I wish to express my thanks to Mr. M. Z. Mahmoud for his kind help during the work.

Y. S. Sabet

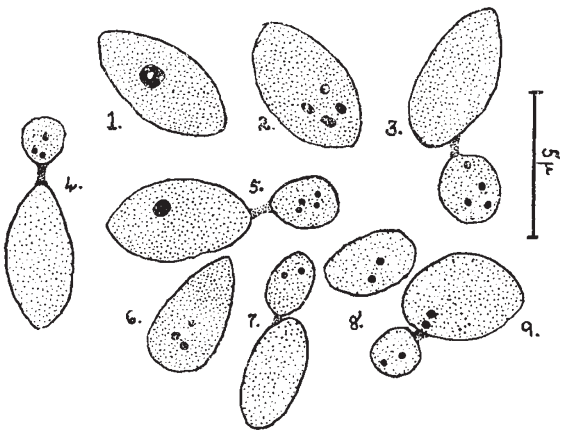
Botany Department, Faculty of Science,
Fouad I University, Cairo. March 1.

Staining the Chromosomes of Yeast by the Feulgen Technique

In spite of the recent controversy[1] whether the Feulgen reaction stains the 'chromosomin' or the desoxyribose nucleic acid in the chromosome, the value of the above test for the identification of chromatin and chromosomes still remains unimpaired. This is more so in the case of the yeast, where volutin granules could easily be confused with chromatin. Kater[2] describes and figures metachromatic granules both in parent and bud during metaphase and anaphase. On the other hand, Caspersson and Brandt[3] showed that while in the resting cells the nucleic acid is confined to the volutin granules, in growing cells these granules disappear and the nucleic acid has a homogeneous distribution in the cytoplasm. The only cytological method of differentiating desoxyribose nucleic acid from the ribose type is said to be by Feulgen's nucleal reaction (Mirsky[4]). Both volutin and chromatin stain by ordinary staining procedures, but only chromatin is stained by the Feulgen technique. Whatever may be the interpretation of the phenomena of nucleal reaction[5], the stained structure in the preparation is identified as chromatin. It appeared to us that a demonstration of the chromosomes of yeasts by the Feulgen technique would establish beyond doubt that yeasts have chromosomes and that these chromosomes conform in their behaviour to similar structures in higher plants.

The material studied was a strain of distillery yeast, the behaviour of the chromosomes of which was interesting[6]. The method of handling the yeast is very important for any successful demonstration of the chromosomes[7]; especially is this the case for demonstration of the chromosomes by the Feulgen technique.

A thin layer of wort in a conical flask was inoculated lightly with a loop of the material from a wort tube culture. After 24 hours a layer of resting cells would usually be found at the bottom of the flask. The supernatant fluid was then poured out, three times its volume of wort added and the flask well shaken in order to ensure uniform distribution. In smears made 50–55 minutes later, under standardized conditions, metaphase and anaphase stages could be seen in the control preparations. Therefore, fresh wet smears were made 50–55 minutes after changing wort, fixed for 30 minutes in osmic vapour[8], dried and stored in 70 per cent alcohol. Seven minutes hydrolysis was found to be ideal, and the slides were kept in a good sample of the stain for 24–48 hours. The slides were counterstained with light green.



In finished slides, the pictures illustrated in the figures were seen. Fig. 1 shows the nucleus in a cell proceeding to division, while the metaphases of the diploid, triploid and the tetraploid are seen in Figs. 8, 6 and 2. The migration of one pair of chromosomes of the diploid to the bud is illustrated in Fig. 9, while Fig. 5 is that of a parent showing a reconstituted nucleus while the chromosomes still remain discrete in the bud. The nucleus of the parent cell appears to lose its power of being stained as it becomes quiescent, and two such examples are illustrated in Figs. 3 and 7, where the attached buds still show discrete chromosomes. As in the control preparations, here also the chromosomes appear lightly glistening.

A comparison of the above with the results already reported[6] would show the similarity of the pictures obtained in Bouin or Carnoy iron hæmatoxylin preparations and in Feulgen's nucleal reaction.

We are very grateful to Sir J. C. Ghosh and Mr. M. Sreenivasaya for their active interest and encouragement.

M. K. Subramaniam
B. Ranganathan

Fermentation Technology Section,
Indian Institute of Science, Bangalore. Nov. 5.

[1] Gulland, J. M., Barker, G. R., and Jordan, D. O., *Ann. Rev. Biochem.*, **14**, 175 (1945).
[2] Kater, J. McA., *Biol. Bull.*, **52**, 436 (1927).
[3] Caspersson, T., and Brandt, K., *Protoplasma*, 35 (1941).
[4] Mirsky, A. E., "Advances in Enzymology", **3**, 1 (1943).
[5] Stedman, E., and Stedman, E., *Nature*, **152**, 267 (1943).
[6] Subramaniam, M. K., and Ranganathan, B., *Nature*, [**157**, 50 (1946)].
[7] Subramaniam, M. K., *Proc. Nat. Inst. Sci.*, in the press.
[8] Robinow, C. F., *Proc. Roy. Soc.*, B, **130**, 299 (1942).

