CHROMOSOME COUNTS IN A POLYPLOID SERIES OF SACCHAROMYCES[1]

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Received for publication September 10, 1956

Direct determination of chromosome number in Saccharomyces is advantageous in the further development of yeast genetics. The enumeration of centromeres by deductions from tetrad analysis sets a minimum chromosome number (Lindegren, 1949; Lindegren and Lindegren, 1951*b*), but interchromosomal linkage (Shult and Lindegren, 1955) introduces complexities into the deduction of chromosomal numbers from genetic data. Experimental information bearing on the degree of ploidy arises from (1) morphological studies (Winge, 1935; Lindegren, 1949) and the characteristic growth patterns of the different members of the ploidy series (Townsend and Lindegren, 1954), (2) genetical analysis (Lindegren and Lindegren, 1951*a*; Roman *et al.*, 1951), (3) biochemical tests (Ogur *et al.* 1952; and Ogur, 1954*a*, *b*), and (4) irradiation data (Lucke and Sarachek, 1953), and although they are in general agreement, they do not reveal the chromosome number.

The present work describes the direct investigation of the chromosome number of diploid, triploid, and tetraploid Saccharomyces. The problem was made feasible by the convergence of several lines of experience in this laboratory:

(1) The perchloric acid-Giemsa technique of chromatin staining has yielded interpretable nuclear structures in yeast.

(2) Study of sporulation in yeast has indicated that the perchloric-Giemsa stained structures behave in a manner characteristic of chromosomes in higher organisms during meiosis I yielding discrete, rodlike, countable, chromatinic bodies.

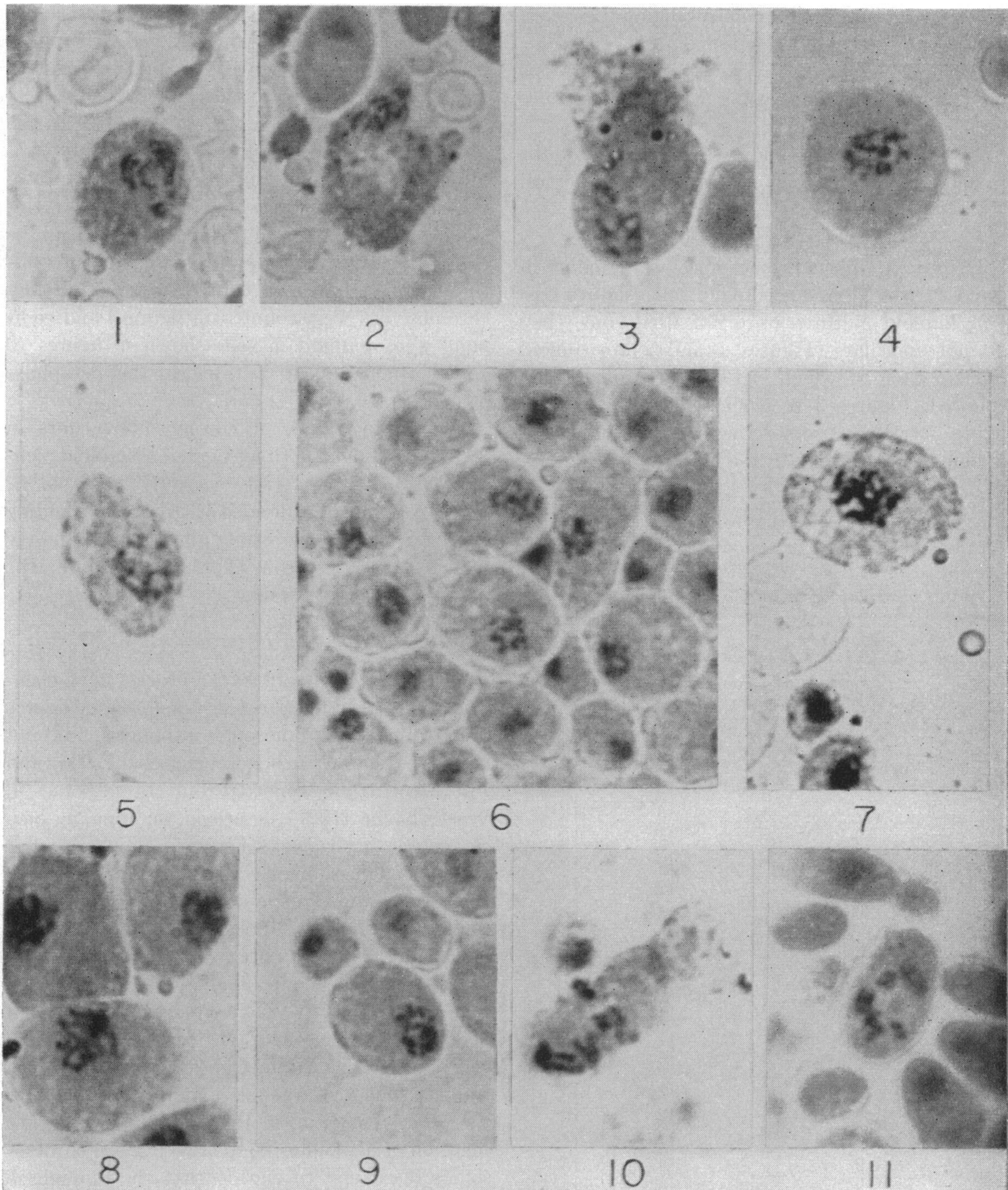
(3) The availability of sporulating diploid, triploid, and tetraploid cultures has made it possible to count chromosomes at several levels of ploidy and to estimate the haploid number as the mean chromosome count divided by the degree of ploidy.

