ABNORMAL MITOSES IN TETRAPLOID YEASTS

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Introduction

The unsatisfactory nature of the basic morphological criteria employed by yeast geneticists was elaborately discussed in a few recent contributions (Duraiswami and Subramaniam, 1950; Subramaniam, 1950 *a*, *b*, *c*; 1951) and it was indicated that for any ordered progress in the future a fruitful association with cytology is imperative. The demonstration of spontaneous and induced tetraploidy in yeasts (Subramaniam, 1945, 1947, 1951; Prema Bai and Subramaniam, 1947; Royan and Subramaniam, 1952) necessitated the belief that segregation in tetraploids if assumed as occurring in diploids—as has been done by Winge and Roberts (1950)—would appear really unique.

Winge (1951 *a*) states: "The fact is that a regular reduction of the chromosome complement does not occur in meiotic divisions of autotetraploids and, furthermore, that multipolar spindles lead to a random distribution of the chromosomes so that only rarely will well balanced cells arise" (p. 91). Roman, Hawthorne and Douglas (1951) in a paper which appeared a few months before that of Winge (1951 *a*) visualized the possibility that the exceptional ascus observed by them may have arisen from a tetraploid cell or that "the diploid spores may have been the consequence of an extra division in spore formation". They add: "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 can be obtained" (p. 81). Winge (1951 *b*) admitted that Roman, *et al.* (1951), "had in their material one tetraploid ascus out of a total of 64 giving four diploid spores" (p. 236). While our cytological proof for an induction of polyploidy and the recovery of the diploid from such autotetraploids is brushed aside by Winge on the plea that autotetraploids cannot form normal spores, the results of Roman, *et al.*, are accepted even though they offered no cytological evidence. We would like to emphasize that the observations of Roman, *et al.*, are a confirmation of our earlier suggestion (Subramaniam and Krishna Murthy, 1949; Duraiswami and Subramaniam, 1950).

Lindegren and Lindegren (1951) following up the observations of Roman, *et al.* (1951), now report the existence of tetraploidy in *Saccharomyces*. They

admit a somatic doubling of chromosomes preceding spore formation (p. 890) and confirm our suggestion (Duraiswami and Subramaniam, 1950; Subramaniam, 1950 *a*, 1951) that diploid spores can develop parthenogenetically—a phenomenon denied by Winge (1935)—and that they can in turn form spores. Incidentally their paper also offers support for our claim that the difference between " polyhaploids " and " real haploids " is not merely one of " terminological interest " as claimed by Winge (1951 *a*, p. 90).

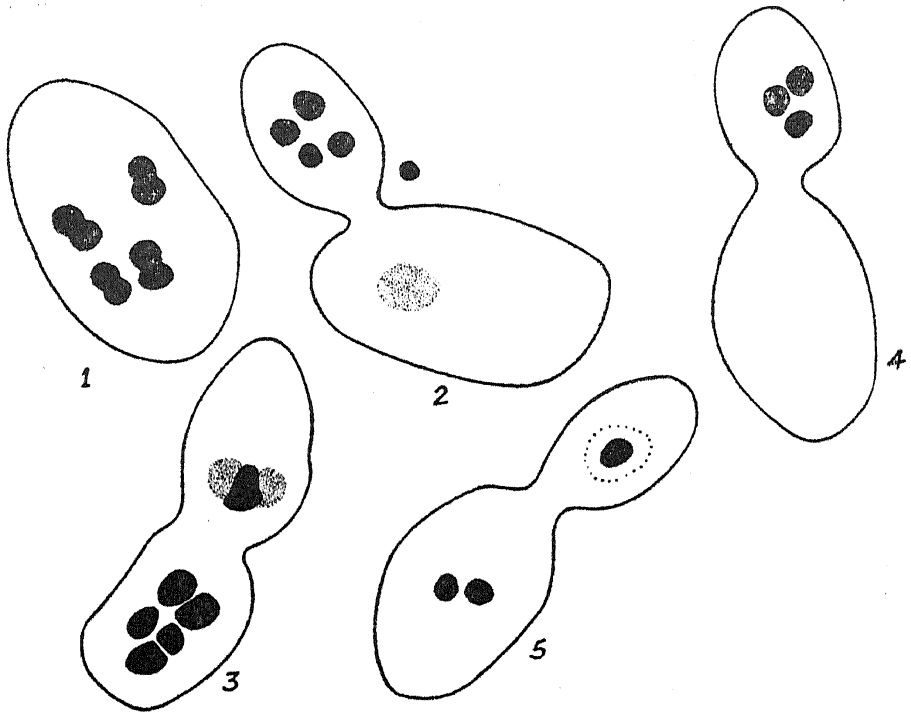
We stated (Subramaniam, 1950 *a*, 1951) that morphological criteria are valueless to differentiate between tetraploids, diploids and haploids. This is borne out by the following statement of Lindegren and Lindegren (1951). "Cultures 11294 and 11296 do not sporulate but the genotypes shown below may be inferred from the data obtained from 11295 and 11297" (p. 889). Winge (1935, 1951 *b*) asserted that his so-called " diploids " could be differentiated from the " haploids " by the ability of the former to form spores. When as reported by Lindegren and Lindegren (1951) tetraploids sporulate and some diploids do not, we presume that in their earlier work they have included diploids and polyploids under their so-called " diploids ".

