WHITHER YEAST GENETICS?

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CYTOLOGY could progress independently of genetics. Genetics on the contrary is dependent on cytology especially since varying grades of polyploidy have been shown to occur in higher plants. For the past fifteen years, yeast genetics has been pursuing a solitary course. The lack of correlation between genetics and cytology has resulted in a very interesting situation. There are as many alternative explanations for the same phenomenon as there are workers!

Roman, *et al.*[1] found an exceptional ascus, the spores in which developed directly into vegetative cultures and gave rise in turn to spores which showed a 2 : 2 segregation. This observation is believed to be consistent with the polyploidy hypothesis and they suggest that the irregular ratios reported in the so-called "diploids" (Winge and Roberts[2]; Lindegren[3]) are capable of a rational explanation. Such a behaviour by some strains is not something unique. Postulating a "direct diploidization" (*parthenogamy* of other workers[4]) Winge and Laustsen[5] adduced the above as evidence for an "inbreeding degeneration". Lindegren[6] also records a similar experience. One of his "illegitimate" diploids which ought to have been homozygous gave a 2 : 2 segregation. He remarks: "This suggests that the illegitimate diploid was heterozygous and indicates that copulation in the single ascospore culture had occurred after mutation made the haplophase heterogeneous" (p. 120). Fowell's[7] interpretation of the same phenomenon is at variance with that of the others. He found a baker's yeast producing spores and some of the "haploid" cultures obtained by direct germination of the spores, giving rise in turn to spores. Evidences[7] are offered to show (p. 184) that both the so-called "diploid" and "haploid" cultures can sporulate. Information, however, is not available regarding the segregation ratios in the spores of the so-called "haploids".

The results in Table I thus demonstrate the inadequacy of even the elementary criteria employed by Winge[8] and Lindegren.[9] To evaluate the basic causes responsible for such divergence of views one has to go back to the earlier publications. Winge[8,5] and Lindegren[9] differentiate "haploids" from "diploids" on pure morphology. The necessity for cytological confirmation, though realized by both these investigators, is discarded on specious grounds. There appears to be no unanimity regarding the relative importance of the characteristics themselves. Skovsted[10] from Winge's laboratory revealed the limitations of these criteria when he stated that transformation of a haploid into a diploid is "much easier to confirm on morphological character than the change from diploid to tetraploid" (p. 250). If polyploidy does not occur in yeast, morphology may have been of some value. The alternative interpretation offered by Roman, *et al.*[1] dissipates any hope of classifying yeasts into "haploids" and "diploids" without accurate cytological confirmation. Let us now assess the value of the characters on which so much reliance has been placed.

Spore Germination

Since polyploids and diploids sporulate, the direct germination of a spore is no *prima facie*

TABLE I

Actual observations	Starting material	→ Ascus → Spores →	Veg. cells from direct germination	→ Spores →	2 : 2 segregation
Interpretations :					
Roman, *et al*	Tetraploid	Diploid	Diploid	Haploid	2 : 2
Fowell	Diploid	Haploid	Haploid	?	No details
Winge	Diploid	Haploid	Nuclear fusion giving diploid	Poor viability explained as due to "Inbreeding degeneration"	
Lindegren	Diploid	Haploid	Copulation between cells of the same mating type—"illegitimate" diploid	Haploid	2 : 2 segregation due to mutations before fusion of haploid cells

evidence for haploidy. There is an unsubstantiated assumption in yeast literature that *regular meiosis* precedes spore formation. The observation of Fowell[7] that "haploids" do sporulate and that many of the asci are 4-spored necessitates either the belief, (*a*) that they are not "haploids" or (*b*) that meiosis is not regular. Since Roman, *et al.*[1] reported a 2 : 2 segregation one has to presume that the so-called "haploids" of Fowell,[7] Lindegren[3] and Winge[2] may not be *real haploids* at all.

Cell Size and Shape

Ignoring the statement of Guilliermond[11] that yeast cells are polymorphic, Winge[5] as well as Lindegren[13] have tried to justify the reliability of cell size and shape as a criterion. Winge and Laustsen[12] admit that "the form and size of the cells are very susceptible to various cultural conditions" (p. 113). Curiously enough Winge and Laustsen[14] as well as Lindegren[9] have offered evidence subsequently that cell morphology is itself gene determined. Ditlevsen[15] in fact describes a "diploid" which seems to show the characteristics of the so-called "haploid" cultures. What is more surprising is that Fowell[7] (p. 183) reports unusually large highly vacuolated cells in his "haploid" cultures confirming the many exceptions recorded in yeast literature (Lindegren,[13] p. 209; Winge,[8] pp. 95 and 97).