MATERIALS AND METHODS

Three Carbondale strains of Saccharomyces (1) diploid strain 14268 x 8256, (2) triploid strain 11296 x 13894 and (3) tetraploid strain 11294 x 11296 were grown for 24 hr at 30 C on agar slants of the following composition: KH_2PO_4, 2.0 g; $(NH_4)_2SO_4$, 2.0 g; $MgSO_4$, 0.5 g; glucose, 20.0 g; basamin (Anheuser-Busch, Inc.), 5.0 g; agar, 20.0 g; and distilled water, 1,000 ml. These cultures were used to inoculate slants of a sporulation medium similar to the acetate medium of Stantial (1928, 1935) and Elder (1933, 1937) which was described by Adams (1949): sodium acetate, 10.0 g; liquid yeast extract (Anheuser-Busch, Inc.), 2.5 ml; glucose, 1.0 g; agar, 30.0 g; and distilled water, 1,000 ml. These cultures were incubated at 24 C for periods of time varying from 16 to 24 hr. From these cultures, cells in almost every phase of sporulation were obtained. Experience has shown that the chromosomes are most clearly distinguishable during the presporulation phase.

The cells were washed from the slants with approximately 5 ml of distilled water, centrifuged, and the supernatant decanted and stained by the perchloric-Giemsa method (Lindegren *et al.*, 1956). Wet mounts were squashed according to a suggestion by Dr. Jack Shultz (personal communication) as follows: The slide and coverslip were coated lightly with silicone grease by polishing with a tissue after having placed a small amount of silicone stopcock grease on each. One or two loopfuls of a cell suspension were applied to the coated side of the slide, the coverslip applied, and blotted with paper. During the blotting operation, considerable pressure was applied with the thumb in a rotary direction. The coverslip was then hammered vigorously with a small rubber mallet. To prevent drying,

[1] This work was supported by a grant from the American Cancer Society administered by the Committee on Growth, National Research Council.



Figures 1–11. Figures 1 and 2. Diploid Saccharomyces in late prophase of meiosis I showing 8 chromosomes.

Figures 3 to 5. Triploid Saccharomyces in late prophase of meiosis I showing 12, 13, and 11 chromosomes, respectively.

Figure 6. Cells in synchrony.

Figures 7 to 11. Tetraploid Saccharomyces cells in prophase and metaphase. Sixteen chromosomes were counted in the cell shown in figure 9, and 8 pairs were counted in figures 10 and 11, respectively.

the preparations were sealed by ringing the coverslip with immersion oil or permount.

Pictures were taken with a light microscope on panatomic X film using a 560 interference filter.

Chromosomes were counted at all focal depths in cells having distinct, scattered chromosomes.

