk do not reveal possible variations in DNA content from one nucleus to another. To overcome this difficulty attempts have been made to determine the DNA content of individual nuclei by quantitative microspectrophotometric methods (Pollister, Swift & Alfert, 1951). Such methods are useful for comparative measurements, and have in general confirmed the very narrow range of variation of the nuclear DNA even though the size of the nucleus and its protein content may vary over a wide range. Thus, both methods of DNA estimation indicate that variations in DNA content fall within limits which are narrow enough to make it almost certainly the least variable of all cell components.

The concept that all cells of an organism contain the same quantity of DNA or a geometric multiple of it has been confirmed in a large number of species. In fungi such geometric series with ploidy have been demonstrated in *Aspergillus* by Heagy & Roper (1952), in *Ustilago maydis* by Holliday (1961), and in *Saccharomyces cerevisiae* by Ogur,

Minchler, Lindegren & Lindegren (1952). The technique which the latter used in the extraction of DNA from yeast was that described by Ogur & Rosen (1949): it has been employed widely in slightly modified forms by many later workers.

During the course of investigations involving the measurement of DNA by the method of Burton (1956, 1968), we isolated a strain of yeast which was genetically diploid but showed a consistently low DNA value. The general properties of this strain are described here together with the effect upon DNA estimation of such parameters as yeast numbers and the presence or absence of pigmentation in adenine-dependent cultures.

METHODS

Yeast strains. The following polyploid series was used. 4n: 38/8d × 38/3d wild-type. 3n: 141/3b × (20 × 20); *a/a/α*, *leu1 trp5*/+/+/+, *met2*/+/+, +/*ade2/ade2*.* 3n: 141/3b × (21 × 21); *a/a/α*, *leu1 trp5*/+/+/+, *met2*/+/+, +/*ade2/ade2*. 2n: 2nJ; wild-type, *a/α*. 2n: 20/20; *ade2/ade2*, *a/α*. n: indicates combined results for five different haploids; all wild-type.

Cultures of haploids mutant at the *ade2* locus, and diploid cultures homozygous at the *ade2* locus formed red colonies on a complete medium. *Ade2* mutants were blocked at a point in the synthetic pathway concerned with adenine production and this led to the accumulation and polymerization of a red pigment, aminoimidazole carboxylic acid ribonucleotide (Levinthal, Fogel & Hurst, 1962).

Media and genetic techniques. The complete medium was that described by Cox & Bevan (1962). Cultures were grown for these experiments on solid medium for three days, at which time there was no cell clumping, no sporulation, and budding was less than 1%. The buds present were of all sizes.

The red coloration in the homozygous *ade2* diploids and *ade2* haploids could be abolished by growing these strains on complete medium supplemented with an excess of adenine to a final concentration of 80 µg/ml.

The sporulation medium used was that described by Hurst & Fogel (1964). The minimal medium used was Difco Yeast Nitrogen Base without amino acids (Difco code 0919-15-3).

After sporulation the ascus walls were treated with an extract of the fruiting bodies of the common cultivated mushroom (Bevan & Costello, 1964), and then the asci were dissected by micromanipulation on open complete-medium plates.

A replica-plating technique was used to test cultures for their various nutrient requirements; the series of minimal-medium plates being suitably supplemented with different combinations of growth factors.

DNA measurements. (1) Extraction. Resting phase yeasts were harvested and washed twice in distilled water. Samples were resuspended in distilled water to a measured volume, and the number of yeasts in each sample was counted in a haemocytometer slide. DNA was extracted by a modified procedure of Ogur & Rosen described by Williamson & Scopes (1960).

The samples were subjected to two preliminary washes in 10% trichloroacetic acid at 4° followed by two washes in 95% ethanol. The DNA was then extracted in two hot extractions

* The symbols used here and in the text are those agreed upon at the Osaka Yeast Genetics Conference 1969 (*Microbial Genetics Bull.* no. 31. *Yeast Genetics Supplement*). The symbols refer to the following phenotypes: *leu* – leucine requiring, *trp* – tryptophan requiring, *met* – methionine requiring, *ade* – adenine requiring, *a/α* – mating type gene. The genetic loci are identified by the italicized numbers.

with 5 % perchloric acid at 70° for 15 min each, the yeast debris was removed by centrifugation, and the supernatant fluids were combined for the estimation of their DNA content.

(2) Estimation. The DNA in 1 ml of the extracts was estimated colorimetrically by the diphenylamine method of Burton (1956). Calf thymus DNA (British Drug Houses Ltd, Godalming, Surrey) was used as a standard. DNA in the extracts was also determined by measuring their u.v. absorbance at 260 nm.

'Analar' grade chemicals were used.

Rationale of variations in procedure. The effect of variation in yeast cell numbers on the DNA estimations was investigated directly by extracting DNA from samples in a dilution series of cells, i.e. a concentrated suspension of yeast was made as sample 1, and gradually reduced portions of this were diluted with appropriate quantities of distilled water to give a series of samples containing different numbers of yeasts in the same volume; such series usually comprised eight samples.

It was conceivable that the extraction procedure would have been more efficient when the sample consisted of a small number of yeasts because of the inefficient circulation of a large number of cells in the extraction liquid. This possibility was tested by diluting the extract obtained from the most concentrated yeast sample, sample 1, by the same amounts as the previous cell-dilution series. This gave a dilution series of the extract parallel to the dilution series of yeasts.