The occurrence of polyploidy in yeasts furnishes the possibility of an array of genetic combinations ignored up till now by Winge and Lindegren. But that is not all. Even during vegetative divisions there are possibilities of the origin of new types by abnormal mitoses in tetraploids (*cf.* Vaarama, 1949) as was pointed out earlier by Ranganathan and Subramaniam (1948)

OBSERVATIONS

The technique of handling yeast for cytological investigations has already been dealt with elsewhere (Subramaniam, 1948 *a*; Duraiswami and Subramaniam, 1951). The distillery yeast described below was shown earlier to be a natural tetraploid (Ranganathan and Subramaniam, 1948). Plate III Photo 1 (Fig. 1) is the full metaphase of the distillery yeast from a Carnoy iron hæmatoxylin preparation. There are four pairs of chromatids. Cytokinesis in yeasts may or may not synchronize with karyokinesis (Subramaniam, 1946; Ranganathan and Subramaniam, 1948; Duraiswami and Subramaniam, 1951; Royan and Subramaniam, 1952). In Plate III Photo 2 (Fig. 2) the mother cell has a resting nucleus while the bud has four chromosomes. This is the normal behaviour. The chromosomes segregate into equal complements.

A considerable percentage of cells, however, show departures from this procedure. During anaphase the eight chromosomes may segregate into groups of five and three or six and two. In Plate III Photo 3 (Fig. 3) is illustrated a late anaphase from a Feulgen preparation in which the mother



cell has five chromosomes while the bud has only three. While cytokinesis synchronizes with karyokinesis in this instance. in Plate III Photo 4 (Fig. 4) it is absent and the bud alone shows three chromosomes. Since, as is evident in Plate III Photo 3 (Fig. 3) the chromosomes lie in different planes it has been found difficult to get a clear photograph showing a mother cell with six chromosomes and the bud with only two. But in Plate III Photo 5 (Fig. 5) is illustrated a normal mitosis of the diploid originating by such an abnormal mitosis. The mother cell shows two chromosomes, while the bud has a nucleus with a chromatin mass. The behaviour of the diploids, triploids; pentaploids and hexaploids originating by the abnormal mitoses of the tetraploid is elaborately discussed and illustrated elsewhere (Ranganathan and Subramaniam, 1952).