Estimation of Genes in Inheritance of Quantitative Characters

It is well known that the estimation of the number of genes controlling the inheritance of quantitative characters is usually very uncertain. Few workers have published calculations of the number of genes involved, and of these few, "Student" is most frequently quoted (for example, Lush[1], Smith[2]). In 1933, in "The Implications of Winter's Selection Experiment", "Student" estimated[3] that a minimum of 100–300 genes was required to allow the response to selection that Winter attained. However, Fisher, in the same year, pointed out in *Nature*[4] that "Student" had admitted to him in private correspondence "that this calculation fails from over-simplification". In reply to Fisher's criticism, "Student" published his "Calculation of the Minimum Number of Genes in Winter's Selection Experiment"[5]; in which paper he reduced the minimum number to 20–40, an amendment which generally appears to be overlooked.

With regard to his final calculation, "Student" listed the assumptions he made, pointing out particularly those which "cast an element of doubt on the whole calculation". Bearing this in mind, I have made an examination of "Student's" method of estimation.

The following formulæ are applied by "Student" to a normally segregating population, where all genes have equal additive effects. Let the effect due to one gene be k; maximum genetic range, $2n\,k$; genetic standard deviation, $\sqrt{2n\,(\overline{pq} - \sigma_p{}^2)}\,k$, where p and q represent the frequencies of the corresponding alleles, that is, $p + q = 1$. When $p = q = \frac{1}{2}$, as in a normal F_2 generation, the genetic deviation becomes $\sqrt{\frac{n}{2}}\,k$. For simplicity in this discussion, it is assumed that $p = q = \frac{1}{2}$.

If the maximum range and genetic standard deviation are known, then the number of genes segregating may be calculated as follows:

$$\frac{\text{maximum range}}{\text{genetic standard deviation}} = \frac{2n\,k}{\sqrt{\frac{n}{2}}\,k} = 2\sqrt{2n}.$$

$$\therefore\ n = \left(\frac{\text{maximum range}}{\text{genetic standard deviation}}\right)^2 \times \frac{1}{8}.$$

"Student" has applied this formula (correcting for varying frequencies of p and q), using a genetic standard deviation calculated after three generations of selection. This clearly does not represent the genetic deviation due to segregation from the whole population, since much variation was released only after several more generations of selection. Now, in the use of the formulæ as shown above, it is necessary that the standard deviation should be calculated from the same data as the maximum range; otherwise n represents different quantities, n_1 and n_2, in each formula, so that the ratio could not be simplified as "Student" has done. By using data extending over only a fraction of the range, "Student" under-estimates his genetic standard deviation, and by treating this as total genetic deviation he over-estimates his minimum number of genes.

The foregoing discussion is based on the assumption that gene effects were additive. This is not necessarily so, as "Student" pointed out. An examination of the data reveals a correlation between mean oil content and standard deviation. Moreover, the high-selection line has progressed much further from the original mean than has the low-selection line. This indicates probable geometric action of genes and environment, while dominance and varying frequencies of alleles may contribute further complications. The formulæ for maximum range and genetic standard deviation no longer hold, thus making the whole calculation useless, as suspected by "Student".

To summarize: "Student", in his second paper, reduced the minimum number of genes from 100–300 to 20–40. In doing so, he has probably over-estimated the number of genes by using only a portion of the genetic standard deviation. Furthermore, he ignores the obvious geometric genetic effects, and possible dominance deviations which make the whole method of approach inaccurate. It would therefore seem advisable to cease quoting "Student's" estimate of the minimum number of genes in Winter's selection experiment, especially as "Student" himself doubted the validity of the calculation.

S. W. Boyce

Wheat Research Institute,
Christchurch,
New Zealand.
March 7.

[1] Lush, J. L., "Animal Breeding Plans" (Ames, Iowa, 1943).
[2] Smith, H. H., *Bot. Rev.*, **10** (1944).
[3] "Student", *Eugen. Rev.*, **24**, 293 (1933).
[4] Fisher, R. A., *Nature*, **131**, 400 (1933).
[5] "Student", *Ann. Eugen.*, **6**, 77 (1934).

Seed Setting of Sugar Cane in South Africa

It has been generally believed that sugar cane in South Africa does not produce viable pollen and hence fails to set seed. For this reason, sugar cane seed has been imported for the purpose of breeding new varieties from other countries. When the flowers of a number of varieties were examined by me in 1944, however, several had pollen grains which seemed normal and which the iodine test showed to be starch-filled. Pollen grains were found germinating on stigmas under natural conditions, and were also successfully germinated on the agar medium described by Sartoris[1]. Seed collected from the varieties Glagah, Co. 205 and Co. 301 gave in all thirty-six seedlings; these are believed to be the first true sugar cane seedlings raised from seed set on the African continent. Although seedlings from Amu Darya which, like Glagah, is a variety of *Saccharum spontaneum*, had previously been obtained at the Experiment Station, and although some