It was possible that when extracts from small numbers of yeasts or dilute extracts were measured, the quantities of DNA were below the level that could be accurately measured by the procedures employed. To test this possibility calf thymus DNA was added to each extract to bring the total DNA in the extracts up to easily assayable amounts.

These various procedures were used for the polyploid series of the strains described above and for 2nL. They were used when all strains had been grown on complete medium and on complete medium supplemented with an excess of adenine. After growth on this latter medium, the strains which had previously been red were white; no difference could be seen in the other strains.

On any one day parallel analyses were run upon at least four different strains. All experiments were repeated at least three times.

Statistical analysis of results. The linear regression analyses of total DNA on number of yeasts analysed, and the curvilinear regression analyses of $\log_{10}$ transformations of DNA per 10^8 cells on number of yeasts analysed were performed by an ICT 1900 computer using the program XDS2/19 I.T.C. 1900 statistical analysis procedure.

The regression of total DNA (Y) on number of yeasts analysed (X) is approximately described by the equation: $Y = a + bX$. The significance of the regression coefficient, b , was given by a t test, and regression lines were plotted for the estimated Y values. The regression coefficient, b , is an estimate of the amount of DNA (μg) per yeast cell.

The regression of DNA per 10^8 cells on number of yeasts analysed was curvilinear even when logarithmic transformations were used for X and Y . This regression was found to be described by a second degree polynomial, $Y = a + bX + cX^2$, which introduces the methods of multiple regression. The significance of b was given by a t test, and regression lines were plotted for the estimated Y values.

To test the hypothesis that the slopes of any two regressions were the same, that is, the population regression lines were parallel, the methods of covariance analysis were employed. The variance between the two sample regression coefficients was measured and compared with the variance within samples using the F test.

Growth studies. (1) Preparation of a synchronously dividing culture. Synchronously dividing cultures were prepared by the rapid method of Williamson & Scopes (1962). The synchronized population was rapidly suspended at a concentration of approximately 1×10^7 cells/ml in a fresh complete medium at 28° , and vigorously aerated. Samples were taken initially and at 30 min intervals for counting in a haemocytometer slide, and for DNA estimations. Samples (30 ml) were taken for DNA measurements initially, but as the yeast numbers increased the sample size was reduced to 20 ml. Samples were treated in the way described by Williamson & Scopes (1960) to halt growth.

(2) Non-synchronous growth. Stationary phase cultures were harvested from complete medium, washed twice in distilled water and resuspended in fresh complete medium at both low and high yeast concentrations. The cultures were vigorously aerated at 28° . Samples were taken initially and at half-hour intervals in the concentrated culture. The yeast cells were counted in a haemocytometer slide; any bud of diameter greater than one-third that of the parental yeast was counted as one cell. Also suitable dilutions were performed and samples plated on to complete medium plates and scored for colony formation after five days' growth at 28° .

Ultraviolet treatment. The u.v. source was a Hanovia IIA mercury discharge tube generating 97% of its light at 253.7 nm. Stationary phase yeast suspensions in 5 ml saline at a concentration of approximately 1×10^7 cells/ml were exposed to u.v. light at an intensity of 22 erg/s/mm² for varying times in an agitated glass Petri dish. The suspensions were shaken vigorously. All manipulations were performed in dim red lighting to avoid photo-reactivation, and incubation was at 28° in the dark.

The lamp was calibrated initially using a chemical actinometer (Hatchard & Parker, 1956), and subsequently by means of a calibrated photoelectric cell.

After irradiation suitable dilutions were prepared and samples plated on complete medium. The plates were scored for visible colony formation after five days' growth at 28° .

Gamma treatment. Cultures were prepared as they were for the estimation of survival after u.v. treatment.

The samples were exposed to γ -irradiation in air in a 4000 Ci Hotspot Mark IV (U.K. Atomic Energy Authority) for periods of up to five min at a dose rate of 17 krad/min.

RESULTS

Origin and genetic analysis. The strain 2nL arose as a single ascospore isolate from the triploid 141/3b $\times$ (20 $\times$ 20): $a/a/\alpha$, $leu1\ trp5/+ +/+ +$, $met2/+ +$, $+/ade2/ade2$.

It was found that 2nL did not mate with either a or α mating type haploids; but it did sporulate. Asci were dissected and the percentage spore germination was about 94%. Twenty-one spore tetrads were analysed for the segregation of the above markers; they revealed that the strain 2nL appeared to be a normal diploid, heterozygous for the genes $leu1$, $trp5$, $met2$ and the mating type locus, and homozygous for $ade2$. The markers segregated regularly, showing the expected linkage relationships (Mortimer & Hawthorne, 1966) except that no recombinants were found between $met2$ and $leu1$. This may indicate that a translocation has occurred between fragment 3 and chromosome VII.

Thirteen tetrads from a spontaneous white form of 2nL were analysed: they demonstrated the heterozygosity of all the markers in this culture. As before there was no recombination between $met2$ and $leu1$.

The spore cultures 8a, 8c and 1a from 2nL were mated with a strain which had nine

Fig. 1. Linear regressions of total DNA content on number of yeasts analysed for all strains. The lines of regression are computer calculated lines of best fit from at least three independent experiments. DNA estimations were made by the diphenylamine reaction.

chromosomes marked. The percentage spore germination from these hybrid diploids was only about 70 %. Analysis of 10 tetrads from each of these hybrid diploids revealed normal gene segregation ratios and that recombination between gene pairs was in agreement with established linkage relationships.