RESULTS

Definite rodlike chromosomes were found in diploid, triploid, and tetraploid cells (figures 1 to 11); haploid cultures were not used since they do not sporulate. Figures 1 and 2 are diploid cells in each of which eight chromosomes were counted. Figures 3 to 6 show triploid cells. In figure 3 twelve chromosomes were counted, but in figure 4 and 5 thirteen and eleven were counted, respectively. These and other discrepancies in number are probably not due to irregular numbers of chromosomes, but to our inability to resolve them clearly. Figure 6 shows the synchrony of chromosome formation in the presporulation phase of meiosis I. Figures 7 to 11 depict tetraploid cells. Figures 7 and 8 show the nature of the chromosomes preceding metaphase. At this stage they are too long and entangled to be clearly resolved, but they soon shorten into distinct rods as shown in figure 9. Sixteen distinct chromosomes were counted in figure 9 and eight pairs were counted in cells shown in figures 10 and 11 which appeared at or near the metaphase of meiosis I.

TABLE 1

Chromosome counts in the first meiotic prophase of sporulating diploid, triploid and tetraploid cells of Saccharomyces

	Diploid	Triploid	Tetraploid
	9	12	15
	9	11	16
	8	11	13
	10	11	12
	11	12	16
	7	13	13
	9	11	15
	8	13	14
	10	12	13
	9	15	16
	11	10	12
	9	13	17
	8	10	15
	8	11	15
	8	11	16
	7	12	16
	8	12	18
	8	12	16
	8	11	14
	9	14	15
	8	10	13
	10		16
			16
			17
			16
			17
Mean count per cell....	8.7	11.7	15.1
Haploid no.*...........	4.35	3.90	3.78

* Mean haploid no. 4.01.

The mean values of chromosome counts in 20 to 30 diploid, triploid, and tetraploid cells were 8.7, 11.7, and 15.1, respectively (table 1). When each mean is divided by the corresponding degree of ploidy, the whole number most closely fitting the series is 4. This is, therefore, the probable haploid number.

DISCUSSION

Wager (1898) described the process of sporulation in several strains of yeasts with figures illustrating the chromosomes although he did not consider them as such. Swellengrebel (1905) and Fuhrmann (1906) reported four chromosomes in yeast. Kater (1927) described budding in *Saccharomyces cerevisiae* as a mitotic process similar to that of Phaseolus. He believed that the size of the chromosomes made attempts to count them impractical, but stated that there were probably at least twice the number reported by Swellengrebel and Fuhrmann. Badian (1937) observed two Feulgen-positive bodies in yeast cells which he called chromosomes. He stated that during cell division the chromosomes divided by longitudinal splitting—one pair going into the bud, the other remaining in the mother cell. At conjugation of haploid cells, the two pairs of chromosomes fused end to end so that the diplophase cells also contained two chromosomes. Beginning in the sporulation cycle with a cell containing two chromosomes, he showed division into four, then into eight chromosomes followed by the formation of two elongated bodies resembling the bodies described by Wager (1898) and Nagel (1946). Each body divided transversely, then longitudinally, to form four pairs of chromosomes. The protoplast divided to form four spores

with each containing two chromosomes. His figures indicate that he observed an optical section of the chromatin coated spindle (Lindegren, *et al.*, 1956).

Sinoto and Yuasa (1941) and DeLamater (1950) reported four chromosomes in the budding phase of the strains of Saccharomyces which they studied. Levan, however, in 1947 stated that there were at least ten chromosomes. Leitz (1951) found three chromosomes in the haplophase and six in the diplophase of cells of *Saccharomyces priorianus*.

Subramaniam (1948, 1951) states that the haploid number of chromosomes in yeast is one and that variations are due to "endopolyploidy." Mundkur (1954) failed to find discrete chromosomal bodies in vegetative cells fixed by freeze-drying and stained with Feulgen and concluded that there are no microscopically demonstrable chromosomes in yeast and that all previous demonstrations were due to harsh chemical fixatives.

Previous work in this laboratory on the vegetative tetraploid cells (Lindegren *et al.*, 1956) failed to reveal countable chromosomes in contrast to the present success with cells at late prophase of meiosis I.

SUMMARY

Observation of sporulating diploid, triploid, and tetraploid strains of Saccharomyces, fixed with modified Carnoy solution, hydrolyzed in perchloric acid, and stained with Giemsa, revealed nuclei with definite rodlike chromosomes. Counts of the chromosomes when correlated to the corresponding degrees of ploidy showed that the haploid number is 4.

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