Mating Type Alleles

The mating type alleles have been brought in as an additional character to identify the so-called "haploids" when they do sporulate (Fowell, p. 184). When he observes unusually large cells in "haploids" Fowell argues that they are still "haploids" because they do not sporulate. But when "haploid" cultures do sporulate he contends that these are still "haploids" because their cells are smaller in size and show a mating reaction when mixed with cultures of the opposite type. Lindegren[9] is well aware of the limitations of this character. His "illegitimate" hybrids are the result of fusion of cells of the same mating type. Roman, *et al.*[1] visualize the possibility that "illegitimate" hybridization between cells of like mating type would give "a certain proportion of *aa* and ^{aa} diploids and the mixing of the two clones by Lindegren's methods should produce tetraploids of the type suggested above" (p. 81).

Sporulation

According to Winge and Laustsen[5] "haploid" yeasts do not sporulate. Lindegren and Lindegren[3] on the other hand observed sporulation in some haploids (p. 128). Winge and Laustsen[12] record asporogenous "diploids" (p. 114) while Fowell[7] agrees with Lindegren that some haploids can sporulate (p. 184). Roman, *et al.*[1] report (p. 80) a 2 : 2 segregation in spores of cultures which would normally have been identified as "haploids". It is this 2 : 2 segregation that appears to have prompted them to give a conventional explanation on the polyploidy hypothesis for the supposed curious segregation claimed in the so-called "diploids".

All the radical theories on "gene conversion", "converter stocks" and "cytoplasmic inheritance" in yeasts have been postulated on the belief that the basic criteria are absolutely correct and incontrovertible. These have been repeatedly asserted without ever considering the possibility of an alternative interpretation. In spite of their divergent views[2,3] on identical problems, Winge and Lindegren have not offered any elucidation even when they recorded several exceptions to their original basic criteria. Entire dependence on morphology is justified on the plea that the confused nature of yeast cytology necessitates such a procedure. Two years back we[16] stated on the basis of purely cytological investigations that much of the work on the genetics of yeasts will have to be re-evaluated in the light of polyploid segregation. That we are right in our approach would be apparent from such a re-appraisal now attempted by Roman, *et al.*[1]

In 1947 and 1948[17,18] we *defined the criteria* for the identification of chromosomes and evaluated some contributions on the cytology of yeasts. Winge and Roberts[19] admitted the validity of our criticisms (p. 311). Winge[20] now states that our cytological investigations are "of a doubtful nature". If as admitted by Winge and Roberts[19] our criteria are acceptable, then our identification of chromosomes based on those criteria ought to be correct. The recent criticism is not based on any *re-definition of nuclei or chromosomes* in yeasts. It appears to be the expression of a personal opinion. The reasons are obvious, in view of the fact that in the paper in which they report the unique phenomenon of the existence of polymeric genes in a strain of whose polyploid nature they admit they are "totally ignorant", they also dispute our demonstration of an induction of polyploidy. Acceptance of our results would render it impossible to characterise their observations as unique.

Winge's[20] criticism that the bodies identified by us as chromosomes are not chromosomes because "they are found scattered in the cytoplasma *at all stages** and in a varying number" is entirely at variance with the facts published by

us.[21,22,23] The pictographic summary published by Subramaniam[21] as far back as 1946 would be sufficient answer that they are not scattered in the cytoplasm *at all stages.*

Induction of polyploidy in yeasts was claimed by Bauch[24] as far back as 1941. It is really surprising that while Winge and Lindegren classify yeast types into "haploid" and "diploid" on the basis of morphology, polyploids identified on the very same morphological criteria by Bauch[24] have received scant attention. The basic cause for all this confusion is lack of organized investigations on the cytology of yeasts. It may be relevant in this connection to remember the salutary comment of Fowell,[7] "There is a deplorable lack of agreement about the identity of chromosomes in yeast, still more about their behaviour during mitosis and meiosis. In the absence of this vital information, it must be considered premature to dismiss all conventional explanations for irregular segregation ratios and even more premature to elaborate unorthodox theories about gene structure and behaviour" (p. 195).