DNA measurements. Preliminary determinations of DNA concentrations by the diphenylamine method, revealed that 2nL contained less DNA than normal diploids and that the spore cultures were similar to each other in DNA content and were below the normal haploid level. In order to examine the possibility that there was some inhibition of the method of DNA measurement specific to these strains, 2nL and the members of two of its spore tetrads, 1 and 8, were analysed according to the methods described above and compared with the polyploid series.

The results of the analyses of regression of total DNA on the number of yeast cells analysed are summarized in Table 1. All regressions are positive and highly significant at the 1 % level except for some of the extracts from red yeasts when measured in the presence of calf thymus DNA, which are negative. Extract dilutions gave similar results to the yeast

Table 1. *Summary of linear regression analyses of total DNA (μg) on number of yeasts analysed*

Strain	DNA measurements made upon the extracts alone						DNA measurements made upon the extracts + standard DNA					
	Cell dilutions			Extract dilutions			Cell dilutions			Extract dilutions		
	<i>b</i>	<i>t</i> *	<i>r</i>	<i>b</i>	<i>t</i> *	<i>r</i>	<i>b</i>	<i>t</i> *	<i>r</i>	<i>b</i>	<i>t</i> *	<i>r</i>
4n	8.186	29.41	1.00	—	—	—	—	—	—	—	—	—
	8.655	109.00	1.00	—	—	—	—	—	—	—	—	—
3n	6.818	34.81	1.00	—	—	—	—	—	—	—	—	—
	5.435	85.77	1.00	—	—	—	—	—	—	—	—	—
2nJ	3.614	24.84	1.00	—	—	—	3.417	6.74	0.94	—	—	—
	3.808	22.85	0.99	—	—	—	3.436	17.90	0.99	—	—	—
	3.704	61.15	1.00	—	—	—	3.249	10.80	0.98	—	—	—
	4.021	50.06	1.00	4.021	94.71	1.00	3.228	18.84	0.99	3.895	24.37	0.99
	3.450	31.79	1.00	3.505	103.10	1.00	3.224	28.37	1.00	3.368	14.91	0.99
20/20 white	3.660	34.73	1.00	—	—	—	3.355	9.38	0.97	—	—	—
	4.628	24.79	1.00	—	—	—	3.289	12.01	0.98	—	—	—
	3.839	44.15	1.00	3.541	43.32	1.00	3.692	20.01	0.99	3.542	12.12	0.98
20/20 red	2.528	8.02	0.96	—	—	—	-17.62	5.23	-0.93	—	—	—
	2.836	61.60	1.00	2.880	17.43	0.99	-3.67	2.79	-0.75	-8.02	7.18	-0.95
2nL white	3.395	37.07	1.00	—	—	—	2.503	5.96	0.92	—	—	—
	2.936	29.67	1.00	2.983	231.5	1.00	2.660	13.52	0.98	3.002	13.05	0.98
2nL red	1.745	13.12	0.99	—	—	—	—	—	—	—	—	—
	0.920	10.44	0.98	—	—	—	-8.58	15.60	-0.99	—	—	—
	1.807	16.26	0.99	—	—	—	-5.21	6.52	-0.94	-13.36	9.60	-0.98
	1.088	7.06	0.94	—	—	—	—	—	—	—	—	—
	1.658	12.50	0.98	1.910	11.25	0.98	—	—	—	—	—	—
n	2.717	15.57	0.99	—	—	—	—	—	—	—	—	—
	2.552	30.57	1.00	—	—	—	1.636	2.18	0.66	—	—	—
	2.177	64.14	1.00	—	—	—	1.583	6.76	0.94	—	—	—
	2.544	19.37	0.99	2.645	39.28	1.00	2.117	7.59	0.96	2.251	11.99	0.98
	2.359	25.74	1.00	2.040	33.18	1.00	2.329	10.28	0.97	2.637	4.70	0.89
2nL/8a white	1.784	29.60	1.00	2.018	41.35	1.00	1.478	5.62	0.92	—	—	—
8b white	1.658	23.05	0.99	1.528	30.64	1.00	1.477	13.60	0.98	—	—	—
8c white	1.926	33.60	1.00	1.945	41.34	1.00	1.885	5.77	0.92	—	—	—
8d white	2.139	35.67	1.00	1.746	27.36	1.00	1.562	11.95	0.98	—	—	—
2nL/8a red	1.038	31.41	1.00	0.827	12.03	0.98	-2.62	3.96	-0.85	-4.39	8.92	-0.97
8b red	1.120	21.14	0.99	0.936	9.97	0.97	-2.91	4.00	-0.85	-5.26	6.31	-0.93
8c red	1.143	15.02	0.99	1.090	8.49	0.96	-3.09	3.77	-0.84	-5.67	7.40	-0.95
8d red	0.954	16.07	0.99	1.123	15.43	0.99	-3.48	7.47	-0.95	-4.65	12.69	-0.98
2nL/1a white	1.414	12.26	0.98	—	—	—	1.557	12.21	0.98	—	—	—
1b white	1.497	14.49	0.99	—	—	—	1.826	17.70	0.99	—	—	—
1c white	1.833	12.71	0.98	—	—	—	1.382	6.68	0.94	—	—	—
1d white	1.446	45.56	1.00	—	—	—	1.476	25.56	1.00	—	—	—
2nL/1a red	1.223	7.76	0.95	—	—	—	-4.23	2.91	-0.77	—	—	—
1b red	1.409	11.48	0.98	—	—	—	-4.19	2.58	-0.73	—	—	—
1c red	1.284	16.16	0.99	—	—	—	-2.30	2.71	-0.74	—	—	—
1d red	1.288	10.76	0.98	—	—	—	-2.30	2.61	-0.73	—	—	—

* Tabulated *t*, d.f. = 6, $P \leq 0.05$, $t = 2.447$; $P \leq 0.01$, $t = 3.707$.*b* = regression coefficient = $\mu\text{g}/\text{DNA}/10^8$ cells; *r* = correlation coefficient.

dilution series within a strain. The addition of calf thymus DNA to each sample did not alter the final result except in the instances mentioned above.