Discussion

The peculiar cytological behaviour of the distillery yeast described by Subramaniam and Ranganathan (1946) has been extensively discussed by Ranganathan and Subramaniam (1948). In that paper we remarked: " It has always been the custom to try to correlate and deduce abnormal behaviour of chromosomes in organisms, tissues or cells from normal patterns observed in innumerable plants and animals " (p. 389). Winge (1951 *a*) ignoring the latter publication selects a few disconnected statements and presents them as follows: " When cells were found with a large number of

bodies as, *e.g.*, 4, 6 and 8 " chromosomes " they believed that they represented the anaphase of the diploid, triploid and tetraploid nuclei although they actually bear little resemblance to a typical anaphase figure. They observed a mother cell with 6 " chromosomes " and its bud with 2 " chromosomes " and this was considered to be due to " lagging " (p. 90).

Lagging of chromosomes was described in yeasts by Levan (1947) and the cytological pictures presented by him bear a strong resemblance to the behaviour of the endopolyploid cells reported by Subramaniam (1948 *b*). Further, Skovsted (1948) from Winge's laboratory suggested chromosome lagging as the probable cause for the deviating yeast types observed after treatment with camphor. It is rather curious that while the above speculation of Skovsted, unsupported as it is by any cytological evidence, has met with the approval of Winge, exception should have been taken to our interpretation of observed abnormal cytological pictures as due to chromosome lagging.

It is difficult to understand Winge's emphasis on the shape and size of chromosomes in yeasts and on what he terms the " anaphase figure ". Chromosomes are defined not on the basis of their size or shape but as " the bodies into which the nucleus resolves itself at the beginning of mitosis and from which it is derived at the end of mitosis " (Darlington, 1932, p. 495). Similarly anaphase is described as " the stage at which the daughter chromosomes move apart in a nuclear division " (Darlington, 1932, p. 493). There is thus no emphasis on any specific type of configuration. We believe that the criteria on the basis of which we identified chromosomes in yeasts are much more acceptable than those of Winge. It would be well to remember that in *Drosophila*, all the chromosomes of a haploid set are not identical either in regard to their size or shape. Without trying the technique of others, Winge (1951 *a*) reproduces some photographs taken some fourteen years back. Not having repeated the experiments of others one can only presume that the views expressed are as old as the photographs themselves.

Lindegren and Lindegren (1951) after substantiating our claims regarding the varying grades of polyploidy remark that their controlled production of triploid and tetraploid yeasts " should not be taken as supporting Subramaniam's views on variations of ploidy " (p. 890). Lindegren's belated admission that triploid and tetraploid yeasts do occur is a tacit admission of the accuracy of our criticism—vigorously contested by Winge (1951 *a*, p. 90)—that since he and Winge analysed segregation in yeasts ignoring the possibility of polyploidy their work was based on a fundamentally erroneous assumption.

SUMMARY

1. The occurrence of tetraploidy in yeasts reported by us several years back finds confirmation in some recent reports regarding the existence of tetraploid *Saccharomyces*. Tetraploidy furnishes the possibility of an array of genetic combinations ignored up till now by Winge and Lindegren.

2. Photomicrographs are presented to illustrate normal and abnormal mitoses in a tetraploid. The origin of diploids, triploids, pentaploids and hexaploids are described.

3. Abnormal mitoses common in tetraploid yeasts offer possibilities regarding the origin of new types. Lindegren's belated admission that triploid and tetraploid yeasts do occur is a tacit admission of the accuracy of our criticism that since he and Winge analysed segregation in yeasts ignoring the possibility of polyploidy, their work was based on a fundamentally erroneous assumption.

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EXPLANATION OF ILLUSTRATIONS

PHOTO 1. (FIG. 1). Full metaphase showing four pairs of chromatids. Carnoy. Iron Hæm Length of cell, 6·1 μ.

PHOTO 2. (FIG. 2). Mother cell with a reconstituted nucleus and bud with four chromosomes. Carnoy. Iron Hæm. Length of mother cell, 6·1 μ.

PHOTO 3. (FIG. 3). Mother cell with five and bud with three chromosomes. Osmic Feulgen. Length of mother cell, 4·9 μ.

PHOTO 4. (FIG. 4). Bud showing three chromosomes at early telophase. Carnoy. Iron Hæm. Length of mother cell with bud, 8·4 μ.