Yeast genetics is thus at the cross roads. Ordered progress in the future depends on a fruitful association with cytology.

* Italics are ours.

1 Roman, H., Hawthorne, D. C., and Douglas, H. C., *Proc. Nat. Acad. Sci., U.S.*, 1951, **37,** 78-84. 2. Wing, O., and Roberts, C., *Nature*, 1950, **165,** 151. 3. Lindegren, C., and Lindegren, G., *Cold Spring. Harb. Symp. Quant, Biol.*, 1946, **11, 115**. 4. Sharp, L. W., *Introduction to Cytology*. 3rd Edn., New York, 1934. 5. Winge, O. and Laustsen, O., *C. R. Lab. Carlsberg. Ser. Physiol*, 1940, **23,** 17. 6. Lindegren, C. C., *Ann. Mo. Bot. Gdn.*, 1945, **32,** 107. 7. Fowell, R. R., *J. Inst. Brewing.*, 1951, **57,** 180. 8. Winge, O., *C. R. Lab. Carlsberg. Ser. Physiol*, 1935. **21,** 77. 9. Lindegren, C. C., *Bact. Revs.*, **1945, 9,** 111. 10. Skovsted, A., *C. R. Lab. Carlsberg. Ser. Physiol*, 1948, **24,** 249. 11. Guilliermond A., *The Yeasts* (Trans. F. W. Tanner), New York, 1920. 12. Winge, O., and Laustsen, O., *C. R. Lab. Carlsberg. Ser. Physiol.* 1937, **22,** 99. 13. Lindegren, C. C., and Lindegren, G., *Ann. Mo. Bot. Gdn.*, 1944, **31,** 203. 14. Winge O., and Laustsen, O., *C. R. Lab, Carlsberg., Ser. Physiol.*, 1339, **22,** 357. 15. Ditlevsen. E., *Ibid.* 1944, **24,** 31. 16. Subramaniam, M. K., and Krishna Murthy, S. N., *Proc. Ind. Acad. Sci.*, 1949, **B30,** 185. 17. Ranganathan, B., and Subramaniam, M. K., *Sci. and Cult.*, 1947, **12,** 478. 18. Subramaniam, M. K., *Proc. Nat. Inst. Sci. India*, 1948, **14,** 315. 19. Winge, O., and Roberts, C., *C. R. Lab. Carlsberg. Ser. Physiol.* 1948, **24,** 263. 20. Winge, O., *Curr. Sci.*, 1951, **21,** 236. 21. Subramaniam, M. K., *Proc. Nat. Inst. Sci. India*, 1946, **12,** 143. 22. —, *Ibid.*, 1947, **13,** 129. 23. Ranganathan, B., and Subramaniam, M. K., *Ibid.*, 1948, **14,** 389. 24. Bauch, R., *Naturwiss.*, 1941, **29**, 687.

HIDDEN WEALTH IN THE WASTE OF MANGANESE MINES

AS there is a boom in the manganese market, jigging operations for concentrating small size ore have been started almost at every mine, since ore fragments of the size of one-fifth of an inch thick are marketable. About 50% of the mines are working the so-called "boulder ore" from dumps and virgin ground.

While large amounts of ore of small size termed "Chili" have been reclaimed from the dumps with the help of the improvised jigs, little attention has been paid so far to the 'beneficiation' of the ore from bigger lumps which constitute the major part of the extensive dumps and the *in situ* ore in the veins of some mines. It has been roughly estimated that about three million tons of ore are recoverable from the waste dumps and probably reserves of about 15 million tons exist in the veins. This refers to low grade ore only in the Nagpur and Chhindwara districts. The waste dumps in the Balaghat and Bhandara districts are even more extensive.

The process of reclaiming the ore from the waste will be quite simple and mechanical. Due to high specific gravity of the manganese ores most of the gangue minerals can be separated and the ore concentrated by making use of any of the methods based on gravity. The smaller mines can employ only crushers and carry out the concentration of ore by jigging. In the case of bigger mines and bigger dumps, beneficiation plants of the heavy media separation type of various capacities can be installed. It is understood that portable units of small size for handling five tons of ore per day are also available.

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