These results are represented diagrammatically in Fig. 1, where it can be seen that 2nL and its spore tetrads 1 and 8 are distinct from the diploid and haploid regression lines. The regression coefficients ($\mu\text{g DNA}/10^8$ cells) of the adenine-dependent strains are greater when extracts are measured from white, pigment-repressed cells than from red cells; this effect can be seen for the diploid 20/20, and 2nL and its tetrads 1 and 8 (Fig. 1). The regression line for white cells of the strain 20/20 coincides with that for the other diploid strain 2nJ.

The slope of the regression lines, as indicated by the coefficient b (Table 1), is different for each strain and dependent upon the ploidy of that strain. DNA values for the same number of cells per strain have been noted in Fig. 1: they approximate to a doubling series with ploidy.

The statistical significance of the differences in these regressions between strains can be demonstrated by covariance analysis. The results presented in Table 2 have been confined to the haploid and the two diploids for simplicity: the triploids and the tetraploid follow the same general pattern. First it is shown that the regressions of total DNA on yeast numbers for extracts from the haploids and the diploid, 2nJ, are significantly different; whereas extracts from white cells of 20/20 are not significantly different from 2nJ. The 1% level of significance was taken throughout. This reinforces the interpretations drawn from Fig. 1 that these regression lines are characteristic of the ploidy of a strain, as demonstrated by the juxtaposition of 2nJ and 20/20 white.

Table 2. *Analysis of covariance between and within yeast strains and treatments*

Subjects for covariance analysis	Variance ratio F^*	Degrees of freedom	Probability
n and 2nJ	$\frac{222.69}{3.95} = 56.36$	1, 12	$\ll 0.01^\dagger$
2nJ and 20/20 white	$\frac{23.54}{3.50} = 6.73$	1, 12	> 0.01
n dilutions of cells and n dilutions of extracts	$\frac{7.76}{1.56} = 4.98$	1, 12	> 0.01
2nJ dilutions of cells and 2nJ dilutions of extracts	$\frac{0.32}{2.66} = 0.12$	1, 12	> 0.01
20/20 dilutions of cells and 20/20 dilutions of extracts	$\frac{5.01}{1.17} = 4.29$	1, 12	> 0.01
n extracts alone and n extracts + standard DNA	$\frac{0.07}{3.47} = 0.02$	1, 12	> 0.01
2nJ extracts alone and 2nJ extracts + standard DNA	$\frac{4.37}{3.29} = 1.33$	1, 12	> 0.01
20/20 (white) extracts alone and 20/20 (white) extracts + standard DNA	$\frac{1.07}{1.95} = 0.55$	1, 12	> 0.01
20/20 white and 20/20 red	$\frac{71.88}{1.03} = 69.78$	1, 12	$\ll 0.01^\dagger$

* Tabulated F , d.f. 1, 12; $P = 0.01$ is 9.33.

† Highly significantly greater than tabulated F .

It is also shown in Table 2 that in every instance there was no significant difference in the regression of total DNA on number of yeasts analysed as measured by a dilution series of extracts from that measured by a dilution series of cells, indicating that DNA was extracted with the same efficiency from small and large numbers of cells. In the Figures the regression lines are drawn from combined results of at least three experiments treated in both ways.

When DNA measurements were made on both yeast dilutions and extract dilutions from white cells of 2nJ, 20/20 and n with equal amounts of calf thymus DNA added to each sample, there was no significant difference between these regression coefficients and those obtained in the absence of additional DNA for the same strain (Table 2). The results are therefore in agreement with the hypothesis that the two regression lines, as plotted in Fig. 2*a*, are parallel. These experiments were duplicated for the strain 20/20 in its red and white forms. Extracts from white yeasts measured with and without additional DNA gave parallel regression lines in agreement with the above findings. However, when red yeasts were analysed in this way, the addition of calf thymus DNA to the sample extracts did not give results paralleling those from red yeasts measured alone, i.e. values slightly lower than those obtained for white cells of this strain. Instead, negative results were obtained, i.e. total DNA estimates were obtained that were less than the amount of added DNA, an apparent loss of DNA having occurred. The results were positive, or above the level of the additional DNA, only when small numbers of cells were analysed. With increasing numbers of cells per sample, or increasing sample concentration, there was a fall and tailing-off in DNA values (Fig. 2*a*). This is reflected in the negative regression coefficients (*b*) for these samples in Table 1. Thus, the presence of the red pigment or some other accumulation product in extracts from adenine-dependent strains depressed the estimates of DNA content, and there was also a severe interaction between such extracts and calf thymus DNA.

Finally it is shown in Table 2 that the regressions of total DNA on yeast numbers for extracts from the red and white forms of 20/20 are significantly different.

2nL and the members of its spore tetrads showed the same general response to these variations in procedure as did the members of the polyploid series. The differences in regression lines between and within strains have been analysed for their significance in Table 3. It is demonstrated that the regressions of total DNA on yeast numbers were significantly different from each other for extracts from white cells of 2nJ, 2nL and n. (It has been shown in Table 2 that 20/20 white is not significantly different from 2nJ.)