PHOTO 5. (FIG. 5). Mother cell with two chromosomes at early telophase while the bud shows a reconstituted nucleus with a chromatin mass. Osmic Feulgen. Length of mother cell with bud, 8·2 μ.

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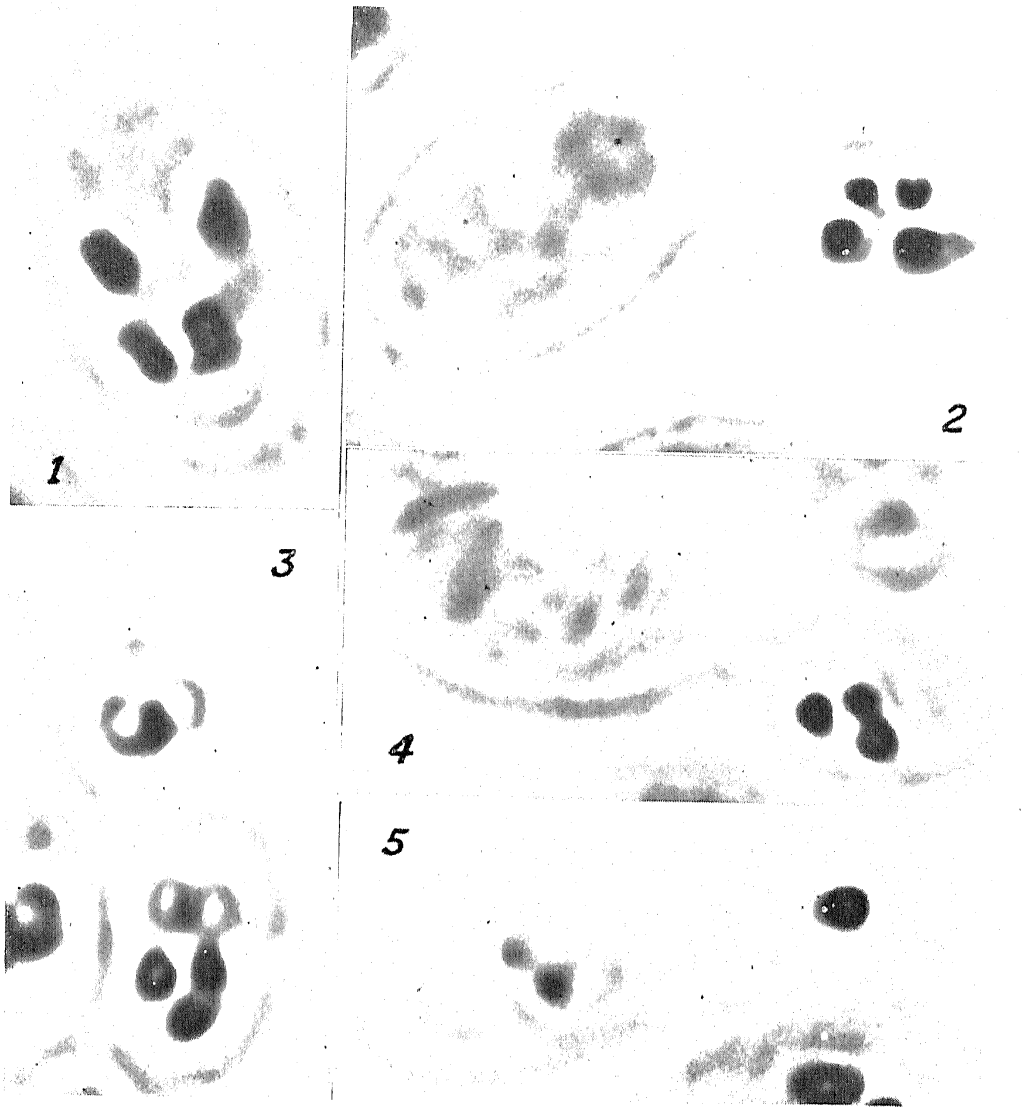
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