The regression lines obtained for the members of tetrad 8 from 2nL are compared in Table 3 and show that with the exception of the regression of the cell dilution series of strain 8d, there is no significant difference between the estimation of DNA content among the haploid segregants from 2nL. There was, however, a significant difference between 8a, taken as a representative of the tetrad, and the haploid n (Table 3).

The comparisons made in Table 3 are drawn from the data for adenine-repressed white cells of tetrad 8; measurements made on red cells show a further depression of the regression line away from the haploid level (Fig. 1).

The estimates obtained after the addition of calf thymus DNA are included for comparison with the other estimates (Fig. 2*b*). They show the red-yeast effect, as described above.

Although the above statistical analyses strongly indicate a direct linear relationship between total DNA and number of yeast cells analysed over a wide range of cell concentrations, examination of the relationship between number of yeast cells analysed and DNA per cell showed that linearity did not hold when small numbers of yeasts were analysed. Curvilinear regression analyses of logarithmic transformations of DNA per 10^8 yeasts on

Fig. 2 (a). Linear regressions of total DNA (μg) on number of yeasts analysed for extracts from the haploid, $2n_J$, and 20/20 red and white yeasts measured alone or with the addition of calf thymus DNA. ■, Yeast extracts measured + calf thymus DNA; □, dilutions of the most concentrated yeast extract measured + calf thymus DNA; ●, yeast extracts measured alone; ○, dilutions of the most concentrated yeast extract measured alone.

Fig. 2 (b). Linear regressions of total DNA content on number of yeasts analysed for red compared with white cultures of the diploid 20/20, $2n_L$ and the four members of tetrad 8 from $2n_L$, measured with and without the addition of calf thymus DNA. ●, Yeast extracts measured alone; ○, dilutions of the most concentrated yeast extract measured alone; ×, yeast extracts measured + calf thymus DNA; +, dilutions of the most concentrated yeast extract measured + calf thymus DNA.

number of yeasts analysed was carried out as described under Methods. The results are summarized in Table 4. The regression coefficients are all negative and the regressions are mainly significant at the 1% level, some only at the 5% level – and not as expected, i.e. zero or parallel to the abscissa. These results are shown diagrammatically in Fig. 3 and demonstrate the effect shown in Table 2 that the regression coefficients, or the slopes, are not greatly different from strain to strain, but they differ in elevation and are therefore

Fig. 3. For legend see opposite page.

separate. It is also shown that the slope is influenced by the presence of the red pigment in strains 20/20, 2nL and its spore tetrads and by the addition of calf thymus DNA to all samples – both cause a steepening of the slopes. The regression lines were all steepest when small numbers of yeasts were analysed; there was a tailing-off effect when more than 3×10^8 yeasts were present in the sample and the lines became almost parallel to the X axis. However, this cell number effect could not be attributed to the inefficient extraction of DNA from a large number of yeasts because of the agreement of results from the dilution series of extracts and cells.

These effects were specific for the diphenylamine reagent. Measurements of the absorbance of extracts at 260 nm provide constant estimates of DNA content over a wide range of cell concentrations (Fig. 4).

Table 3. *Analysis of covariance between and within yeast strains and treatments*

Subjects for covariance analysis	Variance ratio F^*	Degrees of freedom	Probability
2nL white and 2nJ	$\frac{68.85}{5.75} = 11.98$	1, 12	$<0.01^\dagger$
2nL white and n	$\frac{72.27}{4.54} = 15.93$	1, 12	$<0.01^\dagger$
2nL red and 20/20 red	$\frac{70.83}{1.02} = 69.72$	1, 12	$\ll 0.01^\dagger$
2nL white and 2nL red	$\frac{145.26}{4.07} = 35.69$	1, 12	$\ll 0.01^\dagger$
2nL dilutions of cells and 2nL dilutions of extracts	$\frac{0.33}{3.18} = 0.11$	1, 12	>0.01
2nL extracts alone and 2nL extracts+standard DNA	$\frac{9.90}{6.29} = 1.57$	1, 12	>0.01
8a and 8b	$\frac{7.52}{6.34} = 1.19$	1, 12	>0.01
8a and 8c	$\frac{8.17}{2.92} = 2.80$	1, 12	>0.01
8a and 8d	$\frac{52.15}{3.38} = 15.43$	1, 12	$<0.01^\dagger$
8a dilutions of cells and 8a dilutions of extracts	$\frac{8.51}{1.30} = 6.56$	1, 12	>0.01
8d dilutions of cells and 8d dilutions of extracts	$\frac{42.93}{2.89} = 14.86$	1, 12	$<0.01^\dagger$
8a and 8d dilutions of extracts	$\frac{0.28}{1.69} = 0.17$	1, 12	>0.01
8a and n	$\frac{71.29}{2.53} = 28.15$	1, 12	$\ll 0.01^\dagger$

* Tabulated F , d.f. 1, 12; $P = 0.01$ is 9.33.

† Significantly greater than tabulated F .

Fig. 3. Curvilinear regressions of logarithmic transformations of DNA content/ 10^8 yeast cells on number of cells analysed for all strains red and white and in the presence of calf thymus DNA. The lines of regression are computer calculated lines of best fit from at least three independent experiments.

Table 4. *Summary of curvilinear regression analyses of DNA (μg)/ 10^8 yeasts on number of cells analysed*

Strain	DNA measurements made upon the extracts alone						DNA measurements made upon the extracts + standard DNA					
	Cell dilutions			Extract dilutions			Cell dilutions			Extract dilutions		
	<i>b</i>	<i>t</i> *	<i>r</i>	<i>b</i>	<i>t</i> *	<i>r</i>	<i>b</i>	<i>t</i> *	<i>r</i>	<i>b</i>	<i>t</i> *	<i>r</i>
4n	-0.23	12.20	-0.98	—	—	—	—	—	—	—	—	—
	-0.28	10.48	-0.98	—	—	—	—	—	—	—	—	—
3n	-0.46	33.28	-1.00	—	—	—	—	—	—	—	—	—
2nJ	-0.22	4.37	-0.89	—	—	—	-0.46	4.32	-0.89	—	—	—
	-0.27	2.76	-0.78	—	—	—	-0.76	39.57	-1.00	—	—	—
	-0.16	2.19	-0.70	—	—	—	-0.44	4.55	-0.90	—	—	—
	-0.16	5.76	-0.93	-0.31	13.64	-0.99	-0.55	10.99	-0.98	-0.27	3.50	-0.84
	-0.24	3.61	-0.85	-0.41	33.87	-1.00	-0.74	36.16	-1.00	-0.64	16.82	-0.99
20/20 white	-0.22	4.18	-0.88	-0.26	7.42	-0.96	-0.75	26.80	-1.00	-0.73	21.26	-0.99
20/20 red	-0.43	4.65	-0.92	—	—	—	} Negative results					
	-0.56	26.18	-1.00	-0.56	20.73	-0.99						
2nL white	-0.15	2.03	-0.67	—	—	—	-0.61	2.24	-0.70	—	—	—
	-0.15	12.26	-0.71	-0.43	18.92	-0.99	-0.88	17.01	-0.99	-0.70	21.45	-0.99
2nL red	-0.34	4.09	-0.92	—	—	—	} Negative results					
	-0.33	6.10	-0.96	—	—	—						
	-0.48	4.81	-0.91	—	—	—						
	-0.40	13.82	-0.99	—	—	—						
	-0.62	11.85	-0.98	-0.44	6.10	-0.94						
n	-0.46	6.42	-0.97	—	—	—	—	—	—	—	—	—
	-0.53	5.73	-0.93	—	—	—	-0.98	36.34	-1.00	—	—	—
	-0.32	8.70	-0.97	—	—	—	-0.79	13.94	-0.99	—	—	—
	-0.62	7.11	-0.96	-0.44	15.09	-0.99	-0.50	7.17	-0.96	-0.23	2.33	-0.72
	-0.41	3.19	-0.82	-0.52	13.14	-0.99	-0.79	20.51	-0.99	-0.77	11.28	-0.98
2nL/8a white	-0.40	12.17	-0.99	-0.30	7.62	-0.96	-0.97	5.29	-0.62	—	—	—
8b white	-0.24	1.37	-0.52	-0.44	2.26	-0.75	-1.04	2.94	-0.83	—	—	—
8c white	-0.41	3.92	-0.87	-0.53	9.24	-0.97	-0.62	6.35	-0.94	—	—	—
8d white	-0.27	1.52	-0.56	-0.08	0.47	-0.21	-0.94	10.20	-0.98	—	—	—
2nL/8a red	-0.62	9.27	-0.97	-0.79	9.32	-0.97	} Negative results					
8b red	-0.58	22.47	-1.00	-0.52	3.10	-0.81						
8c red	-0.51	0.74	-0.39	-0.53	3.33	-0.86						
8d red	-0.39	1.78	-0.62	-0.24	2.70	-0.77						
2nL/1a white	-0.02	0.32	-0.14	—	—	—	-0.92	5.02	-0.91	—	—	—
1b white	-0.05	0.97	-0.40	—	—	—	-0.42	2.92	-0.79	—	—	—
1c white	-0.28	1.52	-0.56	—	—	—	-0.51	5.04	-0.39	—	—	—
1d white	-0.33	6.37	-0.94	—	—	—	-1.13	2.14	-0.73	—	—	—
2nL/1a red	-0.41	3.92	-0.87	—	—	—	} Negative results					
1b red	-0.42	7.35	-0.96	—	—	—						
1c red	-0.41	2.80	-0.78	—	—	—						
1d red	-0.25	3.52	-0.84	—	—	—						

* Tabulated *t*, d.f. = 6, $P \leq 0.05$, $t = 2.447$; $P \leq 0.01$, $t = 3.707$.
b = regression coefficient; *r* = correlation coefficient.

Fig. 4. Effect of sample size on DNA estimations by u.v. absorbance measurements at 260 nm. Results for white cultures of a wild-type diploid, 2nL and the four members of tetrad 8 from 2nL are compared. They represent the combined results of two experiments each producing eight points.

The quantities of DNA for the same number of cells per strain are compared in Fig. 1, and the mean regression values are summarized in Table 5. They indicate that 2nL had approximately 18 % less DNA than the two stock diploids, and its ascospore cultures had approximately 20 % less DNA than the five stock haploids.

DNA was measured in some hybrid diploids (2nL spores $\times$ n) and their tetrads. These analyses were not extensive, but are summarized in Table 6. The hybrid diploids had normal diploid values for DNA content but there was great variation in DNA content between members of the tetrads derived from such hybrids. One ascospore culture, 4d, from the hybrid diploid (2nL/8a $\times$ n) had a diploid quantity of DNA and behaved as a normal diploid.

Growth studies. (1) Asynchronous. The rates of increase in yeast numbers when stationary phase cells of a diploid, a haploid and 2nL were inoculated into fresh medium at a low cell concentration are shown in Fig. 5. They were similar to each other, but 2nL appeared to tail off more sharply once high yeast concentrations were reached than did either the diploid

Table 5. Summary of mean regressions (μ g DNA/ 10^8 cells)

Strain	Without added DNA		With added DNA	
	No. of analyses	Mean regression (μ g DNA/ 10^8 cells)	No. of analyses	Mean regression (μ g DNA/ 10^8 cells)
4n	2	8.421	—	—
3n	2	6.126	—	—
2nJ	7	3.732	7	3.402
20/20	4	3.917	4	3.470
2nL	3	3.105	3	2.722
n	7 (5 strains)	2.435	6	2.092
2nL/8a-8d	2	1.843	1	1.601
2nL/1a-1d	1	1.548	1	1.560
		1.696		1.581

Fig. 5. Increase in yeast numbers from low yeast concentrations in cultures of 2nL compared with a stock diploid and stock haploid cultures.

or haploid cultures. Since this may have been caused by an exhaustion of the growth medium, stationary phase cells of the same three strains were inoculated into fresh medium at high concentrations. The results are shown in Fig. 6. In a period of time when the stock diploid and haploid cultures increased their population size by a factor slightly greater than four,

Table 6. *Summary of DNA contents of hybrid diploids (2nL spores $\times$ n) and their segregants*

Strain	DNA $\mu\text{g}/10^8$ cells $\pm$ standard error	Strain	DNA $\mu\text{g}/10^8$ cells $\pm$ standard error	Strain	DNA $\mu\text{g}/10^8$ cells $\pm$ standard error
2nL/8a $\times$ n	4.02 $\pm$ 0.89	2nL/8c $\times$ n	4.06 $\pm$ 0.61	2nL/1a $\times$ n	
4a	1.92 $\pm$ 0.86	11a	2.40 $\pm$ 0.32	1a	3.77
4b	1.62 $\pm$ 0.36	11b	1.42 $\pm$ 0.41	1b	4.02
4c	2.84 $\pm$ 0.20	11c	2.94 $\pm$ 0.46	1c	1.38
4d	4.32 $\pm$ 0.45	11d	1.42 $\pm$ 0.34	1d	3.49
2a	2.16	2a	1.48	2a	1.67
2b	2.13	2b	2.48	2b	3.12
2c	1.78	2c	2.93	2c	3.81
2d	2.43	2d	4.02	2d	2.14
5a	3.07	12a	4.47		
5b	2.18	12b	2.44		
5c	2.03	12c	1.96		
5d	2.48	12d	1.58

Fig. 6. Increase in yeast numbers in cultures of 2nL inoculated into growth medium at a high concentration, compared with stock diploid and haploid cultures similarly treated.

2nL only doubled its population size. It was found that both 2nL and the white form induced by the addition of an excess of adenine to the medium behaved in the same way.

When 2nL was inoculated at high cell concentrations, its growth was linear, not exponential, i.e. in this culture the same number of cells divided in each cycle, not the same proportion as in exponential growth.

At the end of the growth period the percentage of cells, without visible buds, in the populations of 2nL and 2n was 2.4% for 2n and 20% for 2nL.

Besides counting, in a haemocytometer slide, the numbers of cells in cultures of strains n, 2n and 2nL, samples were plated after appropriate dilution on to complete medium plates, and the number of viable colony-forming units was estimated. The results are compared in Table 7. There was agreement between the two methods for all three strains, and there was no obvious inviability in the strain 2nL.

(2) Synchronized growth. The results shown in Fig. 7 of the synchronized cell division of a population of yeasts of the strain 2nL, and the total DNA estimations on samples of these dividing yeasts, are based upon only one experiment. Nevertheless, they are included because of the demonstration that the order in which DNA doubling and cell doubling

Fig. 7. Growth study of a synchronously dividing culture of 2nL and DNA content/20 ml of culture. Samples taken initially and at 30 min intervals for yeast counting and DNA estimations.

occurred in this culture was normal (Williamson & Scopes, 1960). They also illuminate the result from the non-synchronized high yeast concentration growth studies, viz. that cell doubling was not complete. However, at such high yeast concentrations this was probably due to medium exhaustion.

The duration of the complete cell cycle was in good agreement with normal behaviour in yeast (Williamson & Scopes, 1960).

Cellular inactivation by u.v. and γ -radiation treatments. The survival curve of each strain after treatment with u.v. and γ -radiations was determined by at least three replicate experiments and the averaged results for each point have been plotted. The survival curves for 2nL are compared with standard survival curves obtained for a series of haploid and diploid strains in Figs. 8 to 10. The survival curve for 2nL after u.v. treatment is compared with the standard haploid survival curve in Fig. 8, and with the standard diploid survival curve in

Table 7. Comparison of plate counts of viable colonies with yeast counts

Strain	Estimated titre of cells by	
	Cell counts in a haemocytometer slide	Plate counts of viable colonies
2n	7.30×10^8	6.05×10^8
	2.03×10^8	2.16×10^8
2nL	4.15×10^8	5.48×10^8
	1.68×10^8	1.44×10^8
n	3.03×10^8	2.43×10^8
	1.70×10^8	1.49×10^8

Fig. 8. The survival curve of 2nL after u.v. irradiation compared with a standard haploid survival curve. The dose rate was 22 ergs/sec/mm² and the vertical bars represent 95 % confidence limits.

Fig. 9. The survival curve of 2nL after u.v. irradiation compared with a standard diploid survival curve. The dose rate was 22 ergs/sec/mm² and the vertical bars represent 95 % confidence limits.

Fig. 9. It has a complex shape, having lost the low-dose shoulder which is characteristic of u.v.-survival curves in many organisms treated under similar conditions, and it is therefore more sensitive to u.v. than a haploid at this low dose range. At approximately the 10% survival level there is a plateau in the survival curve of 2nL, where it is intersected by the haploid survival curve. With increasing dose survival decreases and it is intermediate between haploid and diploid in its sensitivity. It would appear that the characteristic shoulder region of a survival curve has shifted down the curve of 2nL to a higher dose rate than usual.

In Fig. 10 the survival response of 2nL to γ -irradiation is compared with the stock haploid and diploid response. The haploid survival curve is a one-hit response, with a 'tailing-off' effect which is characteristic of haploid yeast (Beam, Mortimer, Wolfe & Tobias, 1954).

Fig. 10. The survival curve of 2nL after gamma irradiation compared with standard haploid and diploid survival curves. The dose rate was 17 krad/min and the vertical bars represent 95 % confidence limits.

The characteristic diploid yeast response to γ -irradiation is the sigmoid curve; 2nL is more sensitive to γ -irradiation than a normal diploid and there is no shoulder in its survival curve.

DISCUSSION

The yeast culture 2nL behaved as a normal diploid during genetic analysis but was distinguishable from other diploid strains on the basis of two separate criteria.

- (1) It had 18 % less DNA than had normal cells, and this low DNA value was independent of the method of DNA estimation, extraction techniques, or any of the parameters which may have influenced estimates of DNA content.
- (2) The culture showed increased sensitivity to both u.v. and γ -irradiation.

Ogur *et al.* (1952) showed that DNA content varied during the budding cycle in yeast and demonstrated that DNA synthesis was completed before visible bud formation. Williamson (1965) also measured the timing of DNA synthesis in the cell cycle and showed that it was

restricted to the first quarter, suggesting that stationary phase yeasts are in G₁. It is possible that a postponement of DNA synthesis in a proportion of the cells of 2nL could account for the low DNA values obtained, but such a misplacement of DNA synthesis at mitosis did not occur in the synchronous culture of this strain.

The growth studies did reveal a disturbed cell division at high cell concentrations although there was no loss of viability. This may have been connected with the low DNA values.

Since the observations of Mirsky & Ris (1951) concerning the wide range in DNA content of gametic nuclei of diploid chromosomal organisms, many other workers have confirmed and extended our knowledge of this variation. Variations in the quantity of DNA/nucleus have been demonstrated within all levels of classification down to the individual (Callan, 1967; Jones & Rees, 1968). Such variations have had various interpretations placed upon them by the different authors. Considering a change from a low to a high DNA content, there are two main hypothetical explanations: (i) that an increase in the number of lateral strands has occurred in the chromosomes; (ii) that extensive longitudinal repetition or accretion of genetic units has occurred.

Both imply that much of the DNA is either repetitive or redundant in the informational sense. Stebbins (1968) put forward a hypothesis associating the quantity of DNA with the genetic regulation of cellular metabolism by means of duplication of genetic information. He also points out that phylogenetic reduction in chromosome number is usually associated with the occurrence of unequal translocations between non-homologous chromosomes. Britten & Kohne (1968) and Britten & Davidson (1969) have discussed repetitive DNA and its possible role in the regulation of development. Walker (1971) has discussed the possible structural importance of satellite DNA to the chromosomes and its possible role in controlling the process of homologous chromosome recognition at meiosis.

If repetitive DNA is at all common in yeast, it would seem likely that parts of it could be lost without lethal effect. Tetrad analysis of 2nL showed that a translocation of *met2* to chromosome VII close to *leu1* and the centromere may have occurred. Such a translocation could have been accompanied by a loss of inessential DNA. The fact that meiosis was undisturbed in 2nL, but of reduced efficiency in the hybrid diploids derived from the crosses between 2nL ascospore cultures and the stock cultures, may indicate a structural role for the missing DNA.

Moustacchi & Williamson (1966) have reported the presence in yeast of satellite DNA bands of different buoyant density from the main nuclear DNA band. One satellite band is associated with the mitochondria, the other is of unknown location. 2nL is not *petite*, but even in *grande* yeasts, the mitochondrial DNA fraction can vary considerably (Moustacchi & Williamson, 1966). The amount of mDNA in normal yeasts is estimated to be in the region of 17% of the total DNA (D. H. Williamson, personal communication). It is certain that there are many copies of mDNA in normal cells. It is possible that 2nL maintains only one or a few copies. This would account both for its *grande* phenotype and for its low DNA content. The other satellite fraction may also be involved in the loss.

The observation that 2nL was intermediate between haploids and diploids in its sensitivity to u.v. and γ -radiations is in agreement with the hypothesis that it has a reduced target or chromosome volume. In classical target theory, lethality is regarded as the inevitable outcome of the accumulation of an appropriate number of physical 'hits' in the target volume (Lea, Haines & Coulson, 1936). No allowance is made for the repair of induced damage. However, the shoulders on many survival curves are now attributed to the existence of repair processes which become saturated (Haynes, 1964, 1966). The absence of shoulders on these survival curves suggests that this strain's sensitivity could equally well be explained

partly or totally by a defective repair system. Thus the lost DNA in this strain may have resulted in its increased sensitivity to radiations.

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